Post-Graduate Degree Programme (CBCS)

in

ZOOLOGY

(M.Sc. Programme)

SEMESTER-II

CHORDATE BIOLOGY, BIOSYSTEMATICS AND TAXONOMY

ZCORT-205

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

Kalyani, Nadia

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CONTENT WRITER:

Sl. No.	Name of Content Writer	Designation	Unit
1.	Dr. Subhabrata Ghosh	Assistant Professor of Zoology, Directorate of Open and Distance Learning, KU	Unit I-X
2.	Dr. Sudeshna Banerjee	Assistant Professor of Zoology, Directorate of Open and Distance Learning, KU	Unit XI-XII
3.	Dr. Subhankar Kumar Sarkar	Associate Professor, Dept. of Zoology, KU	Unit XIII, XIV, XVI-XX
4.	Dr. Kakali Bhadra	Assistant Professor, Dept. Of Zoology, KU	Unit XV

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Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2020 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Manas Kumar Sanyal, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

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Their persistent and coordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

All the Self Learning Materials are self writing and collected from e-book, journals and websites.

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HARD CORE THEORY PAPER (ZCORT–205)

Group A (Chordate Biology)				
Module	**Unit**	**Content**	**Credit**	**Page No.**
ZCORT–207 (Chordate Biology)	I	Blood and cardiovascular system: Blood pressure and baroreceptors, bloodvolume regulation.	**3**	
	II	Cardiac cycle. Myogenic and neurogenic heart, origin and conduction of heart beat, ECG and its implications, neural and chemical regulation of functions of heart.		
	III	Respiratory system: Comparative account of respiratory pigments; transport and exchange of gases.		
	IV	Nervous system: Gross anatomy of brain and spinal cord; cranial nerves, neural control of muscle tone.		
	V	Thermoregulation: Importance of body temperature in animal physiology,heat exchange interactions between animals and environment.		
	VI	Thermoregulation in ectotherms and endotherms, physical, chemical, neuralregulation of body temperature; acclimation and acclimatization.		
	VII	Digestive system: Acquisition of Energy: Types of feeding, Digestion (motility and Secretions), Metabolism, and absorption.		
	VIII	Physiology of gastrointestinal system (mammals) including neural and hormonal regulatory mechanisms.		
	IX	Circulatory systems: General plan, Hemodynamics.		
	X	Cardiovascular response to extreme conditions like exercise, diving and hemorrhage Neural control of cardiovascular system.		
	Group B (Biosystematics and Taxonomy)			

ZCORT–207 (Biosystematics and Taxonomy)	**XI**	Species concept: Biological species concept, difficulties in application of biological species concept.		
	XII	Nomenclature rules, ICZN: The code; amendments and applications; Concept of Type.		
	XIII	Character and character states in taxonomy: Types of character: primitive and advanced, missing, polymorphic, micro, cryptic and internal.		
	XIV	Character state transition, environmental effect and their significances, artifacts and special characters.		
	XV	Taxonomic key: types and their role in classification		
	XVI	Phenetic method of classification a. Numerical phenetics and numerical taxonomy. b. Preparation of data matrix and similarity matrix using distance method (Manhattan distance and Euclidian distance); c. Cluster analysis (different methods)	**3**	
	XVII	Cladistic method of classification - Cladistics and cladogram, terminologies in cladistics.		
	XVIII	Methods of measuring evolutionary transitions c) Homoplasy, parsimony and character conflict.		
	XIX	Polyphasic concept in biosystematics – Biochemical taxonomy, cytotaxonomy and molecular taxonomy and DNA barcoding		
	XX	Phylogenetic trees: construction and analysis; types.		
	Total counseling session 18hrs.			

UNIT-I

Blood and cardiovascular system: Blood pressure and baroreceptors, blood volume regulation.

Objective: In this unit you will learn about blood pressure, how blood volume is regulated and also about different aspects of vertebrate cardiovascular system.

Introduction: The circulatory system is of two types: open of closed. In open circulatory system, blood pumped by the heart passes through large vessels into open spaces or body cavities called sinuses. This type of system is present in Arthropods and Molluscs.

In closed circulatory system the blood pumped by the heart is always circulated through a closed network of blood vessels. This system is more advantageous as the fluid is regulated in better ways. The closed circulatory system is present in Annelids and Chordates.All vertebrates have a muscular heart. Fishes possess a 2-chambered heart with an arterium and a ventricle. Lung fishes and amphibians have a 3-chambered heart with 2 atria and one ventricle. Reptiles except crocodiles have 3-chambered heart with 2 atria and partially divided single ventricle whereas crocodiles, birds and mammals have a 4-chambered heart with 2 atria and 2 ventricles.

In fishes the heart pumps out deoxygenated blood which is oxygenated by the gills and sent to the body parts from where deoxygenated blood is carried to the heart. It is called single circulation.In lung fishes, amphibians and reptiles, the left atrium gets oxygenated blood from the ills/lungs/skin/buccopharyngeal cavity and the right atrium receives the deoxygenated blood from other body parts. But both oxygenated and deoxygenated blood gets mixed up in single ventricle which pumps out mixed blood.

Types of Circulatory Systems:

There are two types of circulatory Systems.

1. Open circulatory System:

It is called open system because the blood vessels open into spaces and not into capillaries. The main blood vessels arising from the heart, pour the blood into tissue spaces called sinuses. This is common in prawns, crabs, spiders and insects.

2. Closed circulatory system:

It is common in Annelids, Invertebrates and all vertebrates including human beings. In this type of circulatory system, blood remains in blood vessels and never coming in direct contact with tissue cells. The strong pumping action of heart makes the blood flow rapidly and with pressure in the arteries.

Importance of the Circulating System:

1. It carries food substances to the all body cells.

2. Carries absorbed oxygen to each cell of the body.

3. Caring back carbon dioxide to the lungs.

4. Helping in removal of harmful chemical substance from the body.

Circulatory System of Human Beings:

Blood:

Blood, as you know, is a liquid connective tissue that circulates in a closed system of blood vessels.An adult man has about five to six liters of blood, while a woman, on an average, has about one liter less.Our blood consists of (i) solid elements—which include red blood corpuscles (RBCs), white blood corpuscles (WBCs), and blood platelets, and (ii) liquid element—the plasma.The corpuscles comprise about 45% and the plasma about 55% of the volume of blood.

Plasma:

Plasma is a straw-coloured liquid in which the RBCs, WBCs and platelets float. It contains mainly water, in which are dissolved various substances such as plasma proteins, food substances (amino acids, glucose, and fats), nitrogenous compounds and ions of sodium, potassium, calcium, magnesium and phosphorus.

Blood corpuscles:

Blood is red in colour due to the presence of RBCs. The RBCs contain the red-coloured respiratory pigment haemoglobin. This iron-protein compound transports oxygen from the lungs to the tissues. RBCs also transport carbon dioxide. WBCs protect the body from infection. Platelets help in the clotting of blood.Visit a diagnostic centre. Give your blood sample. Get it checked for the level of haemoglobin.

The normal range of haemoglobin in humans is 120-180 g/L, or 12-18 g/L, of blood. Check your blood report to see if your haemoglobin level fails in this range. But haemoglobin levels also depend on age, sex and ethnic values of a place. For example, females have a lower normal value of haemoglobin level than males.

A below-normal level of haemoglobin may indicate anemia due to a number of possible causes. Low haemoglobin level could be from actual loss of blood from haemorrhage, vitamin deficiencies, lack of iron in the diet or a disease. This indicates that the person's cells are not getting enough oxygen for energy production. Such anemic persons always feel tired and weak.

You can even obtain the normal range of hemoglobin level in animals such as cows, buffaloes, goats, etc., by visiting a veterinary clinic. This value is lower in these animals than in humans. The normal hemoglobin level in cows lies in the range of (5.9 ± 1.54) g/L of blood.

Blood clotting by platelets:

You must have noticed that after a cut the skin bleeds for a while, and then the blood thickens to form a clot. This process takes place as a result of a series of reactions in the blood. These reactions are started by the release of an enzyme by the circulating platelets.The clot, which forms at the point of the wound, is a microscopic network of insoluble fibrous protein. It minimizes the loss of blood. If blood is lost, it leads to a loss of pressure by the pumping heart.

Functions of blood:

1. Transport of respiratory gases:

Blood carries oxygen from the lungs to the tissues. It also carries carbon dioxide from the tissues to the lungs.

2. Transport of nutrients:

Absorbed in the small intestine enter the blood capillaries. Blood carries these nutrients and distributes them to all parts of the body.

3. Transport of waste products:

Waste products of the body, such as urea, uric acid, etc., are carried by blood to the excretory organs.

4**. Regulation of water content of cells:**

Blood regulates the water content of the cells when the water content in cells increases, blood takes up the excess amount of cellular water. Blood provides water to cells when they need it.

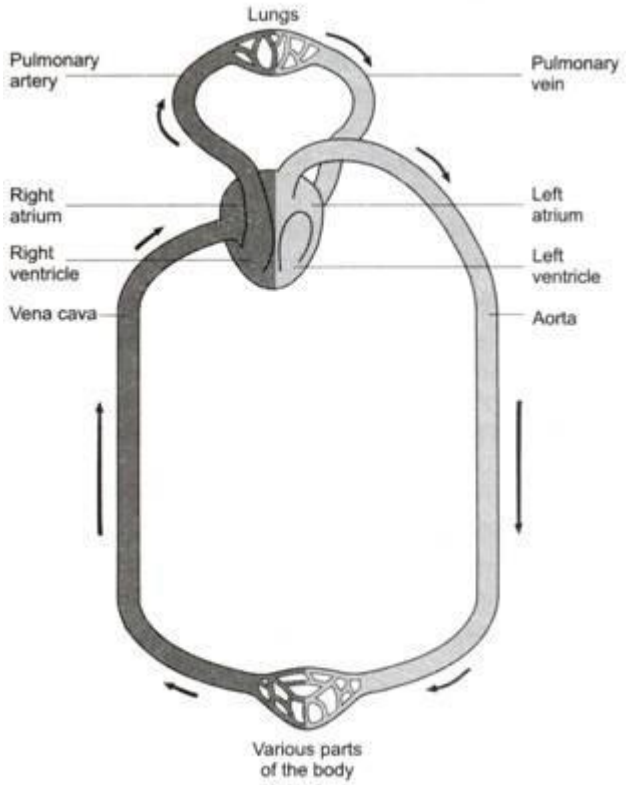
5. Regulation of body temperature:

Increased body temperature resulting from the excess respiration of a particular tissue is equalized by circulation of blood.

6. Defence against infection:

Blood protects the body against infection.

7. Prevention of bleeding:
Clotting blood prevents excess bleeding.



Route of blood circulation

Blood Vessels:

Three types of blood vessels, namely, arteries, veins and capillaries, are involved in blood circulation. They are all connected to form one continuous closed system.

Arteries:
The arteries are wide, elastic and thick-walled vessels as they carry blood away from the heart to the limbs and organs of the body. They have thick, elastic walls to withstand the high pressure of the blood emerging from the heart.

Veins:
Veins bring back blood from the tissues and organs to the heart. The blood in veins flows under less pressure than that in arteries. Therefore, veins do not have thick walls. But veins can accommodate more blood. Veins have valves that allow blood to flow in one direction only.

Capillaries:
Arteries branch out into smaller and thinner blood vessels called arterioles. These divide into still smaller vessels to provide blood to all the cells. The thinnest blood vessels are called capillaries. Their walls have just one layer of squalors cells.

These walls are permeable, so that water and dissolved substances pass in and out, exchanging oxygen, carbon dioxide, dissolved nutrients and waste products with the tissues around the capillaries. The capillaries form a dense network, reaching out to each and every part of the body. The flow of blood is very slow in capillaries. They join to form venules and veins, which return blood from organs and tissues to the heart.

Comparative Anatomy of Hearts

A. Heart of Fish:

a. Location:

Heart is situated ventral to the oesophagus in the pericardial section of the coelom.

b. Covering:

The heart is covered by a transparent protective covering, called pericardium. It is a single layer in fish. Within pericardi-um there is a pericardial fluid, protects the heart from the external injury.

c. Structure: Heart of fishes consists of 3-chambers — a sinus venosus, a single auricle and a single ventricle. No conus arteriosus. The embryonic heart of fishes consists of 4 chambers which include sinus venosus, auricle, ventricle and bulbus cordis but in adult the term conus arteriosus may be used instead of bulbus cordis if it possesses cardiac muscles**.** But according to Hildebrand (1982) , the bulbus arterious is found in the potion of cinus (Conu arteriosus) in teleosts which does not possess cardiac muscle but is highly elastic and passively evens the flow of blood into the afferent branchial arteries.

Sinus Venosus: Sinus venosus is a thin walled sac. It receives deoxygenated blood by two precaval veins or ductus Cuveiri. It opens to the auricle by sinuauricular aperture, guarded by valves.

Auricle: Auricle is a thin walled single chamber of the heart. It opens into the ventricle by auriculoventricular aperture. This aperture is guarded by valves. Sinus venosus and auricle both constitute the receiving chambers of the heart.

Ventricle: Single, conical, thick- walled, forwarding chamber of the heart.

Conus arteriosus: There is no conus arteriosus. The bulbus aorta is a dilated part at the base of the ventral aorta, and not regarded as the part of the heart. It is the part of the arterial system.

B. Heart of Amphibia:

Location: Heart is located mid ventrally in the anterior part of the body cavity.

Covering: The heart is covered by a transparent protective covering, called pericardium. It is a single layer in fish. Within pericardium there is a pericardial fluid, protects the heart from the external injury.

Structures: 5 chambers. Sinus venosus, two auricles, single ventricle and conus arteriosus. Out of 5 chambers, the two auricles and single ventricle are regarded as permanent chambers, and sinus venosus and conus arteriosus are considered as accessory chambers.

Sinus Venosus: It is a dorsally placed, thin walled triangular sac, formed by the union of two precavals and a post caval. It receives deoxygenated blood by three venacavae. It opens into right auricle through sinuauricular aperture which is guarded by sinuauricular valve. It is well developed.

Auricle: There are two unequal sized auricles. The left auricle is smaller than the right. Two auricles and a sinus venosus are the receiving parts of the heart. The two auricles are placed anterior to the ventricle. These auricles are separated internally by inter-auricular septum. Both the auricles are sharply marked off from the ventricle externally by a narrow constriction, called coronary sulcus.The left auricle receives oxygenated blood from the lungs, through two pulmonary veins. The right auricle receives deoxygenated blood from the sinus venosus through sinuauricular aperture. Two auricles open into the ventricle by a common auriculoventricular aperture.This aperture is guarded by membranous valves, called auriculoventricular valves. The valves remain attached with the wall of the ventricle by fine thread-like Chordae tendineae.

Ventricle: Single, thick walled highly muscular forwarding chamber, with the apex pointed towards the caudal end. The inner wall of the ventricle is thrown into muscular ridges, known as columnaecarnae.

Conus Arteriosus: From the base of the ventricle arises a stout tube-like structure called conus arteriosus (Pylangium) which proceeds forward as truncus arteriosus (Synangium). The lumen of the conus arteriosus is divided into two Channels by a spiral valve.The left channel of the spiral valve is known as cavum pulmocutanum and the right one is called cavum aorticum. Each branch of truncus arteriosus gives three arches, known as carotid, systemic and pulmonary. The deoxygenated blood passes

through the cavum pulmocutaneum to the lungs through the pulmonary arch and less oxygenated blood travels through the cavum aorticum to the different parts of the body and cephalic region. The truncus arteriosus is not to be considered as the part of the heart. It is the basal stem of the three main arteries.

Mechanism of Circulation: The right auricle receives deoxygenated blood and left auricle receives oxygenated blood. The two auricles contract and the blood is driven to the ventricle. The blood mixes into the lumen of the ventricle and by contraction, reaches into the conus arteriosus.As the conus arises from right side, a large quantity of deoxygenated blood goes to the cavum pulmocutaneoum, then goes to the pulmocutaneous arteries. The mixed blood from the middle region of the ventricle goes to the systemic arches though the cavum aorticum and lastly the oxygenated blood goes to the carotid arteries. Ultimately the spiral valve helps in the entry of blood into different arches. Therefore, it presents a transitional stage due to separation of auricle.

d. Heart of Reptiles :

Location: Heart is located in the anterior part of the thoracic cavity.

Covering: The heart is covered by a transparent protective covering, called pericardium. It is a single layer in fish. Within pericardium there is a pericardial fluid, protects the heart from the external injury.

Structures: In Calotes, there are 3 permanent chambers — two auricles and an incompletely divided ventricle, but there is no conus arteriosus (Fig. 10.144D). In crocodiles, the heart is completely 4-chambered — the two auricles, and two completely divided ventricles. In crocodiles, no sinus venosus and conus arteriosus (Fig. 10.144E).

Sinus Venosus: The sinus venosus of reptiles represents from larger size (turtles) to small or vestigial in other groups. It is a thin walled triangular receiving chamber placed dorsal to the auricles. It receives deoxygenated blood by three venae cavae, two anterior and one posterior. It opens into right auricle through sinuauricular aperture, guarded by sinuauricular aperture. In crocodile the sinus venosus is absent. In lizards the sinus venosus is the first chamber and contains the pace-maker.

Auricle: There are two auricles, known as right and left auricles. These are thin walled receiving chambers and placed anterior to the venricle. The auricular region is wider than the venricular portion and right auricle is larger than the left.The inner lining of the right auricle is provided with a number of muscular ridges, known as musculipectinati. It receives the deoxygenated blood. The left auricle receives oxygenated blood through a common pulmonary vein. The pulmonary aperture is circular in outline and not provided with valves. Internally the two auricles are separated by an inter auricular septum which extends posteriorly within ventricle and possesses its tip auriculoventricular valves.The two auricles open into the ventricle by a common

auriculoventricular aperture. In the crocodiles all the structures of the auricles are same except two separate auriculoventricular apertures.

Ventricle: Incompletely divided, thick- walled, highly muscular chamber. The lumen of the ventricle is provided with an incompletely divided inter ventricular septum in most groups except crocodiles. In crocodiles this septum is complete. The two sides of the crocodilian heart are connected by an aperture, which connects between the bases of the left and right aortic trunks.The inner cavity of the ventricle in lizards has been arbitrarily divided into three regions, namely cavum pulmonale in the right side, cavum arteriosum in the left side and cavum venosum in the middle. The right part of the inter-ventricular septum is called Cavum ventrale and the left part of the inter-ventricular septum is called cavum dorsale.In crocodiles 4-chambered heart with only two aortic arches are seen. In crocodiles the pulmonary trunk and left aortic arch develop from the right ventricle and only right aortic arch develops from the left ventricle.

Conus arteriosus: Absent

Mechanism of Circulation: The heart of lizard represents a transitional stage which approaches a double circuit stage but has not reached it completely due to lacking of complete separation of the ventricle.In crocodiles the heart is completely 4-chambered. The right part always gets deoxygenated blood and the left part gets oxygenated part. All the apertures are guarded by muscular valves that prevent the back flow of blood. The deoxygenated blood goes to the lungs through the pulmonary aorta from the right ventricle.The oxygenated blood of the left ventricle constitutes the major circuit over the body. The blood circulation represents a double circuit stage, which attains maximum completely among reptiles.

e. Heart of Aves :

Location: Heart is situated in the thoracic cavity between the two lungs and is slightly towards the left side.

Covering: The heart is covered by a transparent protective covering, called pericardium. It is a single layer in fish. Within pericardium there is a pericardial fluid, protects the heart from the external injury.

Structures:4 chambered heart. No conus arteriosus and vestigial sinus venosus. Right ventricle partly covers the left .

Sinus Venosus: Vestigial.

Auricle: Auricles are comparatively thick walled receiving chamber and placed anterior to ventricle. The two auricles are separated internally by inter-auricular septum. The wall of the right auricle bears sinuauricular node or pace-maker and the atrial septum

bears auriculoventricular node (AV node).The right auricle receives deoxygenated blood from three caval veins and the left auricle receives oxygenated blood through four pulmonary veins. The right auricle opens into the right ventricle by a right auriculo ventricular aperture which is guarded by a single muscular flap-like valve. The left auriculoventricular aperture is provided with 3 valve which is composed of two cusps.

Ventricle: Ventricle is a thick-walled, highly muscular forwarding chamber of the heart. It is divided into two by a complete muscular septum. The lumen of the ventricle is thrown into muscular ridges, called columnaecarnae. Only two aortic arches originate from the ventricle. The right ventricle gives rise to pulmonary arch and the left ventricle gives rise to single right aortic arch.The opening of the arches are guarded by three cup-like semi-lumar valves. The right auriculo- ventricular aperture is guarded by a single valve but the left auriculo ventricular aperture is guarded by a membranous valve and provided with two cusps (bicuspids).

Conus Arteriosus: Absent.

Mechanism of Circulation: The heart of pigeon is a double circuit heart and there is no chance of mixing up of deoxygenated and oxygenated blood. The circulation represents an evolutionary advancement in birds over reptiles and its working efficiency has reached maximum.

f. Heart of Mammals:

Location: Heart is situated in the thoracic cavity between the two lungs and is slightly towards the left.

Covering: It is a double layered. The inner layer is called visceral layer which is highly vascularized and outer connective tissue layer, called peritoneal layer.

Structures:4 chambered heart. No conus arteriosus and vestigial sinus venosus. Right ventricle partly covers the left (Fig. 10.144F). The sinus venosus is absent in adult mammals.

Sinus Venosus: Absent. The embryonic sinus venosus is merged with the right atrium in the adult stage.

Auricle: Comparatively thick walled receiving chamber. The right auricle receives deoxygenated blood from the body through the anterior and posterior vena cavae. The three venae cavae open separately into the right auricle. In between the apertures of the two anterior venae cavae and to some extent guarding the posterior vena cava is the Eustachian valve.The blood of the walls of the heart is brought to the auricle by means of an aperture, coronary sinus in the right auricle. The opening of coronary sinus is guarded by a coronary valve. The left auricle receives oxygenated blood from the lungs

through four pulmonary veins. The inter-auricular septum bears an oval- shaped depression, called fossa ovalis. This depression indicates the position of foramen ovale which was the aperture during the embryonic stage.This aperture becomes closed before the birth of the animal. The fossa ovalis is surrounded by an annular-shaped prominent ridge, called annulus ovalis. The two auricles open into the ventricles by separate apertures. The left auriculo-ventricular aperture is provided with bicuspid or mitral valve. The lumen of the left ventricle is provided with a number of muscular ridges, called trabeculae carnae. The right auriculo-ventricular aperture or ostrium is provided with a tricuspid valve which is composed of three cusps.

Ventricle: Ventricle is a thick-walled, highly muscular forwarding chamber of the heart. It is divided into two by a complete muscular septum. The lumen of the ventricle is thrown into muscular ridges, called columnaecarnae. Only two aortic arches originate from the ventricle. The right ventricle gives rise to pulmonary arch and the left ventricle gives rise to single right aortic arch. The opening of the arches are guarded by three cup-like semi-lumar valves. The right auriculo- ventricular aperture is guarded by a single valve but the left auriculo ventricular aperture is guarded by a membranous valve and pro- vided with two cusps (bicuspids). Except the pulmonary trunk arises from the right ventricle and the left aortic arch arises from the left ventricle.

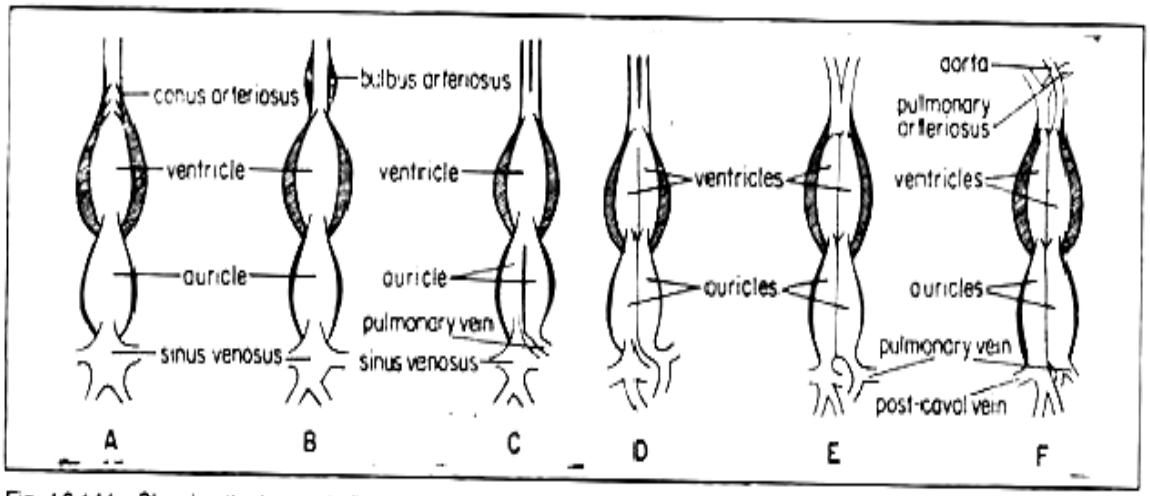


Fig. 10.144 : Showing the basic similarities in the structural plan of vertebrate hearts. A = Elasmobranchs, B = Teleost, C = Amphibia, D = Reptilia (typical), E = Crocodile and F = Birds and Mammals. Note the gradual elimination of sinus venosus and conus arteriosus in course of evolution and acquisition of two auricles and two ventricles.

Conus Arteriosus: Absent.

Mechanism of Circulation: The heart of pigeon is a double circuit heart and there is no chance of mixing up of deoxygenated and oxygenated blood. The circulation represents an evolutionary advancement in birds over reptiles and its working efficiency has reached maximum.

The Human heart:

Structure:

The heart is a muscular, conical and dark red organ that plays the role of a pump in the circulatory system. Its pumping action maintains the circulation of blood.

In man, the heart weighs about 0.43 per cent of the body weight. It is located in the middle of the thoracic cavity, but its apex is tilted towards the left side. The heart is enclosed in the pericardium, a tough, inflexible membrane. Between the heart and the pericardium is a fluid which reduces the friction produced during heartbeat.

The heart is made up of cardiac muscles. These muscles contract with considerable force, squeezing the blood out into the arteries. The heart beats nonstop throughout one's life. It is due to the rhythmic contraction and relaxation of the heart muscles. There are four chambers in the heart—two atria, with thin walls, and two ventricles, with thick walls.

Working of the heart:

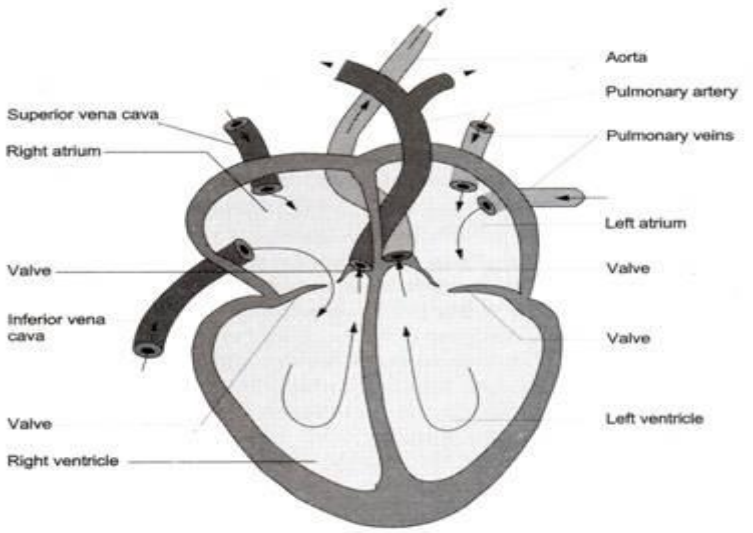
Blood from different parts of the body comes to the right atrium when it expands. This impure blood is brought from the upper part of the body through the superior vena cava and from the lower part of the body through the inferior vena cava.

As the right atrium contracts, the blood goes to the right ventricle, which dilates. The atrioventricular aperture is closed by a valve after the blood transfer. Valves prevent the backflow of blood when the atria or ventricles contract.

When the right ventricle contracts, the blood is forced out to the lungs for oxygenation through the pulmonary artery, guarded by another valve. In the lungs, there is an exchange of oxygen and carbon dioxide. After the blood has received oxygen from the lungs and given off carbon dioxide, the oxygenated blood returns to the left atrium.

Pulmonary veins bring this oxygenated blood from the lungs to the left atrium, as it relaxes. When the left atrium contracts, blood is transferred to the left ventricle, which expands. The aperture between the left atrium and left ventricle is guarded by a valve. The wall of the left ventricle is three or four times thicker than the wall of the right ventricle, as it pumps blood to the body.

When the left ventricle contracts, the oxygen-rich blood is pumped into the aorta for circulation to different parts of the body. The opening of the aorta is also guarded by a valve. Deoxygenated blood is collected from different parts of the body by small veins. These open into larger veins, which bring the blood back to the right atrium.



Internal structure of the human heart

Cardiac cycle:

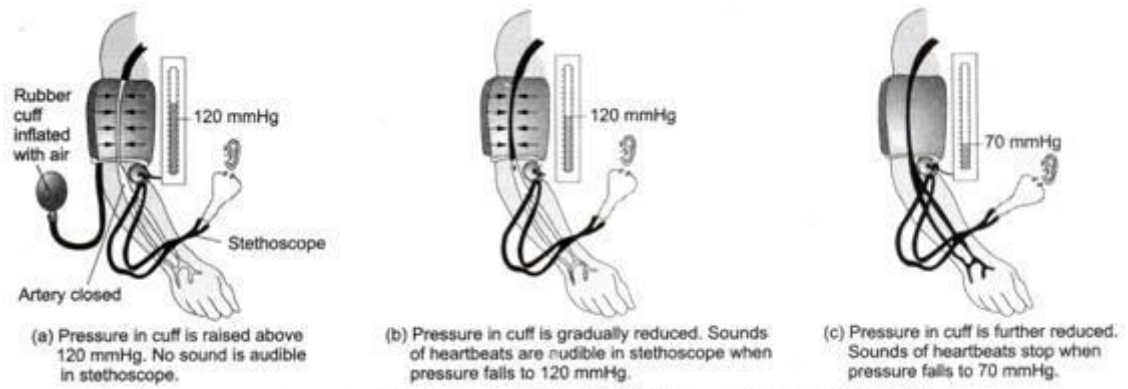
One sequence of the filling of the heart with blood and its pumping is called the cardiac cycle. The phase of contraction of the ventricle is called systole and its relaxation phase is called diastole.

Blood pressure:

As blood flows, it exerts a force on the walls of the blood vessels. This is much greater in the arteries than in the veins. The pressure of flow of blood in the aorta and its main branches is defined as blood pressure. The heart has to develop a high pressure so that blood can be pumped through the arteries, capillaries and veins.

During the ventricular contraction, or systolic phase, it is equal to that exerted by a column of 120 mm of mercury. During the ventricular relaxation, or diastolic phase, it is about 80 mmHg.Thus, the normal blood pressure is said to be '120/80'. However, the blood pressure varies from person to person and is affected by age, sex, heredity, physical and emotional states, and other factors.

An instrument called sphygmomanometer is used to measure blood pressure. Abnormally high blood pressure is called hypertension. It may be associated with a disease or may occur due to anxiety.During hypertension, the arterioles get constricted and increase resistance to blood flow. High blood pressure can cause the rupture of blood vessels, internal bleeding or stroke. If a blood vessel is ruptured in the brain, that part does not get blood, oxygen and nutrients, and loses its function.



Measuring blood pressure (here '120/70') by a sphygmomanometer

Meaning of Blood Pressure:

Blood pressure is defined as the lateral pressure exerted on the vessel wall by column of flowing blood or it is also termed as end arterial pressure.

Systolic blood pressure is defined as maximum pressure that can be recorded in arteries during ventricular systole. In a normal adult of 20 years it ranges from 100 to 140 mm Hg and the mean pressure is 120 mm Hg.

Diastolic blood pressure is the minimum pressure that can be recorded in arteries during ventricular diastole. In a normal adult of 20 years, it ranges from 60 to 90 mm Hg and the mean will be 80 mm Hg.

Mean arterial pressure is the mean pressure in the arteries during a cardiac cycle. It is diastolic pressure plus one-third of pulse pressure. So it will be 80 + 40/3 = about 94 mm Hg.

Pulse pressure is the difference between systolic and diastolic pressure. So it is 120 – 80 = 40 mm Hg.

Blood pressure is the lateral pressure exerted by blood on the vessel walls while flowing through it.

Four terms are in common use:

i. Systolic Pressure (S.P.):

The maximum pressure during systole.

ii. Diastolic Pressure (D.P.):

The minimum pressure during diastole.

iii. Pulse Pressure (P.P.):

The difference between systolic and accepted diastolic pressure.

iv. Mean Pressure (M.P.):
It is roughly the arithmetic mean of the diastolic and the systolic pressure.

But a close approximation to the mean pressure may be obtained by adding the diastolic pressure with one-third of the pulse pressure. In true sense it is the level of the line halving area between the pulse wave contour and the diastolic pressure level.

In adults the relation between the three pressures is as follows:
S.P/D.P./P.P = 3/2/1, viz., if systolic pressure be 120, diastolic pressure should be 80 and pulse pressure 40 mm of Hg.

Basal Blood Pressure:
As blood pressure differs from an individual to another one and under different circumstances it varies in the same individual, it is permissible to use the term normal range of blood pressure. When an individual is with the least possible amount of strain or stress, basal blood pressure is generally considered. It may also be regarded as the lowest pressure necessary in maintaining blood flow sufficient for needs of the body.

When a subject is in reclining state, 5 – 6 hours after last meal, in a comfortably warm room, after resting for at least 30 – 40 minutes and with a mind at possible ease, the basal pressure is obtained. In adult males, the average systolic pressure 125 – 130 mm of Hg ± 15 (viz., from 110 – 145 mm of Hg) and average diastolic pressure, 70 – 90 mm of Hg.

Although it is constant in a given individual, yet basal pressure varies in different ones with the following factors:

I. Physiological Variations:

i. Age:

Blood pressure rises with age. During infancy, the systolic pressure is from 70-90 mm of Hg; childhood, 90-110 mm of Hg; puberty, 110-120 mm of Hg; old age, 140-150 mm of Hg. At any age, a systolic pressure persistently above 150 mm of Hg and a diastolic pressure above 100 mm of Hg should be accepted as high. On the other hand, systolic pressure below 100 mm of Hg and diastolic below 50 should be taken as low in the adults. Recent observations indicate that the pressure which is reached in adolescence does not normally rise with age any more.

The average systolic pressure is 110-120 mm of Hg and diastolic pressure 70-80 mm of Hg. The normal upper limits of systolic and diastolic pressures are placed at 140 and 90 mm of Hg respectively.Average blood pressure and standard deviations in apparently healthy persons (assuming diastolic end point is disappearance of sound) is listed in Table 7.7.

Table 7.7 Average blood pressure and standard deviations in apparently healthy persons

Age group	Males		Females	
	Systolic	Diastolic	Systolic	Diastolic
20–29	124.0 ± 13.2	77.0 ± 9.5	116.5 ± 11.6	73.0 ± 9.4
30–39	126.5 ± 13.9	79.5 ± 10.0	122.0 ± 14.0	76.5 ± 10.4
40–49	129.5 ± 16.0	81.5 ± 10.2	129.0 ± 18.3	81.0 ± 11.1
50–59	136.5 ± 19.0	83.5 ± 11.4	138.0 ± 21.4	84.0 ± 12.0
60–69	142.5 ± 23.5	84.0 ± 11.2	149.0 ± 25.7	85.0 ± 13.4
70–79	145.5 ± 24.0	81.5 ± 14.1	158.5 ± 26.0	84.5 ± 14.2
80–89	145.0 ± 25.0	80.5 ± 12.4	155.5 ± 28.0	82.5 ± 15.2

ii. Sex:
In females both systolic and diastolic pressures are slightly lower than in males up to the age of 45-50 years.

iii. Build:
The systolic pressure is usually high in obese person. In most of the overweight persons the blood pressure is found to be high.

iv. Exercise:
In strenuous exercise the systolic pressure rises and may reach even up to 180 mm of Hg. In moderate exercise there is slight rise of systolic blood pressure.

v. Posture:
The diastolic pressure is slightly higher in the standing position. In the recumbent position the diastolic pressure is lower than in the standing or in the sitting position.

vi. Sleep:
The systolic pressure falls by about 15 to 20 mm of Hg during sleep.

vii. After Ingestion of Meals:
There is a slight rise of systolic pressure.

viii. Emotion of Excitement:
It causes increase of systolic pressure.

II. Significance of Blood Pressure:

i. Systolic Pressure:
It undergoes considerable fluctuations. Excitements, exercise, males, etc., increase it, while sleep, rest etc., and diminish it.

The height of systolic pressure indicates:
(a) The extent of work done by heart,

(b) The force with which the heart is working, and

(c) The degree of pressure which the arterial walls have to withstand.

ii. Diastolic Pressure:
It undergoes much less fluctuations in health and remains within a limited range. Increase of diastolic pressure indicates that the heart is approaching towards its failure. Consequently, variations of diastolic pressure are of greater prognostic importance than those of systolic. Diastolic pressure is the measure of peripheral resistance. It indicates the constant load against which heart has to work.

iii. Pulse Pressure:
It generally varies directly as the stroke volume. But this quantitative relation may not be true in all cases.

III. Normal Function of Blood Pressure:
i. To maintain a sufficient pressure head to keep the blood flowing.

ii. To provide for the motive force of filtration at the capillary bed, thus assuring nutrition to the tissue cells, formation of urine, lymph and so on.

From the above considerations, it is seen that, the height of blood pressure gives correct information's about the state of the circulatory system as a whole and also about the functional condition of the tissue cells and organs.

IV. Measurement and Recording of Blood Pressure:

A. Arterial Blood Pressure:
This can be measured by two methods:
(a) Direct, and

(b) Indirect.

(a) Direct Method:
The artery is exposed and an arterial cannula of which one tapering end is inserted directly into the lumen of the exposed vessel and the other end is connected to the U-shaped mercury manometer that shows the actual blood pressure in mm of Hg. As the mercury column in one limb (that has direct contact with the blood vessel) descends and the other limb of the U-tube ascends, the value in the scale will be doubled so as to get the actual blood pressure. For convenience it is generally considered to be 1 mm in the scale equivalent to 2 mm of Hg of pressure.

Before recording the blood pressure, the mercury levels in both limbs of the U-tube must be adjusted to the 0 mark of the scale. For recording pressure a floating stylus with

a writing pointer that marks on the smoked paper may be used (Fig. 7.85). This method is only suitable in animals and gives the idea about mean pressure. Due to high inertia of the mercury, the blood pressure changes associated with cardiac cycle are damped. Respiratory undulation of mean pressure waves are clearly seen in this direct method.

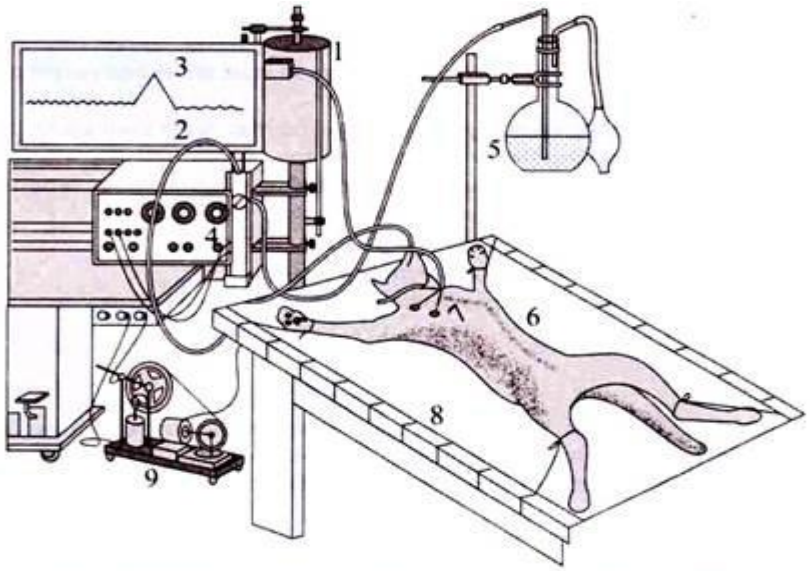


Fig. 7.85 Direct method for measurement of arterial blood pressure. 1=Kymograph. 2=floating stylus for blood pressure recording. 3=stylus for respiration recording. 4=mercury manometer. 5=reservoir. 6=animal (cat). 7=artery cannula. 8=operation table. 9=respiratory pump.

(b) Indirect Method:

In indirect method, the pressure may be measured without any surgical procedure and thus it is very convenient clinically in human being. Riva-Roci (1896) first introduced this indirect method and afterwards Korotkoff (1905) introduced a convenient method by which the systolic and diastolic pressure could be ascertained only through listening to a sound. This is the standard method of recording blood pressure all through-out the world.

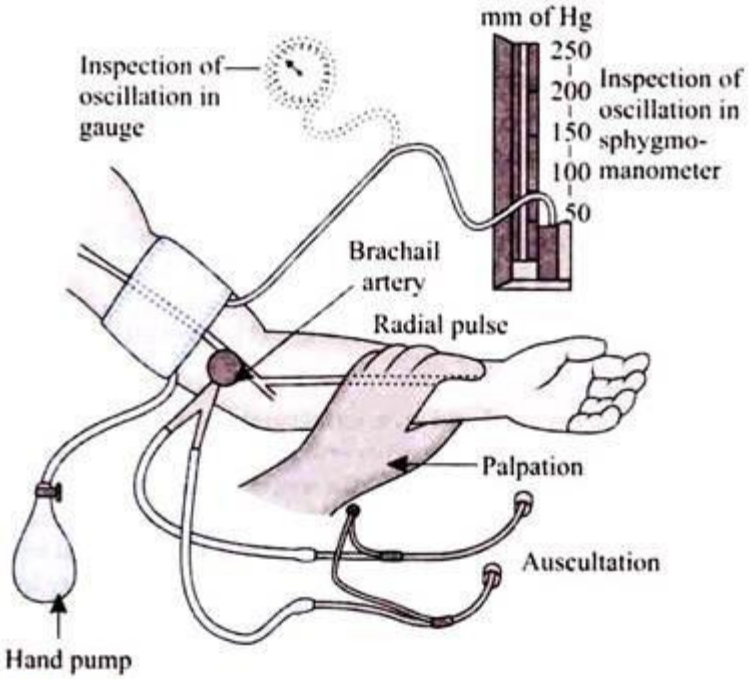


Fig. 7.86 Measurement of arterial blood pressure in human beings

In this method commonly the pressure of the brachial artery is measured. The instrument used is known as Sphygmomanometer.

Three methods:

1. Oscillatory,

2. Palpatory, and

3. Auscultatory.

1. Oscillatory Method:

Inspection of oscillation in spring gauge or mercury manometer is the basis of this method. In this method a pressure cuff is wrapped over the brachial artery and the oscillations that are produced by the pulsations are observed. The instrument is always kept at the heart level. When the cuff pressure is increased and raised above the systolic pressure, the oscillations disappear, but on releasing the pressure gradually, the oscillations become larger and prominent.The pressure head, at which the larger oscillations are seen, is considered as systolic pressure. But on further release of pressure, the oscillations become smaller and disappeared. The pressure, at which the oscillation just becomes smaller or disappears, is known as diastolic pressure.

2. Palpatory Method:

The instrument is kept at the level of the heart and the cuff is tied round the upper arm. Pressure is raised to 200 mm of Hg and then gradually released. When the pulse just

appears at the wrist, the pressure is noted. This is the systolic pressure. This method is not accurate. By this method the diastolic pressure cannot be determined.

3. Auscultatory Method:
The instrument is kept at the level of the heart and the cuff is tied round the upper arm. Pressure is raised to 200 mm of Hg and then gradually released. Variations of sounds are heard with a Stethoscope placing its chest piece on the brachial artery, a little below the cuff.

The sounds are heard due to occurrence of turbulence in the flow of blood through the narrowed blood vessels when the manometric pressure just coincides with the systolic blood pressure. Due to giving air pressure in the cuff, the vessel is pressed and blood flow is obliterated.

But while releasing the air pressure gradually, blood just begins to flow through the narrowed blood vessels and the pattern of flow is changed from streamline flow (silent) to turbulent flow (noisy). When the pressure is further released, normal streamline flow sets in and the sound is no longer heard. At this point manometric pressure coincides with the diastolic blood pressure.

So as the pressure is released the following variations of sounds are heard:
i. First Phase:
Sudden appearance of a clear tapping sound. This indicates systolic pressure. It persists while the pressure falls through 15 mm of Hg.

ii. Second Phase:
The tap sound is replaced by a murmur persisting for another 15 mm of Hg.

iii. Third Phase:
The murmur is replaced by a clear loud gong sound lasting for the next 20 mm of Hg.

iv. Fourth Phase:
The loud sound suddenly becomes muffled and rapidly begins to fade. This point indicates diastolic pressure.

v. Fifth Phase:
Absence of all sounds

Factors Controlling Arterial Blood Pressure:

i. Pumping Action of the Heart:
Effectual contraction of the heart is the main factor for controlling the cardiac output, blood pressure and flow within the blood vessel. Because in each effectual contraction of the ventricle certain amount of blood is ejected out into the aorta. The driving force of blood is mainly created by the pumping action of the heart. The efficiency of the heart is considered upon how much amount of blood is driven out by the heart into the aorta in each beat.

ii. Cardiac Output:
Alterations of cardiac output will alter blood pressure. Cardiac output depends upon venous return, force and frequency of heart beat. Blood volume affects blood pressure directly, by mainly modifying the cardiac output.

iii. Peripheral Resistance:
It is the resistance which blood has to overcome while passing through the periphery. The chief seat of peripheral resistance is the arterioles and to a smaller extent the capillaries (vide below).

Peripheral resistance depends on the following:
(a) Velocity of blood,

(b) Viscosity of blood,

(c) Elasticity of arterial walls, and

(d) Lumen of blood vessels.

Resistance is directly proportional to the first two and inversely to the last two factors:

(a) Velocity:
A rapidly flowing stream will have more frictional effect than a slower one. Hence, pressure is high in the aorta but low in the capillaries.

(b) Viscosity:
Other factors remaining constant, a more viscid blood will have a higher friction than a lesser one. For this reason, plasma transfusion is sometimes more effective to maintain blood pressure than ordinary saline.

(c) Elasticity:
Due to elastic properties in the arteries can dilate and accommodate considerable amount of blood with relatively less rise of blood pressure. In old age, the arterial walls become stiff. Hence, blood pressure rises.

(d) Lumen of the Vessel:
Peripheral resistance is inversely proportional to the lumen of the vessel. In other words, smaller the vessel, higher will be the resistance. One should expect therefore that the capillaries, having the smallest lumen, should have the highest pressure.

But this is not the case. Because the velocity of blood being lowest in the capillaries, the frictional effect is very low. Hence, the pressure is also low. The seat of peripheral resistance is found to be chiefly in the arterioles, where the velocity is fairly high and the lumen is narrow.

$$\text{Peripheral resistance} \propto \frac{\text{Mean arterial pressure}}{\text{Cardiac output}}$$

Mean arterial pressure can be expressed in dynes per square centimetre by multiplying the pressure in mm of Hg by 1,332.

iv. Elasticity of the Arterial Walls:
In normal diastolic pressure arterial walls are stretched but due to the presence of elastic tissues in their walls, they tend to recoil. Due to elasticity of the arterial walls the blood flow is pulsatile in the arteries. In the capillaries and venules the flow is continuous. In old age the expansion of the arterial walls becomes limited due to sclerotic changes and the blood pressure rises.

v. Blood Volume:
Increase in blood volume will raise both the systolic and diastolic blood pressures due to the increased quantity of blood in the arterial system and greater stretching of the arterial walls.

vi. Viscosity of the Blood:
Alteration in blood viscosity will affect the diastolic pressure by its effect on the peripheral resistance. The intramolecular friction is greater when the viscosity is high.

B. Venous Pressure:
It is the pressure which is exerted by the blood within the veins. Average venous pressure of human being in recumbent position is about 60-120 cm of H_2O. The venous pressure can be measured by inserting a needle directly into the anticubital vein and by connecting the needle to a water manometer (Fig. 7.87). Venous pressure is a valuable index in determining the efficiency of the heart.

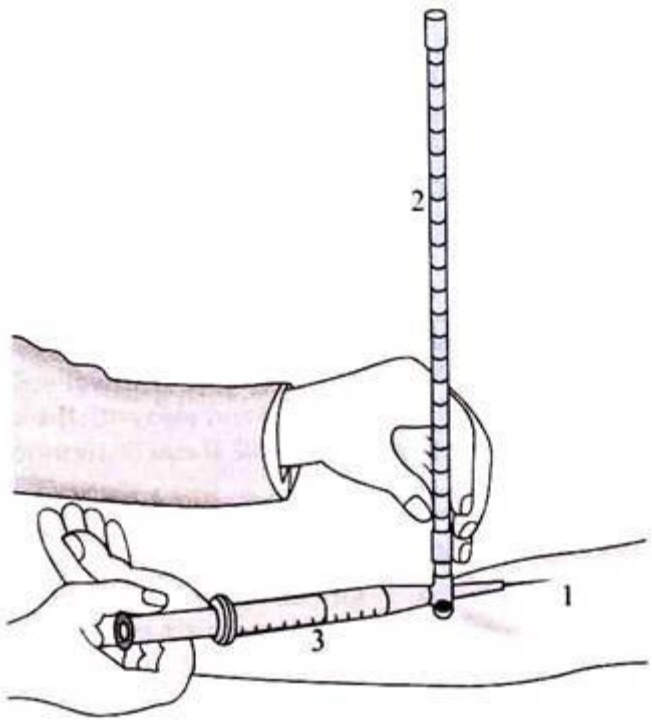


Fig. 7.87 Use of water manometer for measurement of venous pressure. 1=anti-cubital vein. 2=manometer. 3=syringe.

Poiseuille's Law:

In 1841 the French Physician J.L.M. Poiseuille studied the factors regulating the flow of viscous fluid through the capillary tubes. He showed that resistance to blood flow in any blood vessel proportionally varies directly with the viscosity of the blood and also with the length of the blood vessel, and inversely with the fourth power of the radius of the blood vessel.

It can be represented by the following formula:

$R = \eta l/r^4 \times 8/\pi$, where R stands for resistance to blood flow, η for viscosity of blood, l for length of blood vessels, r for radius of the blood vessel, 8 for Hagen's integration and π factor for a cylindrical tube.

Taking this value for resistance in the formula it is found that blood flow proportionally varies directly with the blood pressure and the fourth power of the radius of the blood vessel, and inversely with the viscosity of the blood and length of the blood vessel.

The following formula, known as poiseuille's law, expresses the above relations:

$BF = K\ BP/R$ or $BF = BP \times (\pi/8) \times (1/\eta) \times (r^4/l)$, where BF stands for blood flow, BP for blood pressure, r for radius of the blood Vessel, η for viscosity of blood, l for length of the blood vessel and π for 3.14. This law is not applicable when the arterial pressure falls below 20 mm of Hg.

Adjustment of Blood Pressure:
In normal individual the constancy of the internal environment is being adjusted by the well-organised controlling system—which is called Milieu interieur after Claude Bernard and Homoeostasis after Cannon. Adjustment of blood pressure, according to the needs of the body, may be carried out by the several complex reflexes whose centres are lying in the cerebral cortex formatio reticularis, hypothalamus, medullary and spinal vasomotor centres. The (I) efferent and (II) afferent pathways constituting the above reflexes are lying within the sympathetic and parasympathetic nervous systems whose activities are modified by the hypothalamus and other centres.

I. Efferent Pathways of this Self-adjustment or Homoeostasis of Blood Pressure:
These are the vagi and the sympathetic nerves which control the blood pressure by:
(a) Modifying the cardiac activity,

(b) Altering the cardiac output, and

(c) Altering the lumen of the blood vessels.

The relative activities of the vagi and the sympathetic of the efferent pathways are under the control of vasomotor systems, which are described below:
Vasomotor System:
This system consists of:
i. Vasomotor centre,

ii. Vasoconstrictor nerves, and

iii. Vasodilator nerves.

They supply vasomotor nerves—mainly to the arterioles but to some extent to the capillaries and venules. This vasomotor centre is highly developed in higher animals and human beings. In infants and children it is imperfect. By regulating the radius of the blood vessels this system takes part in adjusting blood pressure and blood supply to a particular part. It also plays an immense role in heart regulation.

i. Vasomotor Centre (V.M.C.):
Vasomotor centre is situated on the floor of the fourth ventricle in the reticular formation at the level of the calamus scriptorius. It extends from the lower part of the pons to the obex and forms a diffuse network of neurones. After section of the brain stem at the level of the calamus scriptorius there is fall of blood pressure.

There are practically two areas in the reticular formation of the medulla:
a. Pressor centre—which causes rise of blood pressure.

b. Depressor centre—which causes fall of blood pressure.

The depressor centre is not the vasodilator centre. This centre causes inhibition of the vasoconstrictor tone. The depressor centre relays the inhibitory impulses to the pressor centre. Pressor and depressor centres form one functional unit and it is defined as the vasomotor centre. The vasomotor centre discharges impulses which pass down the lateral white column of the spinal cord in the cervical, thoracic and lumbar segments of the spinal cord and form synaptic connections with the lateral horn cells of the spinal cord.

Vasomotor Reflexes:

a. Depressor Reflex:

Blood pressure falls due to diffuse dilatation of the arterioles. Rise of blood pressure stimulates the baroreceptors of the carotid sinuses and aortic arch, and causes slowing of the heart and arteriolar dilatation. The vasodilatation is due to inhibition of vasoconstrictor effect of the sympathetic.

b. Pressor Reflex:

Blood pressure rises due to diffuse constriction of the arterioles. Diminution of blood pressure fails to stimulate the baroreceptors of the carotid sinuses and aortic arch, and the parasympathetic inhibitory tone over the heart and blood vessels is withdrawn. Blood pressure is raised reflexly through overactivity of the sympathetic. Vasoconstriction of the arterioles is due to activity of the vasoconstrictor centre. Reflex vasoconstriction also occurs due to stimulation of chemoreceptors during the fall of blood pressure.

Control of V.M.C:

Vasomotor centre is under the superior control of cerebral cortex and hypothalamus (Fig. 7.88).

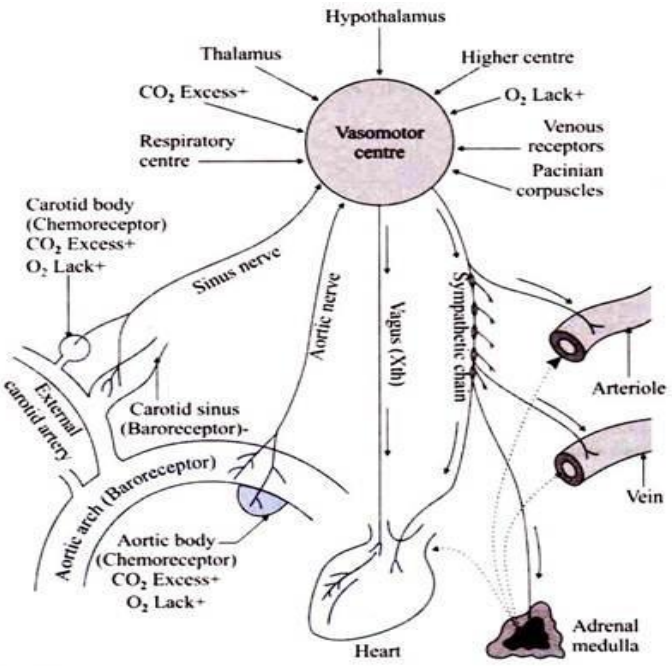


Fig. 7.88 Different factors that influence the vasomotor centre (Diagrammatic representation).

Factors influencing V.M.C. have been described as follows:

a. Higher Centre (Including Hypothalamus):
Emotion generally stimulates, causing vasoconstriction. But shock may depress the centre—leading to a sudden fall of blood pressure and fainting (vasovagal attacks).

b. Respiration:
During inspiration systemic blood pressure is generally decreased but increased during expiration. This is due to the decrease of left ventricular cardiac output during inspiration. Reverse is the effect during expiration. There is no evidence of direct respiratory centre—effect on vasomotor centre.

c. CO_2 Excess:
Excess stimulates. The action is mainly on the centre but partly reflexly through the sino-aortic nerves.

d. O_2 Lack:
Generally stimulates V.M.C. The effect is mainly reflex through the sino-aortic nerves and slightly direct on the centre.

e. Sino-Aortic Nerves:
Variations of blood pressure, CO_2 tension, O_2 tension, etc., reflexly regulate the activity of the vasomotor centre through the sino-aortic nerves. Normally, a stream of inhibitory impulses is carried up by these nerves depressing the vasomotor centre. When blood pressure rises, V.M.C. is depressed, vasodilatation occurs and further rise of blood

pressure is checked. When blood pressure falls, the centre is released causing vasoconstriction and raising blood pressure. [Sino-aortic nerves also control cardiac centre, respiratory centre and adrenaline secretion]

f. Other Afferents:
Local vasomotor tone is altered by afferent nerves originating from different baroreceptor and chemoreceptor areas, distributed all throughout the body. The baroreceptors are located in the right atrium, in the left atrium and left ventricle, in the pulmonary arch of aorta, in the junction of the superior thyroid artery and common carotied artery, junction of the subclavian artery and common carotid artery, and all throughout common carotid artery in between the superior thyroid artery and subclavian artery, mesenteric blood vessels (Pacinian corpuscles), thoracic arch of the aorta and in the central vein (venous receptor).

The chemoreceptors are located in the ventricular cavity and all throughout the blood vessels wall. Reactive hyperaemia is the consequence of local chemoreceptor activity on the blood vessels wall by the accumulated metabolites. Heat dilates and cold constricts the skin vessels, reflexly.

ii. Vasoconstrictor Nerves:
The fibres pass along the sympathetic outflow from the first thoracic to the second lumbar segments.

Brief details are as follows:
a. To the Skin and Muscles:
Pass out through the grey rami communicants—to the mixed spinal nerves— and finally distributed through ordinary motor and sensory nerves. The distribution is strictly unilateral, stopping sharply at the midline.

b. To the Head and Neck:
Come from the first to the fourth thoracic segments—enter the superior cervical ganglion from which postganglionic fibres arise and pass along the carotid artery and its branches.

c. To the Fore Limbs:
Arise from the fourth to tenth thoracic segments—enter the stellate ganglion from which the postganglionic fibres arise and pass along the spinal nerves and supply the blood vessels.

d. To the Hind Limbs:
Arise from the eleventh thoracic to the second lumbar segments—relay in the lower lumbar and upper sacral ganglia, the postganglionic fibres accompany the nerves of the sacral plexus.

e. To the Abdominal Viscera:
From the lower thoracic and upper two lumbar segments—pass through the splanchnic nerves to coeliac ganglion-the postganglionic fibres pass along the blood vessels.

f. To the Thoracic Viscera:
Heart receives constrictor fibres through the vagus; lungs form the sympathetic.

iii. Vasodilator Nerves:

There are three types of vasodilator nerves:
1. Parasympathetic (Craniosacral) Vasodilators:
i. Cranial:
a. Chorda tympani—to the sub-maxillary or sub-mandibular gland,

b. Lesser superficial petrosal-to the parotid gland, and

c. Lingual—to the vessels of tongue.

ii. Sacral:
Nervi erigentes—to the vessels of genitalia.

2. Sympathetic Vasodilators:
Sympathetic fibres are mostly vasoconstrictor in nature. But some vasodilators are also present.

For instance:
i. The dilator fibres of the coronary vessels come through the sympathetic.

ii. Sympathetic dilator fibres have been demonstrated in the peripheral nerves in human beings.

iii. Stimulation of the last anterior thoracic root produces dilatation of the kidney vessels.

iv. Stimulation of the right splanchnic nerve sometimes causes vasodilatation and fall of blood pressure.

3. Antidromic Vasodilators:
Antidromic vasodilators in the posterior spinal root (Fig. 7.89). When posterior spinal root is cut, distal to the ganglion and the peripheral end is stimulated—although the nerve is afferent, yet the vessels in the periphery-both skin and muscles, dilate (axon reflex). In the skin, it is due to liberation of histamine and as such produces the typical triple response; dilatation, flare and wheal. In the muscle it liberates acetylcholine and thereby causes vasodilatation.

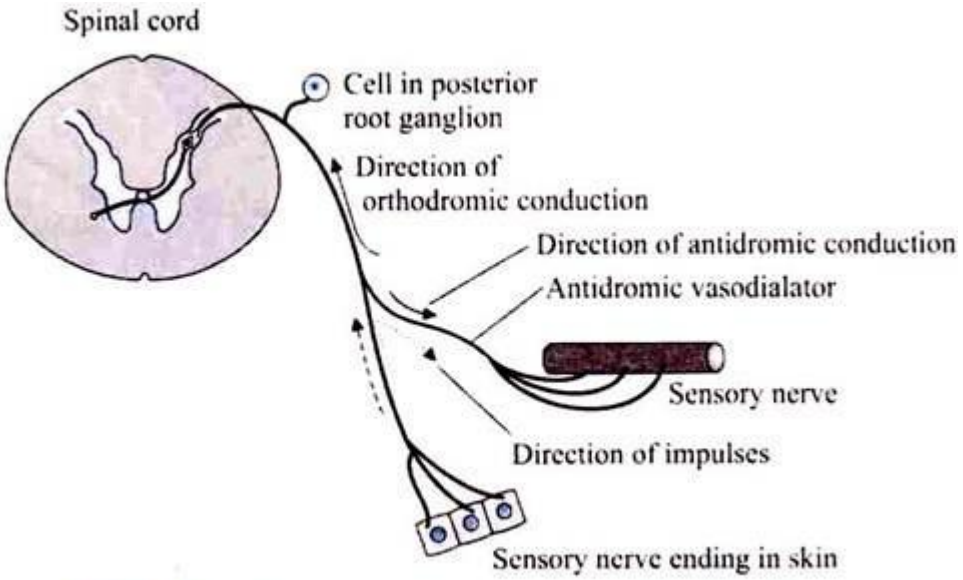


Fig. 7.89 Antidromic nerve fibres in the posterior nerve root.

II. Afferent Pathways:

They are lying in two sets of receptors that carry information of the instantaneous circulatory status to the centre.

These sensory receptors are:

(1) Chemoreceptors, and

(2) Baroreceptors distributed all throughout the cardiovascular system.

The relative roles of the different afferent pathways have been described under separate headings, viz.:

(a) Sino-aortic mechanisms controlling systemic blood pressure and flow, and

(b) Vasocular receptors other than Sino-aortic—controlling mostly the local blood pressure and flow.

(a) Role of Sino-Aortic Mechanism in the Regulation of Normal Blood Pressure:

From the above, it is evident that blood pressure can be adjusted according to the needs of the body in various ways. Of all the factors, the Sino-aortic mechanism plays the chief role. The Sino-aortic mechanism is carried on by baroreceptors and chemoreceptors. This mechanism regulates blood pressure by adjusting the heart rate, vasomotor centre, and secretion of adrenaline and noradrenaline. It also adjusts respiratory centre in such a way that the functions of heart and respiration may run parallel.

Sino-Aortic Mechanism:

A. Baroreceptors:

This includes carotid sinus and aortic arch (Fig. 7.90).

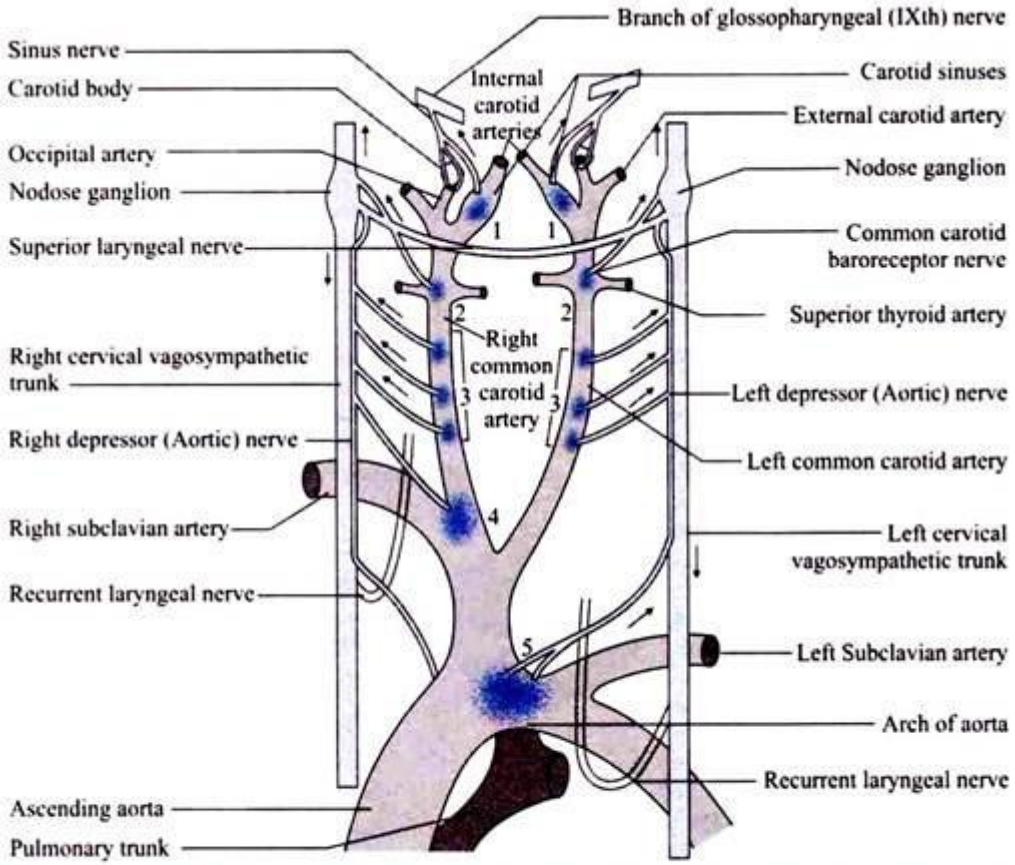


Fig. 7.90 Diagram represents the distribution of different baroreceptors on the walls of the blood vessels. 1=carotid sinus. 2=baroreceptors at the junction of the superior thyroid artery and common carotid artery. 3=baroreceptor areas on the wall of the common carotid arteries. 4=baroreceptors at the junction of the subclavian artery and common carotid artery.5=aortic arch baroreceptors.

i. Carotid Sinus:

It is a dilatation at the root of internal carotid artery, often involving the common carotid. The exact location varies in different species. The wall of the sinus is thinner due to less muscle fibres in the media. In the deeper parts of adventitia, an extensive network of afferent nerve fibres is present. The fibres end in free nerve terminals and characteristic minisci. These pressor receptors are sensitive to stretch (distortion effect) being stimulated by rise of blood pressure.

The sinus nerve (afferent) arises from the carotid sinus and carotid body, passes along the glossopharyngeal nerve and ends in the medulla in close relation with respiratory, cardiac and vasomotor centres.

ii. Aortic Arch:

Afferent nerves and stretch receptors—similar to those in the carotid sinus—are also present in the adventitia of aortic arch, the roots of great vessels and even the adjoining parts of left ventricle. They serve the same function as the carotid sinus.

Aortic Nerve:
This nerve arises from the arotic body, the aortic arch and the basal part of left ventricle. It is a purely afferent nerve. Its course varies in different species but in human beings it mostly passes in the vagus. Like the sinus nerve it ends in medulla being closely related to cardiac, vasomotor and respiratory centres.

B. Chemoreceptors:
This includes carotid (G.karas =sleep) body and arotic bodies (Fig. 7.92).

i. Carotid Body (Fig. 7.91):
It is a small nodule situated on the occipital artery, a branch of the external carotid artery very close to the carotid sinus. It consists of clumps of large polyhedral cells (Glomus cells), richly supplied with blood vessels and nerves. The vessels arise from the carotid artery. Some of the cells stain with chromic acid and belong to the chromaffin system but do not contain adrenaline. Numerous afferent nerve fibres surround the cell clumps and even the individual cells, and terminate in special chemoreceptors. They are sensitive to chemical changes in blood.

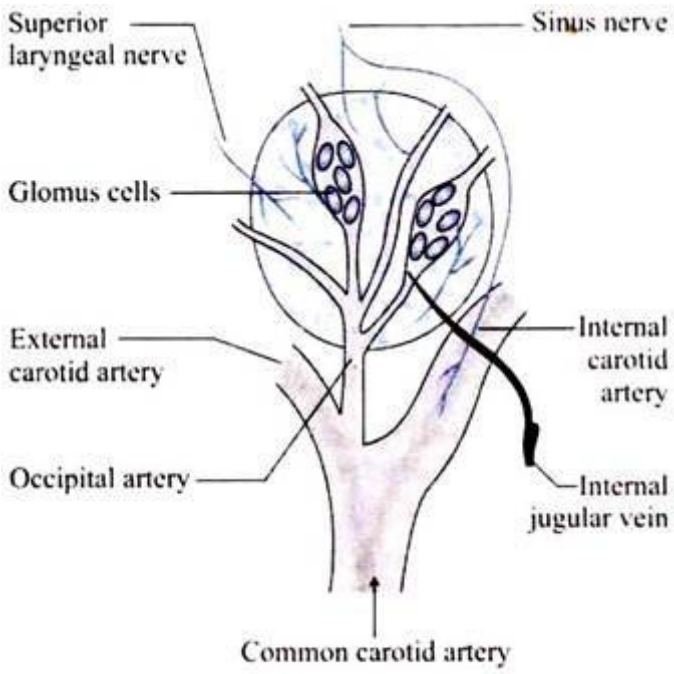


Fig. 7.91 Carotid body (Glomus caroticum) showing blood vessels and nerve supply.

ii. Aortic Bodies:
Four groups of aortic bodies have been shown in cat.

These are small nodular structures, supplied by a special blood vessel and situated:

(a) On the thorax between the pulmonary trunk and ascending aorta,

(b) On the ventral surface of the root of the right subclavian artery,

(c) On the ventral surface of the root of the left subclavian artery, and also

(d) On the ventral surface of the aortic arch (Fig. 7.92). Afferent pathways from these chemoreceptor areas are lying in the arotic nerves and vagi. Their structures, nerve endings and functions are similar to those of carotid body.

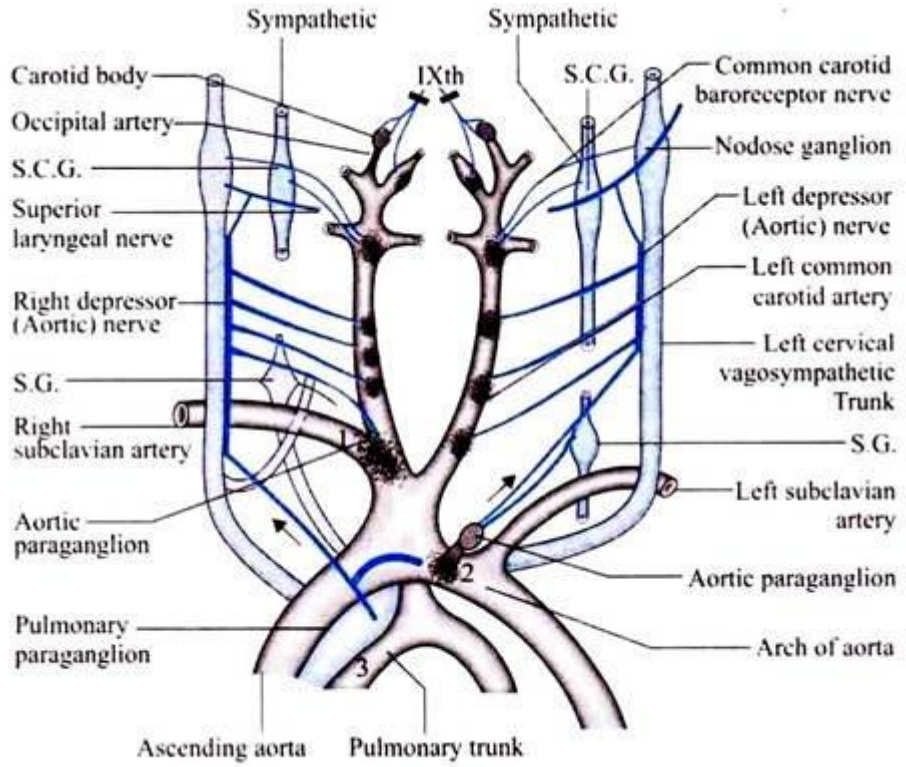


Fig. 7.92 Distribution of chemoreceptors at areas of the blood vessels. 1,2,3 are the chemoreceptor areas.

By perfusion experiments, the effects of chemical changes in blood, as brought about through the Sino-aortic chemoreceptors. It is seen that CO_2 excess, O_2 lack and increased H-ion concentration stimulate respiration (mainly), increase heart rate, produce vasoconstriction and raise blood pressure.

After haemorrhage or in enfeebled circulation a rhythmic blood pressure wave (vasomotor wave) is often encountered. These vasomotor waves are due to chemoreceptor activities under such state. These waves were observed by Mayer (1876) and known as Mayer's wave. Following inactivation of chemoreceptors, these waves disappear completely.

Mechanisms of Stimulation of Chemoreceptors:

It has been claimed that for the stimulation of chemoreceptor nerve endings, the liberation of acetylcholine plays as the chemical intermediary (vide Chemical regulation of respiration)

Experimental Observations:

To study the functions of the Sino-aortic nerves various experiments have been performed.

For instance:

i. Section and Stimulation:

Section and stimulation of the sinus and carotid nerves.

ii. Perfusion Experiments:

Perfusion experiments in which the carotid sinus region Is isolated and perfused with blood or other fluids whose pressure and composition can be varied at will.

iii. Electrophysiological Study:

Electrophysiological study shows that in normal arterial pressure the sinus nerves discharge impulses, the frequency of which rises with systolic pressure and diminishes with diastolic pressure. Rise in systolic pressure increases, the frequency of impulse discharge.

iii. Cross-Circulation Experiments:

Heymans and his associates have studied the functions of the carotid sinus and sinus nerve by cross-circulation experiments (Fig. 7.93).

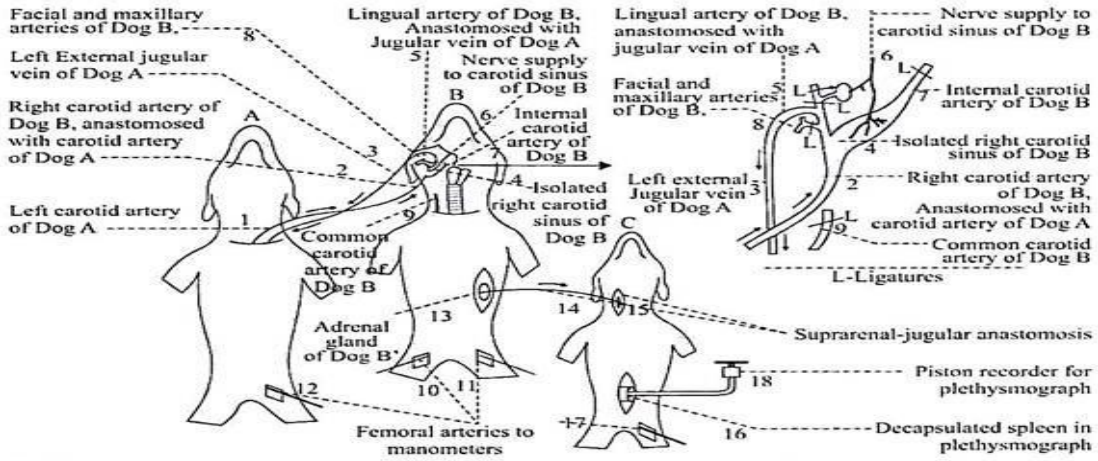


Fig. 7.93 Scheme of perfusion of the isolated carotid siuus of dog B, by dog A, and an anastomosis between the supra-renal vein of dog B and the jugular vein of dog C. The blood from dog A flows through the carotid sinus of dog B and back to dog A, Via anastomosis between the lingual artery of dog B and the external jugular vein of dog A (after *Heymans*). *By the permission of copyright owner* THE WILLIAMS & WILKINS COMPANY, BALTIMORE

The carotid sinus of the second dog B was isolated (the nerve supply remaining intact) and perfused with the blood of the first dog A. When the arterial pressure of the dog A was raised the arterial pressure of the dog B was diminished. Again when the arterial pressure of the dog A was lowered, the arterial pressure of the dog B was increased by secretion of adrenaline as evidenced from splenic contraction in dog C which got adrenaline from dog B through anastomoses of the suprarenal vein and the jugular vein.

The followings are the complete observations:

On raising the pressure in the carotid sinus, the following reflex effects are produced:

i. Slowing of the heart rate.

ii. Peripheral vasodilatation preferably in the splanchnic bed so as to increase the total vascular capacity.

iii. Fall of blood pressure.

iv. Diminished adrenaline secretion.

v. Slowing or stoppage of respiration.

vi. Diminished tone in voluntary muscles.

vii. Various changes in the viscera, viz., increased volume and movement of the stomach, decreased tone of urinary bladder, etc., caused by disturbed activity of the autonomic system. Fall of sinus pressure or section of the sinus nerve produces opposite effects. Stimulation of the central cut end of the sinus nerve also produces similar effects.

Raising the aortic pressure causes the following effects:

i. Slowing of the heart rate—mainly due to the stimulation of cardio-inhibitory centre and partly to the inhibition of accelerator centre.

ii. Inhibition of the vasomotor centre causing vasodilatation.

iii. Depressed adrenaline secretion.

iv. Fall of blood pressure.

v. Depressed respiration.

Fall of aortic pressure of section of the aortic nerve—produces reverse effects. Stimulation of the central cut end of the aortic nerve produces similar effects. Sometimes stimulation of the central cut end of vagus or aortic nerve may raise blood pressure by reflex cardiac acceleration and vasoconstriction. This proves that these nerves also carry some pressor fibres.

For the last few years there is considerable progress regarding the studies in connection with changes in the circulation and arteriolar resistance in blood pressure. In hypertension of the Sino-aortic origin there occurs vasoconstriction and stimulation of the sympathetic nerves of the heart. After occlusion of the common carotid arteries, hypertension occurs with decrease in the volume of spleen, kidney, limbs, etc.

Thus, it is evident that the functions of the sinus and aortic nerves (Buffer nerves) are very similar

The effects of the various factors are summarised as follows:

Table 7.8 Effect of various factors on sinus and aortic nerves

Factors	Respiration	Heart rate	Vasomotor	Adrenaline secretion	Blood pressure
❑ *On Baroreceptors*					
• B.P rise	Slow	Slow	Dilatation	Depressed	Rise checked
• B.P fall	rapid	Rapid	Constriction	Stimulated	Fall checked
❑ *On Chemoreceptors*					
• B.P. fall	Rapid	Rapid	Constriction	Stimulated	Raised
• CO_2 excess	Rapid	Rapid	Constriction	Stimulated	Raised
• CO_2 fall	Slow	Slow	Dilatation	Depressed	Lowered
• O_2 lack	Rapid	Rapid	Constriction	Stimulated	Raised
• H-ion concentration changes	Effects same as CO_2 changes.				

From the above observations, the functions of Sino-aortic nerves may be described as follows:

i. Reflexly maintain the vagal tone and thus exert tonic inhibitory control on the heart.

ii. Reflexly maintain the inverse relation between blood pressure and heart rate and thus keep the variations of blood pressure within an optimum range (hence called Buffer nerves).

iii. Exert tonic inhibitory action on respiratory centre and vasomotor centre (vasoconstrictor).

iv. Reflexly regulate the activity of the respiratory, cardiac, vasomotor centres and adrenaline secretion and thus bring about a perfect coordination among them.

v. Changes in viscera, viz., variations of movement, tone, etc., may be reflexly produced through autonomic nerves.

(b) Vascular Receptors other than Sino-Aortic for the Control of Blood Pressure and Flow:

The Sino-aortic mechanisms are meant for the maintenance of systemic blood pressure and flow. But the vascular receptors other than Sino-aortic are responsible mostly for control of local blood pressure and blood flow.

These are as follows:

A. As Baroreceptors:

i. At the Junction of Superior Thyroid Artery and Common Carotid Artery:

These baroreceptors mainly control the pressure and flow of the thyroid gland. When the systemic pressure is raised, these baroreceptors may be stimulated and impulse is carried through the common carotid nerve (C. C. N.) and is reflexly produced dilatation of the thyroid blood vessels. The presence of these baroreceptors has been demonstrated by Green (1956) in cats.

ii. Several Baroreceptor Areas in the Wall of Right and Left Common Carotid Arteries:

Several baroreceptor areas in the wall of right and left common carotid arteries between the level of the superior thyroid artery and subclavian bifurcation—have been demonstrated by Green (1956). Afferent impulses from these areas are carried through the branches of the aortic nerves.

iii. Baroreceptor Areas at the Junction of the Right Subclavian Artery and Common Carotid Artery:

These baroreceptor areas have been demonstrated by Heyman's (1956) in cats and also in other mammals. Rise of pressure in these areas produces reflex systemic hypotension and hypopnoea. Afferent impulses are carried from these areas via the branch of the aortic nerves.

iv. Baroreceptors of the Pulmonary Arch of Aorta:

These baroreceptors are present in the pulmonary conus of bifurcation. It has been claimed by Aviado and Schmidt (1955) that reflex bradycardia and hypotension are produced if these receptors are stimulated due to rise of pressure.

v. Receptors in Thoracic Aorta:

The presence of baroreceptors has been claimed by Gruhzit and Moe (1953) and also others. Rise of blood pressure may produce reflex vasodilatation in the innervated limb through the stimulation of these baroreceptors.

vi. Mesenteric Baroreceptors:

Gammon and Bronk (1935) first detmonstrated the presence of mesenteric baroreceptors. The Pacinian corpuscles are the actual baroreceptors of the mesenteric blood vessels. These receptors are not directly related to the level of systemic blood pressure, but to a degree of distention of the mesenteric blood vessels.

It has been observed by Heymans and his colleagues (1937) under isolated cross-circulation technique that increases pressure in mesenteric blood vessels produces reflex vaso-dilatation of the spleen. But the specific functions of these baroreceptors are not yet clear. These receptors do not play any important role in the regulation of

systemic blood pressure but they may play in the regulation of blood flow in the abdominal viscera.

vii. Peripheral Vascular Receptors:
Presence of other peripheral vascular receptors has been observed by many. Yamado and Burton (1954) have observed decrease of flow in the finger if negative pressure is exerted on it. It is claimed that this reflex decrease of blood flow is through venous-arteriolar reflex—causing constriction of the arterioles due to (a) distention and (b) increase of transmural pressure of the veins.

In congestive heart failure there is general occurrence of peripheral vasoconstriction (in the fingers and nose) is mostly due to reflex effect of increased central venous pressure. Ganglionic blocking agents and sympatholytic drugs relieve these conditions. The venous-arteriolar reflexes may play an important role in relieving the venous congestion because with the increase of venous congestion, the arterioles are constricted so that the outflow from the veins will exceed inflow from the constricted arterioles.

The presence of veni-venimotor reflexes have also been described by Wallis and others (1963). They have observed vasoconstriction in the haemodynamically isolated venous segment due to venous congestion produced by blowing up a cuff on the arm. Local anaesthesia abolishes this response.

viii. Löven Reflex:
This is nothing but axon reflex. If any portion of the vessel is dilated then the neighbouring vessel is constricted. This was first observed by Lovett.

ix. Bainbridge Reflex:
Bainbridge (1915) showed that intravenous administration of saline or blood produced reflex acceleration of the heart. He claimed this to be reflex arising from the stretch receptors present in the venous side of the heart (great vein and also right atrium) and bilateral sectioning of the vagi abolished the response.

His observation has been criticised by many but Jones (1962) has observed that reflex acceleration of the heart rate due to venous engorgement following infusion of saline or blood is dependent upon pre-existing heart rate of the animal. He claimed that if the heart rate is initially high (above 130 per min) then the reflex effect will be bradycardia instead of tachycardia.

x. Right Atrial Receptors (A and B):
Aviado and Schmidt have claimed that increasing the perfusion pressure in the right atrium produces bradycardia. This reflex effect is abolished by atropine or vagotomy. They suggest that the Bainbridge effect is the cause of stimulation of chemoreceptors supplied by the vago-depressor trunk which might well be activated by the changes in

gas content, acidity, tonic balance and viscosity of the blood associated with the massive intravenous infusion.

xi. Pulmonary Deflation Receptors:
Paintal (1955a) has claimed that these receptors are stimulated by congestion of lungs during rapid venous return and produce reflex bradycardia. These receptors are also a part of pulmonary depressor chemo-reflex.

xii. Reflexes from the Inflation of the Lungs:
The physiology of these reflexes has been studied by Irving (1939) and Scholander (1963). They have described these reflexes to be an important line of defence against death due to asphyxia of the divers. They observed bradycardia and selective vasoconstrictions in non-vital organs (limbs, kidneys and mesenteries) after diving. They claimed that these reflex effects are due to stimulation of stretch receptors in the lungs.

xiii. Left Atrial and Left Ventricular Receptors:
Paintal (1955b) has shown that these receptors are excited by the increased pressure in the left side of the heart and produce bradycardia.

B. As Chemoreceptors:

i. Bezold and Jarisch Reflex:
Bezold and Hirt (1868) and Jarisch (1938) observed profound bradycardia, hypotension and apnoea following intravenous injection of veratrine alkaloid. They considered to be the direct effect of the drug on this cardiac receptors in left ventricle (mostly) that causes reflex cardiac and respiratory effects.

Jarisch has claimed that these are proprioceptive receptors and are normally responsive to stretch of the ventricular wall. Paintal (1955b) has shown that veratrine and related substances may stimulate the ventricular receptors and also some of atrial receptors ('A' and 'B'). He also claimed that these drugs do not act directly on these receptors but act through changing the ionic status of the receptor areas.

ii. Pulmonary Depressor Chemoreflex and Pulmonary Respiratory Chemoreflex:
Brodie (1900) observed bradycardia and hypotension following intravenous administration of egg-white and serum into cats. He claimed that these effects are due to stimulation of receptors of the pulmonary vascular bed. These have been investigated by many. Dawes and Mott (1950) also observed bradycardia and hypotension after administration of phenyl diguamide.

They claimed that these effects were due to direct stimulation of the pulmonary vascular receptors. Paintal (1955a) has claimed these pulmonary depressor

chemoreflex, pulmonary respiratory chemoreflex to be responsible for the stimulation of the pulmonary deflation receptors.

iii. Reactive Hyperaemia:
Chemoreceptors are present all throughout the vascular wall and are stimulated by the local accumulation of metabolites. Hyperaemia caused by locally accumulated metabolites acting on the blood vessels are called reactive hyperaemia. It is a kind of auto-regulation of blood circulation of the organ itself.

iv. Abdominal Chemoreceptors:
It has been described by a group of workers that certain peripheral (abdominal) vascular chemoreceptors are present which, when stimulated by strongly irritant chemicals, may produce reflex respiratory effects. But the presence of these receptors has not yet been substantiated and Heymans and his colleagues have shown that these effects are not observed if the drugs are administered in the isolated organs.

Summarily, it can be argued that nature's mission is to protect the vital organs like heart and brain from any un-physiological conditions. Whenever there is any rise of blood pressure or fall of pressure, the normal range of blood pressure and blood flow of the vital organs are maintained by redistribution of blood. This redistribution of blood is made by withdrawing or heightening the vasomotor tone.

In condition of increased blood pressure, there is depression of sympathetic tone along with activation of parasympathetic tone—causing peripheral vasodilatation so as to shift the blood to the splanchnic bed (peripheral bed); but in condition of decreased blood pressure, there is increase of sympathetic tone along with decrease of para-sympathetic tone—causing profound vasoconstriction in the splanchnic bed (other peripheral bed) so as to shift the blood to the vital organs.

Chemical Control of Blood Pressure Influenced by Vasomotor Mechanism:
Many substances produced in the body are known to increase or decrease blood pressure by influencing the vasomotor mechanism.

Some of these are:
i. CO_2:
Tonic activity of the vasoconstrictor area may be due to stimulating action of CO_2 in blood. During early stage of asphyxiation this may bring about a great increase in blood pressure. It is observed that over-ventilation of lungs, as by voluntary deep inspiration and expiration for 3 or 4 minutes, causes a feeling of giddiness. As large amounts of CO_2 are expelled from blood by over-ventilation, the vasoconstrictor area is derived from proper stimulation by CO_2. As a result, a fall in blood pressure and vasodilatation in splanchnic area occur.

ii. Epinephrine:
If it is injected into blood, epinephrine constricts the cutaneous and abdominal arterioles, and this result in a very sharp rise of blood pressure, but the elevated pressure does not stand for a long time. By application locally, it is used in minor operation on the eye, nose, etc. In contrast, there is a dilatation of the coronary and skeletal muscle arterioles.

iii. Ephedrine:
It is much weaker in action than in that of epinephrine. It is much used in bad colds and hay fever because of its constricting action. When inhaled or applied locally by drops or a spray, it brings about an immediate shrinkage of the congested blood vessels of the nasal mucosa. After 3 or 4 hours, the opening of nasal passages by vasoconstriction becomes freer and more comfortable for breathing.

iv. Histamine:
It causes a marked dilatation of capillaries and arterioles in lowering of blood pressure.

v. Alcohol:
It causes a marked dilatation of blood vessels as a depressant on the vasomotor centre. On a cold day after consuming alcohol the cutaneous vessels are especially affected.

vi. Tobacco:
Smoking increases both systolic and diastolic blood pressure. There is an increase of pulse rate materially and a decrease of temperature of extremities. So the use of tobacco may be injurious in arteriosclerosis and also in .cardiac diseases associated with arteriosclerosis or even high blood pressure.

vii. Vasopressin:
Though it is an internal secretion of the posterior pituitary, it causes an increase of blood pressure. But this rise in pressure is not as great as that of epinephrine, yet this pressure continues for a long time.

viii. Acetylcholine:
Direct action of acetylcholine on coronary blood vessels is dilatation.

Factors Influencing Blood Pressure:

Factors influencing blood pressure are:

i. Age

ii. Sex

iii. Body build

iv. Posture

v. Exercise

vi. Emotional aspects

Factors that maintain the normal blood pressure are:
i. Cardiac output

ii. Peripheral resistance

iii. Blood volume

iv. Viscosity of the blood

v. Elasticity of the blood vessel

Cardiac output:
Systolic blood pressure depends entirely on the cardiac output. An increase in the cardiac output increases the systolic pressure and a decrease in the output will have the opposite effect.

Peripheral resistance affects the diastolic pressure and has a direct relationship. The seat of resistance is the arterioles. Arterioles contain large number of smooth muscle fibers that are supplied by sympathetic vasoconstrictor fibers. Vasoconstriction increases the resistance offered to blood flow and, therefore, the diastolic blood pressure is increased.

Blood volume:
If blood volume is reduced, it decreases the blood pressure. Decreased blood volume decreases the systemic filling pressure. This in turn will decrease the venous pressure, decrease the venous return and cardiac out put and, therefore, the blood pressure.

Blood pressure:
i. Lateral pressure

ii. End arterial pressure

iii. Normal BP 120/80 mm Hg

120 mm Hg—systolic BP

80 mm Hg—diastolic BP

Pulse pressure = Systolic-Diastolic

= 120-80

= 40 mm Hg

Mean arterial BP = Diastolic + rd of pulse pressure

= 80 + (13 or 14)

= 94 mm Hg

Measurement of Blood Pressure:
1. Direct:
Inserting a needle into an artery

2. Indirect:
i. Sphygmomanometer

a. Palpatory method

b. Auscultatory method

Viscosity of the blood:
Increased viscosity offers increased resistance to blood flows and, therefore, increases the blood pressure. This is usually seen in cases of polycythemia vera.

Elasticity of the blood vessels:
As age advances, the amount of elastic fiber decreases in the vessel wall. The blood vessel becomes more rigid tubes; the distensibility of the blood vessel is reduced. As a result, the systolic blood pressure is increased and the diastolic pressure is decreased.

However, in practice, what is seen is an increase in the diastolic pressure. This is because of atherosclerotic changes, the tube (blood vessel) diameter decreases and, therefore, offers greater resistance to blood flow.

Measurement of blood pressure can be done both by direct and indirect methods. But the direct method is not done during routine measurement of blood pressure as the technique is invasive and needs insertion of needle into an artery to determine blood pressure. Hence the indirect method is preferred.

Indirect methods are:
i. By palpatory method

ii. By auscultatory method.

By palpatory method, only the approximate systolic pressure can be measured. Diastolic blood pressure cannot be measured by this method.

Auscultatory method is the most accurate method of measuring the blood pressure. Both systolic and diastolic pressures can be measured by this method. Instrument used to measure blood pressure is known as sphygmomanometer.

In palpatory method, an approximate systolic pressure is obtained whereas in auscultatory method an accurate systolic and diastolic pressure can be obtained.

While determining blood pressure by auscultatory method, the sounds heard using stethoscope is known as Korotkoff sound. Korotkoff sounds are produced due to turbulence created by flow of blood through the partially obstructed blood vessel.In the normal course, the blood flow through the blood vessel is laminar or silent as there is no obstruction. In laminar flow, the central most layer of blood will be flowing at maximal velocity and the velocity of flow of the layer of blood nearer to the wall of vessel will be least.

In a completely occluded vessel, there is no flow beyond the area of occlusion. When the vessel is partially opened (occlusion is removed partially), now blood has to pass through the narrow area. This creates turbulence beyond the area of partial obstruction.

This turbulence is responsible for production of Korotkoff sound. When the occlusion is completely removed, the turbulent flow is replaced by laminar flow. Hence there will be no more sound production.

Regulation of Blood Pressure:

This can be discussed as under:

i. Local mechanisms:

ii. Spinal cord in the regulation

iii. Medulla oblongata in the regulation

iv. Higher centers in the regulation

Or

i. Immediate mechanisms

ii. Intermediate mechanisms

iii. Long-term mechanisms

Local mechanisms include the production of vasodilator or vasoconstrictor substances. The vasoconstrictor substances are noradrenaline, angiotensin II, 5-hydroxytryptamine and others. The vasodilator substances are bradykinin, histamine, and adrenaline in

certain regions. Hypoxia, hypercapnea, warmth, acidosis, etc. also bring about vasodilatation. These substances mainly alter the peripheral resistance and, therefore, the diastolic blood pressure.

Spinal Cord in the Regulation of Blood Pressure (Fig. 3.33):

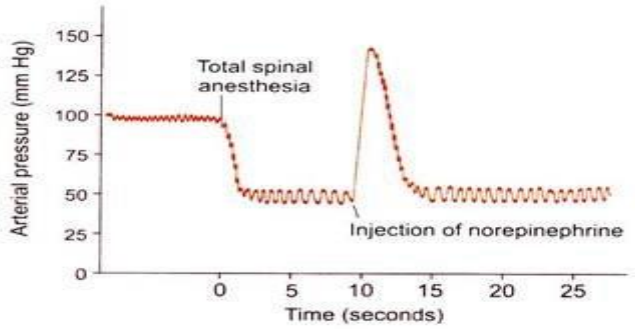


Fig. 3.33: Role of sympathetic nerve activity in the maintenance of normal blood pressure

Complete transverse section of the spinal cord (at the level of T1 segment) is followed by a marked fall in blood pressure. During the recovery phase, the lateral horn cells recover, send vasoconstrictor impulses to the blood vessels, blood vessels constrict, the blood pressure improves though it may not come back to normal.

During the state of spinal shock, carbon dioxide breathing will stimulate the lateral horn cells directly and increases the blood pressure. Influence of VMC is mediated through the lateral horn cells and the impulses from VMC on the lateral horn cells are always excitatory.

Role of Medulla Oblongata in the Regulation of Blood Pressure:

Medulla oblongata is the most important region of the central nervous system that is involved in the regulation of blood pressure. Collectively, these neurons are called as the vasomotor center (VMC). Stimulation of these neurons will give rise to vasoconstriction, which increases the peripheral resistance and increases the blood pressure.

On the other hand, when this neuronal activity is decreased it leads to vasodilation and, therefore, decreases the peripheral resistance and hence decreases the blood pressure.

Factors controlling the activity of the vasomotor center are (Fig. 3.34):

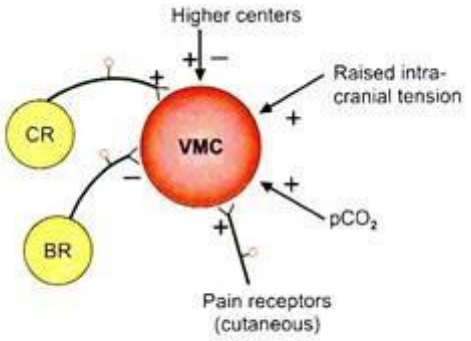


Fig. 3.34: Various factors influencing activity of vasomotor center present in the brainstem

i. Impulses coming from the baroreceptors

ii. Impulses from the chemoreceptors

iii. Impulses from the higher centers

iv. Impulses from the pain receptors and from joint receptors

v. Impulses from the visceral receptors.

Baroreceptor Mechanism in the Regulation of Blood Pressure:

i. Baroreceptor mechanism is the most important mechanism in the regulation of blood pressure.

ii. Carotid sinus and arch of aorta contain the baroreceptors (Fig. 3.35).

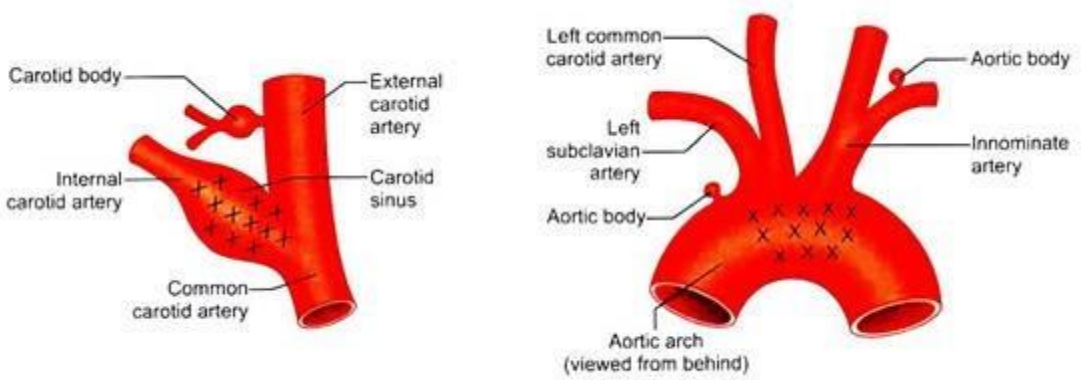


Fig. 3.35: Location of baroreceptors and chemoreceptors

iii. Carotid sinus is located at the bifurcation of the carotid artery and at the commencement of the internal carotid artery.

iv. Aortic arch receptors are located in the arch of the aorta.

v. A branch of the glassopharyngeal nerve supplies carotid sinus and the aortic nerve a branch from the vagus nerve supplies the aortic arch (Fig. 3.36).

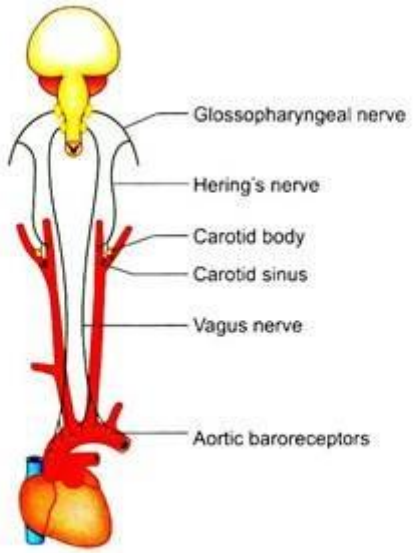


Fig. 3.36: Afferent nerves carrying impulses from baroreceptors

vi. These receptors are stretch receptors. An increase in the blood pressure further stretches the receptors area resulting in production more number of impulses (Fig. 3.37).

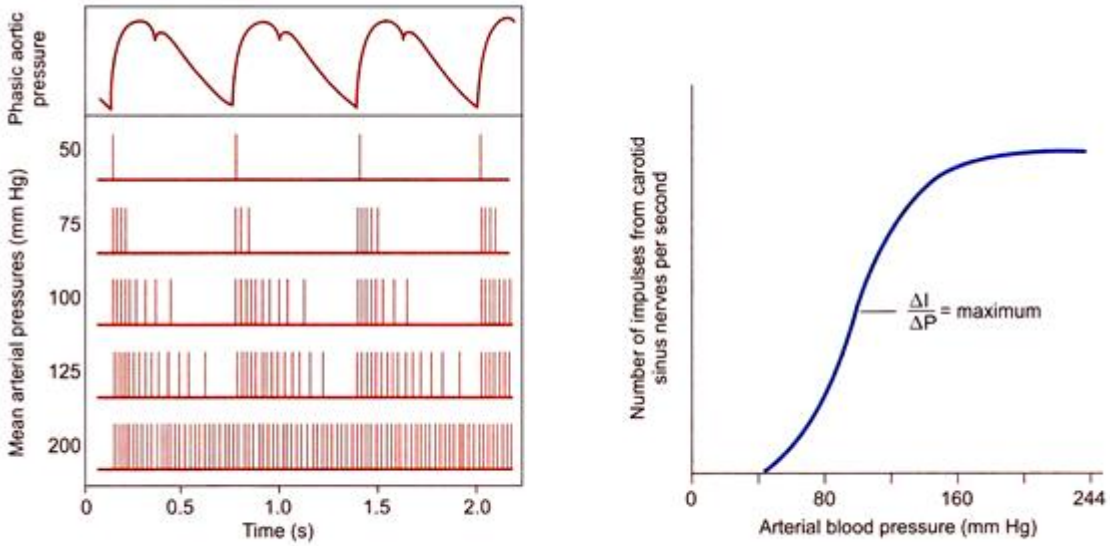


Fig. 3.37: The relationship between mean arterial pressure and frequency of impulse discharge in the sinus nerve

vii. More number of impulses are produced and these impulses reach the following centers (Fig. 3.37):

a. Vasomotor center

b. Cardioinhibitory center

c. Respiratory center

Role of Vasomotor Center (Fig. 3.38):

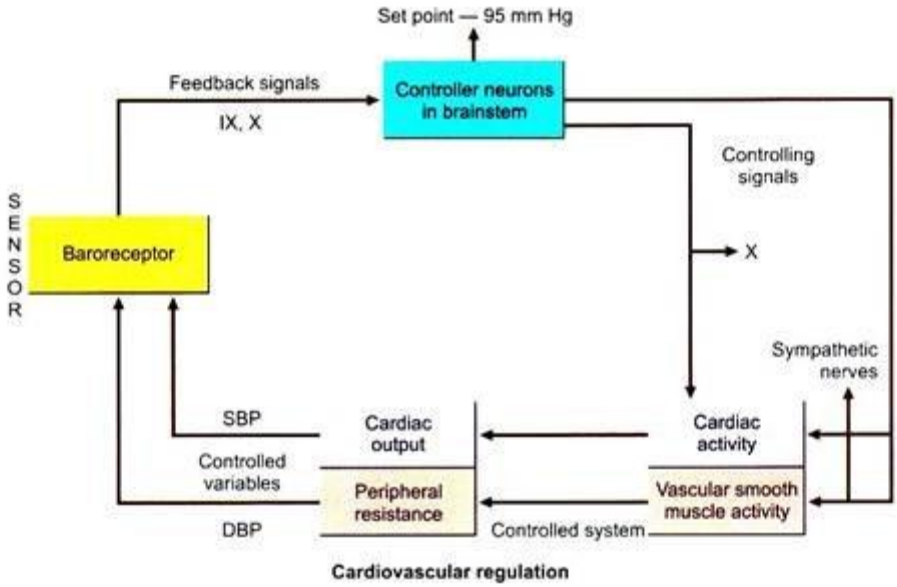


Fig. 3.38: Feedback circuit indicating the role of baroreceptors in the regulation of BP by altering the cardiac output and peripheral resistance

i. Impulses going to the vasomotor center from the baroreceptors are inhibitory in nature and therefore, the activity of the vasomotor center is inhibited. This in turn decreases the number of impulses going to the lateral horn cells in the spinal cord. The activity of the lateral horn cells is decreased.

Vasoconstrictor impulses going to the arterioles are reduced, leading to vasodilatation, decreased peripheral resistance and a decrease in diastolic blood pressure.

ii. Inhibition of vasomotor center also decreases the venomotor tone; blood gets pooled in the venous compartment. This decreases the venous return, decreases the cardiac output and, therefore, the blood pressure.

iii. Inhibition of vasomotor center decreases the amount of catecholamine secretion from the adrenal medulla which in turn decreases the peripheral resistance and cardiac output.

iv. Due to decreased sympathetic activity, the heart rate and force of contraction of the heart are also reduced, reducing the systolic blood pressure.

The loss of function of baroreceptor (Fig. 3.39, when baroreceptor area is denervated or when baroreceptor area is not prefused—Figs 3.40 and 3.41) on BP variations has been depicted.

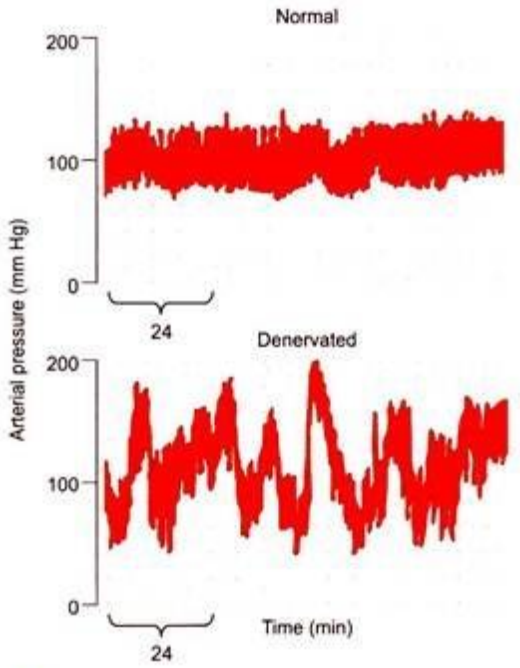


Fig. 3.39: Variation in blood pressure even under resting conditions when the carotid sinus baroreceptor area is denervated when compared to the normally innervated carotid sinus baroreceptor area

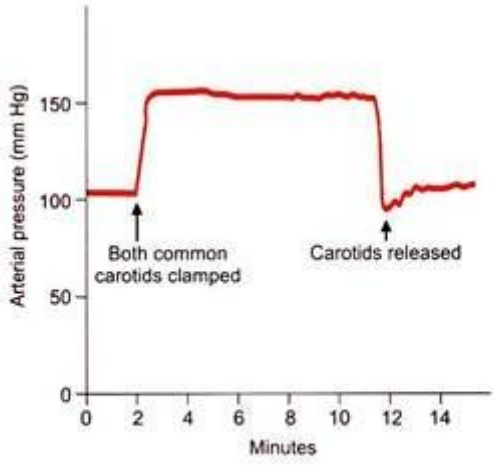


Fig. 3.40: Increase in blood pressure when the common carotids of both the sides are occluded thereby preventing blood flow through the carotid sinus and consequent non-stimulation of carotid sinus baroreceptors

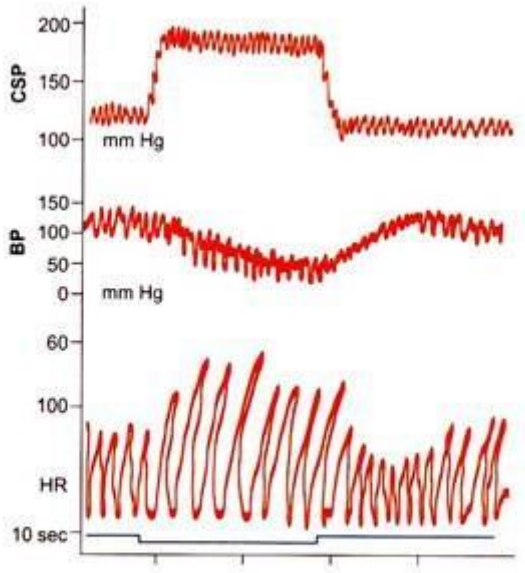


Fig. 3.41: Decrease in blood pressure and decrease in heart rate when the blood pressure in the carotid sinus region is increased

Role of Cardioinhibitory Center:

Impulses coming from the baroreceptors are excitatory to the cardioinhibitory center. Stimulation of cardioinhibitory center increases the vagal tone, which in turn decreases the heart rate and force of contraction of the heart. This will lead to decreased cardiac output.

Marey's law states that heart rate is inversely proportionate to blood pressure. Whenever the blood pressure is increased, acting through the cardioinhibitory center, it reflexly lowers the heart rate and blood pressure.

Role of Respiratory Center:

Impulses coming from the baroreceptors are inhibitory to the respiratory center. This in turn decreases the rate and depth of respiration. Because of this, the changes in the intrapleural pressure become less. This will lead to decreased venous return. Decreased venous return decreases the cardiac output and, therefore, blood pressure.

Chemoreceptor Mechanism:

i. Chemoreceptors are the carotid bodies and aortic bodies.

ii. Decreased blood pressure decreases the blood flow through the chemoreceptors decreasing the oxygen supply.

iii. Hypoxia, hypercapnia and acidosis stimulate these chemoreceptors.

The impulses from the chemoreceptors in general are going to stimulate the vasomotor center, respiratory center and inhibit the cardioinhibitory center during the course of regulation of blood pressure. Stimulation of VMC increases the peripheral resistance and hence the blood pressure.

CNS Ischemic Response:

If the blood pressure falls to a greater extent, the blood flow to the brain is markedly reduced, metabolic waste products accumulate, the resulting hypercapnea and acidosis stimulate the vasomotor center directly and more powerfully. Peripheral blood vessels under go marked vasoconstriction and increases the blood pressure to a greater extent.

Stretch receptors present in the low pressure areas of the cardiovascular system:

i. Stretch receptors are present in the walls of great veins, right atrium.

ii. An increase in the blood volume, distension of the venous compartment. This leads to the receptors getting stretched and stimulated.

iii. Impulses travel to the higher centers and reflexly bring about the following changes:

a. Peripheral arteriolar dilation decreased peripheral resistance, therefore, a fall in the blood pressure.

b. Afferent arteriolar dilation leads to increased hydrostatic pressure in the glomerular capillary network leading to increased glomerular filtration rate and increase the fluid loss.

c. Decreased release of ADH will increase the urinary output. This in turn decreases the blood volume and blood pressure.

Atrial natriuretic factor:

A chemical substance released from the atrial muscle fibers due to distension of the atrium. This can occur whenever the blood volume is increased or whenever the venous return is increased. Any time this hormone is released it brings about peripheral vasodilation, increased excretion of water and salt. This in turn decreases the blood pressure.

Factors Regulating Blood Pressure- Intermediate Mechanisms:

Fluid shift mechanism:

Any time the blood pressure falls, the pre-capillary sphincter contracts, this decreases the hydrostatic pressure in the capillaries. All along the capillaries, the colloidal osmotic pressure remains high and, therefore, the fluid shifts from the extravascular compartment to the intravascular compartment. This increases the blood volume and blood pressure.

Renin-angiotensin mechanism (Fig. 3.42):

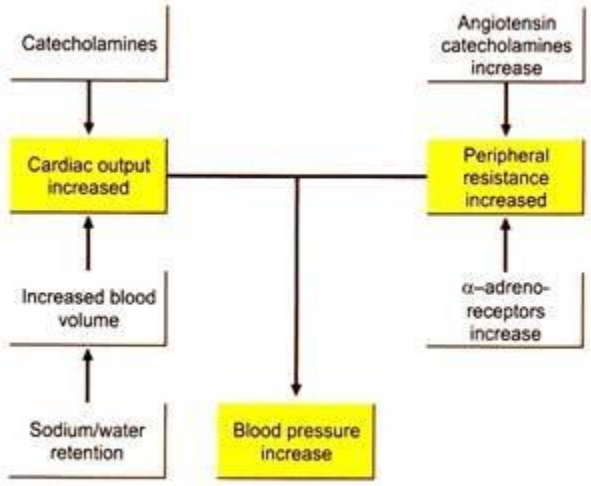


Fig. 3.42: Role of catecholamines and angiotensin in the regulation of blood pressure

Decreased blood flow to the kidney due to a fall in the blood pressure will bring about the release of renin from the juxtaglomerular cells. This converts angiotensinogen to angiotensin I which is converted to angiotensin II.

This is a powerful vasoconstrictor substance which brings about constriction of the arterioles, increasing the peripheral resistance and blood pressure. This mechanism also increases the production of aldosterone. This hormone acts on the renal tubules, increasing the reabsorption of salt and water, increasing the blood volume and blood pressure.

ADH mechanism:

Any time when the blood volume is increased, the blood pressure is increased. The volume receptors are stimulated. Impulses arising from these receptors reach the hypothalamus and inhibit the secretion of ADH. More amount of water is lost from the kidneys, lowering the blood volume and blood pressure.

Long-term Regulation of Blood Pressure:

Kidneys play a very important role in the long-term regulation of blood pressure. Thus the fluid volume is maintained and, therefore, the blood volume and blood pressure is maintained.

Increased blood pressure in person with normal kidney excretes more of salt and water known as pressure natriuresis and pressure diuresis. The efficiency of the kidney in this respect is infinite (Fig. 3.43).

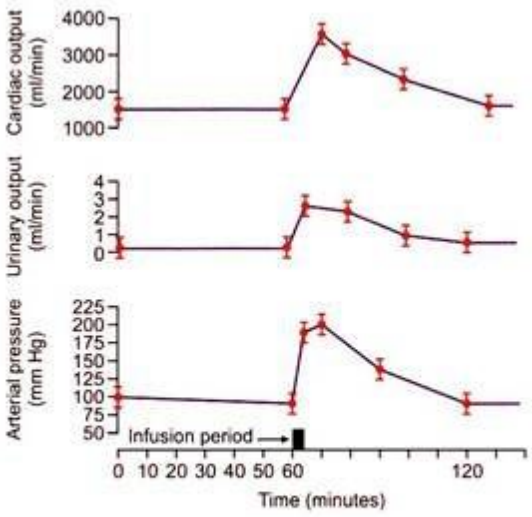


Fig. 3.43: Role of kidney in the regulation of blood pressure in long-term

Baroreceptors of carotid sinus and aortic arch show the property of adaptation. Sustained increase in blood pressure occurs in essential hypertension. Sustained increase of blood pressure will lead to resetting of the baroreceptors due to the property of adaptation. Therefore, the baroreceptors fail to lower the blood pressure.

Probable Questions:

1. Differentiate open and close circulatory system.
2. Discuss functions of blood.
3. Discuss different types of blood vessels.
4. Write the characteristics of fish heart.
5. Write the characteristics of amphibian heart.
6. Write the characteristics of reptilian heart.
7. Write the characteristics of avian heart.
8. Write the characteristics of mammalian heart.
9. What is blood pressure? How it is measured?
10. Define SP,DP, MP PP.
11. How human heart works?
12. What factors determine blood pressure?
13. Discuss the Factors Controlling Arterial Blood Pressure.
14. How Medulla oblongata controls blood pressure?
15. How baroreceptors regulates blood pressure?

Suggested Readings:

1. Animalphysiology-MohanP.Arora.
2. Textbookofmedicalphysiology/ArthurC.Guyton,JohnE.Hall.
3. Ganong'sreviewofmedicalphysiology.

UNIT-II

Cardiac cycle. Myogenic and neurogenic heart, origin and conduction of heart beat, ECG and its implications, neural and chemical regulation of functions of heart

Meaning of Cardiac Cycle:

SA node, the pacemaker has the property of automaticity and rhythmicity. Because of this, it produces action potentials, which spread all along the atrial and ventricular muscle fibers. This in turn brings about depolarization and repolarization. Following this, various changes occur in the heart, which is repeated from beat to beat. These events are known as the events of the cardiac cycle.

The events are (Fig. 3.24):

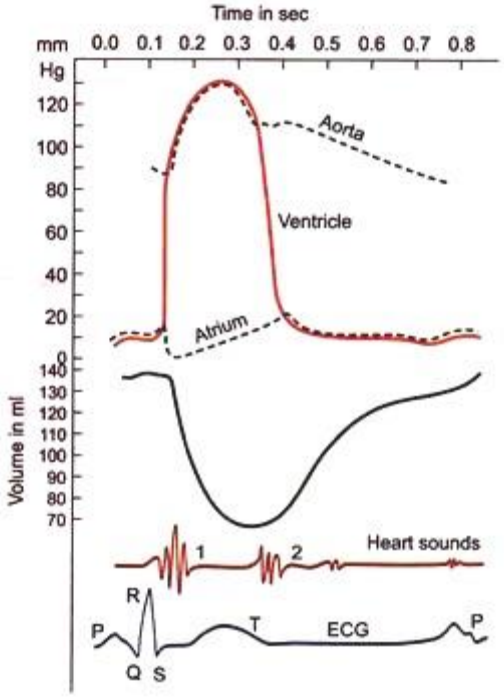


Fig. 3.24: Electrical, pressure, volume and acoustic changes during a cardiac cycle

i. Mechanical changes in the form of contraction (systole) and relaxation (diastole) in atria and ventricles.

ii. Hemodynamic changes that is pressure and volume changes in atria and ventricles.

iii. Acoustic changes that is the production of heart sounds.

Duration of Cardiac Cycle:

Duration of cardiac cycle depends on the heart rate per minute. For example, if the heart rate is 60 per minute, the cardiac cycle duration will be 1 sec. There is an inverse relationship between the duration of cardiac cycle and heart rate. Hence when the heart rate is increased to 120 beats per minute, the cardiac cycle duration will be about 0.5 sec.Duration of atrial and ventricular systole and diastole will be as follows: when the heart rate is about 75 times per minute the duration of systole and diastole in different chambers will be (Table 3.5);

Table 3.5: Events during cardiac cycle

Cardiac cycle (0.8 sec)	
a. Atrial cycle	
Systole 0.1 sec	
Diastole 0.7 sec	
b. Ventricular cycle	
Systole 0.3 sec	
Diastole 0.5 sec	
Mechanical events	
a. Atrial	
Systole	Volume change
	Pressure change
b. Ventricular	
Systole	Volume change
	Pressure change
Diastole	Volume change
	Pressure change

Atria:

a. Systole is about 0.1 sec.

b. Diastole is about 0.7 sec.

Ventricular:

a. Systole is about 0.3 sec.

b. Diastole is about 0.5 sec.

Phases of Cardiac Cycle:

From the above, it is obvious that the systole of atrial chambers will be followed by the systole of ventricular chambers. The ventricular and atrial systole will never coincide. Part of ventricular diastole and atrial diastole occur simultaneously. That is part of atrial diastole will occur when the ventricles are also in diastolic phase.

The further events of the cardiac cycle occurring in the ventricular chambers are discussed on the basis of intraventricular pressure changes. The pressure recording and the volume changes in the ventricular chambers can be made out with the help of cardiac catheterization (Fig. 3.25).

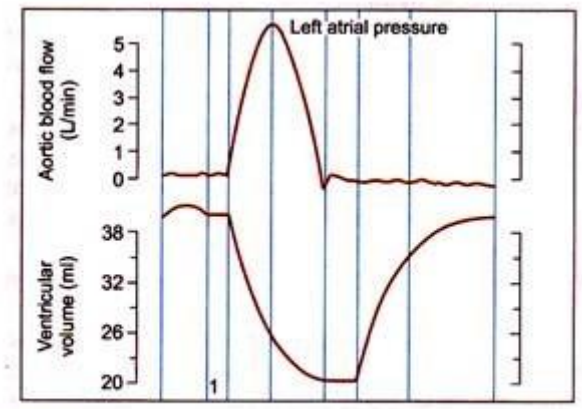


Fig. 3.25: Pressure in aorta and left ventricular volume change during a cardiac cycle

At the end of the atrial systole, the ventricular systole starts. The ventricular chamber is filled with blood. The left intraventricular pressure is about 5-8 mm Hg. As the chamber begins to contract, the intra ventricular pressure begins to rise. Blood in the ventricle tries to go back into the atrium.

This is prevented by the closure of atrioventricular valves and this is responsible for the production of the 1st heart sound. The semilunar valves, which are present at the beginning of aorta, are yet to open, as the aortic pressure is around 80 mm Hg. Hence the ventricle now contracts isometrically as a closed chamber. Because of this, the intraventricular pressure rises sharply.

So, the phase of ventricular systole during which both the AV and SL valves are in closed state, and which gives raise to sharp increase of intra ventricular pressure is known as isovolumetric contraction/isovolumetric ventricular contraction. The duration of this phase is about 0.05 sec.

The ventricular pressure rapidly rises from about 5 mm Hg to about 80 mm Hg. When the intraventricular pressure rises beyond 80 mm Hg, the SL valves are forced open and this leads the next sub-phase of ventricular systole, which is known as maximum ejection phase.

a. Maximum Ejection Phase:

i. Duration of this phase is about 0.11 sec.

ii. The ventricular fibers contract isotonically.

iii. The pressure in the chamber increases gradually to about 120 mm Hg.

iv. Approximately, 70% of the stroke volume is pumped out from the ventricle into the aorta during this phase.

b. Reduced Ejection Phase:

i. Duration is about 0.14 sec.

ii. Some of the ventricular muscle fibers have already started relaxing.

iii. About 30% of the stroke volume is pumped out into the aorta during this phase.

iv. The intraventricular pressure slowly starts decreasing.

c. Protodiastole Phase:

i. Duration is about 0.04 sec.

ii. This is the time interval from the end of ventricular systole to the closure of the SL valves.

iii. When once the intraventricular pressure falls below the aortic pressure, blood from the aorta tends to flow back into the ventricle.

iv. This is prevented by the sudden closure of aortic valves.

v. The closure of aortic valves is responsible for the production of the 2nd heart sound.

The time interval between the 1st and 2nd heart sounds is known as clinical systole.

d. Isovolumetric Relaxation Phase:

i. Duration is about 0.08 sec.

ii. AV valves which were closed at the beginning of ventricular systole are still in the closed state and the SL valves have also got closed.

iii. Now the ventricular muscle starts relaxing, and the ventricle relaxes as a closed chamber. Therefore, the pressure in the ventricle falls sharply without any alterations in the volume of blood in the ventricle

iv. The pressure falls rapidly to as low as zero mm Hg.

Right from the moment the closure of AV valves, the blood that is returning to the heart from the venous compartment keeps getting accumulated in the atria. This is responsible for slow raise of pressure in the atrium. The increase of pressure in the atrium continues until the AV vales open which occurs at the end of isovolumetric ventricular relaxation and this will lead to the next phase namely the initial rapid filling phase.

e. Initial Rapid Filling Phase:

i. Duration is about 0.09 sec.

ii. During this phase, there is pressure gradient between the atrium and the ventricle.

iii. Due to this, blood starts flowing from the atrium to ventricle.

iv. The sudden rush of blood from the atrium to ventricle is responsible for the production of the 3rd heart sound.

v. About 70% of ventricular filling occurs during this phase.

vi. Since no active contraction of the muscle is involved for ventricular filling, only a pressure gradient facilitates this, the ventricular filling occurs by a passive process.

Clinical significance:

Conditions like atrial fibrillations wherein atrial muscles stop contracting. Due to the passive process of filling of ventricle, cardiac output does not fall considerably.

Diastasis (slow filling phase):

i. Duration is about 0.19 sec.

ii. As blood flows from the atrium to ventricle, the pressure falls in the atrium.

iii. Rushing of blood from the atrium into the ventricle, blood accumulates in the ventricle and increases the intraventricular pressure.

iv. Due to this, the pressure gradient between the atrium and ventricle gradually decreases. This stops the further blood flow from the atrium to the ventricle during this phase.

Though there is no blood flow, this phase has lot of practical significance, when there is increase or decrease of heart rate; cardiac cycle duration will also change. Normally, the duration of the ventricular systole does not get altered much when compared to the duration of the ventricular diastole.

Even in the ventricular diastole, it is the duration of the diastasis that is affected. When there is an increase of heart rate, the duration of the diastasis is compromised. Therefore, in spite of an increase in the heart rate, ventricular filling remains fairly normal.

f. Final Rapid Filling Phase:

i. Duration is about 0.1 sec.

ii. This corresponds to the phase of atrial systole.

iii. Now the active contraction of atrial muscle pumps blood from atrium to ventricle.

iv. About 25% of ventricular filling occurs during this phase.

v. Blood flow into the ventricle causes the production of 4th heart sound.

Phonocardiogram: Refers to graphical recording of heart sounds.

Heat sounds can be heard using a stethoscope at specified areas on the precardial region. Almost anyone who is trained better, can hear the 1st and 2nd heart sounds and sometimes the 3rd sound also. But the 4th sound can only be graphically recorded. The heart sounds are affected in conditions like stenosis of valves, incompetence of valves, etc.

At times, in very rare cases, the 1st heart sound may split due to asynchronous closure of mitral and tricuspid valves.

The 2nd heart sound is replaced or followed by murmur in aortic incompetence.

Some of the important features of 1st and 2nd heart sounds have been shown in Table 3.6.

Table 3.6: Differences between 1st and 2nd heart sounds

	1st heart sound	*2nd heart sound*
Produced due to	Closure of AV valves	Closure of SL valves
Duration (sec)	0.12–0.16	0.1–0.14
Frequency (cycles/sec)	Less than 40	More than 40
Pitch	Low	High
Heard better in	Mitral and tricuspid areas	Aortic and pulmonary areas
Indicates	Beginning of ventricular systole	Beginning of ventricular diastole
Carotid pulse relationship	Corresponds to carotid pulse	Follows carotid pulse

Definition of ECG:

This is the graphic records of the variations in electrical potential caused by electrical activity of the heart muscle and detected at the body surface, as a method for studying the action of the heart muscle.

As the cardiac impulse passes through the heart, electrical currents spread into the tissues surrounding the heart, and a small proportion of these spreads all the way to the

surface of the body. If electrodes are placed on the skin on opposite sites of the heart, electrical potentials generated by these currents can be recorded; the recording is known as an electrocardiogram and abbreviated as ECG or EKG.

Characteristics of Normal ECG:

The normal ECG is composed of 'P' wave, a 'QRS' complex, and a 'T' wave. The QRS complex is often three separate waves, the Q wave, the R wave and the S wave.

The P wave is caused by electrical potentials generated as the atria depolarize prior to contraction. The QRS complex is caused by potentials generated when the ventricles depolarize prior to contraction, that is as the depolarization wave spreads through the ventricles.

Therefore, both the P waves and the components of the QRS complex are **"depolarization waves."** The T wave is caused by potentials generated as the ventricles recover from the state of depolarization. This wave is known as "repolarization wave." Thus, the electrocardiogram is composed of both depolarization and repolarization waves.

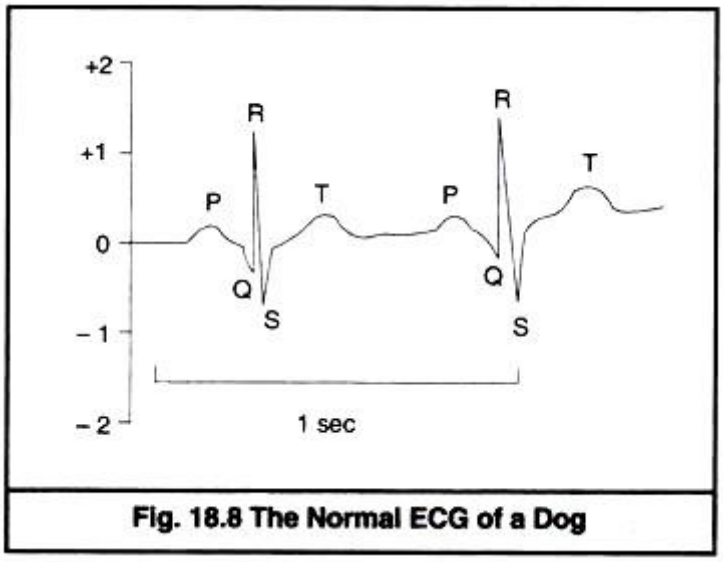


Fig. 18.8 The Normal ECG of a Dog

Meaning of Electrocardiogram (ECG):

At every beat, the heart is depolarized to trigger its contraction. This electrical activity is transmitted throughout the body and can be picked up on the skin. This is the principle behind the ECG. An ECG machine records this activity via electrodes on the skin and displays it graphically. An ECG involves attaching 10 electrical cables to the body, one to each limb and six across the chest.

ECG terminology has two meanings for the word "lead":

i. The cable used to connect an electrode to the ECG recorder.

ii. The electrical view of the heart obtained from any one combination of electrodes.

The standard ECG uses 10 cables to obtain 12 electrical views of the heart. The different views reflect the angles at which electrodes "look" at the heart and the direction of the heart's electrical depolarization.

Leads of Electrocardiogram (ECG):

I. Limb Leads:

Three bipolar leads and three unipolar leads are obtained from three electrodes attached to the left arm, the right arm, and the left leg, respectively. (An electrode is also attached to the right leg, but this is an earth electrode.)

The bipolar limb leads reflect the potential difference between two of the three limb electrodes:

i. The limb leads form the points of Einthoven's triangle (an equilateral triangle used as a model of the standard limb leads used in electrocardiography).

ii. Einthoven's law — The potential differences between the bipolar leads measured simultaneously will have the values II = I + III.

iii. Lead I = LA ↔ RA, Lead II = LL ↔ RA, Lead III = LL ↔ LA (Fig. 6.17).

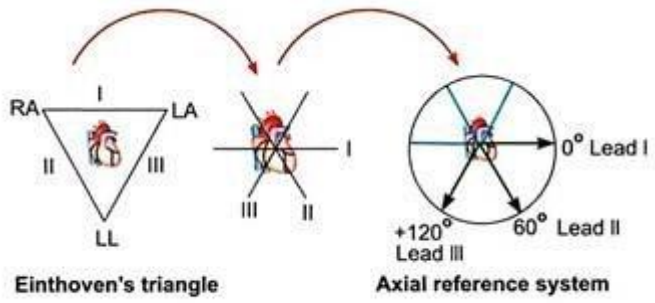


Fig. 6.17: ECG leads

II. Augmented Limb Leads:

The unipolar leads reflect the potential difference between one of the three limb electrodes and an estimate of zero potential — derived from the remaining two limb electrodes. These leads are known as augmented leads.

The augmented leads and their respective limb electrodes are:

i. aVR lead — Right arm

ii. aVL lead — Left arm

iii. aVF lead — Left leg

III. Chest Leads:

Another six electrodes, placed in standard positions on the chest wall, give rise to a further six unipolar leads — the chest leads (also known as precordial leads), V_1-V_6. The potential difference of a chest lead is recorded between the relevant chest electrode and an estimate of zero potential—derived from the average potential recorded from the three limb leads.

Placement of Electrodes:

Electrode label — Electrode placement

RA — On the right arm, avoiding bony prominences

LA — In the same location that RA was placed, but on the left arm this time

RL — On the right leg, avoiding bony prominences

LL — In the same location that RL was placed, but on the left leg this time

V_1 — In the fourth intercostal space (between ribs 4 and 5) just to the right of the sternum
V_2 — In the fourth intercostal space (between ribs 4 and 5) just to the left of the sternum
V_3 — Between leads V_2 and V_4
V_4 — In the fifth intercostal space (between ribs 5 and 6) in the midclavicular line
V_5 — Horizontally even with V_4, but in the anterior axillary line
V_6 — Horizontally even with V_4 and V_5 in the midaxillary line

Planes of View:

The limb leads looking at the heart in a vertical plane, whereas the chest leads look at the heart in a horizontal plane. In this way, a three-dimensional electrical picture of the heart is built up.

ECG leads and their respective views of the heart.

View — Lead

Inferior — II, III, aVF

Anterior — I, aVL, V_1–V_3
Septal — V_3, V_4
Lateral — V_4 –V_6

The Electrocardiogram (ECG) Trace:
The ECG machine processes the signals picked up from the skin by electrodes and produces a graphic representation of the electrical activity of the patient's heart.

The basic pattern of the ECG is logical:
i. Electrical activity towards a lead causes an upward deflection

ii. Electrical activity away from a lead causes a downward deflection

iii. Depolarization and repolarization deflections occur in opposite directions.

Normal Results:
Schematic representation of normal ECG. A typical ECG tracing of the cardiac cycle (heartbeat) consists of a P wave, a QRS complex, a T wave, and a U wave. The baseline voltage of the electrocardiogram is known as the isoelectric line.

Waves and Intervals:

i. P Wave:
It is due to atrial depolarization. Its duration is 0.1 sec and just precedes the atrial systole. Its amplitude is about 0.1-0.3 mV. The cardiac impulse reaches the AV node at the summit of P wave.

ii. QRS Complex:
The QRS complex reflects the rapid depolarization of the right and left ventricles. They have a large muscle mass compared to the atria and so the QRS complex usually has much larger amplitude than the P wave. Q wave is a small downward deflection which represents septal depolarization. R is a prominent positive wave and S is a small negative wave. Both R and S are due to depolarization of ventricular muscle. Duration of QRS complex is 0.08 sec and its amplitude is about 1 mV.

iii. T Wave:
It is due to the ventricular repolarization. It is a broad wave of variable duration and of low amplitude. Its duration is 0.27 sec and amplitude is about 0.15-0.5 mV.

iv. U Wave:
U Wave is rarely seen, and thought to possibly be the repolarization of the papillary muscles.

PR Interval:
PR interval is measured from the beginning of the P wave to the beginning of the QRS complex. The PR interval reflects the time the electrical impulse takes to travel from the sinus node through the AV node and entering the ventricles. The PR interval is therefore a good estimate of AV node function. The normal duration is 0.12-0.16 sec and does not

exceed 0.2 sec. If it is more than 0.2 sec, it indicates conduction delay. PR interval corresponds to the A-C interval of the jugular pulse tracing.

QT Interval:
This is measured from the beginning of the QRS complex to the end of the T wave. The average duration is 0.4 sec. A prolonged QT interval is a risk factor for ventricular tachyarrhythmias and sudden death.

ST Interval:
The ST interval is measured from the J point to the end of the T wave. The average duration is 0.32 sec.

J Point:
The point at which the QRS complex finishes and the ST segment begins. Used to measure the degree of ST elevation or depression present.

ST Segment:
The ST segment connects the QRS complex and the T wave and it is isoelectric. Its duration is 0.05 sec.

Electrical Axis:
The heart's electrical axis refers to the general direction of the heart's depolarization wave front (or mean electrical vector) in the frontal plane. With a healthy conducting system the cardiac axis is related to where the major muscle bulk of the heart lies. Normally this is the left ventricle with some contribution from the right ventricle. It is usually oriented in a right shoulder to left leg direction, which corresponds to the left inferior quadrant of the hexaxial reference system, although -30° to +90° is considered to be normal.

If the left ventricle increases its activity or bulk then there is said to be "left axis deviation" as the axis swings round to the left beyond -30°, alternatively in conditions where the right ventricle is strained or hypertrophied then the axis swings round beyond +90° and "right axis deviation" is said to exist. Disorders of the conduction system of the heart can disturb the electrical axis without necessarily reflecting changes in muscle bulk.

Normal	–30° to 90°		
Left axis deviation	–30° to –90°	May indicate left anterior fascicular block or Q waves from inferior myocardial infarction	Left axis deviation considered normal in pregnant women and those with emphysema
Right axis deviation	+90° to +180°	May indicate left posterior fascicular block, Q waves from high lateral myocardial infarction, or a right ventricular strain pattern	Right deviation is considered normal in children and is a standard effect of dextrocardia

Specific Arrhythmias:

i. Sinus Bradycardia:

Low sinus rate less than 60 beats/min.

ii. Sinus Tachycardia:

High sinus rate of 100-180 beats/min as occurs during exercise or other conditions that lead to increased SA nodal firing rate.

iii. Sick Sinus Syndrome:

A disturbance of SA nodal function that results in a markedly variable rhythm (cycles of bradycardia and tachycardia).

iv. Atrial Tachycardia:

A series of 3 or more consecutive atrial premature beats occurring at a frequency more than 100/min usually due to abnormal focus within the atria and paroxysmal in nature, therefore appearance of P wave is altered in different ECG leads. This type of rhythm includes paroxysmal atrial tachycardia (PAT).

v. Atrial Flutter:

Sinus rate of 250-350 beats/min.

vi. Atrial Fibrillation:

Uncoordinated atrial depolarizations.

vii. Junctional Escape Rhythm:

SA node suppression can result in AV node-generated rhythm of 40-60 beats/min (not preceded by P wave).

viii. AV Nodal Blocks:
A conduction block within the AV node (or occasionally in the bundle of His) that impairs impulse conduction from the atria to the ventricles.

First-Degree AV Nodal Block:
The conduction velocity is slowed so that the P-R interval is increased to greater than 0.2 seconds. It can be caused by enhanced vagal tone, digitalis, beta-blockers, calcium channel blockers, or ischemic damage.

Second-Degree AV Nodal Block:

The conduction velocity is slowed to the point where some impulses from the atria cannot pass through the AV node (Fig. 6.24). This can result in P waves that are not followed by QRS complexes. For example, 1 or 2 P waves may occur alone before one is followed by a QRS. When the QRS follows the P wave, the PR interval is increased. In this type of block, the ventricular rhythm will be less than the sinus rhythm.

There are two subtypes of second-degree AV blocks:

I. Mobitz I and

II. Mobitz II.

I. Mobitz I:
In Mobitz I (Wenkebach block), the PR interval gradually increases over several beats until it is sufficiently prolonged (that is, AV conduction is sufficiently impaired) that the impulse fails to pass into the ventricles (i.e. a P wave will not be followed by a QRS).

II. Mobitz II:
Mobitz II occurs when the PR interval is fixed in duration, but some P waves are not followed by a QRS (as illustrated). If complete heart block develops suddenly, there occurs a delay before ventricles start beating at their own rate. During this period the systemic blood pressure falls to a very low level and blood supply to brain becomes inadequate. If ventricular standstill lasts for few seconds, it causes dizziness, and fainting, called Stokes-Adams syndrome, or if it is more prolonged, it leads to loss of consciousness, convulsions and death.

Third-Degree AV Nodal Block:

Conduction through the AV node is completely blocked so that no impulses are able to be transmitted from the atria to the ventricles. QRS complexes will still occur (escape rhythm), but they will originate from within the AV node, bundle of His, or other ventricular regions. Therefore, QRS complexes will not be preceded by P waves.

Furthermore, there will be complete asynchrony between the P wave and QRS complexes. Atrial rhythm may be completely normal, but ventricular rhythm will be

greatly reduced depending upon the location of the site generating the ventricular impulse. Ventricular rate typically range from 30 to 40 beats/ min.

i. Supraventricular Tachycardia (SVT):
Usually caused by re-entry currents within the atria or between ventricles and atria producing high heart rates of 140-250, the QRS complex is usually normal width, unless there are also intraventricular conduction blocks (e.g. bundle branch block).

ii. Ventricular Premature Beats (VPBs):
Caused by ectopic ventricular foci; characterized by widened QRS, often referred to as a premature ventricular complex, or PVC.

iii. Ventricular Tachycardia (VT):
High ventricular rate caused by aberrant ventricular automaticity (ventricular foci) or by intraventricular re-entry can be sustained or non-sustained (paroxysmal) usually characterized by widened QRS (>0.14 sec) rates of 100 to 280 beats/min, life-threatening.

iv. Ventricular Flutter:
Very rapid ventricular depolarizations >250/min, since wave appearance; leads to fibrillation.

v. Ventricular Fibrillation:
Uncoordinated ventricular depolarizations; leads to death if not quickly converted to a normal rhythm or at least a rhythm compatible with life.

Bundle Branch Block (BBB):
A problem in the bundle of His presents in an identical fashion to a combined block of both bundles, i.e. complete heart block. However, a more common occurrence is an isolated left or right bundle branch block. The patterns of the ECG are characteristic, but highly variable; the hallmark is a wide QRS complex. In left bundle branch block (LBBB), the pattern is best detected in V_6 where there is an "M" pattern, while in V_1 there is a "W" pattern.
In right bundle branch block (RBBB), the pattern is best detected in V_1 where there is an RSR complex, while in V_6 there is a QRS complex.

Ventricular Pre-Excitation:

Wolff-Parkinson-White syndrome (WPW syndrome) Pre-excitation is defined as an early depolarization of the ventricular myocardium that occurs prior to any conduction through the AV node. The most common condition in which this is seen is WPW syndrome, where there is an accessory AV pathway called the bundle of Kent.

The anomalous conducting system can be located anywhere around the mitral or tricuspid rings. Conduction through the accessory connection is faster and is independent of the heart rate. Consequently, the ventricular myocardium is activated from two directions – through the normal system and through the accessory pathway.

The resulting QRS complex is a product of fusion of the two distinct activation wave fronts. Since conduction over the accessory pathway is faster, the initial part of the QRS complex represents ventricular activation through this route (delta wave—Fig. 6.27).

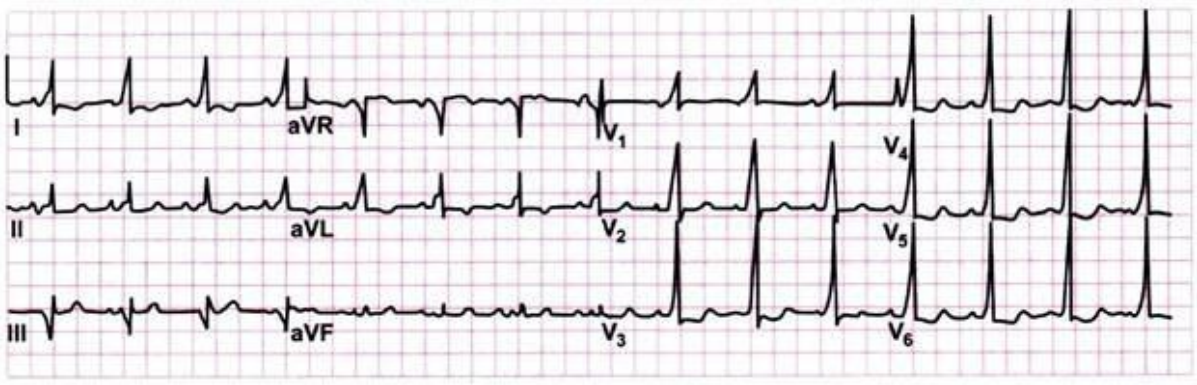


Fig. 6.27

Hyperkalemia:

Note the tall, tented T waves in ECG (Fig. 6.28).

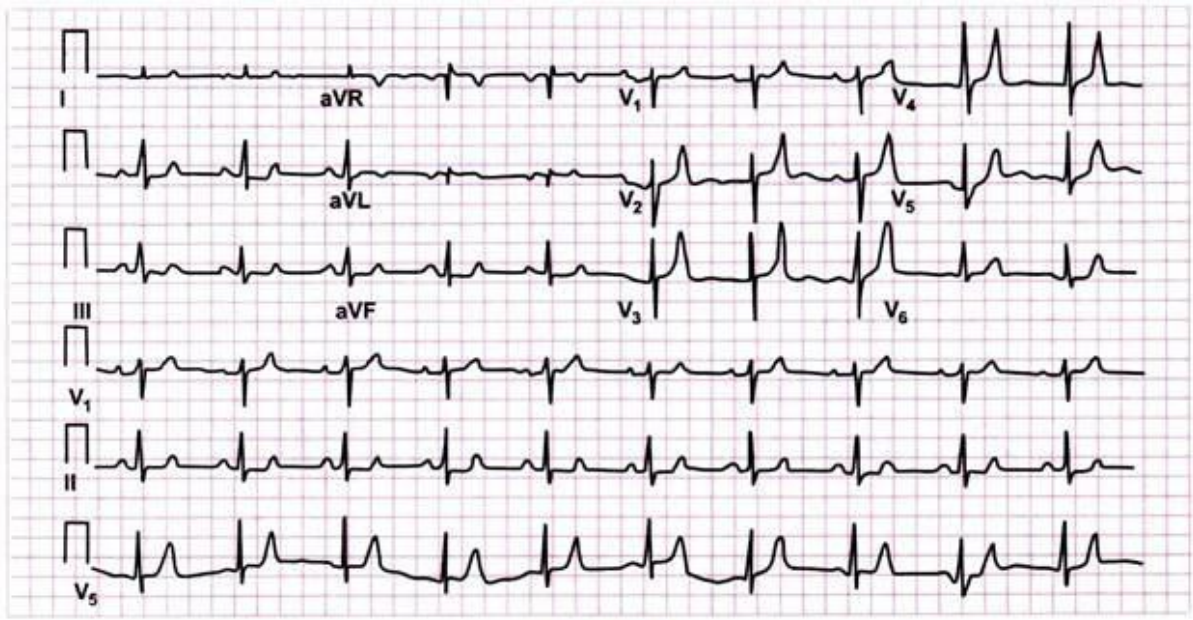


Fig. 6.28

Sequence of Electrocardiogram (ECG) Changes:

Sequence of Changes in Myocardial Infarction:

The ECG sequence shown in Fig. 6.29 gives you an idea as to how ST elevation would develop with this process of necrosis.

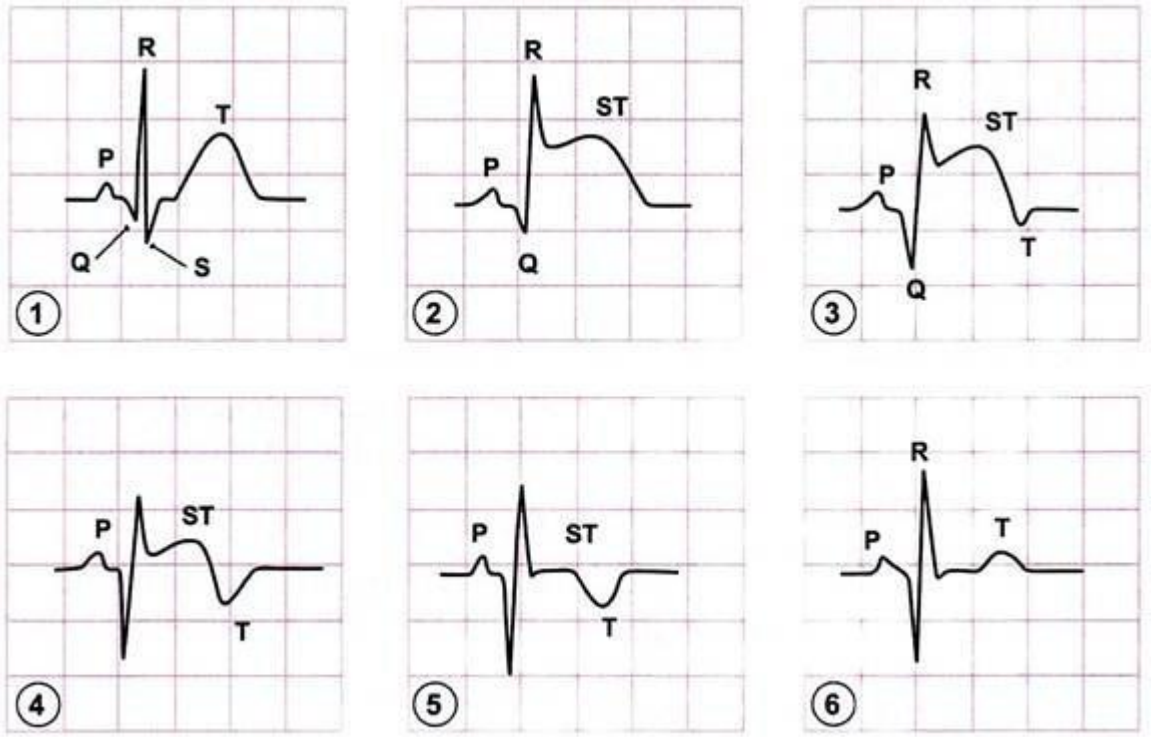


Fig. 6.29: Sequence of ECG changes in myocardial infarction

Image:

Sequence of changes in evolving anterior myocardial infarction.

Let's work through the sequence in numerical order:

1. This picture shows a normal sinus complex. The ST segment is on the isoelectric line. At the onset of pain the ECG would be normal but the ST segment would soon start to change. In this picture, the T wave has grown taller.

2. Within an hour the ST segment would be noticeably elevated, indicating the onset on myocardial necrosis (tissue death).

3. If thrombolysis is administered, we would be looking for specific changes on the ECG. In this picture, the ST elevation has reduced by more than 50% from picture 2. You can also see the T wave invertion is much deeper. This is a good sign of reperfusion.

4. In this picture you can see the ST segment is back on the isoelectric line but the T wave remains inverted.

5. Six, in some cases, after a few months the ECG looks relatively normal. Compare picture 6 with picture 1. They look much the same but for the deep Q wave in picture 6. A deep Q wave is an indicator myocardial tissue death and will remain on the ECG.

Myogenic and Neurogenic Heart:

Neurogenic and Myogenic hearts • In animals with open circulatory system the heart is usually sac-like or tubular. It has ostia or lateral openings which get closed when heart contracts and opens when heart relaxes. When heart relaxes vaccum is created to suck blood in the heart. Hence these hearts are known as suction pumps. • In most of the suction pump hearts the beating rhythm is set through nerve impulses. Such hearts are known as Neurogenic hearts.

In higher animals with closed ciruclatory system 2, 3 or 4 chambered hearts are seen with muscular ventricles which pumps the blood in the body with pressure and hence heart are known as pressure pumps. • In pressure pump heart the rhythm is set in specialised muscle cells within the heart. They are known as Myogenic hearts. • Most of the embryonic hearts are myogenic which later on may be become myogenic or neurogenic.

Probable Questions:

1. What is cardiac cycle? Discuss different phases of cardiac cycle?
2. Define ECG? What is the characteristics of normal ECG?
3. Discuss different waves and intervals of ECG?
4. Discuss two subtypes of second-degree AV blocks.
5. What is third degree AV block?
6. Differentiate myogenic and neurogenic heart.

Suggested Readings:

1. Animalphysiology-MohanP.Arora.
2. TextbookofmedicalphysiologyArthurC.Guyton,JohnE.Hall.
3. Ganong'sreviewofmedicalphysiology.

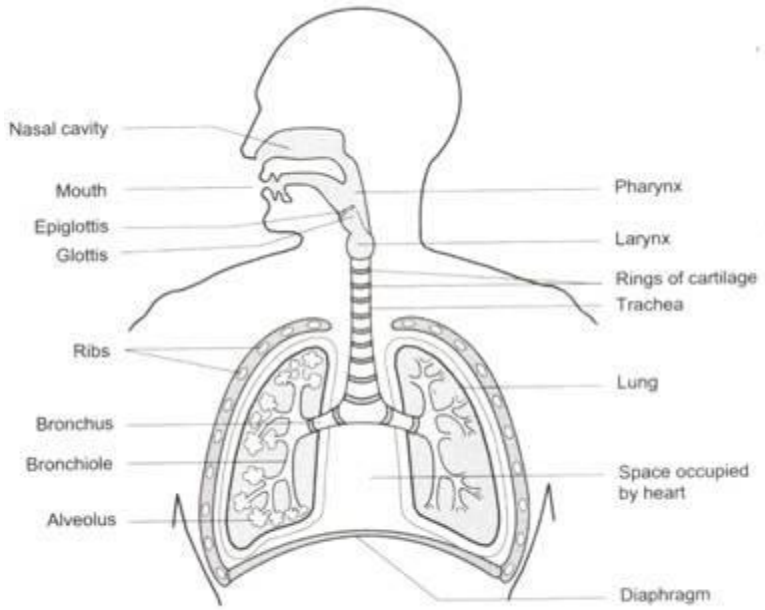
UNIT-III

Respiratory system: Comparative account of respiratory pigments; transport and exchange of gases

Objective: In this unit we will learn about comparative anatomy of respiratory system in different groups of vertebrates. We will also learn about Comparative account of respiratory pigments and how the respiratory gases are exchanged.

Human Respiratory System and it's Mechanism:

The human respiratory system consists of a pair of lungs and a series of air passages leading to the lungs.The entire respiratory tract (passage) consists of the nose, pharynx, larynx, trachea, bronchi, and bronchioles.



Human respiratory system

Air enters the nose through the nostrils. When air passes through the nose, it is warmed, moistened and filtered. The hairs present in the nose filter out particles in the incoming air. The air is moistened by the mucus present in the nose, and it is warmed by the blood flowing through the capillaries in the nose.

The respiratory tract from the nose to the bronchioles is lined by mucous membranes and cilia. The mucus and cilia act as additional filters.

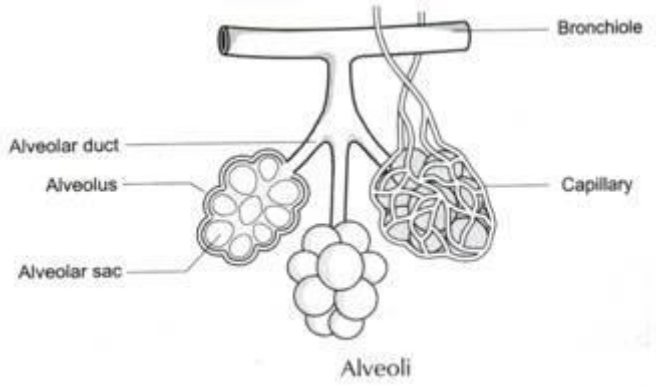
Behind the nose lies the pharynx (throat). There are two passages here—one for food and the other for air. The air passes from the pharynx to the larynx, or the voice box. The opening leading to the larynx is called glottis. It is protected by a lid called epiglottis, which prevents food from entering the passage to the lungs.

From the larynx the air goes to the trachea, or the windpipe. The trachea is about 11 cm long. It is guarded by 16-20 C-shaped cartilage rings, which prevent the trachea from collapsing. The trachea divides into two tubes called bronchi. Each bronchus divides and branches out in the form of thinner tubes called bronchioles.

The bronchioles enter the lungs and divide further into finer branches called alveolar ducts. These open into extremely thin-walled, grape-shaped air sacs called alveoli. Each alveolus is covered by a web of blood capillaries.

The lungs are a pair of spongy organs lying in the chest cavity formed by the ribs. The actual exchange of gases between the air and the body takes place in the capillary-covered alveoli inside the lungs. Here, oxygen from the air in the alveoli goes into the blood, and the carbon dioxide in the blood goes out.

The oxygen binds to the haemoglobin molecules present in the red blood corpuscles and is taken to different parts of the body.



Alveoli

The total surface area through which the exchange of gases can take place increases because of the millions of alveoli in the lungs. Their total surface area can be about a hundred times that of the body. The large surface area allows sufficient oxygen intake needed for releasing the large amount of energy required by us.

Mechanism of Breathing:

There are two main steps in breathing: inspiration and expiration:

Inspiration:

Inspiration (inhalation) is the process of breathing in, by which air is brought into the lungs.

Inspiration involves the following steps:

i. The muscles attached to the ribs on their outer side contract. This causes the ribs to be pulled out, expanding the chest cavity.

ii. The muscle wall between the chest cavity and the abdominal cavity, called diaphragm, contracts and moves downwards to further expand the chest cavity.

iii. The abdominal muscles contract.

The expansion of the chest cavity creates a partial vacuum in the chest cavity. This sucks in air into the lungs, and fills the expanded alveoli.

Expiration:

After the exchange of gases in the lungs, the air has to be expelled. Expulsion of the air from the lungs is called expiration. In this process, muscles attached to the ribs on their inner side contract, and the diaphragm and the abdominal muscles relax. This leads to a decrease in the volume of the chest cavity, which increases the pressure on the lungs. The air in the lungs is pushed out and it passes out through the nose.

When we breathe out, not all of the air in the lungs gets expelled. Some of it remains in the lungs. This keeps the lungs from collapsing and allows more time for the exchange of gases.

Functions of respiratory system can be broadly classified into:

a. Respiratory

b. Non-respiratory.

a. Respiratory Function:

It is to provide adequate volume of oxygen to the tissues. Normal person at rest needs about 250 ml of oxygen per minute. Atmospheric air enters the lungs during inspiration. Oxygen from the air diffuses through the alveoli into pulmonary capillary blood. The oxygenated blood reaches the left ventricle and from there it gets pumped to reach all parts of the body.

About 200 ml of carbon dioxide is produced in the body every minute because of tissue metabolism. From the tissues, carbon dioxide enters the blood to reach the lungs for the process of excretion. When deoxygenated blood reaches the lungs, carbon dioxide gets diffused from the pulmonary capillaries into the alveoli. The air from the alveoli is expelled out from the lungs by the process of expiration.

b. Non-Respiratory Functions:

1. Regulation of acid-base (pH) balance.

2. Mast cells present in the lungs produce heparin, which acts as an anticoagulant.

3. Macrophages in the alveoli have phagocytic function.

4. Converting enzyme present in the lungs play a role in converting angiotensin I to angiotensin II, which is a powerful vasoconstrictor.

5. The passage of the air through the larynx is essential for vocalization and has role in communication by speech.

6. Plays a minor role in body temperature regulation.

Mechanisms of Respiration:

Respiration has two phases namely inspiration and expiration. During normal quiet inspiration due to contraction of muscles of inspiration, the chest and lungs expand. The pressure inside the alveoli (intra- alveolar pressure) falls below the atmospheric pressure.

Due to the pressure gradient developed in the direction of the alveolus, air moves from the atmosphere into the lungs. Because inspiration is brought about by the contraction of the muscles, the process of inspiration is an active one.

However, the process of expiration is normally a passive process. The relaxation of the muscles of inspiration and the recoiling of the elastic fibers present in the lungs is more than enough to bring about the expiration.

During expiration, since the alveoli are trying to recoil, the intra-alveolar pressure becomes more than the atmospheric pressure and hence air can be driven out of the lungs into the atmosphere. In forced expiratory states, expiration needs the active contraction of certain muscles. Hence in such states even expiration becomes an active process.

a. Muscles of inspiration:

Diaphragm and external intercostals are the muscles of inspiration during a normal quiet breathing. However, during forced inspiration, contraction of sternocleidomastoid, scalene, serratus anterior and platysma muscles is very much required. These muscles are known as accessory muscles of inspiration.

b. Muscles of expiration:

The normal quiet expiration is a passive process. However, in forced expiration, even this phase becomes an active process and requires active contraction of certain muscles. The muscles that are involved in forced expiration are known as accessory muscles of expiration and they are internal intercostals and muscles of the anterior abdominal wall.

Thorax is separated from the abdominal cavity by the diaphragm, a dome-shaped muscle. The thorax has three different diameters namely vertical, transverse and anteroposterior. During inspiration, the thoracic volume gets increased because of increase in the diameters of chest. The increase in the thoracic volume decreases the intra-alveolar pressure.

The most important muscle of inspiration is diaphragm supplied by the phrenic nerve. This is responsible for about 70% increase in the thoracic volume and the rest volume increase in thorax is contributed by the contraction of external intercostals supplied by the intercostal nerves.

This type of respiration is called as abdominothoracic type. In case the external intercostals play a major role in expansion of thorax, the type of respiration is known as thoracoabdominal type.

Contraction of the diaphragm alters the vertical diameter whereas the contraction of the external intercostals increases the anteroposterior and transverse diameters of the thoracic cavity.

Role of Lungs in Excretion:

Human lungs regularly remove about 18 L of CO_2 per hour and about 400 ml of water per day in normal resting condition. Water loss through the lungs is small in hot humid climate and large in cold dry climate. Thus CO_2 and water (both are metabolic wastes produced during oxidation of food in the cells) are removed via lungs.

Role of Skin in Excretion:

In many aquatic animals ammonia is mainly excreted out into the surrounding water by diffusion through the skin. Human skin has two types of glands: sudoriferous (sweat) glands and sebaceous (oil) glands.

(i) Sudoriferous glands (Sweat glands) secrete an aqueous fluid called sweat. Sweat contains water (99.5%), NaCl, urea, lactic acid, amino acids and glucose. Sweat does not contain uric acid.

The volume of sweat varies from negligible to 14L a day, depending upon activity and temperature. When sweat evaporates, it provides cooling to the body. Normal pH of sweat is 4.5. Sweat production is also influenced by atmospheric temperature.

(ii) Sebaceous glands (Oil glands) secrete an oily or wax-like secretion called sebum. It keeps the skin oily. Sebum removes some lipids like waxes, sterols, other hydrocarbons and fatty acids from the body.

Role of Liver in Excretion:

Urea is formed in the liver which is eliminated through kidneys. Liver cells also degrade the haemoglobin of worn out red blood corpuscles into bile pigments (bilirubin and bi-liver- din). Liver cells also excrete cholesterol, certain products of steroid hormones, some vitamins and many drugs. Liver secretes these substances in the bile. The bile carries these substances to the intestine and are passed out with faeces.

Role of Intestine in Excretion:

The epithelial cells of the intestine (colon) excrete certain salts such as iron and calcium. These salts are eliminated with the faeces.

Role of Salivary glands in Excretion:

Salivary glands excrete substances like mercury, potassium iodide, lead and thiocyanate. In aquatic animals like fish, gills remove carbon dioxide. Gills of many bony fish also excrete salt.

Mechanism of Breathing:

It means the inflow (inspiration) and outflow (expiration) of air between atmosphere and the alveoli of the lungs. It is affected by the expansion and contraction of lungs. There are mainly two processes by which the lungs are expanded or contracted.

(i) The downward and upward movement of the diaphragm which increases and decreases the diameter of the thoracic cavity (chest cavity).

(ii) The elevation and depression of the ribs, which lengthens and shortens the thoracic cavity.

1. Inspiration:

It is a process by which fresh air enters the lungs. The diaphragm, intercostal muscles and abdominal muscles play an important role.

(i) Diaphragm:

The diaphragm becomes flat and gets lowered by the contraction of its muscle fibres thereby increasing the volume of the thoracic cavity in length.

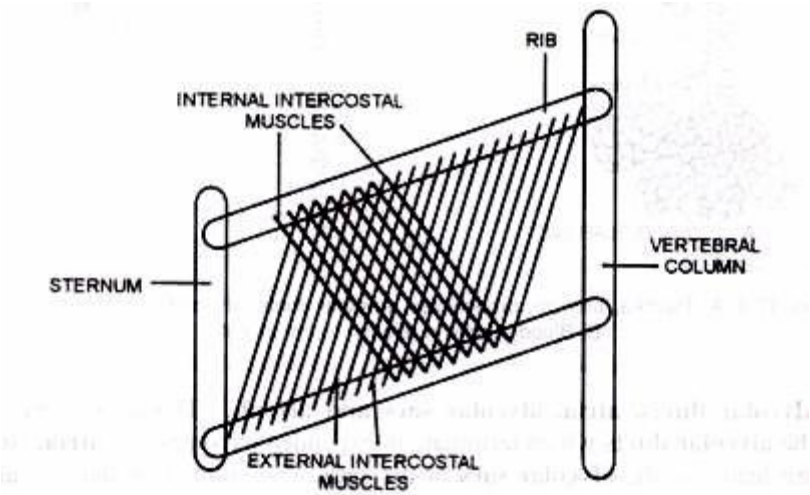


Fig. 17.5. Diagrammatic representation of the position of the intercostal muscles.

(ii) External intercostal muscles:

They occur between the ribs. These muscles contract and pull the ribs and sternum upward and outward thus increasing the volume of the thoracic cavity

(iii) Abdominal Muscles:

These muscles relax and allow compression of abdominal organs by the diaphragm. The abdominal muscles play a passive role in inspiration. The muscles of the diaphragm and external intercostal muscles are principal muscles of inspiration.

Movement of Fresh Air into the Lungs:

Thus overall volume of the thoracic cavity increases and as a result there is a decrease of the air pressure in the lungs. The greater pressure outside the body now causes air to flow rapidly into external nares (nostrils) and through nasal cavities into internal nares.

Thereafter the sequence of air flow is like this:

External nares → Nasal cavities → Internal nares → Pharynx → Glottis → Larynx → trachea → Bronchi → bronchioles → alveolar ducts → alveoli.

From the alveoli oxygen passes into the blood of the capillaries and carbon dioxide diffuses out from the blood to the lumen of the alveoli.

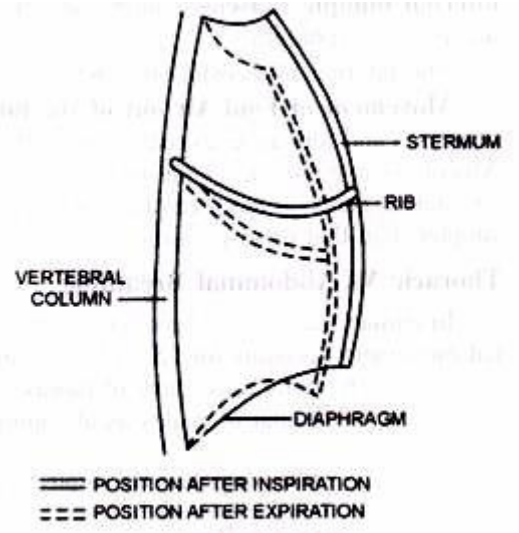


Fig. 17.6. Side view of thorax to show movements during breathing (only one rib shown).

2. Expiration:

It is a process by which the foul air (carbon dioxide) is expelled out from the lungs. Expiration is a passive process which occurs as follows.

(i) Diaphragm:

The muscle fibres of the diaphragm relax making it convex, decreasing volume of the thoracic cavity.

(ii) Internal Intercostal Muscles:

These muscles contract so that they pull the ribs downward and inward decreasing the size of me thoracic cavity.

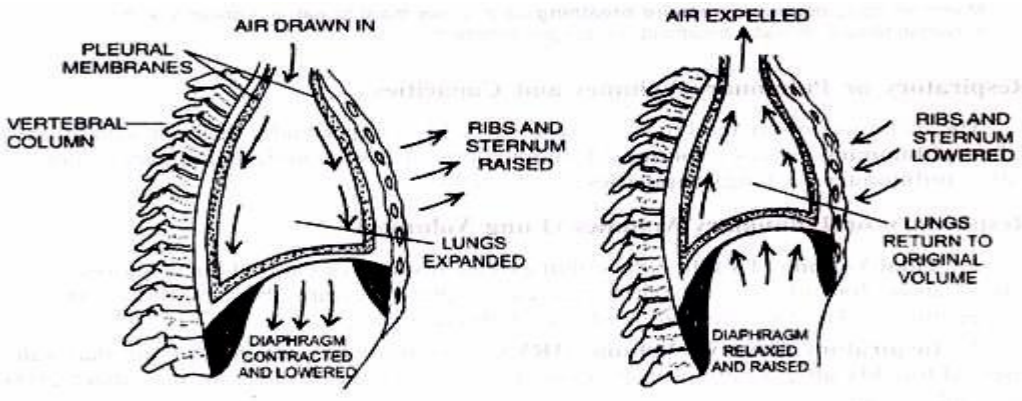


Fig. 17.7. Mechanism of preathing showing inspiration and expiration.

(iii) Abdominal Muscles:

Contraction of the abdominal muscles such as external and internal oblique muscles compresses the abdomen and pushes its contents (viscera) towards the diaphragm. The internal intercostal and abdominal muscles are muscles of expiration.

Movement of Foul Air out of the lungs:

Thus overall volume of the thoracic cavity decreases and foul air goes outside from the cavities of the alveoli in the following manner:

Alveoli → alveolar ducts → bronchioles → bronchi → trachea → larynx → glottis → pharynx → internal nares → nasal cavities → external nares → outside. The process of expiration is simpler than that of inspiration.

Thoracic Vs. Abdominal Breathing:

In human males, lateral movement of thorax constitutes 25% of breathing while abdominal movement accounts for 75% of breathing. In pregnant women, almost the entire breathing is through lateral movement of thorax. Therefore, breathing of women is often regarded as thoracic while that of males as abdominal.

Advantages of Nasal Breathing:

Breathing through nose is healthier because it is a natural process. The air which is inhaled contains dust, bacteria, etc., get filtered in the nose. Thus the air which goes into lungs is cleaner. The conchae of the nose also filter and warm up the air.

Respiratory or Pulmonary Volumes and Capacities:

The quantities of air the lungs can receive, hold or expel under different conditions are called pulmonary (= lung) volumes. Combinations of two or more pulmonary volumes are called pulmonary (= lung) capacities.

Respiratory or Pulmonary Volumes (Lung Volumes):

1. Tidal Volume (TV):

It is the volume of air inspired or expired during normal breath. This is about 500 mL, i.e., a healthy man can inspire or expire about 6000 to 8000 mL of air per minute. The lowest value is of tidal volume.

2. Inspiratory Reserve Volume (IRV):

It is the extra amount of air that can be inspired forcibly after a normal inspiration. Thus it is forced inspiration. It is about 2500 to 3000 ml. of air

3. Expiratory Reserve Volume (ERV):

It is the extra amount of air that can be expired forcibly after a normal expiration. Thus it is forced expiration. It is about 1000 to 1100 ml.

4. Residual Volume (RV):

It is the volume of air which remains still in the lung after the most forceful expiration. It is about 1100 mL to 1200 ml.

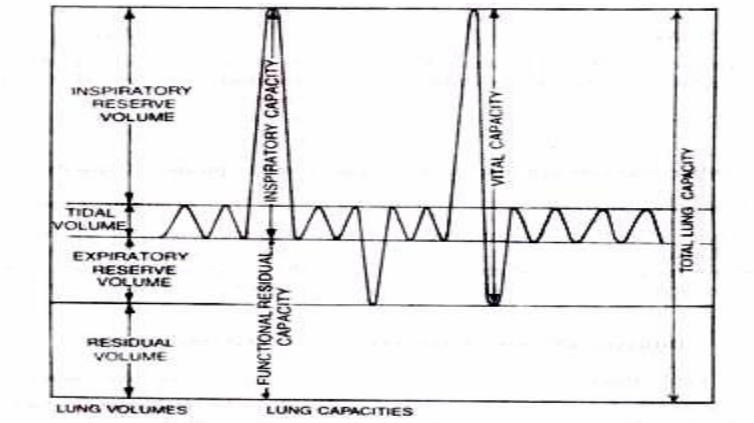


Fig. 17.8. Diagram showing Pulmonary volumes and Pulmonary capacities.

Respiratory or Pulmonary Capacities (Lung Capacities):

1. Inspiratory Capacity (IC):

It is the total volume of air a person can inspire after a normal expiration. It includes tidal volume and inspiratory reserve volume (TV + IRV).

2. Expiratory Capacity (EC):

It is the total volume of air a person can expire after a normal inspiration. This includes tidal volume and expiratory reserve volume (TV + ERV).

3. Functional Residual Capacity (FRC):

Volume of air that will remain in the lungs after a normal expiration is called functional residual capacity. This includes residual volume and the expiratory reserve volume (RV + ERV).

4. Vital Capacity (VC):

The maximum volume of air a person can breathe in after a forced expiration or the maximum volume of air a person can breathe out after a forced inspiration is called vital capacity. This includes tidal volume, inspiratory reserve volume and expiratory reserve volume (TV + IRV + ERV). In fact total lung capacity minus residual volume is called vital capacity. VC varies from 3400 mL to 4800 ml. depending upon age, sex and height of the

individual. The vital capacity is higher in athletes, mountain dwellers than in plain dwellers, in men than women and in the young ones than in the old persons.

5. Total Lung Capacity (TLC):

It is the total volume of air present in the lungs and the respiratory passage after a maximum inspiration. It includes vital capacity and the residual volume (VC + RV). All pulmonary volumes and capacities are about 20 to 25 per cent less in women than in men and they are greater in tall persons and athletes than in small and asthenic (slight build) people.

Respiratory Quotient (RQ):

Respiratory quotient is the ratio of the volume of carbon dioxide produced to the volume or oxygen consumed over a period of time in respiration.

RQ = Volume of CO_2 evolved/Volume of O_2 absorbed

Respiratory quotient varies with different foods utilized in respiration. For glucose, RQ (RQ $6CO_2/6O_2 - 1$), for fats it is about 0.7, for proteins it is about 0.9 and for organic acids it is about 1.3 or 1.4. In anaerobic respiration, there is no consumption of oxygen. Carbon dioxide is produced in most of the cases. Therefore R.Q. is infinity. The respiratory quotient indicates the type of food oxidized in the body of the animal during respiration.

Table 17.1 : Partial Pressure of Respiratory Gases in mm Hg

Gas	*Inspired air*	*Alveolar air*	*Deoxygenated blood*	*Oxygenated blood*	*Expired air*	*Tissues*
Oxygen	159	104	40	95	116	40
Carbon dioxide	0.3	40	45	40	32	45

Respiratory Pigments:

A respiratory pigment is a kind of substance which increases the oxygen carrying capacity ofblood in human (hemoglobin) and in other vertebrates. These are metal containing proteins,combinereversiblywithoxygen.Thefourcommonrespiratorypigmentsare– 1)Hemocyanin,2)Hemoglobin 3)Chlorocruorin and 4)Haemerythrin.

1. Haemoglobin:

A haemoglobin (Hb) molecule is a conjugated protein, because it consists of a simple protein and with a non-protein part. The non-protein part is called prosthetic group. The protein part of the haemoglobin is called globin (96%) and a porphyrin ring with an

iron atom at its centre, called haem or haematin (4%). The globin part consists of 4 polypeptide chains-two alpha (α) chains and two beta (β) chains.

Porphyrins are heterocyclic ring structure containing haem or magnesium. The heterocyclic ring structure is composed of four pyrrole rings which are linket by methine bridges.

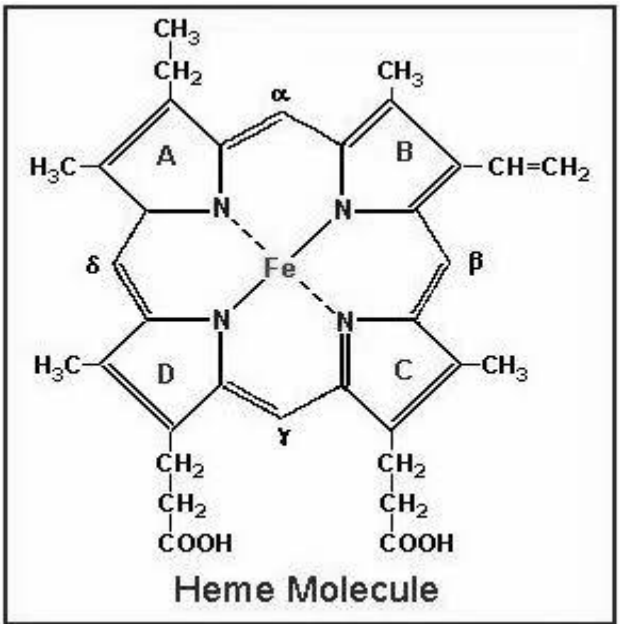
The globin part helps to prevent oxygen from binding tightly to haem; when globin is present, oxygen binds reversibly to haem and can be released to the tissues. In respiratory organs the haemoglobin combines with O_2 which form Oxyhaemoglobin at normal temperature and pressures.

At the low pressure the oxyhaemoglobin dissociates as oxygen and haemoglobin ($HbO_2 \leftrightarrow Hb + O_2$)- Haemoglobin is involved in vertebrates in the transport of respiratory CO_2 (about 10% of the total) as carbamino-haemoglobin in which CO_2 is bound to the globin protein. The molecular weight of a haemoglobin molecule is 64,500 daltons.
The oxygenated form of haemoglobin is scarlet and deoxygenated form is bluish-red. The haemoglobin is present in the erythrocytes in almost all vertebrates except a few Antarctic fish. In the invertebrates they are found in the plasma, coelomic fluid and haemoglobin-containing cells.

In Annelida, the pigment is found in polychaeta (different kinds of respiratory pigments), in Oligochaeta (e.g., Pheretima, Lumbricus, Tubifex etc.), and in Hirudinea (e.g., Hirudo, Hirudinaria, etc.).In Mollusca the haemoglobin is found in the plasma of Gastropods (e.g., Planorbis), and in Bivalvia (e.g., Solen, Area, etc.). In Chiton and in some prosobranchs the haemoglobin is present in the muscles of radula as Myoglobin. In Crustacea the pigment is present in small-sized animals (e.g., Artemia, Daphnia, Triops, etc.).

2. Hemocyanin- Itistheprimaryrespiratorypigmentoroxygentransporterthroughoutthebodyofsomeinvertebrateanimals. (Abbreviation-Hc).Molecularweight-rangesfrom17,000-30,00,000KDa.

Chemistry and Structure-Hb made up of globulin chains (connected together).In normal adultHb (HbA) two □ (141 □□□□□□□ □□□□□ □□□□□□□□□□)and two β(146 amino acid residues) globulinchainsarepresent.Infetus,thebetachainsarenormalyabsent,insteadoftwobetachains,two γ chains are present. Each globulin chains contain an important iron containing porphyrincompound-knownasHeme.Hemegroupcontainsaniron(Fe)ion(coordinatewith4nitrogen atoms) in a heterocyclic ring. The porphyrin ring consists of four pyrrole moleculesjoined bymethanebridge.



Heme Molecule

Oxyhemoglobin-isa formwhenoxygenbindswithhemegroup.

Oxygenetionofhemoglobin-deoxygenetivehemoglobinispurplish,butbrightredinoxygenativestate.

Distribution-all vertebrates possesHb except Lepidocephalous larva.Mollusca-purpura,chiton, etc.Annelida-*Lumbricus,Arenicota,Tubifex.*

Echinodermata-Seacucumber.

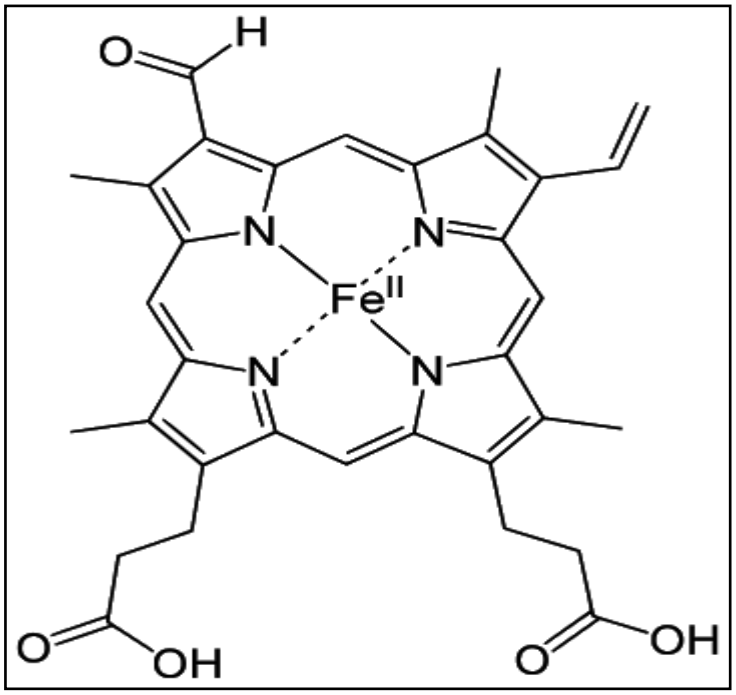
Arthropods-insects (Chironmas and Gastrophilus).Nematoda-*Ascarissp.*

Haemocyanin is a blue-green copper containing respiratory pigments found in some crustaceans, xiphosurans, myriapodes, and in some gastropods, bivalves and cephalopods. Haemocyanin is always found in dissolved condition in plasma. It occurs in two forms— oxidized and reduced forms, and in reduced forms the prism-shaped or needle-shaped crystals are soluble in water.

The oxygen carrying capacity of haemocyanin is lesser than haemoglobin. The mol. weight is variable in different groups of animals. In some crustaceans the mol. weight is about 4,00,000 daltons and in some gastropods it is 13,00,00,000.

Haemocyanin binds a molecule of oxygen between a pair of copper atoms linked to amino acid side chains. In oxygenated condition the haemocyanin is bluish- green but it is colourless in deoxygenated state.In addition,someprotozoa,yeastand rootnodulesofleguminousplantsalsocontainHb.

3.Chlorocruorin-It is a green coloured respiratory pigment containing iron found in the plasma of some polychaetes (e.g., Serpulid, spirorbid, sabellidfanworms). It is also found in oxygenated and reduced forms. The metalloprotein of chlorocruorin is similar to haemoglobin except one vinyl group (CH_2 = CH—) is replaced by formyl (HCO—) group. The mol. weight is 30,00,000 daltons and generally it functions as oxygen carrier.Itisanhemeprotein,similartoerythrocruorin(foundinannelidsandarthropods).Mol ecularweight-20075000Da. Chemistryandstructure-Itconsistsofmanymyoglobinlikesubunits,arrangedinahugecomplexofoverahundredsubu nits.It containsabnormal heme group.Oxygenation-Oxygenatedchlorocruorinturnsfromgreentored.



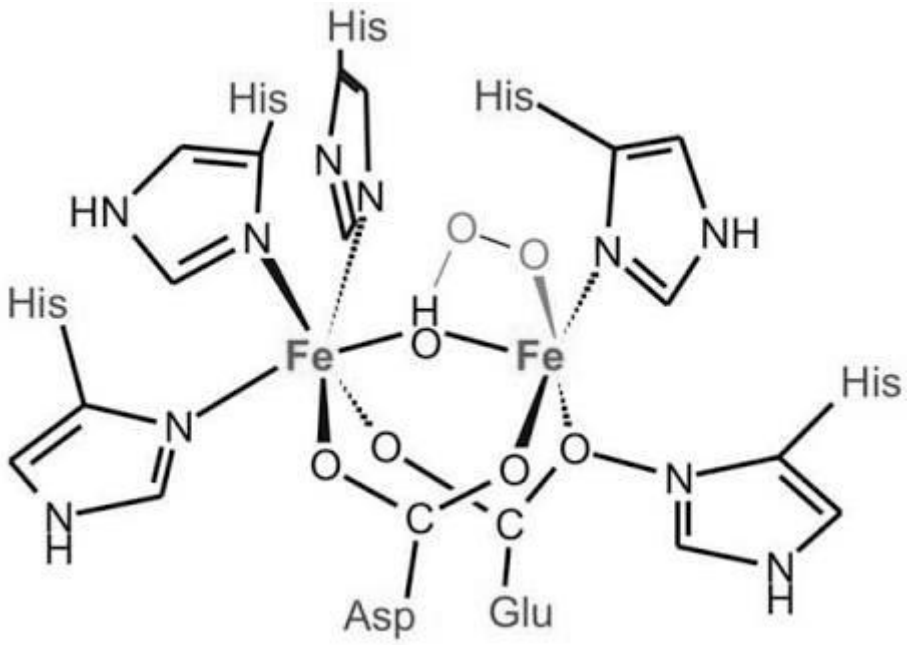
Distribution-restrictedin4familiesofpolychaetes-Sabellidae(allsp.),Surpulidae,Chloronaemidae.

4. Hemerythrin- It is an iron containing respiratory pigment found in the blood corpuscles of some invertebrates (e.g., Sipunculans, Priapulids and inarticulate Brachiopods). It is pink or violet coloured in oxygenated state and colourless in

deoxygenated state. The mol. weight varies from 40,000 to 108,000 daltons and plays the role of oxygen storage.

Thepigmentismainlyresponsibleforoxygentransportinthemarineinvertebratephyla.**Molecularweight-1**,08,000KDa.

Chemistryandstructure-
Thepigmentisahomooctamerorheterooctamerinstructure,composedofalphaandbetatype subunits.Eachsubunithasfouralphahelixfoldbindingabinuclearironcentre.Itlackscooperativebindingoftooxygen,roughly¼0fhemoglobin.Oxygenation-
oxygenatedhaemerythrinisvioletopinkcoloured.Butcolourlesswhendeoxygenated.



Distribution-Sypunculids-allsp.Polychaetes-Megelona,Brachiopods-Lingnea.

HEMOGLOBIN

1. Hemoglobin,theO_2carryingpigmentfound withinthe cytoplasmofRBC.

2. In RBM,Hb begins to appear from the stage of intermediate or early normoblast stage,leadsto newHb synthesis.

3. MatureRBCcannotsynthesizenewHb.

Normalvalues-Innormaladultmaleitisabout15gm/100ml,andinfemale11-13gm/100ml.Overthe ageof75yrs.Hbconcentrationinmales about12.5gm%.

4. **Shape-**spherical.

5. **Molecularweight**-64,500.

Structure-

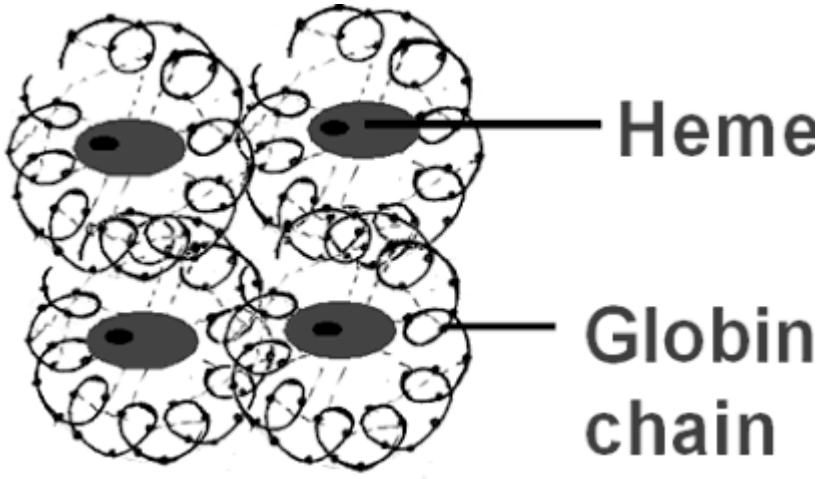
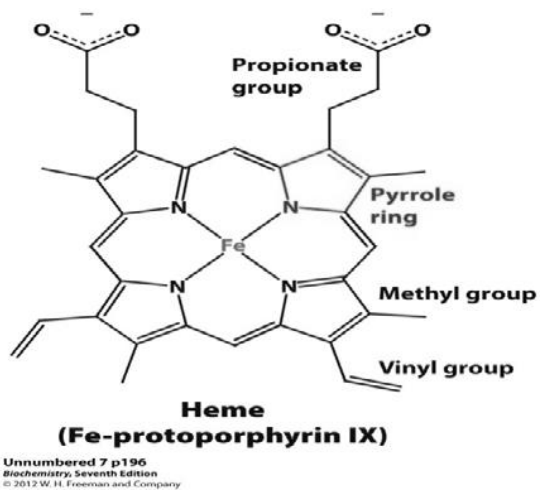


Figure:Structureofhemoglobin.

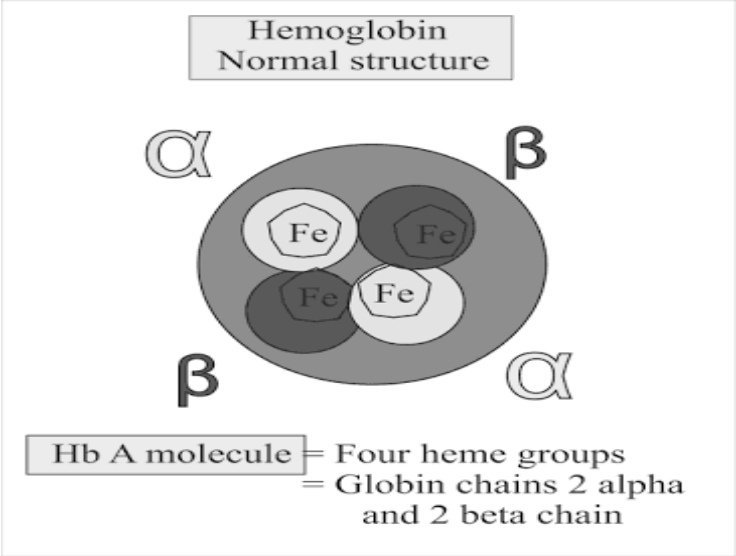


6. Each hemoglobin molecule contains two pairs of polypeptide chains andtwo eachpolypeptide chain, a coloured prosthetic group, called heme, is attached.In each hemegroupthereare4pyrolerings,linkedtogetherbymethinegroup,known asporphyrine.TheparticulartypeofporphyrinfoundinHbiscalledironphotoporphyrin.Each hemepart, there is one iron (held in a porphyrin ring) and witheachion onemoleculeofoxygencan bind.

7. Thepolypeptidechainsaretwoαchains(having141aminoacidseachchain)andtwo β chains (having 146 amino acids in each chains). The 4 polypeptide chains aretogethercalledglobin.SoHemoglobinhastwoparts–heme(4%)and globin(96%).

8. Physiologicalvarietiesofhemoglobin-

Adult haemoglobin (HbA)-In normal adults, 98% Hb is HbA, α_2 β_2,i.e.two alpha andtwo beta chains. About 2% of adult Hb is HbA_2 ($\alpha_2$and δ_2 chains). There are two δchainsinsteadoftwoβchains.Therearesomedifferencesbetweenthetwochains,buttheδchains also have146 amino acid residues.



Foetalhemoglobin(HbF)-Embryo/foetushasdistinctHb,embryosynthesizesζchains which are α like chains and ε chains which are βlike chains. In developmentalstageζ chains are replaced by α chains and the ε chains are replaced by γ chains. HbFhassubunit components $\alpha_2\gamma_2$.

DifferencebetweenAdultandFetalHemoglobin

HbA	HbF
1. Composedoftwoαandtwoβ chains.	2. Composedoftwoαandtwoγ chains.
3. Lifespanisaabout120days.	4. Lifespanislessthan80days.
5. Affinityforoxygenless thanHbF.	6. Greateraffinityforoxy genbinding.

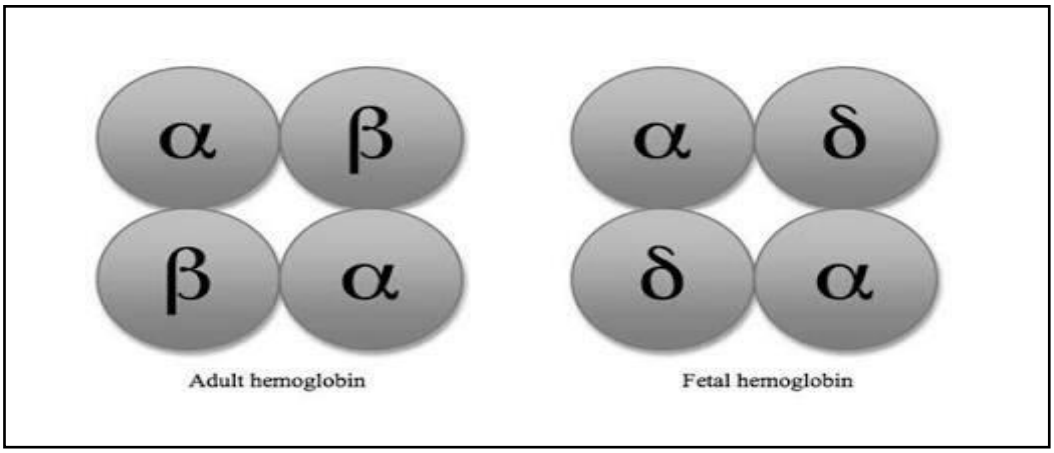


Figure:differencebetweenHbAandHbF.

FunctionsofHb-

1)carriageofoxygen(cofactorhelpsisFe).0xygenatedHbabbreviatedas HbO2.

2) CO_2transport.

3) actasbloodbuffer.

ROLEOFHbINOXYGENTRANSPORT

Normally 97% of O_2 transported from lungs to tissues is carriedin chemical combinationwithHbinthe RBC.3% istransportedinthe dissolvedstate inwater of plasma andbloodcells.

Oxygen molecule can bind loosely or reversibly with the prosthetic group of Hb. WhenPO2 is high oxygen binds withHb, but when PO2 is low, in tissue capillaries oxygen isreleasedfromtheHb.This isthebasisofoxygen transportfrom lungsto tissues.

A few other less common respiratory pigments are:

(i) Pinnaglobin—a brown coloured manganese containing pigment found in the plasma of Pinna (Lamellibranchs).

(ii) Vanadium—It is a green coloured vanadium containing pigment found in the vanadocytes of some sea squirts (Ascidians).

(iii) Molpadin pigment is present in Holothurian Molpadia, and

(iv) Echinochrome is known in sea urchins of echinoderms.

Exchange of Gases:

(A) Exchange of gases between alveoli and blood (Fig. 17.9 & 17.11):

The exchange of gases (i.e., oxygen and carbon dioxide) between lung alveoli and pulmonary capillaries is called external respiration

It occurs as follows:

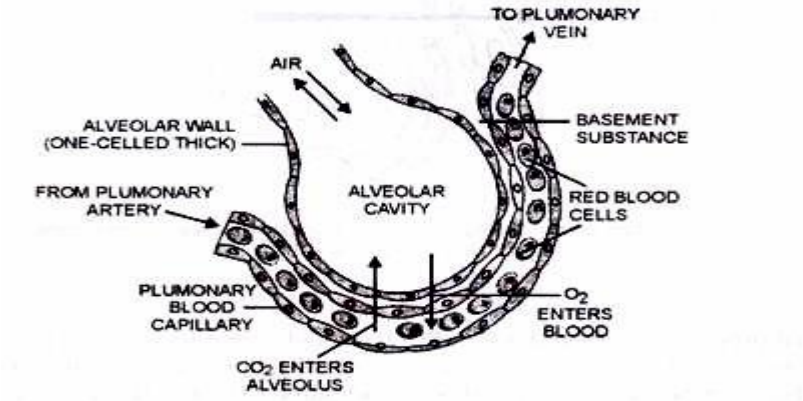


Fig. 17.9. A diagram of a section of an alveolus with a pulmonary blood capillary.

The wall of the alveoli is very thin and has rich network of blood capillaries. Due to this, the alveolar wall seems to be a sheet of flowing blood and is called respiratory membrane (= alveolar-capillary membrane).

The respiratory membrane (Fig. 17.10) consists mainly of:

(a) Alveolar epithelium,

(b) Epithelial basement membrane,

(c) A thin interstitial space

(d) Capillary basement membrane and

(e) Capillary endothelium.

All these layers form a membrane of 0.2 mm thickness. The respiratory membrane has a limit of gaseous exchange between alveoli and pulmonary blood. It is called diffusing capacity. The diffusing capacity is defined as the volume of gas that diffuses through the membrane per minute for a pressure difference of 1 mm Hg. It is further dependent on the solubility of the diffusing gases. In other words at the particular pressure difference, the diffusion of carbon dioxide is 20 times faster than oxygen and that of oxygen is two times faster than nitrogen. The partial pressure of oxygen (PO_2) in the alveoli is higher (104 mm Hg) than that in the deoxygenated blood in the capillaries of the pulmonary arteries (95 mm Hg.). As the gases diffuse from a higher to a lower concentration, the movement of oxygen is from the alveoli to the blood. The reverse is the case in relation to carbon dioxide.

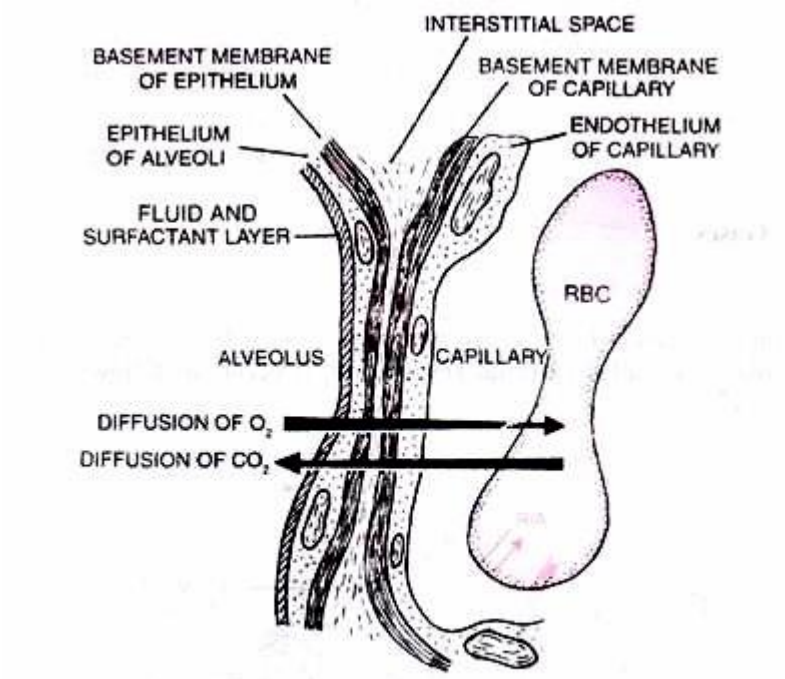


Fig. 17.10. Diagram showing Ultrastructure of Alveolar Respiratory membrane and Red blood corpuscle (RBC).

The partial pressure of carbon dioxide (PCO_2) is higher in deoxygenated blood (45 mm Hg) than in alveoli (40 mm Hg), therefore, carbon dioxide passes from the blood to the alveoli. The partial

pressure of nitrogen (PN_2) is the same (537 mm Hg) in the alveoli as it is in the blood. This condition is maintained because nitrogen as a gas is not used up by the body.

(B) Exchange of gases between blood and tissue cells (Fig. 17.11):

The exchange of gases (i.e., oxygen and carbon dioxide) between tissue blood capillaries and tissue cells is called internal respiration. The partial pressure of oxygen is higher (95mm Hg) than that of the body cells (40 mm Hg) and the partial pressure of carbon dioxide is lesser (40 mm Hg) than that of the body cells (45 mm Hg).

Therefore, oxygen diffuses from the capillary blood to the body cells through tissue fluid and carbon dioxide diffuses from the body cells of the capillary blood via tissue fluid. Now the blood becomes deoxygenated. The latter is carried to the heart and hence to the lungs.

Transport of Gases (Fig. 17.11):

Blood transports oxygen and carbon dioxide.

(A) Transport of Oxygen in the Blood:

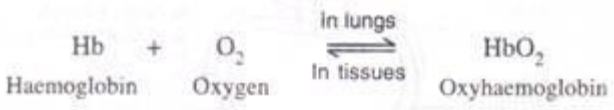
Blood carries oxygen from the lungs to the heart and from the heart to various body parts.

Oxygen is transported in the following manners:

(i) As dissolved gas. About 3 per cent of oxygen in the blood is dissolved in the plasma which carries oxygen to the body cells.

(ii) As oxyhaemoglobin. About 97% of oxygen is carried in combination with haemoglobin of the erythrocytes.

Haemoglobin (Hb) consists of a protein portion called globin and a pigment portion called heme. The heme portion contains four atoms of iron, each capable of combining with a molecule of oxygen. Four molecules of oxygen bind one molecule of haemoglobin. Oxygen and haemoglobin combine in an easily reversible reaction to form oxyhaemoglobin.



Under the high partial pressure, oxygen easily binds with haemoglobin in the pulmonary (lung) blood capillaries. When this oxygenated blood reaches the different tissues, the partial pressure of oxygen declines and the bonds holding oxygen to haemoglobin become unstable. As a result, oxygen is released from the blood capillaries.

A normal person has about 15 grams of haemoglobin per 100 ml of blood. 1 gram of haemoglobin binds about 1.34 ml of O_2. Thus on an average 100 ml of blood carries about 20 ml (19.4 ml exactly) of O_2 Hence under normal conditions, about 5 ml of oxygen is transported to tissues by 100 ml. of blood. During exercise or under strenuous conditions, the muscle cells consume more oxygen. The partial pressure of oxygen in the tissue falls, as a result of which, the blood at the tissue level has only 4.4 ml of oxygen/100 ml of blood. Thus about 15 ml. of oxygen is transported by haemoglobin during exercise.

Oxygen-haemoglobin Dissociation curve (=Oxygen Dissociation Curve):

The amount of oxygen that can bind with haemoglobin is determined by oxygen tension. This is expressed as a partial pressure of oxygen (PO_2). The percentage of haemoglobin that is bound with O_2 is called percentage saturation of haemoglobin.

The relationship between the partial pressure of oxygen (PO_2) and percentage saturation of the haemoglobin with oxygen (O_2) is graphically illustrated by a curve called oxygen haemoglobin dissociation curve (also called oxygen dissociation curve).

Normal Oxygen Haemoglobin Dissociation Curve:

Under normal conditions, the oxygen haemoglobin dissociation curve is sigmoid shaped or 'S' shaped (Fig. 17.12). The lower part of the curve indicates dissociation of oxygen from haemoglobin. The upper part of the curve indicates the acceptance of oxygen by haemoglobin. When the partial pressure of oxygen is 25 mm Hg the haemoglobin gets saturated to about 50%. It means the blood contains 50% oxygen. The partial pressure at which the haemoglobin saturation is 50% is called P_{50}. At 40 mm Hg of partial pressure of oxygen, the saturation is 75%. It becomes 95% when the partial pressure of oxygen is 100 mm Hg.

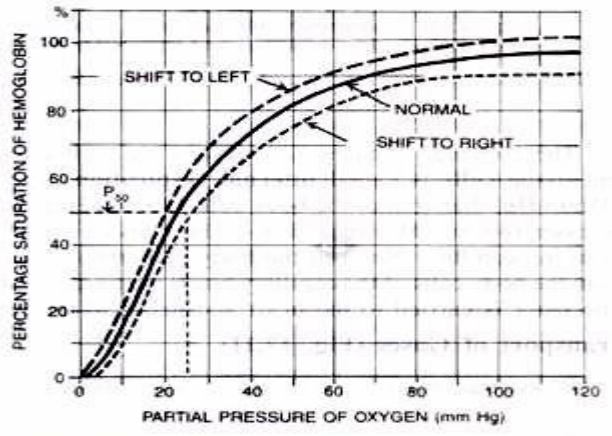


Fig. 17.12. Oxygen-haemoglobin dissociation curve .

Factors Affecting Oxygen Haemoglobin Dissociation Curve:

The oxygen haemoglobin dissociation curve is shifted either to right or left by various factors.

Shift to Right:

Shift to right indicates dissociation of oxygen from haemoglobin.

The oxygen-haemoglobin curve is shifted to right in the following conditions:

(1) Decrease in partial pressure of oxygen.

(2) Increase in partial pressure of carbon dioxide (Bohr effect).

(3) Increase in hydrogen ion concentration and decrease in pH (acidity).

(4) Increased body temperature.

(5) Excess of 2, 3 diphosphoglycerate (DPG). DPG is a by-product in glycolysis. It is present in RBCs.

Shift to Left:

Shift to left indicates acceptance (association) of oxygen by haemoglobin.

The oxygen haemoglobin dissociation curve is shifted to left in the following conditions:

(1) In the foetal blood, because, foetal haemoglobin has more affinity for oxygen than the adult haemoglobin.

(2) In the low temperature and high pH.

Bohr Effect:

An increase in carbon dioxide in the blood causes oxygen to be displaced from the haemoglobin. This is Bohr effect. This is an important factor increasing oxygen transport. It is named after the Danish physiologist Christian Bohr (1855-1911). The presence of carbon dioxide decreases the affinity of haemoglobin for oxygen and increases release of oxygen to the tissues. The pH of the blood falls as its CO_2 content increases so that when the PCO_2 rises the curve shifts to the right and the P_{50} rises. As stated in the oxygen haemoglobin dissociation curve, the partial pressure at which the haemoglobin saturation is 50% is called P_{50}.

Factors Influencing Bohr Effect:

All the factors, which shift the oxygen haemoglobin dissociation curve to the right (mentioned above) increase the Bohr effect.

(B) Transport of Carbon dioxide:

In the oxidation of food, carbon dioxide, water and energy are produced. Carbon dioxide in gaseous form diffuses out of the cells into the capillaries, where it is transported in three ways.

(i) Transport of CO_2 in Dissolved Form:

Because of its high solubility, about 7 percent carbon dioxide gets dissolved in the blood plasma and is carried in solution to the lungs. Thus as compared to O_2, a much larger volume of CO_2 is transported in dissolved form. This is about 7% of all the CO_2 transported by blood from tissues to the lungs.

(ii) Transport of CO_2 as Bio-carbonate:

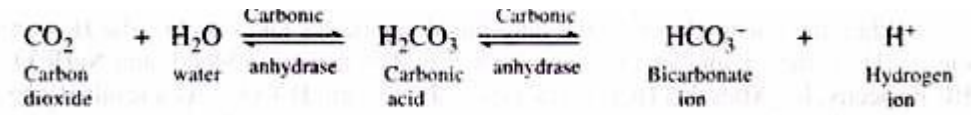
The three main fractions are:

a. A small amount of carbonic acid.

b. The "carbamino-bound" CO_2 which is transported in combination with proteins (mainly hemoglobin).

c. That carried as bicarbonate in combination with cations of sodium or potassium.

The largest fraction of carbon dioxide (about 70%) is converted to bicarbonate ions (HCO_3^-) and transported in plasma. When carbon dioxide diffuses into the RBCs, it combines with water, forming carbonic acid (H_2CO_3). H_2CO_3 is unstable and quickly dissociates into bicarbonate ions and hydrogen ions:



Although this reaction also occurs in plasma, it is thousands of times faster in erythrocytes because they (and not plasma) contain carbonic anhydrase, an enzyme that reversibly catalyzes the conversion of carbon dioxide and water to carbonic acid. Hydrogen ions released during the reaction bind to hemoglobin, triggering the Bohr effect; thus, no oxygen release is enhanced by carbon dioxide loading (as HCO_3~). Because of the buffering effect of hemoglobin, the liberated hydrogen ions cause little change in pH under resting conditions. Hence, blood becomes only slightly more acidic (the pH declines from 7.4 to 7.34) as it passes through the tissues.

Chloride Shift (= Hamburger's Phenomenon):

Since the rise in the HCO_3^- content of red cells is much greater than that in plasma as the blood passes through the capillaries, about 70% of the HCO_3 formed in the red cells enters the plasma. The excess HCO_3 leaves the red cells in exchange for Cl^- (Fig. 17.13). This exchange is called the chloride shift. Because of it, the Cl^- content of the red cells in venous blood is, therefore, significantly greater than in arterial blood. The chloride shift occurs rapidly and is essentially complete in 1 second. Consequently, the red cells take up water and increase in size.

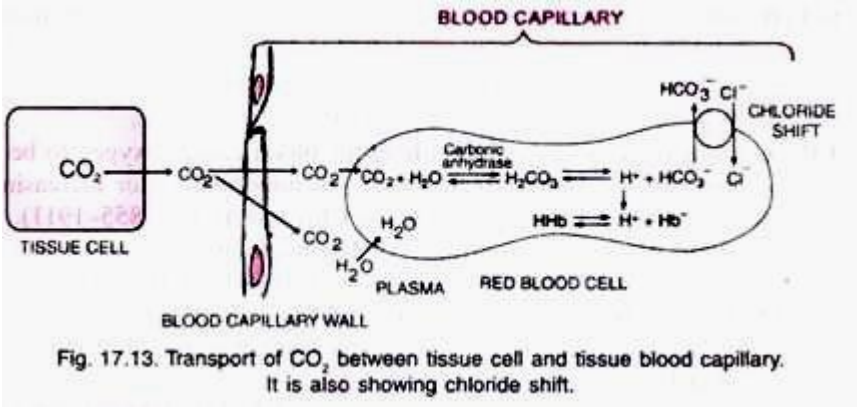


Fig. 17.13. Transport of CO_2 between tissue cell and tissue blood capillary. It is also showing chloride shift.

(iii) Transport of CO_2 as Carbaminohaemoglobin. About 23 per cent CO_2 is carried by haemoglobin as carbaminohaemoglobin. In addition to reacting with water, carbon dioxide also reacts directly with amine radicals (NH_2) of haemoglobin to form an unstable compound carbaminohaemoglobin (Hb CO_2). This is reversible reaction.

CO_2 + Hb (Haemoglobin) ⇋ $HbCO_2$ (Carbominohaemoglobin).

Every 100 mL of deoxygenated blood delivers approximately 4 mL of CO_2 to the alveoli.

Release of Carbon Dioxide in the Alveoli of Lung:

The pulmonary arteries carry deoxygenated blood to the lungs. This blood contains carbon dioxide as dissolved in blood plasma, as bicarbonate ions and as carbaminohaemoglobin.

(i) CO_2 is less soluble in arterial blood than in venous blood. Therefore, some CO_2 diffuses from the blood plasma of the lung capillaries into the lung alveoli.

(ii) For the release of CO_2 from the bio-carbonate, a series of reverse reactions takes place. When the haemoglobin of the lung blood capillaries takes up O_2, the H^+ is released from it.

Then, the Cl^- and HCO_3^- ions are released from KC1 in blood, and $NaHCO_3$ in the RBC respectively. After this HCO_3^- reacts with H^+ to form H_2CO_3– As a result H_2CO_3 splits into carbon

dioxide and water in the presence of carbonic anhydrase enzyme and CO_2 is released into the alveoli of the lungs.

(iii) High PO_2 in the lung blood capillaries due to oxygenation of haemoglobin favours separation of CO_2 from carbaminohaemoglobin.

Haldane Effect:

It was proposed by J.S. Haldane, a Scotish physiologist, 1860-1936. Binding of oxygen with haemoglobin tends to displace carbon dioxide from the blood. This is called Haldane effect. It is far more important in promoting carbon dioxide than is the Bohr effect which promotes oxygen transport. The Haldane effect encourages CO_2 exchange in both the tissues and lungs.

It is quantitatively far more important in promoting CO_2 transport than the Bohr effect in promoting O_2 transport. Thus, Haldane effect and Bohr effect complement each other. In the tissues addition of CO_2 to the blood facilitates unloading of O_2 by Bohr effect. In turn, O_2 unloading favours uptake of CO_2 by Haldane effect.

Effect of CO_2 on Blood pH:

a. CO_2 evolved from the tissues forms carbonic acid. Most of the carbonic acid formed is promptly converted to bicarbonate as shown in the equation below (B^+ represents, principally, Na^+ or K^+).

$$\mathbf{H_2CO_3 \rightleftharpoons H^+ + HCO_3^- + B^+ \rightleftharpoons BHCO_3^- + H^+}$$

b. At the pH of blood (7.4), a ratio of 20: 1 must exist between the bicarbonate and carbonic acid. This ratio is calculated from the Henderson-Hasselbalch equation. Any change in H^+ activity is met by an adjustment in the reaction. Any alteration in the ratio disturbs the acid-base balance of the blood in the direction of acidemia or alkalemia.

Cellular Respiration:

As the name indicates it occurs inside the cells. It takes place in all types of living cells. Respiratory substrates are those organic substances which can be catabolized to liberate energy inside the living cells. The most common respiratory substrate is glucose. Fats are used as respiratory substrates by a number of organisms because they contain more energy as compared to carbohydrates.

However, fats are not directly used in respiration. Instead they are first broken to intermediates common to glucose oxidation, viz., acetyl CoA, glyceraldehyde phosphate. Proteins are used rarely in respiration. Proteins are hydrolysed to form amino acids from which organic acids are produced through deamination. Organic acids enter Krebs cycle, e.g., aspartic acid, glutamic acid.

At other times, proteins are employed as reparatory substrates under starvation conditions only when carbohydrates and fats become unavailable. As stated earlier respiration is of two main types: anaerobic and aerobic. In anaerobic respiration food is oxidised without using molecular oxygen. Less energy is produced in anaerobic respiration. In aerobic respiration organic food is completely oxidised with the help of oxygen into carbon dioxide and water. 686 Kcal of energy is also liberated per mole of glucose.

Aerobic respiration consists of four steps:

(i) Glycolysis:

It is a first step which is common to both anaerobic and aerobic modes of respiration. It occurs in cytoplasm and does not require oxygen. Glycolysis consumes ATP molecules. No carbon dioxide is released in glycolysis. Water and ATP molecules are released,

(ii) Krebs Cycle:

It is the second step in respiration. It operates inside mitochondria and uses oxygen and, therefore, occurs only in aerobic respiration. It does not consume ATP but liberates ATP molecules. Water and carbon dioxide are produced during Krebs cycle,

(iii) Electron Transport Chain (ETC):

It is a series of coenzymes and cytochromes that take part in the passage of electrons from a chemical to its ultimate acceptor. The enzymes involved in electron transport chain are components of the inner mitochondrial membrane. Thus ETC occurs in mitochondria. Oxygen is the ultimate acceptor of electrons.

It becomes reactive and combines with protons to form metabolic water $2H^+ + O^{2-} \rightarrow 2H_2O$]. (iv) Oxidative Phosphorylation. It is the synthesis of energy rich ATP molecules with the help of energy liberated during oxidation of reduced co-enzymes (NADH, $FADH_2$) produced in respiration. The enzyme required for this synthesis is called ATP synthase. ATP synthase is located in F_1 or head piece of F_1 or elementary particles. The particles are present in the inner mitochondrial membrane. The net gain from complete oxidation of a molecule of glucose in muscle and nerve cells is 36 ATP molecules. However, in aerobic prokaryotes, heart, liver and kidneys, 38 ATP molecules are produced per glucose molecule oxidised.

ENVIRONMENTAL INFLUENCES

a. Availability of oxygen:

Insufficient oxygen in atmosphere (in high altitude and polluted area), causes inadequate oxygenation of blood in the lungs, leads to **Hypoxia**. In this condition the oxygen binding capacity of hemoglobin is influenced by the partial pressure of oxygen in the environment. The partial pressure of oxygen falls to 60 mmHg or below. causes problems with blood flow in the tissues, leads to breathing problem.

The respiratory function of hemocyanin containing blood of LibiniaemarginataandOcypodeguadratawas studied, exposed to hypoxic condition. During progressive hypoxia convection initially increases on both sides of the gill in *L.emarginata*, while in *O.quadrata*cardiac output decreases. Blood pH increases with decreasing ambient Po_2 below 60 torr in *L. emarginata*inducing a greater hemocyanin O_2 affinity.

b. CO Poisoning:

CO binds with Hb hundred times tighter than oxygen and disrupts the oxygen carrying capacity of blood. The ranges of CO –4-6ppm at a resting level.

In urban areas 7-13ppm and for smokers-20-40ppm. The level of 40ppm is equivalent to a reduction in hemoglobin levels of 10 g/L. It removes the allosteric shift of the oxygen dissociation curve and shifting the foot of the curve to the left. So the Hb is less likely to release its oxygens at the tissues. So the poor supply of oxygen occurs.

c. Higher concentration of CO_2:

In polluted areas, there are excess carbon dioxide. Increase in carbon di oxide, increases alveolar Pco_2, rises about 60 to 75 mmHg. Due to greater binding affinity of carbon dioxaide with Hb leads to rapid and deep breathing, called air hunger leads to dyspnea.

If partial pressure of carbondioxaide increases 80-100 mmHg, the person becomes lethargic and even semi comatose. Above 120 mmHg rising partial pressure depresses the respiration and causes respiratory death.

d. Temperature:

Decreased temperature causes the oxygen hemoglobin dissociation curve shift to leftward, tends to lower oxygen supply in the tissues. Exposure of slightly increasing temperature on hermit crab associated problem of dehydration. Changes in enthalpy (>39 kj/mol) changes the hemocyanin concentration, which affect the oxygen affinity.

e. Other factors:

Some inflammatory agents (air born viruses, bacteria, moulds) infects the membrane of pulmonary and walls of alveoli, raptures the red blood cells to leak out of blood into the alveoli leads to infection spreads. This infection causes reduction in the area of respiratory membrane and decreased ventilation perfusion ratio. The both causes low blood oxygen and high concentration of carbon dioxide, which causes respiratory problems.

Probable Questions:

1. What is the mechanism of human respiratory system?
2. Discuss inspiration and expiration.
3. Discuss respiratory function of respiratory system.
4. Discuss non-respiratory function of respiratory system.
5. Discuss the role of lungs in excretion.
6. Discuss the muscles which help in expiration.
7. Discuss the muscles which help in inspiration.
8. Define TV,IRV, VC,VRV, IC, EC, FRC.
9. Describe respiratory quotient.
10. Describe the role of Hemocyanin in gas transport.
11. Describe the role of Chlorocruorin in gas transport.
12. Describe the role of hemerythrin in gas transport.
13. Discuss structure and function of Hemoglobin.
14. Differentiate HbA and HbF.
15. Describe oxygen dissociation curve.
16. When oxygen dissociation curve shift to right?
17. When oxygen dissociation curve shift to left?
18. Describe different mode of CO2 transport.
19. What is Haldane effect and Bohr effect?
20. What are the environmental influences on gas transport?

SuggestedReadings/References-

1. Animalphysiology-MohanP.Arora.
2. Textbookofmedicalphysiology/ArthurC.Guyton,JohnE.Hall.
3. Ganong'sreviewofmedicalphysiology.

UNIT-IV

Nervous system: Gross anatomy of brain and spinal cord; cranial nerves,neural control of muscle tone.

Objective: In this unit you will learn about gross anatomy of brain and spinal cord. You will learn also about cranial nerves and how nerves control muscular activity.

Location and Protective Coverings of the Brain:

The brain is the anterior most part of the central neural system which is lodged in the cranial cavity (cranium) of the skull. The human brain weighs from 1220 to 1400 grams. The human neural system has about 100 billion neurons, majority of them occur in the brain. The brain is covered by three membranes or meninges (sing, meninx). The innermost membrane, the piamater is thin, very delicate and vascular and invests the brain closely.

The next is arachnoid membrane (also called arachnoid mater), which is a thin "spider webby" structure from which it gets its name. The outermost membrane, the duramater is the tough fibrous membrane adhering closely to the inside of the skull. Between the arachnoid membrane and piamater is a space known as sub-arachnoid space. The space which is present between the arachnoid and duramater is called subdural space. The sub-arachnoid space is filled with cerebrospinal fluid.

This fluid serves as a pad to cushion the central nervous system from shocks. It also provides a medium for exchange of food materials, wastes, respiratory gases and other materials. The subdural space contains a little fluid which is not cerebrospinal fluid. The membranous areas between the cranial bones of the foetal skull are called fontanels.

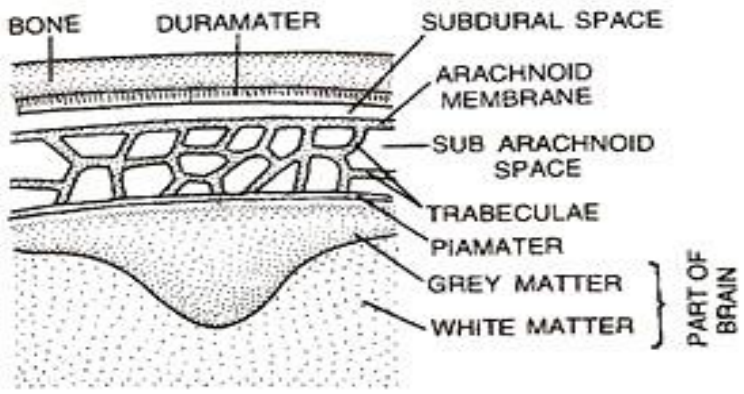


Fig. 21.3. Diagram showing meninges, grey matter and white matter of brain.

Structure and Functions of Human Brain:

The human brain is divisible into three parts:

1. Fore brain or Pros encephalon includes olfactory lobes, cerebrum and diencephalon.

2. Mid brain or Mesencephalon comprises corpora quadrigemina and crura cerebri.

3. Hind brain or Rhomb encephalon consists of cerebellum, pons varolii and medulla oblongata.

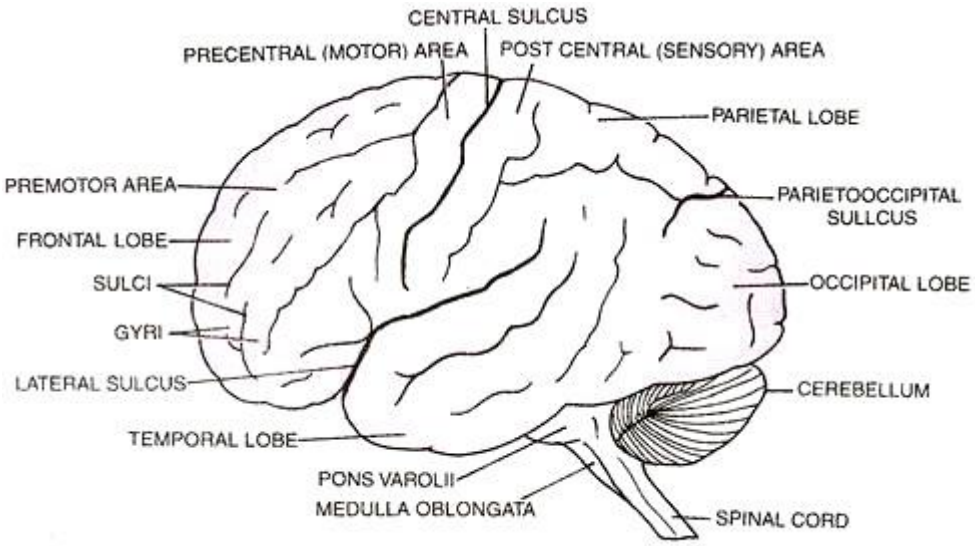


Fig. 21.4. Human brain in lateral view.

Fore Brain:

(i) Olfactory lobes:

The anterior part of the brain is formed by a pair of short club- shaped structures, the olfactory lobes. Each lobe consists of two parts, an anterior olfactory bulb and a posterior olfactory tract. They are fully covered by the cerebral hemispheres and are, therefore, only visible in the ventral view of the brain. A pair of olfactory nerves arises from the olfactory lobes.

Function:

Olfactory lobes are concerned with the sense of smell.

(ii) Cerebrum:

The cerebrum is the largest and most complex of all the parts of the human brain. It consists of left and right hemispheres connected by a large bundle of myelinated fibres, the corpus callosum and other smaller fibre bundles.

Anteriorly the corpus callosum is folded back to form the genu. Posteriorly the corpus callosum curves ventrally to form rounded splenium which joins a fibrous strip called fornix. The fornix is a paired structure, one of which is present in each hemisphere. Left cerebral hemisphere is smaller than the right.

Cerebral Cortex:

The outer portion of cerebrum is called the cerebral cortex that makes up the grey matter of the cerebrum. The surface of the cortex is greatly folded. The upward folds, or gyri (sing, gyrus), alternate with the downward grooves, or sulci (sing, sulcus).

Beneath the grey matter there are present millions of medullated nerve fibres, connecting the neurons of the cerebral cortex with those located elsewhere in the brain. The large concentration of medullated nerve fibres gives this tissue an opaque white appearance. Hence they are collectively called White matter.

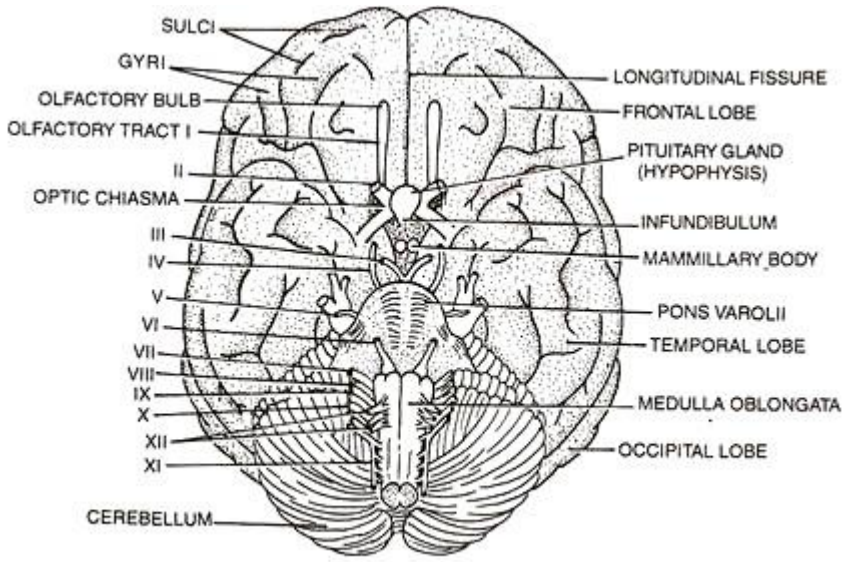


Fig. 21.5. Human brain in ventral view. The numbers I to XII indicate the cranial nerves.

Lobes:

A very deep fissure, the longitudinal fissure, separates the two cerebral hemispheres. Each cerebral hemisphere of the cerebrum is divided into four lobes: frontal, parietal, temporal and occipital lobes. The central sulcus separates the frontal lobe from the parietal lobe. The lateral sulcus separates the frontal lobe from the temporal lobe. The parieto-occipital sulcus separates the parietal lobe from the occipital lobe.

Functional Areas of the Cerebrum:

In each cerebral hemisphere, there are present three types of functional areas:

(a) Sensory areas:

They receive impulses from the receptors.

(b) Association areas:

They interpret the input, store the input and initiate a response in light of similar past experience. Thus the associated areas are involved in memory, learning and reasoning,

(c) Motor areas:

They transmit impulses to the effectors.

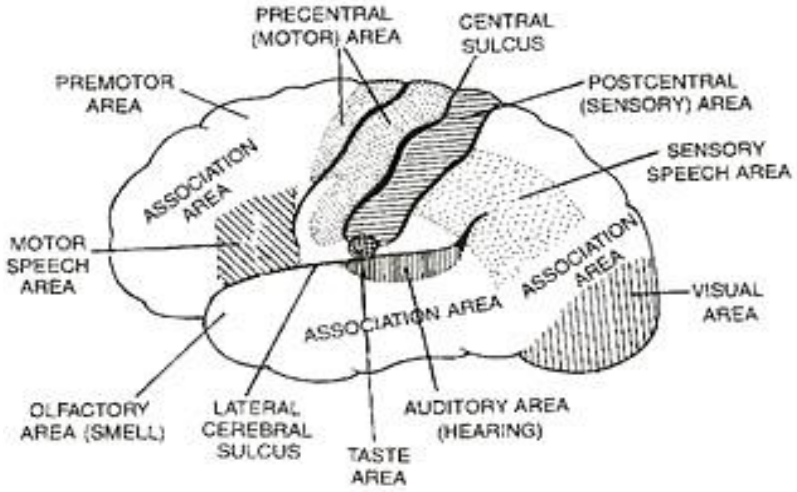


Fig. 21.6. A cerebral hemisphere (half of the cerebrum) showing the functional areas.

The pre-central (motor) area lies in the frontal lobe immediately anterior to the central sulcus. The nerve cells are called pyramidal cells which initiate the contraction of voluntary muscles. The post central (sensory) area (somaesthetic area) lies in the parietal lobe immediately posterior to the central sulcus. It perceives sensations of pain, temperature, pressure and touch.

The sensory speech area is situated in the lower part of the parietal lobe and extends into the temporal lobe. It perceives the spoken word. The auditory (hearing) area lies immediately below the lateral sulcus in the temporal lobe. It is the centre for hearing. Wernicke's area is usually located in the left temporal lobe that plays a role in understanding speech and writing words.The visual area lies in the greater part of occipital lobe. It is the

centre for sight. The olfactory (smell) area lies deep within the temporal lobe. It receives the impulses from the nose via olfactory nerve and interprets them.

The taste area lies in the parietal lobe above the lateral sulcus in the post central (sensory) area. The nerve impulses from the tongue are interpreted here. The motor speech area (also called Broca's motor speech area) lies in the frontal lobe. Other functional areas of the cerebrum include the visual association area in the occipital area, parietal association area in the parietal lobe, the frontal association area in the frontal lobe and temporal association area in the temporal lobe. (An association area is a portion of the cerebral cortex that neither receives direct sensory stimuli nor directly initiates motor impulses; instead, it appears to process and interpret sensory impulses).

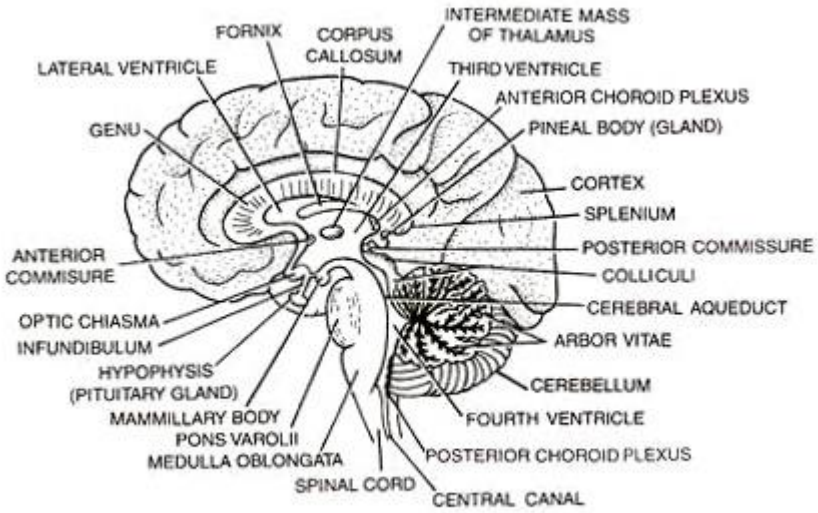


Fig. 21.7. Median section of human brain.

Basal Ganglia (Basal Nulcei):

Basal ganglia are the scattered masses of grey matter, submerged in the subcortical substance of cerebral hemispheres. The corpus striatum, the largest nucleus in the basal ganglia is a mass of grey matter situated at the base of the cerebral hemispheres in close relation to the thalamus. The lenticular nucleus is also a part of basal ganglia.

The main functions of the basal ganglia are:

(i) Control of the movements during voluntary motor activity,

(ii) Control of reflex muscular activity,

(iii) Control of muscle tone,

(iv) Control of automatic associated movements and

(v) Role in arousal mechanism. Parkinson's disease occurs due to damage of the basal ganglia. Wilson's disease is due to damage of the lenticular nucleus.

Functions:

Each lobe of cerebral hemisphere performs specific functions:

(a) In the frontal lobe creative ideas occur,

(b) In the temporal lobe sounds are interpreted so that one can understand what is being spoken,

(c) In the parietal lobe feelings about touch, hot and cold and pain are registered. It is this area that allows to accurately follow directions on map, reading a clock or dressing a person,

(d) The occipital lobe is where eyes see, and interpret what is seen.

A summary of major functions of cerebral lobes has been given below.

Cerebral Lobe	Major Functions
Frontal lobe	Inner monitoring of complex thoughts and actions, creative ideas, translation of perceptions and memories into plans of muscle movement, reality testing by judgement, *controls intellectual ability* to abstract, reasoning, decision making, expression of emotions, willpower and personality.
Parietal lobe	Registration of sensory perception of touch, pain, heat and cold, knowledge about position in space, taking in information from environment, organising it and communicating to rest of brain.
Temporal lobe	Decoding and interpretation of sound, language comprehension, smell, memory and emotion.
Occipital lobe	Decoding and interpretation of visual information; shape and colour.

(iii) Diencephalon:

Its main parts are epithalamus, thalamus and hypothalamus. Epithalamus is thin and not formed of nervous tissue. Its anterior part is vascular and folded to form the anterior choroid plexus.

Just behind the anterior choroid plexus, the epithelium forms a short stalk, the pineal stalk which has a rounded body, the pineal body, at its tip. The pineal body is an endocrine gland and, therefore, secretes a hormone, named melatonin. The thalamus, which lies superior to the mid brain is composed primarily of grey matter.The optic nerves which come from the eyes, form a crossing, the optic chiasma in front of the hypo-thalamus. The hypophysis (pituitary gland) is directly attached to the hypothalamus by a stalk, the infundibulum. The

pituitary gland is an endocrine gland and secretes certain hormones. Behind the infundibulum, a pair of small rounded eminences, the mammillary bodies are present. They are like nipple and hence their name.

Functions of Hypothalamus:

Although hypothalamus is relatively small (4 grams, about 1/300 of the total brain mass) yet it is highly vascular. It integrates and controls the visceral activities. It maintains homeostasis. It provides anatomical connection between the nervous and endocrine systems by its relationship to the pituitary gland.

Through connections with pituitary gland, it controls growth and sexual behaviour. Hypothalamus is thermoregulatory centre. Hence it is called "thermostat" of the body.

It keeps body temperature at roughly 37°C by means of a complex thermostat system. It is also associated with behavioural activities. Appetite, thirst and satiety (feeling of being satisfied) centres are located in the hypothalamus. It also influences respiration and heart beat.

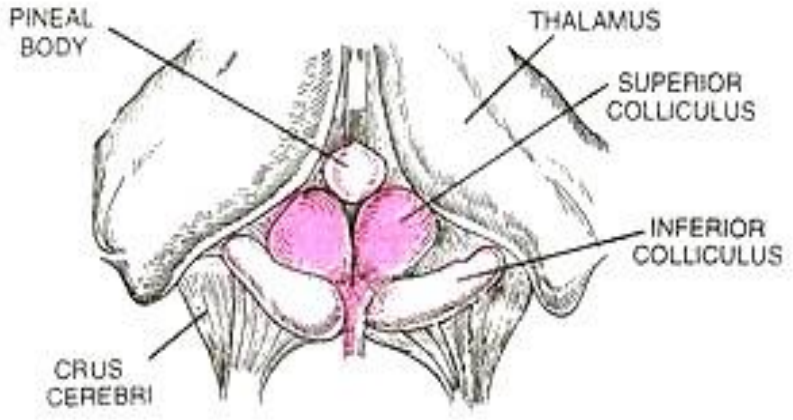


Fig. 21.8. Part of Human brain in posterior view to show some structures in detail.

Mid Brain:

(i) Corpora quadrigemina:

The upper or superior surface of the mid brain has two pairs of rounded protrusions collectively called the corpora quadrigemina; one pair is called superior colliculi and the other pair is called inferior colliculi. The superior and inferior colliculi of each side are termed the corpora bigemina.

Functions:

The superior colliculi are concerned with the sense of sight. However, the inferior colliculi are concerned with hearing.

(ii) Cerebral peduncles (Crura cerebri):

These are two bundles of fibres which lie on the lower or inferior surface of the mid brain.

Function:

They relay impulses back and forth between the cerebrum, cerebellum, pons and medulla.

Hind Brain:

(i) Cerebellum:

The second largest part of the human brain is the cerebellum (means simply "little cerebrum"). It is well developed in human brain. It consists of two lateral cerebellar hemispheres and central worm shaped part, the vermis.

Like the cerebrum, the cerebellum has its grey matter on the outside, comprising three layers of cells and fibres. The middle layer contains characteristically large flask shaped Purkinje cells. The Purkinje's cells rank among the most complex of all neurons. The cerebellum also has Golgi cells, basket cells and granule cells.

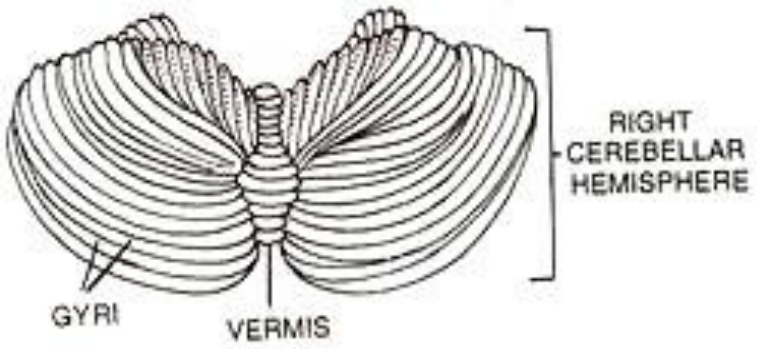


Fig. 21.9. The cerebellum viewed from below.

A cross section of the cerebellar hemispheres shows a branching tree like arrangement of grey and white matter called the arbor vitae ("tree of life").

Functions:

The cerebellum controls rapid muscular activities, such as running, typing and even talking. All activities of the cerebellum are involuntary, but may involve learning in their early stages. Alcohol affects the cerebellum. Since, alcohol is a depressant, it interferes with the functions of the cerebellum.

(ii) Pons Varolii:

It is situated in front of the cerebellum below the mid brain and above the medulla oblongata. It consists mainly of nerve fibres which form a bridge (pons— bridge) between

the two hemispheres of the cerebellum and of fibres which pass between the higher levels of the brain and the spinal cord. Pneumotaxic centre is present in pons varolii.

Functions:

Pons varolii relays impulses between the medulla oblongata and more superior part of the brain, between the hemispheres of the cerebellum and between the cerebrum and cerebellum. The pneumotaxic centre limits inspiration.

(iii) Medulla oblongata:

It extends from the pons varolii above and is continuous with the spinal cord below. Its shape is like a pyramid. The medulla oblongata has a very thin, non-vascular folded structure on its lower side called the posterior choroid plexus.

Functions:

Medulla oblongata receives and integrates signals from spinal cord and sends resulting signals to the cerebellum and thalamus. It contains centres that regulate heart rate, blood pressure, breathing, swallowing, salivation, sneezing, vomiting and coughing and some other involuntary movements.

Brain Stem:

The mid brain, pons varolii and medulla oblongata are collectively called the brain stem, connecting the fore brain and spinal cord.

Limbic System:

Components of Limbic System:

Certain components of the cerebrum and diencephalon constitute the limbic system (limbus L. a border or edge or fringe of a part).

Its main components are the following:

(i) Hippocampus:

Its shape roughly resembles the sea horse. It is located inside the temporal lobe

(ii) Amygdala or Amygdaloid nucleus (L., Gr. amygdale – almond):

It is almond shaped and is located in the tip of the temporal lobe,

(iii) Septal nuclei:

These are located within the septal area formed by the regions under corpus callosum and the paraterminal gyrus (a cerebral gyrus)

(iv) Mamillary bodies:
These are present behind the infundibulum.

(v) Basal ganglia:
They are scattered masses of grey matter.

Functions of limbic system:
(a) It is sometimes called the "emotional brain" because it controls emotional behaviour expressed in the form of joy, sorrow, fear, fight, friendship, liking and disliking,

(b) It controls food habits necessary for survival of the individual,

(c) It also controls sex behaviour necessary for survival of the species.

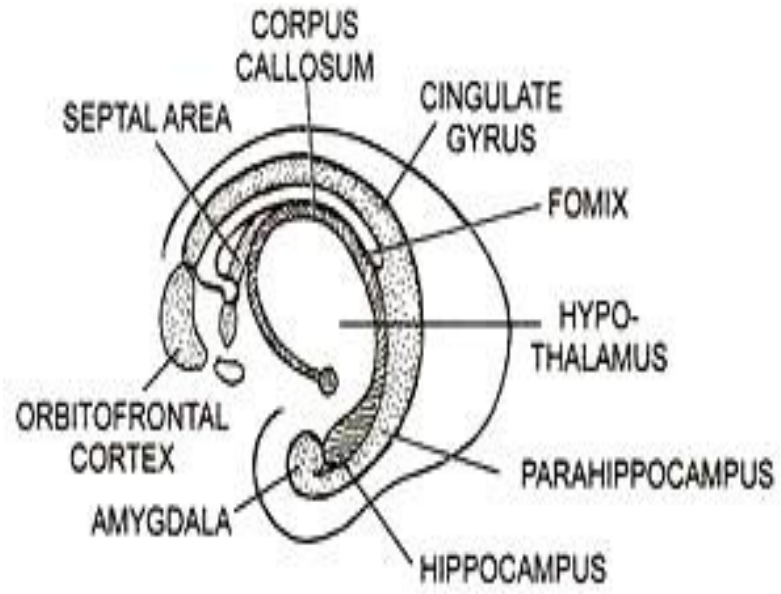


Fig. 21.10. The Limbic System

Ventricles of the Brain:
The ventricles consist of four hollow, fluid filled spaces inside the brain. A lateral ventricle lies inside each hemisphere of the cerebrum. Each lateral ventricle is connected to the third ventricle by an interventricular foramen (foramen of Monro).

The third ventricle consists of a narrow channel between the hemispheres through the area of the thalamus. It is connected by the cerebral aqueduct or aqueduct of Sylvius or iter in the midbrain portion of the brainstem to the fourth ventricle in the pons and medulla. The fourth ventricle is continuous with the central canal of the spinal cord.

Three openings in the roof of the fourth ventricle, a pair of lateral apertures (foramina of Luschka) and a median aperture (foramen of Magendie) allow cerebrospinal fluid to move upward to the subarachnoid space that surrounds the brain and spinal cord. Ventrolateral wall of each paracoel appears striated and hence called corpus striatum.

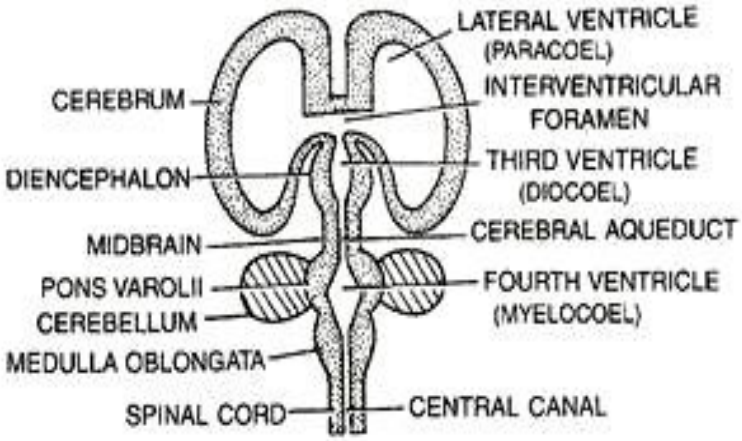


Fig. 21.11. Schematic representation of the ventricles of human brain.

Cerebrospinal Fluid (CSF):

The cerebrospinal fluid is secreted by anterior choroid plexus and posterior choroid plexus and is found inside the ventricles of the brain, the central canal of the spinal cord and in the subarachnoid space around the brain and spinal cord.

The cerebrospinal fluid performs the following functions:

(i) Protection of the Brain and Spinal cord:

CSF protects the delicate brain and spinal cord by providing shock-absorbing medium. It acts as cushion jolts to the central nervous system.

(ii) Buoyancy to the Brain:

Since the brain is immersed in the CSF, the net weight of the brain is reduced from about 1.4 kg to about 0.18 kg. Thus the pressure at the base is reduced.

(iii) Excretion:

CSF carries harmful metabolic wastes, drugs and other substances from the brain to the blood.

(iv) Endocrine Medium for the Brain:

Certain hormones are released into CSF. These hormones are carried to different parts of the brain by CSF where they may act.

Mammalian Characters in Human Brain:

(i) Olfactory lobes are small and solid.

(ii) Cerebral hemispheres are quite large in size and divided into lobes.

(iii) Corpus callosum is also found.

(iv) Optic lobes are solid and further divided into corpora quadrigemina.

(v) Pons varolii is present.

(vi) Cerebellum is very much folded and solid.

Comparative anatomy of Brain

a. Structure of Brain in Amphibia:

1. Forebrain:

The two olfactory lobes are fused together anteriorly. Olfactory peduncle is absent. These lobes are responsible for the sense of smell.

2. Cerebral Hemispheres:

Cerebral hemispheres are divided by a longitudinal fissure. The roof of the hemisphere is thick, smooth and nervous. Corpora striata are poorly developed and are connected by anterior commissures. Lumen of each hemisphere is reduced by the thickening of its lateral and median wall. Cerebrum is the centre for intelligence, conscience and control of voluntary muscles.

On the dorsal side of the diencephalon epiphysis is small. Pineal body of unknown function. Anterior choroid plexus is present. Ventral side of the diencephalon bears an x- shaped optic chiasma. Presence of infundibulum and pituitary body. The lateral sides of the diencephalon are thick to form optic thalami.

3. Midbrain:

Optic lobes are large and hollow ovoid bodies and two in number. Crura cerebri are longitudinally placed nerve fibres-like structures placed ventrally. Optocoels are present.

4. Hind Brain:
Cerebellum is small and does not overlap the structures in front and behind. It represents a thin transverse band-like structure.

5. Medulla Oblongata:
Medulla oblongata is thick and gradually tapers behind as a spinal cord. It gives rise 4 pairs of cranial nerves. The roof of the medulla oblongata is formed by a thin vascular membrane, called the posterior choroid plexus. The corpora restiformia are absent in medulla oblongata. It regulates the breathing, heartbeat, and metabolism.

b. Structure of Brain in Reptiles:
1. Forebrain:
Olfactory lobes are small and connected to the brain by olfactory peduncle.

2. Cerebral Hemispheres:
Cerebral hemispheres are larger than amphibians. The two hemispheres are elongated and are separated medially by a deep fissure. The dorsal surface of the hemispheres is smooth and thin but the lateral and ventral walls are thick that constitute the corpora striata.

Due to accumulation of large amount of grey matters the roof of cerebral hemispheres is called neopallium. On the dorsal surface of the diencephalon there are tub projections, called parietal organ, situated at the anterior of the pineal body. Another projection, called paraphysis, which is present in reduced condition. On the ventral side hangs infundibulum and pituitary body. Lateral ventricles of the cerebral hemispheres are less spacious. Hippocampal lobes are differentiated. Anterior commissures and corpus albicans are absent.

3. Midbrain:
Optic lobes are more developed than those of amphibia and two in number, called corpora bigemina. They are hollow with optocoels. Crura cerebri at the floor and poorly developed.

4. Hind Brain:
Cerebellum is very small, semicircular and flap-like. It contains ventricle. Floccular lobes and pons varolii are absent. The surface of the cerebellum is smooth.

5. Medulla Oblongata:
Medulla oblongata tapers gradually and joins with the spinal cord. Its roof is thin and vascular that forms the posterior choroid plexus.

c. Structure of Brain in Aves:

1. Forebrain:

Olfactory lobes are reduced. Olfactory bulbs and tract are much reduced.

2. Cerebral Hemispheres:

Cerebral hemispheres exhibit considerable increase in size. These two lobes are separated by a median dorsal fissure. Corpora striata are prominent that reduces the lateral ventricles to very narrow spaces.

The corpus striatum is differentiated into hyper-striatum, meso-striatum and palaeo-striatum. The roof of cerebral hemispheres is called neopallium. The neopallium is un-convoluted. The diencephalon is inconspicuous and completely covered by cerebral hemispheres and cerebellum.

3. Midbrain:

Two large optic lobes with optocoels much reduced. Crura cerebri are well developed.

4. Hind Brain:

Cerebellum is more advanced than the reptilian cerebellum. It is divided two lateral and one median lobe. The median lobe is called vermis and lateral lobes are called flocculi. It is solid because the 4th ventricle does not extend it. Generally surface is extensively folded.

5. Medulla Oblongata:

Medulla tapers and joins with the spinal cord. Posterior choroid plexus is completely covered by cerebellum. Ventral flexure is highly developed.

d. Structure of Brain in Guinea-Pig:

1. Forebrain:

Olfactory lobes are small and club- shaped. Olfactory peduncle is absent.

2. Cerebral Hemispheres:

Cerebral hemispheres are very large. They occupy two-thirds of the whole brain. The two cerebral hemispheres are connected by a band of nerve tracts that connect ventrally, called corpus callosum. Each hemisphere is subdivided into 4 lobes, called frontal, parietal, temporal and occipital, by grooves, called Sylvian fissure. Neopallium is highly developed. Presence of corpus albicans.

3. Midbrain:

Optic lobes are small and four in number (corpora quadrigemina) Optocoels are highly developed.

4. Hind Brain:

Cerebellum is more complex and highly advanced than the cerebellum of pigeons. It is large, elongated and solid. It is subdivided into 5 lobes — namely a median vermis, two lateral lobes and two flocculi. The surface of cerebellum is thrown into numerous folds, called gyri and grooves between the folds called sulci.

On the ventral side the two halves of the cerebellum are connected by a broad band, known as pons varolii.

5. Medulla Oblongata:

The lateral walls and floor of the medulla are highly thickened. Its roof forms highly vascularized posterior choroid plexus.

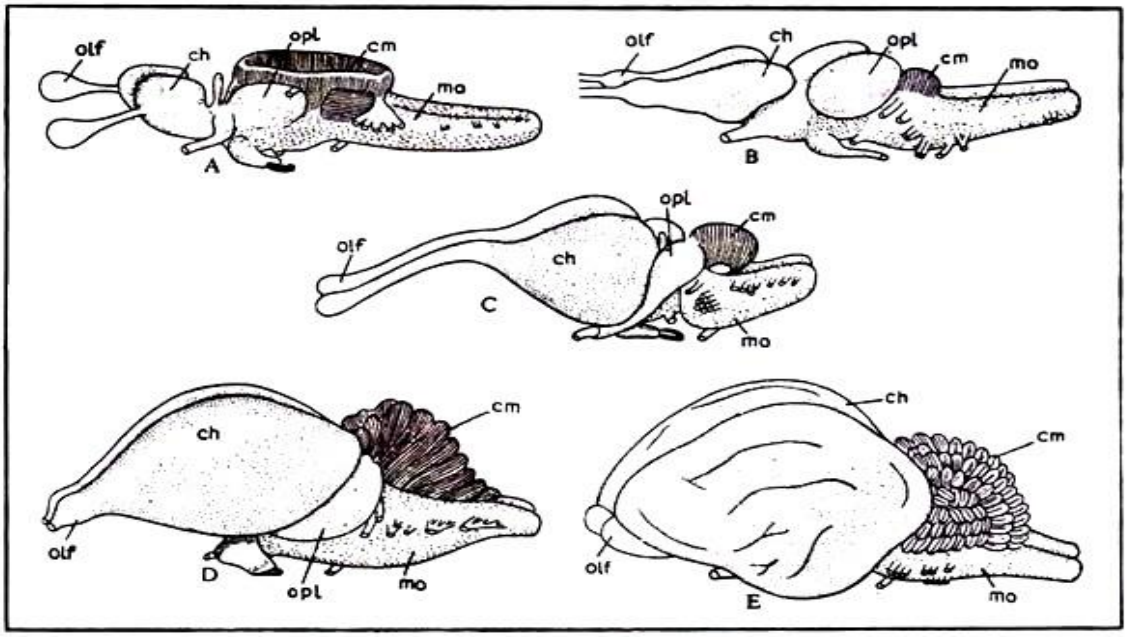


Fig. 10.154 : Showing the basic similarities in the structural organisation of brain in different vertebrates. Brain of fish (A), amphibia (B), reptile (C), bird (D) and mammal (E). Note the gradual aquisition of complexities in cerebral hemisphere, cerebellum and optic lobes. olf = olfactory lobe, ch = cerebral hemisphere, cm = cerebellum, opl = optic lobe and mo = medulla oblongata.

Cranial Nerves:

There are twelve pairs of cranial nerves in man. They are numbered by Roman numeral I to XII. A cranial nerve arises from the brain by two roots, a dorsal and a ventral root.These two roots do not unite but appear like separate nerves. The cranial nerves are generally medullated (having a myelin sheath).

The following table shows the origin, nature and distribution of cranial nerves:

Cranial Nerves of Man

No.	*Nerve*	*Origin*	*Branch if any*	*Nature*	*Distribution*
I.	OLFACTORY	Olfactory lobe	—	Sensory	Nasal epithelium.
II.	OPTIC	Optic lobe	—	Sensory	Retina of eye
III.	OCULO-MOTOR	Floor of mid-brain	—	Motor	Muscles of eye ball except superior, oblique and external rectus. Muscles of upper eye lid.
IV.	TROCHLEAR	From the junction between optic lobes and cerebellum	—	Motor	Superior oblique eye ball muscle.
V.	TRIGEMINAL	Side of the medulla oblongata	Three branches	Mixed	
			(*a*) **Opthalmic**	Sensory	Upperlip, nose, lower eye lid.
			(*b*) **Maxillary**	Sensory	Skin of snout, nasal cavity, palate, lower
			(*c*) **Mandibular**	Motor	lip, skin of lower jaw, tongue.
VI.	ABDUCENS	Ventral side of medulla	—	Motor	External rectus muscle of eye ball
VII.	FACIAL	Lateral side of medulla	Three branches	Mixed	
			(*a*) **Palatine**	Sensory	Roof of buccal cavity and nasal chamber.
			(*b*) **Hyomandibular**	Motor	Muscles of lower jaw, neck, pinna.
			(*c*) **Chorda tympani**	Motor	Salivary glands and taste buds of tongue.
VIII.	AUDITORY	Floor of medulla	Two branches	Sensory	
			(*a*) **Vestibular**	Sensory	Internal ear
			(*b*) **Cochlear**	Sensory	Cochlea
IX.	GLOSSOPHARYNGEAL	Side of medulla	—	Mixed	Tongue and muscles of pharynx
X.	VAGUS	Sides of medulla from vagus ganglion	Five branches		
			(*a*) **Superior laryngeal**	Motor	Muscles of larynx
			(*b*) **Recurrent laryngeal**	Motor	Muscles of larynx
			(*c*) **Cardiac**	Motor	Muscles of heart
			(*d*) **Pneumo gastric**	Mixed	Lungs, oesophagus
			(*e*) **Depressor**	Motor	Muscles of diaphragm.
XI.	SPINAL ACCESSORY	Sides of medulla and spinal	—	Motor	Muscles of neck and shoulder
XII.	HYPO-GLOSSAL	cord Ventral side of medulla	—	Motor	Muscles of neck and tongue.

The nerve fibres which carry impulses or sensations or stimuli are of 3 kinds. They are sensory, motor and mixed nerve fibres. The sensory or afferent nerve fibres carry impulses or sensations from the receptors like skin, eye, ear etc. to the central nervous system (brain and spinal cord).

The motor or efferent nerve fibres carry impulses or sensations from the central nervous system to the effectors like muscles and glands. Certain nerves contain sensory and motor fibres, and hence are mixed type. All the body functions can be conveniently divided into two categories: somatic functions and visceral functions.

Somatic functions are performed by the help of the body wall (skin and muscles) and visceral functions are carried on by internal organs like the digestive, circulatory, urinogenital, and respiratory or endocrine glands. Accordingly the nervous system has four functional components and four kinds of nerves.

They are as follows:
(a) Somatic sensory nerves carry impulses from somatic receptors such as skin, eyes, nose, body walls and joins to central nervous system.

(b) Somatic motor nerves carry impulses from central nervous system to voluntary muscles.

(c) Visceral sensory nerves carry sensations from the viscera to the central nervous system.

(d) Visceral motor nerves carry impulses from the central nervous system to the involuntary muscles of alimentary canal, glands and other visceral organs.

Spinal Nerve:

There are 31 pairs of spinal nerves in man — arising in pairs from the spinal cord. Out of these 8 pairs are Cervical, 12 pairs Thoracic, 5 pairs Lumbar, 5 pairs Sacral and 1 pair Coccygeal nerve (Fig. 1.14 and 1.16).

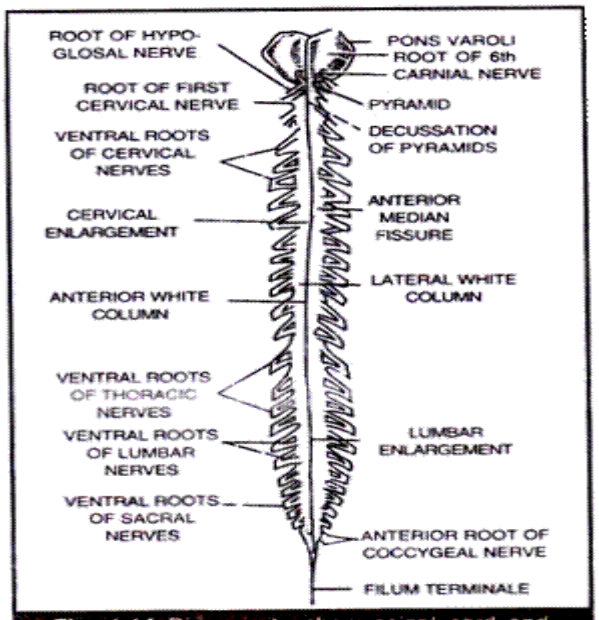


Fig. 1.14 Diagram to show spinal cord and spinal nerves of man.

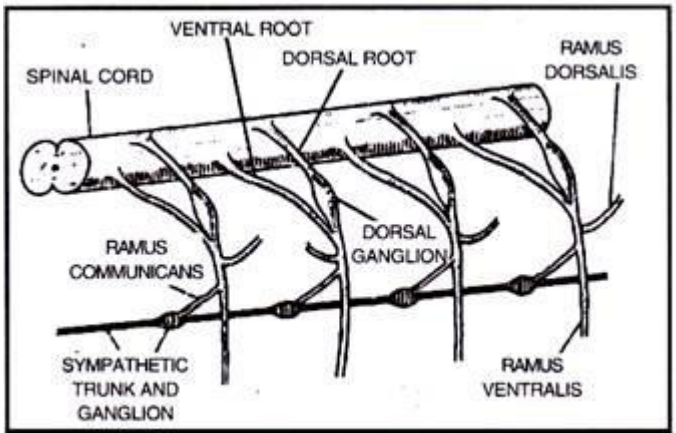


Fig. 1.16 Pattern of spinal nerves.

The spinal nerves are of mixed type and arise in pairs from the spinal cord by two roots, a dorsal root and a ventral root (Fig. 1.14 and 1.16). The dorsal or sensory root consists of afferent fibres which may be somatic sensory or visceral, sensory. It also bears a ganglion. The ventral root consists of efferent fibres which may be somatic motor or visceral motor fibres. These two roots unite to form a spinal nerve which comes out through a small aperture between vertebrae called inter-vertebral foramen guarded by a silvery- white calcareous gland known as glands of Swammerdam.

Soon after its emergence, the spinal nerve trunk divides immediately into three branches as follows:

(a) Ramus dorsalis contains somatic sensory fibres and supplies the skin and muscles of dorsal body wall.

(b) Ramus ventralis is the thick, main nerve and contains somatic motor fibres.

(c) Ramus communicans contains visceral sensory and visceral motor fibres and later joins autonomic nervous system and spinal cord.

Structure of Human Spinal Cord

Location and Coverings (Meninges):

It is a posterior part of central nervous system which runs mid-dorsally within the vertebral column. It lies in the neural canal of the vertebral column. The spinal cord is surrounded by the same three protective membranes (meninges) as found in the brain, viz., a thin innermost piamater, the middle webby arachnoid membrane (arachnoid mater) and the outer tough duramater.

The subarachnoid space is filled with cerebrospinal fluid. There is an additional space, the epidural space above the Dura mater. The epidural space contains fatty and connective tissues and veins.

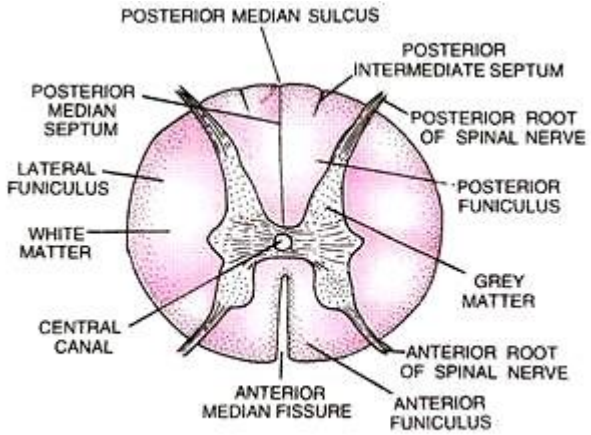


Fig. 21.12. T.S. Human spinal cord.

External Structure:

The spinal cord extends from the medulla oblongata. It is continuous, to the level of the second lumbar vertebra. In an adult the spinal cord is from 42 to 45 cm long. Its diameter varies at different levels, being enlarged in the cervical and lumbar regions.

The cord is also flattened. The cervical enlargement extends from the fourth cervical to the first thoracic vertebrae; it is the region from which nerves supplying the arms arise. It may seem strange that the lumbar enlargement should be in the thoracic region; this is the case because the spinal cord grows at a slower rate than the vertebral column. By adulthood the area within the vertebral column below the second lumbar vertebra contains spinal nerves that branch from the spinal cord at higher levels.

These spinal nerves are collectively called, the cauda equina, or “horse’s tail”. The spinal cord ends as the conus medullaris. The conus medullaris ends at the level of the intervertebral disc between the first and second lumbar vertebral in adults. Actually the conus medullaris is a conical portion of lower spinal cord.From the conus medullaris a fine connective tissue

filament, the filum terminate, extends down to the coccygeal region. The filum terminate consists mostly of pia mater.

The spinal cord does not extend to the coccygeal region because during development the vertebral column elongates more rapidly than the spinal cord. The filum terminate anchors the spinal cord within the vertebral column. In-fact, the filum terminale is a long slender filament at the end of the spinal cord.

Internal Structure:

The internal anatomy of the spinal cord is best seen in cross section. Two indentations, the posterior median sulcus and the anterior median fissure, separate the spinal cord into left and right symmetrical halves. The inner butterfly-shaped area is the grey matter of the spinal cord.

Grey matter is so named because it lacks myelin and therefore, appears grey in an unstained preparation. Surrounding the grey matter are bundles of myelinated nerve fibres, called fasciculi or white columns, which together form the white matter of the spinal cord.In each segment of the spinal cord a spinal nerve arises from each side of the cord. Each spinal nerve connects with the cord through two nerve roots.

The dorsal nerve root consists of a bundle of sensory axons (carrying incoming signals) whose cell bodies are located in the dorsal root ganglion. These axons extend into the posterior horn of the grey matter, where they often form synapses with other neurons, some of which are called intemeurons. Interneurons, short neurons confined to the grey matter of the cord, form synapses with other intemeurons and with the motor neurons whose cell bodies are located in the anterior horn of the grey matter.Aggregations of motor axons (carrying outgoing signals) from these cell bodies form the ventral nerve roots. The lateral horns lie between the anterior and posterior horns. The hollow central canal contains cerebrospinal fluid.

Spinal Tracts:

Along the white matter of the spinal cord there are two kinds of fasciculi, or bundles of axons, the ascending tracts, which carry sensory impulses to the brain, and the descending tracts, which carry motor impulses from the brain to the spinal nerves at various levels of the cord.

The human spinal cord is made up 31 segments. From each of these segments, a pair of spinal nerves takes origin.

There are 31 pairs of spinal nerves. Of 31 pairs:
i. 8 belong to cervical segments

ii. 12 belong to thoracic segments

iii. 5 belong to lumbar segments

iv. 5 belong to sacral segments

v. 1 belongs to coccygeal segment.

In a transverse section of spinal cord, in the central part will be H-shaped grey matter and this will be surrounded by white matter. The grey matter and white matter on either side have continuity through commissures (Fig. 9.14).

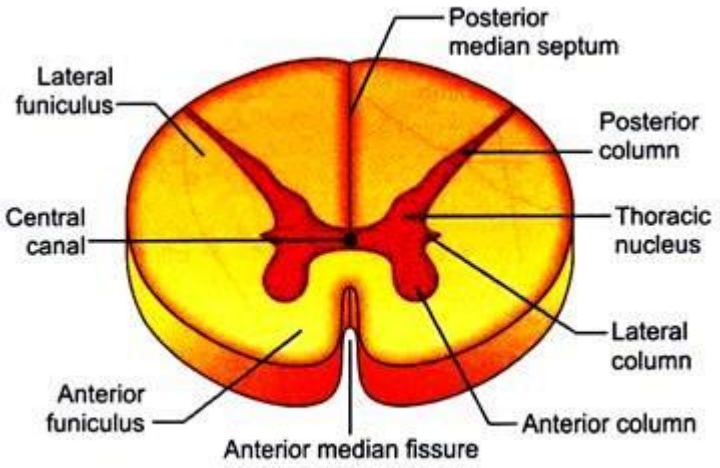


Fig. 9.14: Diagram of transverse section of spinal cord

In the grey matter, nerve cell bodies are present whereas the white matter is composed of compactly packed nerve fibers in the form of tracts. The tracts which carry impulses from peripheral parts of body towards higher parts of CNS through spinal cord are called ascending tracts. Likewise, tracts which carry information from higher parts of CNS to motor neurons present in spinal cord are called descending tract (Fig. 9.15).

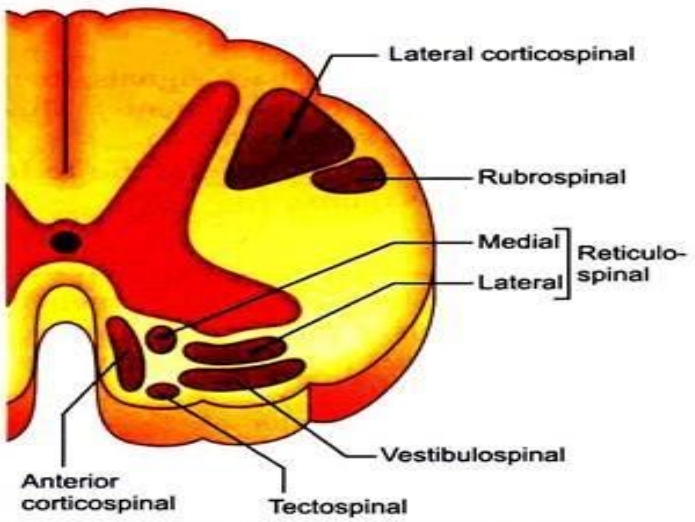


Fig. 9.15: Transverse section of spinal cord showing some of the important ascending and descending tracts

Structural and functional integrity of both grey matter and white matter areas is essential for normal functioning of nervous system. The cell bodies in grey matter area and tracts in white matter are both present in a bilaterally symmetrical way in spinal cord.

Ascending Tracts:

i. Carry information from spinal cord to higher parts of CNS.

ii. They are essential for sensory perception as impulses should reach brain from peripheral parts of body.

iii. Most of these tracts end in sensory areas of cerebral cortex.

iv. Some of the important ascending tracts are dorsal column tracts, lateral spinothalamic tract, anterior/ventral spin thalamic tract.

Dorsal Column Tract or Posterior Column Tract or Tract of Goll and Burdach or Fascicules Gracilis and Cuneatus:

Sensations carried by these tracts are:

1. Fine touch

2. Tactile localization

3. Tactile discrimination (2 point discrimination)

4. Pressure sensation

5. Vibratory sensation

6. Proprioception or sense of position and joint movement and is also known as kinesthetic sensation.

7. Stereognosis.

The above sensations from peripheral parts of body are carried by posterior column tracts to cerebral cortex. Nerve fibers carrying these sensations are A beta fibers.

Receptors involved will be:

a. Merkel's disk

b. Meissner's corpuscle

c. Pacinian corpuscle

d. Ruffini's end organ

e. Receptors in and around joints

Touch sensation has dual pathways. Fine touch sensation is carried by posterior column tracts and crude touch sensation is carried by anterior or ventral spinothalamic tract.

From the receptors, A beta fibers carry impulse. When posterior nerve fibers reach spinal cord, the nerve trunk of posterior spinal nerve separates into two divisions, namely medial and lateral divisions between dorsal root ganglion and spinal cord. The fibers going to contribute for formation of posterior column tracts enter spinal cord through medial division.

These fibers reach posterior funiculus of spinal cord and ascend up on same side of spinal cord. In the brainstem, at the level of medulla oblongata, these fibers synapse in two different nuclei, namely gracile and cuneate nuclei. First order neurons are posterior root ganglion cells.The second order fibers take origin from the nuclei of gracile and cuneate and cross midline and to reach opposite side. These fibers that cross are known as internal arcuate fibers (80%). Internal arcuate fibers will be contributing for sensory decussation. Approximately, 20% of fibers which takes origin from gracile and cuneate nuclei, do not cross midline and these are known as external arcuate fibers.

They carry impulses to cerebellum of same side by passing through inferior cerebellar peduncle. The internal arcuate fibers as they ascend up further to form medial lemniscus after crossing. Fibers of medial lemniscus synapse in ventroposterolateral nucleus present in thalamus. Neurons extending from gracile and cuneate nuclei to thalamus are called as second order neurons.Fibers which take origin from ventroposterolateral nuclei pass

through posterior limb of internal capsule to end in sensory area (3, 1, 2) of cerebral cortex (primary somesthetic area) present in postcentral gyrus. These fibers which end in cerebral cortex are known as thalamic/sensory radiation fibers and constitute third order neurons (Fig. 9.16).

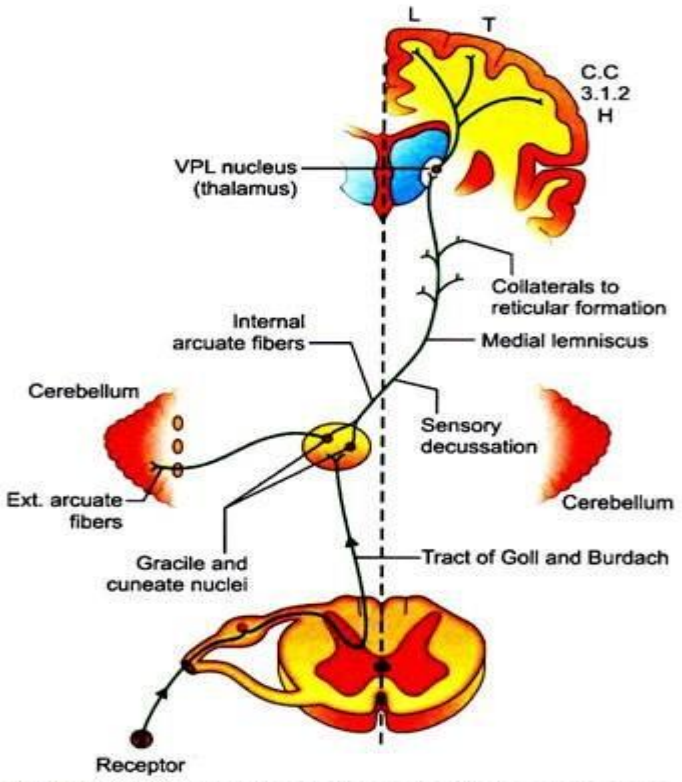


Fig. 9.16: Pathway carrying fine touch, tactile localization, etc. from peripheral parts of the body

Sensory Homunculus:

In uncrossed tract, sacral segmental fibers will be medial most and cervical fibers will be lateral most. Knowledge of topographical arrangement afferent fibers in different ascending tracts is essential in certain spinal cord lesions especially when lesion is due to degeneration of tissue around spinal canal (syringomyelia).

Stereognosis:

It is ability of person to identify some familiar/known objects even with closed eyes. The impulses for this sensation will be carried by posterior column tracts.

The person is able to identify object based on:

i. Shape

ii. Size

iii. Texture of object

Fine touch, tactile localization, and tactile discrimination, etc. from face.

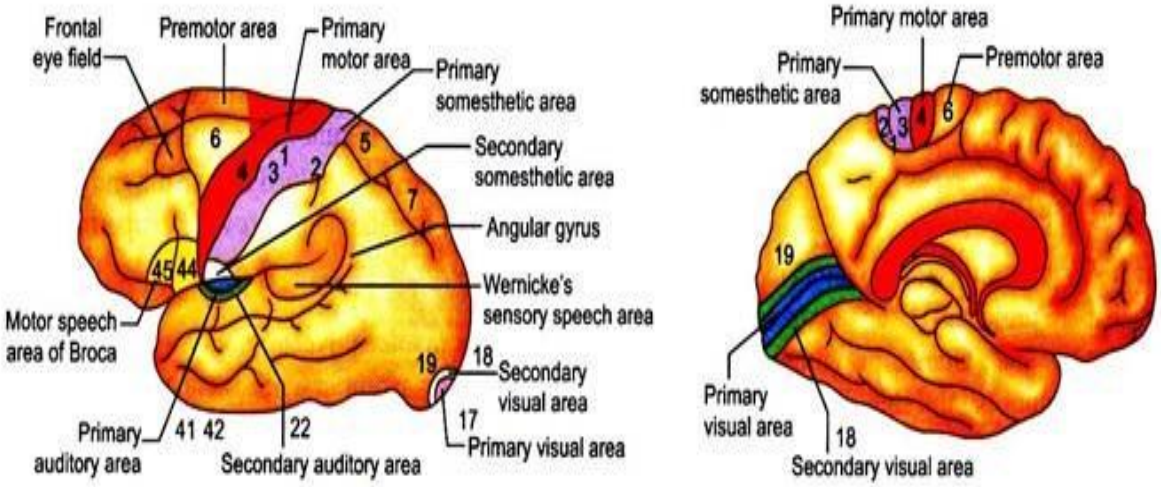


Fig. 9.17: Cerebral cortex showing some of the important areas

Fibers carrying sensations from receptor belong to 5th cranial nerve (carry sensory fibers from face). These fibers reach Gasserian ganglion. From ganglion, fibers enter pons to synapse in chief sensory nucleus. From chief sensory nucleus, 2nd order fibers take origin and cross the midline to reach the opposite side. These fibers ascend upward as trigeminal lemniscus. They synapse in ventroposteromedial nucleus of thalamus.

From ventroposteromedial nucleus, 3rd order fibers originate. They end in lateral most part of cerebral cortex area number 3,1, and 2 present in post-central gyrus where face is represented (Fig. 9.19).

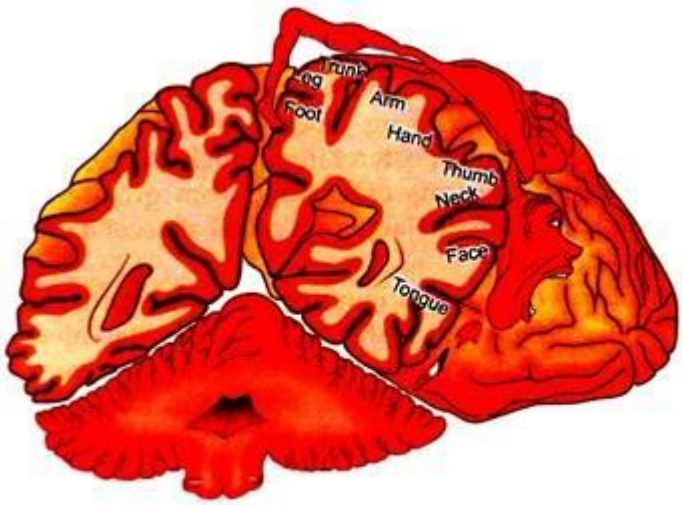


Fig. 9.18: Body representation in sensory cortex

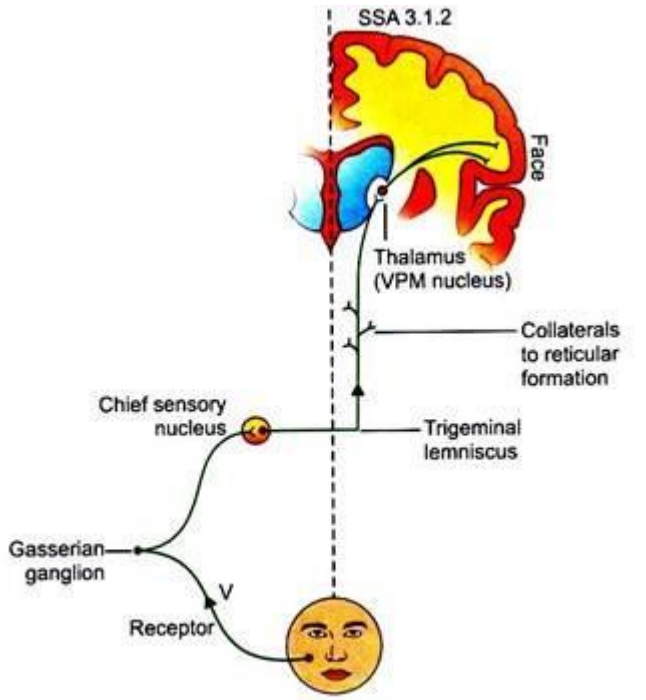


Fig. 9.19: Pathway for fine touch, tactile localization, etc. from face

Functions of Spinal Cord:

(i) The stimuli are passed from and to the brain through the spinal cord.

(ii) It is the centre of spinal reflex action.

Reflex action:

What happens when you touch something hot or your finger is pricked by a needle? You immediately pull your hand away, without even thinking why you are doing so. Such sudden involuntary responses to stimuli are examples of reflex action. The response may be different when your conscious thought process is involved. For example, when a doctor pricks you with an injection needle to inject a medicine into your arm, you do not withdraw your arm immediately.Your conscious thinking tells you that the medicine is being administered to cure your disease. In this case, a message from the spinal cord goes to the cerebrum, the thinking part of your brain, and your thinking brain directs your arm to bear the pain and not pull away.

The spinal cord is the centre of reflex action. Reflex actions are produced by reflex arcs, which may be formed anywhere along the spinal cord, nearest to the receptor and effecter. A reflex arc is formed by a sensory nerve and a motor nerve joined by a connecting nerve present in the spinal cord.As the impulses do not have to travel all the way to the brain and back, the detection of stimuli and the completion of responses are faster.

Reflex action is an extremely quick action, which does not involve any thinking by the brain. If someone hits your leg with a hammer the leg is immediately withdrawn. In this type of reflex action the impact of the hammer (stimulus) received by the receptor is sent to the spinal cord through the sensory nerve. The message is received by the connecting nerve in the spinal cord.The connecting nerve then sends a response through the motor nerve to the muscles (effectors) to pull the leg away. Thus, reflex action is a sudden, involuntary motor response to a stimulus. The flow of food in the alimentary canal, blinking in strong light or in response to a sudden movement in front of the eye, sneezing, coughing, yawning, hiccupping, shivering, etc., are also reflex actions.

Peripheral nervous system:

The peripheral nervous system includes 12 pairs of cranial nerves arising from the brain and 31 pairs of spinal nerves arising from the spinal cord. The nerves from the brain and the spinal cord connect the skeletal muscles and control their activity according to the directions and demands of the body. These nerves are, therefore, related to voluntary acts, i.e., they act according to our will.

Autonomic nervous system:

The autonomic nervous system controls and integrates the functions of internal organs like the heart, blood vessels, glands, etc., which are not under the control of our will.

The autonomic nervous system has two subdivisions: sympathetic and parasympathetic. The organs receive both sympathetic and parasympathetic nerves. The two types of nerves have opposite effects on the organs, i.e., if one is stimulatory, the other is inhibitory.

Meaning of Autonomic Nervous System:

It is the part of nervous system, which regulates functioning of visceral organs. Two different limbs of autonomic nervous system are sympathetic and parasympathetic nervous system components.Sympathetic takes origin from lateral horn cells of thoracolumbar segments of spinal cord whereas parasympathetic takes origin from cranial (in brain) and sacral (in spinal cord) regions. In parasympathetic component, nerves included are cranial nerves 3, 7, 9 and 10 and also pelvic nerve from sacral segments of spinal cord (Fig. 9. 56).

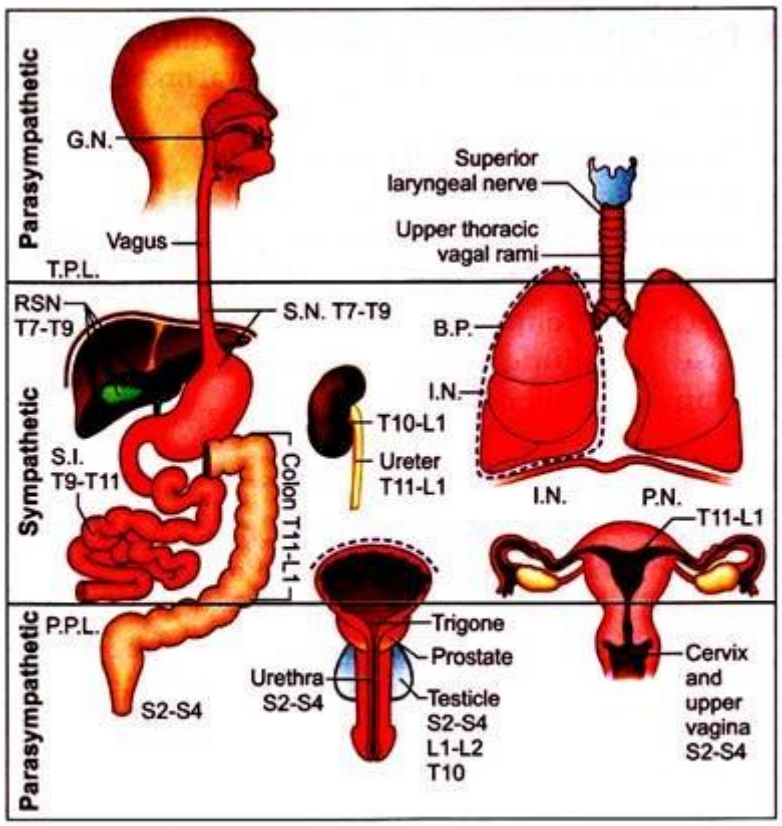


Fig. 9.56: Parts of the body that are influenced by sympathetic and parasympathetic activity

i. Centres for autonomic nervous system are present in hypothalamus.

ii. The anterior hypothalamus controls activity of parasympathetic and posterior hypothalamus that of sympathetic nerves.

iii. In any autonomic nervous system pathway, there are two neurons along efferent pathway. The preganglionic and postganglionic. The neurotransmitter at pre- and post-ganglionic regions in parasympathetic part is acetylcholine.

In sympathetic, at preganglionic region, it is acetylcholine whereas in post-ganglionic region in most of parts of body it is noradrenaline. In some parts, at the postganglionic region of sympathetic nervous system, the neurotransmitter liberated is ACh.

iv. Acetylcholine can act through nicotinic or muscarinic receptors present in pre- and post-ganglionic regions respectively.

v. Noradrenaline can act through alpha or beta receptors.

vi. Alpha receptors are of two types namely alpha 1 and alpha 2 and likewise even beta receptors are of two types namely beta 1 and beta 2.

Difference between Sympathetic Nerve Fibre and Parasympathetic Nerve Fibre:

1. Sympathetic nerve arise from thoraco-lumber (T_1 to L_3) region of the spinal cord.

2. Ganglia are nearer to CNS. The ratio of pre and postganglionic fibres is generally 1: 20 or more. So, the post ganglionic fibers are longer.

3. Neurotransmitters are ACh (in ganglia) and norepinephrine (at neuro-effector junctions).

4. Sympathetic activity increases in stress and emergency.

5Parasympathetic nerves arise from the craniosacral (III, VII, IX, X, S_2 – S_4) region of the CNS.

6. Ganglia are away from CNS, and on or close to the organs. The ratio is generally 1: 1. So, the postganglionic fibres are shorter.

7. Neurotransmitters are ACh in both the ganglia and neuroeffector junctions.

8. Parasympathetic activity predominates during rest.

In most of the places (e.g., in smooth muscles of the gut, bladder, and heart) the sympathetic and parasympathetic systems produce opposite effects; but in some other organs only one system operates.

The sweat glands and most blood vessels, for example, have only a sympathetic innervation and the ciliary muscle of the eye has only a parasympathetic innervation. On the other hand, the two systems may produce similar, rather than opposite effects in an organ (e.g., salivary glands).

Functions of Autonomic Nervous System:

1. Regulation of functions of visceral organs, like heart, gastrointestinal tract, etc., and thereby help to regulate heart rate, blood pressure and gastrointestinal secretion and motility.

2. In lungs, bring about bronchodilation when acts through sympathetic nerve and vice versa effect, when parasympathetic nerve is acting.

3. Sweat glands are supplied by sympathetic cholinergic fibers and are involved in regulation of body temperature. In addition to this, during thermoregulation, goose pimples are formed due to piloerrection which is brought about by contraction of erector pilorum muscle.

4. Secretions from adrenal medulla are regulated by activity of sympathetic nerves (pelvic splanchnic nerve).

5. In eyes, pupillary dilation is by sympathetic nerve stimulation and constriction by parasympathetic nerve stimulation.

6. Defecation and micturition are possible due to activity of parasympathetic nerves stimulation.

7. In male reproductive system, erection of penis is due to parasympathetic stimulation and ejaculation of semen is because of sympathetic nerve activity.

How does the nervous tissue cause the muscles to act?

When an electrical signal from a nerve cell reaches a synapse it causes the axon bulb to release a chemical. This chemical, which is discharged at the junction between the nerve cell and the muscle cell, causes the cell membrane of the muscle cell to move some ions in the muscle cell. This triggers a series of changes, ultimately causing the muscle to contract or relax.

Probable Questions:

1. How does the nervous tissue cause the muscles to act?

2. What are the Functions of Autonomic Nervous System?

3. Describe Difference between Sympathetic Nerve Fibre and Parasympathetic Nerve Fibre.

4. What is autonomic nervous system?

5. Write a short note on Reflex action.

6. Describe the external and internal structure of spinal cord.

7. What are the differences between reptilian, avian and mammalian brain?

8. Write a short notes on ventricles of brain.

9. Give the structure and function of cerebellum.

10. Give the structure and function of mid brain.

11. What are the main functions of basal ganglia?

12. Give the structure and function of fore brain.

13. write a short note on protective coverings of brain.

14. State the names and distribution of any four cranial nerves.

15. Classify cranial nerves on the basis of their function and distribution.

Suggested Readings:

1. Eckert Animal Physiology by David Randall, Warren Burggren, Kathleen French
2. Textbook of Animal Physiology by Anjali Mishra.
3. Principles of Animal Physiology by Moyes and Schulte
4. Introduction to Animal Physiology by Kay Ian

UNIT-V

Thermoregulation: Importance of body temperature in animal physiology, heat exchange interactions between animals and environment

Objective: In this unit you will learn about Importance of body temperature in animal physiology, heat exchange interactions between animals and environment.

Thermoregulation:

Thermoregulation is the ability of an organism to maintain a core body temperature, which is 37° C (98°F) within an optimal physiological range. The hypothalamus, a portion of a brain which plays an important role in regulating body temperature by acting as a thermostat. Thermoregulation is also called as the heat regulation.

Example: Human beings living in a climate of varying temperature and are able to maintain constant body temperature. In both animals and birds, the balance in heat gain and loss is provided by the hair, feathers, and fat skin layers.

We might have come across the term cold blood and warm-blooded animals. Based on the temperature regulation and their adaptations towards balancing the gain and loss in the body heat, these animals are classified into:

Ectothermic Animals

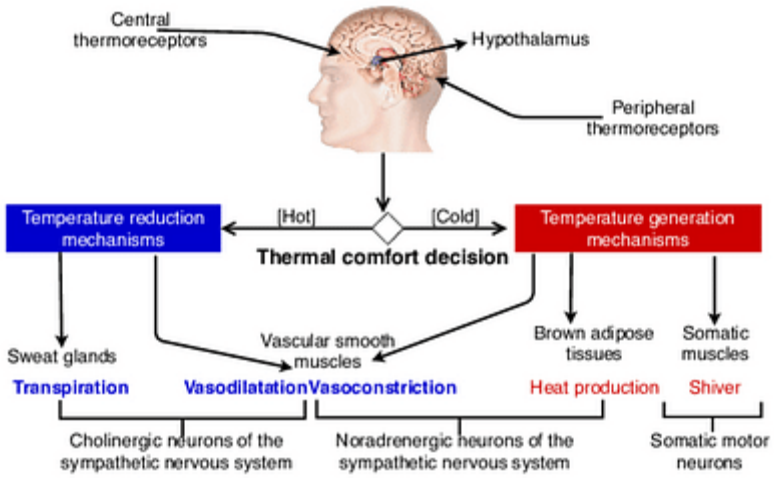
They are commonly called cold-blooded animals. They gain most of their heat from external sources. They produce a very less amount of heat to keep their body warm and has a low metabolic rate. Examples of ectothermic animals include amphibians, fish, lizards, other reptiles, etc.

Endothermic Animals

They are commonly called as warm-blooded animals. They develop most of the heat mainly from their body's metabolisms and produce a required amount of heat to keep their body warm. These animals have a very high metabolic rate. Examples of Endothermic animals include all mammals and birds.

Thermoregulation Explanation

Body temperature has an impact on bodily functions. In general, when body temperature rises, so does enzyme activity. Enzyme activity doubles with every 10 degrees Celsius increase in temperature, up to a point. With high heat (about 50 Degree C for mammals), body proteins, including enzymes, begin to denature and lose their activity. With a few exceptions, enzyme activity decreases by 50% for every 10 degree centigrade drop in temperature until the point of freezing. Some fish can resist freezing solid and then defrost to normal.



Thermoregulation

In human beings, A healthy internal body temperature is contained within a restricted range. The typical person's body temperature ranges between 98°F (37°C) and 100°F (37.8°C). Temperature is something that your body can adjust to. However, reaching extremes of body temperature might impair your body's capacity to operate. For example, “hypothermia” occurs when your body temperature dips to 95°F (35°C) or below. This disease has the potential to cause cardiac arrest, brain damage, and even death. If your body temperature increases to 107.6°F (42 °C), you risk brain damage or death.

The nervous system plays a very important role in thermoregulation. The hypothalamus regulates body temperature by triggering reflexes that produce vasodilation and sweating when the body is too warm, or vasoconstriction and shivering when the body is too cold. It reacts to substances produced by the body. When phagocytic leukocytes kill a bacteria, substances known as endogenous pyrogens are released into the bloodstream. These pyrogens go to the hypothalamus, where they reset the thermostat. This permits the body's temperature to rise, resulting in what is generally referred to as a fever.

Working of Thermoregulation

Sensors in your central nervous system (CNS) transmit information to your hypothalamus when your internal temperature changes. It responds by sending messages to numerous organs and systems throughout your body. They react through a number of methods.

If your body needs to cool down, the following methods can help:

- Sweating: Sweating is produced by your sweat glands and evaporates to cool your skin. This aids in the reduction of your internal temperature.
- Vasodilation is the widening of the blood vessels under your skin. This boosts blood flow to your skin, which is colder, and away from your warm inside organs. This allows your body to shed heat via heat radiation.

If your body needs to warm up, the following methods can help:

- Vasoconstriction is a narrowing of the blood vessels beneath the skin. This reduces blood flow to your skin, causing heat to be retained near the heated interior body.
- Thermogenesis: Heat is produced by your body's muscles, organs, and brain in a number of ways. Shivering, for example, causes muscles to generate heat.
- Hormonal thermogenesis occurs when your thyroid gland produces hormones that stimulate your metabolism. This increases the quantity of energy and heat produced by your body.

Mechanism of Thermoregulation

The hypothalamus is a small section or a portion of a human brain, which is mainly involved in secretion or release of all hormones from their respective glands and controlling several body functions. The mechanisms of thermoregulation are also controlled by this Hypothalamus.

When there is a small variation in the internal body temperature, the sensors in the central nervous system sends the message to the hypothalamus and in response, the hypothalamus sends signals to various cells, muscles, and other systems in our body.

If our body needs to warm up, the mechanisms of thermoregulation include:

1. Vasoconstriction: As the blood vessels under the skin receive signals they become narrower to decrease the blood flow and retain heat to warm the inner body.

2. Thermogenesis: This process is mainly seen in all warm-blooded animals. The body's organs produce heat in a variety of ways to keep the body warm.

3. Hormonal thermogenesis: In this mechanism, the thyroid gland regulates to release hormones in order to increase the body's metabolism, which produces a more amount of heat to maintain a stable internal body temperature.

If our body needs to cool down, the mechanisms of thermoregulation include:

1. Sweating: Here the sweat glands receive signals to release sweat and it cools our skin as it evaporates. This helps by lowering the internal temperature.

2. Vasodilatation: In this process, the blood vessels present beneath the skin expand and increases the blood flow, which cools by releasing the body's heat through heat radiation.

Increasing heat production—thermogenesis:

Endotherms have various ways of increasing metabolic heat production, or **thermogenesis**, in response to cold environments.

One way to produce metabolic heat is through muscle contraction—for example, if you shiver uncontrollably when you're very cold. Both deliberate movements—such as rubbing your hands together or going for a brisk walk—and shivering increase muscle activity and thus boost heat production.

Nonshivering thermogenesis provides another mechanism for heat production. This mechanism depends on specialized fat tissue known as **brown fat**, or brown adipose tissue. Some mammals, especially hibernators and baby animals, have lots of brown fat. Brown fat contains many mitochondria with special proteins that let them release energy from fuel molecules directly as heat instead of channeling it into formation of the energy carrier ATP

Controlling the loss and gain of heat

Animals also have body structures and physiological responses that control how much heat they exchange with the environment:

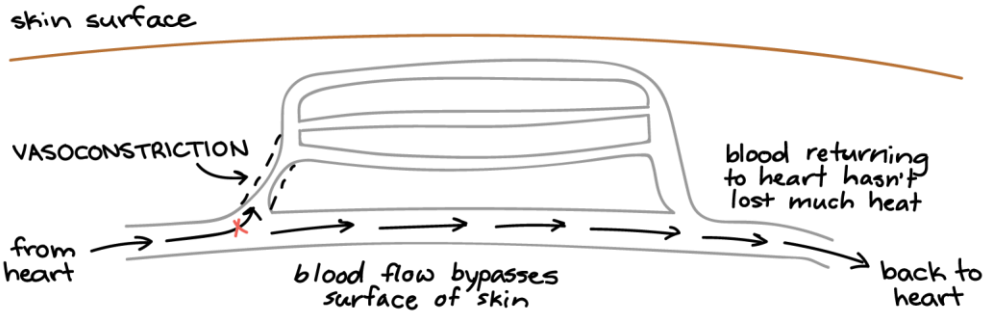
- Circulatory mechanisms, such as altering blood flow patterns
- Insulation, such as fur, fat, or feathers
- Evaporative mechanisms, such as panting and sweating

Circulatory mechanisms

The body's surface is the main site for heat exchange with the environment. Controlling the flow of blood to the skin is an important way to control the rate of heat loss to—or gain from—the surroundings.

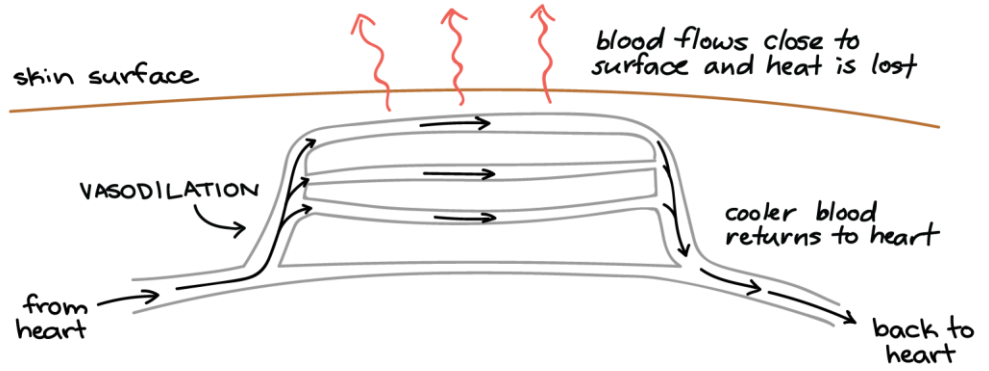
Vasoconstriction and vasodilation:

In endotherms, warm blood from the body's core typically loses heat to the environment as it passes near the skin. Shrinking the diameter of blood vessels that supply the skin, a process known as **vasoconstriction**, reduces blood flow and helps retain heat.



A bed of capillaries near the surface of the skin is fed by a blood vessel that can be vasoconstricted—narrowed—or vasodilated—expanded—to control flow of blood through the capillaries. When it is cold, this blood vessel is vasoconstricted, and the blood coming from the heart does not enter the capillary bed, instead traveling through an alternative "shunt" blood vessel that lets it bypass the skin surface. Thus, the blood returning to the heart has not lost much heat.

On the other hand, when an endotherm needs to get rid of heat—say, after running hard to escape a predator—these blood vessels get wider, or dilate. This process is called **vasodilation**. Vasodilation increases blood flow to the skin and helps the animal lose some of its extra heat to the environment.



A bed of capillaries near the surface of the skin is fed by a blood vessel that can be vasoconstricted—narrowed—or vasodilated—expanded—to control flow of blood through the capillaries. When it is hot, this blood vessel is vasodilated, and the blood coming from the heart enters the capillary bed, avoiding an alternative "shunt" blood vessel that would let it bypass the skin surface. As it travels close to the skin, the blood loses heat to the cooler environment and is thus cooled by the time it exits the capillary bed on its way back to the heart.

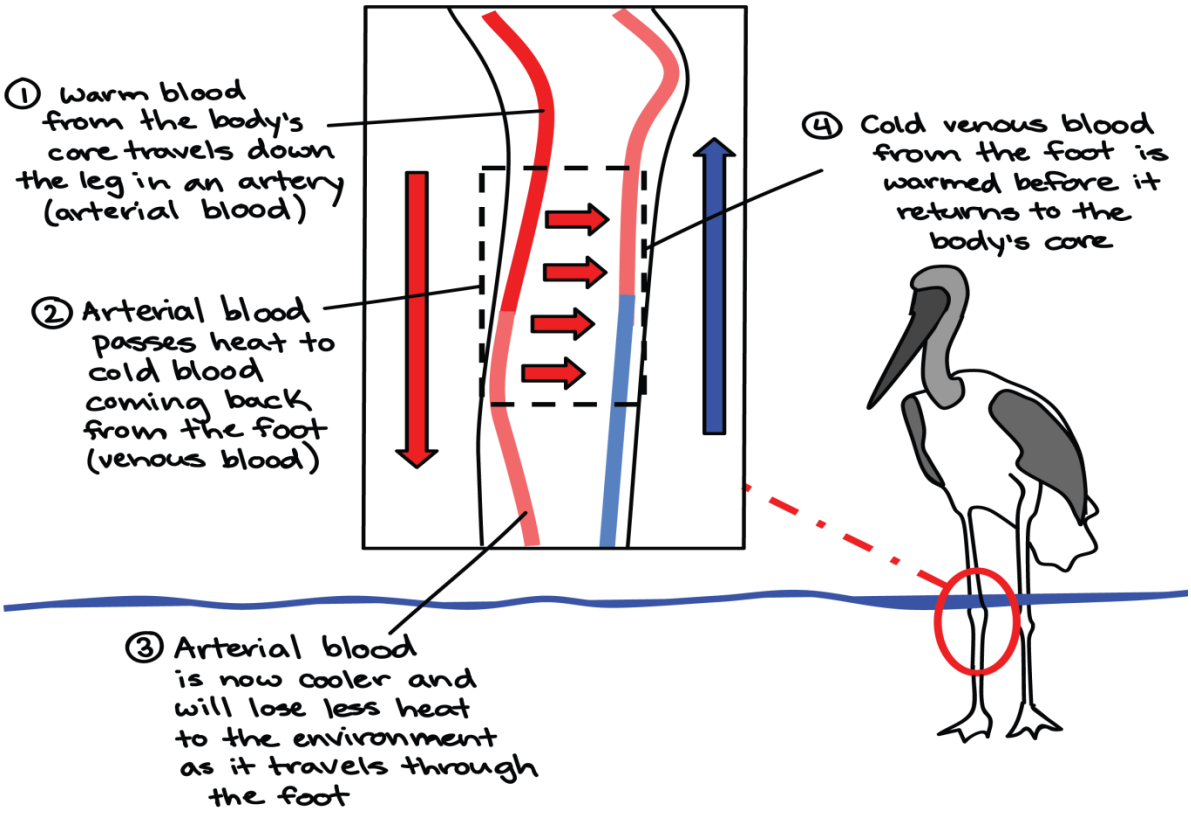
Furry mammals often have special networks of blood vessels for heat exchange located in areas of bare skin. For example, jackrabbits have large ears with an extensive network of blood vessels that allow rapid heat loss. This adaptation helps them live in hot desert environments.

Some ectotherms also regulate blood flow to the skin as a way to conserve heat. For instance, iguanas reduce blood flow to the skin when they go swimming in cold water to help retain the heat they soaked up while on land.^{5,6}5,6start superscript, 5, comma, 6, end superscript

Countercurrent heat exchange

Many birds and mammals have **countercurrent heat exchangers**, circulatory adaptations that allow heat to be transferred from blood vessels containing warmer blood to those containing cooler blood. To see how this works, let's look at an example.

In the leg of a wading bird, the artery that runs down the leg carries warm blood from the body. The artery is positioned right alongside a vein that carries cold blood up from the foot. The descending, warm blood passes much of its heat to the ascending, cold blood by conduction. This means that less heat will be lost in the foot due to the reduced temperature difference between the cooled blood and the surroundings and that the blood moving back into the body's core will be relatively warm, keeping the core from getting cold.



Insulation:

Another way to minimize heat loss to the environment is through **insulation**. Birds use feathers, and most mammals use hair or fur, to trap a layer of air next to the skin and reduce heat transfer to the environment. Marine mammals like whales use blubber, a thick layer of fat, as a heavy-duty form of insulation.

In cold weather, birds fluff their feathers and animals raise their fur to thicken the insulating layer. The same response in people—goosebumps—is not so effective because of our limited body hair. So, most of us wear a sweater!

Evaporative mechanisms:

Land animals often lose water from their skin, mouth, and nose by evaporation into the air. Evaporation removes heat and can act as a cooling mechanism.

For instance, many mammals can activate mechanisms like sweating and panting to increase evaporative cooling in response to high body temperature.

- In sweating, glands in the skin release water containing various ions—the "electrolytes" we replenish with sports drinks. Only mammals sweat.

- In panting, an animal breathes rapidly and shallowly with its mouth open to increase evaporation from the surfaces of the mouth. Both mammals and birds pant, or at least use similar breathing strategies to cool down.^88start superscript, 8, end superscript

  In some species, such as dogs, evaporative cooling from panting combined with a countercurrent heat exchanger helps keep the brain from overheating

Working of Thermoregulation

Sensors in your central nervous system (CNS) transmit information to your hypothalamus when your internal temperature changes. It responds by sending messages to numerous organs and systems throughout your body. They react through a number of methods.

If your body needs to cool down, the following methods can help:

- Sweating: Sweating is produced by your sweat glands and evaporates to cool your skin. This aids in the reduction of your internal temperature.
- Vasodilation is the widening of the blood vessels under your skin. This boosts blood flow to your skin, which is colder, and away from your warm inside organs. This allows your body to shed heat via heat radiation.

If your body needs to warm up, the following methods can help:

- Vasoconstriction is a narrowing of the blood vessels beneath the skin. This reduces blood flow to your skin, causing heat to be retained near the heated interior body.
- Thermogenesis: Heat is produced by your body's muscles, organs, and brain in a number of ways. Shivering, for example, causes muscles to generate heat.
- Hormonal thermogenesis occurs when your thyroid gland produces hormones that stimulate your metabolism. This increases the quantity of energy and heat produced by your body.

Importance of Thermoregulation

Thermoregulation is the set of mechanisms that an organism uses to keep its internal temperature constant. We have already discussed how in so many ways thermoregulation regulates body temperature and functioning. We will discuss more in detail the importance of thermoregulation in survival below:

1. When the temperature outside changes, organisms must maintain homeostatic control over their bodies. A constant temperature is essential because the enzymes in our bodies only operate at specific temperatures, usually about 37°C. Temperature fluctuations also have an effect on the fluidity of cell membranes. When an organism's core body temperature is too high or too low, some physiological systems might shut down, resulting in the organism's death.

2. Thermoregulation is a critical component of human homeostasis. The majority of body heat is produced by deep organs, including the liver, brain, and heart, as well as by skeletal muscle contraction. Humans have adapted to a wide range of climates, including hot humid and hot desert. High temperatures put the human body under severe strain, putting it at risk of damage or even death. One of the most frequent reactions to hot temperatures is heat exhaustion, which is a disease that can occur when exposed to high temperatures and results in symptoms such as dizziness, fainting, or a fast heartbeat.

3. Thermoregulatory capabilities are tightly linked to energy balance, and animals are continuously striving to reduce the energy costs of normothermia. When temperatures rise, physiological processes become more active, resulting in higher rates of energy consumption. Species can, however, make behavioural changes to minimise autonomic labour and, as a result, the energy costs of thermoregulatory responses. As a result, during cold exposure, thermogenesis-induced metabolic expenditures can be minimised, while hyperthermia associated with dehydration can be avoided during hot exposure.

4. When migration and metabolic changes are not viable choices, resident homeotherms can tolerate high temperatures. Reindeer (Rangifer tarandus) are famous for their ability to remain active in extremely cold (-23°C) settings, even bearing young in the dead of winter. Their dense fur aids in insulation, and regional heterothermy helps to store heat in the body core. Furthermore, unlike other vertebrates, their thermoneutral zone extends considerably deeper into lower temperatures.

5. Thermogenesis occurs in the blooms of several plants in the Araceae family, as well as in the cones of cycads. Furthermore, the holy lotus (*Nelumbo nucifera*) can thermoregulate itself, staying 20°C (36°F) above air temperature when blossoming. Heat is generated by breaking down the starch stored in their roots, which necessitates the consumption of oxygen at a pace comparable to that of a soaring hummingbird. One proposed reason for plant thermoregulation is that it protects the plant against cold temperatures. The skunk cabbage, for example, is not frost-resistant, yet it begins to develop and blossom while there is still snow on the ground.

Probable Questions:

1. Define thermoregulation.
2. write down the difference between ectothermy and endothermy.
3. What is thermogenesis. Briefly describe the mechanism of thermogenesis.
4. Describe counter current heat exchange.
5. Define acclimation and acclimatization.
6. describe behavioral adaptations for heat loss.
7. Describe physiological changes during acclimation and acclimatization.
8. Describe the role of vasoconstriction and vasodilation in thermoregulation.
9. Write down the response to high and low temperature with a chart.

Suggested Readings:

1. Eckert Animal Physiology by David Randall, Warren Burggren, Kathleen French
2. Textbook of Animal Physiology by Anjali Mishra.
3. Principles of Animal Physiology by Moyes and Schulte
4. Introduction To Animal Physiology by Kay Ian

UNIT-VI

Thermoregulation in ectotherms and endotherms, physical, chemical, neural regulation of body temperature; acclimation and acclimatization

Objective: In this unit we will discuss about acclimation and acclimatization. We will also discuss about how brains sense temperatures and also about behavioural thermoregulation.

ACCLIMATION & ACCLIMATIZATION

Acclimation: When an animal is subjected to changed condition in laboratory, animal shows compensatory changes to new environment. These changes are known as **acclimation**. In other words acclimation is adjustment to laboratory conditions.

Types of acclimation: In case of temperature regulation acclimation is of two types

i) **Cold Acclimation**
ii) **Hot or warm Acclimation**

Acclimatization – Compensatory adjustment of organism to change in the environment, in nature or natural condition is known as acclimatization.

Adjustment to seasonal changes in temperature comes under acclimatization.

• Normally when an animal is exposed to a **Ta** lower than normal then initially the body temperature **Tb** drops, thereafter the animal compensates and tries to bring the **Tb** to normal.

• When exposed to hot **Ta** the **Tb** initially increases and then the animal tries to bring it to normal through compensation.

• when an animal from normal temperature ta is subjected to cold ta, then there is initial drop in body temperature as shown by position 1. If the animal is exposed to hot environment there is initial rise in body temperature shown by pattern 1. If the body temperature (tb) from 1 settles to 0 then the compensation is known as "**perfect compensation**" & acclimation as **"Perfect Acclimation".**

• If the temperature settles between 1 & 0 i.e. 2 then compensation is not full proof so known as '**partial compensation**' & hence known as "**partial Acclimation**"

• If the body temperature (tb) settles to pattern 4 i.e. in cold Ta the body becomes warmer than normal & in hot Ta it become cooler than normal then the compensation is known as "**over shoot**" or "**excess compensation**".

• And if the body temperature as shown from pattern 1 changes to 3 i.e. in cold it further drops & in hot environment it further rises then the compensation is not there or it indicates "no compensation." The animals, depending upon their genetic ability show different type of compensation. However it is difficult to generalize about compensation because there are too many variations in acclamatory compensation.

• Many animals show compensatory changes in particular range of temperature.

• So also the acclimation depends on physiological state of animal such as hormonal state, health, injuries etc.

• Not only the animals show different type of acclamatory compensation. It is found that in an animal different tissues show different type of acclamatory compensations. This is of course is related to the importance of the tissue in the body.

• As shown in diagram below a fish trout when acclimated to 16 to 80^0C temperature following Precht‟s pattern was seen in which brain showed perfect acclimation, liver showed over shoot compensation, whole body showed partial compensation & gills showed no compensation.

• It is generally observed that the animals which show torpidity & hibernation or are poor in compensatory acclimation. Whereas the animals that remain active in extreme environment are better in compensatory acclimation.

• The animals which live in tropics & polar environment enjoy stable environment & hence are poor in compensatory acclimation as compared to those living in temperate environment which have more temperature fluctuations. Changes that occur during Acclimation & Acclimatization.

Physiological changes that take place in Acclimation & Acclimatization:

• In cold acclimation protein & enzyme secretion is more as compared to hot acclimation. All the changes during acclimation are not understood except the following:

i) Protein & phospholipid synthesis & turnover.

ii) Synthesis of isozymes suitable for new environment.

iii) Modification of membrane lipids.

• Normally membrane lipids become unsaturated fats so that they remain fluid in cold condition.

• Likewise animals show behavioral responses (refer to Gradient selection in poikilotherms)
Cold Acclimation
• When birds or mammals are kept at low temperature in laboratory, initially their body temperature falls down but then they show rise in metabolic rate, rise in O_2 consumption. This change is proportional to change in temp.

• As oxidation is faster, the food intake rises. The enzymes in liver, muscles and mitochondria rise. Glucose, pentose, fatty acid pathways are mobilized. They become faster. Adrenal & thyroid secretion rises. Most important is that the peripheral circulation rises to keep skin warm & in them normally insulation by fur coat does not change.

Cold Acclimatization:

• This is a slow process & is seasonal. The change is prolonged & gradual & hence the compensatory changes are different. In nature when change occurs, the animal shows changes in thickness of fur coat. They reduce the peripheral circulation & both these prevent heat loss.

• This is because in environment with approach of winter there is scarcity of food.

• It is found that the thickness of fur rises in large animals.

• In them fat is mobilized & unsaturated fat gets deposited in joints & extremity so that the flexibility is maintained.

• Their CNS, tissue, nerves gradually gain resistance to stand the cold. Sparrow gain resistance at rate 60 drop / month. Sensitivity of tissues is increased. There is higher sensitivity of the tissues to neurotransmitter or the transmitter subs.

• Along with these the animal show behavioral changes in them e.g., Dog, rats, bees show aggregation phenomenon in cold season.

The organization of the thermoregulatory system :

Feed-forward and feedback regulation of body temperature:

Body temperature is not a single value but varies depending on where it is measured. In studies of thermoregulation, it is common to divide the body into two compartments: (1) the external shell, which includes the skin and largely fluctuates in temperature along with the environment, and (2) the internal core, which includes the central nervous system and viscera and has relatively stable temperature (Jessen, 1985; Romanovsky et al., 2009).

The core temperature is the regulated variable in the thermoregulatory system (Hensel, 1973) and is maintained by a combination of feedback and feed-forward mechanisms (Kanosue et al., 2010). Feedback responses are those that are triggered when core temperature deviates from the defended range: for example, exercise generates heat that can increase internal temperature by several degrees Celsius (Fuller et al., 1998; Walters et al., 2000). Such changes in internal temperature are detected by specialized thermoreceptors located throughout the body core, including the viscera, brain, and spinal cord (Jessen, 1985). Localized heating or cooling of any of these internal structures induces global feedback responses that oppose the applied temperature change.

Feed-forward mechanisms are triggered in the absence of any change in core temperature and instead enable preemptive responses to anticipated thermal challenges. The most common example of feed-forward control is the detection of a change in air temperature by thermoreceptors in the skin, which triggers thermoregulatory responses that precede and prevent any change in core temperature (Nakamura and Morrison, 2008, 2010; Romanovsky, 2014). Although feed-forward and feedback signals convey different kinds of information about body temperature, they are thought to converge on a common set of neural substrates in the preoptic area (POA) of the hypothalamus.

Physiologic versus behavioral thermoregulation

Body temperature is regulated by two types of mechanisms, physiologic and behavioral (Figure 1). Physiologic effectors are involuntary, mostly autonomic responses that generate or dissipate heat. The primary physiologic responses to cold exposure are brown adipose tissue (BAT) thermogenesis and skeletal muscle shivering, which generate heat, and the constriction of blood vessels (vasoconstriction), which prevents heat loss. Exposure to warmth triggers a complementary set of autonomic responses, including suppression of thermogenesis and facilitation of heat loss through water evaporation (e.g. sweating) and dilation of blood vessels (vasodilation). Different species sometimes use different strategies to achieve the same physiologic effect. For example, humans achieve evaporative heat loss primarily by sweating, whereas dogs rely on panting and rodents spread saliva on their fur

(Jessen, 1985). Likewise the effects of vasodilation are enhanced in species that have specialized thermoregulatory organs, such as the rat tail or rabbit ears, that can rapidly dissipate heat due to their large surface area. Despite these superficial differences, the major classes of physiologic responses are thought to be governed by a common set of neural substrates that are conserved across mammals. Behavior is also an important mechanism for body temperature control. Whereas physiologic responses are involuntary, thermoregulatory behaviors are motivated, meaning that they are flexible, goal-oriented actions that are learned by reinforcement and driven by the expectation of reward (Carlton and Marks, 1958; Epstein and Milestone, 1968; Weiss and Laties, 1961). The most basic thermoregulatory behaviors are cold and warm-seeking, in which animals move between microenvironments in their habitat in order to alter the rate of heat loss or absorption. More complex thermoregulatory behaviors include nest or burrow making, in which animals create their own thermal microenvironment (Terrien et al., 2011); social behaviors such as huddling between conspecifics (Batchelder et al., 1983); and human behaviors such as wearing clothing or using air-conditioning. The engagement of specific thermoregulatory mechanisms is hierarchical, meaning that different effectors become activated at different temperature thresholds. In general, behavioral responses are utilized in preference to autonomic effectors, and autonomic effectors are activated in a stereotyped sequence. This sequence is thought to reflect the "cost" of activating different responses, either in terms of their energy use or the trade-offs they require with competing physiologic systems. For example, heat challenge triggers vasodilation at lower temperatures than sweating, possibly because sweating results in water loss that upsets fluid balance (Costill and Fink, 1974). Similarly cold challenge activates vasoconstriction before shivering or BAT thermogenesis, in accordance with the relative energy cost of these different mechanisms. The existence of these distinct temperature thresholds has been interpreted as evidence that the thermoregulatory circuit contains multiple effector loops, each of which operates to some extent independently (McAllen et al., 2010; Satinoff, 1978).

Interactions between thermoregulation and other physiologic systems

The core temperature defended by the thermoregulatory system (the "balance point" or "set point") is not a fixed value but fluctuates in response to internal and external factors. Many of these factors are unrelated to temperature per se and instead reflect interactions with other physiologic systems. One example is fever, which is the controlled increase of body temperature that occurs most commonly in response to an infection (Figure 2). Fever is triggered by bacterial lipids and other molecules ("pyrogens") that directly or indirectly induce the production of prostaglandin E2 (PGE2) by endothelial cells lining the POA (Evans et al., 2015). PGE2 is thought to inhibit the activity of POA neurons that function to reduce body temperature, thereby producing a regulated hyperthermia that increases the likelihood of surviving an infection.

Sleep is a second example of a physiologic process that modulates, and is modulated by, the thermoregulatory system (Krueger and Takahashi, 1997). The onset of sleep tracks closely the rate of decline in body temperature, and, during sleep, entry into epochs of rapid eye-movement (REM) is accompanied by near complete inhibition of thermoregulatory responses in many species (Krueger and Takahashi, 1997). Overlaid on these effects of sleep are slower timescale, diurnal fluctuations in body temperature that arise from circadian rhythms (Heller et al., 2010). Sleep, circadian rhythms, and body temperature are all controlled by dedicated neural circuits in the anterior hypothalamus, but the interconnections between these circuits have not been defined.

Thermoregulation is also tightly interconnected with the energy and fluid homeostasis systems, due to the substantial demands that thermoregulatory effectors place on bodily resources. For example, cold-induced thermogenesis consumes approximately 60% of total energy expenditure when mice are maintained at an ambient temperature of 4°C (Abreu-Vieira et al., 2015). To satisfy this energy need, mice exposed to cold will double their daily food intake (Bauwens et al., 2011) and, if supplied with adequate food, can live and proliferate at cold temperatures indefinitely. However when food is scarce, mice sacrifice the defense of body temperature and go into torpor, a state of regulated hypothermia and inactivity in which core body temperature can fall below 31°C (Webb et al., 1982).

Similarly, in dehydrated animals, evaporative heat loss is attenuated in favor of thermoregulatory effectors that do not require water (Baker and Doris, 1982; Fortney et al., 1984; Morimoto, 1990). How these trade-offs between competing physiologic needs are resolved in the brain is an important open question (Nakamura et al., 2017)

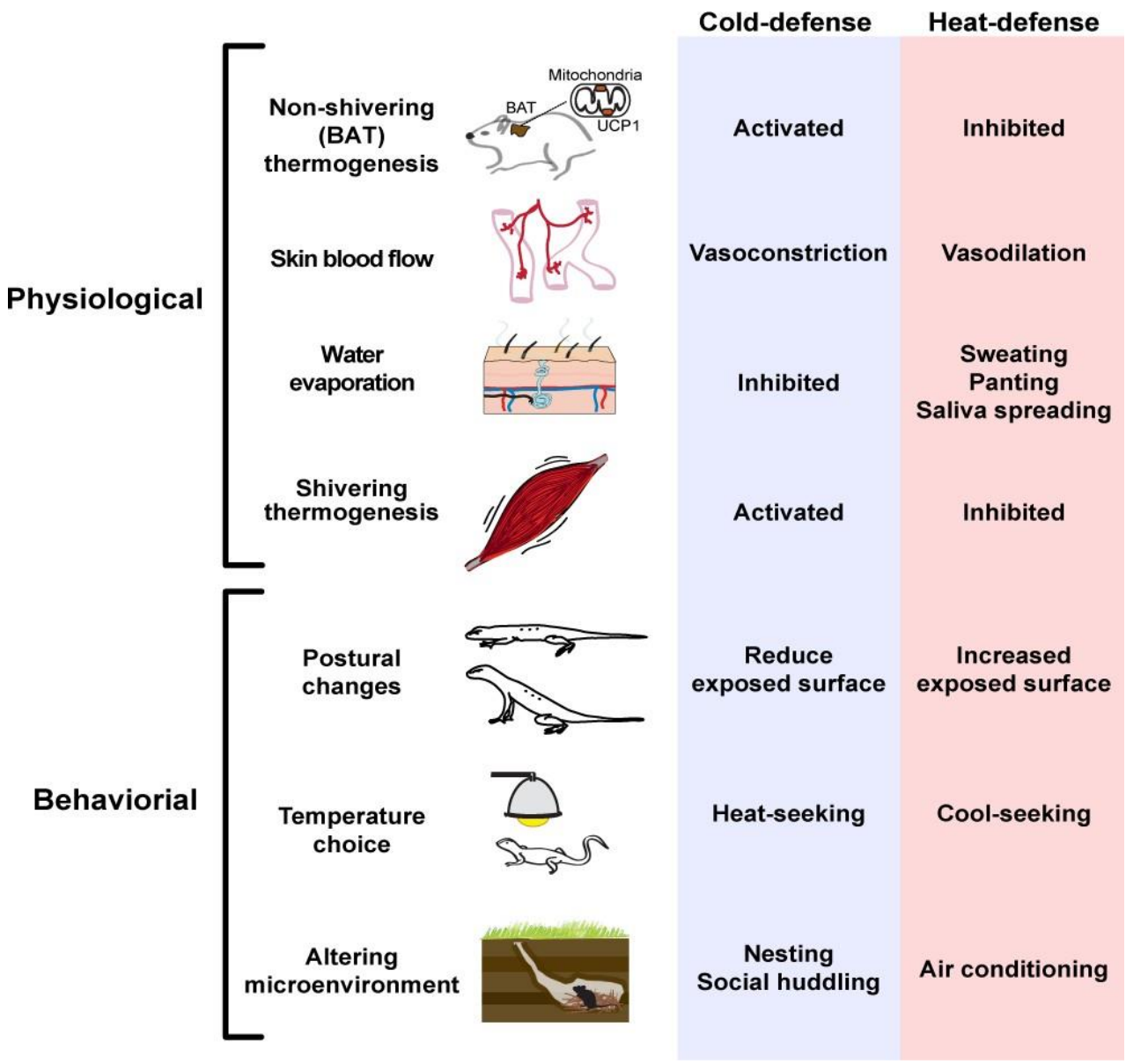


Figure1.Typesofthermoregulatoryeffectors

Examplesofphysiologicalandbehavioralstrategiesforcontrollingbodytemperature

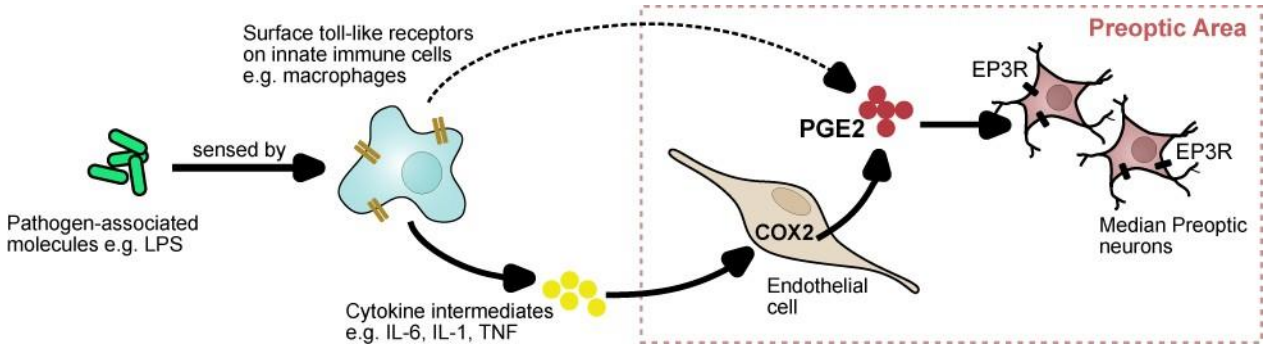


Figure2.Thegenerationoffever : The presence of molecules associated with pathogens like bacteria and viruses is sensed byinnate immune cells in the blood and lead to the production of pyrogenic intermediates likecytokines and prostaglandins that act on the preoptic area. In the preoptic area, COX2expression in endothelial cells result in local PGE2 production, which is the dominantsourceof fever-inducing PGE2. PGE2actsthroughEP3receptorsexpressedinthemedianpreoptic(MnPO)toeffectchangesinbodytemperature.LPS–lipopolysaccharide;COX2–cyclooxygenase2.

Sources of input into the thermoregulatory system

The primary input into thermoregulatory system comes from sensory neurons that measure the temperature of the body. Most of these sensory neurons have cell bodies located in peripheral ganglia and axons that extend out to measure the temperature of key thermoregulatory tissues (e.g. the skin, spinal cord, and abdominal viscera; discussed below). A separate set of sensory neurons are located within the brain itself and measure the temperature of the hypothalamus.

Peripheral temperature sensing

Peripheral temperature sensing is mediated primarily by two classes of sensory neurons that are activated by innocuous warmth (~34-42°C) or col d (~14-30°C). These neurons have cell bodies located in trigeminal ganglion (for innervation of the head and face) and dorsal root ganglia (DRG; for innervation of the rest of the body). They are pseudounipolar, meaning that their axons split into two branches, one of which innervates the skin or viscera and the other projects to the dorsal horn of the spinal cord or to the spinal trigeminal nucleus in the brainstem. Peripheral thermosensation has been comprehensively reviewed elsewhere (Ma, 2010; Vriens et al., 2014). Here we briefly outline the key facts relevant to thermoregulation.

Cold-sensing—TRPM8 is the primary peripheral cold sensor in the thermoregulatory system. This channel is activated *in vitro* by mild cooling (<26-28° C) and its expression is required for cold perception (Bautista et al., 2007; Dhaka et al., 2007; McKemy et al., 2002; Peier et al., 2002). TRPM8 is expressed in essentially all cold-sensitive neurons, and ablation of these TRPM8+ cells abolishes the behavioral and neural responses to cooling (Knowlton et al., 2013; Pogorzala et al., 2013; Yarmolinsky et al., 2016). As would be expected for thermosensor with a role in thermoregulation, treatment with TRPM8 agonists causes hyperthermia, whereas TRPM8 antagonists cause hypothermia (Almeida et al., 2012; Gavva et al., 2012). In addition, TRPM8 antagonists block the ability of environmental cold to induce Fos in brain regions that mediate thermoregulation (Almeida et al., 2012). Thus TRPM8 and the neurons that express it delineate a labelled line that communicates cold information from the periphery into the CNS.

Warm-sensing—The molecular identity of the peripheral warm sensor is controversial. Several TRP channels have been proposed to play this role, including TRPV1, TRPV3, TRPV4, and TRPM2, but there is conflicting evidence for and against all of these candidates. At the level of the sensory neurons, the cells that mediate warm- sensing are a subset of TRPV1+ primary afferents. Treatment of these cells with a TRPV1 antagonist blocks their activation *in vivo* by innocuous warmth (Yarmolinsky et al., 2016), which is counterintuitive given that TRPV1 is activated *in vitro* only at higher temperatures (>42° C). However this may reflect the presence of co-agonists or post-translational modifications that can lower the TRPV1 temperature threshold *in vivo* (Tominaga et al., 1998; Vellani et al., 2001). Peripheral TRPV1 antagonists induce hyperthermia, whereas TRPV1 agonists induce hypothermia, consistent with a role in thermoregulation ((Gavva, 2008; Gavva et al., 2007; Hori, 1984; Steiner et al., 2007) but see counterarguments in (Romanovsky et al., 2009). Nevertheless the fact that TRPV1 knockout mice have normal body temperature indicates that, at a minimum, other channels can compensate for its thermoregulatory function (Caterina et al., 2000; Iida et al., 2005; Szelenyi et al., 2004). Knockout of other TRP channels, alone or in combination, has yielded inconsistent effects in some cases and in others the thermoregulatory phenotype has not been fully characterized (Huang et al., 2011; Song et al., 2016; Tan and McNaughton, 2016; Vriens et al., 2014).

Tissues that provide thermoregulatory input—The relative contribution of different tissues to the overall body temperature signal has been investigated by manipulating their temperature and then measuring the thermoregulatory response (Figure 3). This has identified four tissues that provide particularly important input: the skin, spinal cord, abdominal viscera, and brain (Cabanac, 1975; Jessen, 1985). In general, the POA is the most thermosensitive site (i.e. largest effector response per degree of warming or cooling), whereas the skin undergoes the largest temperature fluctuations. These inputs from different tissues are summed to determine the magnitude of the thermoregulatory response; this summation can be simply additive or more complex, depending on the context.

Skin temperature functions both as an input that activates thermoregulatory effectors (e.g. shivering when the air is cold) and as a discriminative signal that guides behavior (e.g. this object is warm). For this reason different parts of the skin contribute to thermoregulation in different ways. Non-hairy (glabrous) skin in mammals is restricted to a few sites, such as parts of the hands, feet, and face, that are more important for discriminating the temperature of external objects. Hairy skin covers the majority of the body and, due to this larger surface area, contributes relatively more of the input signal that drives thermoregulatory effectors (Romanovsky, 2014). However there are exceptions to this rule. For example, heating of the face (Nadel et al., 1973) or the scrotum (Waites, 1962) drives panting and sweating to a greater extent than would be predicted based on their surface area, whereas heating of the extremities (e.g. arms and legs) has proportionally less effect.

Outside of the brain, the spinal cord is the most well characterized contributor to the core body temperature signal, and numerous studies have shown that selectively heating or cooling the spinal cord can trigger appropriate thermoregulatory responses (Cabanac, 1975; Jessen, 1985). The thermosensitivity of the spinal cord is thought to be mediated by the same sensory neurons that measure the temperature of the skin and viscera (Brock and McAllen, 2016). This is possible because the axons of these primary sensory afferents terminate in the dorsal horn of the spinal cord, such that heating or cooling of the spinal cord potentiates neurotransmitter release from their thermosensitive terminals.

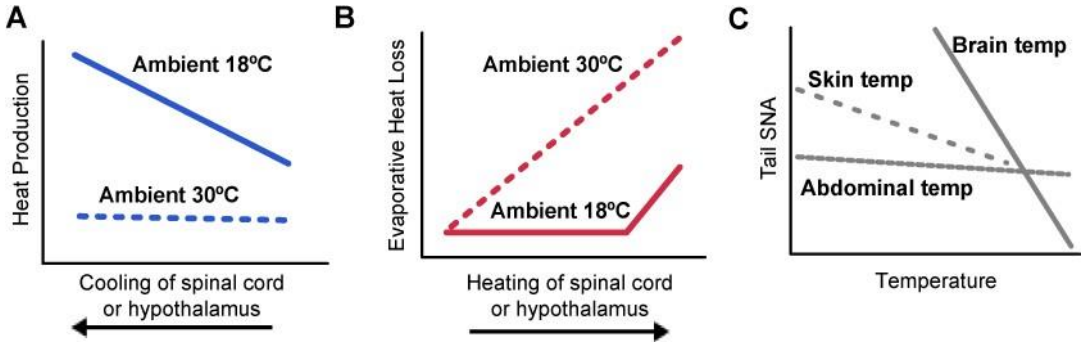


Figure3.Interactionbetweencore(brainorspinalcord)andambient(skin)temperatureinthecontrolof thermoregulatory effectors

A and B. Adapted from (Jessen and Ludwig, 1971). Spinal cord (SC) and hypothalamic (Hypo) temperatures in the dog were independently manipulated at varying ambienttemperaturesandresultanteffectsofheatproductionandevaporativeheatlossareshown.

C. Adapted from (Shafton et al., 2014). Changes in rat tail sympathetic nerve activity(SNA),whichisameasureofvasoconstriction(lowSNA–vasodilation)asabdominal,skinor brain temperature is altered.

Temperature sensing in the brain

In addition to peripheral tissues, the temperature of the brain itself is an input into the thermoregulatory system (Figure 3). The most sensitive site in the brain is a hotspot in the midline POA, located between the anterior commissure and optic chiasm, that when heated elicits dramatic and coordinated heat defensive responses such as panting, sweating, vasodilation, and cold-seeking behavior (Andersson et al., 1956; Carlisle, 1966; Carlisle and Laudenslager, 1979; Hemingway et al., 1954; Magoun et al., 1938). Cooling of this structure has the opposite effect, promoting vasoconstriction, BAT thermogenesis, shivering, and operant responses for heat (Hammel et al., 1960). These observations suggest that the midline POA contains intrinsically thermosensitive neurons that are important for body temperature control.

Brain temperature can increase by 2-3°C in response to exercise or fever, which provides a context in which POA warm-sensing may be important (Fuller et al., 1998; Walters et al., 2000). On the other hand, acute exposure to environmental heat or cold does not affect brain temperature in most animals (Bratincsak and Palkovits, 2005; Hammel, 1968; Hammel et al., 1963; Hellstrom and Hammel, 1967; Nakamura and Morrison, 2008, 2010). In addition to sensing local brain temperature, POA neurons also receive information about peripheral temperature via an ascending neural pathway (Figure 3), and 25-50% of the POA neurons that are activated by local brain warming are also activated by warming of the skin or spinal cord (Boulant and Hardy, 1974; Wit and Wang, 1968). Thus many POA cells integrate central and peripheral thermal information.

While it is likely that intrinsically thermosensitive POA neurons play a role in thermoregulation, it is important to emphasize that thermosensitive neurons are found in many brain regions, and most of these neurons presumably have no role in body temperature regulation (Barker and Carpenter, 1970; Eisenman et al., 1971; Hellon, 1986). For example, cooling of the HVC, a premotor nucleus in the zebra finch, can selectively slow the bird's song speed, even though songbird singing is not naturally controlled by changes in brain temperature (Long and Fee, 2008). To definitively establish the physiologic relevance of intrinsic POA thermosensing, it will likely be necessary to identify and disrupt the brain temperature sensor.

Molecules that sense brain temperature—If warm-sensitivity is defined as an increase in spontaneous firing rate of >0.8 action potentials per second per degree Celsius, then approximately 20% of POA neurons are warm-sensitive in vitro (Boulant, 2006; Nakayama et al., 1978) or in vivo (Hellon, 1970; Knox et al., 1973; Nakayama et al., 1963). This warm-sensitivity is an intrinsic property of POA neurons (Kelso and Boulant, 1982) and has been proposed to be mediated by either a heat-activated non-selective cation current

(Kobayashi et al., 2006) or a heat-inactivated potassium current (Boulant, 2006). In neither case has the identity of the relevant ion channel been established.

Two TRP channels, TRPV1 and TRPM2, have been proposed to function as warm-sensors in the brain. The case for TRPV1 is based on the fact that central injection of the TRPV1 agonist capsaicin can induce hypothermia (Hori, 1984; Romanovsky et al., 2009). However the site of action of capsaicin in these experiments is unclear, since TRPV1 is expressed at extremely low levels in the brain and is absent from the POA (Cavanaugh et al., 2011). The evidence supporting a role for TRPM2 includes the fact that TRPM2 knockout mice have an exacerbated fever response, and the fact that neurons from these knockout mice have attenuated thermosensitivity in vitro (Song et al., 2016). However TRPM2 KO mice have normal core body temperature, and TRPM2 is broadly expressed in the brain and periphery (Song et al., 2016; Tan and McNaughton, 2016), suggesting that TRPM2 is unlikely to be the molecule that confers warm-sensitivity on a specific subset of neurons. For both of these candidates, a critical test will be to measure whether deletion of the channel in the brain can abrogate the thermoregulatory response to POA warming.

Thermoregulatory Behaviors

Animals engage in voluntary behaviors that alter their local thermal environment. These include warm and cold seeking, nesting and burrowing, huddling, basking, postural extension, and saliva spreading, as well as more complex strategies used by humans (Terrien et al., 2011). Thermoregulatory behavior is ancient and widespread: it occurs not only in endotherms (birds and mammals), but also in reptiles, fish, and many invertebrates that rely almost exclusively on behavior to respond to changes in external temperature.

Thermoregulatory behavior is also motivated, at least in mammals, which means that temperature can serve as a reward that trains animals perform new tasks. For example, rats exposed to cold will learn to lever press to turn on a heat lamp (Carlton and Marks, 1958; Weiss and Laties, 1961), whereas rats exposed to heat will lever press to turn on a cold shower (Epstein and Milestone, 1968) or a cooling fan (Lipton, 1968). This suggests that thermoregulatory behaviors are driven by the same motivational systems that subserve other behaviors, such as eating and drinking, that arise from homeostatic needs.

The neural circuitry underlying these behavioral responses is poorly understood (Almeida et al., 2015). The POA is sufficient but not necessary for activation of most thermoregulatory behaviors. The evidence for sufficiency includes the fact that cooling of the POA stimulates operant responses for heat (Gale et al., 1970; Laudenslager, 1976; Satinoff, 1964), whereas warming of the POA inhibits those responses (Carlisle, 1966; Carlisle and Laudenslager, 1979; Laudenslager, 1976). Optogenetic stimulation of warmth-activated POAPACAP/BDNF neurons promotes cold-seeking behavior and inhibits nest building in the cold (Tan et al.,

2016), whereas chemogenetic stimulation of POALepR neurons promotes postural extension, a behavioral strategy for heat dissipation (Yu et al., 2016). It is important to note that, in all of these cases, the behavioral and autonomic responses to POA manipulation function in the same direction, indicating that they are part of a coordinated, homeostatic response.

Nevertheless, lesions that ablate the POA leave most thermoregulatory behaviors intact (Almeida et al., 2006; Carlisle, 1969; Lipton, 1968; Roberts and Martin, 1977;Satinoff and Rutstein, 1970). In fact POA lesions often enhance operant responding for thermal rewards, likely in order to compensate for the loss of autonomic thermoregulation (Carlisle, 1969). In general, lesioning experiments have failed to identify any forebrain brain region that is necessary for thermoregulatory behaviors in the way that the POA is necessary for autonomic responses, although a few special cases have been identified. These include a requirement for the POA in the postural extension induced by warmth (Roberts and Martin, 1977); for the DMH in cold-seeking induced by systemic inflammation, but not other stimuli (Almeida et al., 2006; Wanner et al., 2017), and for the MnPO in cold-seeking in response to salt challenge (Konishi et al., 2007).

The failure of POA lesions to block thermoregulatory behaviors has generally been interpreted to imply that the POA is "not involved" in these responses (Almeida et al., 2015). However this seems unlikely given the broad sufficiency of POA stimulation for orchestrating a variety of thermoregulatory behaviors. An alternative explanation is that the POA circuitry is complex, containing many intermingled cell types, and for this reason it is difficult to interpret the results of lesioning experiments that lack cell-type-specificity. There is ample precedent for this. For example, non-specific lesions of the hypothalamic arcuate nucleus (ARC) are famous for causing hyperphagia and obesity, suggesting the ARC functions as a "satiety center" (Choi and Dallman, 1999). However specific ablation of one ARC cell type (AgRP neurons) causes starvation (Luquet et al., 2005). In the future, it will be important to use approaches for cell-type-specific manipulation to re-investigate the role of the POA and downstream structures in the control of thermoregulatory behaviors.

Probable Questions:

1. Define acclimation and acclimatization.
2. describe behavioral adaptations for heat loss.
3. Describe physiological changes during acclimation and acclimatization.
4. How brain can sense temperature and how fever occurs?
5. What is warm sensing and cold sensing?

Suggested Readings:

1. Eckert Animal Physiology by David Randall, Warren Burggren, Kathleen French
2. Textbook of Animal Physiology by Anjali Mishra.
3. Principles of Animal Physiology by Moyes and Schulte
4. Introduction To Animal Physiology by Kay Ian

UNIT-VII

Digestive system: Acquisition of Energy: Types of feeding, Digestion (motility and Secretions), Metabolism, and absorption

Objective: In this unit we will discuss about different modes of feeding in animals. We will also discuss about digestive system of vertebrates and digestion process.

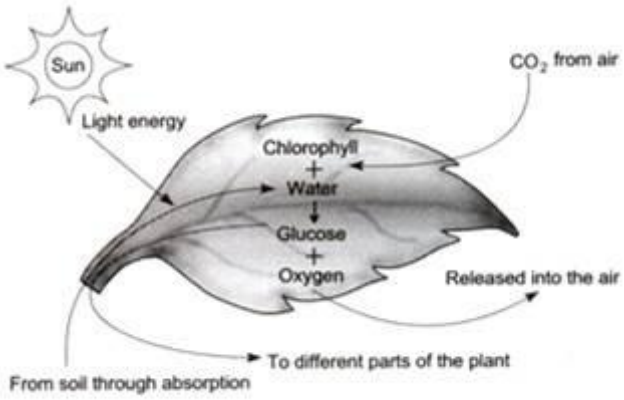
Introduction:

Plants and animals do not obtain food by the same processes. Plants and some bacteria have the green pigment chlorophyll to help synthesize food, while animals, fungi and other bacteria depend on other organisms for food.

Based on this, there are two main modes of nutrition: autotrophic and heterotrophic.

1. Autotrophic nutrition:

The term 'autotroph' is derived from two Greek words—autos (self) and trophe (nutrition). In autotrophic nutrition, an organism makes its own food from simple raw materials.



A summary of nutrition in green plants

Photosynthesis:

Green plants, which are autotrophic, synthesize food through the process of photosynthesis. Photosynthesis is a process by which green plants, having chlorophyll, synthesize the simple sugar (glucose) from the simple raw materials water and carbon dioxide using the energy of sunlight. Oxygen is released in this process. The overall equation of photosynthesis is

$$6CO_2 + 12H_2O \xrightarrow[\text{Chlorophyll}]{\text{Sunlight}} C_6H_{12}O_6 + 6H_2O + 6O_2$$

The sugar produced is stored in the form of starch in plants. (In animals food is stored in the form of glycogen.) These food reserves provide energy as and when required by the organism. Since autotrophic plants are able to produce food, they are also known as producers.

2. Heterotrophic nutrition:

The word 'heterotroph' is derived from two Greek words—heteros (other) and trophe (nutrition). Unlike autotrophs, which manufacture their own food, heterotrophic organisms obtain food from other organisms. As heterotrophs depend on other organisms for their food, they are called consumers. All animals and non-green plants like fungi come under this category.

Consumers which consume herbs and other plants are called herbivores, and those which consume animals are called carnivores. After taking complex organic materials as food, heterotrophs break them into simpler molecules with the help of biological catalysts, or enzymes, and utilize them for their own metabolism.

Depending upon the mode of living and the mode of intake of food, heterotrophs may be parasitic, saprophytic or holozoic.

a. Parasitic:

Parasitic organisms, or parasites, live on or inside other living organisms, called hosts, and obtain their food from them. The host does not get any benefit from the parasite. Different parasites, like Cuscuta (akash-bel), Cassytha (amar-bel), hookworms, tapeworms, leeches, etc., have different modes of feeding, depending upon habit, habitat and modifications.

b. Saprophytic:

Saprophytic organisms, or saprophytes, derive their food from dead organisms. They secrete enzymes that are released on food material outside their body. These enzymes break down

complex food into simple forms. Common examples of saprophytes are fungi (moulds, mushrooms, yeasts) and many bacteria.

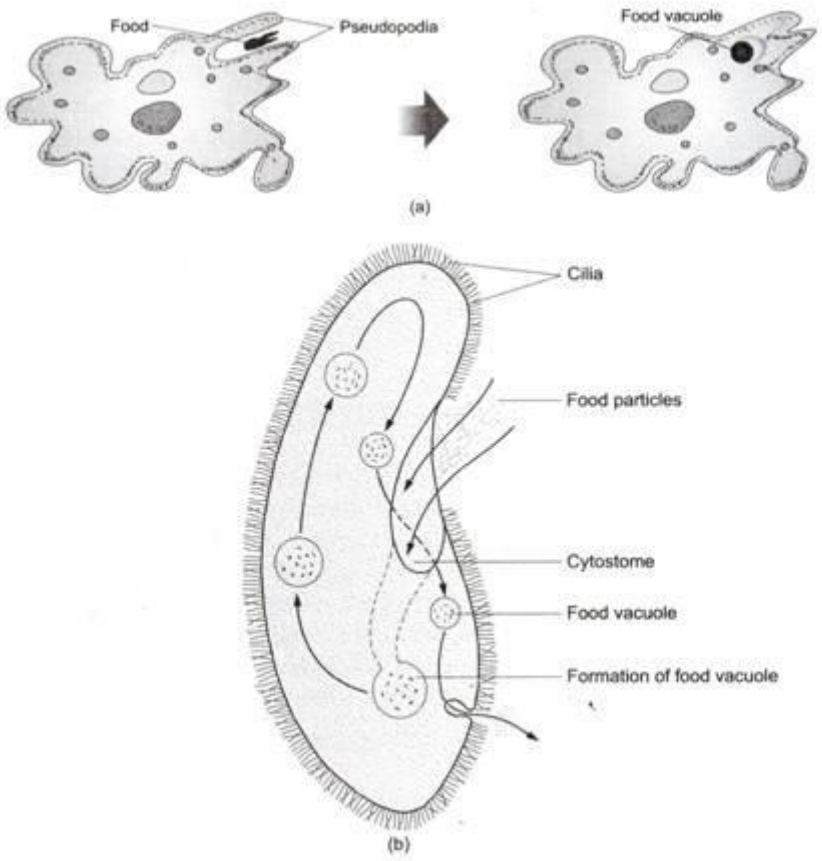
c. Holozoic:
In holozoic nutrition complex organic substances are ingested (taken in) without their being degraded or decomposed. After intake, such food is digested by enzymes produced within the organism. Digested food is absorbed into the body and the undigested product is egested (expelled) from the body. This kind of nutrition is found mainly in non-parasitic animals—simple ones like Amoeba and complex ones like human beings.

How Organisms Obtain Nutrition:

Different organisms obtain food in different ways. Nutrition in unicellular organisms, like Amoeba, involves ingestion by the cell surface, digestion and egestion.

Amoeba takes in complex organic matter as food. Amoeba first identifies its food. It then throws out a number of small pseudopodia (projections of cytoplasm, also called false feet). These pseudopodia enclose the food particle and prevent it from escaping. The food enclosed in the cell membrane forms a food vacuole.

The complex food is broken down into simpler molecules with the help of digestive enzymes of the organelle called lysosome. The digested food is distributed in the cytoplasm and the undigested food is egested through the cell membrane.



(a) *Amoeba* sends out pseudopodia to engulf food. (b) Feeding in *Paramoecium*

In Paramecium, a unicellular organism with a specific shape, food is ingested through a special opening, the cytostome (cell mouth). Food is brought to this opening by the lashing movement of cilia that cover the entire surface of the cell.

Different mode of feeding in animals:

Feeding behavior of an organism refers the process of obtaining and consuming food. Ingestion refers to consumption of the food taken by an organism. The nutritional or medicinal content of the food will be extracted to make the consumed food useful to the body. Such a process is accomplished once the substance gets into the gastrointestinal tract. However, in single-celled organisms, ingestion takes place once the substance goes through the cell membrane. Different organisms have specific adaptations to capture food and transform it into digestible particles. The adaptation may include specialized claws, special mouthparts and teeth, distinct beak shapes, sensory tissues, ability to camouflage and digestive system. This article explore some of the common modes of ingestion in different animals and the examples of animals in each category.

1. **Filter Feeding:** Filter feeding is where an organism passes water through specialized filtering organs. The organisms feed by straining suspended particles and digestible matter from the water. Such organisms are ecosystem engineers by clarifying and purifying water. Some of the organisms that utilize this method of

ingestion are the sponges, clams, baleen whales, and some bird species such as the flamingos and some ducks. Most forage fish species and crustaceans especially Mysidacea constantly apply the filter feeding technique. Cnidarians such as moon jellyfish use their grid fibers to motion food particles from the water into their bodies. The krill, a marine organism feeds exclusively through this technique.

2. **Deposit Feeding:** Deposit mode of feeding refers to a situation whereby an organism, either aquatic or dry land creatures, feeds on minute specks of organic deposits that have been drifted by water. These organisms are referred to as detritivores since they obtain their nutrients from detritus. Deposit feeding can also involve getting nutrients from digestible particles suspended in the soil. Such feeders are dominant organisms in regions with finer sediments or muddy sediments. Some do shoveling on the surface or subsurface while others live in barrows which are either single or U-shaped. Some of the deposit predators use tentacles to either gather particles or in an actual feeding. Examples of such feeders include flounders, haddock, fiddler crabs, snails, sea cucumbers, and eels. In a food web, these organisms mainly play the role of decomposers.

3. **Fluid Feeding:** Fluid feeders thrive on nutrients obtained from fluids of other organisms. The organisms can be hematophagy and feed on blood, nectarivore and feed on nectar, or plant sapsuckers. The living host may or may not be affected by the predator. All animals in this category have a common characteristic of having a sharply pointed mouth to enable it to pierce the skin or wall of the target plant or animal to extract the fluid. They also have sucking ability such as the hummingbird which has a long pointed beak. Other examples in this category include mosquitoes, aphids, bees, and hummingbirds.

4. **Bulk Feeding:** Bulk feeding is whereby the predator gets the required nutrients by eating all of the prey. Some animals may exhibit the behavior by eating the prey in small pieces by first chewing then swallowing while others swallow the prey whole. The food is then broken down into smaller particles and nutrients extracted during the digestion process. The undigested food is then removed out of the body through the process of excretion. The technique is common in macroscopic animals. Most carnivores, herbivores, and omnivores employ this mode of eating. Bulk feeding involves moving to where the target source of food is located and taking a bite. This can be done in one attack and swallow or in

repeated motions. Bulk feeding is the most efficient and effective technique on land. Major examples of animals that are adapted to bulk feeding are human beings, lions, snakes, and most bird species. While some snakes will take a simple bite, large snakes such as anacondas will swallow their prey whole.

5. **Ram Feeding:** Also known as lunge feeding, the ram feeding mode involves the hunter moving forward underwater with its mouth wide open. As the predator moves, it engulfs the prey. Such prey remains fixed in its position while the predator moves jaws past the target to capture it. The motion of the head of the predator may apply a bow wave approach hence push the prey from the jaws though this can be avoided by allowing water to pass through the jaws. To prevent the prey from escaping, adaptations such as expandable throat like in the case of some baleen whales and the snapping turtles is applied. Another adaptation that makes ram feeding successful is allowing water to flow through the gills of the predator as commonly seen in herring and sharks. The garfish and water snakes have an evolved narrow pointed snout. The *Manta alfredi* are sea creatures known to swim against the tidal current to catch prey. As it moves it keeps the mouth wide open and sieves zooplankton from the water.

6. **Suction Feeding:** Suction feeding is a common technique in marine living organisms. The prey particles in the fluid are ingested into the predators by sucking it into the mouth. The hunter expands its oral cavity or throat capacity volume causing a difference in pressure between the interior of the mouth and exterior environment. Upon opening the mouth, the pressure difference makes the water flow into the mouth of the predator carrying the target food item with it. Suction feeding involves little or no body movement towards the prey to enable capturing it easy. A creature known as the Grouper is famous for capturing the prey by sucking it into the mouth. Other examples of this derived character belong to fish spices in the Teleostei clade. Some of the fish species employ both ram and suction feeding mode depending on the convenience. Suction feeding is mainly created when the fish opens its mouth and mostly involves little or no movement at all unlike ram feeding where the predator must be in motion.

Human Digestive System

The alimentary canal and the glands associated with digestion constitute the human digestive system.

1. Alimentary Canal:
It comprises the following parts:

I. Mouth:
Human mouth consists of two parts.

(a) Vestibule:
The vestibule is a slit-like space bounded externally by lips and cheeks and internally by the gums and teeth.

(b) Oral Cavity (Buccal Cavity):
It is inner portion of the mouth which has the following parts.

(i) Palate:
The roof of the oral cavity (buccal cavity) is called palate. Anterior part of the palate is known as hard palate which bears transverse ridges, the rugae. The posterior part of the palate is smooth and is termed the soft palate. The hinder free part of the soft palate freely hangs down as a small flap, the uvula.

(ii) Tongue. The tongue is attached to the floor of the mouth by a fold called the lingual frenulum. An inverted V-shaped furrow termed the sulcus terminalis divides the upper surface of the tongue into anterior oral part and posterior pharyngeal part. The apex of the sulcus terminalis projects backward and is marked by a small median pit, named the foramen caecum.

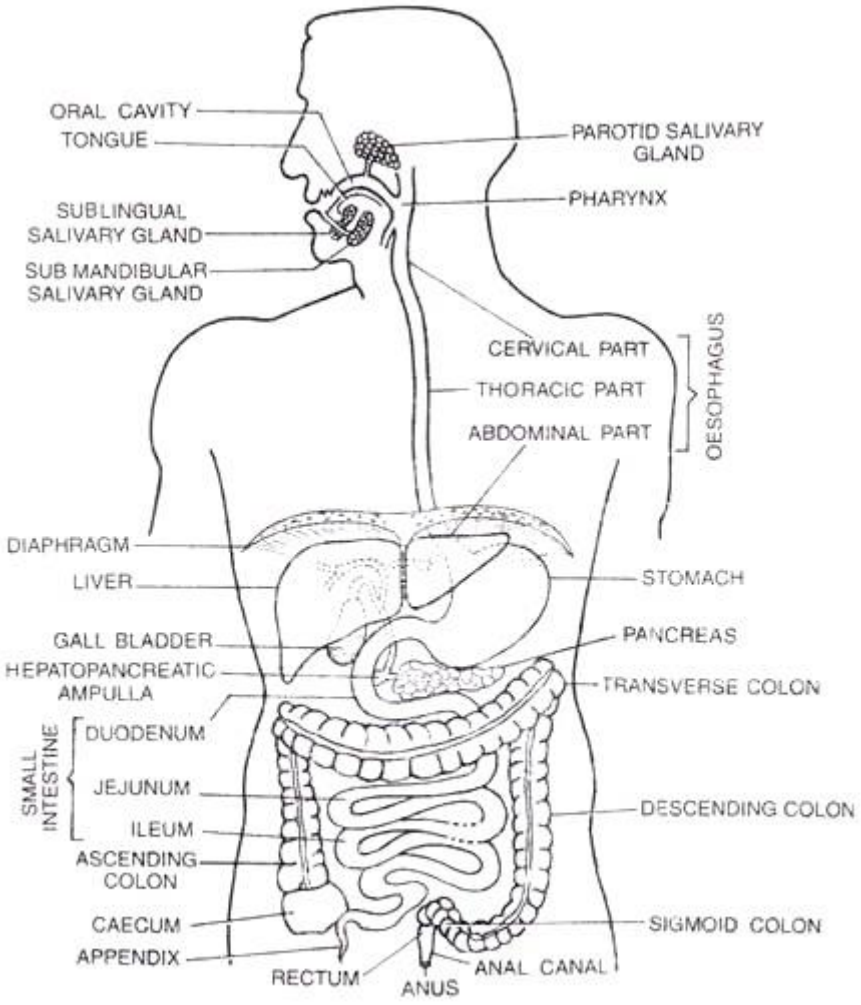


Fig. 16.1. Human digestive system.

Papillae:

The upper surface of the tongue has four types of papillae (little projections).

(a) Vallate papillae or Circumvallate papillae are usually about 8 to 12 in number. Each vallate papilla contains up to 100 taste buds. These papillae are the largest of the four types of papillae.

(b) Filiform papillae are the smallest and most numerous of the four types. They are conical. They are found mainly near the centre and most of the upper surface of the tongue. These papillae contain tactile (touch) receptors but not taste buds.

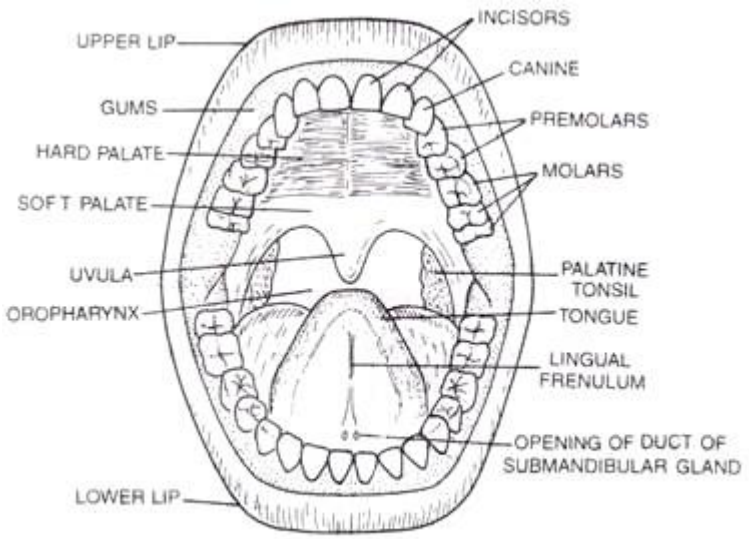


Fig. 16.2. Human oral cavity.

(c) Fungiform papillae are much less numerous than the filiform papillae. They are rounded but smaller than vallate but larger than filiform papillae. They are most numerous near the tip of the tongue. Each fungiform papilla contains about five taste buds.

(d) Foliate papillae are not developed in human tongue. These are leaf-like and are situated at the sides of the base of the tongue. On each border there are four or five vertical folds. Their taste buds degenerate in early childhood.

Human tongue has four taste areas (sweet, salt, sour and bitter). Areas of sweet and salt can overlap.

Functions of the Tongue:

The tongue acts as an accessory digestive organ.

(i) It helps in chewing the food.

(ii) It aids in swallowing the food,

(iii) It acts as a brush to clean the teeth,

(iv) It plays a role in speech,

(v) It is an organ of taste.

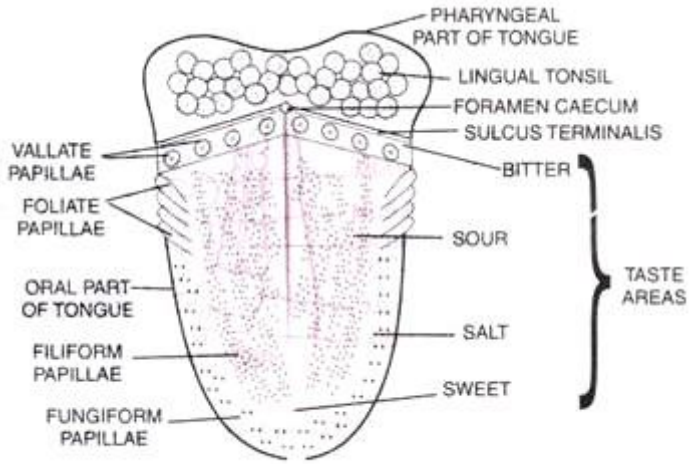


Fig. 16.3. Upper surface of human tongue.

Teeth:

(a) Characteristics:

Men have diphyodont (two sets of teeth— milk or deciduous and permanent), thecodont (teeth are embedded in the sockets of the jaw bones) and heterodont teeth (different types of teeth). There are present four kinds of teeth— incisors, canines, premolars and molars.

Incisors:

They are usually specialized for cutting.

Canines:

They lie immediately behind the incisors. They are also used for cutting the food.

Premolars and molars:

These are called cheek teeth which are broad, strong crushing teeth. Third molars in human being are called wisdom teeth. The latter are vestigial in human beings.

(b) Number:

The milk or deciduous or temporary teeth are 20 in number; 10 each in the upper jaw and in the lower jaw. The milk teeth begin to erupt when the child is about 6 months old and should all be present by the end of 24 months. The permanent teeth begin to replace the milk teeth in the 6th year of age. These teeth are 32 and usually complete by 18-25 years.

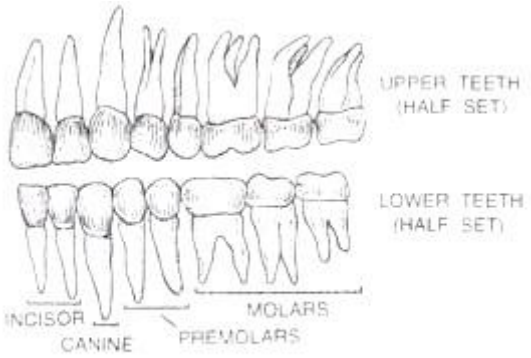


Fig. 16.4. Arrangement of human permanent teeth.

(c) Dental Formulae:

Milk teeth of man include 8 incisors, 4 canines and 8 molars (premolars are absent). Molars of milk teeth are shed off and premolars of permanent teeth take their place. The permanent teeth are 8 incisors, 4 canines, 8 premolars and 12 molars. Thus 12 teeth (8 premolars and 4 molars) are monophyodont (teeth which grow only once in life). Dental Formulae of milk teeth and permanent teeth of human are given below.

212/212 x 2 = 20

2123/2123 x 2 = 32

Milk teeth Permanent Teeth:

The dental formula gives half of the total number of teeth. This is doubled to determine the full number.

(d) Structure:
A typical tooth consists of three regions; crown— the part which projects above the gums, the neck— the part which is surrounded by gum and the root— the part which is embedded in the bone.

The incisors and canines have one root, the upper first premolars have two roots and the upper second premolars and lower premolars usually have only one root. The upper molars have three roots and the lower molars have two roots.

A human tooth consists of the following parts:
Enamel:
It is the hardest substance of the human body. It covers the dentin in the crown.

Dentin:
It has numerous fine canaliculi that pass radially from the pulp cavity towards the enamel.

Cement:
It covers the root of the tooth.

Periodontal Ligament:
It is made up of collagen fibres and covers the cement. It fixes the tooth in its socket.

Pulp Cavity:

Dentin encloses the pulp cavity that contains a mass of cells, blood vessels and nerves which constitute the pulp. Narrow extensions of the pulp cavity called root canals, run through the root of the tooth.

Apart from the connective tissue cells of the pulp and of the periodontal membrane and the cementocytes in cement, there are two main types of cells. These are dentine forming odontoblasts and enamel forming ameloblasts.

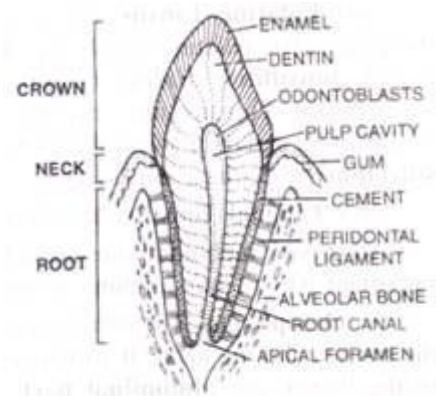


Fig. 16.5. Vertical section of human incisor.

II. Pharynx (Throat):

It is divided for descriptive purposes into three parts; the nasopharynx, oropharynx and laryngopharynx.

(i) The nasopharynx (nasal part of the pharynx) lies behind the nasal cavities, above the soft palate. The Eustachian tube (also called auditory tube) connects nasopharynx with the middle ear.

(ii) The oropharynx (oral part of the pharynx) lies behind the oral cavity (buccal cavity). The nasopharynx and oral cavity open into the oropharynx which is a common passage for both food and air.

(iii) The laryngopharynx (laryngeal part of the pharynx), is the most inferior portion of the pharynx. It leads into the oesophagus behind and into the larynx in front.

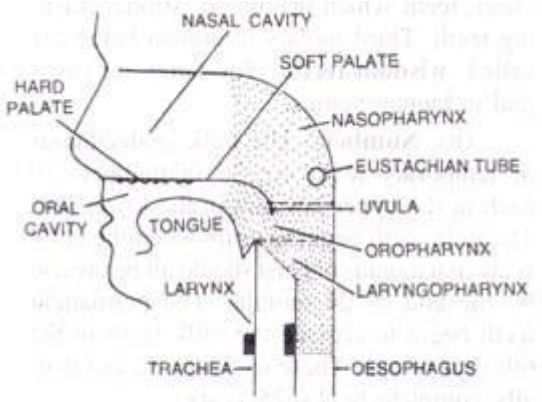


Fig. 16.6. Diagram showing three parts of pharynx.

Function:
The pharynx is a common passage for food and air.

Waldeyer's Ring:

The lymphatic tissues of the pharynx and oral cavity are arranged in a ring like manner, which are collectively called Waldeyer's ring (- Waldeyer's lymphatic ring).

The ring mainly consists of the following:
(i) Pharyngeal Tonsil is attached to pharynx. In children pharyngeal tonsil may become enlarged and is then referred to as the adenoids. The resulting swelling may be a cause of obstruction to normal breathing.

(ii) Tubal Tonsils are situated around the Eustachian tube.

(iii) Palatine Tonsils are attached to the palate. The palatine tonsils are often infected (tonsillitis) leading to sore throat. Such enlarged tonsils may become a focus of infection and their surgical removal (tonsillectomy) becomes necessary.

(iv) Lingual Tonsil is attached to pharyngeal part of the tongue.

All these lymphoid tissues are active in production of immunoglobin. A which forms an important part of our immune system.

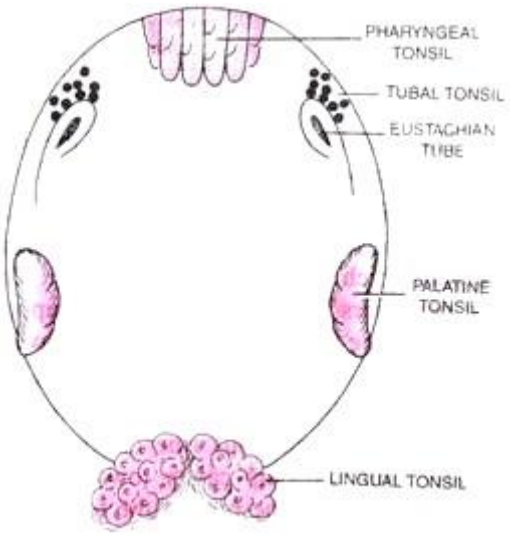


Fig. 16.7. Waldeyer's Ring.

III. Oesophagus:

The human oesophagus or food pipe is about 25 cm long. It lies behind the trachea and the heart. It comprises three parts: cervical part in the neck, thoracic part in the thorax and abdominal part in the abdomen. The oesophagus passes through the diaphragm and opens into the stomach.

Function:

The oesophagus transfers food from the pharynx to the stomach.

IV. Stomach (= Gaster):

It is the widest organ of the alimentary canal. The stomach is J-shaped organ. The lesser curvature lies on the posterior surface of the stomach. The greater curvature is on the anterior surface of the stomach.

The fold of peritoneum which attaches the stomach to the posterior abdominal wall extends beyond the greater curvature. This is called the greater omentum which stores fat. The stomach has four parts: cardiac part, fundus, body and pyloric part.

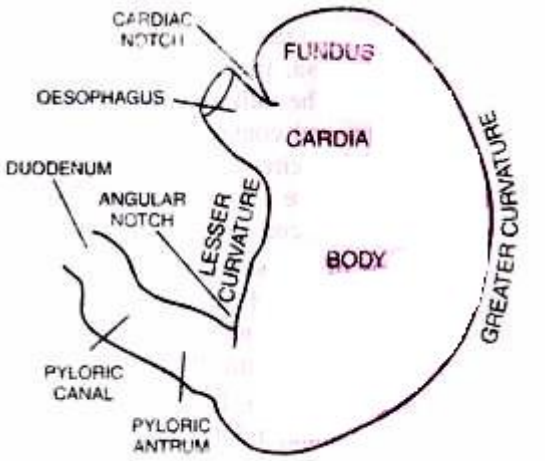


Fig. 16.8. Human Stomach.

(i) Cardiac Part (= cardia):
It is so called because it is present near the heart. The gastro esophageal sphincter (= cardiac sphincter) lies in the opening between oesophagus and stomach. It is not a true valve. It is a functional sphincter.

(ii) Fundus:
It is commonly filled with air or gas.

(iii) Body:
It is the main part of the stomach.

(iv) Pyloric Part (Pylorus):
It is the posterior part of the stomach.

The pyloric part is divided into the pyloric antrum and the pyloric canal. The latter opens into the duodenum. The pyloric sphincter guards the opening between the stomach and the duodenum and periodically permits partially digested food to leave the stomach and enter the duodenum.

Functions of the Stomach:

It stores food for some time. It churns and breaks up food and mixes the pieces with gastric juice. Partial digestion of food (proteins and fats) takes place here. It produces Castle's intrinsic factor (a glycoprotein) which is necessary for the absorption of vitamin B_{12} to be absorbed in the intestine.
It secretes pro-enzymes— pepsinogen and pro-rennin and enzymes gastric lipase and gastric amylase. It also secretes gastrin (hormone). Alcohol, aspirin, some lipid-soluble drags, moderate amounts of sugar and water are absorbed by the stomach wall.

V. Small Intestine:

It is so named because it has small diameter. Length is correlated with the height of the individual but not with weight. It is the longest part of the alimentary canal. It is about 6.25 metres long. It comprises three parts; duodenum, jejunum and ileum.

(i) Duodenum:

It is so called because it is about as long as the breadth of 12 fingers. It is about 25 cm long and is the shortest, widest part of the small intestine. It is somewhat C-shaped. The hepatopancreatic ampulla (ampulla of Vater) opens into the duodenum. This ampulla receives both bile duct from the liver and main pancreatic duct from the pancreas. Iron is mainly absorbed in the duodenum.

(ii) Jejunum:

It has a diameter of about 4 cm. Its wall is thick. It is redder and more vascular. It is the middle part of the small intestine and is about 2.5 metres long.

(iii) Ileum:

It has a diameter of 3.5 cm. Its wall is thinner than that of the jejunum. It is the longest part of small intestine and is about 3.5 metres long. Both the jejunum and ileum are greatly coiled. They are suspended by mesentery.

Small nodules of lymphatic tissue can be seen along the entire length of the small intestine. In some places, particularly along the ileum, these nodules are clustered together in groups called Peyer's patches or lymph nodules.

Peyer's patches are a distinguishing characteristic of the ileum, which produce lymphocytes (type of WBCs). Finger-like projections of the mucosa, the villi are present in the small intestine. Villi are absent over the Peyer's patches.

The villi increase the surface of the small intestine. Each villus is covered with epithelium and contains a lymph capillary (lacteal) and blood capillaries. The entire small intestine has circular folds of the mucous membrane, the plicae circulares ('Valves' of Kerkring). These folds are more prominent in the jejunum. They further increase the absorptive surface considerably.

Functions of the small intestine:

The small intestine completes digestion of proteins, carbohydrates, fats and nucleic acids. It absorbs nutrients into the blood and lymph. It secretes certain hormones such as cholecystokinin, secretin, enterogastrone, duocrinin, enterocrinin and villikinin. It also secretes digestive enzymes.

VI. Large Intestine:

Its diameter is larger than that of the small intestine. Hence it is so named. It is about 1.5 metres long and is divisible into three parts; caecum, colon and rectum.

(i) Caecum and vermiform appendix:

The caecum is a pouch-like structure which is about 6 centimetres. The vermiform appendix (commonly called the appendix) is an outgrowth of the caecum.

It is a slightly coiled blind tube of about 8 centimetres long. Its wall contains prominent lymphoid tissue. Appendix is thought to be vestigial. The inflammation of vermiform appendix is called appendicitis. The caecum and appendix are well developed in herbivorous mammals like rabbits.

(ii) Colon:

The caecum leads into the colon, which is divided into four regions; the ascending, transverse, descending and sigmoid colon (pelvic colon is its former name). Ascending colon is the shortest part of the colon. The colon has three longitudinal bands called taeniae coli and small pouches called haustra (sing, haustrum).

(iii) Rectum:

The sigmoid colon opens into the rectum. The rectum comprises the last 20 centimetres of the digestive tract and terminates in the 2-centimetre long anal canal. The opening of the anal canal is called anus.

The anus has an internal anal sphincter composed of smooth muscle fibres and an external anal sphincter comprised of striped (voluntary) muscle fibres. Structures formed due to enlargements of veins of anal columns in anal canal as well as anus are called haemorrhoids or piles.

Functions of the large intestine:

The chief functions of the large intestine are the absorption of water and the elimination of solid wastes. However, moderate quantities of vitamin K and vitamin B complex are manufactured by bacteria in the large intestine.

Histology of Human Gut (Alimentary Canal):

The wall of alimentary canal consists of four basic layers.

From the outer surface inward to the lumen (cavity) the layers are as follows:

1. Visceral peritoneum (= Serosa):

It is made up of squamous epithelium and areolar connective tissue. It is continuous with the mesentery. Since the oesophagus lies outside the coelom, its outer wall is not covered by peritoneum (serosa) but by an irregular coat of dense elastic fibrous connective tissue called adventitia external (= external adventitia).

2. Muscularis (Muscular coat):

It is composed of outer longitudinal and inner circular muscle fibres. In the stomach an additional layer of oblique muscle layer is found inner to the circular muscle fibres.

These muscle fibres are un-striped (smooth). In between the longitudinal and circular muscle fibres there is a network of nerve cells and parasympathetic nerve fibres, called the Auerbach's plexus (= myenteric plexus). The Auerback s plexus controls peristalsis.

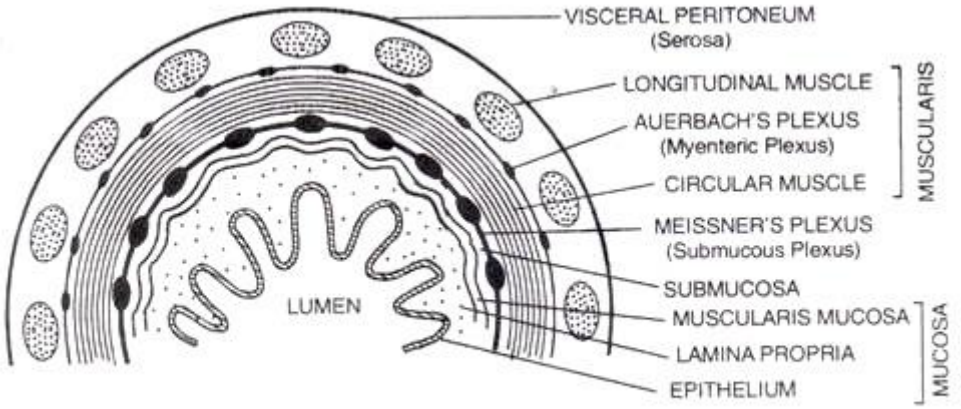


Fig. 16.9. Diagrammatic representation of transverse section of part of gut (alimentary canal).

3. Sub-mucosa:

It consists of loose connective tissue richly supplied with blood and lymphatic vessels and in some areas with glands. Another network of nerve cells and sympathetic nerve fibres, called Meissner's plexus (= sub-mucosal plexus) is present between the muscular coat and the mucosa. This plexus controls the secretion of intestinal juice.

4. Mucosa (= Mucous membrane):

It is the innermost layer lining the lumen of the alimentary canal. It is so named because it secretes mucus to lubricate the inner lining of the gut. This layer forms irregular folds (rugae) in the stomach.

Mucosa is composed of three layers:
(i) The muscular is mucosa consists of outer longitudinal and inner circular muscle fibres, both are un-striped.

(ii) The lamina propria consists of loose connective tissue, blood vessels, glands and some lymphoid tissue.

(iii) The epithelium forms gastric glands in stomach, and villi and intestinal glands in small intestine.

In upper one third of the oesophagus both Auerbach and Meissner's plexuses are absent.

Digestive Glands

I. Salivary Glands:

Salivary glands discharge their secretion into the oral cavity. In man, the salivary glands are three pairs— parotid, sublingual and submandibulor glands,

(i) Parotid glands. These are the largest salivary glands which are situated near the ears. Their ducts open into the oral cavity near the upper second molars. The duct of parotid gland is called Stenson's duct,

(ii) Sublingual glands. These are smallest salivary glands which are located beneath the tongue and their ducts called sublingual ducts or ducts of Rivinus which open into the floor of the oral cavity,

(iii) Submandibular (also called sub maxillary) glands.

These are medium sized salivary glands which are located at the angles of the lower jaw. Their ducts open into the oral cavity near the lower central incisors.

The duct of submandibular gland is called Wharton's duct. The parotid salivary glands secrete much of salivary amylase or a-amylase (= ptyalin). Sub-lingual and sub-mandibular salivary glands secrete salivary amylase and mucus. Salivary amylase is absent in herbivores.

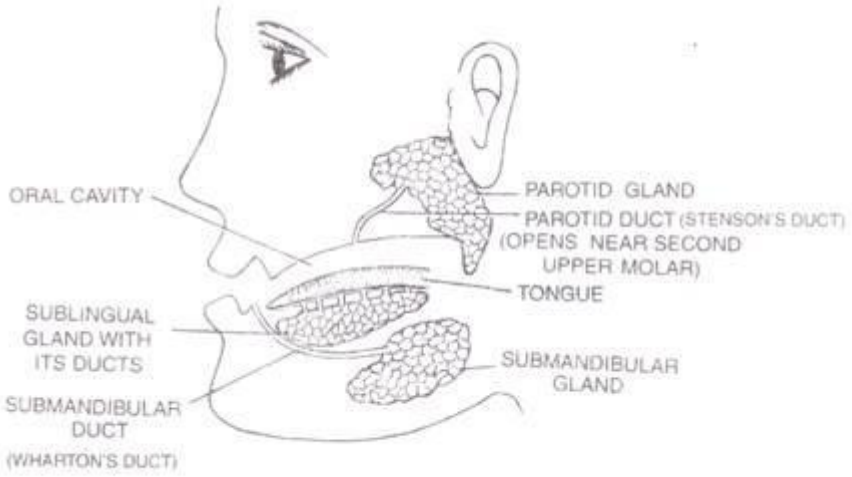


Fig. 16.10. Human Salivary Glands

The disease mumps is a viral infection that may involve one or both parotid salivary glands. The fluids secreted by the salivary glands constitute saliva. Saliva is slightly acidic (pH 6.8). About 1,000-1500 ml of saliva is secreted per day.

Saliva is a mixture of water and electrolytes (Na^+, K^+, CI^-, $HC0_3^-$), derived from blood plasma, mucus and serous fluids (watery constituent of saliva), and salivary amylase or ptyalin (enzyme) and lysozyme (antibacterial agent). Ions of thyocyanate are also present in the saliva.

II. Gastric Glands:

These are numerous microscopic, tubular glands formed by the epithelium of the stomach. Gastric glands have three major types of cells.

(i) Chief cells or Peptic cells (= Zymogenic cells) are usually basal in location and secrete gastric digestive enzymes as pro-enzymes or zymogens; pepsinogen and pro-rennin.

The chief cells also produce small amount of gastric amylase and gastric lipase. Gastric amylase action is inhibited by the highly acid condition. Gastric lipase contributes little to digestion of fat. Pro-rennin is secreted in young mammals. It is not secreted in adult mammals.

(ii) Oxyntic cells (= Parietal cells) are large and are most numerous on the side walls of the gastric glands. They are called oxyntic cells because they stain strongly with eosin. They are called parietal cells as they lie against the basement membrane. They secrete hydrochloric acid and Castle intrinsic factor.

(iii) Mucous cells (= Goblet cells) are present throughout the epithelium and secrete mucus.

The secretions of these cells form gastric juice with pH 1.5-2.5 (very acidic). Infant's gastric juice pH is 5.0. About 2,000-3,000 ml of gastric juice is secreted per day. The gastric juice contains two pro-enzymes— pepsinogen (pro-pepsin) and pro-rennin, and enzymes gastric lipase and gastric amylase, and mucus and hydrochloric acid.

The epithelium of gastric glands also has the following two types of cells:

(i) Endocrine cells are usually present in the basal parts of the gastric glands. These are argentaffin cells and Gastrin cells (= G-cells). Argentaffin cells produce serotonin (its precursor is 5-hydroxytryptamine, 5-HT), somatostatin and histamine. Gastrin Cells (= G-cells) are present in the pyloric region and secrete and store the hormone gastrin.

Serotonin is a vasoconstrictor and stimulates the smooth muscles. Somatostatin suppresses the release of hormones from the digestive tract. Histamine dilates the walls of blood vessels. Gastrin stimulates the gastric glands to release the gastric juice.

(ii) Stem cells are undifferentiated cells that are also present in the epithelium of the gastric glands. They multiply and replace other cells. They increase in number when the gastric epithelium is damaged (e.g., when there is a gastric ulcer) and play an important role in healing.

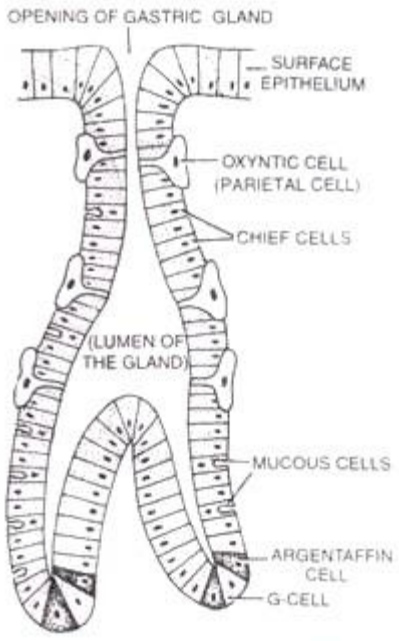


Fig. 16.11. Diagram showing gastric gland.

III. Liver (= Hepar):

It is the largest gland of the body. The liver lies in the upper right side of the abdominal cavity just below the diaphragm. It is heavier in males than females. In males it generally weighs 1.4-1.8 Kg and in females 1.2-1.5 Kg.

The liver is divided into two main lobes— right and left lobes separated by the falciform ligament. The latter is a membrane that is continuous with the peritoneum. The right lobe of the liver is further differentiated into right lobe proper, a quadrate lobe and a caudate lobe on the posterior surface.

Internally, the structural and functional units of liver are the hepatic lobules containing hepatic cells arranged in the form of cords. Each lobule is covered by a thin connective tissue sheath called the Glisson's capsule. Glisson's capsule is the characteristic feature of mammalian liver. The mammalian liver also contains Kupffer cells that are phagocytic cells and eat worn out WBCs, RBCs and bacteria.

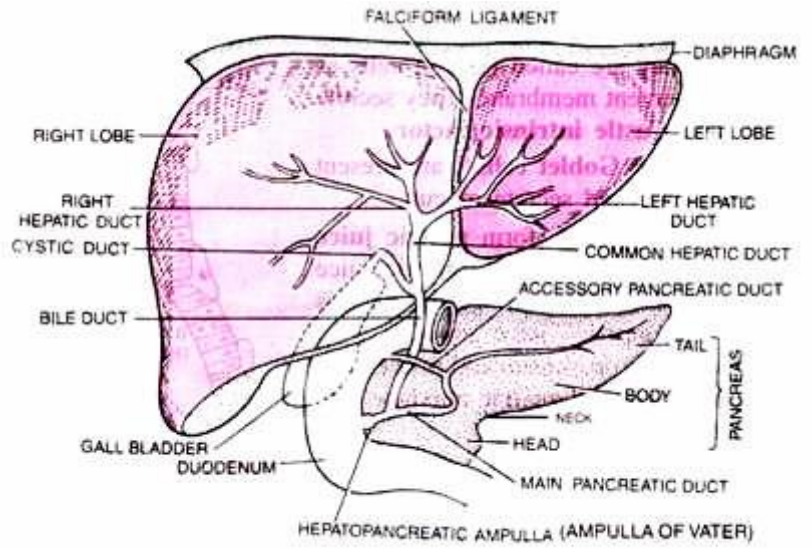


Fig. 16. 12. Liver and pancreas with associated structures.

Fat storage cells are also present. The plates of liver cells are separated from the endothelial lining of the sinusoid by a narrow perisinusoidal space of Disse. Some fat cells may also be seen in the space of Disse. Blood vessels and bile ductules present in the portal canals are surrounded by a narrow space of Mall.

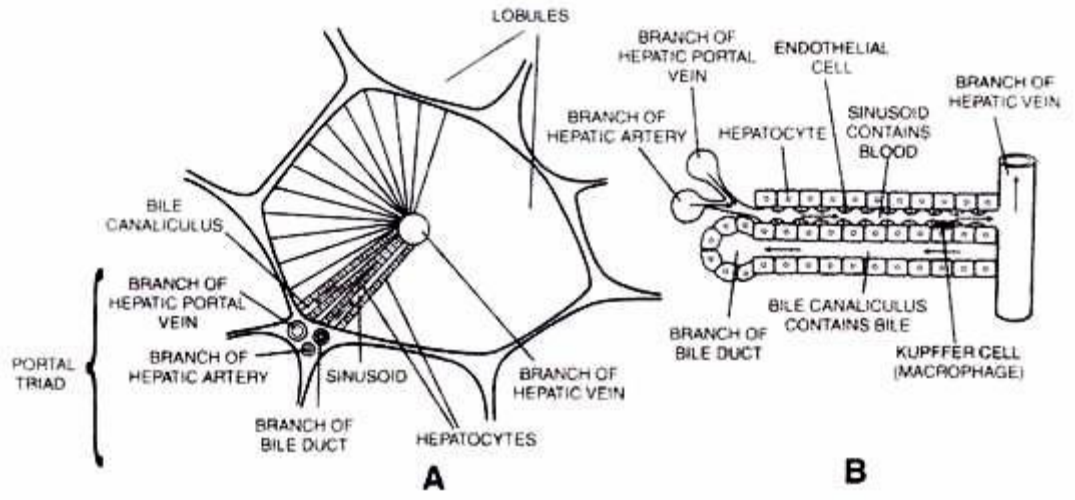


Fig. 16.13. A, Diagram of a Transverse section of a liver lobule (Arrows indicate flow of blood in sinusoid and the flow of bile in canalculi). B, A simplified diagram of part of a liver lobule.

Bile is secreted by the liver cells (hepatocytes). Bile enters bile canaliculi or bile capillaries (a net work of tubular spaces between the liver cells). The bile canaliculi empty into small Hering's canals walled by cuboidal epithelium. These canals pour bile into interlobular bile duct (=bile ductule) walled by columnar epithelium.

Gall Bladder:

A pear shaped sac like structure is attached to the posterior surface of the liver by connective tissue. It stores bile secreted by the liver. Rat and horse do not have gall bladder.

Ducts:

The right and left hepatic ducts join to form the common hepatic duct. The latter joins the cystic duct which arises from the gall bladder. The cystic duct and common hepatic duct join to form bile duct which passes downwards posteriorly to join the main pancreatic duct to form the hepatopancreatic ampulla (= ampulla of Vater).

The ampulla opens into the duodenum. The opening is guarded by the sphincter of Oddi. The sphincter of Boyden surrounds the opening of the bile duct before it is joined with the pancreatic duct.

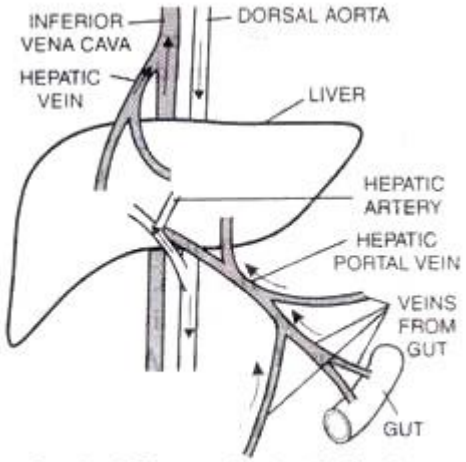


Fig. 16.15. Diagram showing the blood supply to liver from two sources.

Blood Supply:
Blood enters the liver from two sources. From the hepatic artery it gets oxygenated blood and from the hepatic portal vein it receives deoxygenated blood. Blood in the hepatic artery comes from the aorta. Blood in the hepatic portal vein comes directly from the intestine containing newly absorbed nutrients. The hepatic portal vein also brings blood from the spleen to the liver. Liver has high power of regeneration.

Functions of Bile:
Bile is a watery greenish fluid mixture containing bile pigments, bile salts, cholesterol and phospholipids.

Bile serves the following functions:
(i) Neutralization of HCI:
Its sodium bicarbonate neutralizes HC1 of chyme (semi-fluid food that comes from the stomach).

(ii) Emulsification:
Sodium glycocholate and sodium taurocholate break the large fat droplets into the smaller ones. This process is called emulsification.

(iii) Absorption of fat and fat-soluble vitamins:
Its salts help in the absorption of fat (fatty acids and glycerol) and fat-soluble vitamins (A, D, E and K) in the small intestine.

(iv) Excretion:
Bile pigments (bilirubin and biliverdin) are excretory products.

(v) Prevention of Decomposition:
Bile is alkaline hence it prevents the decomposition of food by preventing the growth of bacteria on it.

(vi) Stimulation of Peristalsis:
Bile increases peristalsis of the intestine.

(vii) Activation of Lipase:
Bile contains no enzyme but activates the enzyme lipase.

Obstruction of the hepatic or bile duct by gall stones or due to other causes is common. Jaundice occurring as a result of such obstruction is called obstructive jaundice. In this disease the bile is absorbed into the blood instead of going to the duodenum and cause yellowing of eyes and skin.

IV. Pancreas:

The pancreas is soft, lobulated, greyish- pink gland which weighs about 60 grams. It is about 2.5 centimetres wide and 12 to 15 centimetres long, located posterior to the stomach in the abdominal cavity.

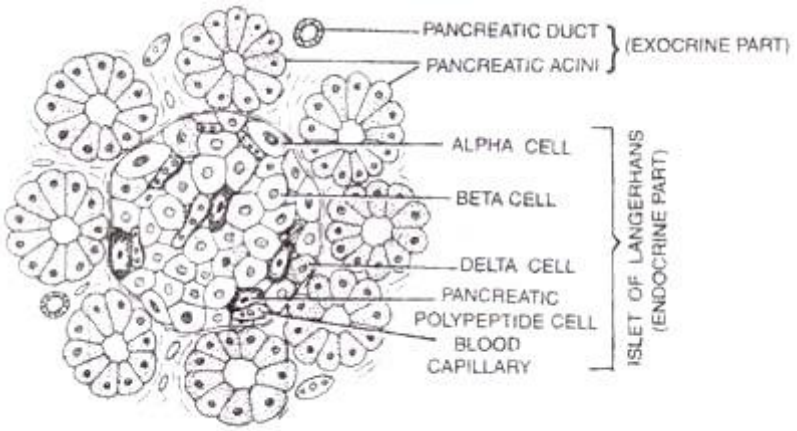


Fig. 16.16. Section of Pancreas.

External Structure of Pancreas:

The Pancreas comprises the head, neck, body and tail. The head lies in the curve of the duodenum, the neck follows the head, the body behind the stomach and the tail reaches the spleen lying in front of the left kidney.

The main pancreatic duct (= duct of Wirsung) is formed from smaller ducts within the pancreas. The main pancreatic duct opens into the hepatopancreatic ampulla (= ampulla of

Yater). An accessory pancreatic duct (= duct of Santorini) is also present in the pancreas and opens directly into the duodenum.

Internal Structure of Pancreas:

It consists of two parts: exocrine part and endocrine part.

(i) Exocrine part:

The exocrine part of the pancreas consists of rounded lobules (acini) that secrete an alkaline pancreatic juice with pH 8.4. About 500-800 ml of pancreatic juice is secreted per day. The pancreatic juice is carried by the main pancreatic duct into the duodenum through the hepatopancreatic ampulla.

The accessory pancreatic duct directly pours the pancreatic juice into the duodenum. The pancreatic juice contains sodium bicarbonate, three pro-enzymes; trypsinogen, chymotrypsinogen and procarboxypeptidase and some enzymes such as elastase, pancreatic a-amylase, DNase, RNase and pancreatic lipase. The pancreatic juice helps in the digestion of starch, proteins, fats and nucleic acids.

(ii) Endocrine part:

The endocrine part of the pancreas consists of groups of islets of Langerhans. The human pancreas has about one million islets. They are most numerous in the tail of the pancreas. Each islet of Langerhans consists of the following types of cells which secrete hormones to be passed into the circulating blood.

(a) Alpha cells (= α-cells):

These cells are more numerous towards the periphery of the islet and constitute 15% of the islet of Langerhans. They produce glucagon hormone which converts glycogen into glucose in the liver. Thus glucagon is diabetogenic hormone.

(b) Beta cells (= β-cells):

These cells are more numerous towards the middle of the islet and constitute 65% of the islet of Langerhans. They produce insulin hormone which converts glucose into glycogen in the liver and muscles. Deficiency of insulin causes diabetes mellitus.

(c) Delta cells (= δ-cells):

These cells are also found towards the periphery of the islet of Langerhans and constitute 5% of the islet of Langerhans. They secrete somatostatin (SS) hormone which inhibits the secretion of glucagon by alpha cells and secretion of insulin by beta cells. This hormone also slows absorption of nutrients from the gasrointestinal tract.

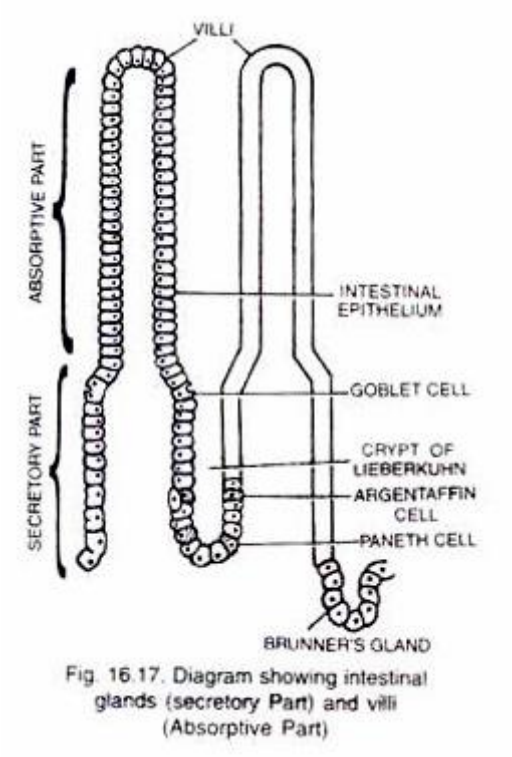


Fig. 16.17. Diagram showing intestinal glands (secretory Part) and villi (Absorptive Part)

Somatostatin secreted by argentaffin cells of gastric and intestinal glands suppresses the release of hormones from the digestive tract. Somatostatin is also secreted by the hypothalamus of the brain where it inhibits the release of growth hormone (somatotropin) by the anterior lobe of pituitary gland. That is why it is also called growth inhibitory hormone.

(d) Pancreatic polypeptide cells (= PP cells or F-cells):

Apart from the three main types of cells described above, the PP cells are also present in the pancreas, which constitute 15% of the Islet of Langerhans. These cells secrete pancreatic polypeptide (PP) which inhibits the release of pancreatic juice. Thus the pancreas performs two main functions i.e., secretion of pancreatic juice which contains digestive enzymes and production of hormones.

V. Intestinal Glands :

These are formed by the surface epithelium of the small intestine. These are of two types: crypts of Lieberkuhn and Brunner's glands.

(i) The crypts of Lieberkuhn are simple, tubular structures which occur throughout the small intestine between the villi. They secrete digestive enzymes and mucus. The mucus is secreted by the goblet cells (= mucous cells) whereas water and electrolytes are secreted by enterocytes present on the intestinal crypts. These crypts have at the base paneth cells and argentaffin cells.

(a) Paneth cells are found particularly in the duodenum. These cells are present in the bottom of crypts of Lieberkuhn. These cells are rich in zinc and contain acidophilic granules. The function of these cells is not certain but there is evidence that they secrete lysozyme (antibacterial substance). Paneth cells are also capable of phagocytosis.

(b) Argentaffin cells synthesize secretin hormone and 5-hydroxytryptamine (5-HT).

(ii) The Brunner's glands are found only in the duodenum and are located in the submucosa. They secrete a little enzyme and mucus. The mucus protects the duodenal wall from getting digested. Digestion of most of nutrients takes place in the duodenum under the action of various enzymes. The Brunner's glands open into the crypts of Lieberkuhn.

The secretion of intestinal glands is called intestinal juice or succus entericus with pH 7.8. About 2,000-3,000 ml of intestinal juice is secreted per day. The intestinal juice contains many enzymes— maltase, isomaltase, sucrase, lactase, α- dextrinase, enterokinase, aminopeptidases, dipeptidases, nucleotidases, nucleosidases and intestinal lipase.

In addition to the glands mentioned above the entire alimentary canal has mucous glands that produce mucus. The mucus lubricates the digestive tract and food. Human digestive system has many accessory organs. Tongue, salivary glands, liver, gall bladder and pancreas are some important human accessory digestive organs.

Swallowing or Deglutition :

The food is tasted in the oral cavity and mixed with saliva. Tongue manipulates food during chewing and mixing with saliva. This collection of food, the bolus (mass of food) is then pushed inward through the pharynx into the oesophagus.

This process is called swallowing or deglutition. Swallowing involves coordinated activity of tongue, soft palate, pharynx and oesophagus.

Swallowing is conveniently divided into three stages:

(i) The Voluntary stage:

The tongue blocks the mouth. The bolus is forced to move from the oral cavity into the pharynx (oropharynx). This represents the voluntary stage of swallowing.

(ii) The Pharyngeal stage:

With the passage of the bolus into the pharynx, the involuntary pharyngeal stage of swallowing begins. The palate closes off the nose and the epiglottis seals off the glottis of larynx. Thus breathing is temporarily interrupted. The bolus is passed from the pharynx into the oesophagus.

(iii) The Oesophageal stage:

This also represents the involuntary stage of swallowing. The bolus passes through the laryngopharynx and enters the oesophagus in 1 to 2 seconds. The respiratory passage then reopens and breathing resumes. Swallowing is controlled by a swallowing centre located in the medulla oblongata and lower pons varolii of the brain.

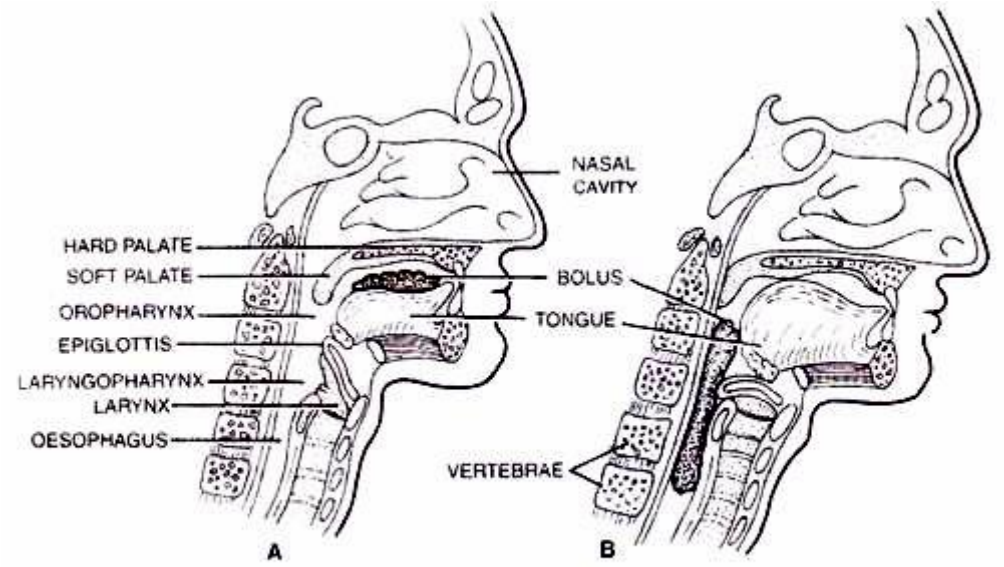


Fig. 16.18. Swallowing. A. Position of structures prior to swallowing. B. During swallowing, the tongue rises against the palate, the nose is closed off, the epiglottis seals off the larynx and the bolus is passed into the oesophagus.

Peristalsis:

During the oesophageal phase of swallowing, food is pushed through the oesophagus by involuntary muscular movements called peristalsis.

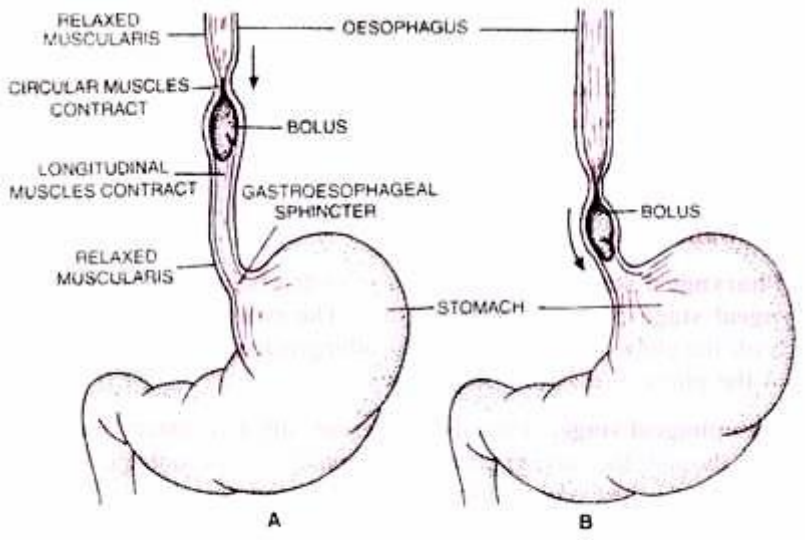


Fig. 16.19. Peristalsis in oesophagus.

Peristalsis is produced by involuntary contraction of circular muscles in the oesophagus lying just above and around the top of the bolus and simultaneous contraction of the longitudinal muscles lying around the bottom of and just below the bolus.

Contraction of the longitudinal muscles shortens the lower part of the oesophagus, pushing its walls outward so that it can receive the bolus. After this circular muscles of the oesophagus relax. The contractions are repeated in a wave that moves down the oesophagus, pushing the food towards the stomach. There is least peristaltic movement in the rectum of human being.

Probable Questions:

1. Discuss different types of heterotrophic nutrition.
2. What is autotrophic nutrition?
3. Discuss different types of feeding strategies in animals with examples.
4. Write the structure of teeth of human.
5. What is dental formula? How it is calculated?
6. What is the function of the tongue?
7. Discuss Waldeyer's Ring with diagram.
8. Discuss different parts of stomach with diagram.
9. Discuss different parts of small intestine with diagram.
10. Discuss different parts of large intestine with diagram.
11. Discuss structure and function of salivary gland.
12. Discuss structure and function of liver.
13. Discuss structure and function of gall bladder
14. Discuss structure and function of Pancreas.
15. Discuss different stage of swallowing?

SuggestedReadings/References-

1. Animalphysiology-MohanP.Arora.
2. TextbookofmedicalphysiologyArthurC.Guyton,JohnE.Hall.
3. Ganong'sreviewofmedicalphysiology.

UNIT-VIII

Physiology of gastrointestinal system (mammals) including neural and hormonal regulatory mechanisms

Objective: In this unit we will discuss Physiology of gastrointestinal system (mammals) including neural and hormonal regulatory mechanisms

Physiology of digestion

Digestion:

Digestion is the process of gradual break down of foods that we eat in a soluble form suitable for absorption. For example, meat, even when cooked, is chemically too complex to be absorbed from the alimentary canal.so, it first digested before absorption.

Digestion releases its constituents: glucose,amino acids, mineral salts, fat and vitamins, which are ready for absorption.

The activities of the digestive system can be grouped under five main headings.

1. Ingestion: This is the taking of food into the alimentary tract, i.e. eating and drinking.

2. Propulsion: This mixes and moves the contents along the alimentary tract.

3.Digestion.: This consists of:
• **mechanical digestion:** breakdown of food by, e.g. mastication (chewing)
• **chemical** digestion: breakdown of food into small molecules by enzymes produced by digestive glands.

4. Absorption: This is the process by which digested food substances pass through the walls of alimentary canal into the blood and lymph capillaries to use by body cells.

5. Elimination: undigested and unabsorbed substances are excreted from the alimentary canal as faeces by the process of defaecation.

Mechanism of digestion

1. Mechanical digestion

i. Mastigation:

The teeth are admirably designed for chewing. The anterior teeth (incisors) provide a strong cutting action, and the posterior teeth (molars) provide a grinding action.

Chewing is important for digestion of all foods, but it is especially important for mostfruitsand raw vegetables because they have indigestible cellulose membranes around theirnutrient portions that must be broken before the food can be digested.

Furthermore, chewing aids the digestion of food for another simple reason: Digestive enzymes act only on the surfaces of food particles; therefore, the rate of digestion is dependent on the total surface area exposed to the digestive secretions

.

In addition, grinding the food to a very fine particulate consistency prevents excoriation of the gastrointestinal tract and increases the ease with which food is emptied from the stomach into the small intestine, then into all succeeding segments of the gut.

ii. Swallowing (deglutition):

Swallowing is a complicated mechanism, principally because the pharynx serves respiration and swallowing both.The pharynx is converted for only a few seconds at a time into a tract for swallowing of food.Tongue helps in mixing of saliva with the food.Saliva moistens and lubricates the food, which changes into semisolid form called bolus. The bolus is then swallowed through Oesophagus to the stomach.Peristalsis movement of alimentary canal also helps in swallowing.

iii. Churning in stomach:

The wall of stomach undergoes periodic movement as well as contraction producing churning movement called peristalsis, which results in breakdown of complex food into simpler form.

The bolus after mixing with gastric juice, turn into fine soluble form known as chime.

2. Chemical digestion

It involves the breaking of covalent chemical bonds in organic molecules by digestiveenzymes.

Carbohydrates are broken down into monosaccharides, proteins are broken down into amino acids, and fats are broken down into fatty acids and glycerol.

i. Digestion of Carbohydrates

Ingested carbohydrates consist primarily of polysaccharides, such as starches (rice, bread), disaccharides, such as sucrose (table sugar) and lactose (milk sugar); and monosaccharides, such as glucose and fructose (found in many fruits).

During the process ofdigestion, polysaccharides are broken down into smaller chains and finally into disaccharides and monosaccharides. Disaccharides are broken down into monosaccharides.

a) digestion of carbohydrates in mouth:

Carbohydrate digestion begins in the oral cavity with the partial digestion of starchesbysalivary amylase.

About 30 percent of starch is hydrolysed here by this enzyme amylase (optimum pH 6.8) into a disaccharide – maltose.

Lysozyme present in saliva acts as an antibacterialagent that prevents infections.

b) digestion of carbohydrates in stomach and intestine:

A minor amount of digestion occurs in the stomach through the action of gastric amylaseand gelatinase.Carbohydrate digestion is continued in the intestine by pancreatic amylase.A series of disaccharidases enzymes that are released by intestinal epithelium digest disaccharides into monosaccharides.

ii. Digestion of Proteins

Proteins are taken into the body from a number of dietary sources.

Pepsin secreted by the stomach catalyzes the cleavage of covalent bonds in proteins to produce smaller polypeptide chains.

a) digestion of protein in stomach and intestine:

Gastric pepsin digests as much as 10%–20% of the total ingested protein.

The mucosa of stomach has gastric glands.

Gastric glands have three major types of cells namely –
(i) mucus cells: which secrete mucus;

(ii) peptic or chief cells; which secrete the proenzyme pepsinogen; and

(iii) parietal or oxyntic cells; which secrete HCl and intrinsic factor (factor essential forabsorption of vitamin B12).

The stomach stores the food for 4-5 hours.The food mixes thoroughly with the acidic gastric juice of the stomach by the churning movements of its muscular wall and is called the chyme.

The proenzyme pepsinogen, on exposure to hydrochloric acid gets converted into pepsin.Pepsin then converts proteins into proteoses and peptones (peptides).

The mucus and bicarbonates present in the gastric juice play an important role in lubrication and protection of the mucosal epithelium from excoriation by the highly concentrated hydrochloric acid.HCl provides the acidic pH (pH 1.8) optimal for pepsins.Rennin is a proteolytic enzyme found in gastric juice of infants which helps in the digestion of milk proteins.

b) digestion of protein in intestine:

The bile, pancreatic juice and the intestinal juice are the secretions released into the small intestine.Pancreatic juice and bile are released through the hepato-pancreatic duct.

The pancreatic juice contains inactive enzymes – trypsinogen, chymotrypsinogen, procarboxypeptidases.Trypsinogen is activated by an enzyme, enterokinase, secreted by the intestinal mucosa into active trypsin, which in turn activates the other enzymes in the pancreatic juice.Pancreatic proteinases (all secreted in their inactiveforms) digest peptides into amino acids.

Trypsinogen is activated by enterokinase (secreted by duodenum) into trypsin, which in turn activates the other 3 enzymes -- chymo-trypsinogen becomes chymotrypisn, proaminopeptidase becomes aminopeptidase, and procarboxypeptidase becomes carboxypeptidase.

iii. Digestion of Lipids

Lipids are molecules that are insoluble or only slightly soluble in water.Lipids include triglycerides, phospholipids, cholesterol, steroids, and fat-soluble vitamins.The first step in lipid digestion is emulsification, which is the transformation of large lipid droplets into much smaller droplets.The emulsification process increases the surface area of the lipid exposed to the digestive enzymes by decreasing the droplet size.

Emulsification is accomplished by bile salts secreted by the liver and stored in the gallbladder.Lipase digests lipid molecules .The vast majority of lipase is secreted by the pancreas. A minor amount of lingual lipase is secreted in the oral cavity, is swallowed with the food, and digests a small amount (<10%) of lipid in the stomach.

The stomach also produces very small amounts of gastric lipase.The primary products of lipase digestion are free fatty acids and glycerol and few cholesterol and phospholipids.

3. Absorption

Absorption is the process by which the end products of digestion pass through the intestinalmucosa into the blood or lymph.It is carried out by passive, active or facilitated transport mechanisms.

Water moves by osmosis; small fat soluble substances, e.g. fatty acids and glycerol, are able to diffuse through cell membranes; while others are generally transported inside the villi by other mechanisms.

i. Passive transport:

Small amounts of monosaccharides like glucose, amino acids and some electrolytes like chloride ions are generally absorbed by simple diffusion.The passage of these substances into the blood depends upon the concentration gradients.

ii. Active transport:

Active transport occurs against the concentration gradient and hence requires energy.Various nutrients like amino acids, monosaccharides like glucose, electrolytes like Na+ are absorbed into the blood by this mechanism.Some substances like glucose and amino acids are absorbed with the help of carrier proteins. This mechanism is called the facilitated transport.Fatty acids and glycerol being insoluble, cannot be absorbed into the blood. They are first incorporated into small droplets called micelles which move into the intestinal mucosa. They are re-formed into very small protein coated fat globules called the chylomicronswhich are transported into the lymph vessels (lacteals) in the villi. These lymph vesselsultimately release the absorbed substances into the blood stream. The absorbed substancesfinally reach the tissues which utilise them for their activities. This process is called assimilation.

4. Defaecation

The digestive wastes, solidified into coherent faeces in the rectum initiate a neural reflexcausing an urge or desire for its removal.The egestion of faeces to the outside through the anal opening (defaecation) is a voluntary process and is carried out by a mass peristaltic movement.

DIGESTIVE JUICES:

There are five digestive juices, viz., saliva, gastric juice, pancreatic juice, succus entericus (intestinal juice) and bile, secreted from salivary, gastric, pancreatic, intestinal and hepatic gland respectively, which are poured in the alimentary canal at its different levels successively from oral to aboral side.

The term, mechanism of secretion is meant by- (a) How the glands respond to secrete by the stimuli and how its flow and composition are maintained, (b) How the glands modify their secretion as regards their flow and composition by various types of stimuli and site of stimulation.

A. Salivary Secretion:

Purely a reflex process; two types of reflexes present; conditioned and unconditioned. Conditioned reflex is proved by the fact that even sight and smell of food stimulate secretion. Other conditioned stimuli can be established.

The stimulus for unconditional reflex arises chiefly in the mouth. But it also arises from the oesophagus (oesphago-salivary reflex), from the stomach (gastro-salivary reflex), as well as from other viscera such as gravid uterus.

B. Gastric Secretion:

Experiments:

i. Sham feeding.

ii. Pavlov's pouch.

Phases:

Three phases:

(a) Cephalic (or nervous),

(b) Gastric, and

(c) Intestinal.

(a) Cephalic Phase:

Starts immediately after taking food. It is a reflex process involving both conditioned and unconditioned reflexes. The juice secreted is called appetite juice. It is constant in composition and does not vary with the nature of food. In man it is small in amount but is important.

(b) Gastric Phase:

Starts half an hour after the entry of food in the stomach. The stimulus is chemical. The chemical substance is manufactured by the pyloric mucous membrane from some products of protein digestion and is known as gastrin. Gastrin enters blood stream carried to the gastric glands and stimulates their secretion independent of all nerves.

Largest quantity of gastric juice is secreted during this phase. This part of gastric juice varies in quality and quantity with the nature of food. Proteins increase both the amount and the HCl content. Fats inhibit both. Bread stimulates a secretion having the greatest digestive power. Water, coffee, spices stimulate.

(c) Intestinal Phase:

Starts when food enters the duodenum. It is small in amount and is independent of nerves; consequently, the stimulus is chemical but its exact nature is not known. Presence of fat in the duodenum inhibits gastric secretion. This, according to Ivy, is due to the liberation of an inhibitory hormone from the intestine called enterogastrone.

The three phases are closely interrelated. Cephalic phase initiates appetite juice, which digests the proteins partly. Form these products of digestion gastrin are manufactured which initiates the second phase. After this when gastric digestion has proceeded to the required stage, stomach empties into duodenum and thereby intestinal phase starts. Thus each phase initiates the next.

Even in fasting condition stomach secrets at the rate of 10-60 ml per hour. Its cause is not known. It acts as an antiseptic against pathogenic bacteria that may be swallowed with saliva in empty stomach.

C. Pancreatic Secretion:

Two phases:

(a) Nervous.

(b) Chemical.

(a) Nervous Phase:

Starts 1-2 minutes after taking food. The reflex is purely unconditioned. Unlike gastric juice there is no conditioned reflex here. The stimulus for this secretion arises both in the mouth (during chewing) as well as from the stomach after the food is swallowed.

(b) Chemical Phase:

Starts when stomach empties into duodenum. This is due to hormone-like substances called secretin and pancreoxymin. Secretin thus enters blood stream, goes to pancreas and

stimulates the secretion. The secretin fraction stimulates the secretion of water, alkali and other salts of pancreatic juice; whereas, pancreozymin increases the enzyme content.

On the whole, juice stimulated by secretin is rich in alkali but poor in enzyme. Whereas secretion produced by the stimulation of the vagus is poor in alkali but rich in enzyme. Pancreatic secretion varies with the type of food. Meat stimulates a 'secretin' type of juice, fat stimulates the 'vagus' type; whereas, bread elicits a mixed type of secretion.

D.Bile Secretion:

Experiments:

(1) Altercursive intubation.

(2) Triple intubation.

Mechanism:

Bile secretion by liver is active and continuous, total amount 800 -1,000 (15 ml per Kg body weight) per day. The rate of secretion increases one hour after taking food remains high for 2 – 5 hours and then falls. At the end of intestinal digestion it comes to the fasting level. The mechanism involves only chemical stimuli, nerves are of no practical importance.

The chemical stimuli are the following:

i. Bile Salts:

Chief cholagogues.

ii. Foodstuffs:

Fats and proteins stimulate. It has been noted that bile secretion increases about one hour after meal, remains high for about 2-5 hours and then declines.

iii. Humoral:

Cholecystokinin which stimulates bile secretion by contraction of gall-bladder and hepatocrinin which stimulates the liver cells to secret bile.

E. Succus Entericus:

Experiment:

Thiry-Vella fistula.

Mechanism:

During fasting, no secretion. Flow starts one hour after food, maximum in the third hour, then gradually declines.

Mechanism involves two factors:
(i) Mechanical, and

(ii) Chemical.

(i) Mechanical Factor:
When the mucosa is mechanically irritated secretion takes place. It is independent of the vagus and the sympathetic (splanchnic) but depends upon local nerve plexuses. Presence of food in the intestine acts as a normal stimulus.

(ii) Chemical Stimulus:
(a) Some products of digestion, particularly of proteins,

(b) Enterocrinin, and

(c) Duocrinin which acts on duodenum only.

All three independent of nerves, act as important stimuli in the normal process of secretion of intestinal juice. Enterocrinin is a hormone manufactured by intestinal mucosa which is believed to act as important stimulus in the normal process of secretion of intestinal juice. It is obvious that all these factors only operate so long as intestinal digestion and absorption continue. Consequently, after this period— (normally 2-5 hours)—the flow will cease.

Gastric secretion has divided into four phases:

1. Nervous Phase 2. Gastric Phase 3. Intestinal Phase

4. Interdigestive Phase.

1. Nervous Phase:
A pouch of Pavlov is prepared in a dog and upon the same animal oesophagus is divided, as done in the experiment of sham feeding. The food, swallowed by the animal, comes out through the cut end of the oesophagus and does not enter stomach. In spite of it, it is found that the stomach secretes after a latent period, of about 5-10 minutes and continues for as long as $1^{1/2}$ hours. When the vagi are cut this secretion fails to occur.
i. Stimulation of the vagus produces a secretion rich in pepsin and HCl also some mucus, the most powerful action is possibly on acid secretion. The gastric cells are stimulated by acetylcholine released after vagal action. There is also a possibility that increased amount of histamine, liberated at the mucosa of stomach after vagal stimulation, stimulates the parietal cells.

Vagal stimulation also causes vasodilatation of the gastric mucosa. Under certain conditions vagal stimulation can also stop or diminish gastric secretions. Stimulation of the vagus also increases the release of gastrin and augments the response of the cells of stomach to other type of stimuli.

ii. Stimulation of the sympathetic nerves, supplying the stomach causes vasoconstriction, but its effects on gastric secretion are not constant.

iii. Hypothalamus exerts undoubted influence upon gastric secretion. Stimulation of hypothalamus increases gastric secretion by augmenting vagal activity. Hypoglycaemia has similar effect mediated in an identical way. Experimental lesions of hypothalamus have been found to produce gastric haemorrhages, erosions and even perforations. It is believed that some such lesion may be associated with the causation of gastric ulcer.

iv. These show that the initial phase of gastric secretion is a reflex process and this type of secretion is called appetite juice by Pavlov.

On further analysis it is seen that two types of reflexes are involved in it:

i. Unconditioned Reflex:

The sensory stimulus for the unconditioned reflex arises in the mouth during chewing and swallowing of the food. The sensory nerves are the fifth, seventh and ninth cranial nerves. The motor nerve is the vagus.

ii. Conditioned Reflex (Psychic Reflex):

The existence of conditioned reflex is proved by the fact that sight or smell of the accustomed food stimulates gastric secretion. Various other conditioned stimuli can be established which can arouse gastric secretion even when no food is actually given to the dog, i.e., without the contact of food in the mouth. The sensory nerves are those of special senses, viz., vision, smell and hearing. Motor nerve is the vagus.

The Appetite Juice:

It has got the following characters:

i. It is rich in pepsin, acid in reaction and contains mucus.

ii. The composition of appetite juice is constant and does not vary with the type of food.

iii. The quantity varies with the intensity of appetite.

iv. The secretion of psychic juice may be inhibited by shock, fear, anxiety, etc.

v. In animals it forms a considerable part of the total gastric secretion but in man the quantity is probably much less and is not essential.

vi. Its importance lies in the fact that it helps to initiate the second phase of gastric secretion.

2. Gastric Phase (Hormonal):

At the end of sham feeding, gastric secretion elicited by cephalic phase dies away. But if food enters the stomach, further secretion of gastric juice takes place. The gastric phase of secretion is mediated by local and vagal reflex response to distention and also by the hormone gastrin released by the mucosa of the pyloric area.

Thus when the stomach is completely denervated, this secretion is not affected. This proves that this secretion is addition to a nervous reflex and mechanical irritation of food on the gastric mucosa is due to a chemical stimulus. By further experiments it has been proved that a chemical excitant is actually operating in this phase and is called gastrin (Fig. 9.33).

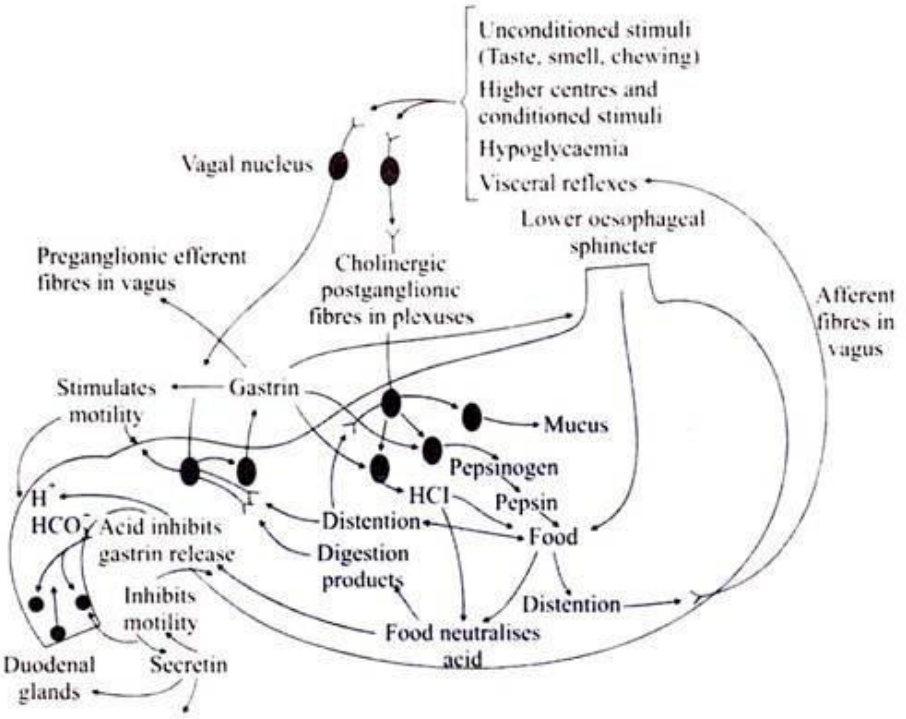


Fig. 9.33 Action of hormones controlling gastric secretion (Schematic representation). From *physiology of the Digestive Tract*, by Horace W. Davenport, Year Book Medical Publishers, Inc. Used by permission.

The following experimental facts can be put forth to uphold the gastrin theory:

i. Acid extract of pyloric mucosa, on injection, stimulates gastric secretion.

ii. At the height of gastric secretion, a substance is found to be present in the venous blood of stomach which can excite gastric secretion.

iii. Ivy and Farrell cut out a small pouch from the body of the stomach and grafted it in the mammary region of a pig. The mammary gland being highly vascular, the graft easily sets there. The wound in the stomach is adequately sutured. With such a preparation it is seen that, when the second phase of gastric secretion, is taking place in the main stomach, the grafted pouch also secretes gastric juice.

Since there is no nervous connection between the stomach and the grafted pouch and there is no separate nervous connection (vagus motor) of the pouch, secretion in the latter must be due to a chemical excitant which is carried to the pouch through blood stream.

iv. Komarov has isolated a protein derivative from the pyloric mucosa possessing strong stimulating effect on gastric secretion.

v. After resection of the pyloric part of stomach this phase of gastric secretion is greatly reduced.

vi. When coagulated egg albumin, raw meat, undigested starch or fat, is introduced into the stomach through the gastric fistula or through the oesophagotomy wound of a sleeping dog (to avoid secretion of psychic juice) no secretion takes place.

This proves that these substances neither have any mechanical effect nor carry the necessary chemical stimulus. But when meat extracts, liver extracts and partly digested meat, egg-white, etc., be introduced in the stomach, gastric secretion is stimulated. It has been demonstrated that stimulation of the vagus causes release of gastrin. This gastro-intestinal hormone is also liberated through a local reflex mechanism mediated through cholinergic nerves other than the vagus.

From these observations it can be concluded that gastrin is manufactured by the pyloric mucosa from some products of protein digestion. This substance enters the blood stream, brought back to the gastric glands and stimulates their secretion.

Nature and Action of Gastrin:

It is polypeptide in nature, two gastrins, gastrin I and II, differing in amino acid sequence have been isolated. Both of them stimulate gastric secretion.

On injection:

(a) Gastrin stimulates gastric secretion—which is rich in acid but poor in pepsin,

(b) it stimulates bile secretion, and

(c) It also stimulates pancreatic secretion to a slight extent.

The gastric phase of secretion constitutes the main part of gastric juice and continues for about three hours. Unlike psychic juice, this part of secretion and varies in quality and quantity according to the type of foodstuff.

The variations are as follows:

Response to Food:

i. Meat increases both the quantity and the HCl content.

ii. Bread stimulates a secretion having the greatest digestive power.

iii. Fat inhibits secretion both in quality and quantity. [It also inhibits the movements of stomach] This depressing effect may be due to a chemical substance called enterogastrone. [see below.] The inhibitory effects of fats are more strongly exerted from the duodenum than from the stomach.

iv. Water, tea, coffee, spices, condiments, vegetable juices, etc., stimulate gastric secretion.

v. Mechanical distention of stomach by gas, such as with aerated waters, stimulates gastric secretion (and movements).

3. Intestinal Phase:

It was observed that the presence of certain food substance in the small intestine excites gastric secretion. The latent period is 2 – 3 hours but continues for 8 -10 hours. When water, meat extract, peptone and partly digested proteins etc., enter the duodenum in the process of digestion or are directly introduced into the duodenum (through a duodenal fistula), this secretion occurs.

When these parts are completely denervated this phase of gastric secretion is not affected. This proves that it is due to a chemical stimulant, a hormone or secretagogue absorbed with the food from the intestine, the exact nature of the stimulus is not known.

Gastric secretion can also be inhibited by the presence of certain substances in the duodenum.

For instance:

(a) Introduction of alkali directly into duodenum inhibits gastric secretion, and

(b) Presence of fats in the duodenum inhibits gastric secretion (both the gastric and intestinal phases).

This inhibitory action of fat is due to the liberation of an intestinal hormone called enterogastrone. It inhibits gastric secretion and gastric motility. Such an inhibitory agent has been detected in the blood of fat-fed animals and has been extracted from the intestinal mucosa.

Urogastrone is another inhibitory substance similar to, but not identical with enterogastrone. It has been isolated both from the urine of a normal male and from that of a pregnant women. It exerts a specific inhibitory effect on gastric secretion (for this reason its therapeutic use in the treatment of gastric ulcer has been recommended). Its role in the normal process of gastric secretion is not known.

4. Interdigestive Phase:

Hydrochloric acid secretion has been found to take place at regular intervals, even in fasted man and dog. They all act by stimulating the nucleus of the vagus.

It has been observed that both hormonal and nervous mechanisms are involved in such secretion, the latter being mediated through the vagus. Recently, it is believed that the interdigestive phase is a part of intestinal phase and partly due to spontaneous secretion of saliva.

Hormones of Gastrointestinal Tract:

1. **Gastrin:** This hormone is secreted by gastrin cells (= G-cells) in the pyloric region of the stomach. It stimulates gastric glands to secrete and release the gastric juice. It also stimulates gastric mobility.

2. **Enterogastrone:** (= Gastric Inhibitory Peptide— GIP). It is secreted by the duodenal epithelium. It inhibits gastric secretion and motality. It slows gastric contraction, hence it is also called gastric inhibitory peptide.

3. Secretin: It was the first hormone to be discovered by scientists. It is secreted by the epithelium of duodenum. It releases bicarbonates in the pancreatic juice. It increases secretion of bile. It decreases gastric secretion and motality.

4. Cholecystokinin pancreozymin (CCK-PZ): The word cholecystokinin is derived from three roots: Chol meaning bile, cyst meaning bladder and kinin meaning to remove. The word pancreozymin is derived from pancreas and zymin, which means enzyme producer. This hormone is secreted by the epithelium of entire small intestine. It stimulates the gall bladder to release bile and pancreas to secrete and release digestive enzymes in the pancreatic juice.

5. Duocrmin: It is secreted by the duodenal epithelium and stimulates the Brunner's glands to release mucus and enzymes into the intestinal juice.

7. **Enterocrinin:** It is secreted by the epithelium of entire small intestine. It stimulates the crypts of Lieberkuhn to release enzymes into the intestinal juice.

8. **Vasoactive Intestinal Peptide (VIP):** It is secreted by the epithelium of entire small intestine. It dilates peripheral blood vessels of the gut. It also inhibits gastric acid secretion.

9. **Villikinin:** It is secreted by the epithelium of entire small intestine. It accelerates movement of villi.

10. **Somatostatin (SS):** Somatostatin secreted by the Delta cells of islets of Langerhans of the pancreas inhibits the secretion of glucagon by aplha cells and insulin by beta cells Somatostatin produced by argentaffin cells of gastric and intestinal glands suppresses the release of hormones from the digestive tract.

11. **Pancreatic Polypeptide (PP):** It is secreted by the pancreatic polypeptide cells (also ailed PP cells or F-cells) of islets of Langerhans. It inhibits the release of pancreatic juice from the pancreas.

Probable Questions:

1. Briefly discuss five activities of digestive system.
2. Discuss the mechanism of digestion.
3. How carbohydrates are digested in the mouth?
4. How proteins are digested in the small intestine and stomach?
5. How absorption took place?
6. What is defecation?
7. Discuss the hormones of digestive system.
8. Discuss different stages of gastric secretion.

SuggestedReadings/References-

1. Animalphysiology-MohanP.Arora.
2. TextbookofmedicalphysiologyArthurC.Guyton,JohnE.Hall.
3. Ganong'sreviewofmedicalphysiology.

UNIT-IX

Circulatory systems: General plan andHemodynamics

Objective: In this unit we will discuss about circulatory system of vertebrates and haemodynamics.

Regulation of Blood Flow:

1. Increased carbon dioxide tension (increased pCO_2) is the most important factor.CO_2 is a power full vasodilator of the cerebral blood vessels. Increasing the CO_2 content of the inspired air (3-5%) almost doubles the blood flow to the brain. Voluntary hyperventilation decreases the pCO_2, and brings about vasoconstriction and decreases the cerebral blood flow. This gives rise to dizziness.

2. Increased H^+ concentration of the CSF increases the cerebral blood flow.

3. Hypoxia (decreased pO_2) also increases the cerebral blood flow.

4. A rise in the intracranial tension compresses the blood vessels supplying the brain. This decreases the cerebral blood flow (Monro-Kellie doctrine).

5. Stimulation of sympathetic/parasympathetic never fibers has very little effect on cerebral blood flow.

Monro-Kellie doctrine:

Since the three compartments are placed in rigid box (cranium) expansion of any one of the compartment can occur only at the expense of compromise of the other two compartments. When the CSF compartment expands (due to increased accumulation of CSF) the vascular compartment is pressed upon. This decreases cerebral blood flow.

Coronary Blood Flow:

i. Blood flow through the coronaries supplies the heart muscle (myocardium).

ii. The right and the left coronary arteries take their origin from the root of the aorta.

iii. Normal coronary arterial blood flow is about 250 ml/minute.

iv. Arteriovenous oxygen difference is highest even under resting conditions. It is about 14 ml (20-6 ml)/100 ml.

v. Therefore, whenever there is an increased demand for oxygen by the heart muscle it is met with only by increasing the coronary blood flow.

vi. The venous blood from the myocardium is drained into the coronary sinus and the anterior cardiac veins.

vii. There is variation in blood flow during cardiac cycle. More blood flows through the coronary vessels during ventricular diastole than during systole. This is more so with respect to left coronary artery (Fig. 3.45).

viii. The volume flow variation is more phasic in the endocardial region when compared to epicardial region.

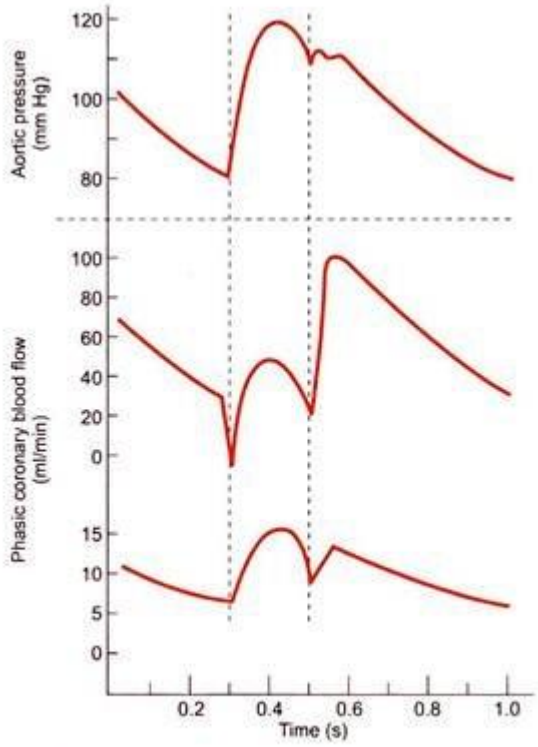


Fig. 3.45: Phases of cardiac cycle and blood flow through the right and left coronary arteries

Determination of Coronary Blood Flow:

i. By applying Fick's principle.

ii. Nitrous oxide is the substance of choice. Radioisotope thalium (Tl-201) can also be used.

iii. The venous blood from the myocardium is drained into the coronary sinus and the anterior cardiac veins.

CBF (coronary blood flow) = $Q/A_C - V_C$ ml/minute
Wherein

Q is the quantity of nitrous oxide taken up by brain tissue.

A_C is the concentration of the substance in arterial blood.
V_C is the concentration of substance in venous blood.

Factors Influencing the Blood Flow:

1. Coronary blood flow is subjected to an auto- regulation.

2. The pressure head (aortic pressure minus coronary sinus pressure)

3. Phasic blood flow

4. Chemical factors (blood gases), the most important one being the oxygen supply (hypoxia) and decreasing in oxygen tension (fall in pO_2). Any hypoxic situation will be promptly followed by an increase in blood flow.
5. Sympathetic stimulation

Left Coronary Flow:

i. During isometric contraction phase as the intraventricular pressure is suddenly increasing, the blood vessels are compressed upon and, therefore, the blood flow decreases.

ii. During maximum and reduced ejection phase, the cardiac muscle fibers contract and the intraventricular pressure increases to 120 mm Hg. The endocardial blood vessels are compressed and hence blood flow decreases.

iii. During the same time epicardial blood vessels are not compressed to a great extent. The total blood flow remains low (about 40 ml/minute)

iv. During diastole, as the intraventricular pressure rapidly falls, the compressor force on the blood vessels decreases and this leads to an increase in blood flow.

Right coronary arterial blood flow remains high both during ventricular systole and diastole, because the blood vessels supplying the right heart are not subjected to greater compression. This is because the pressure changes in the right ventricle during a cardiac cycle remains low (10-25 mm Hg).

Chemical Factors Mechanism involved:

1. Hypoxia produced leads to production of adenosine an end product of anaerobic metabolism and adenosine is a powerful vasodilator substance to the coronary blood vessels. The effect of hypoxia is not direct one but it is through the production of adenosine.

2. An increase in pCO_2 or an increase in H^+ will also bring about coronary vasodilatation, and increase in the blood flow.

Sympathetic Stimulation (Fig. 3.46):

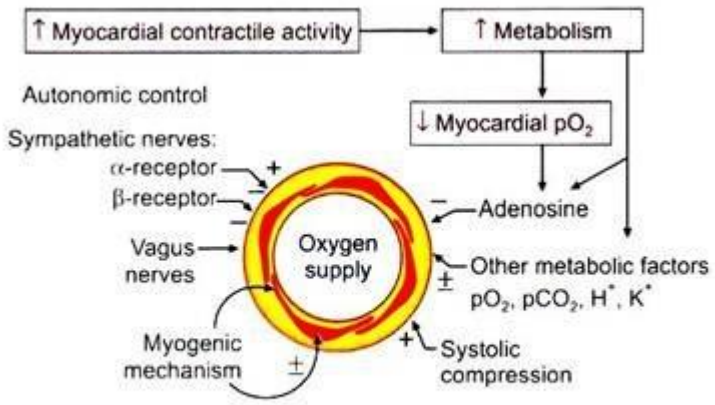


Fig. 3.46: Factors affecting the lumen diameter of coronary vessels

Coronary arteries contain both and receptors. Stimulation of receptors will bring about coronary vasoconstriction. Stimulation of receptor results in coronary vasodilatation. However, stimulation of sympathetic fibers to the heart is associated with coronary vasodilatation.

Sympathetic fiber stimulation to the heart increases the force of contraction and, therefore, metabolism of the cardiac musculature. Metabolic end products bring about coronary vasodilatation. Therefore, the net effect of sympathetic stimulation is coronary vasodilatation and increase in blood flow.

Acetylcholine (ACh):

Parasympathetic (ACh is the neurotransmitter liberated by these nerve terminals) nerve stimulation is associated with coronary vasodilatation and increase in the blood flow.

Other coronary vasodilators include:

i. Potassium

ii. Lactate

iii. Prostaglandin

iv. And NO (nitric oxide), nitroglycerin, nitrates are used clinically as coronary vasodilators.

Auto-Regulation of Blood Flow:

Mean arterial pressure determines the blood flow through the vascular region. At the level of organ or tissue, it is the perfusion pressure, which is nothing but pressure difference between the beginning of the flow (P_1, arterial end pressure) and at the end of flow (P_2, venous pressure).

There is a direct relationship between this perfusion pressure ($P_1 - P_2$) and the blood flow. In most of the tissues or organs, the pressure difference ($P_1 - P_2$) will be around 70 mm Hg.

Organs and tissues in which autoregulation of blood flow occurs are:

1. Coronary flow (blood flow through the myocardium)

2. Cerebral flow (blood flow through brain)

3. Renal flow (blood flow through kidney)

4. Skeletal blood flow, etc.

The above organs have well-developed auto- regulatory mechanism to maintain the flow constant within a particular range of pressure. This is termed as autoregulation of blood flow.

By definition, autoregulation of blood flow states that it is the ability of an organ or tissue to regulate its own blood flow despite a change in blood pressure.

Critical Closing Pressure/Critical Opening Pressure (Fig. 3.52):

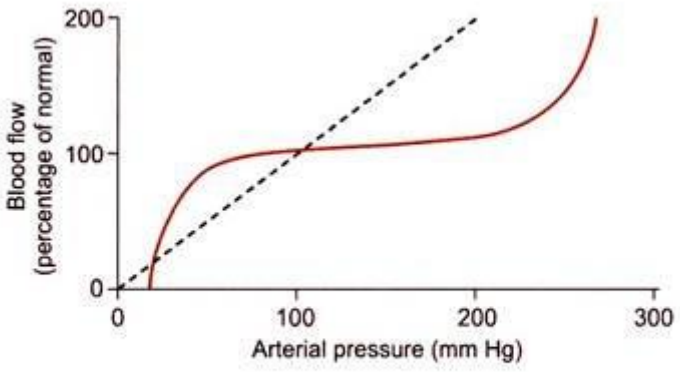


Fig. 3.52: Flow of blood in a rigid tube (hatched lines) and flow of blood through blood vessel (solid line)

Critical closing pressure is the minimum mean arterial pressure that is essential to keep the arteries in a distended state. If the pressure in the vessel is below the minimal value, the blood vessels collapse. Normally, the critical closing pressure is around 20 mm Hg. Below this pressure, since the blood vessels collapse, the blood flow through the organ stops completely.

As the pressure increases above the critical closing pressure, the volume of blood flow also increases proportionately till a limit. So they will have a direct relationship. However, when once the pressure exceeds a particular value, in spite of an increase in pressure, there will not be any further increase in blood flow. This is termed as autoregulatory ability of the organ to regulate the blood flow.Most of the organs have the ability to autoregulate their flow between a pressure range of 60 and 180 mm Hg. Beyond 180 mm Hg, the autoregulatory mechanisms fail and hence there would be further increase of blood flow proportionate to the increase in pressure. Autoregulation of blood flow is seen even in a denervated isolated organ. This suggests that the nerve supply is not responsible for autoregulation mechanism.

There are many theories which try to explain the mechanism of auto-regulation of blood flow.

They are:

1. Myogenic theory

2. Tissue metabolite theory

3. Tissue fluid pressure theory

4. Renin-angiotensin theory, etc. (this theory is applicable only in kidneys)

1. Myogenic Theory (Fig. 3.53) According to This Theory:

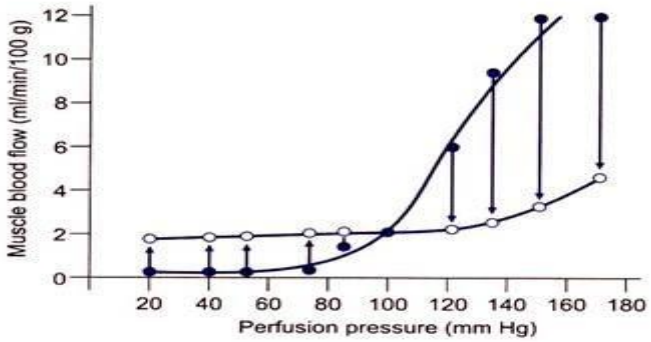


Fig. 3.53: Autoregulation of blood flow based on myogenic theory

i. When there is an increase in pressure, initially there will be an increase of blood flow.

ii. ii. When blood flow increases, the smooth muscles present in the walls of the blood vessels get stretched.

iii. iii. The stretching of the muscle will act as a stimulus (mechanical stimulus) to the muscle.

iv. iv. The muscle starts responding to the stimulus by contracting.

v. v. Contraction results in the narrowing of the lumen diameter. Decreased lumen diameter increases the resistance to the flow (decrease in the radius of the lumen) and restricts the increase in the flow.

vi. vi. Greater is the stretch; greater is the contraction of the smooth muscle fibers. Therefore, the total blood flow remains the same.

vii. The role of myogenic theory in regulation of blood flow can be experimentally proved. When papavarine is injected, papavarine brings about the paralysis of smooth muscles.

viii. Hence, after injection of papavarine, when the perfusion pressure is increased, there will be increase of blood flow without any autoregulation. It proves the role of smooth muscle fibers of blood vessels in the auto regulatory mechanism. This theory holds good for almost all organs.

2. Tissue Metabolite Theory:

i. At any given time, there will be some amount of metabolites in the tissues, e.g. the pCO_2 in the tissues will be generally around 45 mm Hg.

ii. These metabolites exert vasodilator effect. Hence some amount of vasodilatation is maintained by these metabolites.

iii. When there is increase in perfusion pressure, to start with, there will be slight increase of blood flow.

iv. This brings about increased washout of metabolites from the tissues.

v. So the concentration of metabolites in the tissue is reduced.

vi. This reduces the vasodilator effect and leads to a little more vasoconstriction.

vii. Decreased lumen diameter will regulate the blood flow.

This theory holds good for all organs.

3. Tissue Fluid Pressure Theory:

i. There will be constant movement of fluids between blood and tissues at the level of capillaries.

ii. Exchange of fluid occurs at the level of capillaries because of the capillary dynamics.

iii. When there is increase of perfusion pressure, initially there will be increase of blood flow.

iv. This increases the hydrostatic pressure both at the arterial and venous end of capillaries.

v. Because of this, more fluid goes out at the arterial end of the capillary and less fluid returns at the venous end of the capillaries.

vi. This leads to increased accumulation of fluid in the tissue spaces.

vii. This in turn leads to compression of blood vessels.

viii. So blood flow is regulated.

This theory is applicable in the case of encapsulated organs, like kidney, liver, etc.

4. Renin-Angiotensin Theory:

i. When there is increase in perfusion pressure, there will be increase of blood flow to the kidney.

ii. This increases the filtration pressure in the nephrons.

iii. So the volume of filtrate is more in the nephrons.

iv. Because of this, more sodium will reach the distal convoluted tubules in the nephrons.

v. The amount of sodium reaching the distal convoluted tubules will be sensed by the macula densa of distal convoluted tubules.

vi. This leads to more renin getting released from juxtaglomerular cells.

vii. Renin acts on angiotensinogen present locally and brings about formation of angiotensin I.

viii. This angiotensin I is converted to angiotensin II (by converting enzyme present in the endothelial cell lining the blood vessels).

ix. Increased angiotensin II brings about the constriction of the arterioles (especially the afferent arteriole) in the kidneys.

x. This decreases the lumen diameter and the blood flow to the kidney is regulated.

This theory is applicable only to the kidneys.

Probable Questions:

1. How coronary blood flow is determined?
2. Which factors influence blood flow?
3. Discuss about sympathetic stimulation.
4. Discuss myogenic theory for autoregulation of blood flow.
5. Discuss Tissue Metabolite Theory.
6. Discuss Tissue Fluid Pressure Theory.
7. Discuss Renin-Angiotensin Theory.

SuggestedReadings/References-

1. Animalphysiology-MohanP.Arora.
2. TextbookofmedicalphysiologyArthurC.Guyton,JohnE.Hall.
3. Ganong'sreviewofmedicalphysiology.

UNIT-X

Cardiovascularresponse to extreme conditions like exercise, diving and hemorrhage.Neural control of cardiovascular system

Objective: In this unit we will discuss about how cardiovascular system is controlled by nervous systemand effect of exercise, diving and hemorrhage on circulatory system.

Introduction:

Homeostasis can be defined as the maintenance of the physical and chemical properties of the extracellular fluid in all body tissues. It is dependent, among other things, on effective cardiovascular and respiratory regulatory mechanisms that ensure that the delivery of O_2 to all regions of the body is sufficient to match the metabolic demands of each region. This is particularly critical in the case of the heart and skeletal muscles, whose metabolic activity can vary greatly in different circumstances. For example, during maximal exercise in humans, O_2 demand can increase to a level up to 50-fold greater than resting levels. This is achieved by an enormous increase in blood flow (up to 20-fold) together with a 2- to 3-fold increase in O_2 extraction from the blood. These effects are produced by a combination of local and neural mechanisms, as shown in Fig. 1. Local mechanisms, which include metabolic, endothelial, and myogenic components, result in vasodilation in metabolically active skeletal muscle vascular beds, leading to large increases in local blood flow provided the perfusion pressure (arterial pressure) is maintained or increased. Similarly, the increase in O_2 extraction from the blood also depends on both local factors (e.g., local acidosis, which shifts the hemoglobin-oxygen saturation curve to the right) as well as central regulatory mechanisms that maintain the arterial blood P_{O_2} (Pa_{O2}) despite large changes in metabolic activity.

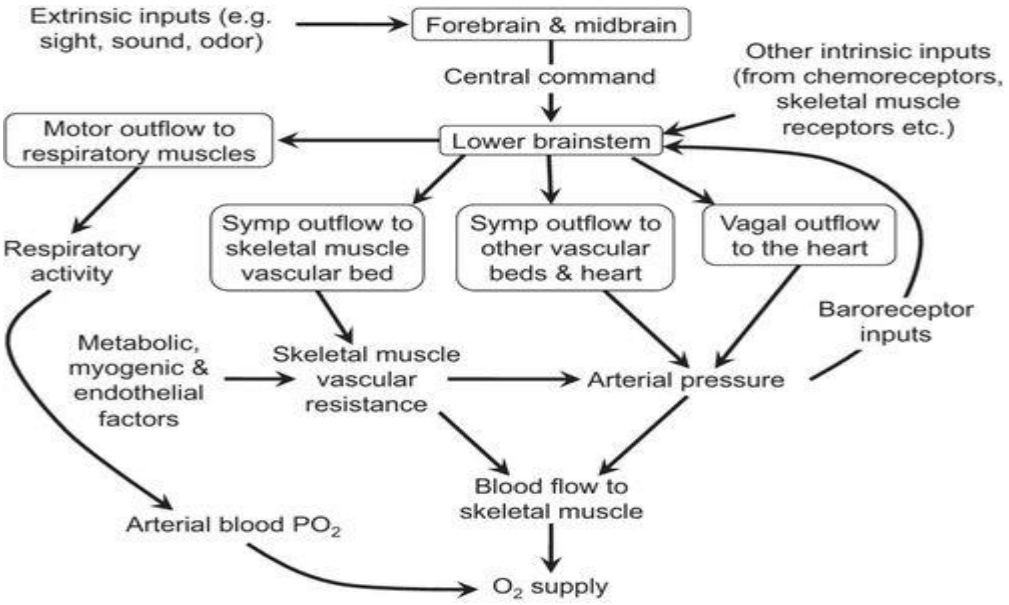


Fig. 1. Flow diagram illustrating how feedforward (central command) and feedback (reflex) mechanisms operate together to regulate the O_2 supply to particular regions (skeletal muscle in this example) to match the metabolic demands of that region and thus maintain homeostasis.

Apart from physical exercise, coordinated cardiovascular and respiratory mechanisms regulate the O_2 supply to all tissue during other behaviors, such as defensive behavior or sleep. In addition, such regulatory mechanisms are also required to maintain homeostasis in the face of challenges such as hypoxia, dehydration, or changes in ambient temperature.

As shown in Fig. 1, arterial blood pressure is regulated by autonomic nerves, consisting of sympathetic nerves that innervate the heart and blood vessels, and vagal parasympathetic nerves, which innervate the heart. Sympathetic outflow, in turn, is regulated by sympathetic premotor neurons located in the lower brain stem and hypothalamus, whereas vagal cardiac outflow originates primarily from the nucleus ambiguus in the medulla oblongata. The activity of the sympathetic premotor neurons and cardiac vagal neurons is controlled by two general mechanisms: *1*) reflex effects arising from stimulation of a wide variety of peripheral receptors and *2*) feedforward control, or "central command," from descending inputs arising from higher centers in the brain (Fig. 1).

One of the most important cardiovascular reflexes is the baroreceptor reflex, and an example of its operation is shown in Fig. 2*A*. In this example, recordings were made of mean arterial pressure, heart rate, and renal sympathetic nerve activity in a conscious rat during treadmill exercise. Changes in arterial pressure were induced by systemic injection of a

vasoconstrictor (phenylephrine) and a vasodilator (sodium nitroprusside), resulting in reflex changes in heart rate and renal sympathetic nerve activity.

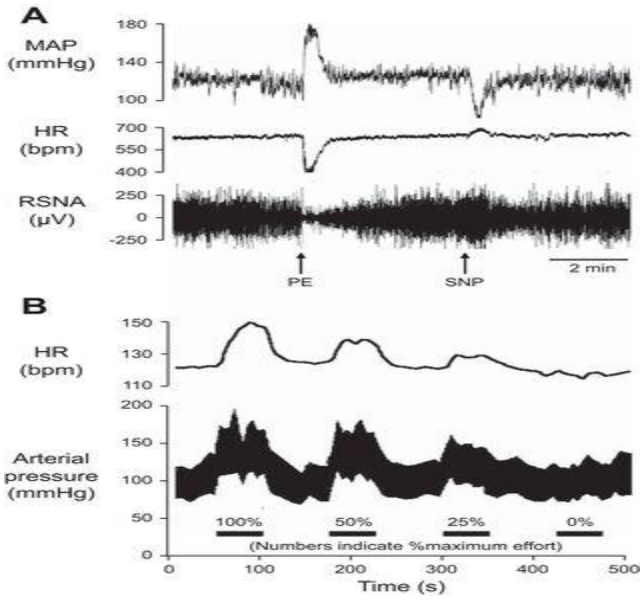


Fig. 2.*A*: example of baroreflex control of the cardiovascular system. Changes in mean arterial pressure (MAP) were induced in a conscious exercising rat by systemic injection of the vasoconstrictor phenylephrine (PE) or the vasodilator sodium nitroprusside (SNP), resulting in reflex changes in heart rate (HR) and renal sympathetic nerve activity (RSNA). [Modified from Miki et al. with permission.] *B*: example of central command. Recordings of arterial pressure and HR [in beats/min (bpm)] were made in a paralyzed, mechanically ventilated, but conscious human subject, who was asked to attempt to contract leg muscles. The numbers indicate the effort as a percentage of the maximum. Note that there were graded increases in arterial pressure and HR according to the degree of effort, despite the lack of any afferent feedback from the paralyzed muscle.

In contrast to reflex or feedback control, feedforward control (central command) does not require inputs from peripheral receptors. A classic example of such control is shown in Fig. 2*B*. Recordings of arterial pressure and heart rate were made in a paralyzed, mechanically ventilated, but conscious, human subject, who was asked to attempt to contract leg muscles. The numbers indicate the effort as a percentage of the maximum. Note that there were graded increases in arterial pressure and heart rate according to the degree of effort, despite the lack of any afferent feedback from the paralyzed muscles.

These two general mechanisms of feedback and feedforward control are not, however, entirely independent. In particular, as shall be described in more detail below, cardiovascular reflexes such as the baroreceptor reflex can be powerfully modulated by central command signals arising from the forebrain or midbrain.

Central Mechanisms Subserving Homeostatic Reflexes

To maintain cardiovascular homeostasis, several key physiological variables must be regulated: arterial blood pressure, the O_2 content of the blood, blood volume, and body temperature. The following sections will briefly describe the reflex mechanisms that regulate these variables.

Blood pressure.

The baroreceptor reflex is the principal mechanism regulating arterial pressure, at least in the short term. For example, a decrease in arterial pressure is sensed by baroreceptors located in the walls of the carotid sinus and aortic arch (Fig. 3*A*). The baroreceptors are stretch receptors located on the terminal arborizations of afferent fibers, so a decrease in arterial pressure results in a decreased firing rate of baroreceptor afferent fibers. Inputs from baroreceptor afferent fibersreflexly inhibit the sympathetic outflow to the heart and blood vessels and reflexly excite the cardiac vagal outflow via central pathways in the brain stem and spinal cord. Therefore, a decrease in baroreceptor firing rate results in a reflex increase in sympathetic vasomotor activity, which increases total vascular resistance, and an increase in sympathetic cardiac activity together with a reflex decrease in cardiac vagal activity, which together results in an increase in heart rate and cardiac contractility, and thus cardiac output (Fig. 3*A*). The reflex increases in total peripheral resistance and cardiac output together help to restore arterial pressure (Fig. 3*A*). The most important component of the reflex response is the reflex change in total peripheral resistance, which accounts for ~80% of the reflex change in arterial pressure at rest and virtually 100% during exercise (Fig. 3*B*).

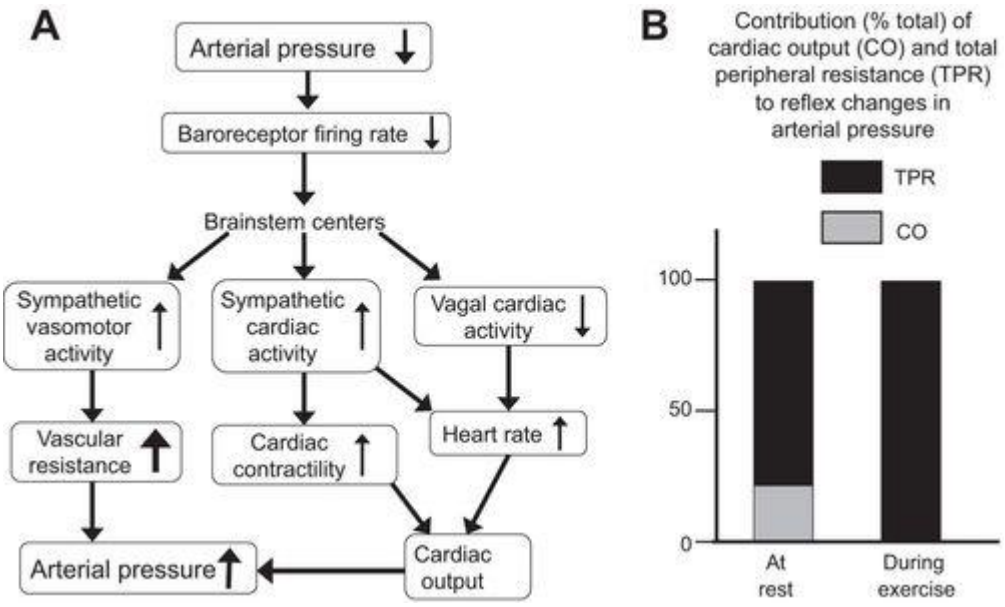


Fig. 3.*A*: flow diagram showing the sequence of events after a decrease in arterial pressure, leading to a reflex compensatory restoration of arterial pressure. *B*: histogram showing that the reflex increase in total peripheral resistance (TPR) is the major factor contributing to the reflex response both at rest and during exercise. CO, cardiac output.

The functional properties of the baroreceptor reflex in any particular situation can be represented by a sigmoidal curve that shows the input-output relationship for the reflex, where the input is the mean arterial pressure and the output is the reflexly controlled variable, e.g., renal sympathetic activity or heart rate (Fig. 4). To determine this curve, changes in mean arterial pressure are induced (e.g., by infusing a vasodilator or vasoconstrictor drug, as shown in Fig. 2*A*), and reflex changes in the output (e.g., renal sympathetic activity or heart rate) are then measured. The sigmoidal curve that best fits the relationship between the input and output is then determined (e.g., Fig. 4*B*).

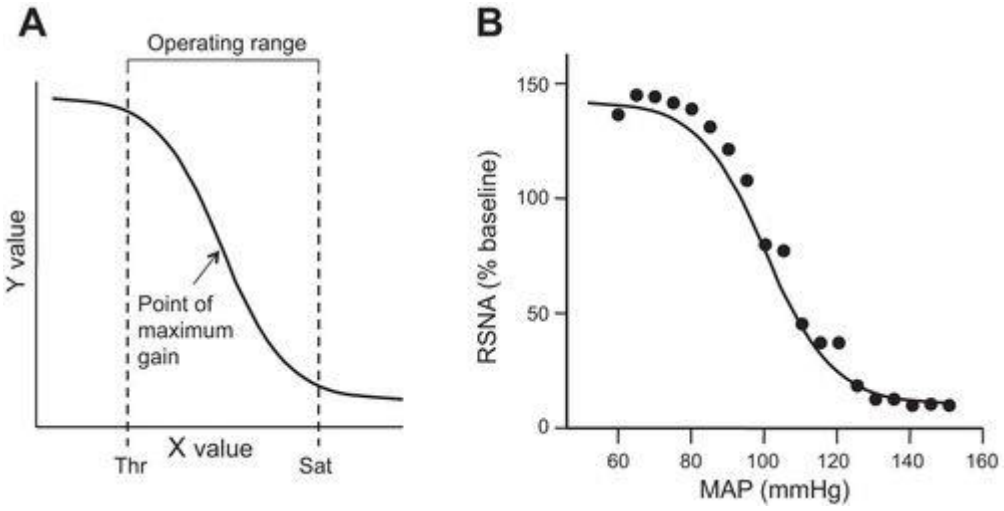


Fig. 4.*A*: the standard sigmoidal curve that is used to represent the input-output relationship for the baroreceptor reflex. The curve represents the following function: $Y = A_1/\{1 + \exp[A_2(X - A_3)]\} + A_4$, where X is the input (typically MAP) and Y is the output (e.g., sympathetic activity or HR) and A_1, A_2, A_3, and A_4 are the parameters that define the specific curve in any particular situation. The gain or sensitivity of the reflex at any value of X is represented by the slope of the curve and is maximal at the midpoint of the Y range (i.e., between the maximum and minimum values of Y). The threshold (Thr) value of X is the point at which the value of Y is 5% of the Y range below the maximum value of Y, and the saturation (Sat) value of X is the point at which the value of Y is 5% of the Y range above the minimum value of Y. The operating range of X lies between the Thr and Sat values and is thus the range of X over which changes in X evoke significant reflex changes in Y. [Modified from McDowall et al. .] *B*: example of a baroreflex sigmoidal function curve that best fits the reflex relationship between MAP and RSNA. In this experiment, changes in MAP were induced by

injections of vasoconstrictor and vasodilator drugs, and the corresponding reflex changes in RSNA were measured (solid circles).

The precise characteristics of the sigmoidal baroreflex function curve are defined by *1*) the maximum and minimum values of the reflexly controlled output; *2*) the maximum gain or sensitivity of the reflex, i.e., where the slope of the curve is maximal; and *3*) the operating range of the reflex, which is defined as the range of mean arterial pressure over which changes in pressure can produce significant reflex changes in the output (Fig. 4*A*).

The baroreceptor reflex is operational at all times, although the functional properties of the reflex can vary under different behavioral conditions. For example, the maximum gain of the baroreceptor-sympathetic reflex is increased both during exercise (Fig. 5*A*) and psychological stress (Fig. 5*B*). Furthermore, the reflex is reset so that it operates over a higher range of mean arterial pressure and sympathetic activity during exercise and stress (Fig. 5, *A* and *B*). Similarly, the baroreceptor-heart rate reflex is reset to a higher operating range of both mean arterial pressure and heart rate during exercise (Fig. 5*C*), with little change in gain. The effect of such baroreflex resetting is that during behaviors where an increase in arterial pressure is physiologically advantageous (e.g., exercise or defensive behavior), the baroreflex continues to be highly effective in regulating arterial pressure at this increased level.

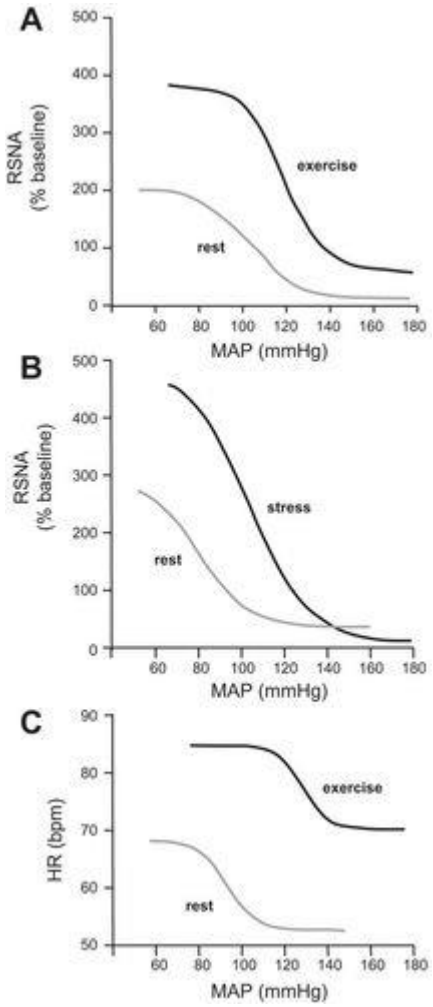


Fig. 5.*A*: baroreflex function curves showing the relationship between MAP and RSNA in conscious rats at rest and during exercise. Note that the maximum gain is increased and the operating range is shifted to higher values of MAP during exercise. [Modified from Miki et al. with permission.] *B*: baroreflex function curves showing the relationship between MAP and RSNA in conscious rats at rest and during psychological stress (air jet stress). Note that the maximum gain is increased and the operating range is shifted to higher values of MAP during psychological stress, similar to the changes observed in exercise. *C*: baroreflex function curves showing the relationship between MAP and HR in human subjects at rest and during exercise. Note that the operating range of the reflex is shifted to higher values of MAP during exercise but with little change in the maximum gain of the reflex.

It is well known that mean arterial pressure and heart rate show parallel diurnal variations, such that, in humans, both of these variables tend to be minimal during the early hours of the morning (i.e., during the sleep phase) and maximal after waking during the morning period. These variations in arterial pressure and heart rate can be explained as a continuous

modulation or resetting of the baroreflex, which thus serves to regulate arterial pressure at a level that is optimal for each phase of the sleep-wake or activity cycle.

Studies over the last 35 yr have identified the essential central pathways that mediate the baroreceptor reflex, and these are shown in Fig. 6. Primary afferent fibers from arterial baroreceptors located in the carotid sinus and aortic arch, which run in the glossopharyngeal nerve (cranial nerve IX) and vagus nerve (cranial nerve X), respectively, terminate in the nucleus tractus solitarius (NTS) in the dorsomedial medulla. From the NTS, second-order neurons project directly to cardiac vagal motoneurons in the nucleus ambiguus or to interneurons in the caudal ventrolateral medulla (CVLM). The latter group are GABAergic neurons, which project to and inhibit sympathetic premotor neurons in the rostral ventrolateral medulla (RVLM). RVLM sympathetic premotor neurons are tonically active, and their tonic activity is critical in maintaining sympathetic vasomotor tone and resting arterial pressure. Furthermore, the tonic activity of RVLM sympathetic premotor neurons under resting conditions also permits both reflex decreases and increases in sympathetic activity in response to altered input from the arterial baroreceptors.

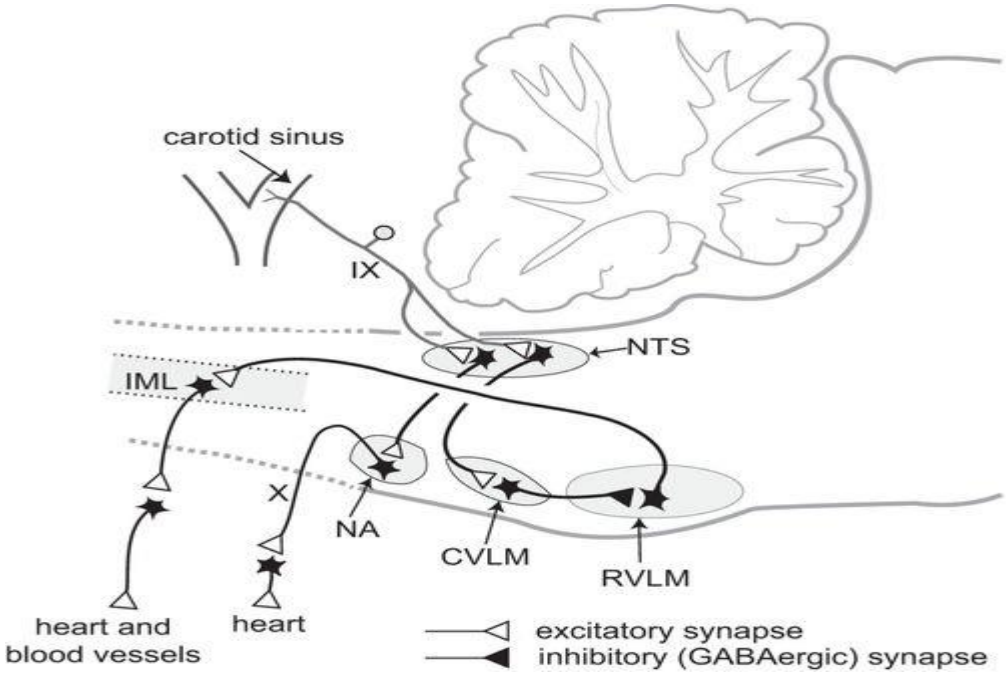


Fig. 6. Schematic diagram showing the essential pathways within the lower brain stem that subserve the baroreflex control of the sympathetic outflow to the heart and blood vessels and of the vagal parasympathetic outflow to the heart. CVLM, caudal ventrolateral medulla; IML, imtermediolateral cell column; NA, nucleus ambiguus; NTS, nucleus tractus solitarius; RVLM, rostral ventrolateral medulla; X, vagus nerve.

Some of the neurons within the baroreflex circuitry shown in Fig. 6 receive inputs from nuclei at higher levels of the brain, including the midbrain periaqueductal gray (PAG),

dorsomedial and paraventricular nuclei in the hypothalamus, central nucleus of the amygdala, medial prefrontal cortex, and insular cortex. Although the precise functions of these inputs has not been determined, it is likely that they include inputs that reset the baroreceptor reflex during different behaviors.

Blood O_2 level:

Nearly all O_2 in the blood is attached to hemoglobin. In arterial blood, >95% of hemoglobin molecules are bound to O_2, forming oxyhemoglobin, provided the Pa_{O2} is >90 mmHg. The principal mechanism that helps to maintain Pa_{O2} under hypoxic conditions (e.g., when atmospheric P_{O2} is reduced at high altitudes or when normal breathing is prevented, such as during submersion in diving animals) is the arterial chemoreceptor reflex. Chemoreceptors located in the carotid and aortic bodies are activated primarily by a decrease in Pa_{O2} (Fig. 7*A*). The main reflex effects of chemoreceptor activation are *1*) an increase in respiratory rate and depth that increases alveolar ventilation and *2*) cardiovascular effects that reduce blood flow to peripheral tissues and that also decrease heart rate and thus cardiac work, thus conserving the available O_2 (Fig. 7*B*).

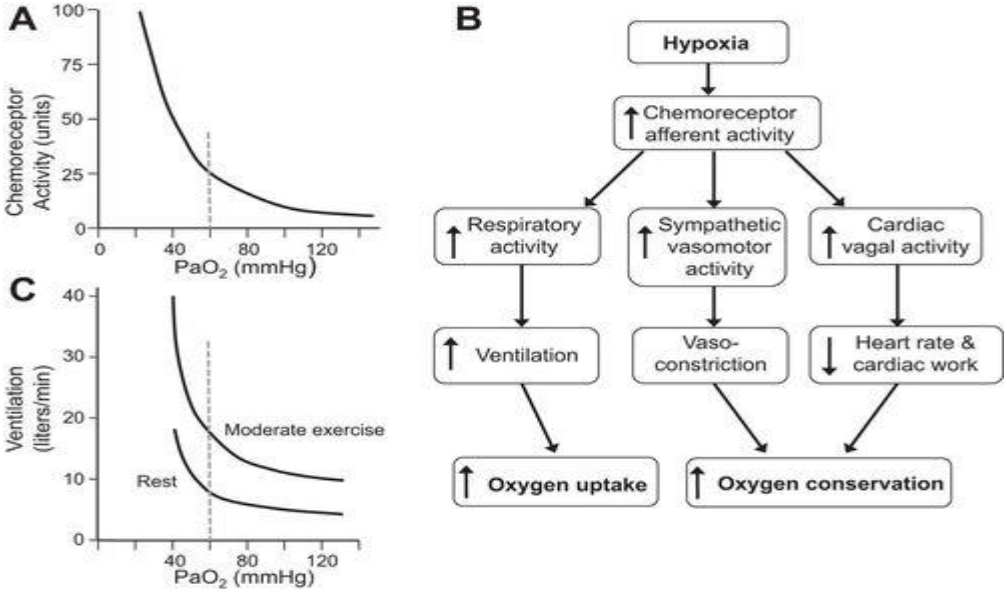


Fig. 7.*A*: curve showing the relationship between the arterial blood P_{O2} (Pa_{O2}) and the activity of a single carotid body chemoreceptor afferent fiber. *B*: flow diagram showing the reflex effects of chemoreceptor stimulation by arterial hypoxia, leading to an increase in ventilation (provided that respiratory activity can increase, as during exposure to high altitude) as well as cardiovascular reflex changes that tend to conserve the available O_2. *C*: curve showing the chemoreflex relationship between Pa_{O2} and alveolar ventilation at rest and exercise in human subjects. Note that the reflex effects on ventilation are enhanced during exercise.

Under resting conditions, carotid body chemoreceptor activity and the reflex ventilatory response do not start to increase markedly until Pa_{O2} decreases to ~60 mmHg (Fig. 7, *A* and *C*). This corresponds to the point at which the percentage of hemoglobin binding O_2 also starts to decrease rapidly, so the result is that the chemoreflex ventilatory response reflects the degree of hypoxia of arterial blood. The reflex ventilatory response to chemoreceptor stimulation is not constant but is enhanced during exercise (Fig. 7*C*), as a consequence of increased peripheral chemosensitivity. This is a further example of reflex operating properties being altered according to the behavioral state.

The essential central pathways mediating the chemoreceptor reflex are shown in Fig. 8. Chemoreceptor primary afferent fibers arising from the carotid and aortic bodies, which run in cranial nerves IX and X, respectively, terminate on secondary interneurons in the NTS. The secondary interneurons, in turn, project to a number of targets, including respiratory neurons that drive the ventilatory response as well as sympathetic premotor neurons in the RVLM that drive the sympathetic component of the reflex. In regard to the latter, there is now strong evidence that chemoreflex sympathoexcitation is mediated by both a direct input from the NTS to sympathetic premotor neurons in the RVLM as well as by indirect inputs via neurons within the central respiratory network, including respiratory neurons in the preBötzinger complex and dorsolateral pons (Fig. 8)

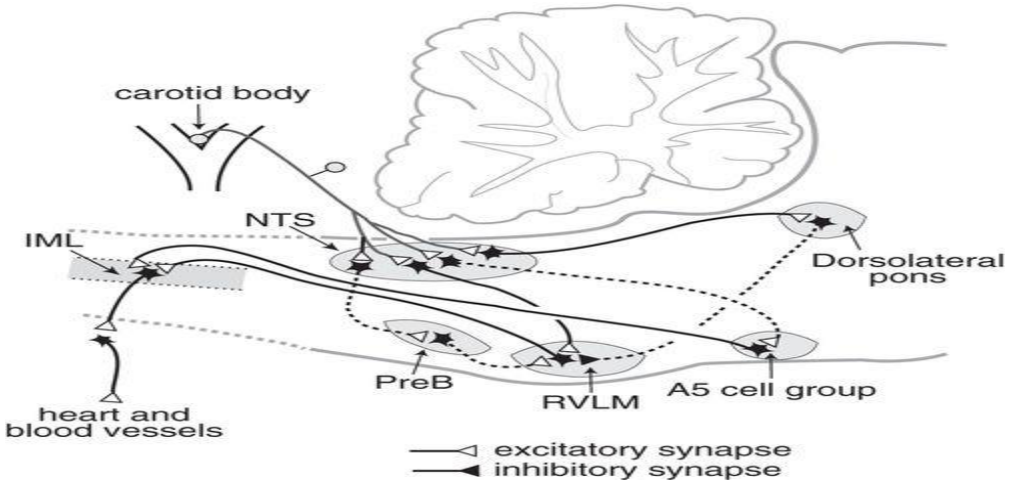


Fig. 8. Schematic diagram showing the essential pathways within the lower brain stem that subserve the chemoreflex control of the sympathetic outflow to the heart and blood vessels. The solid lines indicate direct connections that have been clearly identified, whereas the dashed lines may be direct or indirect. PreB, pre-Bötzinger cell group. For other abbreviations, see Fig. 6.

Apart from the chemoreceptor reflex, all air-breathing vertebrates have a diving reflex (also called nasopharyngeal reflex), which is another reflex that acts to conserve the available O_2. This reflex is particularly powerful in diving animals (Fig. 9*A*). The reflex is triggered by

activation of nasopharyngeal receptors, which leads to a reflex apnea, intense widespread peripheral vasoconstriction (except in the brain and heart), and a profound bradycardia (Fig. 9*B*). The cardiovascular reflex effects conserve the available O_2, which is thus preferentially provided to the brain and heart, two critical regions that cannot sustain an O_2 debt. The same pattern of reflex respiratory and cardiovascular effects is also evoked in nondiving animals, in response to stimulation of nasopharyngeal receptors by a noxious substance, such as smoke. Under those circumstances, cessation of ventilation combined with O_2 conservation will also increase the probability of survival.

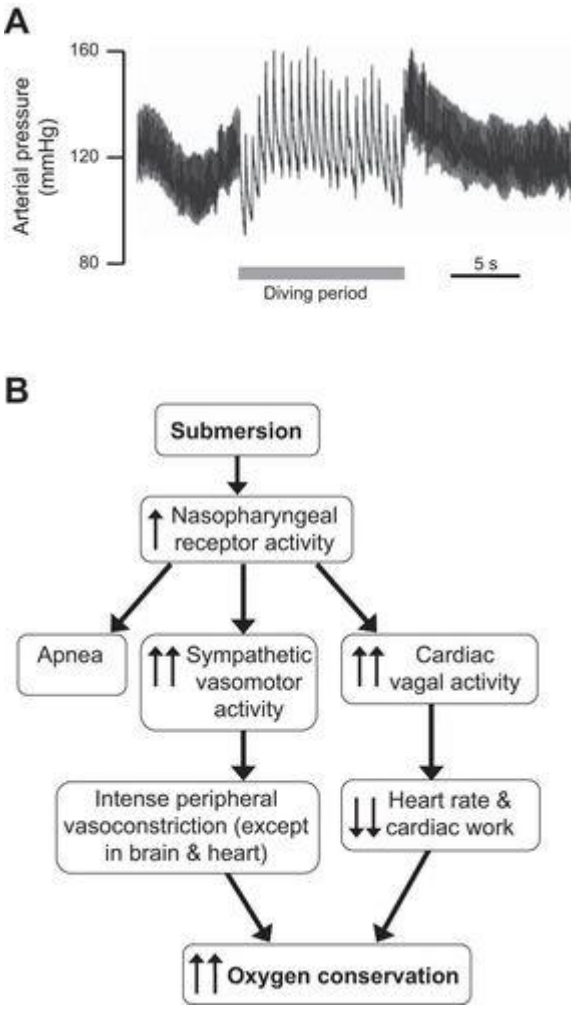


Fig. 9.*A*: example of the extreme bradycardia evoked during voluntary diving in a rat. Note that despite the extreme bradycardia (decrease in HR of ~80%), the arterial pressure is maintained, due to intense vasoconstriction. *B*: flow diagram showing the reflex effects of nasopharyngeal stimulation submersion, leading to cardiovascular reflex changes that conserve the available O_2.

Interactions between reflexes.

In most situations, more than one reflex is activated in response to a particular challenge, and hypoxia is a good example of this. For example, in diving animals, the first effect of submersion is the activation of nasopharyngeal receptors that then trigger the diving reflex, including apnea as well as the cardiovascular effects described above. The resultant hypoxia, in turn, triggers the chemoreceptor reflex (Fig. 10). The interaction between the two reflexes reinforces the vasoconstriction and bradycardia, but the normal ventilatory response to chemoreceptor stimulation is suppressed by inputs from nasopharyngeal receptors (Fig. 10).

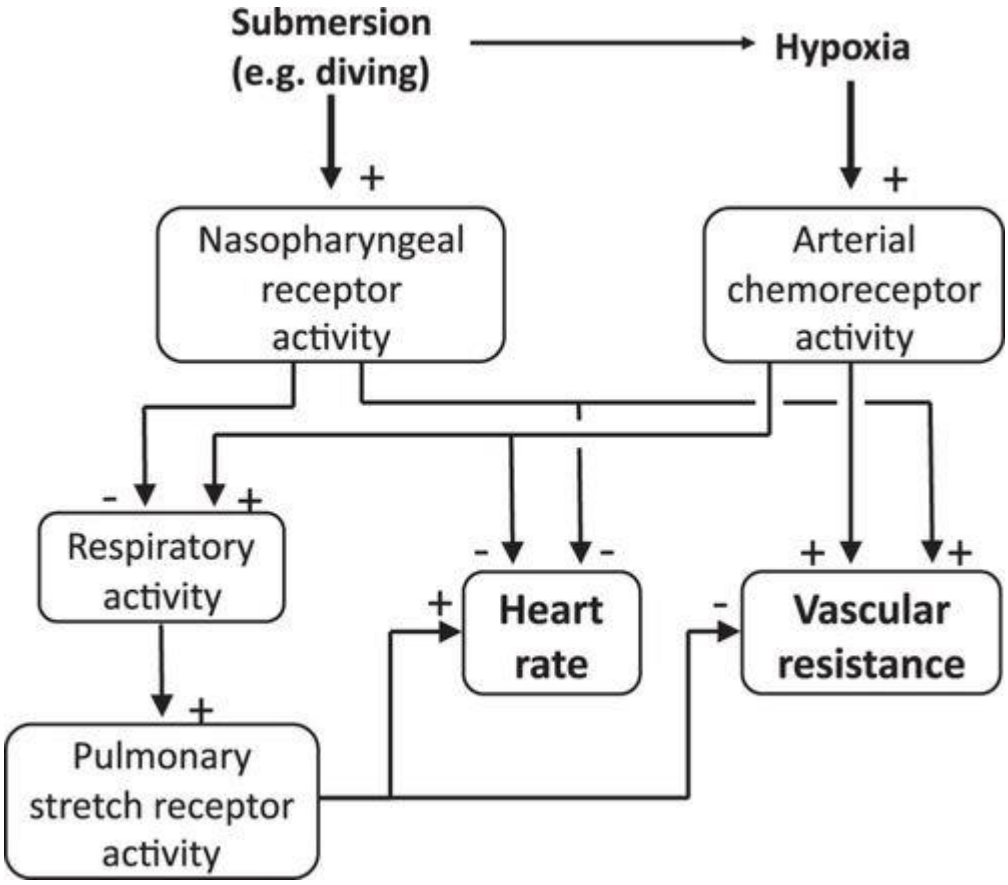


Fig. 10. Flow diagram illustrating the interaction between reflexes arising from inputs from arterial chemoreceptors, pulmonary stretch receptors, and nasopharyngeal receptors. When hypoxia occurs under conditions where respiratory activity can increase (e.g., exposure to a high altitude), the reflex decrease in HR and the reflex increase in vascular resistance (in skeletal muscle and visceral beds) is opposed by the secondary reflex effects arising from the activation of pulmonary stretch receptors, which tends to increase O_2 uptake. In contrast, when hypoxia occurs under conditions when respiratory activity cannot increase (e.g., during submersion), the primary reflex response to chemoreceptor stimulation is not opposed by these secondary effects. Furthermore, under such conditions, nasopharyngeal receptors may be stimulated, triggering reflex effects that reinforce the primary effects of

chemoreceptor stimulation, leading to greater reflex bradycardia and peripheral vasoconstriction and thus a greater degree of O_2 conservation.

In contrast, under conditions where hypoxia occurs without activation of nasopharyngeal receptors, (e.g., high altitude), chemoreceptor activation does reflexly increase ventilation, which then activates another reflex arising from pulmonary stretch receptors, innervated by afferent vagal fibers. The pulmonary stretch receptor reflex tends to increase heart rate and decrease vascular resistance, opposing the primary effects of chemoreceptor stimulation (Fig. 10). Thus, the net effect on cardiovascular and respiratory function depends on interactions between a number of reflexes, which ensures that the pattern of reflex cardiovascular and respiratory responses is optimal for the particular environmental challenge faced by the animal.

As shown in Fig. 11, the NTS and RVLM are key components of the central pathways mediating the nasopharyngeal reflex as well as baroreceptor and chemoreceptor reflexes (see above). Furthermore, inputs from a wide range of receptors that reflexly affect cardiovascular function also project to the NTS, either directly or indirectly via other relay nuclei (Fig. 11). These receptors include cardiopulmonary receptors (that respond primarily to changes in blood volume), vestibular receptors that are critical for othostatic reflexes, receptors in skeletal muscle that are activated during exercise (sometimes called "ergoreceptors"), and skin nociceptors. In addition, inputs from some of these receptors also project to the RVLM via other pathways that bypass the RVLM (Fig. 11). Ultimately, however, all of the inputs from the receptors shown in Fig. 11 converge on sympathetic premotor neurons in the RVLM. Thus, the RVLM is a major site at which interactions between different inputs regulating sympathetic activity occurs. In addition, it is a likely site, together with the NTS, at which inputs from higher centers modulate baroreceptor, chemoreceptor, and other cardiovascular reflexes (Fig. 11).

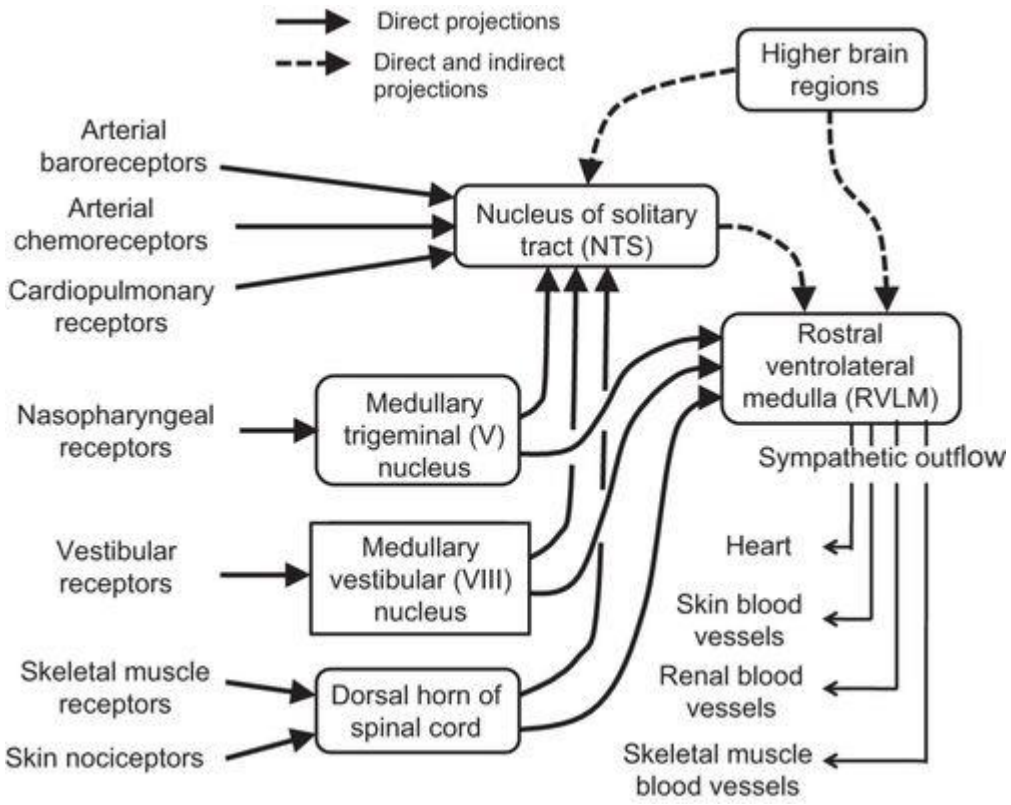


Fig. 11. Schematic diagram summarizing the essential central connections within the brain stem of different reflexes regulating the sympathetic vasomotor outflow. Note that there are direct inputs from all receptors to the NTS and that the RVLM is also a site of convergence of signals from all receptors, which are conveyed by direct inputs or indirect inputs via the NTS. There are also inputs to the NTS and RVLM from higher brain regions, which can modify the reflex responses arising from the various peripheral receptors. Finally, note that there are separate descending outputs from the RVLM, each of which exclusively or preferentially regulates the sympathetic outflow to blood vessels in different regions. This allows for a differentiated control of the sympathetic outflow according to the pattern of inputs from peripheral receptors and higher brain regions.

The reflex effects of activation of these various inputs on the sympathetic outflow are not uniform (Fig. 12). For example, baroreceptor stimulation results in reflex vasodilation in skeletal muscle vascular beds and a modest vasodilator effect on the skin blood vessels, whereas chemoreceptor stimulation has a similar effect on skin blood vessels, but evokes a powerful vasoconstrictor effect on skeletal muscle vascular beds. Such differentiated effects on the sympathetic outflows to different vascular beds reflect the fact that there are subgroups of sympathetic premotor neurons in the RVLM that preferentially or exclusively control different sympathetic outflows (Fig. 11).

Receptor activated	Muscle vasoconstrictor	Skin vasoconstrictor	Cardiac sympathetic
Baroreceptors	↓↓↓	↓	↓↓
Chemoreceptors	↑↑	↓	↓

Fig. 12. Examples of the different patters of reflex activation of the sympathetic outflow to different vascular beds in response to stimulation of arterial baroreceptors and chemoreceptors.

Blood volume.

The essential central pathways subserving the reflexes described above are contained within the lower brain stem, although they can be powerfully modulated by descending inputs from higher brain regions. In contrast, the central regulatory mechanisms defending the body against a decrease in blood volume (e.g., as a result of hemorrhage or dehydration) are located in the forebrain as well as the lower brain stem and include neural, hormonal, and behavioral components. The signals that activate compensatory responses to a decrease in blood volume are also complex, including those that are an immediate consequence of the hypovolemia as well as secondary effects that result from the hypovolemia .

For example, hypovolemia caused by dehydration results in increased blood osmolarity as well as reduced atrial and arterial pressures (as a consequence of the reduced blood volume and venous return) (Fig. 13). Apart from the reflex changes in sympathetic activity resulting from unloading of cardiopulmonary and arterial baroreceptors, the reduced arterial pressure also activates the renin-angiotensin system (Fig. 13). The increased levels of osmolarity and ANG II in the blood act on receptors on neurons in the circumventricular organs in the anterior wall of the third ventricle [especially the organum vasculosum lamina terminalis (OVLT) and subfornical organ (SFO)]. These neurons in the OVLT and SFO have direct and indirect (via the median preoptic nucleus) connections to the hypothalamic supraoptic nucleus (SON) and paraventricular nucleus (PVN), and thus activation of these neurons leads to increased sympathetic activity and vasopressin release from the pituitary (Fig. 13). In addition, these signals also trigger an increase in drinking (Fig. 13). The combined effect of all these compensatory responses is to minimize fluid loss and increase fluid intake, thus restoring fluid homeostasis.

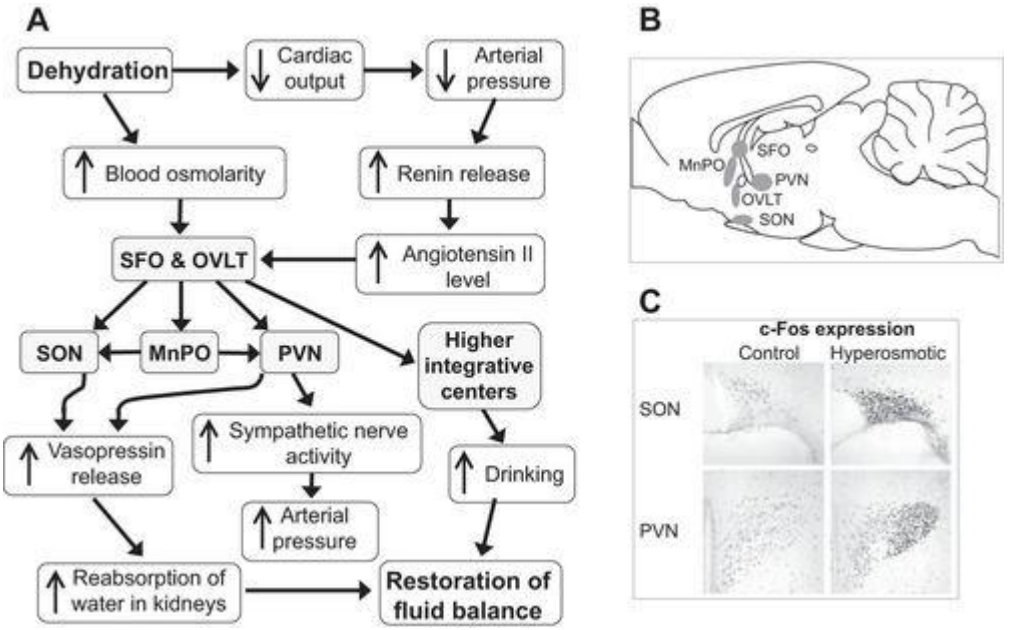


Fig. 13.*A*: flow diagram showing the sequence of events following dehydration, which leads ultimately to compensatory cardiovascular, hormonal, and behavioral responses that restore fluid balance. The subfornical organ (SFO) and organum vasculosum lamina terminalis (OVLT) are key components of these central mechanisms via their projections to the paraventricular nucleus (PVN), median preoptic nucleus (MnPO), and supraoptic nucleus (SON) in the hypothalamus. *B*: sagittal section of the rat brain indicating the locations of the nuclei referred to in *A*. *C*: examples of increased neural activity, indicated by c-Fos expression, within the SON and PVN induced in a rat after dehydration compared with a control rat.

Apart from ANG II and Na^+, other circulating substances (e.g., relaxin, leptin, and cytokines) can also activate OVLT and/or SFO neurons, and there is now strong evidence that both these circumventricular organs, together with the median preoptic nucleus, are critical sites at which these circulating substances can affect cardiovascular function. For example, circulating relaxin acts on receptors in the OVLT and SFO to stimulate vasopressin release. Second, infusion of leptin, a hormone derived from adipose tissue, induces an increase in renal sympathetic nerve activity, whereas blockade of leptin receptors in the SFO prevents this effect. Third, circulating proinflammatory cytokines act on the brain to increase blood pressure, heart rate, and sympathetic activity, and these effects are blocked by lesions of the SFO. It should be noted, however, that these results do not necessarily imply that circulating leptin or cytokines exert their effects exclusively via the SFO. For example, blockade of leptin receptors in the hypothalamic arcuate nucleus also prevents the increase in renal sympathetic nerve activity evoked by circulating leptin whereas Yu et al. found that circulating proinflammatory cytokines can also increase sympathetic activity via increases in

prostaglandin production in perivascular macrophages located in hypothalamic regions outside the circumventricular organs.

Taken together, however, the results of many studies over many years have led to the conclusion that the OVLT, SFO, and median preoptic nucleus, which collectively is referred to as the lamina terminalis, have a pivotal role in cardiovascular regulation. This region also plays an important role in maintaining increased sympathetic activity in at least some forms of experimental hypertension.

Central Mechanisms Coordinating Cardiovascular Responses with Different Behaviors: Central Command

The above examples of central cardiovascular mechanisms all involve reflexes with feedback from peripheral receptors. As stated in the Introduction, the other general mechanism of central cardiovascular control is central command, or feedforward control. Such cardiovascular responses are components of more complex and highly coordinated responses, which typically include appropriate respiratory and behavioral components.

Defensive behavior.

The ability to respond rapidly and appropriately to a threat in the external environment is critical for survival, and so it is not surprising that highly complex brain systems have evolved that subserve such defensive responses. Such responses may be triggered by a wide variety of stimuli, which may be either unconditioned salient stimuli arising from the external environment (e.g., sight, sound, or odor of a predator or prey) or else conditioned stimuli (e.g., stimuli that are normally innocuous but which an animal has learned is indicative of a threat or other stimulus that requires immediate action).

A general scheme illustrating the brain regions that are involved in generating these coordinated responses is shown in Fig. 16. Signals relating to the stimulus (e.g., sight, sound, or touch) reach the cortex, amygdala, and hippocampus via thalamic relay nuclei. The amygdala also receives inputs from the cortex and hippocampus. The amygdala, which consists of several interconnected nuclei, plays a critical role in generating cardiovascular and respiratory responses to unconditioned and conditioned alerting stimuli . The input to the amygdala from the hippocampus (Fig. 16) is essential for the expression of physiological responses to conditioned stimuli but not unconditioned stimuli. Inputs arising from unconditioned salient stimuli that project to the thalamus then project to the amygdala directly or indirectly via the cortex (Fig. 16). The direct input from the thalamus is believed to generate rapid responses to simple external stimuli (e.g., a sudden loud noise), whereas more complex stimuli require cortical processing.

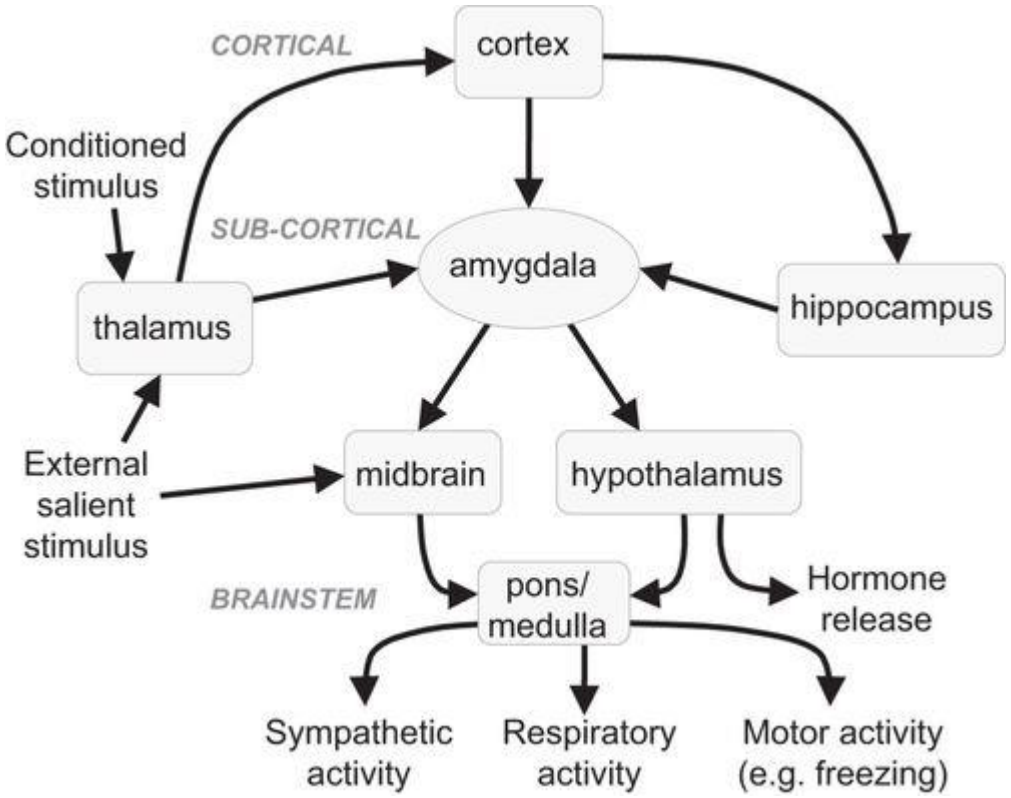


Fig. 16. Flow diagram showing the major central connections at cortical, subcortical, and brain stem levels that subserve the autonomic, respiratory, and somatomotor responses to both conditioned and unconditioned stimuli that signal a real or potential threat in the external environment.

The output pathways from the amygdala to cardiovascular, respiratory, and somatomotor nuclei in the lower brain stem responses include synapses in hypothalamic and midbrain regions (Fig. 16). One of these regions is the DMH and adjacent perifornical area (PeF), which, like the amygdala, have a critical role in generating cardiovascular and respiratory responses to alerting or stressful stimuli. Apart from the amygdala, there are also inputs to the DMH/PeF from the cortex and brain stem (Fig. 17) that also may signal alerting or stressful stimuli. The output pathways from the DMH/PeF have not been completely identified but include direct descending projections to sympathetic premotor neurons in the medullary raphe pallidus that regulate the sympathetic outflows to the heart, skin blood vessels, and BAT. These sympathetic outflows are activated in response to alerting or stressful stimuli as well as in response to a cold stress, as discussed above. In addition, there are output pathways from the DMH/PeF to other sympathetic premotor neurons that regulate the sympathetic outflow to renal, splanchnic, and other visceral blood vessels. These premotor neurons are not within the RVLM, but there is evidence that they are located more medially, within the rostral ventromedial medulla (RVMM). Thus, in summary, the sympathetic premotor neurons that drive the sympathetic outflow during arousal or defensive behavior appear to be distinct from the sympathetic premotor neurons within the

RVLM that mediate the baroreceptor, chemoreceptor, and other homeostatic cardiovascular reflexes.

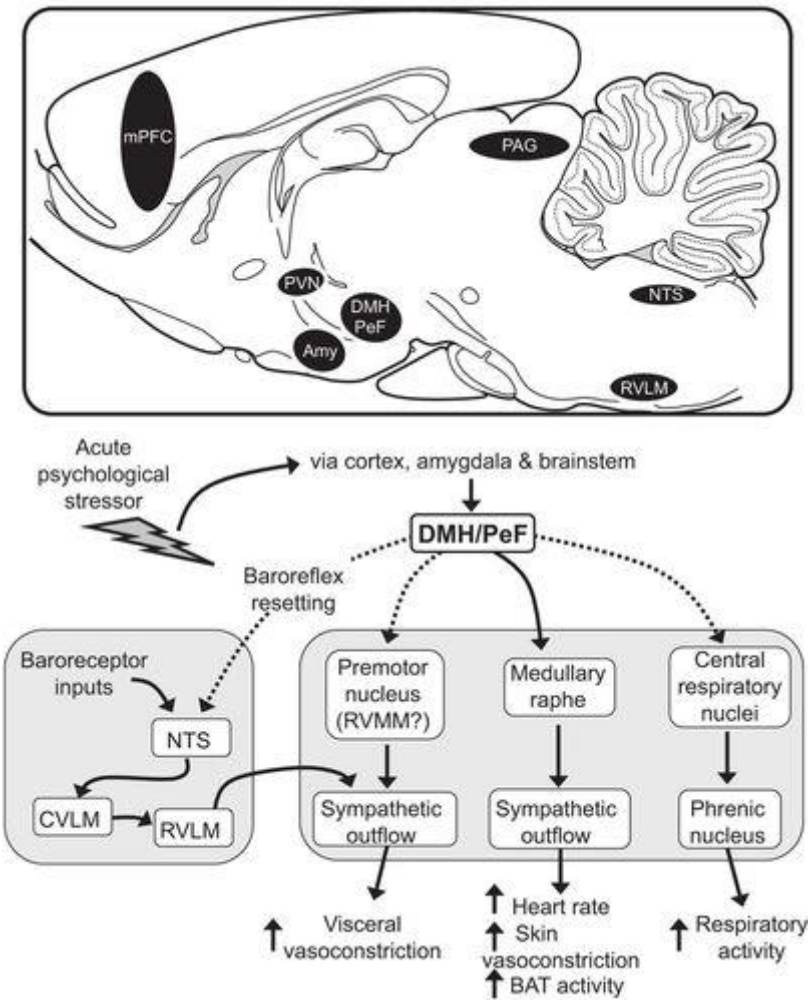


Fig. 17. Flow diagram showing the major pathways that subserve the cardiovascular and respiratory responses to an acute psychological stressor. Note that the DMH and perifornical area (PeF) are key components of these pathways, and they receive inputs from the cortex, amygdala, and brain stem that signal the real or perceived threatening stimulus. Note also that the sympathetically mediated vasoconstriction is dependent on two mechanisms: *1*) central command subserved by sympathetic premotor neurons located outside the RVLM, possibly in the rostral ventromedial medulla (RVMM), and *2*) baroreflex resetting, mediated by descending inputs from the DMH/PeF. The solid lines indicate direct connections that have been clearly identified, whereas the dashed lines may be direct or indirect. Amy, amygdala; mPFC, medial prefrontal cortex; PAG, periaqueductal gray. For other abbreviations, see previous figures.

As also discussed above, however, the baroreceptor reflex is reset during defensive behaviors, such that the sympathetic outflow continues to be regulated but within a higher operating range of arterial blood pressure and sympathetic activity. The DMH/PeF contains neurons that, when activated, reset the baroreceptor-sympathetic reflex in this way, probably via descending pathways to the NTS (Fig. 17) .

It is well established that the PAG in the midbrain is another brain region that can coordinate a wide variety of behavioral responses associated with appropriate cardiovascular and respiratory changes. The PAG is organized into longitudinal columns, including dorsolateral, lateral, and ventrolateral columns. The dorsolateral PAG and lateral PAG columns regulate what has been termed an active coping strategy, consisting of freezing and/or flight, associated with increases in blood pressure and heart rate, visceral vasoconstriction, skeletal muscle vasodilation, and increased ventilation (Fig. 18). Conversely, the ventrolateral PAG column regulates what has been termed a passive coping strategy, consisting of behavioral quiescence, associated with decreases in blood pressure and heart rate as well as sympathoinhibition (Fig. 18).

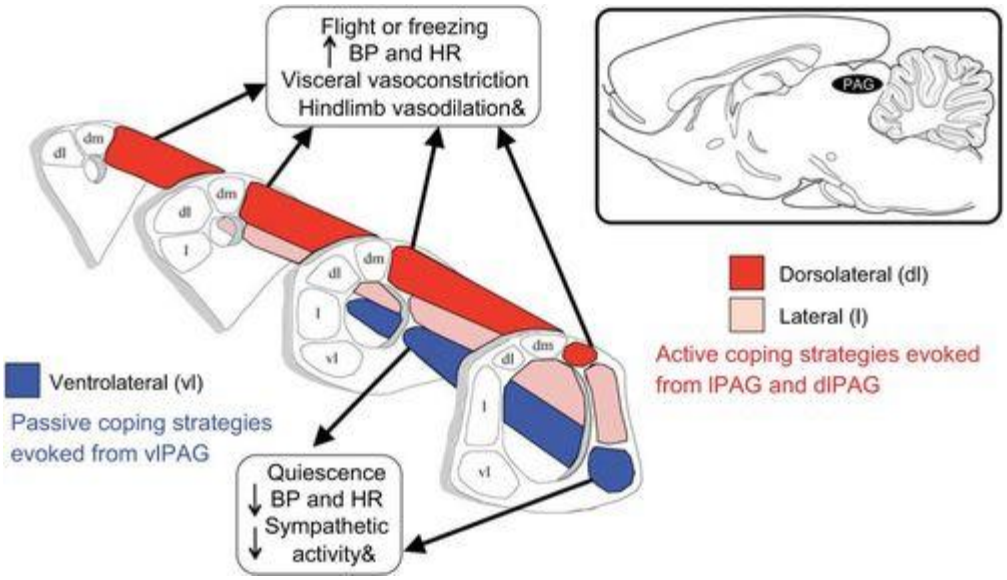


Fig. 18. Schematic diagram showing the longitudinal columns within the midbrain PAG that mediate different types of defensive responses. Neurons in the lateral (l) and dorsolateral (dl) columns generate active coping responses, characterized by flight or freezing and increases in blood pressure (BP) and HR, whereas neurons in the ventrolateral (vl) column generate passive coping responses, characterized by quiescence and decreases in BP and HR.

The precise pattern of responses generated by the PAG naturally depends on the pattern of inputs to the PAG. For example, inputs from visceral nociceptors trigger passive coping responses, whereas inputs from somatic nociceptors (e.g., a painful stimulus to the skin)

trigger active coping responses. It is also important to note that active coping responses triggered by physical stimuli (such as the above example of a painful stimulus to the skin) are generated by activation of neurons within the lateral PAG, whereas those triggered by emotional or psychological stressors (e.g., sight, sound, or odor of a predator or a perceived emotional stressor) generate a similar pattern of behavioral, cardiovascular, and respiratory responses but via activation of the dorsolateral PAG (Fig. 19).

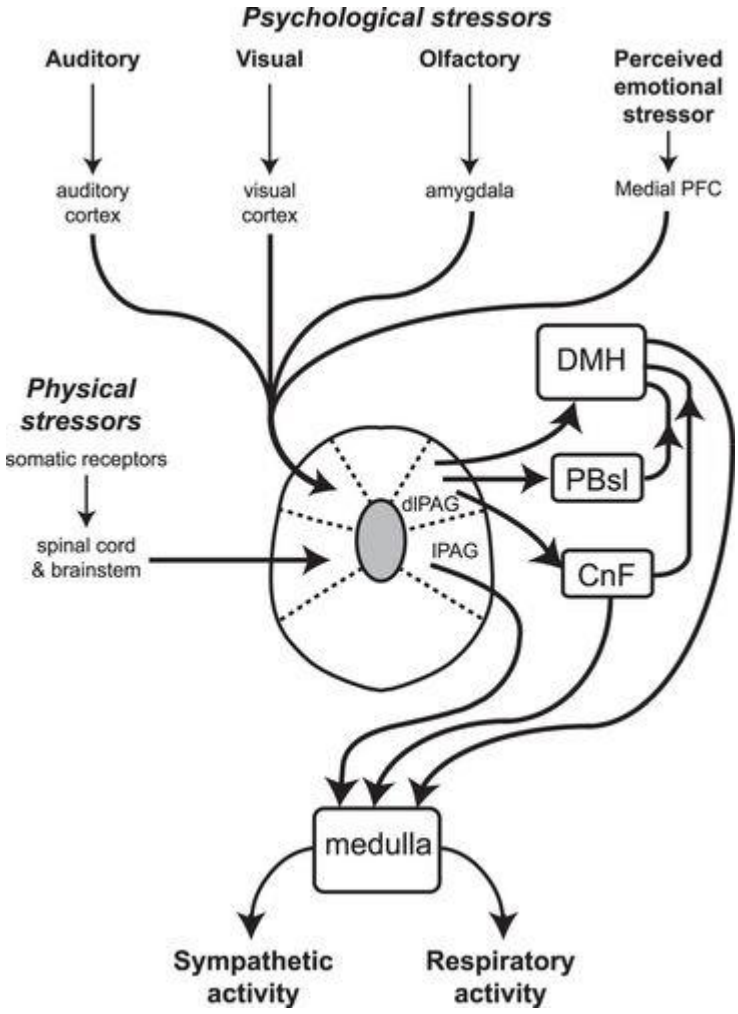


Fig. 19. Schematic diagram showing major inputs to the dlPAG and lPAG and the proposed output pathways subserving the coordinated changes in sympathetic vasomotor and respiratory activity regulated by the dlPAG and lPAG. The lines with arrows indicate connections that are either direct (monosynaptic) or indirect (polysynaptic). Neurons in the dlPAG are activated primarily by inputs related to psychological stressors, whereas those in the lPAG are activated primarily by inputs related to physical stressors. Note that the dlPAG projects to the DMH via both direct and indirect [via the superior lateral parabrachial nucleus (PBsl) or cuneiform nucleus (CnF)]. The cardiovascular and respiratory responses generated from the dlPAG are dependent on its connections with the DMH, whereas the

responses generated from the lPAG are mediated by direct descending projections to the medulla.

These differences in inputs to the lateral PAG and dorsolateral PAG are also reflected in differences in outputs (Fig. 19). Whereas neurons in the lateral PAG descend directly to the medulla where they synapse with neurons regulating somatomotor, cardiovascular, and respiratory responses, there are no direct descending projections to the medulla from the dorsolateral PAG (Fig. 19). There are, however, ascending projections from the dorsolateral PAG to the DMH (Fig. 19), and this projection is essential for the expression of cardiovascular and respiratory responses generated from the dorsolateral PAG. Thus, the DMH is a site of convergence of inputs related to psychological stressors that are relayed via the dorsolateral PAG as well as those from the cortex and amygdala, as discussed above.

A further component of the brain mechanisms that subserve the cardiovascular and respiratory responses associated with defensive behavior is the basal ganglia/colliculi system. The basal ganglia/colliculi system is phylogenetically ancient and independent of the cortex and DMH/PeF and is capable of responding to threats that require immediate stereotyped responses. In contrast, the defense systems described above that include the DMH/PeF and cortex as important components appear to be better adapted to integrating responses to more sustained threats that require cognitive appraisal.

Exercise.

The cardiovascular and respiratory changes associated with exercise have been well described, both in animals and humans. It is well established that central command plays a major role in generating these responses (Fig. 2*B*), but reflexes also have an important role.

There are many similarities in the pattern of cardiovascular and respiratory changes associated with exercise and psychological stress (e.g., in both cases, there are increases in blood pressure, heart rate, and cardiac output, vasoconstriction in the renal and splanchnic beds, and vasodilation in skeletal muscle beds). In addition, in both exercise and psychological stress, the baroreflex is reset in a similar way, as described above (e.g., Fig. 5). This naturally raises the question as to whether the cardiovascular and respiratory responses to exercise and those to stress are driven, at least in large part, by the same central mechanisms. Relatively little is known about the brain regions responsible for central command during exercise, although studies in animals have indicated that the DMH and immediately adjacent regions are activated during exercise, as is the case in psychological stress. Furthermore, neurons that contain the peptide orexin (also called hypocretin) in the DMH/PeF are activated during both exercise and stress, and it is thought that orexin neurons facilitate cardiorespiratory responses in both exercise and psychological stress.

Probable Questions:

1. Discuss the role of baroreceptors in controlling blood pressure.
2. How nervous system control blood flow?
3. Discuss interaction between different reflexes.
4. How blood volume is regulated?
5. How exercise affects blood pressure?

SuggestedReadings/References-

1. Animalphysiology-MohanP.Arora.
2. Textbookofmedicalphysiology/ArthurC.Guyton,JohnE.Hall.
3. Ganong'sreviewofmedicalphysiology.

UNIT XI

Species concept: Biological species concept, difficulties in application of biological species concept

Objective:

In this unit we will discuss about Species concept: Biological species concept, difficulties in application of biological species concept.

Introduction:

Linnaeus (1735) conceived "species" as an unchangeable unit. This monotypic or static concept prevailed till the 19th century. The system of classification followed by Linnaeus is an artificial system. While defining a species only the morphological characters were considered by him. Later Lamarck (1809) and Darwin (1859) put forwarded their evolutionary thoughts. As a result the monotypic concept was replaced by polytypic or dynamic concept. The latter concept states that the species undergo modification in course of time, in order to adapt themselves to the ecological niches and may gradually form another species under favourable conditions.

But in recent years, the approach of biology has radically changed. Today, while ascribing characters, physiological, genetic, ecological and phylogenetic points are taken into consideration. These new insights have moulded the idea of species.

Dobzhansky (1937) has defined the species as **"a group of individuals which while passing through the ordeal of evolution has been physiologically and genetically incompatible of inbreeding with other group of individuals"**. Emerson (1941) proposed that "a species is that which has evolved by reproductive isolation and a genetically distinct group of natural population". Mayr (1963) called a species as **"groups of actually or potentially interbreeding natural populations which are morphologically distinct and reproductively isolated from the neighbouring natural groups".**

Authorities on this line, however, laid much less emphasis on morphological distinctness but have given much emphasis on reproductive isolation. Mayr's definition of the species is based upon the biological parameters, such as reproductive isolation and a common gene pool, hence it is called biological species concept. Simpson (1961) viewed species as "a lineage (an ancestral-descendant sequence of populations) evolving separately from others and with its own evolutionary role and tendencies".

✓ **Some major species concepts are:**

- Typological (or Essentialist, Morphological, Phenetic) species concept
- Evolutionary species concept
- Biological species concept

1. Biological Species Concept:

K. Jordan (1905) first gave the definition of biological species concept. Later Mayr proposed the biological species concept in 1940, 1942, 1949. According to this concept, "a species is a group of interbreeding natural population that is reproductively isolated from other such groups". Mayr explained that a species has three following properties.

These are:

1. Reproductive community:

The individuals of a species seek each other as potential mates for the purpose of reproduction and the members form a reproductive community.

2. Ecological unit:

The members of a species differ each other for many features but all members together form a unit, interact as a unit with other species in any environment.

3. Genetical unit:

The members freely interbreed consisting of an intercommunicating gene pool, whereas the individual is merely a temporary vessel holding a small portion of the contents of gene pool.

This definition of biological species concept has accepted by Dobzhansky (1951) and Hanson (1981) especially for two reasons— gene pool and reproductive isolation.

Dobzhansky, Ayala, Stebbins and Valentine (1977), have postulated more or less same definition. According to them, a species as a single or more Mendelian populations between which the gene exchange is limited or prevented by reproductive isolating mechanisms.

Most modern taxonomists and evolutionists consider the biological species concept as the widely accepted species concept because the maximum workers apply this concept during their work. This concept has no fixity, and always changeable and has the potentiality for modifications required by the evolution.

- **Shortcomings of the Biological Species Concept:**

(i) Lack of information:

Due to lack of proper information systematizes face some problems when applied to some cases.

(a) The morphological differences are observed due to sexual dimorphism, age differences and genetical polymorphism and individual variation can be unmasked through the study of life history and through the population analysis. The taxonomists mostly work on preserved museum specimens. So reproductive isolation is not verified in the preserved specimens. Again biological species concept is not applicable in fossil specimens.

(b) The closely related two populations live in a continuous area but show preferences for different habitats. In this case, two populations fail to interbreed due to living in different habitats. So it is difficult to apply the biological species concept on these populations because these populations are either distinct species or failure of interbreeding due to living in different habitat. An example of drongo birds is recorded in central Africa. Species A, *Dicrurus ludwigii* are found in the evergreen rainy forest areas and species B, *D. adsimilis* are found in the open grassy land areas. They live in twoecological niches with a distance of 50 m apart and do not interbreed.

(ii) Apomictic or asexual groups:

Biological species concept is not applicable in apomictic species (i.e., asexually reproducing groups) that do not fulfil interbreeding criterion which is the most important characteristic feature in biological species concept. Apomictic groups show uniparental reproduction by parthenogenesis, apomixes and budding, etc.

Uniparental reproduction is seen in lower invertebrates and lower vertebrates. The descendants of apomictic groups are termed agamospecies or binoms, paraspecies but Ghiselin (1987), Mayr (1988a) stated that these are not considered as 'species'. To solve this dilemma, Simpson (1961), Mayr (1963, 1969) and M.J.D. White (1978) discussed the problem on the basis of discussion of Dougherty (1955) and Stebbins (1966).

Attempts to define agamospecies or asexual species with or without using the word population have not been successful. There are well defined morphological discontinuity among the uniparentally reproductive organisms. These discontinuities are produced by natural selection among the various mutants which occur in asexual clones.

(iii) Sibling or Cryptic species:

Biological species concept is not applicable in sibling or cryptic species because members of sibling or cryptic species are all alike, not separated morphologically but reproductively isolated populations.

(iv) Incompleteness of speciation:

Evolution is a gradual and continuous process. To attain a new species, especially three attributes are necessary, such as reproductive isolation, ecological difference and morphological differentiation. There are many species which represents an incomplete

stage during speciation. To apply the bio- logical species concept in these cases becomes difficult.

(v) Hybridization:

According to biological species concept, two good species fail to interbreed. If the reproduction isolation breaks down, the two good species interbreed and produce fertile hybrid.

Probable questions:

1. Define species.
2. Describe different mode of speciation with a schematic diagram.
3. Describe biological species concept with suitable examples.
4. What are the shortcomings of Biological Species concept?
5. What do you mean by sibling species, Apomictic species and Incompleteness of speciation?

Suggested Readings:

1. Darwin, C. 1859. On the Origin of Species. London: John Murray (always seek out the first edition,facsimile version, and avoid later editions).
2. Dobzhansky, T. 1937. Genetics and the Origin of Species. New York: Columbia Univ. Press (thereare several later editions, and the title changed in the last).
3. Fisher, R. A. 1930. The Genetical Theory of Natural Selection. Oxford: Oxford Univ. Press.
4. Mayr, E. 1942. Systematics and the Origin of Species. New York: Columbia Univ. Press.
5. Simpson, G. G. 1944. Tempo and Mode of Evolution. New York: Columbia Univ. Press .
6. Otte, D. and J. A. Endler (eds.). 1989. Speciation and its Consequences. Sunderland, MA: Sinaue

UNIT XII

Nomenclature rules, ICZN: The code; amendments and applications; Concept of Type

Objective:

In this unit you will discuss about nomenclature rules, ICZN: The code; amendments and applications; Concept of Type.

Introduction:

- **Zoological Classification:**

Classification is necessary to avoid confusion in the animal kingdom. It is a part of taxonomy by which we can make an orderly arrangement on the basis of the relationships. So Zoological classification can be defined as the ordering of animals into groups or sets on the basis of their relationships (Simpson, 1961). Mayr (1957, 1969) has also given a similar type of definition. According to Mayr and Ashlock (1991), a biological classification is the **"ordered grouping of organisms according to their similarities and consistencies with their inferred descent".** This definition makes classification natural because it reflects the evolutionary pathway of the organisms.

- **Purpose of Classification:**

(i) Identification of the animals and to arrange the different types of animals into groups on the basisof relationships;

(ii) To express the degree of genetic relationships or affinity between the different types of animals.

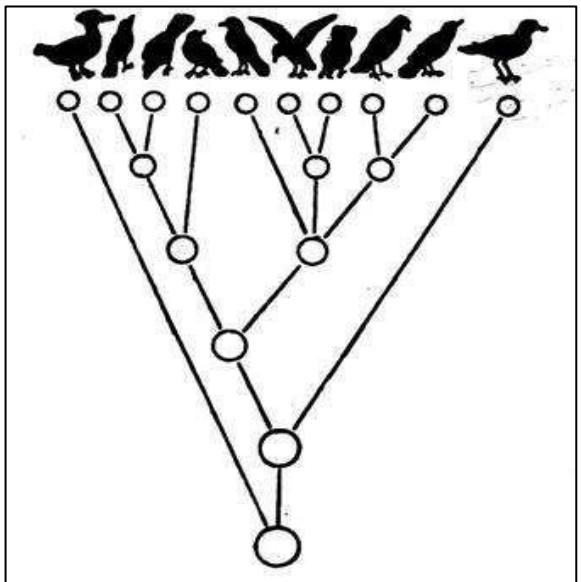


Fig. 8.1: Classification of ten different birds. Note that the classification in one hand indicates interrelationship and at the same time their origin.

- **Codes and Rules of Nomenclature:**

There are five codes of nomenclature:

(i) International Code of Botanical Nomenclature (ICBN),

(ii) International Code of Zoological Nomenclature (ICZN),

(iii) International Code of Bacteriological Nomenclature (IC Bac N),

(iv) International Code of Viral Nomenclature (ICVN) and

(v) International Code of Nomenclature for Cultivated Plants (ICNCP).

International conferences are held from time to time to update the codes and resolve the controversies,if any.

- **The rules of nomenclature framed under these codes as well as the rules set by Linnaeus are asfollows:**

1. Each organism is given only one name consisting of two words, generic and specific.
2. Though the codes are separate for plants, animals, bacteria, etc. and the same generic name can be given to different organisms belonging to these domains, it should be avoided. However the same specific name can be given to organisms belonging to different genera. Two species belonging to the same genus cannot have similar specific names.
3. The generic name is written first. It is followed by specific name and then the name of the discoverer in full or in abbreviation.
4. The specific name can be single or compound. Usually it begins with a small letter.
5. The scientific name is printed in italics. It is underlined in handwritten description. An exception is made when the biological name is written as title of paragraph.
6. The name of the author is kept in Roman script.
7. The original names were taken from Latin and Greek languages. New names are now derived either from Latin language or are Latinized. This is because Latin language is dead and, therefore, it will not change in form or spellings with the passage of time.
8. Barring obvious error or misprint, a scientific name retains its original spellings.
9. No names are recognised prior to those used by Linnaeus in 1753 for plants in "Species Plantarum" and in 1758 for animals in the 10th edition of "Systema Naturae".
10. The names of families and subfamilies should be based on name of type genus.
11. The names of subfamilies, families and other categories are not printed in italics.

They can, however, be written in bold letters.

12. When a species is transferred or revised the name of the original worker is retained but in parenthesis, e.g., *Syzygium cumini* (L) Skeels.
13. In publishing a new name the type specimen of the material is kept.

A new scientific name is thought of on the basis of its characteristic, a personality or place. The selected name is such that it has no resemblance with any previously published name.

International Code of Zoological NomenclatureBrief History of International Code of Zoological Nomenclature:

The need for a code to give a scientific name to every species was first realized by British Association for the Advancement of Science in 1842, when a set of rules were framed by it. This was also felt by American Association for the Advancement of Science in 1877. Then similar learned bodies in different countries like France, Germany and Soviet Union developed codes for their respective countries.In 1889, at the International Congress of Zoology in Paris, discussions were made to find out some common code of nomenclature. First version of the code .was adopted in the Vth International Congress of Zoology in Berlin in 1901. In the XVth session held in London in 1958, the codes were rewritten and published on 6th November, 1961 and the updated version of the code (1961) was made available in 1964 (2nd edition).

This code is concerned only up to naming of superfamily and did not satisfy the zoologists. The latest edition (4th edition) of the code was published in 1999 and its effective use has started from 2000.The International Zoological Congress elects a judicial body, called International Commission of Zoological Nomenclature which interprets or recommends the provisions of the code for classification or nomenclatural problems of the animals.

Again the International Code of Zoological Nomenclature (ICZN) formed by the International Commission of Zoological Nomenclature to see the rules and principles of nomenclature and the application of these rules for both living and fossil animals.

- **Parts of International Code of Zoological Nomenclature:**

The International Code of Zoological Nomenclature contains three main parts:

(i) The Code proper,

(ii) The Appendices and

(iii) The Official glossary.

The code proper includes a preamble followed by 90 articles which cover mandatory rules withoutany explanation.

There are three Appendices, of which the first two cover the status of recommendations and the third part of the Appendices is the constitution of the commission. The glossary contains the terms used in the codes with detailed definition.

➢ Rules of Zoological Nomenclature:

At present the naming of the animal is governed by the International Code of Zoological Nomenclature. There are many rules (Articles) concerning the Zoological Nomenclature.

Of these rules, some important ones are cited below:

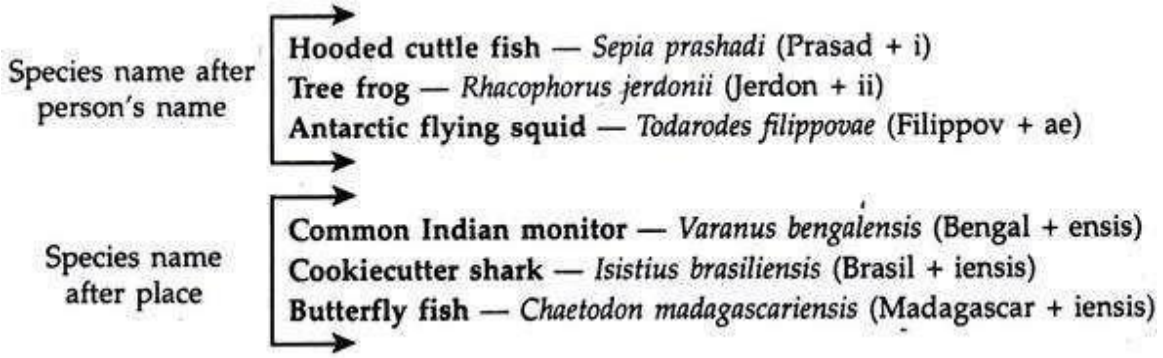
1. Zoological nomenclature is independent of other system of nomenclature. The scientific name of animals and plants must be different, and the generic name of a plant and an animal may be same, but this system is to be avoided. e.g., the generic name of banyan or fig tree is Ficus and the fig shell (a kind of gastropod shell) is Ficus. The scientific name of fig tree is *Ficus carica* or *F. indica*, etc., but the scientific name of the fig shell is *Ficus ficus* or *Ficus gracilis,* etc.
2. The scientific name of a species is to be binomial (Art. 5.1) and a subspecies to be trinomial.

   e.g., the scientific name of Indian bull frog is *Rana tigrina*. It is binomial. The scientific name of Indian lion is *Panthera leo persica*. It is trinomial. Such a system of naming by three Latin or Latinised words is known as trinomial nomenclature. Sometimes it becomes imperative to recognise subspecies within a species and is given a third specific name.
3. The first part of a scientific name is generic (L. Genus = race) and is a single word and the first alphabet or letter must be written in Capital letter. The genus must be a noun in the nominative singular. The generic part assigns a Latin noun, a Latinized Greek or a Latinized vernacular word.
4. The second part of a name is species (L. species = particular kind) name and may be a single word or a group of words. The first alphabet or letter of the species name must be written in small letter. The species name must be adjective form in nominative singular agreeing in gender with genus name which is in noun form;

Ending in species name	Ending in genus name	Full name of the species
Masculine ending (–i)	(–i/–us/–es)	Common mongoose (*Herpestes edwardsi)* River lapwing (*Vanellus duvaucelli*)
Feminine ending (–a/–e)	(–a/–e)	Golden cuttle fish (*Sepia esculenta*) Humpnosed viper (*Hypnale hypnale*)
Neuter ending (–um/–us, etc.)	(–um/–us, etc.)	Tusk shell (*Dentalium elephantinum*) Common crane (*Grus grus*) Lesser black-backed gull (*Larus fuscus*)

e.g.: The specific name (species part) indicates distinctness while generic part shows relationship.

5. If the species names are framed after any person's name, the endings of the species are i, ii and ae, or if the species name are framed after geographical place, the endings of the species are 'ensis', 'iensis', e.g.:



6. First part of a compound species-group name is a Latin letter and denotes a character of the taxon, connected to the remaining part of the name by a hyphen (-), e.g., Sole (a kind of flat fish) — *Aseraggodes sinus*-arabici. L. Sinus = recess China-rose (a kind of coloured rose)—*Hibiscus rosa-sinensis*. L. rosa = rose

7. If a subgenus taxon is used, it is included within parenthesis in between genus and species part and is not included in binomial and trinominal nomenclature, e.g.:

Name	Genus	Subgenus	Species	Subspecies
Fan shell (Bivalvia)	*Atrina*	*(Servatrina)*	*pectinata*	*pectinata*
Dussumieri's half beak (Osteichthyes)	*Hemirhampus*	*(Reporhampus)*	*dussumieri*	

8. The person who first publishes the scientific name of an animal, is the original author of a name, may be written after the species name along with the year of publication. The author's name may be in its abbreviated form. Lion—*Felis leo* Linnaeus, 1758 Lion—*Felis leo* Linn., 1758 or *Felis leo* L., 1758

9. Comma is only used between author's name and the year of publication (Art. 22. A. 2.1), e.g., the scientific name of Common octopus is Octopus vulgaris Cuvier, 1797. No punctuation marks are considered one to other ends of the name, e.g., "Octopus vulgaris Cuvier, 1797" (Not considered). No diacritic mark, apostrophe (i') and hypen (-) are used in names. In German word the umlaut sign is removed from a vowel and the letter 'e' is inserted after the vowel, e.g., mulleri becomes muelleri.

10. If the original generic name given by the first author who also reported the species name, transfers the species part from one genus to the other, the name of the

original author is put within parenthesis, e.g., Tiger: *Felis tigris* Linnaeus, 1758. At first almost all the members of the cat family were placed under thegenus-Felis.

Later the genus Felis was divided into two genera, the genus of the larger cats (tiger, lion, leopard, etc.) is Panthera and smaller cats such as jungle cat, fishing cat, golden cat, etc. are placed under the genus Felis, e.g.: Lion—*Felis leo* Linnaeus, 1758 – Lion—*Panthera leo* (Linnaeus, 1758)Jungle cat—*Felis chaus*

11. The names are not acceptable before the publication of Linnaean treatise, Systema Naturae (10th edition) which was published on 1st January, 1758 except the Nomenclature of spiders which starts in 1757. The book Aranei suecici was published by C. Clerck in 1757.

12. The scientific names must be either in Latin or Latinized or so constructed that they can be treated as a Latin word.

13. The scientific names must be italicized in printed form, or underlined in hand written or in typed forms, e.g. Indian leopard—*Panthera pardus fusca* (Meyer) [in printed form] Indian leopard—*Panthera pardus fusca* [in handwritten or typed forms]

14. All taxa from subgenera level and above must be uninominal (Art. 4.1, 4.2) and are plural nouns for names above genus, and singular nouns for genus and subgenus. Taxon 'species' may be used as singular or plural.

15. In case of animals some rules and practices are applied on the basis of zoological codes (Art. 29.2) for the formation of suprageneric taxa from superfamily to tribe, e.g.

Taxon level	Endings of the name	Examples
Superfamily	—oidea (for vertebrates) or —acea (for invertebrates)	Hominoidea Genus *Homo* (Latin) = man Genitive *Hominis* Root *Homin*—of *Homo*
Family	—idae	Hominidae [Homin + idae]
Subfamily	—inae	Homininae [Homin + inae]
Tribe	—ini	
Subtribe	—ina	

16. A family name should be based on the basis of type-genus, e.g., Chitonidae—Chiton (type genus) + idae = Chitonidae.

17. Two species under a same genus should not have the same name.

18. Nomenclature of a hybrid/hybrids cannot be considered because the hybrids are normally individuals but not population. Thus such names have no status in nomenclature. Hybrids are typically sterile and become synaptic failure during meiosis. They are prevented from back crossing with either parental species.

19. A name published without satisfying the conditions of availability (nomen nudum

= naked name) has no standing in zoological nomenclature and is best never recorded, even in synonymy.

20. A scientific valid name which is not used about 50 years in literature, then as per zoological code's provision the unused senior valid scientific name is treated as obliterated name and junior name which is used continuously in literature (atleast by 10 authors in 25 publications) becomes the accepted official name.

Remark: The disadvantage of the binominal system is its instability and the name of a species changes every time and is transferred to a different genus (Mayr and Ashlock, 1991).

21. As per the zoological code's provision (Art. 18), the species and subspecies parts of a name may be same spelling and even the second or the third component of the name repeats the generic name (tautonomy), e.g.: Scandinavian red fox—*Vulpes vulpes vulpes*

22. Synonyms are the different names for a same animal or a taxon (species or genus). If the several scientific names are given to a single animal by different scientists, the senior-most name is selected by law of priority. The senior-most or earliest name is called senior synonym (Art. 10.6) and is considered as valid species and the rest of the names are called junior synonyms and are treated as invalid species.

The leopard cat was named *Felis bengalensis* by Kerr and the same animal was named by Grey, *Felis chinensis*. Again this animal was named as *Prionailurus bengalensis* by Kerr. So the first name is Senior synonym and valid and the rest names are junior synonyms and are invalid.

The whale shark was named Rhiniodon typus by Smith in 1828 and the same was named *Rhinodon typicus* by Muller and Henle in 1839, *Micristodus punctatus* by Gill in 1865 and *Rhinodon pentalineatus* by Kishinouye in 1891. Here the first name is considered as senior synonym (*Rhiniodon typus*) and valid, the rest are junior synonyms and are invalid.

23. Homonyms mean when identical names are given to two or more different taxa. According to the zoological code (Art. 52.2) when two or more homonyms are found, the seniormost (oldest) homonym (Art. 52.2) is used and the junior-most homonyms are replaced with new names, e.g., Cuvier proposed the genus Echidna in 1797 for the spiny anteater.

Forster already proposed the genu Echidna in 1777 for morey eels. According to Law of Priority, Forster s genus claimed senior homonym and Cuvier's genus considered as junior homonym. Illiger replaced the Cuvier's name as Tachyglossus for spiny anteater in 1811.

24. Principle of priority:

Of all the rules of zoological nomenclature, it is the most controversial part to choose the correct name when two or more names of a single taxon are

discovered. Arbitrariness in nomenclature prevails since the period from 1780-1850. The taxonomists of different countries specially in Europe were unable to consult the names of different taxa during the period of French revolution and Napoleonic wars.

A large number of synonyms appeared on these days. The continuous change of names of different taxa could be prevented when priority was adopted as a basic principle of nomenclature.

Reasons for the Changes of Name:

1. **Changes dictated by scientific progress:**

(i) Change of the generic part of binomial (binominal).

(ii) Change of specific name.

(iii) Synonymising of currently accepted species names.

(iv) Analysis of species complex.

2. **Changes dictated by rules of nomenclature:**

(i) Discovery of an earlier (senior) synonym.

(ii) Discovery of an earlier (senior) homonym.

(iii) Discovery of an earlier genotype fixation.

(iv) Discovery of inapplicable type-specimen.

✓ Law of Priority:

The Law of Priority includes that any name given to a species or genus for the first time (from1st January 1758 till this day) will be accepted provided:

(i) The specific name is accompanied by an indication or in descriptional figures.

(ii) The author has followed the system of Linnean binominal nomenclature.

(iii) The author has published his contention in a scientific book or journal which has been properly printed and widely circulated.

(iv) In case of a name proposed as a substitute for a name which is invalid by reason of being ahomonym, with a reference to the name which is thereby replaced.

(v) In case of the generic or sub-generic name, it should accompany the genotype or sub-generic typefixation.

The Law of Priority in zoological nomenclature is a basic law of International Code and promotes stability. A zoological name and name of a taxon become valid if they belong to the category of seniorsynonym and senior homonym.

The Law of Priority in zoological nomenclature applies only from subspecies to family category but not to the higher categories. Priority of the zoological name and taxon are considered from the date of publication. Priority means the oldest date, month and year of the publication.

- **Binomial and trinomial nomenclature of animals**

Binomial Nomenclature:

The scientific method of naming plants and animals by applying two components where the first component is generic and the second part is specific, is called binomial nomenclature. It was evolved by Linnaeus and adopted by the International Code of Zoological Nomenclature.

Scientific names of a few common animals are given below:

Rohu—*Labeo rohita,* Pigeon—*Columba livia,*Tiger—*Panthera tigris*.

Trinomial Nomenclature:

Sometimes it becomes imperative to recognize subspecies within a species and is given a third specific name. Such system of naming is known as Trinomial nomenclature. The scientific name of the lion is *Panthera leo* (Linn.). The same species of the specimen collected in different countries shows minor differences from the original form. So a third sub-specific name becomes necessary in many cases.

The scientific name of the Indian lion is designated as *Panthera leo* persica (Linn.). A trinomen is used to recognise a subspecies. International Rules of Zoological Nomenclature recognised the trinomial nomenclature. The adoption of Latinised names for the organisms and the scheme of classification according to hierarchy are the two main themes of the classificatory secret of Linnaeus. The selection of Latin as the language of nomenclature is quite reasonable because it remains unchanged through generations and is not subjected to grammatical changes as it happens in other vernacular languages.

- **Concept of types**

A nomenclatural type (typus) is that element to which the name of a taxon is permanently attached, whether as the correct name or as a synonym. The nomenclatural type is not necessarily the most typical or rep¬resentative element of a taxon

The type (holotype, lectotype, or neotype) of a name of a species or infraspecific taxon is a single specimen conserved in one herbarium or other collection or institution. In fossil plants, the type always is a specimen. One whole specimen is to be considered as the nomenclatural type.

With respect to the designation of lectotypes, neotypes and epitypes, for purposes of priority, designation of a type is achieved only if the type is definitely accepted as such by the typifying author, if the type element is clearly indicated by direct citation including the term "type" (typus) or an equivalent, and, on or after 1 January 2001, if the typification statement includes the phrase "designated here" (hic designatus) or an equivalent

1. Holotype

A holotype is the one specimen used by the author, or designated by the author as the nomenclatural type. As long as the holotype is extant, it fixes the application of the name concerned.

2. Isotype

An isotype is any duplicate of the holotype; it is always a specimen. In fossil plants it can only be applied when a number of small plant fragments are found together that clearly belong together but that are not attached to each other. So an isotype always comes from the same locality and collection as the holotype.

3. Syntype

A syntype is any specimen cited in the protologue when there is no holotype, or any one of two or more specimens simultaneously designated as types (see Art. 37 note 1: When the type is indicated by reference to a gathering that consists of more than one specimen, those specimens are syntypes). The term is relevant for names published in old times when the type concept did not yet exist, and when authors occasionally cited and/or illustrated the specimens that they used. Example: In 1858 Bronn described *Phylladelphia strigata* Bronn, an enigmatic leaf from the Triassic of Raibl, without indicating a holotype. He based his description on a number of specimens of which he figured two. These are the syntypes (see also Kustatscher & van Konijnenburg-van Cittert, 2008).

4. Paratype

A paratype is a specimen cited in the protologue that is neither the holotype nor an isotype, nor one of the syntypes if two or more specimens were simultaneously designated as types. It usually provides information additional to the holotype. Example: *Scolopendrites grauvogelii* van Konijnenburg-van Cittert et al, 2006. Here the paratype gave details about the morphology of the sporangia and in situ spores, which were not available in the holotype.

N.B. In most cases in which no holotype was designated there will also be no paratypes, since all the cited specimens will be syntypes. However, when an author designated two or more specimens as types (Art. 9.4), any remaining cited specimens are paratypes and not syntypes.

N.B. Which specimen is the holotype, and which specimens are isotypes, syntypes and/or paratypes, can only be seen in the protologue, or derived from the protologue.

The protologue is everything associated with a name at its valid publication, i.e. description or diagnosis, illustrations, references, synonymy, geographical data, citation of specimens, discussion, and comments.

5. Lectotype

A lectotype is a specimen designated from the original material as the nomenclatural type, in conformity with Art. 9.9 and 9.10, if no holotype was indicated at the time of publication, or if it is missing, or if it is found to belong to more than one taxon.

In lectotype designation, an isotype must be chosen if such exists, or otherwise a syntype if such exists. If no isotype, syntype or isosyntype (duplicate of syntype) is extant, the lectotype must be chosen from among the paratypes if such exist. If no cited specimens exist, the lectotype must be chosen from among the uncited specimens, which comprise the remaining original material, if such exist. Examples: *Stachyopitys preslii* Schenk 1867: the syntypes in Schenk's publication were found to belong to two taxa (Kirchner & van Konijnenburg-van Cittert,1996); hence a lectotype had to be designated. For Scytophyllum waehneri (Stur) Kustatscher et al. nov. comb., only uncited specimens from the original material still existed; hence a lectotype had to be designated (see Kustatscher et al. (2011)).

So, a lectotype always comes from the original material but is designated afterwards.

6. Neotype

A neotype is a specimen selected to serve as nomenclatural type if no original material is extant, or as long as it is missing. A lectotype always takes precedence over a neotype, with one exception: When a holotype or a previously designated lectotype has been lost or destroyed and it can be shown that all the other original material differs taxonomically from the destroyed type, a neotype may be selected to preserve the usage established by the previous typification (Art. 9.14). Example: All original material of *Pterophyllum brevipenne* Kurr ex Schenk was destroyed, hence a neotype had to be designated (Pott et al., 2007). So, a neotype always comes from a later collection of material than described in the original publication, and is designated afterwards.

7. Epitype

An epitype is a specimen selected to serve as an interpretative type when the holotype, lectotype, or previously designated neotype, or all original material associated with a validly published name, is demonstrably ambiguous and cannot be critically identified for purposes of the precise application of the name of a taxon. When an epitype is designated, the holotype, lectotype, or neotype that the epitype supports must be explicitly cited, since it only has standing as long as that type is accepted. An epitype is often selected when additional information on a species becomes available later on, e.g., cuticle characters when so far only leaf gross morphology was known. Example: Bosma et al. (2009) designated an epitype for *Aachenia debeyi* Knobloch (a Late Cretaceous conifer cone scale assigned to the Doliostrobaceae), because it showed a preserved cuticle that was hitherto unknown, and that differentiated it from other taxa.

Probable Questions:

1. Define classification. State its purpose.
2. Briefly discuss about codes of nomenclature.
3. Briefly describe the International Code of Zoological Nomenclature.
4. Describe the parts of International Code of Zoological Nomenclature.
5. Write the rules of zoological nomenclature.
6. What is principle of priority? Give examples.
7. What is law of priority?
8. Give short notes on Binomial and trinomial nomenclature.
9. Define holotype, neotype, lectotype.

Suggested Readings:

1. Hillis, D., C. Moritz, and B. Mable. 1996. Molecular Systematics, second edition. Sunderland, MA:Sinauer.
2. Maddison, W. P. and D. R. Maddison. 1992. Macclade, Analysis of Phylogeny and Character Evolution, version 3. Sunderland, MA: Sinauer (Part 2, chapters 3 6, deal with modern phylogenetic theory).
3. Sanderson, M. J., and L. Hufford. 1996. Homoplasy, the Recurrence of Similarity in Evolution. SanDiego: Academic Press (a collection of essays by many authors).

UNIT XIII

Character and character states in taxonomy: Types of character: primitive and advanced, missing, polymorphic, micro, cryptic and internal

Objective:

In this unit you will discuss about Character and character states in taxonomy: Types of character: primitive and advanced, missing, polymorphic, micro, cryptic and internal

Introduction:

Taxonomy, or systematics, is the science of classification of organisms. The term *taxonomy* is derived from the Greek *taxis*, arrangement, and *nomos*, law, and was proposed by de Candolle (1813) for the theory of plant classification. *Systematics* stems from the Latinized Greek word s*ystema,* as applied to the systems of classification developed by the early naturalists, notably Linnaeus *(Systema naturae,* 1735).

In modern usage both terms are used interchangeably in the fields of plant and animal classification. There is no one definition either of taxonomy or of systematics. Indeed many workers have used these two terms more or less synonymously. Yet, it is possible to make a valid and useful distinction between them as, for example, is suggested by Hawksworth and Bisby (1988). Taxonomy in this sense includes a range of different areas from the description and naming of new taxa (nomenclature), the arrangement of organisms into a convenient classificatory system (classification), and the construction of identification systems for particular groups of organisms. Systematics, may be considered as a rather broader topic of which taxonomy is only a part, albeit a substantial one. Thus systematics includes traditional taxonomy with the addition of theoretical and practical aspects of evolution, genetics and speciation. It is also often helpful to identify separately the explicit study of the evolutionary relationships between organisms. This aspect of systematic is usually referred to as phylogenetics.

Taxonomy and systematics encroach either directly or indirectly on many other areas of science including fields as diverse as agriculture, horticulture, medicine, pharmacology, anthropology, archaeology and petrology as well as the traditional areas of botany, zoology and microbiology. Within each of these, they provide names for organisms and a framework within which these are classified. On top of this, the hypotheses that systematics generates about the evolutionary relationships between organisms form the basis of the comparative method, a central technique for drawing unbiased conclusions about the relationships among characters and between characters and environmental factors.

In the 1960s, the call for a more objective taxonomy led to the largely separate development of two new and more rigorous areas of systematics. One of these, known from its inception as **numerical taxonomy** but nowadays frequently and perhaps more appropriately referred to as **phenetics,** was largely developed and popularized by Sneath and Sokal (1973). As the name implies, numerical taxonomy consists of applying various mathematical procedures to numerically encoded character state data for the organisms under study. The products of these operations were often taken to be "unbiased' indicators of the similarity or difference between the taxa, which were in turn used to arrange taxa in a hierarchy.

The phenetic approach of numerical taxonomy is based on the assumption that the more similar two taxa are, then the more closely related they are likely to be. Although at first sight, this might appear to be a reasonable assumption, on closer inspection this can be shown to be invalid. In numerical taxonomy, character states are each assigned a numerical value and the resulting sets of values (data matrices) obtained for several characters over a group of individuals or taxa is then processed with the aim of using them to obtain unbiased taxonomic inferences. For example, by use of an appropriate algorithm it is possible to use the numerical data to define groups of **OTUs** (operational taxonomic unit) based on overall similarity, a process referred to as **phenetic clustering. Phenetic clustering techniques** are superior to cladistic methods for forming the basis of a classification. Briefly, they argue that natural (i.e. **monophyletic)** groups may not share any one single defining character as it is often purely a matter of chance whether or not a member of the group will show a character reversal such that it loses the diagnostic feature. For this reason, Sokal and Sneath advocated the use of a **polythetic** system for defining classificatory groups such that possession of at least some minimum number of a set of characters will justify placement of a taxon in that group while not requiring that any included taxon must display all the character states in the polythetic set.

Taxonomic Characters:

The essence of original taxonomic research is the analysis of material and the synthesis of the results into a classification. Although in practice these steps are often combined, they are really two separate operations. The first consists of finding and evaluating differences, the second of discovering points of resemblance. In both cases we are dealing with certain attributes of organisms which are known as taxonomic *characters.* Organisms differ from one another in many ways. Differences may be insignificant, as in identical twins, clones, and parthenogetic offspring, but more often they are extensive and numerous. Individuals of the human species differ in innumerable points, some well marked and easily described such as size and hair color, and some elusive and difficult to describe. Even greater is the number of differences between individuals representing two different species. Such individuals differ in an infinite number of characters and yet may retain certain features in common.

Mayr et al (1953) defined a taxonomic character as '*any attribute of an organism or of a group of organisms by which it differs from an organism belonging to a different taxonomic category (or resembles an organism belonging to the same category)*'.

Taxonomic characters thus have a double function:
(1) They have a diagnostic aspect as indicators of difference (emphasis on differentiating properties is particularly strong in the lower taxonomic categories).
(2) They function as indicators of relationship (this property makes them especially useful in the study of the higher categories).
Differences between organisms belonging to the same taxonomic category (male vs. female, immature vs. adult form, etc.) are not taxonomic characters.

The raw data of both numerical taxonomy and phylogenetic methods are generally taxa versus character matrices which can he manipulated in many different ways according to requirements. In this case the word **character** is used to refer to a particular attribute of a specimen that can take two or more different forms or **character states**. According to Watrous and Wheeler (1981) ' ... a character is an original form plus all of its subsequent modifications'.
The taxonomic characters can be roughly grouped into- Morphological characters, Physiological characters, Behavioral characters, and Ecological & distributional characters.

The kinds of available taxonomic characters may be somewhat arbitrarily classified under five headings: (1) morphological, (2) physiological, (3) ecological, (4) ethological, (5) geographical. Within these five classes we can distinguish additional subdivisions.

KINDS OF TAXONOMIC CHARACTERS

1. Morphological characters
    a. General external morphology
    b. Special structures *(e.g.,* genitalia)
    c. Internal morphology (= anatomy)
    d. Embryology
    e. Karyology (and other cytological differences)
2. Physiological characters
    a. Metabolic factors
    b. Serological, protein, and other biochemical differences
    c. Body secretions
    d. Genie sterility factors
3. Ecological characters
    a. Habitats and hosts
    b. Food
    c. Seasonal variations
    d. Parasites

e. Host reactions

4. Ethological characters
   a. Courtship and other ethological isolating mechanisms
   b. Other behavior patterns
5. Geographical characters
   a. General biogeographical distribution patterns
   b. Sympatric-allopatric relationship of populations

Morphological Characters. *General External Morphology.* Since external morphology has traditionally provided a primary and evident source of of taxonomic characters. They range from such superfidal features as plumage and pelage characters of birds and mammals, through linear scale counts of fish and reptiles, to the highly conservative and phylogenetically significant sutures and sclerites of the arthropod body. Animals with an external skeleton (arthropods, mollusks, etc.) present in general the greatest array and most useful range of external structural characters.

Genitalic Structures. Because of the fact that reproductive isolation is a *sine qua non* at the species level, *differences in genitalia* have been employed in many groups as the last court of appeal in delimiting specieS.

It has even been suggested by Dufour and others that a lock-and-key relationship exists as regards the copulatory structures of the males and females of those species with sclerotized genitalia. Such appears to be the case in certain groups of insects, *e.g.,* the Fulgoridae. On the other hand, genitalic characters have been found to vary in the same manner as other characters (Jordan, 1905). In general, it may be said that genital differences must be evaluated just like other characters. In groups where their significance has been proved they are usually very useful, because genitalic structures appear to be among the first to change in the course of speciation.

Internal Morphology. Anatomy provides an ambulant source of taxonomic characters in practically all groups of higher animals. However, the extent to which such characters have been used varies greatly from group to group, generally in inverse ratio to the abundance and usefulness of the external morphological characters. In many groups of vertebrates selected portions of the internal skeleton *(e.g.,* the skull) are routinely preserved and used in identification, but in general both the hard and soft parts of the internal anatomy of most animal groups are used primarily as a source of characters for the elucidation of higher categories. Paleontologists, of course, must deal almost exclusively with hard parts, and as a result they have focused attention on many useful skeletal characters in groups of animals with an internal skeleton.

Embryology. Comparative embryology offers taxonomic characters of great phylogenetic significance. Thus cleavage patterns, gastrulation, and other embryological phenomena may be characteristic for whole phyla or for series of phyla and thus assist greatly in the understanding of our highest categories. On the other hand, in such groups as insects, the total (holoblastic) cleavage of the Collembola (springtails) emphasizes the wide gap

which separates this group from the other Apterygota (primitively wingless insects) and the Pterygota, in spite of the secondary reappearance of this cleavage type in a few highly specialized parasitic Hymenoptera near the top of the insect series.

Karyology. Karyological and other cytological characters may be useful to the taxonomist, though the degree of differentiation and limits of variation in chromosomal structure must be tested in each group before: the significance of such characters can be determined. The simplest cytological character is chromosome number. This is determined by relatively simple technic involving the crushing or smearing of the test on a slide. Chromosome numbers have been recorded for thousands animals, and the results of such studies have been used as evidence of phylogenetic relationship. Chromosome morphology is being used by the plant taxonomist to a ever increasing extent. Karyology seems to be equally promising in many genera and families of animals. Dobzhansky, Patterson, and Sturtevant, as well as several other authors, have made substantial contributions in recent years to our knowledge of chromosomal variation in *Drosophila.* Such closely related species as *Drosophila pseudoobscura,* and *D. persimilis* are diagnosed more easily by their chromosome configuration than by any other feature. In the genus *Sciara* also the chromosomes have excellent diagnostic value. In a study of the Finnish bugs of the family Lygaeidae, all the genera and nearly all the 56 cytologically investigated species could be identified by their chromosome alone. Some of these cytological differences interfere with chromosome pairing and thus serve as isolating mechanisms. Gene arrangements on chromsomes have been used to analyze populations of *Drosophila, Anopheles* and *Tendipes* (= *Chironomus),* and the presence of supernumerary chromosomes to study populations of grasshoppers.

Physiological Characters. Physiological characters have been very unevenly exploited for taxonomic purposes. Yet in constancy, diversity, and significance they probably far exceed morphological characters. They have, however, the disadvantage that in most cases their study requires living organisms. Thus the most suitable subjects for this·approach have been forms with a short life cycle, small body size, or other features which make for ease of laboratory experimentation or observation. However, the array of physiological characters in general increases with the complexity of the organism. We can never hope for a complete comparative physiology for taxonomic purposes (any rnore than we can hope for a complete comparative morphology). Nevertheless, physiological characters are coming into greater use, not only as a supplement to morphological characters, but as a means for checking conclusions based on other kinds of data and as an aid in the development of sound classifications.

Metabolic factors. Up to the present time, the microbiologists, especially the bacteriologists, who have had little morphology to rely on, have made the greatest use of physiological characters both in the development of a classification and for purposes of identification. Thus enzymatic activity is an important taxonomic character, and both anabolic and catabolic reactions are used. Cell chemistry is important in the differential

ability of certain bacteria to react to certain stains (as Gram-positive or Gram-negative). Metabolic requirements are of great importance, as, for instance whether the bacteria are aerobic or anaerobic, and how cultural growth patterns and coloration develop on standardized media.

Serological Protein and Other Biochemical Differences. These have been receiving increasing attention as taxonomic tools. Serology is concerned with the nature and interactions of antigens and antibodies. Antigens are substances capable of inducing the formation of antibodies when introduced into the blood stream of other animals. Antibodies obtained from the blood sera of immunized animals are serum globulins which are produced in response to the introduction of a foreign antigen. These are the principal substances concerned in serological reactions.

Ecological Characters. Through the work of field naturalists an experimental ecologists during recent decades, it has been well established that each species of animal has its own range of tolerance of habitat, food, breeding season, and other ecological factors. No two species with identical ecological requirements can coexist in the same place (Gause's rule). Likewise it has been shown for genera and the still higher categories that each occupies a separate adaptive plateau or adaptive zone. In view of these properties of the taxonomic categories, it should be possible to define them ecologically and to diagnose them with the help of ecological characters. This is, indeed, the case, Lack showed, for instance, that each genus of Galapagos finches occupies a separate ecological zone. *Geospiza* is a ground finch (chief food, seeds); *Camarhynchus,* a tree finch (chief food, insects); and *Certhidea,*a warbler finch (chief food, small insects). Although at the present time most genera and other higher categories are defined on purely morphological grounds, it is probable that more naturally defined genera, families, etc., will result from augmenting the definition of these categories with ecological characters. Ecological characters are of even greater practical importance in the diagnosis and separation of sibling species. The three closely related nickets of the *Nemobius fasciatus* group can be identified principally by their habitats and songs. In southern Michigan *N. fasciatus* (DeGeer) lives in dry grasslands, *N. socius* Scudder in marshes, and *N. tinnuli1s* Fulton in sunny oak-hickory forests. The various species of cave swiftlets *(Collocalia)* apparently can be better identified by the composition of their nests than by morphological characters of the birds. The six European species of the *Anopheles maculipennis* group differ more in ecological than in morphological characters.

Ethological Characters. Just as morphological characteristics change from species to species and from genus to genus, thus supplying material for a taxonomic analysis, so behavior patterns change from group to group. It may be too early to speak of a science of comparative ethology, but beginnings have been made in the analysis of the unit elements of which behavior patterns of some animal groups are composed and the comparison of their evolutionary modification from species to species. This was done by

Lorenz for most species of river ducks (Anatini), by Spieth for the species of the *Drosophila willistoni* group, and by Jacobs for grasshoppers. It has been found that the behavior pattern is on the whole composed of homologous elements within a given taxonomic group, but that there is great variety in the manifestations of these elements, and that many of the modifications are species-specific.

Courtship and Other Isolating Mechanisms. Differences in mating habits are especially important behavior characters, since they are more likely to result in reproductive isolation and consequent speciation. For example (Mayr, 1942), the slugs are a group of animals which, although morphologically very similar, tend to have color phases and varieties, most of which had originally been described as good species. No two taxonomists could agree as to which of these forms were good species and which were not.

Geographical Characters. Geographical characters are among the most useful of tools for clarifying confused taxonomic pictures and for testing taxonomic hypotheses. Most sound classifications show some correlation with geographic or associated ecologic features. Essentially the taxonomist is interested in two kinds of geographical characters, (1) general biogeographic patterns, which are especially useful in the arrangment and interpretation of higher categories, and (2) the allopatric and sympatric relationship, which is most helpful in determining whether not two populations are conspecific.

A. Types of Character:

1. Primitive and Advance character

The terms primitive character and advance character are first time used by Sporne (1948). The primitive character means 'one which possessed by a present-day taxon and was also possessed by its ancestors.'

An advanced character is 'one which possessed by a present-day taxon and not possessed by its ancestors, that is, it replaced an ancestral character during evolution.'

A primitive (or ancestral) character, trait, or feature of a lineage or taxon is one that is inherited from the common ancestor of a clade (or clade group) and has undergone little change since. Conversely, a trait that appears within the clade group (that is, is present in any subgroup within the clade but not all) is called advanced or derived. A clade is a group of organisms that consists of a common ancestor and all its lineal descendants.

A primitive trait is the original condition of that trait in the common ancestor; advanced trait indicates a notable change from the original condition. These terms in biology contain no judgement about the sophistication, superiority, value, or adaptiveness of the named trait. "Primitive" in biology means only that the character appeared first in the common ancestor of a clade group and has been passed on largely intact to more recent

members of the clade. "Advanced" means the character has evolved within a later subgroup of the clade.

2. Missing characters

3. Polymorphic characters

In this case, one character becomes eliminated. If a population is polymorphic at a given gene locus, becomes divided into two or more populations. It subsequently diverges and ultimately forms a new species. Each of the daughter population or species may still display the same range of alleles. Such characters typically include certain chromosomal modifications such as inversions, translocations, enzyme polymorphisms etc. Without studying these characters, it is impossible to ascertain how long polymorphism may persist in a population.

The example of this phenomenon is the expression of certain chemicals by an organism. For many complex chemicals, a number of enzymes are necessary the enzymes are responsible for the production of many intermediate products. The end product of the said chemical is produced when all the genes are normal. Any one mutation of the gene cascade is enough to lose the ability of an organism, to synthesize the chemical. So, loss of expression of the chemical will be more possible event. This loss of one character is termed as 'Dollo character.'

4. Micro characters

A separate worry about the use of microcharacters in taxonomy concerns particular classes of microcharacter that might be especially likely to show homoplasy. These arguments centre on their small size and consequent reduced complexity which could mean that they are under the control of relatively few genes, and because with lower complexity they display fewer characters that would enable homology to be distinguished from analogy (Rieger and Tyler, 1979). Erdtmann (1954), for example, showed that while the morphology of pollen grains undoubtedly carries a considerable amount of phylogenetic information, there arc also plenty of examples in which distantly related plants produced remarkably similar pollen morphologies.

5. Cryptic & Internal characters

It is a widespread joke that traditional taxonomists spend all of their time counting the bristles on flies' legs or some other similar uninspiring enterprise. Unfortunately, there is a bit more of an element of truth in this than many practising taxonomists would care to advertise. However, what often gets overlooked is the reason why some of us feel at times that we have to perform these potentially mind-numbing tasks. The reality is that evolution has not provided every good biological species with a neat and conspicuous name-tag, and therefore in order to be able to discriminate reliably between species it may be necessary to search for the minutest of features. When the distinguishing

features are really obscure, they tend to be referred to as **cryptic characters** for obvious reasons, and the pairs or groups of species that they separate, **cryptic species.**

Probably because the classical zoology of the nineteenth century included a large element of comparative anatomy, internal organization has had an important role in taxonomy since its early days. Although more so for some groups than others. Techniques of dissection have not changed radically for many years, and if anything, training in this area has been on the decline in recent times. However. dissection may sometimes be unavoidable for the reliable identification of some animals and for many others the arrangements of internal or concealed organs may provide a wealth of new phylogenetically informative characters to supplement those available from external study. For some groups, such as nematode and nemertean worms. The extreme uniformity of external characters makes internal anatomy the only realistic path to identification or to phylogeny, and the use of male genitalia in many insect groups for separating species is widely known. However. even among the vertebrates. much of phylogenetic interpretation is dependent on detailed internal anatomy including the structure of the heart, the circulatory system, and the brain (Kemp, 1988).
Investigations of internal anatomy generally require fresh or specially preserved material and few museum specimens fit the bill. Birds and mammals are normally skinned perhaps with some skeletal remains being retained but until recently soft tissues have seldom been kept. Insects, especially the larger ones, are usually dry-mounted on entomological pins, and so forth. Nevertheless, even in long-dead dry insect specimens it may be possible to obtain some information on internal anatomy by softening and careful dissection. Modern techniques of preservation can make a big difference and one of the most important advances has been the development of critical point drying, a process in which liquid-preserved material can be dehydrated and dried without the solvent evaporating and causing distortion. Specimens dried in this way can preserve many details of soft tissue anatomy that can be revealed by delicate dissection.

Significance of Taxonomic Characters: The significance of taxonomic characters can be summarized herein as under-

i. The taxonomic characters are the expression of biology of taxon, as such its knowledge is also essential.

ii. The taxonomic characters which evolve slowly are most useful in the recognition of higher taxa whereas, those which changes rapidly are most useful in lower taxa.

iii. The same phenotypic character may vary in value and constancy from taxon and even within single phyletic series.

iv. The taxonomic characters, which are subject to parallel evolution, i.e., involving loss or reduction, should be used with utmost care.

Probable Questions:

1. Define taxonomy.
2. Write down the differences between Taxonomy and systematic.
3. Define OTUs (operational taxonomic unit).
4. What do you mean by Taxonomic characters?
5. Describe Physiological Characters of taxonomic purposes.
6. Describe Ecological Characters of taxonomic purposes.
7. Describe Ethological Characters of taxonomic purposes.
8. What do you mean by Primitive and Advance characters?
9. What do you mean by cryptic characters?
10. Write down the Significance of Taxonomic Characters.

Suggested Readings:

1. Hennig, W. (1966). *Phylogenetic Systematics.* University of Illinois Press, Urbana, Chicago, London, vii + 263 p.
2. Kapoor, V. C. and Kapoor, M. (2012). *Theory and Practice of Animal Taxonomy*. Oxford and IBH. 7th ed.
3. Kitching, I. J., Forey, P. L., Humphries, C. J. and Williams, D. (1998). *Cladistics: Theory and Practice of Parsimony Analysis* (*Systematics Association Special Volumes*). 2nd ed. OUP Oxford.
4. Mayr, E. and Ashlock, P. D. (1991). *Principles of Systematic Zoology.* 2nd ed. McGraw- Hill.
5. Quicke, D. L. J. (1993). *Principles and Techniques of Contemporary Taxonomy*. Blackie Academic and Professional.

UNIT XIV

Character state transition, environmental effect and their significances, artifacts and special characters

Objective:

In this unit you will discuss about Character state transition, environmental effect and their significances, artifacts and special characters.

Introduction:

Character state transition:

When a taxon shows two-character states, i.e. one plesiomorphic and one apomorphic character state, only two types of character state transitions can take place: Plesiomorphic to apomorphic or apomorphic to plesiomorphic. It is known as character state reversal.

In the case, when more than two-character states occur, more though has to be given to assess the possible character state transitions. In order to access character state transitions, we have to determine the type of character state an organism has. It is needed to be considered whether a character state symmetrically contribute the tree length or not. It is as follows:

a. **Ordered character or Wagner character:** In evolution, a character has changed from one state through a range of intermediates to a new final state. For example, in moths, the taxon with non-functional wings may consider to be an intermediate state in between the long winged and wingless forms.
b. **Unordered character or Fetch character:** The case where all transitions seem alike or the case where it may be impossible to determine the transitional forms, the character state is known as unordered characters. In this case, the transitional forms contribute an equal amount in tree length. It is also called non additive character.
c. **Dollo character:** Characters that seems to evolve in one direction, is called Dollo character. It is named after Dollo's law. According to this law, in evolution, characters once lost are never regained. It is also known as Dollo parsimony. In this type of character, an advanced state is only evolved once.

Environmental effect and their significances:

a. Artifacts and special characters: Formation of typical type of architecture of the artifacts of a particular species is a genetically controlled behaviour. It may be a combination of differences in the environment and experience of the constructor. So, types and features of artifact are available in the construction of diagnostic key to artifacts. Different groups of spiders can be identified and differentiated by observing their web patterns. Different groups have their unique pattern of web design.

Artifacts are capable of yielding considerable number of characters for phylogenetic interpretation. Wenzel (1991) in his study on nest architecture of neotropical social wasps, used artifacts in phylogenetic interpretation.

Cognitive artifacts: Cognitive artifacts are physical objects made by humans for the purpose of aiding, enhancing or improving cognition. Many cognitive artifacts rely on numeracy skills.

Sometimes even structures that are not made by humans, may act as cognitive artifacts. Examples of cognitive artifacts include a string tied around the finger as a reminder, a calendar, a shopping list and a computer. The behaviours of actors in a social setting can serve as cognitive artifacts.

Animal artifacts: Many organisms produce artifacts of specific type. Some of the commonly found artifacts are burrows, caddis fly case, nest of bird, hive of bees and wasps, nests of termites, etc. These artifacts sometime used as taxonomic character for the grouping of animals. They may be collected or photographed for future studies. Differences between animal artefacts reflect a combination of differences in the environment, experience of the constructor and/or underlying genetically controlled behaviours. Whilst all of these may be of interest to the taxonomist if the job at hand is the construction of a diagnostic key to artefacts, it is the last that is paramount if the objective is to use the artefacts to gain a better understanding of the phylogeny. Artifacts are in fact often capable of yielding considerable numbers of characters for phylogenetic interpretation as has been beautifully exemplified by the studies of Wenzel (1991) on nest architecture of neotropical social wasps.

Probable Questions:

1. Discuss about Character state transition?
2. What do you mean by Dollo character?
3. What do you mean by Fetch character?
4. Define Wagner character?
5. Discuss about Environmental effect and their significances in taxonomy

Suggested Readings:

1. Hennig, W. (1966). *Phylogenetic Systematics.* University of Illinois Press, Urbana, Chicago, London, vii + 263 p.
2. Kapoor, V. C. and Kapoor, M. (2012). *Theory and Practice of Animal Taxonomy*. Oxford and IBH. 7th ed.
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4. Mayr, E. and Ashlock, P. D. (1991). *Principles of Systematic Zoology.* 2nd ed. McGraw- Hill.
5. Quicke, D. L. J. (1993). *Principles and Techniques of Contemporary Taxonomy*. Blackie Academic and Professional.

UNIT XV

Taxonomic key: types and their role in classification

Objective:

In this unit you will discuss about Taxonomic key: types and their role in classification

Introduction:

Taxonomic key:

A taxonomic key is a tool that is used to identify different types of organisms. The key consists of a series of choices, based on observed features of the plant/animal specimen. It provides a choice between two contradictory statements resulting in the acceptance of one and the rejection of the other.

A single pair of contradictory statements is called a couplet and each statement of a couplet is termed a lead. By making the correct choice at each level of the key, one can eventually arrive at the name of the unknown plant/animal.

Types of Taxonomic Keys:
There are basically two types of keys viz. Dichotomous and Poly clave/Synoptic keys.

1. Dichotomous Keys: Keys in which the choices allow only two (mutually exclusive) alternative couplets are known as dichotomous keys. In constructing a key, contrasting characters are chosen that divide the full set of possible species into smaller and smaller groups i.e., the statements typically begin with broad characteristics and become narrower as more choices are required. Each time a choice is made, a number of species are eliminated from consideration and the range of possible species to which the unknown specimen may belong is narrowed. Eventually, after sufficient choices have been made, their range reduces to a single species and the identity of the unknown plant is revealed. Dichotomous comes from the Greek root dich meaning "**two**" and temnein meaning "**to cut** ". Couplets can be organized in several forms. The couplets can be presented using numbers (numeric) or using letters (alphabetical). The couplets can be presented together or grouped by relationships. There is no apparent uniformity in presentation of dichotomous keys.

Types of Dichotomous Keys: There are two types of dichotomous keys. They differ in the method by which the couplets are organized and how the user is directed to successive choices.

i. **Indented keys (Yoked keys):** Indents the choices (leads) of the couplet an equal distance from the left margin. The two choices of the couplet are usually labelled 1 and 1′ or la and lb. It is not necessary that the choices are numbered, but it helps. The user goes to the next indented couplet following the lead that was selected.

This type of key is advantageous in the sense that the relationship of various divisions is quite apparent to the eye and can be used in reverse also. It serves good purpose for keys to higher taxa or comparative keys.

ii. **Bracketed keys:** Provides both choices side-by-side. The choices of the couplet must be numbered (or lettered). It is very helpful if the previous couplet is given. This key has exactly the same choices as the first example. The choices are separated, but it is easy to see the relationships. While this key might be more difficult to construct, it gives more information to the user.

When properly constructed one can quickly and easily run through this key both forward and backward. It is also more economical in terms of space because it is un-indented.

Advantages of dichotomous keys:

a. Similar specimens are grouped together
b. It is harder to get lost your place.
c. They are faster to use.
d. It is easier to retrace your steps if you make a 'wrong turn'.

Disadvantages of dichotomous keys: A key may be difficult to use at times because-

a. The key may not include all potential variations in the species.
b. The key may rely on features not present in that season.
c. The key may not include 'all' species of interest.
d. One may misinterpret a feature or make a mistake.

2. Poly Clave Keys: Another type of key, which is relatively a new alternative to dichotomous keys and becoming increasingly popular, especially because of the ease of computerizing them, is termed multiple access or poly clave or synoptic key. The advantage of these keys is that they allow the user to enter the key at any point.

This key is based on the identification of organisms by a process of elimination. In a written poly clave key, there is a series of characters and character states. Each state is followed by a number or code for the species that possess that feature.

The user needs to select any character and then copy down the list of species that possess the feature. Then the user has to select another character and eliminate any

species that is not common to both lists. This process has to be continued until the specimen is identified.

It's easy to imagine how these keys are computerized. Consider a series of standard playing cards. Imagine each card has four holes punched into it along the top margin. If the card is a spade, we cut the first hole through the margin; if a club, the second hole is notched to the margin; a heart the third hole is notched; and finally, if it is a diamond the fourth hole is notched.

Further imagine that along the bottom of the card we punch 14 holes (2 – 10, J, K, Q, A) and cut a notch for the appropriate number. Thus, the Queen of Hearts will have a notch cut into the third hole on the top of the card, and the Queen notch on the bottom of the card.

Now, let's use our punch card deck of cards to identify an unknown card. Shuffle another deck of cards and pick any card. Let's assume that this "**unknown**" card is the Ace of Spades. To identify this unknown, we analyse the characters and two are obvious, suit and number.
Let's start with suit – take a long needle and stick it through the "**spades hole** ". Since, only spades are notched, the other suits will remain on the needle and spades will drop out of the deck.
Now, collect the spades cards and put a needle through the next character, the Ace and, viola, the Ace of Spades falls out. This is the general principle of how the computerized version of poly clave keys work. The main difference is that a computer allows for countless holes (characters) and notches (states) to be included and does the needlework for us.

Advantages of Poly clave keys:
The advantages of a poly clave (multiple-access) keys are:

a. They are easy to use.
b. They allow multi-entry i.e. the user can start anywhere. This is a significant advantage because the user can rely on characters that are most easy to observe, rather than having to deal with characters that may not be present in the specimen or are poorly developed.
c. They are order-free i.e. the user can work in any direction with any direction with any character.
d. They are easily computerized. In fact, these keys are most used in this form. Paper versions are typically large and unwieldy because each character needs to list all possible taxa.

Disadvantages of Poly clave keys: It is based on specimen and their availability. They have generally been written only for a limited number of taxonomic groups.

Probable Questions:

1. Define taxonomic key with example.
2. Describe elaborately about different types of Taxonomic Keys.
3. What is Dichotomous Keys?
4. What do you mean by Bracketed keys?
5. Write short notes on Poly Clave Keys.
6. Write down the advantages of Poly clave keys.

Suggested Readings:

1. Hennig, W. (1966). *Phylogenetic Systematics.* University of Illinois Press, Urbana, Chicago, London, vii + 263 p.
2. Kapoor, V. C. and Kapoor, M. (2012). *Theory and Practice of Animal Taxonomy*. Oxford and IBH. 7th ed.
3. Kitching, I. J., Forey, P. L., Humphries, C. J. and Williams, D. (1998). *Cladistics: Theory and Practice of Parsimony Analysis* (*Systematics Association Special Volumes*). 2nd ed. OUP Oxford.
4. Mayr, E. and Ashlock, P. D. (1991). *Principles of Systematic Zoology.* 2nd ed. McGraw- Hill.
5. Quicke, D. L. J. (1993). *Principles and Techniques of Contemporary Taxonomy*. Blackie Academic and Professional.

UNIT XVI

Phenetic method of classification - Numerical phenetics and numerical taxonomy; Preparation of data matrix and similarity matrix using distance method (Manhattan distance and Euclidian distance); Cluster analysis (different methods

Objective:

In this unit you will discuss about Phenetic method of classification - Numerical phenetics and numerical taxonomy; Preparation of data matrix and similarity matrix using distance method (Manhattan distance and Euclidian distance); Cluster analysis (different methods).

Methods of classification-

Biological classification is always hierarchical, eg. several genera like *Vulpes* sp., *Canis aureus* (golden jackel), *Canis lupus* (grey wolf) combine to make a family-Canidae; several families combine to make order- Carnivora; several orders combine to make class- Mammalia; several classes combine to make phylum- Chordata and several phyla combine to create kingdom- Animalia. Now question arises what procedures or methods should be applied for the biological classification? In 1950's two new methods or approaches to systematic arose which are used to classify species into groups-

1. **Phenetic**

2. Cladistic or phylogenetic methods

Phenetics (as numerical taxonomy) emerged in the late 1950s. Its origin was associated with, Charles Michener, Arthur Cain, and especially Robert Sokal and Peter Sneath. In biology, phenetics (Greek: phainein - to appear) also known as taximetrics, is an attempt to classify organisms based on overall similarity, usually in morphology or other observable traits, regardless of their phylogeny or evolutionary relation. It is closely related to numerical taxonomy, which is concerned with the use of numerical methods for taxonomic classification.

Phenetic method groups species according to their observable attributes i.e. the species are grouped by their similarity with respect to observable attributes. If two species look more like each other than either resembles any other species, they will usually be grouped together in a phenetic classification. The full classification consists of a hierarchy of levels, such that the members of different groups at higher and higher levels have decreasingly similar appearances. Example- A wolf and a dog (Same genus)

look phenetically more alike than do a wolf and a dolphin (same class). Almost any observable attributes of organisms can be used for this purpose. Fossil vertebrates can be classified phenetically by the shape of their bones, modern species of fruit flies by the pattern of their wing venation and birds by the shape of their beaks or colour pattern of their feathers, even some species can be grouped according to the number, shape or banding pattern of chromosomes by the immunological similarity of their proteins or by any other measurable phenotypic property.

Phenetic analysis does not distinguish between plesiomorphies- traits that are inherited from an ancestor and apomorphs- traits that evolved new in one or several lineages. Consequently, phenetic analysis is liable to be misled by convergent evolution and adaptive radiation.

Numerical phenetics and numerical taxonomy-

Charles Michener and Robert Sokal in 1950 used numerical algorithms to create diagram of overall similarity among species. Such a diagram or phenogram was intended as an objective basis for classification and their approach came to be known as phenetics. Numerical phenetics is the methodology of assembling individuals into taxa on the basis of an estimate of unweighted overall similarity. Numerical taxonomists recommended measuring as many characters as possible. The more characters that are measured, the more likely it is that peculiar individual characters will be averaged out and the better founded and more natural the classification will be. The different measurement of distance (distance measurement by Euclidean distance or MCD) can give different hierarchies, forcing the pheneticist to make a subjective choice.

Claims made by numerical phenetics-

- Application of the method requires no previous knowledge of the studied taxon and its literature, only the ability to make observations and quantifications.

- Because these methods produce strictly repeatable results, any beginner could make classifications as good as those of a specialist.

- After a decade or so the application of phenetic methods, all important questions in systematics would be answered and work could be done by technicians trained solely to collect data from specimens.

Measurement of phenetic classifications-

A. Distance methods-

Preparation of data matrix and similarity matrix using distance method (Manhattan distance and Euclidean distance)

Suppose that we wish to classify a group of fly species. We shall start with the simple case of two characters such as the length of a certain wing vein and length of the tibia of

the hind-leg. Suppose five species have been measured for the above two characters. The x-axis is the measurement of each species for length of a wing vein and the y-axis is the measurement for length of tibia. The distance between two species on the graph or matrix is the phenetic distance between them. If species 1 and 2 differ by x units in character A and y units in character B, then Euclidean distance = $\sqrt{x^2+y^2}$ i.e. measured by Pythagoras theorem and MCD (mean character distance) = (x+y)/2.

Manhattan distance- For phylogenetic character data, raw distance values can be calculated by simply counting the number of pairwise differences in character states i.e. The distance between two points is the sum of the (absolute) differences of their coordinates.

Given below are the measurements of two characters (A and B) among three species.
Calculate the Euclidean distance and the mean character distance for the three species pairs.

	Species 1	**Species 2**	**Species 3**
Character A	2	4	3
Character B	2	3	5

Solution:

species 1 and **2** differ by *x* units in character A: 4 – 2=2 = x units

and *y* units in character B: 3 – 2 = 1 = y units

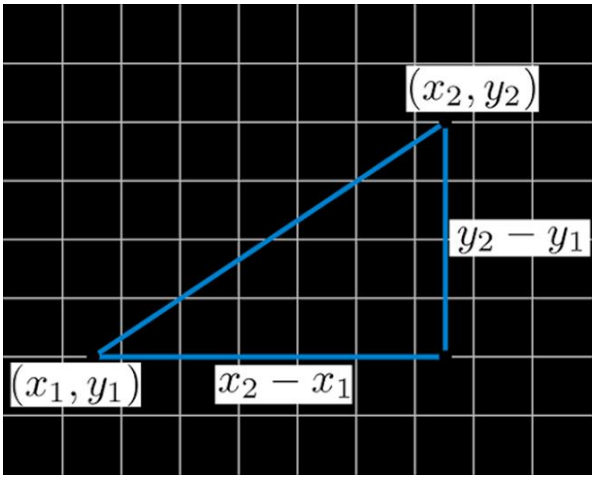
so the points of representation in graph will be (2,1)

then Euclidean distance between Species 1 & 2 = $\sqrt{x^2+y^2}$ i.e. measured by Pythagoras theorem = $\sqrt{(2)^2 + (1)} = \sqrt{4+1} = \sqrt{5} =$ **2.23**

and MCD (mean character distance) = (x+y)/2 = (2+1)/2= 3/2= **1.5**

Similarly, do it among **species 1** and **3 and species 2** and **3**

And comment which two species are close to each other….



B. Cluster method-

Cluster analysis or clustering is the task of grouping a set of objects/ species in such a way that objects/ species in the same group are more similar to each other than to those in other groups.

The cluster analysis method is called the unweighted pair-group method using arithmetic averages (UPGMA) and is a commonly used distance method. This is based on averaging and cross-averaging method also known as average linkage. According to this the lowest similarity coefficient close together. Table 1 shows the data matrix based on Manhattan distance between the taxa. Figure 1 describe the phenogram made, based on Manhattan distance of demonstration insects.

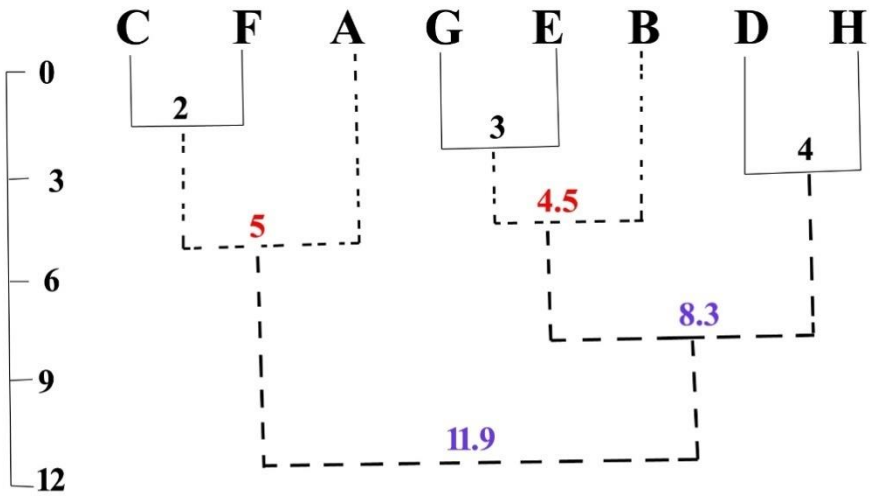


Fig. 1 Phenogram. Manhattan distance of the demonstyration insects. Primary clusters are indicated by solid lines. Secondary clusters by dotted lines and tertiary clusters by dashed lines. The scale on the left is a distance measure.

The first step in cluster analysis is to find **primary clusters**, i.e. those which consist of only two taxa. Since distance measures signify greater similarity with smaller numbers

(shorter distances), the first primary cluster is found by locating the lowest number in the rearranged matrix. C and F form a cluster at value 2, and G & E form a second primary cluster at value 3. Although taxa B & F show a difference of 4, F is already a member of a primary cluster. D & H, however can and do form a third primary cluster to group at value 4. In any case, a primary cluster always consists of two taxa that are closer to one another than either is to any other taxon.

Secondary clusters contain more than two taxa but only one primary cluster. Starting with a primary such as C-F, one scans the columns or rows for C & F for an unclustered taxon with the lowest average distance value to C & F, here that is 5 and 5 for taxon A. If A does not yet belong to another cluster, indicated by another value of 5 or less in the A column, it joins the cluster C-F to form a secondary cluster at an average value of 5 [(5+5)/2= 5]. A comparison of pairs of values in the columns of the primary cluster G-E reveals that unclustered taxon B is closest with values 5 and 4. These two values are now averaged, and taxon B joins G-E to form a secondary cluster at the level 4.5. Although taxon G is most similar to the last remaining primary cluster, D-H, with values of 5 and 7, taxon G is already a member of a secondary cluster, and one must conclude that the primary cluster D-H is not part of a secondary cluster.

Table 1. Data Matrix

Sl no.	Characters/trait	Taxon							
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	**PnLb** (pronotum medially without/with an incomplete/ complete transverse grooves producing lobes)	0	1	0	3	1	0	1	2
2	**CsMg** (costal wing margin curved in/ out at base)	0	1	0	1	1	0	1	1
3	**NkSw** (Neck curved in/out behind eyes for about/ more than length of eye)	1	2	0	1	3	0	2	1
4	**ClyL** (Clypeus projecting beyond rest of head)	0	1	0	0	1	0	0	0
5	**ColC** (Collar complete/ incomplete)	1	1	1	1	1	1	1	0
6	**PnEx** (pronotal expression absent from posterior angle/ rounded / acute apically	0	0	2	0	0	1	0	0
7	**CrVn** (cross vein absent/ present in wing membrane)	0	0	0	1	1	0	1	0
8	**MmbL** (wing membrane shorter / longer than head and pronotum together)	0	1	0	2	1	0	1	2
9	**PnHm** (pronotal hind margin straight/ lightly curved/ deeply curved)	0	2	0	1	2	0	1	1
10	**OcP** (ocelli on an/ less than imaginary line drawn between posterior eye margins)	0	1	0	1	1	0	1	2
11	**A3Sw** (antennal segment 3 not swollen/ swollen apically)	0	0	0	1	0	1	0	1

12	**aFSp** (forefemur with two/ one spines apical to large spine)	1	0	0	0	0	0	0	0
13	**bFSp** (spine near base of forefemur absent/ present)	0	1	0	0	0	0	0	0
14	**TbSp** (foretibia without/ with a spine)	0	1	1	0	0	2	0	0

While many taxa with sequentially higher distances may join a secondary cluster, no secondary

Cluster can contain more than one primary cluster.

Tertiary clusters are clusters of clusters. They are formed joining several primary and secondary clusters together by cross averaging values to complete the analysis. In this case, pairs BEG and DH cluster at 8.33, the smallest average value of the three possible pairs. The two remaining clusters, ACF and GEBDH (Table 2), are joined in the final phenogram by the same cross averaging process. That is, the similarity coefficients of A with each member of group GEBDH are summed, the same sum is figured for C and F and the average of the 15 similarity co-efficients is calculated. The resulting average is 11.9, the level at which the last separate clusters are. The number of values to be averaged at any stage of clustering is the product of the

numbers of taxa in the two groups that are to be clustered. By the time the final phenogram is complete, every number in the distance matrix has been used. Table 3 shows the minimatrices determining the tertiary clusters.

Table 2. Tertiary cluster

	G	E	B	D	H
A	8	11	11	11	11
C	11	14	12	14	14
F	11	14	12	12	12
178/15 = 11.9					

Table 3. Minimatrices determining tertiary clusters

	A	C	F		A	C	F		B	E	G
B	11	12	12	D	11	14	12	D	10	8	5
E	11	14	14	H	11	14	12	H	10	10	7
G	8	11	11	74/ 6 = 12.33				50/6 = 8.33			
104/9 = 11.56											

Difference in the application of phenetic and cladistic classification-

- Phenetic classification ignores evolutionary relations and classifies species by their similarity in appearance. Numerical phenetic classification groups species using as many characters as possible and averaging them regardless of their evolutionary meaning. Whereas cladism ignores phenetic relations and classifies species by their common ancestry. It selects, from all characters shared between species, one special class of characters- the shared derived characters- and use only those characters to group the species; all other kinds of characters are ignored in cladistic classification.
- Phenetic classification is ambiguous (vague, hidden) because more than one way of measuring phenetic similarity exists and the different measures can disagree whereas cladism is unambiguous because only one phylogenetic tree of all living things exists.
- Phenetic classification has the advantage that it is not subject to revision when new phylogenetic ideas are put forward, whereas phylogenetic inference is uncertain.

Weak points of phenetic methods-

1. **Practical difficulties:**

- This method is **laborious**, and time consuming because of their insistence on the equal weighting of all characters. And due to assembly of large number of characters the true characters become diluted with useless characters as well.
- Their studies depend upon the availability of large number of characters, but some taxa do not have rich supply of characters and **morphological variability** is not available, e.g Arthropods and sponges.
- **phenetics classification** can't be improved gradually, because addition of new characters requires a new analysis.
- They do not provide any solid **contributions to the classification** of any mature group or to the classification of higher taxa.

2. **Inability to meet the claims of objectivity and repeatability**:

- This method is unable to avoid **subjectivity** (personal choice) in the selection of characters that are analysed and in the handling of variations. They do not have any meaning full OTUs (Operational taxonomic units) to assemble phena and thus a failure to apply biological criteria at the species level leads to the unreasonable classification.
- The choice of **clustering method** is also based upon personal preference, as a consequence, different clustering methods yield different fits by cophenetic

correlation coefficients to the same resemblance matrix. This imparts a great deal of subjectivity to the phenetic approach.

- The choice of **different algorithms yields different results**, which is against their claim that if two systematics independently working on same group would achieve the same results.
- They claim that if larger number of characters is used the selection of particular characters become irrelevant, but adding new characters derived from different components of the phenotype or genotype, almost changelessly affects the classification.

3. **Other unsubstantiated assumptions:**

    - Pheneticists are inconsistent in application of their own **non weighting principle**, as giving all characters the same weight is, of course also a method of weighting. Sokal and Sneath devote an entire section of their work to the principles governing the proper selection of characters.

    - They have the assumption that the amount of unweighted phenetic similarity reflects an equal amount of underlying similarity of genotype and can be safely used for ranking. This assumption proved wrong later on by evidences, e.g they forced to assign **sibling species in single OTU**.

    - Pheneticists state that similarity is to be decent from a common ancestor. but a phenogram cannot provide a good estimate of Phylogeny. Because parallel, convergent and reversed characters are treated as equivalent similarities in purely phenetics method. Only cladistic analysis can detect homoplasy and reversals and treated them as differences. **Homoplasy and homology are not the important component of phenetics methodology**. Moreover, Sokal acknowledged this that classification based upon unweighting characters would be affected by parallelism, convergence and by unequal evolutionary rates and hence would not yield monophyletic lineages.

    - The numerical values of phena cannot be compared in different taxa because they are based on very different set of characters and thus do not permit to establish **universal scale**.

4. **Weakness in the theoretical foundation of phenetics:**

    - Phenetics are strict **empiricists** and reject theoretical concepts. They don't consider factors that are responsible for the causation of a group. Pheneticists fail to test their taxa for monophyly. pheneticists do not recognize that taxa are historical groups but consider supra-specific taxa as classes and are not concerned with their reality.

    - Different characters, parts of body, stages, have different evolutionary rates, as a consequence it will lead to the different similarity estimates if

either one of the character or other is used. it is a mistake to assume that **random character selection will reflect equivalent differences in genotype** as external morphotypes reveals only very small and biased portion of genotype. e.g larvae and adult of the mosquito have hardly contained any correlation and similarity but no one shed light on their genotypes. Hence phenetic techniques will not have any compatibility with classifications when they are based on different sets of characters.

- Pheneticists are not be able to distinguish between **ancestral and derived groups**, however, the term **pre groups and ex groups** were used by pheneticists
- The strict inductionism (laws induced from set of data) of numerical phenetics represent a step back from **hypotheticodeductive methodology** (scientific inquiry proceeds by formulating a hypothesis) allow for no meaningful testing, predictions and interpretations. They lack all properties of theory and therefore cannot be tested.

Some commonly used terms in systematics-

- ✓ Plesiomorphy: An ancestral character state.
- ✓ Symplesiomorphy: An ancestral character state shared by two or more taxa.
- ✓ Apomorphy: A derived character state.
- ✓ Synapomorphy: A derived character state shared by two or more taxa.
- ✓ Autapomorphy: A derived character state possessed by only one of the taxa under consideration.
- ✓ Node: A branch point in a phylogenetic tree.
- ✓ Sister groups or sister taxa: Two groups with the same immediate common ancestor.
- ✓ Cladogram: The branching pattern revealed by cladistic analysis is presented in a diagram called cladogram that is based on the pattern of synapomorphies. Each branching point (node) in a cladogram represents a speciational event, which potentially give rise to a new holophyletic taxon. No branch of a cladogram can be based on pleisomorphic characters.
- ✓ Parsimony: It is a non-parametric statistical method commonly used in computational phylogenetics for estimating phylogenies. Under parsimony, the preferred phylogenetic tree is the tree that requires the least evolutionary change to explain some observed data.
- ✓ Homoplasy: A homoplasy is a similarity in a character that two taxa have acquired independently. The taxonomist encounters three forms of homoplasy-

- ✓ Convergence- It is usually a superficial similarity of two distantly related taxa; the similar characters are not homologous. The wings of birds and those of insects are an example, but so are the wings of birds and those of bats.
- ✓ Parallelism- Parallelism means that taxa began with the same initial conditions and independently underwent the same changes. The classification of the cichlid fishes of the African lakes, for instance had to be drastically revised when it was discovered that the same jaw articulation had evidently been acquired independently by different lineages. Parallelism is rampant among passerine birds in which similar morphological types evolved independently on most of the major continents. Example includes flycatchers, warblers, finches, titmice, shrikes, tree creepers and nectar feeders.
- ✓ Reversals- The loss during phylogeny of previously acquired characters is apparently far more common than formerly realized. A lost apomorphy mimics a plesiomorphy.

Outlook-

The popularity of phenetic methods has greatly declined since the rise of cladistics. Indeed, it is widely believed that a method which ignores the necessity of weighting characters and the recognition of monophyletic groups cannot lead to astable and sound classification. In combination with other methods or as a check against other approaches, phenetic methods are still much in use, but it is recommended that phonetically delimited groups always subsequently be tested for monophyly. Indeed, the method of so-called evolutionary classification consists essentially of a provisional phenetic delimitation of taxa followed by 'purification' by monophyly testing when necessary.

Interestingly, the methods of numerical phenetics have found their greatest use outside the field of animal taxonomy. In the classification of objects whose groups are not the result of a causation but simply are due to the joint possession of similar characteristics, the methods of numerical phenetics are often most helpful.

The future of numerical phenetics-

In recent years the decline in the popularity of phenetics seems to have been halted, indeed reversed, because of three developments. First, morphological characters are increasingly supplemented by a wealth of molecular characters that can be used for distance measures. Second, clustering is no longer the obligatory method in numerical phenetics nor do distance algorithms necessarily require an assumption of an equal rate of evolutionary changes in all branches of the tree. Three, the widespread view that taxa should be relatively homogenous has led to an increased use of phenetic methods.

An eventual synthesis of phenetics with other approaches to classification would be facilitated if pheneticists adopted three modifications of their philosophy:

1. The weighting of characters whenever the data allow for this,

2. The testing of provisionally recognized taxa for monophyly and

3. The recognition that species have reality in nature and are not purely subjective, arbitrary inventions of the taxonomist.

Probable questions:

1. What do you mean by phonetics?
2. What do you mean by cladistics?
3. What are the differences between phonetics and cladistics?
4. Discuss about the measurement processes of phonetics classification.
5. Discuss about the cluster method of measurement of phenetics classification.
6. What is Secondary cluster?
7. What is Plesiomorphy and symplesiomorphy?
8. Describe the drawbacks of phenetic methods.

Suggested Readings/ Reference:

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3. Evolution by Mark Ridley
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UNIT XVII

Cladistic method of classification- Cladistics and cladogram, terminologies in cladistics

Objective:

In this unit you will discuss about Cladistic method of classification- Cladistics and cladogram, terminologies in cladistics.

Introduction:

- **Cladistics**

Wittering (1966) in his book "Phylogenetic Systematics", effectively founded the subject known as Phylogenetic systematics now called as "Cladistics". The term Cladistics was coined by E. Mayr in 1969. Cladistics is a methodology that attempts to analyse phylogenetic data objectively, in a manner parallel to that in which taxometrics seeks to introduce objectivity into phenetics and phenetic classification.

Cladistics is concerned with the current methods that are available for estimating phylogenies primarily through the analysis of character versus taxa data matrices but one section deals with the use of taxon to-taxon distance measures from a phylogenetic perspective. Three rather different methods based on cladistic principles have been more or less widely employed for this purpose. These are parsimony analysis, compatibility analysis and maximum likelihood analysis. The first of these has been by far the most widely accepted for a variety of practical and theoretical reasons and will be largely concentrated on in the discussion that follows. However, while the three above-named types of procedure encompass nearly all of current cladistic practice, it should not be imagined that the azimuth of tree-constructing methods has been reached. Many problems still remain. Unequal evolutionary rates along different evolutionary paths and differing degrees of homoplasy in different characters pose their own problems, while for analysis of macromolecular sequences many issues concerning alignment, deletions and insertions, and bias in nucleotide composition have yet to be fully resolved. Yet more difficulties can potentially result from hybridization events and introgression (horizontal gene transfer) since all current methods of cladistic analysis presuppose that characters are only inherited in simple ancestor/descendant lines.

Cladistics can be defined as the study of the pathways of evolution. In other words, cladistics is interested in such questions as: (1) how many branches there are among a group of organisms; (2) which branch connects to which other branch; and what the branching sequence is.

The methods of Wagner, Henning etc. are known as Parsimony methods whereby they utilise the principle of parsimony. Not only cladistics but phenetic taxonomists also used principle of Parsimony to relate extent Operational Taxonomic Units (OTUs) without hypothesizing a priori. Cladistics methods differ fundamentally from taxometrics in the deductive (a priori) reasoning is used to determine routes of evolutionary change.

The basic units that are manipulated in cladistics are often known as Evolutionary units (EUs), equivalent to the phenetic OTUs. Once a set of data relating to Plesimorphous versus apomorphous character-states has been accumulated for all the EUs, a data matrix can be constructed.

In cladistics, the organisms are ranked and classified according to the "recency of common descent". Taxa based on entirely shared derived (synapomorphic) characters originated from a common ancestor. It is a method of phylogenetic analysis to identify monophyletic lineages or clades. Julian Huxley used the term 'clade' in 1958 and Cain and Harrison introduced the term 'cladistic' in 1960. Cladistic method was first used by Willi Hennig in 1966.

Character:	eye color	**Character states:**	blue, brown, green
	mammary glands		present, absent
	number of legs		0, 2, 4, 6, 8, etc.
Molecular Characters	nucleotide bases		A, C, T, G
	amino acid codons		ACC, CGT, GAT, etc.

The whole concept is based on Darwinism. According to this concept each valid taxon is derived from a common ancestor but the common characteristic features of a realm of the biological world do not always include a common ancestry. Organisms are classified using a combination of phylogenetic relationship and overall similarity. This type of taxonomy considers taxa more important rather than single species.

- **Cladogram**

A cladogram is a diagrammatic representation which shows the relationship of the closely related organisms. It is a type of a phylogenetic tree. But it only shows the relationships between clades with the common ancestor. As an example, a cladogram shows human are more closely related with chimpanzees than gorilla, but it does not show the evolutionary time and the exact distance from the common ancestor.

Cladograms or dendrograms are normally based upon the minimal or most parsimonious way in which the EUs can be connected to account for the data in the text table. In case of fossils, the cladograms are wholly hypothetical and are known together with the single hypothetical ancestor, as Hypothetical Taxonomic Unit (HTUs). When

the hypothetical ancestral taxon is known, the evolutionary polarity is decided rooted tree or dendrogram is formed. When polarity of characters is not decided, the dendrogram obtained is not directional; this is called Unrooted tree or Network. Network can become rooted by deciding a posteriori.

A phylogenetic tree can be read like a map of evolutionary history. Many phylogenetic trees have a single lineage at the base representing a common ancestor. Scientists call such trees rooted, which means there is a single ancestral lineage (typically drawn from the bottom or left) to which all organisms represented in the diagram relate. Notice in the rooted phylogenetic tree that the three domains—Bacteria, Archaea, and Eukarya—diverge from a single point and branch off. The small branch that plants and animals (including humans) occupy in this diagram shows how recent and miniscule these groups are compared with other organisms. Unrooted trees don't show a common ancestor but do show relationships among species.

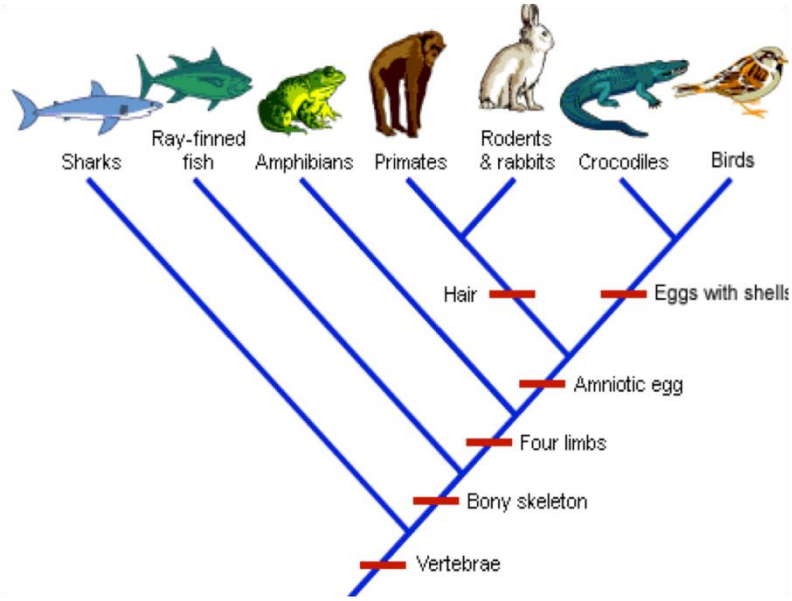


Fig: A cladogram of vertebrates

Phylogeny

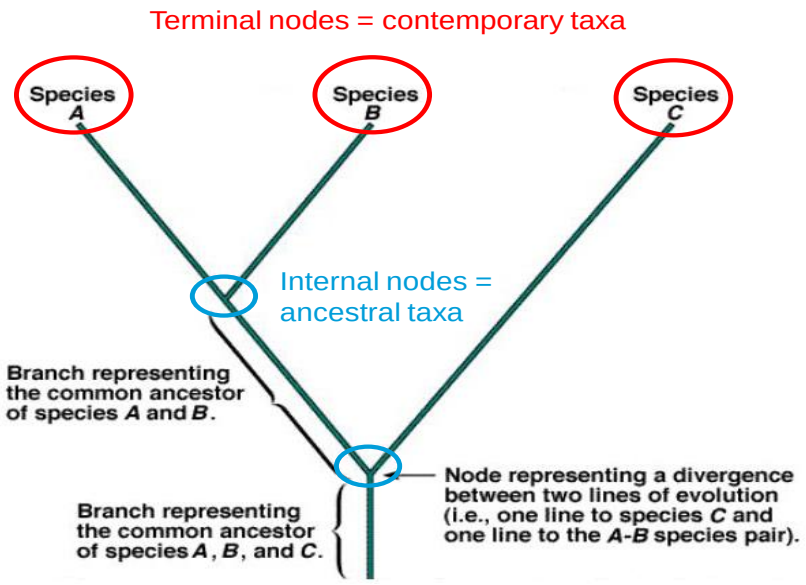


Figure: Different parts of a phylogenetic tree

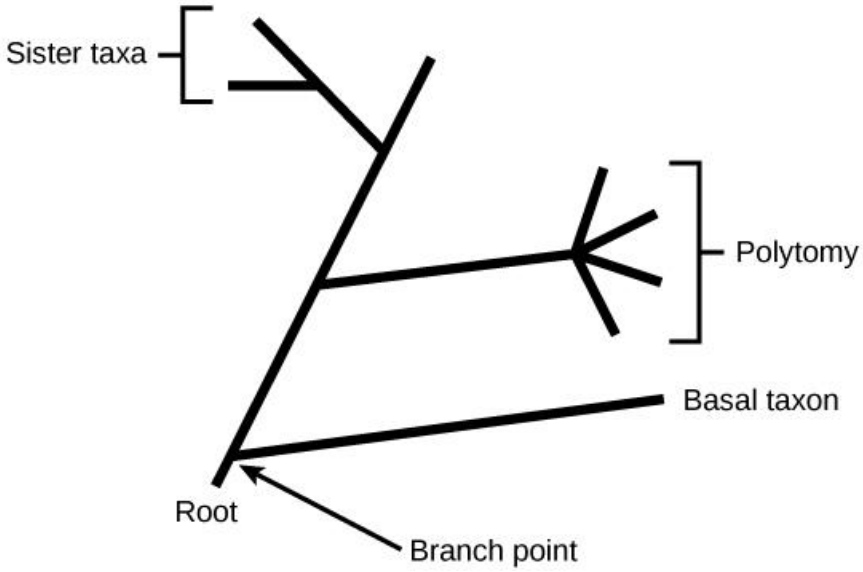


Figure: The root of a phylogenetic tree indicates that an ancestral lineage gave rise to all organisms on the tree. A branch point indicates where two lineages diverged. A lineage that evolved early and remains unbranched is a basal taxon. When two lineages stem from the same branch point, they are sister taxa. A branch with more than two lineages is a polytomy.

In a rooted tree, the branching indicates evolutionary relationships. The point where a split occurs, called a **branch point**, represents where a single lineage evolved into a distinct new one. A lineage that evolved early from the root and remains unbranched is called **basal taxon**. When two lineages stem from the same branch point, they are called **sister taxa**. A branch with more than two lineages is called a **polytomy** and serves to illustrate where scientists have not definitively determined all of the relationships.

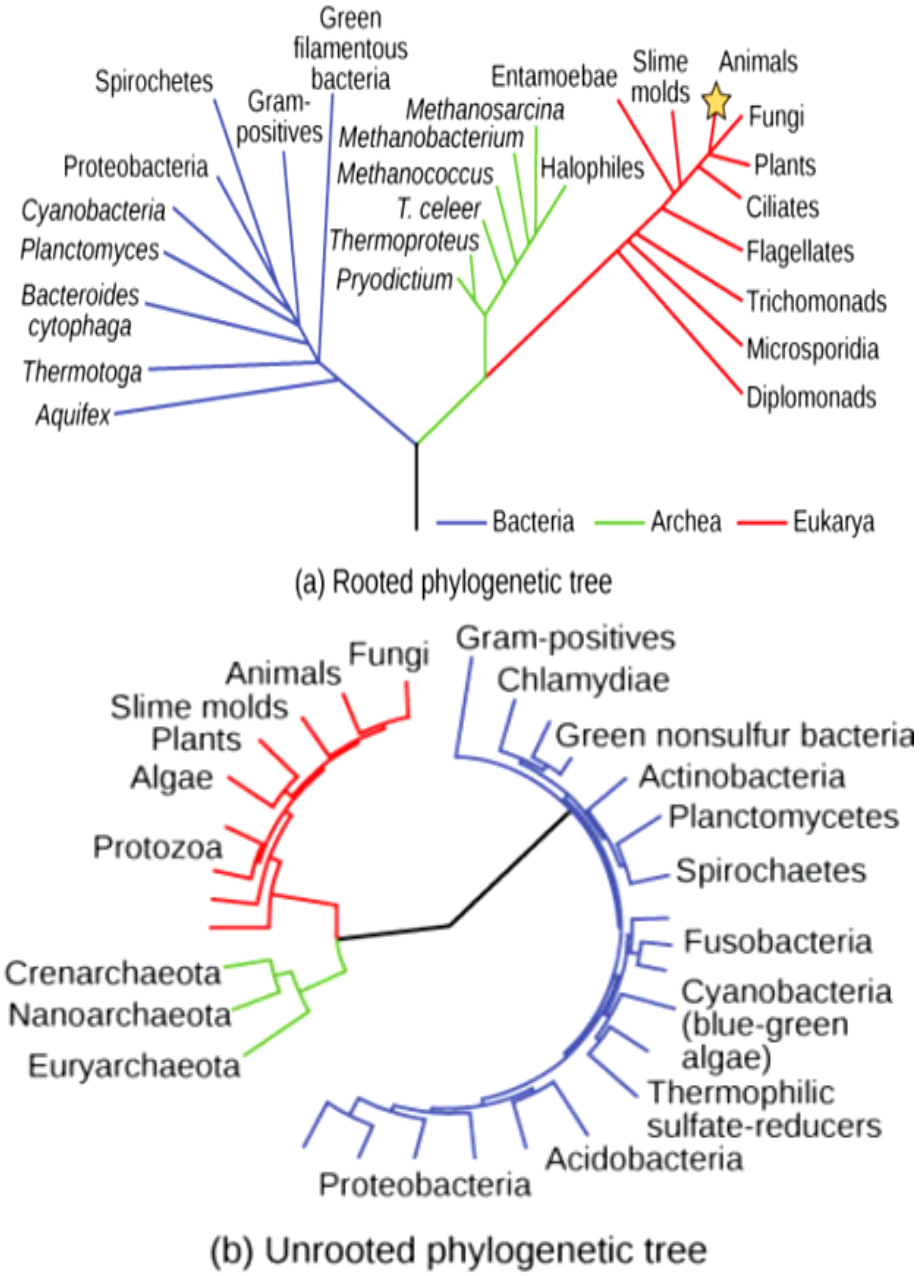


Figure: Both of these phylogenetic trees shows the relationship of the three domains of life—Bacteria, Archaea, and Eukarya—but the (a) rooted tree attempts to identify when

various species diverged from a common ancestor while the (b) unrooted tree does not. (Source: modification of work by Eric Gaba)

The diagrams above can serve as a pathway to understanding evolutionary history. The pathway can be traced from the origin of life to any individual species by navigating through the evolutionary branches between the two points. Also, by starting with a single species and tracing back towards the "trunk" of the tree, one can discover that species' ancestors, as well as where lineages share a common ancestry. In addition, the tree can be used to study entire groups of organisms.

Many disciplines within the study of biology contribute to understanding how past and present life evolved over time; these disciplines together contribute to building, updating, and maintaining the "tree of life." Information is used to organize and classify organisms based on evolutionary relationships in a scientific field called systematics.

It is important to note that although sister taxa and polytomy do share an ancestor; it does not mean that the groups of organisms split or evolved from each other. Organisms in two taxa may have split apart at a specific branch point, but neither taxa gave rise to the other.

Another point to mention on phylogenetic tree structure is that rotation at branch points does not change the information. For example, if a branch point was rotated and the taxon order changed, this would not alter the information because the evolution of each taxon from the branch point was independent of the other.

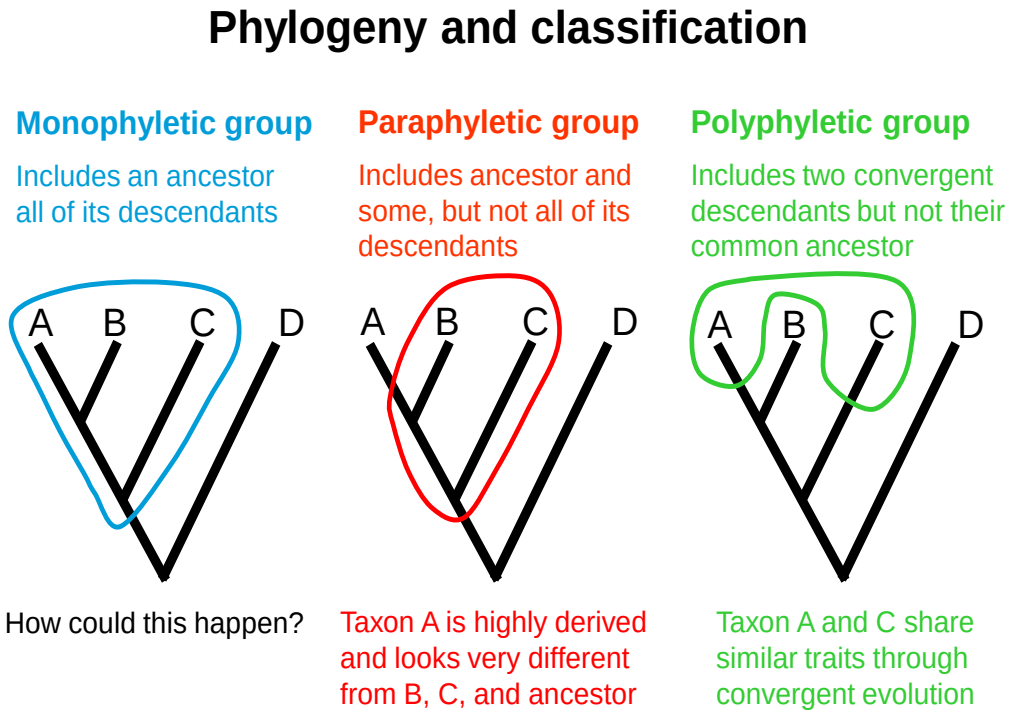
Data may be collected from fossils, from studying the structure of body parts or molecules used by an organism, and by DNA analysis. By combining data from many sources, scientists can put together the phylogeny of an organism; since phylogenetic trees are hypotheses, they will continue to change as new types of life are discovered and new information is learned.

✓ Terminologies in Cladistics:

The following terms, coined by Hennig, are used to identify shared or distinct character states among groups:

Taxa are nested on the basis of their characteristics. Some taxa include related organisms while some taxa consist of unrelated organisms. Ancestor and descendants are grouped under taxa. Monophyletic, paraphyletic and polyphyletic are such groups used in phylogenetic studies.

A monophyletic taxon is defined as a group that consists of the most recent common ancestor of a group of organisms and all of its descendants. A paraphyletic taxon is defined as a group that consists of the most recent common ancestor and some of its descendants. A polyphyletic group is defined as a group of unrelated organisms that lacks a most recent common ancestor.



A **plesiomorphy** ("close form") or **ancestral state** is a character state that a taxon has retained from its ancestors. When two or more taxa that are not nested within each other share a plesiomorphy, it is a **symplesiomorphy** (from *syn-*, "together"). Symplesiomorphies do not mean that the taxa that exhibit that character state are necessarily closely related. For example, Reptilia is traditionally characterized by (among other things) being cold-blooded (i.e., not maintaining a constant high body temperature), whereas birds are warm-blooded. Since cold-bloodedness is a plesiomorphy, inherited from the common ancestor of traditional reptiles and birds, and thus a symplesiomorphy of turtles, snakes, and crocodiles (among others), it does not mean that turtles, snakes and crocodiles form a clade that excludes the birds.

An **apomorphy** ("separate form") or **derived state** is an innovation. It can thus be used to diagnose a clade – or even to help define a clade name in phylogenetic nomenclature. Features that are derived in individual taxa (a single species or a group that is represented by a single terminal in a given phylogenetic analysis) are

called **autapomorphies** (from *auto-*, "self"). Autapomorphies express nothing about relationships among groups; clades are identified (or defined) by **synapomorphies** (from *syn-*, "together"). For example, the possession of digits that are homologous with those of *Homo sapiens* is a synapomorphy within the vertebrates. The tetrapods can be singled out as consisting of the first vertebrate with such digits homologous to those of *Homo sapiens* together with all descendants of this vertebrate (an apomorphy-based phylogenetic definition). Importantly, snakes and other tetrapod's that do not have digits are nonetheless tetrapod's: other characters, such as amniotic eggs and diapsid skulls, indicate that they descended from ancestors that possessed digits which are homologous with ours.

A character state is **homoplastic** or "an instance of **homoplasy**" if it is shared by two or more organisms but is absent from their common ancestor or from a later ancestor in the lineage leading to one of the organisms. It is therefore inferred to have evolved by convergence or reversal. Both mammals and birds are able to maintain a high constant body temperature (i.e., they are warm-blooded). However, the accepted cladogram explaining their significant features indicates that their common ancestor is in a group lacking this character state, so the state must have evolved independently in the two clades. Warm-bloodedness is separately a synapomorphy of mammals (or a larger clade) and of birds (or a larger clade), but it is not a synapomorphy of any group including both these clades. Hennig's Auxiliary Principle states that shared character states should be considered evidence of grouping unless they are contradicted by the weight of other evidence; thus, homoplasy of some feature among members of a group may only be inferred after a phylogenetic hypothesis for that group has been established.

The terms plesiomorphy and apomorphy are relative; their application depends on the position of a group within a tree. For example, when trying to decide whether the tetrapods form a clade, an important question is whether having four limbs is a synapomorphy of the earliest taxa to be included within Tetrapoda: did all the earliest members of the Tetrapoda inherit four limbs from a common ancestor, whereas all other vertebrates did not, or at least not homologously? By contrast, for a group within the tetrapod's, such as birds, having four limbs is a plesiomorphy. Using these two terms allows a greater precision in the discussion of homology, in particular allowing clear expression of the hierarchical relationships among different homologous features.

It can be difficult to decide whether a character state is in fact the same and thus can be classified as a synapomorphy, which may identify a monophyletic group, or whether it only appears to be the same and is thus a homoplasy, which cannot identify such a group. There is a danger of circular reasoning: assumptions about the shape of a phylogenetic tree are used to justify decisions about character states, which are then used as evidence for the shape of the tree. Phylogenetics uses various forms of parsimony to decide such questions; the conclusions reached often depend on the dataset and the methods. Such is the nature of empirical science, and for this reason,

most cladists refer to their cladograms as hypotheses of relationship. Cladograms that are supported by a large number and variety of different kinds of characters are viewed as more robust than those based on more limited evidence.

Probable questions:

1. Define cladistics.
2. Define cladogram.
3. What are the differences between cladistics and cladogram?
4. What is phylogeny?
5. Elaborate the term sister taxa with example.
6. What do you mean by monophyletic taxon?
7. Discuss about plesiomorphy and apomorphy.
8. What do you mean by synapomorphy?
9. What are paraphyletic, polyphyletic and monophyletic groups within a phylogeny?

Suggested Readings/ Reference:

1. Principles and Techniques of Contemporary Taxonomy by D.L.J. Quicke. Blackie Academic and Professional. Chapman & Hall. 1993
2. Methods and Principles of Systematic Zoology by E. Mayr, E.G. Linsley, and R. L. Usinger. Mcgraw-Hill book company, Inc. 1953.
3. https://www.mun.ca/biology/scarr/Character_Conflict.html

UNIT XVIII

Methods of measuring evolutionary transitions - Homoplasy, parsimony and character conflict

Objective:

In this unit you will discuss about Methods of measuring evolutionary transitions - Homoplasy, parsimony and character conflict.

Introduction:

Evolutionary transitions are documented by creating a branching structure, termed as phylogeny or tree that illustrates the relationships between the sequences. The synthetic or evolutionary method of classification thus combines components of cladistics and of phenetics, but in a rather different manner. It agrees with cladistics; in the postulate that as complete as possible a reconstruction of phylogeny must precede the construction of a classification since groups that are not composed of descendants of a common ancestor are artificial and of low predictive value. More generally it agrees also with the cladists in the careful weighting of characters. It rejects, however, the "divisional" process of classification ("downward" classification), which is most evident in the cladists' definition of "monophyletic." Evolutionary classification rejects most of the conceptual axioms of phenetics, but agrees with it in the actual procedure of grouping by a largely phenetic approach. However, in contrast to the unweighted approach of the pheneticists, it bases its conclusions on the careful weighting of characters.

Homoplasy

Homoplasy is the similarity in trait that is not parsimoniously explained by descent from a common ancestor. Thus, the trait is said to have evolved as a result of convergence or a reversal. When trait has been gained or lost independently in separate lineages over the course of evolution, it is called Homoplasy.

Homoplasy is the type that occurs in more closely related phylogenetic groups. Homoplasy can arise from both similar selection pressure acting on adapting species and the effects on genetic drift.
According to cladistic interpretation, homoplasy is very common due to the redundancy of the genetic code. Homoplasy may be the result of random nucleotide substitutions accumulating over time. For example, evolution of warm-blood ness in both mammals and birds, though this trait is absent in the common ancestor. Mammals and birds are

both warm-blooded and share a common tetrapod ancestor. This is an example of Homoplasy. It is an evolved trait that is not present in a common ancestor but evolved independently by both birds and mammals. Therefore, evidence indicates that the trait of warm-bloodedness must have evolved separately within each clade.

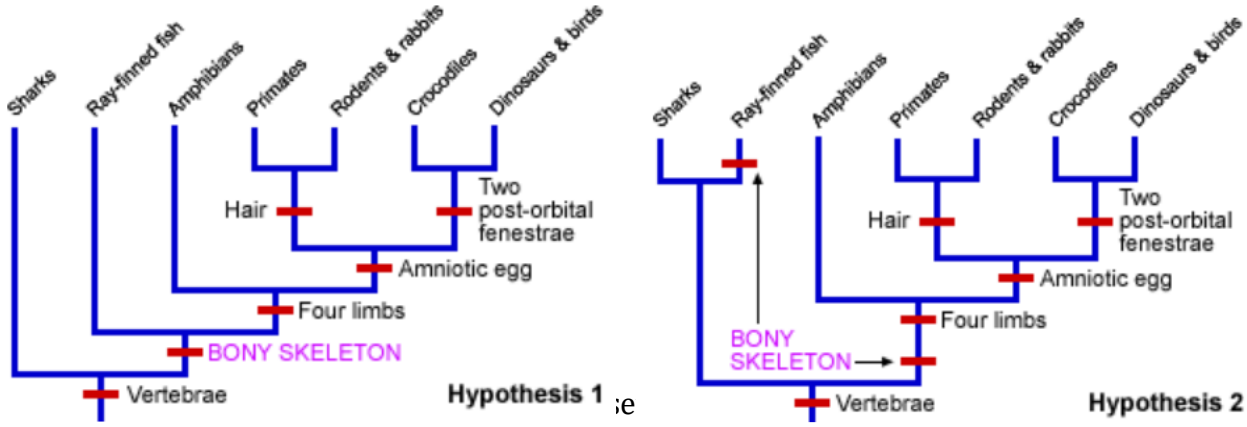
Types of Homoplasy:

a. **Parallel Homoplasy:** Derived trait, when present in two groups (or species) without a common ancestor due to convergent evolution is called parallel homoplasy.
b. **Reverse Homoplasy:** A trait when present in an ancestor but not in direct descendants that reappears in later descendants is called reverse homoplasy.

Parsimony

The parsimony principle is basic to all science and tells us to choose the simplest scientific explanation that fits the evidence. In terms of tree-building, that means that, all other things being equal, the best hypothesis is the one that requires the fewest evolutionary changes.

For example, we could compare these two hypotheses about vertebrate relationships using the parsimony principle:



Evolutionary changes, with a bony skeleton evolving independently, twice. Although both fit the available data, the parsimony principle says that Hypothesis 1 is better—since it does not hypothesize unnecessarily complicated changes.
Cladistics analysis depends on the principle of parsimony when selecting the best tree diagram to represent the relationships between the taxa at the tips-

a. Parsimony assumes that the simplest solution is likely the best.

b. In terms of tree diagrams, the most parsimonious tree is the one that minimizes the number of character changes along the branches i.e., the less number of steps the better parsimony.

c. Parsimony works well for morphological characters having multiple, complete character with relatively low rates of evolution.

d. One of the basic tenets of much of cladistic analysis, but one which has nevertheless been subject to substantial criticism from time to time, is that the most likely explanation of a taxonomic data set is the one which requires the least number of evolutionary changes or, more specifically, character state transitions. Such explanations take the form of trees connecting the taxa under consideration and the shortest trees derived from the data set, i.e. the one(s) that require the fewest character state transitions to have taken place, are known as the most parsimonious ones. Methods used to find these most parsimonious solutions are accordingly referred to as parsimony analysis.

e. One of the most important assumptions of parsimony analysis is that character state transitions are intrinsically unlikely events. Otherwise, if they were considered as probable events, it would be a non-sense to try to find evolutionary hypotheses that minimise them. Conversely, if a data set is suspected of being heavily biased towards characters undergoing rapid and homoplaseous change, then parsimony analysis should not be expected to provide accurate phylogenetic estimates.

Compatibility analysis

Whereas variation in some characters may truly reflect the evolutionary history of a group **(true characters),** others, due to homoplasy, do not **(false characters).** In practice many characters usually show some homoplasy and so different characters will often support different phylogenetic hypotheses even though only one hypothesis can be correct. The problem is that frequently there is no totally reliable means of distinguishing between true and false characters. A possible solution to this was suggested by Le Quesne (1964) who argued that: 'Variation in any combination of true character will always be compatible, that is, it will always support the same phylogenetic hypothesis, whereas, if two character state distributions are incompatible, then at least one of the characters must be false.' To minimize the effects of false characters, Le Quesne proposed that analysis should be restricted to characters that are compatible with one another and thus the raw data set should be pruned down so as to leave the largest set of characters that are mutually compatible. In its underlying philosophy this is not very different from parsimony analysis in that both aim to 'minimize' homoplasy (Felsenstein, 1983).

The essence of performing compatibility analysis is to find a set of mutually compatible characters, usually called a **clique,** from among all the available ones. The larger this set

is relative to the number of taxa, then the more likely it is on average that relationships among the taxa will be fairly well resolved. The larger the clique relative to the total number of characters, then the more confidence, rightly or wrongly, there may be in the data set. Unfortunately, if the compatibility idea is accepted, perhaps because it intuitively seems to be conservative, the largest clique will quite often be found to contain only a small number of the original characters. For example, Meacham and Estabrook (1985) surveying 22 published data sets found that the proportion of the total number of characters represented in the largest clique ranged from 0.16 to 0.96 with a mean of 0.46. Thus, on average nearly half of the characters scored were discounted in the subsequent tree construction process, and any phylogenetic information they contained would not be used. Many proponents of compatibility analysis see this as a distinct advantage in that if the largest clique comprises only a few characters then the data may not be up to the job of indicating the group's phylogeny. One way of still following a compatibility procedure that in part overcomes the problem of discarding a good proportion of potentially informative data is to keep applying the analysis to the progressively smaller subsets of taxa that were indicated as monophyletic by the previous analysis. Given that there may be a tendency for parallelisms to occur within groups of fairly closely related taxa this may be reasonably acceptable.

Maximum likelihood and related methods

While much evidence suggests that parsimony analysis does quite a good job of estimating phylogenies, it has been shown that there are circumstances in which it will fail. Specifically, parsimony will fail consistently if evolutionary rates along different evolutionary branches are sufficiently dissimilar and if the assumption that change is rare is violated.

More reliable estimates of phylogeny can be made if information about evolutionary processes can be included in the analysis. In these models, evolutionary change is assumed to be a purely chance process and as a result each possible phylogeny for a set of taxa must have a certain probability of being correct given a particular set of data. Thus the preferred tree should be the one that is most likely to have given rise to that particular data set, and the approach that finds this tree is referred to as a **maximum likelihood method.**

Under some circumstances, for example if change is not rare, maximum likelihood will yield quite different trees to maximum parsimony. At present, and probably for the foreseeable future, maximum likelihood treatments are likely to be largely limited to analyses of a very few molecular characters where the evolutionary transitions comprise a small and tightly defined group of changes whose probabilities can be estimated with some accuracy. Consequently, most applications of maximum likelihood methods have dealt directly with nucleotide sequence data.

One drawback of maximum likelihood approach is that it is demanding of computer time.
A particularly awkward problem that has dogged phylogenetic analysis using sequence data is that some of the changes that occur along different diverging branches of the evolutionary tree will by chance be parallel.

However, unlike some other sorts of data, there is no way of reexamining molecular data to try and distinguish an homologous change from an analogous one (in this case a parallel change). One possible way of minimizing the effects of parallel change is only to consider relatively conserved sequences, another is only to count **transversions** (relatively rare DNA base changes in which a pyrimidine is replaced by a purine or vice versa), and to carry out parsimony the resulting new pair of bases will be identical or different (i.e. two pyrimidines could equally change to two adenines or two guanines as they could to an adenine and a guanine).

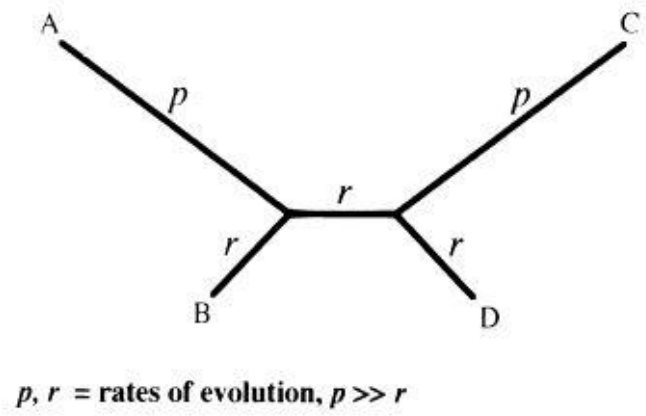


Figure 3.1 An illustration of unequal evolutionary rates leading to four taxa that under the condition of rate *p* being much greater than *r* will cause parsimony analysis to fail to lead to the correct evolutionary interpretation. The reason for this is that with enough changes along the branches leading to A and C, chance will mean that some will give rise to analogous apomorphous states in both taxa. Hence, if *p>>q* then the number of apomorphics shared by A and C will exceed those shared by A and B or by C and D. This model is particularly relevant to molecular data where homoplasy is likely to be common and parallel changes truly indistinguishable. (After Felsenstein. 1978.)

However, if the change was due to a single informative apomorphic event, then the resulting bases will always be the same. Using this inequality, Lake's process then counts which of the possible unrooted four-taxon trees is supported or is countered by a particular distribution of transversions. Because it analysis on these changes - this process has been called **transversion parsimony.** However, the latter results in discarding much potentially useful information. Recently, Lake (1987) has proposed an

alternative method which similarly only considers transversions but does so in a different way. Lake's technique, referred to as **Lake's method of invariants or evolutionary parsimony,** is restricted to sets of four sequences (taxa) and further considers only base positions in the sequence where two taxa have purine residues and the other two have pyrimidines. The idea behind Lake's method is that if this distribution results from a parallel change then it is equally likely that makes a distinction between different purines or pyrimidines, Lake's method uses more information than transversion parsimony.

However, the value of Lake's method is limited by the relative frequency of **transition** substitutions (DNA base changes in which a pyrimidine or purine is replaced by another pyrimidine or purine, respectively). If transitions are too common, then they will be likely to mask informative changes.

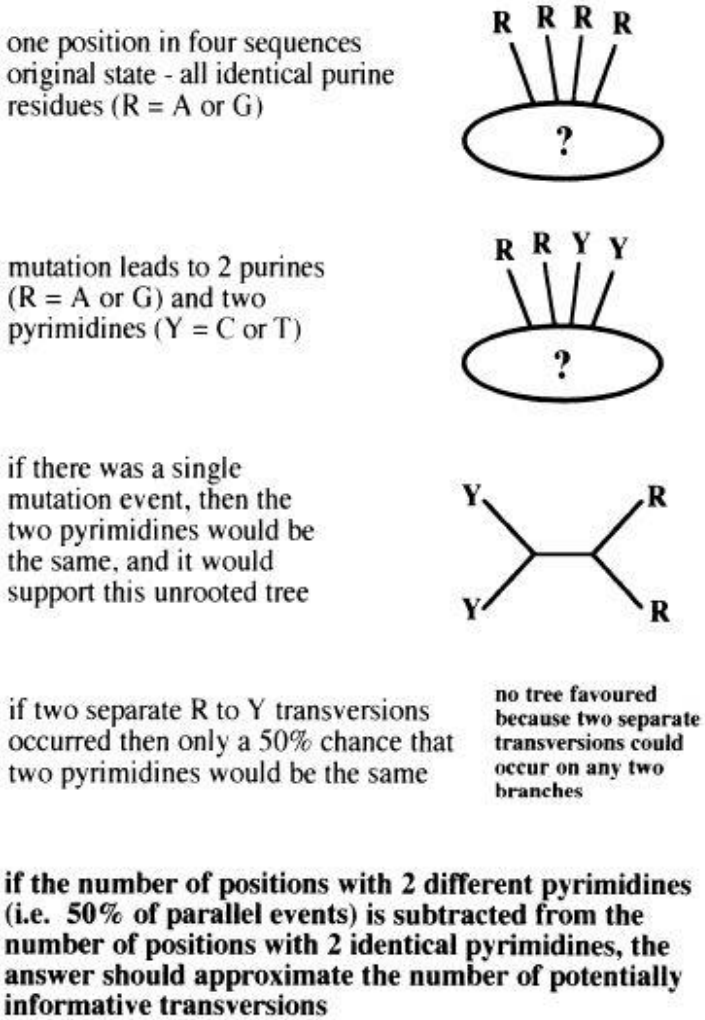


Figure: Basis of Lake's method of invariants, a technique for inferring evolutionary relationships for sets of four taxa based on their nucleotide sequences. Abbreviations: A, adenine; C, cytosine: G. guanine: T. thymine: R, either purine; Y, either purimidine.

Character conflict

Since two taxa may differ by an almost unlimited number of characters, the working taxonomist is forced to make a very restricted selection. The ultimate classification may depend entirely on this selection. For instance, Rosen et al. (1981), on the basis of the characters they selected, came to the conclusion that the Dipnoi (lungfishes) are the sister group of the tetrapods, while Holmes (1985) partly on the basis of different characters, concluded that the traditional classification according to which some group of rhipidistians is the sister group of the tetrapods is far better documented. The difficulty lay in the correct identification of the synapomorphies. Similarly, the claim that birds and mammals are sister groups was based on a strict cladistic analysis (Gardiner 1982) that used a very different set of characters than did the traditional classification, in which the birds are derived from archosaurian ancestors and the mammals from the therapsids, two very different groups of reptiles; this classification was confirmed by Kemp (1988) and Gauthier, Kluge, and Rowe (1988). Nor has strictly cladistic analysis been able to establish the Phylogeny of the lice, Mallophaga and Anoplura (Lyal 1985 versus Kim et al. 1987). These cases show that a cladistic analysis does not guarantee a correct cladogram unless it is based on valid apomorphies.

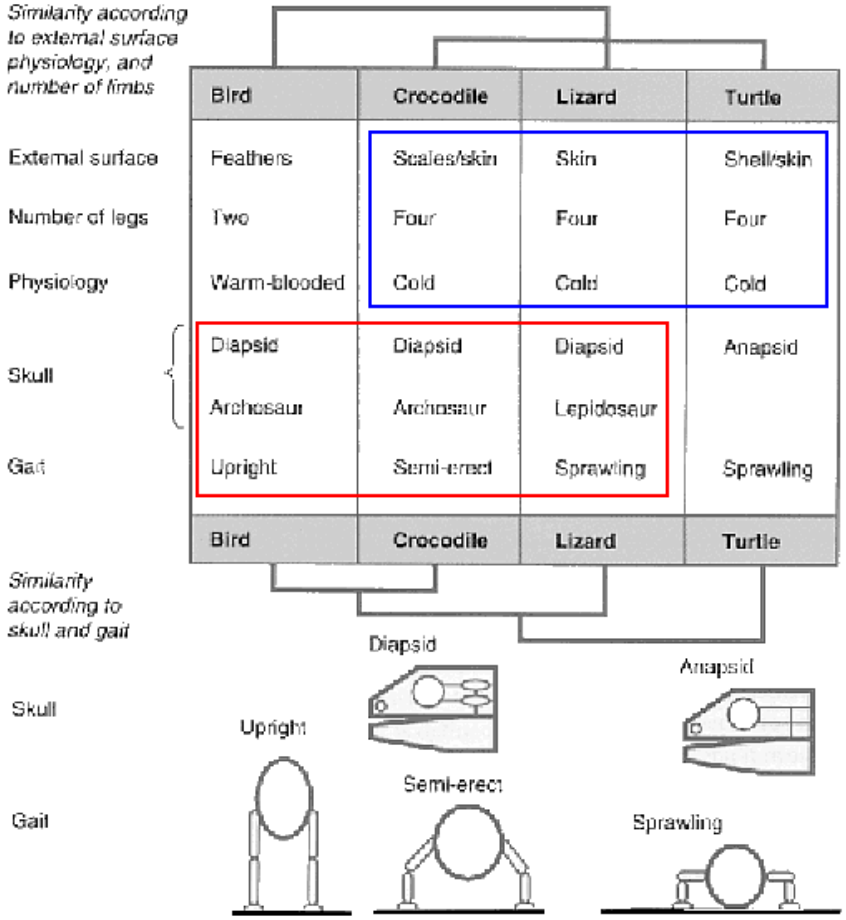


Figure: Character conflict in classification of Reptiles & Birds. Leg number, physiology, and external surface link the reptilian groups. The gait and the anatomy of the skull link crocodiles and birds. Though both have diapsid skulls, Archosaurs and Lepidosaurs differ in that the latter lack a lower temporal arch. (after Ridley 1996)

Probable questions:

1. Define Homoplasy.
2. Define parsimony.
3. Elaborate about maximum likelihood method**.**
4. What do you mean by Character conflict?
5. Define clique.

Suggested Readings/ Reference:

1. Principles and Techniques of Contemporary Taxonomy by D.L.J. Quicke. Blackie Academic and Professional. Chapman & Hall. 1993
2. Methods and Principles of Systematic Zoology by E. Mayr, E.G. Linsley, and R. L. Usinger. Mcgraw-Hill book company, Inc. 1953.
3. https://www.mun.ca/biology/scarr/Character_Conflict.html

UNIT XX

Phylogenetic trees: construction and analysis; types

Objective:

In this unit you will discuss about Phylogenetic trees: construction and analysis; types

Introduction:

A phylogenetic tree can be read like a map of evolutionary history. Many phylogenetic trees have a single lineage at the base representing a common ancestor. Scientists call such trees rooted, which means there is a single ancestral lineage (typically drawn from the bottom or left) to which all organisms represented in the diagram relate. Notice in the rooted phylogenetic tree that the three domains—Bacteria, Archaea, and Eukarya—diverge from a single point and branch off. The small branch that plants and animals (including humans) occupy in this diagram shows how recent and miniscule these groups are compared with other organisms. Unrooted trees don't show a common ancestor but do show relationships among species.

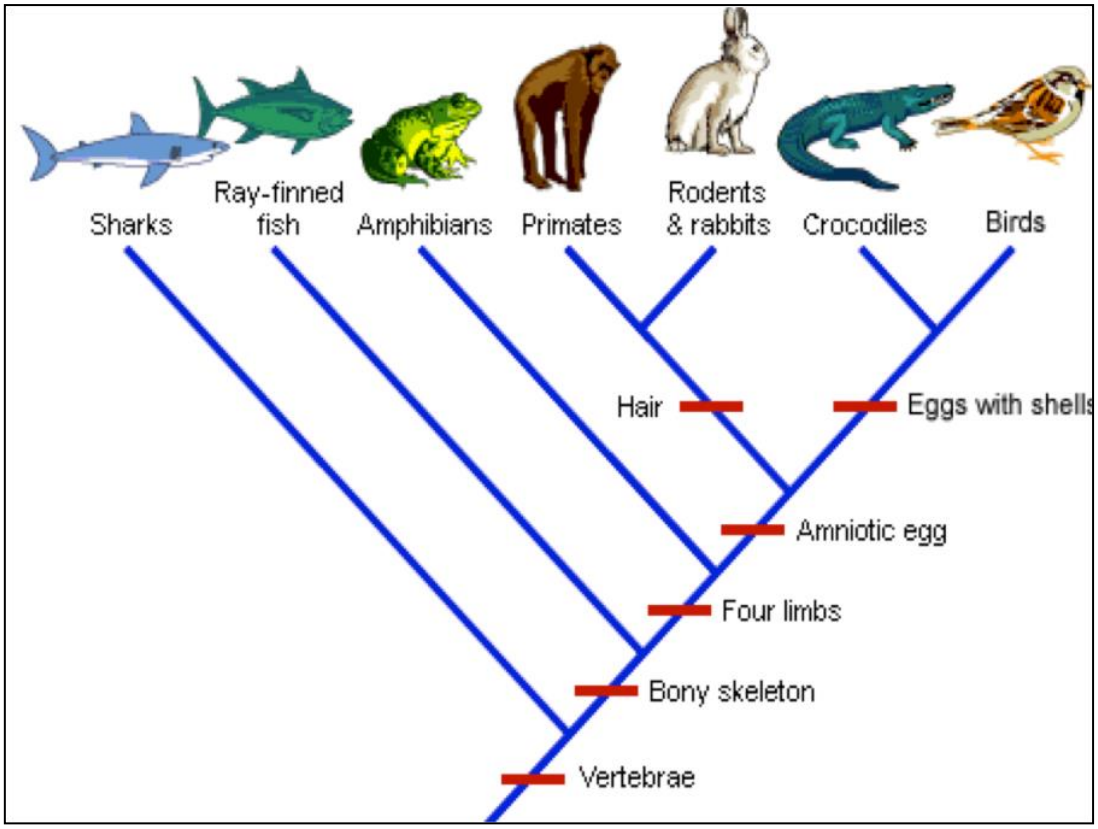


Fig: A phylogenetic tree of vertebrates

In a rooted tree, the branching indicates evolutionary relationships. The point where a split occurs, called a **branch point**, represents where a single lineage evolved into a distinct new one. A lineage that evolved early from the root and remains unbranched is called **basal taxon**. When two lineages stem from the same branch point, they are called **sister taxa**. A branch with more than two lineages is called a **polytomy** and serves to illustrate where scientists have not definitively determined all of the relationships.

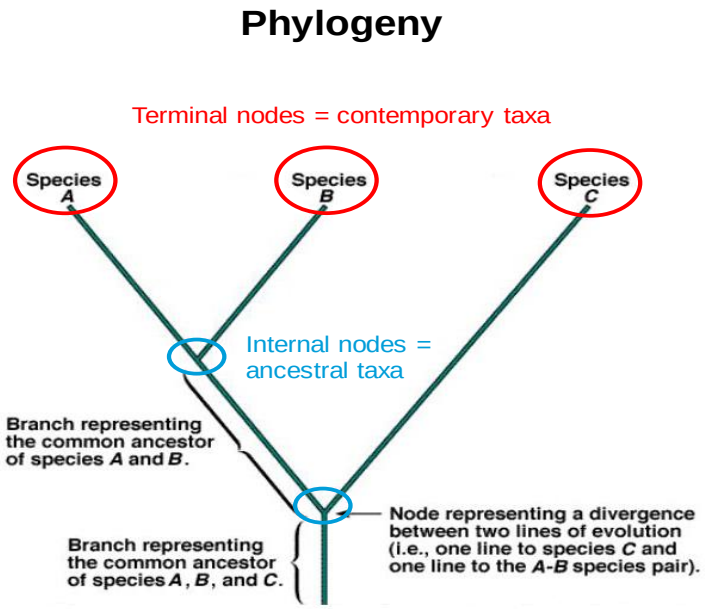


Figure: Different parts of a phylogenetic tree

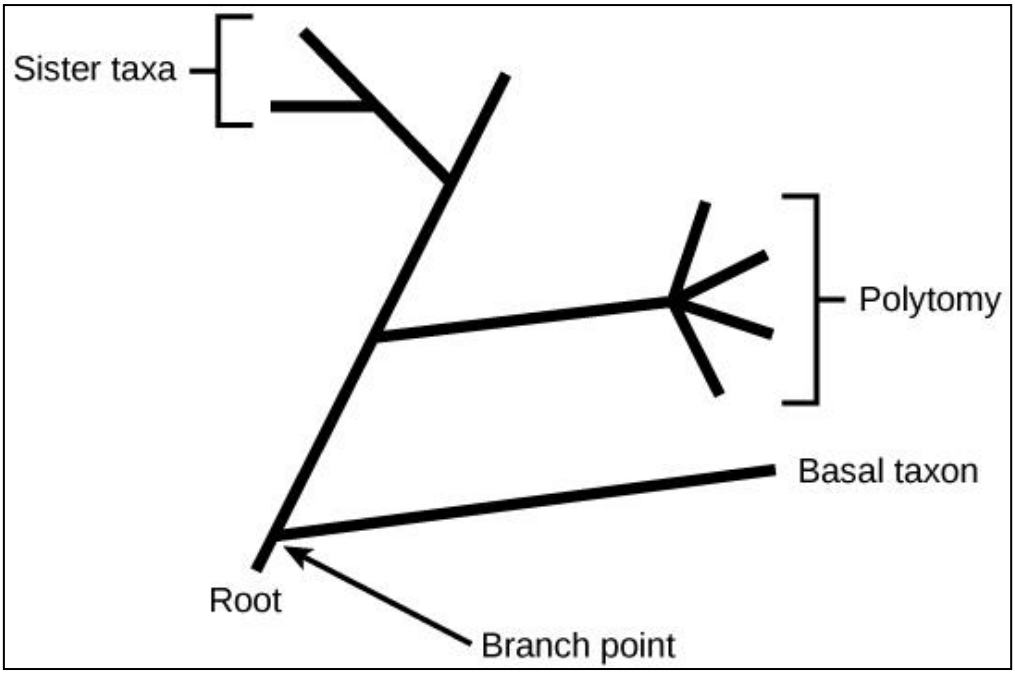
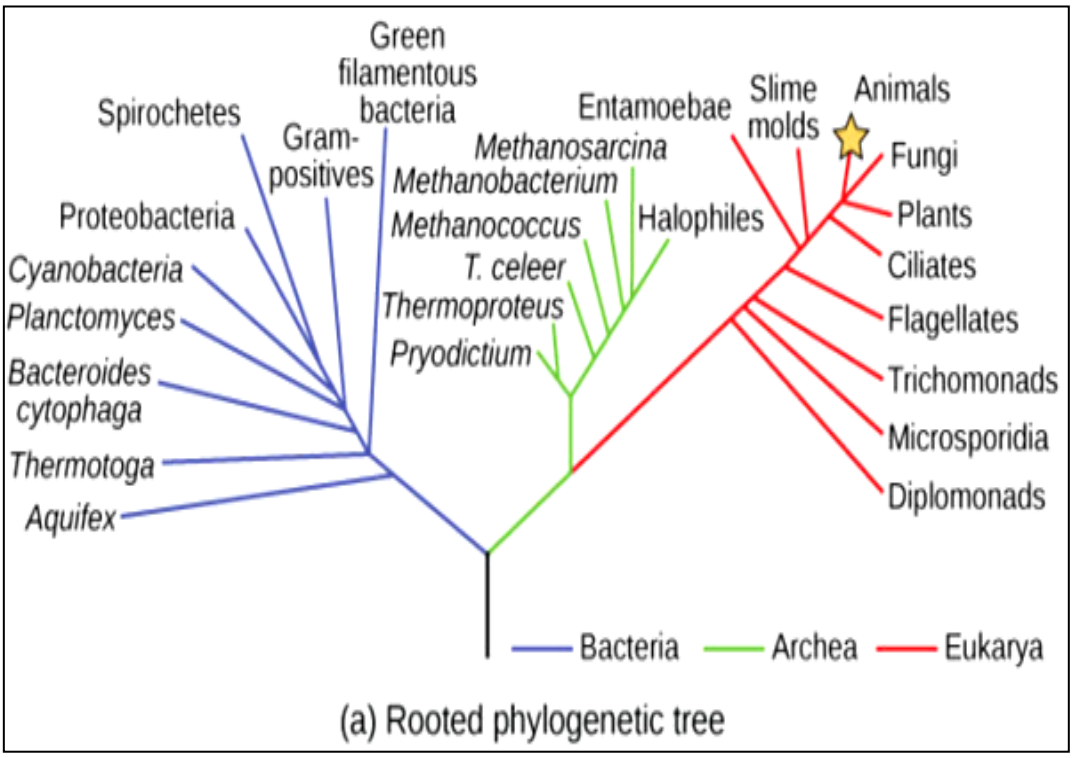


Figure: The root of a phylogenetic tree indicates that an ancestral lineage gave rise to all organisms on the tree. A branch point indicates where two lineages diverged. A lineage that evolved early and remains unbranched is a basal taxon. When two lineages stem from the same branch point, they are sister taxa. A branch with more than two lineages is a polytomy.



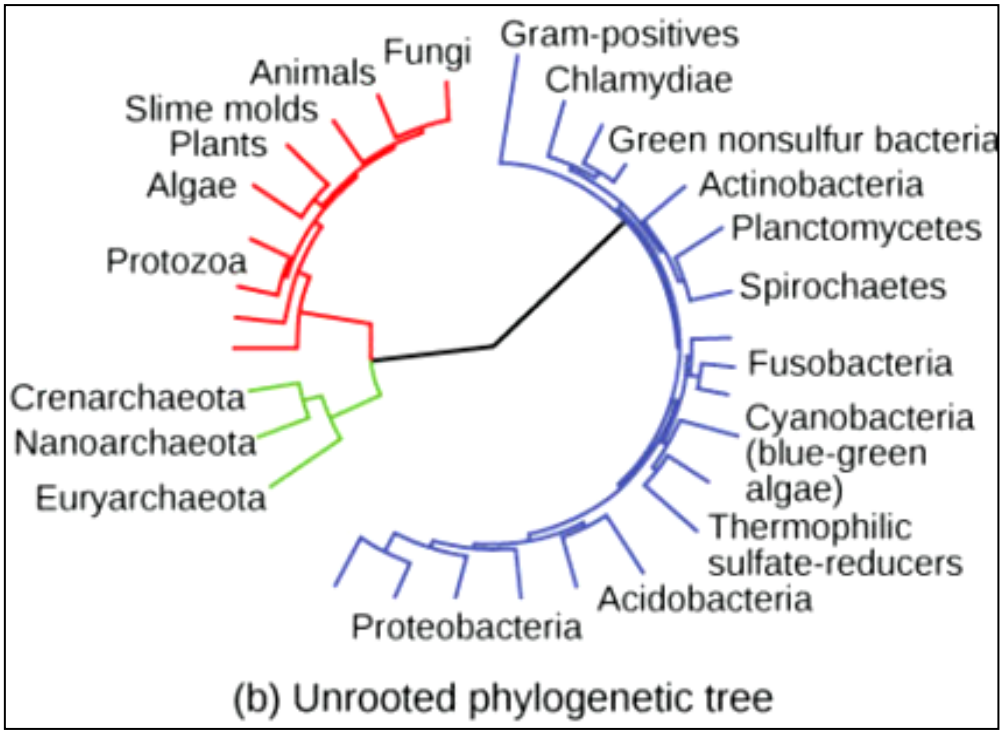


Figure: Both of these phylogenetic trees shows the relationship of the three domains of life—Bacteria, Archaea, and Eukarya—but the (a) rooted tree attempts to identify when various species diverged from a common ancestor while the (b) unrooted tree does not. (Source: modification of work by Eric Gaba)

The diagrams above can serve as a pathway to understanding evolutionary history. The pathway can be traced from the origin of life to any individual species by navigating through the evolutionary branches between the two points. Also, by starting with a single species and tracing back towards the "trunk" of the tree, one can discover that species' ancestors, as well as where lineages share a common ancestry. In addition, the tree can be used to study entire groups of organisms.

Many disciplines within the study of biology contribute to understanding how past and present life evolved over time; these disciplines together contribute to building, updating, and maintaining the "tree of life." Information is used to organize and classify organisms based on evolutionary relationships in a scientific field called systematics.

It is important to note that although sister taxa and polytomy do share an ancestor; it does not mean that the groups of organisms split or evolved from each other. Organisms in two taxa may have split apart at a specific branch point, but neither taxa gave rise to the other.

Another point to mention on phylogenetic tree structure is that rotation at branch points does not change the information. For example, if a branch point was rotated and the taxon order changed, this would not alter the information because the evolution of each taxon from the branch point was independent of the other.

Data may be collected from fossils, from studying the structure of body parts or molecules used by an organism, and by DNA analysis. By combining data from many sources, scientists can put together the phylogeny of an organism; since phylogenetic trees are hypotheses, they will continue to change as new types of life are discovered and new information is learned.

Probable questions:

1. What is phylogenetic tree? Give an example.
2. What are 3 things that can be determined by looking at phylogenetic trees?
3. What is branch point? Site an example.
4. What are the 3 main branches of a phylogenetic tree?
5. What are the different types of phylogenetic trees?
6. What is rooted phylogenetic tree?
7. What are the differences between rooted and unrooted phylogenetic tree?
8. What is the difference between a branch and a node on a phylogenetic tree?
9. What is polytomy?
10. What are the limitations of phylogenetic trees?

Suggested Readings/ Reference:

1. Principles and Techniques of Contemporary Taxonomy by D.L.J. Quicke. Blackie Academic and Professional. Chapman & Hall. 1993
2. Methods and Principles of Systematic Zoology by E. Mayr, E.G. Linsley, and R. L. Usinger. Mcgraw-Hill book company, Inc. 1953.

Disclaimer :

The study materials of this book have been collected from books, various e- books, journals and other e-sources.

Post-Graduate Degree Programme (CBCS)

in

ZOOLOGY

(M.Sc. Programme)

SEMESTER-II

ADVANCED PARASITOLOGY, VECTOR BIOLOGY AND FISH BIOLOGY

ZCORT-206

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

Kalyani, Nadia

West Bengal, India

CONTENT WRITER:

Sl. No.	Name of Content Writer	Designation	Unit
1.	Dr. Subhabrata Ghosh	Assistant Professor of Zoology, Directorate of Open and Distance Learning, KU	Unit XI-XX
2.	Dr. Sudeshna Banerjee	Assistant Professor of Zoology, Directorate of Open and Distance Learning, KU	Unit I-X

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Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2020 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Manas Kumar Sanyal, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every member of PGBOS (DODL), University of Kalyani. Heartfelt thanks are also due to the Course Writers-faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and coordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

All the Self Learning Materials are self writing and collected from e-book, journals and websites.

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3	Prof. Kausik Mondal, Professor, Dept. of Zoology, University of Kalyani	Member
4	Dr. Kakali Bhadra, Associate Professor, Dept. Of Zoology, University of Kalyani	Member
5	Dr. Subhabrata Ghosh, Assistant Professor of Zoology, DODL, University of Kalyani	Member
6	Dr. Sudeshna Banerjee, Assistant Professor of Zoology, DODL, University of Kalyani	Member
7	Director, DODL, University of Kalyani	Convener

HARD CORE THEORY PAPER (ZCORT–206)

Group A (Advanced Parasitology, Vector Biology)				
Module	**Unit**	**Content**	**Credit**	**Page No.**
ZCORT–206 (Advanced Parasitology, Vector Biology)	I	Protozoans as parasites and causal agents of diseases	**3**	
	II	Physiology and metabolism of Haemo flagellates.		
	III	Physiology and immunopathogenesis of *Plasmodium vivax* and *P. falciparum*.		
	IV	Physiology of cestodes, trematodes and nematodes.		
	V	Fish parasites and its control		
	VI	Parasites of edible oysters		
	VII	Mode of transmission, pathogenicity and prevention of tuberculosis, cholera		
	VIII	Mode of transmission, pathogenicity and prevention of tetanus, rabies and dengue		
	IX	Life cycle, medical importance and control of disease-causing vectors: *Anopheles* sp., *Culex* sp., *Aedes* sp.		
	X	Life cycle, medical importance and control of disease-causing vector: i. Black fly		
ZCORT–206 (Fish Biology)	**Group B (Fish Biology)**			
	XI	Excretion and osmoregulation in fish.		
	XII	Reproduction in fish: reproductive strategies, oviparity, viviparity, ovo-viviparity, maturity stages, breeding cycle		
	XIII	Structure and physiology of endocrine glands in fishes		
	XIV	Electroreception in fish		
	XV	Determination of age of fish by scale and hard parts		
	XVI	Poisonous and venomous fish.		

	XVII	Fish migration: Types, Theories and Significances		
	XVIII	Parental care in Fish		
	XIX	Respiratory organs of Fishes: Water breathing, air breathing		
	XX	Swim Bladder in Fish		
	Total counseling session 18hrs.			

Group-A: ADVANCED PARASITOLOGY, VECTOR BIOLOGY

UNIT I

Protozoans as parasites and causal agents of diseases

Objective:

In this unit we will discuss about the Protozoans as parasites and causal agents of diseases.

Introduction:

Protozoa are unicellular, eukaryotic, heterotrophic organisms. They are either free-living or parasites. There are around 65000 species of protozoans categorized in different groups. They lack a cell wall. There are many different cell organelles, which perform various tasks performed by different organs in higher animals, e.g. mouth, anus, intestinal tract, etc.

There are many protozoa that cause various diseases in animals and humans, e.g. *Plasmodium* (malaria parasite), *Trypanosoma* (sleeping sickness), *Trichomonas* (trichomoniasis), etc.

The protozoa have many stages in their life cycle. Some of the stages of the life cycle are infectious.

The cyst stage is dormant and resistant to environmental stress, the trophozoite stage is reproductive and causes disease.

➢ General Characteristics of Protozoa

Habitat- Protozoa are found in the aquatic environment. They live in freshwater or oceans. Some are free-living and some are parasitic in plants and animals. Mostly they are aerobic but some are anaerobic and present in the rumen or human intestine.

Some of the species are found in extreme environments like hot springs. Some of them form resting cyst to overcome dry environments.

Size and Shape- The size and shape of Protozoa vary greatly, from microbial (1µm) to large enough and can be seen by the naked eye. The shell of unicellular foraminifera can have a diameter of 20 cm.

They lack a rigid cell wall, so they are flexible and found in various shapes. Cells are enclosed in a thin plasma membrane. Some of the species have a hard shell on the outer surface. In some of the protozoans especially in ciliates, the cell is supported

by **Pellicle,** which may be flexible or rigid and give organisms the definite shape and help in locomotion.

Cellular Structure- They are unicellular having a eukaryotic cell. The metabolic functions are performed by some specialized internal structures.

- They mostly have one membrane-bound nucleus in the cell
- The nucleus has diffused appearance due to scattered chromatin; the vesicular nucleus contains a central body called endosome or nucleoli. Nucleoli of apicomplexans have DNA, whereas amoeboids lack DNA in their endosome
- Ciliates have micronucleus and macronucleus
- The plasma membrane encloses the cytoplasm and other locomotory projections like flagella, pseudopodia and cilia
- Some of the genera have a membranous envelope called pellicle, which gives a definite shape to the cell. In some of the protozoans, epibiotic bacteria attach to the pellicle by their fimbriae
- The cytoplasm is differentiated into outer ectoplasm and inner endoplasm, ectoplasm is transparent and endoplasm contains cell organelles
- Some of the protozoa have cytostome for ingesting food. Food vacuoles are present, where ingested food comes. Ciliates have a gullet, a body cavity which opens outside
- The central vacuole is present for osmoregulation, that removes excess water
- Membrane-bound cell organelles, like mitochondria, Golgi bodies, lysosomes and other specialized structures are present

Nutrition- Protozoa are heterotrophic and have holozoic nutrition. They ingest their food by phagocytosis. Some of the protozoan groups have a specialized structure called **cytostome** for phagocytosis.

The pseudopodia of amoeboids help in catching the prey. Thousands of cilia present in ciliates drive the food-laden water into the gullet.

The ingested food comes to the food vacuole and gets acted on by lysosomal enzymes. The digested food gets distributed throughout the cell.

Locomotion- Most of the protozoa species have flagella, cilia or pseudopodia. Sporozoa, which don't have any locomotory structure, have subpellicular microtubules, which help in the slow movement.

Life Cycle- The life cycle of most of the protozoa alternates between dormant cyst stage and proliferating vegetative stage, e.g. trophozoites.

The cyst stage can survive harsh conditions without water and nutrients. It can remain outside the host for a longer duration and get transmitted.

The trophozoite stage is infectious, and they feed and multiply during this stage.

Reproduction- Mostly they reproduce by asexual means. They multiply by binary fission, longitudinal fission, transverse fission or budding.

In some of the species, sexual reproduction is present. The sexual reproduction is by conjugation, syngamy or by gametocytes formation.

➢ Examples of Diseases caused by Protozoa

Most of the protest diseases in humans are attributed to protozoa. Protozoa induces sickness in humans when it turns into human parasites. A majority of the prevalent and fatal diseases are caused by protozoan infections in humans such as Malaria, amoebic dysentery and African sleeping sickness.

These are capable of multiplying in humans, contributing to their survival, enabling the development of dangerous infections from one entity only. The transmission of protozoa found in the intestine of humans to another human usually takes place via the fecal-oral path, such as, through contaminated water or food or person-to-person contact. Those protozoa found in the tissue or blood of humans is passed to other humans by an arthropod vector.

Many of the protozoans are parasites and are disease causing pathogens. Find below the common diseases caused by protozoans.

1. Malaria

Cause: Genus *Plasmodium* (In humans, malaria is brought about by four different species of the organism: *Plasmodium malaria*, *Plasmodium falciparum*, *Plasmodium vivax*, and *Plasmodium ovale*).

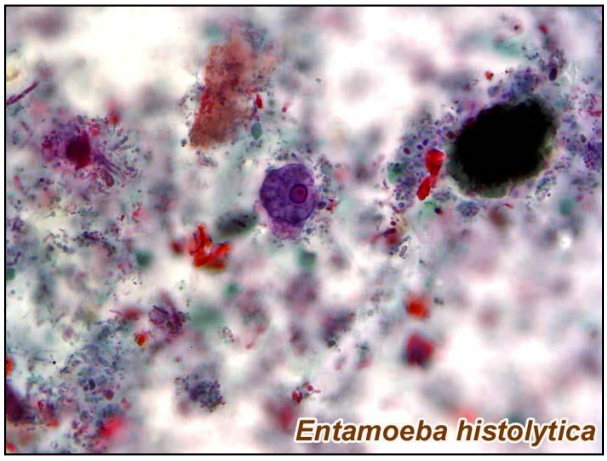
Vector: The parasite is only carried by the female *Anopheles* mosquito. Usually, it bites during dusk and dawn.

Nature: Malaria is an illness that needs an incubation period of more than 7 days. Hence, if the illness developed in less than a week after exposure, it is safe to say that it is not malaria.

Symptoms: Among all types of malaria, the most severe is the one that is caused by the species *Plasmodium falciparum*. Some of the most common symptoms include fever, headache, muscle pain, vomiting, abdominal pain, and cough. In worse cases, organ failure, convulsions, and the collapse of the circulatory system may occur.

Treatment: Malaria is preventable using antimalarial drugs such as chloroquine, quinine, mefloquine, etc.

2. Amoebiasis



Cause: Amoebiasis of the *Entamoeba* genus. The parasite *Entamoeba histolytica* is the most common.

Vector: none

Nature: Also known as *amoebic dysentery*, amoebiasis is a type of gastrointestinal infection that is often linked to poor sanitation and socioeconomic status rather than the weather or climate.

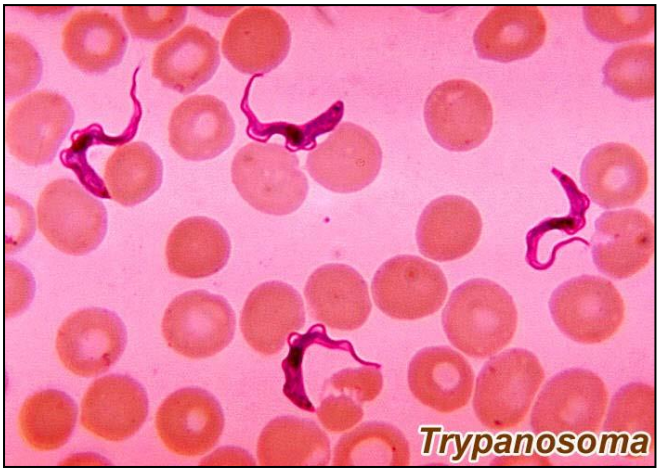
Symptoms: Most of the time, amoebiasis starts with abdominal pain that eventually develops into loose bowel movements. Aside from that, fever, nausea, loss of appetite and bloody stools are also an indication of amoebiasis.

Treatment: To treat amoebiasis, most doctors prescribe metronidazole or tinidazole.

3. Trypanosomiasis or African Sleeping sickness

Cause: *Trypanosoma brucei*. In humans, two types of this species can infect humans: *Trypanosoma brucei gambiense* (TbG) and *Trypanosoma brucei rhodesiense* (TbR).

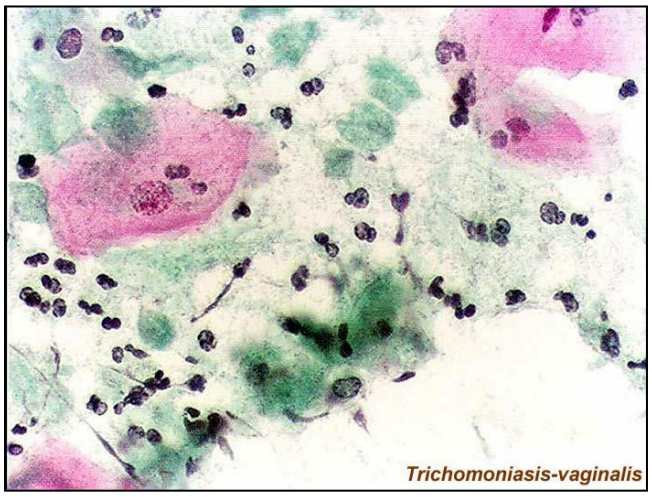
Vector: The protozoa are transmitted by the blood-sucking insect called tsetse fly (genus *Glossina*).



Symptoms: Some of the most common symptoms of the disease include a headache, high fever, irritability, muscle and joint pain, and swollen lymph nodes. In worse cases, some people get skin rash and develop neurological problems. If left untreated, the infected patient may die within a few months.

Treatment: While the type of treatment differs depending on the type of trypanosomiasis infection, the most common medicine recommended during its early stages are pentamidine, suramin, nifurtimox, eflornithine, and melarsoprol.

4. Trichomoniasis



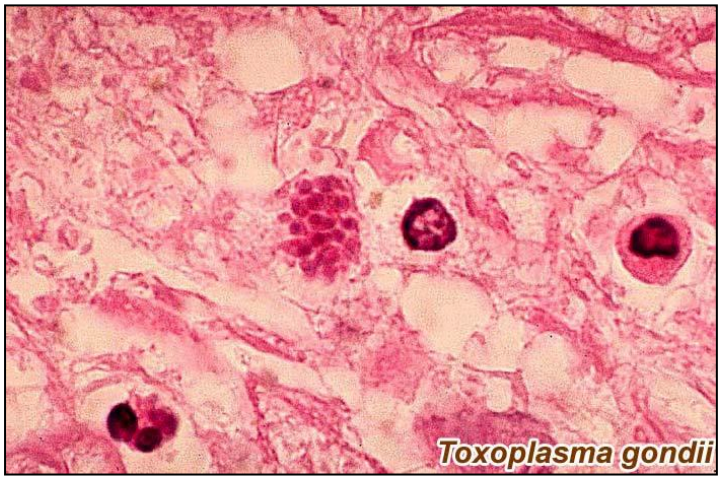
Cause: Trichomoniasis is a sexually-transmitted disease that is caused by the protozoan *Trichomoniasis vaginalis*.

Vector: None. The parasite is transmitted from person to person during sexual intercourse. In men, the inside of the penis (urethra) is the most commonly affected. On the other hand, in women, the lower genitalia (includes the vagina, cervix, vulva, and the urethra) is the most commonly affected.

Symptoms: Most of the time, trichomoniasis occurs with no noticeable symptoms. Both men and women with the disease may experience itching and burning sensation in the affected areas, as well as having weird discharge.

Treatment: Regarding treatments available, this disease is considered to be one of the most treatable diseases in the United States.

5. Toxoplasmosis



Cause: Toxoplasmosis is a type of infection from one of the world's most common protozoan *Toxoplasma gondii*.

Vector: The parasite can be ingested via contact with contaminated water and soil in various areas. Aside from that, the eggs (oocysts) of the parasite may also attach to the fur of animals that have come in contact with contaminated cat feces.

Symptoms: People infected with the disease may develop flu-like symptoms, and experience blurred vision and redness of the eyes. Aside from that, infected people may also experience body pain and fatigue.

Treatment: Some of the most common prescription drugs for the disease include *pyrimethamine* (Daraprim) and the antibiotic *sulfadiazine*.

6. Balantidiasis

Cause: Balantidiasis (also known as balantidiosis) is defined as large-intestinal infection with *Balantidium coli,* which is a ciliated protozoan (and the largest protozoan that infects humans). *B coli* are known to parasitize the colon, and pigs may be its primary reservoir.

Vector: Balantidiasis is a zoonotic disease transmitted from pigs to humans and nonhuman primates via the fecal-oral route. Humans acquire this infection through water and food contaminated with pig or human feces. Human-to-human transmission is also possible.

Symptoms: People infected with the disease may develop symptoms like persistent diarrhea, dysentery, abdominal pain, weight loss, nausea, and vomiting. If left untreated, perforation of the colon can occur.

Treatment: Three medications are used most often to treat *Balantidium coli*: tetracycline, metronidazole, and iodoquinol.

7. Giardiasis

Cause: Giardiasis is an infection in your small intestine. It's caused by a microscopic parasite called *Giardia lamblia*.

Vector: Giardiasis spreads through contact with infected people. And you can get giardiasis by eating contaminated food or drinking contaminated water. Pet dogs and cats also frequently contract giardia.

Symptoms: Some people with giardia infection never develop signs or symptoms, but they still carry the parasite and can spread it to others through their stool. For those who do get sick, signs and symptoms usually appear one to three weeks after exposure and may include:Watery, sometimes foul-smelling diarrhea that may alternate with soft, greasy stools, Fatigue, Stomach cramps and bloating, Gas, Nausea, Weight loss

Treatment: Metronidazole is the most commonly used antibiotic for giardia infection. Side effects may include nausea and a metallic taste in the mouth. Don't drink alcohol while taking this medication.

8. Leishmaniasis

Cause: Leishmaniasis is a vector borne disease that is transmitted by sand flies and caused by obligate intracellular protozoa of the genus *Leishmania*. There are 3 main forms of leishmaniasis – visceral (also known as kala-azar, which is and the most serious form of the disease), cutaneous (the most common), and mucocutaneous.

Vector: The parasite lives and multiplies inside the female sand fly. This insect is most active in humid environments during the warmer months and at night, from dusk to dawn. Domestic animals, such as dogs, can serve as reservoirs for the parasite. Transmission may occur from animal to sand fly to human.

Humans can also transmit the parasite between each other through a blood transfusion or shared needles. In some parts of the world, transmission may also occur from human to sand fly to human.

Symptoms: People can carry some species of *Leishmania* for long periods without becoming ill. Symptoms depend on the form of the disease.

- **Cutaneous leishmaniasis**

The main symptom of this condition is painless skin ulcers. Cutaneous symptoms may appear a few weeks after being bitten by an infected sand fly. However, sometimes symptoms won't appear for months or years.

- **Mucocutaneous leishmaniasis**

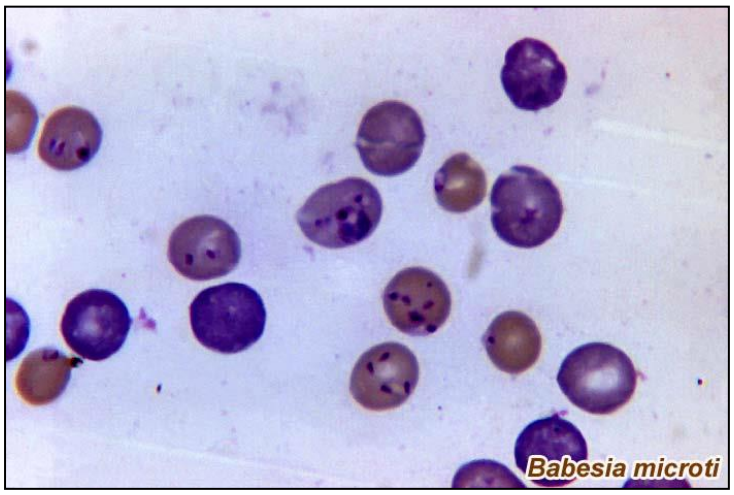
In people with the mucocutaneous form of the disease, symptoms usually appear one to five years after the skin lesions. These are primarily ulcers in their mouth and nose or on their lips. Other symptoms may include: runny or stuffy nose, nosebleeds, difficulty breathing

- **Visceral leishmaniasis**

Symptoms often don't appear for months after the bite with this type of leishmaniasis. Most cases are apparent two to six months after the infection occurred. Common signs and symptoms include: weight loss, weakness, fever that lasts for weeks or months, enlarged spleen, enlarged liver, decreased production of blood cells, bleeding, other infections, and swollen lymph nodes.

Treatment: Antiparasitic drugs, such as amphotericin B (Ambisome), treat this condition.

9. Babesiosis



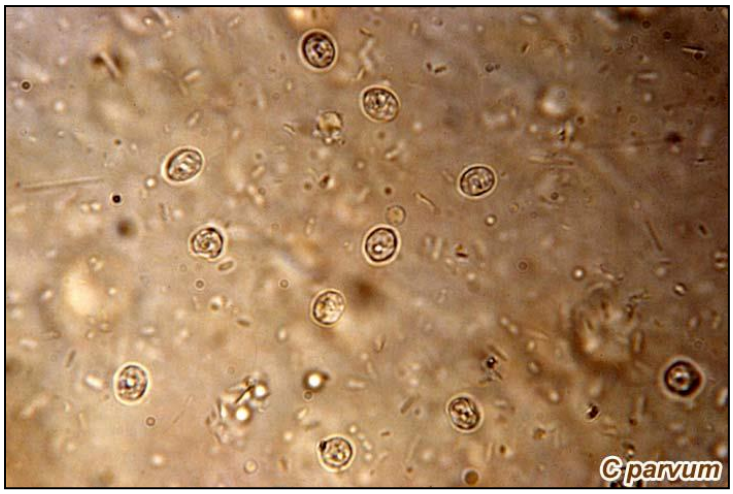
Cause: The disease is caused by the microscopic red blood cell-infecting parasite *Babesia microti*.

Vector: The protozoan is transmitted through the bite of the infected tick *Ixodes scapularis*. Most of the time, it is transmitted when the said tick is in the nymph stage.

Symptoms: Most of the time, people infected with the protozoan do not exhibit any noticeable symptoms. In some cases, they develop fever, headache, fatigue, body pain, nausea, and loss of appetite. Babesiosis can be sometimes life-threatening especially for some people who have weak immune systems.

Treatment: Patients with babesiosis can be treated within 7 to 10 days, with a combination of prescription drugs. Such combinations may be *clindamycin* and *quinine*, or *azithromycin* and *atovaquone*.

10. Cryptosporidiosis



Cause: In humans, the disease is caused by the apicomplexan protozoan members of the genus *Cryptosporidium*. The most common species found in humans are *C.parvum* and *C.hominis*.

Vector: Unlike other parasites, Cryptosporidium species do not need a vector and have the ability to complete their life cycle on their own.

Symptoms: In humans, the disease is characterized by the infection of the small intestine, particularly the *jejunum* and the *ileum*. As a result, an infected patient experiences watery diarrhea that could last up to two weeks. In severe cases, the infection could reach the stomach and the lungs.

Treatment: At present, there is still no prescribed medication for the disease. Most of the time, the recovery period is dependent on the immunity of the patient. Usually, it takes about two weeks to recover without medication.

Probable questions:

1. Name the causative agent of malaria. Discuss the symptoms and treatment of malaria disease.
2. Write short notes on Amoebiasis.
3. Name the causative agent and vector of Trypanosomiasis.
4. Discuss the symptoms of Balantidiasis.
5. Write short notes on Leishmaniasis, Babesiosis, Cryptosporidiosis.

Suggested readings/ references:

1. Chandler, A. C. and Read. C. P. (1961). *Introduction to Parasitology*, 10th ed. John Wiley and Sons Inc.
2. Cheng, T. C. (1986). *General Parasitology*. 2nd ed. Academic Press, Inc. Orlando.U.S.A.
3. Cox, F. E. G. (1993). *Modern Parasitology*. 2nd ed. Blackwell Scientific Publications. Lea and Febiger, Philadelphia
4. Noble, E. R. and Noble G. A. (1989). *Parasitology. The Biology of animal Parasites.* 6th ed.
5. Roberts, L. S., Janovy, J. and Nadler S. (2013) *Gerald D. Schmidt &Lary S. Roberts'Foundation of Parasitology*. 9th ed. McGraw-Hill International

UNIT II

Physiology and metabolism of Haemoflagellates

Objective:

In this unit you will know about Physiology and metabolism of Haemoflagellates.

Introduction

Haemoflagellates are protozoan flagellates in the family Trypanosomatidae that are parasitic in the blood of many species of domestic and wild animals and birds, and of humans; they include the genera Leishmania and Trypanosoma, several species of which are important pathogens.

Leishmaniasis is a disease caused by the protozoa *Leishmania* and is most commonly transmitted by infected sandflies. It has been historically widespread in tropical climates across multiple continents including Europe, Africa, Asia, and America. In humans, these parasites replicate intracellular and present classically with a visceral or cutaneous disease.

1. Leishmaniasis:

Kala-azar, also known as Dum-dum fever, is a serious oriental disease of man. It is found in India, China, Mediterranean countries and parts of Africa and South America. Its causative agent is a pathogenic flagellate, known as *Leishmania donovani* which is transmitted by the bite of small blood sucking sandflies called *Phlebotomus argentipes* (Fig 1)). *Leishmania* species undergo multiplication as promastigotes *Phlebotomus argentipes*, but they are injected into a vertebratehost when the sand fly feeds, and they undergo additional multiplication, as amastigotes, in a variety of tissues.

The species concerned are as follows:

Indian vector: *P. argentipes*; Chinese vectors: *P. chinensis*, and *P. sergenti*; Mediterranean vectors: *P. perniciosus* (Italy and Sicily); Tropical American vector: *P. intermedius*; East African vector: *P. martini*.

A. Life cycle in Man:

(a) Infection:

L. donovani is transmitted to man by the sand-fly, *Phlebotomus argentipes*. In case of this parasite sand fly acts as vector. The insect vector which has fed on some suitable fruit or plant juice after feeding on infected human blood meal, shows an enormous number of parasites in its buccal cavity and pharynx.When such a vector bites a man, it introduces the parasites in the skin wound by its proboscis. Some authors are of opinion that the

Indian vector (Sand fly) does not bite but spreads infection by being crushed possibly by slapping.

(b) Multiplication:

The parasites administered by the vector into human body are the promastigote or leptomonad form. Some of them, entering the blood circulation directly become destroyed while those entering the cells of R. E. system (liver, bone marrow, lymph node, spleen) change into amastigote or leishmanial forms. Multiplication by binary fission goes on continuously till the cells become packed with the parasites. The host cell is thereby enlarged and eventually ruptures (50 to 200 or even more may be found embedded in the enlarged host cell) (Fig 1).

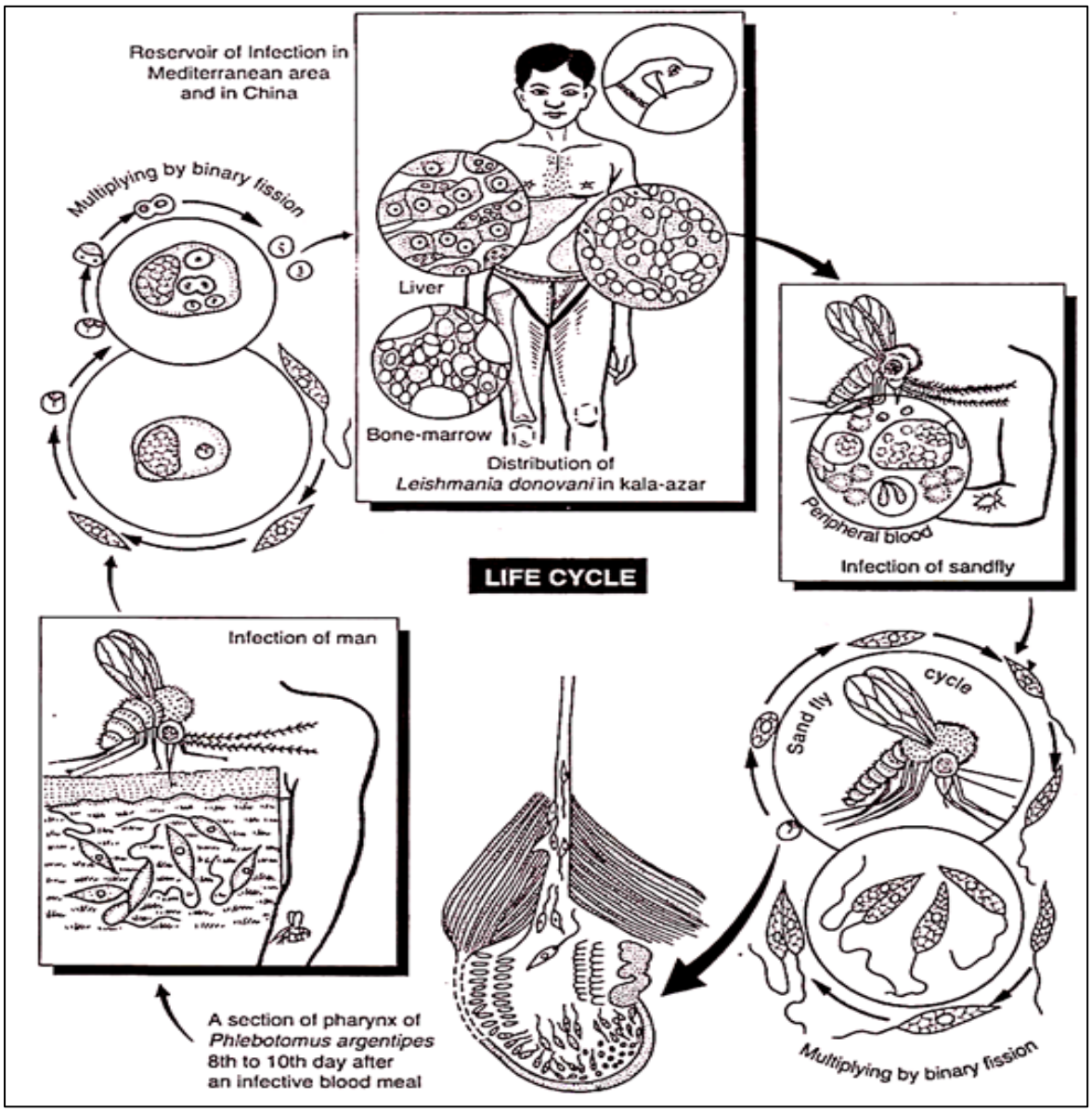


Fig 1: Life cycle of *Leishmania donovani* in man and sand fly

(c) Spread of infection:

When the number of parasites reaches upto 200 or even more, the host cell ruptures. The parasites liberated as a result of the rupture into the circulation are again either taken up by or invade fresh cells and the multiplication cycle is repeated so that the entire R. E. system becomes progressively infected. In the blood stream, some of the free amastigotes are phagocytosed by the neutrophil granulocytes and monocytes. A blood sucking insect draws these free amastigote forms as well as those within the monocyte during its blood meal. These heavily parasitized cells wander through the general blood circulation leading to a general infection.

B. Life cycle in vector (Sand fly):

(a) Transfer to vector:

When the vector sucks blood of an infected person, it receives free amastigotes as well as parasitized neutrophils and monocytes along with the blood meal.

(b) Development in the vector:

In the midgut of sand fly, the amastigote form becomes elongated and acquires a free flagellum, thus developing into promastigote form which again multiplies by binary fission producing an enormous number of flagellates. This multiplication proceeds in the midgut of this insect vector and the flagellates tend to spread forwards to the anterior part of digestive tract of (pharynx and buccal cavity) sand fly. A heavy pharyngeal infection of this insect vector is usually found between 6 to 9 days of its infective blood meal. This type of development is called anterior station development. The transmission into a new host is thereby effected when such a heavily infested sand-fly bites the host (but salivary glands are not infected).

▪ Types of leishmaniasis

Leishmaniasis comes in three forms: cutaneous, visceral, and mucocutaneous. Different species of the *Leishmania* parasite are associated with each form. Experts believe that there are about 20 *Leishmania* species that can transmit the disease to humans.

I. Cutaneous leishmaniasis

Cutaneous leishmaniasis causes ulcers on your skin. It's the most common form of leishmaniasis. Treatment may not always be necessary depending on the person, but it can speed healing and prevent complications.

II. Mucocutaneous leishmaniasis

A rare form of the disease, mucocutaneous leishmaniasis is caused by the cutaneous form of the parasite and can occur several months after skin ulcers heal. With this type of leishmaniasis, the parasites spread to your nose, throat, and mouth. This can lead to partial or complete destruction of the mucous membranes in those areas. Although mucocutaneous leishmaniasis is usually considered a subset of cutaneous leishmaniasis, it's more serious. It doesn't heal on its own and always requires treatment.

III. **Visceral leishmaniasis**

Visceral leishmaniasis is sometimes known as systemic leishmaniasis or kala azar. It usually occurs two to eight months after being bitten by a sand fly. It damages internal organs, such as your spleen and liver. It also affects your bone marrow, as well as your immune system through damage to these organs. The condition is almost always fatal if it's not treated.

- **Symptoms of leishmaniasis**

People can carry some species of *Leishmania* for long periods without becoming ill. Symptoms depend on the form of the disease.

I. **Cutaneous leishmaniasis**

The main symptom of this condition is painless skin ulcers. Cutaneous symptoms may appear a few weeks after being bitten by an infected sand fly. However, sometimes symptoms won't appear for months or years.

II. **Mucocutaneous leishmaniasis**

In people with the mucocutaneous form of the disease, symptoms usually appear one to five years after the skin lesions. These are primarily ulcers in their mouth and nose or on their lips. Other symptoms may include: runny or stuffy nose, nosebleeds, difficulty breathing

III. **Visceral leishmaniasis**

Symptoms often don't appear for months after the bite with this type of leishmaniasis. Most cases are apparent two to six months after the infection occurred. Common signs and symptoms include: weight loss,weakness, fever that lasts for weeks or months, enlarged spleen, enlarged liver, decreased production of blood cells, other infections, swollen lymph nodes. It is followed by general weakness, emaciation, anaemia and a peculiar darkening of the skin.

2. Trypanosomiasis

African Trypanosomiasis, also known as "sleeping sickness", is caused by microscopic parasites of the species *Trypanosoma brucei*. It is transmitted by the tsetse fly (*Glossina* species), which is found only in sub-Saharan Africa. The parasite is classified into three subspecies: *Trypanosoma brucei gambiense, T. brucei rhodesiense,* and *T. brucei brucei*. The later subspecies is not pathogenic to humans. These subspecies cannot be differentiated morphologically. *T. b. gambiense* is distributed in western and central Africa which causes chronic disease whereas, *T. b. rhodesiense* found in eastern and southern Africa and responsible for acute severe disease.

The epidemiology of these parasite species also differs and depends on the distribution of their vectors, *Glossina palpalis* and *Glossina morsitans* respectively.

Morphology:

T. gambiense are microscopic, elongate, flattened and have fusiform body pointed at both ends and covered by a membranous pellicle which maintains the form of body. It measures about 10 µm to 40 µm in length and 2.5 µm to 10 µm in width. A single flagellum arises from a basal body situated near the posterior end and curves in a spiral form round the body forming undulating membrane, thrown into 3 or 4 folds depending upon the length of the parasite. The undulating membrane is believed to be an adaptation for locomotion in the blood. The flagellum is free at the anterior end. The nucleus is large and oval, situated in the centre of the body and the cytoplasm contains numerous greenish refractile granules called volutin granules. These granules store food particles mainly glycogen and phosphate. At the base of the flagellum is located the basal granule or blepharoplast close to which is another granule, the parabasal body.

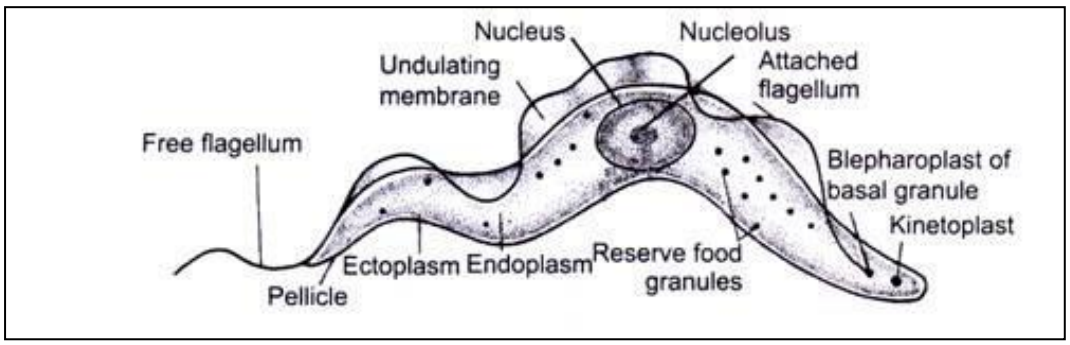


Fig: Morphology of *Trypanosoma gambiense*

Life cycle:

T. gambiense is a digenetic parasite which requires two hosts for completing the life cycle (figure 6). The primary host is humans and the intermediate host is blood sucking insect Tsetse fly of the genus *Glossina*. The mammals like pig, antelopes and buffaloes often act as reservoir host harbouring the parasite. When Tsetse fly sucks the blood from infected individual or wild mammal, it carries Trypanosomes to its mid gut where they divide asexually by longitudinal binary fission. Here the parasite changes their morphology and give rise to metacyclic forms which are short and stumpy. At this stage, the fly is said to be infective. When the infected tsetse fly bites a healthy human host, it releases these metacyclic trypanosomes in the blood stream of host and repeats the life cycle. Sexual reproduction is unknown in T. *gambiense*. It is essentially a parasite of connective tissue in human where it multiplies readily. It consumes large amount of glucose and invades the regional lymph nodes through the lymphatic systems and also invades the blood system causing parasitaemia. It finally localizes in the brain. It is to be noted that African sleeping sickness is a disease which affects the central nervous system.

A. Life cycle in human:

Infection: The infection by parasite is initiated when tsetse fly harbouring the infective metacyclic form, bite the healthy individual. When the fly bites, it releases trypanosomes into blood stream which develop into long slender form and multiply asexually by binary longitudinal fission at the site of inoculation. These become 'stumpy' via 'intermediate' forms. Consequently the parasites invade the blood stream and causes parasitaemia. The trypomastigote forms, mainly the short stumpy forms are taken up by the tsetse fly along with its blood meal and undergo a series of complex biological development inside the insect host before becoming infective to man**.**

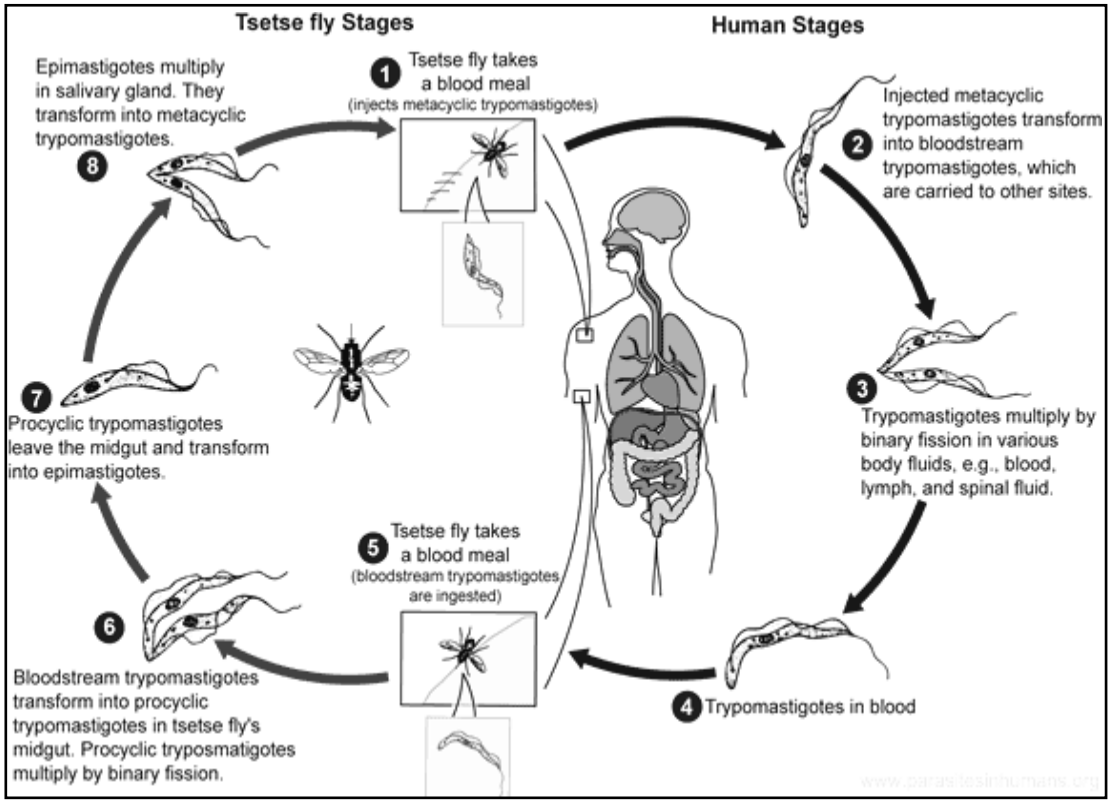


Fig: Life cycle of *Trypanosoma* sp.

Multiplication: All stages of parasites in humans are extracellular as they are present in the blood cells. In human blood, the metacyclic forms which are devoid of free flagellum become transformed into long slender forms equipped with long flagella. These stages can freely swim by beating of their free flagellum along with the vibratile movements of the undulating membrane. They multiply asexually by longitudinal binary fission and obtain energy by anaerobic process of glycolysis.

Metamorphosis: When absorption of glucose ceases due to antibodies which are produced in blood, is hampered glycolysis. As a result, the trypanosomes stop dividing and shrink to short stumpy forms, which are lacking free flagellum. These stumpy forms do not feed and ultimately die if they are not sucked up by tsetse

fly along with the blood meal from infected human.

Relapse of infection: It has also been reported that some of the long and slender forms of trypanosomes do not undergo any transformation, but change their antigen in blood to which the host has produced the antibodies. These unaltered slender forms continue to survive and multiply in blood leading to future relapses of the infection

B. Life cycle in tsetse fly:

Transfer to tsetse fly: When tsetse fly feed on the blood of an infected person, it also sucks short stumpy forms of parasite along with the blood. It is the stumpy forms which continue development in the vector. Development in mid gut: Further developments of stumpy forms proceeds in the insect mid gut within peritrophic membrane. In the mid gut parasite transforms into long slender form and multiply asexually by longitudinal binary fission. The kinetoplast moves farther from the posterior end of body. The energy yielding process is related to mitochondrial oxidation of pyruvic acid. Development in salivary gland: After sometime, the long slender forms migrate into salivary glands via oesophagus and mouthparts of insects. Here, they metamorphose into the crithidial forms with shortened body, reduced free flagellum and the kinetoplast in front of the nucleus. The mitochondria develop an extensive network of cristae and parasite respires more economically as blood glucose gradually declines. The crithidial forms multiply in the lumen of salivary glands and transform into slender metacyclic forms. When the tsetse fly bites a healthy person, it transfers the metacyclic stage into his blood where they initiate another infection. Sleeping sickness: *T. gambiense* causes the disease of West African sleeping sickness. It is different from American sleeping sickness or Encephalitis which is caused by filterable virus.

Mode of infection:

Inoculative method: by the bite of the infective tsetse fly, *Glossina*: Both male and female suck the blood and can transmit the infection. They bite by daylight, usually in the early morning and evening. The metacyclic stage is introduced by the tsetse fly with the saliva into the subcutaneous pool of blood on which it feeds. Some of the parasites may enter the blood stream directly and majority of them entangled in the tissue space. The initial growth of trypomastigotes occurs in the tissue space which form a favourable nidus or possibly here the organisms can escape the action of antibodies which might be developed. It is to be noted that while the trypomastigotes are multiplying in the subcutaneous tissue, the organisms are either absent or present in small numbers only in the peripheral blood. It has been suggest that although unlikely yet the connective tissue damage caused by the trypomastigotes may be due to an exaggerated immune response (autoimmune

reaction or massive release of kinin) rather than to any direct effect (mechanical damage due to motility) of this relatively non toxic organism. The presence of trypomastigotes in the subcutaneous connective tissue excites host's immune response in two ways.

a) By producing large amount of non specific immunoglobulins which are however not capable of sensitizing the antigen. Antibodies are produced in response to the secretion of an exo-antigen of the trypomastigotes.

b) By heavily infiltrating the site of infection with macrophages, the cells competent to deal with the invaders. The neutrophils take peculiarly little interest in the defense and are therefore not much in evidence.

Thus it will be seen that there is no lack of mobilization of the hosts defensive mechanism but it is the cellular defense which plays the dominant role. The macrophages could be seen to remove the living trypomastigotes in the tissue space. The release of kinins may help to attract macrophages, it also increases the capillary permeability of tissues and may explain the oedematous swollen subcutaneous tissue at the site of infection. Furthermore, trypanosomes are surrounded by a coat that is composed of variant surface glycoproteins (VSG). These proteins act to protect the parasite from any lytic factors that are present in human plasma. The host's immune system recognizes the glycoproteins present on the coat of the parasite leading to the production of different antibodies (IgM and IgG). These antibodies will then act to destroy the parasites that circulate around the blood. However, from the several parasites present in the plasma, a small number of them will experience changes in their surface coats, resulting in the formation of new VSGs. Thus, the antibodies produced by the immune system will no longer recognize the parasite leading to proliferation until new antibodies are created to combat the novel VSGs. Eventually the immune system will no longer be able to fight off the parasite due to the constant changes in VSGs and infection will arise.

Symptoms:

i. Bite of tsetse fly causes local irritation which subsides after few days.

ii. A trypanosomal chancre may develop at the site of inoculation of trypomastigotes introduced by the bite of the infected tsetse fly. It is a hard painful nodule and fluid withdrawn from it contains actively dividing trypomastigotes.

iii. The symptom can appear after several months or a year in Gambian form but symptoms may appear after two weeks in case of Rhodesian form.

iv. It is characterized by the infection of blood stream, involvement and enlargement of lymph nodes and eventually invasion of the central nervous system.

v. The early symptoms are fever, loss of nocturnal sleep, severe headache, and feeling of oppression.

vi. Lymph node enlargement, particularly of the posterior triangle of the neck is a feature of Gambian disease whereas invasion of CNS is very rapid in case of 'rhodesian' form.

vii. As the CNS is involved, the symptoms of meningo-encephalitis develop resulting in classical sleeping sickness. In due course, the patient fall asleep, first at regular interval and then lies prostrate in coma. Finally, the patient becomes thin and exhausted, accompanied by signs of malnutrition. Disruption of the sleep cycle is an important symptom of this stage that gave the disease the name 'sleeping sickness'.

The person infected from disease experience unsystematic and uneven 24-hour rhythm of the sleep-wake cycle. The patient sleeps in daytime and at night time shows periods of wakefulness. Other neurological symptoms of the disease include tremor, confusion, paralysis, general muscle weakness, hemiparesis and paralysis of a limb. Parkinson like movements may also arise due to non-specific movement and speech disorders. The person infected from sleeping sickness may also exhibit psychiatric signs like aggressive behaviour, irritability, psychotic reactions or apathy which can sometimes dominate the clinical diagnosis. If the disease is not treated, it can invariably become fatal, with progressive mental deterioration that leads to coma, systemic organ failure and finally death.

viii. The infection can be spread from pregnant mother to her child because the trypanosomes are able to cross the placenta and cause the disease to the fetus.

The mechanical transmission is also possible through other blood sucking insects.

Accidental infections may also be possible in the laboratories due to pricks from contaminated needles.

Physiology and metabolism of *Leishmania* and *Trypanosoma*

Members of the genus *Leishmania* are like most, if not all, trypanosomids essentially aerobic organisms, but endowed with the faculty of enduring for certain periods experimentally imposed anoxic conditions. In nature they probably do not encounter severe oxygen deficiencies either in the intermediate host, or in the final host, the probability being that at least in the latter the parasites have as ready access to oxygen

as the host cells. The oxygen supply in the deeper layers of skin lesions, for example, may differ materially from those characteristic of normal tissues;

Leishmania donovani forms aerobically lactic and succinic acids, as well as acetic and pyruvic acids, while anaerobically only the excretion of lactic and succinic acid has been found. These end products are undoubtedly derived from the utilization of exogenous carbohydrates. There is no indication that leishmanias store any larger amount of a genuine reserve carbohydrate such as· glycogen. In fact, the only polysaccharide so far described from *Leishmania donovani* yielded upon acid hydrolysis glucose, galactose and arabinose. It is interesting to note that the spectrum of carbohydrates available to *Leishmania* is broader than that reported utilized by some trypanosomids, such as *Trypanosoma lewisi*. Glycerol which is readily consumed by *Trypanosoma rhodesiense*, in fact preferentially to glucose, has been shown by quantitative chemical procedure to be utilized only in small amounts by the human leishmanias.

- **Carbohydrate metabolism**

The pathways by which the end products of carbohydrate metabolism are formed have hardly been investigated in *Leishmania*. Only a single enzyme, hexokinase, has been described from the flagellates under consideration. Cell-free extracts of *Leishmania donovani* rapidly phosphorylated glucose, fructose, mannose, galactose, and d-glucosamine, with only very weak activity against ribose and glycerol. Apparently only a single enzyme is involved in hexose phosphorylation, as the mutual inhibition excerted by the various sugars indicates. An appropriately low pH the organisms are able to utilize practically all intermediates of the Krebs cyele. It has furthermore been found that malonate inhibits succinate oxidation by *Leishmania brasiliensis* and that fluoroacetate inhibits the respiration of *Leisbmania enrietti*.

It must furthermore be recalled that *Leishmania* excretes under aerobic conditions succinic acid, a typical intermediate of the Krebs cyele. It is clear that the reactions of the tricarboxylic acid cycle would soon come to a complete stand still unless oxaloacetic acid, the motor of the sequence, were resynthesized by an auxiliary reaction and re-fed into the sequence. It is possible, granting that *Leishmania* may possess a Krebs cyele, that as in *Trypanosoma cruzi* aerobic carbon dioxide fixation is involved.

- **Lipid metabolism**

Some interesting data on the lipid chemistry of *Leishmania* have become available. Types of sterol found in flagellates are ergosterol from *Leishmania tropica* and cholesterol in *Leishmania donovani*. In *Leishmania tarentolae,* for instance, 21 fatty acids with chain length longer than C_{11} have been found.

- **Nucleic acid metabolism**

The nucleic acids of *Leishmania* have received but little attention up to the present time. It has been established by caesium chloride density gradient centrifugation that the base composition of the deoxyribonucleic acid of *Leishmania tarentolae* corresponds to 54 mole percent of guanine plus cytosine, a value well in the range of those found in

other zooflagellates, but sharply differing from those reported for ciliates and rhizopods. DNA extracted from *Leishmania enrietti* could be resolved into two bands. The major one had a guanosine plus cytosine content of 57 percent: the minor one of 36 percent. It was established that the DNA of the kinetoplast contained essentially only the minor component, with apparent little if any of the major component present. The latter was preferable to the DNA of the nucleus, but it could not be established with certainty whether or not the latter also contained some of the minor component.

Compounds usually associated with the nucleic acids (guanosine, uracil, hypoxanthin, and ribose), in addition to phosphate, were found to leak out rapidly from *Leishmania enrietti* when the flagellate was maintained on a simple medium at elevated temperature, that is, above 30°C instead of the usual 22-23°C. It is probable that this leakage of metabolically significant compounds is due to a general increase in hydrolytic activity at higher temperatures, conceivably due to an activation of lysosome enzymes. It should especially be noted that this leakage is not limited to, but only greatly enhanced, by the elevated temperature. In the range 10 to 40°C leakage increased about 30 fold, while respiration only doubled.

- **Respiration**

The last topic to be considered briefly concerns the respiratory activities of *Leishmania*. Their endogenous respiration is apparently reatively low, but sufficient endogenous reserves are available to maintain the flagellates viable and motile for several h ours as we have repeatedly observed in my laboratory. In the presence of an utilizable substrate the rate of oxygen consumption or the rate of anaerobic CO_2 production can rise rather sharply. With glucose as substrate, for instance, the oxygen consumption of *Leishmania donovani* and *L. enrietti* is raised approximately 3 to 8 times above the endogenous rate while the anaerobic carbon dioxide production rose even more. The aerobic respiration of various *Leishmania* species is strongly inhibited by cyanide while the data for azide are somewhat contradictory. *Leishmania enrietti* appears not to be susceptible to azide inhibition. While it does seem likely that heavy metal catalysis characterizes the respiration of the flagellates.

- **Comparison between different forms**

In view of the fact that the leishmanias alternate in their life cycle between Leishman-Donovan bodies, and motile leptomonad forms, the question should be raised whether the metabolism of these stages differs to a great extent. Little information on this point is available. All the data summarized so far are derived from studies with the leptomonads, for the simple reason that they are easily cultivated, while the isolation of Leishman-Donovan bodies is a difficult and time-consuming task. The oxygen consumption of the leptomonad stage was thus more susceptible to cyanide and amidine inhibition than that of the Leishman-Donovan bodies. It also appeared probable that the latter contain relatively more oxidizable reserve substances than the former. This seems indicated by the relatively high endogenous respiration of the Leishman-Donovan bodies and by the fact that their respiration is percentage wise less stimulated

by glucose than that of the leptomonads. The metabolism of all members of the genus *Leishmania* is characterized by aerobic fermentations, which lead to the excretion of various partly oxidized metabolic end products. The flagellates utilize several carbohydrates freely, but hardly anything is known about the intermediate carbohydrate metabolism. At suitably low pH they are able to consume intermediates of the Krebs cycle, but the presence of a functional tricarboxylic acid cycle has not yet been demonstrated conclusively. Recent data on lipid metabolism and biosynthesis have revealed interesting paralleles to phytoflagllates, as have newer studies on nucleic acids. The composition of the proteins of *Leishmania* is essentially unknown, but the fact that under certain experimental conditions numerous amino acids are leaked into the medium, indicates that probably all common amino acids are leaked into the medium, indicates that probably aH common amino acids are present. The respiration of the flagellates is probably characterized by heavy metal catalysis, but it is uncertain whether or not they contain a fully functional cytochrome system.

Probable questions:

1. Describe the physiology and metabolism of *Leishmania* and *Trypanosoma*.
2. Discuss the life cycle of *Trypanosoma*.
3. Elaborate the pathology of Trypanosomiasis.
4. Describe the structure of *Trypanosoma.*
5. Describe the life cycle of *Leishmania* with proper diagram.

Suggested readings/ references:

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UNIT III

Physiology and immunopathogenesis of *Plasmodium vivax* and *Plasmodium falciparum*

Objective:

In this unit we will discuss about the Physiology and immunopathogenesis of *Plasmodium vivax* and *Plasmodium falciparum.*

Introduction:

Malaria is one of the most important infectious diseases in the world, and it has been recognized as the most widespread infection in tropical and subtropical areas with high rate of morbidity and mortality. An estimated 3.3 billion people are at risk of malaria in the world, of which 1.2 billion are at high risk and 97 countries had ongoing malaria transmission. It still remains a challenge in the world in general and in Africa in particular. There is an estimated 212 million cases all over the world; most of these cases (82%) were in the WHO African Regions, followed by the WHO Southeast Asia Regions (12%) and the WHO Eastern Mediterranean Regions (5%). Globally, malaria deaths were 429,000 and 90% of these deaths were in the WHO African Regions, followed by the WHO Southeast Asia Regions (7%) and the WHO Eastern Mediterranean Regions.

Plasmodium falciparum is mainly responsible for the enormous deaths (99%) than other *Plasmodium* species. Some studies associate the virulence of *P. falciparum* with its ability to escape the human and vector immune system by different mechanisms. This review is mainly focused on evading, invading, and immune response mechanisms involved in different stages of malaria parasite and on its implication for vaccine development.

Malaria causes disease through a number of pathways, which depend to a certain extent on the species. Malaria is caused by a single-celled parasite of the genus *Plasmodium*; there are five species which infect humans, being *Plasmodium falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*.

Life cycle of *Plasmodium sp.*

All these species are introduced into the human blood stream through the bite of an infected mosquito; the life stage of malaria at this point is called a "sporozoite", and they pass first to the liver, where they undergo an initial stage of replication (called "exo-erythrocytic replication"), before passing back into the blood and invading red blood cells (called "erythrocytes", hence this is the "erythrocytic" part of the cycle). The malaria parasites that invade red blood cells are known as merozoites, and within the

cell they replicate again, bursting out once they have completed a set number of divisions. It is this periodic rupturing of the red blood cells that causes most of the symptoms associated with malaria, as the host's immune system responds to the waste products produced by the malaria parasites and the debris from the destroyed red blood cells. Different species of malaria rupture the red blood cells at different intervals, which lead to the diagnostic cycles of fever which characterise malaria; *P. vivax*, for example, tends to produce cycles of fever every two days, whereas *P. malaria* produces fever every three.

Pathogenesis

In addition, *Plasmodium falciparum* produces unique pathological effects, due to its manipulation of the host's physiology. When it infects red blood cells, it makes them stick to the walls of tiny blood vessels deep within major organs, such as the kidneys, lungs, heart and brain. This is called "sequestration", and results in reduced blood flow to these organs, causing the severe clinical symptoms associated with this infection, such as cerebral malaria.

Clinical manifestations

Plasmodium falciparum infections are induced by the asexual stages of the parasite that develop inside red blood cells (RBCs). Because splenic microcirculatory beds filter out altered RBCs, the spleen can innately clear subpopulations of infected or uninfected RBC modified during falciparum malaria. The spleen appears more protective against severe manifestations of malaria in naïve than in immune subjects. RBC loss contributes to malarial anaemia, a clinical form associated with subacute progression, frequent spleenomegaly, and relatively low parasitemia. Stringent splenic clearance of ring-infected RBCs and uninfected, but parasite- altered, RBCs, may altogether exacerbate anaemia and reduce the risks of severe complications associated with high parasite loads, such as cerebral malaria. The age of the patient directly influences the risk of severe manifestations.

- **Immune Response Regarding Malaria**

An innate immune response is triggered during *Plasmodium* infection as first line of defense, followed by an adaptive immune response, which includes T-cells, B-cells, and antibodies. A mosquito inoculates sporozoite into a host's skin when biting; these can remain in the skin for up to 6 h after inoculation. Such retention affects the place for antigen presentation and the location and type of response so induced.

Dendritic cells (DCs) present antigens, depending on the anatomic environment and the resulting immune response. These cells, through pattern recognition receptors, recognize the pathogen-associated molecular patterns (PAMPs) exhibited by the parasite. The mechanism of action regarding such recognition triggers intracellular signals enabling DC maturation. Three PAMPS have been described in *P. falciparum*: hemozoin, immunostimulatory nucleic acid motifs, and glycosylphosphatidylinositol anchors [glycophosphatidylinositol (GPI) anchors].

The parasite's main source of protein is RBC hemoglobin. Hemoglobin hydrolysis releases lipophilic prosthetic group—heme—which is extremely toxic for the parasite. Heme detoxification is thus necessary and is achieved by converting heme into an insoluble crystalline material called hemozoin (Hz). Regarding *P. falciparum* infection, Hz binds DNA inside host cell phagolysosomes and cytosol, and toll-like receptor (TLR)9 is activated by nucleic acids, NLRP3, AIM2, and other cytosolic sensors.

In terms of AT content in the genome, *P. falciparum* has the highest AT content (82%) and *P. vivax* the lowest AT content (56%). On the other hand, *in silico* analysis has shown that *P. falciparum* contains ~300 CpG and ~6,000 AT-rich motifs and *P. vivax* ~2,000 CpG and ~5,500 AT-rich motifs. The release of CpG *Plasmodium* DNA into phagolysosomes produces an innate immune response activating TLR9.

Some functions described for DCs have been T- and B-lymphocyte activation, immune tolerance, natural killer (NK) cell activation, and macrophage activation. For example, a third of Spz are drained to regional lymph nodes where they become internalized by skin-derived DCs (CD103+) and presented to CD8+ T-cells.

Immune response during the erythrocyte stage is mainly mediated by antibodies while a cellular response predominates during the hepatic stage. CD4+ T-, B-, and NK cells also play an important role in the immune response induced by the parasite during the erythrocyte stage since immunity depends on memory B-cell production and lifespan, following infection.

During *P. vivax* infection, some individuals can acquire immunity naturally; such immunity consists of a cytokine production-mediated cellular immune response, cytokine receptors, and proteolytic enzymes forming part of the host response to infection, as well as IgG antibodies.

Interleukin-10 acts as immunoregulator by controlling the effects of other cytokines produced by CD4+ Th1 and CD8+ T-cells in *Plasmodium* infection. The overproduction of cytokines such as IFN-γ by these cells not only helps to increase phagocytosis for eliminating the parasite but also produces immunopathological effects associated with the disease.

Immunoglobulins can protect or arrest disease progression in different ways; neutralizing anti-Spz antibodies can block Spz from invading hepatocytes. Mrz can be opsonized in the erythrocyte stage by specific antibodies that activate cell-mediated death or prevent the invasion of RBC and block the proteins responsible for binding to molecules on cell surface.

- **Immunopathogenesis of *Plasmodium falciparum***

Malaria causes approximately 212 million cases and 429 thousand deaths annually. *Plasmodium falciparum* is responsible for the vast majority of deaths (99%) than others. The virulence of *P. falciparum* is mostly associated with immune response-evading ability. It has different mechanisms to evade both *Anopheles* mosquito and human host immune responses. Immune-evading mechanisms in mosquito depend mainly on the

Pfs47 gene that inhibits Janus kinase-mediated activation. Host complement factor also protects human complement immune attack of extracellular gametes in Anopheles mosquito midgut. In the human host, evasion largely results from antigenic variation, polymorphism, and sequestration. They also induce Kupffer cell apoptosis at the pre-erythrocytic stage and interfere with phagocytic functions of macrophage by hemozoin in the erythrocytic stage. Lack of major histocompatibility complex-I molecule expression on the surface red blood cells also avoids recognition by $CD8^+$ T cells. Complement proteins could allow for the entry of parasite into the red blood cell. Intracellular survival also assists the escape of malarial parasite. Invading, evading, and immune response mechanisms both in malaria vector and human host are critical to design appropriate vaccine. As a result, the receptors and ligands involved in different stages of malaria parasites should be elucidated.

- **Immune Response to *P. falciparum***

Immune response against the *P. falciparum* malaria parasite is multifaceted and stage specific both in *Anopheles* mosquito vector and human host. Immunological responses could also contribute to the pathophysiology of the disease in human.

***Anopheles* Mosquito's Immune Response**

Plasmodium falciparum malaria parasite is limited by several bottlenecks before establishing infection in its *Anopheles* mosquito vector. This includes the physical, microbiological, and immunological defences of the mosquito immune system. Immunological defence plays a key role when ookinete traverses the midgut and sporozoites migrate to the salivary glands. The interaction of the mosquito immune system is critical to control its vectorial capacity.

Physical Barriers

It is the first line of defence of Anopheles mosquito to *P. falciparum* parasites. The major physical barriers are peritrophic membrane (PM) of the midgut, cuticle of the exoskeleton, and lining of the tracheal respiratory system. Capsule formation around the parasite by mosquito melanin also has a protective role.

Midgut Microbiota

The microbiota found in *Anopheles* mosquitoes, such as *Asaia, Enterobacter, Pseudomonas* and *Pantoea*, induces AMPs, which stimulate a basal innate immune activity against *P. falciparum*infection.

Humoral Immune Response

Complement-like or thioester-containing protein (TEP) 1 that circulates in the Anopheles mosquito haemolymph is the major arm of defence in the humoral immune response of Anopheles mosquito. It forms leucine-rich repeat protein 1 (LRIM1)/anopheles plasmodium-responsive leucine-rich repeat protein (APL) 1/TEP1cut complex and gets accumulated on the ookinete surface for killing. Apolipophorin and apolipoprotein D precursors and fibrinogen-related proteins are

also documentedas players in midgut antiplasmodial defence.

A study conducted in Portugal showed that hemozoin activates transcription of several key immune genes like REL2-F transcription factor that regulates TEP1, APL1, LRRD7, and FBN9 anti- Plasmodium immune factors. Anti-*Plasmodium* response of An. gambiae aga-miR-305 is also demonstrated. Antibodies also avert ookinete motility, penetration of the midgut wall, and formation of oocyst.

Cellular Immune Response

The primary immune cells involved in mosquito innate immune response are hemocytes. Hemocytes include granulocytes, oenocytoids, and prohemocyte subtypes that are involved in phagocytosis, melanization, and hematopoietic progenitors, respectively. Other immune effectors released by hemocytes and fat body into hemolymph are also involved in phagocytosis, secretion of antimicrobial peptides, nodule formation, agglutination, encapsulation, and melanization. Reactive oxygen species (ROS) produced by haemocyte is also involved in mosquito immunity against *P. falciparum*.

Signaling Pathways in Mosquito Antiplasmodial Immunity

This pathway includes Toll, immune deficiency (Imd), Janus kinase (JNK), and signal transducers and activators of transcription (STAT), which contribute to anti-Plasmodium defence. The Toll and Imd pathways target the ookinete stage of the parasite and promote activation of the mosquito TEP1 complement-like system. These pathways are activated when they recognize PAMPs, which activate NF-κB that leads activation of Rel1 and Rel2 in Toll and Imd pathways, respectively. Activation of both pathways is also important for the entry of AMPs to nucleus such as defensins, cecropins, attacin, and gambicin, which have antiplasmodial activity. Rel1 and Rel2 are negatively controlled by Cactus and Caspar, respectively. Potent anti-Plasmodium effectors such as TEP1, APL1, LRRD7, and FBN9 are also controlled by the Imd pathway. Immune-enhanced Anopheles stephensi mosquitoes by Rel2 in the midgut demonstrated better resistance to Plasmodium infection and may give clear direction to design appropriate control strategies. Currently, activation of the Imd and Toll pathways to induce the expression of AgDscam isoforms that have species-specific antiplasmodial responses is indicated. The genes that mediate these pathways and tissues in which they are produced need to be illustrated.

Janus kinase–STAT pathway is also linked with anti-Plasmodium defence but activation of these pathways is not clearly explained. The JNK pathway also regulates the expression of HPX2, NOX5, and TEP1 in haemocytes that promote TEP1-mediated lysis. The STAT pathway targets after parasites cross the midgut and change into the oocysts.

Signal transducers and activators of transcription genes (STAT1/AgSTAT-B and STAT2/AgSTAT-A) mediate immunity against the *P. falciparum* malaria parasite. AgSTAT-A is involved in transcriptional activation of NO synthase that increases reactive NO and transcription of suppressors of cytokine signaling (SOCS), which lessen

the development of parasite. The exact role of AgSTAT- B is not characterized.

- **Immune Response to *P. falciparum* in Human**

The response is complex and targets at different stages of plasmodium parasites. Immune attack involvement is high in the erythrocytic stage in contrast to pre erythrocytic stage, and major immune players in the pre erythrocytic and erythrocytic stages are $CD8^+$ T cells and antibodies, respectively.

Skin as Physical Barrier

The skin is the first critical physical barrier and acts as first line of defence against many pathogens and is also true for *P. falciparum* malaria parasites. After inoculation, sporozoites stay in the skin for several hours and are activated into a state of readiness for the hepatic stages. Antibodies found in the skin tissues also inhibit sporozoite motility in the dermis. Approximately 50% of the sporozoites do not leave the inoculation site. As a result, this early stage could play a key role in vaccine design.

The sporozoite proteins (SPECT1 and SPECT2) were reported to be necessary to pass the skin barrier, cell traversal, and migration to the liver. This allows for sporozoites to evade destruction by phagocytes, and growth is arrested in nonphagocytic cells in the host dermis.

Immune Response to Pre-erythrocytic-Stage Parasites

Immune response at the pre-erythrocytic stage is targeted on free sporozoites and infected hepatocytes. Antibodies against free sporozoites and circumsporozoite protein (CSP) are important to prevent invasion of hepatocytes by neutralizing proteins required for cell traversal and invasion. It also activates complement fixation, phagocytosis, and lysis by cytotoxic NK and NKT cells. It also recognizes parasite neoantigens at the surface of infected hepatocytes and kills through an antibody-dependent cell-mediated mechanism by Kupffer cells and NK cells.

CD8+ T cells producing interferon-γ are mainly involved in killing of intrahepatic parasites. Other cells like NK, NKT, and γδT cells also kill intrahepatic parasites through secretion of type I interferons and IFN-γ. Killing of infected hepatocytes and blocking of invasion by $CD8^+$ T cells and antibodies, respectively are bottleneck phases that could be targeted by vaccine.

Immune Response to Erythrocytic Stage of Infection

Adaptive immunity against erythrocytic-stage *P. falciparum* is more complex than other stages. The release of merozoites from hepatocytes to invade RBCs is responsible for initiation of the erythrocytic stage. At this stage, the targets are free merozoites and intraerythrocytic parasites (schizonts). Humoral or antibody and T cell responses are important to control merozoites and intraerythrocytic parasites, respectively. Antibodies can opsonise merozoites for uptake or to inhibit invasion of RBCs.

The role of $CD8^+$ T cells in the erythrocytic stage is negligible. $CD4^+$ T helper cells are

also important to produce proinflammatory cytokines that activate macrophages. They also mediate an activation of specific B cell clones.

Others like NK cells and γδT cells are also involved in the immune response. IFN-γ, perforin, and granzyme produced by NK cells are responsible to kill P. falciparum-infected RBCs.

Immune Response against Gametocyte

Antibodies kill gametocyte through complement-mediated lysis and prevent sequestration and maturation of gametocytes in the host. Antibodies derived from host during blood meal are also highly responsible for complement-mediated killing of gametocytes and prevent gamete fusion in mosquito. Nitric oxide produced by macrophages is also important to kill gametocytes.

- ## Immune Evasion

Immune Evasion Mechanisms of *P. falciparum* in *Anopheles* Mosquito

Immune evasion is a strategy used to avoid immune response attack. Similarly, *P. falciparum* parasites evade mosquito immune response to transmit to a new host. The main and critical *P. falciparum* gene used for evasion of *Anopheles* mosquito immune response is Pfs47. It inhibits JNK- mediated apoptosis by preventing activation of several caspases. Moreover, being deficient in caspase-S2 also prevents protein nitration in the mosquito midgut cells. Pfs47 gene also inhibits NOX5 and HPX2.

Host complement factors (FH) have also an influence on mosquito midgut stages. The FH receptor in *P. falciparum* gamete is glideosome-associated protein 50 (GAP50) that protects the gametes from complement attack. Blocking of this receptor may also shed light for vaccine development. Immune- modulatory peroxidase (IMPer) is also crucial to form dityrosine network, which helps parasites to inactivate NOS.

- ## Immune Evasion in Human

Immune Evasion Strategies of Liver Stage *P. falciparum* Malaria Parasites

Free sporozoites and intrahepatic parasites must pass the hurdle of host immune response in order to enter the erythrocytic stage. Research showed that sporozoites actively pass through Kupffer cells (KCs, 24%) and endothelial cells (ECs, 53%); some sporozoites can cross the gaps between an EC and a KC, but it is puzzling how sporozoites safely pass through KCs, which kill other microorganisms. To pass this barrier, the CSP binds to KC surface proteins, and this interaction produces high levels of intracellular cAMP/EPAC that prevents the formation of ROS. Sporozoites' contact with KC also downregulates the inflammatory Th1 cytokines and upregulates the anti-inflammatory Th2 cytokines. In some cases, the binding of sporozoites also induced KC apoptosis and reduced the expression of major histocompatibility complex (MHC)-I. This

results in induction of T cell tolerance. The CSP antigen of sporozoites could also be responsible for the reduction of KC MHC-I expression. Sporozoites are able to manipulate the KC functions.

Once inside the hepatocyte, parasitophorous vacuole prevents lysosomal degradation. Host heme oxygenase-1 (HO-1) also enhances the development of intrahepatic parasites by modulating the host inflammatory response. Sporozoite also interferes with the mTOR pathway.

Intra-erythrocytic Immune Evasion

The success of evasion depends on merozoites and infected red blood cell (iRBC) surface proteins. Mostly, immune evasion by intraerythrocytic parasites is the result of antigenic diversity and sequestration. Intracellular survival also assists the parasite escape by avoiding direct interaction with the immune cells. Lack of MHC-I molecule expression on the surface RBCs also helps parasite to void recognition by CD8+ T cells. They also create rosettes that help them to bind on RBC epitopes and avoid immune recognition.

Expression of variable antigenic surface proteins on iRBCs helps them to evade host immune response. Antigenic diversity is mostly developed from multicopy gene families and polymorphic alleles. PfEMP1 is one of the highly polymorphic proteins, encoded by approximately 60 copies of var genes. It has different variable domains that establish their binding to various ligands on endothelial cells. A particular var gene, known as var2csa mediates cytoadherence of iRBC to syncytiotrophoblasts of the placenta.

The second immune evasion mechanism at this stage is sequestration, mediated by PfEMP-1, RIFIN, and STEVOR multigene families. These allow iRBC adherence to vascular endothelium that protects from clearance of the parasites by spleen. Endothelium receptors such as EPCR, CSA, CD36, and ICAMs are also important for sequestration. Rosette formation and adherence are important for immune evasion through sequestration and responsible for the occurrence of cerebral malaria.

IgM, which is not specific for these parasites, also binds to PfEMP-1 molecules through their Fc portion (Fc) and promotes rosetting that may facilitate sequestration by preventing splenic elimination.

Phagocytic functions of macrophages are also hindered by P. falciparum malaria pigment or hemozoin. Macrophages that have hemozoin cannot phagocytose more iRBC and reduce the production of radical oxygen intermediates. *P. falciparum* infection also activates checkpoint inhibitormolecules.

Immune Evasion by Merozoites

The mechanisms used for merozoite evasion include antigenic proteins. Principally, merozoite surface proteins (MSPs), PfAMA1, PfEBA, and PfRHs are involved in merozoite evasion. Among them, MSPs are highly polymorphic and play a key role to evade

immune attack. Merozoite proteins show strong homology to the host protein that makes it difficult to be recognized by antibody.

Evading Mechanisms of Gametocytes

Antibodies are also enhancing transmission to the mosquito. Expression of Var, Rif, and Stevor proteins that provides an immune evasion is also indicated recently.

- **Immune Response to *Plasmodium vivax***

Malaria caused by *Plasmodium vivax* continues being one of the most important infectious diseases around the world; *P. vivax* is the second most prevalent species and has the greatest geographic distribution. Developing an effective antimalarial vaccine is considered a relevant control strategy in the search for means of preventing the disease. Studying parasite-expressed proteins, which are essential in host cell invasion, has led to identifying the regions recognized by individuals who are naturally exposed to infection. Furthermore, immunogenicity studies have revealed that such regions can trigger a robust immune response that can inhibit sporozoite (hepatic stage) or merozoite (erythrocyte stage) invasion of a host cell and induce protection.

P. vivax Pre-erythrocyte Phase Protein Antigenicity and Immunogenicity

P. vivax CSP

One of the predominant surface proteins in Spz is the CSP; it is expressed during the preerythrocyte phase and plays a fundamental role during hepatocyte invasion. This protein is a candidate for a vaccine against malaria in the preerythrocyte phase since various studies have shown that anti-CSP antibodies block hepatocyte invasion.

Three allele variants of the *P. vivax* circumsporozoite protein (*Pv*CSP) have been described: VK210, VK247, and vivax-like CSP-P, which differ at repeat region sequence level. VK210 has greater global distribution, being found in countries like Brazil, India, Thailand, and Peru, while VK247 is found in some regions of Colombia and Brazil, and vivax-like CSP-P in Brazil, Indonesia, Madagascar, and PNG.

Antigenicity studies in people exposed to the disease in different endemic regions have found variable prevalence in individuals responding to different *Pv*CSP fragments (87) (Table 1). Preclinical studies and phase I clinical assays (Table 3) have been carried out regarding *P. vivax* with long synthetic peptides (LSP) having more than 70 *Pv* amino acids from *Pv*CSP amino terminal (N), carboxyl terminal (C), and repeat (R) regions linked to tetanus toxoid peptide (87). Immunized non-human primates from the genus *Aotus* spp. produced specific antibody response recognizing both LSP and CSP since the first immunization (87). LSP has also induced a Th1-type immune response

characterized by increased IFN-γ and reduced IL-4 production in T-lymphocytes stimulated *in vitro*.

P. vivax Erythrocyte Phase Protein Antigenicity and Immunogenicity

Merozoite Surface Protein-1 (MSP-1)

The MSP family has been the most studied candidate from the erythrocyte asexual phase when developing an effective vaccine against malaria. The MSP-1 belongs to this family, being one of the most studied and currently important for both *P. falciparum* and *P. vivax*.

The MSP-1 analog in *P. vivax* is encoded by the *Pv*200 gene, having a 200-kDa molecular weight. The proteolytic processing profile is thought to be similar than for *P. falciparum* MSP-1, leading to 4 fragments: 83, 30, 38, and 42 kDa; further cleavage of the last one (C-terminal region) produces 33 and 19 kDa polypeptides, which are released to the blood stream. A 19-kDa portion remained bound to the recently formed ring phase following reticulocyte invasion.

The 19-kDa C-terminal fragment has been one of the most studied from MSP-1 (*Pv*200). An increase in antibodies was observed in sera, which became increased with the second vaccination, this being attributed to a booster for helping epitopes in $Pv200_{19}$. The response was T-cell dependent, suggesting that an immune response to a vaccine based on this protein could be boosted by natural infection.

C-terminal region contains two immunogenic epidermal growth factor (EGF)-like domains which induce T-cell and antibody responses against *P. vivax* during natural infection in humans. .C-terminal region, specifically $PvMSP\text{-}1_{19}$, have shown that these two EGF-like domains function as a binding portion in *Pv*MSP-1 interaction with erythrocytes.

The decrease of antibodies directed against the C-terminal region could have contributed toward cases of reinfection in high-risk areas.

The major responses in Turkey (where *P. vivax* is the only *Plasmodium* species present in the area) were IgG, IgM, and IgA to a lesser extent. It is worth highlighting the fact that $PvMSP\text{-}1_{19}$ was highly antigenic in individuals who are naturally exposed to the infection and, since no other Plasmodia are infecting in that area, the response observed cannot be attributable to a crossed reactivity.

Rosa et al. characterized the $MSP\text{-}1_{19}$ recombinant protein's antigenic and immunogenic properties together with two T-helper epitopes (the universal pan allelic DR epitope and a new internal MSP-1 epitope from the 33-kDa C-terminal region). It was seen that T-helper epitopes did not modify protein recognition by human IgG. The complete recombinant protein was immunogenic in marmosets (*Callithrix jacchus jacchus*), but only when Freund's adjuvant was used.

The MSP-1 protein's 42-kDa fragment has also been studied due to its potential as vaccine candidate; its immunogenicity was evaluated in mice. High IgG1, IgG2a, and

IgG2b antibody levels were observed while IgG3-type response was low. A high proliferative response also found high IL-2, IL-4, IL-10, and IFN-γ levels being detected in culture supernatants. Greater prevalence of recognition of *Pv*MSP1$_{19}$ than *Pv*MSP1$_{42}$ was found when a naturally acquired humoral immune response was reported.

An MSP-1 paralog has been identified recently (*Pv*MSP1-P); its immune response was characterized using different protein fragments (83, 30, 38, 42, 33, and 19 kDa). The NT (83 kDa) fragment and two from the C-terminal region (33 and 19 kDa) were recognized by sera from infected patients living in endemic areas.

Due to its colocation of IgG with MSP1, it has been thought that it played a similar role in erythrocyte invasion; however, analyzing the sequences has suggested different roles for each protein. Cytoadherence assays demonstrated that MSP1-P could be an essential adhesion molecule regarding *P. vivax* invasion to erythrocytes, is immunogenic in humans, and is a potential vaccine candidate against *P. vivax*.

Merozoite Surface Protein-3 (MSP-3)

The *P. vivax* merozoite surface protein-3 (*Pv*MSP3) is a member of the MSP family characterized by having a highly polymorphic alanine-rich central domain. It has a relatively conserved N- and C-terminal domain and two central blocks of seven repeats forming tertiary supercoiled helices in their structure. It is expressed in schizonts and is associated with Mrz surface during the erythrocyte phase.

Its homolog in *P. falciparum* has been studied as a vaccine candidate in preclinical and phase I assays, protection was associated with reduced parasitemia by cytophilic antibodies inducing antibody-dependent cell-mediated inhibition of parasite growth mechanism. It has been shown to be highly immunogenic in *P. vivax*, having a high prevalence of antibodies directed against *Pv*MSP-3α block II.

Merozoite Surface Protein-9 (MSP-9)

The *P. vivax* merozoite surface protein-9 (*Pv*MSP-9) is also a potential vaccine candidate. Some studies have shown that this protein is conserved among *Plasmodium* species infecting humans, rodents, and primates. Furthermore, antibodies produced against *Pv*MSP9 homologs in *P. cynomolgi* and *P. knowlesi* can inhibit Mrz invasion of erythrocytes.

The *P. vivax, P. knowlesi*, and *P. cynomolgi msp-9* genes encode a hydrophobic signal peptide and repeat motifs upstream of the stop codon and a C-terminal region having two species-specific blocks of repeat amino acids (*Pv*MSP9-RI and *Pv*MSP9-RII). Together with *P. falciparum* has four cysteine residues close to the NT giving the MSP-9 family's structural and functional characteristics. This protein is expressed during schizogony and is organized on Mrz surface during schizont development and segmentation.

Duffy Binding Protein (DBP)

Plasmodium vivax Mrz requires antigens from the Duffy blood group as surface receptor for invading human reticulocytes.. *P. vivax* DBP adhesion to its receptor on erythrocytes [Duffy antigen receptor for chemokines (DARC)] is essential for the parasite to continue developing during the asexual phase in human blood. *Pv*DBP is a 140-kDa protein, which is located in the micronemes; it has been divided into four important regions: a peptide signal sequence (region I), two cysteine-rich regions separated by a non-homologous hydrophilic region (region II, identified as the erythrocyte-binding domain, and region VI), and transmembrane domain (region VII. *Pv*DBP is a main target to use as vaccine candidate since its importance during parasite invasion and its ability to induce antibodies against the parasite's asexual phases.

Serological evaluation in a PNG endemic area has shown that a humoral immune response was common and increased with age, suggesting a possible booster effect regarding antibody response in some cases by repeated exposure to the infection. A similar pattern has been observed in an endemic region of Colombia where a positive correlation was found between increased antibody response and patients' age. Also, an immunologic boost for DBP was found, even in endemic areas having a low transmission level. The forgoing shows that DBP_{II} was naturally antigenic in people residing in endemic regions.

Children having high antibody levels against DBP_{II} has been associated with delayed reinfection time with the same *P. vivax* variant; however, such association was not observed when evaluating MSP1–19. In other studies have been observed that naturally acquired neutralizing antibodies against DBP are short-lived, increasing with acute infection, and are strain specific.

Apical Membrane Antigen-1 (AMA-1)

The AMA-1 in *Plasmodium* is a transmembrane protein, which is localized in the micronemes. It seems to be essential during cell host invasion and is present in all *Plasmodium* species.

Eight disulfide bonds have been identified in the AMA-1 ectodomain of 66 kDa, defining three different subdomains (DI, DII, and DIII). Immune responses induced by AMA-1 from different *Plasmodium* species have shown potent parasite-inhibitory effects both in animals and *in vitro* thus suggesting AMA-1 as a potential vaccine candidate.

Plasmodium vivax AMA-1 ectodomain (*PV*66/AMA-1) has been shown to be highly immunogenic in rhesus monkeys, inducing high IgG antibody titers; however, these suffer a rapid decline. A slight reduction in parasitemia has been observed in *P. cynomolgi*-challenged animals previously immunized with *PV*66.

Reticulocyte-Binding Proteins (RBP)

Reticulocyte-binding proteins include *Pv*RBP1 and *Pv*RBP2 and their variants *Pv*RBP1a and b and *Pv*RBP2a, b, and c, and other family members. RBP1 is a homodimer bound by

disulfide bonds, binds non-covalently to RBP2, and forms a protein complex. They are colocalized in the apical zone in Mrz micronemes and contain a transmembrane domain toward the C-terminal extreme, possessing repeat regions in *Pv*RBP2 and reticulocyte-binding domains.

It is thought that RBPs could participate in reticulocyte invasion since no infection by *P. vivax* has been observed in mature erythrocytes. Their reticulocyte-binding ability has also been reported, but the specific receptors have yet to be identified. It has recently been found that only *Pv*RBP2b binds specifically to reticulocytes. Due to their participation in infection, they have been studied as erythrocyte phase vaccine candidates, aimed at blocking Mrz invasion of reticulocytes.

Conclusions

This study manly focused on the evasion, invasion, and immune response mechanisms of *P. falciparum and P. vivax* involved both in *Anopheles* mosquito vector and human host. Based on this, molecular and cellular immune molecules and cells are discussed. The receptors and ligands involved in each stage are also elucidated that gives an indication for vaccine development.

Probable questions:

1. Describe the overall immunopathology and immunity of *Plasmodium* sp.
2. Describe the immunopathology and immunity of *Plasmodium falciparum.*
3. What is immune evasion? Discuss Immune Evasion Mechanisms of *P. falciparum* in *Anopheles* Mosquito
4. Describe the Immune Response to *P. falciparum* in Human.
5. Describe the *Anopheles* Mosquito's Immune Response to *P. falciparum.*
6. Describe the immunopathology and immunity of *Plasmodium vivax*
7. Describe *P. vivax* Pre-erythrocyte Phase Protein Antigenicity and Immunogenicity.
8. Discuss the roll of different types of Merozoite Surface Protein in immunopathology and immunity of *Plasmodium vivax.*

Suggested readings/ references:

1. https://www.hindawi.com/journals/jir/2018/6529681/
2. http://www.bloodjournal.org/content/117/2/381?sso-checked=true
3. https://www.malariasite.com/pathophysiology/
4. https://www.karger.com/Article/Pdf/402535
5. https://academic.oup.com/femsre/article/40/2/208/2570118

UNIT IV

Physiology of cestodes, trematodes and nematodes

Objective:

In this unit, we will discuss about Physiology of cestodes, trematodes and nematodes.

Introduction:

Physiology is the branch of biology relating to the function of organs and organ systems, and how they work within the body to respond to challenges.

The phylum Platyhelminthes (flatworms) is comprised of parasitic worms characterized mainly by their flattened, bilaterally symmetrical body. This phylum includes the four classes: (1) Turbellaria, (2) Trematoda, (3) Monogenea, and (4) Cestoda.

- ## Physiology of cestodes

Cestodes are a group of thousands of parasites called tapeworms. It's because their bodies have a tape-like, segmented shape to it. Cestodes have a head, called a scolex, which has suckers. These suckers are used to attach to a person's intestinal tract. Some cestodes also have hooks on their head as well.

Although cestodes can be found in a person's digestive tract, ironically they don't have one themselves. They absorb nutrients through a skin-like covering instead. Cestodes don't have a body cavity too. Instead, their insides are filled with spongy cells that suspend their internal organs.

And when it comes down to reproduction, cestodes are monecious. In other words, they are hermaphrodites. They are also oviparous, or egg-laying.

Some of the most common cestodes include:

- *Diphylobothrium latum*
- *Taenia solium*
- *Taenia saginata*
- *Hymenolepis diminuta*
- *Echinococcus granulosus*
- *Hymenolepus nana*

- ### Morphological/Structural Characteristics

Generally, the body of Cestodes is divided into three distinct parts.

These include:

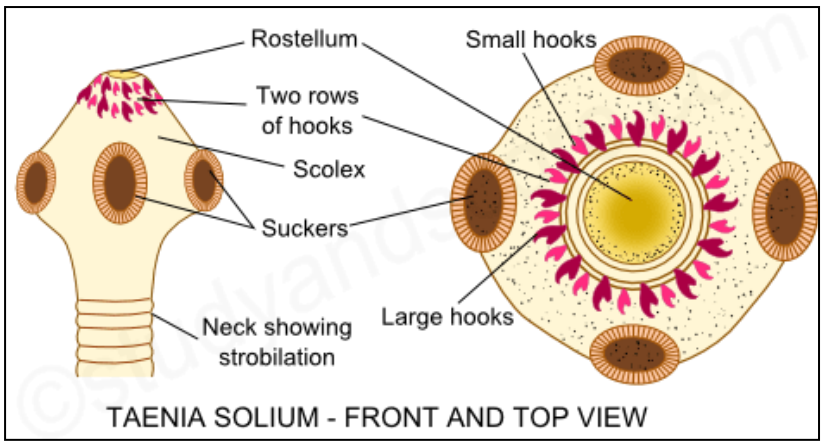
The Scolex

The scolex is the anterior part of cestodes. It's a specialized segment that consists of hooks and suckers that anchor the parasite to the walls of the small intestine. On the scolex, the hooks (which vary in number from one species to another) protrude from a muscular structure known as the rostellum.

This structure has been shown to be retractable, capable of protruding and retracting into a small pouch known as the rostellar pouch.

While some species have both hooks and suckers on their scolex (e.g. *Taenia solium* which has between 22 and 32 hooks and four suckers), others do not have a rostellum (and thus hooks) and therefore have to rely on suckers to attach onto the walls of the small intestine in order to continue absorbing nutrients.

For other species, like catfish tapeworms, the ovoid suckers are also used to cling onto fish given that they do not have hooks. By comparing the structure of the scolex, presence or absence of hooks etc, it's possible to distinguish different types of cestodes.



TAENIA SOLIUM - FRONT AND TOP VIEW

Neck

The neck of a cestode is the thin segment that is immediately attached to the scolex. According to recent findings, fragmentation of cestodes is influenced by signals operating in the neck region. Through these signals, stem cells in the body of the organism are activated resulting in fragmentation.

Proglottid

Proglottids are individual segments of the cestodes attached to the neck region. As the parasite grows, the number of proglottids increase allowing the organism as a whole to grow to several meters in length (the length is dependent on the species).

Together, these segments make up the strobilus. A mature proglottid contains both male and female reproductive organs and is therefore capable of reproduction. Those closest to the scolex are the youngest segments (and thus immature) while those located further away from the scolex are the mature ones (ready for fragmentation). Also known as gravid segments, these proglottids are filled with eggs.

As previously mentioned, cestodes do not have a gut (as well as a mouth). They also lack a body cavity and are therefore acoelomate. While they lack a mouth and digestive system, cestodes simply absorb nutrients through their surface membrane.

Based on close microscopic studies, the entire surface of the strobilus was shown to be covered with tiny wrinkles as well as projections that increase the surface area for nutrients absorption.

Here we discuss the physiology of *Taenia solium* as it is very common cestode.

External Feature of *Taenia Solium*:

A fully-developed *Taenia Solium* may attain a length of 3-5 metres. Its anteroposterior ends are clearly distinguishable but it is difficult to differentiate the dorsal from the ventral surface. The body is ribbon-like (Fig. 69A) and consists of a distinct head or scolex at the anterior region.

The tip of the head bears a conical elevation—the rostellum which can be retracted or extended. The rostellum bears 28 to 33 hooks arte of two types- larger and smaller and they alternate with each other. Each hook parts (fig 69B) has three a base or guard, a conical blade at the tip and a handle projected from the middle.

The hooks are arranged in two rows. When the contractile rostellum is withdrawn the hooks become anteriorly directed and get fixed into the host tissue. The head bears in the middle four cup-like suckers or Acetabulum.

Rostellum and suckers act as organs of attachment to the intestine of the host. Behind the head there is a narrow and small tubular region—the neck or the zone of proliferation. The rest of the body or tape is called strobila. The strobila is segmented in appearance.

The chain like strobila is made up of numerous segments or sexual units called proglottids. The proglottids progressively increase in size and mature towards the posterior extremity. The youngest or newly formed proglottid occupies a position just beneath the neck while the oldest one is at the posterior end. The number of proglottids varies from 800-850 in a full-grown worm.

A proglottid from the middle region of the strobila offers a rectangular outline. The surface is lined by cuticle (recently renamed as epidermis). It is thick and perforated at

intervals by fine canals, at the bottom of which either gland cells or nerve endings are situated.

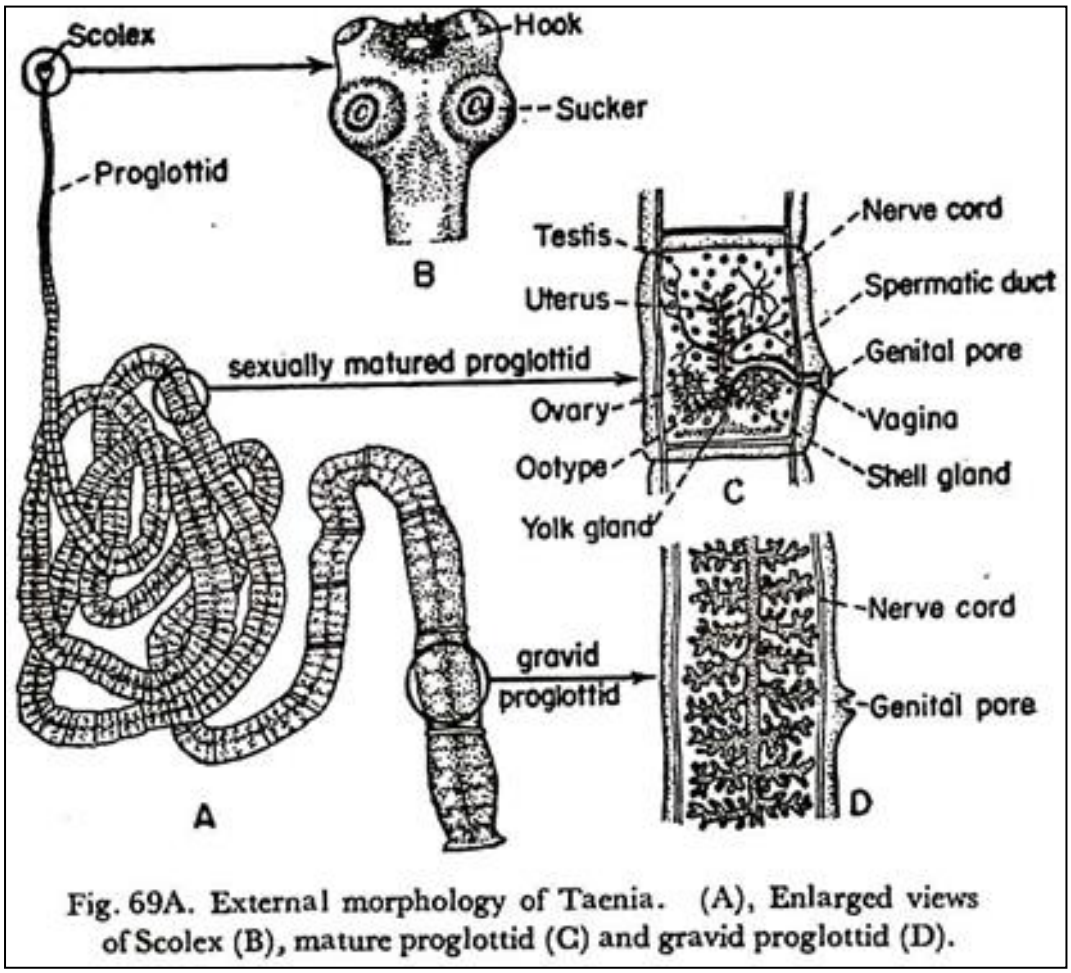


Fig. 69A. External morphology of Taenia. (A), Enlarged views of Scolex (B), mature proglottid (C) and gravid proglottid (D).

It is followed by longitudinal and circular layers of muscles. The circular muscles divide the parenchyma cells into an outer cortex and inner medullary regions (Fig. 69C).

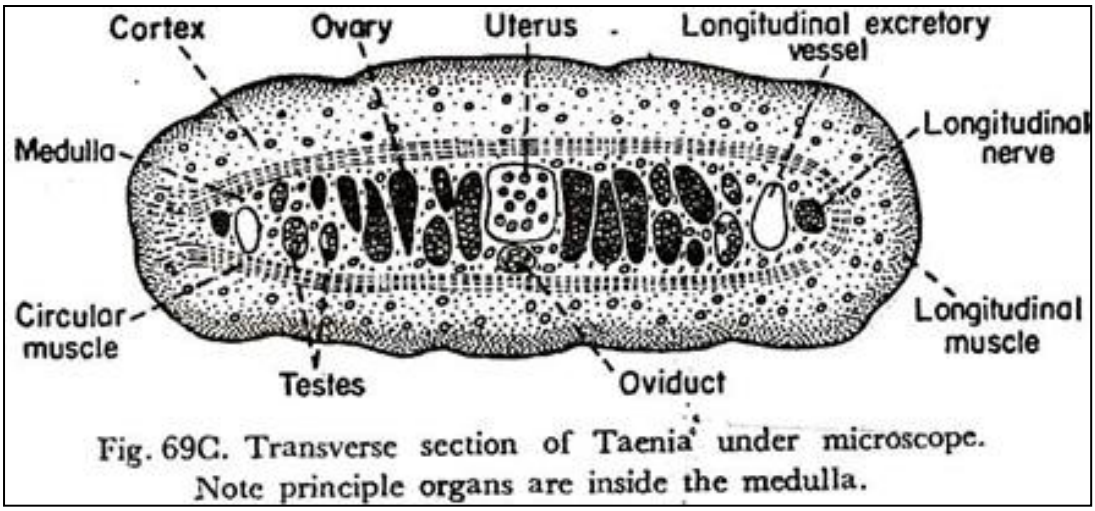


Fig. 69C. Transverse section of Taenia under microscope. Note principle organs are inside the medulla.

Towards each lateral margin is found the longitudinal nerve and just median to them lies the longitudinal pair of excretory vessels. A transverse excretory canal is situated at a posterior position of the proglottid.

The anterolateral borders of the medulla are housed with testes and posterior lateral borders are with ovary. The male and female genital ducts open to a chamber called genital atrium which is situated in the middle of one of the lateral margins. The atrium opens to the exterior through an aperture called genital pore.

The body is covered by a thick epidermis. The epidermis is many-layered and perforated. It is impregnated with calcium carbonate. The epidermis remains sunk iii the parenchyma. Longitudinal muscles run under the epidermis.

The parenchyma is divided into an outer cortical zone and inner medullary zone by the circular muscles. Nervous, reproductive and excretory organs are situated within the medullary zone.

For a long time it was regarded that the body of tapeworm is covered by a thick cuticle. But recently electron microscopic studies have revealed that the outer layer of the body of all cestodes contains mitochondria and remains continuous with processes emerging from the cytoplasm of some underlying cells.

Alimentary System of *Taenia Solium*:

It is completely absent. This absence is due to the parasitic life of the tapeworm. It absorbs nutrients from the intestinal contents of the host.

- Tapeworm completely lacks alimentation in all stages of life-history.
- It is clear that tapeworms absorb nutrients from their hosts and that such movement of materials from the hosts into parasites is an essential element of host-parasite relationships.
- The soluble nutrients like glucose, amino acids, glycerol, etc., diffuse directly through the general body surface (teguments0.
- The absorptive surface of a parasite is generally increased by the microvilli or teguments.
- Some of the tissue fluid s from the host are probably absorbed by scolex of tapeworm which is anchored deeply into the intestinal mucosa.
- The stored food consists of mainly glycogen and some lipid substances. The glycogen content of *T. solium,* by net weight, is 2.17%.

Respiratory System of *Taenia Solium*

- The respiration in tapeworm is mainly anaerobic type or anoxybiotic type as there is no free oxygen available in the human intestine.

- The principal reserve food, glycogen is the main source of energy which undergoes glycolysis to produce carbon dioxide and fatty acids and other organic acids are also produced.
- The carbon dioxide is diffused out through the general body surface, while fatty acids are removed through the excretory system.
- The tapeworm also consumes free oxygen when available.
- The rate of consumption is maximum in anterior proglottids and declines gradually towards the posterior end.

4. Excretory System of *Taenia Solium*:

It consists of an Excretory canal and flame cells.

Excretory canal

- There are, on each side, two lateral longitudinal excretory canals or collecting tubules of which one is dorsal and the other ventral .
- They get connected by a network of tubules or ring-like vesicle called, **nephridial plexus** in scolex.
- Dorsal canals are thin and are confined to the anterior region of the body.
- Ventral canals are large and extend along the entire length of the body.
- Two ventral canals are connected by a transverse canal at the posterior part of each proglottid (except the last).
- In the last proglottids, they join to form a pulsatile bladder or caudal vesicle, opening to the exterior by a single excretory pore, but when this proglottid is shed off, the **caudal vesicle** is lost and the terminal of two ventral canals behave as independent excretory pores.
- Each longitudinal excretory canal receives numerous secondary canals all along its length.

Flame cells

- A **flame cell** is irregular-shaped, with granular cytoplasm and a nucleus.
- These are scattered throughout the parenchyma from which they remove metabolic wastes.
- A bundle of cilia or flame arises from basal granules near the nucleus.
- cilia are enclosed into a funnel-shaped lumen formed by the terminal blind end of a capillary.
- The long cilia of flame cells have the flickering movement which maintains hydrostatic pressure by which wastes is driven into the excretory canal.

Physiology of excretion

- The flame cells are scattered all over the parenchyma cell. With the help of flagellar movements, the excretory products in a fluid state enter into parenchyma to flame cells.
- The longitudinal canals are lined internally by cuticle, while the secondary canal is non-ciliated and capillaries have a ciliated lining.
- The cilia set up the hydrostatic pressures which drive out the excretory products through the excretory canal and out of excretory pores.
- The fluid content of the body is also regulated by this system(osmoregulation) in the case of platyhelminths but in cestodes, the protonephridia concerned only the excretion.

5. Nervous System of *Taenia Solium*:

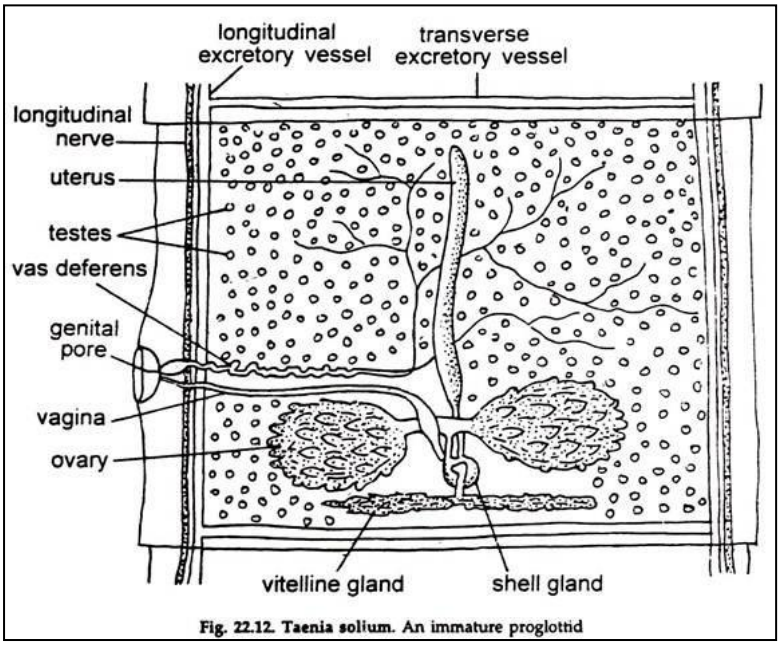


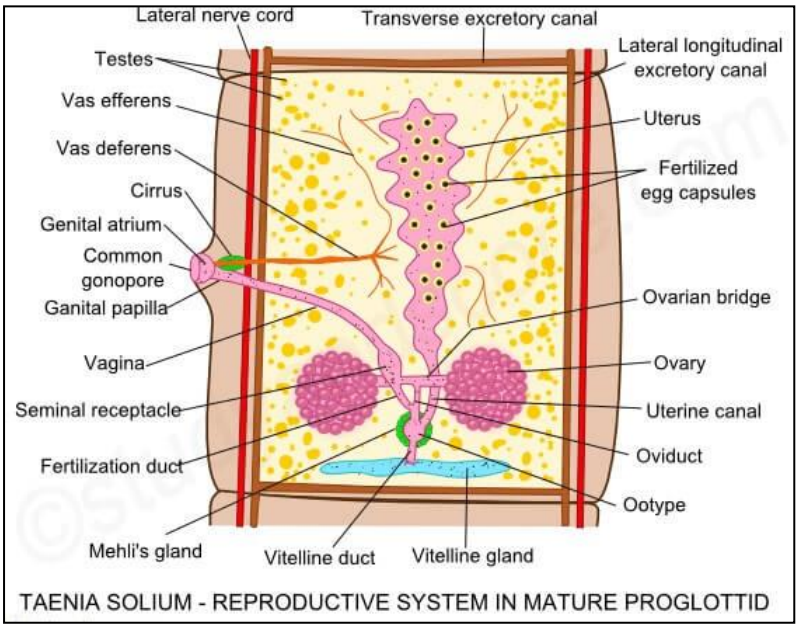
Fig. 22.12. Taenia solium. An immature proglottid

- The nervous system of *T. solium* consists of a pair of **cerebral ganglia** connected by (i) a ring consisting of dorsal and ventral commissures and (ii) a thick ganglionate cross commissures or transverse commissures.
- All the structures together are known as the **brain complex**, which is connected with another **rostellar nerve ring** that has a pair of **rostellar ganglia** in the rostellum. These two rings are interconnected by eight nerves.
- The nerve fibers from cerebral and rostellar ganglia supply the suckers and rostellum.

- 10 longitudinal **nerve cords** arise from the brain complex and run through strobila. Out of these **two lateral longitudinal nerves** are best developed.
- The longitudinal nerve cords are connected in each proglottid by a ring connective situated below the transverse excretory canal.
- Sense organs are absent in tapeworm but free sensory nerve-endings are present throughout the body specially scolex.
- A detached proglottid, passing out with feces hows some movements and sensitivity to stimuli.

6. Reproductive System in Taenia Solium:

Taenia Solium is hermaphrodite. Male and female reproductive organs occur in the same individual. Each proglottid behind the first 200 is equipped with a set of reproductive organs. Male reproductive organs develop first in each proglottid and then female organs make their appearance.



TAENIA SOLIUM - REPRODUCTIVE SYSTEM IN MATURE PROGLOTTID

The Male reproductive organs consist of testes, vasa efferentia, vas deferens, cirrus or penis, and genital atrium.

a. Testes

- Testes are numerous small and are scattered along the length and breadth of proglottids.

b. Vasa efferentia

- From each testis arises a fine ductule, the **vas efferens.**

- Vas efferentia usually gets interconnected with similar ductules from the surrounding of the testes to form a common **sperm duct** or **vas deferens** in the middle of proglottids.

c. Vas deferens

- The vas deferens is a thick, convoluted tube which is transverse in position and extends towards the lateral margin (left or right) of the proglottid.
- It opens in the cirrus.

d. Cirrus and cirrus sac

- The **cirrus** is a thick, muscular, eversible, copulatory organ that is enclosed in a firm **cirrus sheath or cirrus sac.**
- The cirrus opens by the male gonopore into a cup-shaped **genital atrium.**
- The genital atrium opens to the exterior by the **common gonophore** situated at the peak of a tiny protuberance, the **genital papilla.**
- The common gonophores of successive proglottids lie alternatively on both the side.

Female reproductive system

The female reproductive system consists of a bilobed ovary, oviduct, ootype, vagina, uterus, Mehil's glands, and vitelline gland.

a. Ovary

- It is also called **germarium.**
- It is bilobed and lies ventrally in the posterior part of proglottids.
- Each lobe of the ovary is dorso-ventrally flattened and consists of a number of radially-arranged **germinal cords** or **follicles.**
- Both the lobes are connected medially by a transverse, tubular bridge, the **ovarian bridge**, or **isthmus.**

b. Oviduct

- It is short but wide arises from about the middle of the ovarian isthmus.
- It opens into the Ootype.

c. Ootype

- It is a small, rounded chamber formed by the union of the oviduct, uterus, and vitelline duct.
- It is surrounded by numerous unicellular Mehlis's glands which secrete a slimy substance that lubricates the eggs in the uterus.

d. Vagina

- It is a bent narrow tubular structure that arises from the **female genital pore** located behind the male genital pore in the genital atrium.
- It runs obliquely inwards to join the oviduct.
- Before joining the oviduct, the vagina swells up to form a sperm storing sac, the **seminal receptacle**, or **receptaculum seminis.**
- The seminal receptacle stores the sperms temporarily.

e. Uterus

- It is a blind and cylindrical tube that arises from Ootype extending up to the anterior part of proglottids.
- It consists of a proximal short and narrow tubular portion, the **uterine duct,** and distal broad part forming the **uterine expansion.**
- It contains thousands of fertilized eggs, and in gravid proglottids, it forms 7 to 13 lateral branches on each side.
- The branched uterus is the only genital structure persisting in gravid proglottids.

f. Vitelline gland

- It is a large lobulated gland.
- It is situated at the posterior margin of the proglottids.
- It is connected with the Ootype by a short median **vitelline duct.**
- It consists of numerous follicles secreting yolk cells.

g. Mehils's glands

- The Ootype is surrounded by a large number of unicellular glands called Mehil's glands.

▪ Physiology of Trematodes

Trematodes are flatworms classified in the **phylum Platyhelminthes, class Trematoda, subclass Digenea**. In general, trematodes are dorso-ventrally flattened and leaf like in shape. Their bodies are covered with tegument, which is usually armed with scale like spines. They have two suckers: one oral and one ventral.

Flukes are parasitic flatworms that infect the blood vessels, gastrointestinal tract, lungs, or liver. They are often categorized according to the principle organ system they invade:

- *Clonorchis sinensis*, *Fasciola hepatica*, and *Opisthorchis* species: Liver and bile ducts

- *Fasciolopsis buski*, *Heterophyes heterophyes*, and related organisms: Lumen of the gastrointestinal tract
- *Paragonimus westermani* and related species: Lungs
- *Schistosoma* species: Vasculature of the gastrointestinal or genitourinary system

Here we discuss the physiology of *Fasciola hepatica* as it is very common cestode.

Respiration of *Fasciola hepatica*:

Mode of respiration is anaerobic or anoxybiotic. In fact, glycogen is metabolised to carbon dioxide and fatty acids releasing energy in the form of heat.

The process is completed in following steps:

(i) The glycogen undergoes anaerobic glycolysis to form pyruvic acid,

(ii) The pyruvic acid is decarboxylated to form carbon dioxide and an acetyl group,

(iii) The acetyl group then combines with coenzyme A to form acetyl coenzyme A, and

(iv) The acetyl coenzyme A is then finally condensed and reduces to form fatty acids.

The carbon dioxide, thus, produced is diffused out through general body surface and the fatty acids are excreted through the excretory system.

Digestive System of *Fasciola hepatica*:

(i) Alimentary Canal:

The oral sucker encloses a ventral mouth which leads into a funnel- shaped mouth cavity, followed by a round muscular pharynx with thick walls, and a small lumen. The pharynx has pharyngeal glands. F. indica has a short muscular pharynx from which arises an oral pouch which is about half the size of the pharynx.

There is a short narrow oesophagus leading into an intestine which divides into two branches or intestinal caeca or crura each running on one side to the posterior end, and ending blindly. The intestinal caeca give out a number of branching diverticula in order to carry food to all parts of the body since there is no circulatory system. The median diverticula are short and lateral ones are long and branching. There is no anus.

The interior part of the alimentary canal up to the oesophagus is lined with cuticle and serves as a suctorial fore gut; the intestine is lined with endodermal columnar epithelial cells. The caceal epithelium has secretory gland cells.

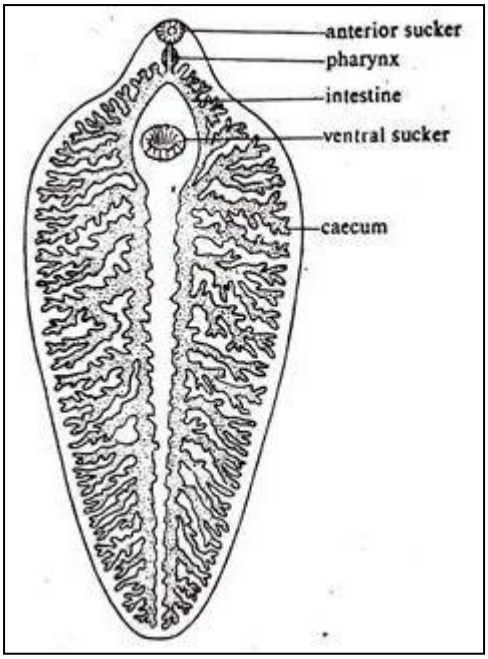


Fig: Digestive system of liver fluke

(ii) Food, Feeding and Digestion:

It feeds on bile, blood, lymph and cell debris. The oral sucker and pharynx together constitute an effective suctorial apparatus. Digestion is extracellular, occurs in intestine. The digested food material is distributed by branching diverticula of intestine to all parts of the body as the circulatory system is not found in this animal. Thus, the digestive system functions as a gastro vascular system.

In fact, the digested nutrients are passed into the parenchyma through intestinal diverticula; from parenchyma they are diffused into the various organs of the body.

Reserve food, mostly in the form of glycogen and fats is stored in the parenchyma. However, monosaccharide sugars like glucose, fructose, etc., are directly diffused into the body of the fluke through general body surface from the surrounding fluid of the host. The indigestible remains of the food, if any, are probably said to be ejected through the mouth.

Excretory System of *Fasciola hepatica*:

The excretory system of *Fasciola hepatica* is concerned with excretion as well as osmoregulation. It consists of a large number of flame cells or flame bulbs or protonephridia connected with a system of excretory ducts.

(i) Flame Cells:

The flame cells, supposed to be modified mesenchymal cells, are numerous, irregular in shape bulb-like bodies found distributed in the mesenchyma throughout the body of Fasciola. The distribution pattern of flame cells follows a specific pattern referred to as **'the flame cell pattern'** (Faust, 1919).

The flame cells are characteristic, each has a thin elastic wall with pseudopodia-like processes, a nucleus and an intracellular cavity having many long cilia arising from basal granules. In living condition, the cilia vibrate like a flickering flame, hence, the name flame cell.

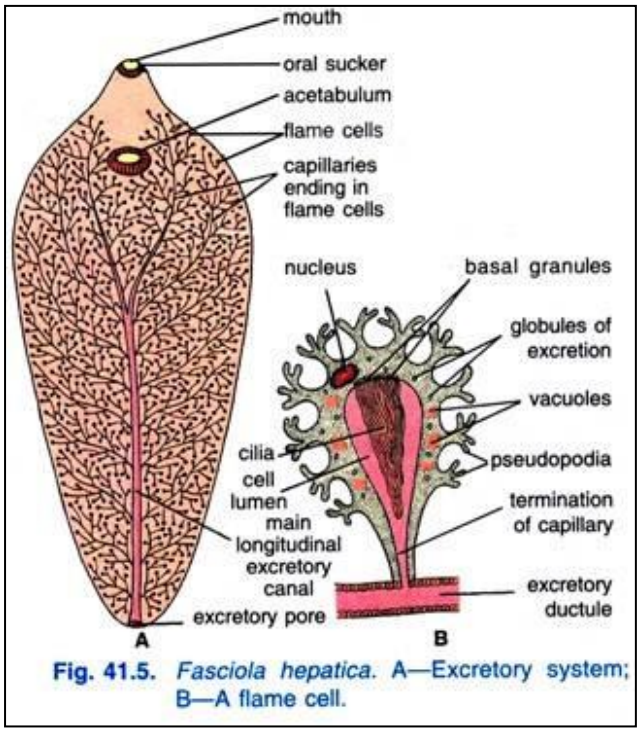


Fig. 41.5. *Fasciola hepatica*. A—Excretory system; B—A flame cell.

(ii) Excretory Ducts:

There is an excretory pore at the posterior end from which arises a longitudinal excretory canal, from this arise four main branches, two dorsal and two ventral, which subdivide into numerous small capillaries which anastomose; the capillaries are continued into the intracellular cavity of flame cells. The longitudinal excretory canal is non-ciliated but the capillaries are lined with cilia.

(iii) Process of Excretion:

The excretory wastes, generally fatty acids and ammonia, are diffused from surrounding mesenchyma into the flame cells and finally collected into their intracellular cavities. The vibrating movement of the cilia causes the flow of wastes from the intracellular cavities of flame cells into the excretory ducts and then into the main excretory canal and finally to the exterior through excretory pore by hydrostatic pressure.

Such an excretory system of flame cells and canals or ducts of various orders with no internal opening and leading to an excretory pore which opens to the exterior is spoken of as a protonephridial system which is excretory but its main function is to regulate the amount of fluid in the animal's body.

Nervous System of *Fasciola hepatica*:

A nerve ring surrounds the oesophagus, it has a pair of cerebral ganglia dorsolaterally, and a ventral ganglion below the oesophagus. Small nerves are given out anteriorly from the ganglia. Posteriorly three pairs of longitudinal nerve cords arise from the ganglia, a dorsal, a lateral, and a ventral pair of nerve cords.

The lateral nerve cords are best developed and they run to the posterior end. Nerve cords are connected by transverse commissures and they give out many small branches, some of which form plexuses. The nerve cells are mostly bipolar. Due to parasitic life, sense organs are lost in adult Fasciola.

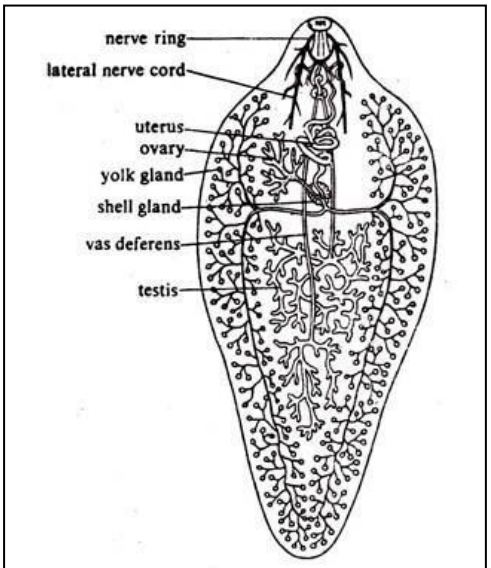


Fig. 1.54 : Nervous and reproductive systems of liver fluke (major part of the lateral nerve cord is removed).

Reproductive System of *Fasciola hepatica*:

Fasciola hepatica is hermaphrodite but usually cross fertilization takes place. The reproductive organs are well developed and complex.

(i) Male Reproductive System of Fasciola hepatica:

The male reproductive system consists of testes, vasa deferentia, seminal vesicle, ejaculatory duct, cirrus or penis, prostate glands and genital atrium.

(a) Testes:

These are two in number, much ramified tubular and placed one behind the other (i.e., with tandem arrangement) in the posterior middle part of the body. In fact, they occupy major space from behind the middle part of the body of Fasciola. The cells lining the wall of testes give rise to spermatozoa.

(b) Vasa Deferentia:

A narrow and slender vas deferens or sperm duct arises from each testis and runs forwards.

(c) Seminal Vesicle:

The two vasa deferentia unite together near the acetabulum (ventral sucker) and become dilated to form a muscular, elongated, broad, bag-like seminal vesicle or vesicula seminalis. It serves the purpose of storing sperms.

(d) Ejaculatory Duct:

The seminal vesicle continues anteriorly into a very narrow and coiled duct called ejaculatory duct.

(e) Cirrus:

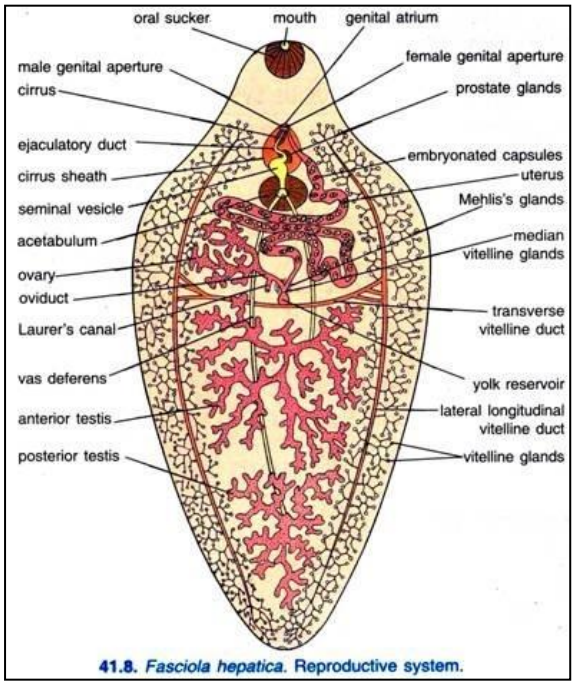
The cirrus (penis) is a muscular and elongated structure into which ejaculatory duct opens. The cirrus opens by male genital aperture in a common genital atrium. The cirrus of F. indica is covered with small spines.

(f) Prostate Glands:

The ejaculatory duct is surrounded by numerous unicellular prostate glands. These glands open into the ejaculatory duct and their secretion (alkaline) helps in free movement of sperms during copulation.

(g) Genital Atrium:

The genital atrium is a common chamber for male and female genital apertures; it opens externally by a gonopore lying ventrally in front of the acetabulum. The cirrus can be everted through the gonopore during copulation. The cirrus or penis, seminal vesicle and prostatic glands are surrounded in a common cirrus sheath or cirrus sac.



41.8. *Fasciola hepatica*. Reproductive system.

(ii) Female Reproductive System of Fasciola hepatica:

The female reproductive system consists of ovary, oviduct, uterus, vitelline glands, Mehlis's glands and Laurer's canal.

(a) Ovary:

The ovary is single, tubular, highly branched and situated to the anterior of testes at the right side in anterior one-third of the body.

(b) Oviduct:

All the branches of ovary open into a short and narrow tube called oviduct. The oviduct travels down obliquely and opens into the median vitelline duct.

(c) Uterus:

From the junction of oviduct and median vitelline duct arises a wide convoluted uterus having fertilised shelled eggs or capsules. The uterus opens by female genital aperture into the common genital atrium on the left side of male genital aperture. The uterus is comparatively small and it lies in front of the gonads.

The terminal part of uterus has muscular walls, referred to as metraterm which ejects the eggs and also sometimes receives the cirrus during copulation.

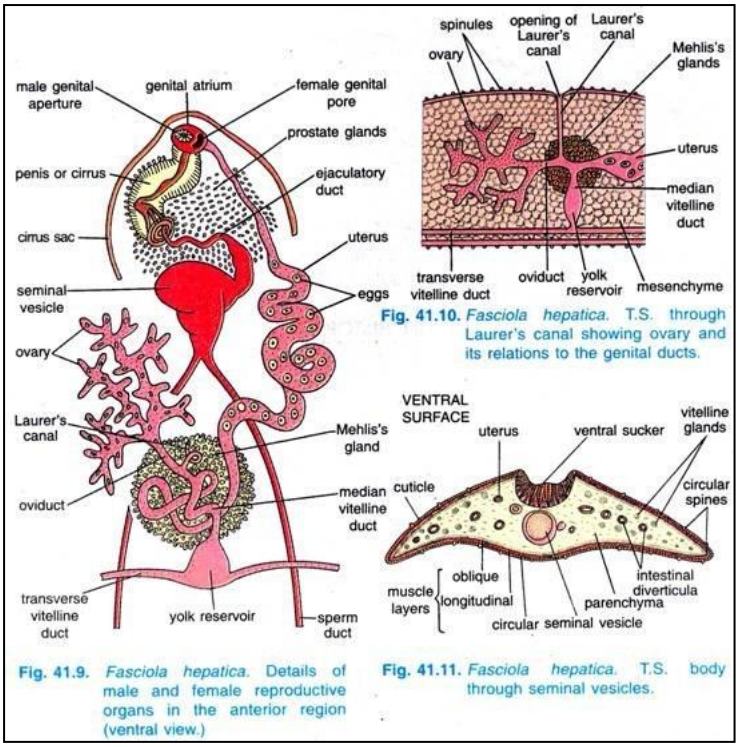


Fig. 41.9. *Fasciola hepatica*. Details of male and female reproductive organs in the anterior region (ventral view.)

Fig. 41.10. *Fasciola hepatica*. T.S. through Laurer's canal showing ovary and its relations to the genital ducts.

Fig. 41.11. *Fasciola hepatica*. T.S. body through seminal vesicles.

(d) Vitelline Glands:

On both lateral sides and also behind the testes do numerous follicles constitute the vilellaria, yolk glands or vitelline glands which produce albuminous yolk and shell material for the eggs. The vitelline glands open by means of minute ducts into a longitudinal vitelline duct on each side.

The two longitudinal ducts are connected together by a transverse vitelline duct placed above the middle of the body. The transverse vitelline duct is swollen in the centre to form the yolk reservoir or vitelline reservoir. From the yolk reservoir a median vitelline duct starts and runs forward to join the oviduct.

(e) Mehlis's Glands:

A mass of numerous unicellular Mehlis's glands is found situated around the junction of median vitelline duct, oviduct and uterus. The secretion of Mehlis's glands lubricates the passage of eggs in the uterus and probably hardens the egg shells, it probably also activates spermatozoa.

The junction of oviduct and median vitelline duct is swollen to form ootype in certain flukes like F. indica, in which the parts of an egg are assembled and the eggs are shaped, but an ootype is lacking in F. hepatica (according to some authorities).

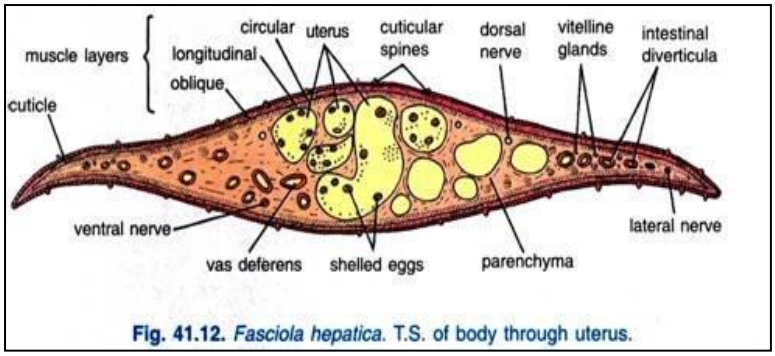


Fig. 41.12. *Fasciola hepatica*. T.S. of body through uterus.

(f) Laurer's Canal:

From the oviduct arises a narrow Laurer's canal, it runs vertically upwards. This canal opens on the dorsal side temporarily during breeding season and acts as vestigial vagina to serve as copulation canal.

▪ Physiology of Nematodes

Nematode, also called **roundworm**, any worm of the phylum Nematoda. Nematodes are among the most abundant animals on Earth. They occur as parasites in animals and plants or as free-living forms in soil, fresh water, marine environments, and even such unusual places as vinegar, beer malts, and water-filled cracks deep within Earth's crust. The number of named species is about 20,000, but it is probable that only a small proportion of the free-living forms have been identified.

Although numerous nematodes infect humans, six spend the majority of their lifecycle in the bowel lumen and are classified as intestinal nematodes: ***Ascaris lumbricoides*; *Trichuris trichiura* (whipworm); *Ancylostoma duodenale* and *Necator americanus* (the two human hookworms); *Enterobius vermicularis* (pinworm).**

Here we will discuss about the physiology of *Ascaris lumbricoides* which is

the most common nematode.

Ascaris lumbricoides is an endoparasite in the small intestine of man lying freely in the lumen. It has been living in man from time immemorial. It is cosmopolitan in distribution. It is found more commonly in children than in adults. Sometimes it migrates from intestine to stomach and comes out through the mouth or nostrils of the host.

Structure of Phylum Nematoda:

There exists a considerable similarity of organisation and shape in different nematodes. General shape of the body as the name implies is round, cylindrical and tapering at both ends. The length usually varies from 0.4 m (*Ascaris*) to 1 m (*Dracunculus*).

The largest of all nematodes is *Placentonema gigantissima*. The females of this species attain a length of 8.5 m, the diameter being 2.5 cm and they parasitise the placenta of sperm whales. The females of all nematodes are generally larger than the male.

Body Cavity of Phylum Nematoda:

Body cavity is not a true coelom because it is not lined by epithelial layer derived from mesoderm. Some workers have called it 'Pseudocoelom'. According to them, the absence of mesenchyme in between the body wall and digestive tract has stood in a good way for the evolution of a more organised digestive system. The pseudocoelom is filled with a fluid and the fluid acts as a 'hydrostatic skeleton'.

Digestive System of *Ascaris lumbricoides*:

(i) Alimentary Canal:

It consists of the mouth, a short pharynx or oesophagus forming the foregut; a long tubular intestine or the midgut and a short rectum or hindgut.

(ii) Mouth:

As already referred to, the mouth is a triradiate aperture situated at the anterior tip surrounded by three lips or labia.

(iii) Pharynx:

The terminal mouth leads into a cylindrical thick-walled pharynx or oesophagus which has a posterior swelling called end bulb which is provided with valves, the pharynx has muscular walls having radial muscle fibres which dilate the lumen.

Internally it is lined by cuticle which, at the margin of mouth, is continued with the cuticle of the body wall. The pharynx has 3 large branching gland cells which open by cuticular ducts into the lumen; these are in fact, pharyngeal or oesophageal glands.

The cavity of the pharynx has three deep longitudinal grooves lined by cuticle, and in a transverse section the lumen appears triradiate, connective tissue fibres arise from each

of the three internal grooves and go to the cuticle covering the pharynx, they maintain the triradiate shape of the lumen. This much constitutes the stomodaeum or foregut.

(iv) Intestine:

The pharynx opens posteriorly into a thin-walled dorsoventrally flattened intestine or midgut which extends almost the entire length of the body. It is formed of a single layer of columnar epithelial cells lined externally by a thin layer of cuticle. The free inner margin of each cell is produced into several finger-like projections, the microvilli (Kessel et al., 1961).

They form a sort of tightly packed brush border which increases the surface area. The intestine has no muscle layer.

(v) Rectum:

The intestine is followed by the, hindgut or rectum which is also flattened dorsoventrally. Its wall consists of tall columnar cells and lined internally by cuticle and externally by few muscle fibres. In male the rectum opens out by cloaca because it receives the ejaculatory duct but in female the rectum opens out by a transverse slit-like aperture, the anus.

The anal aperture is guarded by anterior and posterior lips and is provided with a few special dilator muscles running from the rectum to the body wall, called depressor ani. Their contraction from time to time causes the faecal matters to be discharged out. The rectum also has large unicellular rectal glands; 3 in the female and 6 in the male.

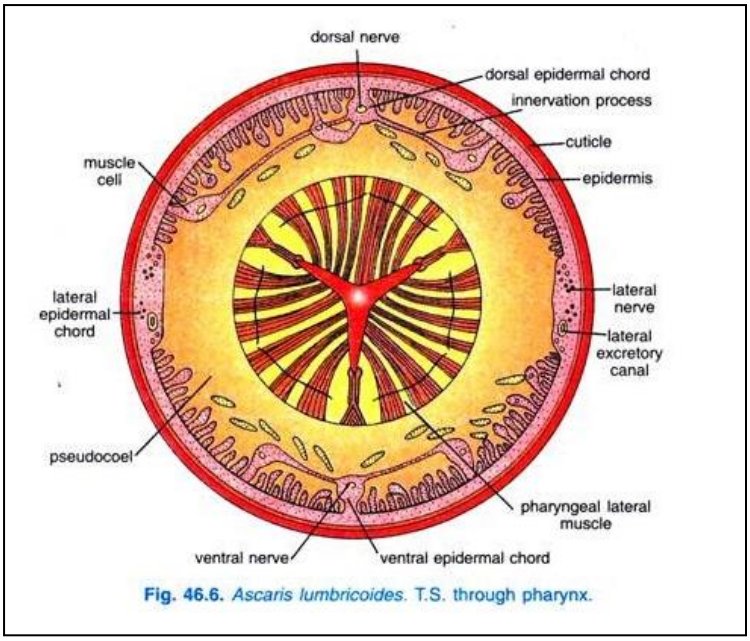


Fig. 46.6. *Ascaris lumbricoides*. T.S. through pharynx.

(vi) Food, Feeding and Digestion:

Food of *Ascaris lumbricoides* comprises blood, tissue exudes and partly or fully digested food of the host. Food is sucked in by the suctorial action of the pharynx.

Digestion is extracellular which occurs in the intestinal lumen; the process of digestion is facilitated by the enzymes like proteases, amylase and lipase secreted by the gland

cells of the pharynx. The digested nutrients are absorbed in the intestinal wall and finally distributed by the pseudocoelomic fluid.

Excess food is generally stored as reserve glycogen and fat in the intestina wall, muscles and syncytial epidermis. Intracellular digestion has also been reported to occur in the cells of intestinal wall as they engulf solid particles to digest intracellulary. The undigested wastes, if any, are defecated out by the contraction of special muscles of rectum through anus or cloaca.

Excretory System of Ascaris:

Excretory Organs:

In marine nematodes, the excretory system consists of one or two large renette gland cells lying ventrally in the pseudocoel near the junction of the pharynx and intestine, a duct arises from each renette cell, these ducts join and open by an excretory pore mid-ventrally.

There is much evidence to support the view that from this glandular system arose a tubular excretory system which is like an H with two longitudinal excretory canals connected by a bridge, the transverse canal from which arises a common excretory canal leading to an excretory pore.

In *Ascaris lumbricoides* there is a longitudinal excretory canal in each lateral line, the anterior limbs of the H are reduced, and the transverse canal is branched to form a network from which arises a short common excretory canal to open by a minute ventral excretory pore just behind the lips. The canals are more developed on the left side than on the right.

The canals are lined by a firm membrane and covered with a layer of cytoplasm; they are intracellular excavations in the single giant cell whose nucleus lies on the transverse canal. The excretory system has no internal openings, cilia, flame cells, or nephridia.

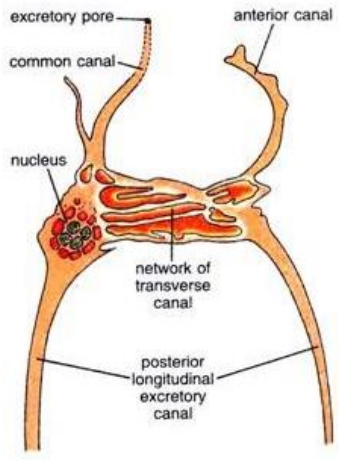


Fig. 46.7. *Ascaris lumbricoides.* Excretory system.

Physiology:

Excretory physiology of *Ascaris lumbricoides* is very poorly understood. However, the nitrogenous waste chiefly comprises urea which diffuses into the pseudocoelomic fluid.

The excretory canals are said to secrete this urea which is eliminated through the excretory pore; some ammonia and urea are also passed out along with the faecal matters. Observations are also there to suggest that *Ascaris lumbricoides* excretes more urea when water is scarce.

Respiratory System of *Ascaris lumbricoides*:

There are no respiratory organs, but parasites carry on anaerobic or anoxybiotic respiration as the oxygen content in the intestine of host is very poor. It obtains energy by the breaking down of glycogen into CO_2 and fatty acids which are excreted though the cuticle like those of flatworm parasites, e.g., Fasciola and Taenia.

The chief fatty acids produced as excretory wastes are butyric, valerianic and caproic acids. It also consumes oxygen when available. The small amount of hemoglobin iii the body wall and pseudocoel fluid takes up the oxygen even when it is present in low tension, so that aerobic respiration may also take place.

Nervous System of *Ascaris lumbricoides*:

The nervous system of *Ascaris lumbricoides* (Fig.46.8 and 46.9) is well developed and complicated and like the excretory system it is also situated in the body wall, i.e., it is hypodermic. However, it consists of circumpharyngeal ring or nerve ring and nerves originating from it.

There is a circumpharyngeal ring around the pharynx, it is made of nerve fibres and some diffusely arranged nerve cells. Associated with this ring are many ganglia; there is an unpaired dorsal ganglion, and close to it is a pair of sub-dorsal ganglia. On each side of the ring is a lateral ganglion which is divided into six ganglia. On the lower side of the ring is a pair of large-sized ventral ganglia.

Each ganglion has a fixed number of nerve cells. From the circumpharyngeal ring arise six small nerves anteriorly, each having a ganglipn, they are arranged radially and go to the sense organs of the anterior end (papillae and amphids).

Posteriorly six long nerves arise from the ring and run to the posterior end; of these six nerves one is a mid-dorsal nerve and one is a mid-ventral nerve, the former lies in the dorsal line and the latter in the ventral line. The mid-ventral nerve is the main nerve and it is ganglionated along the anterior length, it may be called the nerve cord. Near the anus it has an anal ganglion which sends nerves to the tail.

The other four posterior nerves are thinner, they are one pair of dorsolateral nerves and a pair of ventrolateral nerves, they lie on the sides close to the excretory canals. The

dorsal and ventral nerves are connected by a number of transverse commissures, and the ventral nerve and lateral nerves are joined together by many ventrolateral commissures. Posteriorly the innervation is more complicated in males than in females.

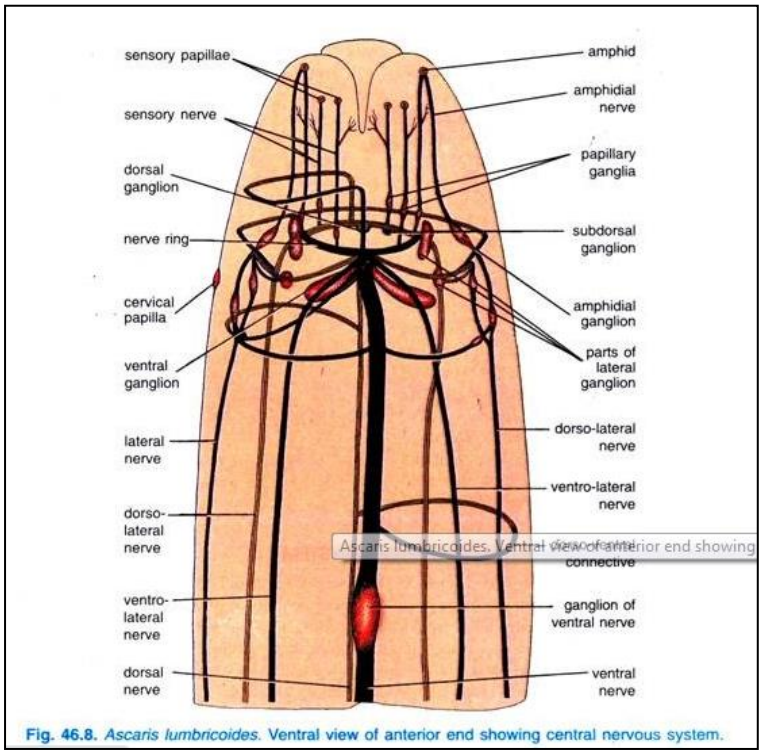


Fig. 46.8. *Ascaris lumbricoides*. Ventral view of anterior end showing central nervous system.

Reproductive System of *Ascaris lumbricoides*:

In *Ascaris lumbricoides*, like other nematodes, sexes are separate, i.e., dioecious and readily distinguishable externally, i.e., sexual dimorphism is well distinct.

The male is smaller in size than the female, its tail is curved, while female's tail is straight, it bears cloaca and a pair of spicules or penial setae but female possesses anus and spicules absent, male possesses pre- and post-anal papillae which are absent in female. The male system is reduced to a single tube but the female system is double.

Male Reproductive Organs:

These are confined to the posterior part of the body and consist of testis, vas deferens, seminal vesicle, ejaculatory duct and penial setae.

(i) Testis:

Testis is monorchic, i.e., single in *Ascaris lumbricoides* but it may be diorchic, i.e., two testes in some nematodes. However, the testis of Ascaris lumbricoides is a long, thread-like, coiled tube.

Its wall is composed of a single layer of cuboidal cells being surrounded by the basement membrane. Its central axis is solid cytoplasmic rachis; the rachis is

surrounded by clusters of amoeboid cells in various stages of their development. In fact, these are developing sperms.

(ii) Vas Deferens:

The testis continues distally into a short and thick coiled tube of the same diameter, the vas deferens. However, it is distinguished from the testis in possessing a central lumen in place of the solid rachis.

(iii) Seminal Vesicle:

The vas deferens joins posteriorly with a much thicker, wider somewhat muscular and straight tube called seminal vesicle. It lies below the intestine in the posterior one- third of the pseudocoel.

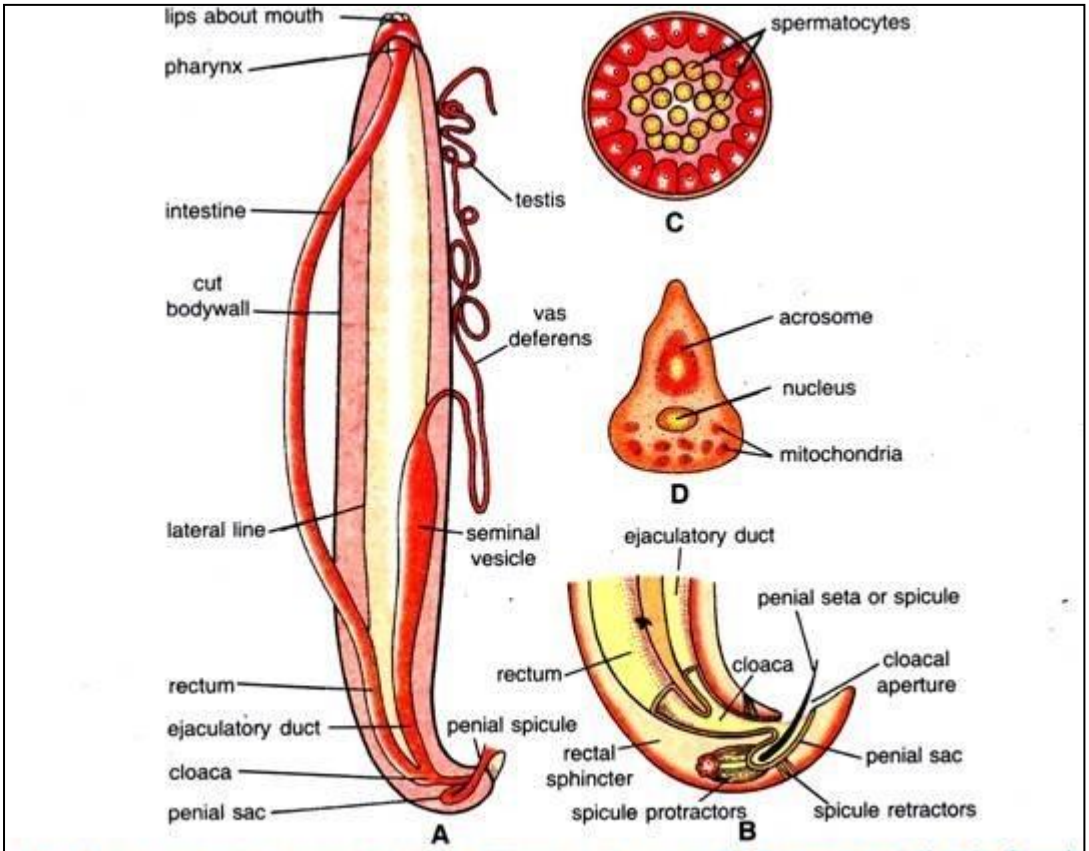


Fig. 46.11. *Ascaris lumbricoides*. A—Male reproductive system; B—Posterior end of male *Ascaris* in lateral view showing cloaca and spicule; C—T.S. vas deferens; D—A sperm.

(iv) Ejaculatory Duct:

The seminal vesicle narrows at its posterior end to form a short, but muscular ejaculatory duct which opens into the rectum to form the cloaca. This duct bears a

number of prostatic glands whose secretions help in copulation. The cloaca opens out by cloacal aperture.

(v) Penial Setae:

Dorsal to the cloaca is- a pair of muscular sacs, the penial sacs or spicule pouches, the two spicule pouches unite and join the cloaca. The pouches contain a pair of spicules or penial setae which are cuticular with a cytoplasmic core. The spicules can be protruded and retracted through the cloacal aperture by the action of special sets of protractor and retractor muscles.

They serve in copulation to open the female genital pore, and, thus, help to transfer sperms, their function is aided by a chitinous plate, the gubernaculum lying in the wall of the cloaca.

Female Reproductive Organs:

These are confined in the posterior two-third of the body and consist of ovaries, oviducts, uteri and vagina.

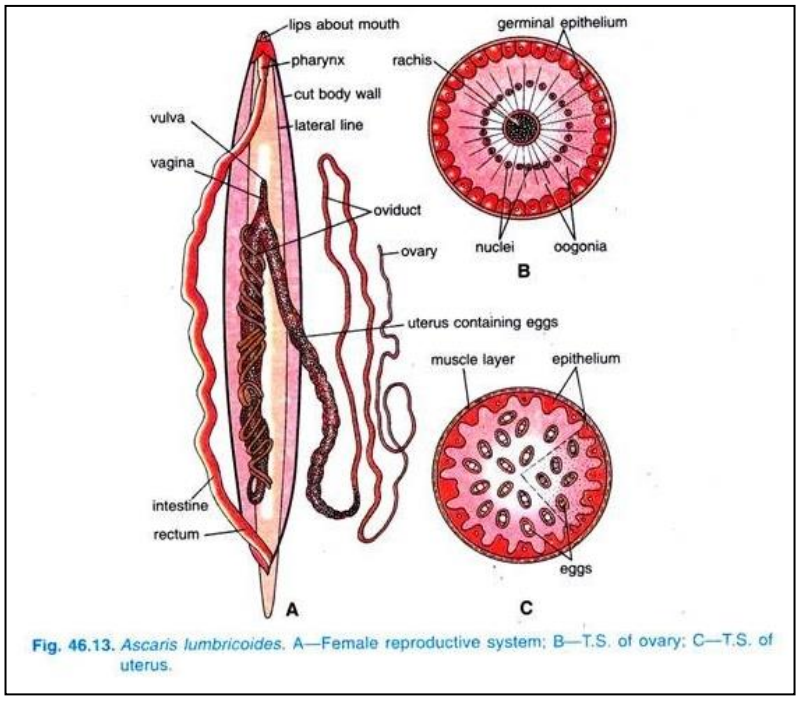


Fig. 46.13. *Ascaris lumbricoides*. A—Female reproductive system; B—T.S. of ovary; C—T.S. of uterus.

In fact in *Ascaris lumbricoides*, like most of the nematodes, there is a set of two parallel tracts of female reproductive organs, i.e., an ovary, oviduct and a uterus in one tract; this condition is called didelphic, although monodelphic (one tract) and polydelphic (many tracts) conditions are also found.

(i) Ovaries:

The paired ovaries of *Ascaris lumbricoides* are long, thread-like and highly twisted tube-like and terminate blindly in the pseudocoel. Internally it has a single layer of cuboidal epithelial cells, a cytoplasmic central rachis and externally a basement membrane

surrounding the epithelial cells. The rachis is encircled round by group of developing ova.

(ii) Oviducts:

The ovaries are continued posteriorly into somewhat broader oviducts having similar structure to ovaries but in place of solid rachis there is a lumen in the oviducts.

(iii) Uteri:

Each oviduct is further continued into still broader, thicker and muscular uterus; it has a thick inner layer of circular muscles and a thin outer layer of oblique muscles. The first part of uterus serves as seminal receptacle where sperms, after copulation, are stored and where fertilization occurs, the remaining uterus stores fertilised eggs, and its cells produce yolk and material for egg shells.

(iv) Vagina:

The two uteri unite and open into a short of gonad. Maturation of median, muscular vagina lined with cuticle. The vagina the gonad not shown. Opens by a transverse gonopore or vulva which lies mid-ventrally about 1/3 of the length from the anterior end.

In some nematodes the end part of the uterus or the end part of the vagina, when present, forms muscular ovejectors which by peristaltic movement force the eggs one by one through gonopore.

Formation of Gametes in *Ascaris lumbricoides*:

Gonads may be hologonic or telogonic. In hologonic gonads germ cells arise along their entire length. In *Ascaris lumbricoides* the gonads are telogonic in which germ cells arise at the proximal end only which is called germinal zone or the zone of proliferation. Next part of the gonad is a growth zone where gametogonia enlarge. In the ovary, the elongated developing eggs are arranged radially around a central cytoplasmic rachis.

In the testis the developing amoeboid sperms are packed around the central rachis.

In the last of part the gonads gametocytes are formed and become free from the rachis, here they undergo maturation division to form eggs or sperms. The last part of gonads where developing gametes undergo maturation are ‘referred to as the maturation zone. As referred to, the sperms are amoeboid in shape, while eggs are elliptical in shape.

Sense Organs of *Ascaris lumbricoides*:

Due to parasitic mode of life, *Ascaris lumbricoides* has developed sense organs which are very simple. They are either as minute elevations or pits in the cuticle of the body.

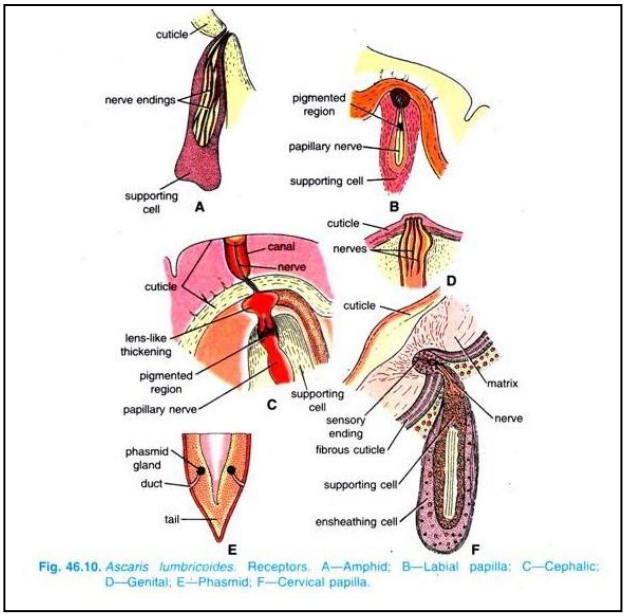


Fig. 46.10. *Ascaris lumbricoides*. Receptors. A—Amphid; B—Labial papilla; C—Cephalic; D—Genital; E—Phasmid; F—Cervical papilla.

However, they are as follows:

(i) Labial Papillae:

The labial papillae are four, two on the dorsal lip and one each on the ventrolateral lips, each is a double sense organ. Each labial papilla consists of a fine fibre of sensory nerve surrounded by many supporting cells. These are gustatory or taste organs.

(ii) Amphids:

The amphids are two, situated one each, on the ventrolateral lips. These are small pits containing glandular and nerve cells supplied by amphidial nerve from the lateral or amphidial ganglia (Fig. 46.10 A). These are olfactory chemoreceptors.

(iii) Phasmids:

The phasmids are unicellular glands situated one on each side of the tail behind the anus. These are pit-like and chemoreceptors.

(iv) Cervical Papillae:

The cervical papillae are a pair of small pits situated just a little behind the oral lips in the lateral sides of the body. These are bulb-like nerve endings with supporting cells. These are probably tactile in function.

(v) Cephalic Papillae:

The cephalic papillae are also pit-like being formed of nerve fibres surrounded by supporting cells but the nerve fibre has a lens-like expansion just beneath the cuticle and then narrows to form canal which widens before opening at the surface.

(vi) Genital Papillae:

The genital papillae are found in males. These consist of nearly 50 pairs of preanal and 5 pairs of postanal papillae . These are also formed of 1-3 nerve fibres embedded in supporting cell. These are also tactile in function and help during copulation.

The various receptor organs of nematodes are of taxonomic importance.

Probable question:

1. Define scolex?
2. Discuss the morphological characteristics of cestode.
3. What is proglottid?
4. Discuss alimentary system of cestode (*Taenia solium*) with diagram.
5. What is flame cell? Discuss the structure of flame cell with diagram.
6. Describe the physiology of excretion.
7. Write down male reproductive system of cestode (*Taenia solium*).
8. What is ootype?
9. What is Mehli's gland? Describe its function.
10. Discuss the digestive System of *Fasciola hepatica* with diagram.
11. Discuss the Process of Excretion in detail in trematoda.
12. What is genital atrium?
13. Describe the role of Vitelline Gland.
14. Describe the Nervous System of Phylum Nematoda emphasizing *Ascaris lumbricoides* with diagram.
15. Describe the sense organs of *Ascaris lumbricoides*.

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UNIT V

Fish parasites and its control

Objective:

In this unit we will discuss about Fish parasites and its control.

Introduction:

The fish production system in India has two main sources viz. culture fisheries- the fish grown in captivity and capture fisheries- the fish caught from the wild stock. There are also some intermediate forms of production such as culture-based fisheries, enhanced capture fisheries and capture based aquaculture. In culture based fisheries, fishes are stocked in reservoirs and lakes and caught back after they grow by utilizing natural food. Enhanced capture fishery means augmenting the fish stock in capture fisheries by stocking support. Under capture-based aquaculture fish seed collected from wild are grown in captivity. The major sources of fish production in India are: freshwater aquaculture, mariculture and coastal aquaculture with intermediate culture system based on culture based fisheries and enhanced capture fisheries of reservoirs/wet lands, marine capture fisheries of open sea, and inland capture fisheries of rivers estuaries and lakes.

At present, the three Indian major carps viz. catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) constitute the main contributor (90%) for freshwater aquaculture production with about half of the total fish production in the country followed by silver carp, grass carp and common carp.

Increased interest in fish culture has also increased awareness of and experience with parasites that affect fish health, growth, and survival. Like any other culture species, fish culture also has to deal with various pathogens like virus, bacteria, fungi and parasites.

Here is a brief chart of the parasites discussed below.

I. Protozoa

A. Ciliates

1. *Ichthyophthirius multifiliis*
2. *Chilodonella*
3. *Cryptocaryon*
4. Trichodina
5. *Ambiphyra*

B. Flagellates

1. *Hexamita / Spironucleus*
2. *Ichthyobodo*
3. *Cryptobia, Trypanoplasma spp., Trypanosoma spp*

C. Myxozoa
D. Microsporidia
E. *Coccidia*
II. Cnidaria
1. *Myxobolus cerebralis*
III. Monogenean Trematodes
1. *Gyrodactylus*
2. *Dactylogyrus*
IV. Digenean Trematodes
1. *Diplostomum spathaceum*
2. *Posthodiplostomum minimum*
V. Nematodes
1. *Camillanus*
VI. Parasitic Crustacea
1. *Argulus*
VII. Dinoflagellates
1. *Amyloodinium ocelatum*

I. Protozoan Parasites

Most of the commonly encountered fish parasites are protozoans. With practice, these can be among the easiest to identify, and are usually among the easiest to control. Protozoans are single-celled organisms, many of which are free-living in the aquatic environment. Typically, no intermediate host is required for the parasite to reproduce (direct life cycle). Consequently, they can build up to very high numbers when fish are crowded causing weight loss, debilitation, and mortality. Five groups of protozoans are described in this publication: ciliates, flagellates, myxozoans, microsporidians, and coccidians.

A. Ciliates

1. *Ichthyophthirius multifiliis:*

This is probably the most common parasite of all fishes. The common name for this parasite and disease is "Ich" or "white spot". The mature parasite reaches approximately 1 mm in diameter and is commonly observed in the gills and/or skin as coalescing white spots, hence the common name. The trophont or mature stage of the parasite has a large "horseshoe" shaped nucleus, and the entire surface of the parasite is covered in cilia. The life cycle of this parasite is direct, but is spent, in part, off of the host. The trophont is within the epidermis of the host, until it leaves the fish, encysts demonstrates mature cyst of this parasite) and divides to produce many host-seeking tomites. The tomites penetrate the skin and gills of the fish to complete the life cycle. The life cycle is temperature dependent with a shorter life cycle occurring at warmer water temperatures.

Fish with a cutaneous infection will "flash", i.e., turn over and expose their white underside, whereas fish with a gill infection will "pipe", i.e., come to the surface of the water and "breathe" through their mouth. Gill lesions include epithelial hyperplasia with the presence of mature trophonts within the gills. Cutaneous lesions also exhibit focal epidermal hyperplasia, with parasites being located beneath the hyperplastic epidermis.

2. *Chilodonella*:

This is a motile ciliated protozoal parasite which causes disease in the skin and gills of fish. It is typically heart-shaped with the posterior end being broader and slightly notched. It measures approximately 20-40 m in width and 30-70 m in length and its surface is covered with cilia. There is a large macronucleus in the posterior portion of this organism and a smaller micronucleus is near or within the macronucleus. This parasite has been attributed to death of fish due to respiratory and osmoregulatory imbalances associated with severe gill parasitism. Diagnosis is dependent upon demonstration of the organism within the affected organs by either cytology or histopathology.

3. Cryptocaryosis:

Cryptocaryon irritans, parasite of gills and skin, is the causative agent of this disease. External signs consist of white spots and mucous excess or ulcers on the skin and impairment of respiratory function. Gill histopathology consists of inflammation, haemorrhages, hyperplasia and lamellar destruction. This ciliate is a typical marine fish parasite affecting commercial and ornamental fish and producing high mortality in culture conditions. Outbreaks appeared mainly at high temperatures. Some treatment and control measures are similar to those recommended for ichthyophthiriasis, though quinine derivatives and low salinity baths have also been used. Diagnosis is based on macroscopical examination followed by microscopical examination for confirming the presence of the ciliate. The parasite can also be found in histopathological studies.

4. Trichodina:

There are three genera which form the Trichodina complex: *Trichodina, Trichodonella* and *Tripartiella*, however, all three are commonly referred to as "Trichodina". All are approximately 100 m in diameter and have a saucer to "frisbee" shape and are ringed with cilia around its entire surface. They have a circular arrangement of tooth-like structures (denticular ring) within the body which provides them a characteristic appearance in fresh gill and skin cytology preparations (photo). Fish with severe gill infections of trichodina will have respiratory and osmoregulatory difficulty and may "pipe" as well as "flash" if there is cutaneous involvement. Fin erosions and/or ulcerations can be observed in chronic cutaneous infections. Diagnosis of this parasitic disease is dependent upon identification of the parasite within the skin or gill cytologic preparations or histopathology.

Fish trichodinids include mainly Trichodina spp., *Trichodinella* spp. and *Tripartiella* spp. These peritrichid ciliates are more commensals than genuine ectoparasites, but can produce different damages in massive infections. The fish show a grey-blue turbid layer on the skin. Respiratory function can be impaired in gill infections. Trichodinids parasitize a lot of freshwater and marine fish species. Diagnosis is mainly based on microscopical examination of fish or gill scraping preparations. Hygiene in hatcheries and quarantine for ornamental fish are recommended for prevention. This ciliatosis can be treated with formaldehyde in baths. In freshawter ornamental fish and fry, baths of salt solutions can be applied, with variable success.

5. ***Ambiphyra*:**

These are ciliated protozoan organisms which are thought to be free-living, but have been known to parasitize fish. They are sessile organisms with a cylindrical to conical body with oral cilia and a permanent motionless equatorial ciliary fringe. They range in size from approximately 60-100 m and adhere to the epithelium of the skin and/or gills. Disease and death of fish have been associated with chronic infections of the gills due to mechanical blockage of respiratory epithelium. Diagnosis of this parasite is dependent upon identification of this organism within the skin or gill scrapings or histopathology.

B. Flagellates

1. Hexamitiasis

Hexamita spp. is parasites of the intestine and gall bladder of freshwater fish, mainly salmonids but also cyprinids and ornamental fish. Hexamitiasis, typical of weak fish, is frequent as a secondary infection. Affected fish can show nervous behaviour, and internally the intestine may appear pale. Mortalities can occur in fry and ornamental fish. Diagnosis is manly based on the direct observation of the flagellate in fresh intestinal scrapings or histopathology study.

2. *Ichthyobodo* spp. (Costiasis)

Ichthyobodo sp. (also known as *Costia*) are the agents of this disease of the gills and skin. *I. necator* is the species parasitizing salmonids in freshwater, but a different species is considered to be present in marine fish. Affected fish appear thin and lethargic, and may show a grey-whitish pellicle on skin, epidermic erosion or even haemorhages or ulcers, as well as gill hyperplasy and edema. Costiasis is widely distributed in different fish species, mainly in larval and juvenile stages, and mortality can occur in fry or ornamental fish with moderate to severe infections. Besides direct mortalities, indirect damage due to decreased health condition and gill lesions must be considered.

Diagnosis is based on microscopical examination and histopathology. Prevention relies on hygienic measures. Costiasis can be treated with formaline 1:4000 or 1:6000 in baths with a good aeration.

3. *Cryptobia* spp., *Trypanoplasma* spp., *Trypanosoma* spp.:

Some species of these genera parasitize internal organs of fish. *Cryptobia iubilans* is the only pathogenic intestinal species, common in aquaria cichlid fish. *Trypanoplasma spp*. and *Trypanosoma* spp. include parasites of the bloodstream and of tissues, with indirect life cycles (leeches are the main vectors). The best known is *Trypanoplasma salmositica* (frequently referred as *Cryptobia samositica*) producing cryptobiasis of salmonids. Clinical signs consist of exophthalmia, splenomegaly, hepatomegaly, abdominal distension with ascites, anemia and anorexia. Mortality is dependent on fish stocks and species, but may be high in juveniles. The disease has severe impact in salmonid cultures in North America. An experimental protective vaccine has been developed. Other pathogenic species, *Trypanoplasma borreli,* parasitizes mainly cyprinids in Europe and North America. The genus *Trypanosoma* includes numerous species of both freshwater and marine fish. Some freshwater species are pathogenic for cyprinids.

C. Myxozoa (myxosporidiosis):

The class Myxosporea (phylum Myxozoa) includes numerous genera and species, most of them parasites of fish. Some species are well know pathogens for freshwater fish. In the last years Myxosporea have been increasingly reported in cultured marine fish. They are characterised by a spore with one to several valves, one or more infective sporoplasms and one to several polar capsules with a coiled polar filament inside.

The most pathogenic species belong to the genera *Ceratomyxa*, *Myxobolus, Myxidium, Spaherospora*, *Enteromyxum, Kudoa, Tetracapsuloides* and *Sphaerospora*.

In freshwater fish the most significant diseases are whirling disease, PKD, sphaerosporosis and ceratomyxosis (produced by *Ceratomyxa shasta*). Myxosporea reported from cultured marine fish include species of the genera *Ceratomyxa, Enteromyxum, Kudoa, Lepthoteca, Sphaerospora and Sinuolinea*. Other myxosporeans reported occasionally in the survey are *Leptotheca* sp. and *Polysporoplasma sparis* from the kidney of *S. aurata,* and *Sinoulinea* sp. from the urinary bladder of turbot, the three reported only by one laboratory from Spain, mainly in routine or occasional samplings. The species of *Leptotheca* has been described as *L. sparidarum* from seabream an dentex.

D. Microsporidia:

Microsporea are represented in fish by different genera, mainly *Enterocytozoon*, *Glugea*, *Loma*, *Pleistophora* and *Tetramicra*. In freshwater fish, *Pleistophora* and *Loma* are relatively frequent. Among cultured marine fish, there have been several reports of *Plesitophora senegalensis* in gilthead seabream, whereas *Glugea* sp. and *Tetramicra brevifillum* have been found in turbot. Pathological concern of microsporidiosis in fish is dependent on location and infection intensity. Variable loses in turbot cultures have been related to *Tetramicra* infections. Diagnosis is based on the direct detection of the parasite at microscope, mainly the spores, but

ultrastructural studies are necessary for identification at the specific (or even generic) level. A PCR based assay has been recently developed for *T. brevifillum*. Among chemicals tested for treatment, toltrazuril has apparently given better results than fumagillin and amprolium.

E. Coccidia:

Different coccidia (Apicomplexa) are known from among freshwater and marine parasites, but their pathological significance for the cultures is very variable. The genera *Eimeria, Goussia* and *Cryptosporidium* include the species more frequently reported from cultured fish. In freshwater fish, *G. carpelli* parasitizes different cyprinids and *E. anguillae* is typical of eels. In marine fish, *E. sparis* and *G. sparis* have been reported from *Sparus aurata* and *E. dicentrarchi* and *E. bouixi* from *Dicentrarchus labrax*. Fish *Cryptosporidium* spp. include species from seabream, seabass, turbot, and aquarium fish, affecting mainly larvae and juvenile, with deleterious effects not always very evident, but resulting in poor condition. *C. molnari* is more frequent in seabream than in seabass. The species of turbot is probably a new one. Diagnosis of fish coccidia is mainly based on histopathology and/or on fresh examination at microscope. Immunodiagnostic methods are available for some human and animal species, but not for fish species. Control of animal coccidians is based on the use of different coccidiostatics or coccidiocides, but information regarding fish coccidia is very scarce. Furazolidone, amprolium chloride and furanace, among others, have been tried to treat different fish coccidia.

II. Cnidaria

1. *Myxobolus cerebralis* (Whirling disease)

The causative agent is *Myxobolus cerebralis* (synonym *Myxosoma cerebralis*). Clinical signs include dark coloration of the posterior part of the body and abnormal swimming in spiral, followed by skull deformation and spinal curvature. Almost all salmonid species can be infected, but susceptibility is very variable according to the species. *Oncorhynchus mykiss* and other *Oncorhynchus* spp. Are very susceptible, while *Salmo trutta* is rather resistant. The involvement of an intermediate oligochaete host in the life cycle of this myxosporean was demonstrated 18 years ago. This knowledge has facilitated preventive measures, consisting of the use of concrete or plastic ponds or tanks and their frequent cleaning for avoiding the presence of oligochaetes and thus the transmission of the disease.

This is very important, considering the limited efficacy of treatments assayed till now (fumagillin, toltrazuril) for this myxosporea and other species. In some European countries, the incidence of whirling disease has clearly decreased, whereas in USA whirling disease is widely distributed and is still an important pathological problem. Diagnosis is based on the histological examination of skull cartilage, or their enzymatic

digestion followed by microscopical observation of the typical spores. A PCR assay has also been developed.

III. Monogenean Trematodes

This is a group of trematodes which complete their entire life cycle on the host. The adults attach to the host by a *haptor* or *opishaptor* which is a specially adapted structure on the posterior end of the parasite. This organ has hooks which allow the parasite to attach firmly to the host fish. These parasites usually cause minimal damage to fish, but will infest the skin, fin and gill of pond fishes. Severe infestations may be responsible for poor respiration and/or emaciation. The two most common monogenetic trematodes include: *Dactylogyrus* and *Gyrodactylus.*

1. *Gyrodactylus*

Gyrodactylus — Also known as Skin Fluke. Fish suffering from infestations of gill flukes may suffer respiratory problems as the flukes begin to damage the delicate gill tissues. Secondary bacterial infection often occurs in fish left suffering from these parasites, due to the physical damage caused by the anchors.

Chemical control of both types of fluke can be achieved with Chloramine T, Malachite Green Formalin and Masoten, or Potassium Permanganate.

In order to kill all generations, repeat treatments may be necessary, the frequency being dependent on temperature and chemical

2. *Dactylogyrus* - The Gill Fluke

Gill and Skin flukes are two of the family of monogenetic trematode genera, all of which are characterized by the large grappling hooks which are used to attach themselves to their victims.

Flukes are parasite affecting fish. They range from 0.05 to 3.00mm long and there are actually a huge number of species in the genus.

IV. Digenetic Trematodes

This group of parasites has a complex life cycle with several successive larval generations, alternating sexual and asexual generations and changes of hosts to develop into the adult in its primary host. The life cycles of trematodes involving fishes may either use fishes as the primary hosts or as intermediate hosts. Adult trematodes may infest the intestine or gall bladder of fishes. A few of the more common digenetic trematodes are listed below.

1. *Diplostomum spathaceum*

The life cycle of this parasite begins as an adult trematode in the intestine of gulls or other fish-eating birds. The body of the adult is 0.3-0.5 cm in length and distinctly

divided into a flattened anterior forebody and a cylindrical and narrower hindbody. Eggs are shed and passed in the feces of the bird to the water. The eggs hatch in approximately 21 days into free-swimming ciliated miracidia. The miracidia infest aquatic snails as the first intermediate host by penetration of the snail's hepatopancreas. The miracidia then become a mother sporocyst, followed by one or more daughter sporocysts. Each daughter sporocyst produces many cercariae which are released into the water. These cercariae seek a second intermediate host by penetrating the fins, skin, gills or cornea of small fishes. Primary host fish which ingest the initially infected fish (second intermediate host) become infected and the life-cycle is completed when the host fish are ingested by fish-eating birds.

2. *Posthodiplostomum minimum:*

This trematode has several synonyms including: *Neodiplostomum minimum, Neodiplostomum orchilongum* and *Postodiplostomum orchilongum.* The life cycle of this trematode is very similar to that of *D. spathaceum* above, although, infectivity of cercariae to fishes lasts no more than 24 hours after release from the snail. Each cercaria actively raises a scale and enters under the scale pocket, causing irritation to the fish. Blood, congestion and hemorrhage occur at the bases of fins or other places of cercarial penetration. The trematodes migrate from the point of entry to visceral organs of the fishes, usually within one to three hours after penetration. Metacercariae are located in any organ of the fishes' body, but are generally more numerous in the liver, kidney, heart, spleen and other organs of abdominal viscera. With many of the digenetic trematodes, the metacercariae within the skin results in increased melanin deposition, hence the term "black spot disease". Visible white or yellow spots in the visceral organs, usually no larger than 1 mm in diameter are often referred to as "white grubs" or "yellow grubs" and could be caused by several trematode species. Diagnosis of digenetic trematode infections is dependent upon identification of the genus and species of the trematode within infected fish.

V. Nematodes

Nematodes, also called roundworms, occur worldwide in all animals. They can infect all organs of the host, causing loss of function of the damaged area. Signs of nematodiasis include anemia, emaciation, unthriftiness and reduced vitality. Three common nematodes affecting fish are described.

1. *Camillanus*

Camillanus is easily recognized as a small thread-like worm protruding from the anus of the fish. Control of this nematode in non-food fish is with fenbendazole, a common antihelminthic. Fenbendazole can be mixed with fish food (using gelatin as a binder) at a rate of 0.25% for treatment. It should be fed for three days, and repeated in three weeks.

VI. Parasitic Crustacea

Parasitic crustacea are increasingly serious problems in cultured fish and can impact wild populations. Most parasitic crustacea of freshwater fish can be seen with the naked eye as they attach to the gills, body and fins of the host.

1. *Argulus*

Argulus — Also known as Fish lice and is easy to detect with the naked eye especially against the background of fins. It lives as an ectoparasite within the gill-chamber of fresh-water fishes. It does not remain permanently fixed and either swims freely or crawls over the surface of the body. The leaf-like body includes a flat and oval cephalothorax and a small bilobed abdomen. Two compound eyes are distinctly visible and near each eye two short antennae are present. Size varies from between 1mm and 5mm. Short first maxillae and styli- form mandibles work inside a siphon which together with a poison spine in front acts as the piercing organ. The second maxillae are transformed as suckers. The maxillipeds are modified for clasping and four pairs of thoracic legs are swimming appendages. Breeding takes place outside the body of the host and fertilization is internal.

Attaching themselves to the fish by suckers which damages the skin, they also inject a poison into the body of the fish which causes inflammation, bleeding and potentially secondary bacterial infection.

Chemical treatments recommended to eradicate these parasites are either Masoten, Dimilin or Paradex.

VII. Dinoflagellates

1. Amyloodiniosis

Also known as "velvet disease", the causative agent is *Amyloodinium ocelatum*, an ectoparasite on the skin and gills of different fish species. Appart from the velvet appearance, clinical signs consist of anorexia and scratching. Histopathological lesions include gill inflammation, haemorrhages and hyperplasy. Massive infections are frequently associated to mortalities, both in mariculture and sea aquaria, mainly at high temperatures. The infection is very common in Mediterranean fish, though other fish species are affected, including tropical and aquarium fish. Other dinoflagellates (as *Piscinoodinium* spp.) parasitize different freshwater fish.

Diagnosis is mainly based on microscopic fresh and histological examination, though an ELISA test is available. No effective control measures are known for Mediterranean fish.

Freshwater (2-4 minutes) or copper sulphate (0.75 mg/l, 12-14 days) baths have been suggested as an aid to control the trophonts or dinospores, respectively. In Pacific threadfin (*Polydactylus sexfilis*) recent findings suggest the suitability of hydrogen

peroxide as treatment in juvenile fish. Some evidences suggest the development of immunity against re-infections, and specific antibodies have been demonstrated in the sera of infected fish.

Conclusion

Most fish health problems occur because of environmental problems: poor water quality, crowding, dietary deficiencies, or "stress". The best cure for any fish health problem is prevention. Good water quality management and proper fish husbandry techniques will eliminate most parasites described here.

Probable questions:

16. Discuss about different causes for fish health problems.
17. Name different protozoan parasites responsible for fish health problem.
18. Name the causative agent of "white spot" disease.
19. What are the symptoms of Cryptocaryosis.
20. Discuss briefly about Trichodina infection in carps.
21. Name one fish parasite responsible for intestine and gall bladder infection of freshwater fish.
22. Discuss the role of Myxozoan parasite in fish infection.
23. Write short notes on Whirling disease and white spot disease.
24. Name two most common monogenetic trematodes in fish infection.
25. Discuss the life cycle of *Diplostomum spathaceum*.
26. How *Argulus* is responsible for creating infection in fishes.

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UNIT VI

Parasites of edible oyster

Objective:

In this unit, we will discuss about parasites of edible oyster.

Introduction:

In India substantial oyster resources exist which remain unutilized. Pioneering attempts were made by James Hornell in 1910 in developing oyster culture in the Chennai in India.

Along the Indian coast there present vast stretches of areas of brackish waters, estuaries, many of which harbour natural populations of oysters, which suggest the suitability of the habitat for oyster culture along the Indian coast.

Being filter feeders, the oysters convert primary production in the water into nutritious seafood.

The oyster resources if cultured and harvested in regulated manner may contribute to the well being of the people that depend on this resource thus helping in improving socio-economic conditions of the coastal communities and protecting the coastal as well as marine environment.

In West Bengal, the Sundarbans region is suitable for attempting oyster culture. Here the local people artificially culture of *Saccostrea cucullate* and *Crassostrea madrasensis*at four different sites of Chotomollakhali Island of Sundarbans

But there are many technical and biological problems for wide range successful oyster culture. The rate of the oyster mortality is high in this region which poses a great problem to the oyster culture in Sundarbans.

Many oysters are died before reaching the market size. This mortality of oyster may be due to the diseases, parasitic infection, harmful organisms, heavy metal deposition or climatic changes.

Recently histopathological studies have thrown some light on the possible factors responsible formany instances of large scale mortalities.

Some viruses are suspected to be responsible for the cause of diseases and mortalities.

Dermo isa dreaded disease caused by a protozoan parasite, *Perkinsusmar inus*inflicted heavy mortality among East American oysters. Gill disease and shell disease attributed to pathogenic fungus,caused heavy mortalities among Japanese and European oysters.

Oyster beds affected by the above disease have revived after a long time. Large and thick oyster beds often are prone to be affected by diseases. Predators and foulers like

fishes, starfishes, gastropods, crabs, polychaetes etc. are also a menace to oyster farmers. Sudden changes in hydrological parameters like temperature, salinity cause much damage to the oyster population.

To develop the oyster resources it is necessary to ensure that the areas of oyster beds are free from pollution since oysters accumulate heavy metals and pathogens

Oysters also play a significant role as a good bioindicator and biomonitoring species

Oysters also act as "ecosystem engineers" (Lawton and Jones, 1995; Jones et al., 1994) and indirectly modulate the availability of resources to other species, by causing a physical change in biotic and abiotic materials.

The word oyster is used as a common name for a number of distinct groups of bivalve molluscs whichlive in marine or brackish habitats. The valves are highly calcified..

The edible oysters belong to phylum Mollusca and Class Bivalvia. There is over hundred species of edible oysters have been distributed all over the world mainly in all temperate and tropical coasts

Molluscs including bivalves and oysters form a low-cost subsistence food and pharmaceuticals of the coastal people, especially for the fishing communities.

Among shellfishes, oysters are considered to be a valuable food items as they constitute rich source of many of the elements, essential for providing a balanced diet as it provides provide highly nutritious food for human consumption as they store large quantities of glycogen and fat.

The oysters are known to show large variations in their meat quality depending on their physiologicalconditions and associated environmental factors.

- **Distribution of edible oysters in India**

The edible oysters occur in all tropical seas, mainly between tidal levels or in shallow waters near the estuaries. The distribution of oysters along the Indian coast shows a distinct pattern.

*Crassostrea madrasensis*is found along the east coast from Orissa to Tamil Nadu. Along the west coast it is more dominant in the south than in the north.

*C. gryphoides*is the main oyster species in the northwest region especially in the Gulf of Kutch. Mixed populations of *C. grvphoides* and *C. rivularis*are seen along the northwest coast.

Saccostrea cucullate has wider distribution and is found along with all the species of the genus

Crassostrea occurring in India.

Apart from this, oyster populations dominated by *S. cucullata*are also seen especially in Karnataka, Maharashtra and Gujarat. This species is also widely distributed in the inshore waters of Andaman and Nicobar islands.

Crassostrea gryphoides and *Saccostrea cucullata*—these are the two important species of edible oysters in the Sundarbans region, West Bengal.

Phylum: Mollusca

Class: Bivalvia

Subclass: Pteriomorphia

Order: Ostreoida

Family: Ostreidae

Genus: *Crassostrea*

DendostreaLopha

Ostrea Ostreola SaccostreaStriostrea

- **Description of the study site**

Two sampling sites have been selected each in the western and central sectors of Indian Sundarbans, a gangetic delta at the apex of the Bay of Bengal. On this background two sampling stations (Kaikhali in central sector and Frasergunj in western sector) have been selected to analyze the concentrations of some metals in the edible oyster species.The host oyster species have been collected from two sites of Sundarbans namely, Kaikhali and Frasergunj. *Crassostrea gryphoides* present in both the sites but *Saccostrea cucullata* has found only in Frasergunj.

oyster species in Sunderban

Saccostrea cuculata Crassostrea madrasensisCrassostrea gryphoides

About ***Crassostrea gryphoides*** **Schlotheim, 1813**

Crassostrea gryphoides shell valves are variable in shape, more elongated and more deeply cupped and hence are called cupped oysters. They contribute a great portion of animal protein in the food market. Its tender flesh forms a cheaper, nutritious and easily digestible food source.

About ***Saccostrea cucullata*** **Born, 1778**

Saccostrea cucullate commonly known as the rock oyster due to its occurrence on rocky substratum are found on both east and west coasts but are more common on the west coast. They also are consumed as food but nowhere does it form beds large enough for exploitation.

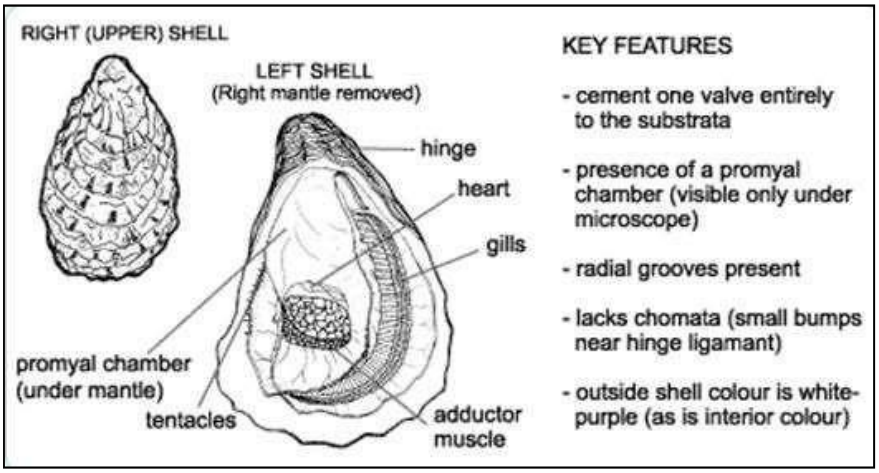
- **Utilization of oyster:** The oysters are either sold in the fresh shell on condition or as shucked meat in the domestic markets. Apart from marketing live oysters, different methods have been developed to preserve it without losing its quality. Some of the products like smoked, dried, frozen or canned oyster meat are sold in the metro cities. Oyster meat is nutritious and is relished in most nations.

Oyster meat also has nutritional and medicinal properties. The oyster meat consists of protein, glycogen and fat.

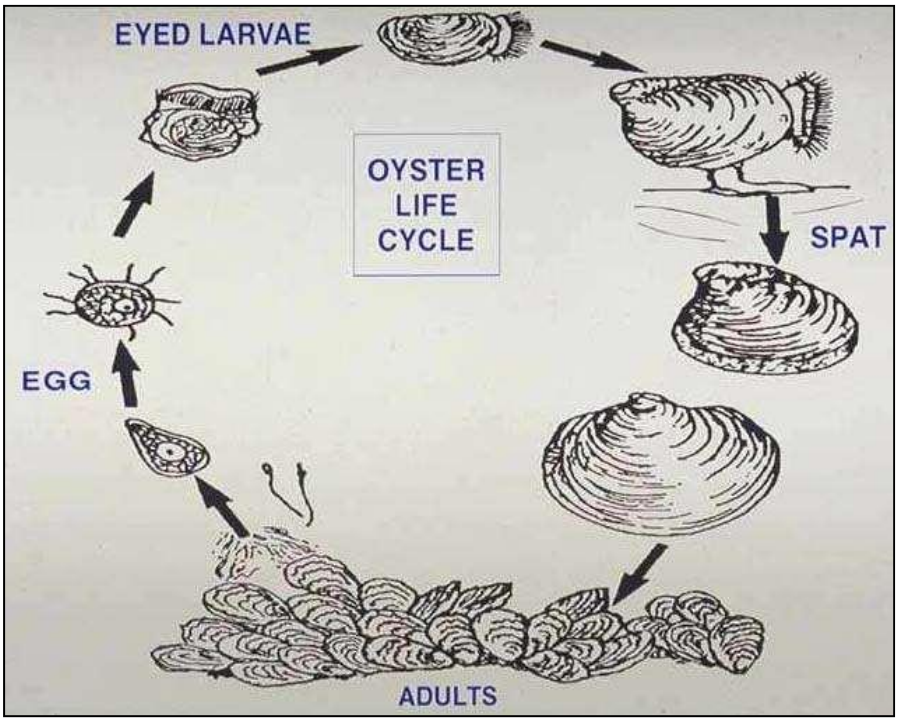
Oyster powder contains wide range of minerals and vitamins and the amino acid taurine which has complex medicinal properties. It has been found to be good for skin care, numerous heart ailments, blood pressure, liver problems, arthritis and rheumatism, diabetes, water retention and premenstrual tension.

Apart from meat, the **oyster shells** fetch a substantial return as by-product. The shell constitutes about eighty five percent of the total weight of oyster and contains fifty two to fifty five percent of calcium oxide and is used in the manufacture of calcium carbide, lime, fertilizers and cement. Further the **oyster shells** are useful as spat collectors of oyster spats. The shells are also broken to pieces of suitable size and used as poultry grit.

One of the major ecological functions of oysters is their potential to improve water quality by filteringplankton and other suspended particles from the water column.



Life History



- Reach sexual maturity at 1 year of age.
- Young oysters are male but change to female after first spawning season.
- Water temperatures above 25^{o}C trigger spawning in the.
- Salinity above 10 ppt.
- Spawn between March and November

Even though sedentary bivalves are able to withstand the wide variations in environmental conditions that commonly occur in their natural habitats, their survival are strongly influenced by fluctuations of environmental factors such as temperature, salinity, pH, food availability, nutrient levels, current speed, water depth etc.

The effect of increasing winter water-temperatures and modifications to regional salinity patterns in the spread and variation in prevalence and intensity of disease in marine bivalves.

Human-mediated climate change might also affect disease prevalence

Increased ocean temperature also caused pathogen range expansions. Climate change had been implicated in the northward expansion of Dermo and possibly MSX diseases of oysters.

Eastern oyster disease *Perkinsus marinus*) extended its range during a winter warming trend .Disease in both terrestrial and marine ecosystems could increase with future climate warming

Increased global acidification could impede calcareous shell formation, perhaps

exacerbated by increased water temperature, particularly in molluscs, thereby have an impact on mollusc culture.

- **Protozoan Parasites of Oyster**

Oysters are subject to various protozoan diseases.

Dermo is caused by a protozoan parasite *Perkinsus marinus.*

MSX (Multinucleated Sphere X) is caused by the protozoan Haplosporidium nelsoni.

Disease is clearly a major factor affecting the abundance of shellfish stocks. *Haplosporidium nelsoni*

and *H. costale* had been reported to cause mortalities in the oyster, *Crassostrea virginica*

P. marinus, a water-borne pathogen most likely acquired through oyster feeding, commonly found intissues of the digestive system

P. marinus caused mass mortalities of the oyster *Crassostrea virginica* along the east coast ofAmerica and in the Gulf of Mexico

QX disease and *Mikrocytosroughleyi* caused winter mortality

- **Dermo disease**

Dermo disease was first identified as the cause of extensive oyster mortalities in the Gulf of Mexico nearly fifty years ago.

It was originally thought to be a fungus and was named *Dermocystidium marinum.* Even though subsequent research led to its reclassification as a protozoan parasite *Perkinsus marinus* in 1978, the disease is still commonly referred to as "Dermo".

Favourable environmental conditions are temp >20°C and salinity >15ppt

Depletes host energy resources causing decreased growth and reproduction and increased mortality

Rapidly proliferates and spreads via hemolymph (oyster blood) through tissues causing deterioration and organ failure

- **Transmission of *P. marinus***

The main method of transmission occurs when infective stages of the parasite, free in the water column, are ingested by oysters and then invade the lining of the digestive system.

Natural infections are most often caused by parasites released from the disintegration of dead oysters.

P. *marinus.*

It may also be distributed by scavengers feeding on dead oysters or by *Booneaimpressa,* a parasitic snail that feeds on oyster hemolymph and acts as a vector in the

transmission of the disease between live oysters.

Lifecycle of *Perkinsus marinus* has two distinct phases of the lifecycle:

1) the developmental stage
2) zoosporulation stage

The developmental stage within the host as a trophozoite in which the flagellum is lost to become a rounded immature form. This matures to form a large vacuole with a vacuoplast. Immature trophozoites free themselves through a tear in the cell wall and ultimately spread throughout the host's body. Zoosporulation occurs in which a mature trophozoite enlarges, loosing its vacuoplast. A discharge tube and pore develops in the cell wall. Palintomy occurs again, which results in numerous biflagellate zoospores moving through the discharge tube into the seawater.

- **MSX diseases**

It is often associated with a visible brown red discolouration of gill and mantle tissues.

Sporulation of *H. nelsoni*is prevalent in juvenile oysters (1-2 yrs) but sporadic in adults and occurs exclusively in the epithelial tissues of the digestive tubules. Infections appear and continue throughoutthe summer (mid-May to the end of October)

MSX disease was first recognized as the cause of massive oyster mortalities (90–95%) in lower Delaware Bay in 1957.

The causative agent, a single celled parasite, *Haplosporidium nelsoni,* was originally given theacronym "MSX" because it was observed as a Multinucleated Sphere with unknown affinity ("X").

Distribution of MSX disease is fairly uniform over a large area within the higher-salinity (greater than 15 ppt – parts of salt per thousand parts of water) portion of affected estuaries

- **Transmission of *H. nelsoni***

The earliest infections of *H. nelsoni* are found in the oyster's gill. The infective stage is water-borne.

The means by which MSX disease is transmitted is not known.

Probable question:

1. Explain the different types of protozoan parasites of edible oyster and their mode of transmission.
2. Write short notes on MSX disease.
3. Discuss the life cycle of oyster with diagram.
4. Elaborate the mode of transmission of Dermo disease.

Suggested readings/ references:

1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4087319/
2. https://www.ncbi.nlm.nih.gov/pubmed/28752231
3. https://link.springer.com/article/10.1007%2Fs00436-017-5566-z
4. http://eprints.cmfri.org.in/12069/

UNIT VII

Mode of transmission, pathogenicity and prevention of tuberculosis and cholera

Objective:

In this unit, we will discuss about Mode of transmission, pathogenicity and prevention of tuberculosis and cholera

Introduction:

Tuberculosis (TB) is a dangerous and highly contagious bacterial disease caused by Mycobacterium tuberculosis. It primarily affects the lungs, but if left untreated, it might spread to different parts of the body.

In most forms of the disease, the bacillus spreads slowly and widely in the lungs, causing the formation of hard nodules (tubercles) or large cheese like masses that break down the respiratory tissues and form cavities in the lungs. Blood vessels also can be eroded by the advancing disease, causing the infected person to cough up bright red blood.

During the 18th and 19th centuries, tuberculosis reached near-epidemic proportions in the rapidly urbanizing and industrializing societies of Europe and North America. Indeed, "consumption," as it was then known, was the leading cause of death for all age groups in the Western world from that period until the early 20th century, at which time improved health and hygiene brought about a steady decline in its mortality rates. Since the 1940s, antibiotic drugs have reduced the span of treatment to months instead of years, and drug therapy has done away with the old TB sanatoriums where patients at one time were nursed for years while the defensive properties of their bodies dealt with the disease.

Types of Tuberculosis (TB)

There are two different types of tuberculosis:

- Pulmonary Tuberculosis.
- Extrapulmonary Tuberculosis.

Pulmonary Tuberculosis

It is an endemic infection affecting the lungs. It is further classified into:

- **Primary Tuberculosis Pneumonia**: Associated symptoms include coughing and high fever. It can also arise in patients with HIV/AIDS.

- **Miliary Tuberculosis**: This form of TB is named so because of a distinctive pattern seen on a chest radiograph, where many small spots are distributed throughout the lung fields, bearing an appearance similar to millet seeds. The infection can eventually spread to the extrapulmonary organs, such as the spleen, liver, and kidneys.
- **Latent Tuberculosis Infection**: This infection is mainly seen in those patients having the bacteria within their body, but does not display any of the symptoms of the disease. This infection is primarily detected through the tuberculin skin test.

Extrapulmonary Tuberculosis

It is usually seen in immunocompromised patients. There are several types:

- Tuberculosis Meningitis
- Osteal Tuberculosis
- Lymph Node Disease
- Renal Tuberculosis
- Adrenal Tuberculosis.

Pathogenicity is the quality or state of being pathogenic, the potential ability to produce disease, whereas virulence is the disease producing power of an organism, the degree of pathogenicity within a group or species.

Symptoms of Tuberculosis

TB bacteria or Mycobacterium tuberculosis multiply once it gets into the lungs. It can cause severe symptoms such as:

- Coughing up blood and mucus from deep inside the lungs
- A bad cough that lasts three weeks or longer.
- Weakness or fatigue.
- Sweating at night.
- Pain in the chest.
- Weight loss.
- No appetite.
- Chills and Fever.

Toxin Production:

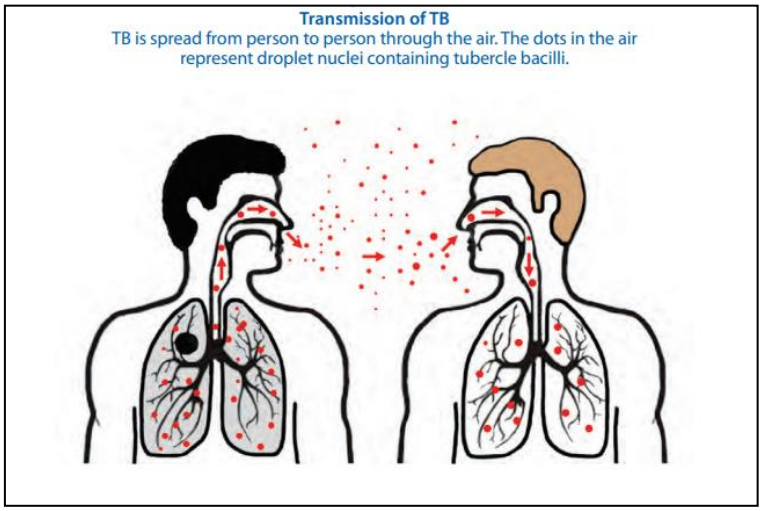
Myco. tuberculosis does not produce an exotoxin. It contains toxic substances which are liberated when it is lysed. In 1890, Robert Koch isolated a substance known as "tuberculin" from tubercle bacilli. The tuberculin is an extract containing the specific protein of tubercle bacilli. The tuberculin reaction is due to the development of tissue hypersensitivity — or bacterial allergy — and is used in men and animals to find if they have or have had tuberculosis in an active or latent form.

Tuberculin is originally obtained from a six week old culture in glycerol broth evaporated to one-tenth of its volume, sterilised by heat and filtered (old tuberculin — OT). Various other methods have been employed in its preparation. The specific tuberculin protein can now be separated from other constituents and products of culture in a synthetic medium and then purified. This purified protein derivative (PPD) is preferable to old tuberculin as it is consistent in composition. It is used in dry state, from which it can be diluted by addition of a borate buffer solution.

It should be noted that the tuberculin prepared from the human and bovine types are indistinguishable as they contain the same specific substance. Tuberculin's are standardised in such a way that a dilution of 1:10,000 of OT is equivalent to 1 tuberculin unit (TU), while 0.000028 mg of PPD equals 1 unit. A common practice in using tuberculin is to test first with 3 or 5TU and if the individual gives no reaction, retest with a dose of 100TU.

Transmission of Tuberculosis:

Tuberculosis is transmitted **through the air**, not by surface contact. Transmission occurs when a person inhales droplet nuclei containing M. tuberculosis, and the droplet nuclei traverse the mouth or nasal passages, upper respiratory tract, and bronchi to reach the alveoli of the lungs.



This organism is expectorated in sputum and expelled in droplets during coughing and speaking and there have been instances of explosive outbreaks of tuberculosis in school

children and others exposed to an infective teacher or singer. But, since very small droplets that can be inhaled directly from the infective patient are less likely to carry tubercle bacilli than larger droplets or sputum, infection may occur more often indirectly from the dried dust particles than directly from moist droplets or droplet nuclei.

The tubercle bacilli can survive slow drying for days or weeks, if protected from the bactericidal day light or sunlight. The spread of infection from infected cases to susceptible contacts by contaminated dust or fomite would be facilitated in overcrowded, badly lit rooms or buildings. Primary infection may occur at any stage, if it occurs in early life (0-3 years) it is often associated with signs and symptoms of the disease. At school age (5-15 years), infection usually occurs in an in apparent form, but in adolescents or young adults, it is again more likely to result in clinical disease.

Infection occurs earlier and is more likely to result in clinical disease among susceptibles living in close contact with open cases, but many personal and environmental factors (age, malnutrition, other respiratory diseases, hormonal dysfunction, pregnancy, stress, genetic constitution etc.) may contribute to overt tuberculosis.

Workers exposed to the inhalation of dust containing silica have a high incidence of tuberculosis. Nurses, medical students, doctors and workers in the pathology laboratory and in the hospital are more exposed and tend to have a higher than an average rate of infection.

In lung tuberculosis, the sputum is the main source of infection; in tuberculosis of kidneys and bladder, the bacilli may be excreted in the urine in plenty; in intestinal tuberculosis, faeces may contain tubercle bacilli and similarly, the pus in the tuberculosis abscess may also contain tubercle bacilli.

It has been estimated that a patient may discharge in 24 hours 500 millions to 3 billions bacilli in his sputum. A spray of sputum from a coughing or sneezing tuberculosis patient may contain tubercle bacilli may get infected with tuberculosis by coming in contact with the patient.

A careless person with active pulmonary tuberculosis may kiss a child, contaminate the floor or furniture with sputum or expectorate into the street or other public place which is a very common practice in under-developed countries. So, children playing in the street or room, creeping on the floor may get the tubercle bacilli on their hands and ultimately into their mouth. The hand to mouth transmission is very common among the children. In ancient times, children were also infected by drinking unpasteurized milk from tuberculosis cows.

While handling food, the fingers soiled with the sputum may contaminate the food. Flies which crawled on the tuberculosis sputum may contaminate the food. Improperly washed spoon, plate or common drinking cup may also carry the tubercle bacilli.

Tubercle bacilli are ubiquitous in nature. Particularly in urban area they are widespread in city streets, theatres and other public places and may result into an imbalanced urban health. Everything that keeps one person in good conditions (good food, sufficient rest, and recreation along with the chemotherapy) may pave ways towards the recovery and good health of the tuberculosis patients.

Measles, whooping cough, influenza, frequent child bearing, continuous strain and fatigue, poor living and unhygienic conditions, alcoholism and malnutrition are the predisposing causes of tuberculosis. Only the public health nurse can educate and influence the public to lead an healthy happy life.

Socio-economic conditions are also related to tuberculosis in a community. Tuberculosis is very common amongst the poor people than among the rich people. Low standards of living are: lack of isolation, rest, sunlight, fresh air and cleanliness; lack of medical, nursing care and lack of sufficient nutritious food.

Prevention of Tuberculosis:

The outcome of mycobacterial infection depends on the host immune response. In most individuals, infection with M. tuberculosis induces an immune response sufficient for the protection against progression to the primary disease.

Bacille Calmette-Guerin (BCC) vaccine reproduces minimal infection but does not impose a disease risk. BCG vaccine, which is derived from a strain of M. bovis attenuated through years of serial passage in culture, was first used in 1921 to protect against tuberculosis in humans. Many BCG vaccines are currently administered to 100 million young children each year throughout the world.

These vaccines are derived from the original strain but vary in cultural characteristics and ability to induce sensitization to tuberculin. There are differences in techniques and methods of producing them as well as various routes of vaccine administration.

Diagnosis

Tuberculin Test:

Direct microscopy of smears from sputum, pus, spinal or pleural fluid, urine, faeces, lymph nodes etc. stained by Ziehl-Neelsen method can reveal the presence of tubercle bacilli. Tubercle bacilli are plenty in lesions of rapid caseation. In miliary tuberculosis, they are relatively rare. Smears stained with aura mine can be examined by Fluorescence microscopy. Coughing may be induced by passing a swab onto the posterior pharynx, the expectoration on it can be used to prepare a smear.

Tuberculin test in man is carried out on the skin by different techniques; those most commonly used are Mantoux, Heaf and jelly tests. Mantoux test is performed by injecting intradermally 0.1 ml of the appropriate dilution of tuberculin, the test is positive when there is an area of induration measuring 5 mm in diameter in 2-3 days after injection.

In Heaf test, a multiple puncture spring release gun is used to prick the previously applied tuberculin into the skin. A positive reaction may range from 4-6 discrete papules to solid induration. These two tests are usually done on the forearm. In the jelly test, a tuberculin jelly is applied in a form of "V" in the intra-scapular area of the back and covered with a plaster; the control is required only for this jelly test, but not with other two tests.

Only Mantoux and Heaf tests were found to be reliable and acceptable to large scale epidemiological investigations, when compared to these tests and Von Pirquet scarification test; Heaf test is generally preferred to Mantoux test. The tuberculin test may be used in epidemiology to determine the incidence of tuberculosis infections in a community.

A positive reaction in a young child may also be useful for case finding among the family contacts; in immunization campaign in order to separate the positive and negative reactors and to assess the response to vaccination by simple testing afterwards.

Recent Polymerase Chain Reaction (PCR) is 95% sensitive and 93% specific when compared to culture, direct microscopy and gas chromatography. Very recently, molecular biological technology—DNA probe and polymerase chain reaction (PCR) — can detect even a single Myco. tuberculosis in clinical specimen with 100% sensitivity and specificity.

Treatment of Tuberculosis:

Before effective drugs were available, half of the patients with active pulmonary TB died within 2 years, and only a quarter were cured. With the advent of anti-TB chemotherapy, protracted bed rest and lengthy isolation became unnecessary, and in theory at least, successful treatment was a reasonable goal in all adults.

Mycobacterium is naturally resistant to most common antibiotics and chemotherapy agents. This is probably due to their highly hydrophobic cell envelope acting as an efficient permeability barrier. Due to the discovery of the effective antlmycobacetrial agentsethambutol (EMB., Fig. 6.1c), INH, pyrazinamide (PZA., Fig. 6.1 d), RIF and streptomycin (STR., Fig. 6.1e) between 1950 and 1970s, and reduction in poverty, there was a drastic decrease in the number of TB cases especially in developed countries, however, since 1980s, the number of TB cases throughout the world has been increasing rapidly due to the emergence of MDR- TB.

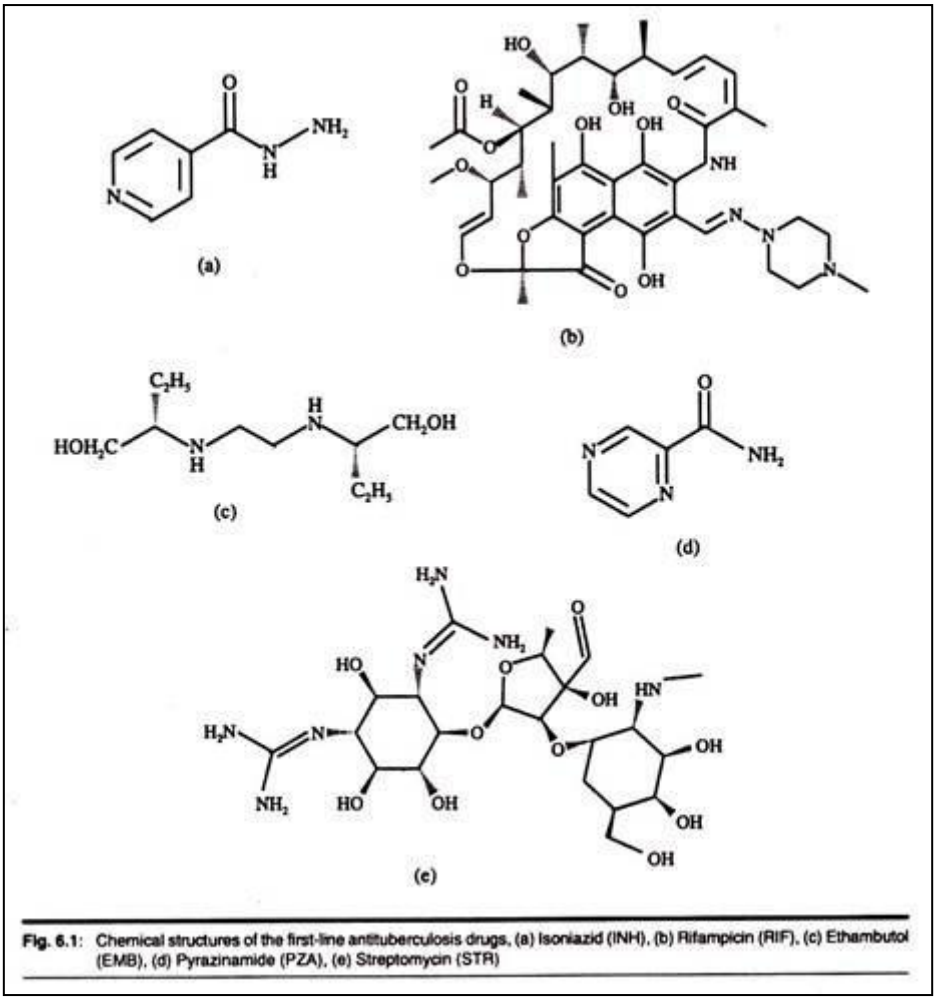


Fig. 6.1: Chemical structures of the first-line antituberculosis drugs, (a) Isoniazid (INH), (b) Rifampicin (RIF), (c) Ethambutol (EMB), (d) Pyrazinamide (PZA), (e) Streptomycin (STR)

The MDR forms of the disease, defined as forms resistant to two or more existing TB-drugs, are often fatal and are difficult and expensive to treat. The situation has recently been complicated by the association of TB with HlV in sub-Saharan Africa and many developing countries. The situation is exacerbated by the increasing emergence of extensively drug-resistant (XDR).

Reliable treatment therapy for TB treatment takes a period of 6 – 9 months with first line drugs (EMB, INH, PZA, RIF and STR). In the case of acquired drug resistance only second-line drugs (capreomycin, cycloserine, kanamycin and ethionamide) can be used and these have significant side effects with approximately 50% cure rate.

The current therapies reduce the pulmonary bacterial burden but the treatment periods of 6 months for non-immune suppressed individuals and at least 9 months for immune suppressed patients are required for reliable treatment efficacy.

However, floroquinolones such as ofloxacin, norfloxacin can be used which are safer than the above-mentioned second-line drugs but have the disadvantage of being very expensive. Emergence of drug-resistant mycobacterial strains is alarming these days. This occurs when a single drug is given alone and when the viable bacterial population in the lesions is large. The occurrence of drug resistance is widely thought to be due to

the overgrowth of sensitive organisms by mutant resistant bacilli present in wild strains before they were ever in contact with the drug concerned.

There have been no new anti- TB drugs introduced in the past 30 years. Thus, there is an urgent need to search for and develop new effective and affordable anti-TB drugs.

- **Cholera**

Cholera is an infectious disease that causes severe watery diarrhoea, which can lead to dehydration and even death if untreated. It is caused by eating food or drinking water contaminated with a bacterium called *Vibrio cholerae*.

It has been one of the diseases most feared by mankind. It is endemic to the Indian subcontinent where it is estimated to have killed more than 20 million people this Century. During the 19th Century there were a number of pandemics of 'Asiatic cholera' spread from the Indian subcontinent throughout Europe and the Americas.

The disease is most common in places with poor sanitation, crowding, war, and famine. Common locations include parts of Africa, south Asia, and Latin America. If you are traveling to one of those areas, knowing the following cholera facts can help protect you and your family.

Cholera Causes

Vibrio cholerae, the bacterium that causes cholera, is usually found in food or water contaminated by feces from a person with the infection. Common sources include:

- Municipal water supplies
- Ice made from municipal water
- Foods and drinks sold by street vendors
- Vegetables grown with water containing human wastes
- Raw or undercooked fish and seafood caught in waters polluted with sewage

When a person consumes the contaminated food or water, the bacteria release a toxin in the intestines that produces severe diarrhoea.

It is not likely you will catch cholera just from casual contact with an infected person.

Cholera Symptoms

Symptoms of cholera can begin as soon as a few hours or as long as five days after infection. Often, symptoms are mild. But sometimes they are very serious. About one in 20 people infected have severe watery diarrhoea accompanied by vomiting, which can quickly lead to dehydration. Although many infected people may have minimal or no symptoms, they can still contribute to spread of the infection.

Signs and symptoms of dehydration include:

- Rapid heart rate

- Loss of skin elasticity (the ability to return to original position quickly if pinched)
- Dry mucous membranes, including the inside of the mouth, throat, nose, and eyelids
- Low blood pressure
- Thirst
- Muscle cramps

If not treated, dehydration can lead to shock and death in a matter of hours.

Cholera toxin

Cholera**,** an acute infection of the small intestine caused by the bacterium *Vibrio cholerae* and characterized by extreme diarrhoea with rapid and severe depletion of body fluids and salts. Once the bacterium infects the intestine, it secretes the enterotoxin from its external coating. The enterotoxin binds to a receptor on the cells of the lining of the small intestine. Part of the toxin then enters the intestinal cells. The toxin increases the activity of an enzyme that regulates a cellular pumping mechanism that controls the movement of water and electrolytes between the intestine and the circulatory system. This pump effectively becomes locked in the "on" position, causing the outflow of enormous quantities of fluid—up to one litre (about one quart) per hour—into the intestinal tract. All of the clinical manifestations of cholera can be attributed to the extreme loss of water and salts.

Pathogenesis and Clinical Features of Vibrio:

Cholera usually has an incubation period of between one and three days and can vary from mild, self-limiting diarrhoea to a severe, life-threatening disorder.

The infectious dose in normal healthy individuals is large when the organism is ingested without food or buffer, of the order of 10^{10} cells, but is considerably reduced if consumed with food which protects the bacteria from stomach acidity.

Studies conducted in Bangladesh indicate that 10^{3}-10^{4} cells may be a more typical infectious dose. Individuals with low stomach acidity (hypochlorohydric) are more liable to catch cholera.

Cholera is a non-invasive infection where the organism colonizes the intestinal lumen and produces a potent enterotoxin. In severe cases, the hyper-secretion of sodium, potassium, chloride, and bicarbonate induced by the enterotoxin results in a profuse, pale, watery diarrhoea containing flakes of mucus, described as rice water stools.

The diarrhoea, which can be up to 201 day $^{-1}$ and contains up to 10^{8} vibrios ml^{-1}, is accompanied by vomiting, but without any nausea or fever.

Unless the massive losses of fluid and electrolyte are replaced, there is a fall in blood volume and pressure, an increase in blood viscosity, renal failure, and circulatory collapse. In fatal cases death occurs within a few days. In untreated outbreaks the death

rate is about 30-50% but can be reduced to less than 1 % with prompt treatment by intravenous or oral rehydration using an electrolyte g^{-1} lucose solution.

The reported incubation period for *V. parahaemolyticus* food poisoning varies from 2 h to 4 days though it is usually 9-25 h. Illness persists for up to 8 days and is characterized by profuse watery diarrhoea free from blood or mucus, abdominal pain, vomiting and fever. *V. parahaemolyticus* is more entero-invasive than *V. cholerae*, and penetrates the intestinal epithelium to reach the lamina propria. A dysenteric syndrome has also been reported from a number of countries including Japan.

Pathogenicity of *V. parahaemolyticus* strains is strongly linked to their ability to produce a 22 kDa, thermo-stable, extracellular haemolysin. When tested on a medium known as Wagatsuma's agar, the haemolysin can lyse fresh human or rabbit blood cells but not those of horse blood, a phenomenon known as the Kanagawa reaction. The haemolysin has also been shown to have enterotoxic, cytotoxic and cardio-toxic activity.

Most (96.5%) strains from patients with *V. parahaemolyticus* food poisoning produce the haemolysin and are designated Kanagawa-positive (Ka+) while 99% of environmental isolates are Ka —. Volunteer feeding studies have found that ingestion of 10^7-10^{10} Ka- cells has no effect whereas 10^5-10^7 Ka+ cells produce illness. A number of other virulence factors have been described but have been less intensively studied.

V. vulnificus is a highly invasive organism that causes a primary septicaemia with a high fatality rate (≈50%). Most of the cases identified occurred in people with preexisting liver disease, diabetes or alcoholism. Otherwise healthy individuals are rarely affected and, when they are, illness is usually confined to gastroenteritis.

In foodborne cases, the symptoms of malaise followed by fever, chills and prostration appear 16-48 h after consumption of the contaminated food, usually seafood's, particularly oysters. Unlike other *vibrio* infections, *V. vulnificus* infections require treatment with antibiotics such as tetracycline.

Vibrio's Association with Foods:

Cholera is regarded primarily as a waterborne infection, though food which has been in contact with contaminated water can often serve as the vehicle. Consequently a large number of different foods have been implicated in outbreaks particularly products such as washed fruits and vegetables which are consumed without cooking.

Foods coming from a contaminated environment may also carry the organism, for example sea-foods and frog's legs. In the current pandemic in South and Central America, an uncooked fish marinade in lime or lemon juice, ceviche has been associated with some cases.

V. parahaemolyticus food poisoning is invariably associated with fish and shellfish. Occasional outbreaks have been reported in the United States and Europe, but in Japan it is the commonest cause of food poisoning. This has been linked with the national

culinary habit of consuming raw or partially cooked fish, although illness can also result from cross-contamination of cooked products in the kitchen.

Though the organism is only likely to be part of the natural flora of fish caught in coastal waters during the warmer months, it can readily spread to deep-water species through contact in the fish market and it will multiply rapidly if the product is inadequately chilled.

Treatment

The rapid loss of fluid from the bowel can, if untreated, lead to death—sometimes within hours—in more than 50 percent of those stricken. However, with proper modern treatment, mortality can essentially be prevented, with rates kept to less than 1 percent of those requiring therapy. This treatment consists largely of replacing lost fluid and salts with the oral or intravenous administration of an alkaline solution of sodium chloride. For oral rehydration the solution is made by using oral rehydration salts (ORS)—a measured mixture of glucose, sodium chloride, potassium chloride, and trisodium citrate. The mixture can be prepackaged and administered by nonmedical personnel, allowing cholera to be treated even under the most adverse conditions. ORS can generally be used to treat all but the most severely dehydrated patients, who require intravenous rehydration.

The administration of antibiotics such as tetracycline during the first day of treatment usually shortens the period of diarrhea and decreases the amount of fluid replacement required. It is also important for patients to resume eating as soon as they are able in order to avoid malnutrition or to prevent existing malnutrition from becoming worse.

Vaccines have been developed against cholera, but they have not been considered effective for the prevention of cholera in large populations or during epidemics. Both the CDC and the World Health Organization have specific guidelines for who should be given this vaccine.

Prevention

A safe and clean supply of water is the key to cholera prevention. Adequate chlorination of public water supplies and, in some cases, the distribution of chlorine tablets to households with instructions for their proper use is often effective measures.

If chemical disinfection is not possible, people can be instructed to boil water before drinking it. Be sure to use bottled, boiled, or chemically disinfected water for the following purposes

- Drinking
- Preparing food or drinks
- Making ice
- Brushing your teeth
- Washing your face and hands

- Washing dishes and utensils that you use to eat or prepare food
- Washing fruits and vegetables

To disinfect your own water, boil it for one minute (or 3 minutes at higher elevations) or filter it and use a commercial chemical disinfectant. You should also avoid raw foods, including the following:

- Unpeeled fruits and vegetables
- Unpasteurized milk and milk products
- Raw or undercooked meat or shellfish
- Fish caught in tropical reefs, which may be contaminated

If you develop severe, watery diarrhea and vomiting -- particularly after eating raw shellfish or traveling to a country where cholera is epidemic -- seek medical help immediately. Cholera is highly treatable, but because dehydration can happen quickly, it's important to get cholera treatment right away.

Probable question:

1. What is pulmonary tuberculosis?
2. What is Miliary Tuberculosis?
3. Name the causative agent of tuberculosis? Write down the symptoms of tuberculosis.
4. Write short notes on tuberculin.
5. Discuss the mode of transmission of Tuberculosis.
6. Write down the diagnostic processes of tuberculosis disease.
7. Describe the reasons of cholera outbreak during pandemic.
8. Write short notes on Cholera toxin.
9. Describe the Pathogenesis and Clinical Features of Vibrio.
10. Discuss elaborately about the prevention technique of cholera.

Suggested readings/ references:

1. Chandler, A. C. and Read. C. P. (1961). *Introduction to Parasitology*, 10th ed. John Wiley and Sons Inc.
2. Cheng, T. C. (1986). *General Parasitology*. 2nd ed. Academic Press, Inc. Orlando.U.S.A.
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UNIT VIII

Mode of transmission, pathogenicity and prevention of tetanus, rabies and dengue

Objective:

In this unit, we will discuss about mode of transmission, pathogenicity and prevention of tetanus, rabies and dengue.

A. Tetanus:

Tetanus, also called **lockjaw**, is a serious infection caused by *Clostridium tetani*. This bacterium produces a toxin that affects the brain and nervous system, leading to stiffness in the muscles. If *Clostridium tetani* spores are deposited in a wound, the neurotoxin interferes with nerves that control muscle movement.

The infection can cause severe muscle spasms, serious breathing difficulties, and can ultimately be fatal. Although tetanus treatment exists, it is not uniformly effective. The best way to protect against tetanus is to take the vaccine.

- Tetanus is caused by the *Clostridium tetani* bacterium.
- The early symptoms of tetanus include diarrhoea, fever, and headache.
- Earlier diagnosis predicts better outcomes.

Tetanus is a serious bacterial infection. The bacteria exist in soil, manure, and other environmental agents. A person who experiences a puncture wound with a contaminated object can develop the infection, which can affect the whole body. It can be fatal. Tetanus is a medical emergency. It will need aggressive wound treatment and antibiotics.

In the United States, there are about 30 cases a year. These are mostly people who have not been vaccinated against tetanus or who have not kept up their booster shots every 10 years.

- **Causes**

Tetanus is caused by the *Clostridium tetani* bacterium. *Clostridium tetani* spores are able to survive for a long time outside of the body. They are most commonly found in animal manure and contaminated soil, but may exist virtually anywhere.

When *Clostridium tetani* enter the body, they multiply rapidly and release tetanospasmin, a neurotoxin. When tetanospasmin enters the bloodstream, it rapidly spreads around the body, causing tetanus symptoms. Tetanospasmin interferes with the signals travelling from the brain to the nerves in the spinal cord, and then on to the muscles, causing muscle spasms and stiffness.

Clostridium tetani enters the body mainly through skin cut or puncture wounds. Thoroughly cleaning any cut helps prevent an infection from developing.

Common ways of contracting tetanus include:

- ♦ wounds that have been contaminated with saliva or faeces
- ♦ burns
- ♦ crush injuries
- ♦ wounds that include dead tissue
- ♦ puncture wounds

Rare ways of contracting tetanus include:

- surgical procedures
- superficial wounds
- insect bites
- compound fractures
- intravenous drug use
- injections into the muscle
- dental infections

- **Mode of Transmission**

Tetanus is spread by the direct transfer of C. tetani spores from soil and excreta of animals and humans to wounds and cuts. It is not transmitted from person to person.

- **Symptoms**

Tetanus symptoms usually emerge about 7 to 10 days after initial infection. However, this can vary from 4 days to about 3 weeks, and may, in some cases, may take months.

In general, the further the injury site is from the central nervous system, the longer the incubation period. Patients with shorter incubation times tend to have more severe symptoms.

i. Muscle symptoms include spasms and stiffness. Stiffness usually starts with the chewing muscles, hence the name lockjaw.

ii. Muscle spasms then spread to the neck and throat, causing difficulties with swallowing. Patients oftenhave spasms in their facial muscles.

iii. Breathing difficulties may result from neck and chest muscle stiffness. In some people, abdominal andlimb muscles are also affected.

iv. In severe cases, the spine will arch backward as the back muscles become affected. This is more common when children experience a tetanus infection.

v. Most individuals with tetanus will also have the following symptoms:

- ✓ bloody stools
- ✓ diarrhoea
- ✓ fever

- ✓ headache
- ✓ sensitivity to touch
- ✓ sore throat
- ✓ sweating
- ✓ rapid heartbeat

- **Treatment**

Wounds should be thoroughly cleaned to prevent infection.

Any cut or wound must be thoroughly cleaned to prevent infection. A tetanus-prone wound should betreated by a medical professional immediately.

A wound likely to develop tetanus is defined as:

- ♦ a wound or burn that requires surgical intervention that is delayed for over 6 hours
- ♦ a wound or burn that has a considerable amount of removed tissue
- ♦ any puncture-type injury that has been in contact with manure or soil
- ♦ serious fractures where the bone is exposed to infection, such as compound fractures
- ♦ wounds or burns in patients with systemic sepsis
- ♦ Any patient with a wound listed above should receive tetanus immunoglobulin (TIG) as soon as possible, even if they have been vaccinated. Tetanus immunoglobulin contains antibodies that kill *Clostridium tetani*. It is injected into a vein and provides immediate short-term protection against tetanus.

TIG is just short-term and does not replace the long-term effects of vaccination. Experts say that TIG injections can be safely administered to pregnant and breastfeeding mothers.

Doctors may prescribe penicillin or metronidazole for tetanus treatment. These antibiotics prevent the bacterium from multiplying and producing the neurotoxin that causes muscle spasms and stiffness.

Patients who are allergic to penicillin or metronidazole may be given tetracycline instead.In treating muscle spasms and stiffness, patients may be prescribed:

- Anticonvulsants, such as diazepam (Valium), relax the muscles to prevent spasms,reduce anxiety, and work as a sedative.
- Muscle relaxants, such as baclofen, suppress nerve signals from the brain to the spinalcord, resulting in less muscle tension.
- Neuromuscular blocking agents block the signals from nerves to muscle fibers and are useful in controlling muscle spasms. They include pancuronium and vecuronium.

- **Surgery**

If the doctor thinks the tetanus prone wound is very large, they may surgically remove as much of the damaged and infected muscle as possible (debridement). Debridement is the act of removing dead or contaminated tissue, or foreign material. In the case of a tetanus-prone wound, the foreign material may be dirt or manure.

- **Nutrition**

A patient with tetanus requires a high daily calorie intake because of increased muscle activity.

- **Ventilator**

Some patients may need ventilator support to help with breathing if their vocal cords or respiratory muscles are affected.

- **Prevention**

Most cases of tetanus occur in people who have never had the vaccine or who did not have a booster shot within the previous decade.

- **Vaccination**

The tetanus vaccine is routinely given to children as part of the diphtheria and tetanus toxoids and acellular pertussis (DTaP) shot. The DTaP vaccine consists of five shots, usually given in the arm or thigh of children when they are aged:

- ✓ 2 months
- ✓ 4 months
- ✓ 6 months
- ✓ 15 to 18 months
- ✓ 4 to 6 years
- ✓ A booster is normally given between the ages of 11 and 18 years, and then another booster every 10 years. If an individual is travelling to an area where tetanus is common, they should check with a doctor regarding vaccinations.

B. Rabies:

Rabies is a viral infection that mainly spreads through a bite from an infected animal. It is an RNAvirus of the rhabdovirus family. Without early treatment, it is usually fatal.

The virus can affect the body in one of two ways:

- It enters the peripheral nervous system (PNS) directly and migrates to the brain.
- It replicates within muscle tissue, where it is safe from the host's immune system. From here, it entersthe nervous system through the neuromuscular junctions.

Once inside the nervous system, the virus produces acute inflammation of the brain. Coma and deathsoon follow.

There are two types of rabies.

I. **Furious, or encephalitic rabies:** This occurs in 80 percent of human cases. The person ismore likely to experience hyperactivity and hydrophobia.

II. **Paralytic or "dumb" rabies:** Paralysis is a dominant symptom.

- **Transmission:**

Rabies is most common in countries where stray dogs are present in large numbers, especially in Asia and Africa. It is passed on through saliva. Rabies can develop if a person receives a bite from an infected animal, or if saliva from an infected animal gets into an open wound or through a mucous membrane, such as the eyes or mouth. It cannot pass through unbroken skin.

In the U.S., raccoons, coyotes, bats, skunks, and foxes are the animals most likely to spread the virus. Bats carrying rabies have been found in all 48 states that border with each other.

Any mammal can harbour and transmit the virus, but smaller mammals, such as rodents, rarely become infected or transmit rabies. Rabbits are unlikely to spread rabies.

- **Symptoms:**

Rabies progresses in five distinct stages:

- ✓ incubation
- ✓ prodrome
- ✓ acute neurologic period
- ✓ coma
- ✓ death

Incubation period:

This is the time before symptoms appear. It usually lasts from 3 to 12 weeks, but it can take as little as5 days or more than 2 years.

The closer the bite is to the brain, the sooner the effects are likely to appear.

By the time symptoms appear, rabies is usually fatal. Anyone who may have been exposed to thevirus should seek medical help at once, without waiting for symptoms.

Prodrome:

Early, flu-like symptoms include:

- a fever of 100.4 degrees Fahrenheit (38 degrees Celsius) or above
- headache

- anxiety
- feeling generally unwell
- sore throat and a cough
- nausea and vomiting
- Discomfort may occur at the site of the bite. These can last from 2 to 10 days, and they worsen over time.

Acute neurologic period:

Neurologic symptoms develop, including:

- confusion and aggression
- partial paralysis, involuntary muscle twitching, and rigid neck muscles
- convulsions
- hyperventilation and difficulty breathing
- hypersalivation or producing a lot of saliva, and possibly frothing at the mouth
- fear of water, or hydrophobia, due to difficulty swallowing
- hallucinations, nightmares, and insomnia
- priapism, or permanent erection, in males
- photophobia, or a fear of light
- Toward the end of this phase, breathing becomes rapid and inconsistent.

Coma and death:

If the person enters a coma, death will occur within a matter of hours, unless they are attached to aventilator.

Rarely, a person may recover at this late stage.

- **Why does rabies cause a fear of water?**

Rabies used to be known as hydrophobia because it appears to cause a fear of water. Intense spasms in the throat are triggered when trying to swallow. Even the thought of swallowing water can cause spasms. This is where the fear comes from.

The excess saliva that occurs is probably due to the impact of the virus on the nervous system. If the individual could swallow saliva easily, this would reduce the risk of spreading the virus to anew host.

- **Diagnosis:**

At the time of a bite, there is usually no way to tell for sure whether an animal is rabid, or whether it has passed on an infection.

Lab tests may show antibodies, but these may not appear until later in the development of the disease. The virus may be isolated from saliva or through a skin biopsy. However, by the time a diagnosis is confirmed, it may be too late to take action.

For this reason, the patient will normally start a course of prophylactic treatment at once, without waiting for a confirmed diagnosis.

If a person develops symptoms of viral encephalitis following an animal bite, they should be treated as if they may have rabies.

- **Treatment**

If a person is bitten or scratched by an animal that may have rabies, or if the animal licks an open wound, the individual should immediately wash any bites and scratches for 15 minutes with soapy water, povidone iodine, or detergent. This might minimize the number of viral particles.

Then they must seek medical help at once.

After exposure and before symptoms begin, a series of shots can prevent the virus from thriving. This is usually effective.

Strategies include:

A fast-acting dose of rabies immune globulin: Delivered as soon as possible, close to the bitewound, this can prevent the virus from infecting the individual.

A series of rabies vaccines: These will be injected into the arm over the next 2 to 4 weeks. These willtrain the body to fight the virus whenever it finds it.

It is not usually possible to find out whether the animal has rabies or not. It is safest to assume theworst and begin the course of shots.

A small number of people have survived rabies, but most cases are fatal once the symptoms develop.There is no effective treatment at this stage.

A person with symptoms should be made as comfortable as possible. They may need breathingassistance.

- **Prevention:**

Rabies is a serious disease, but individuals and governments can and do take action to control andprevent, and, in some cases, wipe it out completely.

Strategies include:

- ✓ regular antirabies vaccinations for all pets and domestic animals
- ✓ bans or restrictions on the import of animals from some countries
- ✓ widespread vaccinations of humans in some areas

- ✓ educational information and awareness

In rural Canada and the U.S., agencies have dropped baits containing an oral vaccine to reduce the number of wild raccoons with rabies.

In Switzerland, the authorities distributed vaccine-laced chicken heads throughout the Swiss Alps. The foxes immunized themselves by consuming the vaccine, and the country is now almost free of rabies.

Individual precautions

Individuals should follow some safety rules to reduce the chance of contracting rabies.

- ♦ Vaccinate pets: Find out how often you need to vaccinate your cat, dog, ferret, andother domestic or farm animals, and keep up the vaccinations.
- ♦ Protect small pets: Some pets cannot be vaccinated, so they should be kept in a cage orinside the house to prevent contact with wild predators.
- ♦ Keep pets confined: Pets should be safely confined when at home, and supervised whenoutside.
- ♦ Report strays to the local authorities: Contact local animal control officials or policedepartments if you see animals roaming
- ♦ Do not approach wild animals: Animals with rabies are likely to be less cautious thanusual, and they may be more likely to approach people.
- ♦ Keep bats out of the home: Seal your home to prevent bats from nesting. Call an expertto remove any bats that are already present.

The World Health Organization (WHO) calls rabies a "100-percent vaccine-preventable disease." They note that at least 70 percent of dogs in an area must be vaccinated to break the cycle of transmission.

In the U.S., vaccinations control rabies in domestic dogs. Nevertheless, between 30,000 and 60,000 people seek rabies postexposure prophylaxis every year, following contact with suspect animals. Hundreds of thousands of animals undergo tests and observation.

Between 60 and 70 dogs and around 250 cats are reported rabid each year in the U.S. Most of these have not been vaccinated, and they were exposed to the virus through wild animals, such as bats.

- **Travelling:**

The prevalence of rabies varies widely in different countries. In nations without a feral dog population, the rates are significantly lower.

Rabies is present in 150 countries and in all continents except Antarctica and the Arctic. Islands such as New Zealand, Australia, Mauritius and the Seychelles, are helped by their natural isolation.

Africa and Asia are the continents where rabies is most common. India has the highest

number of cases.

In recent years, the prevalence of rabies in South America and the Caribbean has fallen significantly, due to rabies control programs. Official figures show that in 1990 there were 250 cases, but by 2010, there were fewer than 10.

Anyone who is travelling to an area where rabies is prevalent, or who is participating in activities where they are likely to come into contact with wild animals that may have rabies, such as caving or camping, should ask their doctor about vaccinations.

C. Dengue:

Dengue is a mosquito-borne viral disease that has rapidly spread to all regions of WHO in recent years. Dengue virus is transmitted by female mosquitoes mainly of the species *Aedes aegypti* and, to a lesser extent, *Ae. albopictus*. These mosquitoes are also vectors of chikungunya, yellow fever and Zika viruses. Dengue is widespread throughout the tropics, with local variations in risk influenced by climate parameters as well as social and environmental factors.

Dengue is caused by a virus of the Flaviviridae family and there are four distinct, but closely related, serotypes of the virus that cause dengue (DENV-1, DENV-2, DENV-3 and DENV-4). Recovery from infection is believed to provide lifelong immunity against that serotype. However, cross-immunity to the other serotypes after recovery is only partial, and temporary. Subsequent infections (secondary infection) by other serotypes increase the risk of developing severe dengue.

Dengue has distinct epidemiological patterns, associated with the four serotypes of the virus. These can co-circulate within a region, and indeed many countries are hyper-endemic for all four serotypes. Dengue has an alarming impact on both human health and the global and national economies. DENV is frequently transported from one place to another by infected travellers; when susceptible vectors are present in these new areas, there is the potential for local transmission to be established.

Global burden

The incidence of dengue has grown dramatically around the world in recent decades. The number of dengue cases reported to WHO increased over 8 fold over the last two decades, from 505,430 cases in 2000, to over 2.4 million in 2010, and 5.2 million in 2019. The total number of cases seemingly decreased during years 2020 and 2021, as well as for reported deaths. However, the data is not yet complete and COVID-19 pandemic might have also hampered case reporting in several countries.

Mode of Transmission

- ✓ **Transmission through mosquito bite**

The virus is transmitted to humans through the bites of infected female mosquitoes, primarily the *Aedes aegypti* mosquito. Other species within the Aedes genus can also act as vectors, but their contribution is secondary to *Aedes aegypti*.

After feeding on an DENV-infected person, the virus replicates in the mosquito midgut, before it disseminates to secondary tissues, including the salivary glands. The time it takes from ingesting the virus to actual transmission to a new host is termed the extrinsic incubation period (EIP). The EIP takes about 8-12 days when the ambient temperature is between 25-28°C. Variations in the extrinsic incubation period are not only influenced by ambient temperature; a number of factors such as the magnitude of daily temperature fluctuations, virus genotype, and initial viral concentration can also alter the time it takes for a mosquito to transmit virus. Once infectious, the mosquito is capable of transmitting virus for the rest of its life.

- ✓ **Human-to-mosquito transmission**

Mosquitoes can become infected from people who are viremic with DENV. This can be someone who has a symptomatic dengue infection, someone who is yet to have a symptomatic infection (they are pre-symptomatic), but also people who show no signs of illness as well (they are asymptomatic).

Human-to-mosquito transmission can occur up to 2 days before someone shows symptoms of the illness, up to 2 days after the fever has resolved,

Risk of mosquito infection is positively associated with high viremia and high fever in the patient; conversely, high levels of DENV-specific antibodies are associated with a decreased risk of mosquito infection (Nguyen et al. 2013 PNAS). Most people are viremic for about 4-5 days, but viremia can last as long as 12 days.

- ✓ **Maternal transmission**

The primary mode of transmission of DENV between humans involves mosquito vectors. There is evidence however, of the possibility of maternal transmission (from a pregnant mother to her baby). While vertical transmission rates appear low, with the risk of vertical transmission seemingly linked to the timing of the dengue infection during the pregnancy. When a mother does have a DENV infection when she is pregnant, babies may suffer from pre-term birth, low birth weight, and fetal distress.

- ✓ **Other transmission modes**

Rare cases of transmission via blood products, organ donation and transfusions have been recorded. Similarly, transovarial transmissions of the virus within mosquitoes have also been recorded.

Signs and symptoms

While majority of dengue cases are asymptomatic or show mild symptoms, it can manifest as a severe, flu-like illness that affects infants, young children and adults, but seldom causes death. Symptoms usually last for 2–7 days, after an incubation period of 4–10 days after the bite from an infected mosquito [25]. The World Health Organization classifies dengue into 2 major categories: dengue (with / without warning signs) and severe dengue.

Dengue

Dengue should be suspected when a high fever (40°C/104°F) is accompanied by 2 of the following symptoms during the febrile phase (2-7 days):

- severe headache
- pain behind the eyes
- muscle and joint pains
- nausea
- vomiting
- swollen glands
- rash.

Severe dengue

A patient enters what is called the critical phase normally about 3-7 days after illness onset. During the 24-48 hours of critical phase, a small portion of patients may manifest sudden deterioration of symptoms. It is at this time, when the fever is dropping (below 38°C/100°F) in the patient, that warning signs associated with severe dengue can manifest. Severe dengue is a potentially fatal complication, due to plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment.

Warning signs that doctors should look for include:

- severe abdominal pain
- persistent vomiting
- rapid breathing
- bleeding gums or nose
- fatigue

- restlessness
- liver enlargement
- blood in vomit or stool.

If patients manifest these symptoms during the critical phase, close observation for the next 24–48 hours is essential so that proper medical care can be provided, to avoid complications and risk of death. Close monitoring should also continue during the convalescent phase.

Diagnostics

Several methods can be used for diagnosis of DENV infection. Depending on the time of patient presentation, the application of different diagnostic methods may be more or less appropriate. Patient samples collected during the first week of illness should be tested by both methods mentioned below:

Virus isolation methods

The virus may be isolated from the blood during the first few days of infection. Various reverse transcriptase–polymerase chain reaction (RT–PCR) methods are available and are considered the gold standard. However, they require specialised equipment and training for staff to perform these tests.

The virus may also be detected by testing for a virus-produced protein, called NS1. There are commercially-produced rapid diagnostic tests available for this, and it takes only ~20 mins to determine the result, and the test does not require specialized laboratory techniques or equipment.

Serological methods

Serological methods, such as enzyme-linked immunosorbent assays (ELISA), may confirm the presence of a recent or past infection, with the detection of anti-dengue antibodies. IgM antibodies are detectable ~1 week after infection and remain detectable for about 3 months. The presence of IgM is indicative of a recent DENV infection. IgG antibody levels take longer to develop and remains in the body for years. The presence of IgG is indicative of a past infection.

Treatment

There is no specific treatment for dengue fever. Patients should rest, stay hydrated and seek medical advice. Depending on the clinical manifestations and other circumstances, patients may be sent home, be referred for in-hospital management, or require emergency treatment and urgent referral[25].

Supportive care such as fever reducers and pain killers can be taken to control the symptoms of muscle aches and pains, and fever.

- The best options to treat these symptoms are acetaminophen or paracetamol.
- NSAIDs (non-steroidal anti-inflammatory drugs), such as ibuprofen and aspirin should be avoided. These anti-inflammatory drugs act by thinning the blood, and in a disease with risk of hemorrhage, blood thinners may exacerbate the prognosis.

For severe dengue, medical care by physicians and nurses experienced with the effects and progression of the disease can save lives – decreasing mortality rates to less than 1% in majority of the countries.

Prevention and control

If you know you have dengue, avoid getting further mosquito bites during the first week of illness. Virus may be circulating in the blood during this time, and therefore you may transmit the virus to new uninfected mosquitoes, who may in turn infect other people.

The proximity of mosquito vector breeding sites to human habitation is a significant risk factor for dengue. At present, the main method to control or prevent the transmission of dengue virus is to combat the mosquito vectors. This is achieved through:

- Prevention of mosquito breeding:
    - Preventing mosquitoes from accessing egg-laying habitats by environmental management and modification;
    - Disposing of solid waste properly and removing artificial man-made habitats that can hold water;
    - Covering, emptying and cleaning of domestic water storage containers on a weekly basis;
    - Applying appropriate insecticides to water storage outdoor containers;

- Personal protection from mosquito bites:
    - Using of personal household protection measures, such as window screens, repellents, coils and vaporizers. These measures must be observed during the day both inside and outside of the home (e.g.: at work/school) because the primary mosquito vectors bites throughout the day;
    - Wearing clothing that minimises skin exposure to mosquitoes is advised;

- Community engagement:
    - Educating the community on the risks of mosquito-borne diseases;

    - Engaging with the community to improve participation and mobilization for sustained vector control;

- Active mosquito and virus surveillance:
    - Active monitoring and surveillance of vector abundance and species composition should be carried out to determine effectiveness of control interventions;
    - Prospectively monitor prevalence of virus in the mosquito population, with active screening of sentinel mosquito collections;
    - Vector surveillance can be combined with clinical and environment surveillance.

In addition, there is ongoing research amongst many groups of international collaborators in search of novel tools and innovative strategies that will contribute in global efforts to interrupt transmission of dengue. The integration of vector management approaches is encouraged by WHO to achieve sustainable, effective locally adapted vector control interventions.

Probable questions:

1. What is lockjaw? What is the causative agent of tetanus?
2. Mention the symptoms of tetanus? How tetanus can be treated?
3. How rabies can be transmitted?
4. Explain the different stages of progression of rabies.
5. What are the preventive measures to control rabies?
6. Discuss the mode of transmission of Dengue
7. Write down the dialogistic techniques of Dengue fever
8. How transmission of Dengue can be controlled?

Suggested readings/ references:

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2. http://www.biologydiscussion.com/human-diseases/clostridium-tetani-properties-prevention-and-treatment-with-diagram/30908
3. https://www.medicalnewstoday.com/articles/181980.php
4. https://www.cdc.gov/vaccines/hcp/vis/vis-statements/rabies.html

UNIT IX

Life cycle, medical importance and control of disease-causing vectors: *Anopheles* sp., *Culex* sp., *Aedes* sp.

Objective:

In this unit you will know about Life cycle, medical importance and control of disease-causing vector**s:** *Anopheles* sp., *Culex* sp., *Aedes sp.*

Introduction

Anopheles is a genus of mosquito first described and named by J. W. Meigen in 1818. The *Anopheles* mosquito is known universally as the Malaria Mosquito species because it is considered the primary vector of the disease. It is also considered a transmitter of heartworm in dogs. About 460 species are recognised; while over 100 can transmit human malaria, only 30–40 commonly transmit parasites of the genus *Plasmodium*, which cause malaria in humans in endemic areas. When resting, the stomach area of the Anopheles mosquito points upward, rather than being even with the surrounding surface like most mosquitoes.*Anopheles gambiae* is one of the best known, because of its predominant role in the transmission of the most dangerous malaria parasite species (to humans) – *Plasmodium falciparum.*

The life cycle of *Anopheles*sp.

Life cycle of *Anopheles* mosquito is discussed below

Copulation:

Mosquitoes copulate while flying during the night. It is believed that the pitch of sound produced during flight is higher in females, and this helps the male mosquitoes to locate the female mosquitoes and copulate. The female Anopheles mosquito will mate several times in her short lifespan, producing eggs after she has found a blood meal. Although she only lives a few weeks to a month at most, she will have been able to produce thousands of eggs during that time.

Oviposition:

After copulation the female Anopheles lays about 40 to 100 after midnight in standing water of some pond, ditch, pool, puddle, lake, well, water-storage tanks etc., or even in water containers in our houses. A blood-meal by the female is necessary before oviposition. Female *Anopheles* lays its eggs singly.

Like all mosquitoes, anophelines go through four stages in their life cycles: egg, larva, pupa, and imago. The first three stages are aquatic and together last 5–14 days, depending on the species and the ambient temperature. The adult stage is when the female *Anopheles* mosquito acts as malaria vector. The adult females can live

up to a month (or more in captivity), but most probably do not live more than two weeks in nature.

The life cycle of *Anopheles* has following four stages; egg, larva, pupa and adult (Fig 1).The first three stages are aquatic and last 5-14 days, depending on the species and the ambient temperature. The adult stage is when the female *Anopheles* mosquito acts as malaria vector. The adult females can live up to a month (or more in captivity) but most probably do not live more than 1-2 weeks in nature.

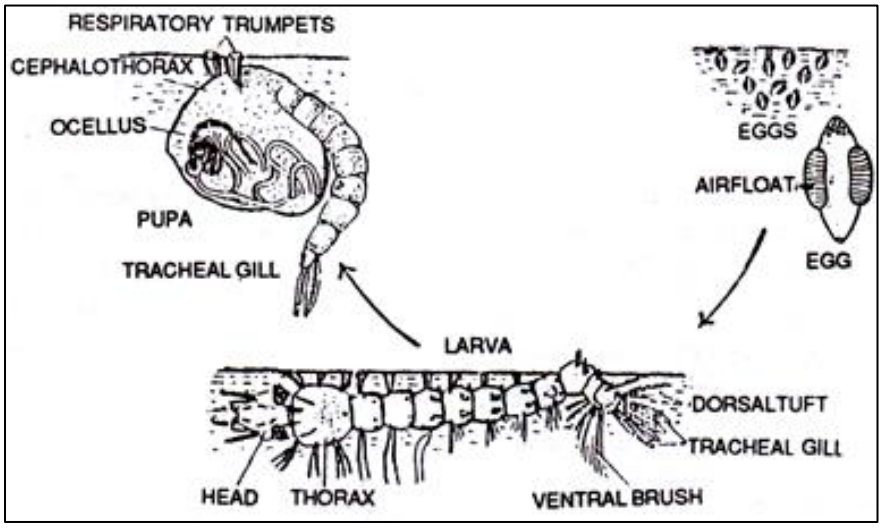


Fig 1: Life cycle of *Anopheles* sp.

The Eggs:

1. The eggs are whitish and boat-shaped.

2. 50 to 80 eggs are laid on water-surface singly and are floating horizontally through air floats.

3. In 24 hrs. they hatch into larvae.

The Larva:

1. The larvae are free swimming.

2. They lie horizontally parallel to the surface of water.

3. Their body is elongated, somewhat cylindrical and is divisible into head, thorax and abdomen.

4. The head bears paired antennae, eyes, feeding brushes and mandibulate mouth parts.

5. Thorax is broad un-segmented and bears tufts of hair for swimming in water.

6. Abdomen is long, 9 segmented and is having palmate hairs.

7. The 8th segment bears a small siphon and paired spiracles on the dorsal surface.

8. Ninth segment bears two pairs of tracheal gills and two tufts.

9. It hatches out into pupa after 4 moultings.

The Pupa:

1. The body of pupa is comma shaped (,) and is covered over with a transparent puparium.

2. In pupa the head and thorax are united into cephalothorax which has become greatly distended and bears paired respiratory siphons, compound eyes, jointed antennae and rudiments of other appendages.

3. The abdomen is long, 9 segmented, curved and flexed completely below cephalothorax and bears palmate hairs and a pair of paddles attached to the 8th segment for swimming.

4. It metamorphoses into adult after a short- free swimming life.

Adult:

The duration from egg to adult varies considerably among species, and is strongly influenced by ambient temperature. Mosquitoes can develop from egg to adult in as little as five days, but it can take 10–14 days in tropical conditions. Like all mosquitoes, adult *Anopheles* species have slender bodies with three sections: head, thorax and abdomen.

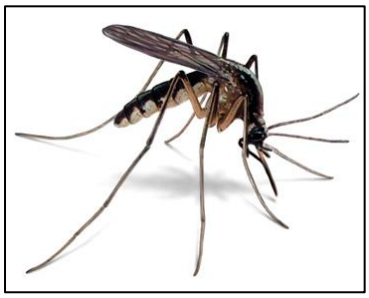


Fig: *Anopheles* sp.

The *head* is specialized for acquiring sensory information and for feeding. It contains the eyes and a pair of long, many-segmented antennae. The antennae are important for detecting host odours, as well as odours of breeding sites where females lay eggs. The head also has an elongated, forward-projecting proboscis used for feeding, and two maxillary palps. These palps also carry the receptors for carbon dioxide, a major attractant for the location of the mosquito's host.

The *thorax* is specialized for locomotion. Three pairs of legs and a pair of wings are attached to the thorax.

The *abdomen* is specialized for food digestion and egg development. This segmented

body part expands considerably when a female takes a blood meal. The blood is digested over time, serving as a source of protein for the production of eggs, which gradually fill the abdomen.

Anopheles mosquitoes can be distinguished from other mosquitoes by the palps, which are as long as the proboscis, and by the presence of discrete blocks of black and white scales on the wings. Adults can also be identified by their typical resting position: males and females rest with their abdomens sticking up in the air rather than parallel to the surface on which they are resting.

Adult mosquitoes usually mate within a few days after emerging from the pupal stage. In most species, the males form large swarms, usually around dusk, and the females fly into the swarms to mate. Males live for about a week, feeding on nectar and other sources of sugar. Females will also feed on sugar sources for energy, but usually require a blood meal for the development of eggs. After obtaining a full blood meal, the female will rest for a few days while the blood is digested and eggs are developed. This process depends on the temperature, but usually takes 2–3 days in tropical conditions. Once the eggs are fully developed, the female lays them and resumes host-seeking.

Breeding Habits

The female *Anopheles* mosquito will lay her eggs in a wide range of locations. Malaria mosquito breeding grounds include fresh water or salt-water, vegetative or non-vegetative, shady or sunlit. Ground pools, small streams, irrigated lands, freshwater marshes, forest pools, and any other place with clean, slow-moving water are all considered prime Malaria mosquito breeding grounds for egg-laying.

Females, particularly fertilized females, may survive winter by hibernating in caves, which means the malaria breeding cycle can virtually last year-round in some locations. Eggs are capable of surviving cold temperatures; however, freezing usually kills eggs. Learn about the malaria mosquito bite, as well as the malaria mosquito species.

Relationship between *Anopheles* sp mosquito and malaria disease

Only certain species of mosquitoes of the *Anopheles* genus—and only females of those species—can transmit malaria. Malaria is caused by a one-celled parasite called *Plasmodium*. Female *Anopheles* mosquitoes pick up the parasite from infected people when they bite to obtain blood needed to nurture their eggs. Inside the mosquito the parasites reproduce and develop. When the mosquito bites again, the parasites contained in the salivary gland are injected and pass into the blood of the person being bitten.

Malaria parasites multiply rapidly in the liver and then in red blood cells of the infected person. One to two weeks after a person is infected the first symptoms of malaria appear: usually fever, headache, chills and vomiting. If not treated promptly with effective medicines, malaria can kill by infecting and destroying red blood cells and by clogging the capillaries that carry blood to the brain or other vital organs.

There are four types of human malaria: *Plasmodium vivax, P. malariae, P. ovale* and *P. falciparum.* are the most common forms. Falciparum malaria—the most deadly type—is most common in sub-Saharan Africa, where it causes more than 400 000 deaths a year.

In recent years, some human cases of malaria have also occurred with *Plasmodium knowlesi* – a species that causes malaria among monkeys and occurs in certain forested areas of South-East Asia.

Malarial parasite (*Plasmodium vivax*)

Plasmodium vivax is a protozoal parasite and a human pathogen. This parasite is the most frequent and widely distributed cause of recurring malaria. Although it is less virulent than *Plasmodium falciparum*, the deadliest of the five human malaria parasites, *P. vivax* malaria infections can lead to severe disease and death, often due to splenomegaly (a pathologically enlarged spleen).*P. vivax* is carried by the female *Anopheles* mosquito; the males do not bite.

In a study by the London School of Hygiene & Tropical Medicine researchers found that female mosquitoes carrying malaria parasites are significantly more attracted to human breath and odours than uninfected mosquitoes. The research team infected laboratory-raised *Anopheles gambiae* mosquitoes with Plasmodium parasites, leaving a control group uninfected. Then tests were run on the two groups to record their attraction to human smells. Female mosquitoes are particularly drawn to foot odours, and one of the tests showed infected mosquitoes landing and biting a prospective host repeatedly. The team speculates that the parasite improves the mosquitoes' sense of smell. It may also reduce its risk aversion.

Systemic Position

Kingdom: Animalia

Phylum: Protozoa

Class: Sporozoa

Order: Haemosporidia

Genus: *Plasmodium*

Species: *Vivax*

Malaria is one of the most widely known diseases since time immemorial. It is caused by a pathogenic protozoan of blood, Plasmodium.

Four species of *Plasmodium*, viz., *P. vivax, P. falciparum, P. malariae* and *P. ovale* are so far known to infect human beings causing different types of malaria. Female *Anopheles* mosquito serves as the carrier or vector hosts and transmits plasmodium from person to person. *Plasmodium* is an intracellular parasite in RBCs of man.

It is also reported from birds, reptiles and various mammals. Plasmodium is widely distributed in tropical and temperate countries the world over. Plasmodium vivax requires two hosts to complete its life cycle- a primary or definite host and a secondary

or intermediate host. Such a two host life cycle is digenetic. Intermediate host is female Anopheles. In human body the parasite multiplies asexually while in female anopheles it undergoes a sexual cycle followed by an asexual multiplication called sporogony.

Asexual cycle in man:

The normal adult or trophozoite phase of plasmodium occurs in RBCs of human beings. The parasite first invades the liver cells for asexual multiplication.

The life cycle of plasmodium in man is can be studied under the following heads:

(i) Exoerythrocytic cycle:

When an Anopheles mosquito bites a human to suck blood. Plasmodium is inoculated into human blood in the form of a minute infective stage called Sporozoites (fig 2). The injected sporozoites invade the hepatocyte cells in the liver. In the liver cell, a sporozoite actively feeds on its cytoplasm and grows into a large (about 45 in diameter) and spherical adult like form called cryptozoite.

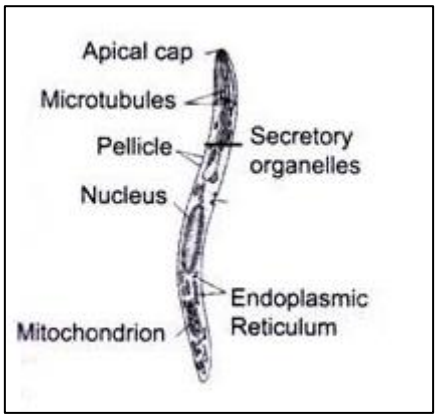


Fig 2: Sporozoite of *P. vivax*

This form multiply into thousands of cryptomerozoites by multiple fission called schizogony (exoerythrocytic schizogony). In such a multiplication repeated nuclear divisions first result into multinucleate organism, and then divides by cytoplasmic segregation around the tiny daughter nuclei. Due to the pressure of cryptomerozoites, the body of cryptozoites as well as the host liver cell ruptures liberating the cryptomerozoites into liver sinusoids. Some of these invade fresh liver cells to continue exo-erythrocytic schizogony, while others remain in blood sream and invade erythrocytes (RBC) to initiate erythrocytic cycle.

(ii) Erythrocyic cycle:

This cycle takes place in RBCs after the RBCs are invaded by cryptomeromerozoites. After invading an erythrocyte, a cryptomeromerozoite soon becomes a rounded, disc like structure called trophozoites (fig 3). As it grows, a contractile vacuole appears in its centre, pushing the cytoplasm and nucleus to a thin peripheral layer and the parasite attains a ring Ike appearance to represent the signet ring stage.

After some time, the vacuole disappears and the parasite assumes an amoeboid shape. The trophzoites actively feed upon the haemoglobin of RBCs and increases in size till the entire corpuscle gets filled with it. This forms the schizont stage and its cytoplasm contain yellowish-brown pigment granules, the haemozoin. It is formed by the decomposition of haemoglobin. The schizont undergoes asexual multiplication termed as schizogony or merogony.

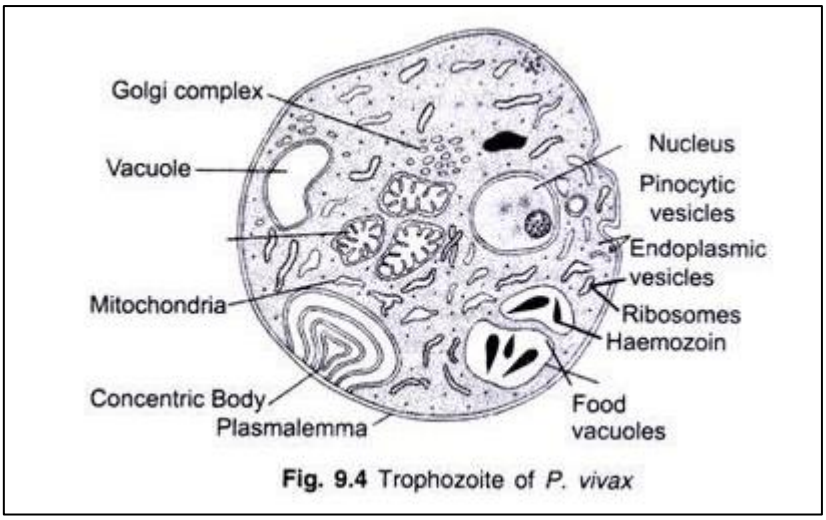


Fig. 9.4 Trophozoite of *P. vivax*

Fig 3: Trophozoite of *P. vivax*

(iii) Schizogony or merogony:

The nucleus or the schizont divides by multiple fission to from 6-24 daughter nuclei which migrate towards the periphery. After some time the totally exhausted erythrocyte bursts liberating the merozoites and the toxic waste (haemozoin granules) into the plasma of blood. These attack the fresh R.B. Cs. And repeat the erythrocytic schozogony. One erythrocytic cycle is completed within 48-72 hours.

As the parasite continues to destroy the R.B.Cs. of the host, the host becomes anemic and its toxin accumulates in the plasma. After about 5 successive erythrocytic cycles the malarial symptoms develop for the first time and the host suffers from paroxysm of chill and fever which are now repeated at the end of each schizogony. Thus the parasite passes a latent period of about 10 to 15 days since its inoculation in the body of host. This period is known as incubation period.

(iv) Formation of gametocytes:

As a result of repeated schizogony in the blood stream, the parasite becomes so potential that its existence is threatened due to lack of fresh R.B.Cs. and the resistance of the host. Consequently, the parasite prepares to enter the new host by the formation of gametocytes. Some of the meozoites, after entering the R.B.Cs. neither form trophozoites nor multiply by binary fission but grow slowly and become compact bodies, the gametocytes. These are of two types:

The more numerous, but small in size and with a large centrally placed nucleus, are the microgametocytes, potentially male. The less numerous but larger in size and with a

greater amount of dense cytoplasm and a small nucleus are the macro or mega gametocytes, potentially female. The mature gametocytes are unable to develop further in the body of primary host and can survive only for two days. They reach the superficial blood vessels and wait for the bite of female Anopheles.

Sexual Life-Cycle in *Anopheles*:

When *Anopheles* sucks the blood of a diseased man, the parasite under different stages of development enters its alimentary canal. But only the gametocytes are able to survive, while others are digested. The gametocytes are set free by the rupture of R.B.Cs. and develop further to form gametes.

(i) Development of male gametes:

The nucleus of microgametocyte divides repeatedly to form 6 to 8 haploid nuclei, as one of these divisions is a reduction division. Each nucleus is surrounded by a little of cytoplasm and metamorphoses into a male gamete. Each has a small body with a nucleus and a cytoplasmic flagellum. By the lashing movement of their flagella the male gametes swim in the stomach fluid.

(ii) Development of female gametes or microgamete's:

The nucleus of the macrogametocyte undergoes reduction divisions forming two nuclei. One of them protrudes out as a polar body and the other comes to lie in a protuberance which is known as reception cone. Thus the macrogamete is formed.

(iii) Syngamy or fertilization:

The actively moving male gamete is attracted by the macrogamete and penetrates it through the reception cone. The nuclei of the two fuses together forming the synkaryon. Syngamy is anisogamous and the zygote thus formed is inert and round.

(iv) Ookinete:

Soon the rounded zygote elongates and assumes the vermiform appearance and becomes motile. It is now known as vermicule or ookinete (fig 4). Its anterior en4 is pointed and with this it penetrates the stomach wall to come to lie in the sub-epithelial tissue underneath the outer limiting membrane. It becomes rounded, secretes a thin membranous cyst and is known as sporont or oocysty. It feeds by absorption and increases in size.

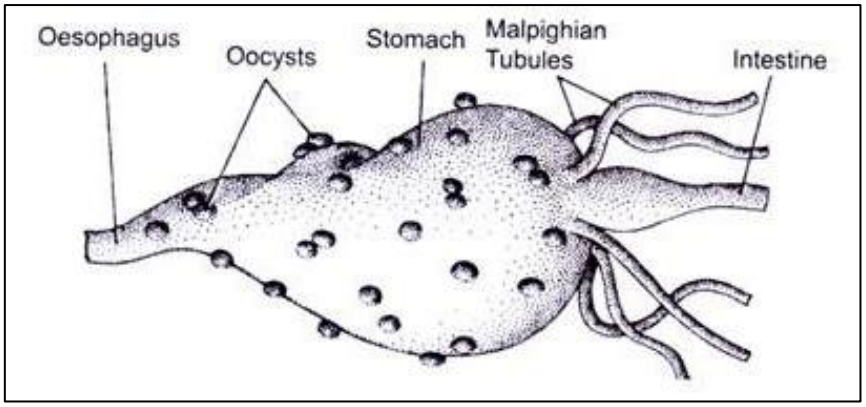


Fig 4: Oocyst in infected mosquito

Sporogony:

The nucleolus of the fully mature oocyst undergoes multiple fission by mitosis producing a large number of daughter nuclei. These get surrounded by fragments of cytoplasm. The irregular uni-nucleate bodies thus formed are known as sporoblasts. The nucleus in each sporoblast divides repeatedly by mitosis.

The nuclei form spindle-shaped sporozoites. These are liberated in the haemocoel or body cavity by the repture of cyst wall. The sporozoites now move to different body organs and also the salivary gland. With the entrance of parasite in the salivary glands the female Anopheles becomes infective and is able to inoculate the parasite into the blood-stream of healthy persons.

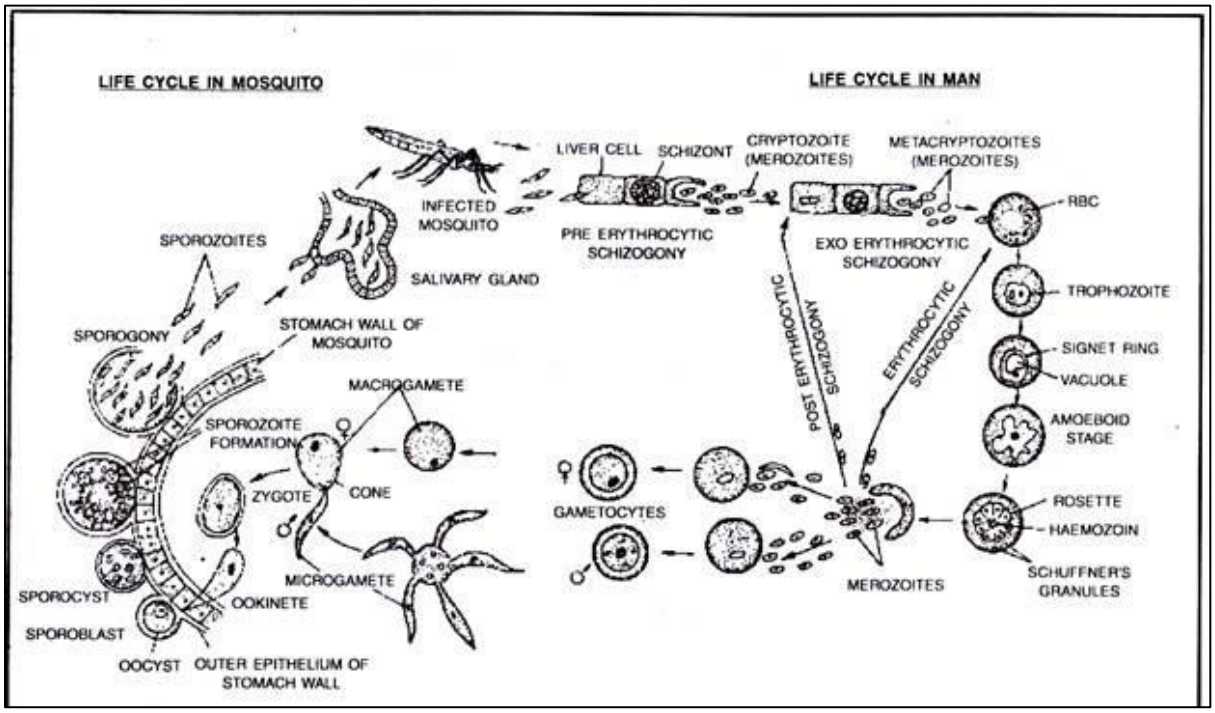


Fig : Life cycle of *Plasmodium* sp.

Mode of transmission of malaria

The female anopheles mosquito is the vector for human malaria. Some 60 species of this mosquito have been identified as vectors for malaria, and their distribution varies from country to country.

The infection is transmitted by the bite of an infected female mosquito – Anopheles. *An. culicifecies* in Rural area &*An. stephensi* in urban area. The mosquito usually bites during dawn & dust time. The mosquito becomes infected by biting a patient with malaria infection. When a mosquito bites an infected individual, it sucks the gametocytes, the sexual forms of the parasite, along with blood. These gametocytes continue the sexual phase of the cycle and the sporozoites fill the salivary glands of the infested mosquito. Once the mosquito becomes infected, it remains so far life. The female mosquitoes can survive upto 4 weeks under normal temperature i.e. 28°C to 30°C and humidity i.e. 60 to 80%. When this female mosquito bites the man for a blood meal, which it needs to nourish its eggs, it inoculates the sporozoites into human blood stream, thus spreading the infection.

Other modes of transmission

Rarely malaria can spread by the inoculation of blood from an infected person to a healthy person. In this type of malaria, asexual forms are directly inoculated into the blood and pre–erythrocytic development of the parasite in the liver does not occur. Therefore, this type of malaria has a shorter incubation period and relapses do not occur.

1. Blood transfusion (Transfusion malaria)

- This is fairly common in endemic areas.Following an attack of malaria, the donor may remain infective for years (1–3 years in *P. falciparum*, 3–4 years in *P. vivax*, and 15–50 years in *P. malariae*).
- Most infections occur in cases of transfusion of blood stored for less than 5 days and it is rare in transfusions of blood stored for more than 2 weeks. Frozen plasma is not known to transmit malaria.
- The clinical features of transfusion malaria occur earlier and any patient who has received a transfusion three months prior to the febrile illness should be suspected to have malaria.
- Donor blood can be tested with indirect fluorescent antibody test or ELISA, and direct examination of the blood for the parasite may not be helpful.
- In endemic areas, it is safe to administer full course of chloroquine to all recipients of blood transfusion.

- In transfusion malaria, pre–erythrocytic schizogony does not occur and hence relapses due to dormant hepatic forms also does not occur. Therefore, treatment with primaquine for 5 (or14) days is not indicated.

2.Mother to the growing fetus (Congenital malaria)

Intrauterine transmission of infection from mother to child is well documented. Placenta becomes heavily infested with the parasites. Congenital malaria is more common in first pregnancy, among non– immune populations.

3. Needle stick injury

Accidental transmission can occur among drug addicts who share syringes and needles.

*Culex*sp.

Culex is a genus of mosquitoes, several species of which serve as vectors of one or more important diseases of birds, humans, and other animals. The diseases they vector include arbovirus infections such as West Nile virus, Japanese encephalitis, or St. Louis encephalitis, but also filariasis and avian malaria. They occur worldwide except for the extreme northern parts of the temperate zone, and are the most common form of mosquito encountered in some major U.S. cities, such as Los Angeles.

Habit and Habitat

Culex pipiens is found in temperate regions all over the world, and *Culex fatigans* throughout the tropics and sub-tropics. *Culex* lives in houses, in cities and farms, and is abundant also in rural areas. They are most abundant during spring, but hibernate during un-favorable climatic conditions, the adults hide in hollows of trees, caves, crevices, barns, etc.

The life span of male mosquitoes is seldom more than three weeks, they die after fertilizing the females. The females live from four weeks to several months, but they die when all their eggs are laid. *Culex* has several generations in a year.

Life Cycle of *Culex* sp.

The life cycle of *Culex*is discussed here with the help of a diagram. The Life Cycle of *Culex* has following stages: egg, larva, pupa and adult (Fig 5)

The Eggs:

1. They are laid in rafts of about 300 eggs held, together through sticky substance for floating along the surface of water.

2. The eggs are brownish in colour and somewhat oval in shape.

3. They contain air bubbles trapped in the sticky substance to provide buoyancy.

4. They hatch after 24-48 hours into free swimming larvae.

The Larvae:

1. They are the first free swimming stages in the life cycle.

2. The larva lies at an angle to the surface of water in resting condition.

3. The body is elongated and somewhat cylindrical and is divisible into head, thorax and abdomen.

4. The head bears paired antennae, eyes, feeding brushes and mandibulate mouth parts.

5 Thorax is broad, un-segmented and bears tufts of hair for wriggling in water.

6. Abdomen is without palmate hairs.

7. The 8th segment bears a long tubular respiratory siphon at the tip of which lies a spiracle.

8. Ninth segment bears two pairs of tracheal gills and two tufts.

9. It hatches out into pupa after 4 moultings.

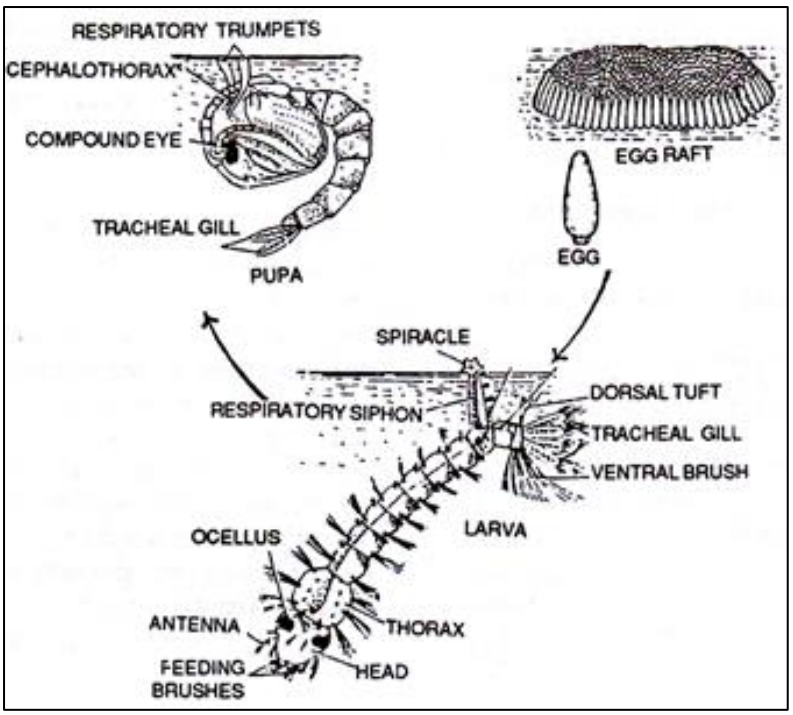


Fig 5: Life cycle of *Culex* sp.

The Pupa:

1. The pupa develops from larva after seven days.

2. The body is comma shaped (,) and is covered over with a transparent puparium.

3. In pupa the head and thorax have united into cephalothorax which has become greatly distended and bears paired short respiratory siphons, compound eyes, long and jointed antennae and rudiments of appendages.

4. The abdomen is long, 9 segmented and curved but not flexed below cephalothorax. It, however, bears a pair of paddles & a pair of tracheal gills attached to ninth segment and palmate hairs on all segments.

5. It metamorphoses into adult after a short free swimming life.

Adult

Depending on the species, the adult *Culex* mosquito may measure from 4–10 mm (0.2–0.4 in). The adult morphology is typical of flies in the suborder Nematocera with the head, thorax, and abdomen clearly defined and the two forewings held horizontally over the abdomen when at rest. As in all Diptera capable of flight, the second pair of wings is reduced and modified into tiny, inconspicuous halteres.

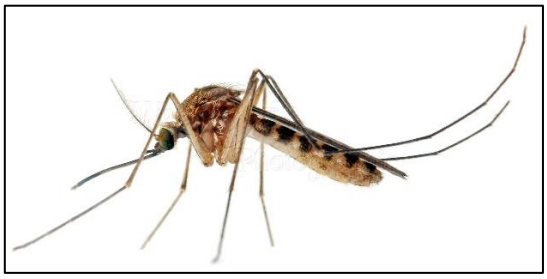


Fig: *Culex* sp.

1. Head:

Head is globular and highly mobile on a slender neck. There are two very large black compound eyes, there are no ocelli. The top of the head has an epicranium below which is a clypeus which is thick and projects in front. There are two filiform antennae, each with 15 joints, the basal segment is the scape which is concealed by a very large globular second segment. The bristles are longer and much more numerous on the antennae of males giving them a bushy appearance. In the female the antennae have rings of few, short bristles, thus, sexes can be distinguished readily by the antennae. The head bears two maxillary palps and a proboscis.

Mouth Parts:

The proboscis is a straight, long tube formed by a fleshy ventral labium which has a deep groove on its upper side, in this groove is a long pointed and ventrally-grooved labrumepipharynx. At the distal end of the labium is a pair of small tactile labella which are reduced labial palps.The groove of the labium also contains five needle-like stylets in a female Culex, they are two mandibles, two maxillae, and a hypo pharynx. The mandibles are finer than the maxillae, but both have saw-like edges on their tips. The hypo pharynx is also needle-like and has a fine salivary duct running through it and opening at the tip, through this duct saliva is poured to prevent coagulation of blood of the victim.

In the male the labrum-epipharynx and the labium are the same as in the female, but the mandibles and maxillae are very short and functionless and the hypo pharynx is fused with the labium.

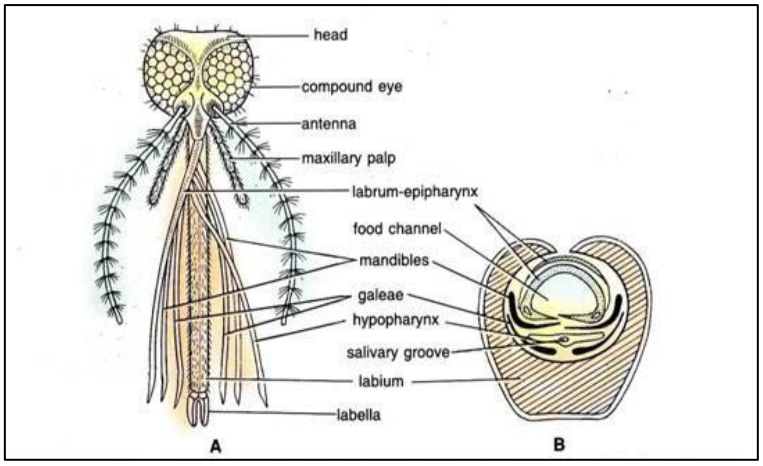


Fig 6: Culex sp. A – Diagrammatic head and mouth parts of a female; B – T.S. of mouth parts

2. Thorax

Thorax is arched, it has mesothorax which is very large and its tergum has three sclerites, a scutum, a trilobed scutellum and a post-scutellum. Prothorax and metathorax are very small. On the thorax there are two pairs of spiracles. From the mesothorax arise a pair of membranous functional wings which are long and narrow.

From the thorax arise three pairs of legs which are very long and slender, they are fragile and have the usual parts of an insect leg, but the coxae are short and tarsi long with five joints ending in a pair of simple claws, below each claw is a pad-like pulvilus. The legs also have many scales and bristles.

3. Abdomen

Abdomen consists of 10 segments of which the first is vestigial and fused to the metathorax; the second to the eighth are clearly seen, each has a pair of spiracles; the ninth and tenth segments are partly telescoped into the eighth. In the female the 10th segment is blunt and bears a pair of cerci, between them is a small post-genital plate which is part of the tenth sternum.

Filariasis

Causes:

The disease filariasis is caused by filarial worms *Wuchereria bancrofti* and *Wuchereria malayi*. They belong to the Phylum – Nemathelminthes and class- Nematoda.The adult worms are slender and thread like in appearance and live in the lymph gland and lympth vessels of man.

The female measures 100 mm in length and 0.25 mm in diameter whereas the males are only 40 mm in length and 0.1mm in diameter. The tail of male is coiled and with two unequal spicules. These two spicules help in the process of copulation. The female is larger than the male (Fig. 7).

Their body colour is creamy white. The anterior end of the parasite is swollen slightly and without lips. Certain species of *culex* mosquito (*Culex fatigans*, *C. quinquefasciatus*) bring about transmission of the parasites from infected person to healthy persons.

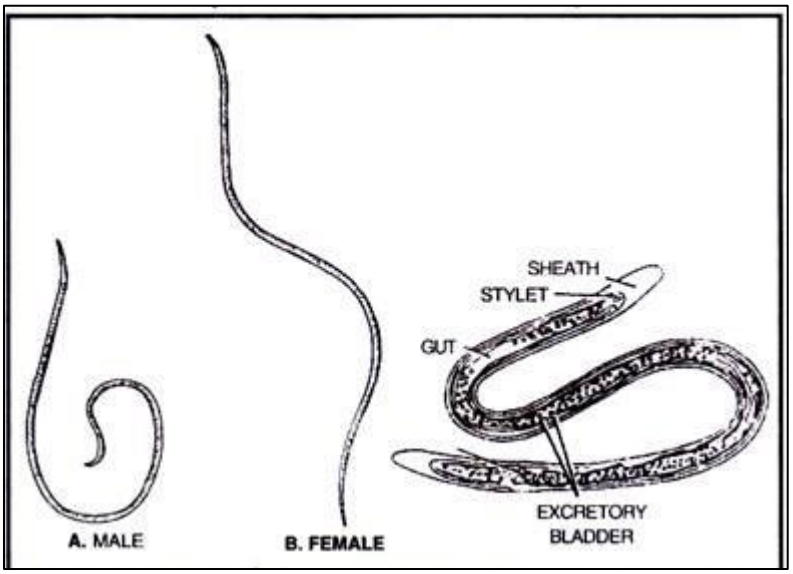


Fig 7. *Wuchereria* sp. A-Detailed structure of male; B-Detailed structure of female and microfilariae of *Wuchereria*

Life Cycle:

Like malarial parasite the life cycle of *Wuchereriabancrofti* is digenetic involving two hosts: man and female *Culex*mosquito (Fig 8). Following mating the female worm lays eggs which hatches to give rise to larvae or juveniles called microfilariae. Each microfilaria is enclosed in a dedicate sheath and measures about 0.22 mm in length. The microfilariae show nocturnal periodicity i.e., they appear in peripheral circulation of man between 10 P.M. and 4 A.M. (to be sucked by mosquito) but live in deeper blood vessels during the day time.

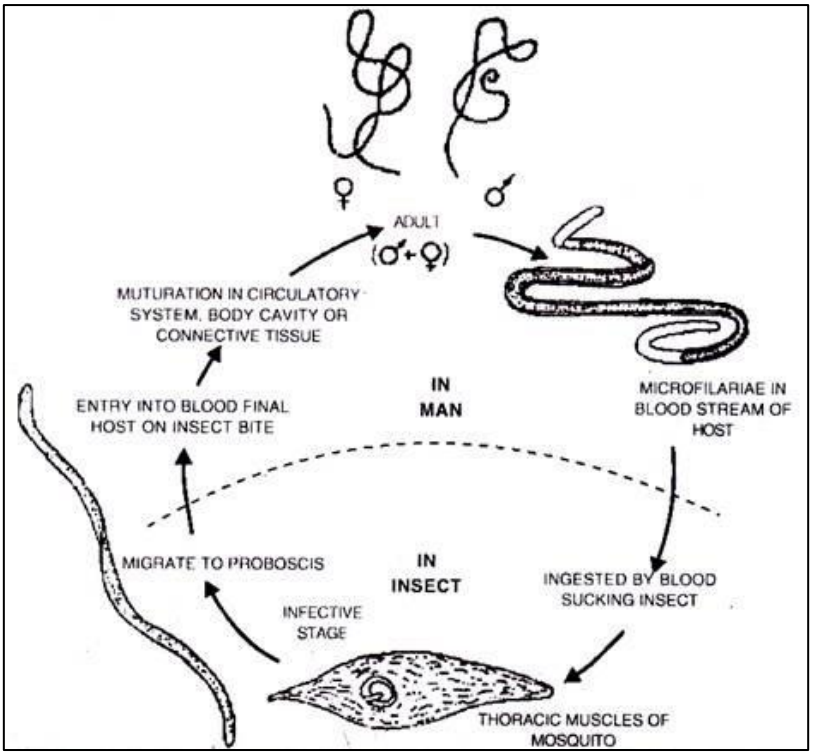


Fig 8: Life cycle of *Wuchereria* sp.

Further development of microfilariae occurs if they are sucked by the mosquito species of *Culex, Anopheles* and *Aedes* which act as vectors. In the mosquito gut the microfilariae lose their sheath and migrate to thoracic muscles and undergo two moultings (cast off old skin) there. It is the infective stage which requires two weeks time for development.

Then they migrate to the labium of mosquito when the infected mosquito bites a healthy person the microfilariae get under the skin and later migrate into the lymphatic system of man. There they undergo third and fourth moult and develop into adult. In the host body the adults live up to about 4 to 5 years.

Mode of infection of filarial disease

Filarial disease is usually transmitted to man through mosquito biting. The disease can be accidentally transmitted through blood transfusion, when the donor is infected with microfilariae. The entry of the infective stage, microfilaria in the human body is not through direct inoculation into the blood stream by the mosquito during its blood meal.

Instead, when a mosquito with infective larvae in its proboscis feeds on a person, the larvae get deposited usually in pairs on the skin near the puncture site. The larvae then enter through the wound or puncture. Subsequently they reach the lymphatic channels and settle down at some spots like scrotal sac or inguinal lymph nodes or abdominal lymphatics and begin to develop into an adult worm within a period of 5-18 months.

Symptoms of filarial disease

i. Filarial disease is accompanied with fever and headache.

ii. Owing to virulent filarial infection, the microfilariae block the passage of lymph-gland and as a result lymph cannot go back to the circulatory system, causes tremendous unequal swelling of scrotum, legs, mammary glands etc. This condition of swelling is known as elephantiasis.

iii. The skin of the affected region becomes rough and fissured.

iv. In extreme cases, the connective tissue of the affected parts becomes abnormal when the condition becomes further complicated.

Prevention of filarial disease

The filarial disease could be prevented by adopting several prophylactic measures:

i. Eradication of the vector mosquito by using allethrin or other insecticides.

ii. Destruction of mosquito larvae in the breeding ground can be done by using various larvae ides.

iii. Sterilization of male mosquito .should be done by artificial means.

iv. Protection against mosquito bite by using various devices like mosquito-net, mosquito repellent mat or cream etc.

v. As a measure of biological control mosquito larvae eating fishes like Gambusia. Gold fish etc. may be cultured in the breeding habit of mosquito.

vi. Reducing the rate of infection amongst hosts by preventing them from biting the infected individuals.

Treatment of filarial disease

i. No proper or satisfactory treatment is yet known.

ii. Ivermectin (Mectizan) is very effective against the microfilariae, a single dose is being able to reduce skin micro-filarial counts to undetectable levels within seven days.

iii. Treatment of filaria patient with proper drugs, viz. Mel. W (an arsenical preparation) on adult worms has given encouraging result; Hetrazan compounds on micro-filarial worm and paramelaminyl phenyl stibonate on infective larva and immature adult worm etc. that are used to make the blood free of microfilariae.

iv. Edematous limbs are sometimes successfully treated by applying pressure bandages, which force the lymph out of the swollen area of filarial patient.

v. Surgical removal of elephantoid tissue is often possible.

Aedes sp.

Aedes is a genus of mosquitoes originally found in tropical and subtropical zones, but now found on all continents except Antarctica. Some species have been spread by human activity: *Aedes albopictus*, a particularly invasive species, was recently spread to the New World, including the United States, by the used-tire trade. First described and named by German entomologist Johann Wilhelm Meigen in 1818, the generic name comes from the Ancient Greek ἀηδής, aēdēs, meaning "unpleasant" or "odious". The type species for *Aedes* is *Aedes cinereus*.

Some species of this genus transmit serious diseases, including dengue fever, yellow fever, the Zika virus, and chikungunya.

Life Cycle of *Aedes aegypti*

Aedes aegypti is a so-called holometabolous insect. This means that the insects goes through a complete metamorphosis with an egg, larvae, pupae, and adult stage. The adult life span can range from two weeks to a month depending on environmental conditions. The life cycle of *Aedes aegypti* can be completed within one-and-a-half to three weeks (Fig 9)

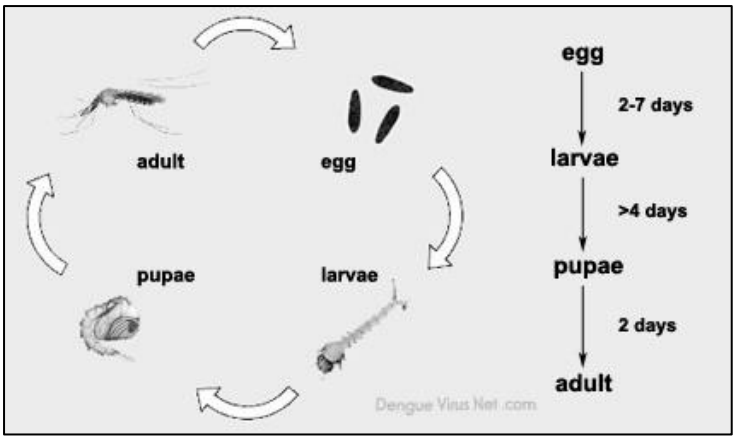


Fig 9: Life cycle of Aedes aegypti: there is an aquatic phase (larvae, pupae) and a terrestrial phase (eggs, adults)

Egg

After taking a blood meal, female *Aedes aegypti* mosquitos produce on average 100 to 200 eggs per batch. The females can produce up to five batches of eggs during a lifetime. The number of eggs is dependent on the size of the blood meal. Eggs are laid on damp surfaces in areas likely to temporarily flood, such as tree holes and man-made containers like barrels, drums, jars, pots, buckets, flower vases, plant saucers, tanks, discarded bottles, tins, tyres, water cooler, etc. and a lot more places where rain-water collects or is stored. The female *Aedesaegypti* lays her eggs separately unlike most species. Not all eggs are laid at once, but they can be spread out over hours or days, depending on the availability of suitable substrates. Eggs will most often be placed at varying distances above the water line. The female mosquito will not lay the entire clutch at a single site, but rather spread out the eggs over several sites.

The eggs of *Aedes aegypti* are smooth, long, ovoid shaped, and roughly 1mm long. When first laid, eggs appear white but within minutes turn a shiny black. In warm climates eggs may develop in as little as two days, whereas in cooler temperate climates, development can take up to a week. Laid eggs can survive for very long periods in a dry state, often for more than a year. However, they hatch immediately once submerged in water. This makes the control of the dengue virus mosquito very difficult.

Larvae

After hatching of the eggs, the larvae (fig 10) feed on organic particulate matter in the water, such as algae and other microscopic organisms. Most of the larval stage is spent at the water's surface, although they will swim to the bottom of the container if disturbed or when feeding. Larvae are often found around the home in puddles, tires, or within any object holding water. Larval development is temperature dependent. The larvae pass through four instars, spending a short amount of time in the first three, and up to three days in the fourth instar. Fourth instar larvae are approximately eight millimeters long. Males develop faster than females, so males generally pupate earlier. If temperatures are cool, *Aedes aegypti* can remain in the larval stage for months so long as the water supply is sufficient.

Fig 10: *Aedes aegypti* larvae stage

Pupae

After the fourth instar, the larvae enters the pupal stage (fig 11). Mosquito pupae are mobile and respond to stimuli. Pupae do not feed and take approximately two days to develop. Adults emerge by ingesting air to expand the abdomen thus splitting open the pupal case and emerge head first.

Fig 11: Aedes aegypti pupae stage

Adult

Adult *Aedes* mosquitoes are distinguished from other types of mosquitoes by their narrow and typically black body, unique patterns of light and dark scales on the abdomen and thorax, and alternating light and dark bands on the legs. Females are further distinguished by the shape of the abdomen, which usually comes to a point at its tip, and by their maxillary palps (sensory structures associated with the mouthparts), which are shorter than the proboscis. *Aedes* mosquitoes characteristically hold their bodies low and parallel to the ground with the proboscis angled downward when landed

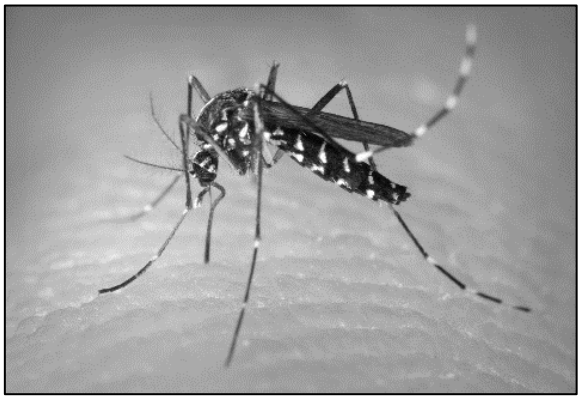


Fig: *Aedes* sp.

Role in disease transmission

Members of the genus *Aedes* are known vectors for numerous viral infections. The two most prominent species that transmit viruses are *A. aegypti* and *A. albopictus*, which transmit the viruses that cause dengue fever, yellow fever, West Nile fever, chikungunya, eastern equine encephalitis, and Zika virus, along with many other, less notable diseases. Infections with these viruses are typically accompanied by a fever, and in some cases, encephalitis, which can lead to death. A vaccine to provide protection from yellow fever exists, and measures to prevent mosquito bites include insecticides such as DDT, mosquito traps, insect repellents, and mosquito nets.

Dengue virus transmission

Dengue viruses are transmitted to humans through the bites of infective female *Aedes* mosquitoes. Most commonly, the mosquitoes involved are *Aedes aegypti* and *Aedes albopictus*, two species which can also transmit other mosquito-borne viruses, including zika and chikungunya. Other infection routes are reported from mother to child as well as blood transmission (figure 11).

	Dengue	Zika	Chikungunya
Mosquitoes	*Aedes aegypti* *Aedes albopictus*	*Aedes aegypti* *Aedes albopictus*	*Aedes aegypti* *Aedes albopictus*
From mother to child	Evidence of transmission from an infected mother to her fetus	Rarely around time of birth, but it is possible that the virus could be passed to her fetus during pregnancy	Rarely from mother to newborn around the time of birth
Breastfeeding	No evidence	No evidence	No evidence
Blood	Rare cases known of transmission via blood transfusions from infected donors	Spread of the virus through blood transfusion have been reported	No evidence, but in theory possible
Sexual	No evidence	Spread of the virus through sexual contact have been reported	No evidence

Fig 11: Comparisment of Dengue, Zika and Chikungunya transmission routes

The main transmission is through mosquitoes that generally acquire the virus while feeding on the blood of an infected person. After virus incubation for eight to ten days, an infected mosquito is capable, during probing and blood feeding, of transmitting the virus for the rest of its life. There is no way to tell if a mosquito is carrying the dengue virus. Infected female mosquitoes may also transmit the virus to their offspring by transovarial (via the eggs) transmission, but the role of this in sustaining transmission of the virus to humans has not yet been defined.

Infected humans are the main carriers and multipliers of the virus, and serving as a source of the virus for uninfected mosquitoes. The virus circulates in the blood of infected humans for two to seven days, at approximately the same time that they have a fever (see also clinical symptoms). Aedes mosquitoes may acquire the virus when they feed on an individual during this period. In parts of South East Asia and Africa, the transmission cycle may also involve jungle primates that act as a reservoir for the virus (fig 12).

Dengue is most widely transmitted by the mosquito named *Aedes aegypti*. The *Aedes albopictus* mosquito and other *Aedes* species also transmit disease in specific areas. *Aedes polynesiensis*, *Aedes scutellaris* and *Aedes pseudoscutallaris* in the Pacific Islands and New Guinea. *Aedes polynesiensis* in the Society Islands and *Aedes niveus* in the Philippines.

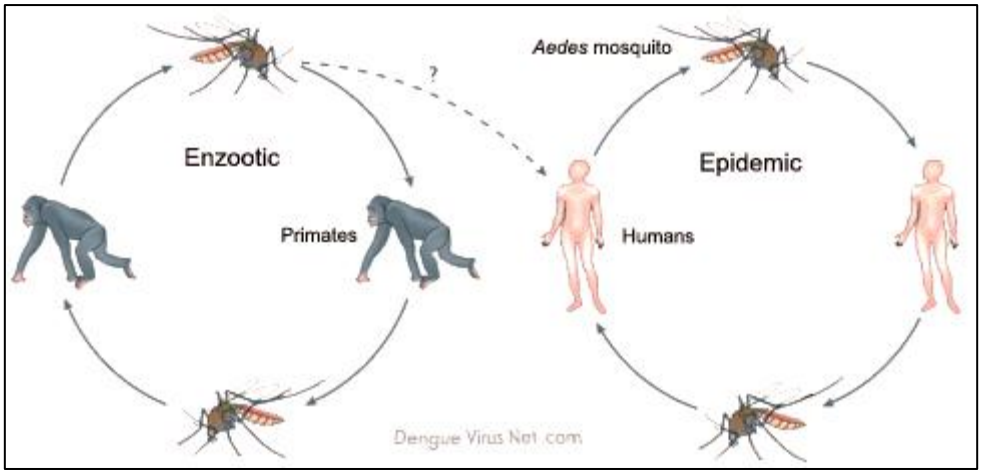


Fig 12. Transmission of dengue viruses.

The *Aedes* mosquito prefers to breed in water-filled receptacles, usually close to human habitation. They often rest in dark rooms (e.g. in bathrooms and under beds) and breed in small pools that collect in discarded human waste (see figure 3). Although they are most active during daylight hours, biting from dawn to dusk, mosquitoes will feed throughout the day indoors and during overcast weather. Dengue virus transmission follows two general patterns: epidemic dengue and hyperendemic dengue.

Epidemic dengue transmission occurs when dengue virus is introduced into a region as an isolated event that involves a single viral strain. If the number of vectors and susceptible pediatric and adult hosts is sufficient, explosive transmission can occur, with an infection incidence of 25-50%. Mosquito-control efforts, changes in weather, and herd immunity contribute to the control of these epidemics. This is the current pattern of transmission in parts of Africa and South America, areas of Asia where the virus has re-emerged, and small island nations. Travelers to these areas are at increased risk of acquiring dengue during these periods of epidemic transmission.

Hyperendemic dengue transmission is characterized by the continuous circulation of multiple viral serotypes in an area where a large pool of susceptible hosts and a competent vector (with or without seasonal variation) are constantly present. This is the predominant pattern of global transmission. In these populations, antibody prevalence increases with age and most adults are immune. Hyperendemic transmission appears to be a major risk for Dengue Haemorrhagic Fever (DHF). Travelers to these areas are more likely to be infected than are travelers to areas that experience only epidemic transmission.

The dengue fever is an acute febrile illness clinically characterized by haemorrhagic phenomenon and a tendency to develop a shock syndrome which may be fatal. The diagnostic symptoms are acute onset, high continuous fever lasting to 2-7 days, with various haemorrhagic manifestations like petechiae, purpura, achymosis epistaxis, gum-bleeding, hematemesis and/or melena, the enlargement of liver and shock manifested by rapid and weak pulse, narrow pulse pressure or hypotension, etc.

The patient is restless with cold clammy skin. Incubation period in man is 4-10 days. The mosquito becomes infected only during the first 3 days of patient's illness. Incubation period in mosquito varies from 8 to 11 days. Once infected, the mosquito remains so for its life and when the mosquito introduces saliva into the man's skin during feeding it transmits infection.

There is no transovarial transmission with respect to the mosquitoes. Although in India, it is becoming endemic in some parts, it still exists in epidemic form in certain parts of South-east Asia like Thailand, Myanmar and Malaysia. The preventive measures include the vector control and the screening of all early cases so as to avoid the mosquitoes becoming infective.

Morphological differences between *Anopheles, Culex* and *Aedes* mosquito

	Anopheles	***Culex***	***Aedes***
Adult			
1.	When it sits, the body makes an acute angle of 45° with the substratum.	When it sits, the body placed more or less parallel to the substratum.	It sits more or less parallel to the substratum.
2.	Wings are provided with black and white bands.	Wing are devoid of any striation or bands.	Wings are large and thick and are provided with black and white bands.
3.	Scanty hairs are present on the antenna.	Dense hairs are present on the antenna.	Hairs are dense and coarse on the antenna.
4.	Both body and legs are narrow.	Both body and legs are comparatively broad.	Both body and legs are comparatively broad.
5.	Produce a peculiar sound during flight.	No sound is produced during flight.	Less sound is produced during flight.
Eggs			
6.	Eggs are small, boat like with air float.	Eggs are elongated and are devoid of air float.	Eggs are small and are devoid of air float.
7.	Eggs occur singly and no raft is formed.	Eggs occur in cluster forming a raft.	Eggs float separately and forming a raft.
8.	Number of eggs vary from	Number of eggs vary from	Number of eggs vary

	200-300.	200-800.	from 200-large number.
Larva			
9.	It floats horizontally placing its body parallel to the surface of water.	The larva floats obliquely with its head facing downwards.	Floats like *Culex* larva.
10.	Spiracles of 8th abdominal segment open on swellings placed side by side. Respiratory siphon is reduced in the larva.	Spiracles of 8th abdominal segment are placed on a pair of long tubular respiratory siphon.	There is tubular respiratory siphon on 9th abdominal segment.
Pupa			
11.	The pupa is green.	Colorless.	Colorless.
12.	Dorsal respiratory tube is small and flat.	Dorsal respiratory tube is narrow and elongated.	Same as *Culex* pupa
13.	Tail fin is large.	Tail fin is small.	Tail fin is medium.

Mosquito Control Measures

- **Personal protection:**

The eggs developing within the female mosquito need human blood for nourishment and so the female mosquito bites humans. By personal protection against mosquito bites, this blood meal can be denied, leading to reduction in mosquito eggs and hence mosquito population. Personal protection by covering the body with clothes and use of mosquito nets and repellents will further help in preventing mosquito bites.

Closure of windows and doors to prevent entry of mosquitoes inside house;

Protection of humans against mosquito bite by using bed nets (insecticide treated) and mosquito repellent.

- **Source reduction:**

High humidity and ambient temperature between 20-30ºC provide ideal conditions for breeding of Anopheline mosquitoes. Common sites of breeding for *Anopheles* mosquitoes include rainwater pools and puddles, borrow pits, river bed pools, irrigation channels, seepages, rice fields, wells, pond margins, sluggish streams with sandy margins, hoof prints, tyre tracks etc. Water stagnation due to construction of

dams, reforestation, shrimp farming, fish ponds etc., and have also been identified as possible sites of *Anopheles* breeding. *An. stephensi* is a well-adapted urban vector, being a container breeder, making use of man-made sites such as building-construction sites, wells, garden ponds, cisterns, overhead tanks, ground level cement tanks, water coolers, tyres, barrels and tins, intra-domestic containers etc. Prevention of water logging, destroying unwanted water collections and keeping the water containers closed, sources of egg laying (Source Reduction) can be denied and breeding of mosquitoes can be prevented.

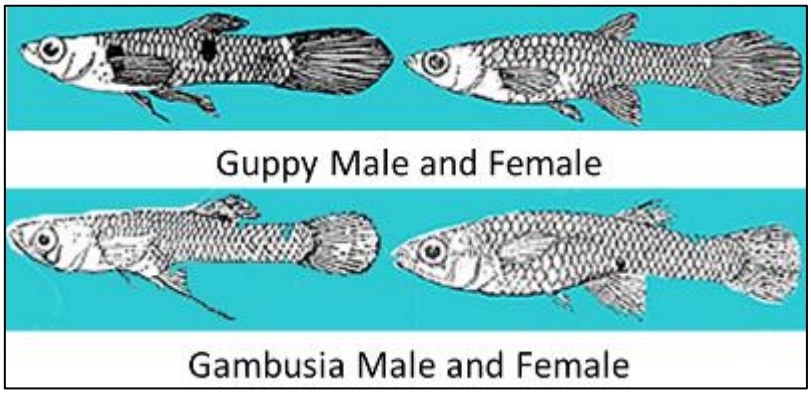
- **Killing of the developing larvae and pupae:**

The best method of mosquito control is preventing the development of the eggs into adult mosquitoes, by reducing the sources of breeding. These anti larval measures are not only simple and cost effective, but also environment friendly.

Different types of chemical (insecticides) or biological (Guppy, *Poecilia reticulate* or Gambusia, *Gambusia affinis* fish or bacteria or fungii*)* larvicides (Themiphos and Fenthion are the two commonly used larvicidal agents. Oils may be applied to the water surface, suffocating the larvae and pupae.) can be used on such breeding grounds to kill the developing larvae and pupae.

- Bacteria such as *Bacillus sphaericus* and *Bacillus thuringiensis var israelensis* are also effective larvicides. However, they need to be re-introduced every 15 days and their culture may need expertise.

- MermitidNematod (*Romanomermisculicivorax*), Notonectid (Bug), Ambylospora (Protozoa), Coelomomyces (Fungus), Nuclear Polyhedrosis (Virus), and Cyclopoid copepods (Crustacean) are the other biological larvicides found to be effective.



- **Killing of the adult:**

Sprays are used to instantly kill the adults and residual sprays, on their resting places such as walls, are used for residual mosquitocidal effect.

a. Preventing egg laying: The easiest, cheapest and most environment-friendly method to control malaria is by preventing the mosquito from laying eggs. This is done by avoiding or eliminating the clean water collections.

To add to the problem, construction workers tend to harbour the malarial parasite, due to frequent infections owing to their poor standards of living. Thus, construction sites not only provide for mosquito breeding but also supply the parasites. This is the reason why malaria tends to be more common in cities where construction activities are in full swing.

Method # 1. Personal Protection:

(a) In mosquito-infected area protective clothing may be used, such as will cover the exposed parts of the body, especially after sunset,

(b) Mosquito repellents are also useful, like mosquito cream, citronella, odomos and Indalone which keep mosquitoes away. Repellent No. 448 of the American navy is very effective for long periods,

(c) While sleeping fine mesh mosquito nets prevent them from biting and bed rooms or houses could be screened to prevent entry of mosquitoes,

(d) Painting walls with creosote repels mosquitoes.

Method # 2. Destruction of Adults:

(a) Killing of mosquitoes can be done be spraying liquid insecticides like flit or D.D.T., the latter not only kills mosquitoes but also makes them leave a house,

(b) Fumigation of dwellings with sulphur dioxide is also useful,

c) A mixture of water and 10% D.D.T. in oil sprayed from the air is very effective in killing large number of mosquitoes in towns, ponds, marshes and forests.

Method # 3. Destruction of Larvae:

It is easier and more effective to kill mosquitoes in their larval forms than as adults, and several methods are used with success,

(a) Oiling:

The breeding places of mosquitoes are sprayed with petroleum oils, the oil film formed on the surface of water does not asphyxiate the larvae, as is commonly believed, but is toxic to them, the oiling must be repeated to kill those larvae and pupae which will hatch later.

(b) Panama Larvicide:

Panama larvicide is a mixture of caustic soda, resin, and phenol in water, it has been used most effectively in the Panama Canal region. The Panama larvicide mixes well with water and kills both the larvae and the algae on which they feed. One part of Panama larvicide is sufficient for 10,000 parts of water.

(c) Paris Green:

Paris green is a powder of arsenic mixed with fine dust, one part of powder with 100 parts of dust. This can be thrown in the wind and it will cover the surface of a pond; it is insoluble in water and remains floating and is eaten by surface feeding larvae of Anopheles', it will kill the larvae but not pupae. It is effective only against those larvae which feed on surface.

(d) Natural Enemies:

Fishes, minnows and *Gambusia* live on larvae and pupae of mosquitoes, and their introduction in a breeding place is helpful, but for this the brush and floating vegetation must be cleared so that the fish can reach the larvae.

(e) Chemical Larvicide:

One part of D.D.T. emulsion in thirty million parts of water is used most extensively as a spray to kill larvae, but it takes 50 hours. Planes can be used for this purpose on large areas.

Method # 4. Elimination of Breeding Places:

For those mosquitoes which breed in rain-filled containers and cisterns, like Aedes, emptying of water is effective. For large ponds and swamps digging a sloping ditch removes large volumes of water. Small ponds can be filled up with mud. In India cycles of 5 wet days followed by 2 to 4 dry days were found to be highly effective in controlling Anopheles in fields.

Method # 5. Preventive Medicine:

Daily doses of quinine are effective against mosquito bites, but a successful vaccine has yet to be found for yellow fever.

Probable questions:

1. What is the primary vector of malaria?
2. Describe the life cycle of *Anopheles* sp. With suitable diagram.
3. Write down the description of adult *Anopheles* mosquito.
4. Which type of malaria is most deadly type? Write the name of causative agent of this type of malaria.
5. Write the name of four types of malaria with its respective causative agents.
6. What is sporozoite?
7. What is cryptomerozoite?
8. What do you mean by scizont?

9. Describe the mode of malaria transmission.
10. Name the *culex*sp found in temperate region and tropical region.
11. Describe the morphology of adult *Culex* sp.
12. Describe the life cycle of *Wuchereria bancrofti* with diagram.
13. Discuss briefly the mode of transmission of filariasis.
14. What are the symptoms and treatment of filarial disease?
15. Describe the mode of transmission of those diseases which are transmitted by *Aedes* mosquito.
16. Describe the mode of transmission of Dengue virus.
17. Describe transovarial transmission with example.
18. Describe the mosquito control measures.

Suggested readings:

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UNIT X

Life cycle, medical importance and control of disease-causing vector - Black fly

Objective:

In this unit you will know about Life cycle, medical importance and control of disease-causing vector Black fly

Introduction

Black flies, known also as "buffalo gnats" and "turkey gnats," are very small, robust flies that are annoying biting pests of wildlife, livestock, poultry, and humans. Their blood-sucking habits also raise concerns about possible transmission of disease agents. You are encouraged to learn more about the biology of black flies so that you can be better informed about avoiding being bitten and about their public health risk.

Common name: black flies

Scientific name: *Simulium* spp

Distribution

Black flies are found in many parts of the US and Canada, including Florida. Populations in Florida normally are not present in numbers large enough to be noticed by humans. *Simulium slossonae* is found in Florida from Dade country north to Duval County, and west to EscambiaCounty. In some areas it is present all year long. In South Carolina, population peaks have been recorded in late July, late September, and late October, but large numbers have been found in Florida between April and November. Overall, Florida has 18 different black fly species, the most common with populations present from August through July. Only female black flies feed on blood. Males feed mainly on nectar.

Black flies can occur in enormous numbers. Immature stages develop in oxygenated water sources, therefore adults are usually associated with slow moving streams, creeks, or rivers where the immature stages develop. Flowing water does not necessarily imply white water rapids, but water must be moving. Water in lakes and ponds that is not flowing is unsuitable for black fly development. *Simulium slossonae* prefers fairly small, slow moving streams with an average velocity of 1.5 ft per second. Water is often tea-colored, with ample vegetation, light shade, and a pH of 4.4 to 4.5. *Simuliumslossonae* makes good use of temporary streams that flow seasonally.

Types of Black Flies

Black flies are true flies (Order Diptera) in the family Simuliidae, which includes more than 1,700 species worldwide. In North America, 255 species in 11 genera have been identified, but additional species remain to be discovered and named. Very little is known about black flies in Indiana, and there are no estimates of the number of species

in the state. For perspective, 12 species have been documented in Illinois, while over 30 species have been documented in both Minnesota and Wisconsin, where black fly habitats are more abundant.

Morphology

Black flies range in size from 5 to 15 mm, and they are relatively robust, with an arched thoracic region (Fig 1). They have large compound eyes, short antennae, and a pair of large, fan-shaped wings. Most species have a black body, but yellow and even orange species exist.

Fig 1: Adult black fly

Life Cycle

Black flies undergo a type of development known as "complete metamorphosis" (Fig 2). This means the last larval stage moults into a non-feeding pupal stage that eventually transforms into a winged adult.

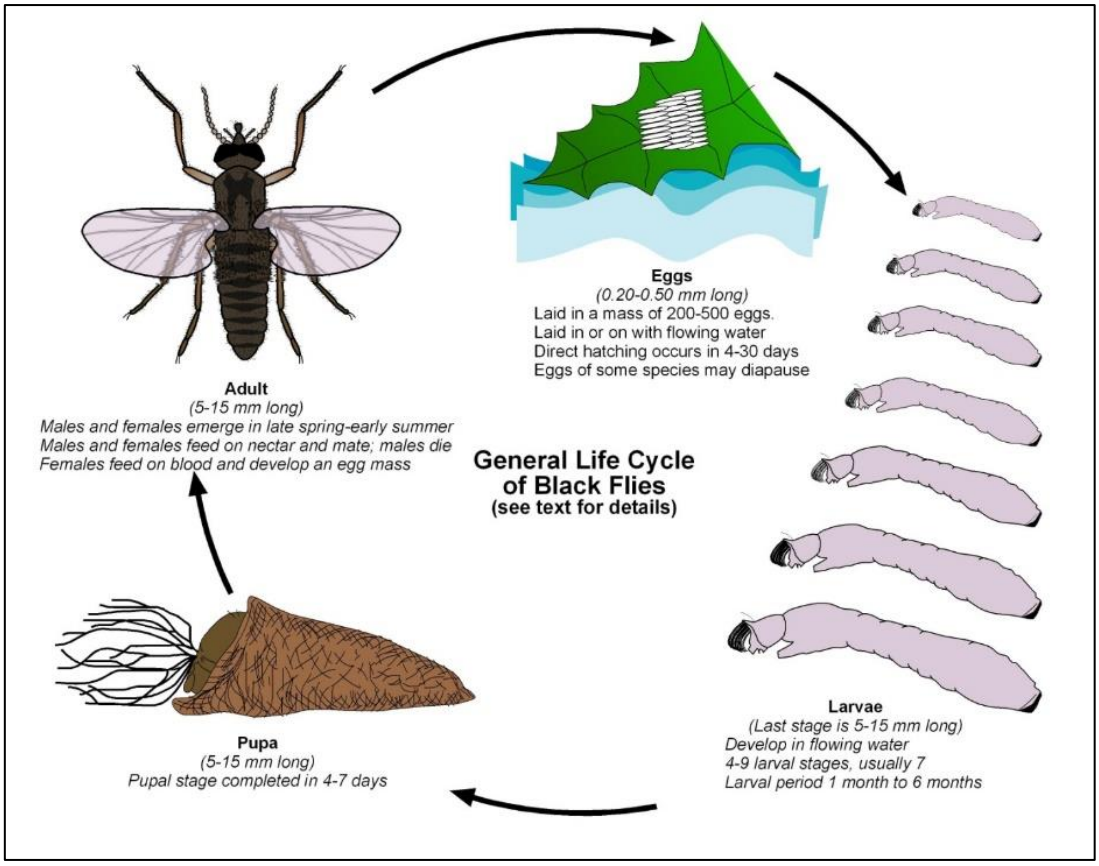

Fig 2: Black fly life cycle

Egg:

Adult black flies are small insects that measure 1 to 5 mm in length, and possess a shiny thorax (middle of the fly) that ranges in colour from black to various shades of grey or yellow. After taking a blood meal, females develop a single batch of 200-500 eggs. Most species lay their eggs in or on flowing water, but some attach them to wet surfaces such as blades of aquatic grasses. The length of time it takes an egg to hatch varies greatly from species to species. Eggs of most species hatch in 4-30 days, but those of certain species may not hatch for a period of several months or longer.

Larva:

The number of larval stages ranges from 4-9, with 7 being the usual number. The duration of larval development ranges from 1-6 months, depending in part on water temperature and food supply.Larvae pass through six stages before reaching the pupal stage. The life cycle stage that passes though winter is the last stage larva attached underwater to rocks, driftwood, and concrete surfaces such as dams and sides of man-made channels.

Larvae remain attached to stationary objects in flowing water, held on by silken threads extruded from glands located at the end of the bulbous abdomen. Depending on species, mature larvae range from 5-15 mm in length and may be brown, green, gray, or nearly black in color. They possess a large head that bears two prominent structures known as "labral fans" that project forward (see Figure 2). Labral fans are the primary

feeding structures, filtering organic matter or small invertebrates out of the water current.

Pupa:

The pupal stage is formed the following spring or summer, typically in the same site as the last stage larva, but may occur downstream following larval "drift" with the current. Adults emerge from the pupal stage in 4-7 days and can live for a few weeks. Adults of most species are active from mid-May to July. The number of generations completed in one year varies among species, with some having only one generation, but most species that are major pests complete several generations per year.

Pupae remain attached to stationary objects in flowing water as well. They typically are orange and appear mummy-like because the developing wings and legs are tightly attached to the body. Pupae of many species produce a delicate, silken "cocoon" of varying density, weave, and size that partially or nearly entirely encloses them; other species produce hardly any cocoon at all.

Black fly larvae and pupae develop in flowing water, typically non-polluted water with a high level of dissolved oxygen. Suitable aquatic habitats for black fly larval development vary greatly and include large rivers, icy mountain streams, trickling creeks, and waterfalls. Larvae of most species typically are found in only one of these habitats.

Adult:

Simulium slossonae adults may fly four to eight miles from breeding sites in search of hosts, then return after feeding to breed and lay eggs. In parts of Africa, adult female black flies may travel more than forty miles from aquatic breeding sites to find blood meals. So, the biting problem at a particular location may be generated at some distance away, even in Florida.

Feeding habits of adult black flies

It is estimated that females of 90% of the black fly species require a blood meal for the development of eggs. Those of most species feed on mammals, while others feed on birds. Females of some black fly species feed on only one host, whereas others are known to feed on over 30 different host species. No North American species feed exclusively on humans. Male black flies are not attracted to humans, and their mouthparts are not capable of biting.

Females of most species of black flies feed during the day, usually biting on the upper body and head. Unlike certain species of mosquitoes and biting midges, black flies do not enter human structures to seek blood meals.

Damage by Black fly

Black flies have preferences for a wide range of individual host species. Adult females feed on the blood of humans, cattle, horses, sheep, goats, poultry, other livestock and wild mammals and birds. Each black fly species may prefer one type of host over

another. The black fly common name sometimes indicates host specificity, for example the turkey gnat. Black flies are daytime biters preferring low wind conditions. They are not restricted to shaded or humid sites, and usually do not go indoors. They are attracted to hosts from a distance by smell, heat, and by sight. The female flies swarm around and crawl on the host preferring the head, hair, and ears as well as any skin that is exposed or that they can crawl onto. *Simuliumslossonae* is primarily a bird feeder and probably preys on wild turkeys to some extent. It is the primary vector of the protozoan blood parasite, *Leucocytozoonsmithi*, in Florida. This parasite is restricted only to birds, especially turkeys. *Simuliumslossonae* will feed on domestic turkeys as well as chickens and other poultry. Several cases of chicken mortality attributed to black fly feeding were reported in Florida during the first three months of 1998.

Female black flies are blood feeders whose bites can itch and persist for several days. The flies bite by cutting into the skin and feeding on the pool of blood that forms in the hole they make. Anticoagulants injected into the feeding site by black flies can cause mild to severe allergic reactions in sensitive individuals. Strong reactions include fever, nausea and allergic dermatitis. Large black fly populations and strong bite reactions can be life threatening and have been reported to kill domestic animals. Black fly bites are very painful because of the hole that is cut in the skin, the anticoagulants and other materials that are injected, and the immunological differences between insect and hosts' tissues.

Some people are very attractive to black flies and have strong feeding reactions. Others appear to repel black flies and are bitten little if at all. Although *Simuliumslossonae* is primarily a bird feeder, large swarms are attracted to people. Fortunately in Florida, most of the black flies attracted to people do not bite. Anyone showing strong allergic reactions should consult a physician for immediate treatment. Treatments for bites include antipruritic lotions or creams.

- ## Onchocerciasis

Onchocerciasis, also known as river blindness, is a disease that affects the skin and eyes. It's caused by the worm *Onchocercavolvulus*.*Onchocerca volvulus* is a parasite. It's spread to humans and livestock through the bite of a type of blackfly from the genus *Simulium*. This type of blackfly is found near rivers and streams. That's where the name "river blindness" comes from.

Causes

You can develop river blindness if you're bitten repeatedly by infected female blackflies. The blackfly passes the larvae of the worm *Onchocercidae* through the bite. The larvae move to the subcutaneous tissue of your skin, and mature into adult worms over 6 to 12 months. The cycle repeats when a female blackfly bites a person infected with onchocerciasis and ingests the parasite.

Adult worms can live for 10 to 15 years and may produce millions of microfilariae during that time. Microfilariae are baby or larval worms. Symptoms appear when

microfilariae die, so symptoms can continue to worsen the longer you are infected. The most extreme, longest-lasting cases result in blindness.

Mode of transmission (onchocerciasis)

The parasites that cause onchocerciasis are transmitted from human to human through the bites of blackflies, which belong to *Simulum* species. Blackflies breed in fast-flowing rivers and streams, with good vegetation nearby. Unlike mosquitoes and sand flies, they bite during the day when people are active in the area.

The adult worms mate in the infected person, and the eggs hatch into microscopic worms called microfilaria, which burrow through the body tissues. The person's immune system attacks the microfilaria, causing inflammation and damage in the surrounding tissues. Sight defects and eventually blindness develops when the microfilaria are embedded in the person's eye. When a female blackfly bites an infected person during a blood meal, the microfilaria are transferred from the person to the fly.Over the course of one to three weeks, the microfilaria develop inside the blackfly to form infective larvae. These are then passed on to other people when the blackfly takes another blood meal. The microfilaria migrate to the skin, lymph nodes and eyes of the infected person, causing inflammation and tissue damage.

In the human host, the larvae migrate into the skin, and nodules (swellings) form around them. They slowly mature into adult worms, which can live for 15 years in the human body. After mating, the female worm releases around 1,000 microfilaria a day into the surrounding tissue. Microfilaria live for one to two years, moving around the body. When they die, they cause an inflammatory response which leads to the clinical manifestations and complications such as blindness.

Symptoms

There are different stages of onchocerciasis. In earlier stages, you may not have any symptoms. It can take up to a year for symptoms to appear and the infection to become apparent.

Once the infection becomes severe, symptoms may include:

- skin rashes
- extreme itching
- bumps under the skin
- loss of skin elasticity, which can make skin appear thin and brittle
- itching of the eyes
- changes to skin pigmentation
- enlarged groin
- cataracts
- light sensitivity

- loss of vision
- Nodding disease, which is a rare form of epilepsy, has been associated with onchocerciasis. It's relatively rare, affecting somewhere around 10,000 children in eastern Africa. Trials are being conducted to learn whether or not doxycycline could help reduce the neuroinflammation that occurs.

Risk factors

You're at increased risk for onchocerciasis if you live near fast-running streams or rivers in intertropical areas. That's because blackflies live and breed in these areas. Ninety percent of cases are in Africa, but cases have also been identified in Yemen and in six countries in Latin America. It's unusual for casual travelers to contract the disease because repeated bites are necessary for the infection to be transmitted. Residents, volunteers, and missionaries in areas of Africa are at the greatest risk.

Diagnosis

There are several tests used to diagnose onchocerciasis.

1. Usually, the first step is for a clinician to feel the skin to try to identify nodules. Your doctor will do a **skin biopsy**, known as a skin snip. During this procedure, they'll remove a 2- to 5-milligram sample of the skin. The biopsy is then placed in a saline solution, which causes the larvae to emerge. Multiple snips, usually six, are taken from different parts of the body.
2. An alternative test is called the **Mazzotti test**. This test is a skin patch test using the drug diethylcarbamazine (DEC). DEC causes the microfilariae to die rapidly, which can lead to severe symptoms. There are two ways that clinicians may use DEC to test for onchocerciasis. One way is by giving you an oral dose of the medication. If you're infected, this should cause severe itching within two hours. The other method involves putting DEC on a skin patch. That will cause localized itching and a rash in people with river blindness.
3. A more rarely used test is the **nodulectomy.** This test involves surgically removing a nodule and then examining it for worms. An enzyme-linked immunosorbent assay (**ELISA**) test can also be performed, but it requires expensive equipment.
4. Two newer tests, **polymerase chain reaction (PCR)** and **rapid-format antibody card tests**, show promise.

   i. PCR is highly sensitive, so it only requires a small skin sample — about the size of a small scratch — to perform the test. It works by amplifying the DNA of the larvae. It's sensitive enough that even very low-level infections can be detected. The drawback to this test is cost.

   ii. The rapid-format antibody card test requires a drop of blood on a specialized card. The card changes color if antibodies to the infection are detected. Because it requires minimal equipment, this test is very useful in the field, meaning you don't

need access to a lab. This type of test is being widely used and efforts are underway to standardize it.

Treatment

The most widely used treatment for onchocerciasis is ivermectin (Stromectol). It's considered safe for most people and only has to be taken once or twice a year to be effective. It also doesn't require refrigeration. It works by preventing the female blackflies from releasing the microfilariae.

In July 2015, controlled trials were conducted to learn whether or not adding doxycycline (Acticlate, Doryx, Vibra-Tabs) to the ivermectin would be more effective in treating onchocerciasis. The results were unclear, in part due to issues in how the trials were conducted.

Control

Followings techniques helps in controlilng of Black fly

i. Control of black flies is difficult because of the number of potential breeding sites. However, satisfactory control has been attained in some states by treating streams with the natural product, *Bacillus thuringiensis* var. *israeliensis*.

ii. The breeding sites in streams as well as the potential migration from these sites by the adult female black fly limits the use of chemical pesticides to regulate populations. Breeding site treatments have been used in Africa, but generally have not proven effective. Treatment of breeding sites (streams, rivers, etc.) would involve techniques similar to those used by mosquito control districts for treatment of mosquito larvae in aquatic habitats.

iii. Control of black flies is difficult, typically aimed at the larval stages, and usually involves aerial applications of insecticides or physically altering the habitat of pest species. The most effective control programs are conducted by state agencies or by professional pest control companies contracted by the state. Any effect is limited in duration, however, in large part because females of pest species are capable of flying long distances from the larval developmental site, and they soon re-infest treated areas.

iv. Fogging for black flies would have to be done during the day when these insects are actively feeding and when fogging is least effective. These techniques do not seem effective against black flies in the north-eastern United States.

v. Black flies are small enough to pass through window screen or come indoors on or in the hair. They do, however, prefer to bite out of doors. Long sleeve shirts, long pants and fine screen netting overhead help prevent feeding.

vi. Repellents containing "DEET" formulations are not very effective for prevention of black fly bites. Individuals wearing DEET may actually have more black flies attracted to them than individuals not wearing DEET.

vii. Some protection is given by herbal-based treatments with an active ingredient of geraniol. Permethrin products designed specifically to repel ticks also work for black flies as a clothing treatment, but can only be applied to fabrics, such as hats and shirts, and not to skin. Because black flies feed only during the day, our best advice is to limit your exposure to black flies. If this is not possible, try the available repellents in the hope that one of them will be effective for you in protecting against the black flies' bites.

Probable question:

1. How many types of black flies are there?
2. Describe the life cycle of black fly.
3. Describe the larval stage of black fly with diagram.
4. Discuss the biting nature of female black fly.
5. Describe the causative factor, mode of transmission and prophylaxis of river blindness.
6. Write short note on public health risk of black fly.

Suggested readings:

1. Bernays, E.A. and Chapman, R.F. (). Host Selection by Phytophagous insects. Chapman and Hall, New York, USA
2. Gullan, P.J. and Cranston, P.S. (). The Insects: An Outline of Entomology. Wiley Blackwell.
3. Hati, A.K. (2010). Medical Entomology. Allied Book Agency, Kolkata.
4. Lehane, M.J. (2005). The Biology of Blood Sucking Insects. 2nd Edn. Cambridge Univ Press.
5. Nation, J.L. Insect Physiology and Biochemistry. CRC Press, USA
6. Snodgrass, R.E. Principles of Insect Morphology. Cornell Univ. Press, USA
7. Wilson, E.O. The Insect Societies. Harvard Univ. Press, UK

HARD CORE THEORY PAPER (ZCORT- 206)

Group B:Fish Biology

Module	Unit	Content	Credit	Page No.
ZCORT - 206 (Advanced Parasitology and Fish Biology)	XI	Excretion and osmoregulation in fish.	3	
	XII	Reproduction in fish: reproductive strategies, oviparity, viviparity, ovo-viviparity, maturity stages, breeding cycle		
	XIII	Structure and physiology of endocrine glands in fishes		
	XIV	Electroreception in fish		
	XV	Determination of age of fish by scale and hard parts		
	XVI	Poisonous and venomous fish.		
	XVII	Fish migration: Types, Theories and Significances		
	XVIII	Parental care in Fish		
	XIX	Respiratory organs of Fishes: Water breathing, air breathing		

	XX	**Swim Bladder in Fish**		

Total Counselling time 18 hours

Unit-XI

Excretion and Osmoregulation in Fish

Objective: In this Unit you will learn about Excretion and Osmoregulation in Fish

Introduction:

The process of elimination or removal of harmful substances i.e. metabolic nitrogenous wastes from the body is known as excretion and the organs associated with the removal of excretory substances known as excretory system. On the other hand, osmoregulation is the process of maintaining an internal balance of salt and water in body. Physiological systems of fishes operate in an internal fluid environment that may not match their external fluid environment.Relative concentrations of water and solutes internally must be maintained within fairly narrow limits. Since the quantity and variety of salts in both fresh and sea waters aredifferent from that in the body fluids of fish, all fish (except the myxinoids) must perform the function of osmotic regulation, i.e. fishes are homeosmotic. The physiological adjustments which are made in performing this function are truly remarkable.

Organs involved in Excretion and osmoregulation:

The functions of excretion and osmoregulation are usually closely related in animals and are performed by the same structures. In fish these structures are the gills and the kidneys.The primary excretory organ in fishes, as in other vertebrates, is the kidney. In fishes some excretion also takes place in the digestive tract, skin, and especially the gills (where ammonia is given off) but kidneys first evolved as osmoregulatory organs in fishes to remove water (freshwater) or conserve water (marine) The kidney, gills, and skin play an important role in maintaining a fish's internal environment and checking the effects of osmosis.

a. Gills:

The gills are filamentous tissues supported by a gill arch and protected by the body wall or an operculum. The blood supply consists of afferent arteries, from the ventral aorta, which divide into capillaries in the gill filaments and come together again to form the efferent arteries leading to the dorsal aorta. Although the gills function chiefly as a respiratory organ, they do very important work in excretion and osmotic regulation.

There are great variations in the external structure of the kidney in fishes. The shape varies according to species. The kidneys occupy dorsal position in the body cavity and are placed just ventral to the vertebral column. In teleost, the kidney is distinguished into head and trunk regions. Head kidney is non-excretory and endocrine in function whereas trunk kidney (posterior kidney) is excretory in nature.

The trunk kidney or body kidney, like other vertebrates, contains renal tubules (nephrons) and interstitial lymphoid tissue. The number of renal tubules varies in different fishes. In teleosts, the trunk kidney consists of a large number of nephrons. The functional unit of kidney is nephron.Each nephron consists of two parts, the renal corpuscles (Malpighian body) and the renal tubule (urinary tubule), the renal corpuscle or Bowman's capsule is double layer cup-like structure of uriniferous tubule which contains tuft of capillaries known as glomerulus.The remaining segment of urinary tubule (renal tubule) is divided into proximal convoluted segment the intermediate and distal segments.The distal segment is absent in marine fishes. The segment of Henle found in higher vertebrates is also absent in the fishes. The glomerulus and Bowman's capsule together constitutes the renal or Malpighian capsule. It is a filtration apparatus of kidney.The glomerular capillaries, which are the vascular part of corpuscle, is the afferent arteriole which divides and forms capillary loops. The loops reunite and leave the capsule as efferent arteriole. The renal corpuscle contains an additional group of cells known as mesangial cells. The function of mesangial cells are not known, although experimental data show that they can remove large proteins from the glomerular basal lamina. Juxtaglomerular cells are present in the wall of afferent arterioles. These cells contain secretory granules and are specialized muscular cells.

The glomeruli of freshwater teleosts are numerous and large in size. In marine teleosts, glomeruli are reduced in size and number. In extreme cases, the glomeruli disappear completely from the kidney of some marine fishes (Sea horse, pipe fish). The renal tubules are thin and short in the neck segment and consist of single layer of low epithelial cells with long cilia. The proximal convoluted are provided with cuboidal epithelial cells. The nuclei are large, round or oval. The cytoplasm contains secretary granules.The intermediate segment is well-developed in carp kidney but absent in several species of fishes. The distal convoluted segment could be distinguished because of coarse granules in the cytoplasm. The distal convoluted segments are absent in the kidney of marine fishes.

Excretion and Urine formation:

Carbon di oxide enters into the bicarbonate equilibrium system and most is excreted at the gills. Protein digestion yields nitrogenous compounds in addition to carbon di oxide and water. In teleost fishes these nitrogenous wastes takes the form of ammonia, a potentially toxic substance. Thus, teleports are primarily "ammoniotelic". Despite its toxicity ammonia has many advantages over urea or uric acids as the chief excretory product of nitrogen metabolism.First the small molecular size and high lipid solubility permits non ionized ammonia (NH_3) to diffuse easily across the gills. Second, ionized ammonia (NH_4^+) is exchanged for Na^+ at the gills for maintenance of relative alkalinity and internal ion balance. Third conversion of ammonia to either urea or uric acid requires energy. Thus in contrast to terrestrial forms, less energy is required to complete nitrogenous compound catabolism.

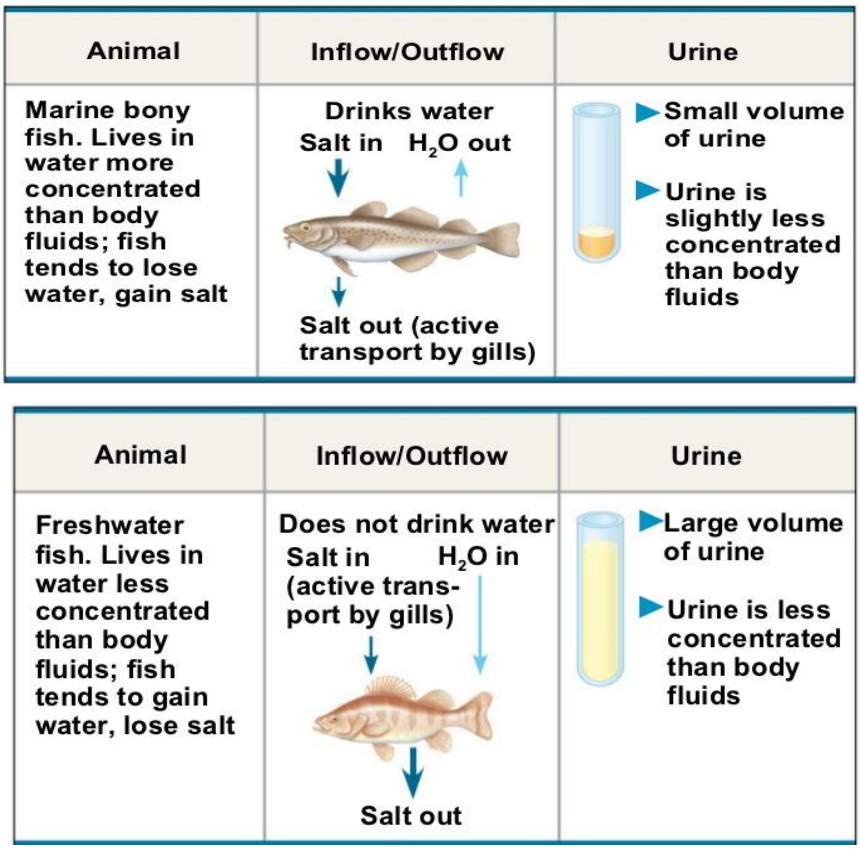

Fig: Urine concentration of Salt water and Fresh water fish

The freshwater teleosts have to excrete large amount of water which is taken through mouth. The urine of freshwater fishes contains creatine, unidentified nitrogenous compounds some of which are amino-acids, little amount of urea and ammonia.The urine is copious and has very low in concentration of electrolyte. Urine contains nitrogen amounting to 2 to 25% of total nitrogen excreted by freshwater fishes. The bulk is removed out through gills as ammonia.Marine fishes produce scanty urine, which contain Ca^{++}, Mg^{++}, SO_4^{--}, SO_4^{--} and PO_4^{--}. In addition to the creatine, creatinine and TMAO (i.e., tri-methylamine oxide) are also excreted out. Ammonia, urea and monovalent electrolytes (Na^+, Cl^-), however, are excreted mainly through gills.

Osmoregulation in Fishes

Life in an aqueous medium poses many challenges for fishes. Furthermore, the greatest challenge to the fishes is the maintenance of water and electrolyte homeostasis in the face of a broad (and sometimes rapidly changing) array of salinities. To maintain solute concentrations within limits compatible with life, fish have developed remarkable strategies for osmoregulation. According to habitat, fishes can be distinguished as marine and fresh water.The marine fishes fall into two distinct groups, (a) Those whose osmotic concentration is the same as or slightly above sea water, e.g., hagfish, elasmobranchs, Latimeria etc. This group has no major problem of water balance, because its inside and outside concentrations are equal, there is no osmotic water flow, (b) Those whose osmotic concentrations are about one third of that of sea water, e.g., lampreys, teleosts, etc. These are hyposmotic animals. They

live in constant danger of losing water to the osmotically more concentrated medium.The fresh water fishes, on the other hand, have internal concentrations greater than that of their external medium. Thus, they are hyperosmotic to the medium. Therefore, the osmotic problems and the means to solve them differ drastically among fishes of different habitats.

Osmoregulation in teleost fishes, whether they live in freshwater or sea, its physiological activity is very closely related to their survival, yet in-spite of the importance of osmoregulation surprisingly little is known about how fish deals with physiological problems inherent in living in hypo-osmotic and hyperosmotic environments.The ability of some fish (e.g., .salmon) to regulate in both environments during migration is of great interest. The classical review of osmoregulation in aquatic animals has been done by Krogh (1939), and Pyefinch (1955).

In fishes the kidneys play an important role in osmoregulation, but major portion of the osmoregulatory functions are carried out by other organs such as the gills, the integument and even the intestine. Osmoregulation may be defined as **"the ability to maintain a suitable internal environment in the face of osmotic stress".**
As a consequence there is always difference between the optimal intracellular and extracellular concentrations of ions. In the fish body, number of mechanisms takes place to solve osmotic problems and regulate the difference.

Of which most common are:

(i) Between intracellular and extracellular compartment

(ii) Between extracellular compartment and the external environment. Both are collectively called 'osmoregulatory mechanisms', a term coined by Rudolf Hober.

Problems of Osmoregulation:

Generally fish lives in an osmotic steady state in spite of frequent variations in osmotic balance. That is, on the average, the input and output being equal over a long period sum up to zero (Fig. 10.1).

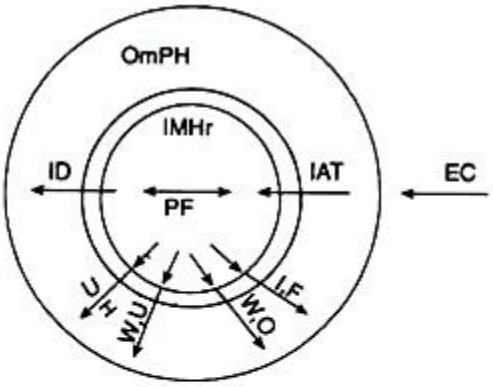

Fig. 10.1 : Principal process of osmoregulation in freshwater fishes. EC, ecological condition; I, ion; IAT, ion active transfer; ID, ion diffusion; IMHr, inner medium hypertonic; O, osmosis; OmPH, outer medium hypotonic; PF, physiological factor; U, urine; W, water.

The osmotic exchanges that take place between the fish and its environment may be of two types:

(i) Obligatory Exchange:

It occurs usually in response to physical factors over which animal has little or no physiological control and

(ii) Regulatory Exchange:

These are the exchanges which are physiologically well controlled and help in the maintenance of internal homeostasis.

3. Factors Affecting Obligatory Exchanges:

i. Gradient Between the Extracellular Compartment and the Environment:

The greater the ionic difference between the body fluid and external medium, the greater the tendency for net diffusion to low concentrations. Thus, a bony fish in a sea water is affected by the problem of losing water into the hypertonic sea water.

ii. Surface/Volume Ratio:

Generally the animal with small body size desiccates (or hydrates) more rapidly than a larger animal of the same shape.

iii. Permeability of the Gills:

Fish gills are necessarily permeable to water and solutes as they are the main site of exchange of oxygen and carbon dioxide between the blood and the water. Active transport of salts also takes place in the gills. Euryhaline fishes (who have tolerance of wide range of osmolarity) are well adapted to saline water by reduced permeability to water.

iv. Feeding:

Fishes take water and solute along with the feeding. A gill takes high quantity of salt than water at the time of feeding on seashore invertebrates, these fishes, therefore, must have some special device to excrete excess of salt. However, a freshwater fish ingests large amount of water than salt and thus needs special means of salt conservation.

Osmoregulators and Osmoconfirmers:

Osmoregulators are those animals who can maintain the internal osmolarity different from the medium in which they live. The fishes, except the hagfish which migrates between fresh and saline waters, the changing osmotic stress due to environmental changes is overcome with the help of endocrine mechanism (Table 1).

Table 1: Approximate composition of extracellular fluids of teleostean fishes (concentration in millimoles per litre of water)

Species	Habitat	Milli	Na^+ mole	K^+	Ca^{++}	Mg^{++}	Cl^-	SO_4^{--}
Paralichthys (flounder)	Sea-water	337	180	4	3	1	160	0..2
Carrassius	Fresh-water	293	142	2	6	3	107	–

Osmoconformers are those animals who are unable to control osmotic state of their body fluids but confirms to the osmolarity of the ambient medium. Majority of fishes either live in freshwater or in salt water (a few live in brackish water).Due to various physiological processes, metabolic wastes are removed from the body in vertebrates by gut, skin and kidneys. But in fishes and aquatic animals their gills and oral membranes are permeable both to water and salts in marine environment, salt is more in water against the salt inside the body fluid, hence water moves out due to the process of 'osmosis'.

The 'osmosis' may be defined as **"if two solutions of different concentrations are separated by a semipermeable membrane, the solvent from the less concentrated part will move through the membrane into more concentrated solution."** Hence to compensate the loss of water marine fishes drink water.

The salt will enter the body due to concentration gradient and so salt will be more inside the body. On the other hand, in freshwater fishes, the salt will go out to the environment as the salt concentration will be more inside the body fluid. The water will move inside the body due to osmosis through partially permeable membrane.

This means solvent will pass into more concentrated solution, but solute will also pass in the opposite direction. There will be, however, a difference in the rate dependent upon the relative permeability for two types of molecules usually solvent pass rapidly.

According to habitat, fishes can be distinguished as (i) Marine, and (ii) Fresh water.

(i) The marine fishes fall into two distinct groups, (a) Those whose osmotic concentration is the same as or slightly above sea water, e.g., hagfish, elasmobranchs, Latimeria etc. This group has no major problem of water balance, because its inside and outside concentrations are equal, there is no osmotic water flow, (b) Those whose osmotic concentrations are about one third of that of sea water, e.g., lampreys, teleosts, etc. These are hypoosmotic animals. They live in constant danger of losing water to the osmotically more concentrated medium.

(ii) The fresh water fishes, on the other hand, have internal concentrations greater than that of their external medium. Thus, they are hyperosmotic to the medium. Therefore, the osmotic problems and the means to solve them differ drastically among fishes of different habitats. A list of some fishes with habitat and osmotic characteristics is given in Table below.

Table 8.9 : Concentrations of major solutes (in millimoles per litre) in sea water and in the blood plasma of some aquatic vertebrates

	Habitat	Solute			Osmotic concentration (mOsm/litre^{-1})
		Na	K	Urea[a]	
Sea water		~450	10	0	~1000
Cyclostomes					
Hagfish (*Myxine*)	Marine	549	11		1152
Lamprey (*Petromyzon*)	Marine				317
Lamprey (*Lampetra*)	Fresh water	120	3	<1	270
Elasmobranchs					
Ray (*Raja*)	Marine	289	4	444	1050
Dogfish (*Squalus*)	Marine	287	5	354	1000
Fresh-water ray (*Potamotrygon*)	Fresh water	150	6	<1	308
Coelacanth (*Latimeria*)	Marine	197	7	350	954
Teleosts					
Goldfish (*Carassius*)	Fresh water	115	4		259
Toadfish (*Opsanus*)	Marine	160	5		392
Eel (*Anguilla*)	Fresh water	155	3		323
	Marine	177	3		371
Salmon (*Salmo*)	Fresh water	181	2		340
	Marine	212	3		400
Amphibians					
Frog (*Rana*)	Fresh water	92	3	~1	200
Crab-eating frog (*R. cancrivora*)	Marine	252	14	350	830[b]

a When no value is listed for urea, the concentration is of the order of 1 mmol per liter and osmotically insignificant. Values for ray, dogfish, and coelacanth include trimethylamine oxide

b Values for frogs kept in a medium of about 800 mOsm per liter, or four-fifths of normal sea water

A. Osmoregulation in Cyclostomes (Lampreys and Hagfishes):

The cyclostomes have two groups, Lampreys, which are anadromous, i.e. live both in sea and in fresh water, while Hag- fishes are strictly marine and stenohaline. The Lampreys, weather fresh-water or marine, have osmotic concentrations about one-quarter to one-third the concentration of sea-water. Their main problem is similar to that of teleost fish. The hagfishes are the only true vertebrates whose body fluids have salt concentrations similar to that of sea-water. In fact, the normal Na^+ concentration in hagfish blood exceeds that in their surroundings. Therefore, they have pronounced ionic regulation as an isosmotic animal.

B. Osmoregulation in Marine Elasmobranchs:

The common examples of marine elasmobranchs are sharks and rays. The salt concentration in their body fluid is roughly one- third the level of the sea-water, but they still maintain osmotic equilibrium. This is achieved by adding to the body fluids large amount of organic compounds primarily urea. Addition of different organic compounds in the body fluid/blood makes the osmotic concentration equal or slightly above the sea-water. In elasmobranchs urea is a normal component of all body fluids; this is abnormal for other vertebrates. In marine elasmobranchs, the tissue cannot function normally in the absence of such a high urea concentration. Urea is the end product of protein metabolism in vertebrates. Generally it is excreted through kidney, but the shark kidney actively reabsorbs this. The use of urea for maintaining osmotic equilibrium helps these animals to keep salt concentration much lower than those of sea-water. But urea can pose problems in the body functioning. It is known that urea destabilizes many proteins, especially enzymes. This problem is solved in elasmobranch by the presence of another organic substance trim-ethylamine (TMAO). TMAO inhibits the effect of urea on enzymes.Although the elasmobranchs have solved the osmotic problem of life in the sea by being isosmotic, they are still capable of extensive ionic regulation. They have sodium concentration much lower than that of sea-water. This means that sodium tends to diffuse from sea-water into the body.Sodium enters into the body primarily through the thin epithelium of the gill and then through the ingested food. This enhances the sodium level in the body, which must have to be excreted or eliminated from the body. Part of the sodium excretion is undertaken in the kidney. Major excretion of Na^+ is performed by a special rectal gland. This is a small gland that opens via a duct into the posterior part of the intestine, the rectum. The gland secretes a fluid with high sodium and chloride concentrations, which is higher than the sea-water concentration.The elasmobranch blood is usually slightly more concentrated than sea-water. This higher concentration inside causes a slight osmotic inflow of water via the gills. In this way, the elasmobranchs slowly gain water osmotically, and this water is used for the formation of urine and for the secretion of rectal gland.The excess osmotic concentration is due to the presence of urea. But retention of urea solves the otherwise difficult osmotic problem of maintaining a low salt concentration while living in sea.

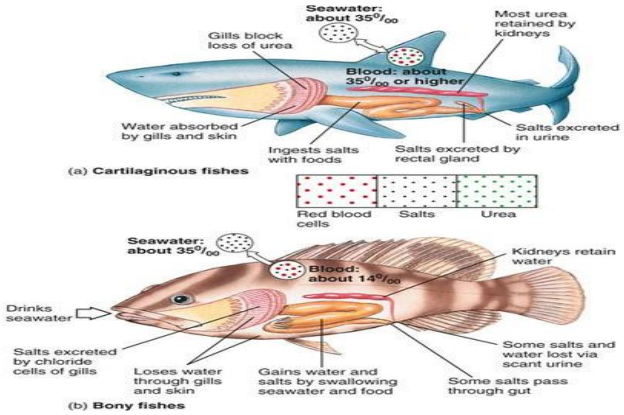

Fig: Osmoregulation in marine elasmobranch and bony fishes

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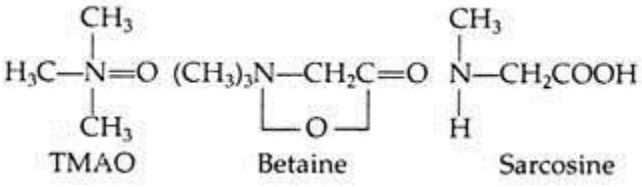
Some other urea inhibiting compounds are betaine and sarcosine:



Although the elasmobranchs have solved the osmotic problem of life in the sea by being isosmotic, they are still capable of extensive ionic regulation. It is clear from Table 8.9, that they have sodium concentration much lower than that of sea-water. This means that sodium tends to diffuse from sea-water into the body.Sodium enters into the body primarily through the thin epithelium of the gill and then through the ingested food. This enhances the sodium level in the body, which must have to be excreted or eliminated from the body.

Part of the sodium excretion is undertaken in the kidney. Major excretion of Na^+ is performed by a special rectal gland. This is a small gland that opens via a duct into the posterior part of the intestine, the rectum. The gland secretes a fluid with high sodium and chloride concentrations, which is higher than the sea-water concentration.

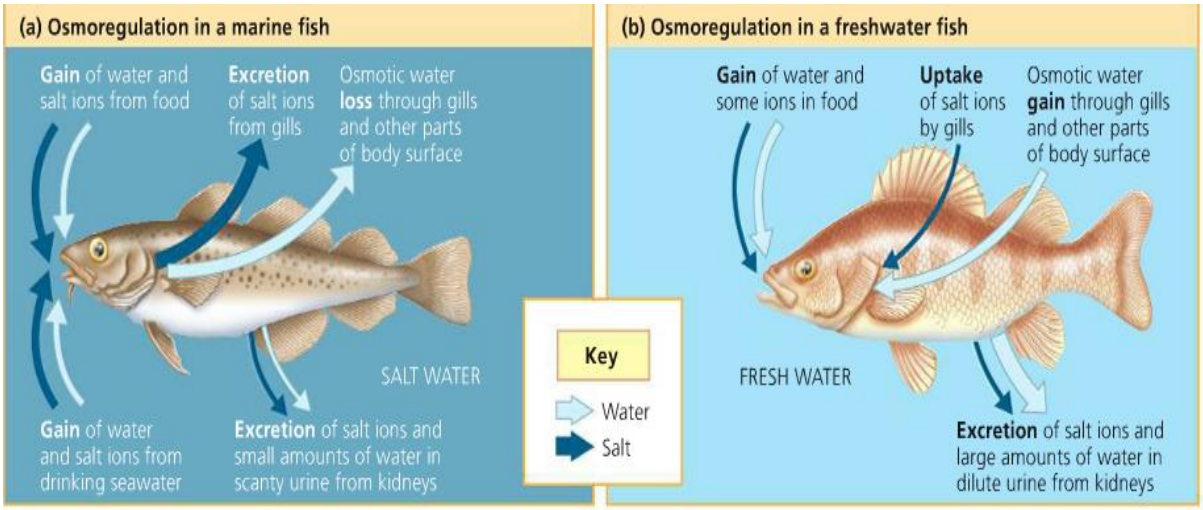
The elasmobranch blood is usually slightly more concentrated than sea-water. This higher concentration inside causes a slight osmotic inflow of water via the gills. In this way, the elasmobranchs slowly gain water osmotically, and this water is used for the formation of urine and for the secretion of rectal gland. The excess osmotic concentration is due to the presence of urea. But retention of urea solves the otherwise difficult osmotic problem of maintaining a low salt concentration while living in sea.

C. Osmoregulation in Freshwater Elasmobranchs:

Majority of elasmobranchs are marine, few of them are also found in freshwater. Some marine species occasionally enters rivers and lakes for various purposes. Among freshwater elasmobranchs, *Carcharhinus leucas* of Lake Nicaragua, four species of elasmobranchs of Perak River in Malaysia and Amazon sting ray Potamotrygon, are remarkable.Their blood concentrations are lower than those of strictly marine forms. The urea concentration is reduced to less than one third of the value of marine sharks.The problem of osmotic regulation is reduced due to the low level of solutes in the blood. The osmotic inflow of water is diminished because lower salt concentration is easier to maintain. The reduced osmotic inflow of water gives less water to be eliminated by the kidney.The urine always contains some solutes; therefore, a low urine flow reduces the urinary salt losses.

Fig: Osmoregulation in marine and freshwater fish

D. Osmoregulation in Teleosts:



Teleost fishes are living both in marine and freshwater. Both types of fishes maintain their osmotic concentration at about the quarter to one-third the level in sea-water. There is another type of fish, which roams both in sea water and fresh water. Therefore, can tolerate a wide range of salinities. These movements are often associated with the life cycle, such as breeding. But this change from one environment to the other requires profound changes in the osmoregulatory processes.

i. Osmoregulation in marine teleosts:

Marine fishes are hypoosmotic with the environment. Their main problem is losing body water to the more concentrated sea-water. The body water comes out through their body surfaces, in particular the large gill surfaces. The gill surface is more permeable to water than general body surface. These fishes compensate their inevitable water loss by drinking sea-water.Drinking of sea-water may restore the water content of the body, but impose another problem. Along with the sea-water, large amount of salts are also ingested and absorbed through the intestine,so salt concentration of the body increases.Now the problem becomes elimination of excess salt. To solve the problem, salts must be excreted in a higher concentration than in the water taken in. The teleost kidney cannot serve this purpose,

because it cannot produce urine that is more concentrated than the blood.Therefore, some other organ must participate in solving this problem of eliminating excess salts. This is done by the gills. So gills have dual function—one, participation in osmoregulation and second, gas exchange. The secretion of salt through gill is an active process, i.e., energy mediated. It takes place from a lower concentration in the blood to a higher concentration in the surrounding medium.The excretion of sodium and chloride in the urine is of minor importance because teleost urine is more dilute than the body fluids.However, the kidney plays a major role in the excretion of divalent ions, magnesium and sulphate. These ions are not eliminated by the gills, which seems to transport only sodium and chloride.

Further studies show that the salt intake is happening not only through drinking sea-water, but also through the general body surface. It is also proved that fish adapted to sea-water are relatively permeable to ions and those adapted to fresh water are relatively impermeable.The ion transport is carried out, not by the general epithelial cells of the gills, but specifically by some large cells known as chloride cells. These cells are also present in the opercular cover of the fish. These cells actively transport chloride ions.

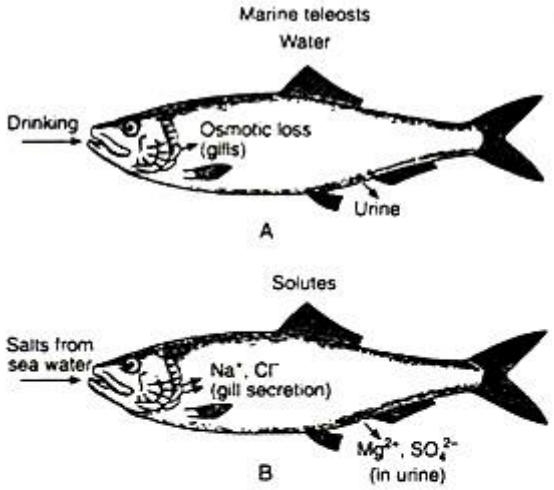


Fig. 8.38 : A marine teleost is osmotically more dilute than the water in which it lives. A. Because of the higher osmotic concentration in the medium, the fish constantly loses water, primarily across the thin gill membranes. Additional water is lost in the urine. B. To compensate for the water loss, the marine teleost drinks substantial amount of sea water. Of the ingested salts, sodium and chloride are absorbed in the intestine and eliminated via the gills by active transport (double arrow, B); magnesium and sulfate are excreted by the kidney

ii. Osmoregulation in fresh-water teleosts:

The osmotic concentration of the blood of fresh water teleosts in much higher than the surrounding water (~300 mOsm/litre). Therefore, their major problem is the osmotic water inflow. Water mainly enters through the highly permeable gills. In freshwater teleosts skin is less important in transporting water inside the body, because it is less permeable.The large volume of water is excreted as urine, which is very dilute and may be produced in quantities up to one-third of the body weight per day. The urine contains 2 to 10 mmol/litre of solutes. Large urine volume also causes a substantial loss of solutes.This loss is replaced by the gills, which is also slightly permeable to ions. Some solutes are taken in with the food, but the main intake is by active transport in the gills. It is evident from the studies that skin plays only a minor, if any, role in active absorption.

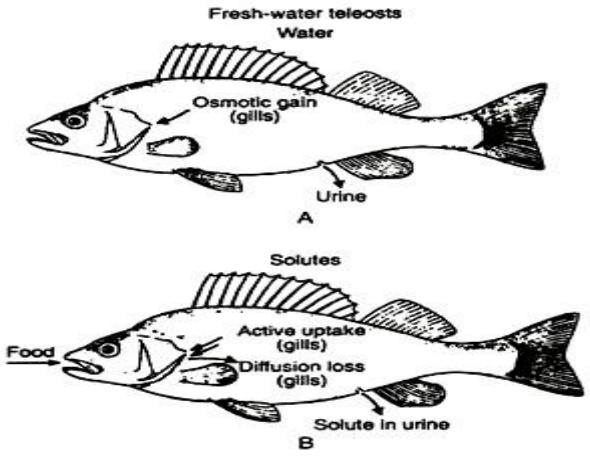


Fig. 8.39 : A fresh-water teleost is osmotically more concentrated than the medium and, therefore, suffers a steady osmotic influx of water, mainly through the gills (A). The excess water is eliminated as urine. Loss of solutes through the gills and in the urine is compensated for primarily through active uptake in the gills (double arrow, B)

Controls of Osmoregulation:

The concentration and dilution of urine is controlled by hormones, which affects the rate of renal filtration by changing the blood pressure and thus control the quantity of urine. Hormones also influence the rate of diffusion and absorption across the gill epithelium. Thyroid gland and suprarenal bodies secrete adrenocortical hormones which control osmoregulation in fishes.

Excretory System in Fish

In vertebrates, the excretory and reproductive organs are morphologically interrelated because certain excretory ducts are used for the discharging of gametes also. So it has been convenient to treat them together as urinogenital system.

In fishes, the association is restricted to the pseudo-copulatory papilla through which both the excretory and generative products leave by a common vent. The association is more intimate in male than in female. Here, these systems are dealt separately as excretory and reproductive organs.

The excretory organs consist of kidneys, ureters and urinary bladder. The urinary bladder is not homologous to that of higher vertebrates (Fig. 11.1a-e).

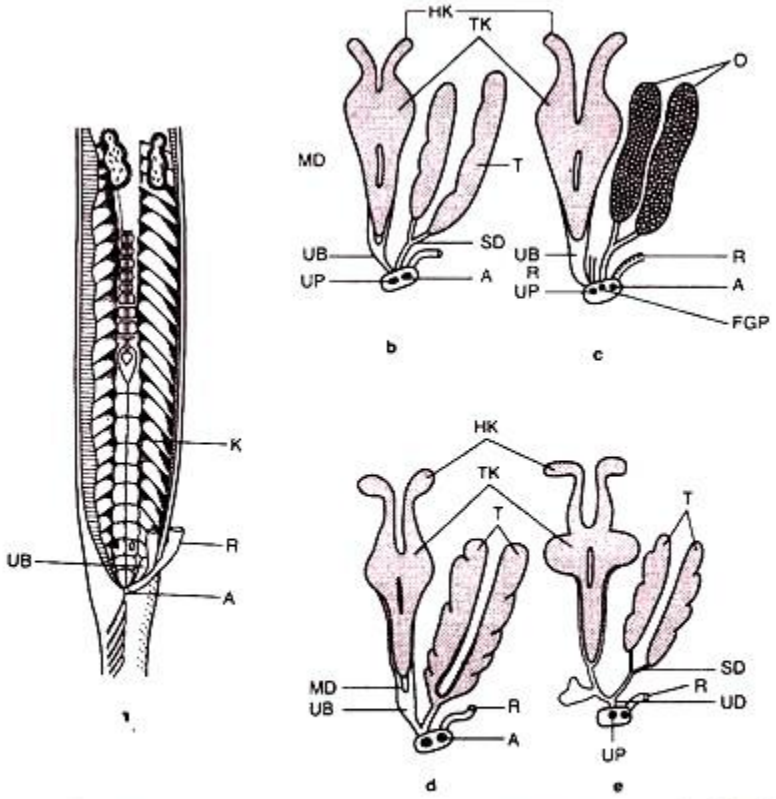


Fig. 11.1a-e : Excretory system of (a) *Xenentodon* (b) *Cirrhina* (male) (c) *Cirrhina* (female) (d) *Labeo* (male) (e) *Barbus* (male). A, anus; FGP, female genital pore; HK, head kidney; MD, mesonephric duct; O, ovary, R, rectum; SD, sperm duct; T, testis; TK, trunk kidney; UB, urinary bladder, UD, urinary duct; UP urinogenital pore.

Kidney:

Kidneys of vertebrates are made up of nephron or kidney tubules. In ancestral vertebrates, kidney possesses one nephron for each of those body segments that lay between the anterior and posterior end of the coelom. The nephron drained into a duct called Wolffian or archinephric duct located posterior to the cloaca. This sort of kidney is known as holonephros because it extends to the entire length of the body. The holonephros is found today in the larvae of certain cyclostomes but not in any adult. In fish and amphibians the most anterior tubules have been lost, some of the middle tubules are associated with tests and there is a

concentration and multiplication of tubules posteriorly.Such a kidney is known as a posterior kidney or opisthonephros. Generally in fishes, the tubules of the anterior region become functional in early life and are designated as pronephors and the tubules present in the posterior regions take up excretory function throughout life. This region of the functional tubules is known as mesonephros (Fig. 11.2a, b).

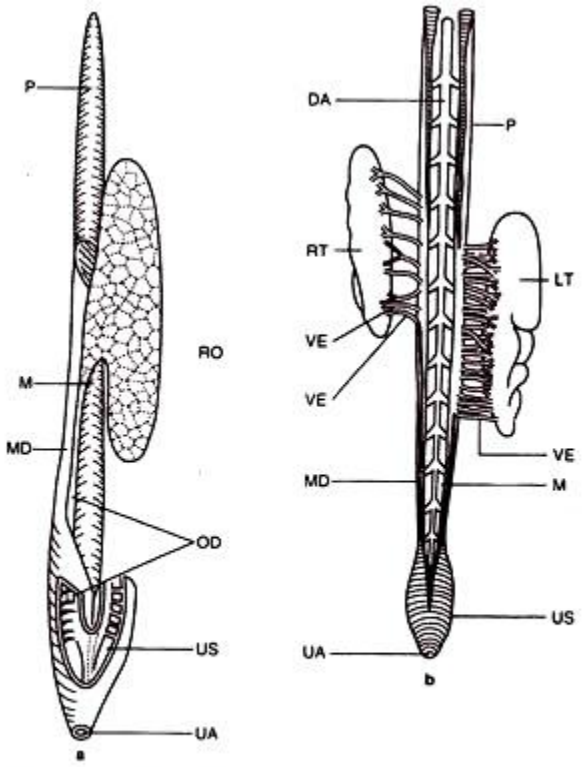


Fig. 11.2a, b : Diagram showing relationship between gonads and excretory organs in (a) Female *Lepisosteus*. (b) Male *Lepisosteus*. DA, dorsal aorta; LT, left testis; M, mesonephros; MD, mesonephric duct; OD, oviduct; P, pronephros; RO, right ovary, RT, right testis; UA, urogenital aperture; US, urogenital sinus; VE, vasa efferentia.

There are great variations in the external structure of the kidney in fishes. The shape varies according to species. The kidneys occupy dorsal position in the body cavity and is placed just ventral to the vertebral column. In teleost, the kidney is distinguished into head and trunk regions.

Such distinction is clearly discernible in carps, but in other fishes the macroscopic differentiation into head and trunk kidneys is not prominent. Head kidney is non-excretory and endocrine in function whereas trunk kidney (posterior kidney) is excretory in nature. So the kidneys of fishes are peculiar in comparison to other vertebrates.

The peculiarities are mentioned as follows:

1. The head kidney is endocrine in nature. It has inter-renal gland homologous to adrenal cortex of mammals. It has chromaffin cells also which are similar to the adrenal medulla of mammals.

2. In the kidneys are embedded yellow bodies called corpuscle of Stannius. It is endocrine in function. These bodies are visible macroscopically in some fishes while in others they are intermingle in the kidney tissues.

3. Head kidney is the site for the development of the blood.

4. Both head and trunk kidneys contain heterotopic thyroid follicles. On the basis of morphology and distinction between head kidney and trunk kidney, Ogawa (1961) classified marine teleost kidney into five categories.

In the first category there is no clear demarcation between head and trunk kidneys and the two kidneys are completely fused throughout (Fig. 11.3a). e.g., rainbow trout and salmon.

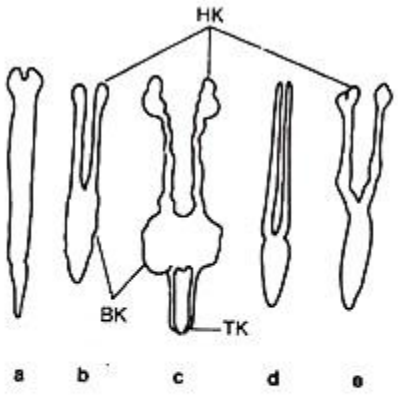


Fig. 11.3a-e : External view of teleostean kidney. (a) rainbow trout. (b) *Ayu*. (c) carp. (d) Eel. (e) yellow tail; BK, body kidney, HK, head kidney; TK, tail kidney.

In the second type there is clear macroscopic demarcation between the head and trunk kidneys. The middle and posterior portions are fused. From the middle fused part are given out two tube like structures anteriorly, which are separate from each other and at the tip of these tubes are present sac-like structure, the head kidneys, e.g., Ayo, Cyprinidae and carps.

In the third type, there is also a clear distinction between the head and trunk kidneys. The kidney is distinguished into head, trunk and tail portions. The tail kidneys are fused, while the trunk and head kidneys are separated and are located at the tip of the anterior most region. The head kidney, is generally globular in shape, e.g., *Notopterus notopterus*. In the fourth type there is no morphological demarcation between head and trunk kidneys. The two kidneys are separate except at the posterior most region where the kidneys are fused.

In the fifth type, the two kidneys are completely separate from each other. The tail kidneys are thin tube-like while the anterior trunk kidneys are thick. There is no morphological distinction between head and trunk kidneys. Regarding freshwater teleost, Ogawa (1961) suggested that kidneys can be grouped into first three of the five types described above.

Kidneys of Some Indian Fishes:

The kidneys of *Clariasbatrachus* are situated in the abdominal cavity. It is placed in retroperitoneal position against the ventral aspects of the vertebral column and dorsal to the alimentary canal and the gonads. They are dark reddish brown in colour and is covered by a

thin transparent membrane. The trunk kidneys are fused throughout their length. They are broadened anteriorly and become gradually narrow posteriorly.

The head kidney are in the form of two triangular lobes which are separated from each other by a very narrow gap. They are not connected with the trunk kidney in *Clarias batrachus*. The triangular head kidneys is present in *Channa marulius, Channa punctatus and Channa gachua*.

The apex of each lobe is pointed and placed anteriorly, while the base is flat and posteriorly directed. The anterolateral side of the trunk kidneys on either side is provided with rounded structures and are known as mesonephric lobes (Fig. 11.4).

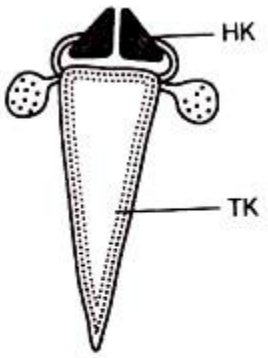


Fig. 11.4 : Structure of kidney of *Clarias batrachus*. HK, head kidney; TK, trunk kidney.

Mesonephric lobes are also present in *Heteropneustes fossilis*. In *Labeo rohita*, the kidneys are also paired structures. They are situated ventrally to the vertebral column. It is very clearly attached by the connective tissue. The peritoneal layer separates them. They are elongated structures which run from the vent and γ reach very close to the gills. They are reddish brown in colour.

The two kidneys are fused in the middle, forming a flattened wing-like middle portion of the trunk kidneys. From this wing-like structure, two tube-like structures are given out anteriorly which are separate from each other. At the anterior end of these tubes are present sac-like structures known as head kidneys.The two head kidneys are separate. Posteriorly, from the middle part of the trunk kidney, the kidneys become narrow. This part is called as tail kidneys. Externally, a depression is visible which indicates that they are two structures.

The tail kidneys are also fused. The trunk kidneys are very big in comparison to the length of a fish. Dorsally, the trunk kidneys show several annulations whereas ventrally it is smooth in outline (Fig. 11.5).

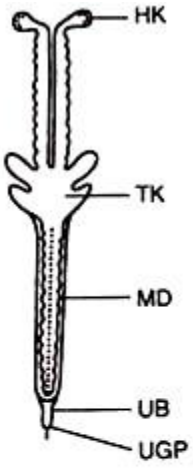


Fig. 11.5 : Morphology of kidney of *Labeo rohita*. HK, head kidney, MD, mesonephric duct; TK, trunk kidney, UB, urinary bladder, UGP, urinogenital pore.

In *Xenentodoncancila*, the trunk kidney in its anterior region shows ladder-like appearance.

Ureter:

The mesonephric ducts or ureters lie closed together in the median line. Anteriorly, they are separate, posteriorly the two mesonephric ducts open separately into urinary bladder. In some species a sac-like enlargement is clearly visible in the posterior region of the ureter.This is known as urinary bladder, but it is not homologous to that of higher vertebrates. The urinary bladder usually opens to the exterior by a common urinogenital aperture in the' male fish but a separate urinary aperture is present in female fish as found in *Mystus*.

Histology of Trunk Kidney:

The trunk kidney or body kidney, like other vertebrates, contains renal tubules (nephrons) and interstitial lymphoid tissue. The number of renal tubules varies in different fishes. In teleosts, the trunk kidney consists of a large number of nephrons. The functional unit of kidney is nephron.Each nephron consists of two parts, the renal corpuscles (Malpighian body) and the renal tubule (urinary tubule), the renal corpuscle or Bowman's capsule is double layer cup-like structure of uriniferous tubule which contains tuft of capillaries known as glomerulus (Fig. 11.6).

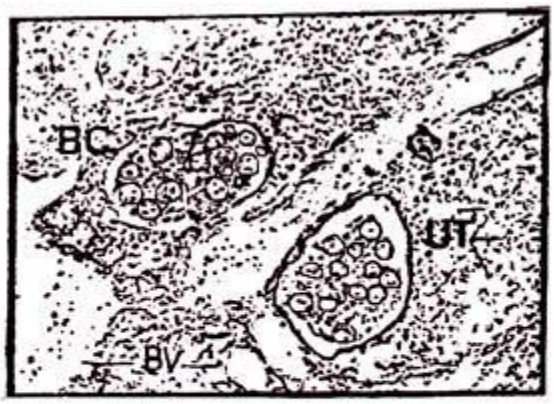


Fig. 11.6 : Microphotograph of transeverse section of kidney of *Clarias batrachus*. X 320. BC. Bowman, capsule; BV, blood vessel; G, glomerulus; UT, uriniferous tubule.

The remaining segment of urinary tubule (renal tubule) is divided into proximal convoluted segment (which further divides into segment I and segment II), the intermediate and distal segments (Fig. 11.7a, b, c).

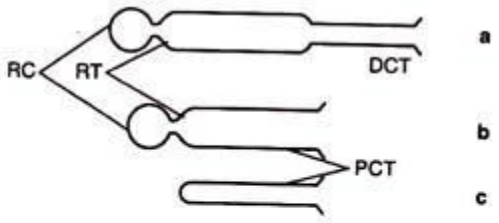


Fig. 11.7a-c : Structure of kidney of teleosts. (a) Freshwater fish. (b) Marine fish. (c) Aglomerular fish. DCT, Distal convoluted tubule; PCT, Proximal convoluted tubule; RC, Renal corpuscles; RT, Renal tubule.

The distal segment is absent in marine fishes. The segment of Henle found in higher vertebrates is also absent in the fishes. The glomerulus and Bowman's capsule together constitutes the renal or Malpighian capsule. It is a filtration apparatus of kidney.

The glomerular capillaries, which are the vascular part of corpuscle, is the afferent arteriole which divides and forms capillary loops. The loops reunite and leave the capsule as efferent arteriole. (Fig. 11.8).

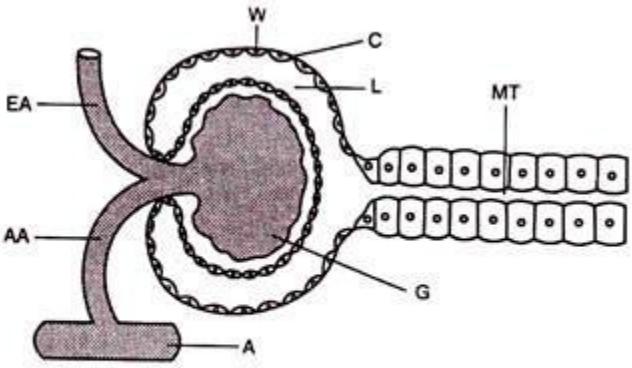


Fig. 11.8 : Diagram of renal corpuscles of fish. A, aorta; AA, afferent arteriole; C, capsule; EA, efferent arteriole; G, glomerulus; L, lumen; MT, mesonephric tubule; W, wall.

The renal corpuscle contains an additional group of cells known as mesangial cells. Mesangial cells are also present in the space between the loops of glomerular capillaries. They are most obviously present at the vascular stalk.The function of mesangial cells are not known, although experimental data show that they can remove large proteins from the glomerular basal lamina. Oguri (1982) reported the presence of juxtaglomerular cells in the wall of afferent arterioles. These cells contain secretory granules and are specialized muscular cells.They are the source of the hormone renin. Renin is the hormone active in increasing blood pressure. In mammals, the juxtaglomerular apparatus is thought to provide feedback information for the control of glomerular filtration.

The glomeruli of freshwater teleosts are numerous and large in size. In marine teleosts, glomeruli are reduced in size and number. In extreme cases, the glomeruli disappear completely from the kidney of some marine fishes. The examples are Sea horse (*Hippocampus coronatus*), Pipefish (*Syngnathsschelegeli*) and frogfish (*Anternnarius tridens*), and these fishes are called aglomerular fishes. The renal tubules are thin and short in the neck segment and consist of single layer of low epithelial cells with long cilia. The proximal convoluted segment I and segment II are provided with cuboidal epithelial cells. The nuclei are large, round or oval. The cytoplasm contains secretary granules.The intermediate segment is well-developed in carp kidney but absent in several species of fishes. The distal convoluted segment could be distinguished because of coarse granules in the cytoplasm. The distal convoluted segments are absent in the kidney of marine fishes.

Ureter:

The function of the ureter is to conduct urine up to urinary bladder. Histologically, it is made up externally by tunica adventitia, the middle layer contains lamina propria and smooth muscles and outermost layer is columnar epithelial cells. Urinary bladder is a thin walled sac-like structure. It is also made up of three layers similar to the ureter.

Head Kidney:

Embryo logically the head kidney originates from pronephros. It is made up of lymphoid tissue containing reticular cells (supporting framework of lymphatic tissue) (Fig. 11.9) and numerous capillaries.

Fig. 11.9 : Microphotograph of transverse section of kidney showing lymphoidal tissue. X 320. BV, blood vessel; N, nerve; LT, lymphoidal tissue.

The chromaffin tissue often shows brown or dark brown pigment granules fixed in bi-chromate solution. The inter-renal gland and chromaffin cells are present in the head kidney. The name inter-renal body was adopted by Balfour in 1878 and since then commonly accepted. In *Xenentodoncancila*, the inter-renal body lies as a compact mass surrounded by a capsule of connective tissue.

It lies at a short distance behind the septum transversum and is an elongated mass about twice the size of a wheat grain. The cells are basophilic with large nuclei. The inter-renal gland of fish is homologous to the adrenal cortex of mammals.In eel (Anguilla japonica) the gland is located in the wall of the post-cardinal vein running closely along the head kidneys. Chromaffin tissues are also present, this tissue is homologous to adrenal medulla of mammals. Both inter-renal and chromaffin tissues are present in many fishes either discrete structure or intermingled in the tissue.

The histochemical examination of teleostean inter-renal cells shows 3-B hydroxysteroid dehydrogenase and glucose 6 phosphate dehydrogenase. These are important in biosynthesis of steroid hormones. The function of chromaffin cells is to secrete, adrenalin and noradrenalin whereas inter- renal secretes corticosteroid. Cortisole is the main corticoid in teleosts but cortisone and corticosteroid are also produced by inter-renal glands. Whether aldosterone is secreted by teleostean inter-renal gland is not clear.

Urine:

The freshwater teleosts have to excrete large amount of water which is taken through mouth. The urine of freshwater fishes contains creatine, unidentified nitrogenous compounds some of which are amino-acids, little amount of urea and ammonia.

The urine is copious and have very low in concentration of electrolyte. Urine contains nitrogen amounting to 2 to 25% of total nitrogen excreted by freshwater fishes. The bulk is removed out through gills as ammonia.Marine fishes produce scanty urine, which contain Ca^{++}, Mg^{++}, SO_4^{--}, SO_4^{--} and PO_4^{--}. In addition to the creatine, creatinine and TMAO (i.e., tri-

methylamine oxide) are also excreted out. Ammonia, urea and monovalent electrolytes (Na^+, Cl^-), however, are excreted mainly through gills.

Corpuscles of Stannius:

The corpuscles of Stanniusis a small ductless gland (endocrine gland) situated partly or completely embedded in the kidney on its dorsal, dorsolateral and ventrolateral side. It was discovered by Stannius (1939), but it is recently considered that they are the subject of physiological interest specially for their role in calcium metabolism.

It is now established that corpuscles of Stannius work in conjuncture with pituitary gland, which produces a distinct hypercalcemic effect, to maintain a relatively constant level of calcium in *Fundulusheteroclitus*. Idler and Freeman (1966) suggested that it is associated with steroidegenic activity, while Oguri (1966) reported that they are responsible in producing some hormone like polypeptides. Chaster Jones (1969) were of the opinion that they had pressor activities and helped in electrolyte metabolism. They also suggested that corpuscle of Stannius might belong to renin angiotensin system.

The corpuscles of Stanniusis situated near the middle portion of mesonephros in salmnoid fishes and *Altherninopsis calciforniensis* but in majority of fishes they are located in the posterior region of the kidney.

There is a great variation regarding the number of corpuscles of Stannius in the kidney, they may be single (Heteropneustessetani, Notoptarusnotopterus, Lepidocephalichthyes, quntea) or may be two in number (Lepadocephalichthyes) as described by Bose and Ahmad (1975).Histologically, the corpuscles of Stannius contain two types of cells while other authors held that there is only one population of cells.

Heterotopic Thyroid Follicle:

The thyroid in fishes is not a discrete organ but fused in the kidney also, hence it is known as heterotopic. The thyroid follicles are scattered in the haemopoietic tissue of the head, mesonephric lobe (air breathing fishes) and in trunk kidneys (Fig. 11.10).

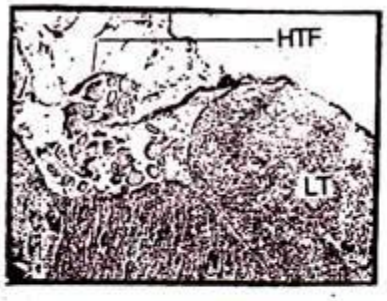


Fig. 11.10 : Microphotograph of transverse section of head kidney of *Clarias* showing heterotopic thyroid follicle. HTF, heterotopic thyroid follicle; LT, lymphoid tissue.

The follicular epithelium is visible and contains colloid which is strongly repeated acidophilic, dense, homogenous and non-vacuolated (Fig. 11.11). An aggregation of a large number of follicles has been present at the junction where the post-cardinal vein opens. It appears that the thyroid follicles are migrating from the pharyngeal region to the kidney.They appear only after the fish becomes two months old. They are present in all parts of the kidney, but the highest concentration is near the cardinal vein. Thick and thin nerves both myelinated as well as un-myelinated are present close to the heterotopic thyroid follicles.

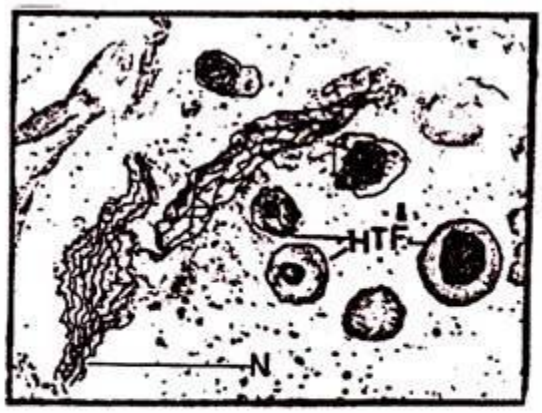


Fig. 11.11 : Microphotograph of transverse section of kidney of *Clarias* showing heterotopic thyroid follicle. X 320. HTF, heterotopic thyroid follicle; N, nerve.

Innervation:

The kidneys are richly innervated by autonomic nervous system. The nerves generally penetrate in the kidney through blood vessels. They divide and re-divide to form nerve plexuses (Fig. 11.12).

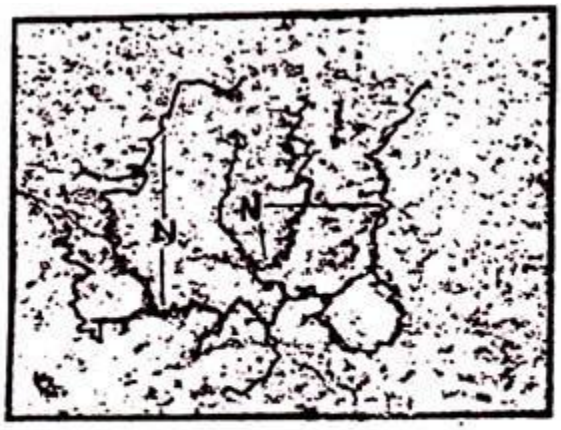


Fig. 11.12 : Microphotograph of section of kidney of *Clarias* showing innervation. X-320. N. Nerve.

Scattered ganglion cells are also present in the trunk kidneys. Both cholinergic and adrenergic nerves are present.

Hormones and Enzymes:

Renin is the hormone secreted from juxtaglomerular cells. The hormone is active in increasing blood pressure and controls the glomerular filtrate. The cholinergic nerve endings secrete an enzyme acetyl cholinesterase (AChE).

The enzyme kinetics of AChE of the head kidney of *Labeo* is 1.11 x 10^{-3} M and V_{max} is 0.222 A/mg proteins/30 minutes. While the Km of the middle portion of trurk and tail kidney is 3.33 x 10^{-3}M and V_{max} is 5.0 A/mg proteins/30 minutes. The lower Km in head kidneys indicates higher enzyme activity (Fig. 11.13).

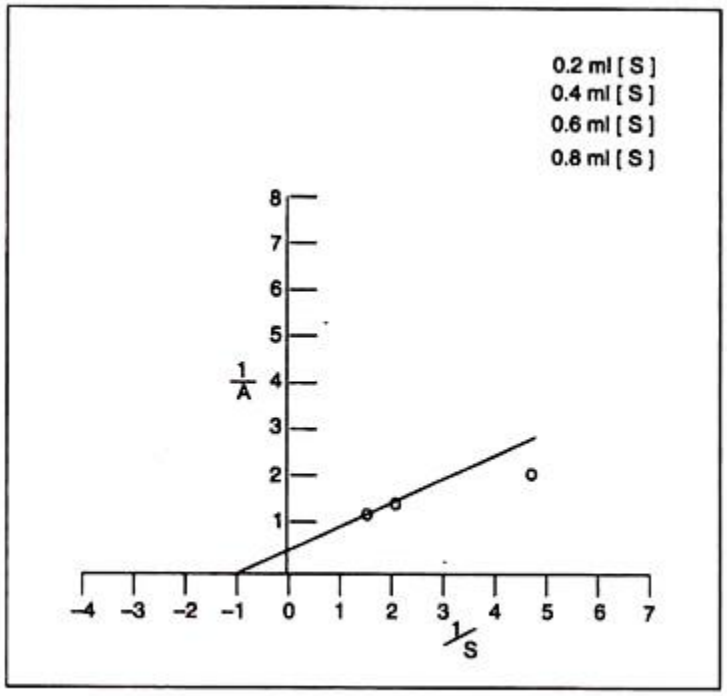


Fig. 11.13 : Lineweaver-Burk plot of AChE activity in the kidney of *Labeo rohita*.

Blood Supply of Kidney:

The kidney of fish receives blood supply by the renal artery and renal portal vein. The renal artery supplies blood to glomeruli, where high blood pressure helps to separate glomerular filtrate. The renal portal veins are connected to capillary network around the kidney

Probable Questions:

1. What are the excretory organs of fishes?

2. How excretion and urine formation occur in fish?

3. Describe osmoregulation in Cyclostomes.

4. Describe osmoregulation in Marine Elasmobranchs.

5. Describe osmoregulation in fresh water Elasmobranchs.

6. Describe osmoregulation in marine Teleost.

7. Describe osmoregulation in fresh water Teleost.

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Unit-XII

Reproduction in Fish:Reproductive strategies, oviparity, viviparity, ovoviviparity, maturity stages, breeding cycle.

Objective: In this unit you will learn about Reproductive strategies, oviparity, viviparity, ovoviviparity, parental care, maturity stages, breeding cycle of fish.

Introduction:

The methods of reproduction in fishes are varied, but most fishes lay a large number of small eggs, fertilized and scattered outside of the body. The eggs of pelagic fishes usually remain suspended in the open water. Many shore and freshwater fishes lay eggs on the bottom or among plants. Some have adhesive eggs. The mortality of the young and especially of the eggs is very high, and often only a few individuals grow to maturity out of hundreds, thousands, and in some cases millions of eggs laid.Males produce sperm, usually as a milky white substance called milt, in two (sometimes one) testes within the body cavity. In bony fishes a sperm duct leads from each testis to a urogenital opening behind the vent or anus. In sharks and rays and in cyclostomes the duct leads to a cloaca. Sometimes the pelvic fins are modified to help transmit the milt to the eggs at the female's vent or on the substrate where the female has placed them. Sometimes accessory organs are used to fertilize females internally-for example, the claspers of many sharks and rays.In the females the eggs are formed in two ovaries (sometimes only one) and pass from the ovaries to the urogenital opening and to the outside. In some fishes the eggs are fertilized internally but shed before development takes place. Members of about a dozen families each of bony fishes (teleosts) and sharks bear live young. Many skates and rays also bear live young. In some bony fishes the eggs simply develop within the female, the young emerging when the eggs hatch (ovoviviparous).Others develop within the ovary and are nourished by ovarian tissues after hatching (viviparous). There are also other methods utilized by fishes to nourish young within the female. In all live-bearers the young are born at a relatively large size and are few in number. In one family of primarily marine fishes, the surfperches from the Pacific coast of North America, Japan, and Korea, the males of at least one species appear to be born sexually mature, although they are not fully grown.

Some fishes are hermaphroditic, an individual producing both sperm and eggs, usually at different stages of its life. Self-fertilization, however, is probably rare. Successful reproduction and in many cases defence of the eggs and young is assured by rather stereotyped but often elaborate courtship and parental behaviour, either by the male or the female or both. Some fishes prepare nests by hollowing out depressions in the sand bottom (cichlids, for example), build nests with plant materials and sticky threads excreted by the kidneys (sticklebacks), or blow a cluster of mucus-covered bubbles at the water surface (gouramis). The eggs are laid in

these structures. Some varieties of cichlids and catfishes incubate eggs in their mouths.Some fishes, such as salmon, undergo long migrations from the ocean and up large rivers to spawn in gravel beds where they themselves hatched (anadromous fishes). Others undertake shorter migrations from lakes into streams or in other ways enter for spawning habitats that they do not ordinarily occupy.

Reproductive strategies in Fish:

Fish species have evolved reproductive methods and attempt physiology that allows them to be successful under a great variety of conditions. The entire combinations of habitat physiology and behaviour, the overall approaches to reproduction will be termed as reproductive strategies. Strategies may require greater number of eggs as mention or fewer eggs with greater opportunity for survival, strategies must ensure survival of a portion of the eggs through force of number, concealment, protection of nest or retention in the body; strategies must plays the earliest feeding stage of the young in the proximity of suitable and ample food and must ensure that the juvenile fish have eventual access to the living species of the adults. There are numerous ways to categorise fishes as to their reproduction, for instances, the simple breakdown into egg layers (oviparous conditions) and live bearers (ovoviviparous, viviparous).

a. Oviparity:

Oviparity or egg laying refers to the situation where the development or the fertilized of egg occurs outside the body of the female i.e. external fertilization. The young hatch when the egg envelope, shell or capsule is broken. Most fishes lay eggs that are heavier than water (Dermal eggs) but some may produce buoyant eggs that may be hydrostatically adjusted by oil inclusion, (embedded water in a large perivitelline space) or a high ratio of surface to volume to float at the surface or at some intermediate depth. Eggs of some species move freely , those of some others attached to each other or to the vegetation by means of tendrils. Demersal egg may be adhesive and deposited in clumps, sticking together through the incubation period. Some fishes engage in mass spawny with no pairing. Numerous males and females release gametes together in a suitable medium. This method of reproduction requires less energy, as the developing embryo depends completely on a concentration of nutrients within the egg called the yolk. This allows more eggs to be produced in some cases, but this lack of parental investment also reduces the offspring's likelihood of survival. Oviparous fish may be further categorized as being either ovuliparous or zygoparous.

Ovuliparity refers to the release of ova from the reproductive tract of the female followed by fertilization or activation in the external environment. Thus, all organisms that have external fertilization and this includes most teleost are said to be ovuliparous. Zygoparity refer to the oviparous condition in which the zygotes (i.e. fertilized ova products of fusion between the eggs and sperm) are retained within the body of the female for a short period of time before being released into the environment. Obviously, zygoparous species display internal fertilization with their being a transfer of male sperm to the reproductive tract of the female. zygoparous reproduction characterizes all skates some sharks and a small number of

teleost.Irrespective of whether the fertilization of the eggs occurs internally or the egg yolk provides externally the nutrient for the developing embryos of oviparous species.

Example: 90% Bony fishes such as Carps (*Labeorohita*, *Catlacatla*), catfishes and Horn sharkes, skates (rays) etc.

b. Viviparity:

Viviparity is a reproductive term in which female retain developing eggs inside their reproductive tract or body cavity and give birth to the off-springs capable of free living existence. Laying of the complete young adult animals, hence the mother delays to release the young individual. So long period is taken for the method of nourishment. Pseudoplacenta which found in expanded pericardial sac, internal fertilization and maternal nourishment occur. Several possible advantages are conferred by this pattern, the first of this is protection. The eggs and embryos are safe from predators.They are protected from adverse water condition, desiccation, anoxia and injurious temperature. There is no need for large number of eggs. Usually fertilization is ensured, so that few eggs are wasted.

Example: Most selachii (themajority of sharks, stingrays, eagle rays, and giant rays) are vivi parous fish.Among bony fish, viviparity is a characteristic ofeelpouts (*Zoarces viviparous)*, Bas s (*Sebastes*), Baikal oil-
fish (*Comephorus baicalensis*),many fish of the familyCyprinodontidae(foureyed fish,*Anableps tetrophthalmus),* and some freshwater fish of the family Hemirhamphidae.

c. Ovoviviparity:

It is intermediates between ovoparity and viviparity. Fish do not release eggs or direct young adults. But eggs sometime remain within the body and release larvae. From the short term retention of eggs to incubating and hatching them internally is only a short step. Many species of frog fish(Family-Scorpaenidae) are ovoviviparous, releasing newly hatched larvae. The rock fishes so little by sexual morphism and have no particular specialization of the ovary where the eggs may remain in incubation. In genus *Dinematichthys* eggs hatch from the ovarian follicles and develop into advance.Larvae in the lumen of ovary in Poeciliidae the young are retained until the juvenile stage is reached.Young remains in the follicle of ovary with sufficient yolk for development.

Breeding cycle:

Breeding cycle means the frequency of spawning. In most cases of animals breeding cycle is cyclic or more or less regularly periodic in nature in fishes. Some fishes survive for short period of life and they breed only once.

1. Silverside (*Labidesthessicculus*): Some species survive for moderately long period but still breed only once in two or five years.
2. *Onchorhynchus* sp. (Pacific Salmon): Breed only once 2-5 years of the life span.
3. Sea lamprey (*Pteromyzon*): Breed once in 5-6

4. *Anguilla* (Freshwater eel): Breed once in 10-14 years
5. *Cyprinuscarpio*: Breed two times in a year and reproduction is very frequent.
6. *Lebistis reticulates:* They breed approx in every 4 weeks or once in a month.
7. Indian major carp: Breed once in a year.
8. Telapia *(Oreochromis mossambica):* Breed 3 times in a day.

Maturity Stages:

Determination of maturity stages record of the stage of maturity of fish examine is often required, since this bears on the condition factor and length weight relationship and may explain what otherwise might appear to be anomalous data. It is also important to know the average size or age at first maturity. One should therefore determine whether each fish is sexually mature, immature, ripe or spent.Lagler (1956) gave clear instruction on the practical procedure for fish living in temperate regions. During the breeding season the reproductive organ should be classified as immature, ripe or spent. Immature means that,there are no easily visible eggs or milt. Ripe means that the gonads contain obvious eggs or sperms and spent means that the fish has spawned. The ovaries of recently spawned fish are often flaccid and blood shots. During the remainders of the year, the fish should be classified as immature, with no egg or milts present or mature when eggs or sperms are clearly apparent.More complex maturity classification have been suggested by Kesteven (1960) based on Buckman (1929) and by Nikolsky (1963)

From Kesteven (1960)	**From Nikolsky (1963)**
1. Virgin:Very small sexual organ closeunder the vertebral column. Testis and ovaries transparent,Colourless to grey. Eggs invisible to naked.	1. Immature: Young individuals which have not yet engaged in reproduction; gonads of very small size.
2. Maturing virgin: Testis and ovaries translucent, grey-red colour. Length half or slightly more than half of the ventral cavity. Single eggs can be seen with magnifying glass.	2. Resting stage: Sexual products have not yet begun to develop. Gonads of very small sizes. Eggs not distinguishable to the naked eye.
3. Developing: Testis and ovaries opaque, reddish with blood capillaries, occupy about half the ventral cavity. Eggs visible to the eye as whitish granules. Testis reddish white. No milt drops appear under pressure. Ovaries orange, reddish.	3.Maturation: eggs distinguishable to the naked eye, a very rapid increasing weight of the gonad is in progress; testis change from transparent to a pale rose colour.

Eggs clearly distinguishable, opaque. Testis and ovaries occupy about two third of ventral cavity.	
4. Gravid: Sexual organs filling ventral cavity. Testes white, drops of milt fall with pressure. Eggs completely round, some already translucent and ripe.	4. Maturity: Sexual products ripe. Gonads have achieved their maximum weight, but the sexual products are still not extruded when light pressure is applied.
5. Spawning: Roe and milt run with slight pressure. Most egg translucent with few opaque eggs left in ovary.	5. Reproduction: Sexual products are extruded in response to very light pressure on the belly; Weight of the gonad decreases rapidly on the start of spawning to its completion.
6. Spawning spent: Not yet fully empty no opaque eggs left ovary.	6. Spent condition: The sexual products have been discharged; genital aperture inflamed; gonads have the appearance of deflated sac, the ovaries usually containing a few leftover eggs and testes; some reduced sperm.
7. Spent: Testes and ovaries empty, red. A few eggs in the state of reabsorption.	7.Resting stage: Sexual products have been discharged; inflammation around the genital aperture has subsided, gonads of very small size, egg not distinguishable to the naked eye.
8. Recovering/ Spent: Testes and ovaries translucent, grey-red. Length half or slightly more than half of the length of the ventral cavity. Single eggs can be seen with magnifying glass.	

Probable questions:

1. Define viviparity, oviparity and ovoviviparity.

2. Describe different types of nests maid by fishes as an outcome of parental care.

3. Describe different types of attachment of eggs in the body of fishes.

4. Describe briefly the breeding cycle in fish.

5. Classify different stage of fish maturation according to Kesteven (1960).

6. Classify different stage of fish maturation according to Nikolsky (1963).

7. Write briefly viviparity, oviparity and ovoviviparity in fishes with examples.

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Unit-XIII

STRUCTURE AND PHYSIOLOGY OF ENDOCRINE GLANDS OF FISH

Introduction:

Endocrine Glands of Fish: The glands that secrete their products into the bloodstream and body tissues along with the central nervous system to control and regulate many kinds of body functions are known as endocrine gland. In fishes various endocrine gland has been found associated with different tasks andfunctions.

Endocrine glands of fishes: Different types of endocrine glands are found in fishes; such as-

- The pituitary gland orHypophysis
- ThyroidGland
- Adrenalgland
- Corpuscles ofStannius
- UltimobranchialGlands
- Urohypophysis
- Pancreaticislets
- Pinealgland

The components of endocrine system can be classified on the basis of their organization, which is as follows:

(A) Discrete Endocrine Glands:

These include pituitary (hypophysis), thyroid and pineal (Fig. 19.1).

(B) Organs containing both endocrine and exocrine functions:

In fishes, it is kidney, gonads (Fig. 19.1) and intestine. Kidney contains heterotopic thyroid follicles, inter-renal, and corpuscles of Stannius.

(C)Scattered Cells with Endocrine Function:

They are known as diffused neuro-endocrines. They are present in digestive tract (Fig. 19.1). They are generally called as paracrines (e.g., somatostatin). There are gastrointestinal peptides whose definite classification as hormone or paracrine agent has not yet been established, these are designated as putativehormones.

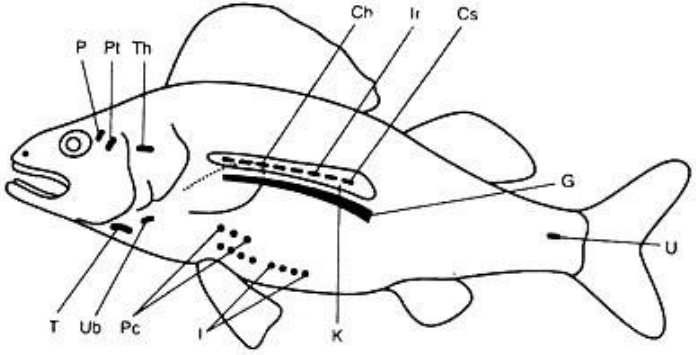

Fig. 19.1 : Schematic diagram to show position of various endocrine glands in fishes. Ch. chromaffin tissue; Cs, corpuscles of Stannius; G, gonad; I, intestinal tissue; Ir, interrenal tissue; K, kidney; P, pineal; Pc, pancreatic islets; Pt, pituitary; T, thyroid; Th, thymus; U, urohypophysis; Ub, ultimobranchial.

Chemically, hormones can be divided into three classes:

(I) Steroid hormones (testosterone &estradiol)

(II) Protein (peptide) hormones (e.g., insulin) and peptide hormones are secreted by hypophysis, thyroid, internal tissue and pancreatictissue.

(Ill) The amino acid analogues are norepinephrine and epinephrine, collectively called catecholamines.

Endocrine glands of increasing complexities are found in cyclostomes, elasmobranchs and Osteichthyes. Elasmobranchs (sharks) possess well developed endocrine glands but these show some interesting differences from those of higher chordates. However, Osteichthyes (bony fishes) have endocrine glands rather more similar to higherchordates.The difference between fish and mammal endocrine glands is probably due to the development and modification of various body systems in these two classes, and also due to exigencies of an aquatic mode oflife.

Mammalian endocrine glands are well advance and well-studied but fish endocrinology is limited to the work on its influence on chromatophores, action of sex cells, function of pituitary and thyroid and control onmigration.Unlike nervous system, the endocrine system is basically related to comparatively slow metabolism of carbohydrate and water by adrenal cortical tissue, nitrogen metabolism by adrenal cortical tissue and thyroid glands and the maturation of sex cells and reproductive behaviour by the pituitary gland and gonadal hormones.

Pituitary Glands:

Origin of Pituitary Glands:

The pituitary gland occupies the same central part in the endocrine signalling system of fish that it has in mammals. This master endocrine gland originates embryo-logically from the two sources. One as ventral down-growth of a neural element from the diencephalon called the infundibulum to join with another, an ectodermal up-growth (extending as Rathke's pouch) from primitive buccal cavity.

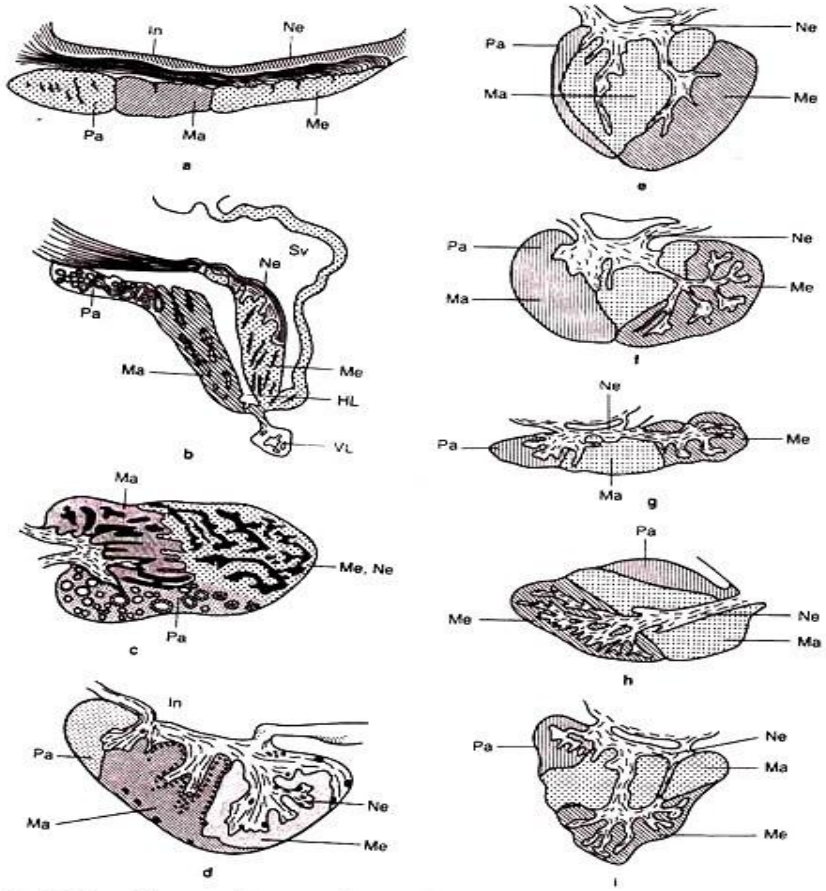

Fig. 19.2a-i : Diagrams of pituitary of various fishes. (a) *Petromyzon*. (b) Dogfish shark (*Squalus*). (c) Trout (*Salmo*). (d) Perch (*Perca*). (e) Rainbow trout. (f) Ayu (*P. altivelis*). (g) Eel. (h) Carp. (i) Yellow tail. HL, lumen of hypophysis; In, Infundibulum; Ma, mesoadenohypophysis; Me, metaadenohypophysis; Ne, neurohypophysis; Pa, proadenophypophysis; SV, saccus vasculosus; VL, ventral lobe; (Source : a-d, Pickford and AtZ, 1957; e-i, Hibiya, 1982).

These two outgrowths are thus ectodermal in origin and enclose mesoderm in between them, which later on supply blood to the pituitary gland, originating from the inter-renal carotidartery.

Location of Pituitary Glands:

The pituitary gland is located below the diencephalon (hypothalamus), behind the optic chiasma and anterior to saccus vasculosus, and is attached to the diencephalon by a stalk or infundibulum (Fig. 19.2). The stalked pituitary is found in *Barbusstigma* and *Xiphophorusmaculatus*. The size of infundibulum varies according to the species. Usually in cyclostomes it is smaller but increases in bony fishes, with prominence in groove or depression of para- sphenoid bone receiving the gland. There is no sella turcica comparable to that found in mammals in *Xiphophorus*. The short, thick- walled, hollow infundibular stalk contains a lumen, which continues with the thirdventricle.

Shape and Size of Pituitary Glands:

The pituitary is an oval body and is compressed dorsoventrally. The size of sexually mature platy-fish has a mean anterior posterior length of 472.9 micra, with mean width of 178micraandmeandepthof360micra.Maleglandsaresmallerthanthoseoffemales.

On ventral aspect the gland gradually tapers caudally from rounded anterior end. The dorsal surface of the pituitary of platy-fish is concave, ventrally it is slightly convex. The pituitary gland is completely enveloped by a delicate connective tissuecapsule.

Anatomy of the Pituitary Glands:

Microscopically, the pituitary gland is composed of two parts:

(i) Adenohypophysis, which is a glandular part originated from the oral,ectoderm.

(ii) Neurohypophysis, which is a nervous part originated from the infundibular region of the brain. Both parts are present in closeassociation.

Pickford and ATZ (1957) divided adenohypophysis into three parts, viz., pro-adenohypophysis, mesoadenohypophysis and metaadenohypophysis while Gorbman (1965) divided adenohypophysis into three parts but called them as rostral pars distalis, proximal pars distalis and parsintermedia.

However, nomenclature is synonyms asfollows:

Pro-adenohypophysis – Rostral pars distalis

Meso-adenohypophysis – Proximal pars distalis
Meta-adenohypophysis –Parsintermedia
(Fig.19.2).

1. **Rostral Pars Distalis (Pro-Adenohypophysis):**

Lying dorsal to the mesoadenohypophysis in the form of thin strip (Fig. 19.2a-i).

2. **Proximal Pars Distalis (Mesoadenohypophysis):**

Lying almost in between the rostral pars distalis and pars intermedia.

3. **Pars Intermedia or Metaadenohypophysis,viz.:**

Lying at the distal tapering end of the pituitary gland (Fig. 19.2a-i). Pituitary are broadly characterized as platybasic and leptobasic. In platybasic form (Eel), the neurohypophysis consists of flat floor of the caudal infundibulum which sends processes into disc-shaped adenohypophysis.

In leptobasic, the neurohypophysis has a fairly well developed infundibulum stalk and the adenohypophysis is globular or egg shaped. There are many intermediate between the two. Both types have similar structures described above (Fig.19.2a-i).

Adenohypophysis:

Earlier workers identified cells of adenohypophysis on the basis of staining procedures. The procedures used were Heidenhain'sazon method, Masson's poncean acid fuschinanilin blue, the periodic acid Schiff's reaction (PAS), aldehyde fuschin technique

(AF) and then they made cellcounts.The cells of pituitary secrete hormones and hormones are stored in granules present in the cytoplasm. The cells are, therefore, classified on the basis of staining properties of granules of these cells. Cell types of adenohypophysis, on the basis of staining reaction, to the mixture of acidic and basic dyes with secretory granules are called as acidophilic and basophilic.

On the basis of binding affinities with ribonucleoprotein the two classes are also classified as chromophobes and chromophils. The chromophobes have little affinity with dye while chromophils stain strongly as they have affinity withdye.Chromophilic cells which take acidic stain are called as acidophils whereas the chromophilic cells which bound basic dye are called basophilic and the cells which do not take any stain are called chromophobes. The acidophilic cells are PAS (periodic acid Schiff) and AF (aldehyde fuschin) negative cells. The basophilic cells are AF and PAS positive.

Recently on the basis of immunocytochemistry, the cells are classified according to hormones released by thepro-adenohypophysis.For example, the cells which take basophilic stain but produce adrenocorticotropic hormones, they are called ACTH cells but if secrete thyroid stimulating hormone, these cells are called thyrotrophs and if they secrete FSH hormones they are called gonadotrops although they are basophilic innature.
The cells of adenohypophysis when stained with periodic and Schiff (PAS) and aldehyde fuschin methods/if do not take stain, they are PAS and AF negative.The teleost hypo-thalamo-pituitary system is unique amongst vertebrates, as there is direct innervation of pars distalis by neurosecretory neurons of hypothalamus and there is loss of modification of the typical vertebrate hypothalamohypophysial portal vascular system for transport of neurohormones to pars distalis.

(a) Pro-Adenohypophysis:

It contains cells which secrete prolactin and corticotropin (ACTH) exclusively in addition to other hormones.

(b) Meso-adenohypophysis:

The mesoadenohypophysis (proximal pars distalis) contains cells which produce gonadotropin (GTH) and growth hormone (GH). Thyrotropin cells may occur in either or both in rostral pars distalis and proximal pars distalis. Acidophils rounded or oval or sometime pyramidalshaped.They are coarsely granular, and give the cytoplasm a splotched appearance. They have round to oval peripheral nuclei. Basophils (cyanophilic) are spherical with large, round,centrally located nuclei. Their cytoplasm is finely granular, chromophobes cells are similar in structure as they are found in pro-adenohypophysis. The basophilic (cyanophilic) cells are PAS positive and AFpositive.

(c) Meta-adenohypophysis:

It also encompasses more neurohypophysial tissue than any other region. The meta-adenohypophysis basophilic cells are PAS positive. However, the granular cells do not show consistent staining reaction with PAS and AFstains.

I. Neurohypophysis:

The neurohypophysis occupies considerable portion of the gland and possesses many interesting and distinctive features. The neurohypophysis comprises connective tissue, neuroglia cells and loosely tangled network of nerve fibres. These nerve fibres are scattered horizontally along the dorsal part of the adenohypophysis and run vertically, which are generously inter-spread with granular material, large irregularly shaped amorphous masses and largenuclei.

They are located in the mid-dorsal region. The amorphous masses are called "Herring bodies", which have an intimate relation with the di-encephalic neuro-secretory cells called nucleus preopticus by means of a fibre tract known as the preoptic neurohypophysial tract. The diencephalon another parts of the brain contain a group of neurons and each group is called nucleus. The NPO and NLT are important as their axons are in association with both adenohypohysis and neurohypophysis (Fig. 19.3). These possess neurosecretory cells. The nucleus preopticus (NPO, preoptic nucleus) is situated on either side of the optic recessus slightly in front of the optic chiasma. The preoptic nucleus (NPO) is further subdivided into two parts.

I- Pars parvocellularis, it is located anteroventrally and consists of relatively small cells.

II- pars magnocellularis, it is situated posterodorsally and comprises relatively largercells.The preoptic nucleus (nucleus preopticus, NPO), their axons and nerve endings in the pituitary are stainable by neurosecretory stains. The neurons with Gormori's chrome alum haematoxylin, aldehyde fuschin and alcian blue, can differentiate the NPO from other nuclei in preoptic region as they are neurosecretory innature.

Blood Supply in Pituitary Glands:

Vascularization of the pituitary has been studied in variety of species. In brook trout, Salvelinus fontinalis and Atlantic salmon, Salmo salar, there is a separate blood supply to the neuro-intermediate lobe from the caudal hypothalamic artery and to the combined rostral proximal pars distalis from hypophysial arteries that branch off the anterior cerebral arteries.

According to Follenius (1963), there is no separate blood supply for rostral proximal pars distalis and pars intermedia in *Salmo gairdneri* but the entire blood supply originated anteriorly from the hypophysial arteries. However, in teleosts, the rostral proximal pars distalis receives blood supply from the extensive looping's of arterioles which are found near the interface with the pars distalis (Fig.19.4).These vessels are invaded into the pars distalis together with the interdegitations of the anterior neurohypophysis. It has been considered that these anterior loops are the rudiment of the hypothalamohypophysial portal system. However, there is no neurovascular connections with these blood vessels, as are typically found in the median eminence of various vertebrates. This hypothesis is argued asa portal system.The function of the hypothalamohypophysial portal system as a means of transport of neurohormones to the pituitary has become redundant and pure vascular in function, probably because the pituitary cells have direct innervation by neurosecretory endings.

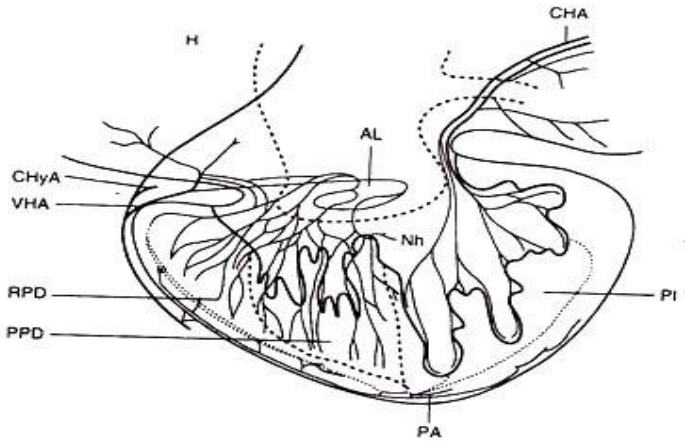

Fig. 19.4 : Para sagittal view of pituitary gland of brook trout showing blood supply. AL, arterial loops; CHA, caudal hypothalamic artery; CHyA, caudal hypophysial artery; H, hypothalamus; Nh, neurohypophysis; PA, peripheral artery; PI, pars intermedia; PPD, proximal pars distalis; RPD, rostral pars distalis; VHA, ventral hypothalamic artery.

In spite of this, a typical but small hypothalamo-hypophysial portal system has been described in variety of teleosts. According to Sathyanesan and colleagues, the branches of hypothalamic arteries form **"primary capillary plexus"** located in the meningeal tissue and the adjacent neural tissue of hypothalamus anterior to the pituitarystalk.This plexus converges into vessels that enter the pituitary or proximal pars distalis or pars intermedia.Thus in teleosts this portal system is the only and primary source of blood for the pars distalis. It has been considered that among teleosts the Cypriniformes or Siluriformes have reduced portal system. It is clear, however, that teleosts have neurohormones secreted more or less directly to the pituitary, and that some have the potential for vascular transportation of neurohormones by a portal system aswell.

Hormones of Pituitary Glands:

There are (seven) various hormones secreted by pituitary but it is generally agreed that one cell type-one hormone concept, is correct. The different hormones secreting cell are not localized in specific region but are spread over in part of the adenohypophysis (Fig. 19.5).

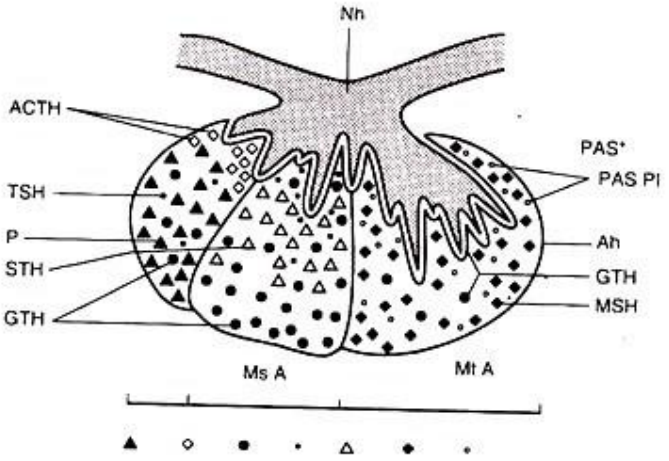

Fig. 19.5 : Section of the pituitary to show various hormone secreting cells in the adenohypophysis (Ah). ACTH, adeno corticotropic cell; GTH, gonado tropic cell; MsA, mesoadenohypophysis MtA, metaadenohypophysis; MSH melanotropic cell; Nh, neurohypophysis; PAS, periodic acid Schiff-positive cell in pars intermediate; P prolactin producing cell; STH, somatotropic cell; TSH; thyrotropic cell.

All hormones secreted by the pituitary are necessarily proteins or polypeptides. There exists a slight difference in the pituitary hormones of the different groups of fishes. The pituitary hormones of fishes are of two types (I) one which regulates the function of other endocrine glands. Such hormones are called tropins or tropichormones.

These are:

1. **Thyrotropin activatesthyroid.**
2. **Adrenocorticotropic hormones activate adrenalcortex.**
3. **The gonadotropin FSH and LH (Leuteotropins, various steroidhormones).**
4. **Growth hormones, somatotropin (actually they are nottropic).**

Hormones which directly regulate the specific enzymatic reactions in the various body cells or tissues are melanin hormones (MH) and melanophore stimulating hormone (MSH), etc. Thyrotropin hormone is secreted from pro-adenohypophysis (rostral pars distalis) and stimulate activity of thyroidhormones.The TSH is secreted under the influence of (TRH), thyroid- releasing hormones from diencephalon in fishes. It is proved that TRH influences the TSH cell activity and thyroid activity in fish. In *Carassus auratus*, crude extract of the hypothalamus or goldfish results decreased radioiodine uptake by the thyroid, which indicates the presence of TRH activity inhypothalamus. In teleosts, the TSH cells have direct innervation by neurosecretory endings, which are adjacent to the cells having no synaptic contact or the endings and may be separated from the TSH cells by a basement membrane. In *Tilapia mossambica*and*Carassius auratus*the TSH cells have direct contact with endings containing elementary neurosecretory granules, and with endings containing vesicles having densegranules.

Gonadotropin:

Gonadotropin (GTH) cells are richly found in the proximal pars distalis (PPD), where they may form a solid ventral rim of cells. Such situation is found in Cyprinoide. In salmonids and eel they are spread throughout rostral pars distalis (RPD) andPPD.Gonadotrops are basophilic cell types and are PAS and AB positive. These cells have irregular and more or less dilated cisternae of granulated endoplasmic reticulum (GER) containing granules with varying electron density.

The gonadotroph (GTH) is under the control of gonadotropin releasing hormone. In many teleosts, unlike mammals, neurosecretory stimuli may pass along the nerve fibres piercing the laminae, that separates the neuro from adenohypophysis, and penetrating into the endocrine parenchyma of pars distalis (Ball, 1981). There are two types of nerve fibres designated as A and B types.Thefibres of A type remain in contact with hormone producing cells, including gonadotrops and even terminate with synapse on these cells. B type fibres form synaptic contact with a large granular vesicle of 60-100 nm diameter, while the A synapse have granules of 100- 200 nmdiameter.

The gonadotropin (GTH) releasing hormone (GnRH) of teleost is similar to luteinizing hormone releasing hormone (LH-RH) is localized in ventral lateral nucleus preopticusperiventricularis (NPP) and posterior lateral nucleus lateral tuberis (NLT) as well as other areas.In hypothalamus, localization of immunoreactive fibre tracts from cells in the NPP and NLP to the pituitary gland suggests that these areas are the origin of endogenous releasing hormone.

Studies of Peter and Crim (1978) on *Carassius auratus* indicate that the nucleus lateralis tuberis (NLT) pars posterior and the NLT pars anterior which are situated in the pituitary stalk, are actively take part in regulation of GTH secretion for gonadal recrudescence (Fig. 19.6).In several fishes GTH secretion is associated with ovulation. In *Carassius auratus*, GTH level becomes higher on the day of ovulation. However, in sockeye salmon, *Oncorhynchus nerka*, high level of GTH found duringspawning.

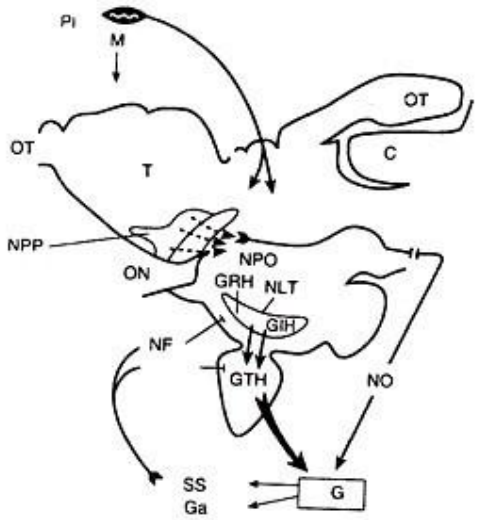

Fig. 19.6 : Diagrammatic representation of the neuroendocrine regulation of gonadotropin (GTH) secretion by releasing hormone (GRH) and one inhibitory hormone (GIH) in gold fish brain. C, cerebellum; G; gonad; Ga, gametes; M, melatonin; NF, negative feedback; NLT, nucleus lateralis tuberis; NPP, nucleus preopticus periventricularis, NPO, nucleus preopticus; NO, neural output; ON, optic nerve; OT, olfactory tract; PI, photoperiod input; SS, sex steroid; T, telencephalon. (Source; Peer and Fryer, 1983).

In fishes there is only one functional gonadotropin is found, which is often regarded as piscian pituitary gonadotropin (PPG). This single gonadotropin has similar properties of two hormones. LH and FSH of mammals. Mammalian luteinizing hormone (LH) promotes release of gametes from nearly mature gonads in fishes and stimulates appearance of secondary sexualcharacters.

This indicates that there must be a similar hormone in fishes also. Salmon pituitary secretes gonadotropins which resembles LH. Furthermore, the gonadotropins from human chorion and urine of gravid mares, have LH like properties which hasten the release of eggs in femalefishes.The presence of follicle stimulating hormone in fishes (FSH), which is the second gonadotropin hormone found in mammalian pituitary gland, is still not confirmed. Recently, prostaglandin, which is hormone-like substance has

been isolated from testis and semen of blue fin tuna (*Thynnus thynnus)* and flounder (*Paralichthysolivaceus*).

Adrenocorticotropic Hormone (ACTH):

It is secreted by ACTH cells located between the rostral pars distalis and the neurohypophysis. Secretion of ACTH from pituitary is stimulated by the hypothalamus through corticotrophin releasing factor (CRF) (Fig. 19.7).Hypothalamic and telencephalic extracts of *Carassius auratus* and longnose suckers,*Catostomus Catostomus*stimulated secretion of ACTH in *Carassius auratus* in vivo.Thenature of this telencephalic hypothalamic CRF is unknown. However, it shows similarity with mammalian CRF.

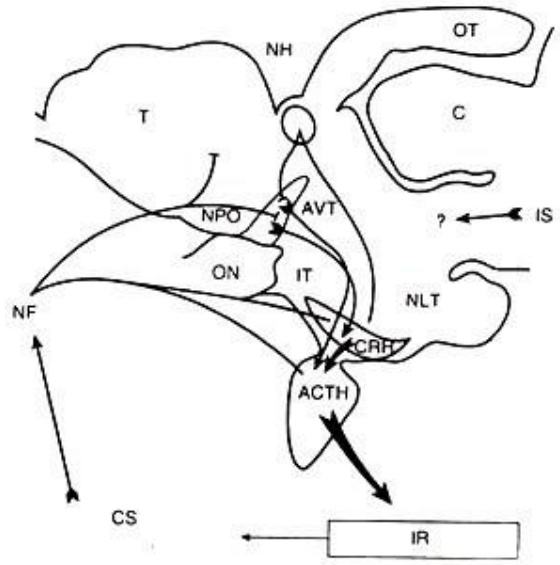

Fig. 19.7 : Diagrammatic illustration of neuroendocrine regulation of (ACTH) by a releasing hormone (RH) and (NPO)→ arrow indicates stimulatory influences; Question (?) indicates unknown pathways. AVT, arginine vasotocin; IT, isotonin; IR, inter renal; IS, input of stress; C, cerebellum; CS, cortico steroid; NF, negative feed back; NH, nucleus habenularis, NLT, nucleus lateralis tuberis; NPO, nucleus preopticus; ON, optic nerve; T, telencephalon.

In *Carassius auratus*, ACTH cells are innervated by aminergic like type B fibres, which originate from nucleus lateralis tuberis (NLT). In teleosts, neurohypophysial peptides may regulate the ACTH secretion.Implantation of cortisol pellets in *Carassius auratus* shows that corticosteroids exert negative feedback effects on the brain to suppress ACTH secretion. Cortisol added to the medium inhibits the activity of ACTH cells and release of ACTH which also suggest direct negative feedback effect of Cortisol on the ACTHcells.

Prolactin:

It is a similar hormone that influences lactation in mammals and is released from pro-adenohypophysis. In some fishes like mummichog (*Fundulusheteroclitus*), prolactin along with the intermedin enhances the laying down of melanin in the melanophores of the

skin.Among the several hormones the prolactin is also involved in electrolytic regulation in teleosts but its importance in maintaining homeostasis varies according to species. The secretion of prolactin from the teleost pituitary is under an inhibitory neuroendocrine control of hypothalamicorigin.

Growth Hormone (GH):

Mesoadenohypophysis secretes a growth hormone which accelerates increase in the body length of fishes. Very little is known concerning its control, mode of action on cell division and protein synthesis in teleosts. It has been reported that teleost GH cells are capable of some spontaneous activity and continue to synthesize and secrete GH invitro.

It is evident that the GH secretion may be influenced by osmotic pressure, as release of GH from cultured *Salmo gairdneri* and *Anguilla Anguilla* pituitaries greater in a medium containing low sodium than in a high sodium medium, relative to plasma sodium levels. However,GHreleasefrom*Poecilialatipinna*pituitaryhasnoeffectofosmoticpressure.Recently a zone of the hypothalamus has been recognized, which is believed to be responsible for control of GH in *Carassius auratus*. In this fish nucleus anterior tuberis (NAT) and sometimes nucleus lateralis tuberis (NLT) forms an area which stimulates GH secretion and is perhaps the origin of a growth hormone releasing hormone(GRH).The hypothalamic control of GH secretion is revealed by ultra-structural studies on the pituitary. In teleosts GH cells of pars distalis have direct synaptoid contact with type B endings as in *Carassius auratus*, which have direct contact without the synaptoid appearance in *Tilapia mossambica*. Very few species like *Oryziaslatipes*conta insynaptoid contact of type A endings on GH cells; in other teleosts, type A fibre may have direct contact with GH cells, but generally the endings are separated from the cells by a basement membrane. Thus it is clear that on neuroendocrine factor reaching the GH cells and probably the GH cells are regulated by a dual hormone.

Melanocyte Stimulating Hormone (MSH) or Intermedin:

MSH is secreted from the meta-adenohypophysis and acts antagonistically to melanin hormone (MAH). MSH expands the pigment in the chromatophores, thus takes part in adjustment of background. It also stimulates the melanin synthesis. Pars intermedia of teleost pituitary comprise two kinds of secretory cells, which can be identified by their staining properties.One cell type is PAS^{+ve} periodic acid Schiff positive and PbH^{-ve} (lead hematoxylin negative). However second cell type is PbH^{+ve} and PAS^{-ve} (Holmes and Ball, 1974). Salmonid seems to have only PbH^{+ve}cells. These cells are source of melanocyte stimulating hormone (MSH) which stimulates melanin dispersion in the melanocytes and darkening of skin. Neuro-intermediate lobe of *Salmo gairdneri*appears to have a melanin concentratingfactor.Several authors have demonstrated the occurrence of MSH and/or its precursor ACTH in PbH cells of several species of teleosts by immunofluorescence techniques. Thus theseobservations confirm earlier correlations of body colour or background adaptation with activity of the PbH cells.

In teleosts, the neuroendocrine control of pars intermedia varies according to species. In fishes like Cymatogastes aggregate, *Anguilla Anguilla*and*Salmo gairdneri*, neurosecretory axons do not enter the pars intermedia but terminate in extravascular channels bordering the pars intermedia or terminate at the basementmembrane.However, other teleosts such as *Carassius auratus*, and *Gillichthys mirabilis* have direct innervation from neurosecretory axons.

Secretion of MSH in teleost may be suppressed by catechol aminergic mechanism. Treatment of 6 OHDA to destroy catechol aminergic nerve terminals also causes activation of the MSH cells in *Gillichthys mirabilis*, darkening of skin or activation of MSH cells in *Anguillaanguilla*, catecholamine inhibits the release of MSH directly, when the former is MIH.Also the catecholamine may affect MSH secretion indirectly by promoting the release of an MIH, or by inhibiting the secretion of an MRH nerve terminals within the pars intermedia.Several histological investigations demonstrate a stimulation of the pars intermedia associated with reproduction. During spawning period pars intermedia of clupea becomes intensely active. In *Carassius auratus* the number of PbH^{+ve} cells increases after spawning in both number and activity during oogenesis and breedingseason.

Oxytocoin and Vasopressin Hormones:

In fishes the neurohypophysis secretes two hormones, i.e., oxytocin and vasopressin, which are stored in hypothalmic neurosecretory cells. These endocrine substances have well known effect on mammalianmetabolism.Vasopressin and antidiuretic (ADH) hormones are responsible for the constriction of blood vessels in mammals and thus stimulates retention of water by their action in kidney. Oxytocin stimulates mammalian uterine muscles and increases the discharge of milk from lactating mammals.The fish pituitary hormones are capable to produce such effects in higher vertebrates but presumably the target organs are specific site of their action in fishes and probably is different from those of higher vertebrates. In fishes they control osmoregulation by maintaining water and saltbalance.

Use of pituitary hormones in induced breeding:

The pituitary hormones have practical applications by injecting and implanting to force or stimulate spawning of certain fishes of great economic value, such as trouts (Salmoninae),catfishes (Ictaluridae), Mullets (Mugliliade), and sturgeons (Acipenseridae). The synthesis of sex hormones in the gonad is controlled by pituitarygonadotropin.Hence pituitary extract containing GTH are taken from sexually mature male or female fish than injected to the same species for inducing and hastening and spawning. For the preparation of pituitary extract closely related species may also be used as donor.

Thyroid Gland of Fishes:

Location of Thyroid Gland:

In many teleosts the thyroid gland is situated in the pharyngeal region in between the dorsal basibranchial cartilages and ventral sternohyoid muscle. The thyroid surrounds anterior and middle parts of first, second and sometimes third afferent branchial arteries of ventral aorta, as found in Ophiocephalus species (Fig.19.8).In *Heteropneustes*it occupies almost the entire length of the ventral aorta and afferent arteries. In *Clariasbatrachus*the thyroid gland is concentrated around the ventral aorta, middle ends of two pairs of afferent arteries and the paired inferior jugular veins.

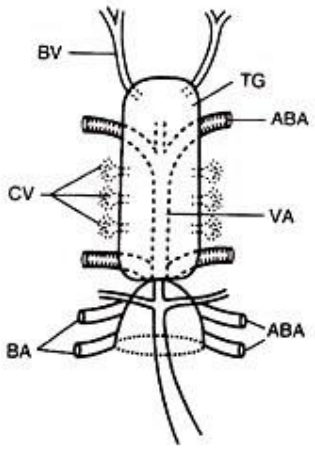

Fig. 19.8 : Ventral view of thyroid gland and their blood vessels in *Heteropneustes*. ABA, afferent branchial artery; BA, branchial vessel; BV, buccal vein; CV, commissural vessel; TG, thyroid gland; VA, ventral aorta.

Shape and Size of Thyroid Gland:

In majority of teleosts the thyroid is un-encapsulated and thin follicles are dispersed or arranged in clusters around the base of afferent branchial arteries.It is thin-walled, sac-like, compact dark brownish and enclosed in a thin-walled capsule of connective tissue in these fishes might be correlated with the air breathing habit because thyroid gland acts here as thermoregulatory to adapt the fish to a semiterrestrial environment of low thermal capacity. In *Heteropneustes* the thyroid gland is an unpaired thin- walled brownish but cylindrical in shape. In *Clarias batrachus* the thyroid gland is not covered by definite wall, i.e., un- capsulated and is elongated in shape.

Histology of the Thyroid Gland:

In teleosts, histologically the thyroid gland consists of a large number of follicles, lymph sinuses, venules and connective tissues. The follicles are round, oval and irregular in shape. Each follicle contains a central cavity surrounded by a wall composed of single layer of epithelial cells. The structure of epithelium varies according to its secretory

activity. Less active follicles generally have thinepithelium.

Epithelial cells are of two types:

(i) Chief cells which are columnar or cuboidal in shape, having oval nuclei and clear cytoplasm.

(ii) Colloid cells or Benstay's cells. They possess droplets of secretory material. The follicles are supported in position by connective tissue fibres, which surrounds them. The central lumen of follicle is filled with colloid containing chromophilic and chromo phobic vacuoles.

Blood Supply in Thyroid Gland:

The thyroid gland is highly vascularized and is generally well supplied with blood. A single buccal vein and two pairs of commissural vessels supply blood to thyroid gland. From its posterior end a pair of veins arise, which merge immediately to form the posterior inferior jugular vein sending blood to the heart.In *Ophiocephalus*, the thyroid gland also receives blood from same vessels. The buccal vein collects blood from the buccal region and after running for a short distance beneath the anterior end of pairs of the thyroid gland opens intoit.The two commissural blood vessels are highly branched and bring blood from the floor of pharynx. One pair opens at the anterior end while the other at the middle of the gland on either side. In *Heteropneustes*the commissural vessels are more than two pairs.

Hormones of Thyroid Gland:

Thyroid hormone is synthesized in the thyroid gland, for which inorganic iodine is extracted from the blood. These inorganic iodine combines with tyrosine. The thyroid hormones of fishes appear to be identical with those of mammals, including, mono- and di-iodo-tyrosine and thyroxin. These hormones are kept stored in the thyroid follicles and are released into blood stream on metabolic demands. The release of the thyroid hormone from the follicle is controlled by the thyrotropic hormone(TSH)of pituitary which in turn is influenced by genetically determined maturation process along with certain factors like temperature, photoperiod and salinity. The thyroid glands in sharks and higher teleosts are diffused in nature. Therefore, it is difficult to remove or inactivate. In spite of the certain deficiency, studies have been made by physiological blocking or radio-thyroidectomy using. In teleosts, there is no respiratory stimulation by thyroxin, which is best known in mammals. Physiologically used small quantity of thyroxin and tri-iodo-thyroxine result in thickening of the epidermis and fading of goldfish (*Carassiusauratus*).
Induced thyroid hyperactivity accelerates the transformation into juvenile smolt stage in salmon but high thyroid the titer retards growth of the larva in the same genus. Induced thyroid hyperactivity in mud skipper (*Periophthelamus*) shows morphological and metabolical changes in response to the more terrestrial existence of fish living mostly outside the water. Thyroid gland of salmons and sticklebacks is known to influence osmoregulation.In salmon the thyroid gland becomes hyperactive during their spawning migration. It has been considered that thyroid influences the growth and nitrogen

metabolism in goldfish, as indicated by high ammonia excreted by them. Thus the action of thyroid is conjugated with other vital processes including growth and maturation and also the diadromous migration offishes.

Adrenal Cortical Tissue or Inter-Renal Tissue:

Location:

In Lamprey (Cyclostomata) the endocrine inter-renal cells are present throughout the body cavity close to the post-cardinal vein. Among the rays they lie in more or less close association with posterior kidney tissue, including some species possessing inter-renal tissue concentrated near the left and in other near the right central border of that organ. In sharks (Squaliformes) they are present between the kidneys. In teleosts the inter-renal cells are multilayered and situated along the post-cardinal veins as they enter the head kidney (Fig. 19.9).

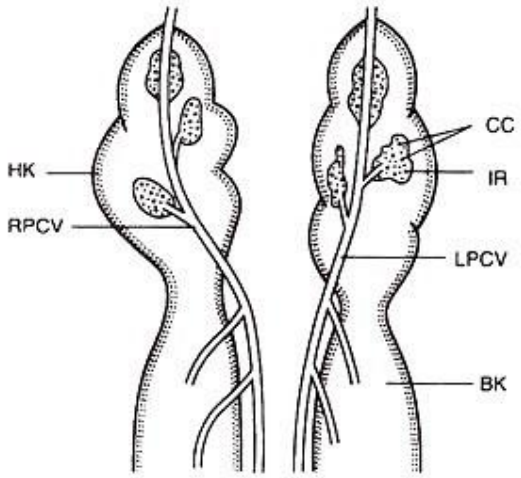

Fig. 19.9 : Digram to show location of interrenal glands in fishes. BK, body kidney, CC, chromaffin cells; HK, head kidney; IR, interrenal tissue; LPCV, left post cardinal view; RPCV, right post cardinal vein. (Source : Habia, T., 1982).

Anatomy:

In some fishes like *Puntius tictoi*nter-renal cells are arranged in form of thick glandular mass while in others like *Channa punctatus* they are present in form of lobules. Each inter- renal cells is eosinophilic and columnar with a roundnucleus.

Adrenal Cortical Hormone:

Adrenal cortical tissue or inter-renal tissue secretes two hormones. These are (i) mineral corticoids concerned with fish osmoregulation, (ii) glucocorticoids, which regulate the carbohydrate metabolism, particularly blood sugar level.*Salmo gairdneri*treated with mineral corticoid excretes higher than normal amount of sodium ions through its gills but conserve more than normal amount of sodium in the kidneys and osmoregulation in thebody.Intramuscular injection of corticosteroid compounds to the oyster toadfish causes increase in blood sugar level thus showing control on carbohydrate metabolism. The cortisone level of blood plasma of salmons rises during spawning period and declines during the more sedentarystages.During the spawning phases 60% of total body protein is catabolized in *Oncorhynchus*, which is correlated with the six fold increase in plasma corticosteroids and rises in liver glycogen.Like higher vertebrates administration of adrenal cortical hormones stimulates lymphocyte release in *Astyanax* and antibody release in European perch. Corticosteroidsstructurallysimilar to androgens and produce androgens side effects. Secretion of adrenocortical hormones is under control of the adrenocorticotropic hormone (ACTH) of hypophysis.

Chromaffin Tissue or Suprarenal Bodies or Medullary Tissue:

In lamprey (Cyclostomata) the chromaffin cells are present in the form of strands along the dorsal aorta as in the ventricle and the portal vein heart. In sharks and rays (Elasmobranchii) these tissues are found associated with the sympathetic chain of nerve ganglia while in bony fishes (Actinopterygii) the chromaffin cells have wide variation in their distribution.They are elasmobranch like, distributed as in flounders (*Pleuronectus*). On the other hand they have true adrenal arrangement as in sculpins (*Cottus*) where chromaffin and adrenal cortical tissue are joined into one organ, similar to the mammalian adrenal gland (Figs. 19.10a, b, c, d).

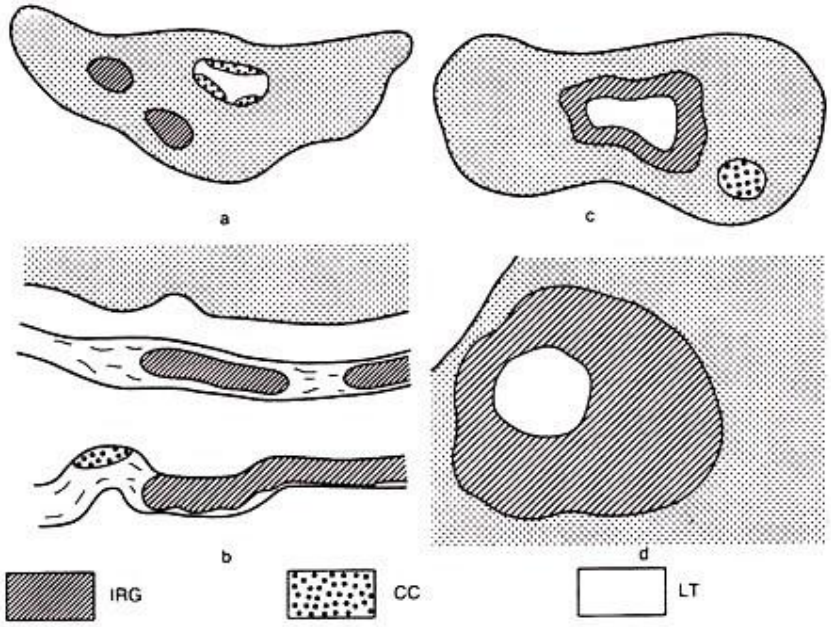

Fig. 19.10a-d : Diagram showing chromaffin tissue in fishes (a) Rainbow trout. (b) Eel. (c) Carp. (d) Yellow tail. IRG, interrenal gland; CC, chromaffin cell; LT, lymphoid tissue. (Source : Hibiya, T., 1982)

Chromaffin tissue of fishes richly contains adrenaline and noradrenaline. Injection of adrenaline and noradrenaline causes changes in blood pressure, bradycardia, branchial vasodilation, diuresis in glomerular teleosts and hyperventilation.

The Ultimo-Branchial Gland:

Typically the gland is small and paired and is situated in the transverse septum between the abdominal cavity and sinus venosus just ventral to the oesophagus or near the thyroid gland. Embryonically the gland develops from pharyngeal epithelium near the fifth gill arch. In *Heteropneustes*, the gland measures 0.4 x 1.5 mm in diameter in average adult of 130 to 150 mm bodylength.Histologically, it consists of parenchyma, which is solid and composed of cell cords and clumps of polygonal cells covered by capillary network. The gland secretes the hormone calcitonin which regulates calcium metabolism.Calcitonin is said to be related with the osmoregulation. Eel calcitonin causes decrease serum osmolarity, sodium and chloride in Japanese eels. The ultimo-branchial gland is under the control of pituitarygland.

The Sex Glands as Endocrine Organs:

The sex hormones are synthesized and secreted by specialized cells of the ovaries and testis. The release of sex hormones is under the control of meso-adenohypophysis of pituitary. In fishes these sex hormones are necessary for maturation of gametes and in addition secondary sex characteristics such as breeding tubercles, colouration and the maturation ofgonopodia.

In elasmobranch (*Raja*) and in salmon the blood plasma contains male hormone testosterone with a correlation between plasma level and the reproductive cycle.

Oryziaslatipes (medaka) and sockey salmon comprise another gonadal steroid, i.e., 11-ketotestosteron, which is 10 fold more physiologically androgenicthan testosterone.Ovary secretes estrogens of which estradiol-17B has been identified in many species in addition of presence of estrone and estriol. In some fishes progesteron is also found but without hormonal function.

There is little information about the influence of gonadial hormones on the reproductive behaviour of fish. Injection of mammalian testosterone and estrone to lamprey causes development of its cloacal lips and coelomic pores, which contribute in reproductive process.Such tests conducted for rays and sharks (Elasmobranchii) could not give any results whereas ethynil testosterone (pregnenolone) which produces mild androgenic and progesterone like effects in mammals and birds, found to be highly androgenic in fishes.Male sex hormones are more similar to those of vertebrates than the ovarian hormones, the former strongly influences ovarian development in a loach, the Japanese weather-fish.

Corpuscles of Stannius:

The corpuscles of Stannius were first described by Stannius in 1939 as discrete gland like bodies in the kidney of sturgeon. The corpuscles of Stannius (CS) are found attached or lodged in the kidneys of fishes particularly holostean and teleost (Fig. 19.11).Corpuscles of Stannius are asymmetrically distributed and often resembles with cysts of parasites but lie different from the latter by higher vascular supply and dull white or pink colour. Histologically, they are similar to the adrenal cortical cells. Their number varies from two to six according tospecies.

The CS. may be flat, oval as in goldfish, trout, salmon. It is made up of columnar cells which are covered by a fibrous capsule. The columnar cells are of two types (i) AF-positive and (ii) AF negative. They are filled with secretory granules. The parenchyma of CS comprises vasculo-ganglionic units consisting of a bunch of ganglion cells, blood vessels and nerve fibres.

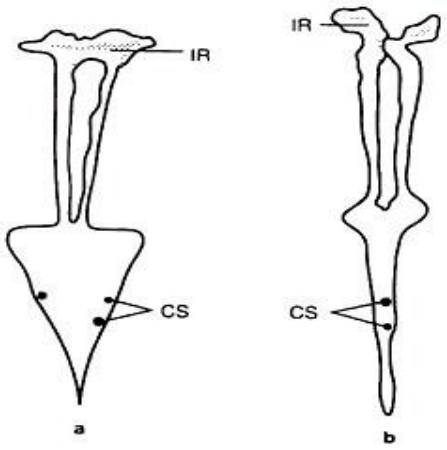

Fig. 19.11a, b : Diagram of kidney of fishes showing corpuscles of Stannius. (a) *Cirrhina mrigala*. (b) *Labeo rohita*. CS, corpuscles of Stannius; IR, interrenal corpuscles.

The number and position of CS vary in different species. Their may be single CS as in *Heteropneustessetani*and *Notopterusnotopterus*(Fig. 19.11a, b), while as many as ten CS are present in some species like *Clariasbatrachus*. In other species their number varies from one to four. According to Garrett (1942), there is a gradual reduction in number of CS that has occurred during evolution of Holostei andTeleostei.

In salmonids the CS is located near the middle part of mesonephros but in majority of fishes they are situated at the posterior end of kidney. Garrett (1942) pointed out that CS moves progressively backward during the course of evolution as a result of body cavity rather than a migration of CS. In *Notopterus notopterus*the CS are present in anterior end of kidney perhaps because most of the body cavity is occupied by the air bladder and also other organs are compactly arranged in limitedspace.ThepresenceoftheCSattheextremeanteriorendofthekidneyin*Heteropneustessetani*is probably ecause this species has wide body space and long archinephricduct.

Therefore, the variation in number and position of CS in teleost species seems to be an embryological speciality. There is only one-cell type present in the CS of pink salmon. However, two-cell types are found in pacific salmon. The corpuscles of Stannius reduce serum level in the *Fundulusheteroclitus*, which have environment containing high calcium, such as sea water. Recently, it has been shown that corpuscles of Stannius work in association with pituitary gland, which exerts hypercalcemic effect, in order to balance relatively constant level of serum calcium.

Intestinal Mucosa:

The intestinal mucosa produces secretin and pancreozymin, which are controlled by nervous system and regulate pancreatic secretion. Secretin affects flow of enzyme carryingliquidsfromthepancreas,whereaspancreozyminacceleratesflowofzymogens. These hormones are usually synthesized in anterior part of the small intestine. In carnivorous fish these hormones are brought into the stomach, containing acidified

homogenate of fish flesh or by injection of secretin into gastric vein which stimulates the secretion ofpancreas.

Islets of Langerhans:

In some fishes like *Labeo*, *Cirrhinus*, and *Channa* small islets are present which are separate from pancreas and are found near gall bladder, spleen, pyloric caeca or intestine. Such islets are often referred to as principal islets. But in some species like *Clarias batrachuss* and *Heteropneustes fossilis* the number of large and small islets is found to be embedded in the pancreatic tissues, similar to the highervertebrates.

In fish the islets are big and prominent and consist of three kinds of cells (Fig. 19.12a,b):

(i) The beta cells which secrete insulin and take aldehyde fuschin stain,

(ii) Another type of cells are alpha cells, which do not take aldehyde fuschin stain and have two types, A_1 and A_2 cells, which produce glucagon. The function of the third type of cells is not known. Insulin is secreted by beta cells and regulates the blood sugar level in fishes.

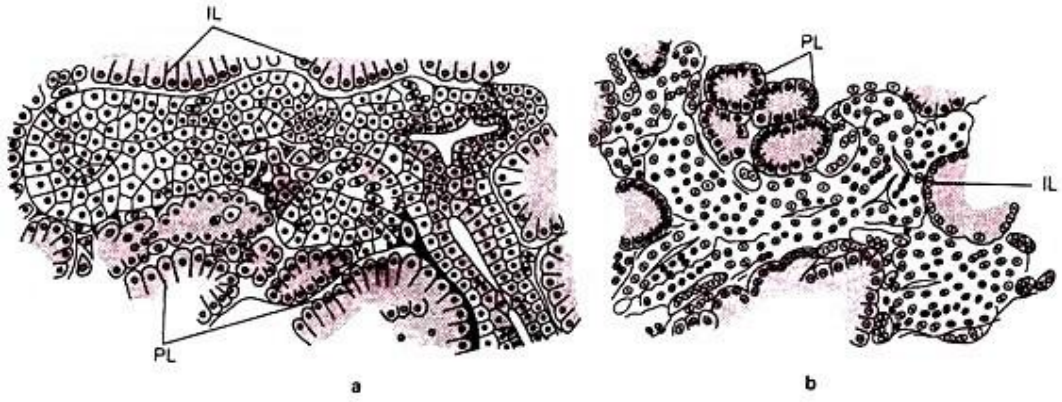

Fig. 19.12a, b : Diagram of pancreas showing endocrine components. (a) *Torpedo marmorata*. (b) *Mustelus laevis*. IL, islets of langerhans; PL, pancreatic lobule.

Pineal Organ:

It is situated near the pituitary. In-spite of being a photoreceptor organ the pineal organ shows endocrine nature of doubtful function. Removal of pineal from *Lebistes* species causes reduced growth rate, anomalies in the skeleton, pituitary, thyroid and corpuscles of Stannius. It has been reported that thyroid and pituitary glands influence the secretion of pineal.

Urophysis:

Urophysis is a small oval body, present in the terminal part of spinal cord (Fig. 19.13a, b, c). It is an organ deposits, which releases materials produced in the neurosecretory cells situated in the spinalcord.These cells together with the urophysis are called the caudal neurosecretory system. This neurosecretory system is found only in elasmobranchs and teleosts but it corresponds to the hypo- thalamo neurosecretory system present invertebrates.

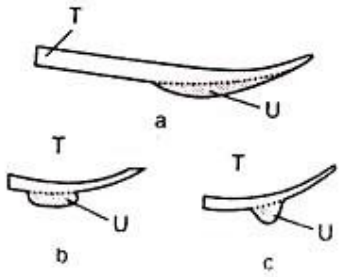

Fig. 19.13a-c : Diagram of urohypophysis of teleosts. (a) rainbow trout. (b) carp. (c) Yellow tail. T, tail; U, urohypophysis (Source : Hibiye, T., 1982)

In caudal neurosecretory system, neurosecretory cells are diffused in terminal part of spinal cord. Axon terminals of these cells assemble at the ventral side of the region and formurophysis with blood capillaries. The neurosecretory cell is a large nerve cell and has basophilic cytoplasm and a polymorphicnucleus.In Ayu (*P. altivelis*) the urophysis is extended like a bow. In carp and yellow tail it is a conspicuous oval body. The urophysis is made up of spinal cord elements like neurosecretory axon, glia, and ependymal and glia fibers and meningeal derivative such as vascular reticulum and reticular fibres. The caudal neurosecretory system is said to be related with osmoregulation. Urophysis extract shows ability to contract smooth muscles of ovary and oviduct of guppy (*Poecilia reticulata*) and the sperm duct of goby (*Gillichthys mirabilis*), suggesting the possibility of involvement in reproduction and spawning.

Probable Questions:

1. Name four major hormones secreted from Pituitary gland of fish. State their functions.

2. What is the main function of thyroid hormones in fishes?

3. Which hormone is secreted from Pineal gland. State its functions.

4. name the hormones secreted from Gonads of fishes. What is their role?

5. write short notes on Chromaffin Tissue or Suprarenal Bodies or Medullary Tissue in fish.

6. Write the names of secreted hormones from Urohypophysis in fishes and what is their functions?

Suggested Readings:

1. Kumar, S, and Tembhre, M (1996) Anatomy and Physiology of Fishes. Vikas Publishing House Pvt. Ltd., India. pp. 152-156.
2. Lagler, K. F., Bardach, J. E., Miller, R. R., and Passino, D. R. (1997) Ichthyology (2nd ed.), New York: John Willy & Sons. pp. 336-346.
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UNIT-XIV

Electroreception in Fish

Objective: In this unit we will discuss about electrolocation in fishes.

Introduction:

Electroreception or electroception is the biological ability to perceive natural electrical stimuli. It has been observed almost exclusively in aquatic or amphibious animals, because water is a much better conductor than air. The known exceptions are the monotremes (echidnas and platypuses), cockroaches and bees. Electroreception is used in electrolocation (detecting objects) and for electrocommunication. Electroreception is the biological ability to perceive natural electrical stimuli. It is observed almost exclusively in aquatic animals, particularly in fish. It is well developed in marine fishes, since salt-water is a much better conductor. Besides fish, it is also used by cockroaches, bees and monotremes (echidnas and platypuses).

Discovery of electroreceptors:

Electroreceptor organs were first identified physiologically in the early 1960s from weakly electric fish by American neuroscientist Theodore H. Bullock and colleagues and by French scientists Thomas Szabo and Alfred Fessard. The existence of electroreceptors had been anticipated in the 1950s by British zoologist Hans W. Lissmann, who was the first to discover continuous weak electric discharges from an electric organ in the tail of a species of African freshwater fish (*Gymnarchusniloticus*). By 1958 he had demonstrated the reason for the discharge by showing that the fish could detect the presence of glass and metal rods or other conducting or nonconducting objects at distances of 10 cm (about 4 inches) or more, even in the absence of visual, mechanical, or chemical cues. Lissmann postulated that the fish was sensing the distortions of its own electric organ discharges as electrical shadows on its skin. He correctly surmised that there were dermal electroreceptors in the fish. He called their behaviour “electrolocation,” after the well-known ability of bats to echolocate objects by detecting returning ultrasonic echoes from their calls.

In the 1960s Dutch scientists Sven Dijkgraaf and Adrianus J. Kalmijn established that sharks and rays, which have dermal sense organs called ampullae of Lorenzini, could sense weak electric currents from their prey organisms such as flatfishes even when the organisms were buried under sand. Dijkgraaf and Kalmijn showed that the ampullae of Lorenzini were essential to this behaviour, which was entirely based on electrosensory cues, and that prey had weak direct current (DC) electric fields surrounding their gills, gut, and skin wounds that gave away their presence to the sharks. When given a choice between a prey fish covered with plastic wrap and a pair of wire electrodes connected to a prey fish in another tank, the shark preferred to dig up the wires. Kalmijn and

colleagues called this ability passive electrolocation, in contrast to the active electrolocation ability of *G. niloticus* discovered by Lissmann.

Occurrence in fish:

Electroreception is found in lampreys,cartilaginous fishes (sharks, rays, chimaeras), lungfishes, bichirs,coelacanths, sturgeons, paddlefishes, catfishes, gymnotiformesandelephantfishes. Theelectroreceptor organs in all these groups are derived embryologically froma mechanoreceptor system. In fishes they are developed from the laterallines.

Electroreceptive fishes can be divided into, as follows,

- Non-electrogenic fish: Fish those are unable to produce electric fields outside the body are called non-electrogenic fish. Most sharks, rays, eels, catfish, and lungfish are non-electrogenic fish.
- Electrogenic fish: Fish those are able to produce electric fields outside the body are called electrogenic fish. They possess a specialised electric organmade up of either disklike modified muscle cells or nerve cells called electrocytes. It is found inTorpedorays, electric skates (Raja), electric catfish (Malapterurus), stargazers (Astroscopidae), South American knifefishes (Gymnotiformes), and African mormyrid form. Strong electric discharges form electric organs used by fish to stun prey or repel predators while weak discharges used in electrolocation and electrocommunication.

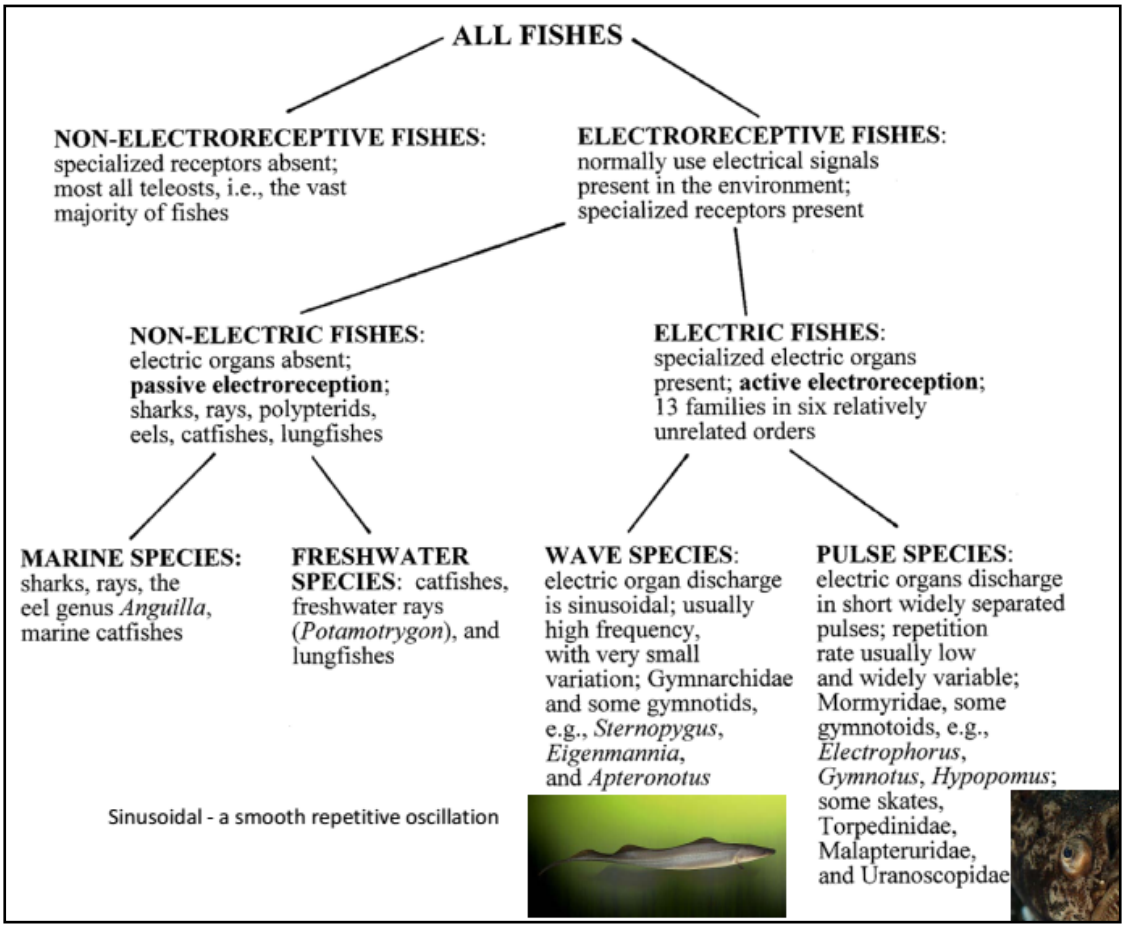

Fig 1: Distribution of electroreception among living fishes.

Function:

Electroreception is used in electrolocation (detecting objects) and for electrocommunication.

A. Electrolocation

Electroreceptive fish use this sense to locate objects around them (Fig: 2A). It is important for feeding and navigation in turbid or murky water and in dark environments, where the animal cannot depend on vision. Many fish use electric fields to detect buried prey.

Types of Electrolocation:

i. Active electrolocation

In active electrolocation, the animal senses its surrounding environment by generating electric fields and detecting distortions in these fields using electroreceptor organs. This electric field is generated by means of a specialized electric organ consisting of modified muscle or nerves. This field may be modulated so that its

frequency and wave form are unique to the species and sometimes, the individual (see Jamming avoidance response). Animals that use active electroreception include the weakly electric fish, which either generate small electrical pulses (termed "pulse-type") or produce a quasi-sinusoidal discharge from the electric organ (termed "wave-type").These fish create a potential which is usually smaller than one volt. Weakly electric fish can discriminate between objects with different resistance and capacitance values, which may help in identifying the object. Active electroreception typically has a range of about one body length, though objects with an electrical impedance similar to that of the surrounding water are nearly undetectable.

ii. Passive electrolocation

In passive electrolocation, the animal senses the weak bioelectric fields generated by other animals and uses it to locate them. These electric fields are generated by all animals due to the activity of their nerves and muscles. A second source of electric fields in fish is the ion pumps associated with osmoregulation at the gill membrane. This field is modulated by the opening and closing of the mouth and gill slits. Many fish that prey on electrogenic fish use the discharges of their prey to detect them. This has driven the prey to evolve more complex or higher frequency signals that are harder to detect. Passive electroreception is carried out solely by ampullary electroreceptors in fish. It is tuned to low frequency signals (below one up to tens of Hertz).

Fish use passive electroreception to supplement or replace their other senses when detecting prey and predators. In sharks, sensing an electric dipole alone is sufficient to cause them to try to eat it.

Example:

1. Sharks and rays (Elasmobranches): Sharks heavily rely on electrolocation for hunting. Some shark embryos and pups "freeze" when they detect the characteristic electric signal of their predators. The electric field sensors of sharks and rays are called the ampullae of Lorenzini (Fig 2B). They consist of electroreceptor cells connected to the seawater by pores on their snouts and other zones of the head. Sharks may also use Earth's magnetic field to navigate the oceans using this sense.

2. Bony fish: Gymnotiformes, specifically, the electric eel (actually a knifefish, not an eel), are able to generate high voltage electric shocks as well as lower voltage pulses for navigation and prey detection in its turbid habitat. The electro-sensory organ found in polypteroids, eels, lungfish and catfish is known as tuberous organ

Evolution and anatomy of electroreception:

Electroreception first appeared in jawless craniates (animals with skulls) such as lampreys that possess an epidermal “end-bud” organ innervated by the lateral line

nerve. The end-bud receptors are sensitive to weak low-frequency (DC to 50 hertz [Hz]) electric fields. Among the earliest jawed vertebrates, the first ampullary electroreceptors were embedded in the skin or at the base of a long conducting canal leading from the skin surface to a specialized patch of modified sensory epithelial cells (in the case of ampullae of Lorenzini). The sensory epithelial cells resemble hair cells of the lateral line or inner ear, and the nerve fibres connecting them to the brain travel in the same nerve bundles with the lateral line nerves. The cells lining the ampullary canals are packed tightly to make a high-resistance insulator around the low-resistance canal lumen, which is composed of a salty gelatinous matrix. Similar to an insulated wire, this arrangement is ideal for conducting electric currents to the sensory cell membranes. All vertebrate electroreceptors follow this basic design.

Electroreception is shared by all the primitive aquatic vertebrates, including some aquatic amphibians, but it was lost in the amniotes as they made the transition to a terrestrial existence. In those monotreme mammals in which the sense reappeared, electroreception is derived from modified mucous glands on the bill or the snout. The mucous glands have low-resistance canals that conduct current from the environment to bare nerve endings of the trigeminal nerve. Electroreception is absent in most modern fishes, with the exception of two independently evolved lineages of teleosts, which include the catfishes and the notopterid knifefishes of Africa. In those groups, electroreceptors evolved independently as ampullary receptors. Tuberous, or alternating current– (AC-) sensitive, electroreceptors also appeared in both of those lineages as subgroups of electric fishes. Those subgroups are the South American knifefishes (Gymnotiformes), which include the electric eel (*Electrophorus electricus*), and the unrelated freshwater mormyrid fishes (Mormyroidea) from Africa. Members of both groups use their tuberous organs for active electrolocation of objects and for electrical communication. Investigations of electroreception among invertebrates have indicated the existence of high behavioral thresholds; for example, the worm *Caenorhabditis elegans* responds to currents that are greater than three volts per centimetre. By comparison, sharks and rays, which have the most-sensitive ampullary receptors, have thresholds as low as 0.02 microvolts per centimetre. Bumblebees detect weak electric fields produced by flowers, though the mechanism and function of electroreception in this case is unknown. The phenomenon was first reported for the species *Bombus terrestris*.

B. Electrocommunication

It is often species specific. Weakly electric fish communicates by modulating the electrical waveform they generate. It is used in mate attraction and territorial displays. Ampullary receptors allow fish to discriminate between own electric discharge as well as those of other fishes, and thus helps in communication with conspecifics (Fig: 2B). Mormyrid electric fish from Africa use tuberous receptors known as Knollen organs to sense electric communication signals.

Example:

1. Agonistic displays: Some species of catfish use their electric discharges only in agonistic displays.

2.Mimicry: A South American riverine fish, belonging to genus *Brachyhypopomu,* mimics the pattern of low voltage electrolocative discharge of the electric eel, may be for protection purpose.

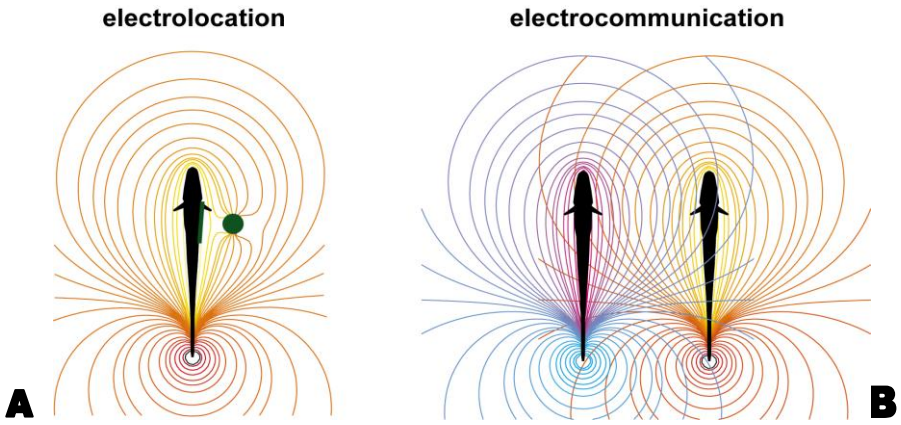

Fig 2. Electrolocation (A) and Electrocommunication (B) in Fish

Types of Electroreception:

Electroreception can be two types – active and passive.

A. Active electroreception: Itinvolves detection of electric fields generated by the fish itself, either by its electric organ or its motion through the earth's magnetic field. Animal use this for orientation.

It has been proposed that sharks can use their acute electric sense to detect the earth's magnetic field by detecting the weak electric currents induced by their swimming or by the flow of ocean currents.Two groups of teleost fishes, the Neotropical knifefishes(Gymnotiformes) and the African elephant fishes (Notopteroidei), are weakly electricand engage in *active e*lectroreception.

Mechanism: In active electrolocation, the animal senses its surrounding environment by generating electric fields and detecting distortions in these fields using electroreceptor organs. This electric field is generated by means of a specialised electric organ possess by some electrogenic fishes (Fig:3). This field may be modulated so that its frequency and wave form are unique to the species and sometimes, the individual. Fish can either generate small electrical pulses (termed "pulsetype") or produce a quasi-sinusoidal discharge (termed "wave-type") from the electric organ. These fish create a potential which is usually smaller than one volt. Weakly electric fish can discriminate between objects with different resistance and capacitance values, which may help in identifying the object.

Active electroreception typically has a range of about one body length, though objects with an electrical impedance similar to that of the surrounding water are nearly undetectable.

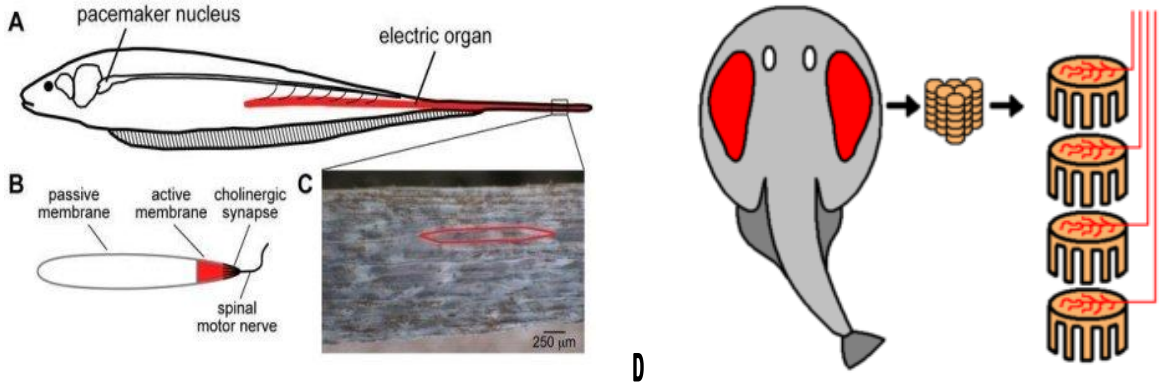

Fig. 3: A. An electric fish showing location of electric organ ; B. An electrocyte; C. A section of electric organ from the tail, with skin removed to expose the electrocytes, which are densely packed within the electric organ. A single electrocyte is outlined in red; D. An electric ray (torpedo) showing location of electric organ and electrocytes stacked within it.

B. Passive electroreception: involves detection of electric currents that originate outside the fish body. In most groups electroreception is passive, where it is usedpredominantly in predation. It is found in elasmobranchs and some catfishes.

Example:

1. Predation: Sharks, particularly hammerheads, can detect a flounder that are buried under 15cm of sand.

2.Communication: Weakly electric marine skates discharge low frequency currents that are picked up by the ampullary receptors of individuals of same species.

Mechanism: In passive electrolocation, the animal senses the weak bioelectric fields generated by other animals and uses it to locate them. These electric fields are generated by all animals due to the activity of their nerves and muscles. A second source of electric fields in fish is the ion pumps associated with osmoregulation at the gill membrane. This field is modulated by the opening and closing of the mouth and gill slits. Many fish that prey on electrogenic fish use the discharges of their prey to detect them. This has driven the prey to evolve more complex or higher frequency signals that are harder to detect. Passive electroreception is carried out solely by ampullary electroreceptors (Fig. 4 B) in fish. It is tuned to low frequency signals **(**below 50 Hz**).** These receptors have a jelly-filled canal leading from the sensory receptors to theskinsurface.Fish use passive electroreception to supplement or replace their other senses when detecting prey and predators. In sharks, sensing an electric dipole alone is sufficient to cause them to try to eat it.

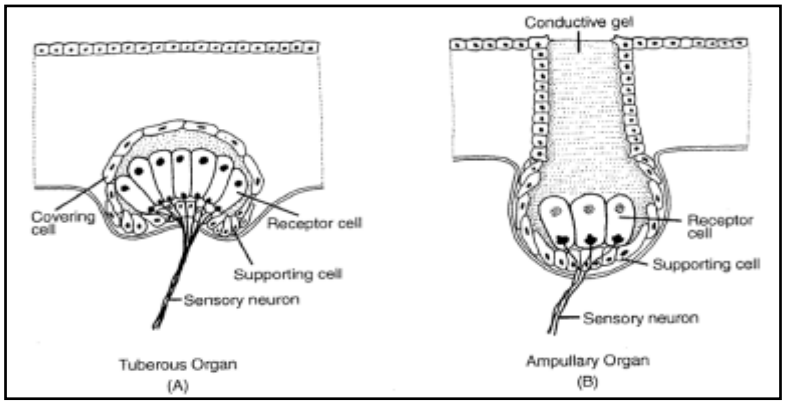

Fig 4: Electroreceptive organs in fish

Bioelectric organ:

Bioelectric organ, also called electric organ, system of tissues specialized for the production and use of electrical power in a living organism. Well developed in a wide variety of fishes, both marine and freshwater, indicating an early evolutionary development, bioelectric organs probably represent a specialization of a common bioelectrical capacity of all living cells. (Various other tissues and organs also possess the capacity to produce electricity—the skin of frogs and the heart, brain, and eye of higher animals including humans). In more than 200 fish species, the bioelectric organ is involved in self-defence or hunting. The torpedo, or electric ray, and the electric eel have especially powerful electric organs, which they apparently use to immobilize or kill prey.

The electric eel has three pairs of electric organs; they constitute most of the mass of the body and about four-fifths of the total length of the fish. This fish is reputed to be able to generate a sufficiently powerful electric shock—600 to 1,000 volts at one ampere—to stun a human. Electric rays have two large, disk-shaped electric organs, one on each side of the body, that contribute to the disklike shape of the body. The electric catfish of Africa, the knife fish of Latin America, and the stargazers probably use their bioelectric organs as sense organs in the detection of other fishes.

The basic element of an electric organ is a flattened cell called an electro plaque. Large numbers of electro plaques are arranged in series and in parallel to build up voltage and current-producing capacity of the electric organ. Fishes deliver a sudden discharge of electricity by timing the nervous impulses that activate individual electro plaques, thereby providing simultaneous action of the entire array.

Probable Questions:

1. Define electroreception in fish.

2. Briefly describe active electroreception with proper examples.

3. Briefly describe passive electroreception with proper examples.

4. What is electrolocation. Give examples.

5. What is electrocommunication. Give examples.

6. Classify fishes n the basis of their electroreceptive property.

7. Describe active and passive electrolocation with examples.

Suggested Readings:

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6. Catania, Kenneth C. (October 2015) Electric Eels Concentrate Their Electric Field to Induce Involuntary Fatiguein Struggling Prey. **CurrentBiology**. ***25*** (22): 1–10. doi:10.1016/j.cub.2015.09.036

UNIT-XV

Determination of age of fish by scale and hard parts

Objective: In this section you will learn about age determination in Fishes by using scale and other hard parts.

Introduction:

Growth is a bio-energetic process and is defined as a change in its length and weight over a period of time. It indicates the health of the individual and of the population and has been extensively studied for a various species of fishes. The growth and age of a fish are closely related to each other and depends on several factors.Determination of age and growth of fishes are one of the important aspects in the development of fisheries. Both age and growth are closely related with one other. As the fish ages, it grows, but after attaining a particular size, growth stops. Age gives an idea about sexual maturity, spawning time, catchable size, growth rate and longevity. Knowledge of all these parameters is essential in fisheries production.

Growth of fish or any organism is the change in length and weight with increase of age as a result of metabolism of nutrition. Hence growth is an index of healthy food and oxygen supply in the water-body. Proper growth of fish also indicates that the water body is devoid of any pollution. The two parameters exhibit growth of a fish are length and weight. The growth in length indicates long term change, whereas growth in weight is more subject to seasonal variation.

a. Absolute growth: means the highest or perfect growth of fish from embryonic to senescence period.

b. Relative growth: means growth comparison from one life period to another. For obvious reasons growth is never similar during any two life periods.

c. Isometric growth: means fish having equality of measure, having the plane of projection equally inclined to three perpendicular axes at right angles to one another. If the fish is following the cube law, the growth is called isometric.

d. Allometric growth: it is lopsided growth. There may be various pattern of this type of growth. For example several fish grow more in length than width and weight.

Knowledge of age and growth of fish has many applications:

1. We can calculate the time of sexual maturity of different species.

2. Further, we can know their spawning time.

3. Growth rate of fish also indicates the suitability of particular species for a particular type of the water-body.

4. Growth rate and age of fish also indicate the size of fish at different stages, e.g., fry, fingerling, and adult of different species.

5. The study of age and growth is helpful in catching fishes by using nets of desirable mesh size.

Factors influencing growth of a fish

- Temperature
- Photoperiod
- Quantity and quality of food available
- Dissolved oxygen
- Ammonia in water
- Salinity
- Age and stage of maturity of fish
- Inter-specific and intra-specific competition
- Stocking density
- Disease

Condition factor or Ponderal Index:

The condition factor or Ponderal index, or co- efficient of correlation expresses the condition of a fish, such as the degree of well being, relative robustness, plumpness or fatness in numerical terms. The condition factor used to determine from length and weight of the fish.

Ponderal index or condition factor $K=100W/L^3$

Where L is length in cms and W is weight of fish in grams. The cube of length is taken because the growth in weight is proportionate to the growth in volume.

Condition factor is generally used by fish biologist as an indication of the health of a fish population. A high value of K shows that plenty of food is available to support both somatic and gonadal development of fish. The value of K differs with season and

influenced by maturity and spawning. The value of K is maximum during spawning season.

Method for Determining Growth

a. Direct method: Growth rate of a fish can be determined directly by rearing the fish under controlled conditions. For this eggs or larva of known age are kept in experimental pond. Length and weight of each are measured at known intervals of time for calculating growth rate.

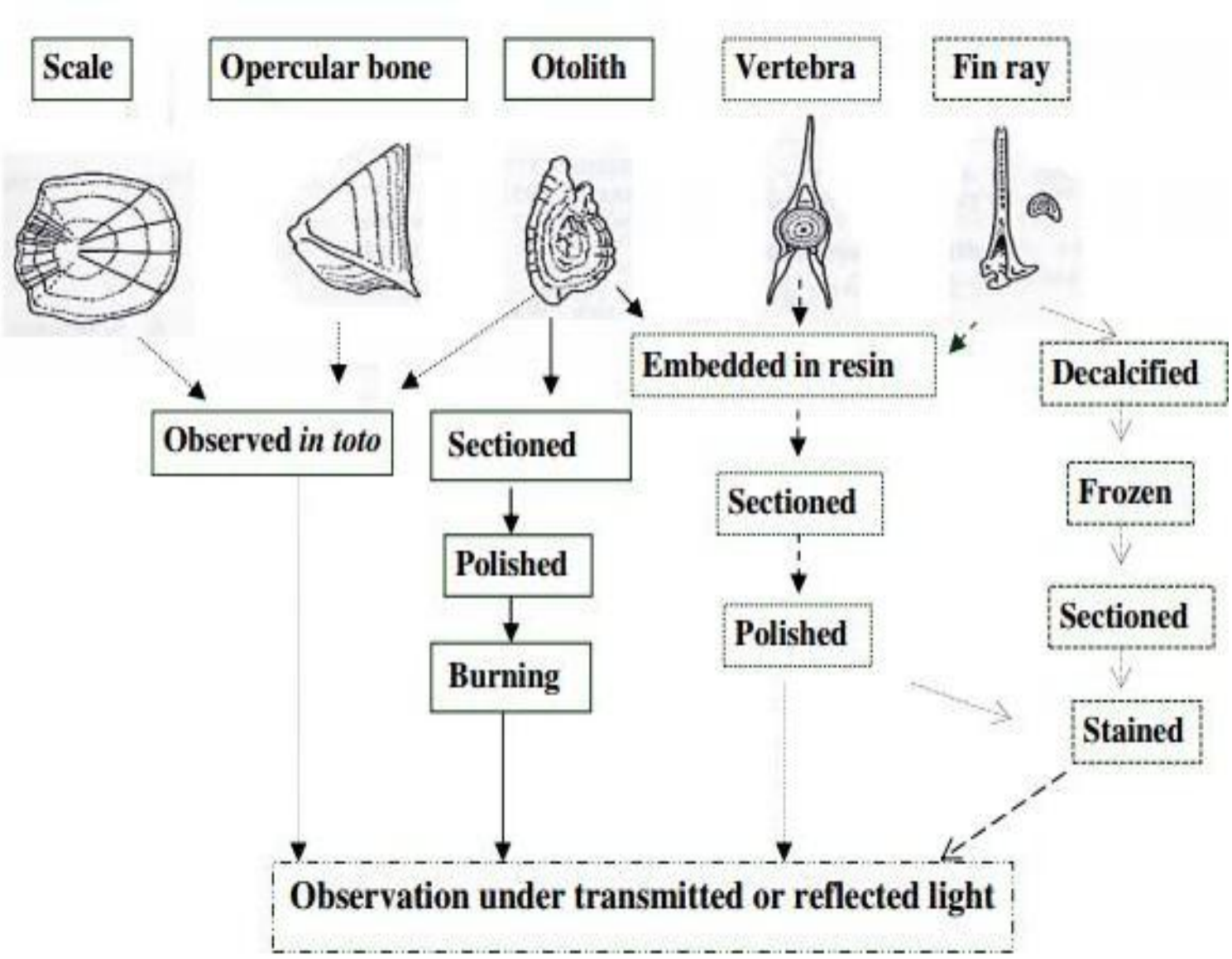
b. Fish marking and tagging: in this method fishes are marked or tagged after the length and weight for identification and are than released in the natural habitat. After the few months these fishes are recaptured and measured again. The change in size during the interval gives the growth rates.

Methods of Age and Growth Determination:

a. By counting rings or annuli on Bones

b. By counting rings or annuli on Otolith

c. By counting rings or annuli on Scales.



Various methods employed for determination of age of fish are:

A. Scale Method:

This method is most commonly used for determination of age of Osteichthyes (bony fish), which are provided with cycloid and ctenoid scales. The structure of scale and its development is useful in the interpretation of growth zones. The structure of scale can be seen very easily under the microscope after washing with dilute solution of caustic soda followed by staining with borax carmine.

A well-developed scale has the following structures:

1. Focus:

It is a clear area in the center, but may be shifted from the center due to irregular growth of anterior or posterior parts of scale caused by unusual overlapping of scales.

2. Circuli:
These are concentric rings present around the focus, they run parallel at regular intervals or distances. They appear as ridges.

3. Grooves:
The grooves are found between the ridges of circuli and they are responsible for maintaining the regular space between them.

4. Radii:
These are grooves found radially, viz., they run from focus to margin of scale. Radii cut the circuli present in their path.

5. Annuli:
These are wide circular troughs found in aged fish over one year. Each trough contains a few incomplete and narrow circuli different from the circuli outside it, which are complete and more widely spaced. The number of annuli represents age of a fish in years (Fig. 14.1).

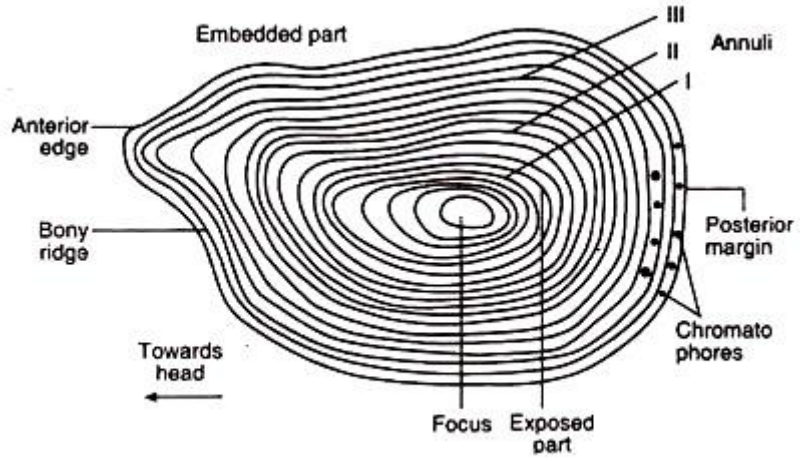


Fig. 14.1 : Scale method fish scale showing growth rings.

At the time of development of scale, focus is established first and represents the original size of the scale. As scale grows older, other structures are added and perform their functions. The grooves and circuli represent growth activity. They also indicate osteoblastic activity as a result of which secreted material is deposited around the focus. In this way every year many such circuli and grooves are formed.

A characteristic bone material, ichthylepidin, is deposited in circuli and thus their height increases which depends on the calcification. Annuli show slow growth in a year but in many fishes, during winters, annuli grow remarkably and are added yearly as fish grows.

Thus annuli are very useful in counting the age of fish and serves as year-marks on the scale for age determination. The annuli are best seen at anterior part of the scale.

Types of Annuli

1. True Annuli:

The true annuli has following characteristic features:

(i) In cycloid scales true annuli is represented by a closely situated circuli, which is covered by widely spaced circuli.

(ii) Two complete circuli surround the trough, which is wider on the anterolateral and posterior side.

(iii) The wide part of the trough contains incomplete circuli that do not grow completely around the scale.

(iv) The trough remains narrow at the anterolateral side. In the ctenoid scales specially, the outer circulus cuts across or crosses over the incomplete circuli lying in the anterior part of the trough.

Annuli are considered as year-marks in the age determination of fish in the case of the following facts.

a. When there is a correlation between the calculated age from the scale and the size of the fish.

b. The length frequency distribution should coincide with the calculated age from the scales.

c. The calculated age should be in agreement with the age determined by other methods.

2. False Annuli:

Sometimes false annuli appear on the scales of fishes due to undesirable factors like retarded growth due to paucity of food, starvation, injury, disease, and fluctuation in temperature. These false annuli resemble the true annuli but they take position on the scale closer to the true annulus of the preceding year than the normal annulus for the next year which appear in case of normal growth (Fig. 14.2).

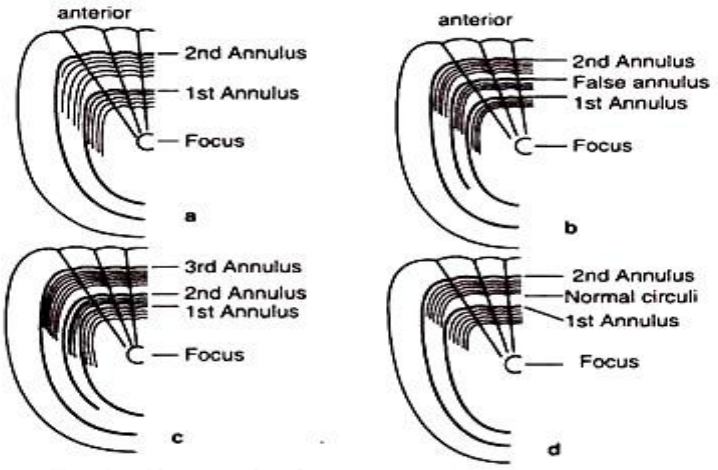


Fig. 14.2a-d : Annuli on scale in four cases of a certain species of fish. a. normal growth for a fish of a certain weight and of 2 years of age, showing annuli; b. abnormal growth for a fish of same weight and of same age, showing false annulus; c. abnormal growth in a 3-years old fish, showing skipped annulus (at 2nd annulus); d. abnormal growth for a 3-year old fish, showing overlapping annulus (at 2nd annulus)

3. Overlapping Annuli:

The position of these annuli in posterior field coincide with the annuli of the preceding year while, in anterior part, it is separated from the preceding year's annuli by 4 or 5 circuli. The overlapping of scales can occur due to a slow growth during the growing period, which is represented by an increase in the length but not in the weight of body.

4. Skipped Annulus:

This type of annulus by position coincides with the annulus of the preceding year, with no normal circuli forming in between. This abnormal function is due to the fact that the fish has not grown during one growing season (one summer) either in length or in weight.

The age of fish can be accurately determined using a scale. The relationship between body length and scale length (or radius) may be plotted as graph using body length on X-axis and scale length on Y- axis. The following formula used to depict the relationship

$$\textbf{Log L= log c + n log S}$$

L = body length

S = scale length

C = intercept on the line on the axis of ordinate n= slope

After the age of the fish has been determined by counting annuli, the fish length at each year can be back calculated by measuring the radius from the focus of the scale to each annulus. Thus the length of a fish at 'n' years

$Ln = a + (L-a)\ (Vn)/Vr$

Ln = calculated length of the fish at n year L= length of the fish at the time of capture

Vn = radius (distance from the focus to the nth annulus)

Vr = scale radius (distance from the focus to edge of the scale)

a = a constant that often fish length at the time of scale formation

Applications of Scale Method:

1. Fish of temperate regions shows clear rings, which are true marks. This is because there is a sharp difference between the temperatures of two seasons—summer—the period of faster growth, and winter—the period of slow growth or no growth. Therefore, the calculation of the age of fish by annuli is most reliable in temperate fish.

2. This method is more reliably applicable in case of salmons, carps, cod and herrings, established a method of estimating age of fish based on scales, which is as given on next page:

Number of Annuli on the Scale	Estimated Age	Designation of Age Group
None	Less than one year old	0+
One	One year old	1
One + a few circuli	More than one year old but less than two years old	1+
Two	Two years old	2
Two + a few circuli	More than two years old, but less than three years old	2+

Limitations of Scale Method:

1. More than one annulus are added in the extreme conditions of life, e.g., extreme cold (causes cessation of feeding), change in food quality or starvation at the time of spawning. These additional rings are called supplementary rings, which cause problem in age determination by the scale method.

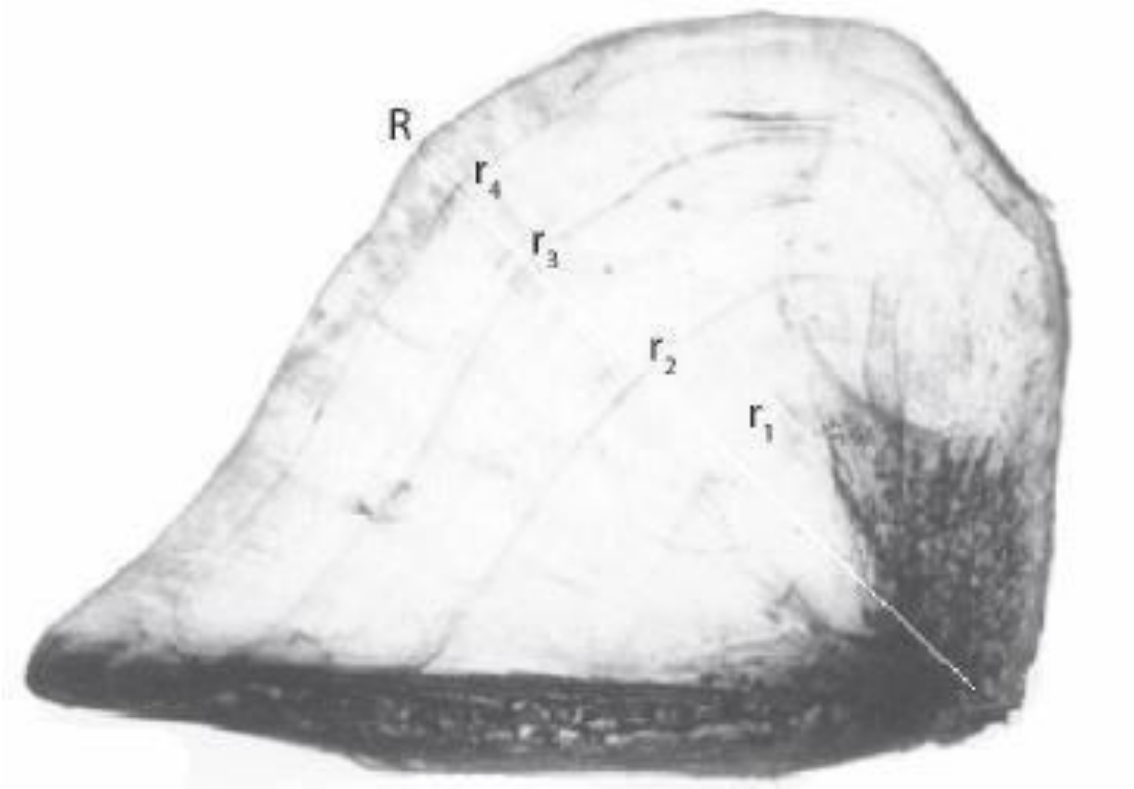
2. This method cannot be applied to those fishes which live in the water with more or less uniform temperatures (tropics). This may be because in these places fishes spawn more than once and, due to fluctuations in food and chemical compositions of water due to rains and floods, formation of annulus may not be an annual feature. Thus, in the fishes of tropical regions, the growth rings do not actually represent year-marks.

B. Bone Method:

Annuli are also present on some bones. The important bones such as operculum, vertebra, supra occipital and scapula are provided with annulations. These annuli are increased in number with the age of fish. The growth rate is different in different seasons. Number of annual rings are helpful in calculating the age of fish.

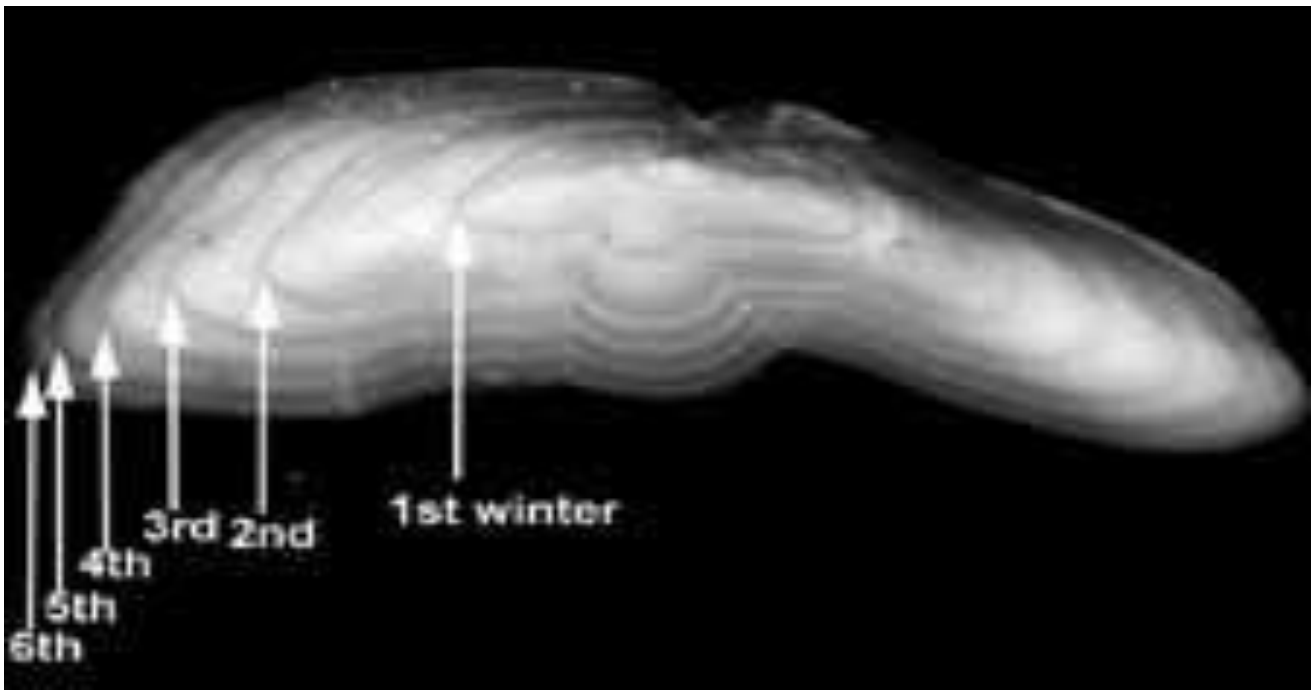
Similarly, the centrum of fish is also helpful in calculating the age of fish. The centrum of fish vertebrae possess rings, which are used in age determination. For counting the rings on the centrum, it is exposed by removing the tissues attached to it using solution of 0.7% pepsin in 0.2% of hydrochloric acid. The rings on the centrum are counted under the microscope.

The choice of calcified structures for aging varies among species, a structure used in one species may not be the same structure used in another. Not all bony structures lay down growth rings equally. Such bony structures used for age estimation are vertebrae, opercula, fin rays, pectoral spines, among others. Preparation for bony parts involves first cleaning by soaking the structure in bleach or boiling to remove soft tissues. Depending on the size, shape, and structure of the calcified aging part it may be examined whole or more likely, sectioned.



C. Otolith Method:

Otoliths are the earbones of a teleost (bony) fish and are present in pairs; fish have three pairs, the lapilli, the sagittae and the astersci. These three pairs of otoliths in teleost fishes differ in form, function, size, shape, and ultrastructure. Otoliths are generally easier to read than scales and are more accurate, being internal and never reabsorbing like scales. Often the sagittae are analyzed for growth as they are the largest of the three otoliths and therefore easiest to remove. When preparing to analyze otoliths, generally if the otolith is <300 mm than it can be analyzed intact, when >300 mm otoliths contain too much three dimensional material and must be sectioned to analyze it more clearly.



D. Known Age Method:

The determination of growth rate through this method requires the knowledge of age, which can be known from time of breeding. The hatchlings are kept in a tank in appropriate conditions for two or three seasons. The growth is measured periodically. The fishes are marked with tags and reintroduced in the water. The fishes are recaptured at regular intervals and idea of growth rate in relation of the time is taken.

E. Length Frequency Distribution Method:

Peterson introduced this method in the nineteenth century. In this method, in the sample of fishes of a particular fish, frequency analysis shows length of individuals of one age varying around the mean length according to normal distribution.Data of the sample is plotted and peaks are counted which represent grouping of fishes of successive lengths. Age groups are thus separated and the age of a given species is determined.

This method is suitable for determining the age of younger fishes of 2 to 4 years. This method is not reliable for calculating the age of older fishes because of overlapping of length frequencies in individuals of different ages.

F. Pectoral Spine or Fin Ray Method:

Spines of the pectoral fin is also useful in age determination. For this 3μ to 4μ thick sections of spines are cut and mounted in glycerin and then observed in the microscope. 1% to 2% of indistinct annuli or fin ray consistency are compared to 15% to 20% of annuli on the scales and, thus age is calculated.

Probable questions:

1. Describe structures of a scale.

2. What are the applications of age determination in fishes.

3. What is known age method of fish age determination?

4. What is otolith method of fish age determination?

5. What is Bone method of fish age determination?

6. What is pectoral fin method of fish age determination?

7. What are the limitations of scale method?

8. Write a short note on True Annuli.

9. Write a short note on False Annuli.

10. Write down the applications of scale method.

Suggested Readings:

A text book of Fish Biology and Fisheries (3rd edition) by- S.S. Khanna & H.R. Sing

UNIT-XVI

Poisonous and Venomous Fish

Objective: In this unit you will know about poisonous and venomous fish.

Introduction:

Biotoxins are mainly of two types; Phytotoxins or plant poisons and Zootoxins or animal poisons. A large variety and number of marine creatures inhabiting the ocean waters pose threat to humans by virtue of their ability either to bite, lacerate or sting or contain toxic substances within their flesh, blood etc. The information regarding the source of adventitious toxicity of fish is not only vital for development of antidotes and rational assessment of the usefulness of the fish species as food source, but it also opens an arena for the exploration of new biologically active chemical substances or biodynamic compounds of therapeutic value. Halstead and Courville (1970), Concon (1988), and Halstead (2001) have classified ichthyotoxic fish into two groups:

a) Poisonous or Phenerotoxic fish which when ingested cause a biotoxication in humans due to a toxic substance present in the fish and

b) Venomous or Acanthotoxic fish that produce poison by means of glandular structures that are equipped with a traumatogenio device to purvey their venoms.

Intermediate to the poisonous and venomous fish are the **crinotoxic fish** that produce a poison by means of glandular structures independent of true venom apparatus.

Divisions of Poisonous Fishes:

Fish poisoning is synonymous to the Icthyotoxism. Halstead has made four divisions of poisonous fishes.

a) Ichthyosarcotoxic fishes: Those fishes which contain a toxin within their musculature, viscera or skin. **Examples:** Cyclostomes, Elasmobranch, Chimaera, Tetraodon etc.

b) Ichthyootoxic fishes: Those fishes which produce a toxin generally confined particularly to female gonads. In such fishes there is a relation between gonadal activity and the production of toxin.

c) Ichthyohaemotoxic Fishes: Those fishes which have a toxin in their blood. Some freshwater eels and several marine fishes make up this group.

d) Ichthyocrinotoxic fishes: Those fishes which produce toxins by glandular secretion but lack a true venom apparatus. Example: Box fishes or trunk fishes and the lampreys.

These may produce a toxic substance inn their skin and subsequently release ths toxin into the environment.

I. Poisonous Fish:

Research in the past several decades has increased our knowledge on the types of toxic fish, the nature and source of toxins and the conditions governing their toxicity. The major problem facing the marine biotoxinologist is the variability and frequent unpredictability of the toxicity of this segment of marine life. The degree of toxicity of these fish may fluctuate periodically. The poisonous substances in fish may be concentrated in specific tissues or organs. Based on the tissue in which the toxin is present in the fish the poisonous fish are further classified into lchthyosarcotoxic (flesh, musculature, viscera or skin) eg: herrings, anchovies, tarpons and pufferfish, lchthyootoxic (roe or gonad) eg: The most dangerous forms are the genera *Barbus, Schizothorax, Tinca* (Cyprinidae) and Stichaeus (Stichaeidae). lchthyohaemotoxic (blood or serum) eg: Those fish having poisonous blood, members of the anguilliform families Anguillidae, Congridae, Muraenidae, and Ophichthidaelchthyo hepatotoxic (liver) eg: sharks.

List of some poisonous fish:

- Species of puffer fish are the most poisonous in the world and the second most poisonous vertebrate after the golden dart frog. The active substance, tetrodotoxin, found in the internal organs and sometimes also the skin, paralyzes the diaphragm muscles of human victims, who can die from suffocation.
- The spotted trunkfish is a coral reef fish that secretes a colorless ciguatera toxin from glands on its skin when touched. The toxin is only dangerous when ingested, and poses no immediate harm to divers. However, predators as large as nurse sharks can die as a result of eating a trunkfish.
- The giant moray is a reef fish at the top of the food chain. Like many other apex reef fish, it is likely to cause ciguatera poisoning if eaten.

II. Crinotoxic fish:

Crinotoxic fish are intermediate to poisonous and venomous fish and include filefish, pufferfish, trunkfish, boxfish, toadfish, gobyfish, catfish etc. They are able to secrete substances from their skin, known as icthyocrinotoxins, that are capable of repelling or incapacitating other marine animals. These secretions are also thought to possess antibiotic activity, protecting fish from the myriad of invading microorganisms in the marine environment, a fact that has prompted recent research on them. These have specialized cells or glands in their skin but lack a parenteral mechanism. Glandular secretions are normally released into the surrounding medium, the water. Crinotoxins are often called mucus toxins. The poison glands of ichthyocrinotoxic fish assist in the

defensive mechanism of the fish by producing warning or repellent substances especially under stress conditions.

III. Venomous fish:

Fish constitute almost half the number of vertebrates on earth, and approximately 22,000 species of fish are contained in some 50 orders and 445 families. Of these, nearly 1,200 species of marine fish, including stingrays, scorpionfish, zebrafish, stonefish, weeverfish, toadfish, stargazers, and some species of shark, ratfish, catfish, surgeonfish and blenny, are known or suspected to be venomous. The vast majority of these fish is non-migratory, slow moving, and tend to live in shallow waters in protected habitats. It has been suggested that this tendency towards inactivity is closely linked with the evolution of venom apparatus.

List of some Venomous fish:

- The most venomous known fish is the reef stonefish with a remarkable ability to camouflage itself amongst rocks. It is an ambush predator that sits on the bottom waiting for prey to approach. Instead of swimming away if disturbed, it erects 13 venomous spines along its back. For defence it can shoot venom from each or all of these spines. Each spine is like a hypodermic needle, delivering the venom from two sacs attached to the spine. The stonefish has control over whether to shoot its venom, and does so when provoked or frightened. The venom results in severe pain, paralysis and tissue death, and can be fatal if not treated. Despite its formidable defences, stonefish have predators.

- The lionfish is a venomous coral reef fish. Unlike stonefish, a lionfish can release venom only if something strikes its spines. Lionfish can aggressively dart at scuba divers and attempt to puncture their facemask with their venomous spines.

- The stargazer buries itself and can deliver electric shocks as well as venom. It is a delicacy in some cultures (cooking destroys the venom), and can be found for sale in some fish markets with the electric organ removed.

- Stingrays can sting and cause an injury with their stinger. Such envenomation can occur to people who wade in shallow water and tread on them. This can be avoided by shuffling through the sand or stamping on the bottom, as the rays detect this and swim away. The stinger usually breaks off in the wound. It is barbed, so it can easily penetrate but not so easily be removed. The stinger causes local trauma from the cut itself, pain and swelling from the venom, and possible later infection from bacteria. Occasionally severed arteries or death can result.

Different types of poisonous and venomous fish with their specific stinging apparatus:

a. Stinging apparatus in the pectoral fin:

In case of some fish the spine has been modified from outer most pectoral fin and is capable of infecting jagged food. At the base of each spine there is a sac with more or less wide opening and this is believed to contain a fluid of viscous nature which is poured out when the spine bought into action. Eg: *Ictalurus* sp., *Clariousbatrachus*

b. Stinging apparatus at operculum and dorsal spine stinger:

Long sharp spine of operculum and spine supporting the 1st dorsal fin associated with venom gland in their tip as in weaver fish has two opercular and five to eight dorsal spines with the glandular tissue surrounding each spine. There is no canal living from the gland and the venom is set free by rapture of the cell throwing down the groove and injected into the wound. Eg: Weever fish

c. Stinging apparatus at gill cover and dorsal spine:

The two spines of the 1st dorsal fin and spine on the gill cover or opercular spine constitute the venom apparatus. Each of these spines is hollowed and perforated just like the venom fang of the snake. The base of each spine is embedded in the center of the poison gland and the secretion is discharged through the spine just like as a hypodermic needle. Eg: *Thalassophryne s*p.

d. Stinger at the base of dorsal fin:

Violy and Formidable scorpion fish of tropic seas have this type of stinger. The venom gland is situated under a heavy skin beneath the bases, towards the tip of dorsal spine and each be continued into a duct situated into the deep groove on either side of the spine. The 5th spine of the dorsal fin penetrate into the skin if someone step on it and the venom is injected into the wound by pressure of the foot on the bag like venom gland. Expulsion of venom takes place when the integumentary sheath is retracted along the spine.Eg: Stone fish(*Synanceia* sp.)

e. Stringer on the each side of the caudal pedancle:

In *Acanthurus sp.* The caudal peduncle stinger is present on each side of the body with venom gland. A pair of knife sharp spine on either side of the caudal peduncle is the main poison apparatus. Venom gland located within the pine sheath is the interesting feature of the fish. The spine is directed forward but when the fish become excited it is extended from the right angles of the body and get ready for injecting.Eg: *Acanthurus sp.*

f. Shoulder stringer with venom gland:

The venom apparatus consists of two solder spines which are situated bilaterally and each of which protrudes through an integumentary sheath. The spines are sharp, conical

structure with cement like material and have concentric groove lamellae. The spine is said to have double groove through which the venom flow. When the pressure is put upon the sheath of the venom containing cell as well as spine the sheath rapture and the venom is forced on the groove of the spine.Eg: *Astroscopus sp.*

g. Stinging apparatus of caudal region:

In case of elasmobranch in sting ray possess one or two spine attached to the upper middle part of the beak like tail. Glandular epitheliam lying in the ventro lateral glandular groove of the spine considered as the primary venom producing area. Each spine along their side bear dozen of bar hooks contain venom glands. Groove turns backward as pierced to the victim and the snake like duct eject poison. Besides sting ray caudal fins are also present in various other fishes.Eg: *Trigon* sp.

h. Stinger in dorsal fin with venomous glandular epithelium in groove:

Venom apparatus composed of dorsal fin spine and associated with glandular tissue that grooved along the base of the spine. The glandular cells are present in the integumentary portion and in the area of anterolateral groove. The venom secretion is holocrine type of secretion.Eg: Spiny dog fish(*Squalusacanthias*)

i. Stinger on dorsal fin with venomous glandular epithelium:

Venom apparatus consists of dorsal spine, glandular epithelium of spine connecting membrane and the enveloping integumentary sheath. Spine has a pronounced keel area and shallow depression. Venom producing tissues lie within the depression. The secretion of the spine is discharged through the groove of the spine into the wounds.

Probable Questions:

1. What is the differences between poisonous and venomous fishes?

2. Classify poisonous fishes.

3. Write short notes on Crinotoxic fishes.

4. Write different types of stinging apparatus in poisonous fishes.

Suggested Readings:

A text book of Fish Biology and Fisheries (3rd edition) by- S.S. Khanna & H.R. Singh

Unit-XVII

Fish Migration : Types, Theories and Significances

Objective: In this unit you will know about types, theories and significance of fish migration.

Introduction:

Migration is the movement of large number of animals from one place to another for feeding, reproduction or to escape weather extremes. When large numbers of fishes come together and move socially it is called **shoaling.** But sometimes migrating fishes exhibit high degree of coordination in their movements and carry out synchronized manoeuvres to produce different types of shapes. This is called **schooling,** as seen in tunas and sardines.

Feeding or alimental migration takes place in fishes for feeding. In high populations fishes exhaust food resources in an area quickly and therefore must migrate constantly in search of new feeding resources. Salmons, cods and sword fish constantly migrate for food from one place to another in the sea.

Spawning migration takes place in breeding season in those fishes which have spawning grounds far away from feeding places. Migratory fishes such as eels and salmons and a large number of riverine fishes spawn in tributaries of river in hills and migrate in large number for laying eggs in these oxygen rich waters.

Juvenile migration involves larval stages of fishes which hatch in spawning grounds and must migrate long distances in order to reach the feeding habitats of their parents.

Recruitment migration takes place when large number of larvae moves from nursery habitat to the habitat of adults which may sometimes be distinctly different. Adults of eels live in rivers in Europe and America but their larval stages live and grown in sea and migrate to reach rivers which may take one to two years.

Seasonal migration takes place in fishes that inhabit arctic areas where in summer climate is conducive and food abundant but as winter approaches temperatures fall below zero and food becomes scarce. Hence fishes must migrate towards subtropical and tropical areas to escape extremes of weather conditions.

Definition of Migration:

Migration of fish is defined as a class of movement which involves a long journey to a definite area for some purpose and impels the migrants to return to the region from which they have migrated. The purpose of the journey is breeding and feeding. Migration is a two-way journey. It includes emigration (outward journey) and

immigration (return journey or inward journey). The fishes are notable for migration for the purpose of spawning. The inherent purpose of migration is not known.

Types of Migration:

Myers (1949) has classified the following types of fish migration.

a. Diadromous migration:
When the migrations occur in between freshwater and marine environments

Diadromous type of migration can be divided into following three-types.

(i) Anadromous migration:
When migration occurs from sea to freshwater for spawning, called anadromous migration; e.g., Atlantic salmon (*Salmo salar*), HILSA shad (*Tenualosailisha*), Toli shad (*Tenualosatoli*), Paradise fish (*Polynemusparadiseus*), Flat head sillago (*Sillaginopsispanijus*), Sturgeon (*Acipenser*) and Salmon (*Oncorhynchus*).

(ii) Catadromous migration:
The journey of freshwater fishes to the sea for spawning, called catadromous migration; e.g., Indian longfin eel (*Anguilla bengalensisbengalensis*), Shortfin eel (*Anguilla bicolorbicolor*), Common freshwater or European eel (*Anguilla anguilla, A. vulgaris*), American eel (*Anguilla rostrata*).

(iii) Amphidromous migration:
Migration of fishes from freshwater to sea and vice versa and is not for the purpose of breeding but for the other purposes (e.g., food). The amphidromous fishes migrate regularly at some particular stage of the life cycle. Marine amphidromy occurs in flat head mullets (*Mugil cephalus*) which spawn in the Indian seas during autumn and early winter and whose young stage spend a short period in brackish water and freshwater. They are able to survive in ponds with salinity at 87%. After spending in fresh- or brackish water they return to marine water.

b. Potamodromous migration:
Migrations of fish that occur entirely in freshwater is called potamodromous e.g., carps and trout. Trouts and carps travel long distances in large shoals in search of suitable spawning grounds and return to feeding areas after spawning.

c. Oceanodromous migration:
Migration which occurs entirely in sea, called oceanodromous migration. Horizontal and vertical distribution is considered in oceanodromous migration. Many fishes undertake short distance migrations throughout their life and some fishes like herrings, cod, tuna and plaice, cover long distance migrations.

Latitudinal Migration:
This is performed by fishes like barracudas *(Sphyraena*) and swordfish (*Xiphius*) of the warm tropical seas. They migrate to north in spring and to south in autumn.

Vertical Migration:

This is performed by many marine and freshwater fishes and is related to light, search of food, protection and also to spawning. The mackeral rises into the surface waters when there is a rich development of plankton. They eat on plankton and go to deep layers after feeding.

The swordfish, which normally lives in surface water move downwards to great depths to feed deep water fishes like scopelids. Many pelagic larvae of marine fishes perform diurnal vertical feeding migrations. They follow the vertical movements of their prey, the planktonic invertebrates which move down to great depth by day and rise to surface by night.Many deep water fishes of the order Scopeliformes rises to spawn in the upper layers. Their eggs develop and often their larvae live feeding on the phytoplankton. Among freshwater fishes the clearest example of vertical spawning migration is that of the Lake Baikal Comephoridae. These fishes are viviparous and rise to surface from great depth of the lake to give birth to their larvae.

Spawning Migration:

This is the migration in fishes for breeding, and so it is related to life cycle. Spawning migration is an adaptation for ensuring the most favourable conditions for the development of the eggs and the larvae. This also gives protection to early stages of fishes from predators.

There are two major types of spawning migrations. Movement from freshwater to saltwater for spawning is called catadromous migration. The reverse movement, that is, from saltwater to freshwater is termed anadromous migration.

(a) Catadromous migration:

The most famous examples of catadromous fish is the eels, *Anguilla rostrata*, the European eel and Anguilla vulgaris, the American eel. For eel, the river serves as the feeding ground while die sea serves as the spawning ground. The stimulus for the start of migration in eel is the ripening of its gonads in rivers.

Before it enters the sea, the eye of the eel becomes enlarged, sometimes becoming four times as large as the eye of freshwater eels. Its face becomes sharper and its colouration changes the back becomes darker, while the belly changes from yellow to a silvery colour. The eel starts its migration in a wellfedcondition.During migration it spends enormous amount of energy so it becomes very thin. The migrating eel does not feed. Its alimentary canal degenerates considerably. Osmotic pressure of its blood rises and size of its swim-bladder decreases.

The eels migrate about 4500 km westwards from Europe or eastward from America and reach the breeding place in the Sargasso Sea off Bermuda. The adult die immediately after spawning in deep waters.The fertilized eggs hatch out into transparent, ribbon like larvae, called the leptocephali. These were erroneously called glass fishes and placed in the genus Leptocephalus. They lead a pelagic life for a year or more and undergoes metamorphosis into elvers (glass eels).

The elvers then start ascending the rivers in shoals and grow for some years to become adult eels. The adult eel, on maturity start moving towards sea, again the cycle is repeated.

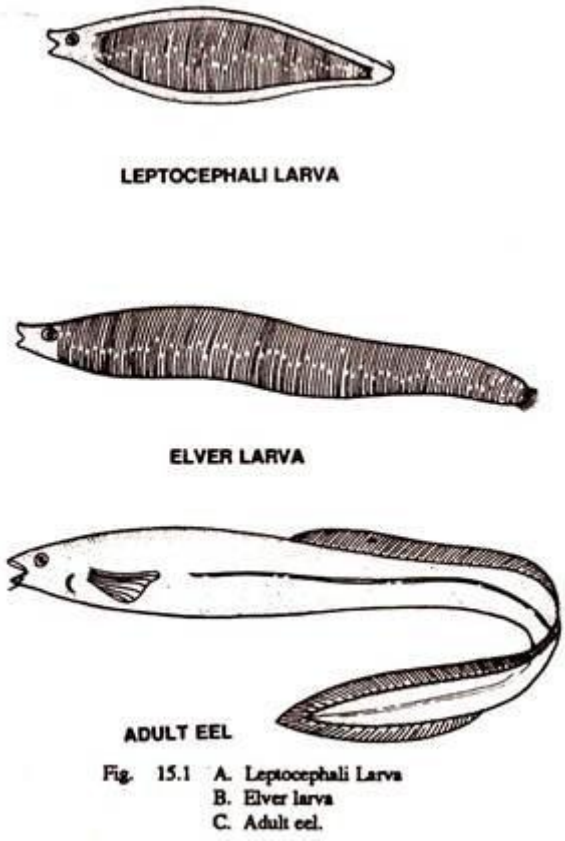


Fig. 15.1 A. Leptocephali Larva
B. Elver larva
C. Adult eel.

Catadromous migration is also performed by certain members of the families Galaxiidae and Gobiidae. But their migration is considerably shorter than those of the eel. They usually pass from the lower reaches of the rivers to the adjacent shallow parts of the sea.

(b) Anadromous migration:

Anadromous migrations are performed by lampreys, sturgeons, salmon, some shads, cyprinoids etc. The best examples are Atlantic salmon (*Salmo salar*) and Pacific salmon (*Oncorhynchus nerka*).

In winter, the both sexes leave their feeding ground at sea to ascend the freshwater mountain streams, reaching the identical spot where they originally grew some years ago. The total distance travelled may be even upto 3600 km, at a speed of 30-40 km. per day.They stop feeding, alimentary tract undergoes changes into a thin thread with

feebly developed pyloric caecae. Change in colour and weight too occurs. Sexual dimorphism becomes evident. The male is characterized by the possession of enlarged front teeth.After the selection of suitable spawning grounds, the salmons segregate into pairs and produce shallow saucer-like nest where spawning takes place.Very young salmons are known as “alevins” and they remain mostly among stones. Alevins develop into next stage called “parr” and finally to adult.

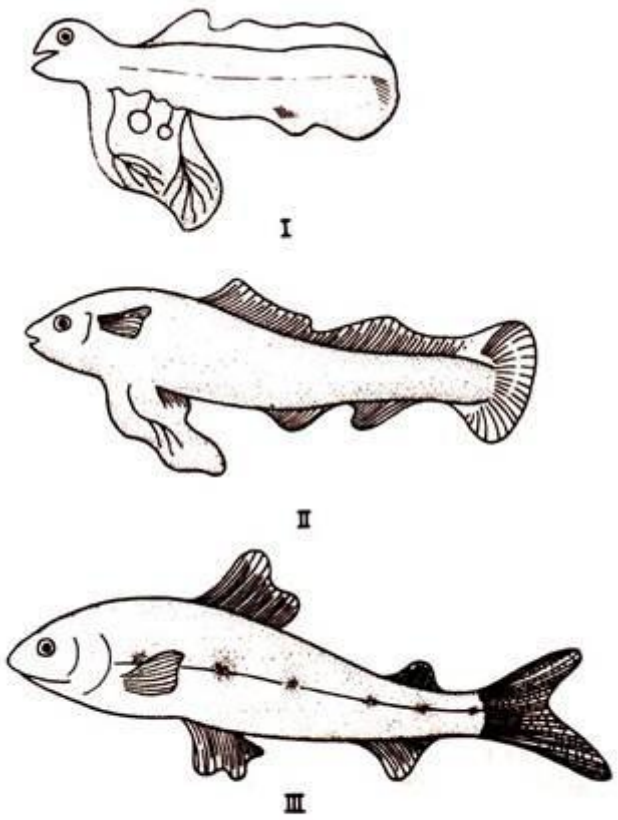


Fig. 15.2 Three stages in the development of Salmon
I & II Alevins
III 'Parr' with marks on its side.

After fertilization, salmons are very exhausted. They are called “kelts”. The males seldom return to the sea. The females recover and after a period in the sea they return to breed again. This process may be repeated several times.Some fishes do not perform significant movement like salmon. They migrate from seas to estuaries or lower reaches of river, for spawning. Such fishes are classified as fluvial anadromous (Semimigratory) fishes. Examples are many whitefishes and cyprinoids.Many freshwater fishes leave the lakes to spawn in the river. This is called as limnodromous migration. One of the common examples of this is the whitefish *Coregonuslavaretus*.

Feeding Migration:

This is the movement from spawning or overwintering grounds to the feeding grounds. Feeding migration can be passive or active. In many fishes the feeding migration even begins in the egg stage. It is a passive feeding migration of eggs and embryos from spawning to feeding ground.Active feeding migration is performed by many marine

fishes like cod. Horizontal feeding migration of cod comprises regular journeys, going from one good feeding ground to another.

Overwintering Migration:

Overwintering and hibernation in fishes are a part of the life cycle of a fish. It is characterized by reduced activity, reduction or stoppage of food consumption, lack of food, poor oxygen condition, low temperature, drought etc. Overwintering do not occur in all fishes.

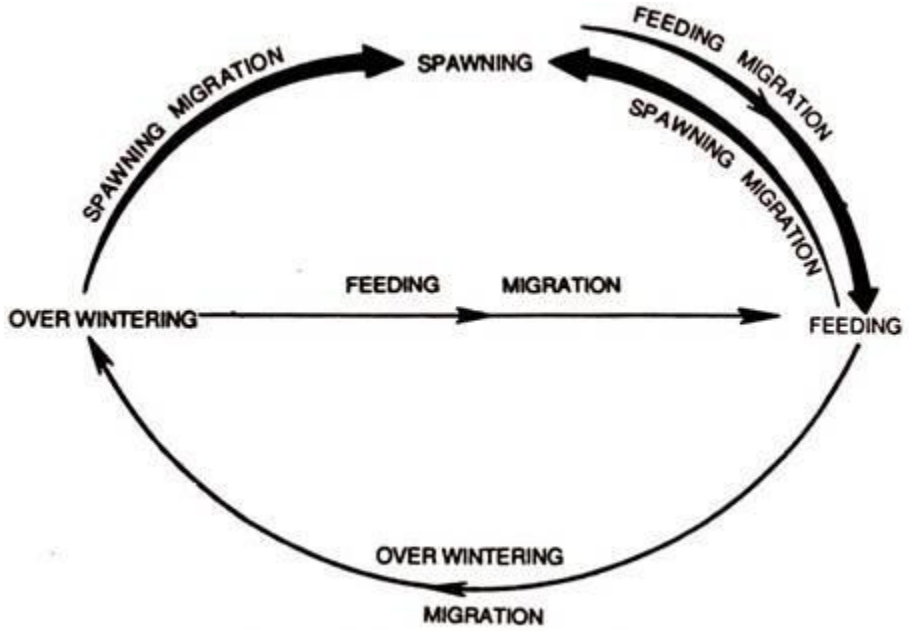


Fig. 15.3 Migration cycle of various fishes.

Overwintering migration is a movement away from feeding to wintering grounds. It occurs only in those fishes which have a wintering ground.

In the wintering ground, fish is in a state of relative inactivity and reduced metabolic rate. It requires protection against predators which are common in feeding ground. Overwintering migration is performed by marine fishes like flatfishes and freshwater fishes like grass-carp.

Shoreward Migration:

In this type of migration there is a temporary movement of fishes from water to land. For example, the common eel travel from one pond to another through moist meadow grass. The mud-skipper, *Periophthalmus* make temporary migration to land by means of modified pectoral fins. The climbing perch, Anabas migrates from water to land and even climbs trees to the height of several feet by means of the strong spines on its pelvic fins and gillcovers.

Examples of Migration:

A. Catadromous migration (Gk. kata = down, dramein = to run)

European Eel Migration:

The life-history of common river eel or the European eel (*Anguilla anguilla or A. vulgaris*), and American eel (*Anguilla rostrata*) will represent a clear idea about the catadromous migration. The common river eels or European eels are found along the shores of Europe and in inland waters of countries near the shores of Europe, some of which inhabit in Iceland, the Mediterranean countries, black sea and the red sea.

The adult eels with their sexual products are not encountered in freshwater, so their biology of reproduction was quite unknown for centuries, From the time of Aristotle the different ichthyologists tried to find out the exact spawning ground of the European river eel.

At last Danish ichthyologist Johannes Schmidt who started his investigation in 1904, ultimately succeeded in 1922 to locate the spawning place of the European river eel. It was found in the Sargasso sea of the Atlantic Ocean.

The life history of the European eel is divided into 4 phases :

a. An ordinary yellow eel representing the growing and feeding form in the river.

b. Changes of the yellow eel into the silvery eel ready for seaward migration for spawning (breeding phase).

c. A pelagic larval phase and

d. The metamorphosis of the pelagic larval phase to elver or young eels.

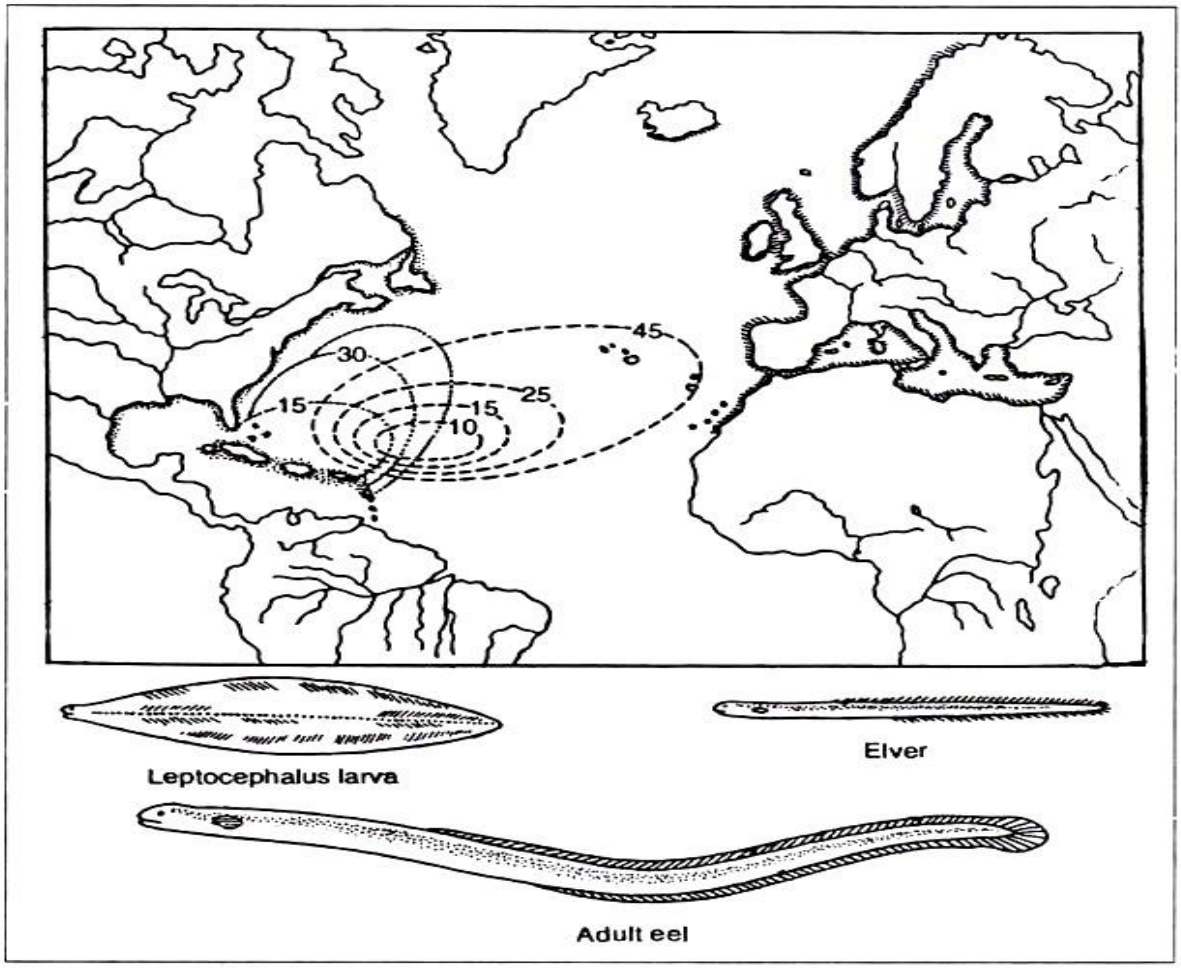


Fig. 6.109 : Life cycle of European eel (*Anguilla vulgaris*) and North American eel (*A. rostrata*). Number and curved lines in the Atlantic Ocean indicate larval distribution and length in millimeters (After Norman).

i. First phase:

The yellow colored variety living in fresh water represents the feeding and growing forms. With the advent of autumn, majority of yellow eels become silvery and prepare to undertake migration forwards the spawning ground, the Sargasso sea of Atlantic Ocean. The eels in the river spend about 10 to 12 years feeding partially on fish.

ii. Second phase:

During the transformation from yellow- colored to silvery colour, the yellow eels stop feeding, eyes become greatly enlarged, the snout becomes sharper with thinner lips and the yellow coloration is replaced by a metallic silvery color. The silver eels are recognized by having matured organs and shrunken digestive tract.

The primary development of gonads is the stimulus for the beginning of migration. The size of the eggs in the ovaries changes before migration. These silver eels first migrate down to the mouth of the rivers and then into the Atlantic Ocean. The European eels probably migrate over 6000 km between its freshwater feeding streams and its spawning ground.

The spawning ground is located in the western part of tropical waters of Atlantic Ocean between 22° and 33° N. latitude and 48° and 65° W. longitude, near Bermuda Islands. Spawning takes place from the end of winter to the middle of summer. After the completion of spawning the parents die.

iii. Third phase:

Eggs are laid in spring at depth of 500 to 700 meters with temperature ranging between 10-12°C. The fertilized eggs float for some time and the young hatch out as the pelagic larvae. The larvae are called Leptocephali.

The leptocephali are flat, glassy, leaf-like body. These tiny creatures are provided with elongated needle-like teeth for feeding. The gut has a straight tubular structure. The eyes are large and silvery. They now begin their long homeward journey. At the end of first summer when they become about 25 mm in length on average, are recorded in the Western Atlantic.

By the second summer the leptocephali reach central Atlantic and the size is about 50-52 mm in length on average. In the third summer they become about 72-75 mm in length and reach in the continental shelf of Europe. The larvae of eel are passively drifted by warm water current of the Atlantic Ocean.

iv. Fourth phase:

During autumn and winter of the 3rd year the leptocephali metamorphose to form elvers or glass eels or young eels. During metamorphosis the larvae stop feeding, their flattened body become cylindrical and the needle-like teeth is replaced by new ones. The young eels when become three years old, measure about 15-20 cm long.

Then they congregate in the mouths of rivers. Here the elvers or young eels ascend the rivers, grow in size and change their color into yellow. The males like to stay in the estuaries and the females ascend the rivers in shoals, specially at night during the spring to reach a suitable resting place. In the yellow eels teeth are lost, intestine shortens and the anal aperture moves forward.

The yellow eels spend 8-10 years on feeding and growing and on maturity, change into silver eels and start their perilous journey towards the Sargasso sea. The well fed silver eels first stop their feeding and leaving the rivers, empty into the Baltic Sea and gradually in the abyssal depths of the Atlantic Ocean.

B. Anadromous Migration:

Migration of HILSA:

Among Indian fish, HILSA or Indian shad (*Tenualosailisha*) represents an example of anadromous migration. Its occurrence is recorded in the coastal waters of Pakistan, India, Sri Lanka, Bangladesh and Myanmar. Its presence in the estuaries, rivers and lakes (e.g., Chilka), mostly during spawning season indicates its anadromous migration.

During spawning season the HILSA population ascends the rivers of the Hooghly, the Ganges, the Mahanadi, the Godavari, the Krishna and the Kaveri and its tributaries in the eastern region of India, and the Narmada, the Tapti and the Kali in the Saurastra coast of the west India.Around the Indian coasts, the Hooghly-Matla estuarine system covers a major portion of the Ganga-Brahmaputra delta and is estimated to be 3,100 sq. miles which is the largest in India.

This estuary zone is mostly famous for HILSA migration. Before the construction of Farakka barrage on the Ganges in West Bengal the HILSA used to migrate up to Delhi during the spawning season covering a distance over 1,250 km. In Bangladesh it ascends in the rivers of Meghna, Padma, Brahmaputra and its deltaic rivers. It also migrates in the upper part of Irrawaddy river of Myanmar.

The marine distribution of HILSA in the Bay of Bengal and in the Arabian Sea is due to several factors, such as vast stretch of continental shelf, low salinity, discharge of huge amount of monsoon freshwater in the coastal region through the rivers, monsoon winds, huge abundance of planktons as food, and other favorable hydrological parameters. Oceanic properties along the Indian coasts are given below in Table 24.

Table 24 : Oceanic Propertis and Continental shelf area along the Indian Coast

Continental shelf area and Oceanic properties	*Range of Variation*
1. Continental shelf area	Along West Bengal and Orissa coast, continental shelf area, ranges from 35 to 20 km from the shore line; along Andhra and Tamil Nadu coast within 5 km; along Gujarat coast more than 100 km from the coast line; along Maharastra coast about 80–100 km.*
2. Rainfall (South-west monsoon remains active from June to September)	
(a) West Bengal, Orissa and Bangladesh	100 – more than 200 cm.*
(b) Saurastra coast	50 – 200 cm.*
3. Surface sea water temperature (°C)	23 – 30 [La Fond (1958), Miller and Jefferies (1967), Wooster, Schaefer and Robinson (1967)]
4. Surface sea water salinity (%)	17 – 36, –do–
5. Depth of euphotic layer (meter)	50 – 65, Krey and Babenerd (1976)
6. Zooplankton biomass (ml. per haul)	5 – 20, Prasad (1968)
7. Phosphate — phosphorus (µg –at/l)	0.2 – 0.6, Wyrtki (1971)
8. Nitrate — Nitrogen (µg –at/l)	0.5 – 1.0, Wyrtki (1971)
9. Primary production ($mgC/m^2/d$)	100 – 500, Krey and Babenerd (1976)
10. Chlorophyll — a (mg/m^3)	0.3 – 0.5, –do–
11. Density of Copepod (no. of per haul)	3,000 – 27,000, Kasturirangan, Saraswathi, Saraladevi, Stephen, Gopalkrishan & Kunjamma (1970).

In the sea, HILSA population is found along the east coast in the vicinity of rivers before the spawning season but in Gujarat and Maharashtra the HILSA is found about 12-16 km off the coast at a depth of about 20 fathoms.

There are two types of HILSA stocks — one estuarine and offshore stocks which are found in the lower region of the estuary and the foreshore areas of the seas, and another is riverine stock which spends throughout the year in the river, mainly in the Ganges

and the Brahmaputra.The estuarine stock migrates upwardly during breeding season and after spawning they return to their natural habitat and spend throughout the year till the next breeding season to come. The riverine stock ascends in the more freshwater river zone during breeding season and after spawning they come back to the lower reaches of the river and spend the rest period until the next breeding season comes.

The life cycle of HILSA is divided into 4 phases:
(i) Egg

(ii) Larval stage or Fry,

(iii) Fingerling or Juvenile stage and

(iv) Adult stage.

The size of adults differs in sexes. The females are larger than males. The size of the sexes differs in different seasons, even in the same river. In the Ganges and in the Padma different sizes of sexes have been reported by different authors. In the Hooghly estuary the mature females are recorded about 250 mm in length.

The adult HILSA are laterally compressed, fusiform animals, having abdomen with a keel about 30-33 scutes. There are very fine numerous, closely set gill-rakes that indicate for planktonic feeding habit, predominantly zooplankton feeder. They mainly consume Cyclops, Daphnia, Moina, rotifers and protozoans. The adult HILSA in the freshwater zone of the Hooghly river are column and bottom feeders.The HILSA is probably polygamous and fertilization is external. The riverine stocks of HILSA become mature in between 1-3 years of age. Matured HILSA spawns once in a year but the time of spawning differs in different parts of India. In Hooghly estuary or freshwater region of the Hooghly river spawning takes place at the onset of evening but in the Narmada spawning takes place in the early morning.

During upstream migration schooling behaviour among HILSA is seen and males move in the upper surface layer of water and females move in the deeper water layer in river during monsoon period.Many authors reported that during spawning period the HILSA do not feed or stop feeding but Pillay (1958) has reported that the appreciable amount of food is available during spawning period, especially of HILSA of the Hooghly region. The feeding intensity is increased considerably after spawning period.In the spawning ground both the sexes discharge their garnets in freshwater. The tailed spermatozoa survive few hours in the water and ova are large, translucent at the marginal zone. The fertilized ova turn transparent after half an hour. The fertilized eggs float in water and color is about light greenish yellow. The hatched ova transform into larva or fry when they become 20-40 mm in length.

The abdomen of the larva possesses 5-7 pre-ventral scutes. At about 40-45 mm in length, the dark blotches on the lateral side are seen that indicate the fingerling or young HILSA stage. The larvae are surface feeders, predominantly zooplanktons which

constitute about 70% in their food composition. When the fry attains 100 mm in size they are called finger- lings or young HILSA.The body of young HILSA becomes laterally compressed with keeled abdomen. The abdomen possesses 30-32 scutes. The body color is silvery along the sides. A row of dark blotches is seen on the lateral sides of the body.When the fingerlings become 150 mm in size, called advanced fingerlings or youngs. At that stage they consume small shrimps and phytoplanktons. The early fingerlings (about 80 mm) are found in the lower reaches of the river and estuary zones. The youngs or advanced fingerlings (above 150 mm) occur along the foreshore areas of the seas.

Two types of anadromous migrations are seen among Indian HILSA species. Breeding or spawning migration is seen during the southwest monsoon when the Indian and Bangladesh rivers are flooded by the monsoon rain. Another type is winter or spring migration which is influenced by the certain changes of the water temperature and rain, mainly seen in the Gangetic delta.The correlation between temperature and movement into freshwater may be a reflection of the energetic cost of migration. In the Indus river of Pakistan and Irrawaddy river of Myanmar, the HILSA migration takes place by the molten snow that creates flood in these rivers.The adult HILSA spends most of the year in their original places except spawning season. The young and adult HILSA can tolerate certain variation of salinity. The Hooghly-Matla estuary is classified as a mixohaline range, in which salinity varies in different zones.

Mystusgulio migrates to the estuarine and freshwater zones of the Hooghly river for spawning, and *Pamapama* also migrates to the estuarine zone of the Hooghly river for both genetic and tropic reasons. Toli shad (*Tenualosatoli*) is found in the western coasts of India, ascends in rivers for spawning.

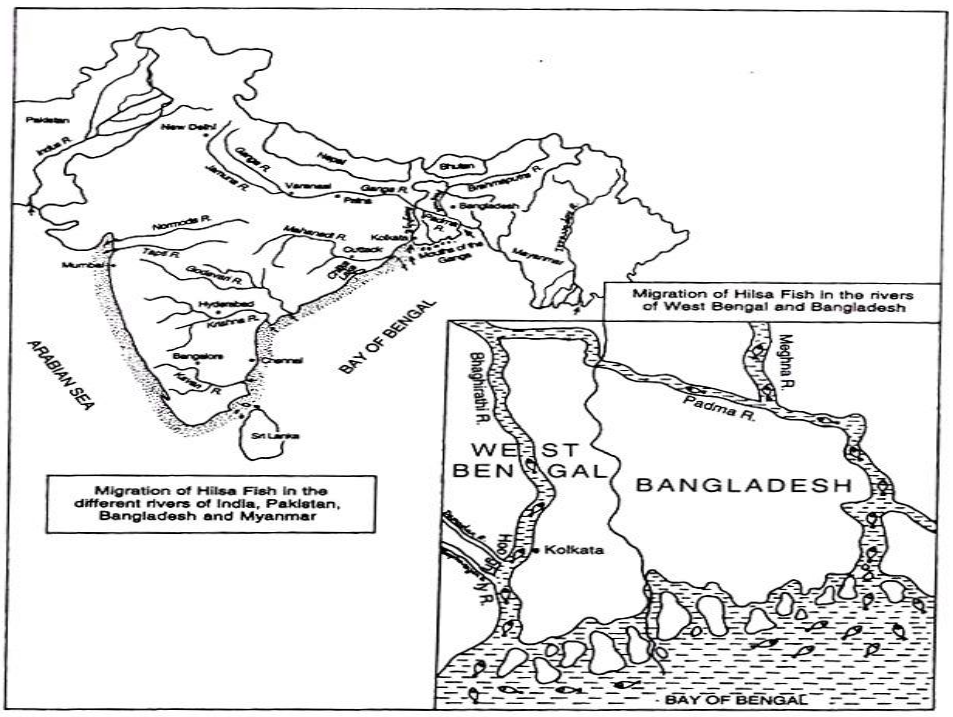


Fig. 6.110 : Migration of hilsa fish.

Causes for Fish Migration:

Fish migration is related to several factors such as physical, chemical or biological.

i. Physical factors:
The physical factors include temperature of water, rainfall, quality of water, water depths, pressure, light intensity, photoperiod, turbidity, tides and currents.

ii. Chemical factors:
The chemical factors include pH of water, salinity, dissolve of O_2 and CO_2, types of dissolved organic and inorganic substances, and taste of water.

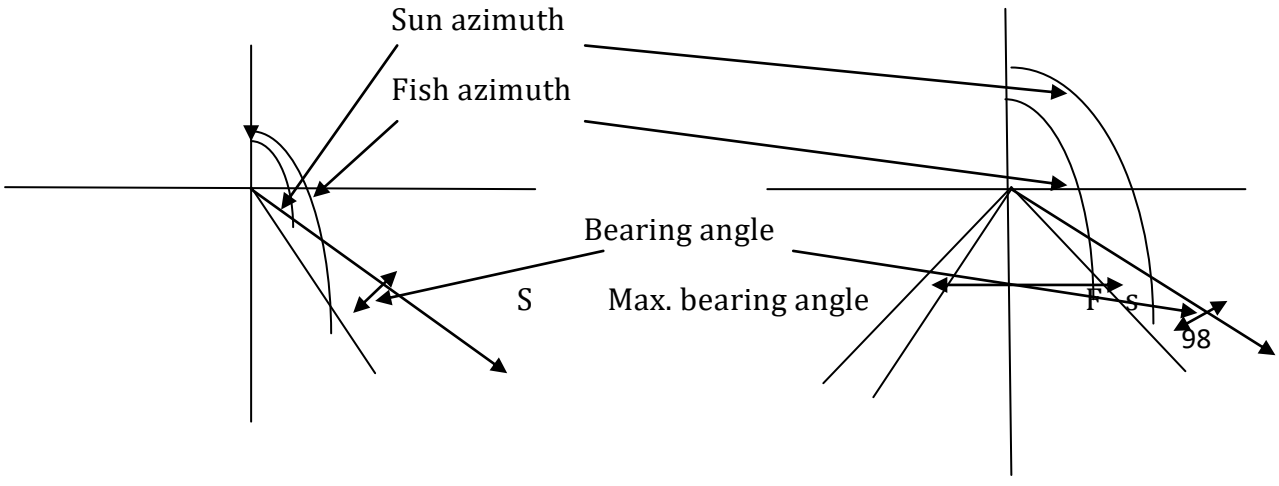
iii. Biological factors:
The biological factors are food, attainment of sexual maturity, endocrine behaviour and competitors and predators.

Theories of migration:

Odor hypothesis / Parent stream theory:
It has been proposed that each stream has a unique organic quality in terms of soil, plants etc. The specific Odor of a certain stream remain with in the brain of fish (Salmon) in their early life and helps to recognize their specific stream in the adulthood.
Mechanism of Sun compass Orientation:
Some fishes use sun as a reference point and able to correct the direction in 'Azimuth' during the day.

In this case 2 cycle period day and year are maintained.

- Azimuth: Sun azimuth can be explain as the relationship between N (North) point and horizontal Component of sun.
- When the fish starts migration, sun exposed to be on left side at noon at the and later on the side. So, adjustment direction (Correction) was done by the fish. Further work has been demonstrated that corrected route was done by the sun azimuth.
- At the fixed depth the distribution of light with azimuth angle in the horizontal place varies with the altitude of the sun. Tyler (1961) gave a clear account of the phenomenon.



(A) (B)

(B) This is a normal phenomenon when a fish is just below the depth of horizontal line, there the difference or bearing angle between sun's azimuth and fishes is short.

(C) The fish cause a correct direction with the help of azimuth. Fish cause change in direction in the angle from (F) and (S) state to F and S situation the bearing angle is maximum than the previous angle and this is advantageous in migration.

Probable Questions:

1. Define migration. Write down the causes for fish migration.

2. Differentiate between Anadromous migration, Catadromous migrationand

Amphidromous migration.

3. Define Feeding Migration, Overwintering Migration and Shoreward Migration.

4. With suitable example illustrate Anadromous migration in fishes.

5. With suitable example illustrate Catadromous migration in fishes.

6. Discuss various theories related to fish migration.

Suggested Readings:

A text book of Fish Biology and Fisheries (3rd edition) by- S.S. Khanna & H.R. Singh

UNIT-XVIII

Parental Care in Fish

Objectives: In this unit we will discuss about different types of migration in fish.

Introduction:

Many species of fishes do not care for their eggs and fingerlings. They leave the spawning grounds after fertilization. For such species of fishes the lacking parental care behaviour is compensated by the production of large number of eggs. Such fishes lay over 300 million eggs at a time.

The cod fish lays over nine million eggs which are scattered at random in the open sea. Carps lay two to four million eggs at random in fresh water and adjoining aquatic vegetation. It has been estimated that about 77 percent fishes show no parental care, another 17 percent of the fish species care for the eggs only, while less than 6 per cent care for eggs and newly hatched young.Fishes that produce limited number of eggs have evolved various grades of parental care behaviour:

A. Deposition of eggs in suitable places.

B. Deposition of eggs into self-made nest.

C. Concealing eggs and young's in or on their body.

D. Viviparity

E. Care of independently swimming young's.

A. Depositing Eggs in Suitable Places:

A number of fish species have developed some design of depositing their eggs in suitably protected places. They do not build nests.

(a) Deposition of eggs in sticky covering:

(i) In carps, eggs are usually laid with some special sticky covering by means of which they are attached to each other or to stones, weeds etc.

(ii) In yellow perch (*Percaflavescens*) eggs are deposited in a rope-like structure (Fig. 5.40A). The eggs are held together by a long floating membrane.

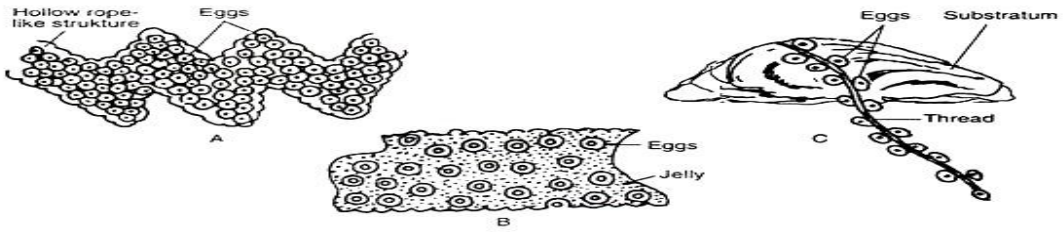


Fig. 5.40 : Deposition of eggs in sticky covering : A. Eggs deposited in a rope-like structure in yellow perch. B. Eggs deposited in a gelatinous outer coat in the case of Angler fish. C. Eggs deposited on a sticky thread secreted from the kidney of flying fishes, skippers, garfishes etc.

(iii) Angler fish (*Lophius*) lay their eggs invested by a gelatinous external coat, that remain together to form a transparent mass of enormous size (Fig. 5.40B).

(iv) Flying fishes, skippers, garfishes etc. secrete a sticky thread-like substance from their kidney, on which the eggs remain attached (Fig. 5.40C). The thread on one end remains adhered to any aquatic substratum while the other end remains free.

(b) Eggs scattered over aquatic plants:

Eggs of fishes such as pikes (*Esoxlucius*), carps (*Cyprinus carpio*), *Carrassius auratus* etc., are scattered over aquatic plants to which they remain attached.

(c) Eggs laid at suitable places:

(i) Anadromous fishes (lives in the sea but migrates to fresh water for breeding) such as Salmo solar, Acipenser, Oncorhyncus etc., lay their eggs in suitable spawning grounds. They dig excavation in gravel substrate, lay their eggs in the pits, cover them with gravel and desert them.

(ii) Sand Gobi *(Pomatoschistosminutus*) lay their eggs in some protected place, where they are guarded by the male who also aerates them by his movement.

(d) Eggs deposited on dead shells of bivalves:

(i) Females of cyprinid family deposit their eggs on the dead shells of mussels.

(ii) Females of European bitterling (*Rhodeusamarus*) deposit eggs in the siphon of a fresh-water mussel by means of a long tube acting as an ovipositor (Fig. 5.41). This ovipositor is a long tube drawn out from the oviduct.

After oviposition male Rhodeus immediately sheds the sperm over the eggs and then guards them. The male Rhodeus, interestingly, are not sexually excited by the presence of the female of its own species, but by the sight of the shell of the mussel in which the eggs have been deposited.

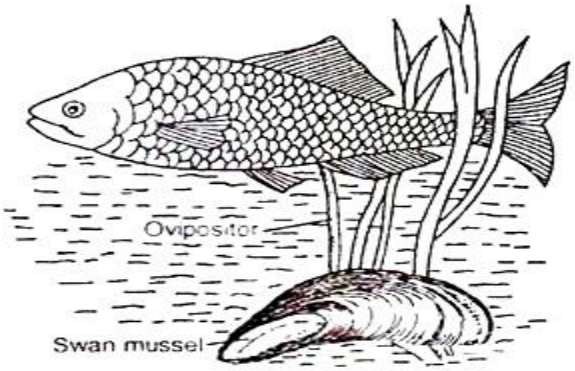


Fig. 5.41 : Oviposition by European bitterling on the shells of a dead mussel

B. Deposition of Eggs Into Self-Made Nest:

Some species of fishes prepare nests of different types for the safe deposition and protection of eggs, and for the development of their young ones. In the building of the nest, only the males or both sexes participate. Various kinds of materials such as pebbles, aquatic vegetation, secretion of their body etc., are used for nest formation.

The different types of nests built are:

(a) Basin-like nests:

(i) Male of Darter (*Etheostomacongregata*), during the spawning season, selects a suitable place called domain, which it defends repelling with vigor any attempt by rival males. Any female Darter entering its domain is allowed to remain.

The female Darter then makes a basin-like depression, sinks into it and deposits the eggs. The eggs are immediately fertilized by the male who covers the fertilized egg by a sticky secretion, secreted from its kidneys. These sticky eggs remain attached to the stone till hatching.(ii) Fresh water sunfishes build a nest by scooping out a shallow basin-like nest at the bottom of the impoundment by carefully removing pebbles and leaving behind large stones (Fig. 5.42). A layer of fine sand remains attached with the eggs. Male sunfish guard the eggs till they hatch.

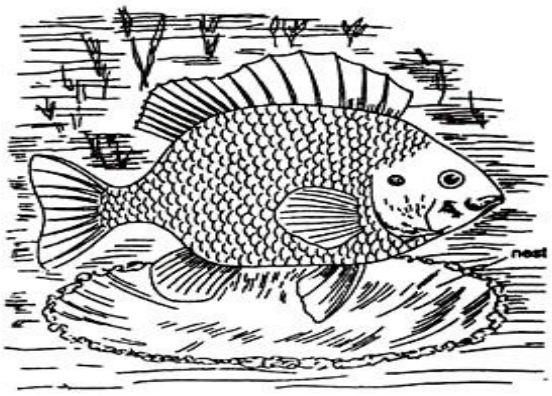


Fig. 5.42 : Basin-like nest of sunfish

(b) **Circular nest** : (i) The male Bowfin (*Amia calva*) prepares a crude pit-like circular nest (Fig. 5.43A) among aquatic vegetation. The male then invites a female. She spawns and the male fertilizes the eggs. The fertilized eggs are then protected by the male who keeps guard over the nest till the young ones are hatched. The young ones are allowed to leave the nest in a boa/ under the protection of the father (Fig. 5.43B).

(iii) Cichlid fishes (*Haplochromis burtoni*) build basin-like nest which is guarded by both the parents.

(iv) In some North American catfishes both the male and female prepare a crude nest in the mud to lay eggs.

(b) Circular nest:

(i) The male Bowfin (*Amia calva*) prepares a crude pit-like circular nest (Fig. 5.43A) among aquatic vegetation. The male then invites a female. She spawns and the male fertilizes the eggs. The fertilized eggs are then protected by the male who keeps guard over the nest till the young ones are hatched. The young ones are allowed to leave the nest in a boa/ under the protection of the father (Fig. 5.43B).

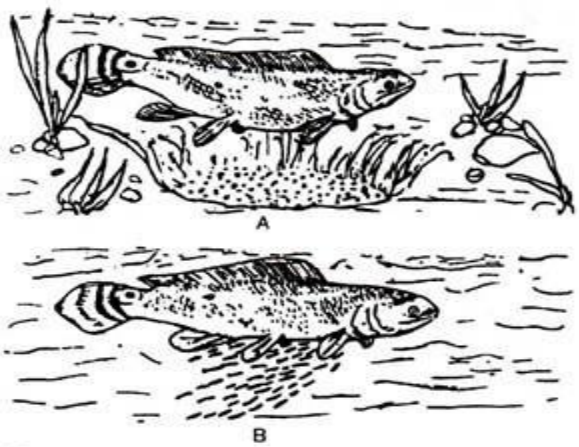


Fig. 5.43 : A. Male Bowfin prepares a circular nest. B. The hatchlings swims in a group under the protection of the father

(ii) Both the male and the female of some cat fishes (Arius) of North America prepare a crude circular nest in the mud, at the bed of the river. The diameter of the nest is about 50 cm and is sometimes provided with a protective cover of logs, stones etc. The fertilized eggs are left uncared.

(c) Hole/Burrow nest:

(i) The African lung fish (Protopterus) prepares a simple nest in the form of a deep hole in swampy places along the river bank. The male prepares the nest surrounded by long aquatic weeds and grasses and after spawning keeps guard over it, occasionally aerating the water by his slow body movement.

(ii) The South American lung fish (*Lepidosiren*) prepares a nest in the form of a burrow in swampy places, varying in length from 1 to 2 metres. The nest consists of a short vertical and a larger horizontal portion in which eggs are deposited. Males take care of the eggs. During this time they develop a long red filament from the pelvic fins which perform the function of aeration without coming out on to the surface to gulp air.

(d) Barrel-shaped nest:

Before the start of courtship the male stickleback (example: three-spined stickleback, *Gasterosteus aculeatus* and ten-spined stickleback, *Pygosteuspungitius*) builds a quite elaborate nest. The male at first selects suitable place in shallow water where the flow of water is continuous but not swift. He then builds a nest by collecting plant fragments, rootlets, weeds and then binds them together with the help of a sticky secretion from its kidney. The various activities of males such as probing, boring, sucking and gluing result in the formation of a compact nest with tunnel to receive the eggs (Fig.5.44)

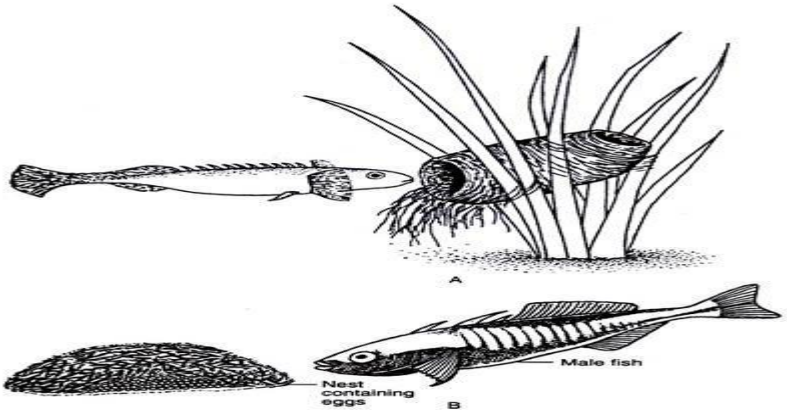


Fig. 5.44 : Barrel shaped nest of : A. Ten-spined stickleback and B. Three-spined stickleback

The male then goes out in search of a mate. The bulging abdomen of the female stimulates the male to perform a zigzag dance around her, displaying his red spot. If the female is ready to lay eggs she responds by curving her head and tail upwards. On reaching the nest the male opens the entrance of the nest with its snout. The female, after depositing two to three eggs within the nest, swims out of the nest through the opposite side of the entrance.

The male then enters the nest and fertilizes the eggs. He then comes out of the back entrance and moves out to seek another female. The same process is then repeated till

sufficient eggs are deposited. The male then guards the nest and keeps the eggs aerated by fanning the nest with his fins and tail. Later, the male stops fanning and keeps close watch over the brood, not allowing any young one to go astray.

(e) Cup-shaped nest:

The male of *Apeltsquadracus* builds an elaborate cup-shaped nest, attached to rooted plants close to the bottom. After a female lays a clutch of eggs, the male builds an extension of the nest up and over the eggs, with a concave upper surface in the extension. A second female lays another clutch of eggs on the new concave floor. This process is repeated several times, until the male has several clutches of eggs stacked vertically within a multi-tiered nest.

(f) Floating nest:

The male Mormyrids (Gymnarchus) constricts a floating nest with the help of aquatic vegetation. The wall of the nest remains projected several centimeters above the water surface.

(g) Foamy nest:

(i) The nest made by American catfishes contains eggs that are suspended in a mass of bubbles and mucus produced by the male.

(ii) The male fighting fish (Beta) builds a nest by blowing bubbles of air and sticky mucus that adhere together forming a floating mass of foam on the surface of water (Fig. 5.45). The fertilized eggs are collected by the male in his mouth, who gives them a coating of mucus and sticks them to the lower surface of the foamy nest. The male then stays on guard and fights till death to defend it.

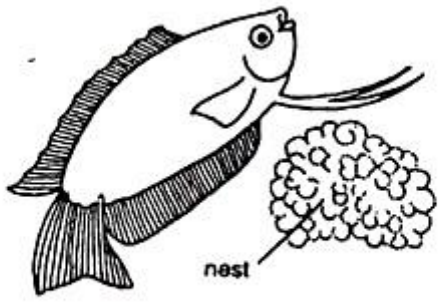


Fig. 5.45 : Male siamese fighting fish (*Beta splendens*) defending his foamy nest

(iii) The male paradise fish (*Macropodus*), also prepares a similar foamy nest. In this species the eggs are lighter and rise up to the nest without the active participation of the father.

C. Concealing Eggs and Young's in or on their Body:

Certain species of fishes have developed many structures in their body to safeguard the eggs and young ones:

(a) Eggs and young's concealed in mouth cavity:

(i) In many cichlids (Example: Tilapia), the female broods the fertilized eggs in her mouth (Fig. 5.46A). After hatching she allows the young to take refuge in her buccal cavity in times of danger.

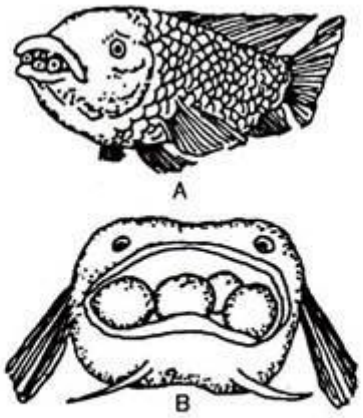


Fig. 5.46 : A. Female *Tilapia* carrying eggs in her mouth. B. The male Brazalian cat fish carrying eggs in a pouch present inside the lower lip

(ii) In most marine cat fishes (*Arius*) and cardinal fishes, the male carries the eggs and young ones in his mouth. The male does not take food during this period.

(iii) In case of the Brazilian cat-fish (*Loricariatypus*), the male has an enlarged lower lip forming a sort of pouch in which labial incubation takes place (Fig. 5.46B). This ensures safety and provides perfect aeration. During this act, the male fish do not take any food. Even after hatching the hatchings remain near the father and enter the buccal cavity of father at the slightest disturbance.

(b) Formation of egg ball: The butter fish (*Pholis*) rolls the eggs into a round ball and then one of them remains on guard by curling around it (Fig. 5.47). It is often the male that guards the egg ball till hatching of young.

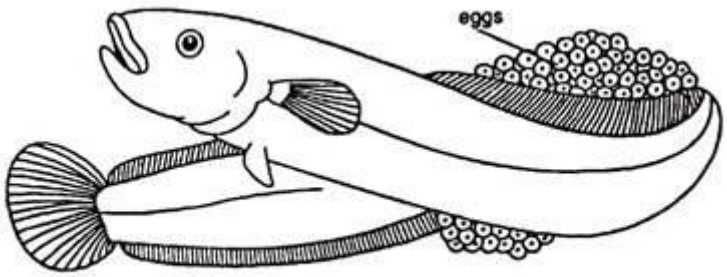


Fig. 5.47 : A butter fish (*Pholis*) curling around the egg ball

(b) Eggs attached to cephalic hook:

The male nursery fish (Kurtus) of New Guinea, carries the mass of eggs on the forehead, held in a cephalic hook (Fig. 5.48).

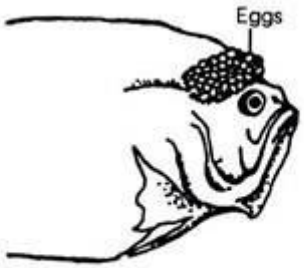


Fig. 5.48 : Male nursery fish carrying egg mass on fore-head

(c) Eggs in integumentary cups:

The cat-fish, Platystacus of Brazil, shows an interesting method of parental care. During the breeding season, the skin of the lower surface of the body of female becomes soft and spongy. Immediately after the eggs are fertilized, the female presses her body against the eggs in such a manner that each egg becomes attached to the skin by a small, stalked cup (Fig. 5.49). The eggs remain fixed in this position till hatching.

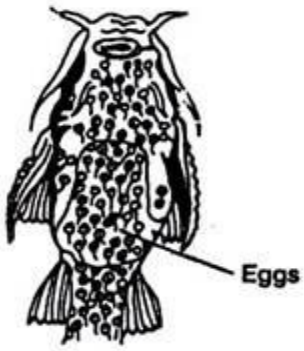


Fig. 5.49 : *Platystacus* bear eggs in integumentary cups

(d) Eggs kept in brood pouches:

(i) In sea horse (Hippocampus) fertilized eggs are transferred by the female into the brood pouch (Fig. 5.50A) of the male. The brood pouch is found on the lower surface of the abdomen. During the males so called 'pregnancy', he provides nutrients and oxygen to the fertilized eggs for several weeks.

Generally, a single large female produces enough eggs in one cycle to fill the brood pouch of nearly three large males. As the sex ratio is 1 : 1, male pouches are in short supply. Thus, males exhibit active mate choice. They favor large ornamental ones that can fill their brood pouch with eggs quickly and sufficiently. After hatching, fry may return to the pouch when in danger,

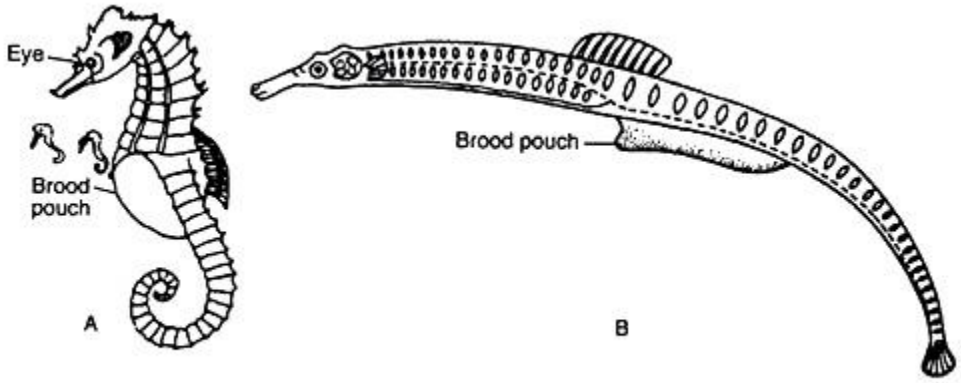


Fig. 5.50 : Brood pouch in the males of A. Sea horse and B. Pipe fish

(ii) In the male pipe-fish (*Syngnathus*), a brood pouch (Fig. 5.50B) is formed by two flaps of skin on the underside of the body on which eggs are placed by the female.

(iii) In the family solenostomidae, pelvic fins of females form the brood pouch. The eggs inside the brood pouch are kept in proper position by numerous long filaments.

(e) Egg capsules:

In oviparous elasmobranchs such as sharks and rays, the fertilized eggs are laid inside protective Horney egg capsules called mermaid purse. The shape of the purse varies in different groups (Fig. 5.51). The capsules remain attached to aquatic weeds by their tendrils. The development of the eggs is completed inside the purse.

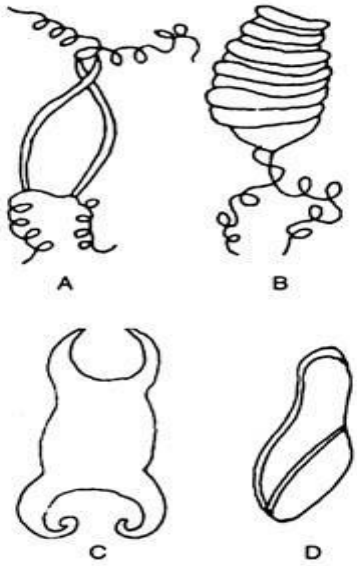


Fig. 5.51 : Different types of mermaid's purse A. shark, B. Pot Jackson shark, C. Skate, and D. Chimaera

Mermaid's Purses:

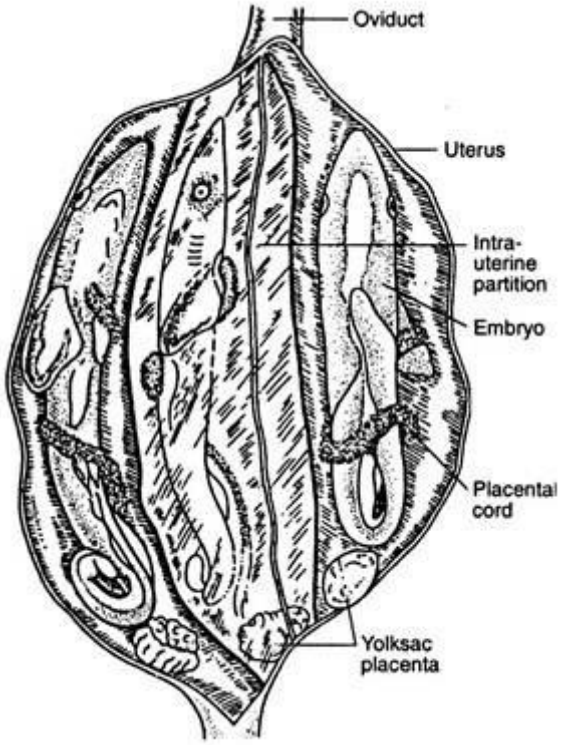
Oviparous sharks (e.g., *Scyllium*) lay fertilized eggs inside the protective horny egg capsules or mermaid's purses, which remain anchored to the sea weeds by their long tendrils. The young hatch out after rupturing the egg case.

D. Viviparity:

The highest degree of parental care is exhibited by those species which are viviparous. They have evolved internal incubation and give birth to young ones, thereby providing maximum protection.

(a) Viviparous Elasmobranchs:

Among the sharks, viviparity has been witnessed in more than a dozen families. In case of *Scoliodon, Mustelus* etc., eggs develop in the uterus. The mucous lining of the uterus forms fluid-filled protective compartments, one for each embryo. Nourishment in the form of embruotrophe or uterine milk is received by each embryo from the uterine tissue through the yolk-sac placenta (Fig. 5.52).



(b) Viviparous Bony Fishes:

(i) In teleosts, species (Example: Zoarces, Gambusia and Poicilia) belonging to the orders Cyprionodonts and Perciformes show internal fertilization and the young ones develop within the ovary but are not attached to its wall.

The embryos develop freely in a sac inside the ovary, feeding upon an "embryotrophic" material, apparently produced by the discharged ovarian follicles. The sac becomes highly vascular and remains as such during the months of pregnancy.

(ii) In the case of shiner-perch (*Cymatogasteraggregata*) the eggs also are fertilized in the ovarian follicles, but are soon released into the ovary cavity and are nourished by a secretion from the ovary (Fig. 5.53). The young are retained in the ovary until they become sexually matured.

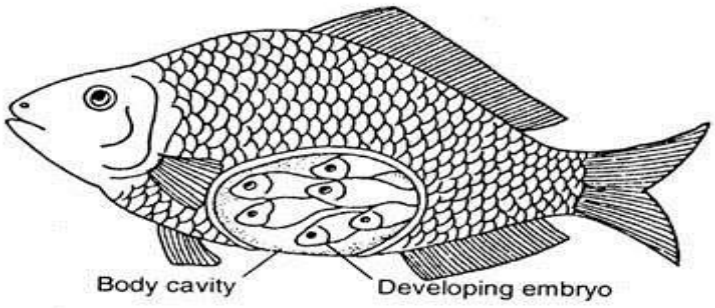


Fig. 5.53 : The body cavity of *Cymatogaster aggregata* is cut open to show fully formed youngs

(c) Ovo-viviparous:

An intermediate condition between oviparous and viviparous is observed in the case of nurse shark (*Ginglymostoma*), called ovo-viviparous. Here the eggs are covered by a horny case and the development takes place in the uterus. The fully developed young ones hatch out by breaking the shell inside the uterus.

E. Care of Independently Swimming Young's:

In the members of some families such as Gasterosteridae, Centachidae and Ictaluridae, parental care does not stop with the caring of the eggs. These fishes defend their young ones by placing them in a safe place, away from predators and enemies.

As has been dealt with earlier, the young ones of Tilapia, seahorse and pipe fish are protected by their parents either in the oral cavity of mother or in the brood pouch of the father. In the case of cichlid fish, both male and female secrete a nutritious substance from their body, which are taken up by their young ones.

Probable Questions:

1. Describe different types of nests maid by fishes as an outcome of parental care.

2. Describe different types of attachment of eggs in the body of fishes.

Suggested Readings:

A text book of Fish Biology and Fisheries (3rd edition) by- S.S. Khanna & H.R. Singh

UNIT-XIX

Respiratory organs of Fishes: Water breathing, air breathing

Objective: m In this unit we will discuss about different kinds of respiratory organs in fishes.

Introduction:

The fishes possess well-developed respiratory organs. The physiological process of respiration in different fishes is essentially similar to that of any higher vertebrate, the only difference in the respiratory process being the organs of respiration.Fishes are the primary aquatic vertebrates. They utilize the oxygen which remains dissolved mostly in water. A few fishes have the power to breathe air. In fishes the respiratory organs are the gills.Besides, some accessory respiratory structures for aerial respiration are encountered in some teleosts. In fishes, where aerial respiratory structures have evolved, the gills in them play a subsidiary role.

The gills constitute the efficient respiratory organs which are specially modified to utilise up to 80% of the oxygen dissolved in water that passes over the gills, whereas in man, the lungs are capable of absorbing about 25% of oxygen from the air drawn into the pulmonary cavities.

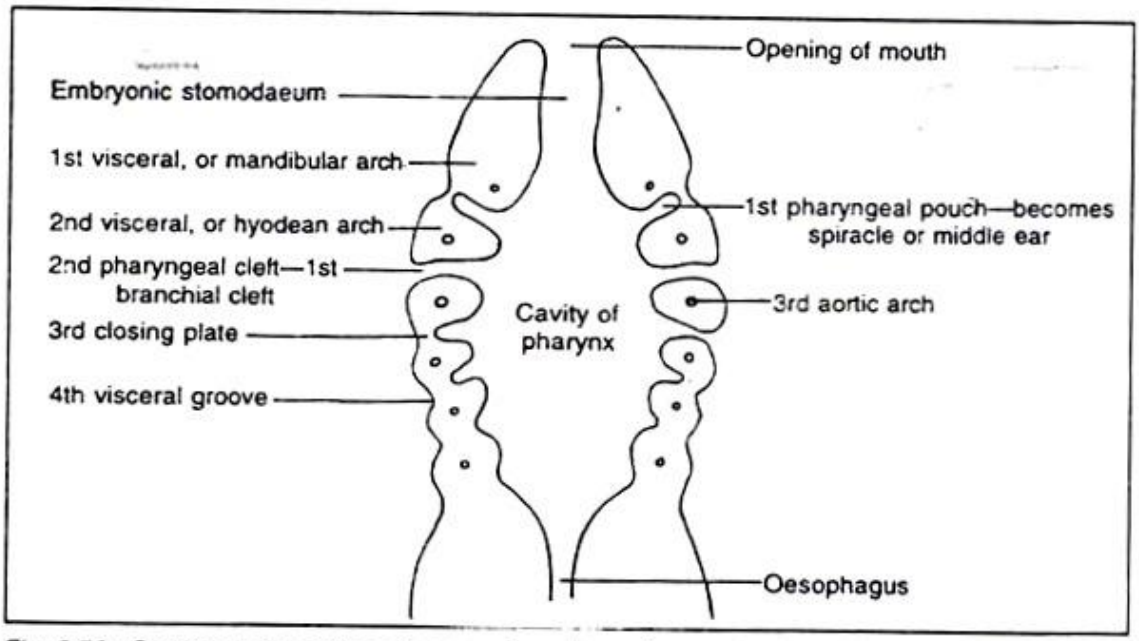


Fig. 6.76 : Sectional view of the pharynx, visceral pouches and arches of the vertebrate embryo.

The efficiency of the gills as the respiratory organs in fishes depends upon two factors:

(a) The structural organization of the gills and the nature of vascularization and

(b) The gills are bathed by the continuous flow of water so that fresh oxygen is always brought in immediate contact with the gills.

Development of Gills:

The larvae of some vertebrates possess gills that develop from the ectoderm and lie outside the body. These are called external gills. Another type of gills lie in the head region, called internal cells. Both types of gills occur in aquatic gnathostomes such as fishes and in the larval stage and adult neotenic amphibians.

The internal gills develop in the pharyngeal region. The side walls of the embryonic pharynx are lined by the endoderm. The paired pouches develop in the endodermal part by invagination and the pouches are called pharyngeal or gill-pouches or visceral pouches (Fig. 6.76). These pouches are six or more in number. The pouches then proceed to the outside through mesodermal layer.At the opposite side of each pouch similar groove-like structure of the ectoderm develops by invagination. These groove like structures, called visceral grooves. The pouches and grooves are joined by the separation of the intervening thin partition, called closing plates. By perforation through the closing plates, slit-like structured develop called gill clefts or branchial clefts or gill-slits.

The external gill-slits are situated on the side of the anterior end of the head between the eyes and the pectoral fins and the slits on the side wall of the cavity of pharynx, called internal gill-slits.The serially arranged pharyngeal or visceral pouches at the two sides are separated by visceral arches and arches contain arteries, called aortic arches. The pharyngeal or gill or visceral pouches are separated by inerbranchial septa which form the walls of the pouches. These septa bear gill-filaments (Fig. 6.76).

According to the structure and arrangement, the gills of the vertebrates are classified into 3 types:

a. Pouched Gills:

This type of gills is found in agnathans (Fig. 6.77). These are spherical or pouch-like muscular gill chambers situated at the side of the pharyngeal region. In Lampreys there are 7 pairs of branchial or gill pouches which remain on each side of the body.

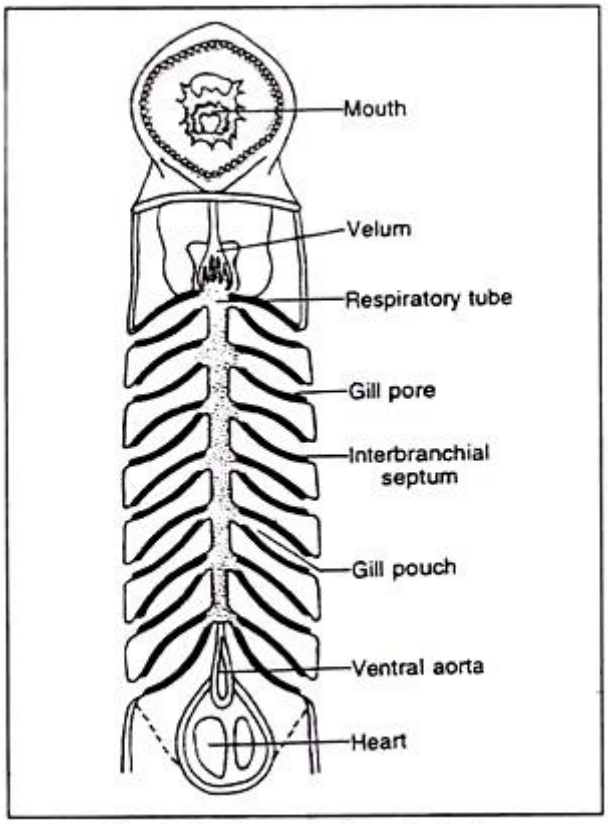


Fig. 6.77 : Structure of pouched gills.

The pouches open directly into the respiratory tube and have no direct connection with the enteric canal. The gill pouches open to the outside by external pores. One end of the respiratory type opens to the buccal cavity and the other end is blindly ended.

The inner wall of the gill pouch is folded 10 form numerous gill lamellae and the outer wall is highly muscular. The gill pouches are separated from each other by inter-branchial septa. In Hagfishes the number of pouches varies from 5 to 15. In Myxine the gill pouches do not open to the exterior like Lamprays. In Myxine there are 6 pairs of pouches and in Eptatretus 13-15 pairs of pouches.

b. Septal Gills:

This type of gills are found in elasmobranchs (Fig. 6.78A). 'Septal gills' are called because the gill pouches are separated by the thick inter-branchial septa or partitions which are strengthened by tough fibrous tissue.

The gill chambers are longer than the pouch gills and communicate internally with the pharynx by larger spaces and also open to the outside by a comparatively narrow slit, the external gill cleft. The inter-brachial septa are very large that extend beyond the gill filaments.

c. Opercular Gills:

This type of gills occurs in bony fishes (Fig. 6.78B). 'Opercular gills' are called because a movable bony gill cover, the operculum contains the gills in an opercular cavity or gill

chamber. The gill bar or septum is usually shorter than the elasmobranchs and may be absent.

Primary Respiratory Organs:

The primary respiratory organs of the fishes are the gills.

Typical Structure of Gill:

Typically each gill is a comb-like structure having series of gill- filaments attached to the gill-arch. Each gill-arch bears a double rows of gill-filaments. The surfaces of each gill-filament are thrown into numerous small folds which increase the sum total surface area of the gills for gaseous exchange. The respiratory area of the gill in fishes depends on the size and number of the gill-filaments.

The development of respiratory area depends on the habit of the fishes. In fast moving forms the respiratory area is more, while in slow moving or sedentary forms the respiratory area of the gill is lesser. The principle of gill-respiration is basically similar in all the fishes but the structure, number and orientation of gills vary considerably.

Location of Gills:

The gill-slits are usually short, but in Cetorhinus (huge Basking shark) these are very large and extend from the upper to the lower surface of the body. The first slit like aperture is designated as spiracle which is situated between the mandibular and hyoid arches. The second is called the hyoid an cleft which lies between the hyoid arch and the first branchial arch.

The rest of the gill-slits are located between the posterior branchial arches. In most of the elasmobranches, the number of the gill-slits is five on each side excluding the spiracle. But in some sharks the gill- slits may exceed the normal number of five pairs. Hexanchus bears six pairs and Heptranchias possesses seven pairs of gill-slits in addition to the spiracles. In most of the elasmobranchs, particularly in the sharks, the gill-pouches open directly to the exterior by independent external gill-slits.

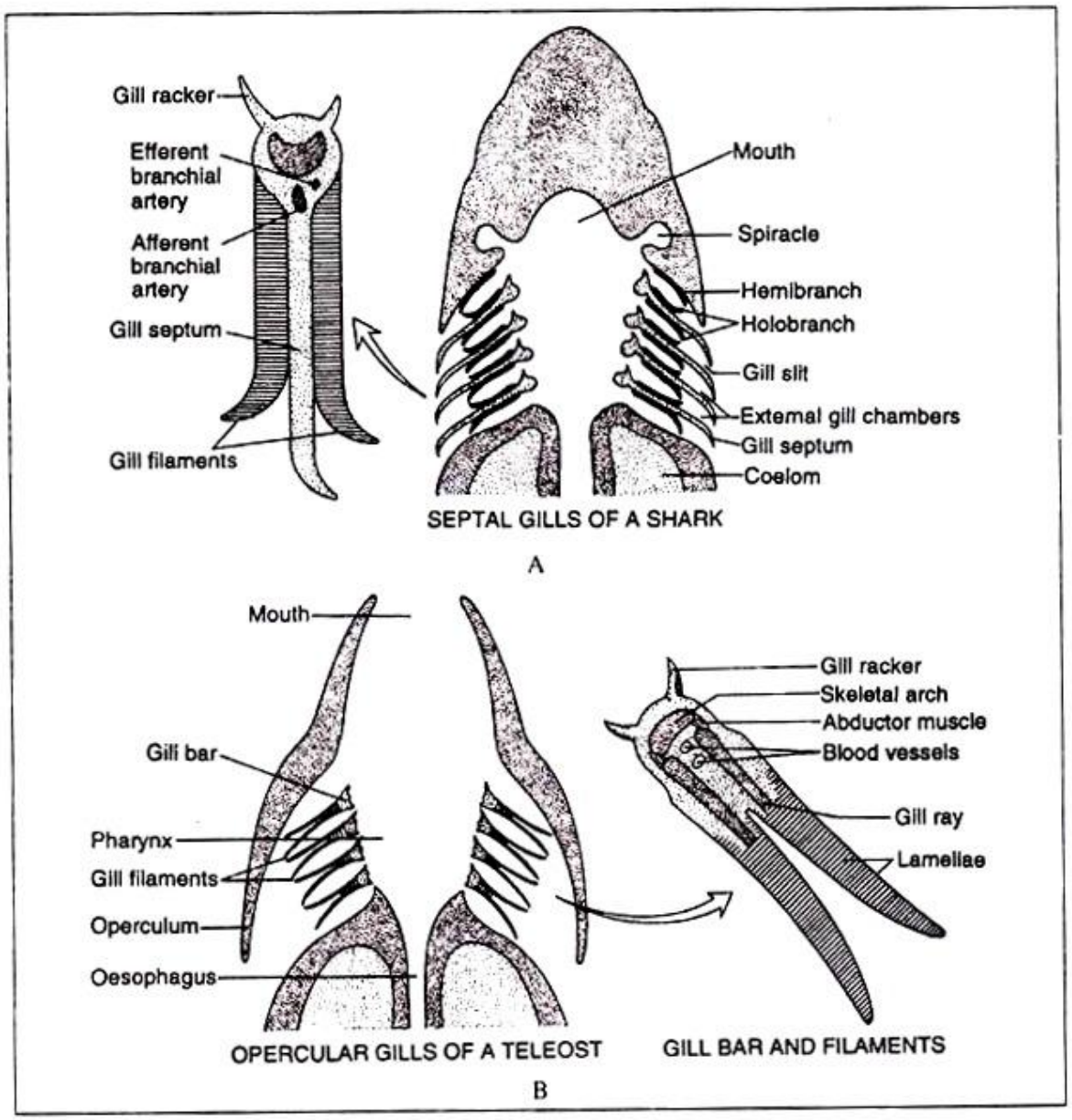


Fig. 6.78 : Types of gills. A. Septal gills of a shark. B. Opercular gills of a teleost.

In the sharks the partitions between the gill- slits are prolonged backward as the folds of skin to cover the external gill-slits. In the bony fishes, the internal pharyngeal gill-slits are present, but these do not open independently to the exterior. All these open into a common branchial chamber which is covered by a movable gill-cover or operculum.

Each branchial chamber opens to the exterior by a large slit or aperture. The opercula of two sides may overlap or fuse ventrally. In some actinopterygians the degree of fusion becomes so great that the opercular openings become greatly reduced to small bilateral slits or round openings as observed in the eels.The operculum is supported by broad, flat bony plates which may or may not be supported by slender branchiostegal rays on the ventral side.

Amongst the elasmobranchs, the holocephalans are operculate. These forms represent a stage which stands midway between the rest of the cartilaginous fishes and the bony fishes. In these forms, the inter-branchial septa are shorter than that in true sharks and the gills are lodged in a common branchial chamber.

The outer side of this chamber is bordered by a flap of skin resembling the operculum of the bony fishes. Each branchial chamber opens to the exterior by a single slit.

Function of Gills:

a. The gills in fishes are mostly respiratory in function.

b. Besides their respiratory role, the gills take part in the elimination of certain waste products and thus help in the maintenance of salt balance.

c. Both freshwater and marine teleosts excrete nitrogenous waste products through the gills in the form of ammonia and urea.

d. The gills of Cyprinus (Carp) and Carassius (Gold-fish) excrete the nitrogenous waste products six to ten times that of kidneys.

Types of Gills based on Location:
Depending on the location of the gills, two types of gills are found in fishes:

1. Internal Gills:

When the gills lie inside the gill-pouches (sharks) or in the branchial chamber (in holocephalans and bony fishes), these are called internal gills.

2. External Gills:

The larval stages of many fishes develop external gills for respiration. The external gills are of two varieties depending on their development.

These are:

(i) True External Gills:

These are independent of the internal gills and develop as the modification of the integument.

(ii) Prolongations from the Internal Gills:

These gills are located outside the body, but these are nothing but the prolongations of the internal gill- filaments which lie on the outer side of the body. True external gills occur in the young stages of Polypterus and Lepidosiren.

In the larvae of Protopterus and Lepidosiren, four pairs of small external gills are present. With the attainment of adulthood, the external gills in these forms are lost except Protopterus where vestiges of the external gills persist even in adult. In young Polypterus (Bichir) a pair of leaf-life external gills are present just above the gill-

opening.The external gills of the second category are found in the embryo of some selachians and some oviparous bony fishes. In the selachian embryos long filamentous external gills develop from the walls of the gill-slits and project out through the external gill-slits. These filamentous structures help in respiration because the sea-water circulates through these structures.

In the viviparous selachians, these structures also help to absorb nutrient. The external gills observed in the young's of some egg laying bony fishes (i.e., Gymnarchus, Clupisdis and many others) are respiratory in function. In Gymnarchus, slender filaments from the internal gills project beyond the opercular edge and function as external gills.

Based on structure:

Based on structure and function, four types of gills are encountered in fishes. These are: hemi branch, pseudo branch, holobranch and lophobranch. The gill-pouches are separated by interbranchial septa. The anterior and posterior walls of each septum bear series of gill- filaments.

Each series of gill-filaments on one side of the interbranchial septum constitute the hemi branch or half-gill. The pseudo- branch is a modified form of the hemi branch.The term pseudo branch is referred to the hemi branch which has lost the original respiratory function. A holobranch or complete gill is composed of two hemibranchs, i.e., a holobranch consists of an interbranchial septum (may be reduced in advanced fishes) with series of vascular gill-filaments developing on its anterior and posterior walls.

Another peculiar type of gill is observed in Seahorses and Pipe-fishes where the gill-filaments become greatly reduced to form rosette-like tufts. These tufts are small and are attached to the greatly reduced gill-arches. Such types of gills are called tuft gills or Lophobranchs.

Fate of Interbranchial Septa:

The interbranchial septa in the primitive fishes are thick and contain tough fibrous tissues. These are supported by many cartilaginous movable segments which constitute the gill-arches. The gill-arches are situated at the inner side of the septa. Each arch appears like a half-hoop.

The lowest pieces of the gill-arches almost join with the counterparts of the opposite and thus support the inner side of the pharynx like series of girders. The inter-branchial septa show the tendency towards reduction in course of evolution (Fig. 6.79).

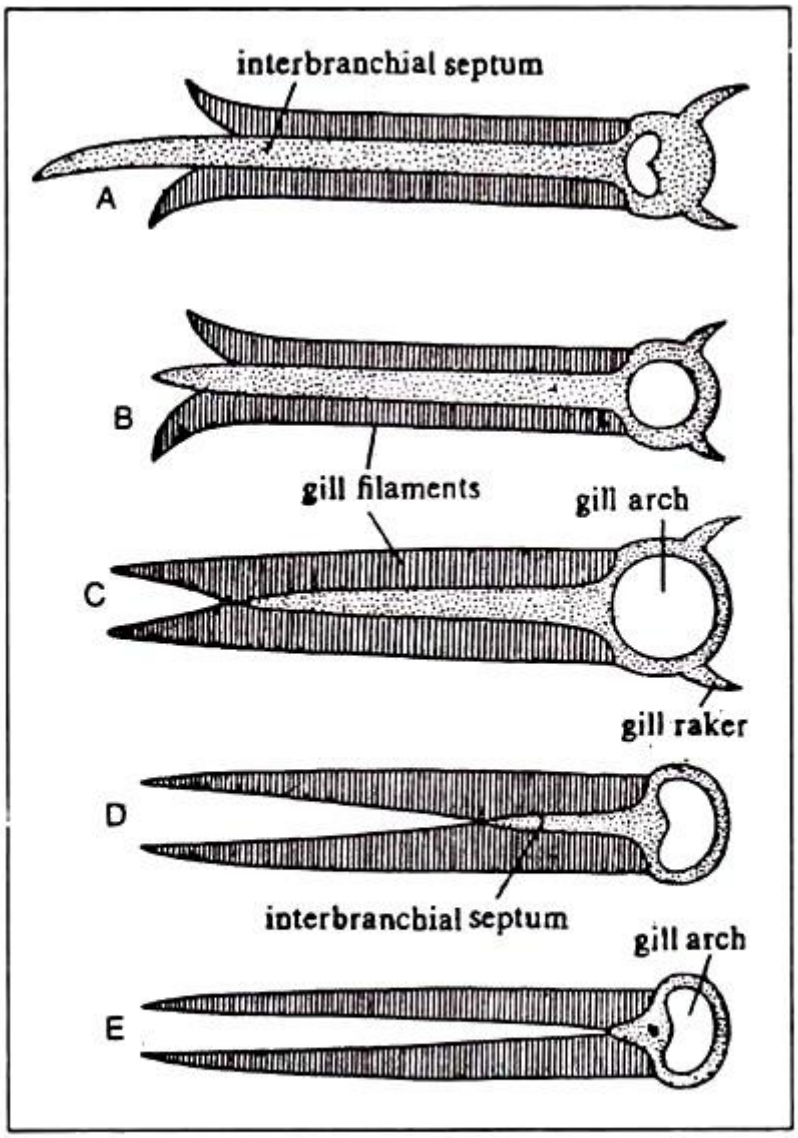


Fig. 6.79 : Showing the fate of the interbranchial septum in different fishes. A. Typical selachian. B. Chimaera. C. Sturgeon. D & E. Teleosts.

In selachians, the interbranchial septa are arranged in such a fashion that a series of independent gill-slits are produced. In these forms the interbranchial septa are larger than the rows of gill-filaments. The interbranchial septa project backward as folds to cover the gill-slits.This condition represents the primitive condition amongst the fishes. But in the remaining fishes these septa becomes reduced in a varying degree. In chimaeras, the inter-branchial septa are slightly shorter than the gill-filaments and the gill filaments project a little beyond the outer edges.

In the primitive bony fishes represented by Sturgeons (Acipenser) the septa become shorter and extend up to the midway. This condition is also observed in *Labeorohita* and *Tenualosailisah*, but in the other bony fishes the septa become progressively shorter as seen in Salmon, *Rita rita, Channa striatus* etc. The condition of the interbranchial septa in Sturgeons exhibits a transitional stage between the chondrichthyan and

teleostean conditions. The rows of gill-filaments or the two sides of the septum are independent of one another but in *Labeorohita*, the adjacent rows of gill- filaments are fused at the tips and bases so that a narrow slit-like aperture is left between these rows of gill-filaments.

Fate of Spiracles:

The spiracle is the slit-like aperture between the mandibular and hyoidean arches. This structure becomes subsequently modified in different fishes. In the sharks, the spiracles are present. The anterior side of the spiracular cleft bears spiracular gill composing of a number of gill-filaments. But in most of the fishes the spiracular gill is represented by a retemirabile or network of blood vessels called pseudo branch.

The pseudobranchs, with all probabilities, are the organs for special sense. In skates and rays, the spiracles are highly developed and are provided with movable valves. The external gill-slits are located ventrally and during rest on sandy bottom, there is every chance of the entry of sand particles inside the gill-pouches along with the respiratory water current.So to prevent the clogging up of gill-filaments by the introduction of foreign particles, these fishes inhale water through the spiracles and expel it by way of the gill-slits. The spiracular openings are closed in adult holocephalans, although these are present in the larval stage. There is no evidence of the existence of pseudo branch in the spiracular opening. In sharks, the spiracles retain a few gill- filaments in adult stages.

In bony fishes, the spiracles are mostly absent, although spiracular pouches may develop. Amia and Lepisosteus lack the spiracle and the spiracular pouch is greatly reduced. In Acipenser, the spiracle and the tube-like spiracular pouch are present. Scaphirhynchus lacks a spiracle. Polypters possesses a wide spiracle and a cellular ridge separates the spiracular pouch and the hyobranchial groove. In crossopterygians, the spiracles are absent. In Latimeria, the spiracular pouch is very deep, but in the lung-fishes, the spiracular pouch is greatly reduced.

Gill-Rakers:

The gill-rakers are specially developed on the inner edge of gill-arches. The gill-rakers are modified dermal denticles and are arranged in double rows. The development of gill-rakers in fishes depends on the particular mode of feeding. In the fishes which devour minute organisms, the gill-rakers are highly developed.

During swimming there is every chance of entry of the small creatures through the internal gill-slits and thus the gill-filaments may either be damaged or clogged. These are avoided by the development of the gill-rakers. The gill-rakers form a sort of sieving apparatus which strains water that bathes the gill- filaments.

The structure of the gill-rakers, varies greatly in different fishes (Fig. 6.80). The hering-like fishes (Hilsa, Cadusia, Gonialosa, Notopterus) are plankton feeders and in them the gill-rakers are slender and extremely elongated. These rakers form a close-set strainer.

In many other filter feeders, the primary gill- rakers bear secondary and tertiary branches and thus appear like a fine gauze.In Esox (Pike) the gill-rakers are reduced to bony knobs which prevent the entry of larger particles. The structure and number of gill-rakers vary considerably even within the closely related forms. In adult Alosa alosa, the lower limb of the arch bears about eighty gill-rakers, whereas Alosa fallax possess only thirty.

The gill-rakers in Cetorhinus (Basking shark) and Rhincodon (Whale shark) measure about 10-12 cm in length. These become flattened to form a closely set structure. This structure recalls the baleen plate of whalebone of whales in structure and function. The gill- rakers are generally absent in other sharks. In crossopterygians the gill-rakers are rudimentary.

Gills in Different Fishes:

The gills in fishes are basically similar. In elasmobranchs, the gills are mostly hemibranchs, whereas in the teleosts the gills are mostly holobranchs. The vestigial mandibular gills (pseudo-branch) are present in some fishes. Most of the sharks possess on each side a mandibular pseudo branch, a hyoidean hemi-branch and four, five or six holobranchs.

In holocephalans, the mandibular pseudo- branch is lacking, but the hyoid arch has a posterior hemi branch. The first, second and third gill-arches bear holobranchs whereas the fourth bears a hemi branch. In sharks, each gill-arch contains one afferent and two efferent vessels, while in holocephalans only one efferent vessel is present.Amongst the Osteichthyes, the actinopterygians possess on each side, a mandibular hemi branch or pseudo branch, four holobranchs (on the first, second, third, and fourth gill-arches). In some forms, a hemi branch may be present.

A hyoidean hemi branch may be lacking in some cases. In crossopterygians, the nature of the gills is slightly different. There is no existence of mandibular pseudo branch in Latimeria, although small hyoidean hemibranchs may be present.

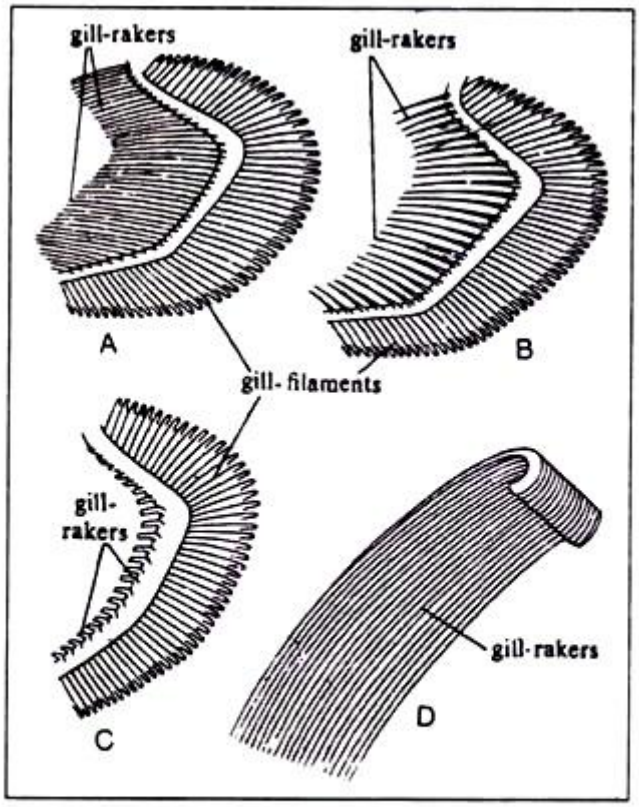


Fig. 6.80 : Showing the gill-rakers in different fishes. A. *Alosa fallax*. B. *Alosa alosa*. C. *Perca fluviatilis*. D. Isolated gill-rakers of *Cetorhinus maximus*.

Like that of other actinopterygians, there are four pairs of holobranchs in Latimeria. But the fifth gill-arch is devoid of any gill. The dipnoans resemble Latimeria in gill arrangement. Neoceratodus contains a hyoidean hemi branch, holobranch on the fourth and fifth arches.

The sixth gill-arch bears an anterior hemi branch. In Lepidosiren, the second, third and fourth gill-arches have holobranchs. The hemibranchs are lacking.

Pseudobranchs:

It is generally considered that the pseudo branch in elasmobranchs and teleosts represents a modified posterior hemi branch of the mandibular gill-arch. They lack respiratory function.

The pseudobranchs become greatly reduced in different fishes. In Trout, the pseudo branch retails the characteristic comb-like appearance.

The gill-filaments become extremely reduced and are covered by the pharyngeal epithelium in case of Perch. In the Cod, the pseudo branch becomes completely covered in the pharyngeal epithelium to form a gland like organ called vaso-ganglion or rete mirabile.

Although the pseudobranchs are embedded deeply in the pharyngeal tissue, these structures retain the fine constituents of the gill-tissue. In Amia, the pseudobranchs are reduced and covered by pharyngeal mucous epithelium. In Catlacatla, the pseudo branch (Fig. 6.81) is attached to the anterior gill.

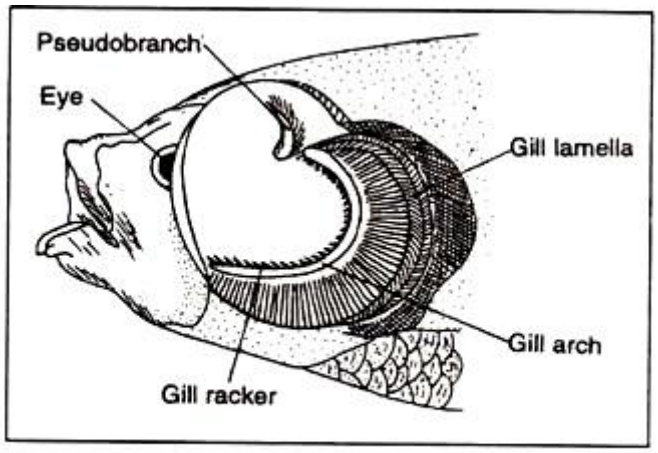


Fig. 6.81 : Position of a pseudobranch of a teleost.

The pseudobranchs may be free or may be covered by mucous epithelium. These receive oxygenated blood from the dorsal aorta. The pseudobranchs lack respiratory function in adults and sub-serve other functions. The mandibular hemibranchs usually help to close the spiracles and get oxygenated blood.

These structures may:
(i) Increase the oxygen concentration in the blood going to the brain or

(ii) Regulate the blood pressure in the ophthalmic artery or

(iii) Act as endocrine organ.

The pseudobranchs are made up largely of acidophilic cells. In Lepisosteus the mandibular pseudo branch remains in close contact with the hyoidean hemi branch and gets blood from the afferent hyoid artery and the efferent artery from the first arch.

Acipenser resembles Lepisosteus except that the mandibular pseudo branch and hyoidean hemi branch lack connection. The pseudobranchs of Amia lack direct afferent or efferent blood connection. The pseudobranchial vessel joins the orbital and ophthalmic vessels. Polypterus lacks mandibular pseudobranchs.

Hyoidean Hemibranchs:

The hyoidean hemibranchs are present in most of the fishes. In the sharks, hyoidean hemibranchs are present. In selachians, the hyoidean hemibranchs have either disappeared or are represented by rudiments. In the holocephalans, hyoidean hemibranchs remain attached with the operculum.

But in most of the actinopterygians, the hyoidean hemibranchs are absent. In Acipenser, Lepisosteus and Polyodon these gills are present, but Amia lacks the hyoidean hemibranchs. In Scaphirhynchus, these hemibranchs are greatly reduced. Polypterus lacks both the mandibular pseudobranchs and hyoidean hemibranchs.

The absence of these gills may possibly be resulted due to the conversion of the swim-bladder into the air- breathing 'lungs'. Amongst the crossopterygians, hyoidean hemibranchs are present in all the living forms except Lepidosiren. In Latimeria the hyoidean hemibranchs are small.

Blood Supply to Gills:

The gills are well-supplied with blood vessels. The flow of blood and the water current pass one another in opposite directions. This arrangement ensures efficient exchange of dissolved substances between the two fluids. If the direction of flow of the two fluids is experimentally reversed, the uptake of oxygen falls from 50% to 9%.

The gill-filaments are provided with folds which permit the blood to come in intimate contact with the water for gaseous exchange. The gills are composed of primary gill-filaments which produce numerous secondary folds (filaments). Each secondary gill-filament has a central core of vascular tissue over lined by a thin layer of connective tissue and mucous epithelium.The vascular central core contains capillary networks and supporting Pilaster cells. In most of the teleosts, each gill- arch contains one afferent and one efferent branchial vessel. But in Labeorohita, Clariasbatraahus, Trichogaster fasciatus, Anabas testudineus and many others, two efferent vessels are present in each gill-arch.

Amongst the cartilaginous fishes, each gill-arch contains one afferent and bilateral efferent vessels. But in the holocephalans only one efferent vessel is present. In lung-fishes each gill-arch contains two efferent arteries.In a typical teleost, each gill-arch contains an afferent branchial and an efferent branchial vessel. Each afferent branchial gives off primary afferent vessels to the primary gill-filaments. Each primary afferent vessel divides into a number of secondary and tertiary branches for the secondary gill-filaments. The afferent vessels break up into capillaries in the secondary gill- filaments.

These capillaries unite and the blood is carried to the primary efferent vessel. The primary efferent vessels run along the margin of the primary gill-filaments and get secondary efferent vessels from the secondary efferent vessels from the secondary gill-filaments.Exchange of gases takes place while the blood passes through the capillaries and the oxygenated blood from the gill-filaments is collected in the main efferent branchial vessels.

Most of the fishes cannot survive out of water because of the failure of respiring in air. But there are many fishes which do survive out of water for a considerable period of time. This is possible by the development of certain specialised organs usually called accessory respiratory organs.

Types of Accessory Respiratory Organs:

1. Suprabrachial Organ:

The supra-branchial organ is a specialised type of respiratory structure encountered in Clariasbatrachus (Fig. 6.83A).

It has a complex structural organisation and consists of the following portions:

(a) An elaborate tree-like structure growing from the upper end of the second and fourth gill-arches of either side. This dendritic organ is composed of numerous terminal knobs, each has a core of cartilage covered by vascular membrane. Each exhibits eight folds which suggest that one such knob is formed by the coalescence of eight gill-filaments.

(b) There are a pair of highly vascularized supra- branchial chambers within which the tree-like structures are contained. The supra-branchial chambers are developed as the vascularized diverticula of the branchial chamber.

(c) The entrance of the supra-branchial chamber is guarded by 'fan'-like structures which are developed by the fusion of the adjacent gill- filaments of the dorsal side of the gill-arches.

The supra-branchial organs, like the gills, are lined by thin outer epithelial layers with intercellular spaces separated by the pilaster cells. The organs and the supra-branchial chambers are supplied by afferent and efferent blood vessels from the gill-arches.

The supra-branchial organs help to breathe in air. The supra-branchial chamber has inhalant and exhalant apertures. These fishes come to the surface of the water and gulp air into the supra-branchial organs. Atmospheric air from the pharyngeal cavity is taken into the supra-branchial chamber by an inhalant aperture located between the second and third gill- arches.

After gaseous exchange the air from the said chamber expels into the opercular cavity by the gill-slit lying between the third and fourth gill-arches. The fan-like structures present in the second and the third gill-arches help to intake the air while the expulsion of the air from the supra-branchial, chamber is caused by the contraction of its wall. Thus the supra-branchial chamber and its contained organs function as 'lung'.

2. Branchial Outgrowths:

In climbing perch (*Anabas testudineus*) there are two spacious sac-like outgrowths from the dorsal side of the branchial chambers (Fig. 6.83B). The epithelium lining these outgrowths is highly vascular and becomes folded to increase the respiratory area.

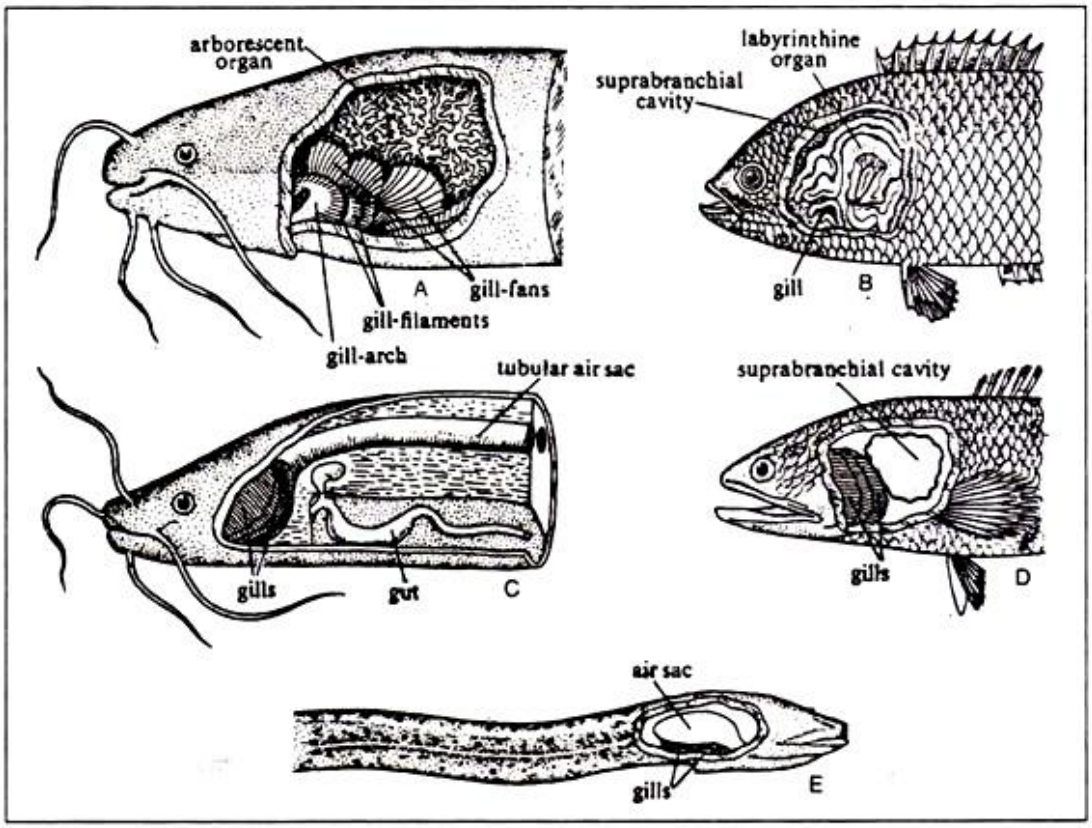


Fig. 6.83 : Accessory respiratory organs in air-breathing teleosts. A. *Clarias batrachus*. B. *Anabas testudineus*. C. *Heteropneustes fossils*. D. *Channa punctatus*. E. *Amphipnous cuchia*.

Each chamber contains a characteristic rosette-like labyrinthine organ. This organ develops from the first epibranchial bone and consists of a number of shell like concentric plates. The margins of the plates are wavy and the plates are covered with vascular gill-like epithelium.

Each branchial outgrowth communicates freely not only with the opercular cavity but also with the buccopharyngeal cavity. Air enters into the outgrowth by way of the buccopharyngeal opening and goes out through the external gill-slits. The entrance is controlled by valves.Anabas can breathe in air by the help of these organs. These fishes have the habit of migration from one pond to the other. Their overland progression is peculiar and is assisted by the operculum and the fins. Each operculum bears sharp spines at the free edge

During travelling the opercula alternately spread out and fix to the ground by the spines and get the forward push from the pectoral fins and the tail. The proverb that the fish can climb the trees seems to be erroneous. The climbing perches are found in the branches of palm or other trees which are possibly brought there by the kites or crows while these fishes migrate over the land.In *Trichogaster fasciatus* the accessory respiratory organs are similar to that of Anabas and consist of supra-branchial chamber, labyrinthine organ and respiratory membrane (Fig. 6.84).

The labyrinthine organ is simpler in construction in comparison to that of Anabas. Each organ assumes a spiral configuration with two leaf-like expansions. Each of these two

expansions is composed of loose connective tissue which is covered by highly vascular epithelium.

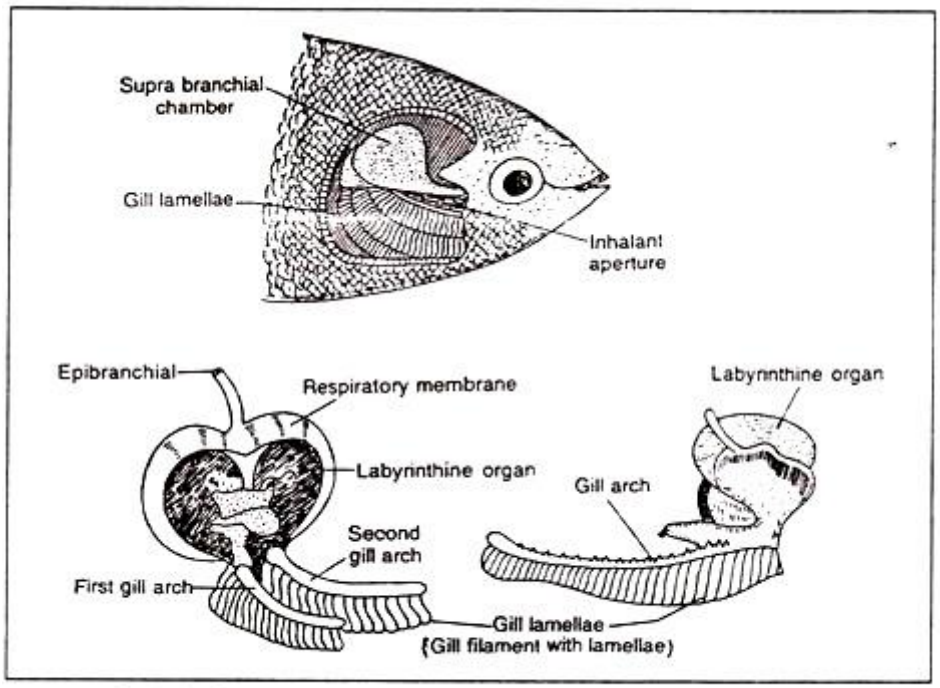


Fig. 6.84 : Gills and accessory respiratory organs of *Trichogaster fasciatus*.

4. Pharyngeal Diverticula:

In the Snake- headed fishes and Cuchia eels, the accessory respiratory organs are relatively simplified. These fishes can survive prolonged drought and their air breathing habit enables them to remain out of water for some time. In both the group of fishes, the pharynx gives a pair of saclike diverticula for gaseous exchange.

In Channa, the accessory respiratory organs are relatively simpler and consist of a pair of air-chambers (Fig. 6.83D). These are developed from the pharynx and not from the branchial chamber as seen in others.The air-chambers are lined by thickened epithelium which is highly vascularized. The air-chambers are simple sac-like structures and do not contain any structure. These chambers function as the lung-like reservoirs. In *Channa striatus* the vascular epithelium lining the chambers becomes folded to form some alveoli. The gill-filaments are greatly reduced in size.

In Cuchia (*Amphipnouscuchia*) the accessory respiratory organs consist of a pair of vascular sac-like diverticula from the pharynx above the gills (Fig. 6.83E). These diverticula open anteriorly into the first gill-slit. These diverticula function physiologically as the lungs.The gills are greatly reduced and a few rudimentary gill-filaments are present on the second of the three remaining gill- arches. The third gill-arch is found to bear fleshy vascular epithelium. In Periophthalmus, a pair of very small pharyngeal diverticula is present which are lined by vascular epithelium.

4. Pneumatic Sacs:

In Heteropneustes fossil is, a pair of tubular pneumatic sacs, one on each side of the body, act as the accessory respiratory organs. These long tubular sacs arise as the outgrowths from the branchial chamber and extend almost up to the tail between the body musculature near the vertebral column (Fig. 6.83C). In Sacco-branchus, similar tubular lung-like outgrowths of the branchial chamber extend back into the body musculature.

5. Buccopharyngeal Epithelium:

The vascular membrane of buccopharyngeal region in almost all the fishes helps in absorbing oxygen from water. But in mudskippers (Periophthalmus and Boleophthalmus) the highly vascularized buccopharyngeal epithelium helps in absorbing oxygen directly from the atmosphere.These tropical fishes leave water and spend most of the time skipping or 'walking' about through dampy areas particularly round the roots of the mangrove trees. The old idea that the mud-skippers use the vascular tail as the respiratory organ is not supported by recent Icthyologists.

6. Integument:

Eels are recorded to make considerable journey through damp vegetation. The common eel, *Anguilla anguilla* can respire through the integument both in air and in water. In *Amphipnouscuchia* and mud- skippers, the moist skin sub-serves respiration.Many embryos and larvae of fishes respire through the skin before the emergence of the gills. The median fin fold of many larval fishes is supplied with numerous blood vessels and helps in breathing. The highly vascular opercular fold of Sturgeon and many Catfishes serves as the accessory respiratory structure.

7. Gut epithelium:

The inner epithelium of the gut essentially helps in digestive process. But in many fishes the gut becomes modified to sub-serve respiratory function. Cobitis (giant loach of Europe) comes above the water-level and swallows a certain volume of air which passes back along the stomach and intestine. In *Misgurusfossilis*, a bulge just behind the stomach is produced which is lined by fine blood vessels.

The bulge acts as the reservoir of air and functions as the accessory respiratory organ. After the gaseous exchange, the gas is voided through the anus. In certain other fishes, *Callichthyes, Hypostomus*and*Doras* the highly vascular rectum acts as the respiratory organ by sucking in and giving out water through the anus alternately.In these fishes the wall of the gut becomes modified. The wall becomes thin due to the reduction of the muscular layers.

8. Swim-Bladder acts as Lung:

Swim-bladder is essentially a hydrostatic organ but in some fishes it functions as the 'lung'. In Amia and Lepisosteus, the wall of the swim-bladder is sacculated and resembles lung. In Polypterus the swim-bladder is more lung-like and gets a pair of pulmonary arteries arising from the last pair of epibranchial arteries.

The swim-bladder in dipnoans resembles strikingly the tetra- pod lung in structure as well as in function. In Neoceratodus, it is single, but in Protopterus and Lepidosiren it is bilobed. The inner surface of the 'lung' is increased by spongy alveolar structures.In these fishes, the 'lung' is mainly respiratory in function during aestivation because the gills become useless during this period. Like that of Polypterus, the 'lung' in dipnoans gets the pulmonary arteries from the last epibranchial arteries.

In Notopterus, the swim-bladder becomes more complex and acts as a lung. Except the hydrostatic, sound production and hearing, a new function like respiration was innovated in Notopterus. In Notopteruschitala the posterior tip of swim-bladder is enlarged which is called caudal extension and the ventral part gives off several finger-like projections, the dorsal side of the gas bladder possesses a specialised striated muscle.The anterior part extends into a projection to the ear. An artery arising from the dorsal aorta forms a network of blood capillaries that spread the entire inner surface of the abdominal and caecal parts of the swim bladder.

The blood capillaries that cover a single epithelial layer helps in the gaseous exchange between the blood and the air of the swim-bladder. This air breathing habit is considered as a secondary adaptation in these fishes.

Functions of Accessory Respiratory Organs:

The accessory respiratory organs contain a high percentage of oxygen. The fishes possessing such respiratory organs are capable of living in water where oxygen concentration is very low. Under this condition these fishes come to the surface of water to gulp in air for transmission to the accessory respiratory organs.If these fishes are prevented from coming to the surface, they will die due to asphyxiation for want of oxygen. So the acquisition of accessory respiratory organs in fishes is an adaptive feature.

Further it has been observed that the rate of absorption of oxygen in such organs is much higher than the rate of elimination of carbon-dioxide. Hence, it is natural that the gills excrete most of the carbon-dioxide. Absorption of oxygen appears to be the primary function of the accessory respiratory organs.

Significance of Accessory Respiratory Organs:

The cause of emergence of the accessory respiratory structures in fishes in addition to the primary respiratory organ is very difficult to interpret. There are two contrasting views regarding the origin of the aerial accessory respiratory structures. First view: some fishes have the natural instinct to make short excursion to the land from the primal aquatic home.

To remain out of water, the development of certain devices to breathe in air becomes necessary. Second view holds that the fishes are forced to ascend the land when the oxygen content of water falls to a considerable extent. The fishes in that particular condition of life gulp in atmospheric air from the land and pass it into the accessory

respiratory structures.If they are prevented by mechanical barriers to come to surface, the fishes will die of suffocation. This habit of swallowing bubbles of air is observed in many bony fishes, especially living in shallow water which dries up periodically or becomes foul by the decomposition of aquatic vegetation.

As a consequence of the air-breathing habit for a considerable span of time, the fishes have developed specialized accessory respiratory organs in addition to the gills.Most of such structures encountered in the fishes assume the shape of reservoir of air and originate either from the pharyngeal or branchial cavities. In extreme cases the reservoir may house special structure for gaseous exchange.

However, the development of such accessory respiratory organs is essentially adaptive in nature to meet the respiratory need and thus enables the fishes to tolerate oxygen depletion in water or to live on land over a varying period of time. The development of the accessory respiratory organs depends directly on the ability to remain out of the water.

Probable Questions:

1. Classify gills on the basis of structure and arrangement.
2. Classify gills on the basis of location.
3. What are the functions of the gills?
4. How blood is supplied to gills?
5. Discuss different types of accessory respiratory organs in fishes.
6. How swim bladder acts as lung?
7. What are the functions of accessory respiratory organs in fishes?
8. What are the significance of accessory respiratory organs in fishes?

Suggested Readings:

A text book of Fish Biology and Fisheries (3rd edition) by- S.S. Khanna & H.R. Singh

UNIT-XX

Swim Bladder in Fish

Objective: In this unit we will discuss about different kinds of swim bladder found in fishes.

Introduction: In most of the fishes a characteristic saclike structure is present between the gut and the kidneys. This structure is called by various names, viz., swim-bladder, or gas-bladder, or air-bladder. In our present discussion, the name of the bladder is followed as the swim-bladder to avoid confusion.The swim-bladder occupies-the same position as the lungs of higher vertebrates and is regarded as homologous to the lungs. It differs from the lungs of higher forms mainly in origin and blood supply.

The swim bladder arises from the dorsal wall of the gut and gets the blood supply usually from the dorsal aorta, while the vertebrate lung originates from the ventral wall of the pharynx and receives blood from the sixth aortic arch.The swim-bladder is present in almost all the bony fishes and functions usually as a hydrostatic organ. Starting as a very insignificant cellular extension from the gut, the swim-bladder in fishes leads the whole group through an evolutionary channel.

Development of Swim-Bladder:

Opinions differ as regards the development of swim bladder in fishes. In teleosts, it originates as an unpaired dorsal or dorsolateral diverticulum of the oesophagus. It starts as a small pouch budded off from the oesophagus. The diverticulum with an opening in the oesophagus becomes subsequently divided into two halves.Of these two, the left one often atrophies except in a few primitive forms. The right half becomes well-developed and take a median position. In dipnoans and Polypteridae, the swim-bladder is modified into the 'lungs' and originates as the down-growths from the floor of the pharynx.

These out-growths have been rotated around the right side of the alimentary canal to occupy the dorsal position. As a consequence of shifting of the position, the original right 'lung' becomes the left one. Spengel advocates the view that the swim-bladder in fishes originates from the posterior pair of the gill-pouches, but definite embryological evidence in support of this idea is lacking.

Basic Structure of Swim-Bladder:

The swim-bladder in fishes varies greatly in structure, size and shape.

a. It is essentially a tough sac-like structure with an overlying capillary network.

b. Beneath the capillary system there is a connective tissue layer called tunica externa.

c. Below this layer lies the tunica interna consisting primarily of smooth muscle fibres and epithelial gas-gland.

d. The swim bladder lies below the kidneys, between the gonads and above the gut.

e. The connection with the oesophagus may be retained throughout life or may be lost in the adult.

Gas Composition of Swim-Bladder:

Biot (1807) and Morean (1876) have shown that the gas secreted by the swim-bladder is mostly oxygen. Nitrogen, and little quantity of carbon-dioxide are also present. Generally the gas composition varies in different species. In salmonids, the maximum amount of gas in the swim-bladder is Nitrogen. Again in many species the composition includes mostly a mixture of oxygen and carbon dioxide.

Types of Swim-Bladder:

Depending on the presence of the duct (ductus pneumaticus) between the swim-bladder and the oesophagus, the swim-bladder in fishes can be divided into two broad categories: Physostomous [Gk. physi = a bladder; stomata, mouth] and Physoclistous types [Gk. clistic = enclosed].

Depending on the condition of the swim-bladder, the teleosts are classified by older taxonomists into two groups Physostomi and Physoclisti. A transitional condition is observed in eels.

A. Physostomous Condition:

The swim-bladder develops from the oesophagus. When the ductus pneumaticus is present between the swim-bladder and the oesophagus, the swim-bladder is called physostomous type (Fig. 6.85A).

A vessel emerging from the coeliacomesenteric artery supplies the swim bladder and the blood from it is conveyed to the heart through a vein joining the hepatic portal vein. This condition is observed in bony ganoid fishes, the dipnoans and soft-rayed teleosts.

B. Physoclistous Condition:

In this condition the ductus pneumaticus is either closed or atrophied (Fig. 6.85B). This type of swim bladder is observed in spiny-rayed fishes. In this type of swim-bladder, there lies an anteroventral secretory gas gland (containing retia mirabilia) and a posterodorsal gas absorbing region called the oval. The oval develops out of the degenerating ductus pneumaticus.The rete mirabilis of the gas gland, the oval and the walls of the bladder are supplied by the coeliacomesenteric artery and also by arteries from the dorsal aorta. But the blood from the different parts of the swim bladder is returned by two routes.

The blood from the gas gland is returned to the heart by the hepatic portal vein, while from the rest of the bladder by the posterior cardinal veins. The bladder, specially the gas gland, gets the lateral branches from the vagus, while the oval is innervated by sympathetic nerves.

C. Transitional Condition:

In Eel (Anguilla), a transitional condition between the physostomous and physoclistous type is present. The swim-bladder retains the ductus pneumaticus which becomes enlarged to form a separate chamber containing the oval (Fig. 6.85C). The gas glands are also present.

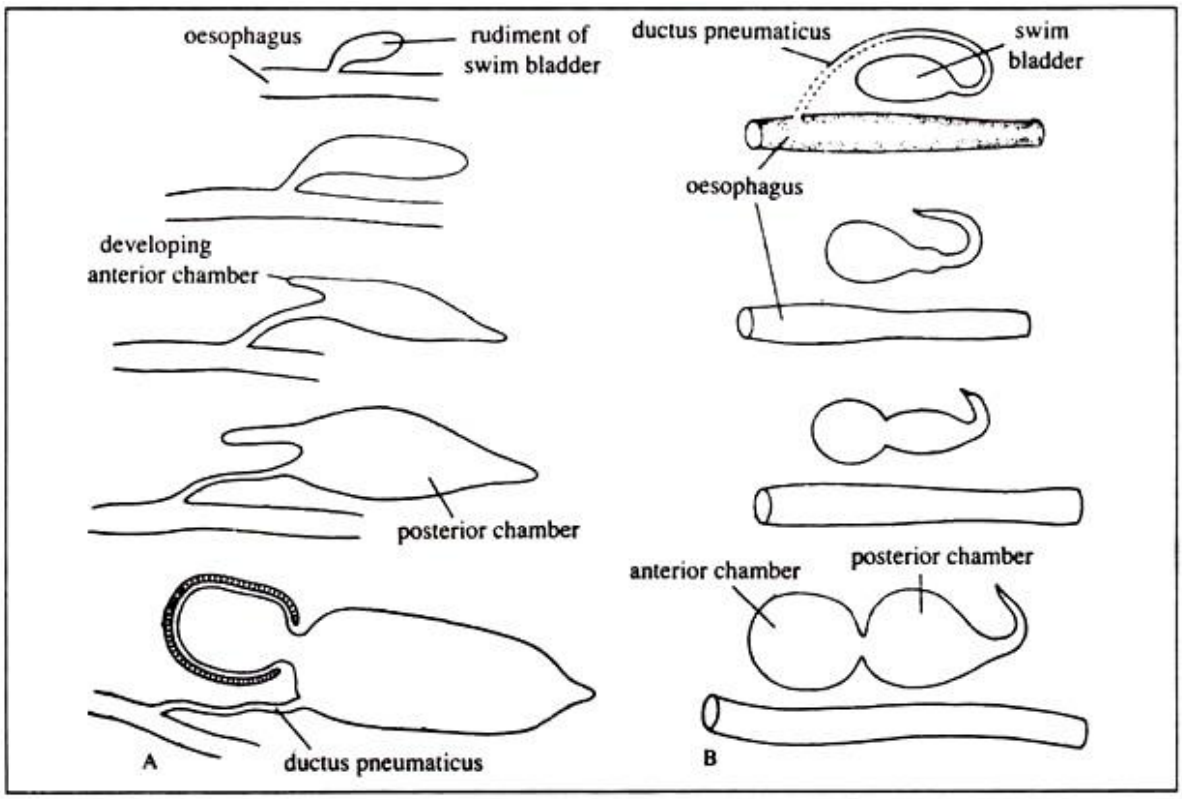


Fig. 6.85 : Showing the derivation of the swim-bladder of the fishes from the gut. A. Stages of formation of physostomous type of swim-bladder in *Catostomus*. B. Stages of formation of the physoclistous type of swim-bladder.

The swim-bladder is supplied with the blood through a branch from the coeliacomesenteric artery while the blood is returned to the heart by a vessel joining the post cardinal vein. The condition represents an intermediate stage when a physostomous condition is on the verge of transformation into the physoclistous state.

Modifications in Swim-Bladder:

In fishes a great diversity in size, shape and function of the swim-bladder is observed. In elasmobranchs, bottom dwelling and deep-sea teleosts the swim-bladder is absent in an adult but a transitory rudiment during development may be present.In flat fishes (Pleuronectidae) swim-bladder is present in the early life when the animals maintain a vertical position. As they tip over one side and assume the lazy adulthood, the swim-bladder becomes atrophied.

In elasmobranchs, the swim-bladder is represented by the transitory rudiment in the embryonic stages. Miklucho-Maclay (1867) has observed a rudimentary dorsal diverticulum from the foregut in the embryos of Squalus, Mustelus ana Caleus. In many fishes, viz., *Heptranchias, Scyllium, Squatina, Pristiurus, Carcharius* and many Rays, small pits are recorded in the oesophageal wall.

Wassnezow (1932) has observed one to six similar oesophageal pits in Pristiurus, Torpedo and Trygon. These pits are located posterior to the fifth pouch. In sharks the swim-bladder is absent in adults, but a hint of a rudimentary swim-bladder is observed during embryonic development. But almost all the teleosts possess the swim-bladder and extreme modifications of the same are encountered because of adaptation to the different modes of living.

Modifications of Physostomous Condition:

The typical physostomous pattern becomes modified in different fishes and the basic trends are:

(1) The formation of paired sacs and

(2) The gradual acquisition of two chambers— an anterior and a posterior.

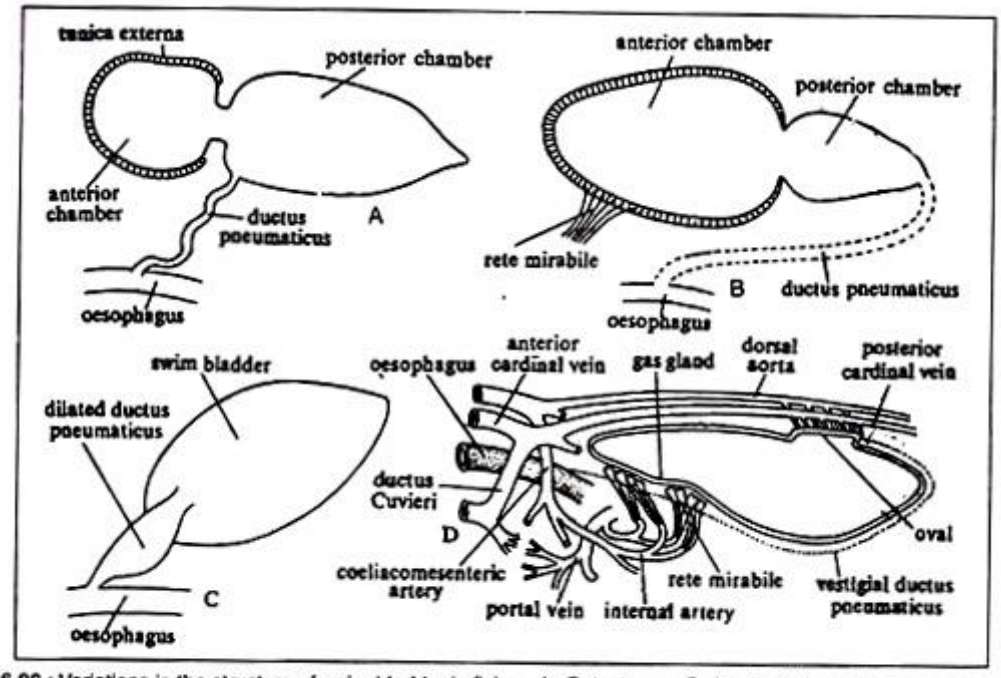


Fig. 6.86 : Variations in the structure of swim-bladder in fishes. A. *Catostomus*. B. A typical physoclistous type of swim-bladder with anterior and posterior chambers. C. Transitional swim-bladder in eel. Note the dilatation of the ductus pneumaticus. D. A physoclistous swim-bladder with oval. Note the circulatory pathways.

The swim-bladder in Polypterus (bichir) (Fig. 6.87A, B) represents the primitive condition. It is a bilobed sac with two unequally developed lobes. The left lobe is shorter and the right lobe is longer. The bilobed sac opens on the floor of the pharynx through a slit-

like glottis. The glottis is provided with muscular sphincter. The internal lining of the bladder is smooth and partly ciliated.The lack of alveolar sacculations and the presence of muscular walls are the two noted feature in the swim-bladder of Polypterus. The walls of the bladder are highly vascular and are lined by two layers of striated muscle fibres.

The bladder is supplied by a pair of pulmonary arteries arising from the last pair of pulmonary arteries arising from the last pair of epibranchial arteries and the corresponding veins enter into the hepatic vein below the sinus venosus.In the dipnoans, the swim-bladder is called the lung and the inner walls are produced into numerous alveoli. The swim-bladder resembles the tetrapod lungs both structurally as well as functionally. In Neoceratodus it is single- lobed, while in Protopterus and Lepidosiren it is bilobed (Fig. 6.87C, D, E).

Other details regarding the structural construction, blood' and nerve supplies have already been dealt in the biology of the lung-fishes.In Sturgeons (Acipenser), the swim-bladder is short and oval in shape. The ductus pneumaticus enters the bladder ventrally and it opens into the gut posterior to the pharynx. The glottis is lacking and the opening into the oesophagus is closed by the simple constriction of the ductus pneumaticus.

The walls of the bladder are fibrous and thick but the inner walls are smooth (Fig. 6.87H). In Acipenser, both the left and right lobes develop from the dorsal side of the oesophagus in the embryonic stage, but the left one becomes completely obliterated and right one gives rise to the adult swim-bladder.

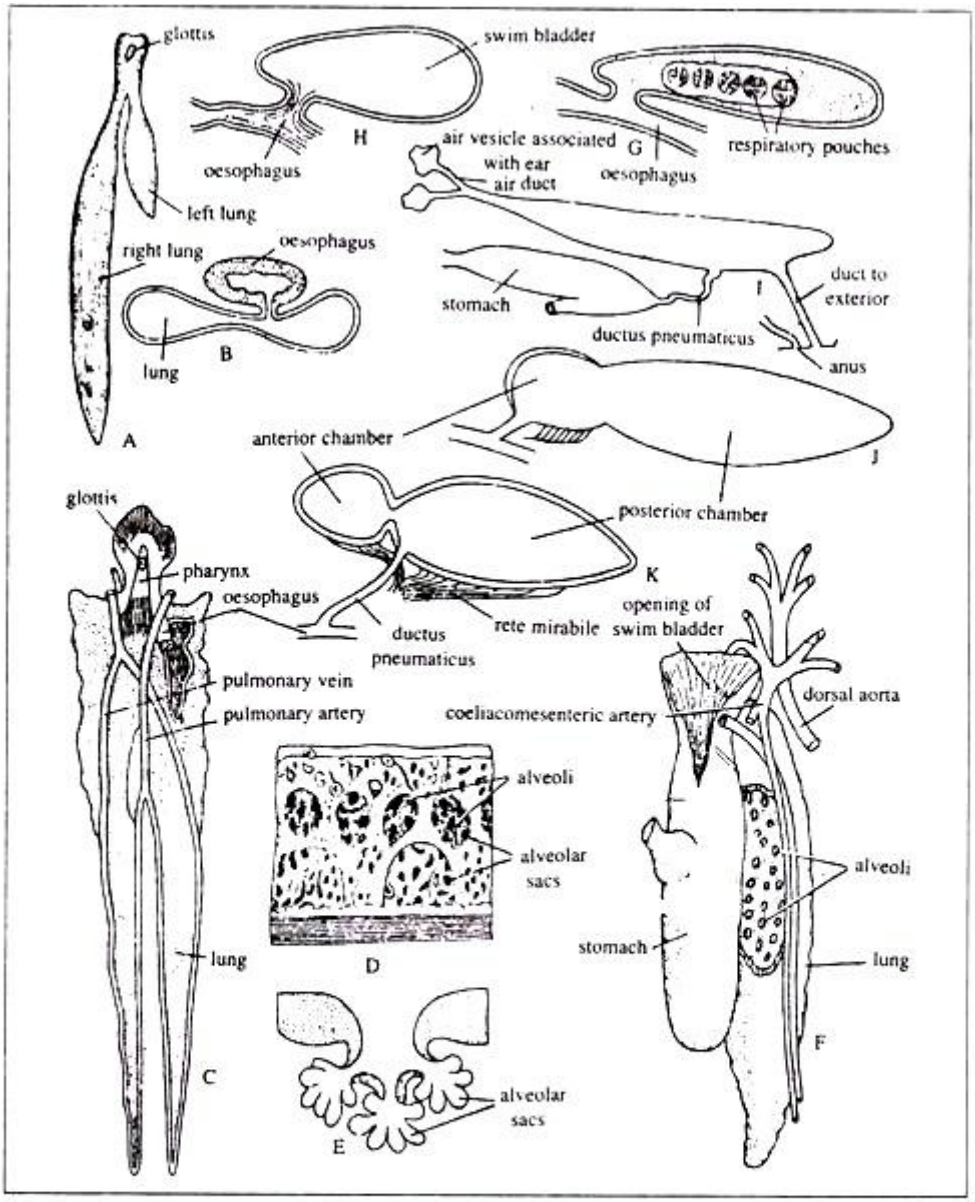


Fig. 6.87 : Variations in the sturcture of swim-bladder in fishes. A. Swim-bladder in *Polypterus*. The swim-bladder is modified as 'lung'. Note that the left 'lung' is smaller in size. B. Sectioal view of the swim-bladder and oesophagus in *Polypterus* showing their relationship. C. Swim-bladder of *Protopterus*. The swim-bladder is modified as the 'lung'. D. A portion of the internal cavity of the 'lung' of *Protopterus* is enlarged to show the location of the alveoli. E. Showing a single alveolus of the 'lung' of *Protopterus*. F. Swim-bladder of *Gymnarchus* and its relative position. The swim-bladder is regarded as 'lung'. A portion of the 'lung' is removed to show the alveoli. G. Swim-bladder of *Amia* and *Lepisosteus*. A portion is cut open to show the internal structures. H. Swim-bladder of *Acipenser*. I. Swim-bladder of *Clupea harengus*. J. Swim-bladder of *Esox*. K. Swim-bladder of a Cyprinoid fish (after various sources). Note that the diagrams are schematic.

In Amia and Lepisosteus, the swim bladder is an unpaired sac extending nearly the entire length of the body cavity. In both the cases rudiment of the left lobe appears during development but persists only for a short time. The ductus pneumatics opens into the oesophagus posterior to the pharynx through a dorsal slit-like glottis.

The walls are highly vascular and exhibit sacculations resembling the pulmonary alveoli (Fig. 6.87G). The sacculations or the respiratory pouches are arranged in two lateral rows. As regards the development of sacculations the swim-bladder of Lepisosteus is more advanced than that of Amia. There are some more minor differences regarding the supply of blood.

The swim-bladder in Amia gets arterial blood from the pulmonary arteries, while that of Lepisosteus gets arterial branches from the dorsal aorta. The blood from the bladder is returned by the left ductus Cuvieri in Amia and by the right post-cardinal in Lepisosteus.Gymnarchus presents an intermediate stage where the efferent branchial arteries from the third and fourth gill-arches join to form a common root for the emergence of the pulmonary and coeliacomesenteric arteries (Fig. 6.87F). Amongst the dipnoans, the swim-bladder of Neocertatodus resembles that of Lepisosteus. The walls are sacculated and act as the lung'.

In *Clupea harengus*, the ductus pneumaticus opens into "the fundus of the stomach and there is a second duct from the posterior part of the swim-bladder opening to the exterior near the anus (Fig. 6.87 I). Similar posterior opening is present in Pellona, Caranx, Sardinella.

Modifications of Physoclistous Condition:

The swim-bladder in all teleosts begins as a physostomous type but in an adult condition the ductus pneumaticus gets degenerated to become a physoclistous type. A typical physoclistous swim-bladder consists of a closed sac having two compartments—an anterior and a posterior. These two compartments are intercommunicated through an aperture called ductus communicans.

The opening and closure of this aperture is regulated by circular and radiating muscles which act as the sphicter. The anterior chamber is formed by circular and radiating muscles which act as the sphincter. The anterior chamber is formed by the enlargement and forward growth of the budding swim-bladder, while the posterior chamber develops as an enlargement of the ductus pneumaticus.This typical structural plan is modified in certain forms. The posterior chamber with retia Mirabella becomes flattened almost to the point of obliteration and is designated 'oval' as seen in the families like Myctophidae, Percidae, Mugilidae.The oval is a thin-walled highly muscular area specialized for the reabsorption of gases (see Fig. 6.86D). The opening of the oval is guarded by circular and longitudinal muscles. This device is of great significance for the fishes undergoing rapid vertical movements.

Histological Modifications:

The morphological modifications of the swim-bladder are accompanied by histological modifications in different fishes, the swim-bladder acts as a hydrostatic organ. It helps fishes to sink or ascend to various depths by altering the gas content in the bladder. In fishes having open ductus pneumaticus, the volume of gas content in the bladder can be changed by swallowing or removing air from the bladder.

But in some physostomous and all physoclistous fishes this process of gas transference is done directly from the blood stream. Inside the bladder there is an oxygen-producing device and an oxygen-absorbing device. The swim bladder is a vascular structure but the degree of vascularization varies in different teleosts.In some species of the families Clupeidae and Salmonidae the capillaries are uniformly present all over the swim-

bladder, but in most cases these highly vascular interlacing and tightly packed capillaries form a mass called rete mirabilis. The anterior chamber of swim bladder shows the tendency to become differentiated into oxygen-producing area called red body.

The oxygen is produced by the reduction of the oxyhaemoglobin in the erythrocytes when brought into close contact with the secreting epithelial cells of the gas gland. The red body consists of internal oxygen-secreting cells (gas gland) and supplied by the blood vessels from the retia Mirabella (sing, rete mirabilis).It forms a complicated structure where the arterial and venous capillaries communicate only after reaching the gas gland. The most primitive condition is observed in Pickerel where the gland is covered by thick glandular epithelium which is thrown into a number of folds. In eels and some other fishes, the red bodies are non- glandular in nature but serve the same physiological function.

The red gland is supplied with blood from the coeliac artery and is returned to the portal vein. The activity of the red gland is controlled by the vagus nerve. In the fishes with functional ductus pneumaticus the gas glands are absent but in eels this function is taken up by the red gland.In the physoclistous fishes, the anterior region is modified for gas production and the posterior region or chamber is specialized for the absorption of gas into the blood. The posterior chamber becomes excessively thin- walled to facilitate gas diffusion.

Beneath the walls, the gas is absorbed directly into the blood. The formation of the oval in some fishes, is a special development for the absorption of gas. The wall of the oval is very thin and highly vascular. Through this epithelial lining oxygen can easily pass to the network of vessels. This gas absorbing region receives blood supply from the dorsal aorta and the blood is returned to the post cardinal vein. The activities are governed by the sympathetic nerves.

The histological differentiation for the gas production and gas absorption is a very significant achievement in fishes. The gas produced by the red body is mostly oxygen and this oxygen is readily absorbed or diffused from the swim-bladder directly into the capillaries. The oval is modified for gas absorption in many fishes.By the alternate process of gas production and gas absorption, the internal pressure and volume of the gas content inside the swim-bladder can be increased or decreased. The red body is usually confined to the anterior chamber, but in fishes where the anterior chamber becomes secondarily associated with the auditory function, the gas gland may be confined to the posterior chamber.

Shape and Size of Swim-Bladder:

The swim-bladder varies extensively in shape and size. In Umbrina (Fig. 6.88A), it is oval shaped and without any appendage. In Atractoscion (Fig. 6.88B), it gives off only one pair of simple diverticula that extends from the anterior side. In Kathala (Fig.

6.88C), the swim-bladder develops a pair of appendage extending in front of transverse septum into head.

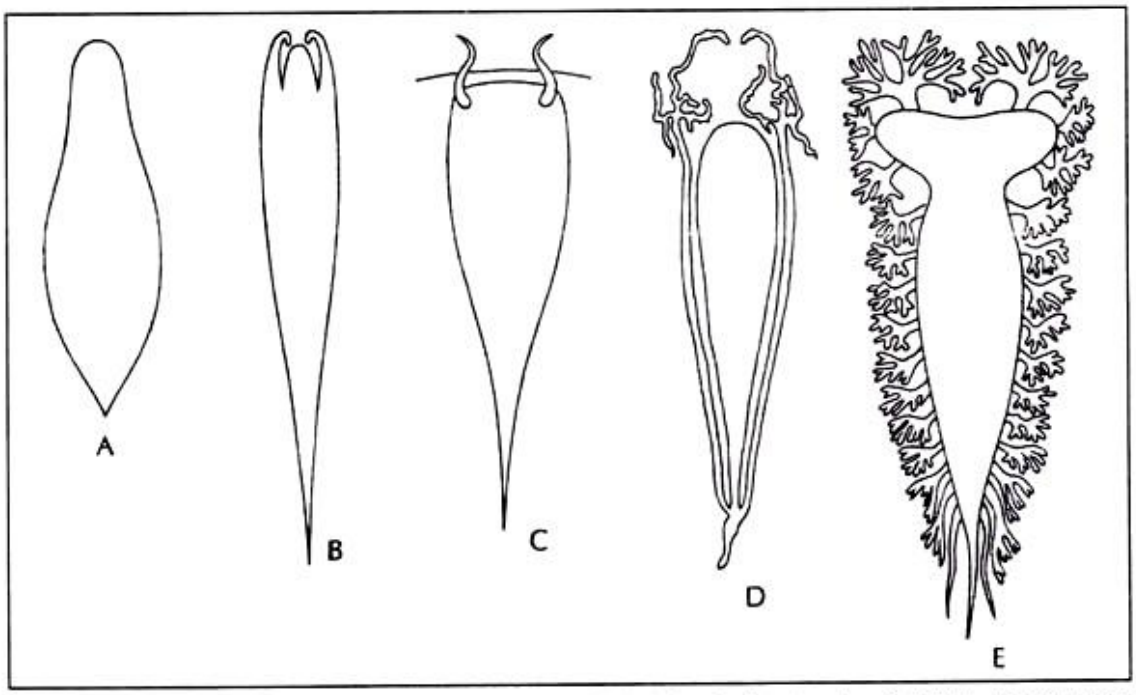


Fig. 6.88 (A–E) : Variations in the shape of swim bladder. A. Umbrina, B. Atractoscion, C. Kathala, D. Otolithoides, E. Johnius.

In some forms it gives off many branched diverticula. In many fishes, the anterior prolongations of the swim-bladder come into close contact with the wall of the space containing the internal ear. In *Clupea*, the narrow anterior end of the swim-bladder enters into a canal in the basioccipital of the skull and divides into two slender branches.

The anterior end of each branch dilates to form a round swelling and lies in close contact with the internal ear. A more or less similar condition is observed in Tenualosa ilisha. In many fishes finger-like diverticula develop from the swim-bladder.In *Gadus* a pair of diverticula originating from the anterior part of the bladder project into the head region. In Otolithus, each anterolateral end of the swim-bladder gives rise to an outgrowth which sends one anterior and a posterior horn.

In Otolithoides (Fig. 6.88D), the appendages attached to posterior end of bladder and at least the main part lying parallel to the bladder. In Corvina lobata, many such branched diverticula develop from the lateral walls of the swim-bladder. In Johnius (Fig. 6.88E), it is hammer-shaped with 12 to 15 pairs arborescent appendages, the first branching in the head and the posterior tip are highly pointed.Usually in most cases, the swim-bladder is divided transversely into an anterior and a posterior chamber as seen in cyprinoids (Fig. 6.87K), Esox (Fig. 6.87J), Catostomus, Pangassius, Corvina, etc. But the longitudinal division of the swim-bladder is rare.

In Arius the swim-bladder is splitted longitudinally. In Notopterus, a longitudinal septum divides the swim-bladder into two lateral chambers. Due to the presence of septum or septa, the internal cavity of the swim-bladder is either completely or partially divided.

Weberian Ossicles:

The perilymphatic sac and the anterior end of the swim-bladder are connected by a series of four ossicles (Fig. 6.89), which are articulated as a conducting chain.

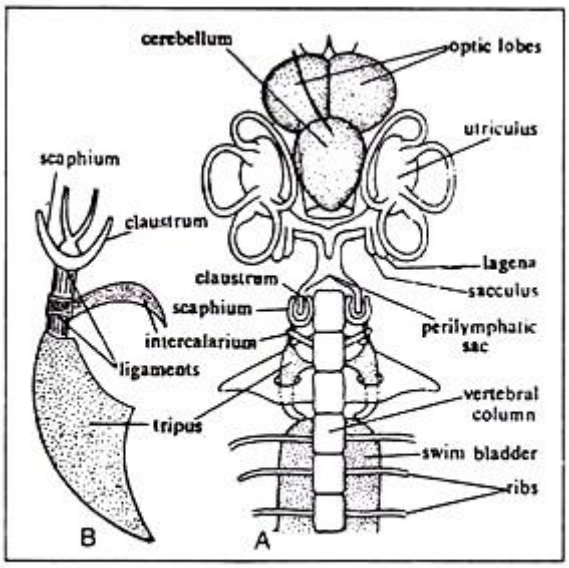


Fig.6.89 : A. Weberian ossicles and their relation with other structures in *Catastomus*. B. Showing the different parts of Weberian ossicles.

Of the four, the tripus, intercalarium and scaphium actually form the chain, while the fourth one, claustrum lies dorsal to the scaphium and lies in the wall of posterior prolongation of the perilymphatic sac. The function of these ossicles is controversial.

It is regarded that the Weberian ossicles either help to intensify sound vibrations and convey these waves to the internal ear of help to understand the state of tension of air pressure in the bladder and transit changes of such pressure to the perilymph to set up a reflex action. There are various views regarding the actual process of derivation of these ossicles.De Beer (1937) and Watson (1939) regarded that these are detached or modified processes of the first three anterior vertebrae. As regards the actual mode of origin of the four ossicles there are differences of opinion.The claustrum is regarded to be modified interspinous ossicle or modified spine of first vertebra or modified neural arch of first vertebra or modified intercalated cartilage or modified neural process of first cartilage. The scaphium is considered to be the modified neural arch of the first vertebra or modified rib of the first vertebra or derived from the neural arch of the first vertebra and also from the mesenchyme.

The intercalarium is derived from the neural arch and transverse process of the second vertebra or from the neural arch of the second vertebra and also from the ossified ligament or from the neural arch of the second vertebra only. The tripus is formed from the rib of the third vertebra and the ossified ligament or from the transverse process of the third vertebra along with ossified wall of the swim-bladder or from the transverse process of the third vertebra and the ribs of third and fourth vertebrae.

Functions of Swim-Bladder:

The swim-bladder in fishes performs a variety of functions.

Hydrostatic Organ:

It is primarily a hydrostatic organ and helps to keep the weight of the body equal to the volume of the water, the fish displaces. It also serves to equilibrate the body in relation to the surrounding medium by increasing or decreasing the volume of gas content.

In the physostomous fishes the expulsion of the gas from the swim-bladder is caused by way of the ductus pneumaticus, but in the physoclistous fishes where the ductus pneumaticus is absent the superfluous gas is removed by diffusion.

Swim-Bladder acts as Adjustable Float:

The swim-bladder also acts as an adjustable float to enable the fishes to swim at any depth with the least effort. When a fish likes to sink, the specific gravity of the body is increased. When it ascends the swim-bladder is distended and the specific gravity is diminished. By such adjustment, a fish can maintain equilibrium at any level.

Swim-Bladder Maintains Proper Centre of Gravity:

The swim bladder helps to maintain the proper centre of gravity by shifting the contained gas from one part of it to the other and this facilitates in exhibiting a variety of movement.

Swim-Bladder helps in Respiration:

The respiratory function of the swim-bladder is quite significant. In many fishes living in water in which oxygen content is considerably low, the oxygen produced in the bladder may serve as a source of oxygen. In a few fishes, specially in the dipnoans, the swim bladder becomes modified into the ‘lung’. The ‘lung’ is capable of taking atmospheric air.

Swim-Bladder as Resonator:

The swim-bladder is regarded to act as a resonator. It intensifies the vibrations of sound and transmits these to the ear through the Weberian ossicles.

Production of sound:

The swim-bladder helps in the production of sound. Many fishes, *Doras, Platystoma, Malapterurus, Trigla* can produce grunting or hissing or drumming sound. The circulation of the contained air inside the swim-bladder causes the vibration of the incomplete septa.The sound is produced as the consequence of vibration of the

incomplete septa present on the inner wall of the swim-bladder. The vibrations are caused by the movement of the contained air of the swim-bladder.

Sound may also be produced by the compression of the extrinsic and intrinsic musculature of the swim-bladder. *Polypterus, Protopterus* and *Lepidosiren* can produce sound by compression and forceful expulsion of the contained gas in the swimbladder. In Cynoscion male, the musculus sonorificus probably helps in compression.

Probable Questions:

1. How swim bladder is developed?
2. Discuss structure of swim bladder.
3. Discuss different types of swim bladder.
4. Discuss different types of modifications of Physostomous Condition of swim bladder.
5. Discuss different types of modifications of Physoclistous Condition of swim bladder.
6. Discuss functions of swim bladder.
7. Discuss about weberin ossicles.

Suggested Readings:

A text book of Fish Biology and Fisheries (3rd edition) by- S.S. Khanna & H.R. Singh

Disclaimer :

The study materials of this book have been collected from books, various e- books, journals and other e-sources.

Post-Graduate Degree Programme (CBCS)

in

ZOOLOGY

(M.Sc. Programme)

SEMESTER-II

IMMUNOBIOLOGY, HUMAN GENETICS & BIOSTATISTICS

ZCORT-207

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

Kalyani, Nadia

West Bengal, India

CONTENT WRITER:

Sl. No.	Name of Content Writer	Designation	Unit
1.	Dr. Sudeshna Banerjee	Assistant Professor of Zoology, Directorate of Open and Distance Learning, KU	Unit I-X
2.	Dr. Subhabrata Ghosh	Assistant Professor of Zoology, Directorate of Open and Distance Learning, KU	Unit XI-XVII
3.	Dr. Subhankar Kumar Sarkar	Associate Professor, Dept. of Zoology, KU	Unit XVIII
4.	Dr. Maharaj Biswas	Assistant Professor, Dept. of Zoology, KU	Unit XIX-XX

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Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2020 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Manas Kumar Sanyal, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every member of PGBOS (DODL), University of Kalyani. Heartfelt thanks are also due to the Course Writers-faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and coordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

All the Self Learning Materials are self writing and collected from e-book, journals and websites.

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5	Dr. Subhabrata Ghosh, Assistant Professor of Zoology, DODL, University of Kalyani	Member
6	Dr. Sudeshna Banerjee, Assistant Professor of Zoology, DODL, University of Kalyani	Member
7	Director, DODL, University of Kalyani	Convener

HARD CORE THEORY PAPER (ZCORT- 207)

Group A (Immunobiology)				
Module	**Unit**	**Content**	**Credit**	**Page No.**
ZCORT–207 (Immunobiology)	I	Basic concepts of Immune System; Primary and Secondary Lymphoid Organs, Cells, Tissues and molecules of Immune System.	**3**	
	II	Innate immunity: Overview, features, epithelial barrier, neutrophils, macrophage functions, inflammation, NK cells, cross talk with adaptive immune system.		
	III	Humoral immune system: Structure and class switching of antibodies.		
	IV	B cell function, maturation and development.		
	V	Complement system and diseases.		
	VI	Antigen presentation: Concept of haptens, determinants, conditions of antigenicity, superantigen, Dendritic cell, MHC, role of APCs.		
	VII	Antigen Recognition: Antigen Receptor: T and B cell Receptor, Structure of Immunoglobulin and T-cell receptor		
	VIII	Antigen Receptor Diversity-Mechanism, Antigen Receptor Maturation and selection.		
	IX	Vaccination and immunization: natural and artificial immunization; active immunization, vaccines.		
	X	Immuno-techniques: Antigen-Antibody Reaction Analysis- Agglutination, Diffusion etc. Isolation and culture of Immune cells, Antigen-Antibody reaction-RIA, ELISA, Visualization of Immune reaction *In vivo* and *vitro*- Immunofluorescence, FISH, GISH,		

		immunohistochemistry.		
ZCORT–207 (Human Genetics and Biostatistics)	**Group B (Human Genetics and Biostatistics)**			
	XI	Immuno-techniques: Antigen-Antibody Reaction Analysis- Agglutination, Diffusion etc. Isolation and culture of Immune cells, Antigen-Antibody reaction-RIA, ELISA, Visualization of Immune reaction In vivo and vitro-Immunofluorescence, FISH, GISH, immunohistochemistry.	**3**	
	XII	Basic concept of human genetics: introduction to the structure of human genome; human genome and mapping.		
	XIII	Human karyotype; karyotype and nomenclature of metaphase chromosome bands.		
	XIV	Chromosome anomalies and Structural Variants.Human genetics and society: genetic testing; human rights; genetic counselling.		
	XV	Molecular Pathology: Loss of function, Gain of function; Mitochondrial disorders.		
	XVI	Genetic analysis of complex traits and disease. Quantitative genetics; variance; heritability and its measurement;inbreeding and cross breeding; QTL.		
	XVII	Measures of Central Tendency		
	XVIII	Measures of dispersion. Concept of Probability and significant test, Probability Distribution (Binomial, Poisson and normal).		
	XIX	Graphical representation of biological data: Box plot analysis, leaf and stem diagram.		
	XX	Test of Hypothesis, Students' t-test and z-test and their application. Analysis of Variance (ANOVA).		
	Total counseling session 18hrs.			

Group-A: IMMUNOBIOLOGY

UNIT I

Basic concepts of Immune System; Primary and Secondary Lymphoid Organs, Cells, Tissues and molecules of Immune System

Objective:

In this unit we will discuss about the basic concepts of immune system; primary and secondary lymphoid organs, cells, tissues and molecules of immune system.

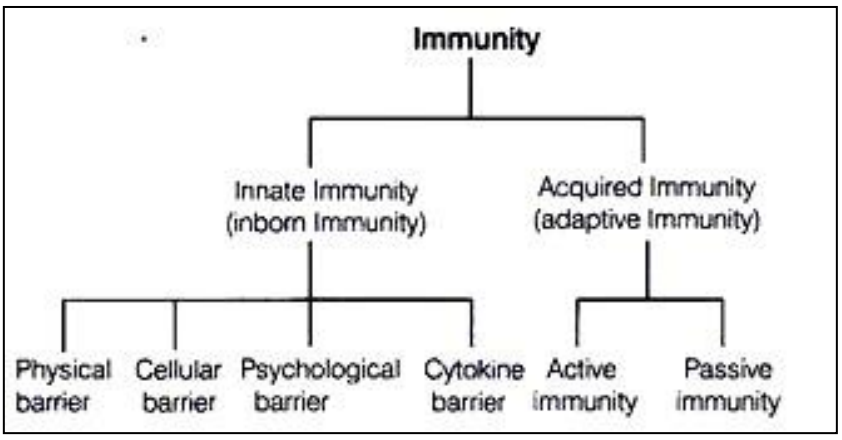
Introduction:

Immunology: The branch of life science which deals with immune reaction is known as immunology. Immunity is the ability of the body to protect against all types of foreign bodies like bacteria, virus, toxic substances, etc. which enter the body.

Any foreign protein, toxin of parasites, bacteria and viruses, when enter into the body, they interfere with host physiological processes and produce harmful effects. The "chemical defense" mechanism of host organism that operates against such effects of parasites and others is called immune reaction or immune response.

The immune system consists of a complex network of cells and molecules, and their interactions. It is specifically designed to eliminate infectious organisms from the body. This is possible since the organism is capable of distinguishing the self from non-self, and eliminates non-self.

Immunity is broadly divided into two types — innate (non-specific) immunity and adaptive or acquired (specific) immunity.



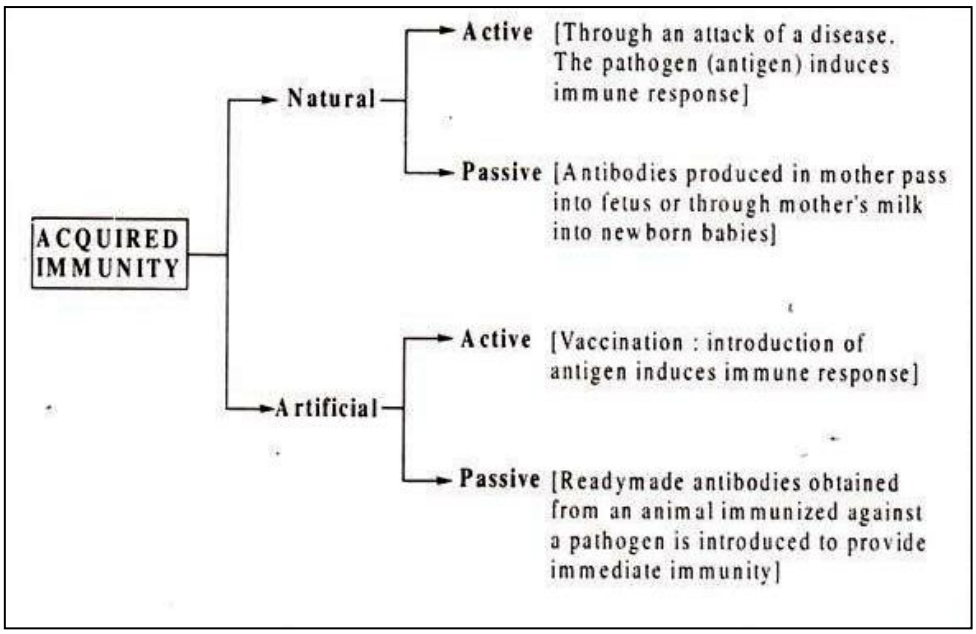


Fig 1: Different types of Acquired immunity

- **Innate Immunity:**

Innate immunity is non-specific, and represents the inherent capability of the organism to offer resistance against diseases. It consists of defensive barriers.

First line of defense:

The skin is the largest organ in the human body, constituting about 15% of the adult body weight. The skin provides mechanical barrier to prevent the entry of microorganisms and viruses. The acidic (pH 3-5) environment on the skin surface inhibits the growth of certain microorganisms. Further, the sweat contains an enzyme lysozyme that can destroy bacterial cell wall.

Second line of defense:

Despite the physical barriers, the microorganisms do enter the body. The body defends itself and eliminates the invading organisms by non-specific mechanisms such as sneezing and secretions of the mucus. In addition, the body also tries to kill the pathogens by phagocytosis (involving macrophages and complement system). The inflammatory response and fever response of the body also form a part of innate immunity.

(i) Physical Barrier:

Skin is the first line of mucous coating on defence. It prevents the entry of the pathogens of the body. Mucous coating on the epithelium lining the respiratory, gastrointestinal and urogenital tracts also help in trapping microbes.

(ii) Physiological Barrier:

Acid in the stomach, saliva in the mouth, tears from the eyes, etc., prevent the entry of microbes.

(iii) Cellular Barrier:

Special types of cells in our body, which kill the disease causing agents. Example are WBCs, Lymphocytes, Polymorpho Nuclear Leukocytes (PMNL—neutrophils, monocytes, macrophages, etc.

(iv) Cytokine Barrier:

Cells which are virus-infected, release types of protein called interferon's. Interferons protect the uninfected cells from further infection.

- ## Acquired Immunity:

It is pathogen specific and is not present from the birth and develops during an individual's lifetime.

This type of immunity is acquired after the birth, either by contracting the disease or by vaccination.

It has the following characters given below:

1. Specificity:

It has the ability to distinguish many different foreign molecules accordingly.

2. Memory:

It is a unique feature, which helps in producing an intensive response when the pathogen attacks the second time.

- **Types of Acquired Immunity:**

Acquired immunity can also be classified as:

(i) Active Immunity:

It is the immunity developed by the body, when it is exposed to the antigens. Antibodies are produced by the body in this case.

Introduction of pathogens or microbes either during immunisation or by any infection induce active immunity. It is slow but long lasting process and has no side effects.

Few examples of this immunity are as follows:

(a) Immunity developed by vaccination (artificial active immunity)

(b) Immunity developed during natural infection (natural active immunity)

(ii) Passive Immunity:

It occurs when antibodies are directly given into the body. It is used when the immune response has to be faster.

Some examples of passive immunity are:

(a) Antibodies received by foetus from mother through placenta. (Artificial passive immunity)

(b) Antibodies in the colostrum (IgA rich), i.e., yellowish fluid secreted by mother during the initial days of lactation. (Natural passive immunity)

(c) It is fast but lasts only for few days.

▪ Physical (or Mechanical) Barriers:

Physical (or mechanical) barriers of the host in cooperation with chemical barriers (secretions) act as the first line of defence against pathogenic microorganisms and foreign materials. These barriers include skin, mucous membranes, respiratory system, gastrointestinal tract, genitourinary tract, eye, bacteriocins, and beta-lysin and other polypeptides.

Skin, mucous membranes, respiratory system, gastrointestinal tract, genitourinary tract, and eyes are the barriers that provide both physical and chemical defence (e.g., gastric juices, lysozyme, lactoferrin, glycoproteins, urea etc.) in cooperation. In addition, bacteriocins and beta-lysin and other polypeptides are the defensive chemicals against microorganisms.

1. Skin:

Intact skin is a very effective physical or mechanical barrier to block the entry of microbial pathogens into the body. With few exceptions the microorganisms fail to penetrate the skin because its outer layer consists of thick, closely packed cells called keratinocytes that produce keratins.

Keratins are scleroproteins comprising the main components of hair, nails, and outer skin cells. These scleroproteins are not easily degradable enzymatically by microorganisms. They resist the entry of microbe-containing water and thus function as physical barrier to microorganisms.

In addition to direct prevention of penetration, continuous shedding of the outer epithelial cells of skin removes many of those microbial pathogens that manage to adhere on the surface of the skin.

2. Mucous membranes:

Mucous membranes of various body systems such as respiratory, gastrointestinal, genitourinary, and eye prevent invasion by microorganisms with the help of their intact

stratified squamous epithelium and mucous secretions, which form a protective covering that resists penetration and traps many microorganisms.

3. Respiratory system:

An average person inhales about 10,000 microorganisms per day usually at the rate of eight microorganisms per minute. These microorganisms are deposited on the moist, sticky mucosal surfaces of the respiratory tract. The mucociliary blanket of the respiratory epithelium traps the microorganism less than 10 μm in diameter and transports them by ciliary action away from the lungs.

Microorganisms larger than 10 μm normally are trapped by hairs and cilia lining the nasal cavity which beat towards the pharynx so that the mucus with its trapped microorganisms is moved towards the mouth and expelled. Coughing and sneezing also help removal of microorganisms from the respiratory tract.

They make clear the respiratory system of microorganisms by expelling air forcefully from the lungs through the mouth and nose, respectively. Salivation also washes microorganisms from the mouth and nasopharyngeal areas into the stomach.

4. Gastrointestinal system:

Microorganisms may manage to reach the stomach. Many of them are destroyed by the gastric juice of the stomach. The gastric juice is a mixture of hydrochloric acid, proteolytic enzymes, and mucus, and is very acidic with a pH 2 to 3. This juice is normally sufficient to kill most microorganisms and destroy their toxins.

Furthermore, the normal microbial population of the large intestine is extremely significant in not allowing the establishment of pathogenic microorganisms in it.

For convenience, many commensalistic microorganisms in the intestinal tract secrete metabolic products (e.g., fatty acids) that prevent “unwanted” microorganisms from becoming established in the tract. In small intestine, however, the microbial pathogens are often killed by various pancreatic enzymes, bile, and enzymes in intestinal secretions.

5. Genitourinary system:

Kidneys, ureters, and urinary bladder are sterile under normal conditions. Kidney medulla is so hypertonic that it allows only few microorganisms to survive.

Urine destroys some microorganisms due to its low pH and the presence of urea and other metabolic end-products like uric acid, hippuric acid, mucin, fatty acids, enzymes, etc. The lower urinary tract is flushed with urine eliminating potential microbial pathogens. The acidic environment (pH 3 to 5) of vagina also confers defence as it is unfavourable to most microorganisms to establish.

6. Eye:

The conjunctiva of eye lines the interior surface of each eyelid and the exposed surface of the eyeball. It is a specialised mucus-secreting epithelial membrane and is kept moist

by continuous flushing action of tears secreted by the lacrimal glands. Tears contain lysozyme and lactoferrin and thus act as physical as well as chemical barriers.

7. Bacteriocins:

The surfaces of skin and mucous membranes are inhabited by normal microbial flora. Of this, many bacteria synthesize and release toxic proteins (e.g., colicin, staphylococcin) under the direction of their plasmids. These toxic proteins are called bacteriocins, which kill other related species thus provide an adaptive advantage against other bacteria.

Inflammation (Inflammatory Response):

Inflammation (L. inflammatio = to set on fire) is an innate (nonspecific) defence response of the body to pathogenic infection or tissue injury and helps localizing the infection or injury in its local area. Many of the classic features of inflammation were described as early as 1600 BC in Egyptian papyrus writings.

In the first century AD, the Roman physician Celsus described the four cardinal signs of inflammation as redness (rubor), swelling (tumor), heat (color) and pain (dolor). In the second century AD, another physician, Galen added a fifth sign: altered function (functio laesa).

▪ Organization of Immune System:

The immune system consists of a network of diverse organs and tissue which vary structurally as well as functionally from each other (Fig. 2). These organs remain spreaded throughout the body. Basically, immune system is a complex network of lymphoid organs, tissues and cells.

The immune system consists of several organs distributed throughout the body (Fig. 2).

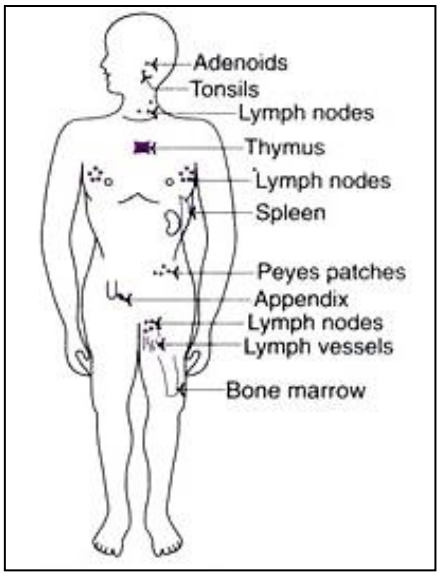


Fig 2 : A diagrammatic representation of human lymphatic system

- ## The Organs of the Immune System:

The immune system is made of the primary lymphoid and the secondary lymphoid organs. The primary lymphoid includes the bone marrow and the thymus, while the others such as the spleen, Peyer's patches of small intestine and the lymph nodes are included in the second category.

These lymphoid organs are categorized as primary and secondary.

1. Primary lymphoid organs:

The primary lymphoid organs are those organs where T lymphocytes and B lymphocytes, mature and acquire their antigen-specific receptors. After maturation, the lymphocytes migrate to secondary lymphoid organs. Primary lymphoid organs include bone marrow and thymus.

(i) Bone marrow:

Bone marrow is the main lymphoid organ where all blood cells including lymphocytes are formed. Maturation of B-lymphocytes occurs here.

(ii) Thymus:

Thymus is the site of T lymphocyte maturation. Thymus is situated near the heart. Thymus is quite large in size at the time of birth but keeps reducing with age. As stated earlier, T-lymphocytes and B-lymphocytes are responsible for cellular and humoral immune response respectively.

2. Secondary lymphoid organs:

After maturation, B lymphocytes and T lymphocytes migrate via blood vascular and lymphatic system to the secondary lymphoid organs where they undergo proliferation and differentiation. The acquired immune response to antigens usually develops in these organs and become effector cells.

In the secondary lymphoid tissues, the lymphocytes do not remain, and move from one lymphoid organ to another through blood and lymph. The secondary lymphoid organs are lymph nodes, spleen, tonsils, Peyer's patches of the small intestine and mucosal associated lymphoid tissues (MALT).

(i) Lymph nodes:

These are small solid structures found at intervals along the lymphatic system. They are composed of lymphoid tissue and act as filters for the lymph, preventing foreign particles from entering the bloodstream. Lymph nodes also produce lymphocytes and plasma cells.

(ii) Spleen:

It is a bean shaped organ which is the largest single mass of lymphoid tissue in the body. In foetus the spleen produces all types of blood cells but in adult it only produces lymphocytes. Macrophages of spleen are phagocytic.

(iii) Tonsils:

Usually there are six tonsils. They act as filters to protect body from bacteria and aid in the formation of white blood cells.

(iv) Peyer's patches:

These are clusters of lymph nodules found in small intestine, especially along the ileum. They produce lymphocytes.

(v) Mucosal-Associated Lymphoid Tissues (MALT):

MALT is significant aggregations of lymphoid tissues which are seen in relation to the mucosa of the major tracts like respiratory, alimentary canal and urinogenital tracts. It constitutes about 50 percent of the lymphoid tissue in human body.

They do not serve as filters of lymph. Larger aggregations extend into the submucosa. However, they are centres of lymphocyte production. Apart from B-lymphocytes and T-lymphocytes, phagocytic macrophages and dendritic cells are present.

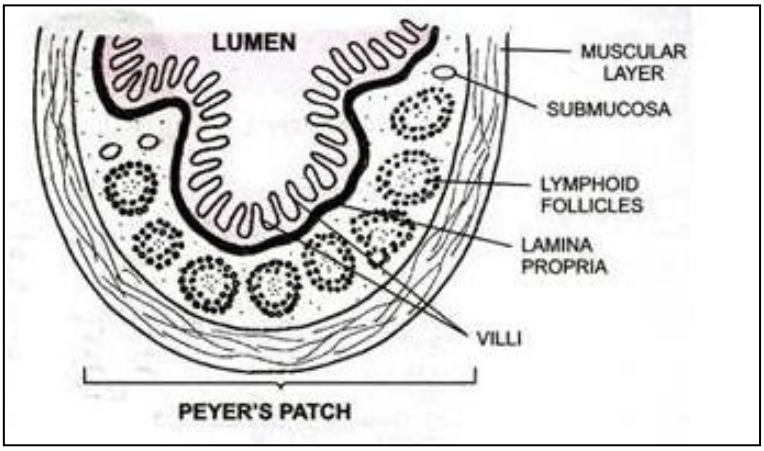


Fig 3: Diagram showing Peyer's patch in the submucosa of small intestine

- **Cells of the Immune System:**

Leukocytes (white blood cells) are immune system cells involved in defending the body against infectious disease and foreign materials. Five different types of leukocytes exist, all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. The innate leukocytes include the phagocytes, mast cells, eosinophils, basophils, and natural killer cells. These cells identify and eliminate pathogens and are important mediators in the activation of the adaptive immune system.

Neutrophils and macrophages are phagocytes that travel throughout the body in pursuit of invading pathogens. Neutrophils are normally found in the bloodstream and are the most abundant type of phagocyte. During the acute phase of inflammation neutrophils

migrate toward the site of inflammation and are usually the first cells to arrive at the scene of infection. Macrophages reside within tissues and produce a wide array of chemicals. They also act as scavengers, ridding the body of worn-out cells and other debris, and as antigen-presenting cells that activate the adaptive immune system. Dendritic cells are phagocytes in tissues that are in contact with the external environment, and are located mainly in the skin, nose, lungs, stomach, and intestines. These cells serve as a link between the bodily tissues and the innate and adaptive immune systems, as they present antigen to T-cells, one of the key cell types of the adaptive immune system.

Mast cells reside in connective tissues and mucous membranes, and regulate the inflammatory response. They are most often associated with allergy and anaphylaxis.

Basophils and eosinophils are related to neutrophils. They secrete chemical mediators that are involved in defending against parasites, and play a role in allergic reactions, such as asthma.

Natural killer cells are leukocytes that attack and destroy tumor cells, or cells that have been infected by viruses.

The cells of the adaptive immune system are special types of leukocytes, called lymphocytes. B cells and T cells are the major types of lymphocytes and are derived from hematopoietic stem cells in the bone marrow.

Here we will discuss seven important cells of immune system. The cells are: 1. Hematopoietic Stem Cell 2. Lymphocytes 3. Monocytes 4. Macrophages 5. Granulocytes 6. Dendritic Cells 7. Mast Cells.

Cell # 1. Hematopoietic Stem Cell:

All blood cells arise from a type of cell called hematopoietic stem cell (HSC) (or stem cell). The stem cells are self-renewing, maintain their population by cell division, and differentiate into other cell types. This process of formation and development of blood cells (red and white blood cells) is called hematopoiesis.

It is remarkable that every functionally specialised, mature blood cell is derived from the same type of hematopoietic stem cell. In contrast to a unipotent cell, which differentiates into a single cell type, a hematopoietic stem cell is multi-potent or pluripotent as it is able to differentiate in various ways and thereby gives rise to various type of blood cells.

In humans, the formation and development of blood cells begins in the embryonic yolk sac during the first weeks of development. The hematopoietic stem cells differentiate into primitive erythroid cells that contain embryonic haemoglobin. In the third month of gestation, hematopoietic stem cells migrate from the yolk sac to the foetal liver and then to the spleen.

Liver and spleen play major roles in hematopoiesis from the third to the seventh months of gestation. In later months, hematopoietic stem cells differentiate in the bone marrow and play major role in hematopoiesis, and by birth there is little or no hematopoiesis in the liver and spleen.

Multi-potent hematopoietic stem cell (or stem cell) in the bone marrow differentiates to form two lineages:

(1) Common-lymphoid progenitor cell and

(2) Common myeloid progenitor cell (Fig. 4).

The progenitor cells, unlike hematopoietic stem cell that is self-renewing, loss the capacity for self-renewal, and are committed to their specific cell linkage.

The common lymphoid progenitor cells give rise to B-lymphocytes (B-cells) that differentiate into antibody secreting plasma cells. T-lymphocytes (T-cells) that become activated T-cells. natural killer (NK) cells, and some dentritic cells.

The common myeloid progenitor cells give rise to erythroblasts that produce erythrocytes (red blood cells), megakaryoblasts that produce platelets (thrombocytes), myeloblasts that produce granulocytes (eosinophils, basophils, neutrophils), monoblasts that differentiate into monocytes which give rise to macrophages and dendritic cells, and an unknown precursor that produces mast cells.

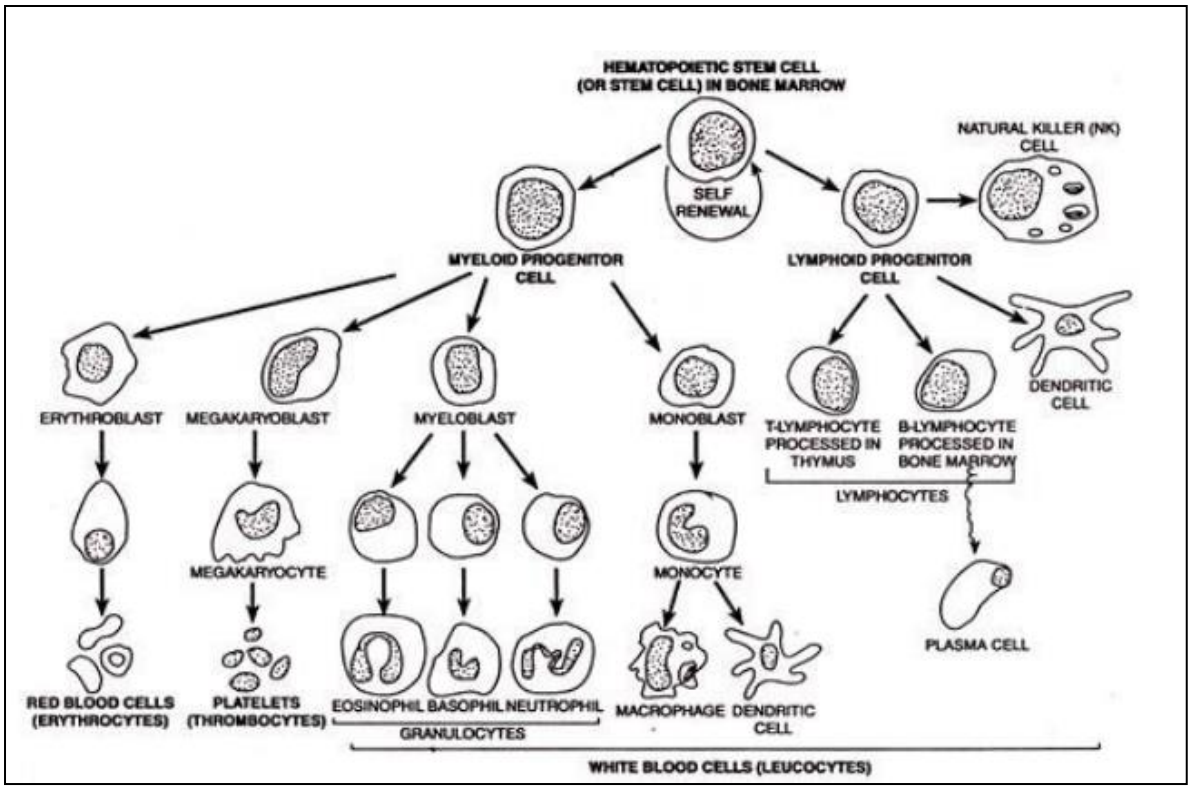


Fig: Haematopoiesis

However, B-lymphocytes (B-cells) T-lymphocytes (T-cells) and natural killer (NK) cells produced by lymphoid progenitor cell lineage and eosinophils, basophils, neutrophils,

macrophages, and dendritic cells produced by myeloid progenitor cell lineage are collectively called white blood cells or leucocytes (Gk. leucos = white, kytos = cell). White blood cells or leucocytes are the cells that are responsible for nonspecific and specific immunity in the body.

Cell # 2. Lymphocytes:

Lymphocytes (L. lympha = water, cyte = cell) are the most important effector cells of many cells involved in specific immune response. These cells are small, round and 5-15 μm in diameter. They are found in peripheral blood, lymph, lymphoid organs, and in many other tissues. Lymphocytes constitute 20% – 40% of the white blood cell (leucocyte) population in the body and 99% of the cells in the lymph.

They may be small (5-8 μm), medium (8-12 μm). and large (12-15 μm). The small lymphocytes are more numerous and may be short-lived with a life-span about two weeks or long-lived with a life-span of three years or more or even for life.

Short-lived lymphocytes act as effector cells in immune response, while long-lived ones function as memory cells. Long-lived lymphocytes are mainly thymus derived. The formation and development of lymphocytes, i.e.. lymphopoiesis takes place in bone marrow, primary or central lymphoid organs, and secondary or peripheral lymphoid organs.

Lymphocytes are approximately 10^{11} in number in a human body; their number ranges from 10^{10} to 10^{12} depending on body size and age. Lymphocytes can be broadly subdivided into three populations: B-lymphocytes or B-cells, T-lymphocytes or T-cells, and null cells (natural killer cells or NK cells are included in this group).

1. B-Lymphocytes or B-Cells:

B-lymphocytes or B-cells derive their name from their site of maturation. They are so named since they were first detected in the bursa of Fabricius of birds and later from bone marrow of a number of mammalian species, including humans and mice. In birds, the multi-potent hematopoietic stem cells originating in the bone marrow migrate to the bursa of Fabricius and differentiate their into antibody synthesizing cells.

The bursa is a small pouch-like organ in the embryonic hind-gut of birds and is absent in mammals. In a number of mammalian species including humans and mice, the B-cells originate in the foetal lever and later migrate to the bone marrow which becomes the site for production of B-cells after embryonic life.

B-lymphocytes do not have the ability to synthesize antibody molecule during undifferentiated stage. During differentiation, each lymphocyte acquires the ability to synthesize antibody molecules when provoked by antigens.

2. T-Lymphocytes or T-Cells:

T-Lymphocytes or T-cells derive their name from their site of maturation in the thymus. They are major players in the cell-mediated immune response and also have an important role in B-cell activation. T-cells themselves do not secrete antibodies (immunoglobulin) like B-cells.

They are immunologically specific and are directly involved in cell-mediated immune responses, can carry a vast repertoire of immunologic memory, and can function in a variety of effector and regulatory way.

Cell # 3. Monocytes:

Monocytes (G. monos = single; cyte = cell) are mononuclear phagocytic leucocytes possessing an oval or kidney-shaped nucleus and granules in the cytoplasm that stain grey-blue (Fig. 5).

Monocytes are produced in bone marrow. During hematopoiesis in bone marrow, granulocyte-monocyte progenitor cells differentiate into pro-monocytes, which-leave the bone marrow and enter the blood where they further differentiate into mature monocytes.

Mature monocytes circulate in the blood stream for about eight hours, enlarge in size, migrate into the tissues, and differentiate into specific tissue macrophages or into myeloid dendritic cells.

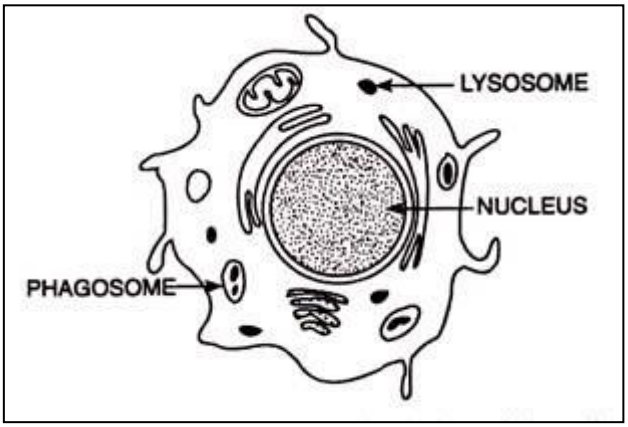


Fig 5: Diagrammatic sketch of typical morphology of a monocyte

Cell # 4. Macrophages:

Macrophages (G. macros = large; phagein = to eat), as noted above, are differentiated from monocytes into the tissues of the body.

Differentiation of a monocyte into a tissue macrophage (Fig. 6) involves a number of changes:

(i) The monocyte enlarges five- to ten-fold,

(ii) Its intracellular organelles increase in both number (especially lysosomes and phagolysosomes) and complexity,

(iii) The cell acquires increased phagocytic ability,

(iv) Produces higher levels of hydrolytic enzymes,

(v) Begins to secrete a variety of soluble factors, and

(vi) Develops ruffles or microvilli on the surface of its plasma membrane.

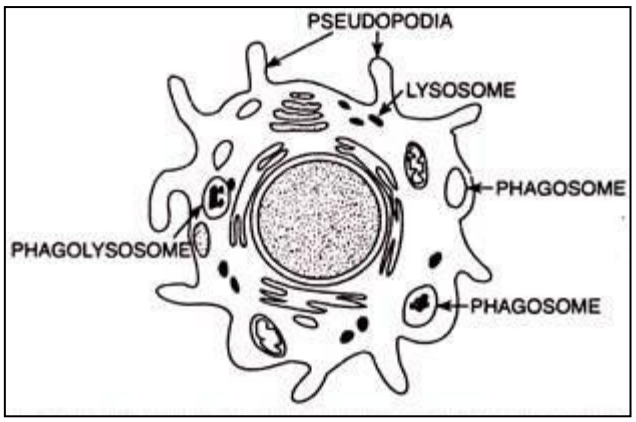


Fig 6: Diagrammatic sketch of typical morphology of a macrophage which are 5 to 10 fold larger than monocytes and contain more organelles especially lysosomes and phagolysosomes

Macrophages are transported throughout the body. Some macrophages reside in particular tissues and become fix macrophages. Others remain motile by amoeboid movement throughout the body and are called free or wondering macrophages.

Macrophages serve different functions i different tissues and are named according to their tissue location, e.g., histiocytes in connective tissues, osteoclasts in bone, microglial cells in the brain, alveolar macrophages in the lung, kupffer cells in the liver, and mesangial cells in the kidney.

Macrophages normally remain in a resting state and are activated for effective functioning. They are activated by a variety of stimuli such as interferon gamma (IFN-γ) secreted by activated T helper (T_H) cells, mediators of inflammatory response, components of bacterial cell walls, etc.

Activated macrophages secrete different types of cytotoxic proteins that help them eliminate large number of pathogens including vims-infected cells, malignant cells, and intracellular bacteria.

Activated macrophages also display class II MHC molecules that allow them to act more effectively as antigen-presenting cells (APCs). Thus, macrophages and T helper (T_H) cells facilitate each other's activation during the immune response.

Macrophages are highly phagocytic and they are capable of ingesting and digesting exogenous antigens (e.g., whole microorganisms and insoluble particles) and exogenous matter (e.g., injured or dead host cells, cellular debris, activated clotting factors).

Cell # 5. Granulocytes:

Granulocytes (Fig. 7) are those white blood cells (leucocytes) which have irregular-shaped nuclei with two to five lobes and granulated cytoplasmic matrix.

Granules of cytoplasmic matrix contain reactive substances that kill microorganisms and enhance inflammation. Granulocytes are also called polymorphonuclear leucocytes (PMNs). Three types of granulocytes are recognised in the body and they are: basophils, eosinophils, and neutrophils.

1. Basophils:

Basophils (G. basis = base; philein = to love) possess bi-lobed irregular-shaped nucleus and cytoplasmic matrix granules that stain bluish-black with basic dyes (e.g., methylene blue). These granulocytes are non-phagocytic cell that function by releasing pharmacologically active substances (e.g., histamine, prostaglandins, serotonin, and leucotrienes) from their cytoplasmic granules upon appropriate stimulation.

Since these pharmacologically active substances influence the tone and diameter of blood vessels, they are collectively termed vasoactive mediators. Basophils possess high-affinity receptors for immunoglobulin-E (IgE) antibody and thereby become coated with these antibodies.

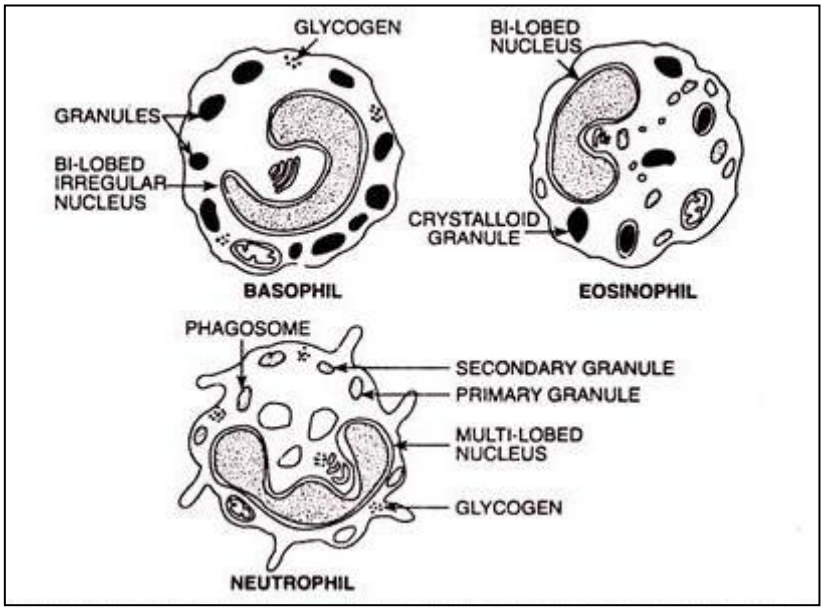


Fig 7: Diagrammatic sketch showing the morphology of granulocytes

Once coated, antigens trigger the basophil cells to secrete vasoactive mediators which are inflammatory and play a major role in certain allergic responses (e.g., eczema, hay

fever, and asthma). Basophils, however, comprise less than 1 % of white blood cells, are non-motile, and remain confined to the blood stream.

2. Eosinophils:

Eosinophils (G. eos = dawn; philein = to love) have a bi-lobed nucleus connected by a slender thread of chromatin and prominent acidophilic granules in cytoplasmic matrix. Eosinophils, like neutrophils, are motile cells that migrate from bloodstream into tissue spaces.

These granulocytes are considered to play a role in the defence against parasitic organisms (protozoans and helminth parasites) by phagocytosis.

They release mainly cationic proteins and reactive oxygen metabolites into the extracellular fluid. These substances damage the plasma membrane of the parasite. Eosinophils constitute only 3-5% of the white blood cells and their acidophilic granules stain red with acidic dyes.

3. Neutrophils:

Neutrophils (L. neuter – neither; philein = to love) possess a three- to five-lobed nucleus connected by slender threads of chromatin, and contain fine primary and secondary granules in cytoplasmic matrix. Neutrophils, like eosinophils, are motile cells that migrate from bloodstream into the tissue.

These granulocytes circulate in the bloodstream for 7 to 10 hours before their migration into the tissues where they enjoy a life span of only a few days. Approximately 60% of the circulating white blood cells (leucocytes) in human are the neutrophils. Like macrophages, the' primary function of neutrophils is phagocytosis of foreign or dead cells and pinnocytosis of pathological immune complexes.

Phagocytosis by neutrophils is similar to that operated by macrophages except that the lytic enzymes and bactericidal substances in neutrophils are contained within primary and secondary granules instead of lysosomes in macrophages. The primary granules are larger and denser and contain peroxidase, lysozyme, and various hydrolytic enzymes.

The secondary granules are smaller and contain collagenase, lactoferrin, and lysozyme. Both primary and secondary granules fuse with phagosome, whose contents are then digested and the remains excreted much as they are in macrophages.

Neutrophils, like macrophages, also use oxygen-dependent and oxygen-independent pathways to generate antimicrobial substances and defensins to kill ingested microorganisms. Neutrophils generate more reactive oxygen intermediates and reactive nitrogen intermediates and express higher levels of defensins than macrophages do.

Cell # 6. Dendritic Cells:

Dendritic cells constitute only 0.2% of WBCs (leucocytes) in the blood and are present in even smaller numbers in skin and mucous membranes of the nose, lungs, and

intestines. They derive their name due to long membrane extensions resembling the dendrites of nerve cells.

Dendritic cells arise from hematopoietic stem cells in the bone marrow via different pathways and in different locations (Fig. 8); they descend through both the myeloid and lymphoid lineages. Stem cell-originated dendritic cells are of four types: Langerhans cells, interstitial dendritic cells, myeloid dendritic cells, and lymphoid dendritic cells.

Despite differences, all the stem cell-originated mature dendritic cells perform the same major function of presenting antigen to T helper (T_H) cells by expressing high levels of both class II MHC molecules and members of the co-stimulatory B-7 family, and thereby play an important accessory role in the specific immune response.

This pattern of functioning makes dendritic cells more potent antigen-presenting cells (APCs) than macrophages and B-lymphocytes, both of which need to be activated before they can function as antigen-presenting cells (APCs).

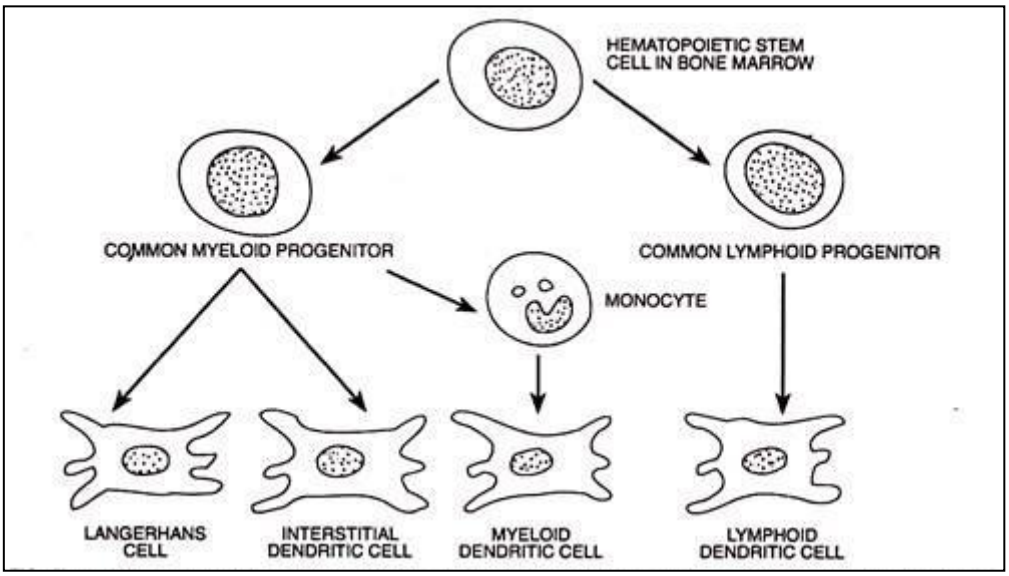


Fig 8: Different types of dendritic cells originated by haematopoietic stem cell in bone marrow. Dendritic cells arise from both the myeloid and lymphoid lineages.

In addition to dendritic cells originated in bone marrow, there are another type of dendritic cells, the follicular dendritic cells, that do not arise in bone marrow and perform their function in a different ways as they do not express class II MHC molecules and do not act as antigen-presenting cells (APCs).

Follicular dendritic cells express high levels of membrane receptors for antibody; which allows the binding of antibody complexes. The interaction of B-lymphocytes with this bound antigen can have important effects on B-lymphocyte responses.

Cell # 7. Mast Cells:

Mast cell precursors originate in the bone marrow and are released into the blood as undifferentiated cells. Mast cells are not differentiated from their precursors until the latter leave the blood and enter the tissues. Mast cells are found in a variety of tissues including the skin, connective tissues of various organs, and mucosal epithelial tissue of the respiratory, genitourinary, and digestive tracts.

These cells, like basophils, possess large numbers of granules in cytoplasmic matrix. The granules in cytoplasm contain histamine and other pharmacologically active substances that contribute to the inflammatory response. Mast cells, together with basophils, play an important role in the development of allergies and hypersensitivities.

- ## Molecules of immune system

1. Antibodies

Antibodies act as the antigen receptor on the surface of B cells and, in response to antigen, are subsequently secreted by plasma cells. Antibodies recognize specific configurations (epitopes, or antigenic determinants) on the surfaces of antigens (eg, proteins, polysaccharides, nucleic acids). Antibodies and antigens fit tightly together because their shape and other surface properties (eg, charge) are complementary. The same antibody molecules can cross-react with related antigens if their epitopes are similar enough to those of the original antigen.

2. Cytokines

Cytokines are a very important set of proteins in the body.Cytokines are polypeptides secreted by immune and other cells when the cell interacts with a specific antigen, with pathogen-associated molecules such as endotoxin, or with other cytokines. These small proteins serve as hormones for the immune system. They are produced in response to a threat and represent the communication network for the immune system. In some cases, cells of the immune system communicate by directly touching each other, but often cells communicate by secreting cytokines that can then act on other cells either locally or at a distance.

Main categories include

- Chemokines
- Hematopoietic colony-stimulating factors
- Interleukins (IL)
- Interferons (IFN-alpha, IFN-beta, IFN-gamma)
- Transforming growth factors (TGFs)
- Tumor necrosis factors (TNF-alpha, lymphotoxin-alpha, lymphotoxin-beta)

Although lymphocyte interaction with a specific antigen triggers cytokine secretion, cytokines themselves are not antigen-specific; thus, they bridge innate and acquired immunity and generally influence the magnitude of inflammatory or immune responses. They act sequentially, synergistically, or antagonistically. They may act in an autocrine or paracrine manner.

3. Complement

The complement system is composed of 30 blood proteins that function in an ordered fashion to defend against infection. Most proteins in the complement system are produced in the liver. Some of the proteins of the complement system coat germs to make them more easily taken up by neutrophils. Other complement components act to send out chemical signals to attract neutrophils to sites of infection. Complement proteins can also assemble on the surface of microorganisms forming a complex. This complex can then puncture the cell wall of the microorganism and destroy it.

Probable questions:

1. List the primary lymphoid organs and summarize their functions in the immune response.
2. List the secondary lymphoid organs and summarize their functions in the immune response.
3. What are the two primary characteristics that distinguish hematopoietic stem cells and progenitor cells?
4. What are the two primary roles of the thymus?
5. What effect would removal of the bursa of Fabricius (bursectomy) have on chickens?
6. Highlight the difference between innate and adaptive immunity.
7. What do you mean by hematopoiesis?
8. Classify the immune cells on the basis of origin.
9. What is antibody dependent cell mediated cytotoxicity?
10. Classify macrophages on the basis of location.
11. What is GALT and MALT?

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UNIT II

Innate immunity: Overview, features, epithelial barrier, neutrophils, macrophage functions, inflammation, NK cells, cross talk with adaptive immune system

Objective:

In this unit we will discuss about Innate immunity: Overview, features, epithelial barrier, neutrophils, macrophage functions, inflammation, NK cells, cross talk with adaptive immune system.

Introduction:

Immunity is the ability of the body to protect against all types of foreign bodies like bacteria, virus, toxic substances, etc. which enter the body.

Immunity is also called disease resistance. The lack of immunity is known as susceptibility.

- **Types of immunity:**

Broadly there are two types of immunity.

1. Innate or natural immunity
2. Acquired immunity

Innate or Natural immunity:

Innate immunity is antigen-nonspecific defense mechanisms that a host uses immediately or within several hours after exposure to almost any microbe. This is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection. Innate immunity can be divided into immediate innate immunity and early induced innate immunity.

Immediate innate immunity begins 0 - 4 hours after exposure to an infectious agent and involves the action of soluble preformed antimicrobial molecules that circulate in the blood, our found in extracellular tissue fluids, and are secreted by epithelial cells. These include: antimicrobial enzymes and peptides; complement system proteins; and anatomical barriers to infection, mechanical removal of microbes, and bacterial antagonism by normal flora bacteria. These preformed innate defense molecules will be discussed in greater detail later in this unit.

Early induced innate immunity begins 4 - 96 hours after exposure to an infectious agent and involves the recruitment of defense cells as a result of pathogen-associated molecular patterns or PAMPS binding to pattern-recognition receptors or PRRs. These

recruited defense cells include: phagocytic cells: leukocytes such as neutrophils, eosinophils, and monocytes; tissue phagocytic cells in the tissue such as macrophages; cells that release inflammatory mediators: inflammatory cells in the tissue such as macrophages and mast cells; leukocytes such as basophils and eosinophils; and natural killer cells (NK cells).

Examples of innate immunity include anatomical barriers, mechanical removal, bacterial antagonism, antigen-nonspecific defense chemicals, the complement pathways, phagocytosis, inflammation, fever, and the acute-phase response. In this current unit we will look at each of these in greater detail.

- Immunity with which an individual is born is called innate or natural immunity.
- Innate immunity is provided by various components such as Skin, mucus membrane, Phagocytic cells etc
- Innate immunity acts as first line of defense to particular microorganisms.

Mechanism of innate immunity:

1. Anatomical barrier
2. Physicochemical barrier
3. Phagocytic barrier or Phagocytosis
4. Inflammatory barrier or Inflammation

Anatomical barriers

Skin and mucus membrane are the examples of anatomical barriers that provides immunity.

Skin and mucus membrane:

- Skin consists of two distinct layer; a thin outer layer called epidermis and thick inner layer called dermis.
- Epidermis consists of mostly dead cell filled with keratin. Dermis is composed of connective tissue, hair follicle, sebaceous gland and sweat gland.
- Skin provides first line of defense by preventing entry of microorganisms. However skin may be penetrated by injury or insects.
- Below skin, the mucus membrane prevents the entry of microorganism to the body. And also it secrets mucus that entraps microorganisms.

Anatomical barriers provide immunity by following ways.

- At first skin and mucus membrane prevent entry of microorganism into host body by mechanical separation. For example, Skin surrounds the host body from external and mucus membrane surrounds the body tracts.

- They also have mechanism to kill the pathogen before entry to body. For example; lysozyme, acidic pH, sebum, high salt concentration in sweat are antimicrobial agents found in skin and mucus membrane.
- Skin and mucus membrane provides first line of defense against microorganism as they are first component to encounter with microorganism.

Physico-chemical barriers

- Physicochemical barrier includes physiological barrier and chemical barrier.
- **Physiological** conditions of body such as normal body temperature, normal body pH etc provides immunity.
- Some species are resistant to certain disease simply because of their higher body temperature. For example, mammals are susceptible to anthrax but birds are resistant to anthrax. It is because *Bacillus anthracis* are killed by higher body temperature of birds (39°C).
- Similarly, body pH also provides immunity. For example acidity of stomach kills most of the ingested bacteria and provides immunity. In infants stomach is less acidic. This is the reason why infants suffer more from gastrointestinal disturbance than adults.
- **Chemical barriers** include various antimicrobial chemicals found in body fluids. For examples, Lysozyme found in tear and mucus kills many Gram +ve bacteria.
- Interferon found in blood and lymph kills viruses. Other antimicrobial chemicals found in body fluids include complement protein, collectins, etc.

Phagocytosis or Phagocytic barrier of immune system (neutrophil macrophage functions)

- Phagocytosis is an important defense mechanism of host to provide immunity. Most of the bacteria that enter into host are killed by phagocytic cells such as Neutrophils, monocytes and macrophages.
- Phagocytosis is an example of endocytosis.
- There are two types of endocytosis; phagocytosis and pinocytosis.

Steps of phagocytosis

1. At first phagocyte approaches to the site of infection
2. Phagocyte extends pseudopodia around bacterial cell.
3. Pseudopodia gradually increase in size and finally fused so that bacteria are engulfed in the form of phagosome or food vacuole.
4. The phagosome and lysosome come nearer to each other and fuse to form phago-lysosome.

5. Inside phago-lysosome ingested bacteria is killed by hydrolytic and digestive enzyme of lysosome.
6. Required materials released from digested bacteria are absorbed into surrounding cytoplasm and undigested residues are excreted out by exocytosis.

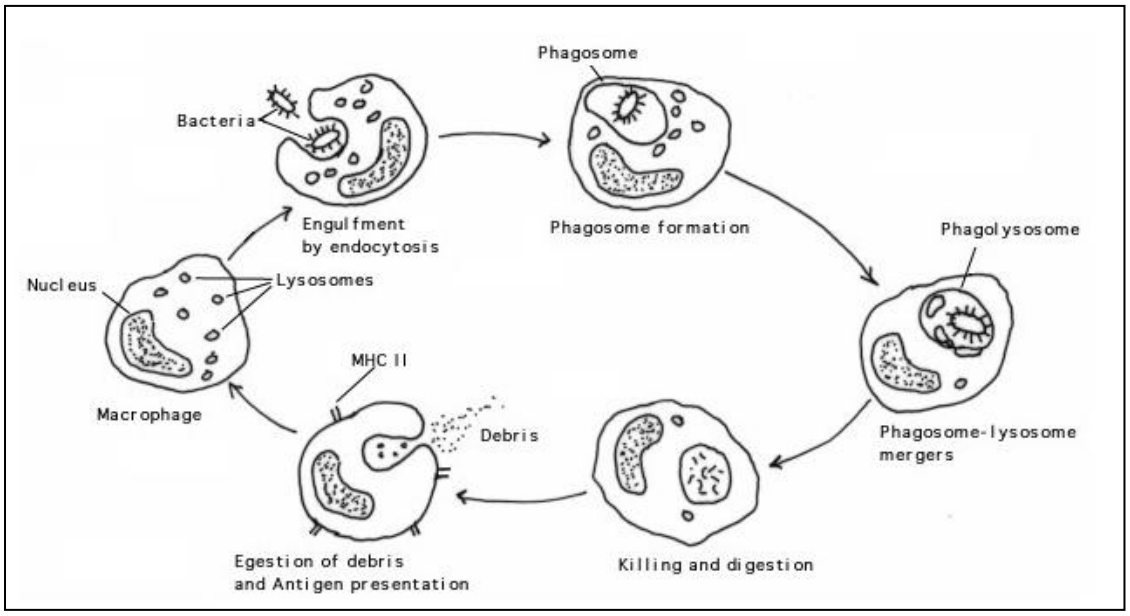

Fig: Steps in phagocytosis

Killing Mechanism of phagocytosis:

Killing of ingested bacteria during phagocytosis occur by two different mechanism

1. Oxygen dependent mechanism
2. Oxygen independent mechanism

1. Oxygen dependent mechanism:

- During phagocytosis, phagocytic cell increases uptake of O2. At the same time rateof pentose phosphate pathway increases to generate more NADPH.
- NADPH reduces molecular O2 to produce various toxic metabolic products such as Hydroxyl free radical, H2O2 and superoxide ions that kill ingested bacteria.
- This process is also known as respiratory burst.
- It is the major mechanism of killing of ingested bacteria during phagocytosis.

2. Oxygen independent mechanism:

- In this mechanism, ingested bacteria are killed by hydrolytic and digestive enzymes of lysozyme.

inflammation, a response triggered by damage to living tissues. The inflammatory response is a defense mechanism that evolved in higher organisms to protect them from infection and injury. Its purpose is to localize and eliminate the injurious agent and to remove damaged tissue components so that the body can begin to heal. The response consists of changes in blood flow, an increase in permeability of blood vessels, and the migration of fluid, proteins, and white blood cells (leukocytes) from the circulation to the site of tissue damage. An inflammatory response that lasts only a few days is called acute inflammation, while a response of longer duration is referred to as chronic inflammation.

Example of acute inflammation is; the response to tissue damage or **Example of** chronic inflammation is; Arthritis, cancer etc.

Main aim of inflammation is to prevent spread of injected microorganism or toxin from site of injection and kill them on spot by phagocytosis.

Characteristics of inflammation:

1. Rubor: redness
2. Tumor: swelling
3. Calor: heat
4. Dolor: pain
5. Functio laesa: loss of function

Steps of inflammatory response

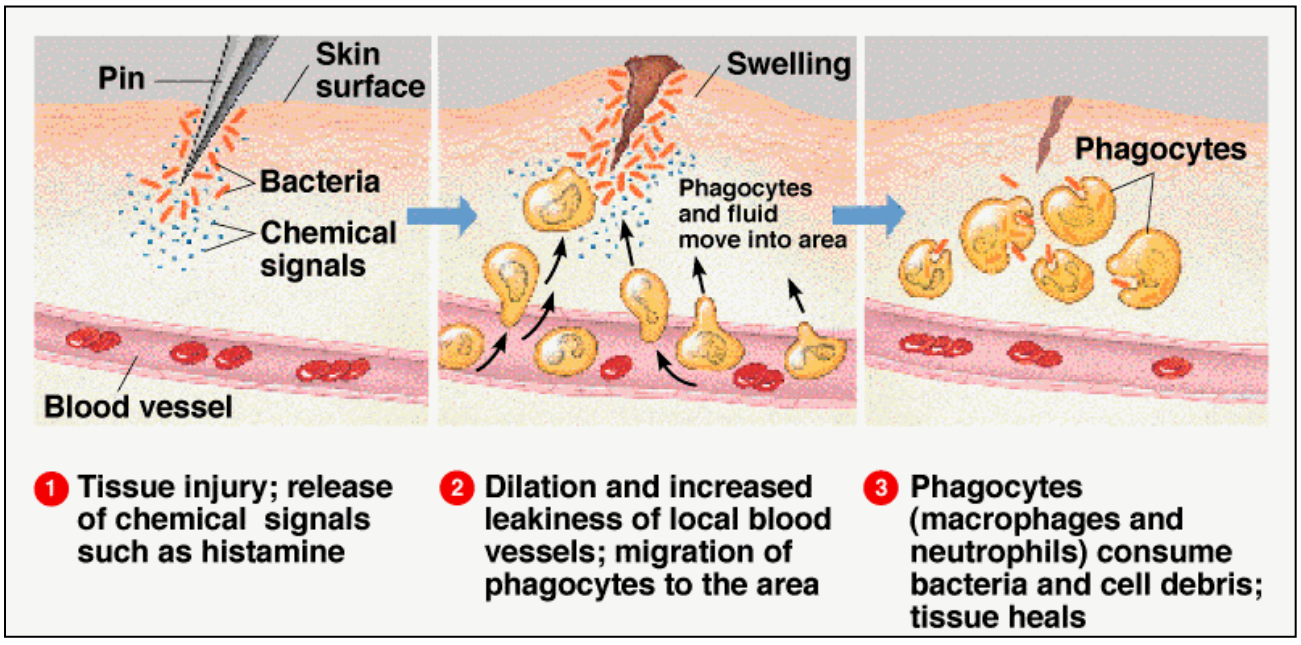

Fig: Inflammation process

Step I: Tissue damage and Release of histamine:

- Tissue damage caused by toxin, microorganism or mechanical injury release histamine.

Step II: Vasodilation:

- Histamine acts on surrounding blood capillaries and causes vasodilation.
- When vasodilation occurs, speed of blood flow decreases so that Neutrophils get chance to settle at the site of infection.

Step III: Increased permeability:

- At the same time histamine increases the permeability of blood capillaries leading to leakage of fluid from blood capillaries. This results in accumulation of fluid causing edema.

Step IV: Extravasation:

- Within few hours, Neutrophil migrates to the site of tissue damage by the process of chemotaxis and passes through capillaries wall and enter into tissue space by the process called extravasation.
- Extravasation completes in 4 steps:
- **Rolling:** neutrophils attach loosely to endothelium by low affinity interaction between glycoprotein-mucin of Neutrophil.
- **Activation of chemotactic stimus:** chemokines are secreted and Neutrophil are attracted.
- **Arrest and adhesion:** ICAMS and integrin stabilize adhesion of neutophil and endothelium
- **Transendothelial migration:** Neutrophil enter through endothelium layer.

Step V: Phagocytosis:

- Neutrophil kills the injected microorganism or toxins by phagocytosis and release molecular mediators that contributes to inflammatory response. At the same time activates effectors cells.

Step VI: Inflammatory response:

- As inflammatory response develops, various cytokines and other inflammatory mediators act on endothelium of local blood vessels, including increased expression of cell adhesion molecules (CAMs). The epithelium is then said to be inflamed.
- Neutrophils are the first cell types to bind to inflamed endothelium and extravasate into tissue.

- ## Natural Killer (NK) cells:

Natural killer (NK) cells are effector lymphocytes of the innate immune system that control several types of tumors and microbial infections by limiting their spread and subsequent tissue damage

The natural killer cell was first described in 1976, when it was shown that the body contains a small population of large, granular lymphocytes that display cytotoxic activity against a wide range of tumour cells in the absence of any previous immunization with the tumor. NK cells were subsequently shown to play an important role in host defense both against tumor cells and against cells infected with some, though not all, viruses. These cells, which constitute 5%–10% of lymphocytes in human peripheral blood, do not express the membrane molecules and receptors that distinguish T- and B-cell lineages. In some cases, an NK cell employs NK cell receptors to distinguish abnormalities, notably a reduction in the display of class I MHC molecules and the unusual profile of surface antigens displayed by some tumor cells and cells infected by some viruses. Another way in which NK cells recognize potential target cells depends upon the fact that some tumour cells and cells infected by certain viruses display antigens against which the immune system has made an antibody response, so that antitumor or antiviral antibodies are bound to their surfaces. Because NK cells express CD16, a membrane receptor for the carboxyl-terminal end of the IgG molecule, called the Fc region, they can attach to these antibodies and subsequently destroy the targeted cells. This is an example of a process known as **antibody-dependent cell mediated cytotoxicity (ADCC)**.

Several observations suggest that NK cells play an important role in host defence against tumours. For example, in humans the Chediak-Higashi syndrome—an autosomal recessive disorder—is associated with impairment in neutrophils, macrophages, and NK cells and an increased incidence of lymphomas. Likewise, mice with an autosomal mutation called beige lack NK cells; these mutants are more susceptible than normal mice to tumor growth following injection with live tumour cells.

There has been growing recognition of a cell type, the NK1-T cell, that has some of the characteristics of both T cells and NK cells. Like T cells, NK1-T cells have T cell receptors (TCRs).Unlike most T cells, the TCRs of NK1-T cells interact with MHC-like molecules called CD1 rather than with class I or class II MHC molecules. Like NK cells, they have variable levels of CD16 and other receptors typical of NK cells, and they can kill cells. A population of triggered NK1-T cells can rapidly secrete large amounts of the cytokines needed to support antibody production by B cells as well as inflammation and the development and expansion of cytotoxic T cells. Some immunologists view this cell type as a kind of rapid response system that has evolved to provide early help while conventional TH responses are still developing.

- **Difference between Innate and Adaptive Immunity**

S.N.	Characteristics	Innate Immunity	Adaptive immunity
1.	Presence	Innate immunity is something already present in the body.	Adaptive immunity is created in response to exposure to a foreign substance.
2.	Specificity	Non-Specific	Specific
3.	Response	Fights any foreign invader	Fight only specific infection
4.	Response	Rapid	Slow (1-2 weeks)
5.	Potency	Limited and Lower potency	High potency
6.	Time span	Once activated against a specific type of antigen, the immunity remains throughout the life.	The span of developed immunity can be lifelong or short.
7.	Inheritance	Innate type of immunity is generally inherited from parents and passed to offspring.	Adaptive immunity is not passed from the parents to offspring, hence it cannot be inherited.
8.	Memory	Cannot react with equal potency upon repeated exposure to the same pathogen.	Adaptive system can remember the specific pathogens which have encountered before.
9.	Presence	Present at birth	Develops during a person's lifetime and can be short-lived.
10.	Allergic Reaction	None	Immediate and Delay hypersensitivity
11.	Used Against	For microbes	Microbes and non-microbial substances called antigens
12.	Memory	No memory	Long term memory
13.	Diversity	Limited	High
14.	Speed	Faster response	Slower response
15.	Complement	Alternative and lectin	Classical pathway

	system activation	pathways	
16.	Anatomic and physiological barriers	Skin, Mucous membranes, Temp, pH, chemicals, etc.	Lymph nodes, spleen, mucosal associated lymphoid tissue.
17.	Composition	The innate immune system is composed of physical and chemical barriers, phagocytic leukocytes, dendritic cells, natural killer cells, and plasma proteins.	Adaptive immune system is composed of B cells and T cells.
18.	Development	Evolutionary, older and is found in both vertebrates and invertebrates.	Adaptive immunity system has been developed recently and is found only in the vertebrates.
19.	Example	White blood cells fighting bacteria, causing redness and swelling, when you have a cut.	Chickenpox vaccination so that we don't get chickenpox because adaptive immunity system has remembered the foreign body.

Probable questions:

1. Define innate immunity?
2. State the differences between innate and adptive immunity.
3. Describe the mechanism of innate immunity?
4. Briefly describe the three major events in the inflammatory response.
5. Innate and adaptive immunity act in cooperative and interdependent ways to protect thehost. Discuss the collaboration of these two forms of immunity.
6. What are the functions of NK cell?
7. Explain the role of macrophages in elimination of foreign particulates.
8. What do you mean by phagocytosis?

9. Which cells are considered as phagocytes?
10. Elaborate the phagocytosis process with diagram.

Suggested readings/ references:

5. Kindt T, Goldsby R, Osborne B, Kuby J, Kuby J. Kuby immunology. 2007. New York: W.H. Freeman.
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UNIT III

Humoral immune system: Structure and class switching of antibodies

Objective:

In this unit, we will discuss about the humoral immune system: Structure and class switching of antibodies.

Introduction:

Humoral immune response is also called B-cell mediated immunity because B-lymphocytes are involved in this response. Humoral immune response is to defend the body against pathogens that may invade body fluids or humor. B cells are antigen specific.

During immune response, B cells, specific for the antigen, enlarge to become lymphoblasts that further differentiate to form plasma cells. The mature plasma cells produce gamma globulins or immunoglobulins called antibodies at a rapid rate of about 2000 molecules per second for each plasma cell. The antibodies secreted into the lymph eventually enter the blood.

The antibody molecule is the basic functional unit of this type of immune response. So this immune response is also called antibody-mediated immune response. The flow chart summarizes the various steps involved in the humoral response

Humoral immune response

The immune system protects the body from potentially harmful substances by recognizing and responding to antigens. Antigens are foreign particles, normally large or small molecules on the surface of cells, viruses, fungi or bacteria. Some non-living substances such as toxins, chemicals, drugs and foreign particles can also be antigens. Substances that contain these antigens are recognized and destroyed by the immune system.

One of the most important immune response is humoral effector response. The effector functions in humoral immunity are mainly mediated by secreted antibodies. It protects body from extra-cellular pathogenic agents by combining with them to form antigen-antibody complex and gradually leads towards their elimination.

Humoral immunity combats extracellular bacteria, fungi and even obligate intra-cellular microbes e.g. viruses; before they infect their target T-cells. Any defect in humoral immunity results in increased susceptibility to infection with bacteria and fungi.

Ways involved in Humoral Immunity:

Humoral effector functions facilitate effective elimination of foreign pathogens from a host animal in a variety of ways.

Antibodies play vital role in elimination of antigenic agents:

(i) The antibody can bind to the surface epitopes of the antigen making it more susceptible to phagocytosis—known as opsonization.

(ii) The antibody molecule can bind to the antigen forming an antigen-antibody complex, which then combines with the complement in a step-wise manner to initiate and facilitate phagocytosis of the antigen.

(iii) The antibody can bind to toxin molecules elaborated by microbes making them nontoxic.

(iv) Antibodies can inactivate free virus particles by combining with the epitopes on viral particles to make them incapable of attachment to host cell membranes.

(v) Binding to potential pathogens at mucous membrane surfaces, preventing colonization.

(vi) Binding to Fc (fragment crystalized) receptors on NK cells or macrophages in antibody dependent cell mediated cytotoxicity (ADCC), confirming specificity for antigen.

Most defenses that are mediated by antibody present in the plasma, lymph and tissue fluids are called humoral immune responses. It protects against extra-cellular bacteria and foreign macromolecules. Transfer of antibodies confers this type of immunity on the recipient. Humoral immune responses have an activation phase and an effector phase.

Phases of humoral immune response occur as follows (Fig. 1):

1. The antigen is taken up by phagocytosis and degraded in a lysosome in an APC, such as a macrophage.

2. A T-cell receptor recognizes processed antigen bound to a class II MHC protein on the macrophage.

3. Cytokines released by the T_H cell and IL-1 released by macrophage stimulate the T_H cell to produce a clone of differentiated cells capable of interacting with B-cells.

Activation phase occurs in lymphatic tissue.

4. B-cells are also antigen presenting cells. Binding of antigen to a specific IgM receptor triggers receptor mediated endocytosis, degradation and display of the processed antigen on class II MHC proteins.

5. When a T_H cell receptor binds to the displayed antigen—MHC II complex on the B cell, it releases cytokines.

6. These cytokines cause the B-cell to produce a clone of B-cells.

7. Now, these B-cells produce antibody secreting plasma cells.

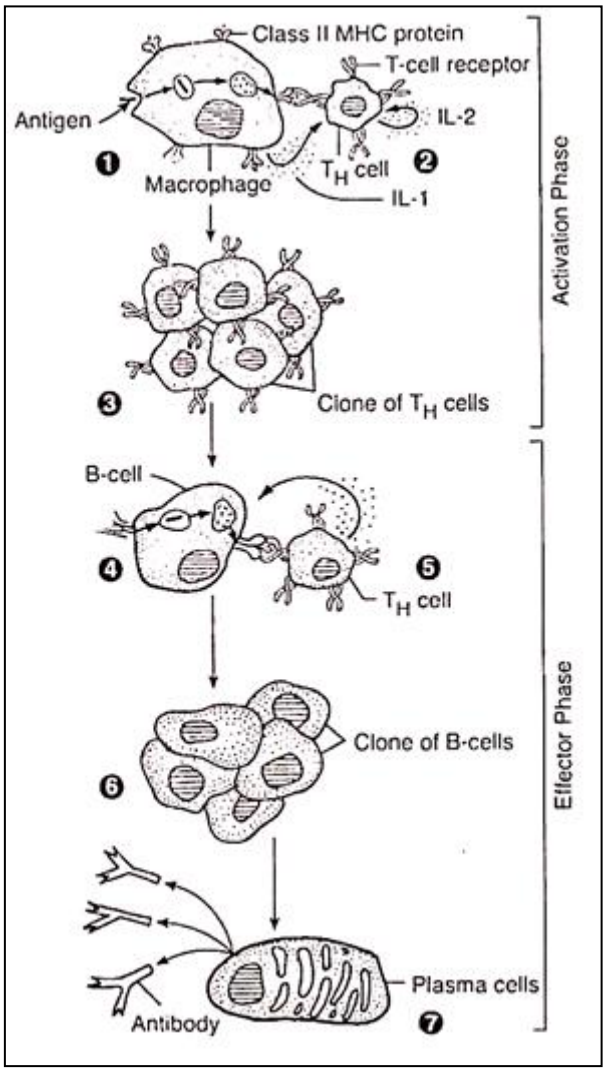

Fig 1: Humoral immune responses-generation of plasma cells

- **Definition of Antibody:**

Antibodies are immunoglobulin's (Igs) which are produced in the body in response to the antigen or foreign bodies. Thus all antibodies are immunoglobulin's but all immunoglobulin's are not antibodies.

- **Location and Formation of antibody:**

The antibodies may be bound to a cell membrane or they may remain free. Antibodies are produced by B lymphocytes and plasma cells. In fact B-lymphocytes get transformed into plasma cells. The mature plasma cell produces antibodies at an extremely rapid rate— about 2000 molecules per second. Antibodies direct the antibody- mediated immunity (=humoral immunity).

- **Types of Antibodies:**

There are five types of antibodies viz:

1. IgA (Ig α);
2. IgD (Ig δ);
3. IgE (Ig δ);
4. IgG (Ig δ) and
5. IgM (Ig μ).

Among the antibodies, IgG forms 80% of the antibodies in the body.

Antibody Structure:

IgG has been studied extensively and serves as a model of basic structural unit of all Igs. An antibody molecule consists of the following parts.

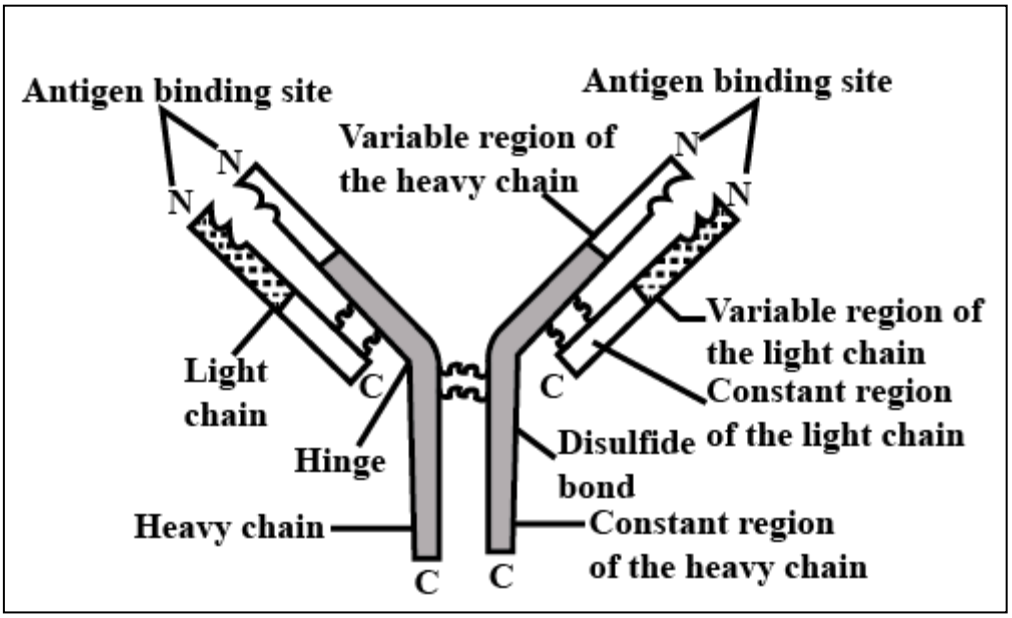

Fig 2: Structure of antibody

(i) Heavy and Light Chains:

An antibody molecule is made up of 4 peptide chains, two small called light chains and two longer called heavy chains. Hence an antibody is represented as H_2L_2. The heavy chain has larger number of amino acids while light chain has smaller number of amino acids. Heavy and light chains may be either lambda or Kappa type.

(ii) Constant and Variable Regions:

There are two different regions the constant region and variable region in each chain of the antibody.

(iii) Disulfide Bonds and Hinge Region:

A disulfide bond joins a light chain with a heavy chain. Two disulfide bonds also link the two heavy chains. This part of the antibody displays considerable flexibility and is called the hinge region. Because the antibody "arms" can move somewhat as the hinge region bends, an antibodycan assume a Y shaped molecule.

(iv) Fragment Antigen Binding (Fab) and Fragment Crystallisable (Fc):

Two identical fragments of Y-shaped molecule possess the antigen-binding sites and is thus named fragment-antigen binding (Fab). The antigen-binding sites bind to the specific antigens in a lock and key pattern, forming an antigen-antibody complex. The third fragment which lacks the ability to bind to antigen and can be crystallized, is, therefore, known as fragment crystallizable (Fc).

The stem of the Y-shaped antibody monomer is called the F_C region, so named because when antibody structure was first being identified, it was a fragment (F) that crystallized (c) in cold storage.

- **<u>Characteristics and Functions of Immunoglobulin's (Igs) or Antibodies:</u>**

Antibodies show the following characteristics and perform different functions.

(i) IgA:

It is the second most abundant class, constituting about 10 to 15 per cent of antibodies of serum. It is mainly found in sweat, tears, saliva, mucus, colostrum (first milk secreted by a mother) and gastrointestinal secretions.

Smaller quantities are present in blood and lymph. IgA has an extra polypeptide called a J- (joining) chain and extra protein known as secretory component. Levels decrease during stress, lowering resistance to infection. Provides localized protection in external secretions (tears, intestinal secretions, etc.) against bacteria and viruses. When IgA is excreted through faeces, it is called copro antibody.

(ii) IgD:

It is mainly found on the surfaces of B cells as antigen receptors, where it activates B cells for antigen recognition. It is about 0.2% of all antibodies in the blood.

(iii) IgE:

It is less than 0.1% of all antibodies in the blood; located on mast cells and basophils releasing histamine from mast cells and basophils. It is involved in allergic and hypersensitivity reactions; provides protection against parasitic worms. This immunoglobulin was discovered in 1966 by Ishizaka.

It exhibits unique properties such as heat lability (inactivated at 56°C in one hour). IgE mediates type I hypersensitivity (anaphylaxis). Prausnitz and Kustner in 1921 demonstrated transmission of IgE-mediated type I hypersensitivity. It is called

Prausnitz-Kustner (PK) reaction. Thus IgE acts as mediator in allergic response.

(iv) IgG:

This is the most abundant class of Ig in the body constituting approximately 80% of the total Igs. It is found in the blood, lymph and intestine. It protects against bacteria and viruses by enhancing phagocytosis, neutralizing toxins and complement activation. It is the only class of antibody to cross the placenta from mother to foetus thereby conferring considerable immune protection in newborns.

(v) IgM:

IgM is about 5 to 10% of all antibodies in the blood. It is also found in lymph. It is the largest Ig which is secreted first by the plasma cells. It is so named because it is a macroglobulin at least five times larger than IgG. IgM is the oldest immunoglobulin class. It activates the B cells. It is also the earliest immunoglobin to be synthesised by the foetus, IgM has a J chain and its each dimer contains polypeptide called a secretory component.

It cannot cross the placental barrier. IgM is 500-1000 times more effective than IgG in opsonisation (to be described ahead), in bacterial action and in bacterial agglutination. But in neutralization of toxins and viruses, it is less active than IgG. It helps in complement activation.

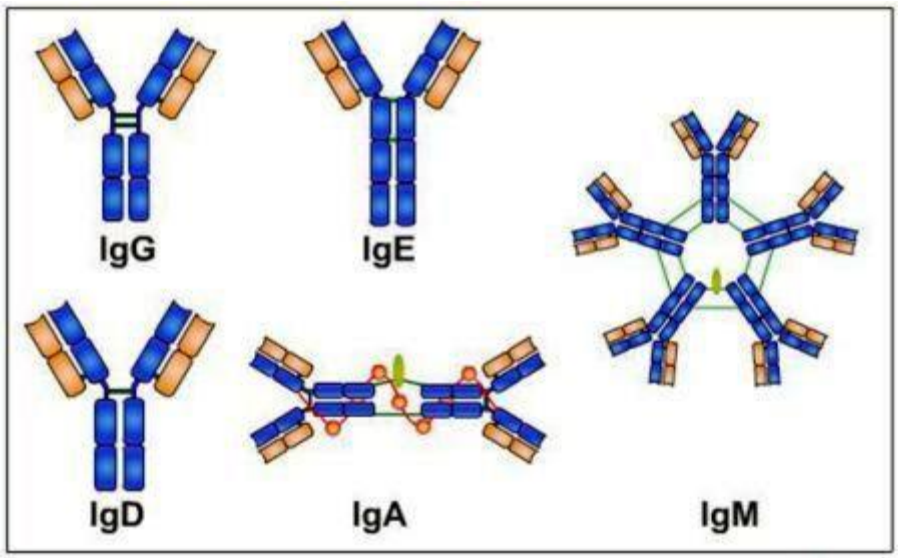

Fig 3: Structure of different types of antibody

CLASS SWITCHING OF ANTIBODIES:

Immunoglobulin class switching, also known as isotype switching, isotypic commutation or class-switch recombination (CSR), is a biological mechanism that changes a B cell's production of immunoglobulin (antibodies) from one type to another, such as from the isotype IgM to the isotype IgG. During this process, the constant-region portion of the antibody heavy chain is changed, but the variable region of the heavy

chain stays the same (the terms "variable" and "constant" refer to changes or lack thereof between antibodies that target different epitopes). Since the variable region does not change, class switching does not affect antigen specificity. Instead, the antibody retains affinity for the same antigens, but can interact with different effector molecules.

Class switching occurs after activation of a mature B cell via its membrane-bound antibody molecule (or B cell receptor) to generate the different classes of antibody, all with the same variable domains as the original antibody generated in the immature B cell during the process of V(D)J recombination, but possessing distinct constant domains in their heavy chains.

Naïve mature B cells produce both IgM and IgD, which are the first two heavy chain segments in the immunoglobulin locus. After activation by antigen, these B cells proliferate. If these activated B cells encounter specific signaling molecules via their CD40 and cytokine receptors (both modulated by T helper cells), they undergo antibody class switching to produce IgG, IgA or IgE antibodies. During class switching, the constant region of the immunoglobulin heavy chain changes but the variable regions, and therefore antigenic specificity, stay the same. This allows different daughter cells from the same activated B cell to produce antibodies of different isotypes or subtypes (e.g. IgG1, IgG2 etc.).

The order of the heavy chain exons are as follows:

μ - IgM, δ - IgD, $\gamma 3$ - IgG3, $\gamma 1$ - IgG1, $\alpha 1$ - IgA1, $\gamma 2$ - IgG2, $\gamma 4$ - IgG4, ε - IgE, $\alpha 2$ - IgA2

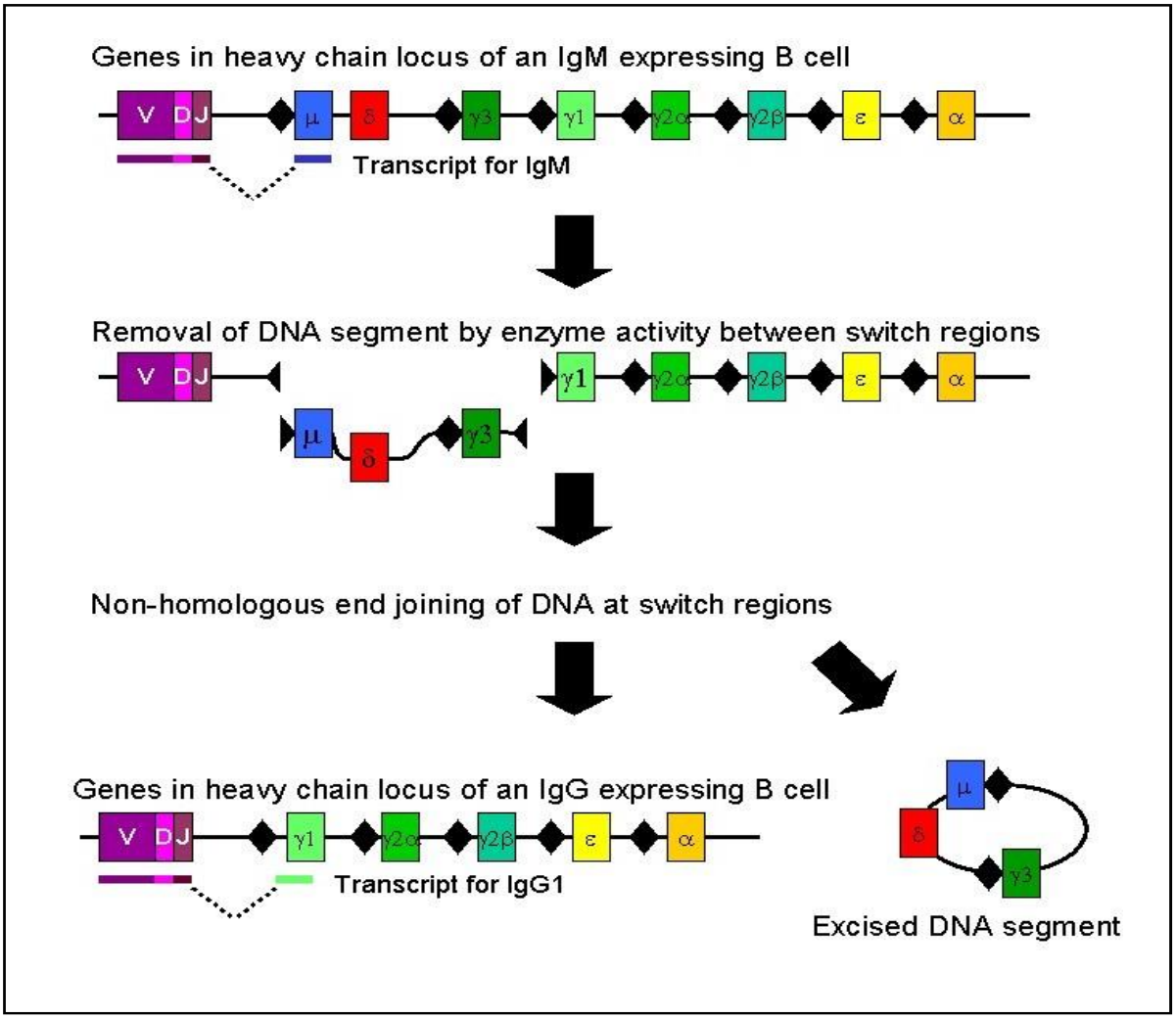

Fig 4: Class switching of antibody molecule

Class switching occurs by a mechanism called class switch recombination (CSR) binding. Class switch recombination is a biological mechanism that allows the class of antibody produced by an activated B cell to change during a process known as isotype or class switching. During CSR, portions of the antibody heavy chain locus are removed from the chromosome, and the gene segments surrounding the deleted portion are rejoined to retain a functional antibody gene that produces antibody of a different isotype. Double-stranded breaks are generated in DNA at conserved nucleotide motifs, called switch (S) regions, which are upstream from gene segments that encode the constant regions of antibody heavy chains; these occur adjacent to all heavy chain constant region genes with the exception of the δ-chain. DNA is nicked and broken at two selected S-regions by the activity of a series of enzymes, including Activation-Induced (Cytidine) Deaminase (AID), uracil DNA glycosylase and apyrimidic/ apurinic (AP)-endonucleases. The intervening DNA between the S-regions is subsequently deleted from the chromosome, removing unwanted μ or δ heavy chain constant region exons and

allowing substitution of a γ, α or ε constant region gene segment. The free ends of the DNA are rejoined by a process called non- homologous end joining (NHEJ) to link the variable domain exon to the desired downstream constant domain exon of the antibody heavy chain. In the absence of non-homologous end joining, free ends of DNA may be rejoined by an alternative pathway biased toward microhomology joins. With the exception of the μ and δ genes, only one antibody class is expressed by a B cell at any point in time. While class switch recombination is mostly a deletional process, rearranging a chromosome in "cis", it can also occur (in 10 to 20% of cases, depending upon the Ig class) as an inter-chromosomal translocation mixing immunoglobulin heavy chain genes from both alleles.

Probable questions:

1. What is humoral immune response and mention the basic components of humoralimmunity.
2. Describe the basic structure of a Ig molecule.
3. Elucidate the structure and function of different Ig molecules.
4. Describe the structure of IgM with diagram.
5. What is the function of IgA antibody?
6. What is class switching of antibody.

Suggested readings/ references:

1. Kindt T, Goldsby R, Osborne B, Kuby J, Kuby J. Kuby immunology. 2007. New York: W.H. Freeman.
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UNIT IV

B cell function, maturation and development

Objective:

In this unit, we will discuss about the B cell function, maturation and development.

Introduction:

B cells are a type of lymphocyte that is responsible for the humoral immunity component of the adaptive immune system. These white blood cells produce antibodies, which play a key part in immunity. Each B cell contains a single round nucleus. Early B cell development and commitment to the B cell lineage occurs in the foetal liver prenatally, before continuing in the bone marrow throughout life.

Lymphocytes account for about 25% of white blood cells, and B cells represent approximately 10% of total lymphocytes.

- **B cell function** :

Lymphocytes are a type of white blood cells. They play a critical role in keeping us healthy. Without them, we can't survive. B cell functions are as follows:

- Antigen presentation to other immune cells
- Cytokine secretion
- Antibody production

I. Antigen-Presenting Cells

- While the most important function of B cells is our humoral (antibody-driven) immunity, inactivated B lymphocytes also act as antigen-presenting cells (APCs).
- Antigen-presenting cells are found all over the body. They attach to an antigen (foreign particle) and process it, producing membrane markers that act as a warning (the antigen-MHC complex in the diagram above) that a T cell can understand.
- B lymphocytes present these antigen-MHC complexes to T cell receptors, causing T cell activation.

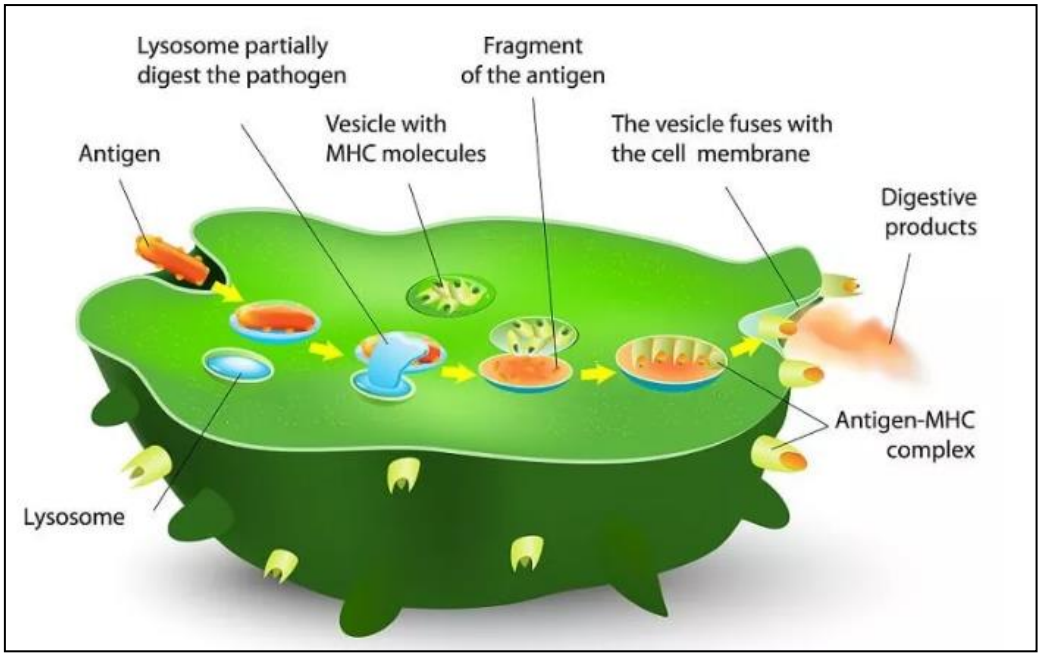

Fig 1: How the APC presents antigens to T cells

- Where the non-specific immune system (the innate immune system) is concerned, this reaction produces the opposite effect – T cell inactivation. This indicates that B lymphocytes also help prevent autoimmune reactions.

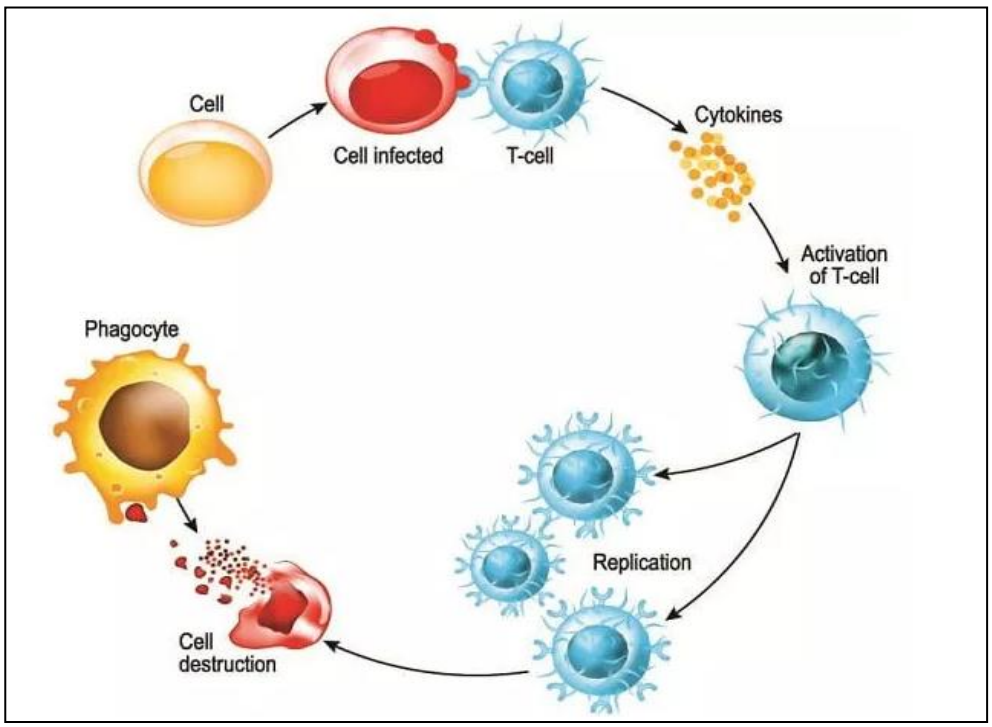

Fig: T cell activation

II. Cytokine Secretion

- Cyto (Greek for cell) and kinos (Greek for movement) describes the action of cytokines – they cause cell movement. Cytokines are signalling molecules and essential for cell-to-cell communication.

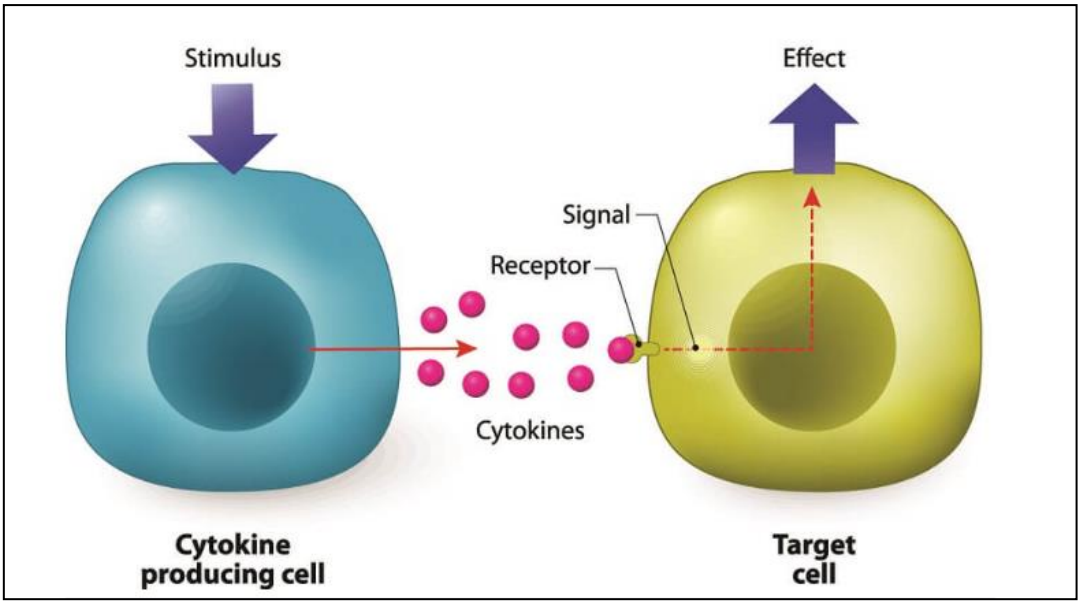

Fig 2: Cytokines – essential for cell to cell communication

- When B lymphocytes release cytokines, they invite white blood cells in the form of phagocytes to the areas where B-cell antibodies have attached to antigens.

III. Antibody Production

- The primary function of B cells is antibody production. To understand this phenomenon, it is important to have some knowledge of the humoral immunity process.

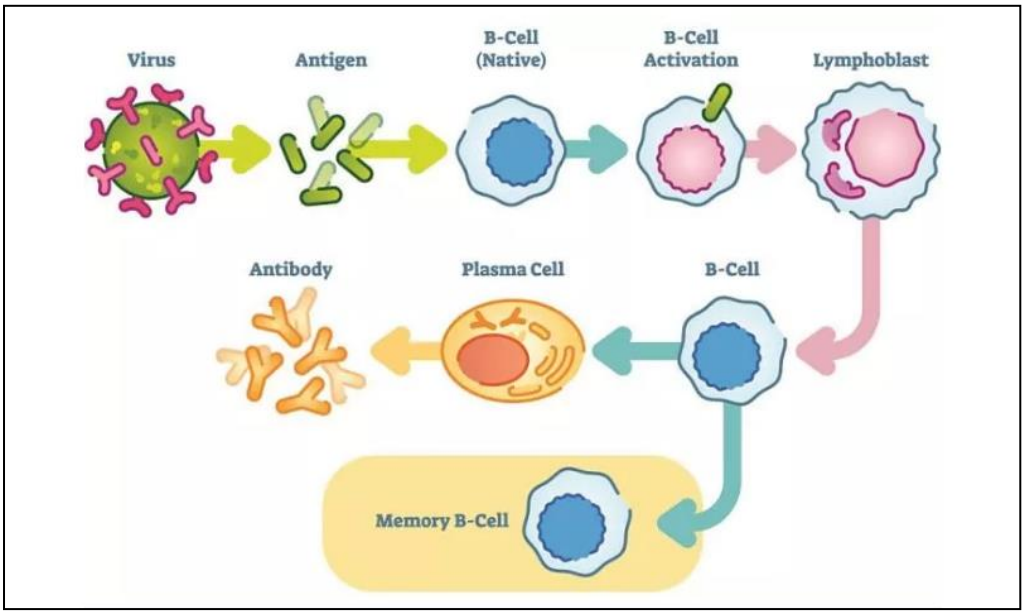

Fig 3: Our humoral immune system

- Humoral immunity begins in the B lymphocyte. While still in the bone marrow, a B cell develops special membrane receptors called B-cell receptors (BCRs). These

proteins are the equivalent of locks that fit antigen keys. B lymphocytes then relocate to the lymphoid organs. Once there, they are called naïve B cells.

- When a naïve B lymphocyte comes into contact with an antigen that fits its receptors, it binds to it and brings it inside its membrane (endocytosis) for processing. This processing is important as it leads to the formation of antigen-MHC complexes that T cells can recognize.
- Contact with an antigen **does not** cause B cell activation. When a T cell binds to the antigen-MHC complex it releases cytokines. These T-cell cytokines activate the B lymphocyte.
- Activation means that the B lymphocyte divides to form one of two types of daughter cells; activated plasma cells or inactive memory cells.
- B lymphocytes, also called B cells, create a type of protein called an antibody. These antibodies bind to pathogens or to foreign substances, such as toxins, to neutralize them. For example, an antibody can bind to a virus, which prevents it from entering a normal cell and causing infection. B cells can also recruit other cells to help destroy an infected cell.
- To understand how B lymphocytes are caused to secrete antibodies during an immune response, let's consider a case in which a person acquires either a bacterial or viral infection.
- Two events must generally occur if B lymphocytes are to be activated (Fig 3).

First, antigens present on the surface of (or released by) the pathogen become bound to antibodies in the plasma membranes of one or more of the millions of clones of B lymphocytes. Binding of the antigen to the surface of the B lymphocytes does not by itself cause activation of the clone. Instead, antigens must also be taken up during nonspecific phagocytosis of antigen-bearing particles by macrophages (i.e., phagocytic cells that act as scavengers in the body's tissues). The antigens taken up by the macrophages are degraded or "processed" and fragments containing antigenic determinants are then displayed at the cell surface.

Macrophages that carry out this process are referred to as antigen-presenting cells. The antigenic determinant is then recognized by one or more clones of T cells possessing T-cell receptors for the antigen. T cells that recognize and are activated by antigen-presenting cells are called T helper cells.

Activated T helper cells then interact with the B lymphocytes to which antigen had already been bound. The interaction between T helper cells and B lymphocytes serves to activate the B lymphocytes causing the rapid proliferation of the clone, thereby yielding plasma cells and memory cells (Fig. 25-12). Only the plasma cells produce and secrete antibodies. The memory cells are kept in reserve and will be called on to respond during a second (or subsequent) infection by the same antigen-bearing pathogen.

Antibodies secreted by plasma cells may have several different effects:

(1) They may interact with free (i.e., soluble) antigens causing precipitation;

(2) They may interact with surface antigens of the pathogen (i.e., particulate antigens) causing agglutination; or

(3) They may promote complement fixation.

B cells or B lymphocytes are part of the adaptive immune response. Once activated, these white blood cells produce antibodies. B lymphocytes have further roles as antigen-presenting cells and cytokine secretors. This cell type is classified into four main groups: transitional, naïve, plasma, and memory B cells.

▪ B-cell maturation:

- The generation of B-cell first occurs in embryo and continues throughout life.
- Before birth, the yolk sac, foetal liver and foetal bone marrow are the major sites of B cell maturation.
- After birth, the generation of mature B-cells occurs in the bone marrow from hematopoietic stem cells (HSC).
- The HSC first divide to form lymphoid progenitor cells which then differentiate into the progenitor B-cells (pro B) which express a transmembrane tyrosine phosphatase called CD45R and signal transducing molecule Igα/ Igβ which are found associated with the membrane bound antibody in later stages of development.
- Pro-B cell also express CD19 (part of co-receptor), CD43 (leukosialin), CD24 (heat stable), and C-kit are present on the surface of Pro-B-cell.
- The pro-B-cells proliferate within bone marrow filling extravascular spaces between large sinusoids in the shaft of a bone proliferation of pro-B-cells to precursor-B-cells (pre-B-cell) require micro-environment provided by the bone marrow stromal cells.
- The stromal cell plays two important roles, they interact directly with Pro-B cell and Pre-B cell and they secrete various cytokines, notably IL-7 that support developmental process.
- Pro-B-cells need direct contact with stromal cells in the bone marrow during the earliest developmental stage.
- This interaction is mediated by several cell adhesion molecules including VLA-4 on Pro-B cell and its ligand, VCAM-1, on the stromal cell.
- After initial contact is made, a receptor on Pro-B cell called C-kit interacts with a stromal cell surface molecule known as stem cell factor (SCF).
- This interaction activates C-kit, a tyrosine kinase and Pro-B cell begins expressing receptor for IL-7.

- The Pre-B-cell express many of same marker that were present on Pro-B-cell, however they cease to express C-kit and CD43 and begin to express CD25.
- The IL-7 secreted by stromal cells drives the maturation process eventually inducing down the regulation of adhesion molecule on Pre-B cell.
- So, the proliferating cell can detach from stromal cells.
- At this stage, Pre-B-cell no longer requires direct contact with stromal cell but continues to requires IL-7 for growth and maturation.

Ig-gene re-arrangement producing immature B-cells:

- B-cell maturation depends on rearrangement of immunoglobulin DNA in the lymphoid stem cells.
- The first Ig-gene re-arrangement to occur in Pro-B-cell stage is a heavy chain DH-JH gene re-arrangement; this is VH-DH-JH rearrangement.
- If the first heavy chain rearrangement is not productive, then VH-DH-JH rearrangement continues on the other chromosome.
- Upon completion of heavy chain arrangement, the cell is classified as Pre-B-cell.
- Continued development of a Pre-B-cell into an immature B-cell requires a productive light-chain gene re-arrangement.
- Only one light chain isotype is expressed on the membrane of a B-cell because of allelic exclusion.
- Upon completion of productive light chain re-arrangement, it commits the immature B-cell to a particular antigenic specificity.
- This specificity is determined by the cells heavy chain VDJ sequence and light chain VJ sequence.
- Immature B cell expresses IgM on its cell surface.
- The bone marrow phase of B-cell development culminates in the production of IgM bearing immature B-cell.
- At this stage of development, B-cell is still not fully functional.
- Thus, antigen induces death or unresponsiveness rather than division and differentiation.
- The co-expression of IgD and IgM on the membrane signals the full maturation.
- This progression involves a change in RNA processing of the heavy chain primary transcript to permit the production of two mRNAs, one encoding the membrane form of the μ chain and other encoding the membrane of the chain.

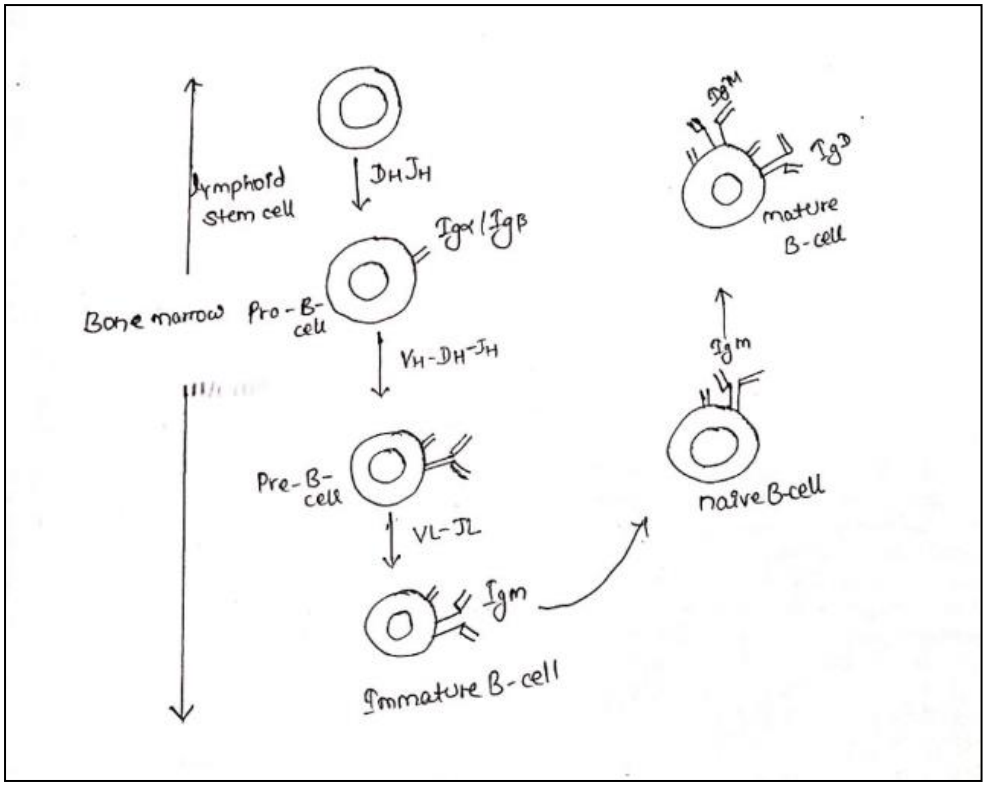

Fig 4: B cell maturation

B-cell proliferation and activation:

- After export of B-cell from the bone-marrow, activation, proliferation and differentiation occur in the periphery and require antigen.
- Depending on the nature of the antigen, B cell activation proceeds by two different routes, one dependent of TH cell, the other not.
- The B cell response to thymus dependent (TD) antigen requires direct contact with TH cell, not simply exposure to TH derived cytokines.
- Antigens that can activate B cells in absence of this kind of direct participation by TH cells are known as thymus independent (TH) antigen.
- The TI antigens are divided into two types 1 and 2 and they activate B-cells by different mechanisms.
- Most TI1 antigens are polyclonal B cell activator i.e. they are able to activate B-cell regardless of their antigenic specificity.
- At high concentration TI-1 antigens will stimulate proliferation and antibody secretion by as many as one third of B-cells.
- It includes bacterial cell wall components including lipopolysaccharide.
- B cells are activated by TI-2 antigens by extensively crosslinking the mIg receptor.
- However, TI-2 antigens contrast to TI-1 antigens in three important respects.

- First, they are not B-cell mitogens and do not act as a polyclonal activators.
- Second, TI-1 antigens activate both mature B-cells and immature B cells. Whereas TI-2 antigen activates mature B cells and inactivates immature B-cells.
- Third, although B cell response to TI-2 antigen does not require direct involvement of TH cells, cytokines derived from TH cells are required for efficient B-cell proliferation and for class switching to isotypes other than IgM.
- It includes highly repetitious molecules like bacterial flagellin.
- Activation of B-cell by soluble protein antigen requires the involvement of TH cells.
- Binding of antigen to B-cell mIg does not itself induce on effective competence without additional interaction with membrane molecule on the TH cell.
- In addition to it, a cytokine mediated progression is required for B-cell proliferation.

B-cell development:

- The development of plasma cell and memory B cells can be divided into three broad stages:
- Generation of mature, immunocompetent B-cells (maturation)
- Activation of mature B-cells and the differentiation of the activated B-cells, into plasma cells and memory B cells.
- These three stages can be divided into two phases:

1. **Antigen independent phase:**
    - This takes place in bone marrow.
    - It involves the maturation of lymphoid progenitors to matured naive B cells.
2. **Antigen dependent phase:**
    - This takes place in lymph node.
    - It involves activation of mature B-cells then they encounter antigen and their differentiation into plasma cells and memory B-cells.

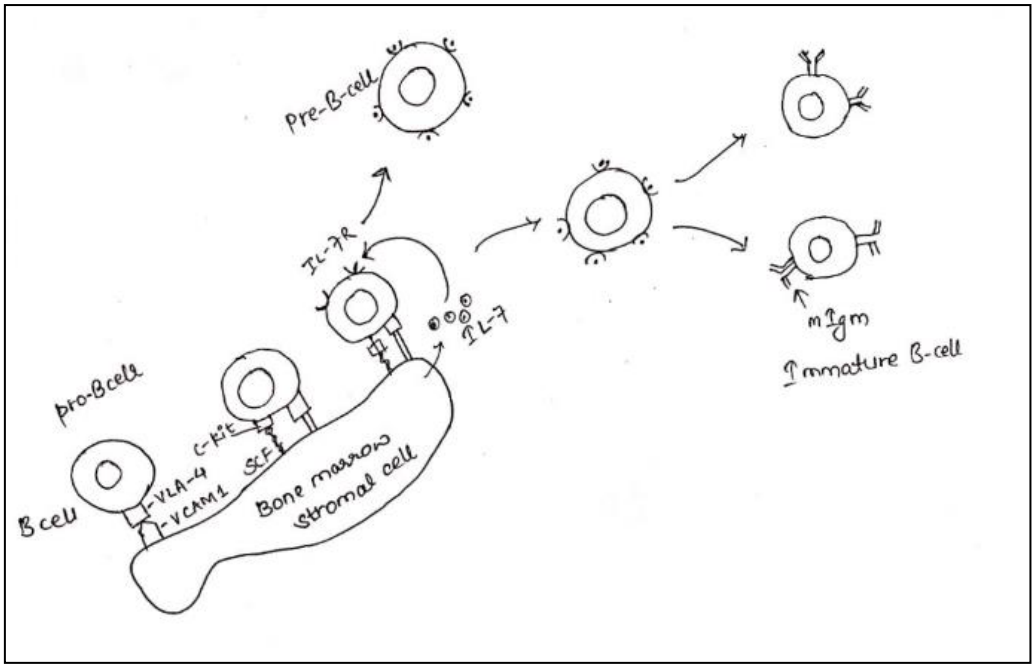

Fig 6: B cell development

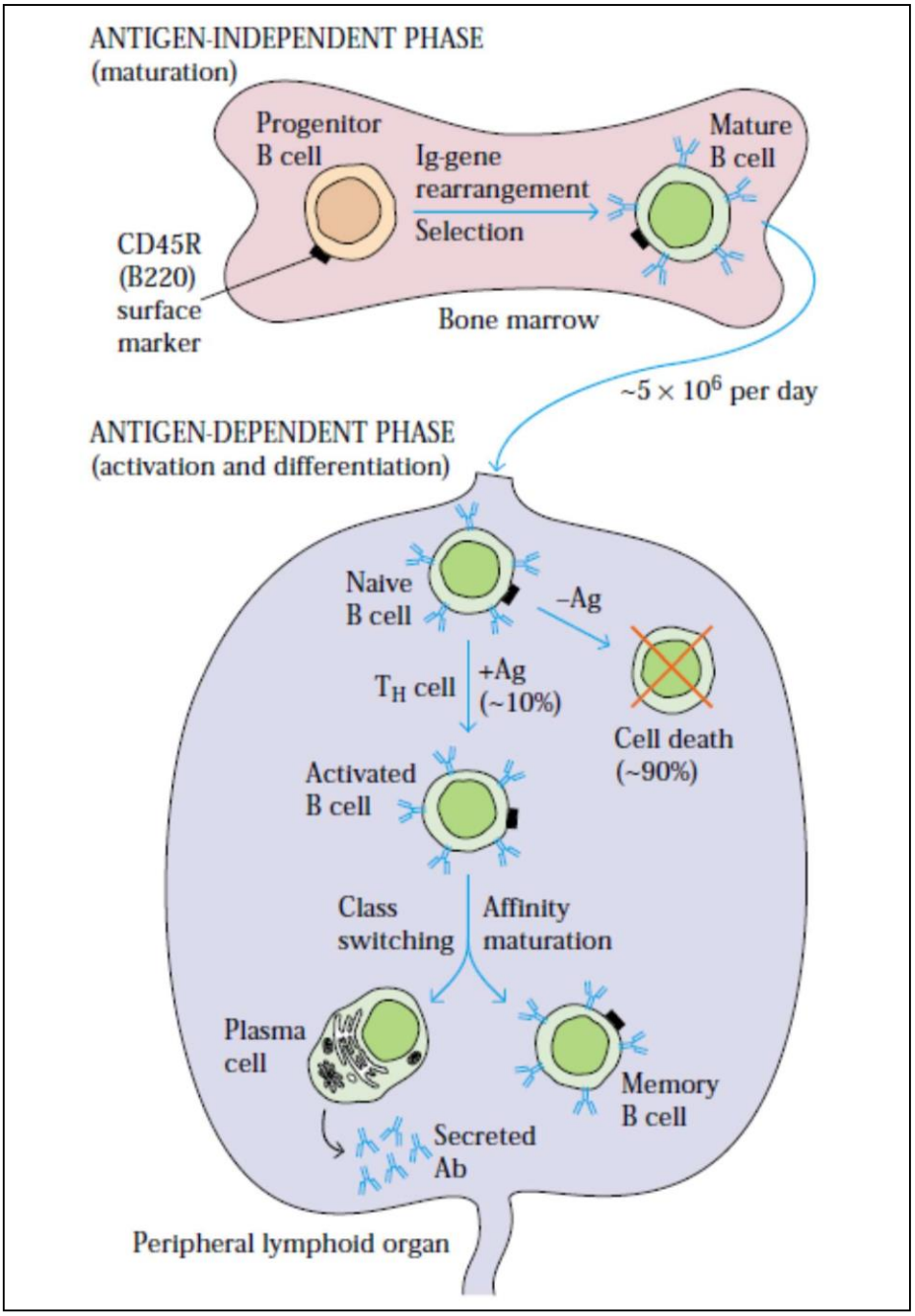

Figure: Overview of B-cell development. During the antigen- independent maturation phase, immunocompetent B cells expressing membrane IgM and IgD are generated in the bone marrow

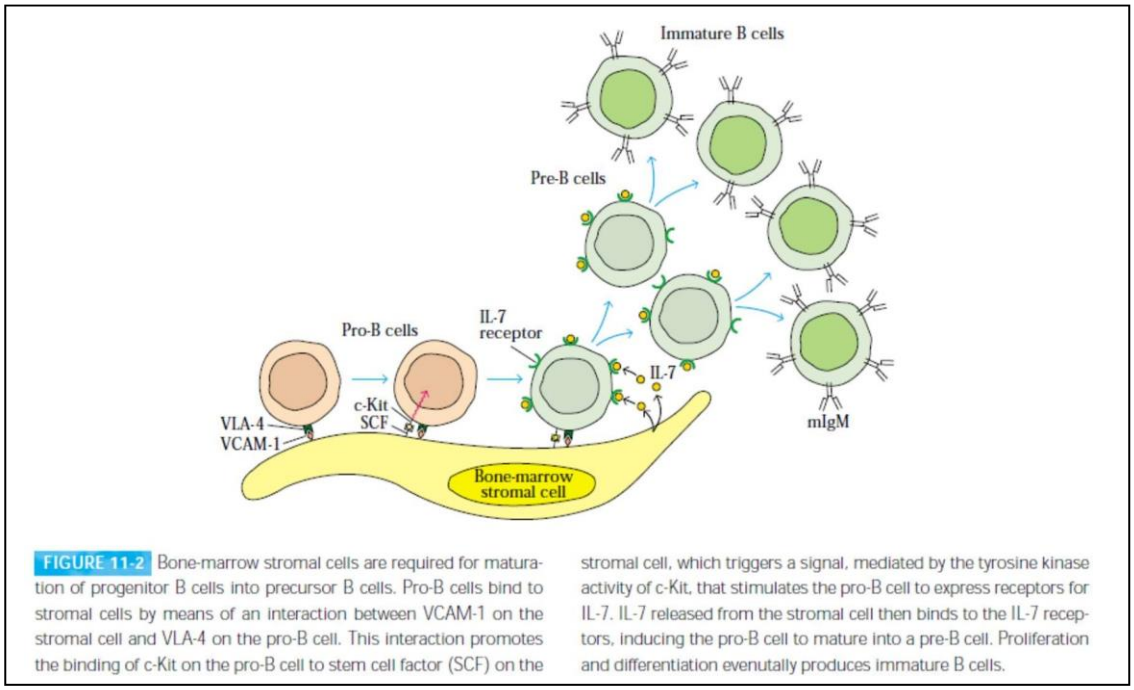

FIGURE 11-2 Bone-marrow stromal cells are required for maturation of progenitor B cells into precursor B cells. Pro-B cells bind to stromal cells by means of an interaction between VCAM-1 on the stromal cell and VLA-4 on the pro-B cell. This interaction promotes the binding of c-Kit on the pro-B cell to stem cell factor (SCF) on the stromal cell, which triggers a signal, mediated by the tyrosine kinase activity of c-Kit, that stimulates the pro-B cell to express receptors for IL-7. IL-7 released from the stromal cell then binds to the IL-7 receptors, inducing the pro-B cell to mature into a pre-B cell. Proliferation and differentiation evenutally produces immature B cells.

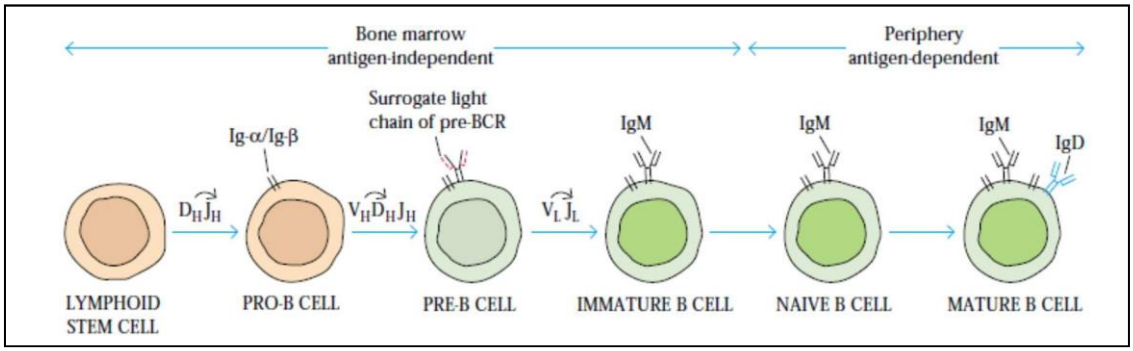

Figure: Development and maturation of B cell.

Probable questions:

1. What are the basic functions of B cell?
2. How maturation and development occur in B cell?
3. What do you mean by pro B cell?
4. What is naïve B cell?
5. Describe the process of antiboby production in humoral immune response.
6. What is plasma cell?
7. What is memory B cell? How it is produced?

Suggested readings/ references:

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3. Murphy, K., Travers, P., Walport, M., & Janeway, C. 2008. Janeway's immunobiology.New York: Garland Science.
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UNIT V

Complement system and disease

Objective: In this unit, we will discuss about the Complement system and diseases.

Introduction:

- Complement system was first discovered in 1890 by Jules Bordet as a heat-labile component of serum that „complemented" or „augmented" its bactericidal properties.
- It is known to be comprised of around 30 soluble proteins, together which constitute around 10% of total serum proteins.
- Most of these proteins are synthesized by liver and remain in inactive form in the absence of infection.
- Evolutionarily, complement system is as ancient as adaptive immune system as even worms and starfishes also have complement system.
- Complement system is the major component of innate system which is encountered by pathogens that enter our body after breaching physical barriers and antimicrobial defenses.
- It consists of three different pathways i.e. classical pathway, alternative pathway and lectin pathway. All these pathways ultimately lead to a common terminal pathway, leading to formation of membrane attack complex which subsequently lyses the target cell.
- Complement system broadly performs three types of functions: host defense against various infections, interface between innate and adaptive immunity and clearance of waste.
- For many of these biological activities of complement system, complement fragments bind various receptors present on different cell types. Additionally, these receptors regulate the complement activity.
- Besides these receptors, various other regulatory proteins are there that keep a check on the activity of complement system.
- The importance of complement system in providing immunity to us lies in the fact that a number of diseases result due to genetic or functional deficiency of different complement proteins.

- Still there are a number of pathogens which have developed evading strategies from complement mediated damage. The mechanism of evasion strategy and type of microbes possessing these are discussed in later part of this unit.

Nomenclature

Complement proteins can be named by:

i) Numerals e.g. C1, C2, C3…C9,

ii) Letter symbols e.g. factor B, factor D,

iii) Trivial names e.g. Decay accelerating factor (DAF), homologous restriction factor (HRF)

- The peptide fragments generated upon cleavage or activation of zymogen complement are denoted by small letters. The smaller fragment resulting from cleavage of a component is designated "a" and the larger fragment is designated as "b" (e.g., C3a, C3b). C2 is an exception to this rule as smaller fragment is called C2b whereas C2a is the larger cleavage fragment. The larger fragments bind to the target near the site of activation, and the smaller fragments diffuse from the site and act as anaphylatoxin.
- Functional complexes are formed by interaction among complement factors. The complexes having enzymatic activity are designated by a bar over the number or symbol e.g. C4b2a (C3 convertase in classical pathway).

The Functions of Complement:

Research on complement now includes more than 30 soluble and cell-bound proteins. The biological activities of this system affect both innate and acquired immunity and reach far beyond the original observations of antibody mediated lysis of bacteria and red blood cells. Structural comparisons of the proteins involved in complement pathways place the origin of this system in primitive organisms possessing the most rudimentary innate immune systems. By contrast, the realization that interaction of cellular receptors with complement proteins controls B-cell activities gives this system a role in the highly developed acquired immune system. Thus we have a system that straddles innate and acquired immunity, contributing to each in a variety

of ways. After initial activation, the various complement components interact, in a highly regulated cascade, to carry out a number of basic functions (Figure 13-1) including:

i. Lysis of cells, bacteria, and viruses.
ii. Opsonization, which promotes phagocytosis of particulate antigens.
iii. Binding to specific complement receptors on cells of the immune system, triggering specific cell functions, inflammation, and secretion of

immunoregulatory molecules.

iv. Immune clearance, which removes immune complexes from the circulation and deposits them in the spleen and liver.

1. The Classical Pathway of Complement:

The classical pathway of complement is initiated by the interaction of antibody with antigen directly (soluble antigen-antibody complexes or immune complexes).

The gradual progress of classical pathway can be mediated by these successive stages called:

(i) Activation of C1 component

(ii) Production of C3 convertase

(iii) Production of C5 convertase and

(iv) Action of membrane attack complex (MAC)

(i) Activation of C1 component:

The initial stage of activation involves C1, C2, C3 and C4. The soluble antigen-antibody complex induces a conformational changes in the fragment crystalized (Fc) portion of the antibody molecule that exposes a binding site for the C1 component of the complement system.

1. C1 is a complex macromolecular protein present in serum in inactive condition. It is a complex of three proteins named—C1q, C1r and C1s, out of which C1q recognizes and binds to the Fc region of the antibody and C1r and C1s remain as inactive proteases with their two subunits each. C1q and two molecules of each C1r and C1s held together is a complex called $C1qr_2s_2$ which is stabilized by Ca^{2+} ions.

2. The structure of C1 is mainly exhibited by C1q; a large molecule composed of 18 polypeptide chains that associate in such a way that forms six collagen-like triple helical arms. The amino-terminal two-thirds of the polypeptides form the stalk and the carboxy-terminal one-third of the polypeptides form the globular flower, which contains the binding site for antibody.

3. Normally, $C1r_2s_2$ complex remains in inactive form and never binds with C1q at that time and shows the configuration 'S'. Each C1r and C1s includes two domains named catalytic domain and interaction domain. Due to action of interaction domain in presence of antigen- antibody complex in the serum it binds with C1q.

4. C1q binds to an antibody Fc region by its globular heads, in terms, activates serine proteases C1r and C1s which are proteolytic enzymes gives serine residues at the active site after being activated.

On binding to antibody, one molecule of C1r is induced to cleave itself, becomes enzymatically active. Gradually it cleaves and activates the second C1r and both C1s molecules. The activated serine protease C1s binds, cleaves and activates the next two

components of the classical pathway i.e. serine protease C4 and C2. Ultimately active CI component is called $C1qr_2s_2$ (Fig 1).

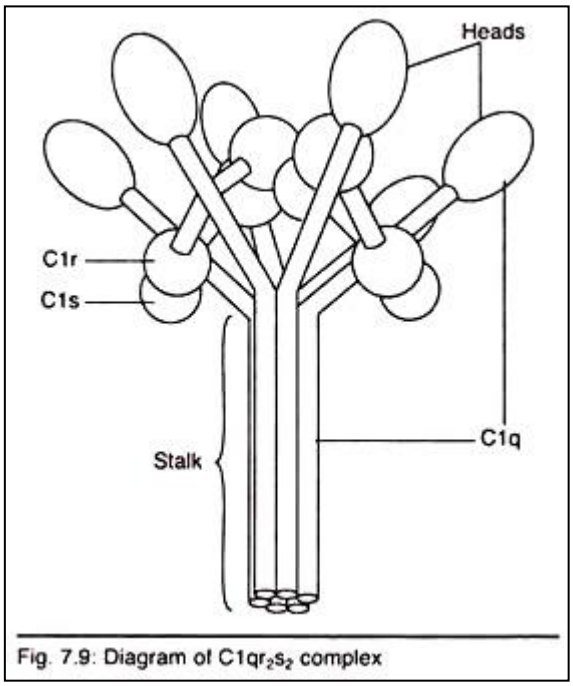

Fig 1: Diagram of $C1qr_2s_2$ component

Activation of classical pathway via IgM and IgG:

The cascade reaction of complement system is only initiated when antibody binds to multiple sites on a cell surface, normally that of a pathogen. When IgM (pentameric) is bound to antigen on a target surface, it requires at least three binding sites for C1q attachment.

In case of IgG molecule, it contains a single C1q binding site in the CH_2 domain of the Fc. As C1q globular head requires at least two Fc sites for a stable C1-antibody reaction, it indicates that two IgG are required to be present on a target surface.

The structural differences between IgM and IgG exert the effect on their activation level. At the activation of Clq binding, IgG requires less amount of time but a good number of IgG molecules are to be present. Whereas IgM activation is delayed one but it is more efficient, even a single IgM molecule can initiate the process.

(ii) Production of C3 convertase:

Active serine protease enzyme $C1qr_2s_2$ has two distinct substrates, C4 and C2. C4 component is a large globular glycoprotein containing three polypeptide chains named α, β and γ. C4 is activated when C1s hydrolyzes a small fragment C4a from the amino terminus of the chain, exposed a binding site on the larger fragment C4b. The C4b fragment attaches to the target surface of the C1 bound to antibody on the pathogen surface.

Besides, active C4 component, the activated C1s protease acts on C2 serine protease, as a result the smaller fragment C2b will be cleaved away from the site of action and C2a larger fragment will remain active at the active site. After that C4b2a active complex is formed which in turn act on the substrate C3 component. C4b2a is called C3 convertase of the classical pathway.

(iii) Production of C5 convertase:

C3 is almost very similar to C4. C3 component is with two types of polypeptide chains — α and β. C3 convertase (C4b2a) helps to cleave the smaller fragment C3a from the amino terminus of the a chain of C3 component.

Even a single C3 convertase molecule can accelerate the production of more than 200 molecules of C3b, and the result is amplification. In due course produced C3b binds with C4b2a to form a tri-molecular complex called C4b2a3b i.e. C5 convertase.

(iv) Action of membrane attack complex (MAC):

C5 convertase acts on C5 protein component, cleaves C5a from the action site and C5b attaches to the antigenic surface. This bound C5b initiates formation of membrane-attack complex (MAC) by taking participation of C6, C7, C8 and C9 components gradually and ultimately forms C5b6789 (MAC) which makes a large pore in the membrane of the antigen and accelerates lysis of it (Fig. 2).

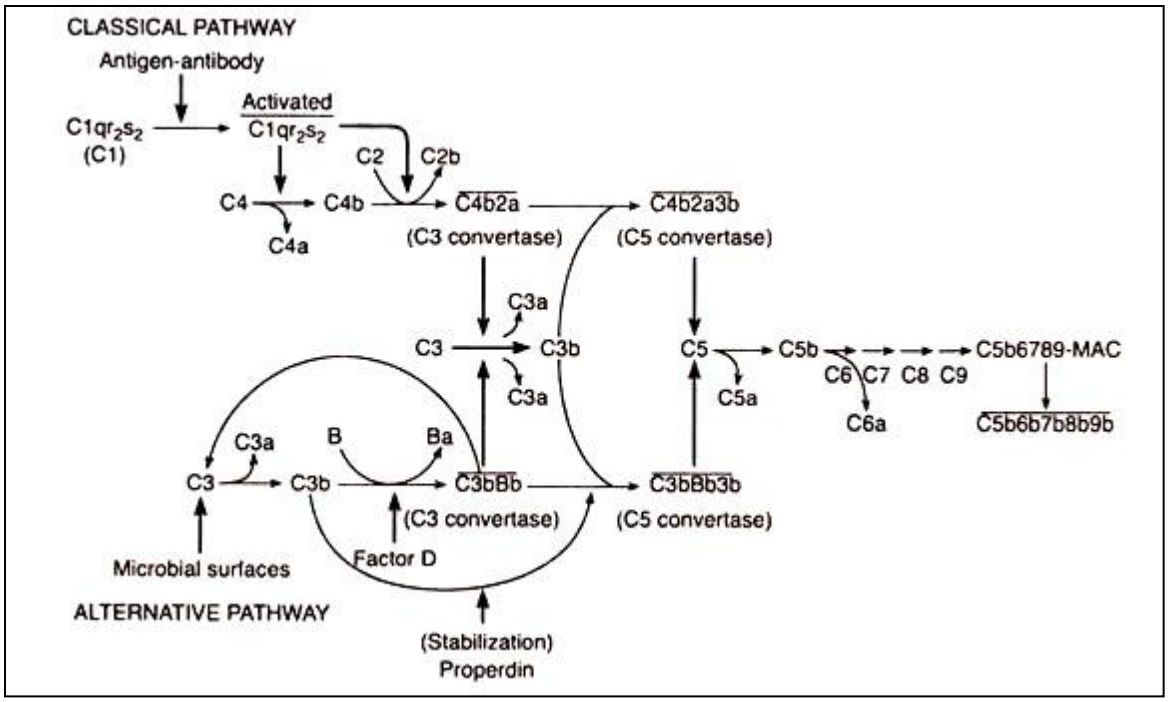

Fig 2: Overview of the complement activation pathways (classical and alternative)

Formation of Membrane Attack Complex

One of the important effects of complement activation is the assembly of the terminal components of complement to form a membrane-attack complex. The end result is a pore in the lipid bilayer membrane that destroys membrane integrity. This is thought to kill the pathogen by destroying the proton gradient across the pathogen cell membrane.

The first step in the formation of the membrane-attack complex is the cleavage of C5by a C5 convertase to release C5b. In the next stages, C5b initiates the assembly of the later complement components and their insertion into the cell membrane. First, one molecule of C5b binds one molecule of C6, and the C5b,6 complex then binds one molecule of C7. This reaction leads to a conformational change in the constituent molecules, with the exposure of a hydrophobic site on C7, which inserts into the lipid bilayer. Similar hydrophobic sites are exposed on the later components C8 and C9 when they are bound to the complex, allowing these proteins also to insert into the lipid bilayer. C8 is a complex of two proteins, C8β and C8α-γ. The C8β protein binds to C5b, and the binding of C8β to the membrane-associated C5b,6,7 complex allows the hydrophobic domain of C8α-γ to insert into the lipid bilayer. Finally, C8α-γ induces the polymerization of 10 to 16 molecules of C9 into a pore-forming structure called the membrane-attack complex. The membrane-attack complex has a hydrophobic external face, allowing it to associate with the lipid bilayer, but a hydrophilic internal channel. The diameter of this channel is about 100 Å, allowing the free passage of solutes and water across the lipid bilayer. The disruption of the lipid bilayer leads to the loss of cellular homeostasis, the disruption of the proton gradient across the membrane, the penetration of enzymes such as lysozyme into the cell, and the eventual destruction of the pathogen.

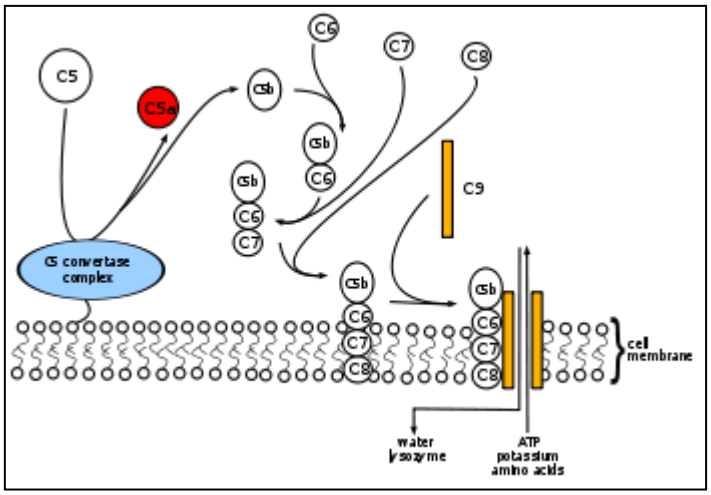

Fig 3: Formation of membrane attack complex

2. *The Alternative Pathway:*

Besides the classical pathway, complement system can be initiated by another method called alternative pathway. Unlike classical pathway the alternative pathway is initiated by the cell-wall constituents of both gram-positive and gram- negative bacteria as foreign particles.

Microbial surfaces directly affect the serine protease C3, gradually cleaving of C3 into C3a and C3b. This conformational change extends its effect on another factor i.e. factor

B. In turn Ba removed from active site keeping Bb towards the C3b in presence of Mg^{++}; forms C3bBb, and considered as C3 convertase of alternative pathway.

Binding of C3b exposes a site on factor B that again serves as the substrate for an enzymatically active serum protein called factor D. Actually factor D cleaves the C3b bound factor B, and helps to form C3bBb. The action of C3bBb is very unstable, becomes stabilized by the presence of another exclusive serum protein properdin in this pathway, helps to increase the convertase activity period.

Formation of C3bBb accelerates the auto- catalyse of more C3 component and forms C3bBb3b as C5 convertase. Though structural basis of C3 and C5 convertase vary in these two pathways of complement system but their mode of action is alike.

Here, C3bBb3b subsequently hydropses the bound C5, C6, C7, C8 and C9 respectively, resulting in Membrane Attack Complex (MAC) formation which binds to the antigenic surfaces of microbes (antigen). MAC gradually displaces the membrane phospholipids, forms a large trans-membrane channel and gradually destroys the membrane and lysis of the antigen occurs.

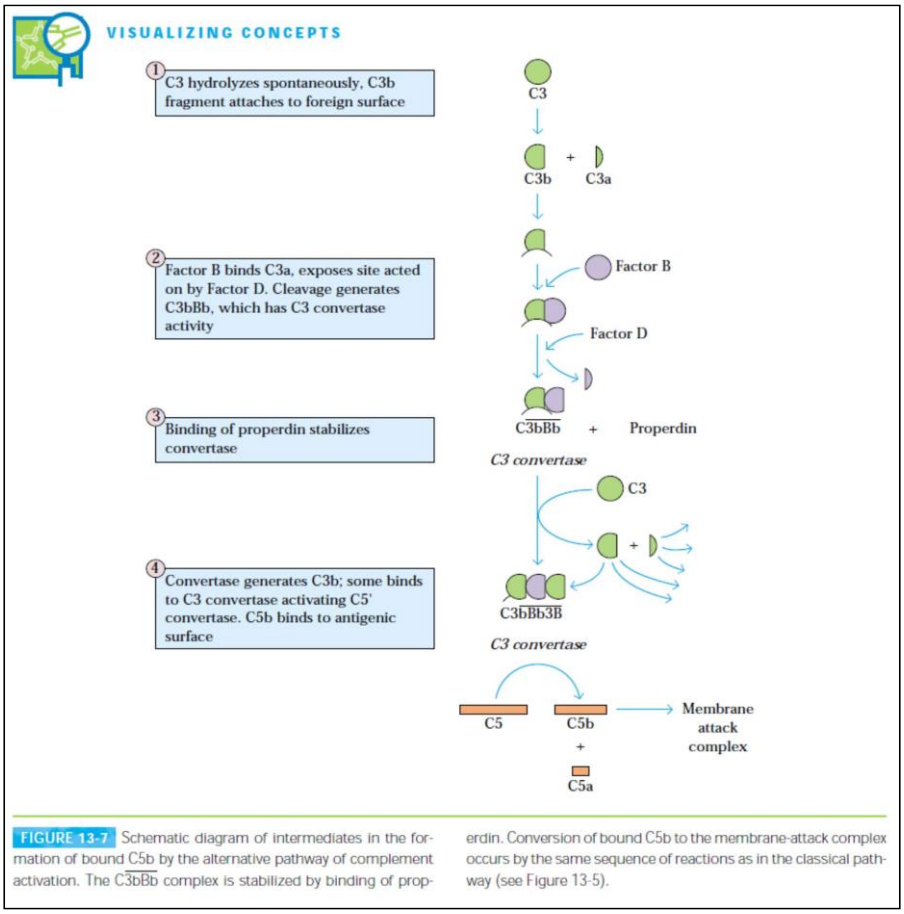

FIGURE 13-7 Schematic diagram of intermediates in the formation of bound C5b by the alternative pathway of complement activation. The C3bBb complex is stabilized by binding of properdin. Conversion of bound C5b to the membrane-attack complex occurs by the same sequence of reactions as in the classical pathway (see Figure 13-5).

3. *The Lectin mediated pathway:*

The third pathway of complement system is lectin-mediated pathway. Lectin-mediated pathway is activated by the binding of mannose-binding protein present in blood plasma to mannose containing proteoglycans on the surfaces of the bacteria and yeast, it forms MBP-MASP (Mannose-binding protein-mannose-associated serum protease). In lectin pathway MBP-MASP acts on the substrate C_4 and C_2 component protein.

- ## Complement Deficiencies

Classical pathway component deficiencies (C1, C4, C2)

Individuals having C1, C4 and C2 deficiencies are prone to immune complex disease, commonly systemic lupus erythematosus (SLE). The reason for this is thought to be that the complement deficiency leads to an inability to clear circulating immune complexes. This in turn leads to their deposition in tissues and an associated inflammatory response. Reduced clearance of apoptotic cells by the complement system may also lead to the development of autoantigens.

C2 deficiency is also associated with recurrent bacterial infection and an increased risk of cardiovascular disease. It is thought to influence the development of atherosclerosis.

I. **MBL deficiency** causes recurrent pyogenic infection in childhood. Deficiency of MBL increases susceptibility to *Saccharomyces cerevisiae* infection as well as pneumococcal and Neisseria infection.

II. **Alternative pathway component deficiencies (properdin, factor B and factor D) -** are prone to pneumococcal and meningococcal infections. With properdin deficiency, there is a particular risk of overwhelming Neisseria infection.

III. **C3 deficiency-** C3 is required for opsonisation (the coating of pathogenic cells with opsonin to facilitate phagocytosis).A defect in the pathway that results in deficiency of C3 can lead to problems with opsonisation.

IV. **MAC deficiencies (C5-C9) -** Recurrent infections are again a feature. Infection with *Neisseria meningitidis* is particularly common. More rare serotypes of this organism such as Y and W135 tend to cause the infection.

V. **Leiner's disease -** This is a paediatric condition associated with a deficiency of C5. It has also been reported with C3 and C4 deficiency. It causes wasting, chronic diarrhoea and widespread seborrhoeic dermatitis.

Probable questions:

1. What do you mean by complement system?
2. What are the basic functions of complement system?
3. Describe the classical and alternative pathway of complement activation.
4. What is membrane attack complex?
5. Summarise the biological effects mediated by complement products.
6. Name the diseases associated with deficiency in complement products

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UNIT VI

Antigen presentation: Concept of haptens, determinants, conditions of antigenicity, superantigen, Dendritic cell, MHC, role of APCs

Objective:

In this unit, we will discuss about the Antigen presentation: Concept of haptens, determinants, conditions of antigenicity, superantigen, Dendritic cell, MHC, role of APCs.

Introduction:

Antigen presentation is the expression of antigen molecules on the surface of a macrophage or other antigen-presenting cell in association with MHC class II molecules when the antigen is being presented to a $CD4^+$ helper T cell or in association with MHC class I molecules when presentation is to $CD8^+$ cytotoxic T cells.

Here we will have a discussion about antigen. Antigens are substances which, when introduced into the body, stimulate the production of antibodies.

Antigen is a substances usually protein in nature and sometimes polysaccharide, that generates a specific immune response and induces the formation of a specific antibody or specially sensitized T cells or both.

Although all antigens are recognized by specific lymphocytes or by antibodies, only some antigens are capable of activating lymphocytes. Molecules that stimulate immune responses are called I**mmunogens.**

Epitope is immunologically active regions of an immunogen (or antigen) that binds to antigen-specific membrane receptors on lymphocytes or to secreted antibodies. It is also called **antigenic determinants.**

Autoantigens, for example, are a person's own self antigens. Examples: Thyroglobulin, DNA, Corneal tissue, etc.

Alloantigens are antigens found in different members of the same species (the red blood cell antigens A and B are examples).

Heterophile antigens are identical antigens found in the cells of different species. Examples: Forrssman antigen, Cross-reacting microbial antigens, etc.

Adjuvants are substances that are non-immunogenic alone but enhance the immunogenicity of any added immunogen.

- **Properties of Antigens**

The properties of antigens are as follows:

1. The antigen should be a foreign substance to induce an immune response.
2. The antigens have a molecular mass of 14,000 to 6,00,000 Da.
3. They are mainly proteins and polysaccharides.
4. The more chemically complex they are, the more immunogenic they will be.
5. Antigens are species-specific.
6. The age influences the immunogenicity. Very young and very old people exhibit very low immunogenicity.

- ## Structure:

Antigenic determinants or epitopes (Gk. epi – upon, topos- place) are components of antigen. Each antigen carries many epitopes. Each Y-shaped antibody molecule has at least two binding sites that can attach to a specific epitope on an antigen. An antibody can also bind to identical epitopes of two different cells at the same time which can cause neighbouring cells to aggregate. Antigens combine with the antibody. The combination is very much like the lock and key analogy.

- ## Chemical Nature of Antigens (Immunogens)

A. Proteins

The vast majority of immunogens are proteins. These may be pure proteins or they may be glycoproteins or lipoproteins. In general, proteins are usually very good immunogens.

B. Polysaccharides

Pure polysaccharides and lipopolysaccharides are good immunogens.

C. Nucleic Acids

Nucleic acids are usually poorly immunogenic. However, they may become immunogenic when single stranded or when complexed with proteins.

D. Lipids

In general lipids are non-immunogenic, although they may be haptens.

- ## Types of Antigen On the basis of order of their class (Origin)

1. Exogenous antigens

- These antigens enter the body or system and start circulating in the body fluids and trapped by the APCs (Antigen processing cells such as macrophages, dendritic cells, etc.)
- The uptakes of these exogenous antigens by APCs are mainly mediated by the phagocytosis

- Examples: bacteria, viruses, fungi etc
- Some antigens start out as exogenontigens, and later become endogenous (for example, intracellular viruses)

2. Endogenous antigens

- These are body's own cells or sub fragments or compounds or the antigenic products that are produced.
- The endogenous antigens are processed by the macrophages which are later accepted by the cytotoxic T – cells.
- Endogenous antigens include xenogenic (heterologous), autologous and idiotypic or allogenic (homologous) antigens.
- Examples: Blood group antigens, HLA (Histocompatibility Leukocyte antigens), etc.

3. Autoantigens

- An autoantigen is usually a normal protein or complex of proteins (and sometimes DNA or RNA) that is recognized by the immune system of patients suffering from a specific autoimmune disease
- These antigens should not be, under normal conditions, the target of the immune system, but, due mainly to genetic and environmental factors, the normal immunological tolerance for such an antigen has been lost in these patients.
- Examples: Nucleoproteins, Nucleic acids, etc.

- **Types of Antigen On the basis of immune response**

1. Complete Antigen or Immunogen

- Posses antigenic properties denovo, i.e. ther are able to generate an immune response by themselves.
- High molecular weight (more than 10,000)
- May be proteins or polysaccharides

2. Incomplete Antigen or Hapten

- These are the foreign substance, usually non-protein substances
- Unable to induce an immune response by itself, they require carrier molecule to act as a complete antigen.
- The carrier molecule is a non-antigenic component and helps in provoking the immune response. Example: Serum Protein such as Albumin or Globulin.
- Low Molecular Weight (Less than 10,000)

- Haptens can react specifically with its corresponding antibody.
- Examples: Capsular polysaccharide of pneumococcus, polysaccharide "C" of beta haemolytic streptococci, cardiolipin antigens, etc

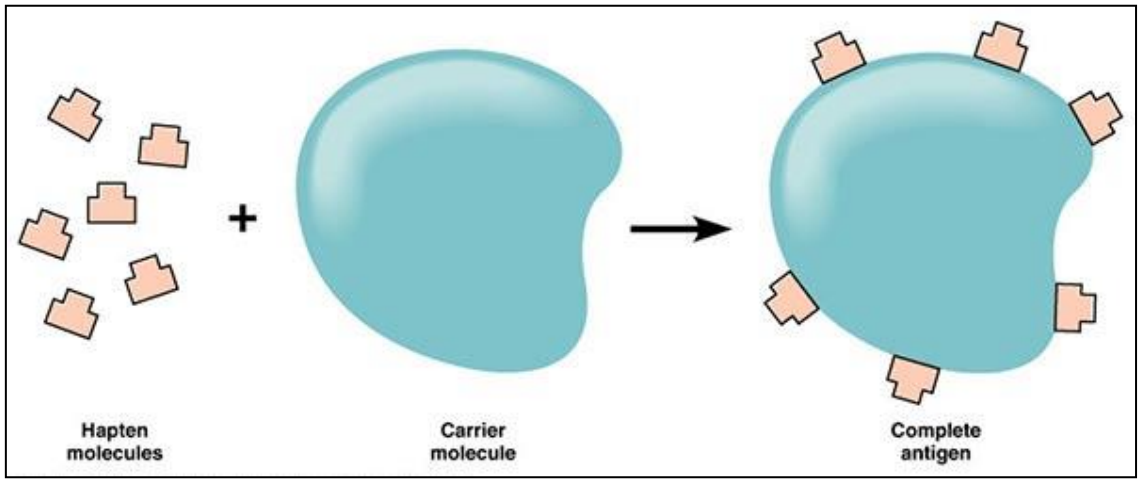

Fig: Hapten

- ## Determinants of Antigenicity

The whole antigen does not evoke immune response and only a small part of it induces B and T cell response.

The small area of chemical grouping on the antigen molecule that determines specific immune response and reacts specifically with antibody is called an ***antigenic determinant.***

- ## Property of antigens/ Factors Influencing Immunogenicity

Immunogenicity is determined by:

1. Foreignness

- An antigen must be a foreign substance to the animal to elicit an immune response.

2. Molecular Size

- The most active immunogens tend to have a molecular mass of 14,000 to 6,00,000 Da.
- Examples: tetanus toxoid, egg albumin, thyroglobulin are highly antigenic.
- Insulin (5700) are either non-antigenic or weakly antigenic.

3. Chemical Nature and Composition

- In general, the more complex the substance is chemically the more immunogenic it will be.
- Antigens are mainly proteins and some are polysaccharides.
- It is presumed that presence of an aromatic radical is essential for rigidity and antigenicity of a substance.

4. Physical Form

- In general particulate antigens are more immunogenic than soluble ones.
- Denatured antigens are more immunogenic than the native form.

5. Antigen Specificity

- Antigen Specificity depends on the specific actives sites on the antigenic molecules (Antigenic determinants).
- Antigenic determinants or epitopes are the regions of antigen which specifically binds with the antibody molecule.

6. Species Specificity

- Tissues of all individuals in a particular species possess, species specific antigen.
- Human Blood proteins can be differentiated from animal protein by specific antigen-antibody reaction.

7. Organ Specificity

- Organ specific antigens are confined to particular organ or tissue.
- Certain proteins of brain, kidney, thyroglobulin and lens protein of one species share specificity with that of another species.

8. Auto-specificity

- The autologous or self antigens are ordinarily not immunogenic, but under certain circumstances lens protein, thyroglobulin and others may act as ***autoantigens.***

9. Genetic Factors

- Some substances are immunogenic in one species but not in another .Similarly, some substances are immunogenic in one individual but not in others (i.e. responders and non-responders).
- The species or individuals may lack or have altered genes that code for the receptors for antigen on B cells and T cells.
- They may not have the appropriate genes needed for the APC to present antigen to the helper T cells.

10. Age

- Age can also influence immunogenicity.
- Usually the very young and the very old have a diminished ability to elicit and immune response in response to an immunogen.

11. Degradability

- Antigens that are easily phagocytosed are generally more immunogenic.
- This is because for most antigens (T-dependant antigens) the development of an immune response requires that the antigen be phagocytosed, processed and presented to helper T cells by an antigen presenting cell (APC).

12. Dose of the antigen

- The dose of administration of an immunogen can influence its immunogenicity.
- There is a dose of antigen above or below which the immune response will not be optimal.

13. Route of Administration

- Generally the subcutaneous route is better than the intravenous or intragastric routes.
- The route of antigen administration can also alter the nature of the response.
- Antigen administered intravenously is carried first to the spleen, whereas antigen administered subcutaneously moves first to local lymph nodes.

14. Adjuvants

- Substances that can enhance the immune response to an immunogen are called adjuvants.
- The use of adjuvants, however, is often hampered by undesirable side effects such as fever and inflammation.
- Example: aluminum hydroxide.

Superantigens

- When the immune system encounters a conventional T-dependent antigen, only a small fraction (1 in 104 -105) of the T cell population is able to recognize the antigen and become activated (monoclonal/oligoclonal response).
- However, there are some antigens which polyclonally activate a large fraction of the T cells (up to 25%). These antigens are called superantigens.
- Examples of superantigens include: Staphylococcal enterotoxins (food poisoning), Staphylococcal toxic shock toxin (toxic shock syndrome),

Staphylococcal exfoliating toxins (scalded skin syndrome) and Streptococcal pyrogenic exotoxins (shock).

- Although the bacterial superantigens are the best studied there are superantigens associated with viruses and other microorganisms as well.
- The diseases associated with exposure to superantigens are, in part, due to hyper activation of the immune system and subsequent release of biologically active cytokines by activated T cells.

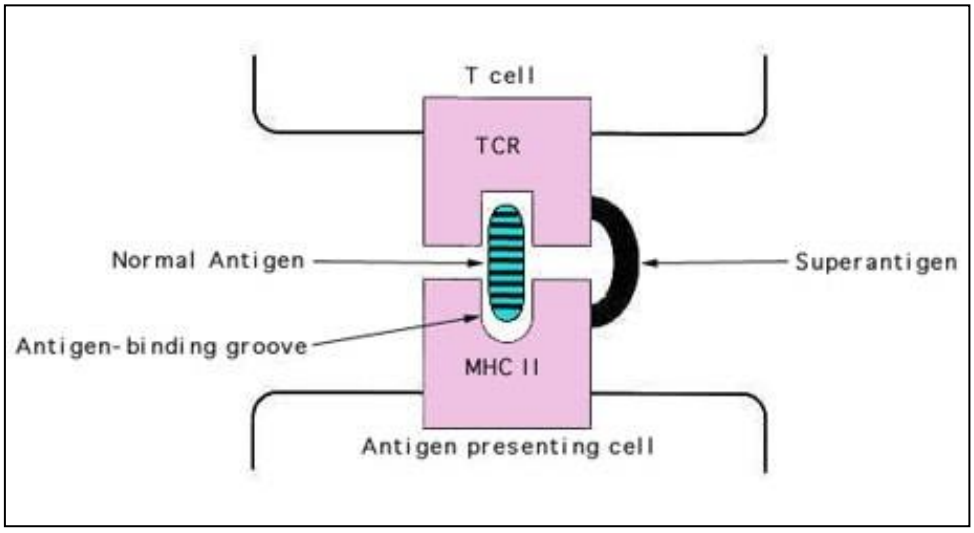

Fig: Structure of superantigen

Antigen Presenting Cells

An Antigen Presenting Cell or APC is a type of cell that displays or presents antigens complexed with major histocompatibility complex (MHC) on their surface, through a process known as antigen presentation. They are very important for the proper functioning of the Helper T cells and Cytotoxic T cells, as a result they are vital for an effective adaptive immune response.

Almost every cell of our body can serve as some form of APC due to the presence of MHC class I molecule, but there are a few cell types that are specialized for the function of antigen presentation. These specialized cells are referred to as the professional APCs. These include Macrophages, Dendritic cells and B cells.

There are three types of antigen presenting cells in the body: macrophages, dendritic cells and B cells.

1. Macrophages:

These are a type of white blood cells that can engulf and digest cellular debris, foreign substances, microbes and even cancer cells. They are present in various forms in different parts of the body such as Kupffer cells, Histiocytes, alveolar macrophages, etc.

They play important role in both innate immune response and adaptive immune response. These cells are also vital for their anti-inflammatory roles and can decrease immune reactions through the release of cytokines.

Macrophages are actually stimulated by the Interferon γ (IFNγ), secreted by the T cell. Once activated, they are capable of expressing MHC class II molecules and other co-stimulatory molecules including B7 complex. They can present phagocytosed peptide fragments to the helper T cells.

2. Dendritic Cells:

Dendritic cells represent the widest range of antigen presentation and are very important for the activation of the naïve T cells. These dendritic cells are able to present antigen to both the helper T cells and cytotoxic cells. These cells are characterized by long cytoplasmic processes. Their primary role is to function as highly effective antigen-trapping and antigen presenting cells. These cells are nonphagocytic in nature. They are found in lymph nodes, spleen, thymus and skin. The different types of dendritic cells are:

(i) Langerhan's dendritic cells in epidermis of skin which trap the organisms coming in contact with body surface.

(ii) Dendritic cells in spleen, which trap the antigen in blood.

(iii) Follicular dendritic cells in lymph nodes which trap the antigen in the lymph.

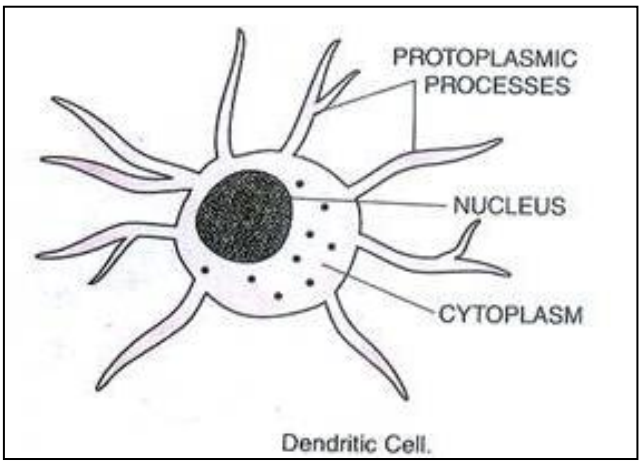

Fig: Dendritic Cell

Another important phenomenon of the dendritic cells is the ability to cross-presentation. In this process they can present exogenous antigens on MHC class I molecules to the cytotoxic T cells, which was normally destined for the helper T cells. They also serve important role in peripheral tolerance that contributes to auto-immune diseases.

In normal condition, the dendritic cells exhibit very low amount of MHC class II and co-stimulatory molecules on their cell surface. These are referred to as the immature

dendritic cells, which are incapable of presenting antigen to helper T cells. The activation of these immature dendritic cells require the recognition of a Pathogen Associated Molecular Pattern (PAMP) by the dendritic cell's Pattern Recognition Receptors (PRR). The activated dendritic cells then phagocytose the antigen and also upregulates the expression of MHC class II molecules and several co-stimulatory molecules, including CD40 and B7, required for T cell activation. The B7 complex can interact with CD28 on the surface of a helper T cell. Now, the fully mature and active dendritic cell moves from tissue to lymph nodes, where it encounters and activate T cells.

3. B-cells:

B-cells express on their surface intra-membrane immunoglobulin (Ig) molecules that function as B cell antigen receptors. Since all the receptors on a single B cell are identical, each B cell can bind only one antigen. This makes them much more efficient antigen-presenting cells than macrophages, which must ingest any foreign material that comes their way.

Descendants of B-cells (plasma cells) produce antibodies. B cells have the ability to internalize antigens that are recognized by the B cell receptors and present it to helper T cells. B cells can also recognize soluble antigens for which their B cell receptor is specific. After they internalize the antigens, they then process the antigens and present the peptides using the MHC class II molecules.

The T Cell Receptor (TCR) of helper T cell, specific for that peptide then bind to the peptide and the B cell marker CD40 binds to CD40L on the T cell surface. Moreover, a B cell can undergo antibody isotype switching, affinity maturation, formation of memory cells when it is activated bya T cell.

Besides this professional Antigen Presenting Cells, all nucleated cells of our body can serve as non-professional APCs as they can use MHC class I molecule coupled to β2 microglubulin to present the endogenous peptides on their cell membrane. The cytotoxic T cells are able to interact with these endogenous peptides bound to MHC class I molecule on the surface of the non-professional APCs.

MHC Molecules and MHC genes:

Immune system has elegant means of recognizing pathogens that have invaded or being taken into host cells as well as tumor antigens produced within cytosol. Proteins from within the cells are digested into short peptide fragments and they are displayed on the cell surface through binding to specialized antigen presenting molecules termed as MHC class I or MajorHistocompatibility Complex class I.

In a similar fashion, peptide derived from proteins ingested from external environment by phagocytosis, are presented by MHC class II molecules. These peptide-MHC

complexes serve as ligand for T cell receptors. The antigen processing and presentation pathway, upon which both activation and regulation of immune response rests, is a complex and fascinating subject.

Discovery of MHC in humans:

The proteins responsible for presenting antigen to T cells are MHC class I and II molecules, which are originally discovered as histocompatibility (transplantation) antigens. Histocompatibility refers to the ability to accept tissue graft from an unrelated donor. The MHC complex locus comprises over 100 separate genes and was discovered when it was recognized that both donor and recipient has to possess the same MHC haplotype to avoid graft rejection. The principal moieties that determine rejection were identified as MHC class I and class II but presently we know that the main purpose of MHC is not the graft rejection. The remaining genes (known as the MHC class III molecules) are sometimes very diverse. They generally encode complement system molecules, cytokines, enzymes and heat shock proteins and other essential molecules in antigen processing.

MHC class I:

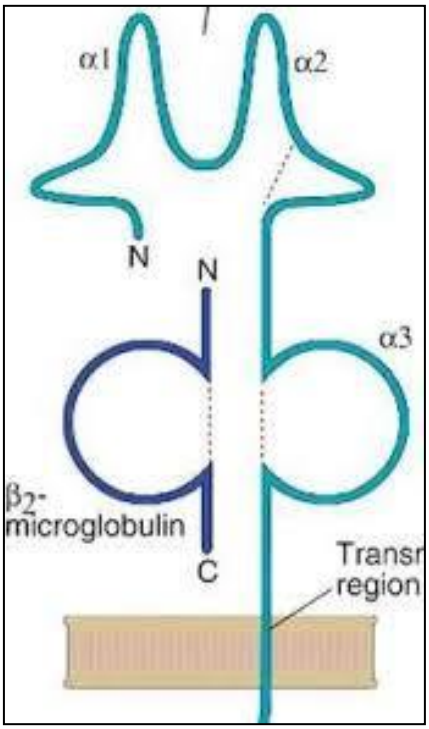

Fig: Structure of MHC I molecule

The MHC class I molecule handle intrinsic antigens and present antigens to cytotoxic T cells by recognizing CD8 marker on the T cells.

The molecule have glycosylated heavy chain of 45 KD. The heavy chains are non-covalently associated with β-microglobulin of 12 KD, which is a peptide also found free in serum. The three extracellular domains designated as α1, α2 and α3 are the main

constituents of the heavy chain. The molecule also possesses a transmembrane region and a cytoplasmic tail. The three extracellular domain comprise 90 amino acids. α2 and α3 domains have interchain disulphide bonds enclosing loops of 63 and 88 amino acids respectively. The α3 domain is structurally hmologus to the Immunoglobulin constant region and contain site that interacts with the CD8 domain of cytotoxic T cells. The extracellular portion of class I heavy chain is glycosylated and the glycosylation depends on the species and haplotype. B microglobulin domain is basically essential for expression of class I MHC molecule. The α1 and α2 components of the heavy chain of MHC class I forms the groove for binding antibody.

MHC Class II:

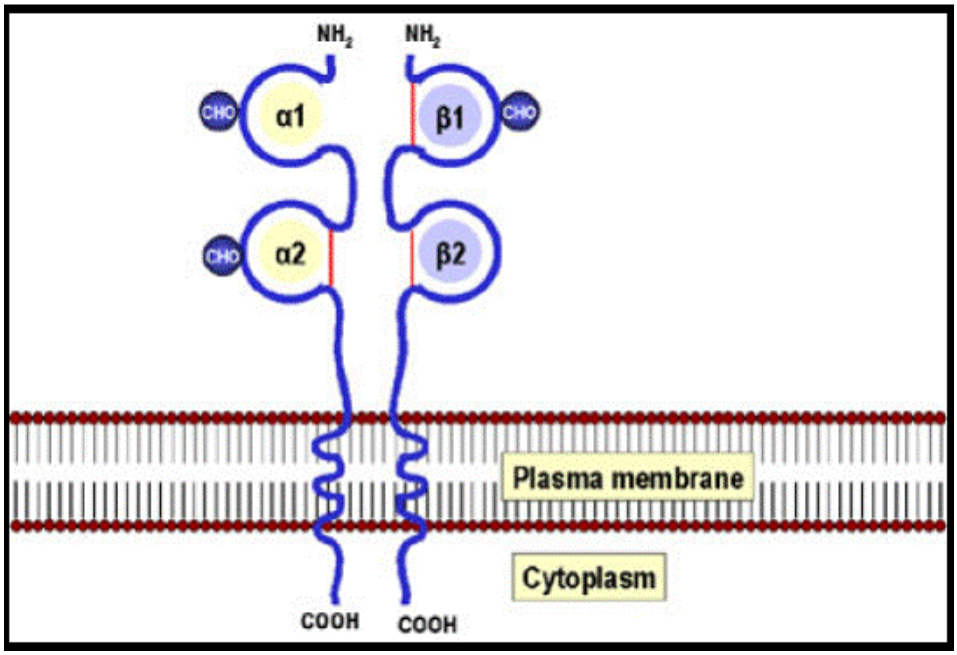

Fig: Structure of MHC II molecule

MHC class II binds to extrinsic antigen and present the antigen to helper T cell, which aid the B cells in generating antibody responses to extracellular protein antigens. The heavy chain of MHC class II consist of α1 and α2, which is a heterodimer and the light chain consist of β1 and β2. The α chains have molecular weight between 30-34 KD and the β chains range from 26-29 KD, depending on the locus involved. The structures of class I and class II MHC molecules are different and these differences are responsible for their functional differences. The binding groove of MHC class II molecule is more open than the binding groove of class I to accommodate longer peptide. On the other hand, MHC class I binds to short fragments of 8-10 amino acids, whereas class II binds to peptide of 13-24 amino acids.

The α and β chains of MHC class II molecules are associated by non-covalent interaction and like MHC class I molecules, the class II molecules are also membrane bound glycoprotein that contain an external domain, a transmembrane segment and the

cytoplasmic anchor segment. The overall 3D structure of MHC class II molecule is more or less similar to class I molecule and the nature of the peptide binding cleft differs from the class I molecule. The peptide binding cleft of the class I molecule is closed at both ends whereas the peptide binding cleft of MHC class II molecule is open at both ends. Moreover, in class I molecules, the peptide binding domain is between α1/α2 whereas in case of class II MHC molecules the peptide binding domain is between the α1/β1.

- **Antigen Processing and Presentation**

Recognition of foreign protein antigen by a T cell requires that peptides derived from the antigen be displayed within the cleft of an MHC molecule on the membrane of a cell. The formation of these peptide-MHC complexes requires that a protein antigen be degraded into peptides by a sequence of events called antigen processing. The degraded peptides then associate with MHC molecules within the cell interior, and the peptide-MHC complexes are transported to the membrane, where they are displayed (antigen presentation).

Class I and class II MHC molecules associate with peptides that have been processed in different intracellular compartments. Class I MHC molecules bind peptides derived from endogenous antigens that have been processed within the cytoplasm of the cell (e.g., normal cellular proteins, tumor proteins, or viral and bacterial proteins produced within infected cells). Class II MHC molecules bind peptides derived from exogenous antigens that are internalized by phagocytosis or endocytosis and processed within the endocytic pathway. This chapter examines in more detail the mechanism of antigen processing and the means by which processed antigen and MHC molecules are combined.

Processing of Antigen Is Required for Recognition by T Cells

The results obtained by K. Ziegler and E. R. Unanue were among those that contradicted the prevailing dogma that antigen recognition by B and T cells was basically similar. These researchers observed that TH-cell activation by bacterial protein antigens was prevented by treating the antigen presenting cells with para formaldehyde prior to antigen exposure. However, if the antigen-presenting cells were first allowed to ingest the antigen and were fixed with paraformaldehyde 1–3 h later, TH-cell activation still occurred. During that interval of 1–3 h, the antigen-presenting cells had processed the antigen and had displayed it on the membrane in a form able to activate T cells. Subsequent experiments by R. P. Shimonkevitz showed that internalization and processing could be bypassed if antigen- presenting cells were exposed to peptide digests of an antigen instead of the native antigen. In these experiments, antigen-presenting cells were treated with glutaraldehyde (this chemical, like paraformaldehyde, fixes the cell, making the membrane impermeable) and then incubated with native ovalbumin or with ovalbumin that had been subjected to partial

enzymatic digestion. The digested ovalbumin was able to interact with the glutaraldehyde-fixed antigen-presenting cells, thereby activating ovalbumin specific TH cells, whereas the native ovalbumin failed to do so. These results suggest that antigen processing involves the digestion of the protein into peptides that are recognized bythe ovalbumin-specific TH cells.

TABLE 8-1 Antigen-presenting cells

Professional antigen-presenting cells	Nonprofessional antigen-presenting cells	
Dendritic cells (several types)	Fibroblasts (skin)	Thymic epithelial cells
Macrophages	Glial cells (brain)	Thyroid epithelial cells
B cells	Pancreatic beta cells	Vascular endothelial cells

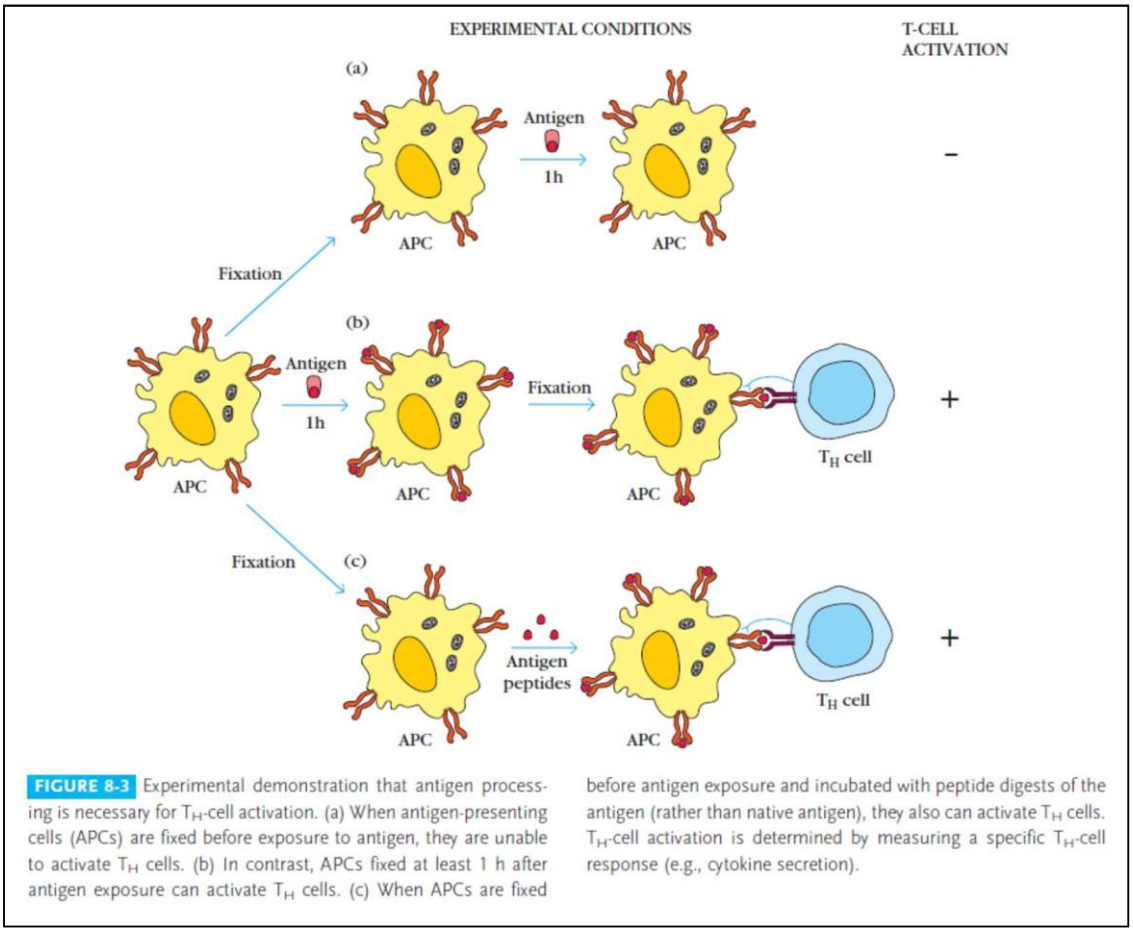

FIGURE 8-3 Experimental demonstration that antigen processing is necessary for T_H-cell activation. (a) When antigen-presenting cells (APCs) are fixed before exposure to antigen, they are unable to activate T_H cells. (b) In contrast, APCs fixed at least 1 h after antigen exposure can activate T_H cells. (c) When APCs are fixed before antigen exposure and incubated with peptide digests of the antigen (rather than native antigen), they also can activate T_H cells. T_H-cell activation is determined by measuring a specific T_H-cell response (e.g., cytokine secretion).

Most Cells Can Present Antigen with Class I MHC; Presentation with Class II MHC Is Restricted to APCs

Since all cells expressing either class I or class II MHC molecules can present peptides to T cells, strictly speaking they all could be designated as antigen-presenting cells. However, by convention, cells that display peptides associated with class I MHC molecules to CD8+ TC cells are referred to as target cells; cells that display peptides associated with class II MHC molecules to CD4+ TH cells are called antigen-presenting

cells (APCs). This convention is followed throughout this text.

A variety of cells can function as antigen-presenting cells. Their distinguishing feature is their ability to express class II MHC molecules and to deliver a co-stimulatory signal. Three cell types are classified as professional antigen-presenting cells: dendritic cells, macrophages, and B lymphocytes. These cells differ from each other in their mechanisms of antigen uptake, in whether they constitutively express class II MHC molecules, and in their co-stimulatory activity:

Dendritic cells are the most effective of the antigen presenting cells. Because these cells constitutively express a high level of class II MHC molecules and costimulatory activity, they can activate naive TH cells. Macrophages must be activated by phagocytosis of particulate antigens before they express class II MHC molecules or the co-stimulatory B7 membrane molecule.

B cells constitutively express class II MHC molecules but must be activated before they express the co-stimulatory B7 molecule. Several other cell types, classified as non-professional antigen- presenting cells, can be induced to express class II MHC molecules or a co-stimulatory signal (Table 8-1). Many of these cells function in antigen presentation only for short periods of time during a sustained inflammatory response. Because nearly all nucleated cells express class I MHC molecules, virtually any nucleated cell is able to function as a target cell presenting endogenous antigens to TC cells. Most often, target cells are cells that have been infected by a virus or some other intracellular microorganism. However, altered self-cells such as cancer cells, aging body cells, or allogeneic cells from a graft can also serve as targets.

Probable questions:

1. What are the properties of an antigen molecule?
2. What is an antigenic determinant?
3. What do you mean by hapten?
4. What is adjuvants?
5. Describe the structure of MHC I and MHC II molecule and state their functions.
6. MHC I molecule is expressed on the surface of all nucleated cells, whereas, MHC II isrestricted to APC- explain why?
7. Write short notes on APC.
8. What is the role of macrophage in humoral immunity?
9. What are the major antigen presenting cells present in the body?
10. What do you mean by Dendritic cell? Where these cells are located inside the body?

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UNIT VII

Antigen Recognition: Antigen Receptor: T and B cell Receptor, Structure of Immunoglobulin and T-cell receptor

Objective:

In this unit, we will discuss about the Antigen Recognition: Antigen Receptor: T and B cell Receptor, Structure of Immunoglobulin and T-cell receptor.

Introduction:

Immune system of our body is important and specially designed to recognize and respond to the variety of antigens. Broadly there are two ways of tackling with the antigen, that is by innate and the adaptive mechanism. Adaptive immune mechanism is initiated after the effort of innate immune responses towards the pathogen is almost over. Adaptive response starts late but lasts for a longer period. The main reason for the late onset is its very precise and species specific recognition of antigen unlike that of innate system where it responds to the general molecular patterns on the bacteria, virus, or other pathogens when they enter the body. The specificity and diversity in adaptive response is attributed to the presence of receptors on B and T lymphocytes as BCR or B cell receptor and TCR or T cell receptors. BCR recognizes the exogenous antigen but TCR recognizes the endogenous antigen complexed with MHC (Major Histocompatibility Complex) present on the membrane of all the nucleated cells. The antigen properties thus differ for each of the receptor and accordingly there are structural variations in their recognition units which consist of BCR, TCR, co receptors and other membrane associated accessory molecules. The recognition unit is a complex of receptors and co-receptors acting as transducer and enhancer to improve the binding ability with the antigen finally resulting in lymphocyte activation. The different structure of the two types of receptors on lymphocytes and the antigens they recognize is dealt with in this module.

- **Recognition Unit of Lymphocytes**

The B and T lymphocytes are the two specialized cells of the adaptive immunity deputed to recognize and eliminate the intruders of the body in different ways. They vary in the nature of antigen they encounter during surveillance before they mount the immune response (Figure 1) as the antigen can be endogenous or exogenous. The specific recognition of the antigen is the most important function of the adaptive immunity which is attributed to the membrane receptors on the two lymphocytes.

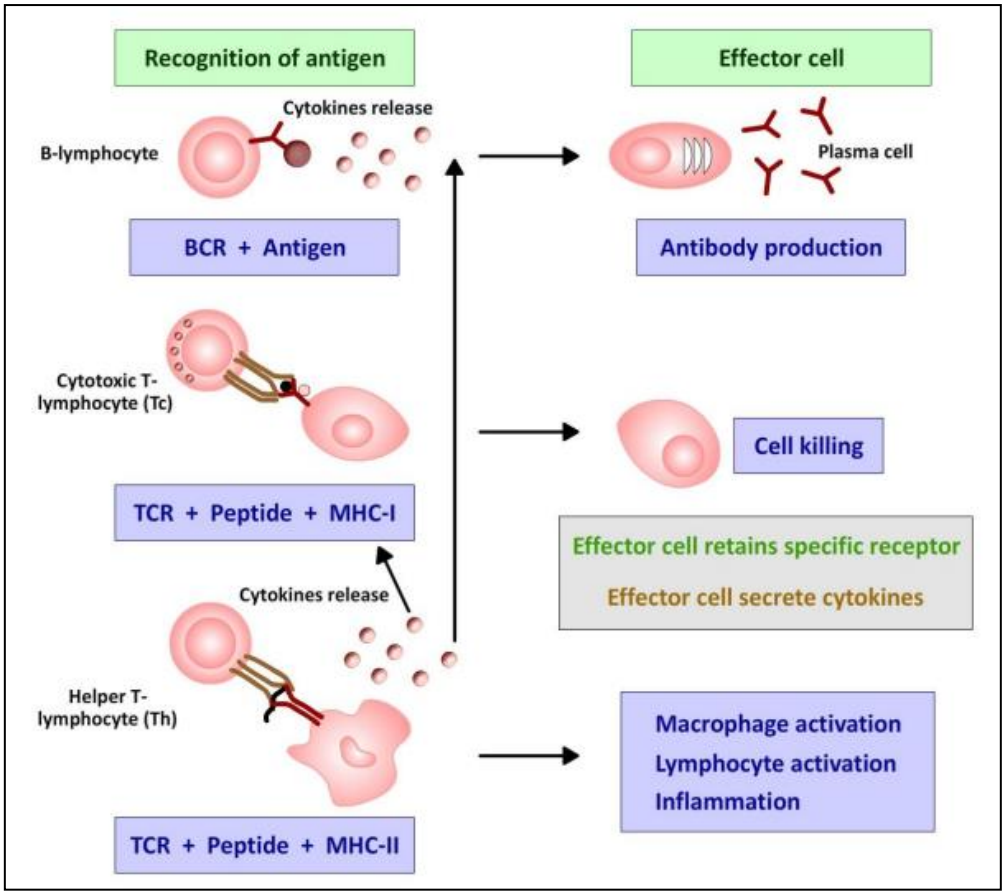

Fig 1: Recognition and Response of different lymphocytes

The receptors on the B cells are called B cell receptors or BCR and those located on the T cells are called T cell receptors or TCR. The BCR recognises exogenous antigen and forms antibody as the effector molecule after signal from T_H/T helper cell through the release of cytokines. The Tc/Tcytotoxic lymphocyte cell when recognizes the antigen complexed with MHCI causes cytotoxicity by converting to CTL (Cytotoxic T Lymphocyte) but the T_H cell complexed with MHCII present on the membranes of APC/ antigen presenting cells, release the cytokines for activation of lymphocytes, macrophage and inflammatory responses (Figure 1) The recognition is followed by response of lymphocytes to the antigen by producing effector molecules (antibody) or effector cells (CTL).The information of the receptors is transduced with the help of membrane co- receptors and the co -stimulatory molecules in the cell interior by protein kinases resulting in cell activation.

- **Structure of BCR (B cell Receptor)**

The BCR complex has two structurally and functionally different components working as a unit. They are BCR or mIg (membrane antibody) and heterodimer of α (alpha) and β (beta) peptide chains (Figure 6). mIg is for the recognition of antigen and αβ heterodimer is for signal transduction. The two functions when performed separately

give the advantage of using the same cellular machinery for transduction on recognizing the variety of antigens.

I. BCR or mIg

Membrane antibody has a monomeric Y shaped structure composed of four polypeptide chains and an anchoring unit so as to to fix it on the lipid rafts of the plasma membrane along with the other accessory molecules required for signal transduction. The IgM and IgD are the membrane receptors where the CH4 Domain of IgM is hydrophobic to insert into the B cell membrane. The Fab component is oriented outside the cell and Fc is noncovalently involved in signal transduction by interacting with co-receptors and accessory molecules. The cytoplasmic tail of membrane antibody/ Fc is insufficient to transduce the signal on antigen recognition (Figure 2).Lipid rafts of the plasma membrane provide a platform for selective interaction of the mIg with the signal molecules which carry the information to the cytoplasm for activation of B lymphocytes. The recognition of antigen requires the antibody crosslinking or clustering which initiates the process of B cell activation. The Y shaped membrane antibody structure has variable light, and heavy chains (VL and VH) as discussed earlier, so variety of antigens are specifically identified and B cell is activated.

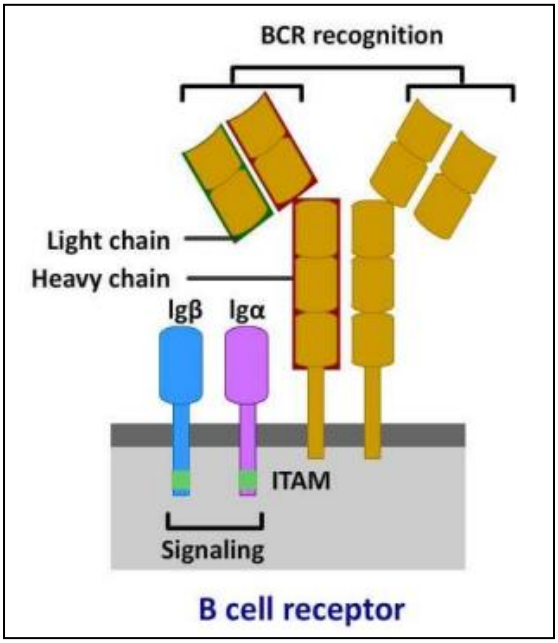

Fig 2: Structure of BCR

II. αβ Heterodimer

It consists of two polypeptide chains alpha and beta which have a long cytoplasmic tail. The cytoplasmic tail of Igα is made of 61 amino acids but that of Igβ has 48 amino acids. If we compare the tail length of mIgM with other secretory antibodies, IgA has 14 aminoacids, IgG and IgE contain 28 amino acids, much shorter than the cytoplasmic tail length of αβ heterodimer. The cytoplasmic tail of the heterodimer has important component ITAM (Immunoreceptor Tyrosine Based Activation motif) which activates enzyme Tyrosine kinase. When an antigen binds, the Tyrosine in these ITAMs becomes

phosphorylated by Tyrosine kinases which are important for transmitting the signal. The efficiency of B cell signal is also increased by the complement activation.

- ## Structure of TCR (T Cell Receptor)

Introduction

T lymphocytes or T cells respond only to peptide fragments of protein antigens that are displayed by self-MHC molecules (major histocompatibility complex). T cell receptor or TCR differs from the B cell receptor in two important ways.

First, the T cell receptor is membrane bound and does not appear in a soluble form as the B cell receptor does; second, the T cell receptor is specific not for antigen alone but for antigen combined with a molecule encoded by MHC.

Further, the T cell receptor remains associated on the membrane with a signal-transducing complex CD3 which is non-covalently linked to the receptor to form the TCR complex. The TCR is a clonally distributed receptor, meaning that clones of T cells with different specificities express different TCRs.

The biochemical signals that are triggered in T cells by antigen recognition are transduced not by the TCR itself but by TCR complex. T cells also express other membrane receptors that do not recognise antigen but participate in responses to antigens; these are collectively called accessory molecules. These molecules deliver signals to the T cell that function in concert with signals from the TCR complex to fully activate the cells.

Antigen recognition by T cells is specific not only for antigen but also for an MHC molecule. T cells were shown to recognise antigen only when presented on the membrane of APC (antigen presenting cell) by a self-MHC molecule. This attribute called self-MHC restriction, distinguishes of antigen recognition by T cells from that by B cells.

Structure of T Cell Receptors:

The antigen receptor of MHC restricted CD4+ helper T cells and CD8+ cytotoxic T cells is a heterodimer consisting of two trans-membrane polypeptide chains. These chains are designated as α and β which are covalently linked to each other by disulfide bonds (Fig. 3). Another group of TCR, found on a small subset of T cells, has y and δ chains.

In the amino terminal, each a chain and β chain consists of the Ig-like variable domain (V), one constant domain (C), hydrophobic trans-membrane region and a short cytoplasmic region. Thus, the extracellular portion of αβ heterodimer in TCR is structurally similar to the antigen binding fragment (Fab) of an Ig molecule.

The α and β chains of V regions of TCR contain short stretches of amino acids where the hyper-variable or complementarity determining regions (CDRs) are located. Three such CDRs in the α chain are juxtaposed to three similar regions in the β chain to form the part of the TCR that specifically recognises peptide-MHC complexes.

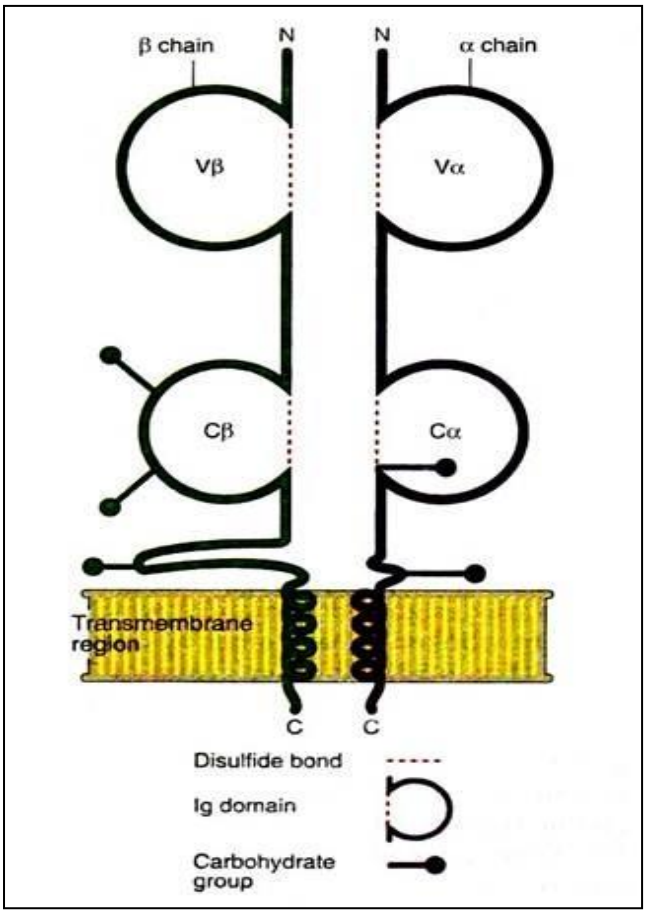

Fig 3: Structure of the T cell receptor. The schematic diagram of the αβ TCR (left) shows the domains of a typical TCR specific for a peptide-MHC complex. The antigen-binding portion of the TCR is formed by the V_α and V_β domains

The C regions of both α and β chains continue into short hinge regions, which contain cysteine residues that contribute to a disulfide bond linking the two chains.

The hinge is followed by a hydrophobic trans membrane portion of 21 or 22 amino acids.

CDRs of T Cell Receptor and their Role in MHC-associated Peptide Recognition:

The affinity of the TCR for peptide-MHC complex is very low. Such low affinity of specific antigen binding is the likely reason that accessory molecules are needed to stabilise the adhesion of T cells to APCs. This structure regulates the TCR-mediated signal transduction. Virtually all αβ TCR-expressing T cells are MHC restricted and express either the CD4 or the CD8 co-receptors. A small population of T cells also expresses markers that are found on NK (natural killer) cells; these are called NK-T cells.

T Cell Receptor Complex: TCR CD3:

T cell receptor and another protein CD3 are located quite close together in the T cell plasma membrane (within 1-1.5 nm of each other).

The expression of CD3 molecule is required for membrane expression of αβ and yδ T cell receptors; thus each heterodimer forms a complex with CD3 on the T cell membrane. Loss of genes encoding either CD3 or TCR chains results in the loss of the entire molecular complex from the membrane.

CD3 is a complex of five invariant polypeptide chains that associate to form three dimers: a heterodimer of gamma and epsilon chains (γε), a homodimer of delta and epsilon chains (δε), and a heterodimer of two zeta chains (ζζ) or a heterodimer of zeta and eta chain (ζη) (Fig. 4).

The ζ and η chains, though encoded by the same gene, may differ in their carboxyl terminal ends because of differences in RNA splicing of the primary transcript. About 90% of the CD3 complexes examined to date have ζζ homodimer than heterodimer ζη as possessed by rest 10% of CD3 complexes.

In CD3 complex, the y, δ and ε chains belong to immunoglobulin superfamily, each containing an extracellular domain followed by a trans membrane region and a cytoplasmic domain of more than 40 amino acid residues.

The zeta and eta (ζ and η) chains have quite different structure, each with a very short extracellular region of only 9 amino acids, a trans membrane region and a long cytoplasmic tail of 113 amino acids in ζ chain and 155 amino acids in η chain.

The trans membrane segment of all CD3 polypeptide chains contains a negatively charged amino acid residue of aspartic acid. Such residues enable the CD3 complex to interact with one or two positively charged amino acid residues in the trans membrane segment of each TCR chain.

The cytoplasmic domains of CD3 Y, ε and δ chains contain one copy of a conserved sequence motif called the immuno-receptor tyrosine-based activation motif (ITAM) in each chain. An ITAM contains two copies of the sequence tyrosine-X-X-leucine (X is an unspecified amino acid) separated by six to eight residues.

ITAM plays a central role in signaling by TCR complex. They are also found in ζ chain of the TCR complex, Iga and IgP proteins associated with membrane Ig molecules of B cells.

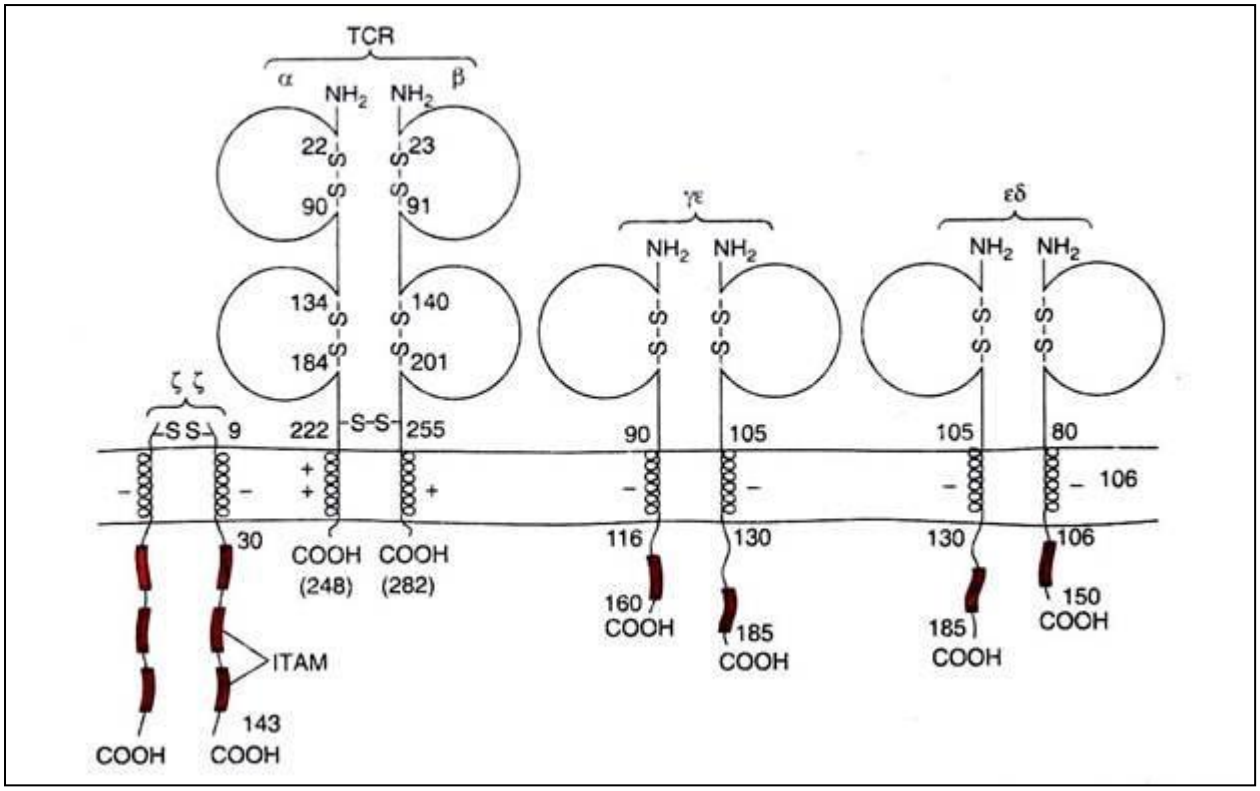

Fig 4: Schematic diagram of the TCR-CD3 complex, which constitutes the T-cell antigen-binding receptor. The CD3 complex consists of the ζζ homodimer (alternately, a ζη heterodimer) plus γε and δε heterodimers. The external domains of the γ, δ, and ε chains of CD3 are similar to the immunoglobulin fold, which facilitates their interaction with the T-cell receptor and each other. The long cytoplasmic tails of the CD3 chains contain a common sequence, the immunoreceptor tyrosine-based activation motif (ITAM), which functions in signal transduction

Functions of CD3 complex:

The CD3 and ζ chains link antigen recognition by the TCR to the biochemical events that lead to functional activation of the T cells. The earliest intracellular event that occurs in T cells after antigen recognition is the phosphorylation of tyrosine residues within the ITAMs in the cytoplasmic tails of CD3 and ζ proteins.

This phosphotyrosines then become the docking sites for adapter proteins and for tyrosine kinase with a kinase called ZAP-70 that binds to the ζ chain. Another kinase that also docks at phosphotyrosine is Fyn that binds to CD3. Subsequent activation of these kinases triggers signal transduction pathway that ultimately lead to changes in gene expression in the T cells.

γδ TCR:

In a few T cell populations, a second type of diverse, disulfide linked heterodimer γδ TCR receptor is expressed instead of αβ TCR. This receptor also remains associated with CD3 and ζ proteins. (This γδ TCR should not be confused with the γ and δ chains of CD3 complex). The TCR γ and δ chains contain extracellular V and C domains, short

connective or hinge regions, hydrophobic trans-membrane segments and short cytoplasmic tails.

The constituents of these segments are almost similar to those of α and β chains. Furthermore, TCR – mediated signaling events typical of αβ expressing T cells are also observed in yδ T cells. However, majority of yδ T cells do not express CD4 or CD8.

The percentages of yδ TCR expressing T cells vary widely in different tissues and species, but overall, less than 5% of all T cells express this receptor. T cells with yδ TCR are a lineage distinct from the αβ-expressing MHC- restricted T cells.

Many δy T cells present in different organs, may have different V regions, indicating that these subsets may be specific for different ligands. One intriguing feature of yδ T cells is their abundance in epithelial tissue of certain species (e.g., small bowel mucosa of mice and chicken). In human only about 10% of intestinal intra-epithelial T cells express the yδ receptors.

The function of the yδ T cells is not clear. yδ T cells do not recognise MHC-associated peptide antigens and are not MHC restricted. Some can recognise small phosphorylated molecules, alkyl amines or lipids that are commonly found in association with "non-classical" class I MHC-like molecules in mycobacteria and other microbes.

Others may recognise protein or nonprotein antigens that do not require processing or any particular type of APCs for their presentation. Some suggest that they may initiate immune responses to a small number of common microbes that frequently encounter at epithelial boundaries between the host and the external environment.

Accessory Molecules of T Cell Receptor:

T cells express several integral membrane proteins that play important role in antigen recognition and T cell activation. Some of these molecules strengthen the interaction between T cells and antigen presenting cells or target cells; some act in signal transduction and some do both. These protein molecules are often collectively called accessory molecules.

(i) CD4 and CD8:

Mature αβ T cells express either CD4 or CD8 membrane protein, but not both. CD4 and CD8 interact with class II and class I MHC molecules, respectively, when the antigen receptor of T cells specifically recognise peptide-MHC complexes on APCs.

Both CD4 and CD8 are trans membrane glycoprotein members of the Ig superfamily, with similar functions but different structures. CD4 is a 55-kDa monomeric protein that contains four extracellular domains (D_1-D_4), a hydrophobic trans membrane segment and a long cytoplasmic tail of 38 basic amino acids (Fig. 6.60). It binds through its two N-terminal domains to non-polymorphic β_2 domain of the class II MHC molecule.

- **Functions of CD4 and CD8:**

CD4 binds to class II MHC molecules and is expressed on T cells whose TCRs recognise complexes of peptide and class II MHC molecules. Most $CD4^+$ class II-restricted T cells are cytokine-producing helper cells (TH cells) and function in host defence against extracellular microbes. CD8 binds to class I MHC molecules and is expressed on T cells whose TCRs recognise complexes of peptide and class I MHC molecules.

Most $CD8^+$ class I-restricted T cells are CTLs (cytotoxic T lymphocytes) which serve to eradicate infections by intracellular microbes. However, in humans, some $CD4^+$ T cells may function as CTLs, but even these are class II restricted. Thus, expression of CD4 or CDS determines the MHC restriction of the T cells and not their functional capabilities.

CD4 and CD8 participate in the early signal transduction events that occur after T cell recognition of peptide MHC complexes on APCs. This signal transduction is mediated by a T cell specific Src family tyrosine kinase called Lak that is non-covalently but tightly associated with cytoplasmic tails of both CD4 and CD8. This kinase is also required for T cell maturation and activation.

CD4 and CDS promote the adhesion of MHC-restricted T cells to APCs or target cells expressing peptide MHC complexes. However, in such strengthening function, both co-receptors need the help of other accessory molecules. The CD4 act as a receptor for the human immunodeficiency virus.

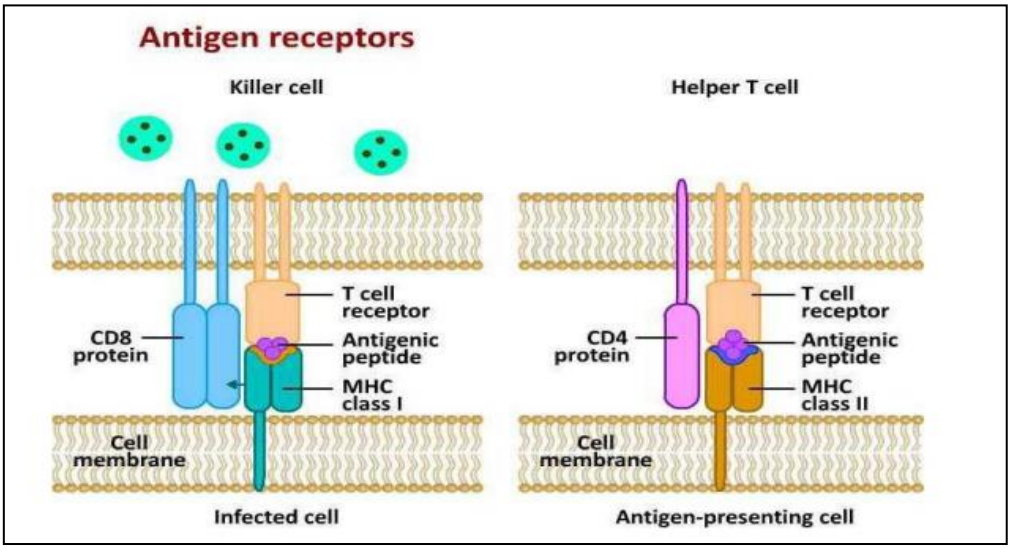

Fig 4: Antigen receptors and T lymphocytes

- **Structure of Immunoglobulin**

IgG has been studied extensively and serves as a model of basic structural unit of all Igs. An antibody molecule consists of the following parts.

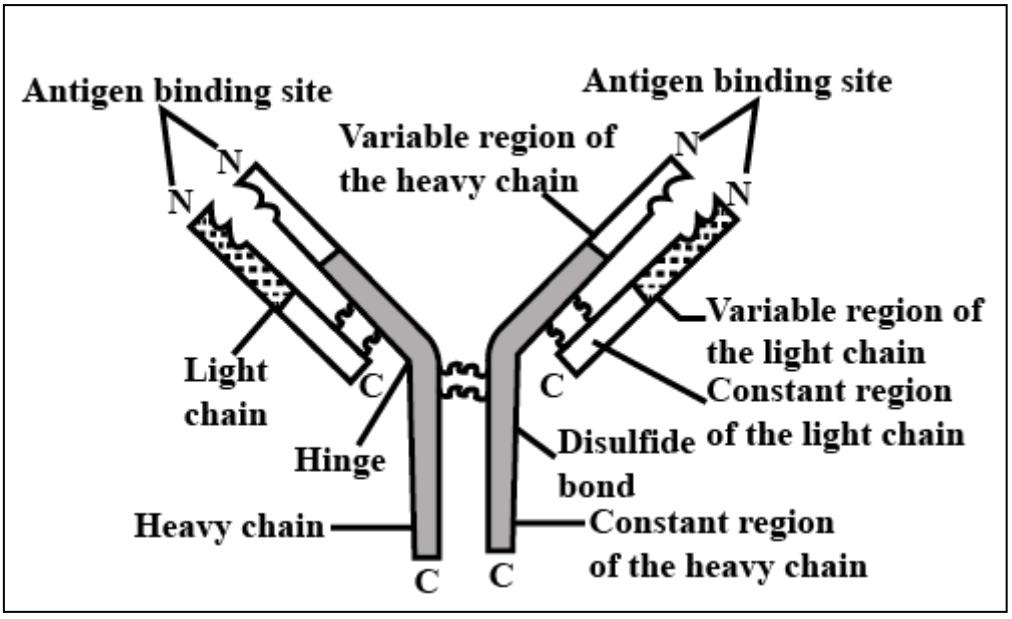

Fig 5: Structure of antibody

(v) Heavy and Light Chains:

An antibody molecule is made up of 4 peptide chains, two small called light chains and two longer called heavy chains. Hence an antibody is represented as H_2L_2. The heavy chain has larger number of amino acids while light chain has smaller number of amino acids. Heavy and light chains may be either lambda or Kappa type.

(vi) Constant and Variable Regions:

There are two different regions the constant region and variable region in each chain of the antibody.

(vii) Disulfide Bonds and Hinge Region:

A disulfide bond joins a light chain with a heavy chain. Two disulfide bonds also link the two heavy chains. This part of the antibody displays considerable flexibility and is called the hinge region. Because the antibody "arms" can move somewhat as the hinge region bends, an antibodycan assume a Y shaped molecule.

(viii) Fragment Antigen Binding (Fab) and Fragment Crystallisable (Fc):

Two identical fragments of Y-shaped molecule possess the antigen-binding sites and is thus named fragment-antigen binding (Fab). The antigen-binding sites bind to the specific antigens in a lock and key pattern, forming an antigen-antibody complex. The third fragment which lacks the ability to bind to antigen and can be crystallized, is, therefore, known as fragment crystallizable (Fc).

The stem of the Y-shaped antibody monomer is called the F_c region, so named because when antibody structure was first being identified, it was a fragment (F) that crystallized (c) in cold storage.

Probable questions:

8. Elaborate the structure of BCR with diagram?
9. Describe the structure of TCR with diagram
10. What are the differences between BCR and TCR?
11. Describe the structure of immunoglobulin.
12. Describe the function of CD4+ T cell

Suggested readings/ references:

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6. Delves, Peter J.; Martin, Seamus J.; Burton, Dennis R.; Roitt, Ivan M. 2011. Roitt'sEssential Immunology. Hoboken, NJ: Wiley-Blackwell.
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UNIT VIII

Antigen Receptor Diversity-Mechanism, Antigen Receptor Maturation and selection

Objective:

In this unit, we will discuss about Antigen Receptor Diversity-Mechanism, Antigen Receptor Maturation and selection.

Introduction:

Each lymphocyte has an antigen receptor of a single specificity, which is determined by genetic mechanisms during lymphocyte development in the bone marrow and thymus. This is known as Antigen Receptor Diversity. These genetic mechanisms generate millions of different variants of the genes that encode the antigen receptors.

✓ Antigen Receptor Diversity-Mechanism

- Virtually all microbes can trigger an antibody response. Successful recognition and eradication of many different types of microbes requires diversity among antibodies, a result of variation in amino acid composition that allows them to interact with many different antigens.
- Antibodies obtain their diversity through 2 processes. The first is called V(D)J (variable, diverse, and joining regions) recombination. During cell maturation, the B cell splices out the DNA of all but one of the genes from each region and combine the three remaining genes to form one VDJ segment.
- The second stage of recombination occurs after the B cell is activated by an antigen. In these rapidly dividing cells, the genes encoding the variable domains of the heavy and light chains undergo a high rate of point mutation, by a process called somatic hypermutation.
- As a consequence of these processes any daughter B cells will acquire slight amino acid differences in the variable domains of their antibody chains. This serves to increase the diversity of the antibody pool and impacts the antibody's antigen-binding affinity.
- Point mutations can result in the production of antibodies that have a lower or higher affinity with their antigen than the original antibody. B cells expressing antibodies with a higher affinity for the antigen will outcompete those with weaker affinities (called affinity maturation).
- **Somatic hypermutation**: a cellular mechanism by which the immune system adapts to the new foreign elements that confront it (for example, microbes). A

major component of the process of affinity maturation, SHM diversifies B cell receptors used to recognize foreign elements (antigens) and allows the immune system to adapt its response to new threats during the lifetime of an organism.

- **V(D)J recombination**: Also known as somatic recombination, this is a mechanism of genetic recombination in the early stages of immunoglobulin (Ig) and T cell receptors (TCR) production of the immune system.

It is the variety in their amino acid composition that allows them to interact with many different antigens. It has been estimated that humans generate about 10 billion different antibodies, each capable of binding a distinct epitope of an antigen. Although a huge repertoire of different antibodies is generated in a single individual, the number of genes available to make these proteins is limited by the size of the human genome. Several complex genetic mechanisms have evolved that allow vertebrate B cells to generate a diverse pool of antibodies from a relatively small number of antibody genes.

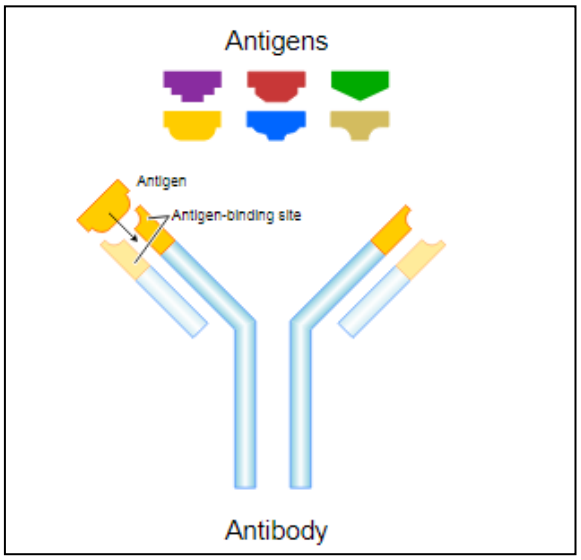

Fig 1: Antibodies bind to specific antigens: Schematic diagram of an antibody and antigens. Light chains are in lighter blue and orange, heavy chains in darker blue and orange. Each antibody binds to a specific antigen; an interaction similar to a lock and key.

- **Antibody Structure**

Antibodies (or immunoglobulin's; we will use the two terms interchangeably) are proteins secreted by B lymphocyte-derived plasma cells in response to the appearance of infectious agents in the body's tissues.

Any substance capable of triggering the production of antibodies is called an antigen, and although antigens can take a variety of different chemical forms, they usually are proteins, polysaccharides, or nucleic acids.

Ag-Ab reaction is the reaction that takes place between antibodies and the foreign antigens that leads to the elimination of the antigen and its source. This reaction is highly specific, that is, a particular antibody usually reacts with only one type of antigen.

Antibodies are typically made of basic structural units—each with two large heavy chains and two small light chains. IgG molecules (Basic antibody molecule) are composed of four polypeptide chains of two different sizes; these are a pair of identical high- molecular-weight chains called heavy chains (or H chains) and a pair of identical low-molecular-weight chains called light chains (or L chains). There are several different types of antibody heavy chains, and several different kinds of antibodies, which are grouped into different isotypes based on which heavy chain they possess.

The H chains have a molecular weight of 50,000 to 55,000 and contain about 450 amino acids, whereas the L chains have a molecular weight of 20,000 to 25,000 and consist of about 214 amino acids. Each L chain is covalently linked to an H chain by a disulfide bridge, and two light chain-heavy chain pairs are covalently linked by two disulfide bridges (Fig. 25-2). There are also 12 intra chain disulfide bridges, four in each H chain and two in each L chain. An asparagine residue in each H chain is bonded to carbohydrate, so immunoglobulin's are also glycoproteins.

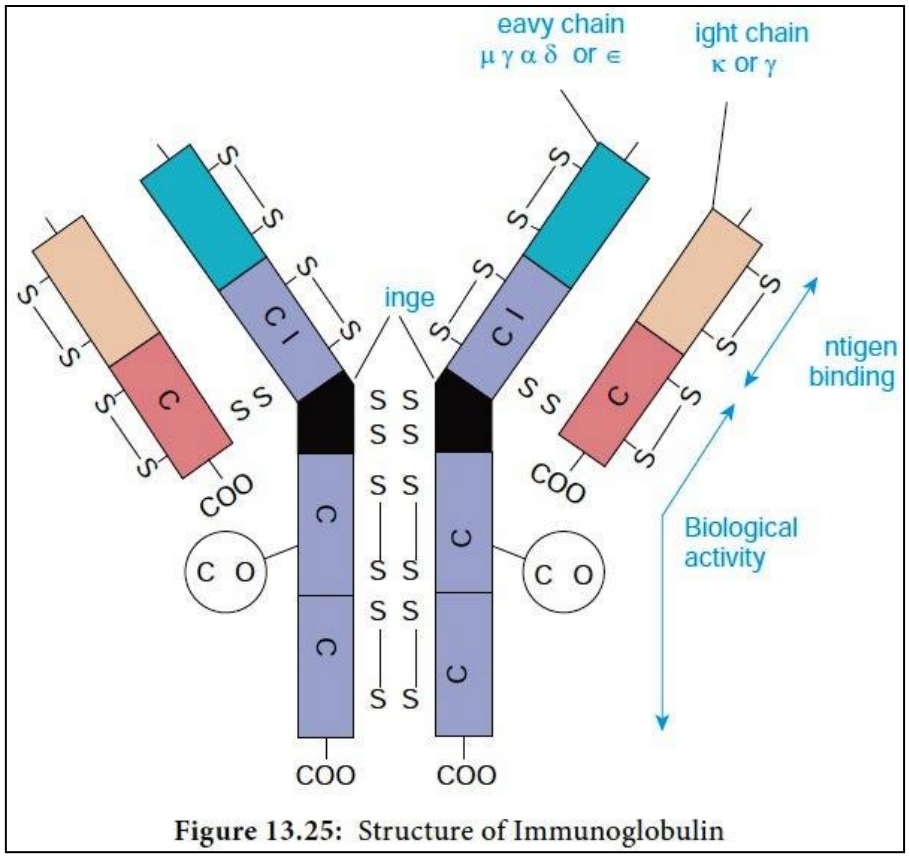

Figure 13.25: Structure of Immunoglobulin

Fig 2: Structure of immunoglobulin

There are two different types of light chains found in immunoglobulin's; they are designated kappa (κ) chains and lambda (λ) chains. The heavy chains of

immunoglobulin's belonging to the IgG class are of the gamma (γ) type, so that an IgG molecule may be represented as either k_2y_2 or λ_2y_2, depending on the light chains that are present. Like IgG molecules, human immunoglobulin's belonging to one of the other four classes also possess k or λ light chains.

However, the heavy chains of immunoglobulin A molecules (IgA) are alpha (α) chains; in IgD, they are delta (δ) chains; in IgE, they are epsilon (ε) chains; and in IgM, they are mu (μ) chains. IgG, IgD, and IgE occur as monomers, but IgA molecules may occur as monomers, dimers, or trimers. IgA dimers are held together by another polypeptide called a J chain ("J" for "joining"). IgG molecules may also be linked by a second component called secretory component. IgM molecules occur as pentamers in which the monomeric units are held together by disulfide bridges and by a J chain.

- **Constant and Variable Domains of an Immunoglobulin:**

An analysis of the primary structures of isolated immunoglobulin's has revealed that they have certain amino acid sequences in common and certain sequences that differ. Amino acid sequences that are common are called constant domains, whereas sequences that vary are called variable domains. Each L chain has one constant and one variable domain, respectively designated C_L and V_L (Fig. 25-4). Because there are two forms of L chains, namely, k and λ, there are also two different constant domains (i.e., constant regions common to k chains and constant regions common to λ chains).

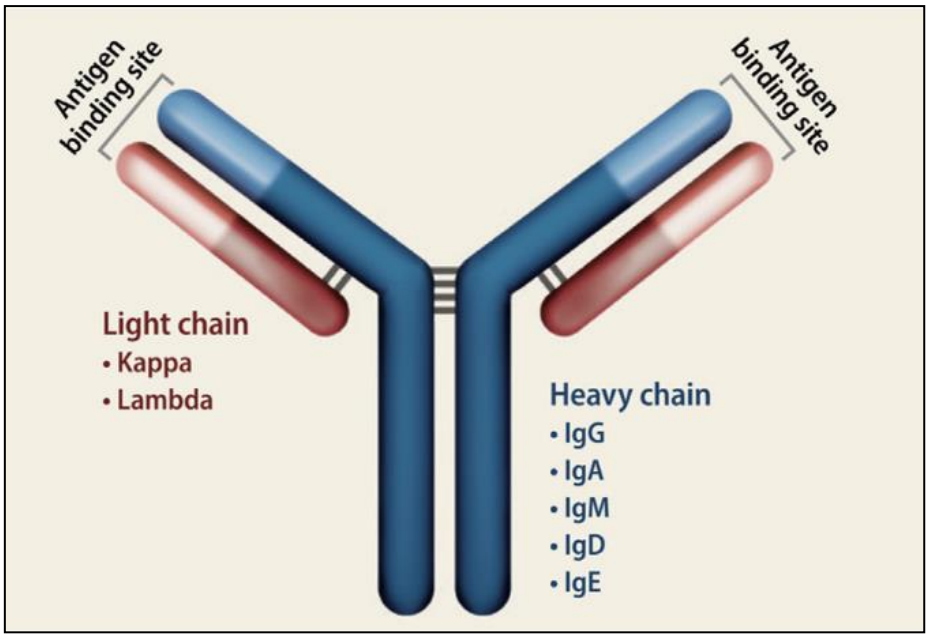

Fig 3: Different types of light and heavy chains in immunoglobulin

Each H chain has one variable sequence (V_H) and three constant regions (C_H1, C_H2, and C_H3). It is now known that the three constant regions of the H chains and the hinge are encoded by separate exons

Five different antibody isotypes are known in mammals, which perform different roles, and help direct the appropriate immune response for each different type of foreign object they encounter.

Though the general structure of all antibodies is very similar, a small region at the tip of the protein is extremely variable, allowing millions of antibodies with slightly different antigen binding sites to exist. This region is known as the hypervariable region, each containing 5 to 10 amino acids. Each of these variants can bind to a different antigen. This enormous diversity of antibodies allows the immune system to recognize an equally wide variety of antigens.

- **Antibody Diversity and the Genome:**

k and λ light chains and α, δ, ϵ y, and μ heavy chains are encoded in separate gene pools, each gene pool containing sets of different C genes, V gene segments, and J gene segments ("J" = "joining"). The k gene pool contains a C gene (C,) and a large number (perhaps several hundred) of V genes (V_k1, V_k2, V_k3 . . . V_kn).

The λ gene pool also contains a C gene (C_λ) and several V genes ($V_\lambda1$, $V_\lambda2$, etc.). The heavy chain gene pool contains many V genes (V_H1, V_H2, V_H3 ... V_Hn) and a sequence of C genes (C_μ, C_δ, C_y, C_ϵ, and C_α) (Fig. 25-6). Each V gene is more appropriately called a V gene segment, because it does not encode an entire variable domain of a chain; this is because there is a small "missing" stretch of DNA called a J gene segment that must be connected to a V gene segment to form a complete V gene.

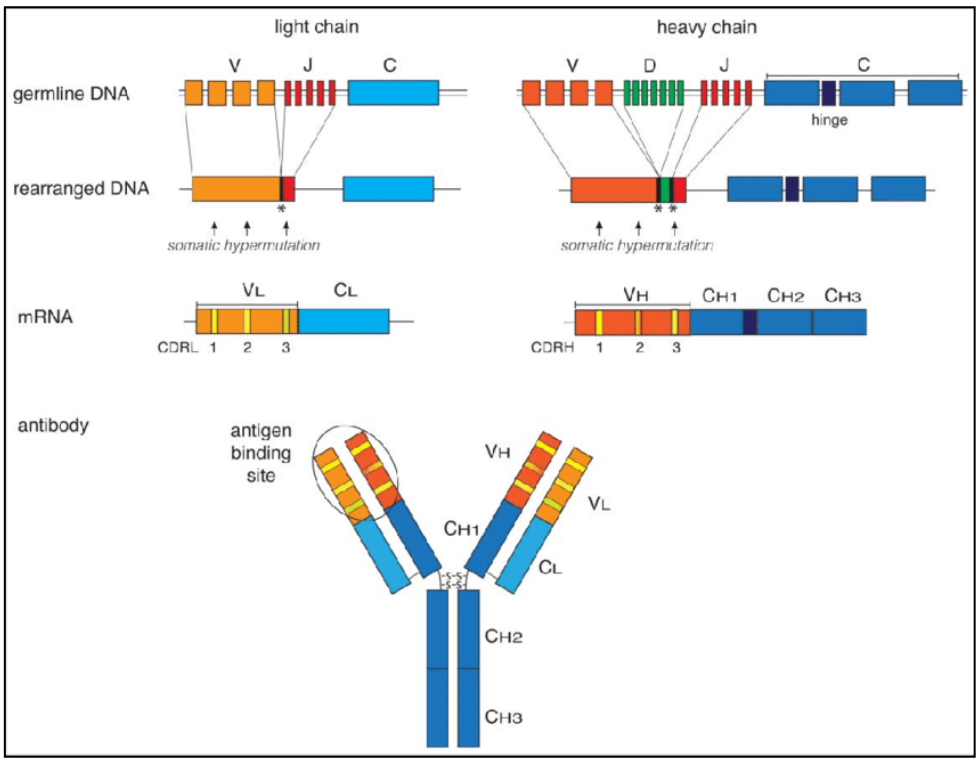

Fig 4: Gene structure of an antibody

Each light and heavy chain gene pool contains four J gene segments. The V gene segments are located hundreds of thousands of nucleotides upstream (i.e., on the 5′ side) of the C gene, whereas the J gene segments are located only a short distance upstream of the C gene and are separated from one another by introns. The assembly of genes expressible as complete variable domains of L chains occurs during the differentiation of lymphocytes into antibody-producing cells and involves the translocation of a V gene segment so that it comes to lie next to a J gene segment. Any V gene segment can be connected to any J gene segment. Primary transcripts may include more than one J gene segment and intron. However, during RNA processing all introns and all J gene segments other than the one whose 5′ side contains the trans located V gene segment are removed.

So, Antibodies obtain their diversity through two processes:

- **V(D)J Recombination**

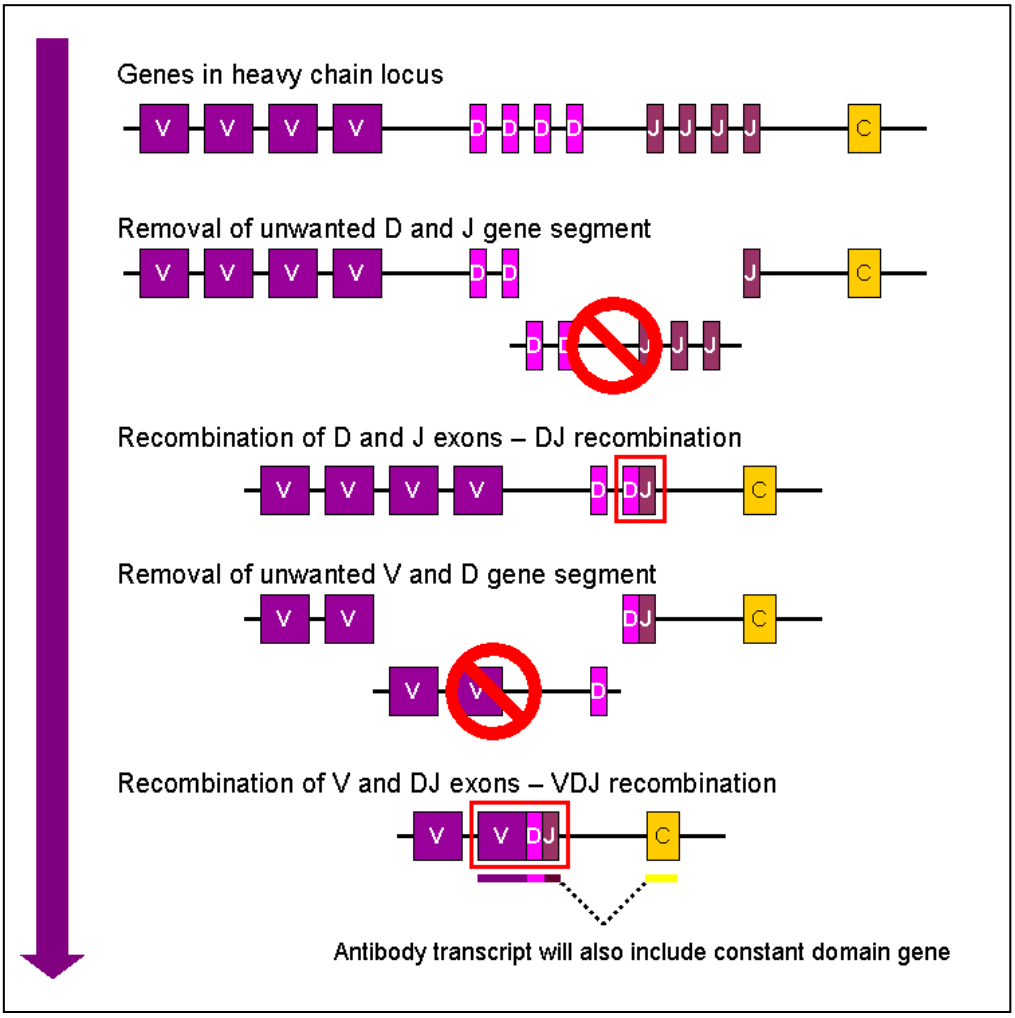

Fig 5: Diagramatic representation of V(D)J Recombination

The first stage is called somatic, or V(D)J, which stands for variable, diverse, and joining regions recombination. Several sets of genes are located within each of the three regions. During cell maturation, the B cell will splice out the DNA of all but one of the

genes from each region and combine the three remaining genes together to form one VDJ segment. This segment, along with a constant region gene, forms the basis for subsequent antibody production.

It is estimated that given the number of variants in each of the three regions, approximately 10,000-20,000 unique antibodies are producible. V(D)J recombination takes place in the primary lymphoid tissue (bone marrow for B cells, and thymus for T cells) and nearly randomly combines variable, diverse, and joining gene segments. It is due to this randomness in choosing different genes that it is able to diversely encode proteins to match antigens.

- **Somatic Hypermutation**

The second stage of recombination occurs after the B cell is activated by an antigen. In these rapidly dividing cells, the genes encoding the variable domains of the heavy and light chains undergo a high rate of point mutation, by a process called somatic hypermutation (SHM). SHM is a cellular mechanism by which the immune system adapts to the new foreign elements that confront it and is a major component of the process of affinity maturation. SHM diversifies B cell receptors used to recognize antigens and allows the immune system to adapt its response to new threats during the lifetime of an organism. Somatic hypermutation involves a programmed process of mutation affecting the variable regions of immunoglobulin genes. SHM results in approximately one nucleotide change per variable gene, per cell division. As a consequence, any daughter B cells will acquire slight amino acid differences in the variable domains of their antibody chains. This serves to increase the diversity of the antibody pool and impacts the antibody's antigen-binding affinity. Some point mutations will result in the production of antibodies that have a lower affinity with their antigen than the original antibody, and some mutations will generate antibodies with a higher affinity. B cells that express higher affinity antibodies on their surface will receive a strong survival signal during interactions with other cells, whereas those with lower affinity antibodies will not, and will die by apoptosis. Thus, B cells expressing antibodies with a higher affinity for the antigen will outcompete those with weaker affinities for function and survival. The process of generating antibodies with increased binding affinities is called affinity maturation. Affinity maturation occurs after V(D)J recombination, and is dependent on help from helper T cells.

Antibody genes also re-organize in a process called class switching, which changes the base of the heavy chain to another. This creates a different isotype of the antibody while retaining the antigen specific variable region, thus allowing a single antibody to be used by several different parts of the immune system.

The genetic basis for the diversity of heavy chain variable domains is somewhat more complex and involves yet another gene segment called a D gene segment ("D" = "diversity"). The formation of an expressible heavy chain variable domain gene requires

transnational events that link any of the 20 D gene segments to both a V gene segment and a J gene segment.

In light of the numbers of V, J, and D gene segments and the variety of combinations of these that are expressible as light and heavy chain variable domains, it is easier to comprehend the enormous potential for immunoglobulin diversity. This diversity is amplified by a lack of precision in the machinery for splicing the DNA and an unexpectedly high mutation rate in the variable domains.

✓ Antigen Receptor Maturation and selection

- Lymphocytes can mount a specific immune response against any foreign antigen because of the enormous diversity of their antigen receptors.
- Each lymphocyte has an antigen receptor of a single specificity, which is determined by genetic mechanisms during lymphocyte development in the bone marrow and thymus. These genetic mechanisms generate millions of different variants of the genes that encode the antigen receptors.
- **Clonal selection theory:** When B and T cells are activated in an antigen-specific manner, they divide and produce identical progeny. Only those lymphocytes that encounter their specific antigen are able to proliferate and differentiate into effector T or B cells. This explains why individuals have the capacity to develop antibodies to virtually any antigen, but only have antibodies that are specific to antigens to which they have been exposed.

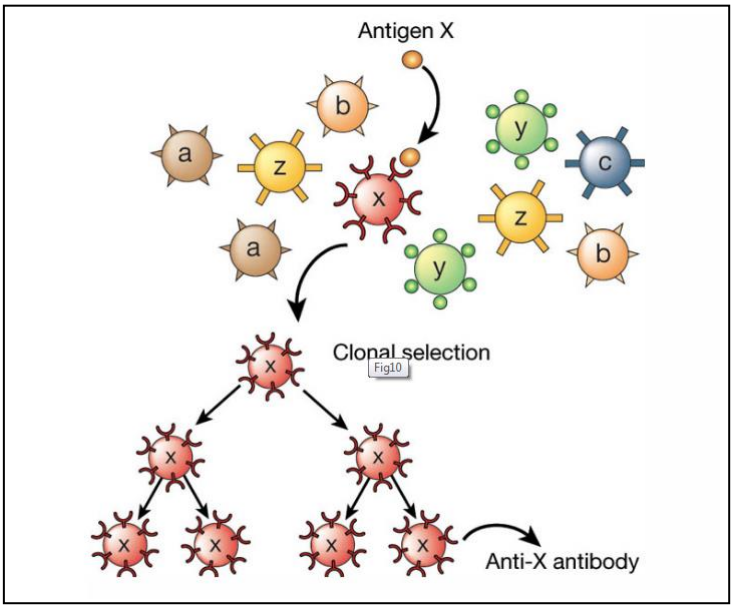

Fig 6: Diagram of Clonal selection and clonal expansion

- **Clonal deletion:** Developing lymphocytes with receptors specific for self-antigens are deleted at an early stage in lymphoid cell development. This allows B and T cells that

are potentially self-reactive to be removed before they can mature, a process known as **central tolerance**.

Probable questions:

11. What is Antigen Receptor Diversity?
12. Briefly discuss the reason behind antigen receptor diversity?
13. Elaborate the mechanism of Antigen Receptor Diversity.
14. What is clonal selection?
15. What is clonal expansion?
16. What is clonal deletion?
17. Describe different types of light chain in immunoglobulin molecule with diagram.
18. What do you mean by V(D)J joining?
19. What is the importance of V(D)J recombination?
20. What do you mean by somatic hypermutation?
21. Briefly discuss the Antibody Diversity and the Genome emphasizing its role on Antigen Receptor Diversity.
22. Describe the process of Antigen Receptor Maturation and selection.

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1. Kindt T, Goldsby R, Osborne B, Kuby J, Kuby J. Kuby immunology. 2007. New York: W.H. Freeman.
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UNIT IX

Vaccination and immunization: natural and artificial immunization; active immunization, vaccine

Objective:

In this unit, we will discuss about the vaccination and immunization: natural and artificial immunization; active immunization, vaccines.

Introduction:

The immune system is a complex system of interacting cells whose primary function is to identify foreign from self and eliminate it, usually referred to as "antigens". The defense system of the body against this antigen usually involves the elicitation of both innate and adaptive branch of immunity. Acquired immunity concerns the production of protein molecules by B lymphocytes, called antibodies (or immunoglobulins), and of specific cells, including T-lymphocytes (also known as cell-mediated immunity) along with the innate immunity involving the cells like macrophages, complement mediated lysis, production of pro-inflammatory cytokines etc. to eliminate the antigen. The most effective immune responses are generally produced in response to a live antigen. However, an antigen does not necessarily have to be alive, as occurs with infection with a virus or bacterium, to produce an immune response. Some proteins, such as hepatitis B surface antigen and other molecules like polysaccharide (long chains of sugar molecules that make up the cell wall of certain bacteria) are easily recognized by the immune system. Immunity can be achieved either by passive immunity or by active processes. Passive immunity is the transfer of preformed antibody produced by one human or other animal to another. Passive immunity provides protection against some infections, but this protection is temporary as the antibodies produced soon degrades during a period of weeks to months, and the recipient will no longer be protected. Active immunity is stimulation of the immune system to produce antigen-specific humoral (antibody) and cellular immunity. Unlike passive immunity, which is temporary, active immunity usually lasts for many years, often for a lifetime. The most tried and tested method to generate active immunity is through vaccines. Vaccines interact with the immune system and often produce an immune response similar to that produced by natural infection, but they do not subject the recipient to the disease and its potential complications. Many vaccines also produce immunologic memory similar to that acquired when infected by the natural disease. A vaccine is defined as a preparation of bacterial, viral or other pathogenic agents or their isolated peptides which is administered with the objective of eliciting the recipient's immunity. First ever encounter to the antigen elicits a primary immune response to the immune-compromised lymphocytes, which peaks at the fourteenth day of antigenic challenge.

This primary immune response leads to formation of IgM type of immunoglobulins, leading to activation of both B and T lymphocytes as well as memory cells are formed. Subsequent exposure to the same antigen leads to the secondary response due to memory cells, which is rapid and the response to the pathogen is by the high –affinity IgG type of immunoglobulins (Fig. 1). It is this rapidity towards secondary exposure to the antigen that protects the host against the potential threat by repeated attack by the same pathogen. Thus vaccine is basically an antigen or its component that can induce the secondary immune responses in the host. We can broadly say that a vaccine aims to introduce immunological memory against a pathogen in the host.

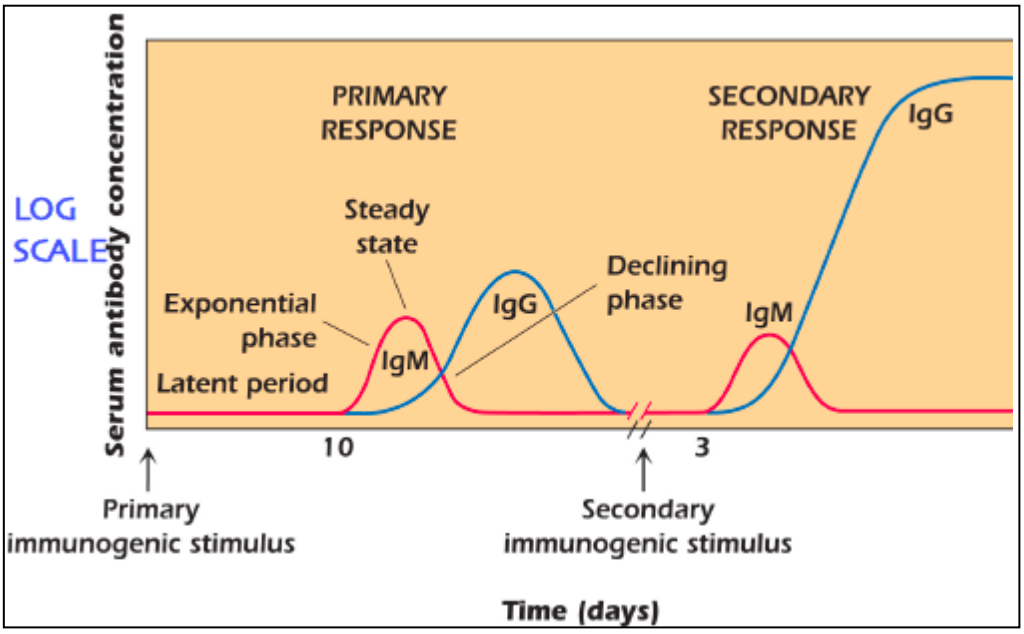

Fig. 1: Graph depicting the antibody titre for IgM and IgG during primary and secondary immune responses after an antigenic challenge

- ✓ A ***vaccine*** is a biological preparation that provides active acquired immunity against a particular disease. A *vaccine* typically contains an antigenic agent that is the disease-causing microorganism in inactivated form or in attenuated form, its toxins, or one of its surface proteins that would generate immune response and provide protection against the disease causing agent during its future attack.
- ✓ **Vaccination:** The act of introducing a vaccine into the body to produce protection from a specific disease. When a sufficiently large percentage of a population has been *vaccinated*, herd immunity results.
- ✓ **Immunization:** A process by which a person becomes protected against a disease through vaccination. This term is often used interchangeably with vaccination or inoculation.

❖ Characteristics of a good vaccine:

1. A vaccine should be able to generate immunological memory. Both T- and Blymphocytes should be formed, hence should be able to generate both arms of immunity i.e. humoral and cell – mediated.

2. It should have the ability to generate the appropriate immunity like cell-mediated immunity should be developed for tuberculosis and viral pathogens, while humoral immunity for all the other bacterial pathogens.

3. It should be able to provide lifelong immunity with a single dose.

4. It should be able to be introduced to the recipient probably through a non-invasive method like through oral administration or nasal spray.

5. Vaccines should be inexpensive, easily manufactured and stable in extreme temperatures or humidity.

6. It should be easy to store and transport

There are two types of immunity: active and passive.

1. **Active Immunity**

Active Immunity results when exposure to a disease organism triggers the immune system to produce antibodies to that disease. Active immunity can be acquired through natural immunity or vaccine-induced immunity.

- **Natural immunity** is acquired from exposure to the disease organism through infection with the actual disease.
- **Vaccine-induced immunity** is acquired through the introduction of a killed or weakened form of the disease organism through vaccination.

Either way, if an immune person comes into contact with that disease in the future; their immune system will recognize it and immediately produce the antibodies needed to fight it. Active immunity is long-lasting, and sometimes life-long.

2. **Passive Immunity**

Passive immunity is provided when a person is given antibodies to a disease rather than producing them through his or her own immune system.

- A newborn baby acquires passive immunity from its mother through the placenta.
- People can also get passive immunity through antibody-containing blood products such as immune globulin, which may be given when immediate protection from a specific disease is needed.

The major advantage to passive immunity is that protection is immediate, whereas active immunity takes time (usually several weeks) to develop. However, passive immunity lasts only for a few weeks or months. Only active immunity is long-lasting.

❖ Active Immunity and Passive Immunity- Differences

Following are the important difference between active and passive immunity:

Active Immunity	Passive Immunity
Active immunity is usually permanent – it is produced by the antibodies of the host in response to direct contact of an antigen	Passive immunity lasts only for a few weeks or months. It is produced by the introduction of antibodies from outside to the host
It produces an immunological memory	It does not produce immunological memory
When the antigens enter the body, antibodies and other specialised lymphocytes are produced	Antibodies are introduced from an external source. For instance, a mother introduces antibodies to a fetus through the placenta and to an infant via mother's milk.
There are no side-effects	It may cause reactions
Immunity does not occur immediately	Immunity develops immediately

❖ Active immunization

Active immunization stimulates the immune system to produce antibodies against a particular infectious agent. Active immunity can arise naturally, as when someone is exposed to a pathogen. For example, an individual who recovers from a first case of the measles is immune to further infection by the measles-causing virus, because the virus stimulates the immune system to produce antibodies that specifically recognize and neutralize the pathogen the next time it is encountered. Active immunization also can be artificially induced through vaccination. Vaccines are preparations containing antigens that stimulate an immune response without causing illness. The purpose of vaccination is to ensure that a large enough number of antibodies and lymphocytes capable of reacting against a specific pathogen or toxin are available before exposure to it occurs. Active immunization is often long-lasting and may be reactivated quickly by a recurrence of the infection or by revaccination.

❖ How Vaccines Work?

Vaccines help develop immunity by imitating an infection. Vaccines contain weakened or inactive parts of a particular organism (antigen) that triggers an immune response within the body. This type of infection, however, does not cause illness, but it does cause the immune system to produce T-lymphocytes and antibodies. Sometimes, after getting a vaccine, the imitation infection can cause minor symptoms, such as fever. Such minor symptoms are normal and should be expected as the body builds immunity. Once the imitation infection goes away, the body is left with a supply of "memory" T-lymphocytes, as well as B-lymphocytes that will remember how to fight that disease in the future. However, it typically takes a few weeks for the body to produce T-lymphocytes and B- lymphocytes after vaccination. Therefore, it is possible that a person who was infected with a disease just before or just after vaccination could develop symptoms and get a disease, because the vaccine has not had enough time to provide protection.

❖ Different Types of Vaccines

The first human vaccines against viruses were based using weaker or attenuated viruses to generate immunity. The smallpox vaccine used cowpox, a poxvirus that was similar enough to smallpox to protect against it but usually didn't cause serious illness. Rabies was the first virus attenuated in a lab to create a vaccine for humans.

Vaccines are made using several different processes. They may contain live viruses that have been attenuated (weakened or altered so as not to cause illness); inactivated or killed organisms or viruses; inactivated toxins (for bacterial diseases where toxins generated by the bacteria, and not the bacteria themselves, cause illness); or merely segments of the pathogen (this includes both subunit and conjugate vaccines).

Sl. No.	**Vaccine type**	**Vaccines of this type on U.S. Recommended Childhood (ages 0-6)**
1.	Live, attenuated	Measles, mumps, rubella (MMR combined vaccine) Varicella (chickenpox), Rotavirus
2.	Inactivated/Killed	Polio (IPV), Hepatitis A, Rabies
3.	Toxoid (inactivatedtoxin)	Diphtheria, tetanus (part of DTaP combined immunization)
4.	Subunit/conjugate	*Haemophilus influenza* type b (Hib), Pertussis (part of DTaP combined immunization), Human papillomavirus (HPV), Pneumococcal Meningococcal
5.	Recombinant	Hepatitis B Vaccine
6.	DNA vaccine	Rabies vaccine, influenza vaccine

Live, attenuated vaccines currently recommended as part of the U.S. Childhood Immunization Schedule include those against measles, mumps, and rubella (via the combined MMR vaccine), varicella (chickenpox), and influenza (in the nasal spray version of the seasonal flu vaccine). In addition to live, attenuated vaccines, the immunization schedule includes vaccines of every other major type—see the table above for a breakdown of the vaccine types on the recommendedchildhood schedule.

The different vaccine types each require different development techniques are described as follows:

1. **Live, Attenuated Vaccines**

Attenuated vaccines can be made in several different ways. Some of the most common methods involve passing the disease-causing virus through a series of cell cultures or animal embryos (typically chick embryos). With each passage, the virus becomes better at replicating in chick cells, but loses its virulence. A virus targeted for use in a vaccine may be grown through— "passaged" through—upwards of 200 different embryos or cell cultures. Eventually, the attenuated virus will be unable to cause disease in humans but will replicate well in human cells, and can be used in a vaccine. All of the methods produce an attenuated live vaccine where the pathogen loses its ability to cause disease in the host but possess all the characteristics to be usedas a vaccine.

When the resulting vaccine virus is given to a human, it will be unable to cause illness, but will still provoke an immune response that can protect against future infection.

One concern that must be considered is the potential for the vaccine virus to revert to a form capable of causing disease. Mutations that can occur when the vaccine virus replicates in the body may result in more a virulent strain. It is worth noting that mutations *are* somewhat common with the oral polio vaccine (OPV), a live vaccine that is ingested instead of injected. The vaccine virus can mutate into a virulent form and result in rare cases of paralytic polio. For this reason, OPV is no longer used in the United States, and has been replaced on the Recommended Childhood Immunization Schedule by the inactivated polio vaccine (IPV).

Protection from a live, attenuated vaccine typically outlasts that provided by a killed or inactivated vaccine.

The advantages of live attenuated vaccines are:

1. One single dose is capable in inducing long-term immunity

2. Provides wide spectrum of immunity

3. Rare incurrence of any allergic reactions or post vaccination lumps.

4. Cost-effective

The disadvantages are:

1. Potential to revert back to virulence form

2. Exacerbate diseased conditions in immune-compromised individuals.

3. In some rare case may lead to abortion or infertility.

2. Killed or Inactivated Vaccines

Inactivated vaccines generally termed as heat killed vaccines are created by inactivating a pathogen, typically using heat or chemicals such as formaldehyde or formalin. This destroys the pathogen's ability to replicate, but keeps it "intact" so that the immune system can still recognize it. Because killed or inactivated pathogens can't replicate at all, they can't revert to a more virulent form capable of causing disease (as discussed above with live, attenuated vaccines). However, they tend to provide a shorter length of protection than live vaccines, and are more likely to require boosters to create long-term immunity.

3. Toxoids

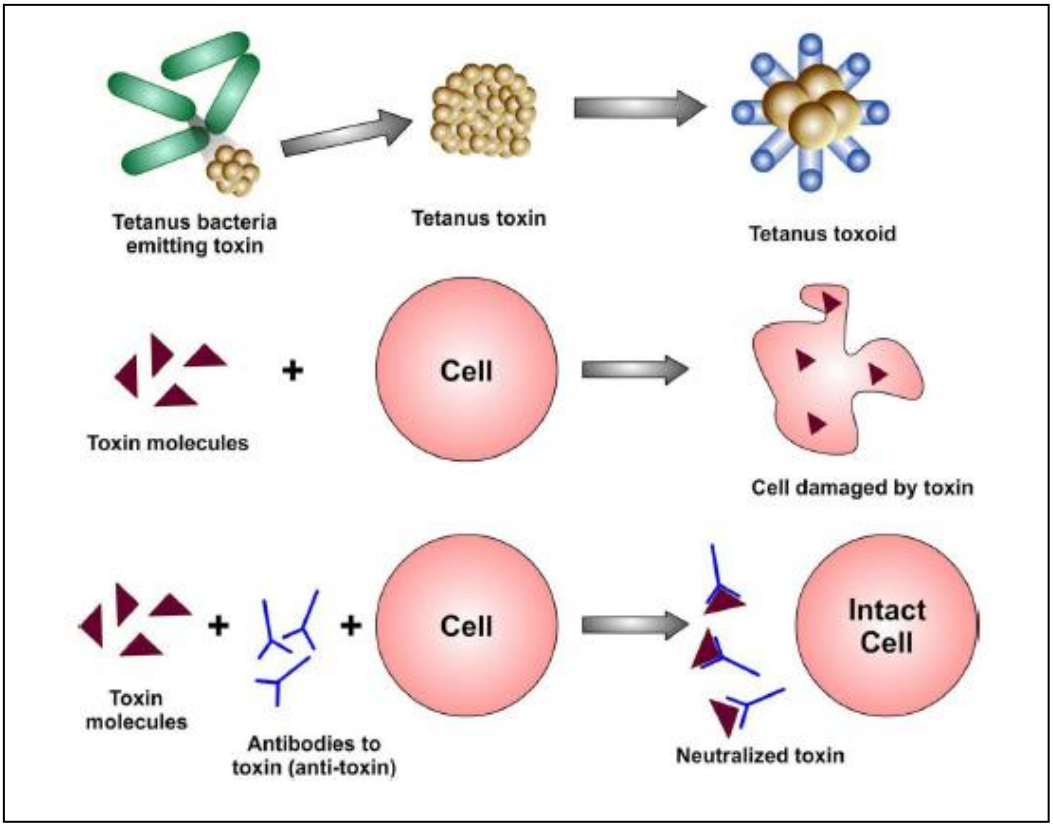

Fig 2: Toxin molecules destroy the cells but vaccinations by toxoids produce antibodies that neutralize the pathogens.

Some bacterial diseases are not directly caused by a bacterium itself, but by a toxin produced by the bacterium. One example is tetanus: its symptoms are not caused by the *Clostridium tetani* bacterium, but by a neurotoxin it produces (tetanospasmin). Immunizations for this type of pathogen can be made by inactivating the toxin that causes disease symptoms. As with organisms or viruses used in killed or inactivated vaccines, this can be done via treatment with a chemical such as formalin, or by using heat or other methods.

Immunizations created using inactivated toxins are called *toxoids*. Toxoids can actually

be considered killed or inactivated vaccines, but are sometimes given their own category to highlight the fact that they contain an inactivated toxin, and not an inactivated form of bacteria.

4. Subunit and Conjugate Vaccines

Both subunit and conjugate vaccines contain only pieces of the pathogens they protect against. Subunit vaccines use only part of a target pathogen to provoke a response from the immune system. This may be done by isolating a specific protein from a pathogen and presenting it as an antigen on its own. The acellular pertussis vaccine and influenza vaccine (in shot form) areexamples of subunit vaccines.

Another type of subunit vaccine can be created via genetic engineering. A gene coding for a vaccine protein is inserted into another virus, or into producer cells in culture. When the carrier virus reproduces, or when the producer cell metabolizes, the vaccine protein is also created. The end result of this approach is a recombinant vaccine: the immune system will recognize the expressed protein and provide future protection against the target virus. The Hepatitis B vaccine currently used in the United States is a recombinant vaccine.

Another vaccine made using genetic engineering is the human papillomavirus (HPV) vaccine. Two types of HPV vaccine are available—one provides protection against two strains of HPV, the other four—but both are made in the same way: for each strain, a single viral protein is isolated. When these proteins are expressed, virus-like particles (VLPs) are created. These VLPs contain no genetic material from the viruses and can't cause illness, but prompt an immune response that provides future protection against HPV.

Conjugate vaccines are somewhat similar to recombinant vaccines: they're made using a combination of two different components. Conjugate vaccines, however, are made using pieces from the coats of bacteria. These coats are chemically linked to a carrier protein, and the combination is used as a vaccine. Conjugate vaccines are used to create a more powerful, combined immune response: typically the "piece" of bacteria being presented would not generate a strong immune response on its own, while the carrier protein would. The piece of bacteria can't cause illness, but combined with a carrier protein, it can generate immunity against future infection. The vaccines currently in use for children against pneumococcal bacterial infections are made using this technique.

5. Recombinant Vaccines

Today, the rise of genetic engineering and molecular biology has had great impact on development and manufacturing process of vaccines. Specific antigenic microbes have high power to arouse the immune response against pathogens. Currently, the sequence of the pathogenic protein antigens could be obtainable by sequencing genes of the main antigen, and producing them synthetically via recombinant DNA technology. Hepatitis B is the first and one of the most successful examples of synthetic vaccines. The surface

antigen of this virus (HBsAg) is very immunogenic and effective, and able to produce high levels of antibody in the body. In the past, for providing hepatitis B vaccine, HBsAg was purified from the plasma of infection carriers and used for vaccination; of course there were some extensive restrictions in purification, such as difficult conditions and contaminated plasma. In order to make recombinant hepatitis B vaccine, recombinant HBsAg is expressed in cells that have a powerful expression system leading to the production of virus-like particles by HBsAg which are highly immunogenic. Other kinds of common vaccines are anti-herpes simplex virus, anti-rotavirus, and anti-HPV vaccines.

6. DNA Vaccine

DNA immunization is a novel technique by which direct injection of genetic material with the foreign gene into a living host leads to the production of that gene product and subsequent immune responses. Transfected muscle cells may produce antigen or foreign proteins resulting in production of B-cells as well they can also directly transfect APC's to elicit an MHC-dependent T- cell mediated response, thus mimicking the similar immune responses induced by pathogens(Fig 6). Production of DNA vaccines starts with E. coli cells which are transformed with the plasmid of interest. Growth of the E. coli is typically done via a fermentation process similar to that used in the manufacturing of certain alcoholic beverages. These cells are grown and stored frozen in a stock of vials called a Master Cell Bank. Cell lysis and release of the plasmid is attained, following the purification of the desired DNA by various chromatographic methods.

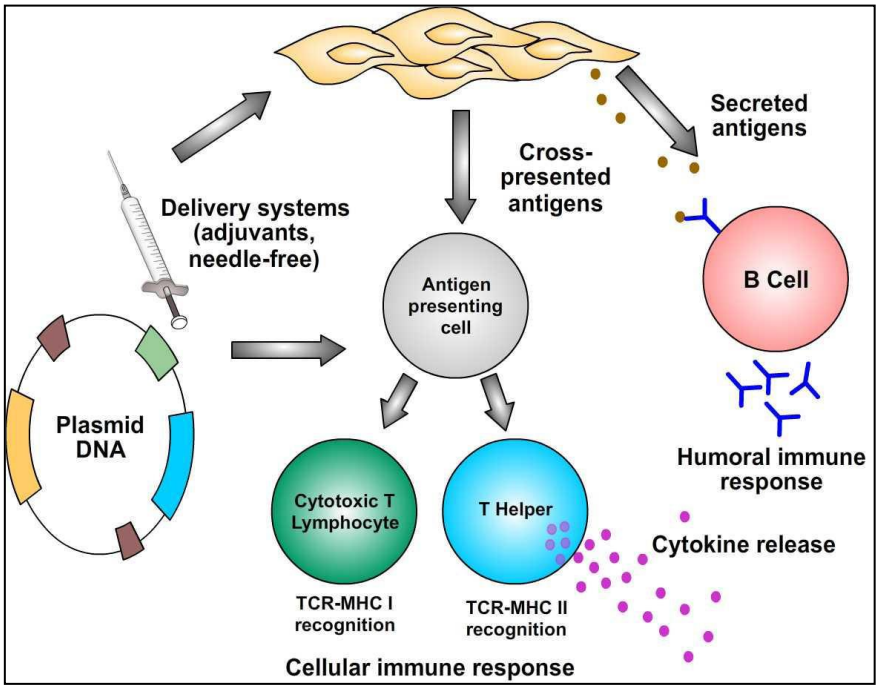

Fig 3: Master cell bank contains the cultures of E. coli cells with the plasmid of interest. Transfection of DNA into the myocytes results in both humoral and cell-mediated immune responses.

Advantages

DNA vaccination has many advantages compared to the conventional vaccine approaches particularly against potentially lethal emerging infectious diseases.

1. DNA vaccines can accommodate a combination of different genes that code for different antigens from one or more different pathogens. In addition they have a significantly shorter production time. This can result in the generation of broad immunity to multiple protein antigens in a short span of duration.

2. DNA vaccines have also been observed to stimulate both antibody and T cell arms of the immune system including those that are specialized to kill viruses or cancer cells (via cytotoxic or killer T cells).

3. The most significant advantage is that DNA vaccines do not require the handling of potentially deadly infectious agents, in light of fast emerging pathogens.

Probable questions:

2. What do you mean by immunization?
3. What is vaccine and vaccination?
4. What do you mean by active immunization?
5. Describe the different types of vaccines administered in human.
6. What is toxoid? Give an example.
7. Explain the mechanism of action of a vaccine.
8. Elaborate the advantages of DNA vaccine.

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UNIT X

Immuno-techniques: Antigen-Antibody Reaction Analysis - Agglutination, Diffusion etc. Isolation and culture of Immune cells, Antigen-Antibody reaction-RIA, ELISA, Visualization of Immune reaction In vivo and vitro- Immunofluorescence, FISH, GISH, immunohistochemistry

Objective:

In this unit, we will discuss different Immuno-techniques: Antigen-Antibody Reaction Analysis - Agglutination, Diffusion etc. Isolation and culture of Immune cells, Antigen-Antibody reaction-RIA, ELISA, Visualization of Immune reaction In vivo and vitro- Immunofluorescence, FISH, GISH, immunohistochemistry.

Introduction:

Immunotechnology focuses on the use of body defence system for the production of immunological agents and diagnosis of several diseases that protect living beings from these diseases. Immunological-based techniques have been extensively utilized for the detection and epidemiological studies of human viral infections. They can detect antiviral antibodies or viral antigens in clinical samples.

Antigen-antibody interaction, or **antigen-antibody reaction**, is a specific chemical interaction between antibodies produced by B cells of the white blood cells and antigens during immune reaction. The antigens and antibodies combine by a process called agglutination. It is the fundamental reaction in the body by which the body is protected from complex foreign molecules, such as pathogens and their chemical toxins. In the blood, the antigens are specifically and with high affinity bound by antibodies to form an antigen-antibody complex. The immune complex is then transported to cellular systems where it can be destroyed or deactivated. The types of antigen - antibody reactions are: Precipitation Reaction, Agglutination Reaction, and Complement Fixation.

- ## Nature of Antigen-Antibody Reactions

a. Lock and Key Concept

An antibody molecule comprises of the Fab portion where the active site consisting the hyper-variable regions of the heavy and light chains is located. The antigenic determinant resides in a cleft formed by the active site of the immunoglobulin molecule

as indicated by X-ray crystallography studies. Thus, the antigen-antibody interactions can be simulated by a key (the antigen) which fits into a lock (the antibody).

b. Non-covalent Bonds

The binding of an antibody and antigen is highly specific and involves weak and reversible non-covalent interactions comprising mainly of van der Waals forces, electrostatic forces, H-bonding and hydrophobic forces. The antigen combines to the antibody at the active site by non-covalent bonds. Multiple bond formation ensures that the antigen will be bound tightly to the antibody.

c. Reversible Nature

Antigen-antibody complexes are strengthened by non-covalent bonds, thus making their nature reversible.

- ## Affinity and Avidity

a. Affinity

The intensity of the reaction between an antigenic determinant and one active site on the antibody molecule defines the affinity of that antibody. Affinity is the equilibrium constant characteristic of a Ag-Ab interaction. It is the sum of the attractive and repulsive forces effective between the antigenic determinant and the active site of a specific antibody. Most antibodies have extremely high affinity and specificity for their antigens.

b. Avidity

Avidity amounts to the total strength by which an antigen binds to multiple antigenic determinants and multivalent antibodies. Avidity is affected by the valence of the antibody as well as that of the antigen and is therefore more than the sum of individual affinities. Hence, affinity is the strength of binding between a single antigenic determinant and its corresponding individual antibody combining site whereas avidity refers to the overall strength of binding between multivalent antigens and antibodies.

- ## Specificity and Cross Reactivity

a. Specificity

Specificity is defined as the ability of a particular antibody active site to recognize and interact with only a single antigenic determinant or the ability of a population of antibody molecules to react with only a single antigen. Antigen-antibody reactions possess extremely high degree of specificity. Antibodies are capable of discriminating between- a. the primary structure of an antigen b. isomeric forms of an antigen c. secondary and tertiary structure of an antigen.

b. Cross Reactivity

Cross reactivity is the capability of a particular antibody active site to react with more than a single antigenic determinant or that of a population of antibody molecules to react with multiple antigens.

Cross reactions are multi specific interactions arising due to sharing of an epitope by a cross reacting antigen and the immunizing antigen or because the epitope is structurally similar to one on the immunizing antigen Cross-reactivity usually occurs among polysaccharide antigens containing similar oligosaccharide residues. For instance, the ABO blood-group antigens are glycoproteins expressed on the surface of erythrocytes. Factors distinguishing the blood-group antigens A and B include fine variations in the terminal sugar residues of these surface proteins. A person with type A blood has anti-B antibodies; a type B person has anti-A; and a type Operson therefore has both anti-A and anti-B antibodies. An individual lacking one or both of these antigens would generate serum antibodies to the missing antigen(s) (Table 1). The serum antibody response is not induced by exposure to erythrocyte antigens but by cross-reacting microbial antigens present on common intestinal bacteria. These antigens intern induce the production of antibodies in individuals lacking the similar blood-group antigens on their erythrocyte surfaces. These antibodies would cross-react with the oligosaccharides on foreign erythrocytes, forming the basis for blood typing and accounting for the essential compatible nature of blood types during blood transfusions.

Table 1: ABO blood type- Antigens present on surface of RBCs act as epitopes for generation of serum antibodies.

Blood type	Antigens on RBCs	Serum antibodies
A	A	Anti-B
B	B	Anti-A
AB	A and B	Neither
O	Neither	Anti-A and Anti-B

• Factors Affecting Measurement of Antigen-Antibody Reactions

Antigen-antibody complexes are formed under specific conditions of temperature and pH. In order to determine whether an antigen-antibody reaction has occurred,the Ag-Ab complexes formed have to be detected by direct or indirect means. A number of factors influence the detection of these complexes.

a. Affinity - Higher affinity of the antibody for the antigen ensures a stable reaction between the two thus facilitating the detection of the complex formed.

b. Avidity - Interactions between multivalent antigens and multivalent antibodies are very stable aiding in their detection.

c. Antigen to antibody ratio - The formation of Ag-Ab complexes is related to the concentration of the antigen and antibody, therefore its detection is directly dependent on the ratio in which they are present at a particular instance.

d. Physical form of the antigen - The physical form of the antigen plays an important role in detection of its interaction with an antibody. Particulate antigens result inagglutination on reaction with an antibody. However, precipitation of the antigen would occur in case it is soluble in nature when large insoluble Ag-Ab complexes are formed.

✓ Antigen-Antibody Interactions

- ***Agglutination***

The word Agglutination comes from the Latin “agglutinare”, meaning "to glue,” referring to clumping of substances.Agglutination is defined as the visible clumping of a particulate antigen when mixed with antibodies specific for it, in the presence of electrolytes at an appropriate temperature and pH.Antibodiesare capable of binding multiple antigen molecules, linking themto create a large lattice like complex which is visible to the naked eye. Antibodies that generate such reactions are called **agglutinins**. All antibodies can theoretically agglutinate particulate antigens but IgM, due to its high valence, is particularly a good agglutinin. Large antigens with multiple epitopes easily adhere to particles such as animal cells or bacteria when combined with specific antibodies resulting in cross-linking. The process of agglutination involves two steps. First step is sensitization and second is lattice formation. Sensitization is the recognition and attachment of specific antibody to corresponding antigen. Temperature,pH and time of incubation influence the reaction. A Lattice is formed by cross linking between sensitized particles. Agglutination reaction used for diagnosis of diseases in lab either uses the particulate or soluble antigens. Example of agglutination reaction using particulate antigens is Salmonella typhi bacteria to detect specific antibody in serum from patient suffering from typhoid fever (Widal test).

Agglutination is a serological reaction similar to precipitation; with the exception of the antigen being large and particulate in case of agglutination.Both reactions are inhibited by antibody excess and this phenomenon is called the **prozone effect**, whereas in case of antigen excess **postzone effect** occurs. If the antigen is an integral part of the surface of a cell or other insoluble particle, the agglutination reaction is known as **direct agglutination**. However, a cell or insoluble particle can be coated with a soluble antigen such as a viral antigen, a polysaccharide or a hapten and the coated cells can be used in an agglutination test for antibody to the soluble antigen in a reaction called **passive agglutination**.

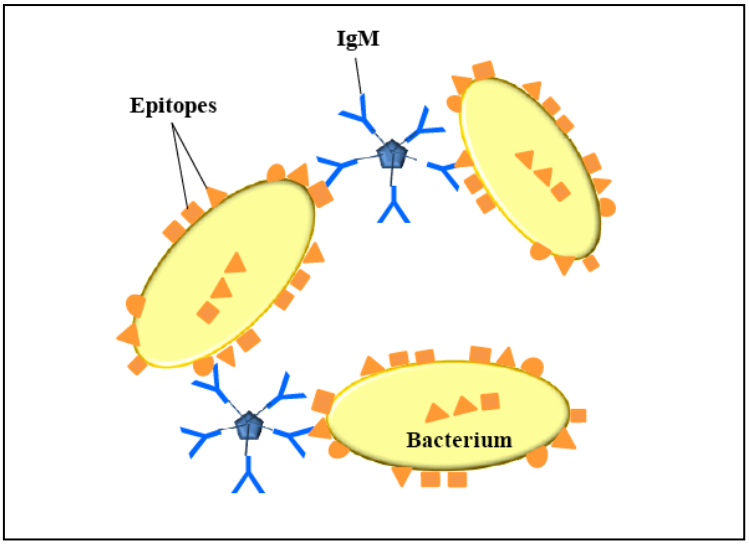


Figure 1: Agglutination – Pentavalent Immunoglobulin IgM is shown to interact and bind with multiple antigenic epitopes on the surface of bacterial cells causing clumping or agglutination reaction

Table 2: Aggutination reaction in ABO blood typing- Serum of individuals would contain different types of antigens on surface of RBCs and corresponding antibodies to these antigens.

	Red blood cells from individuals of type			
Serum from individuals of type	O	A	B	AB
Y O Y Anti-A & Anti-B antibodies	No agglutination	Agglutination	Agglutination	Agglutination
A Y Anti-B antibodies	No agglutination	No agglutination	Agglutination	Agglutination
B Y Anti-A antibodies	No agglutination	Agglutination	No agglutination	Agglutination
A B No antibodies to A or B	No agglutination	No agglutination	No agglutination	No agglutination

✓ Applications of Agglutination Tests

i. Determination of blood group types or antibodies to blood group antigens.

ii. Assessment of bacterial infections. eg. infection with a particular bacterium is indicated by the rise in titer of an antibody to this bacterium.

- ***Precipitation***

The smallest unit of an antigen molecule that can bind with an antibody is known as antigenic determinant or epitope. The corresponding region on the antibody molecule that interacts with the epitope is called paratope. The number of epitopes on the surface of an antigen is known as its valence and it determines the number of antibody molecules that can combine with the antigen at one time. Monovalent antigens are those having a single epitope, however, more than one copy of the same epitope is present on most antigens called polyvalent antigens. Immuno precipitation involves interaction of a soluble antibody with a soluble antigen resulting in the formation of an insoluble product, the precipitate. These reactions consist of lattice (cross-links) formation when the corresponding antigen and antibody combine in optimal ratios. Lattice formation relies upon the valency of both antibody and antigen. Crosslinked complexes result when bi- or polyvalent antigens interact with more than one multivalent antibodies. If the Ag-Ab complexes formed are too large to stay in solution, visible precipitation results. Excess of either component reduces lattice formation and subsequent precipitation hence, these should occur at optimal concentrations. Antibodies that aggregate soluble antigens are called **precipitins.** Precipitation and agglutination reactions differ in size, solubility of the antigen and sensitivity. Antigens are soluble molecules and larger in size in precipitation reactions. Antigen-Antibody lattice formation is governed by the valence of both the antibody and antigen:

- The antibody should be polyvalent (Fab fragments) in order to form a precipitate.
- The antigen should be bi- or poly valent; i.e. it should possess at least two copies of an epitope, or have different epitopes that are capable of reacting with various antibodies in a polyclonal antisera.

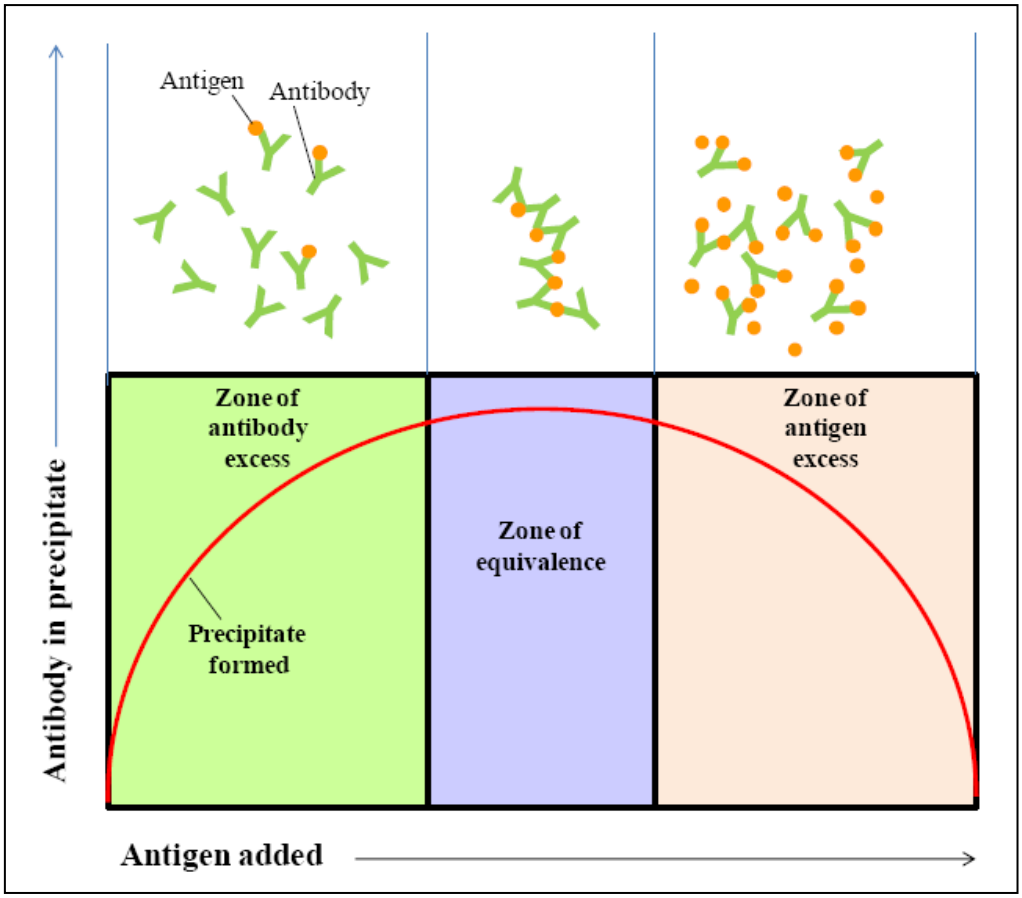


Figure 3: Precipitation curve – Interaction of Antigens with specific antibodies generates the precipitate. Different zones are highlighted according to the concentration of differently distributed components.

Precipitation Reactions in Fluids Yield a Precipitin Curve

When a constant amount of antibody is taken in a series of tubes and increasing amounts of antigen is added to these, variable amounts of precipitates form resulting in a quantitative reaction. Initially, this method was used to determine the amount of antigen or antibody present in a particular sample. After precipitation, the tubes are centrifuged to pellet the precipitate and the amount of precipitate is measured on removing the supernatant. The amount of precipitate when plotted against increasing antigen concentrations yields a **precipitin curve**. Excess of either antibody orantigen interferes with maximal precipitation. Hence, formation of an insoluble antigen-antibody complex occurs within a narrow optimal concentration range known as the **zone of equivalence**. This zone represents the conditions under which antigen-antibody complexes formed are sufficiently large to be precipitated. At equivalence, a large multi-molecular complex is formed which increases in size and precipitates out of solution. On the other hand, outside this zone antigen or antibody excess occurs resulting in the formation of small soluble complexes.

Immunoassay

An immunoassay is a biochemical test used to identify the presence or amount of a particular molecule referred to as an "analyte", in a solution by combining it with an antibody or an antigen. The principal of immunoassays is formation of an immune complex involving the recognition and binding of an antibody to a specific molecule among a mixture of molecules. A key feature of all immunoassays is generation of a measurable signal in response to the binding. Immunoassays utilize a wide range of labels; some emit radiation, result in a visible colour change, fluoresce under light, or could be induced to emit light.

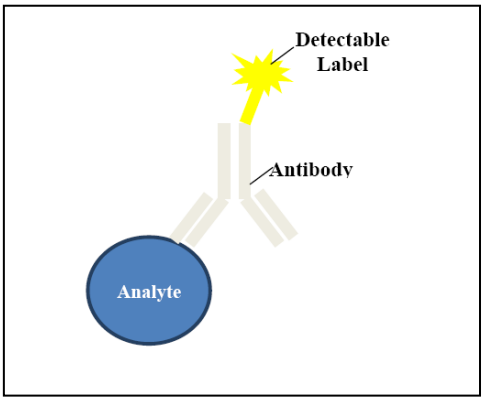


Figure 1: Basic components of an Immunoassay. The analyte specifically binds to the antibody labeled with detectable label

- **Examples of the application of immunoassay include:**
  i. Drug testing
  ii. Hormone testing (insulin in diabetic patients)
  iii. Bacterial or viral testing (AIDS, hepatitis)
  iv. Environmental testing (herbicides, pesticides)
- **Advantages of immunoassays are:**
  i. Inexpensive
  ii. Highly selective
  iii. Low limits of detection
  iv. High-throughput usually
  v. Applicable to the determination of a wide-range of compounds
- ***Categories of Immunoassays***

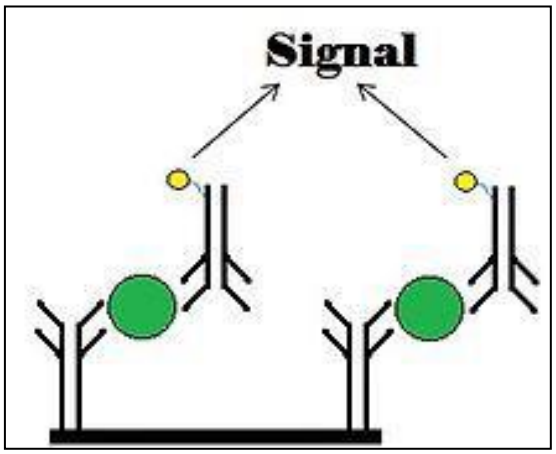
Competitive –A competitive assay or limited reagent assay involves measurement of an unlabeled analyte or antigen by its ability to compete with the labelled antigen in the immunoassay. The assay mixture consists of antibodies saturated by labelled

antibodies, hence higher the reduction in label at the end of assay, greater is the amount of antigen in the test sample.

Non-Competitive

One site Non-competitive - The unknown analyte in the sample are allowed to react with labelled antibodies. After the binding reaction is complete, unbound antibodies are washed away, and the bound labelled antibodies are measured as signals for the complexes formed. Therefore, intensity of the signal is directly proportional to the concentration of unknown antigen.

Two site Non-competitive - An antibody adsorbed on the solid phase surface is allowed to interact with the test sample. The labeled antibodies are in excess in this system and bind specifically to their respective analyte. Subsequently, a second labeled antibody is added causing sandwiching of the target analyte. The quantitation of the labelled antibody helps in determining the concentration of the antigen since the two are directly proportional. The technique is also known as sandwich assay because the analyte is "sandwiched" between two antibodies.



❖ Enzyme-Linked Immunosorbent Assay Enzyme-linked immunosorbent assay (ELISA)

Enzyme-Linked Immunosorbent Assay Enzyme-linked immunosorbent assay, commonly known as **ELISA** or EIA was first developed by Avramais (1966, 1969) and Pierce (1967).In this assay, an antibody coupled enzyme reacts with a colorless substrate called a **chromogenic substrate** to generate a visible coloured reaction product. The enzymes commonly employed for ELISA, include alkaline phosphatase, horseradish peroxidase, and galactosidase. These assays possess high sensitivity and are safe and cost effective. The result generated from an ELISA assay could be qualitative identifying the presence or absence of a particular antigen molecule; semi-quantitative, analyzing relative antigen amounts in assay samples or quantitative, defining precise antigen concentrations with respect to a standard curve. ***There are Numerous Variants of ELISA.***

ELISA assays can be performed in a variety of ways which allow both qualitative and quantitative analysis of either antigen or the antibody. ELISA can be used to identify the presence of antibody or antigen qualitatively. The unknown concentration of a sample can alternatively be determined by a curve based on known concentrations of antibody or antigen. The assay variants are described below –

I. ***Indirect ELISA*** Indirect ELISA is used for both qualitative and quantitative measurements of antibodies. The procedure includes addition of the sample solution containing primary antibody (Ab1) to a microtiter well pre-coated with the antigen, such that the antibody would react with this well bound antigen. Unbound antibody is washed away and this is followed by detection of the antibody bound to the antigen with the help of an enzyme-conjugated secondary anti-isotype antibody (Ab2) which specifically binds to the primary antibody Ab1.Unbound secondary antibody is also washed away followed by addition of substrate for the enzyme. The coloured reaction product is analyzed spectrophotometrically by plate readers.

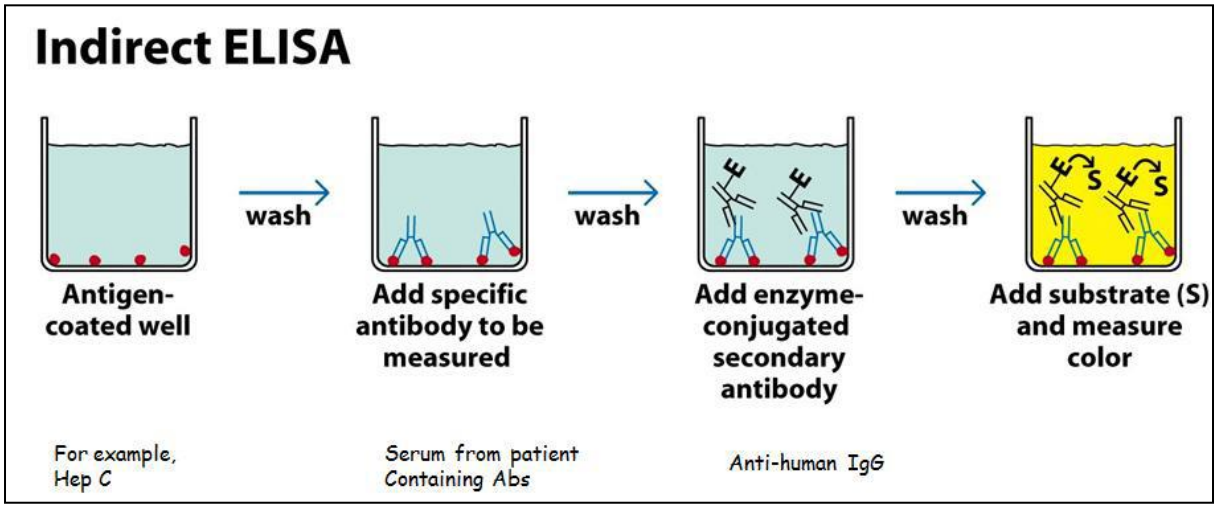


Figure 3: Indirect ELISA - Antigen is immobilized on the surface and sample is added, if antibodies specific to the antigen of interest is present binding would occur and visualized with the help of enzyme conjugated secondary antibody. Immunology, Sixth edition, Kuby, 2007, WH Freeman and company.

Indirect ELISA is preferred for detecting the presence of serum antibodies against human immunodeficiency virus (HIV), the causative agent of AIDS. The recombinantenvelope and core proteins of HIV are used as antigens plated on to microtiter wells.

II. ***Sandwich ELISA*** Sandwich ELISA is used for quantitative or qualitative analysis of antigens. The basis of this technique remains the antigen-antibody interaction however;the antibody instead of the antigen is immobilized on the microtiter well. An antigen sample is then added to the well pre-coated with theimmobilized antibody. The

excess or unbound antigen is washed off using buffers, followed by addition of a second enzyme-linked antibody specific to a second epitope on thebound antigen. Unbound secondary antibody is then washed off and a substrate corresponding to the enzyme on the secondary antibody this added, and the coloured reaction product analyzed spectrophotometrically.

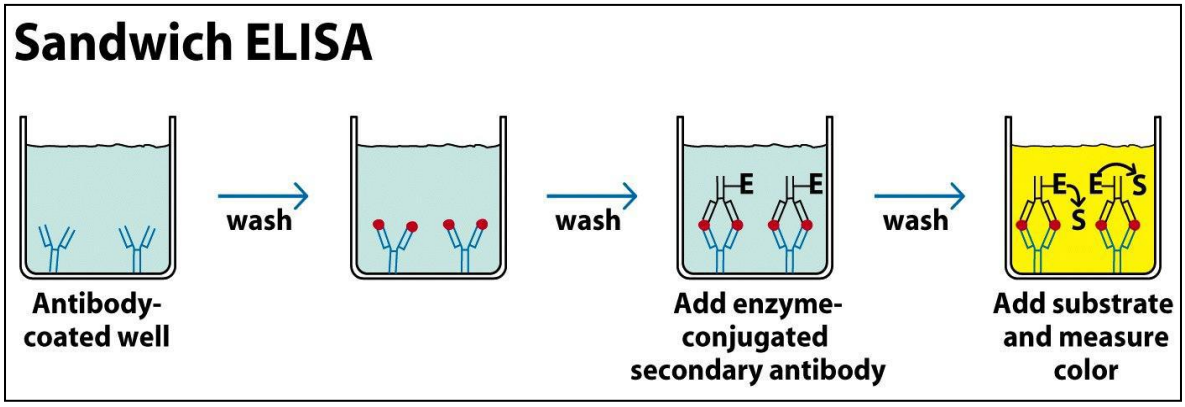


Figure 4: Sandwich ELISA – the antigen of interest is sandwiched between primary antibody immobilized on solid surface and enzyme conjugated secondary antibody. Immunology, Sixth edition, Kuby, 2007, WH Freeman and company.

III. ***Competitive ELISA*** Competitive ELISA is also a variant technique for the quantitation of antigen. The procedure consists of a pretreatment step where the antibody isincubated in solution with a sample containing the antigen. A microtiter plate coated with the same antigen is then incubated with the previously procured antigen-antibody mixture. Greater the amount of antigen in the sample, lesser would be the amount of free antibody available to bind to the antigen-coated well. On addition of an enzyme-conjugated secondary antibody (Ab2) specific for the isotype of the primary antibody, the amount of primary antibody immobilized on the well can be determined like in an indirect ELISA. This competitive interaction suggests that higher the amount of antigen in the original sample, the lower would be the value of absorbance.

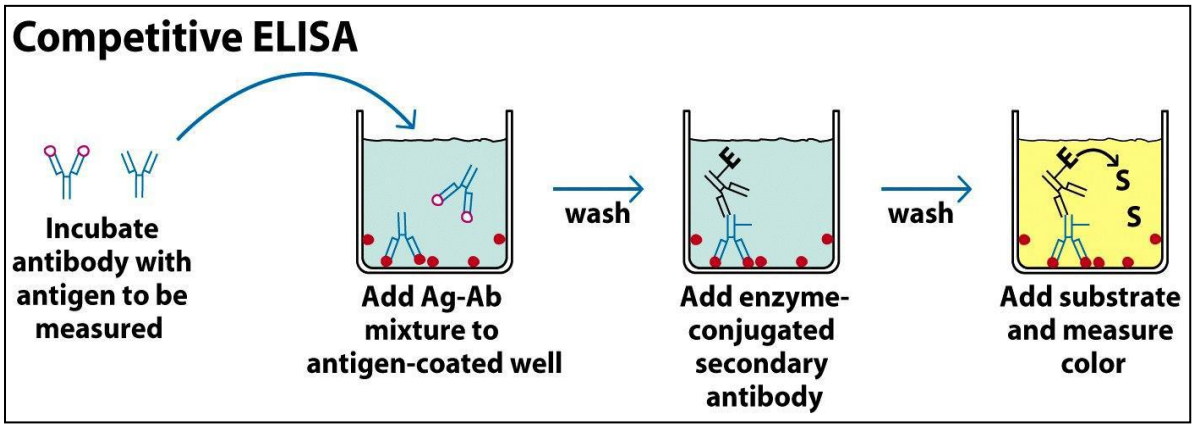


Figure 5: Competitive ELISA - Antigen-antibody mixture is added in addition to the free antibodies and incubated with antigen coated wells. Enzyme conjugated secondary

antibodies when allowed to react with substrate generate coloured product which is quantitated by measuring absorbance.Immunology, Sixth edition, Kuby, 2007, WH Freeman and company.

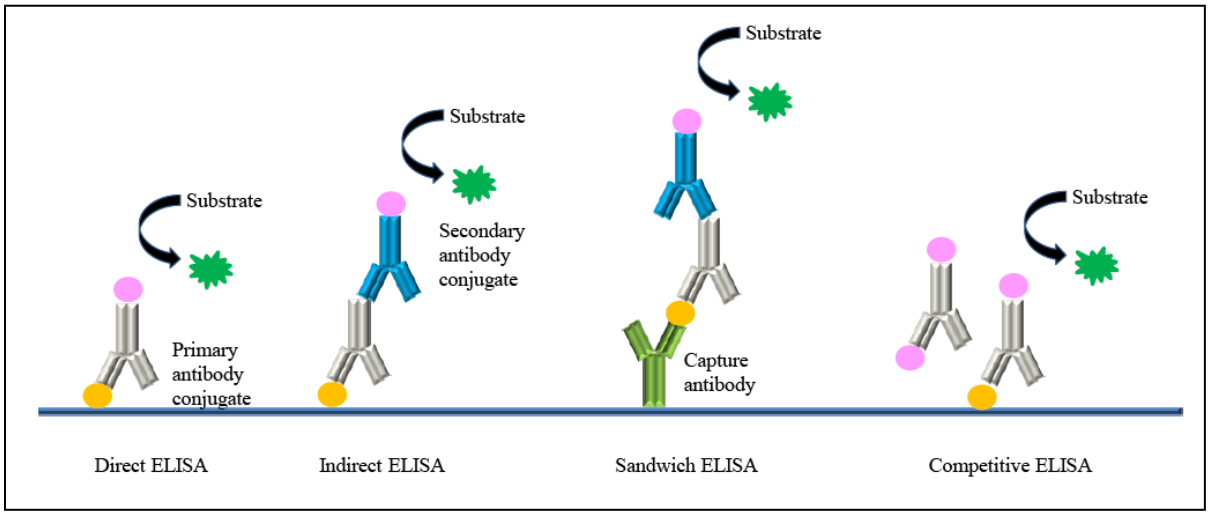


Figure 6: Comparison between different types of ELISA. This overview helps to visualize the differences between different ELISA variants. A particular type of assay can be selected depending upon specific interests.

❖ *Radioimmunoassay – RIA*

Radioimmunoassay (RIA) one of the most sensitive techniques for detecting antigen orantibody was first reported by S. A. Berson and Rosalyn Yalow in 1960, in order to analyze the levels of insulin - anti-insulin complexes in diabetics. This was the first attempt for detection of blood hormones by an *in-vitro* assay. The technique demonstrates high sensitivity and is capable of quantitating hormones, serum proteins, drugs, and vitamins at concentrations as low as 0.001 *micrograms* per milliliter. The basic principle of this technique is competitive binding between the radiolabeled and unlabeled antigen to a high-affinity antibody. First the antibody is allowed to interact with the radio labeled antigen saturating the antigen-binding sites of the antibody. This is followed by addition of large amounts of sample containing unknown amount of unlabeled antigen. The available binding sites on the antibodies are available to both the labeled and unlabeled antigens as the antibody is unable to distinguish between the two. The labeled antigen is progressively displaced from the antibody binding sites with increasing amount of the unlabeled antigen. This reduction in the amount of radio labeled antigen bound to the specific antibody on increasing antigen concentration in the unknown sample is measured in order to quantitate antigen concentrations in the test sample. The primary step for this assay is to ascertain the amount of antibody needed to saturate 50% - 70% of a specific quantity of radioactive antigen in the test mixture. The antibody to antigen ratio is taken such that the labeled antigen displays

more number of epitopes than the total number of antibody binding sites. This ensures competitive binding between unlabeled antigen added to the mixture and the radio labeled antigen against the limited supply of antibody. The bound labeled antigen is quantitated by precipitating the Ag-Ab complex and segregating it from free antigen; and eventually the radio activity of the precipitate is measured.

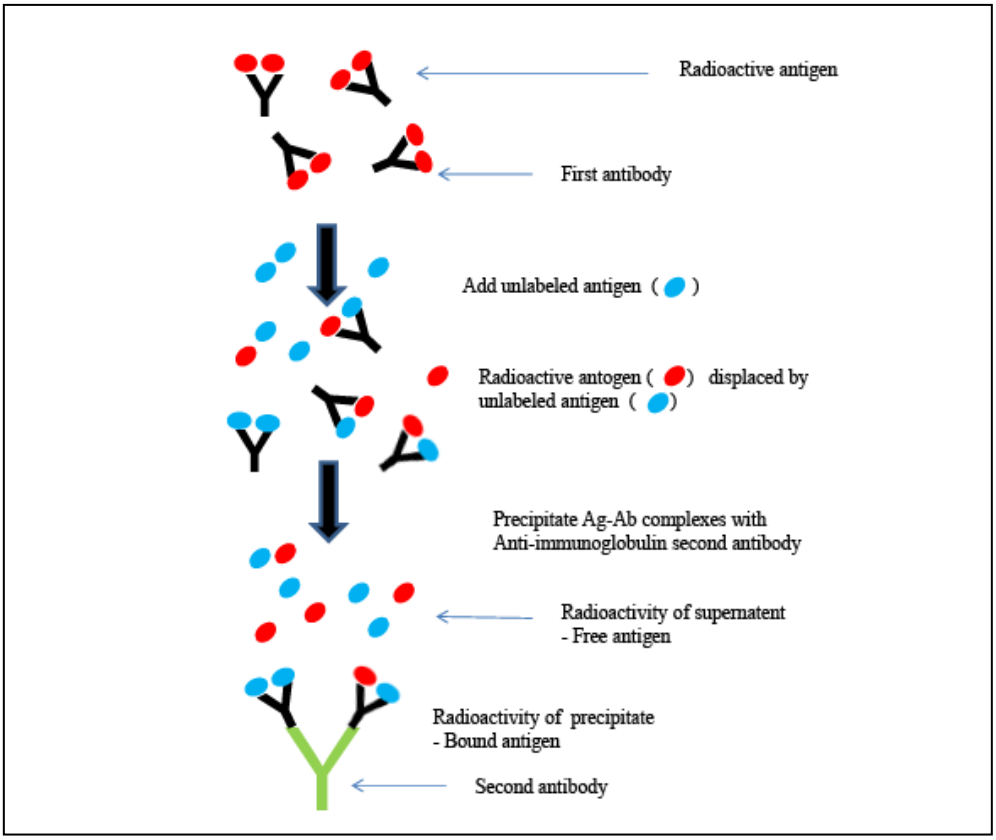


Figure 9: Radioimmunoassay (RIA): Based on competitive binding of radiolabeled and unlabeled antigen to a high-affinity antibody

Immunofluorescence Albert Coons first demonstrated the labeling of antibodies with fluorescent molecules in 1944. These molecules possess the inherent property of absorbing light of a particular wavelength (excitation) and emitting light of another wavelength. Antibodies tagged with a fluorescent dye, or fluorochrome, can be identified by emission of colored light when excited by light of a specific wavelength, when these are a part of immune complexes. This technique also allows detection of antibodies bound to antigen epitopes in cell cultures or tissue sections. Molecules with luminescent properties emit light of a different wavelength on absorbing light of a particular wavelength. Fluorescent materials give off light very promptly due to their atomic structure. The light emitted from luminescent objects can be visualized using a fluorescence microscope equipped with a UV light source. These fluorescent probes are conjugated to the Fc arm of an antibody molecule ensuring that its specificity is not affected. Commonly used fluorochromes are:

i. **Fluorescein** is the most frequently used organic label dye for immunofluorescence. It absorbs blue light (490 nm) and emits a higher wavelength intense yellow-green fluorescence (517 nm).

ii. **Rhodamine** is another organic dye, absorbing light in the yellow-green range (515 nm) and emitting a deep red fluorescence (546 nm). Two-color immunofluorescence assays can be performed using a combination of these two dyes simultaneously as rhodamine emits fluorescence at a longer wavelength than fluorescein. Spatial distribution and comparative assays for two antigens are performed in a single experiment where an antibody specific to one determinant is tagged with fluorescein, and a second antibody recognizing another antigen is labeled with rhodamine. The co-localization of the fluorescein-tagged antibody visualized by its yellowgreencolor, is discretely distinguishable from the red color emitted where the rhodamine-tagged antibody is bound.

iii. **Phycoerythrin** an efficient absorber of light (~30-fold greater than fluorescein) and a brilliant emitter of red fluorescence, is also widely used as an immunofluorescence label.

Applications of immunofluorescence can be wide range, starting with identification of a number of subpopulations of cells in culture, identifying bacterial species, detecting Ag-Ab complexes in disease conditions, detection of complement components, as well as localizing and staining of hormones and other subcellular molecules in situ. It also finds use in analysis of cells in suspension, cultured cells, tissue, beads and microarrays for the detection of specific proteins. A very important application of immunofluorescence is tissue or cell specific antigen localization. The target antigens can be localized in cells or tissues andvisualized by fluorescence microscopy thus, making it a potent tool for associating the molecular architecture of tissues and organs to gross anatomy and physiology.

❖ Fluorescent In Situ Hybridization (FISH):

Fluorescence *in situ* hybridization (FISH) is a kind of cytogenetic technique which uses fluorescent probes binding parts of the chromosome to show a high degree of sequence complementarity. Fluorescence microscopy can be used to find out where the fluorescent probe bound to the chromosome. This technique provides a novel way for researchers to visualize and map the genetic material in an individual cell, including specific genes or portions of genes. It is an important tool for understanding a variety of chromosomal abnormalities and other genetic mutations. Different from most other techniques used for chromosomes study, FISH has no need to be performed on cells that are actively dividing, which makes it a very versatile procedure.

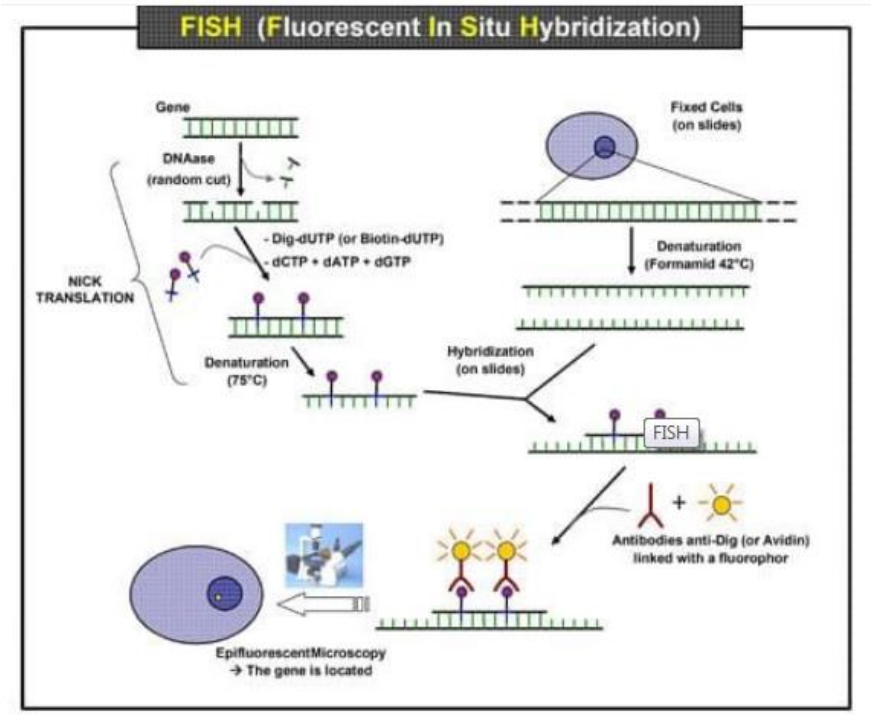


Fig. 1 Scheme of the principle of the FISH experiment to localize a gene in the nucleus.

How does FISH work?

FISH is useful, for example, to help a researcher identify where a particular gene falls within an individual's chromosomes. Here's how it works:

- Make a probe complementary to the known sequence. When making the probe, label it with a fluorescent marker, e.g. fluorescein, by incorporating nucleotides that have the marker attached to them.
- Put the chromosomes on a microscope slide and denature them.
- Denature the probe and add it to the microscope slide, allowing the probe hybridize to its complementary site.
- Wash off the excess probe and observe the chromosomes under a fluorescent microscope. The probe will show as one or more fluorescent signals in the microscope, depending on how many sites it can hybridize to.

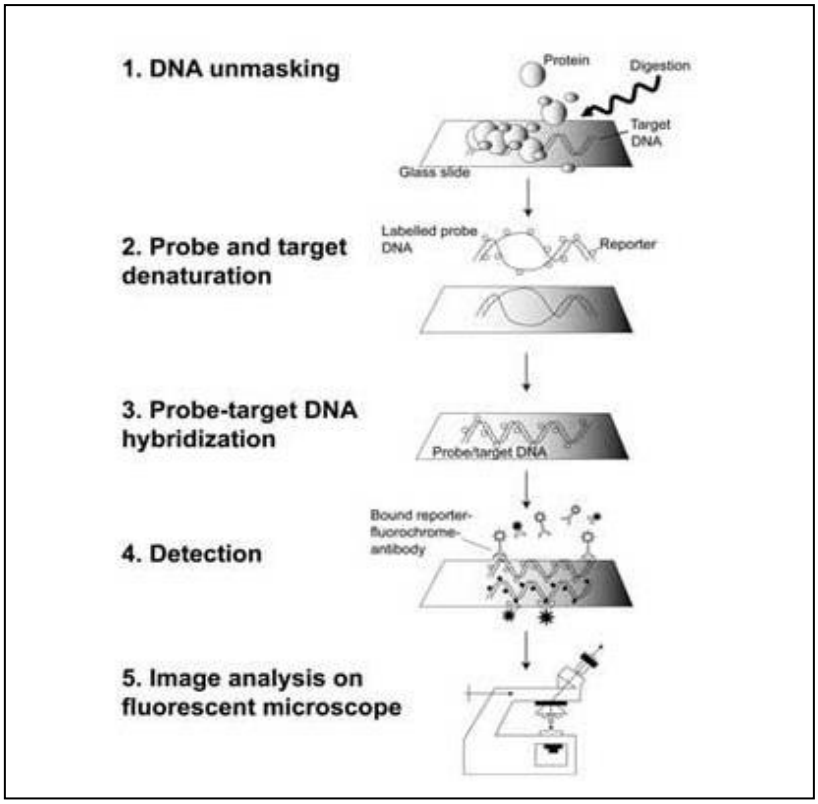


Fig. 2 The five basic steps of FISH. (Oliveira and French 2005)

What is FISH used for?

FISH is widely used for several diagnostic applications: identification of numerical and structural abnormalities, characterization of marker chromosomes, monitoring the effects of therapy, detection of minimal residual disease, tracking the origin of cells after bone marrow transplantation, identification of regions of deletion or amplification, detection of chromosome abnormalities in non-dividing or terminally differentiated cells, determination of lineage involvement of clonal cells, etc. Moreover it has many applications in research: identification of non-random chromosome rearrangements, identification of translocation molecular breakpoint, identification of commonly deleted regions, gene mapping, characterization of somatic cells hybrids, identification of amplified genes, study the mechanism of rearrangements. FISH is also used to compare the genomes of two biological species to deduce evolutionary relationships.

How many types of probes for FISH?

Generally, researchers use three different types of FISH probes, each of which has a different application:

Locus specific probes bind to a particular region of a chromosome. This type of probe is useful when researchers have isolated a small portion of a gene and want to determine on which chromosome the gene is located.

Alphoid or centromeric repeat probes are generated from repetitive sequences found in the middle of each chromosome. Researchers use these probes to determine whether an individual has the correct number of chromosomes. These probes can also be used in combination with "locus specific probes" to determine whether an individual is missing genetic material from a particular chromosome.

Whole chromosome probes are actually collections of smaller probes, each of which binds to a different sequence along the length of a given chromosome. Using multiple probes labeled with a mixture of different fluorescent dyes, scientists are able to label each chromosome in its own unique color. The resulting full-color map of the chromosome is known as a spectral karyotype. Whole chromosome probes are particularly useful for examining chromosomal abnormalities, for example, when a piece of one chromosome is attached to the end of another chromosome.

❖ Genomic in situ hybridization (GISH)

Genomic in situ hybridization (GISH) is an efficacious technique, that is, used for genome differentiation of one parent from the other by utilizing special chromosome-labeling techniques. GISH has a gratuity role in cytogenetics for investigation of evolutionary relationship of crops and identification of inserted region in the parent from the alien species. GISH technique follows the same protocol as in the fluorescent in situ hybridization (FISH) technique. However, genomic and blocking DNA utilization in GISH differentiate it from FISH analysis.

Main steps of the genomic in situ hybridization (GISH) are discussed below.

(A) Direct and indirect probe labeling.

(B) Fragmentation of the blocking DNA.

(C) Slide preparation.

(D) Probe and blocking DNA denaturation in a hybridization mixture.

(E) Addition of the hybridization mixture with the probe and the blocking DNA.

(F) Denaturation of the chromosome DNA.

(G) In situ hybridization of probe and blocking DNA in the target sequence of the chromosome.

(H) Detection of the probe in the chromosome DNA of one parent, in an indirect labeling.

(I) Chromosome DNA molecule of the second parent associated to the unlabeled blocking DNA.

(J) Visualization of hybridization signals associated to a probe (green) in a fluorescence microscope.

Unmarked chromosomes are visualized with a counter-staining (blue). When the probe labeling is direct, the detection step of the GISH can be excluded.

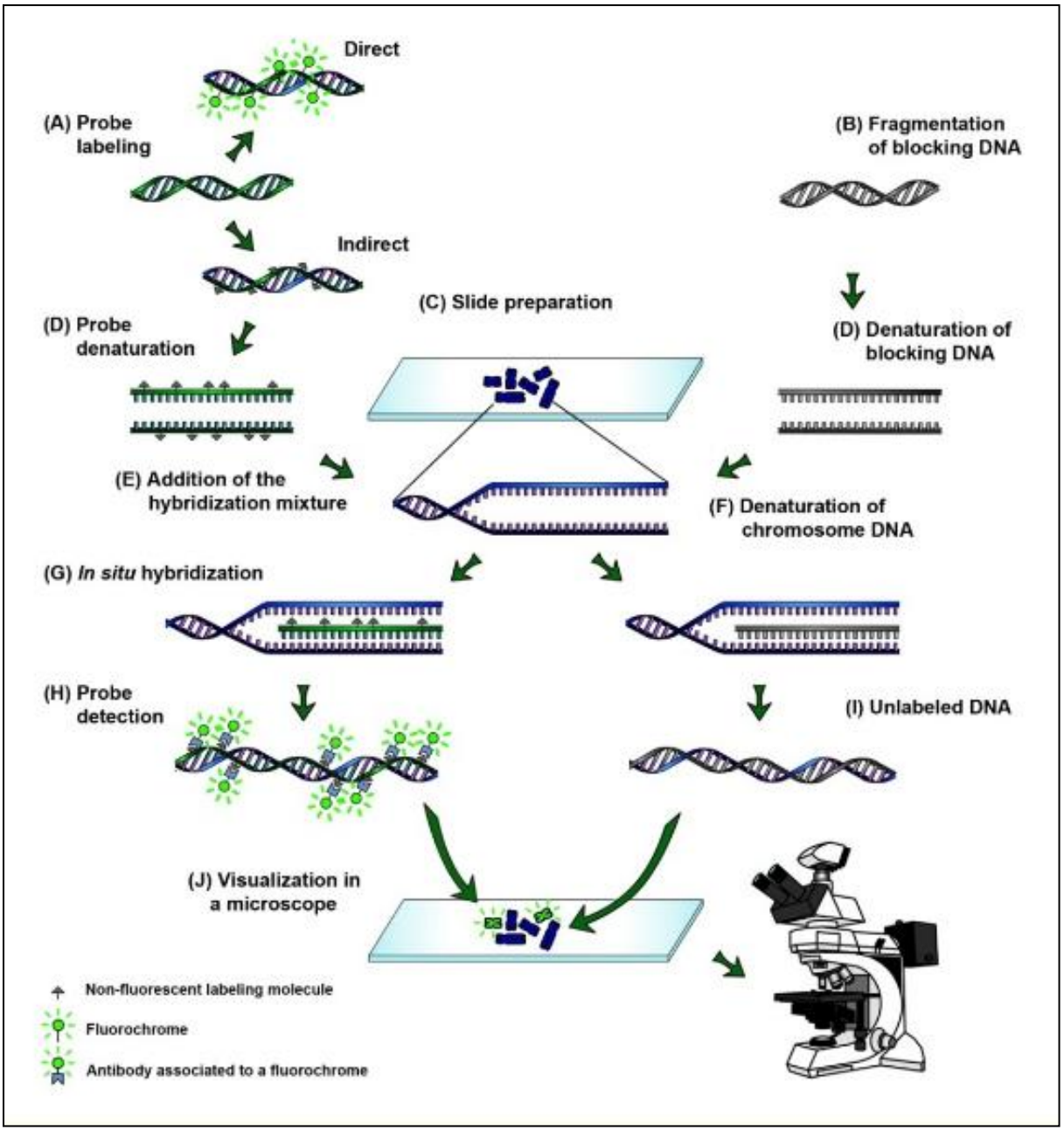


Fig: Main steps of the GISH

Immunohistochemistry (IHC):- a technique used for localizing the proteins in cells of a tissue section.

Immunohistochemistry or IHC refers to the process of localizing proteins in cells of a tissue section exploiting the principle of antibodies binding specifically to antigens in biological tissues. It takes its name from the roots "immuno," in reference to antibodies used in the procedure, and "histo," meaning tissue.

Immunohistochemical staining is widely used in the diagnosis and treatment of cancer. Specific molecular markers are characteristic of particular cancer types. IHC is also widely used in basic research to understand the distribution and localization of biomarkers in different parts of a tissue.

Visualizing an antibody-antigen interaction can be accomplished in a number of ways. In the most common instance, an antibody is conjugated to an enzyme, such as peroxidase, that can catalyse a colour-producing reaction.

Alternatively, the antibody can also be tagged to a fluorophore, such as FITC, rhodamine, or Texas Red. The latter method is of great use in confocal laser scanning microscopy, which is highly sensitive and can also be used to visualize interactions between multiple proteins.

Antibody Types:

The antibodies used for specific detection can be polyclonal or monoclonal. Monoclonal antibodies are generally considered to exhibit greater specificity. Polyclonal antibodies are made by injecting animals with peptide antigens and then after a secondary immune response is stimulated, isolating antibodies from whole serum. Thus, polyclonal antibodies are a heterogeneous mix of antibodies that recognize several epitopes.

Antibodies can also be classified as primary or secondary reagents. Primary antibodies are raised against an antigen of interest and are typically unconjugated (un-labelled), while secondary antibodies are raised against primary antibodies. Hence, secondary antibodies recognize immunoglobulin's of a particular species and are conjugated to either biotin or a reporter enzyme such as alkaline phosphatase or horseradish peroxidase.

Some secondary antibodies are conjugated to fluorescent agents, such as the Alexa-Fluor family, are also frequently used for detection of proteins in IHC procedures. Protein concentration is generally measured by densitometry analysis, where the intensity of staining correlates with the amount of the protein of interest.

Sample Preparation:

In the procedure, depending on the purpose and the thickness of the experimental sample, either thin (about 4-40 μm) slices are taken of the tissue of interest, or if the tissue is not very thick and is penetrable it is used whole. The slicing is usually accomplished through the use of a microtome, and slices are mounted on slides. "Free-floating IHC" uses slices that are not mounted; these slices are normally produced using a vibrating microtome.

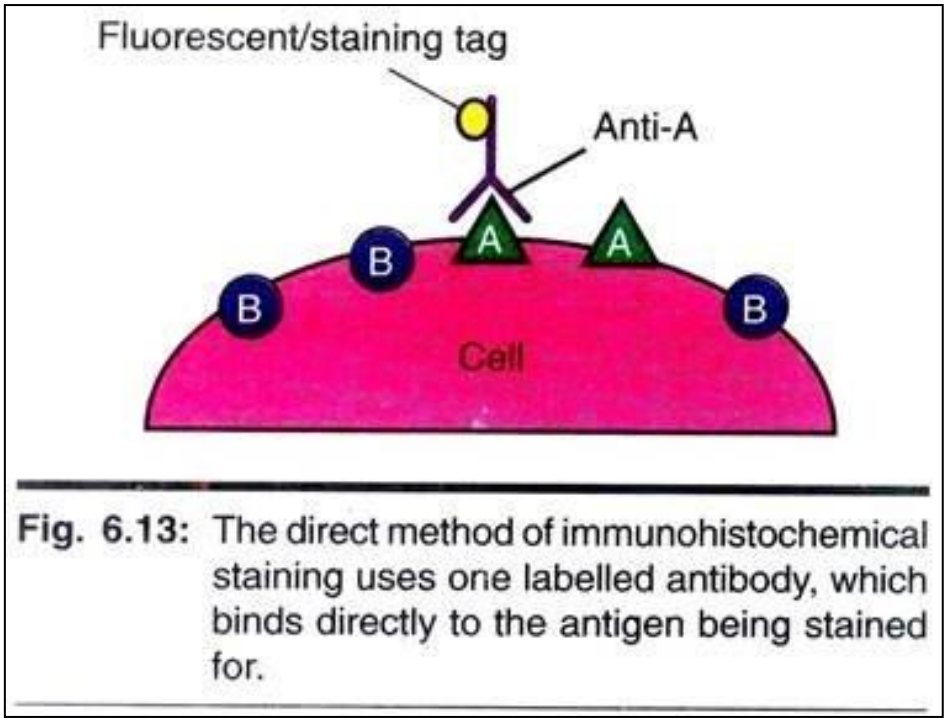


Fig. 6.13: The direct method of immunohistochemical staining uses one labelled antibody, which binds directly to the antigen being stained for.

Diagnostic IHC Markers:

IHC is an excellent detection technique and has the tremendous advantage of being able to show exactly where a given protein is located within the tissue examined. This has made it a widely-used technique in the neurosciences, enabling researchers to examine protein expression within specific brain structures.

Its major disadvantage is that, unlike immuno-blotting techniques where staining is checked against a molecular weight ladder, it is impossible to show in IHC that the staining corresponds with the protein of interest. For this reason, primary antibodies must be well-validated in a Western Blot or similar procedure. The technique is even more widely used in diagnostic surgical pathology for typing tumours (e.g., carcinoma vs. melanoma).

i. Carcinoembryonic antigen (CEA): used for identification of adenocarcinomas. Not specific for site.

ii. CD 15 and CD30 : used for Hodgkin's disease.

iii. Alpha fetoprotein: for yolk sac tumours and hepatocellular carcinoma.

iv. CD117: for gastrointestinal stromal tumours (GIST).

v. Prostate specific antigen (PSA): for prostate cancer.

vi. Estrogens and progesterone staining for tumour identification.

vii. Identification of B-cell lymphomas using CD20.

Probable questions:

23. What do you mean by antigen-antibody reaction?
24. Describe the nature of antigen antibody reaction.
25. What is affinity?
26. What do you mean by cross reactivity?
27. Write short notes on agglutination and precipitation reaction?
28. Describe sandwich ELISA?
29. Describe the process of Radioimmunoassay (RIA) with diagram.
30. What do you mean by Immunofluorescence? Which flurochromes are used in this teachnique?
31. Write down the full form of FISH and GISH.
32. **What is FISH used for?**
33. Write down the main steps of Genomic in situ hybridization with diagram.
34. What do you mean by Immunohistochemistry (IHC)?

Suggested readings/ references:

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Unit-XI

Basic concept of human genetics : introduction to the structure of human genome; human genome and mapping

Objective: In this unit you will learn about Basic concept of human genetics: introduction to the structure of human genome; human genome and mapping.

Introduction:

The 3.2 billion bp of our genetic blueprint is packed into 23 pairs of chromosomes, or 46 DNA molecules. Only a fraction of the genome is occupied by protein-coding exons and the majority of non-exonic sequences consists of repetitive elements. Functional exons contribute merely 2% of a genome, up to 50% of a genome is occupied by repetitive element, the remaining 48% is called unique DNA, most of which probably originated in mobile elements diverged over time beyond recognition. Different evolutionary forces shape the human genome composition and structure. It appears that different mobile elements play a significant role in this process. The human genome is a dynamic entity, new functional elements appear and old ones become extinct as genes that evolve according to birth and death rule similarly to species evolution. This confirms that the theory of evolution is truly universal and applies not only to all organisms but to all levels of life as well. Our genetic material is stored in two organelles: nucleus and mitochondria. This review is focused on the nuclear genome in which 3.2 million bp are packed in 22 pairs of autosomes and two sex chromosomes, X and Y. Human chromosomes are not of equal sizes; the smallest, chromosome 21, is 54 million bp long; the largest, chromosome 1, is almost five times bigger with 249 million bp. Genomic sequences can be divided in several ways. From the functional point of view we can distinguish genes, pseudogenes, and non-coding DNA. Only a minute fraction of the genome — about 3% — codes for proteins. There are many pseudogenes in the human genome (0.5%) but most of the genome consists of introns and intergenic DNA. Almost half of these sequences consist of different transposons; moreover, the remaining non-coding DNA most likely originated from the single copy or in very low copies; 30% of the DNA is moderately repetitive; and 10% is considered highly repetitive.

Sequence Complexity:

The human genome contains various levels of complexity as demonstrated by reassociation kinetics. Such analyses of the human genome estimate that 60% of the DNA is either single copy or in very low copies; 30% of the DNA is moderately

repetitive; and 10% is considered highly repetitive. Various staining techniques demonstrate alternative banding patterns of mitotic chromosomes referred to as karyograms. Although the three broad classes of DNA are scattered throughout the chromosome, chromosomal banding patterns reflect levels of compartmentalization of the DNA. Using the C-banding technique yields dark-staining regions of the chromosome (or C bands), referred to as heterochromatin. These regions are highly coiled, contain highly repetitive DNA, and are typically found at the centromeres, telomeres, and on the Y chromosome. They are composed of long arrays of tandem repeats and therefore some may contain a nucleotide composition that differs significantly from the remainder of the genome (approximately 40–42% GC). That means that they can be separated from the bulk of the genome by buoyant density (caesium chloride) gradient centrifugation. Gradient centrifugation results in a major band and three minor bands referred to as satellite bands — hence the term satellite DNA. The G-banding technique yields a pattern of alternating light and dark bands reflecting variations in base composition, time of replication, chromatin conformation, and the density of genes and repetitive sequences. Therefore, the karyograms define chromosomal organization and allow for identification of the different chromosomes. The darker bands, or G bands, are comparatively more condensed, more AT-rich, less gene-rich and replicate later than the DNA within the pale bands, which correspond to the R bands by an alternative staining technique. More recently, these alternative banding patterns have been correlated to the level of compaction of scaffold-attachment regions (SARs). The human genome may also be compartmentalized into large (> 300 kb) segments of DNA that are homogeneous in base composition referred to as isochores, based on sequence analysis and compositional mapping. L1 and L2 are GC-poor (or 'light) isochore families representing about 62% of the genome. The H1, H2 and H3 (heavy) isochore classes are increasingly GC-rich. There is some correlation between isochores and chromosomal bands. G bands are almost exclusively composed of GC-poor isochores, with a minor contribution from H1. R bands can be classified further into T bands (R banding at elevated temperatures), which are composed mainly of H2 and H3 isochores, and R' (non-T R bands) which are comprised of nearly equal amounts of GC-rich (primarily H1) and GC-poor isochores. Additionally, there are five human chromosomes (13, 14, 15, 21, 22) distinguished at their terminus by a thin bridge with rounded ends referred to as chromosomal satellites. These contain repeats of genes coding for rRNA and ribosomal proteins that coalesce to form the nucleolus and are known as the nucleolar organizing regions.

Gene Distribution :

Genes may be transcribed from either the same or from the opposite strand of the genome, i.e. they may lie in the same (tail-to-head) or opposite orientation (head-to-head or tail-to-tail). Although the vast majority of the human genome accounts for non-exonic sequences, a surprisingly large number of genes occupy the same genomic space.

About 6% of human genes reside in introns of other genes. For example, intron 27th of NF1 gene hosts three other genes that have small introns on their own, suggesting that they are not products of retroposition. Additionally, over 100 gene pairs are overlapping at 3 end, i.e. their 3 UTRs occupy the same region though different strands. TPR and MSF genes map to the same region of chromosome 1. The last exon of the TPR gene is 872 nt long and overlaps completely with the last exon of the MSF gene (200 nt). Interestingly, the very end of the MSF gene overlaps with the intron of the TPR gene. Unlike in plant genomes, most of non-exonic sequences in human genome account for introns (Wong et al., 2000). However, genes are not equally distributed throughout the genome. There is a distinct association between GC-richness and gene density. This is consistent with the association of most genes with CpG islands, the 500–1000 bp GC-rich segments flanking (usually at the 5 end) most housekeeping and many tissue-specific genes. The clustering of CpG islands, as demonstrated by fluorescence in situ hybridization further depicts gene-poor and gene-rich chromosomal segments. As a consequence, more than half of human genes locate in the so-called “genomic core” (isochores H2 and H3) comprising only 12% of the human genome.

Gene Families:

Many genes can be clustered in groups of different sizes based on sequence similarity. The similarity between two genes varies from genes coding identical products to genes in which product similarity is barely detectable and/or limited to short sequence stretches called sequence motives. Genes families arose during the evolution by gene duplications over the different periods of time as reflected in sequence similarity. In general, more similar genes shared a common ancestor later (in nearer past) than genes with a weaker similarity, although gene conversion can result in very similar or identical gene copies regardless of gene duplication time. Gene duplication can occur by different mechanisms, like unequal recombination or retroposition. Not all duplicated genes remain active, some of them end up in genomic oblivion and are called pseudogenes. Some of the pseudogenes can be rescued from the genomic death by capturing a promoter and regulatory elements in the course of evolution as happened with -globin gene which was rescued by an Alu element after 200 million years of silent existence. The histone gene family is an example of very similar genes. It consists of five genes that tend to be linked, although in differing arrays of variable copy numbers dispersed in the human genome. The individual genes of a particular histone family encode essentially identical products (i.e. all H4 genes code for the identical H4 protein). Analysis of individual human genomic clones has identified isolated histone genes, e.g. H4, clusters of two or more histone genes, or clusters of all histone genes, e.g. H3-H4-H1-H3-H2A-H2B. A majority of histone genes form a large cluster on human chromosome 6 (6p21.3) and a small cluster at 1q21. Interestingly, histone genes lack introns; a rare feature for eukaryotic genes. Genes that encode ribosomal RNA (rRNA) total about 0.4% of the DNA in the human genome. The individual genes of a particular

rRNA family are essentially identical. The 28S, 5.8S and 18S rRNA genes are clustered with spacer units in tandem arrays of approximately 60 copies each yielding about 2 million bp of DNA. These clusters are present on the short arms of five acrocentric chromosomes and form the nucleolar organizing regions, hence approximately 300 copies. These three rRNA genes are transcribed as a single unit and then cleaved. 5S rRNA genes are clustered on chromosome 1q. Some genes in the human genome share highly conserved amino-acid domains with weak overall similarity. These often have developmental function. There are nine dispersed paired box (Pax) genes that contain highly conserved DNA binding domains with six -helices. The homeobox or Hox genes share a common 60 amino-acid sequence. In humans there are four Hox gene clusters, each on a different chromosome. However, the individual genes in the cluster demonstrate greater similarity to a counterpart gene in another cluster than to the other genes in the same cluster. There are pseudogenes that are the result of retroposition (retropseudogenes). The pseudogenes lack introns and the flanking DNA sequences of the functional locus and therefore are not products of gene duplication. The generation of these types of elements is dependent on the reverse transcriptase of other retroelements such as LINEs.

Repetitive Sequences:

The human genome is occupied by stretches of DNA sequences of various length that exist in variable copy number. These repetitive sequences may be in a tandem orientation or they may be dispersed throughout the genome. Repetitive sequences may be classified by function, dispersal patterns, and sequence relatedness. Satellite DNA typically refers to highly repetitive sequences with no known function and interspersed repeat sequences are typically the products of transposable element integration, including retrogenes and retropseudogenes of a functional gene.

Microsatellites, Minisatellites, and Macrosatellites:

Microsatellites are small arrays of short simple tandem repeats, primarily 4 bp or less. Different arrays are found dispersed throughout the genome, although dinucleotide CA/TG repeats are most common, yielding 0.5% of the genome. Runs of As and Ts are common as well. Microsatellites have no known functions. However, CA/TG dinucleotide pairs can form the Z-DNA conformation in vitro, which may indicate some function. Repeat unit copy number variation of microsatellites apparently occurs by replication slippage. The expansion of trinucleotide repeats within genes has been associated with genetic disorders such as Huntington disease or fragile-X syndrome. Minisatellites are tandemly repeated sequences of DNA of lengths ranging from 1 kbp to 15 kbp. For example, telomeric DNA sequences contain 10–15 kb of hexanucleotide repeats, most commonly TTAGGG in the human genome, at the termini of the

chromosomes. These sequences are added by telomerase to ensure complete replication of the chromosome. Macrosatellites are very long arrays, up to hundreds of kilobases, of tandemly repeated DNA. There are three satellite bands observed by buoyant density centrifugation. However, not all satellite sequences are resolved by density gradient centrifugation, e.g. alpha satellite DNA or alphoid DNA that constitute the bulk of centromeric heterochromatin on all chromosomes. The interchromosomal divergence of the alpha satellite families allows the different chromosomes to be distinguished by fluorescence in situ hybridization (FISH).

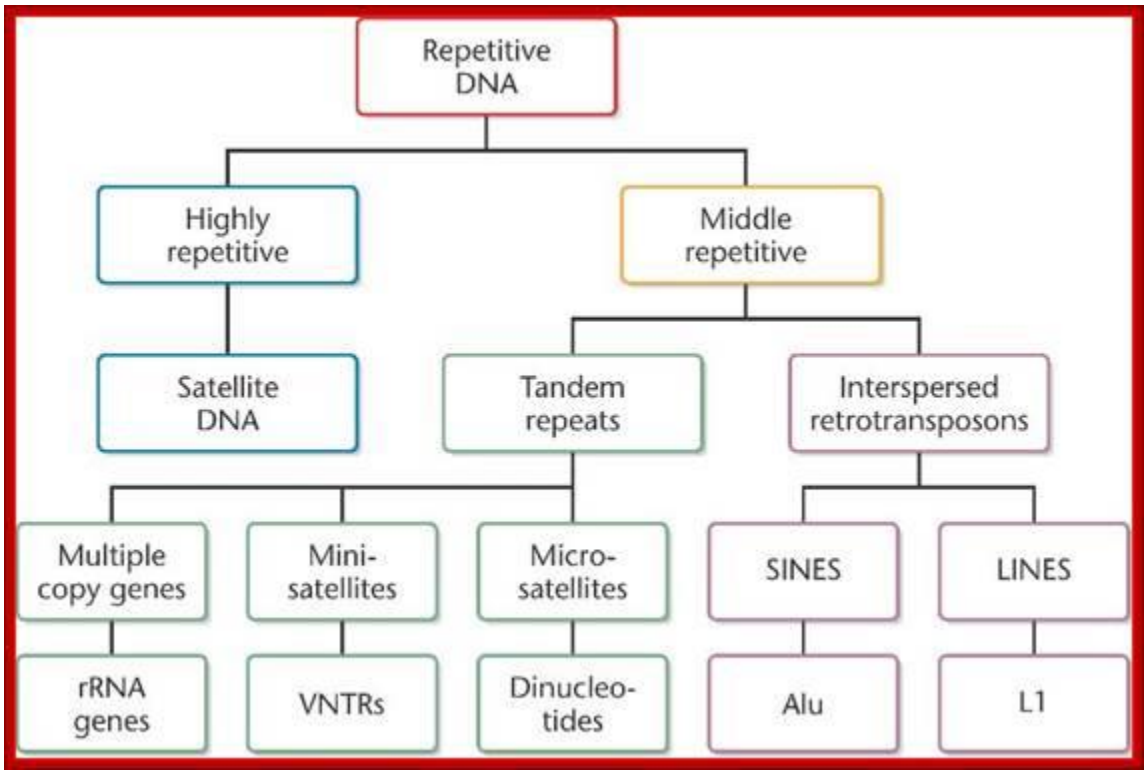


Fig: Flow charts showing various components of repetitive DNA.

Transposable Elements:

The human genome contains interspersed repeat sequences that have largely amplified in copy number by movement throughout the genome. Those sequences (transposable elements or TEs) can be divided into two classes based on the mode of transposition. The Class I elements are TEs which transpose by replication that involves an RNA intermediate which is reverse transcribed back to DNA prior to reinsertion. These are called retroelements and include LTR transposons, which are structurally similar to integrated retroviruses, non-LTR elements (LINEs and SINEs), and retrogenes (see Fig. 5). Class II elements move by a conservative cut-and-paste mechanisms, the excision of the donor element is followed by its reinsertion elsewhere in the genome. Integration of Class I and Class II transposable elements results in the duplication of a short sequence of DNA, the target site. There are about 500 families of such transposons. Most of

transposition has occurred via an RNA intermediate, yielding classes of sequences referred to as retroelements (more than 400 families, e.g. Alu, L1, retrogenes, MIR). However, there is also evidence of an ancient DNA-mediated transposition (more than 60 families of class II (DNA) transposons, e.g. THE-1, Charlie, Tigger, mariner).

Retroelements:

Short interspersed repetitive elements (SINEs) and long interspersed repetitive elements (LINEs) are the two most abundant classes of repeats in human, and represent the two major classes of mammalian retrotransposons. Structural features shared by LINEs and SINEs include an A-rich 3 end and the lack of long terminal repeats (LTRs); these features distinguish them from retroviruses and related retroelements. A full-length LINE (or L1 element) is approximately 6.1 kbp although most are truncated pseudogenes with various 5 ends due to incomplete reverse transcription. There are about 100000 copies of L1 sequences in our genome. Approximately 1% of the estimated 3500 full-length LINEs have functional RNA polymerase II promoter sequences along with two intact open reading frames necessary to generate new L1 copies. Individual LINEs contain a poly-A tail and are flanked by direct repeats. LINE mobilization activity has been verified in both germinal and somatic tissues. The Alu element is estimated at 500000– 900000 copies in the human genome representing the primary SINE family, the most successful transposon in any genome. Sequence comparisons suggest that Alu repeats were derived from the 7SL RNA gene. Each Alu element is about 280 bp with a dimeric structure, contains RNA polymerase III promoter sequences, and typically has an A-rich tail and flanking direct repeats (generated during integration). Although Alu elements are present in all primate genomes, more than 2000 Alu elements have integrated within the human genome subsequent to the divergence of humans from the great apes. The human genome also contains families of retroviral-related sequences. These are characterized by sequences encoding enzymes for retroposition and contain LTRs. In addition, solitary LTRs of these elements may be located throughout the genome. There are several low abundant (10–1000 copies) human endogenous retrovirus (HERV) families, with individual elements ranging from 6 to 10 kb, collectively encompassing about 1% of the genome.

Human Genome and Mapping

Major Characteristics of human Genome:

1. The draft represents about 90% of the entire human genome. It is believed that most of the important parts have been identified.

2. The remaining 10% of the genome sequences are at the very ends of chromosomes (i.e. telomeres) and around the centromeres.

3. Human genome is composed of 3200 Mb (or 3.2 Gb) i.e. 3.2 billion base pairs (3,200,000,000).

4. Approximately 1.1 to 1.5% of the genome codes for proteins.

5. Approximately 24% of the total genome is composed of introns that split the coding regions (exons), and appear as repeating sequences with no specific functions.

6. The number of protein coding genes is in the range of 30,000-40,000.

7. An average gene consists of 3000 bases, the sizes however vary greatly. Dystrophin gene is the largest known human gene with 2.4 million bases.

8. Chromosome 1 (the target human chromosome) contains the highest number of genes (2968), while the Y chromosome has the lowest. Chromosomes also differ in their GC content and number of transposable elements.

9. Genes and DNA sequences associated with many diseases such as breast cancer, muscle diseases, deafness and blindness have been identified.

10. About 100 coding regions appear to have been copied and moved by RNA-based transposition (retro- transposons).

11. Repeated sequences constitute about 50% of the human genome.

12. A vast majority of the genome (~ 97%) has no known functions.

13. Between the humans, the DNA differs only by 0.2% or one in 500 bases.

14. More than 3 million single nucleotide polymorphisms (SNPs) have been identified.

15. Human DNA is about 98% identical to that of chimpanzees.

16. About 200 genes are close to that found in bacteria.

Meaning of Human Genome Project:

The Human Genome Project (HGP) is an International collaborative research programme which started in 1990 and completed in 2003, whose goal was the complete mapping and understanding of the three billion DNA subunits (bases), and to identify all human genes, making them accessible for further biological study.

Aims of Human Genome Project:

The project was aimed for the benefits of humankind, generation of biologists and researchers have been provided with detailed DNA information that will be key to understand the structure, organization and function of DNA in chromosomes.

Human Genome Size:

A genome is an organism's complete set of deoxyribonucleic acid (DNA), a chemical compound that contains the genetic instructions needed to develop and direct the activities of an organism. The human genome contains approx. Three billion base pairs which reside in 23 pairs of chromosomes. Each chromosome contains hundreds and thousands of genes, and ranges in size from about 50000000 to 300000000 base pairs. The total number of genes is 30000 (approx.) and accounts for only 25% of the DNA; the rest is extra-genic DNA.

Human Genome Project Mapping:

Before beginning a sequencing project of the human genome, it was first necessary to produce a good framework map. Two general methods were developed for mapping human genome — standard method and whole genome short-gun method.

The standard method involves finding a segment of the genome and locating where it belongs. Genetic maps based on recombination frequencies between markers are useful in ordering genes. Molecular markers like RFLP, VNTRs (Microsatellites), STSs, SNPs have been used in mapping human genome.

The whole genome shotgun sequencing method involves shearing of genomic DNA followed by cloning, to produce a genomic library.

This is followed by sequencing of cloned DNA fragments at random, followed by shotgun assembly, i.e., the assembly of the fragment sequences into larger units on the basis of their overlaps. Groups of cloned DNA segments that can be aligned in an overlapping fashion to cover a region of the human genome are referred as contigs.

Yeast Artificial Chromosomes (YACs) were initially used as cloning agents when primary task was mapping. However, as the emphasis of the project shifted to sequencing. Bacterial Artificial Chromosomes (BACs) were used.

Human Genome Project had many goals some of the important goals were outlined below:

1. To identify all the approximately 20,000-25,000 genes in human DNA.

2. To determine the sequences of the 3 billion base pairs that makes up human DNA.

3. To store this information in data base.

4. To develop improvised tools for data analysis.

5. To transfer related technologies to other sectors, such as industries.

6. To address the ethical, legal and social issues (ELSI) that may arise from the project.

The methodologies involved two major approaches identifying all genes of the genome and their sequencing. For sequencing, the total DNA from a cell is isolated and converted into fragments of relatively small sizes and cloned in suitable host, this generates a genomic library of the organism. The complete sequencing of the first human chromosome, small chromosome 22, was published in December 1999. Then chromosome 21 was completely sequenced in May 2000. The first draft sequence of entire human genome was published in the famous scientific journal "Nature" on 16th February, 2001.

Individual differences in genomes:

It has to be remembered that every individual, except identical twins, have their own versions of genome sequences. The differences between individuals are largely due to single nucleotide polymorphisms (SNPs). SNPs represent positions in the genome where some individuals have one nucleotide (i.e. an A), and others have a different nucleotide (i.e. a G). The frequency of occurrence of SNPs is estimated to be one per 1000 base pairs. About 3 million SNPs are believed to be present and at least half of them have been identified.

Most of the Genome Sequence is Identified:

About 90% of the human genome has been sequenced. It is composed of 3.2 billion base pairs (3200 Mb or 3.2 Gb). If written in the format of a telephone book, the base sequence of human genome would fill about 200 telephone books of 1000 pages each.

Human Genome Project Sequence:

Sequencing means determining the exact order of the base pairs in a segment of DNA. The primary method used by the HGP to produce the finished version of the human genetic code is map-based or BAC- based sequencing. The human DNA is fragmented into pieces that are relatively large, cloned in the bacteria, stored for replication as required.

A collection of BAG clones containing the entire human genome is called a BAC-library. In this method, each BAC clone is mapped to determine the location of that fragment in human chromosome and then the DNA letters are sequenced from each clone and their spatial relation to sequenced human DNA in other BAC clones. For sequencing, each BAC clone is cut into still smaller fragments that are about 2000 bases in length. These pieces

are called "sub-clones". A "sequencing reaction" is carried out on these sub-clones. With the help of a computer then the short sequences are assembled into contiguous stretches of sequence of the clones.

In a short the whole process can be summarized:

i. Chromosomes, which range in size from 50 million to 250 million bases, must first be broken into much shorter pieces (sub-cloning step).

ii. Each short piece is used as a template to generate a set of fragments that differ in length from each other by a single base that will be identified in a later step (template preparation and sequencing step).

iii. The fragments in a set are separated by gel electrophoresis (separation step).

iv. The final base at the end of each fragment is identified (base-calling step). This process recreates the original sequence of As, Ts, Cs and Gs for each short piece generated in the first step.

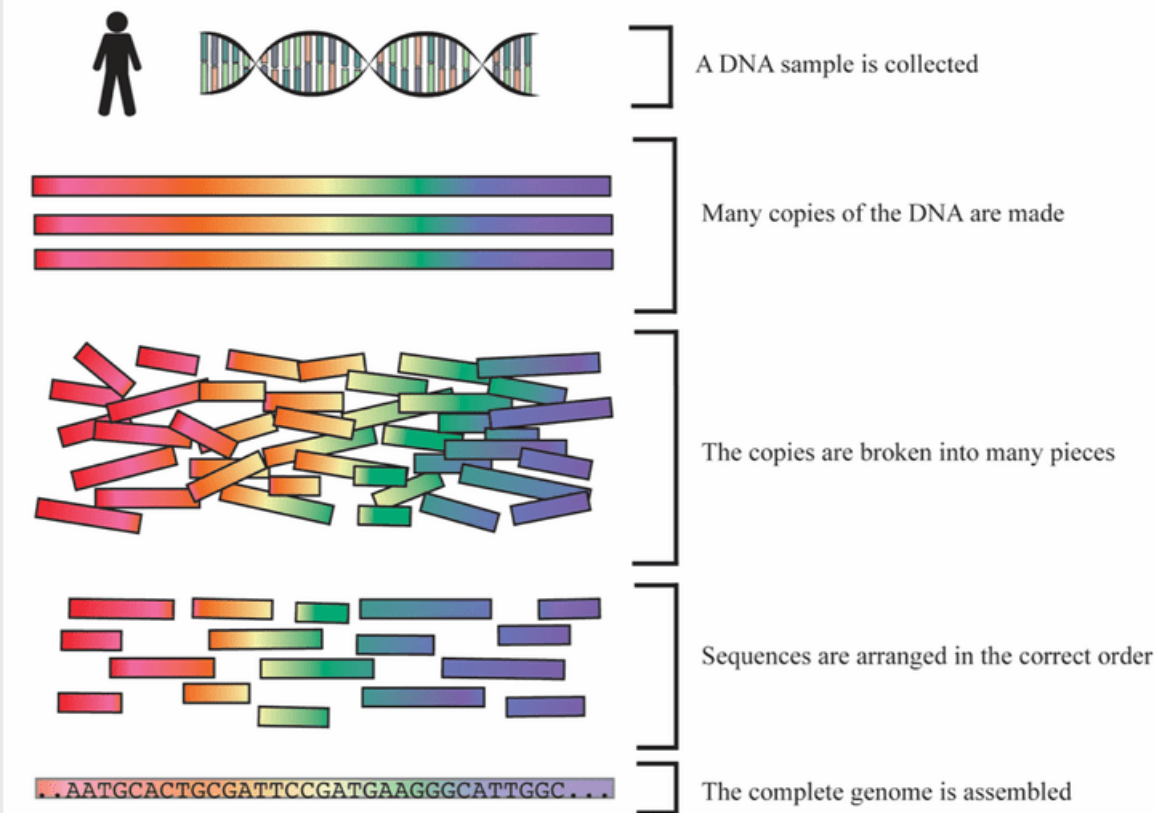
v. After the bases are 'read', computers are used to assemble the short sequences (in blocks of about 500 bases each called the read length) into long continuous stretches that are analysed for errors, gene coding regions, and other characteristics.

vi. Finished sequence is submitted to major public sequence databases, making Human Genome Project sequence data thus freely available to anyone around the world.

The human genome reference sequence do not represent any one person's genome. Rather the knowledge obtained is applicable to everyone because all humans share the same basic set of genes and genomic regulatory regions that control the development.

Researchers collected blood (female) or sperm (male) samples from different races like European, African, American (North, Central, South) and Asian ancestry and a few samples were processed as DNA resources.

Figure 2: Shotgun Whole-Genome Sequencing



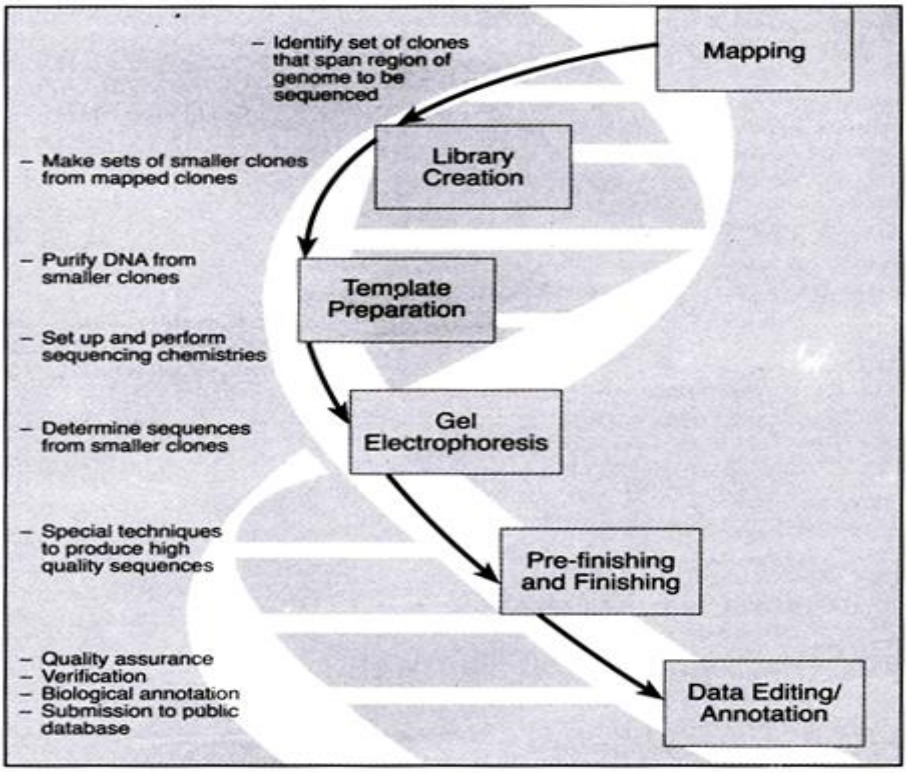


Fig. 18.18: DNA sequencing process

Outcome of Human Genome Project:

i. The human genome contains 3164.7 million chemical nucleotide bases (A, C, T and G).

ii. The average gene consists of 3000 bases, but sizes vary greatly, largest known human gene is **"dystrophin"** – 2.4 million bases.

iii. Total number of genes estimated 30000 approx.

iv. Almost all (99.9%) nucleotide bases are exactly the same in all people.

v. 50% genes are unknown for function.

vi. Less than 2% genomes code for proteins.

vii. Repeated sequences (junk DNA) is 50% of the human genome. This may contribute to create new genes, to modify and reshuffle the existing genes.

viii. A-T rich regions are gene-poor and G-C rich regions are gene-dense. Chromosome-I has the most genes (2968) and the Y chromosome has the fewest (231).

ix. Scientists have identified about 1.4 million locations where single base DNA differences (SNPs) occur in human, these findings will help to localize the disease associated sequences in the chromosomes.

x. Finding the DNA sequences underlying such common diseases as cardiovascular disease, diabetes, arthritis and cancers is being aided by human variation maps (SNPs) generated in HGP.

Benefits/Applications of Human Genome Sequencing:

It is expected that the sequencing of human genome and the genomes of other organisms will dramatically change our understanding and perceptions of biology and medicine. Some of the benefits of human genome project are given.

a. Identification of human genes and their functions:

Analysis of genomes has helped to identify the genes, and functions of some of the genes. The functions of other genes and the interaction between the gene products needs to be further elucidated.

b. Understanding of polygenic disorders:

The biochemistry and genetics of many single- gene disorders have been elucidated e.g. sickle-cell anaemia, cystic fibrosis, and retinoblastoma. A majority of the common diseases in humans, however, are polygenic in nature e.g. cancer, hypertension, diabetes. At present, we have very little knowledge about the causes of these diseases.

The information on the genome sequence will certainly help to unravel the mysteries surrounding polygenic diseases.

c. Improvements in gene therapy:

At present, human gene therapy is in its infancy for various reasons. Genome sequence knowledge will certainly help for more effective treatment of genetic diseases by gene therapy.

d. Improved diagnosis of diseases:

In the near future, probes for many genetic diseases will be available for specific identification and appropriate treatment.

e. Development of pharmacogenomics:

The drugs may be tailored to treat the individual patients. This will become possible considering the variations in enzymes and other proteins involved in drug action, and the metabolism of the individuals.

f. Genetic basis of psychiatric disorders:

By studying the genes involved in behavioural patterns, the causation of psychiatric diseases can be understood. This will help for the better treatment of these disorders.

g. Understanding of complex social trait:

With the genome sequence now in hand, the complex social traits can be better understood. For instance, recently genes controlling speech have been identified.

h. Knowledge on mutations:

Many events leading to the mutations can be uncovered with the knowledge of genome.

i. Better understanding of developmental biology:

By determining the biology of human genome and its regulatory control, it will be possible to understand how humans develop from a fertilized eggs to adults.

j. Comparative genomics:

Genomes from many organisms have been sequenced, and the number will increase in the coming years. The information on the genomes of different species will throw light on the major stages in evolution.

k. Development of biotechnology:

The data on the human genome sequence will spur the development of biotechnology in various spheres.

Probable Questions:

1. How genes are distributed in human genome?

2. What do you mean by gene families?

3. Define microsatellite, macrosatellite and minisatellite.

4. Write a short note on retroelements in human genome.

5. Write the major characteristics of human genome.

6. Write the major goals of Human Genome Project.

7. What are the outcomes of Human Genome Project?

8. What are the applications of Human Genome Project?

Suggested Readings:

1. Principles of Genetics. Snustad and Simmons.

2. Genetics . Verma and Agarwal.

3. Principles of Genetics by Tamarin.

4. Biotechnology by V. Kumaresan

Unit- XII

Human karyotype: karyotype and nomenclature of Metaphase chromosome bands

Objective: In this unit you will learn about Human Karyotype and different banding techniques.

Introduction:

Chromosomal aberrations are abnormalities in the number or microscopically observable structure of chromosomes. The number of chromosomes in human cells is 46 with 22 autosomal pairs (one of each type contributed by the mother and one of each type from the father) and 2 sex chromosomes - 2 X chromosomes for females (one from father and one from mother) or an X and a Y chromosome for males (the X from the mother and the Y from the father). The chromosomes visible only at the metaphase stage of mitosis, 22 homologous pairs of autosomes and two sex chromosomes. Each chromosome has a characteristic size and shape in the "normal" cell. During most of the cell cycle, interphase, the chromosomes are somewhat less condensed and are not visible as individual objects under the light microscope. Mitosis, or nucleus division, is the first part of M-phase and in consists of four stages (prophase, metaphase, anaphase and telophase). However during cell division, mitosis, the chromosomes become highly condensed and are then visible as dark distinct bodies within the nuclei of cells. The chromosomes are most easily seen and identified at the metaphase stage of cell division.

The banding of chromosomes by using dyes was discovered in the late 1960's and before that cytogeneticists depended on chromosome length and position of a constriction to identify the individual chromosomes. The band width and the order of bands is characteristic of a particular chromosome - a trained cytogeneticist can identify each chromosome (1,2,3...22, X and Y) by observing its banding pattern under the microscope. chromosomes are arranged and numbered by size, from largest to smallest. This arrangement helps scientists quickly identify chromosomal alterations that may result in a genetic disorder. Identifying chromosomes has become easier in recent years by using certain staining techniques. One of the most common staining techniques involves Giemsa stain, which gives the chromosomes a banded appearance (hence Giemsa banding or G-banding) G-banding is the treatment of chromosomes in the metaphase stage with trypsin (to partially digest the protein) and stain them with Giemsa. Each homologous chromosome pair has a unique pattern of G-bands, enabling recognition of particular chromosomes. Karyotyping is the process by which doctors and geneticists take pictures of the chromosomes while the cell are undergoing mitosis. The picture is then enlarged. The picture of the chromosomes are then cut up so that each chromosome is removed. The chromosomes are matched up and attached to a paper according to size. The chromosomes pairs are numbered from largest to smallest.

There are 22 pairs of chromosomes which match up exactly. Then the sex chromosomes are paired, in the female (XX) the chromosomes match and in the male (XY) the chromosomes do not match. This technique can be used to assess the “normalcy” of an individual’s chromosomes and to assay for various genetic diseases such as Down’s syndrome Klinefelter’s syndrome. It is estimated that one in 156 live births have some kind of chromosomal abnormality. A chromosome is divided by its centromere into short arm (p) and long arm (q). chromosomes can be classified by the position of their centromere: - Metacentric: If its two arms are equal in length. - Submetacentric: If arms' lengths are unequal. - Acrocentric: If the p arm is so short that is hard to observe, but still present The photograph is enlarged and cut up into individual chromosomes. The homologous chromosomes can be distinguished by length and by the position of the centromere so the chromosomes can be arranged in 7 groups (A, B, C, D, E, F, G). Karyotypes are arranged with the short arm of the chromosome on top, and the long arm on the bottom. In addition, the differently stained regions and sub-regions are given numerical designations from proximal to distal on the chromosome arms. For example, Cri du chat syndrome involves a deletion on the short arm of chromosome 5. It is written as 46,XX,5p-. The critical region for this syndrome is deletion of 15.2, which is written as 46,XX,del(5)(p15.2). Peripheral Blood Karyotyping Medium With Phytohemagglutinin (PHA) is intended for use in short-term cultivation of peripheral blood lymphocytes for chromosome evaluation. It is based on RPMI-1640 basal medium supplemented with L-Glutamine, fatal bovine serum and antibiotics (penicillin and streptomycin). Karyotyping Medium is supplied as frozen medium, which is ready for use after thawing and phytohaemagglutinin supplementation. The blood cell karyotyping method was developed to provide information about chromosomal abnormalities. Lymphocyte cells do not normally undergo subsequent cell divisions. In the presence of a mitogen (PHA), lymphocytes are stimulated to enter into mitosis by DNA replication. After 48-72 hours, a mitotic inhibitor (colcemid) is added to the culture to stop mitosis in the metaphase stage. After treatment by hypotonic solution, fixation and staining, chromosomes can be microscopically observed and evaluated for abnormalities.

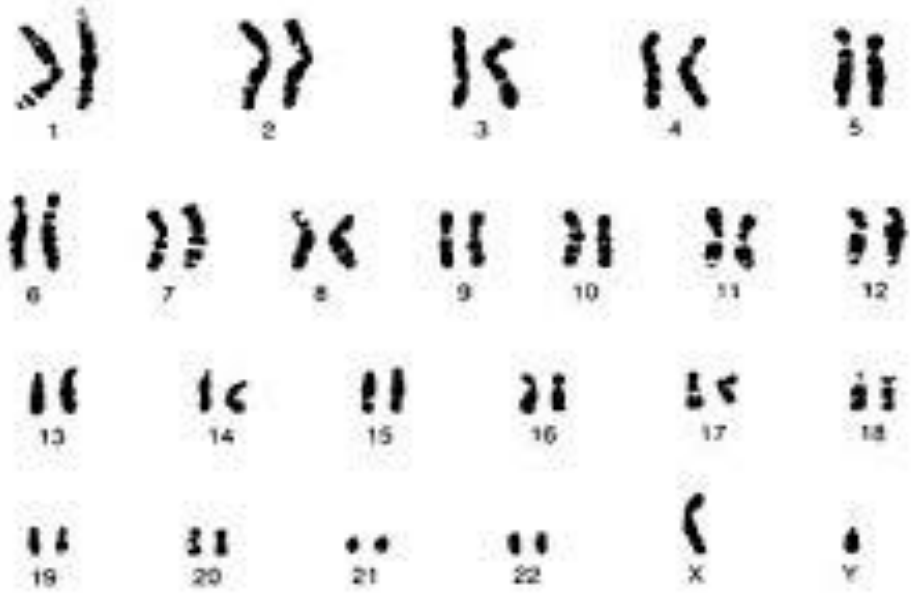


Fig. 21.1 Karyotype of a normal human male showing G-bands

Classification of Chromosomes for Karyotyping:

Chromosomes are arranged into seven groups based on size and centromere location. The centromeres can be found in the middle of the chromosome (median), near one end (acrocentric), or in between these first two (submedian)

Group A: chromosomes 1-3 are largest with median centromere.

Group B: chromosomes 4-5 are large with submedian centromere

Group C: chromosomes 6-12 are medium sized with submedian centromere

Group D: chromosomes 13-15 are medium sized with acrocentric centromere

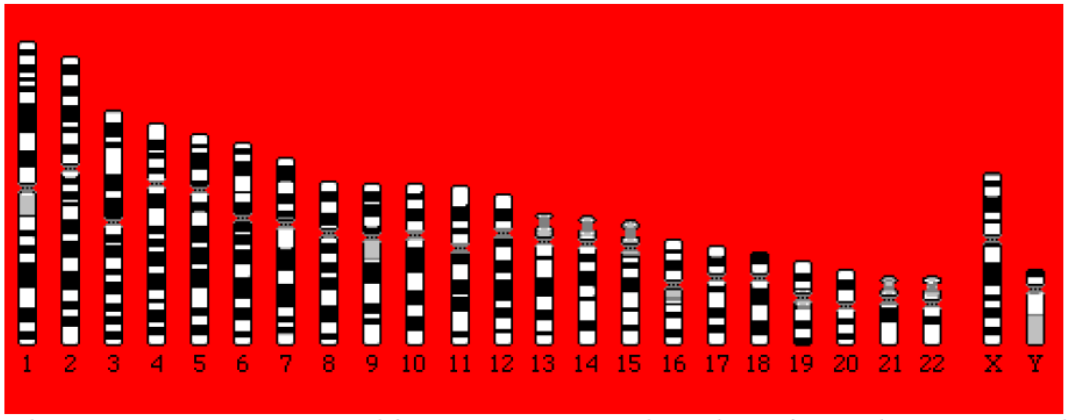
Group E: chromosomes 16-18 are short with median or submedian centromere

Group F: chromosomes 19-20 are short with median centromere

Group G: chromosomes 21-22 are very short with acrocentric centromere.

Chromosome X is similar to group C.

Chromosome Y is similar to group G



Chromosomes are arranged into seven groups based on size and centromere location. The centromeres can be found in the middle of the chromosome (median), near one end (acrocentric), or in between these first two (submedian)

Karyogram:

Photomicrographs of the chromosomes of a single representative somatic metaphase cell are clipped out and arranged in homologous pairs according to their size. If the chromosomes are small and there is difficulty in identifying the individual chromosomes, they are arranged in groups of similar chromosomes.

For example, in human, the 23 pairs of chromosomes had been divided into 7 groups represented by the letters from A to G (Denver System) and numbers (London System); the seven groups are, A (l, 2, 3), B, (4, 5), C (X, 6, 7, 8, 9, 10 11, 12), D (13, 14, 15), E (16, 17, 18), F (19, 20), and G (Y, 21, 22). Thus the X chromosome is placed in the C group, while the Y chromosome is placed in the G group. However, it is now possible to unambiguously identify each of the 23 chromosomes, and even individual chromosome arms, with the help of chromosome banding.

Idiogram or Idiotype:

It is the graphical representation of the karyotype. Generally, the idiogram is prepared to show the haploid chromosome complement of a species; it is prepared from the measurement of somatic metaphase chromosomes. Individual chromosomes must be identified for this purpose. There are techniques by which chromosomes or even specific chromosome segments can be identified. These techniques are fluorescent staining, pulse labelling, chromosome banding, and studying the tertiary constrictions and chromomeres. Chromomere pattern can be studied easily and clearly in pachytene stage in many species and in polytene giant chromosomes of several members of Diptera.

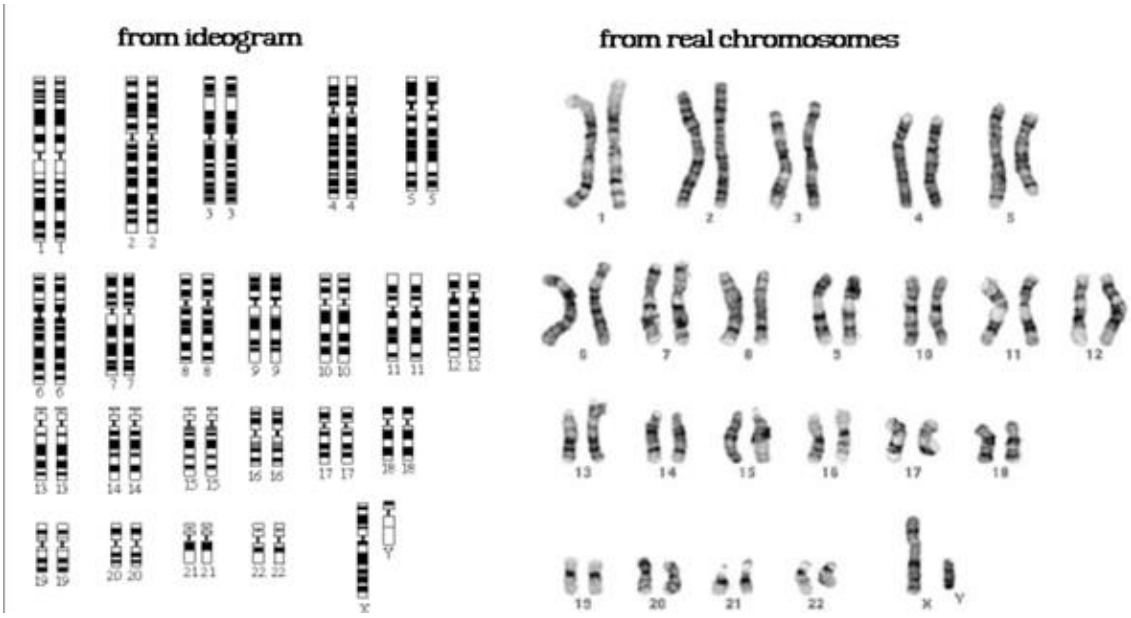
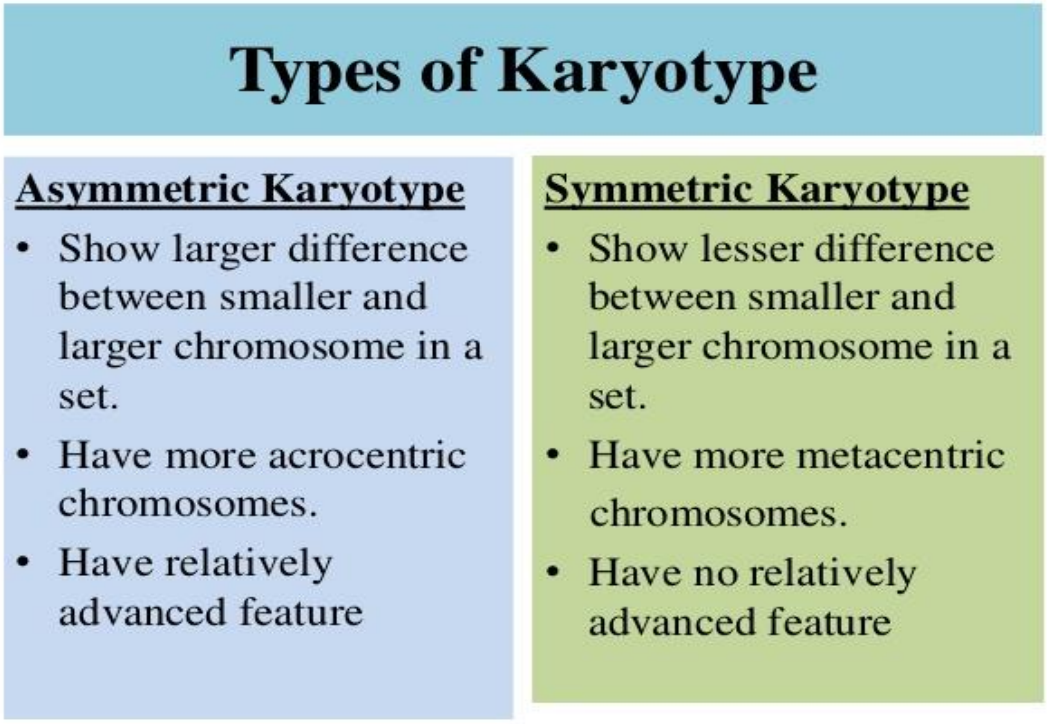


Fig: Idiogram prepared from human chromosomes

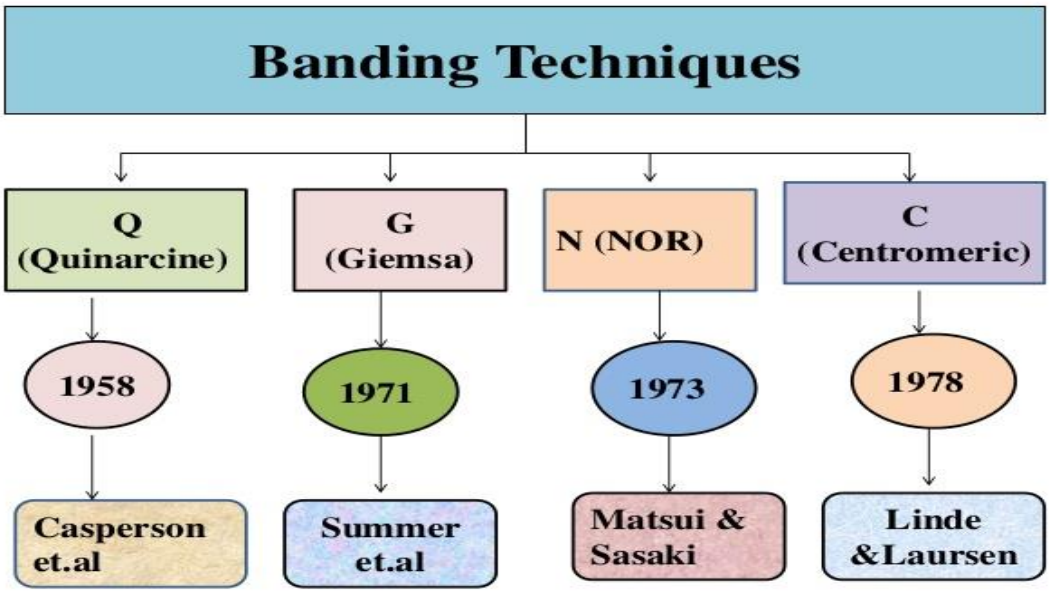


Chromosome Banding:

Chromosome banding is the "lengthwise variation in staining properties along a chromosome…normally independent of any immediately obvious structural variation," and thus excludes patterns such as those seen on polytene chromosomes of Drosophila, which have a morphological component. Although the first observations of what could be called chromosome banding were made at the end of the nineteenth century, modern

chromosome banding methods date from 1968 and can be applied to chromosomes of a wide variety of species with no more than slight modifications. Following the introduction of Q-banding by Caspersson and his colleagues in 1968, Pardue and Gall inadvertently produced differential staining of heterochromatin in their pioneering in situ hybridization studies, leading directly to C-banding, and in 1971 G-banding was discovered by several authors. R-banding was also introduced in 1971. Over the next few years, many other banding techniques, too numerous to mention individually, were introduced, many of them using fluorochromes. Silver staining for nucleolus organizing regions(NORs) was introduced in 1975, methods to show chromosome replication were invented, and the use of autoimmune sera to label kinetochores immunocytochemically was discovered.

Chromosome Banding Techniques:

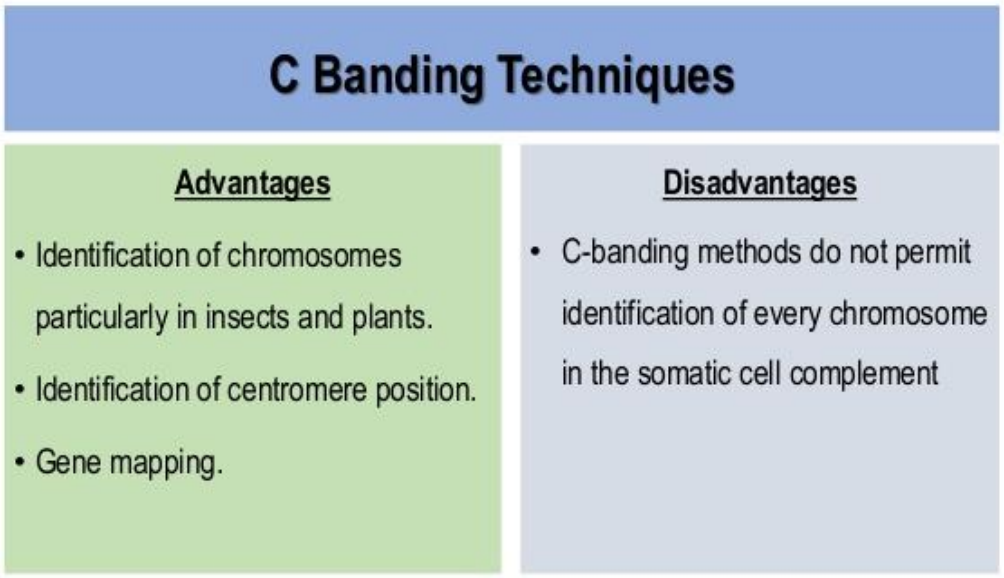


a. C-Banding:

The technique of C-banding originated after the work of Pardue and Gall who reported that constitutive heterochromatin can be stained specifically by Giemsa-solution. Each chromosome possesses a different degree of constitutive heterochromatin which enables the identification of individual chromosomes.

Constitutive heterochromatin is located near the centromere, at telomeres and in the nucleolar organizer regions; it is composed of highly repetitive DNA. C-banding represents the constitutive heterochromatin, and the banding is caused by differential staining reactions of the DNA of heterochromatin and euchromatin. The banding method is a complex technique that involves several treatments with acid, alkali or increased temperature. Denaturation of DNA is caused by these treatments.

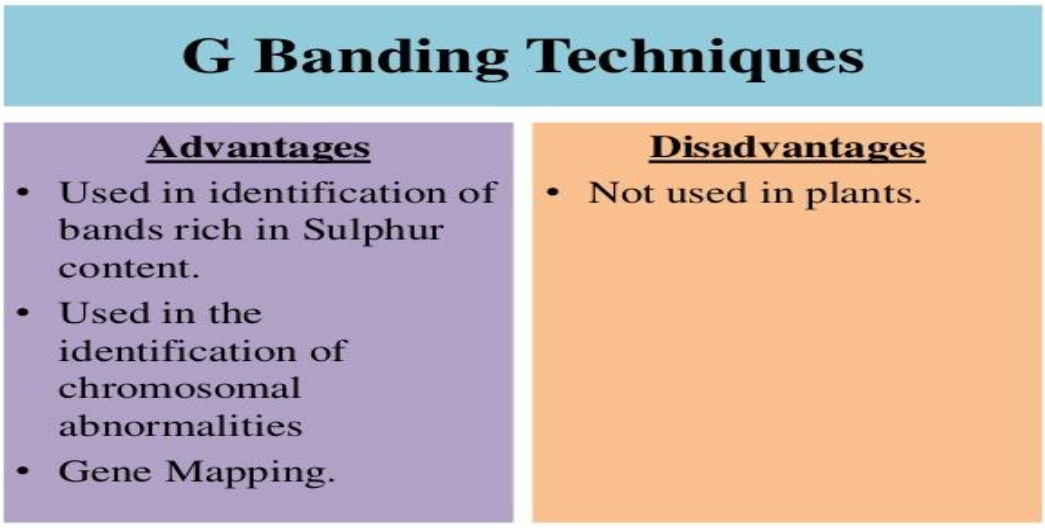
Subsequently, DNA renaturation occurs in treatments with sodium-citrate at 60°C. By these treatments, the repetitive DNA (heterochromatin) re-natures but low repetitive and unique DNAs do not re-nature. This results in differential staining of the specific chromosome regions. Giemsa-C-banding technique has been used to identify chromosomes of various plant and animal species including human. The Y chromosome of mammals is mostly heterochromatic and therefore, the technique of C-banding is quite useful for its identification.



Gupta ,P. K., 2012. Cytogenetics an advanced study, *Chapter 1*: 3-16.

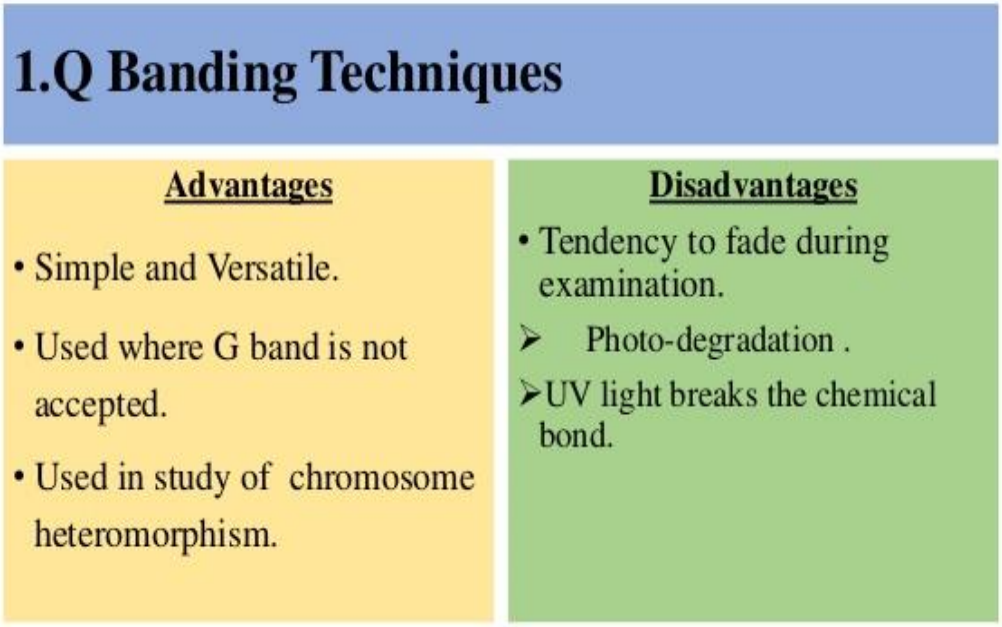
b. G-Banding:

The technique of G-banding involves Giemsa staining following pretreatment with weak trypsin solution, urea or protease. It provides greater detail than C-banding. It was first used for human chromosomes by Summer et al. in 1971. G-bands may reflect a stronger chromatin condensation. However, this technique is not suitable for plant chromosomes.



c. Q-Banding:

The method of Q-banding was developed by Caspersson et al. in 1968. The chromosomes stained with Quinacrine mustard show bright and dark zones under UV light. This technique is used to identify human and mice chromosomes.

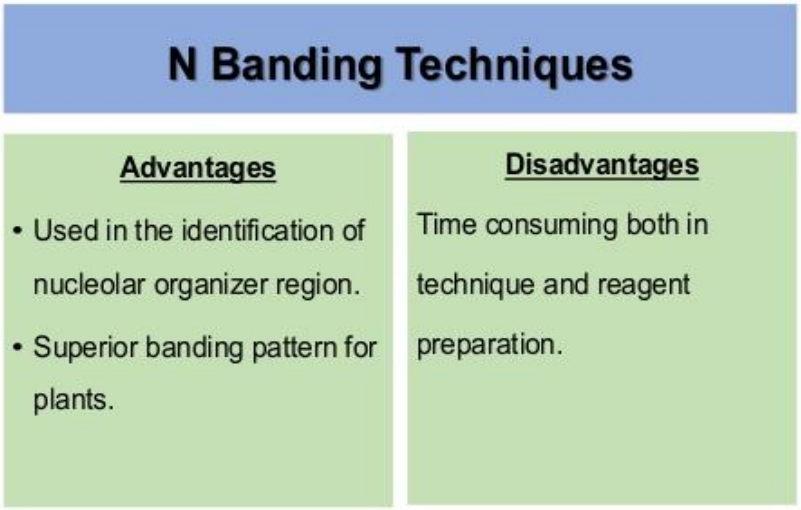


d. N-Banding:

The technique of N-banding was originally described by Matsui and Sasaki in 1973. Briefly, air-dried chromosomes slides are stained for 90 minutes with Giemsa (diluted 1 : 10 in 1/15 M phosphate buffer at pH 7.0) following extraction with 5% trichloroacetic acid at 95°C for 30 minutes and then 0.1 NHCl at 60″C for 30 minutes. The N-bands are

generally located at the secondary constriction, satellites, centromeres, telomeres and heterochromatic segments. It is suggested that the N-bands represent certain structural non-histone proteins specifically linked to the nucleolar organizer region of the eukaryotic chromosomes.

The N- banding patterns have been used for the location of nucleolar regions in the different organisms, such as, mammals, birds, amphibians, fishes, insects and plants. N-banding patterns differ in the chromosomes of different species.In 1980, Islam used this method to identify the barley chromosomes from those of wheat in the reciprocal wheat-barely F, hybrids, and to detect translocations between the wheat and barley chromosomes. He also used this technique to isolate lines possessing a pair of barely chromosomes substituted for particular pair of wheat chromosomes. A modified Giemsa-N-banding technique was developed by Singh and Tsuchiya in 1982 for the identification of barley chromosomes. This method is a combination of acetocarmine staining and Giemsa-N-banding. After processing according to this method, the centromeric region looks like a "diamond-shaped" structure; this is not seen in other techniques. Early metaphase or prometaphase chromosomes are more suitable for this staining as they show better banding pattern than the chromosomes at mid-metaphase in somatic cells.



Other Techniques of Chromosome Banding:

Besides the above, there are other techniques for chromosome banding, e.g., R-banding (Reverse Giemsa banding). H-banding, and T-banding (Terminal banding). Chromosome banding patterns can be used not only for the identification of individual chromosomes of an organism but also to establish evolutionary relationships between different species.

Banding patterns in human, chimpanzee, gorilla and orangutan have indicated that the evolutionary relationship between human and chimpanzee is closer than that between

human and gorilla. It has further indicated that humans have a more distant evolutionary relationship with orangutans.

Uses of Chromosome Banding

G-and R-banding are the most commonly used techniques for chromosome identification (karyotyping) and for identifying abnormalities of chromosome number, translocations of material from one chromosome to another, and deletions, inversions or amplifications of chromosome segments. This has had an invaluable impact on human genetics and medicine and the power of this approach has been augmented by combining cytogenetics with fluorescence in situ hybridization (FISH). The detection of chromosome deletions associated with disorders, very often contiguous gene syndromes, provided some of the

first disease gene localizations in humans. Similarly, translocations have been important in pinpointing the location of disease-associated genes and the characteristic translocations associated with some leukaemia is important,not only for understanding the molecular basis of these cancers, but also for their diagnosis and prognosis. One of the best examples of this is the translocation between human chromosomes 9 and 22 – t(9;22)(q34:q11)– or the Philadelphia chromosome diagnostic of chronic myelogenous leukaemia (CML).

Comparisons of chromosome banding patterns can confirm evolutionary relationships between species and also reveal changes in karyotype that may have been important in speciation. The banding patterns of human, gorilla and chimpanzee chromosomes are almost identical, though human chromosome 2 is the result of a fusion between two great ape chromosomes. There are also extensive similarities between human chromosome bands and those of lower primates.

Evolution of chromosome bands

Whereas Q-, G- and R-banding patterns have only been observed in some eukaryotes, replication banding is almost universal among living organisms possessing chromosomes

large enough to see by microscopy, suggesting that itis a fundamental consequence of, or requirement for, the compartmentalization of complex genomes. Chromosomes from most mammals and birds can be G and R-banded. With amphibia, fish and plants, some species band whereas others do not. The lowest vertebrates with reported good G-banding are the bony fish. Evolutionary analysis of chromosome banding patterns suggests that the first cytogenetically detectable compartmentalization that arose in the genomes of eukaryotes was the temporal control of replication and differences in chromatin packaging and the segregation of some chromosomal domains into heterochromatin. Ability to be G banded(and we will assume here that this is a

reflection of differences in chromatin structure on mitotic chromosomes) followed later. Fluorochrome banding seems to have appeared on the scene last of all.

Probable questions:

1. Define Karyotype and Idiogram.

2. Describe grouping of chromosomes in karyotype.

3. What are the difference between symmetric and asymmetric karyotype.

4. write a note on chromosome banding. Name 4 types of chromosome banding.

5. What do you mean by C banding? state its advantages and disadvantages.

6. What do you mean by Q banding? state its advantages and disadvantages.

7. What do you mean by N banding? state its advantages and disadvantages.

8. What do you mean by G banding? state its advantages and disadvantages.

9. what are the uses of Chromosome banding ?

10. How Chromosomes bands have evolved?

Suggested Readings:

1. Principles of Genetics. Snustad and Simmons.

2. Genetics . Verma and Agarwal.

3. Principles of Genetics by Tamarin.

4. Biotechnology by V. Kumaresan

UNIT-XIII

Chromosome anomalies and Structural Variants.Human genetics and society: genetic testing; human rights; genetic counselling

Objectives: In this unit you will learn about various type of chromosomal anomalies and associated disease. You will also learn about genetic testing, human rights and also about genetic counselling in this unit.

Introduction:

Almost every cell in our body contains 23 pairs of chromosomes, for a total of 46 chromosomes. Half of the chromosomes come from our mother, and the other half come from our father. The first 22 pairs are called autosomes. The 23rd pair consists of the sex chromosomes, X and Y. Females usually have two X chromosomes, and males usually have one X and one Y chromosome in each cell. All of the information that the body needs to grow and develop comes from the chromosomes. Each chromosome contains thousands of genes, which make proteins that direct the body's development, growth, and chemical reactions.

Many types of chromosomal abnormalities exist, but they can be categorized as either numerical or structural. Numerical abnormalities are whole chromosomes either missing from or extra to the normal pair. Structural abnormalities are when part of an individual chromosome is missing, extra, switched to another chromosome, or turned upside down.

Chromosomal abnormalities can occur as an accident when the egg or the sperm is formed or during the early developmental stages of the foetus. The age of the mother and certain environmental factors may play a role in the occurrence of genetic errors. Prenatal screening and testing can be performed to examine the chromosomes of the foetus and detect some, but not all, types of chromosomal abnormalities.

Chromosomal abnormalities can have many different effects, depending on the specific abnormality. For example, an extra copy of chromosome 21 causes Down syndrome (trisomy 21). Chromosomal abnormalities can also cause miscarriage, disease, or problems in growth or development.

The most common type of chromosomal abnormality is known as aneuploidy, an abnormal chromosome number due to an extra or missing chromosome. Most people with aneuploidy have trisomy (three copies of a chromosome) instead of monosomy (single copy of a chromosome). Down syndrome is probably the most well-known example of a chromosomal aneuploidy. Besides trisomy 21, the major chromosomal

aneuploidies seen in live-born babies are: trisomy 18; trisomy 13; 45, X (Turner syndrome); 47, XXY (Klinefelter syndrome); 47, XYY; and 47, XXX.

Structural chromosomal abnormalities result from breakage and incorrect rejoining of chromosomal segments. A range of structural chromosomal abnormalities result in disease. Structural rearrangements are defined as balanced if the complete chromosomal set is still present, though rearranged, and unbalanced if information is additional or missing. Unbalanced rearrangements include deletions, duplications, or insertions of a chromosomal segment. Ring chromosomes can result when a chromosome undergoes two breaks and the broken ends fuse into a circular chromosome. An isochromosome can form when an arm of the chromosome is missing and the remaining arm duplicates.

Balanced rearrangements include inverted or translocated chromosomal regions. Since the full complement of DNA material is still present, balanced chromosomal rearrangements may go undetected because they may not result in disease. A disease can arise as a result of a balanced rearrangement if the breaks in the chromosomes occur in a gene, resulting in an absent or nonfunctional protein, or if the fusion of chromosomal segments results in a hybrid of two genes, producing a new protein product whose function is damaging to the cell.

I) Autosomal Chromosomal abnormalities:

Disorders of the autosomes are much more frequent that disorders of the sex chromosomes (Klinefelter syndrome, Turner syndrome). Typical are numeric abnormalities and we then recognize two types of disorders:

1. monosomy – the carrier lost one copy of a chromosome (45,XY);

2. trisomy – there are one more copy of a chromosome (47,XY).

1. Although the trisomies of chromosomes 18 and 13 were discovered early, there is another trisomy which occurs most – trisomy 21 (Down syndrome).

There are also structural disorders of the autosomes. The most important are deletions. Very well known are deletion of short arm of 5 chromosome (Cri du chat syndrome) and deletion of long arm of 22 chromosome (DiGeorge syndrome).

Most of carriers of autosomal mutations die during their development and usually not born. Especially monosomies in foetus are connected with abortion. Children with trisomy and deletions who are born, suffer from physical and mental retardation and have shorter life at all. The cause of these disorders is usually meiotic nondisjunction – parents have normal karyotype.

a. Trisomy 21 (Down syndrome)

Down syndrome is very well-known trisomy. Trisomy was first described in 1866 by scientist John Down, and later elaborated upon with much greater detail through the Karyotype of Trisomy discovery in 1959..

There are three types of origin. The most common is an *extra chromosome 21* (95%). Sometimes (4% of cases) a small parts of chromosome 21 *translocate* to 14 or 22 chromosome. In 2% of cases we can find *mosaic*. Mosaic means that person has some cells with trisomy and some without. The last two examples have less severe symptoms. The extra chromosome is much worse.

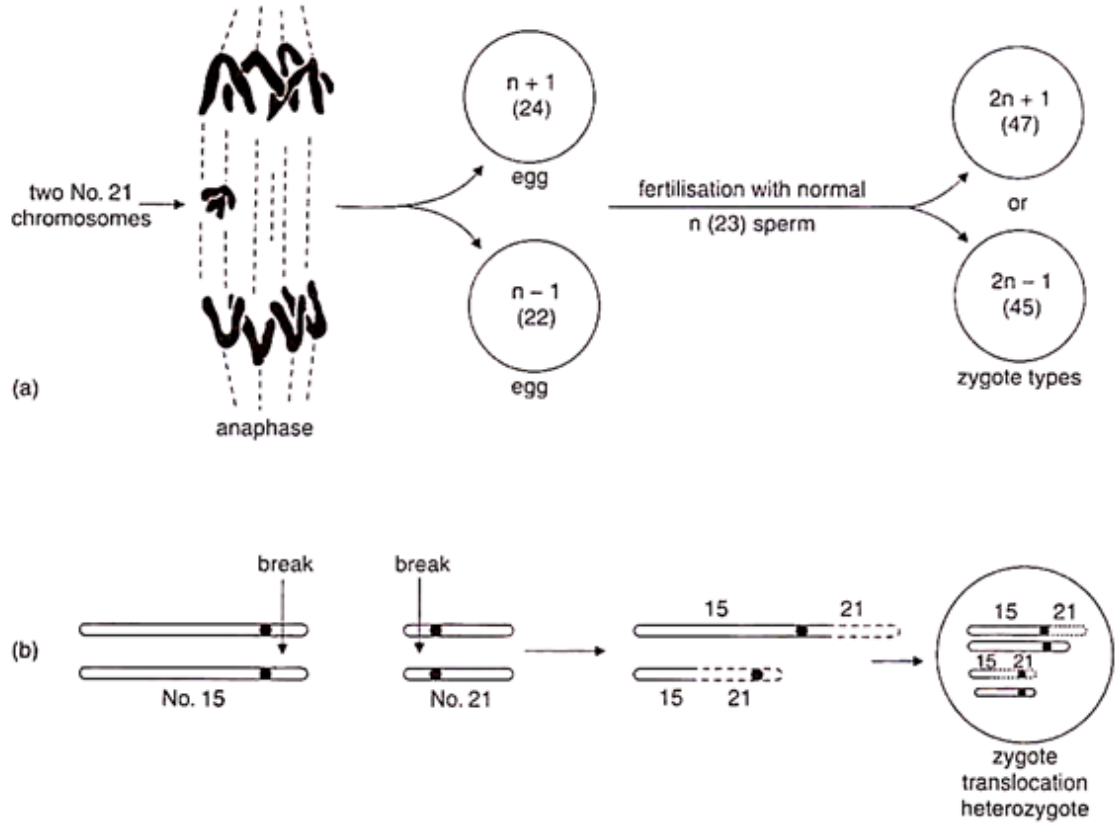


Fig. 21.2 (*a*) Origin of Down's syndrome through nondisjunction during meiosis and (*b*) through translocation.

People with Down syndrome have typical **physical appearance**. They have short body, flat faces, epicanthic folds (part of skin over the inner corner of the eye – the reason why they are sometimes called "Mongoloid"), small low-set ears and relatively large tongue. They have also motor problems because of small muscle tone. Carriers suffer more from leukaemia, infections, cardiac malformations, epilepsy, hypothyroidism and Alzheimer disease. The mental abilities are also worse.

The IQ of people with Down syndrome is about 50. It means that they reach a level of a small child – about 6 years old. On the second side their character is very friendly, warm

and loving. Congenital heart disease and leukaemia occur in many cases. Many have poor muscle tone during infancy. There is a higher incidence of Down's syndrome among children of older mothers.

The patients live for a variable number of years. A Down's male is usually sterile, females are fertile and rarely have produced offspring. The dermatoglyphic pattern (arrangement of lines on palm and fingers) shows in many cases a line called simian crease and distal axial triradius. Frequently all the ten fingers show ulnar loops.

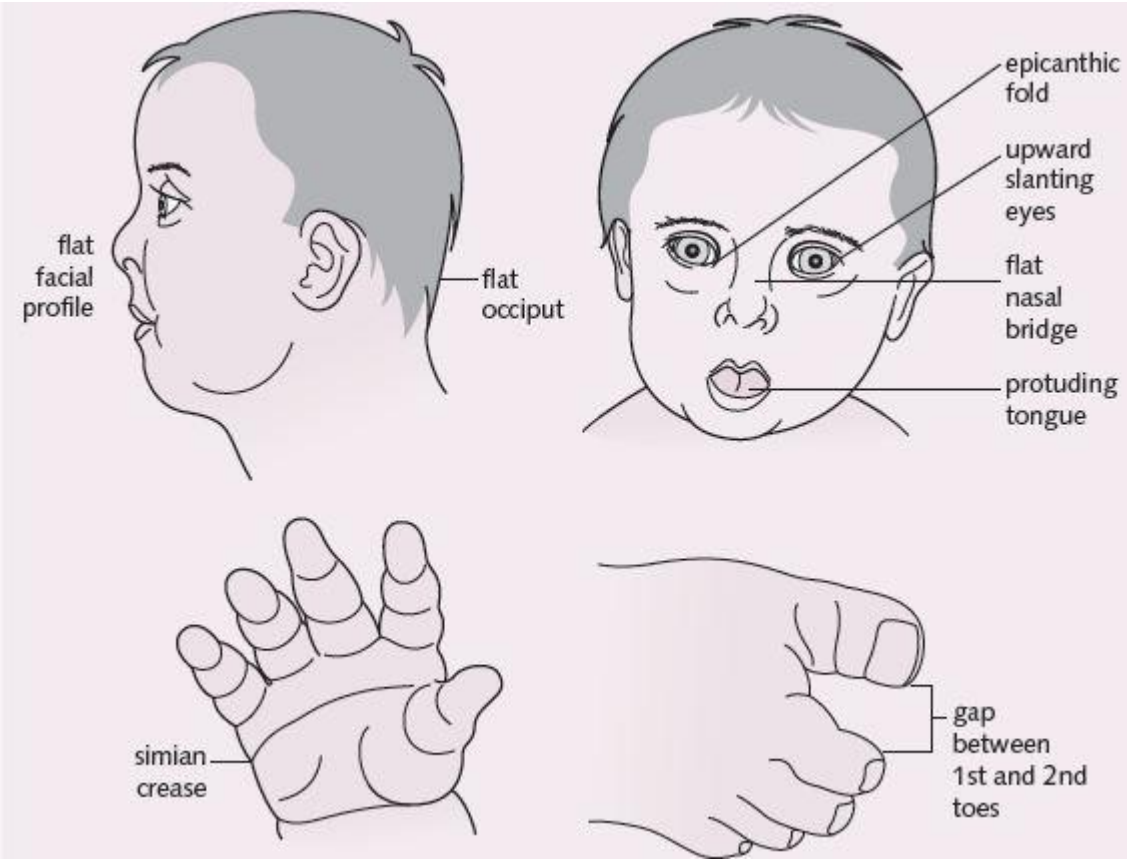


Fig: Symptoms of Down syndrome

The cause of 21 trisomymeiotic nondisjunction. Parents of the children have normal karyotype and no symptoms. Very big influence has the age of the mother. The higher age is connected with higher risks (higher means more than 35 years). The incidence is 1 in 25 live births in women older than 45 years.

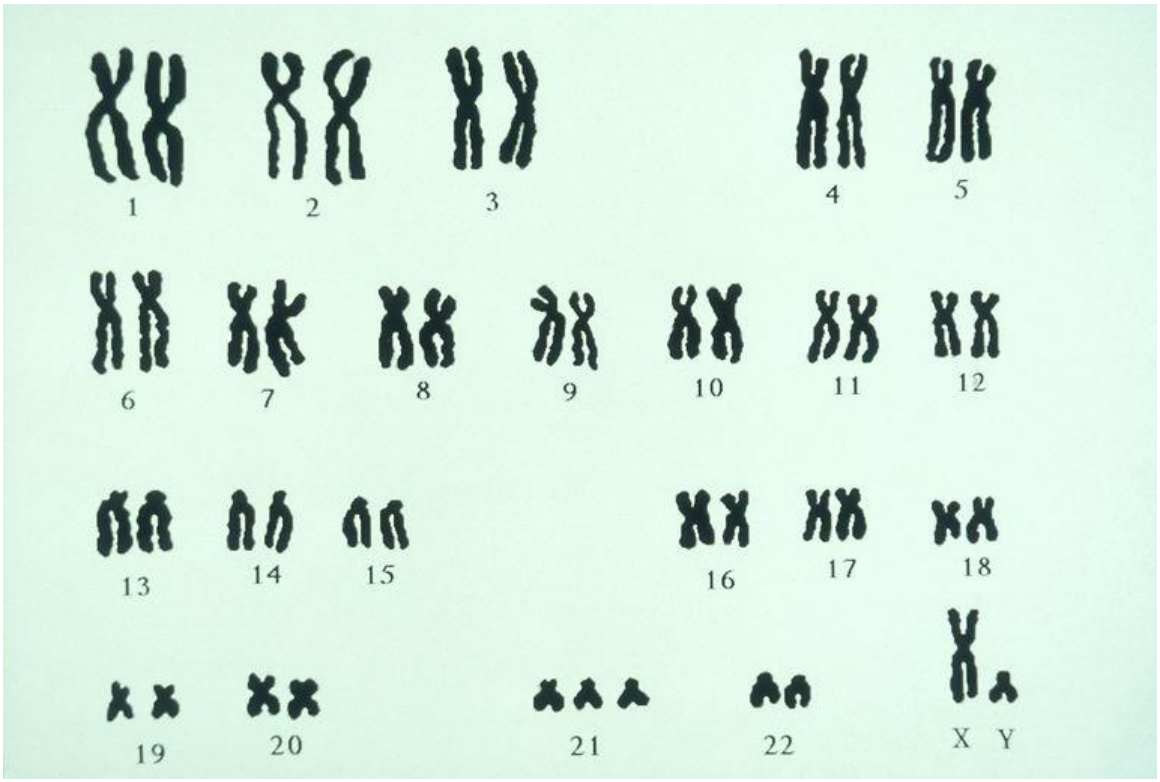


Fig: karyotype of Down Syndrome

As in most genetic diseases there is no cure for a Down's patient. Affected individuals are usually institutionalised. However, after the birth of a Mongol child it is necessary for the parents to have proper genetic counselling to prevent the birth of another child with mongolism.

An accurate diagnosis through karyotype analysis of the affected child and both parents could provide an estimate of the recurrence risk. When mongolism is due to a translocation, the abnormality can be passed on to future generations through the gametes.

A child that inherits the translocation is affected and could in turn produce victims of Down's syndrome. In contrast, Down's syndrome due to non-disjunction, which is a rare event during gametogenesis, is not familial and the condition is not inherited.

b. Trisomy 18 (Edwards syndrome)

Edward and his colleagues in 1960 described a syndrome due to trisomy of an E group chromosome (16-18) and occurring more often in females than in males. The individual may also be a mosaic having a normal cell line and an 18 trisomic line. The incidence is about 1 in 3,500 live births.

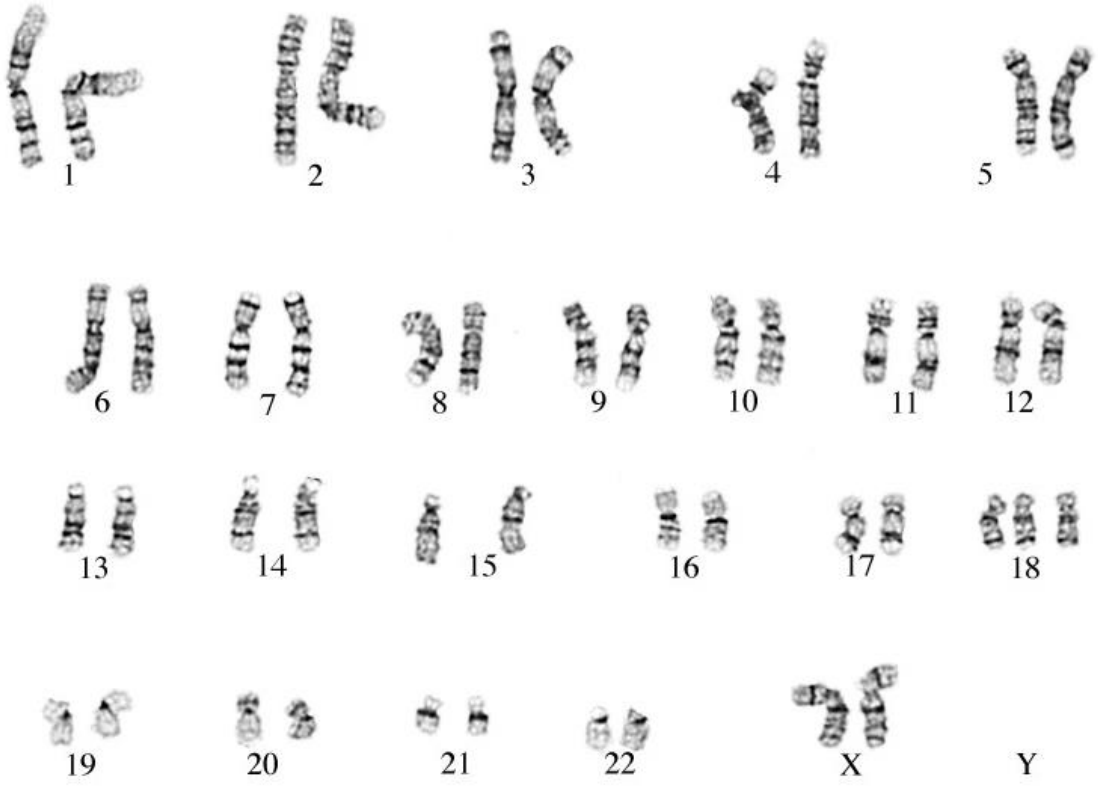


Fig: Karyotype of Edward's Syndrome

The physical and mental growth is much retarded and death usually occurs in early childhood. Patients have hyper-tonicity of skeletal muscles resulting in a peculiar characteristic by which the affected person keeps the fingers tightly clenched against the palm of the hand. The typical features also include micrognathia (small jaws), deformed ears, small sternum and pelvis, a characteristic dermatoglyphic pattern and severe retardation.

The incidence is 1 in 8000. Boys are more affected than girls. As the Down syndrome, Edwards syndrome is also influenced by the age of the mother. Nowadays there is no therapy and the cure is just palliative. Most children with trisomy 18 is not able to survive their first year and they die.

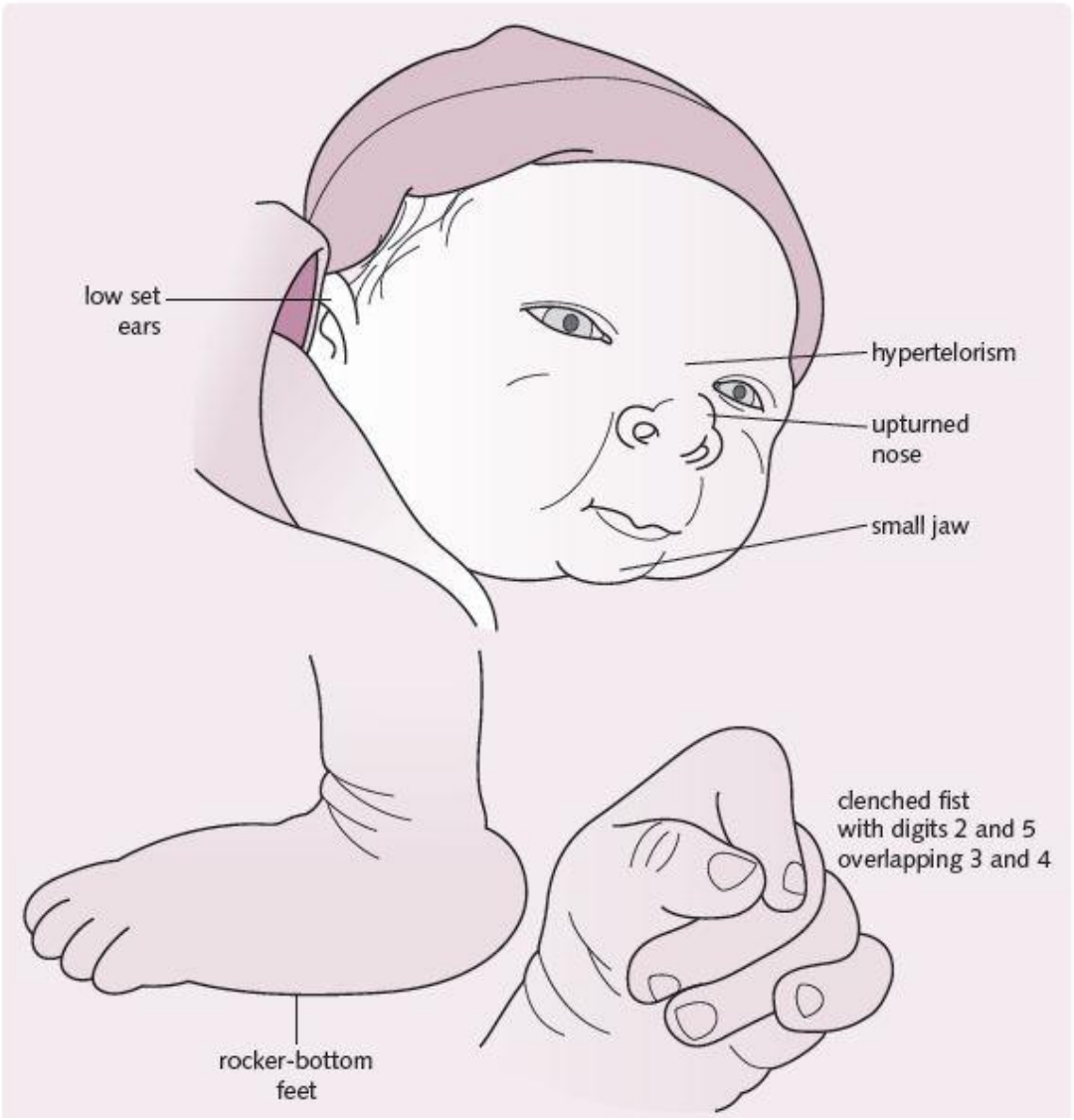


Fig: Symptoms of Edward's syndrome

c. Trisomy 13 (Patau's syndrome)

Bartholin-Patau syndrome, also called autosomal Trisomy 13, is a very severe condition first described by Dr. Klaus Patau in 1960. This genetic disorder is associated with severe intellectual disability and physical abnormalities in many parts of the body. The affected individuals often have heart defects, brain or spinal cord abnormalities, microphthalmia (very small or poorly developed eyes), cleft lip, extra fingers and/or toes, among other features.

Trisomy 13 is a chromosomal condition in which the sex ratio at birth is slightly higher in females than in males. This fact could be related to the decreased survival rate among male foetuses. Like most other trisomies (aneuploidy), Patau syndrome increasing incidence is associated with advanced maternal age, and the additional chromosome

usually arises from nondisjunction in maternal meiosis resulting in three copies of 13 chromosome genetic material. This is a noninheritate case, is the result of random events during meiosis.

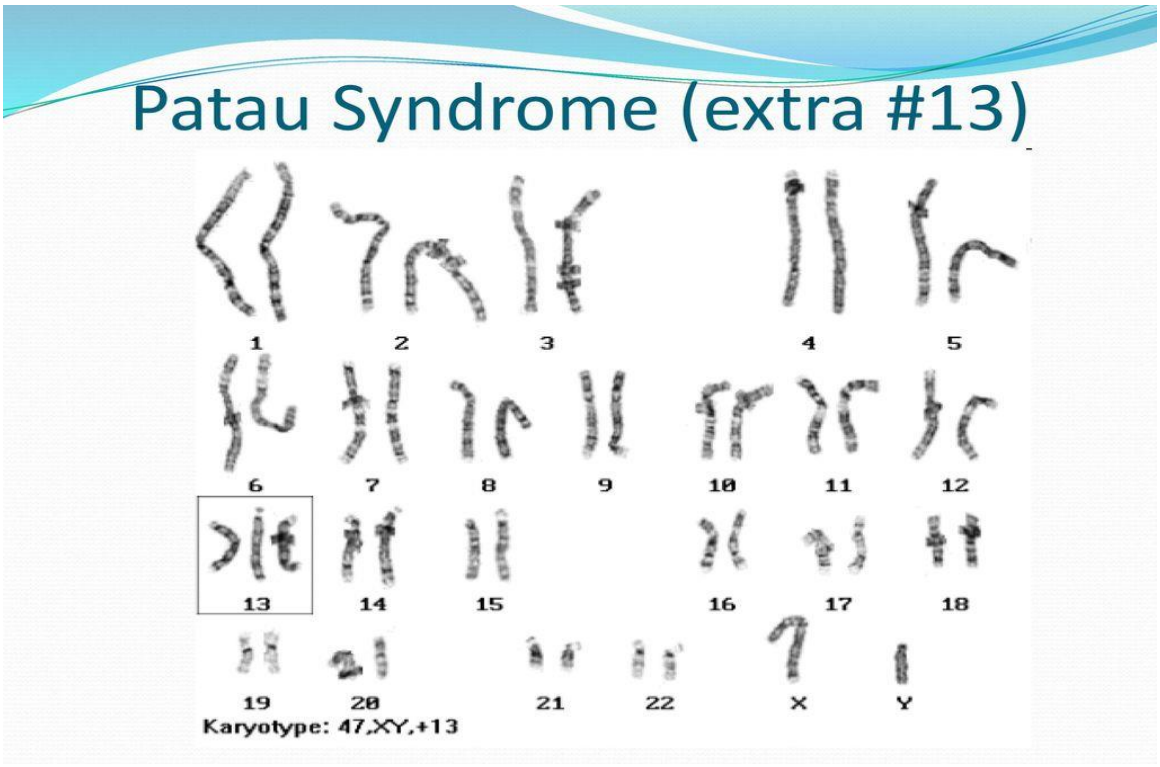


Fig : Karyotype of Patau's Syndrome

The extra genetic material disrupts the normal course of development, causing the characteristic features of trisomy 13. However, some cases derived from germinal mosaicism or by balanced chromosomal rearrangements (Robertsonian translocations), where the extra material is attached to another chromosome, are situations in which the trisomy can be inherited. The person who carries the balanced translocation involving chromosome 13 has an increased chance of passing extra material from chromosome 13 to their children.

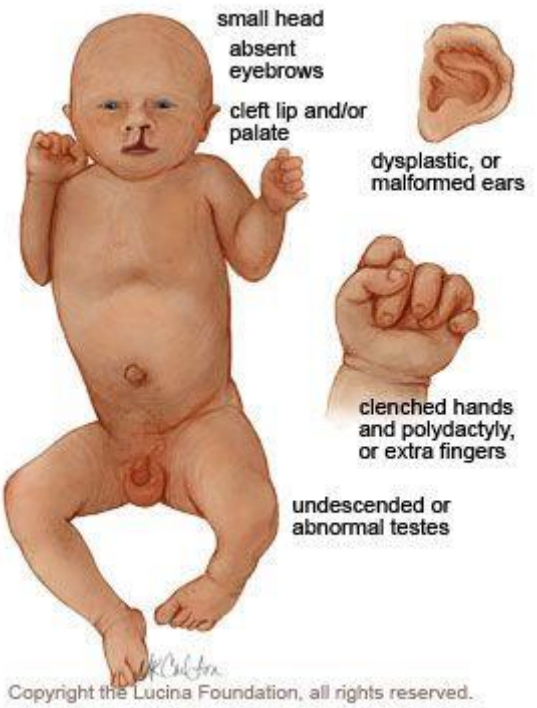
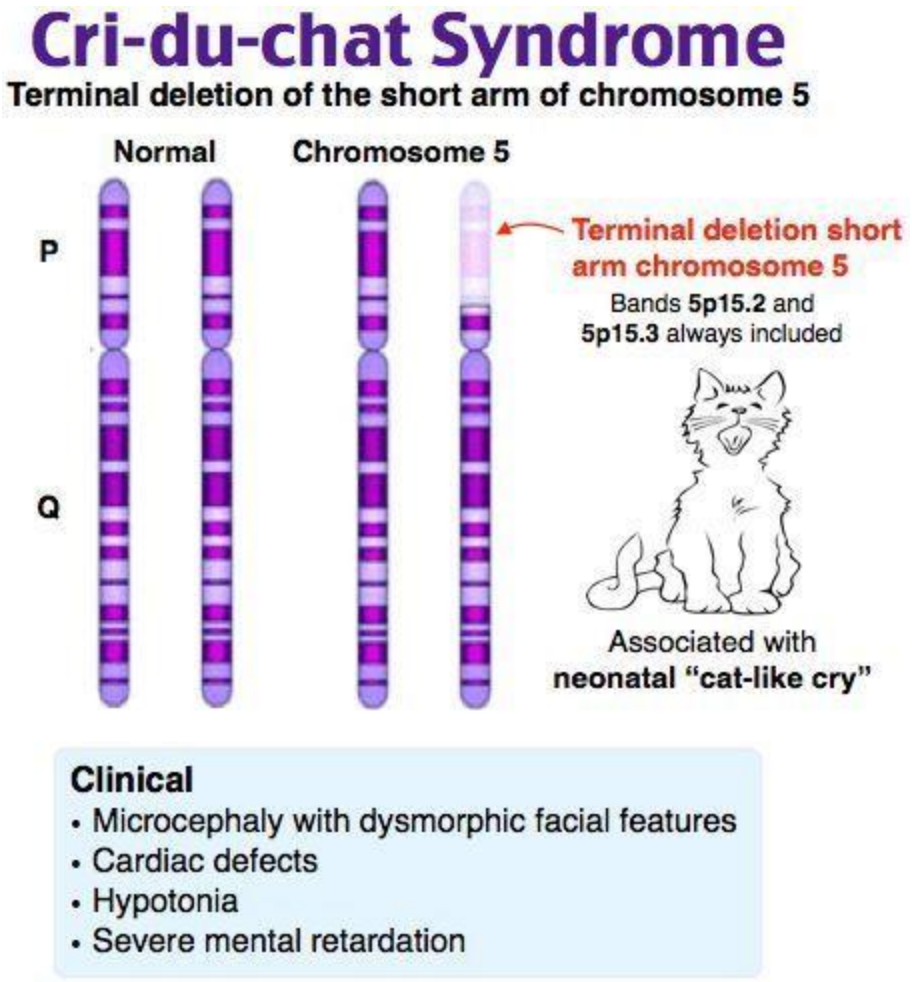


Fig : Symptoms of Patau's Syndrome

d. Cri Du Chat (Cat cry) Syndrome:

An interesting abnormality in which the affected newborn cries in a manner resembling the mewing of a cat, was first described by Lejeune in 1963 in France, hence the name cri du chat (cat cry). A small head, widely spaced eyes, receding chin and congenital heart disease are some other typical features. The condition is due to a deletion in the short arm of the B group chromosome 5, and is very rare.



e. Deletion 22q11.2 (DiGeorge syndrome)

DiGeorge syndrome is caused by the interstitial deletion on the long arm of the 22 chromosome. The deletion can be pretty variable and it is connected with different clinical manifestations. The symptoms are palatal abnormalities, congenital heart disease, facial dysmorphia, microcephaly, hypocalcaemia and thymic hypoplasia with T-cell immunity defect. The treatment is dependent on the symptoms. It is important to start early. In serious cases of immunity in sufficiency the transplantation of bone marrow is necessary.

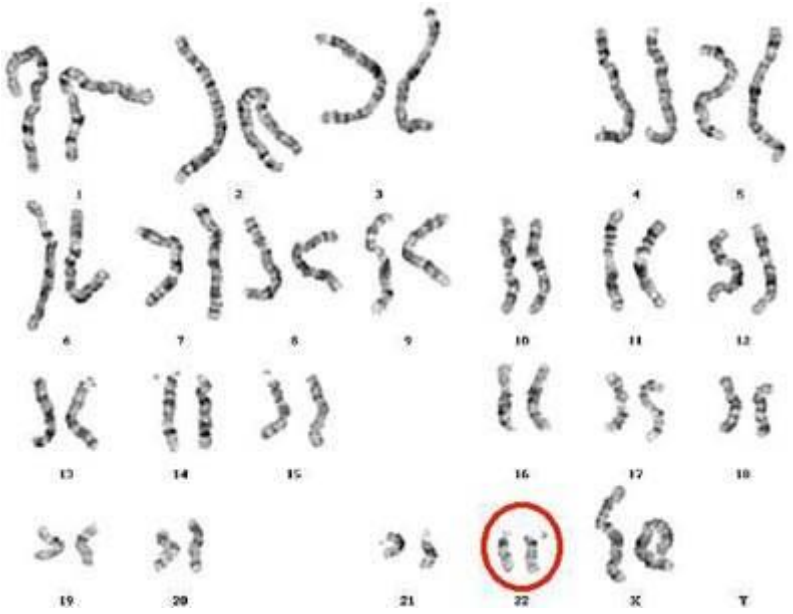


Fig: Karyotype of Di George Syndrome

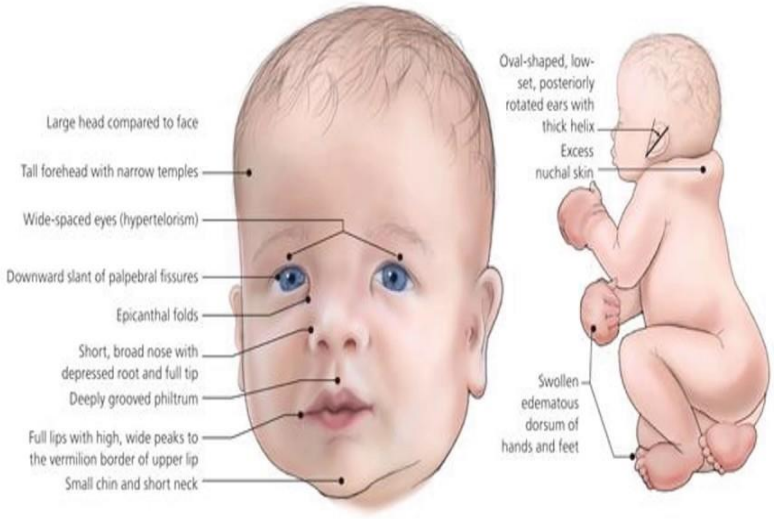


Fig : Symptoms of Di George Syndrome

B. Sex Chromosomal abnormalities:

The majority of known types of chromosomal abnormalities involve sex chromosomes. In frequency of occurrence, they are only slightly less common than autosomal abnormalities. However, they are usually much less severe in their effects. The high frequency of people with sex chromosome aberrations is partly due to the fact that they are rarely lethal conditions. Like Down syndrome and other autosomal problems, sex chromosome gross abnormalities can be diagnosed before birth by amniocentesis and chorionic villi sampling.

Sex chromosome abnormalities are gender specific. Normal males inherit an X and a Y chromosome while females have two X's. A single Y chromosome is sufficient to produce maleness while its absence is necessary for femaleness. Female abnormalities are due to variations in the number of X chromosomes. Male abnormalities are the result of irregular numbers of either the X or the Y chromosome or both.

I. Female Sex Chromosome Abnormalities:

a. Turner syndrome :

It occurs when females inherit only one X chromosome--their genotype is X0 (i.e., monosomy X). If they survive to birth, these girls have abnormal growth patterns. They are short in stature, averaging 4 foot 7 inches as adults, and often have distinctive webbed necks (i.e., extra folds of skin), small jaws, and high arched palates. They generally lack prominent female secondary sexual characteristics. They have exceptionally small, widely spaced breasts, broad shield-shaped chests, and turned-out elbows. Their ovaries do not develop normally and they do not ovulate. The few oocytes that they produce are destroyed by the time they are two. They are in a sense postmenopausal from early childhood and are sterile. However, they can become pregnant and give birth if fertilized eggs from a donor are implanted. Women with Turner's syndrome have a higher than average incidence of thyroid disease, vision and hearing problems, heart defects, diabetes, and other autoimmune disorders. In a few individuals, there is slight mental retardation. Turner syndrome is rare. Current estimates of its frequency range from 1 in 2,000 to 1 in 5,000 female infants. If diagnosed in early childhood, regular injections of human growth hormones can increase their stature by a few inches. Beginning around the normal age of puberty, oestrogen replacement therapy can result in some breast development and menstruation. These treatments allow Turner syndrome women to appear relatively normal.

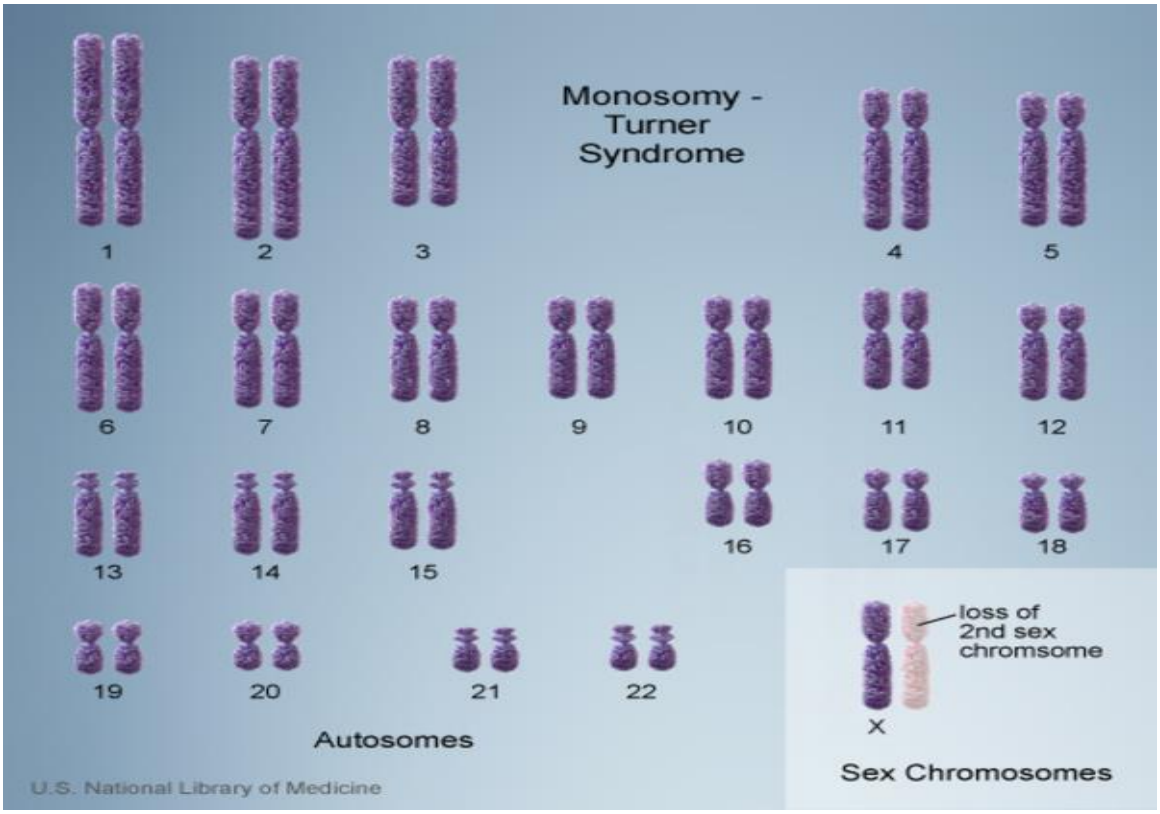


Fig : Karyotype of Turner Syndrome

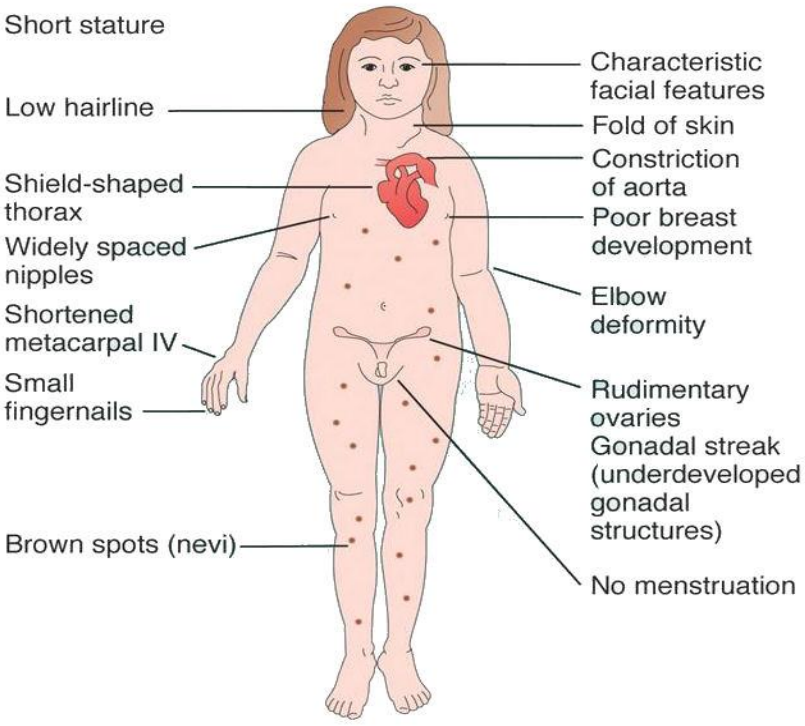


Fig: Symptoms of Turner Syndrome

b. Triple-X syndrome :

It occurs in women who inherit three X chromosomes--their genotype is XXX or more rarely XXXX or XXXXX. As adults, these "super-females" or "metafemales", as they are sometimes known, generally are an inch or so taller than average with unusually long legs and slender torsobut otherwise appear normal. They usually have normal development of sexual characteristics and are fertile but tend to have some ovary abnormalities that can lead to premature ovarian failure. They may have slight learning difficulties, especially in speech and language skills, and are usually in the low range of normal intelligence (especially the XXXX and XXXXX individuals). They frequently are very tall in childhood and tend to be emotionally immature for their size. This sometimes results in teachers and other adults labelling them as troublemakers because they expect more maturity from bigger girls. However, they are usually as emotionally mature as other girls of their age. None of these traits prevent them from being socially accepted as ordinary adult women. Individuals who are genetic mosaics (XX/XXX) have less noticeable symptoms. Triple-X syndrome is less rare than Turner syndrome, but little is known about it. The frequency is approximately 1 in 1,000 female infants and it occurs more commonly when the mother is older.

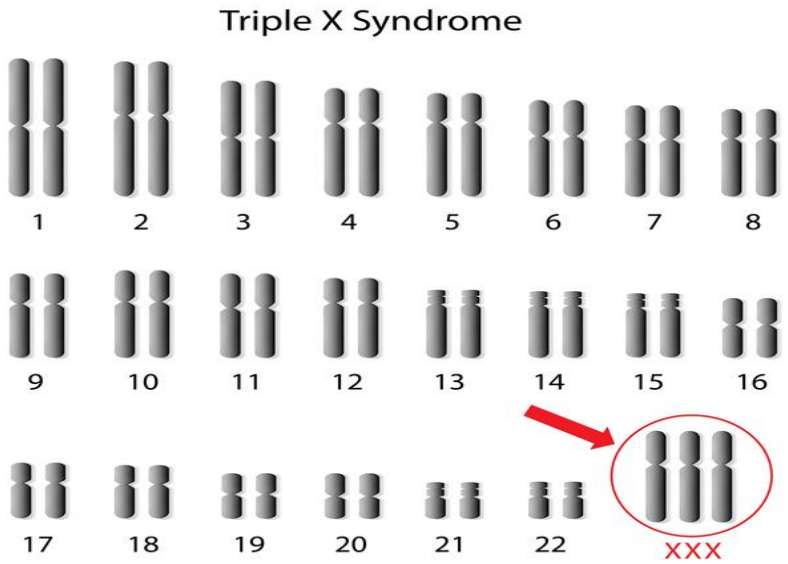


Fig : Karyotype of Triple XXX Syndrome

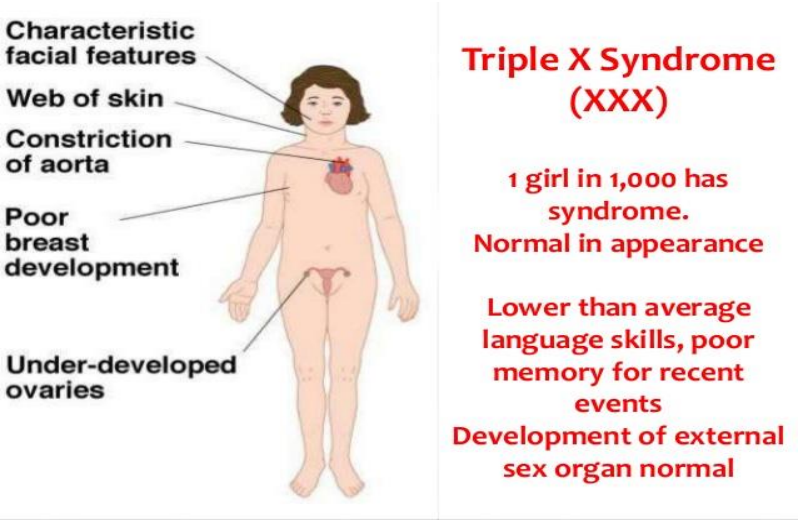


Fig: Symptoms of Triple X Syndrome

II. Male Sex Chromosome Abnormalities:

a. Klinefelter syndrome :

Males inherit one or more extra X chromosomes--their genotype is XXY or more rarely XXXY or XY/XXY mosaic. In severe cases, they have relatively high-pitched voices, asexual to feminine body contours as well as breast enlargement, and comparatively little facial and body hair. They are sterile or nearly so, and their testes and prostate gland are small. As a result, they produce relatively small amounts of testosterone. The feminizing effects of this hormonal imbalance can be significantly diminished if Klinefelter syndrome boys are regularly given testosterone from the age of puberty on. Like triple-X females (described above), many Klinefelter syndrome men are an inch or so above average height. They also are likely to be overweight. They usually have learning difficulties as children, especially with language and short-term memory. If not given extra help in early childhood, this often leads to poor school grades and a subsequent low self esteem. However, most men who have Klinefelter syndrome are sufficiently ordinary in appearance and mental ability to live in society without notice. It is not unusual for Klinefelter syndrome adults with slight symptoms to be unaware that they have it until they are tested for infertility. They are usually capable of normal sexual function, including erection and ejaculation, but many, if not most, are unable to produce sufficient amounts of sperm for conception. Klinefelter syndrome males with more than two X chromosomes usually have extreme symptoms and are often slightly retarded mentally. Men who are mosaic (XY/XXY) generally have the least problems. There is no evidence that Klinefelter syndrome boys and men are more inclined to be homosexual, but they are more likely to be less interested in sex. They have a higher than average risk of developing osteoporosis, diabetes, and other autoimmune disorders that are more common in women. This may be connected to low testosterone

production. Subsequently, regular testosterone therapy is often prescribed. The frequency of Klinefelter syndrome has been reported to be between 1 in 500 and 1 in 1000 male births. This makes it one of the most common chromosomal abnormalities. Males with Down syndrome sometimes also have Klinefelter syndrome. Both syndromes are more likely to occur in babies of older mothers.

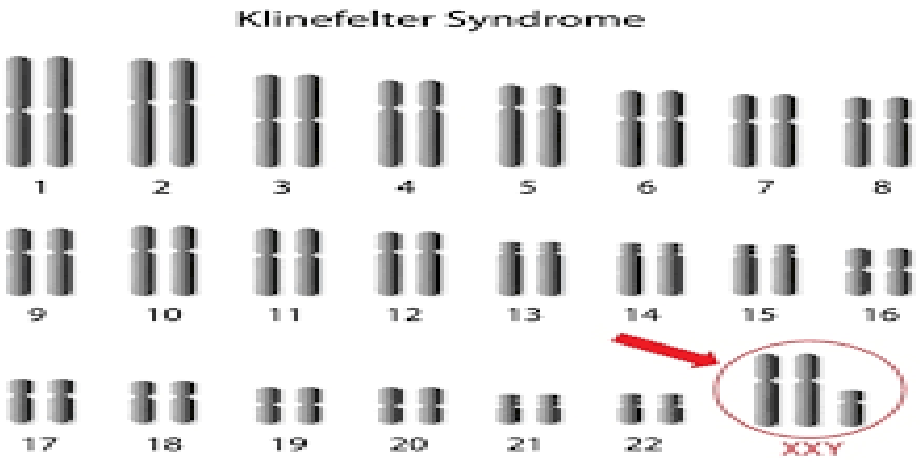


Fig : 47,XXY karyotype showing extra X Chromosome at right side

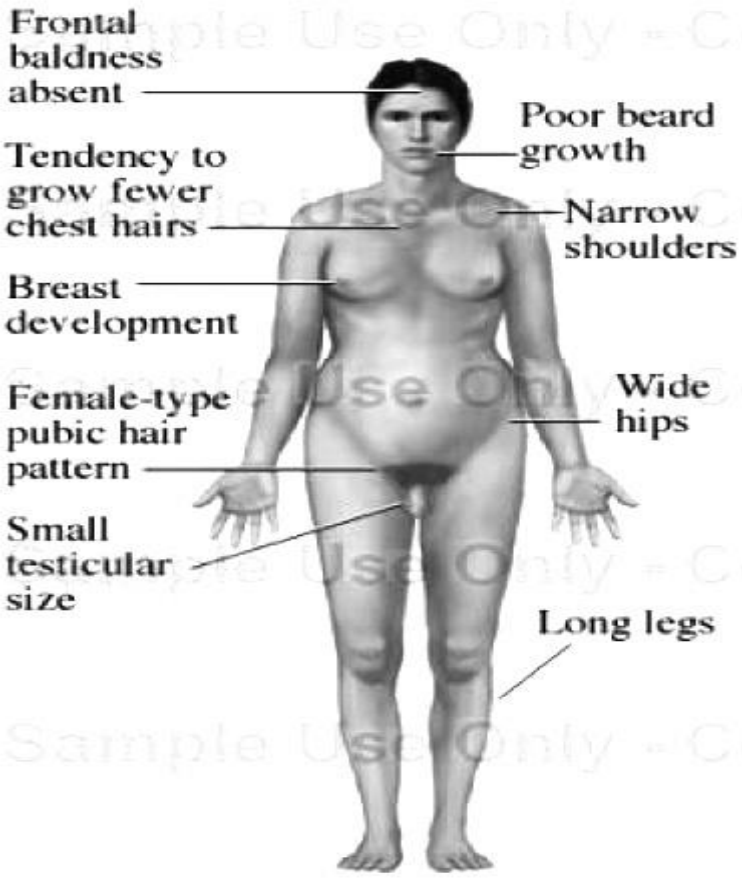


Fig: Symptoms of Klinefelter Syndrome

b. XYY syndrome (Jacobs Syndrome):

n 1965 Jacobs et al found that many of the men kept in institutions for the retarded due to aggressive and antisocial behaviour have 47 chromosomes with XYY sex chromosomes. They are usually tall but not always mentally retarded, frequently show hypogonadism and are sterile.

The presence of two Y chromosomes can be recognized as two brightly fluorescent bodies by proper staining. The discovery of this syndrome received publicity because of the possible association of a chromosome anomaly with human behaviour. Males inherit an extra Y chromosome--their genotype is XYY. As adults, these "super-males" are usually tall (above 6 feet) and generally appear and act normal. However, they produce high levels of testosterone. During adolescence, they often are slender, have severe facial acne, and are poorly coordinated. They are usually fertile and lead ordinary lives as adults. Many, if not most, are unaware that they have a chromosomal abnormality. The frequency of XYY syndrome is not certain due to statistical differences between different studies. It may be as common as 1 in 900 male births to as rare as 1 in 1500 or even 1 in 2,000. XYY syndrome is also referred to as Jacobs syndrome.

Early studies of XYY syndrome done in European prisons initially led to the erroneous conclusion that these men were genetically predisposed to antisocial, aggressive behavior, below average intelligence, and homosexuality. Contributing to the early view that XYY syndrome men have serious personality disorders was the case of Richard Speck. In 1966, he coldly murdered 8 nurses in a Chicago dormitory. At his trial, his lawyer claimed that he was innocent due to uncontrollable urges caused by his XYY genotype. This novel appeal was akin to claiming insanity or severely diminished mental competence. The jury was not convinced and found him guilty of murder. He was sentenced to life in prison where he eventually died. In fact, Richard Speck did not have an XYY genotype. However, some researchers suggest that the high testosterone levels of XYY men can make them somewhat more prone to violence and that this may cause higher rates of wife beating.

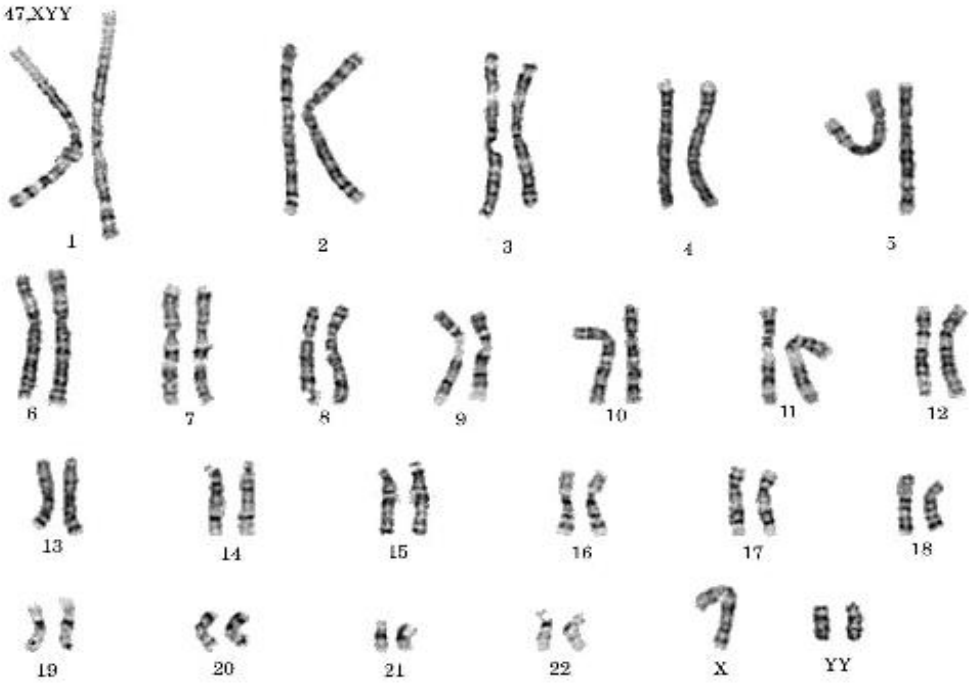


Fig : Karyotype of Jacobs Syndrome

Intersex:

Individuals with both male and female gonadal tissues are called hermaphrodites (from Greek Hermaphrodites, the son of Hermes and Aphrodite). Their karyotype analysis shows that they are mosaics having both X and Y chromosomes in their cell lines. Their buccal smears may or may not show a Barr body. Their external genitalia are often ambiguous, and they are almost always sterile.

The condition of pseudo-hermaphroditism is also included among intersexes. Such individuals are cytogenetically normal with 46, XY (male pseudo-hermaphrodites) or 46, XX (female pseudo-hermaphrodites) chromosomes and normal buccal smears for one sex only. But phenotypically they show both male and female characters.

There are two classes. Male pseudo-hermaphrodites that have testes and either ambiguous or female-like external genitalia. The female pseudo--hermaphrodites have ovaries and either ambiguous or male like external genitalia. The pseudo-hermaphrodites have some defect in the biosynthesis of testosterone in the testes or in the adrenal glands or in both.

GENETIC TESTING AND GENETIC COUNSELLING

Introduction:

The betterment of human society can be achieved by following two inter-related methods:

2. By one of the method we can deal with the already existing human beings. The improvement of already existing human beings can be achieved by improving the environmental conditions, e.g., by subjecting them to better nutrition, better unpolluted ecological conditions, better education and sufficient amount of medical facilities. This type of method of improving the human race is known as euthenics.

3. By another method we can improve the future generations by improving the germplasm of existing individuals. This type of method is known as eugenics. Eugenics believes in artificial selection of physically and mentally sound individuals and discouragement of defective individuals for the inheritance of their defective germplasm to the future generations.

In other words, eugenics seeks the measures to preserve the best type of germplasm and to eliminate defective germplasm from the human society by applying the laws of inheritance to human beings. The primary aim of many of the ancient systems of eugenics was to produce a race of physically perfect human beings. The Greeks had definite ideas regarding eugenics. In Sparta, a physically perfect manhood was the chief aim, whereas the Athenians carried more for the intellectual achievements. Following the doctrine of Greeks and until the 19th century there was little interest in the eugenics. Of the particular importance was the eugenics movement in England in the last part of the nineteenth century.

The movement, spearheaded by persons of outstanding intellect such as Francis Gallon and Karl Pearson, had its objective in the application of biologically sound principles to human populations. Since the biological basis of heredity was unknown, the first objective was to establish the nature of heredity. Galton and Pearson chose to work with human beings and with what they considered important human traits such as intelligence, stature and special abilities. We now know that these characters are very complex traits and are under the control of many genes (polygenes) interacting with environmental variations. So, quite naturally the early investigators made little progress. However, Francis Galton should be credited for being the real founder of the modem movements of eugenics. He defined the eugenics as the study of all the agencies under social control which may improve or impair the inborn qualities of fine generations of humans either physically or mentally.

Darwin also attached great importance to the eugenics and he compared it to a signpost with three directions. One of these indicates the influence of heredity on the fate of

nations. Another point to the rules is that an individual should try to carry out in regard to parenthood based on the law of human heredity. The third aim indicates the regulations to be adopted by the society to encourage racial progress.

Eugenics:

Eugenics is a set of beliefs and practices that aim to improve the genetic quality of a human population by excluding (through a variety of morally criticized means) certain genetic groups judged to be inferior, and promoting other genetic groups judged to be superior. The definition of *eugenics* has been a matter of debate since the term was coined by Francis Galton in 1883.

History of Eugenics:

The concept predates the term; Plato suggested applying the principles of selective breeding to humans around 400 BCE. While eugenic principles have been practiced as early as ancient Greece, the contemporary history of eugenics began in the early 20th century, when a popular eugenics movement emerged in the United Kingdom, and then spread to many countries, including the United States, Canada, and most European countries. In this period, eugenic ideas were espoused across the political spectrum. Consequently, many countries adopted eugenic policies, intended to improve the quality of their populations' genetic stock. Such programs included both *positive* measures, such as encouraging individuals deemed particularly "fit" to reproduce, and *negative* measures, such as marriage prohibitions and forced sterilization of people deemed unfit for reproduction. Those deemed "unfit to reproduce" often included people with mental or physical disabilities, people who scored in the low ranges on different IQ tests, criminals and "deviants," and members of disfavored minority groups.

The eugenics movement became associated with Nazi Germany and the Holocaust when many of the defendants at the Nuremberg trials attempted to justify their human rights abuses by claiming there was little difference between the Nazi eugenics programs and the U.S. eugenics programs. In the decades following World War II, with the institution of human rights, many countries gradually began to abandon eugenics policies, although some Western countries, the United States, Canada, and Sweden among them, continued to carry out forced sterilizations.

Since the 1980s and 1990s, with new assisted reproductive technology procedures available, such as gestational surrogacy (available since 1985), preimplantation genetic diagnosis (available since 1989), and cytoplasmic transfer (first performed in 1996), fear has emerged about the possible revival of a more potent form of eugenics after decades of promoting human rights.

When we consider the future welfare of the human race then the following two factors alarm us greatly:

(i) The declining birth rate among the normal and superior people (those having best germplasm)

(ii) A relative rapid increase of the abnormal and defective individuals (those having defective germplasm).

For the betterment of future generation, it is necessary to increase the population of outstanding people and to decrease the population of abnormal and defective people by applying the principle of eugenics.

The eugenics can be applicable by adopting following two methods:

(A) By encouraging the marriages between desirable persons (constructive method or positive eugenics).

(B) By discouraging the marriages between undesirable persons (restrictive method or negative eugenics).

A. Positive Eugenics:

The positive eugenics attempts to increase consistently better or desirable germplasm and, thus, to preserve best germplasm of the society.

The percentage of desirable traits can be increased by adopting following measures:

a. Early Marriage of those having Desirable Traits:

It is most commonly observed fact that the highly placed persons of the society often have great ambitions for the future life. In achieving their ambitious goals, they often devote the best part of their youth and they are able to marry in their mature age (e.g., 30 to 35 years).

The biological and psychological investigation have revealed that the aged persons often lack in necessary amount of emotional warmth for the sexual activities and moreover, their germplasm also loose its vigour.

Therefore, some laws should be formulated to prevent the late marriages of highly endowed persons by applying high taxation on them and at the same time the young persons having best hereditary traits should be encouraged for early marriage.

b. Subsidizing the Fit:

Because the highly endowed persons lead a well-planned life and to avoid unnecessary difficulties in nursing the children they often prefer to have small number of children. Therefore, the selected young men and women of best eugenic value should be encouraged to increase their birth rate.

c. Eutelegenesis or Germinal Choice:

H.J. Muller has suggested that eugenically sound persons not only should increase their family size but through artificial insemination the outstanding man can serve as father to many more children than would be otherwise possible. The artificial insemination is already widely practiced to permit those women whose husbands are sterile or have some serious hereditary afflictions to bear children.

The sperms and eggs of outstanding persons can be stored for future use by quick freezing and storing them in deep freeze. These germ cells, thus, can be stored for 100 or more years. Very recently the scientists felt the urgent need of establishment of sperm and egg banks to protect these precious germ cells from the radiation. The germ cells could be collected during early adulthood and stored in lead lined containers in the deep freeze. In this state the germ cells would not be subjected to radiation exposure which might affect the donors.

In a modern technique, a woman's ovum is taken out and artificially fertilized in a test-tube. The zygote is then implanted into woman's uterus for the embryonic development. The resulting baby is called test-tube baby.

d. Education:

For the eugenically oriented reforms in the society, the people should be educated about the basic principles of human biology, human genetics, eugenics and sex. The children should be instructed about basic laws of health and they should be encouraged to develop a physically and mentally healthy body.

Moreover, sex should be free from the widespread confusion, narrow minded concepts and religious taboos and ethical bindings because that is a natural biological instinct. The children ignorant about the facts of sex may do more harm to society than otherwise.

e. By Avoiding Germinal Waste:

The wastage of best type of germplasm can be avoided by adopting following measures:

(i) The selection of marriage partners should be made with intelligence.

(ii) The social hindrance which do not allow the teachers, nuns and priests to get married, must be removed. By adopting such measures wastage of best type of germplasm due to lack of opportunities can be prevented.

(iii) The wars must be avoided because in wars the best germplasm of the society is wasted.

f. Improvement of Environmental Conditions:

Both heredity and environment have interrelated role in the development of eugenically better persons. Therefore, every person should get better food, living conditions, education and medical guidance, etc., so his or her hereditary traits can undergo their best development.

g. Promotion of Genetic Research:

Our knowledge about the genetics is not sufficient enough because we still have little information's about various human diseases and metabolic disorders which are generally related with the genes. Therefore, the research in the field of cytogenetics should be increased so that we can learn more and more about the human beings.

h. Genetic Engineering:

During the late 1970s, the science of genetics entered a new era dominated by the use of recombinant DNA technology or genetic engineering (or biotechnology) to produce novel life forms not found in nature.

Through this technology, it has been possible to transfer genes from mammals into bacteria, causing the microbes to become tiny factories for making (in relatively large quantities) proteins of great economic importance such as hormones (including growth hormone and insulin) and interferon's (lymphocyte proteins that prevent replication of a wide variety of viruses). These proteins are produced in such small quantities in humans that the cost of their extraction and purification from tissues has been very expensive, thus, limiting their medical use in prophylaxis (prevention) and therapeutics (treatment) of disease.

By genetic engineering, it has become possible to produce various blood clotting factors, complement proteins (part of immune system) and other substances for the correction of genetic deficiency diseases (euphenics). Recently, experiments have been conducted in which human cells deficient in the synthesis of purines have been obtained from the patients with Lesch- Nyhan syndrome and grown in culture; these cells have been converted to normal cells by transformation with recombinant DNA.

The exciting potential of this technique lies in the possibility of correcting genetic defects-for example, restoring the ability of a diabetic individual to make insulin or correcting immunological deficiencies. This technique is called gene therapy.

B. Negative Eugenics:

The negative eugenics attempts to eliminate the defective germplasm of the society by adopting following measures:

a. Sexual Separation of the Defective:

The defective persons may have various sex-linked diseases such as night blindness, haemophilia, colour blindness, etc., and various other defective traits which may be regulated by dominant or recessive genes.

The increase of germplasm of the persons having such defective traits in the population can be checked by keeping them away and separated from the society. Different states have wisely adopted the restricted measures in segregation of the mental defectives from the society and to place them in mental hospitals.

b. Sterilization:

The sterilization is the best means to deprive an individual from his power of reproduction without interfering with any of his normal functions. The sterilization method is based on surgical operation of sperm duct or vas deferens in males and oviducts or fallopian tubes in females.

The former is known as vasectomy and the latter is tubectomy or salpingectomy. The family planning movement in India has adopted the sterilization as the tool for controlling the rate of rapidly increasing population and in that case the sterilization is euthenical in its application than eugenical.

c. Control of Immigration:

Through immigration there are enough chances that undesirable or defective genes of different races and nationalities may intermingle with the normal germplasm of the population. Therefore, the immigration rules must be strict and the persons with undesirable hereditary traits must not be allowed to migrate from one place to another.

d. Regulation of Marriage:

Presently most human societies are money-minded and for a marriages, relationship like the wealthy or highly placed persons who, however, may have several defective genetical traits, are preferred over those who have economically weak but having eugenically sound hereditary traits. Some rule must be enacted to encourage marriages among desirable mates.

e. Birth Control:

People possessing more of undesired, dysgenic traits should be encouraged to have small families if at all allowed to reproduce. Their foetuses possessing dysgenic traits may be destroyed by abortion.

f. Statutory Ban on Marriage among Close Relatives:

Marriages among close relatives are called consanguineous marriages. The hidden recessive deleterious (e.g., lethal) traits can appear in the progeny in full view if both the parents carry them. The chances of both the parents carrying the hidden traits are more if they are close relatives and have some common ancestors.

Therefore it is advisable not to marry a close relative. If family histories of a rare abnormality are collected and there is no dominance in them, we may generally expect that the anomaly is due to a double dose (i.e., homozygous state) of a recessive gene. If the parents are blood relatives, there will be a reasonable chance that both will be carrying at least one of the deleterious recessives possessed by one or more of their common ancestors. Thus, by marrying a relative, one tremendously increases the chances that a recessive, which has been carried concealed down through the generations, will become expressed in the effective double dose in children. Many societies have some sort of taboo or restrictions on marriages between close relatives, which may have arisen as a result of the observation that such marriages often produced defective offspring.

In most countries, no man is allowed to legally marry his sister, mother, daughter, granddaughter, aunt or niece. Many societies have extended such restrictions to include prohibition of first cousin marriages also.

Consanguineous marriages have influenced world history:

Consanguineous marriages have been sanctioned by royalty. This is the reason for the spread of haemophilic gene to European dynasties by the offspring of Queen Victoria. The death of crown prince of Spain, Prince Alfonso, took place by profuse bleeding after a minor cut which would not have been deadly in a normal individual. This was due to X-linked recessive gene for haemophilia he inherited. This gene does not allow the blood to clot.

This condition was perpetuated in royal families of Europe because of the close intermarriages. Rasputin, “the mad monk of Russia”, obtained strong hold of Russia because he was being considered successful in treating the profuse bleeding (i.e., bleeder’s disease) attacks of Tsarevitch. This monk used his power and played a major role in the effectiveness of Russian revolution which changed the history of the world. Thus, we see how the inheritance of gene has influenced world history.

Genetic Counselling:

Negative eugenics is most widely acceptable when it is practiced through genetic counselling. A voluntary restriction of child bearing by couples with inherited genetic disorders (e.g., albinism, sickle cell anaemia, etc.) can be brought about through proper counselling by well qualified persons in the field.

The term genetic counselling is applied to service, typically available in medical settings, in which the prospective parents are provided with the estimates of the probability that they will produce children with genetically controlled defects. This vital service is intimately related to medical and diagnostic procedures, but the counselling itself does not involve their actual performance.

Medical genetics units are often attached with certain hospitals and medical centres and they provide services of genetic counselling. A genetic counsellor may be a medical doctor or well-trained professional human geneticist.

The most common situation under which people obtain a genetic counsellor's advice is one in which phenotypically normal couple produces a first child suffering from a major defect. Understandingly they wish to know what are the chances for subsequent children to be affected. The genetic counsellor's first task, invariably, is to collect the relevant evidence by making a pedigree study of the man and the women concerned. He then proceeds to estimate the risk or probability of genetic defects among their progeny by applying established principles of inheritance, to the information already collected.

The counsellor, normally, cannot predict anything with certainty and is only in a position to explain the chances of occurrence of a trouble. The final decision is always left to the couple themselves. The role of genetic counsellor will become more important with the perfection of the technique of identification of genetic defects.

It is relatively easy for a trained clinician to identify people suffering from a genetic disease. But the identification of carriers (heterozygotes) for genetic diseases are readily identifiable when they have either a reduced level of the concerned enzyme (e.g., HGPRT enzyme in Lesch-Nyhan syndrome), or a protein/enzyme with an altered charge and, consequently, changed mobility when subjected to electrophoresis (e.g., HbS produced by persons heterozygous for the sickle cell gene). Analysis of family pedigree also provides information of the likelihood of an individual being carrier for a genetic disease.

Once genotype of both the prospective parents become known, it is a simple matter to work out the probability of their child inheriting the disease. If parents heterozygous for a genetic disease decide to produce a child, it is now possible, through appropriate tests done about 6-8 weeks after conception to advise them if their child has inherited the disease.

This is done by obtaining foetal cells from biopsies of trophoblastic villi, which form an external part of human embryo and later form a part of the placenta. Foetal cells may also be obtained by amniocentesis done usually after 18 weeks of pregnancy. The cultured foetal cells may be used for determining their karyotype, levels of critical enzymes and restriction enzyme digestion patterns of DNA.

Such an antenatal (prenatal) diagnosis is now attainable for more than 35 genetic diseases and for a variety of karyotypic defects. The sole purpose of such a diagnosis is premature termination of abnormal foetuses. This approach has helped to reduce the incidence of thalassaemia from 30 to 2 per cent a year in the Cypriot (i.e., a native or inhabitant of Cyprus) community in England. However, antenatal diagnosis may sometimes be misused, e.g., it may be used to selectively abort the foetuses of one set. Some newspaper reports show that some parents in India are using amniocentesis and ultrasound technique (another vital tool for antenatal diagnosis) to selectively abort female foetuses, obviously to save themselves from the hardships and sufferings they have to face to arrange the exceedingly high amounts of dowry for their daughters.

Some other Duties of Genetic Counsellor:

Apart from providing counselling services to high-risk individuals, a genetic counsellor must be able to provide advice to those groups or populations which have a high incidence of certain specific diseases. For example, African people have higher incidence of sickle-cell anaemia, those of Mediterranean descent are prone to thalassaemia, and East European Jews have high probability of carrying the Tay-Sach gene.

The genetic counsellor should also try to wash away the guilt feelings of the parents of the affected child by pointing out that genetic calamities can occur in any family, and the parents are not alone in their problem and no one can escape all the possible undesirable expressions of their genotype.

Genetic counselling and antenatal diagnosis provide definite relief to the possible parents and reduce the frequency of genetically defective individuals in the population. However, it is unlikely that these measures would eliminate the deleterious (=lethal) alleles from a population. This is so because most genetic defects are recessive and they would persist in the population through the heterozygotes; therefore, even such an extreme selection would lead to only a slow decline in their frequency.

Further, it is not likely that all the couples in any society will willingly agree themselves, at least in foreseeable near future, to these procedures. However, genetic counselling has become a routine aspect of medical practice in most developed countries.

Euphenics:

The symptomatic treatment of genetic diseases of human beings is called euphenics. The euphenics deals with the control of several inherited human diseases, especially inborn errors of metabolism in which the missing or defective enzyme has been identified.

One example of this is the condition known as phenylketonuria or PKU, determined by an autosomal recessive gene. Babies with this defect are unable to properly metabolize an amino acid, phenylalanine, the resulting chemical imbalance causes severe mental retardation.Now it is possible to distinguish homozygotes from normal individuals by testing the urine of all new-born babies with ferric chloride. In affected children, the metabolic imbalance caused by the mutation will turn the urine green. Once such a child is detected, a diet free of phenylalanine is prescribed and the child can develop normally.

Although a number of inherited diseases can be treated in a similar euphenic manner, but these constitute only a small fraction of known inherited disease. For the most part, biochemical geneticists could not identify the biochemical errors of many genetic diseases. In other cases, such as albinism, even though the metabolic block leading to an abnormality is known, but, it is not possible to correct it

In future by following euphenic measures, human can get rid of certain fatal genetic diseases:

1. Intake of Missing Enzyme:

One possible euphenic measure for the future would be to supply the known missing enzyme to individuals that would allow their cells to complete the required biochemical reaction.Some attempts to do this have been made without much success. Immunological difficulties are encountered, since the enzymes being supplied are antigenic so the body produces antibodies against them.

2. Cure for Inherited Anaemia:

Scientists, studying the two anaemias in human beings, Cooley's and Lepore anaemia, resulting from abnormally low haemoglobin production in individuals homozygous for mutations at the beta-chain locus, hope that they will someday discover the factors regulating the beta gene activity and thereby increase the amount of beta chain synthesis.

Furthermore if the mechanism regulating haemoglobin synthesis in the foetus and the adult can be detected, they might be able to cure lethal conditions, such as thalassemia

major and sickle cell anaemia, by suppressing synthesis of the abnormal beta chains and allowing foetal or gamma chains to be produced instead.

In fact, individuals have been discovered whose β chain locus for some unknown reason never becomes active. Such individuals function normally with foetal haemoglobin even as adults.

3. Increasing Role of Genetics to Medicine:

The increasing number of human diseases that are being discovered to have a genetic basis lend great importance to the development of such euphenic measures. Three percent of all humans have hereditary diseases which are transmitted in a Mendelian fashion.

Two diseases which account for the deaths of hundreds of thousands a year, cancer and heart disease, are thought to have some heritable component. The future works of immunogenetics can suggest the ways by which an individual having genes for cancer may develop resistance for this disease.A study has shown that some forms of heart diseases may be inherited as an autosomal dominant trait. An understanding of the genetic basis of such heart diseases would alert persons from families with an incidence of the disease to the possibility of incurring heart conditions and perhaps cause them to alter their diets and life habits accordingly. For example, those who may inherit genes for heart trouble would then quit smoking and avoid high fat diets.

Harmful evolutionary effect of euphenics:

To restore affected individuals to normalcy by euphenic measures is the only compassionate goal for scientists to pursue but to do so is to counter the forces of natural selection that are the basis for the evolutionary strength of a species. PKU homozygotes, for example, would normally not reproduce and transmit the harmful mutations to future generations.

Selection against them would be total; however, when they do develop normally and produce progeny, every member of the next generation would be a carrier of the mutation, assuming that the wife of such person being normal. This has to add to our genetic load and to weaken the human species from the evolutionary point of view.

Genetic Testing:

Genetic testing, also known as DNA testing, allows the determination of bloodlines and the geneticdiagnosis of vulnerabilities to inherited diseases. In agriculture, a form of genetic testing known as progeny testing can be used to evaluate the quality of breeding stock. In population ecology, genetic testing can be used to track genetic strengths and vulnerabilities of species populations.

In humans, genetic testing can be used to determine a child's parentage (genetic mother and father) or in general a person's ancestry or biological relationship between people. In addition to studying chromosomes to the level of individual genes, genetic testing in a broader sense includes biochemical tests for the possible presence of genetic diseases, or mutant forms of genes associated with increased risk of developing genetic disorders.

Genetic testing identifies changes in chromosomes, genes, or proteins. The variety of genetic tests has expanded throughout the years. In the past, the main genetic tests searched for abnormal chromosome numbers and mutations that lead to rare, inherited disorders. Today, tests involve analysing multiple genes to determine the risk of developing specific diseases or disorders, with the more common diseases consisting of heart disease and cancer. The results of a genetic test can confirm or rule out a suspected genetic condition or help determine a person's chance of developing or passing on a genetic disorder. Several hundred genetic tests are currently in use, and more are being developed.

Because genetic mutations can directly affect the structure of the proteins they code for, testing for specific genetic diseases can also be accomplished by looking at those proteins or their metabolites, or looking at stained or fluorescent chromosomes under a microscope.

Types of Genetic Testing:

Genetic testing is "the analysis of chromosomes (DNA), proteins, and certain metabolites in order to detect heritable disease-related genotypes, mutations, phenotypes, or karyotypes for clinical purposes". It can provide information about a person's genes and chromosomes throughout life.

I. Diagnostic type testing include:

4. **Cell-free fetal DNA (cffDNA) testing**: It is a non-invasive (for the foetus) test. It is performed on a sample of venous blood from the mother, and can provide information about the foetus early in pregnancy. As of 2015 it is the most sensitive and specific screening test for Down syndrome.

5. **Newborn screening**: Newborn screening is used just after birth to identify genetic disorders that can be treated early in life. A blood sample is collected with a heel prick from the newborn 24–48 hours after birth and sent to the lab for analysis. In the United States, newborn screening procedure varies state by state, but all states by law test for at least 21 disorders. If abnormal results are obtained, it does not necessarily mean the child has the disorder. Diagnostic tests must follow the initial screening to confirm the disease. The routine testing of infants for certain disorders is the most widespread use of genetic testing—millions of babies are tested each year in the United States. All states currently

test infants for phenylketonuria (a genetic disorder that causes mental illness if left untreated) and congenitalhypothyroidism (a disorder of the thyroid gland). People with PKU do not have an enzyme needed to process the amino acid phenylalanine, which is responsible for normal growth in children and normal protein use throughout their lifetime. If there is a buildup of too much phenylalanine, brain tissue can be damaged, causing developmental delay. Newborn screening can detect the presence of PKU, allowing kids to get put on a special diet right away to avoid the effects of the disorder.

6. **Diagnostic testing**: Diagnostic testing is used to diagnose or rule out a specific genetic or chromosomal condition. In many cases, genetic testing is used to confirm a diagnosis when a particular condition is suspected based on physical mutations and symptoms. Diagnostic testing can be performed at any time during a person's life, but is not available for all genes or all genetic conditions. The results of a diagnostic test can influence a person's choices about health care and the management of the disease. For example, people with a family history of polycystic kidney disease (PKD) who experience pain or tenderness in their abdomen, blood in their urine, frequent urination, pain in the sides, a urinary tract infection or kidney stones may decide to have their genes tested and the result could confirm the diagnosis of PKD.

7. **Carrier testing**: Carrier testing is used to identify people who carry one copy of a gene mutation that, when present in two copies, causes a genetic disorder. This type of testing is offered to individuals who have a family history of a genetic disorder and to people in ethnic groups with an increased risk of specific genetic conditions. If both parents are tested, the test can provide information about a couple's risk of having a child with a genetic condition like cystic fibrosis.

8. **Preimplantation genetic diagnosis:** Genetic testing procedures that are performed on human embryos prior to the implantation as part of an in vitro fertilization procedure. Pre-implantation testing is used when individuals try to conceive a child through in vitro fertilization. Eggs from the woman and sperm from the man are removed and fertilized outside the body to create multiple embryos. The embryos are individually screened for abnormalities, and the ones without abnormalities are implanted in the uterus.

9. **Prenatal diagnosis**: Used to detect changes in a foetus's genes or chromosomes before birth. This type of testing is offered to couples with an increased risk of having a baby with a genetic or chromosomal disorder. In some cases, prenatal testing can lessen a couple's uncertainty or help them decide whether to abort the pregnancy. It cannot identify all possible inherited disorders and birth defects, however. One method of performing a prenatal genetic test involves an amniocentesis, which removes a sample of fluid from the mother’s amniotic sac 15 to 20 or more weeks into pregnancy. The fluid is then tested for chromosomal

abnormalities such as Down syndrome (Trisomy 21) and Trisomy 18, which can result in neonatal or fetal death. Test results can be retrieved within 7–14 days after the test is done. This method is 99.4% accurate at detecting and diagnosing fetal chromosome abnormalities. Although there is a risk of miscarriage associated with an amniocentesis, the miscarriage rate is only 1/400. Another method of prenatal testing is Chorionic Villus Sampling (CVS). Chorionic villi are projections from the placenta that carry the same genetic makeup as the baby. During this method of prenatal testing, a sample of chorionic villi is removed from the placenta to be tested. This test is performed 10–13 weeks into pregnancy and results are ready 7–14 days after the test was done.[12] Another test using blood taken from the fetal umbilical cord is percutaneous umbilical cord blood sampling.

10. **Predictive and presymptomatic testing:** Predictive and presymptomatic types of testing are used to detect gene mutations associated with disorders that appear after birth, often later in life. These tests can be helpful to people who have a family member with a genetic disorder, but who have no features of the disorder themselves at the time of testing. Predictive testing can identify mutations that increase a person's chances of developing disorders with a genetic basis, such as certain types of cancer. For example, an individual with a mutation in *BRCA1* has a 65% cumulative risk of breast cancer. Hereditary breast cancer along with ovarian cancer syndrome are caused by gene alterations in the genes BRCA1 and BRCA2. Major cancer types related to mutations in these genes are female breast cancer, ovarian, prostate, pancreatic, and male breast cancer. Li-Fraumeni syndrome is caused by a gene alteration on the gene TP53. Cancer types associated with a mutation on this gene include breast cancer, soft tissue sarcoma, osteosarcoma (bone cancer), leukaemia and brain tumours. In the Cowden syndrome there is a mutation on the PTEN gene, causing potential breast, thyroid or endometrial cancer. Presymptomatic testing can determine whether a person will develop a genetic disorder, such as hemochromatosis (an iron overload disorder), before any signs or symptoms appear. The results of predictive and presymptomatic testing can provide information about a person's risk of developing a specific disorder, help with making decisions about medical care and provide a better prognosis.

11. **Pharmacogenomics:** type of genetic testing that determines the influence of genetic variation on drug response. When a person has a disease or health condition, pharmacogenomics can examine an individual's genetic makeup to determine what medicine and what dosage would be the safest and most beneficial to the patient. In the human population, there are approximately 11 million single nucleotide polymorphisms (SNPs) in people's genomes, making them the most common variations in the human genome. SNPs reveal information about an individual's response to certain drugs. This type of genetic testing can be used for cancer patients undergoing chemotherapy. A sample of

the cancer tissue can be sent in for genetic analysis by a specialized lab. After analysis, information retrieved can identify mutations in the tumour which can be used to determine the best treatment option.

II. Non-diagnostic testing includes:

1. **Forensic testing**: Forensic testing uses DNA sequences to identify an individual for legal purposes. Unlike the tests described above, forensic testing is not used to detect gene mutations associated with disease. This type of testing can identify crime or catastrophe victims, rule out or implicate a crime suspect, or establish biological relationships between people (for example, paternity).

2. **Paternity testing**: This type of genetic test uses special DNA markers to identify the same or similar inheritance patterns between related individuals. Based on the fact that we all inherit half of our DNA from the father, and half from the mother, DNA scientists test individuals to find the match of DNA sequences at some highly differential markers to draw the conclusion of relatedness.

3. **Genealogical DNA test:** To determine ancestry or ethnic heritage for genetic genealogy

4. **Research testing**: Research testing includes finding unknown genes, learning how genes work and advancing our understanding of genetic conditions. The results of testing done as part of a research study are usually not available to patients or their healthcare providers.

Procedure of Genetic Testing:

Genetic testing is often done as part of a genetic consultation and as of mid-2008 there were more than 1,200 clinically applicable genetic tests available. Once a person decides to proceed with genetic testing, a medical geneticist, genetic counsellor, primary care doctor, or specialist can order the test after obtaining informed consent.

Genetic tests are performed on a sample of blood, hair, skin, amniotic fluid (the fluid that surrounds a foetus during pregnancy), or other tissue. For example, a medical procedure called a buccal smear uses a small brush or cotton swab to collect a sample of cells from the inside surface of the cheek. Alternatively, a small amount of saline mouthwash may be swished in the mouth to collect the cells. The sample is sent to a laboratory where technicians look for specific changes in chromosomes, DNA, or proteins, depending on the suspected disorders, often using DNA sequencing. The laboratory reports the test results in writing to a person's doctor or genetic counsellor. Routine newborn screening tests are done on a small blood sample obtained by pricking the baby's heel with a lancet.

Risks and limitations:

The physical risks associated with most genetic tests are very small, particularly for those tests that require only a blood sample or buccal smear (a procedure that samples cells from the inside surface of the cheek). The procedures used for prenatal testing carry a small but non-negligible risk of losing the pregnancy (miscarriage) because they require a sample of amniotic fluid or tissue from around the foetus.

Many of the risks associated with genetic testing involve the emotional, social, or financial consequences of the test results. People may feel angry, depressed, anxious, or guilty about their results. The potential negative impact of genetic testing has led to an increasing recognition of a "right not to know". In some cases, genetic testing creates tension within a family because the results can reveal information about other family members in addition to the person who is tested.[The possibility of genetic discrimination in employment or insurance is also a concern. Some individuals avoid genetic testing out of fear it will affect their ability to purchase insurance or find a job. Health insurers do not currently require applicants for coverage to undergo genetic testing, and when insurers encounter genetic information, it is subject to the same confidentiality protections as any other sensitive health information. In the United States, the use of genetic information is governed by the Genetic Information Non-discrimination Act (GINA) (see discussion below in the section on government regulation).

Genetic testing can provide only limited information about an inherited condition. The test often can't determine if a person will show symptoms of a disorder, how severe the symptoms will be, or whether the disorder will progress over time. Another major limitation is the lack of treatment strategies for many genetic disorders once they are diagnosed. Another limitation to genetic testing for a hereditary linked cancer, is the variants of unknown clinical significance. Because the human genome has over 22,000 genes, there are 3.5 million variants in the average person's genome. These variants of unknown clinical significance means there is a change in the DNA sequence, however the increase for cancer is unclear because it is unknown if the change affects the gene's function.

A genetics professional can explain in detail the benefits, risks, and limitations of a particular test. It is important that any person who is considering genetic testing understand and weigh these factors before making a decision. Other risks include accidental findings—a discovery of some possible problem found while looking for something else. In 2013 the American College of Medical Genetics and Genomics (ACMG) that certain genes always be included any time a genomic sequencing was done, and that labs should report the results.

Human Rights:

Human rights are moral principles or norms that describe certain standards of human behaviour and are regularly protected as natural and legal rights in municipal and international law. They are commonly understood as inalienable, fundamental rights "to which a person is inherently entitled simply because she or he is a human being" and which are "inherent in all human beings", regardless of their nation, location, language, religion, ethnic origin or any other status. They are applicable everywhere and at every time in the sense of being universal, and they are egalitarian in the sense of being the same for everyone. They are regarded as requiring empathy and the rule of law and imposing an obligation on persons to respect the human rights of others, and it is generally considered that they should not be taken away except as a result of due process based on specific circumstances; for example, human rights may include freedom from unlawful imprisonment, torture and execution.

The doctrine of human rights has been highly influential within international law, global and regional institutions. Actions by states and non-governmental organisations form a basis of public policy worldwide. The idea of human rights suggests that "if the public discourse of peacetime global society can be said to have a common moral language, it is that of human rights". The strong claims made by the doctrine of human rights continue to provoke considerable scepticism and debates about the content, nature and justifications of human rights to this day. The precise meaning of the term right is controversial and is the subject of continued philosophical debate; while there is consensus that human rights encompasses a wide variety of rights such as the right to a fair trial, protection against enslavement, prohibition of genocide, free speech or a right to education (including the right to comprehensive sexuality education, among others), there is disagreement about which of these particular rights should be included within the general framework of human rights; some thinkers suggest that human rights should be a minimum requirement to avoid the worst-case abuses, while others see it as a higher standard. In the light of emerging neurotechnologies, four new rights were identified: the right to cognitive liberty, the right to mental privacy, the right to mental integrity, and the right to psychological continuity.

Many of the basic ideas that animated the human rights movement developed in the aftermath of the Second World War and the events of the Holocaust, culminating in the adoption of the Universal Declaration of Human Rights in Paris by the United Nations General Assembly in 1948. Ancient peoples did not have the same modern-day conception of universal human rights. The true forerunner of human rights discourse was the concept of natural rights which appeared as part of the medieval natural law tradition that became prominent during the European Enlightenment with such philosophers as John Locke, Francis Hutcheson and Jean-Jacques Burlamaqui and which featured prominently in the political discourse of the American Revolution and the French Revolution.[6] From this foundation, the modern human rights arguments emerged over the latter half of the 20th century, possibly as a reaction to slavery,

torture, genocide and war crimes, as a realisation of inherent human vulnerability and as being a precondition for the possibility of a just society

Probable Questions:

1. What is the genetic cause of the Down Syndrome? State the symptoms of this syndrome.

2. What is the genetic cause of the Turner Syndrome? State the symptoms of this syndrome.

3. What is the genetic cause of the Klinefelter Syndrome? State the symptoms of this syndrome.

4. What is the genetic cause of the Jacob Syndrome? State the symptoms of this syndrome.

5. What is the genetic cause of the Edwards Syndrome? State the symptoms of this syndrome.

6. What is the genetic cause of the Cri du chat Syndrome? State the symptoms of this syndrome.

7. What is the genetic cause of the Patau Syndrome? State the symptoms of this syndrome.

8. What is the genetic cause of the Di George Syndrome? State the symptoms of this syndrome.

9. Define intersex.

10. Define Eugenics and Euphenics.

11. What is Positive eugenics and negative eugenics?

12. Describe the ways by which positive eugenics is achieved.

13. Describe the ways by which negative eugenics is achieved.

14. Describe genetic counselling. How it may improve human society.

15. Describe euphenic measures by which human can get rid of certain fatal genetic diseases.

16. What is genetic testing? Describe different types of diagnostic genetic testing procedures.

17. Describe different types of Non-diagnostic genetic testing methods.

18. What are the risks associated with genetic testing ?

19. Write an essay on Human Rights and its applications.

Suggested readings:

1. Principles of Genetics. Snustad and Simmons.

2. Genetics . Verma and Agarwal.

3. Principles of Genetics by Tamarin.

4. Biotechnology by V. Kumaresan

UNIT-XIV

Molecular Pathology: Loss of function, Gain of function; Mitochondrial disorders

Objectives: In this nit you will learn about different diseases which are caused due to gain of function and loss of function mechanism. You will also learn about different mitochondrial disorders.

Gain-of-function research (**GoF research** or **GoFR**) is medical research that genetically alters an organism in a way that may enhance the biological functions of gene products. This may include an altered pathogenesis, transmissibility, or host range, i.e. the types of hosts that a microorganism can infect. This research is intended to reveal targets to better predict emerging infectious diseases and to develop vaccines and therapeutics. For example, influenza B can only infect humans and harbor seals. Introducing a mutation that would allow influenza B to infect rabbits in a controlled laboratory situation would be considered a gain-of-function experiment, as the virus did not previously have that function. That type of experiment could then help reveal which parts of the virus are responsible for its host range, enabling the creation of antiviral medicines which block this function.

In virology, gain-of-function research is usually employed with the intention of better understanding current and future pandemics. In vaccine development, gain-of-function research is conducted in the hope of gaining a head start on a virus and being able to develop a vaccine or therapeutic before it emerges. The term "gain of function" is sometimes applied more narrowly to refer to "research which could enable a pandemic-potential pathogen to replicate more quickly or cause more harm in humans or other closely-related mammals."

Some forms of gain-of-function research (specifically work which involves certain select agent pathogens) carry inherent biosafety and biosecurity risks, and are thus also referred to as dual use research of concern (DURC). To mitigate these risks while allowing the benefits of such research, various governments have mandated that DURC experiments be regulated under additional oversight by institutions (so-called institutional "DURC" committees) and government agencies (such as the NIH's recombinant DNA advisory committee). A mirrored approach can be seen in the European Union's Dual Use Coordination Group (DUCG).

Importantly, the US and EU regulations both mandate that an unaffiliated member of the public (or several) be "active participants" in the oversight process. Significant debate has taken place in the scientific community on how to assess the risks and benefit of gain-of-function research, how to publish such research responsibly, and how to engage the public in an open and honest review. In January 2020, the National Science Advisory

Board for Biosecurity convened an expert panel to revisit the rules for gain-of-function research and provide more clarity in how such experiments are approved, and when they should be disclosed to the public.

Experiments that have been referred to as "gain-of-function":

In early 2011, two groups were investigating how flu viruses specific to birds could possibly cross over and create pandemics in humans: one led by Yoshihiro Kawaoka at the University of Wisconsin–Madison in Madison, Wisconsin and another led by Ron Fouchier at Erasmus University Medical Center in the Netherlands. Both groups had both serially passaged H5N1 avian influenza in ferrets, manually taking the virus from one ferret to another, until it was capable of spreading via respiratory droplets. The normally bird-specific virus, through replication over time in the ferrets' lungs, had adopted several amino acid changes that enabled it to replicate in the mammalian lungs, which are a notably colder than those found in birds. This small change also allowed the virus to transmit via droplets in the air made when the ferrets' coughed or sneezed.

Proponents of the Kawaoka and Fouchier experiments cited several benefits: these answered the question of how a virus like H5N1 could possibly become airborne in humans, allowed other researchers to develop vaccines and therapeutics which specifically targeted these amino acid changes, and also demonstrated that there was a linkage between transmissibility in avian viruses and lethality: while the virus had become more transmissible, it had also become significantly less deadly. Various critics of the research (including members of Congress) responded to the publications with alarm. Others called the experiments an "engineered doomsday." Questions were raised by other scientists including Marc Lipsitch of the T. H. Chan School of Public Health at Harvard University about the relative risks and benefits of this research.

In May 2013, a group led by Hualan Chen, director of China's National Avian Influenza Reference Laboratory, published several experiments they had conducted at the BSL3+ laboratory of the Harbin Veterinary Research Institute, investigating what would happen if a 2009 H1N1 circulating in humans infected the same cell as an avian influenza H5N1. Importantly, the experiments had been conducted before a research pause on H5N1 experiments had been agreed upon by the greater virologist community. They used these experiments to determine that certain genes, if reassorted in such a dual-infection scenario in the wild, would allow transmission of the H5N1 virus more easily in mammals (notably guinea pigs as a model organism for rodent species), proving that certain agricultural scenarios carry the risk of allowing H5N1 to cross over into mammals. As in the Fouchier and Kawaoka experiments above, the viruses in this study were also significantly less lethal after the modification.

Critics of the 2013 Chen group study (including Simon Wain-Hobson of the Pasteur Institute and former Royal Society President Robert May) decried this as an unsafe experiment that was unnecessary to prove the intended conclusions, calling Chen's

work "appallingly irresponsible" and also raising concerns about the biosafety of the laboratory itself. Others (including the Director of the WHO Collaborating Centre on Influenza in Tokyo, Masato Tashiro) praised Chen's laboratory as "state of the art." Jeremy Farrar, director of the Oxford University Clinical Research Unit in Ho Chi Minh City, described the work as "remarkable" and said that it demonstrated the "very real threat" that "continued circulation of H5N1 strains in Asia and Egypt" poses.

Loss of Function Disease: Loss-of-function genetic diseases are **caused by the impairment of one protein, with potentially distributed consequences**. For such diseases, the definition of a pharmaceutical target is less precise, and the identification of pharmaceutically-relevant targets may be difficult. An example of a loss of function mutation would be **a nonsense mutation that causes polypeptide chain termination during translation**. Loss of functions mutations are generally recessive. Wild type alleles typically encode a product necessary for a specific biological function. If a mutation occurs in that allele, the function for which it encodes is also lost. The general term for these mutations is **loss-of-function mutations**. The degree to which the function is lost can vary. If the function is entirely lost, the mutation is called a null mutation. If is also possible that some function may remain, but not at the level of the wild type allele. These are called **leaky mutations**.

Loss of function mutations is typically recessive. When a heterozygote consists of the wild-type allele and the loss-of-function allele, the level of expression of the wild type allele is often sufficient to produce the wild type phenotype. Genetically this would define the loss-of-function mutation as recessive. Alternatively, the wild type allele may not compensate for the loss-of-function allele. In those cases, the phenotype of the heterozygote will be equal to that of the loss-of-function mutant, and the mutant allele will act as a dominant.

Although it would be expected that most mutations would lead to a loss of function, it is possible that a new and important function could result from the mutation. In these cases, the mutation creates a new allele that is associated with a new function. Any heterozygote containing the new allele along with the original wild type allele will express the new allele. Genetically this will define the mutation as a dominant. This class of mutations are called **gain-of-function mutations**.

Mitochondrial Diseases:

Mitochondrial disease is a group of disorders caused by **mitochondrial dysfunction**. Mitochondria are the organelles that generate energy for the cell and are found in every cell of the human body except red blood cells. They convert the energy of food molecules into the ATP that powers most cell functions.

Mitochondrial diseases take on unique characteristics both because of the way the diseases are often inherited and because mitochondria are so critical to cell function. A subclass of these diseases that have neuromuscular symptoms are known as mitochondrial myopathies.

History :

The first pathogenic mutation in mitochondrial DNA was identified in 1988; from that time to 2016, around 275 other disease-causing mutations were identified.

Types:

Examples of mitochondrial diseases include:

- **Mitochondrial myopathy**
- **Diabetes mellitus and deafness (DAD)**
  - this combination at an early age can be due to mitochondrial disease
  - Diabetes mellitus and deafness can be found together for other reasons
- **Leber's hereditary optic neuropathy (LHON)**
  - visual loss beginning in young adulthood
  - eye disorder characterized by progressive loss of central vision due to degeneration of the optic nerves and retina
  - affects 1 in 50,000 people in Finland
- **Leigh syndrome, subacute necrotizing encephalomyelopathy**
  - after normal development the disease usually begins late in the first year of life, although onset may occur in adulthood
  - a rapid decline in function occurs and is marked by seizures, altered states of consciousness, dementia, ventilatory failure
- **Neuropathy, ataxia, retinitis pigmentosa, and ptosis (NARP)**
  - progressive symptoms as described in the acronym
  - dementia
- **Myoneurogenic gastrointestinal encephalopathy (MNGIE)**
  - gastrointestinal pseudo-obstruction
  - neuropathy
- **MERRF syndrome**
  - progressive myoclonic epilepsy

    - "Ragged Red Fibers" are clumps of diseased mitochondria that accumulate in the subsarcolemmal region of the muscle fiber and appear when muscle is stained with modified Gömöri trichrome stain
    - short stature
    - hearing loss
    - lactic acidosis
    - exercise intolerance
- **MELAS syndrome**
- **Mitochondrial DNA depletion syndrome**

Causes:

Mitochondrial disorders may be caused by mutations (acquired or inherited), in mitochondrial DNA (mtDNA), or in nuclear genes that code for mitochondrial components. They may also be the result of acquired mitochondrial dysfunction due to adverse effects of drugs, infections, or other environmental causes. Oxalate may enter cells where it is known to cause mitochondrial dysfunction.

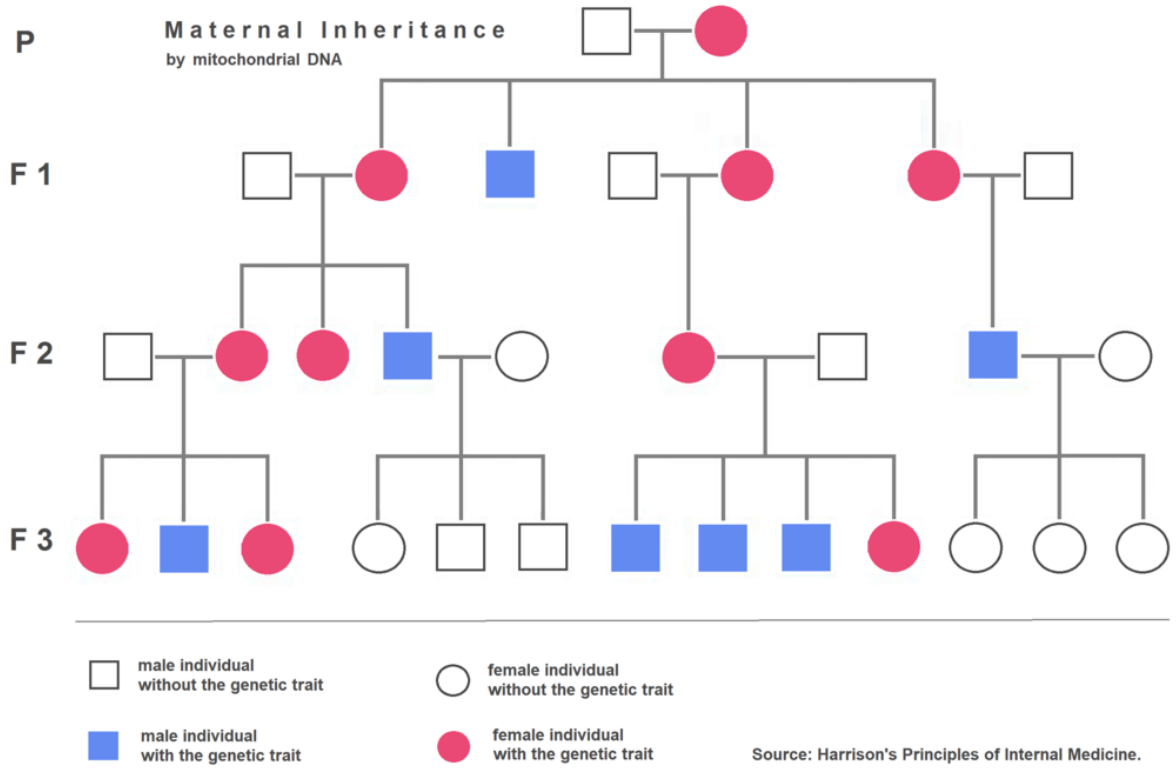
Nuclear DNA has two copies per cell (except for sperm and egg cells), one copy being inherited from the father and the other from the mother. Mitochondrial DNA, however, is inherited from the mother only (with some exceptions) and each mitochondrion typically contains between 2 and 10 mtDNA copies. During cell division the mitochondria segregate randomly between the two new cells. Those mitochondria make more copies, normally reaching 500 mitochondria per cell. As mtDNA is copied when mitochondria proliferate, they can accumulate random mutations, a phenomenon called heteroplasmy. If only a few of the mtDNA copies inherited from the mother are defective, mitochondrial division may cause most of the defective copies to end up in just one of the new mitochondria (for more detailed inheritance patterns, see human mitochondrial genetics). Mitochondrial disease may become clinically apparent once the number of affected mitochondria reaches a certain level; this phenomenon is called "threshold expression".

Mitochondria possess many of the same DNA repair pathways as nuclei do—but not all of them;[13] therefore, mutations occur more frequently in mitochondrial DNA than in nuclear DNA. This means that mitochondrial DNA disorders may occur spontaneously and relatively often. Defects in enzymes that control mitochondrial DNA replication (all of which are encoded for by genes in the nuclear DNA) may also cause mitochondrial DNA mutations.

Most mitochondrial function and biogenesis is controlled by nuclear DNA. Human mitochondrial DNA encodes 13 proteins of the respiratory chain, while most of the estimated 1,500 proteins and components targeted to mitochondria are nuclear-encoded. Defects in nuclear-encoded mitochondrial genes are associated with hundreds of clinical disease phenotypes

including anemia, dementia, hypertension, lymphoma, retinopathy, seizures, and neurodevelopmental disorders.

A study by Yale University researchers explored the role of mitochondria in insulin resistance among the offspring of patients with type 2 diabetes. Other studies have shown that the mechanism may involve the interruption of the mitochondrial signaling process in body cells (intramyocellular lipids). A study conducted at the Pennington Biomedical Research Center in Baton Rouge, Louisiana showed that this, in turn, partially disables the genes that produce mitochondria.



Mechanisms :

The effective overall energy unit for the available body energy is referred to as the daily glycogen generation capacity, and is used to compare the mitochondrial output of affected or chronically glycogen-depleted individuals to healthy individuals. This value is slow to change in a given individual, as it takes between 18 and 24 months to complete a full cycle.

The glycogen generation capacity is entirely dependent on, and determined by, the operating levels of the mitochondria in all of the cells of the human body; however, the relation between the energy generated by the mitochondria and the glycogen capacity is very loose and is mediated by many biochemical pathways. The energy output of full healthy mitochondrial function can be predicted exactly by a complicated theoretical argument, but this argument is not straightforward, as most energy is consumed by the brain and is not easily measurable.

Diagnosis :

Mitochondrial diseases are usually detected by analysing muscle samples, where the presence of these organelles is higher. The most common tests for the detection of these diseases are:

1. Southern blot to detect big deletions or duplications
2. Polymerase chain reaction and specific mutation testing
3. Sequencing

Treatments:

Although research is ongoing, treatment options are currently limited; vitamins are frequently prescribed, though the evidence for their effectiveness is limited. Pyruvate has been proposed in 2007 as a treatment option. N-acetyl cysteine reverses many models of mitochondrial dysfunction. In the case of mood disorders, specifically bipolar disorder, it is hypothesized that N-acetyl-cysteine (NAC), acetyl-L-carnitine (ALCAR), S-adenosylmethionine (SAMe), coenzyme Q10 (CoQ10), alpha-lipoic acid (ALA), creatine monohydrate (CM), and melatonin could be potential treatment options.

Gene therapy prior to conception:

Mitochondrial replacement therapy (MRT), where the nuclear DNA is transferred to another healthy egg cell leaving the defective mitochondrial DNA behind, is an IVF treatment procedure. Using a similar pronuclear transfer technique, researchers at Newcastle University led by Douglass Turnbull successfully transplanted healthy DNA in human eggs from women with mitochondrial disease into the eggs of women donors who were unaffected In such cases, ethical questions have been raised regarding biological motherhood, since the child receives genes and gene regulatory molecules from two different women. Using genetic engineering in attempts to produce babies free of mitochondrial disease is controversial in some circles and raises important ethical issues. A male baby was born in Mexico in 2016 from a mother with Leigh syndrome using MRT.

In September 2012 a public consultation was launched in the UK to explore the ethical issues involved. Human genetic engineering was used on a small scale to allow infertile women with genetic defects in their mitochondria to have children. In June 2013, the United Kingdom government agreed to develop legislation that would legalize the 'three-person IVF' procedure as a treatment to fix or eliminate mitochondrial diseases that are passed on from mother to child. The procedure could be offered from 29 October 2015 once regulations had been established. Embryonic mitochondrial transplant and protofection have been proposed as a possible treatment for inherited mitochondrial disease, and allotopic expression of mitochondrial proteins as a radical treatment for mtDNA mutation load.

Epidemiology:

About 1 in 4,000 children in the United States will develop mitochondrial disease by the age of 10 years. Up to 4,000 children per year in the US are born with a type of mitochondrial disease. Because mitochondrial disorders contain many variations and subsets, some particular mitochondrial disorders are very rare.

The average number of births per year among women at risk for transmitting mtDNA disease is estimated to approximately 150 in the United Kingdom and 800 in the United States.

Probable Questions:

1. What is gain of function disease? Explain.
2. What is loss of function disease? Explain
3. Describe different types of mitochondrial disease.
4. Describe cause of mitochondrial disease.
5. Describe mechanism of mitochondrial disease.
6. How mitochondrial diseases are diagnosed?
7. Discuss treatments of mitochondrial disease.
8. Discuss epidemiology of mitochondrial disease.

Suggested readings:

1. Principles of Genetics. Snustad and Simmons.

2. Genetics . Verma and Agarwal.

3. Principles of Genetics by Tamarin.

4. Biotechnology by V. Kumaresan

UNIT-XV

Genetic analysis of complex traits and disease. Quantitative genetics; heritability and its measurement; inbreeding and cross breeding; QTL

Objective: In this unit you will learn about Genetic analysis of complex traits-complex pattern of inheritance,quantitative traits,threshold traits. In this unit you will learn about Quantitative genetics, variance, heritability and its measurement. You will also have an idea about inbreeding and crossbreeding and QTL

Introduction to Polygenic Traits:

Character or trait refers to any property of an individual showing heritable variation. It includes morphological, physiological, biochemical and behavioural properties. Some characters are governed by one or few genes. Such traits are referred to as qualitative characters or oligogenic characters.

On the other hand, some characters are controlled by several genes. They are known as quantitative characters or polygenic characters. The mode of inheritance of polygenic characters is termed as polygenic inheritance or quantitative inheritance. Since in polygenic inheritance several genes (factors) are involved, it is also known as multiple factor inheritance.

Features of Polygenic Traits:

The term polygene was introduced by Mather in 1941. This term has found wide usage in quantitative genetics replacing the older term multiple gene.

Main features of polygenic characters are briefly presented below:

1. Each polygenic character is controlled by several independent genes and each gene has cumulative effect.

2. Polygenic characters exhibit continuous variation rather than a discontinuous variation. Hence, they cannot be classified into clear-cut groups.

3. Effect of individual gene is not easily detectable in case of polygenic characters and, therefore, such traits are also known as minor gene characters.

4. The statistical analysis of polygenic variation is based on means, variances and co-variances, whereas the discontinuous variation is analysed with the help of frequencies

and ratios. Thus, polygenic characters are studied in quantitative genetics and oligogenic characters in mendelian genetics.

5. Polygenic traits are highly sensitive to environmental changes, whereas oligogenic characters are little influenced by environmental variation.

6. Classification of polygenic characters into different clear-cut groups is not possible because of continuous variation from one extreme to the other. In case of qualitative characters, such grouping is possible because of discrete or discontinuous variation.

7. Generally the expression of polygenic characters is governed by additive gene action, but now cases are known where polygenic characters are governed by dominance and epistatic gene action. In case of oligogenic characters, the gene action is primarily of non-additive type (dominance and epistasis).

8. In case of polygenic characters, metric measurements like size, weight, duration, strength, etc. are possible, whereas in case of oligogenic characters only the counting of plants with regard to various kinds like colour and shape is possible. Thus, metric measurement is not possible in case of oligogenic characters.

9. Transgressive segregants are only possible from the crosses between two parents with mean values for a polygenic character. Such segregants are not possible in case of qualitative or oligogenic traits.

10. The transmission of polygenic characters is generally low because of high amount of environmental variation. On the other hand, oligogenic characters exhibit high transmission because there is little difference between the genotype and phenotype of such character. Thus, polygenic characters differ from oligogenic ones in several aspects (Table 12.1).

TABLE 12.1. Differences between polygenic and oligogenic traits

Polygenic Traits	*Oligogenic Traits*
1. Governed by several genes.	Governed by few genes.
2. Effect of each gene is not detectable.	Effect of each gene is detectable.
3. Usually governed by additive genes.	Governed by non-additive genes.
4. Variation is continuous.	Variation is discontinuous.
5. Separation into different classes is not possible.	Separation into different classes is possible.
6. Highly influenced by environmental factors.	Little influenced by environmental factors.
7. Statistical analysis is based on mean, variances and covariances.	Statistical analysis is based on frequencies or ratios.

In plant breeding both types of characters showing qualitative and quantitative inheritance have equal economic importance.

Similarities between Oligogenic and Polygenic Traits:
East (1916) demonstrated that polygenic characters were perfectly in agreement with Mendelian segregation and later on Fisher (1918) and Wright (1921, 1935) provided a mathematical basis for the genetic interpretation of such characters.

The quantitative characters do not differ in any essential feature from the qualitative characters, as discussed below:
1. Both quantitative and qualitative characters are governed by genes; the former is controlled by polygenes or minor genes and the latter by oligogenes or major genes.

2. Both major as well as minor genes are located on the chromosome in the nucleus.

3. The polygenic traits controlling continuous variation exhibit segregation like major genes controlling discontinuous Mendelian variation.

4. Polygenic characters show variable expression which is due to non-genetic causes i.e., environmental effects. Qualitative characters also exhibit variation in expression but to a lesser degree than polygenic traits.

5. The reciprocal crosses for both types of traits exhibit close agreement in expression of genes.

6. The phenomenon of transgression in polygenes can only be explained by Mendelian principles of inheritance.

7. Polygenes mutate like oligogenes.

8. Dominance and non-allelic interactions are common features of major genes. These features are also observed for polygenes, but are usually complete for major genes and only partial for minor genes.

9. Polygenes exhibit linkage like oligogenes. Many cases of linkage between major genes and polygenes controlling continuous variation have been reported.

Thus, quantitative genetics or biometrical genetics is an extension of Mendelian genetics firmly based on Mendelian principles of heredity.

Analysis of Polygenic Traits:

The method of analysis of quantitative inheritance differs from that of qualitative inheritance in some aspects as given below:
1. It requires various measurements of characters like weight, length, width, height, duration, etc., rather than classification of individuals into groups based on colour or shape.

2. Observations are recorded on several individuals and the mean values are used for genetical studies. Segregation into distinct classes in F_2 generation-is not obtained in the inheritance of quantitative characters. The segregants exhibit continuous range of variation from one extreme (low) to other (high) for such traits.

3. The inheritance is studied with the help of mean, variances and covariance's. These estimates can be worked out from data recorded in replicated experiment.

4. Fisher (1918) was the pioneer worker to interpret the quantitative characters in terms of Mendelian genetics. Now several biometrical techniques are available for the genetic analysis of quantitative characters. The science which deals with the genetic interpretations of quantitative characters has got separate entity as quantitative genetics or biometrical genetics.

Assumptions of Polygenic Traits:

Polygenic inheritance is based on several assumptions.

The six important assumptions are given below:

1. Each of the contributing genes involved in the expression of a character produces an equal effect.

2. Each contributing allele has either cumulative or additive effect in the expression of a character.

3. The genes involved in the expression of characters have lack of dominance. They show intermediate expression between two parents.

4. There is no epistasis among genes at different loci.

5. The linkage is in equilibrium, means there is no linkage.

6. The environmental effects are absent or may be ignored. However, last three assumptions are seldom fulfilled.

There are two types of alleles or genes in the polygenic inheritance, viz:

(1) Contributing alleles and

(2) Non-contributing alleles.

Those alleles which contribute to continuous variation are known as contributing alleles and those which do not contribute to continuous variation are referred to as non-

contributing alleles. Some scientists refer to these as effective and non-effective alleles, respectively.

Examples of Polygenic Traits:

In plant genetics, examples of polygenic characters include yield per plant, days to flower, days to maturity, seed size, seed oil content, etc. Examples of qualitative characters are colour of stem, flower, pollen, etc. and their shapes.

Polygenic inheritance has been reported for various characters both in plants and animals. The most common examples include kernel colour in wheat, corolla length in tobacco, skin colour in man and ear size in maize.

These are briefly described as follows:

a. Kernel Colour in Wheat:

Nilsson Ehle (1908) studied the inheritance of kernel colour in wheat. He found that seed or kernel colour in wheat is governed by one, two and three gene pairs, because in the crosses between red and white kernel varieties, he observed that the F_1 was intermediate between the parental values and in F_2 he observed 3:1,15:1 and 63 : 1 ratios of red and white seeds in different crosses.

The last two ratios indicated that there was duplicate gene interaction; however, in depth study of coloured seeds revealed that there were different grades or shades of colour within the red coloured seeds. The red seeds of 15 : 1 ratio could be easily divided into four classes on the basis of shade of colour, viz., dark red, medium dark red, medium red and light red.

These colours were observed in the ratio of 1 : 4 : 6 : 4 : 1. This suggested that the seed colour in wheat is controlled by genes which show lack of dominance and have small cumulative effects. Here, two types of alleles are involved in the expression of character. Those which contribute to continuous variation and those which do not contribute. The first category of alleles is called effective and second as non-effective. Assume that red seed colour is controlled by two genes R_1 and R_2 and, white seed colour by r_1 and r_2.

From the cross between dark red and white seed parents, Nilsson Ehle observed the following results (Fig. 12.1):

Parents: Dark red × White

Genotypes: $R_1R_1R_2R_2$ × $r_1r_1r_2r_2$

F_1: $R_1r_1R_1r_1$ — Medium Red

F_2	R_1R_2	R_1r_2	r_1R_2	r_1r_2
R_1R_2	$R_1R_1R_2R_2$ [DR]	$R_1R_1R_2r_2$ [MDR]	$R_1r_1R_2R_2$ [MDR]	$R_1r_1R_2r_2$ [MR]
R_1r_2	$R_1R_1R_2r_2$ [MDR]	$R_1R_1r_2r_2$ [MR]	$R_1r_1R_2r_2$ [MR]	$R_1r_1r_2r_2$ [LR]
r_1R_2	$R_1r_1R_2R_2$ [MDR]	$R_1r_1R_2r_2$ [MR]	$r_1r_1R_2R_2$ [MR]	$r_1r_1R_2r_2$ [LR]
r_1r_2	$R_1r_1R_2r_2$ [MR]	$R_1r_1r_2r_2$ [LR]	$r_1r_1R_2r_2$ [LR]	$r_1r_1r_2r_2$ [W]

DR = Dark Red, MDR = Medium Dark Red,
MR = Medium Red, LR = Light Red and W = White

Fig. 12.1. Inheritance of kernel colour in wheat.

Summary of Results

Effective alleles for red colour	*No. of individuals*	*Phenotype*
4	1	Dark red
3	4	Medium dark red
2	6	Medium red
1	4	Light red
0	1	White

Where 4 effective alleles were present, the seed colour was dark red, where 3 such alleles were present, the seed colour was medium dark red, with 2 effective alleles, colour was medium red and with 1 effective allele, seed colour was light red. White seed colour was produced when all the non-effective alleles were present.

2. Corolla Length in Tobacco:

Extreme differences exist in corolla length in *Nicotiana longiflora*. East (1916) studied the inheritance of corolla length in this species of tobacco. He crossed inbred lines of this species with average corolla length of 40 cm and 93 cm.

The F_1 showed intermediate expression for corolla length with 63 cm. In F_2, wide variation for corolla length was observed. The results indicated that five or more genes were involved in the expression of corolla length.

c. Skin Colour Inheritance in Man:

The inheritance of skin colour in man was studied by Davenport. The inheritance of Negro x white mattings can be explained on the basis of two gene difference. Assume that negro colour is governed by A and B genes and white colour by a and b genes.

A cross between negro and white gives birth to a child with medium skin colour called mullatoes (F_1). In F_2 generation, four distinct shades of black colour were observed besides one white (Fig. 12.2). Thus, the phenotypic ratio of 1 : 4 : 6 : 4 : 1 was observed. The individuals having 4, 3, 2, 1 and 0 effective alleles had black (negro) dark, medium, light and white colour, respectively.

The results are presented below:

Parents — Negroes × White

Genotypes — AABB × aabb

F_1 — AaBb Medium Colour Mullato

	AB	Ab	aB	ab
AB	AABB [B]	AABb [D]	AaBb [D]	AaBb [M]
Ab	AABb [D]	AAbb [M]	AaBb [M]	Aabb [L]
aB	AaBb [D]	AabB [M]	aaBB [M]	aaBb [L]
ab	AaBb [M]	Aabb [L]	aaBb [L]	aabb [W]

In bracket B = Black, D = Dark, M = Medium, L = Light and W = White

Fig. 12.2. Inheritance of skin colour in human.

Summary of Results

Effective alleles for skin colour	*Frequency*	*Phenotypes*
4	1	Black (negro)
3	4	Dark
2	6	Medium
1	4	Light
0	1	White

Subsequent studies on skin colour inheritance indicated that as many as six genes are involved in the expression of this character.

Transgressive Segregation:

Appearance of transgressive segregants in F_2 is an important feature of polygenic inheritance. Segregants which fall outside the limits of both the parents are known as transgressive segregants. Transgressive segregation results due to fixation of dominant and recessive genes in separate individuals.

Such segregation occurs when the parents are intermediate to the extreme values of the segregating population. Plant breeders use this principle to obtain superior combinations in segregating material for polygenic characters.

An example of transgressive segregation is presented as follows:

Parents	AAbb	×	aaBB
		↓	
F_1		AaBb	
F_2	AABB and aabb are transgressive segregants		

Environmental Effect:

Polygenic characters are highly sensitive to environmental changes. In other words, they are more prone to genotype x environmental interactions. The main effect of environment is to mask the small differences among different genotypes resulting in continuous variation in the character.

When the contribution of environment is 50 per cent, the distribution becomes roughly similar to normal curve and with 75 per cent contribution, it tends to reach normal distribution. For polygenic traits, generally the environmental variation ranges from 10 to 50 per cent and even more for some traits like yield. The high environmental variation results in overlapping of various classes resulting in continuous, variation.

Partitioning of Polygenic Variability:

The polygenic variation or variability present in a genetic population is measured in terms of variances.

The polygenic variation is of three types, viz:

(1) Phenotypic,

(2) Genotypic and

(3) Environmental.

These are briefly described below:

1. Phenotypic Variability:

It is the total variability which is observable. It includes both genotypic and environmental variation and hence changes under different environmental conditions. Such variation is measured in terms of phenotypic variance.

2. Genotypic Variability:

It is the inherent or genetic variability which remains unaltered by environmental conditions. This type of variability is more useful to a plant breeder for exploitation in

selection or hybridization. Such variation is measured in terms of genotypic variance. The genotypic variance consists of additive, dominance and epistatic components.

3. Environmental Variability:

It refers to non-heritable variation which is entirely due to environmental effects and varies under different environmental conditions. This uncontrolled variation is measured in terms of error mean variance. The variation in true breeding parental lines and their F_1 is non-heritable. Fisher was the first to divide in 1918, the genetic variance into additive, dominance and epistatic components.

a. Additive Variance:

It refers to that portion of genetic variance which is produced by the deviations due to average effects of genes at all segregating loci. Thus, it is the component which arises from differences between two homozygotes of a gene, i.e., AA and aa. Additive genes show lack of dominance, i.e., intermediate expression.

The additive genetic variance is associated with homozygosis and, therefore, it is expected to be maximum in self-pollinating crops and minimum in cross-pollinating crops. Additive variance is fixable and, therefore, selection for traits governed by such variance is very effective.

Additive genetic variance is important for the following major reasons:

1. It is required for estimation of heritability in narrow sense and response to selection is directly proportionate to narrow sense heritability.

2. It is a pre-requisite for selection because this is the only variance which responds to selection.

3. Breeding value of an individual is measured directly by the additive gene effects. The general combining ability (gca) effect of a parent is measure of additive gene effects.

4. Additive genetic variance gets depleted proportionate to the improvement made by selection.

5. In natural plant breeding populations, additive variance is the predominant one closely followed by dominance variance.

b. Dominance Variance:

It arises due to the deviation from the additive scheme of gene action resulting from intra-allelic interaction i.e., interaction between alleles of the same gene or same locus. It is due to the deviation of heterozygote (Aa) from the average of two homozygotes (AA and aa).

Such genes show incomplete, complete or over-dominance. The dominance variance is associated with heterozygosis and, therefore, it is expected to be maximum in cross-pollinating crops and minimum in self-pollinating species.

Dominance variance is not fixable and, therefore, selection for traits controlled by such variance is not effective. Heterosis breeding may be rewarding in such situation. Dominance variance differs from additive variance in several ways. (Table 12.2).

TABLE 12.2. Differences between additive and dominance variance

Additive variance	*Dominance variance*
1. It refers to difference between homozygotes (AA/aa).	It refers to deviation of Aa from the mean of AA and aa.
2. Genes show lack of dominance.	Genes show incomplete, complete or over-dominance.
3. Associated with homozygosity and is more in inbreeders.	Associated with heterozygosity and is more in outbreeders.
4. It is fixable.	It is non-fixable.
5. Selection is very effective as it is fixable.	Selection is ineffective as it non-fixable.
6. It is the chief cause of transgressive segregation.	It is the chief cause of heterosis or hybrid vigour.

c. Epistatic Variance:

It arises due to the deviation as a consequence of inter-allelic interaction, i.e., interaction between alleles of two or more different genes or loci. The epistatic variance is of three types, viz., (i) additive x additive, (ii) additive x dominance, and (iii) dominance x dominance. They differ from each other in several aspects. (Table 12.3).

TABLE 12.3. Comparison of three types of epistatic gene action

Additive × Additive	*Additive × Dominance*	*Dominance × Dominance*
1. It refers to interaction between two loci each exhibiting lack of dominance individually.	It refers to interaction between two loci, one exhibiting lack exhibiting lack of dominance and other dominance individually.	It refers to interaction between two loci each exhibiting dominance individually.
2. This type of epistasis is fixable.	This in non-fixable.	This is also non-fixable.
3. This comes under additive type of gene action.	This comes under non-additive type of gene action.	This comes under non-additive type of gene action.
4. Selection is effective for traits governed by this type of epistasis.	Selection is not effective as this is type of epistasis is not fixable.	Selection is not effective as this is not fixable.
5. This is useful for mass selection and progeny selection.	This type of epistasis is useful for exploitation of heterosis.	This type of epistasis is also suitable for exploitation of heterosis.

(i) Additive x Additive:

In this case both the interacting loci exhibit lack of dominance individually. It is denoted as A x A and is fixable.

(ii) Additive x Dominance:

It refers to interaction between two or more loci, one exhibiting lack of dominance and the other dominance individually. It is denoted as A x D and is non-fixable.

(iii) Dominance x Dominance:

In this type of epistasis both the interacting loci exhibit dominance individually. It is represented as D x D and is non-fixable.

The first type of epistasis is fixable and, therefore, selection is effective for traits governed by such variance. The last two types of epistatic variances are unfixable and, therefore, heterosis breeding may be rewarding for traits exhibiting such variance. In natural plant breeding populations, epistatic variance has the lowest magnitude. Epistatic variance differs in many aspects from dominance variance.

Wright (1935) suggested the partitioning of genetic variance into two components, viz., additive and non-additive (dominance and epistatic components), of which only the additive component contributes to genetic advance under selection.

Mather (1949) divided the phenotypic variance into three components, namely, (1) heritable fixable (additive variance), (2) heritable non-fixable (dominance and epistatic components), and (3) non-heritable non-fixable (Environmental fraction).

In fact, the heritable fixable component of phenotypic variance will include the additive x additive fraction of the epistatic variance as well. Further, the total phenotypic variance may be partitioned as (1) fixable (additive and additive x additive components) and (2) non-fixable (dominance, additive x dominance and dominance x dominance types of epistasis and environmental fraction) components.

TABLE 12.4. Differences between dominance and epistatic variances

Dominance Variance	*Epistatic Variance*
1. It is due to interaction between genes of the same locus.	It is due to interaction between genes of two or more different loci.
2. It is of three types, *viz.*, incomplete, complete and over dominance.	It is also of three types, *viz.*, additive × additive, additive × dominance and dominance × dominance.
3. It is non-fixable.	A × A type is fixable.
4. Magnitude is higher than epistatic variance.	Magnitude is lower than dominance variance.

The above discussion may be summarized as follows:

VP = VG + VE; VG = VA + VD + VI; and VI = VAA + VAD + VDD

Where; VP = phenotypic variance, VG = genotypic variance, VA = additive variance, VD = dominance variance, VI = epistatic variance, VAA = additive x additive variance, VAD = additive x dominance variance, and VDD = dominance x dominance variance.

In homozygous genotypes, the genetic variance is of additive (A) and additive epistatic (AA) types, while in the segregating populations all the three types of genetic variances, viz., additive, dominance and epistasis are observed. In F_2, the phenotypic variance has 1/2D (additive) and 1/4H (non-additive) components.
In a random mating populations with no epistasis and zero inbreeding, the covariance between a parent and its offspring is 1/2 VA; the covariance among half-sibs is 1/4 VA; and the covariance among full-sibs is 1/2 VA + 1/4 VD. These relationships change with the level of inbreeding in the population.

Genetic variability for important agronomic traits in almost all the crops is mainly due to the additive genetic variance. The non-additive variance also exists in nearly all crops and for many important traits, but it is generally smaller in magnitude than the additive component.The variability present in genetic populations can be assessed in four different ways: (1) using simple measures of variability, (2) by variance component analysis, (3) by D_2 statistics, and (4) by metro glyph analysis. For details of these procedures refer Singh and Narayanan (1993).

Significance of Polygenes:

Polygenes are of prime importance to plant breeder for evolution of improved cultivars. Polygenes have great evolutionary significance. They provide variation of fine adjustment and are systems of smooth adaptive change and of speciation.

The potential genetic variability is stored in the form of linked polygenic complexes. Such stores bear mixtures of plus and minus alleles. The potential or hidden variability is released, after inter-mating of such genotypes with other genotypes, due to segregation and recombination.

Mather (1943) has nicely explained the mechanism of storage and release of polygenic variability. It is believed that in natural populations, the best adapted or the fit individuals are those that are close to the population mean for various quantitative traits.

Pleiotropy:

From our study of Mendel's laws, we have learnt that one factor or one gene is responsible for the expression of one character. However, there are also such cases where one gene influences more than one phenotypic character. Such a gene, besides producing its character (i.e., major effect), also affects other characters (i.e., secondary

effect) of the body. Thus, the ability of a gene to have many effects is known as pleiotropy.

The common example of pleiotropy in man is a hereditary disease called sickle-cell anaemia or Cooley's anaemia found among certain African tribes. A recessive gene causes this disease. In homozygous condition the gene causes production of an abnormal haemoglobin. As a result the shape of the red blood cell containing it becomes sickle shaped and distorted.However, in heterozygous condition, the individuals possess both normal and abnormal haemoglobin and have mild anaemia. In Drosophila gene responsible for the size of wing also affects the eye colour, shape of spermatheca, and position of dorsal bristles.

Threshold Traits:

A threshold trait is a trait, which is inherited quantitatively, but is expressed qualitatively. Normally a lot of genes form the basis of a threshold trait, which is why it should be treated as a quantitative trait. A common characteristic of threshold traits and Mendelian traits is that they occur family wise. A threshold character is one for which the phenotypic values are discontinuous but the mode of inheritance is like that of a continuously varying character. The appearance of such characters may be connected with an environmental stimulus.

Many interesting phenotypes have a polygenic basis but are expressed as discrete character states. Examples include wing dimorphism in insects, presence or absence of enlarged horns or other structures in male insects, male size and mating behavior dimorphism in various animal taxa, life-cycle dimorphism in salamanders, trophic dimorphism in salamanders and fish, and reproductive caste dimorphism in eusocial animals. Threshold models provide a quantitative genetic framework for studying the evolution of such dimorphic phenotypes. These models assume that polymorphisms are caused by variation in an unobservable but normally distributed phenotype termed "liability". Individuals with liability above a threshold value express one phenotypic character state (induced) and those with liability below the threshold express the alternate state (uninduced). Quantitative genetic parameters, such as narrow-sense heritability and genetic correlations, can be estimated on this liability scale by considering the joint patterns of induction among relatives.

Predictive evolutionary theory requires that we understand selection and inheritance. If we are interested in the evolution of a threshold trait, then selection must be considered on the liability scale. Classical theory predicts that mass selection on liability should be frequency dependent because the threshold function shields part of the liability variation (the within-morph component) from the purifying effects of selection; the size of the cryptic fraction depends upon the proportion of the population that is induced (the population incidence). However, when populations are partitioned into groups (e.g., demes or family units), random genetic drift will lead to variations among groups

in mean liability and, hence, mean incidence. Group-mean liability can vary continuously and the function that maps fitness to group-mean liability is free to take any shape. In this way, selection can discriminate better between groups than between individuals. The threshold function causes mass selection to be inefficient, leading some to recommend that breeders apply family-level selection to more effectively change the population incidence.

Current models of selection on threshold traits consider only the genes that map directly from individuals' genotypes to phenotypes (i.e., direct genetic effects). Consequently, “family-level selection” in the classical animal breeding literature implies artificial selection on the mean phenotype of family members that results only from direct effects. In reality, the genotypes of social partners (e.g., mothers, siblings, or coresidents) often affect the phenotypes of individuals through indirect genetic effects, which are also known as associative effects. Maternal effects are the best studied and perhaps most widespread type of indirect effect and have been shown to affect the expression of threshold traits in several species, including diapause in cricket eggs sex in reptiles with temperature-dependent sex determination, the presence or absence of horns in male dung beetles, and reproductive caste in some species of ants. Indirect effects arising from both genetic and environmental effects can cause differences in group-mean fitness that lead to group-level selection that may work in concert or in conflict with individual-level selection. Family-level selection in the social evolution literature implies selection among families that have been kept intact so that social interactions contribute to the phenotypes expressed by the social members.

The evolution of traits that are affected by direct and indirect social factors can be understood more fully by partitioning selection into individual and group-level components, using the regression-based method of contextual analysis. This approach has been used to generalize Hamilton's rule, a definition of the conditions necessary for the spread of an “altruistic” trait that has opposing group and individual effects. Despite the special significance given to dichotomous traits in discussion of the evolution of altruism, such as the evolution of discrete queen and worker reproductive castes in eusocial animals, selection on threshold traits has not yet been studied using contextual analysis. Here we use this approach to explore how multilevel selection operates on a threshold trait with an underlying genetic model that includes indirect genetic effects.

Quantative Genetics:

Quantitative genetics is concerned with the inheritance of those differences between individuals that are of degree rather than of kind, quantitative rather than qualitative. These are the individual differences which, as Darwin wrote, "afford materials for natural selection to act on and accumulate, in the same manner as man accumulates in any given direction individual differences in his domestic productions." An understanding of the inheritance of these differences is thus of fundamental significance in the study of evolution and in the application of genetics to animal and plant breeding;

and it is from these two fields of enquiry that the subject has received the chief impetus to its growth.

Virtually every organ and function of any species shows individual differences of this nature, the differences of size among ourselves or our domestic animals being an example familiar to all. Individuals form a continuously, graded series from one extreme to the other and do not fall naturally into sharply demarcated types. Qualitative differences, in contrast, divide individuals into distinct types with little or no connexion by intermediates. Examples are the differences between blue-eyed and brown-eyed individuals, between the blood groups, or between normally coloured and albino individuals. The distinction between quantitative and qualitative differences marks, in respect of the phenomena studied, the distinction between quantitative genetics and the parent stem of "Mendelian" genetics. In respect of the mechanism of inheritance the distinction is between differences caused by many or by few genes. The familiar Mendelian ratios, which display the fundamental mechanism of inheritance, can be seen only when a gene difference at a single locus gives rise to a readily detectable difference in some property of the organism. Quantitative differences, in so far as they are inherited, depend on gene differences at many loci, the effects of which are not individually distinguishable. Consequently the Mendelian ratios are not exhibited by quantitative differences, and the methods of Mendelian analysis are inappropriate.

The methods of study in quantitative genetics differ from those employed in Mendelian genetics in two respects. In the first place,..! since ratios cannot be observed, single progenies are uninformative, and the unit of study must be extended to "populations," that is larger groups of individuals comprising many progenies. And, in the second place, the nature of the quantitative differences to be studied requires the measurement, and not just the classification, of the individuals. The extension of Mendelian genetics into quantitative genetics may thus be made in two stages, the first introducing new concepts connected with the genetic properties of "populations" and the second introducing concepts connected with the inheritance of measurements.

Variance:

Meaning of Variance:

It is very useful measure of dispersions which is commonly used for population data. The arithmetic mean of the squares of deviations obtained from the mean is referred to as variance. It is based on standard deviation and the square of the standard deviation is termed as variance.

$$V = \sigma^2 \text{ or } (S.D.)^2 = \frac{\Sigma(X - \bar{x})^2}{n}$$

$$\text{or } V = \frac{\Sigma d^2}{n} \text{ or } \frac{\Sigma f d^2}{n},$$

Thus, if the spread of a series is known, its standard deviation can be determined:

$$\sigma = \sqrt{variance}.$$

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Co-Efficient of Variation:

Co-efficient of variation is a relative term which measures the relative magnitudes of variations present in observations and is related to the magnitude of their arithmetic mean. Since the standard deviation is independent measure of dispersion obtained from a single series of data, the comparison of variability of two different series of data is not possible directly by standard deviations.

To make it possible Karl Pearson used co-efficient of variation. Co-efficient of variation is defined as the ratio of standard deviation to arithmetic mean and is expressed in percentage. Co-efficient of variation or CV (%) = σ /x × 100, where σ = standard deviation of a series x = arithmetic mean of series.

Thus, when standard deviation of a series is compared to arithmetic mean of the series in terms of percentage, we get co-efficient of variation. It expresses the relative variability of each data series. The co-efficient of variation can be used to compare the homogeneity, consistency and stability of data of two or more series.

The data series with high value of co-efficient of variation is said to be unstable and the series with low values of co-efficient of variation are stable and they show good degree of homogeneity of data.

Example:

Compare the relative variability of two samples with the following data:

Sample A–Arithmatic mean $(\bar{x}) = 30$ and S.D. = 1.5

Sample B–Arithmatic mean $(\bar{x}) = 20$ and S.D. = 2.5

Solution. (*i*) For sample A, co-efficient of variation (CV) $= \frac{\text{S.D. or } \sigma}{\text{mean}} \times 100$

$$= \frac{1.5}{30} \times 100 = 5.0\%.$$

(*ii*) For sample B, CV $= \frac{\sigma}{\text{mean}} \times 100$

$$= \frac{2.5}{20} \times 100 = 12.50\%.$$

Example:

Calculate standard deviation, variance and co-efficient of variation form the following classified data regarding number of pods per plant recorded on 50 plants in a plot.

Number of pods/plant	1 – 3	3 – 5	5 – 7	7 – 9	9 – 11	11 – 13	13 – 15
No. of Plants	2	6	7	16	11	5	3

Solution.

Class (No. of pods per plant)	*Mid-value of class (x)*	*No. of Plants (f)*	$dx = (x - A)$	dx^2	$f.dx^2$	$f.dx$	
1 – 3	2	2	2 – 8 = – 6	36	72	– 12	
3 – 5	4	6	4 – 8 = – 4	16	96	– 24	= – 50
5 – 7	6	7	6 – 8 = – 2	4	28	– 14	
7 – 9	8 = *A*	16	8 – 8 = 0	00	00	00	
9 – 11	10	11	10 – 8 = 2	4	44	22	
11 – 13	12	5	12 – 8 = 4	16	80	20	= 60
13 – 15	14	3	14 – 8 = 6	36	108	18	
Total		$n = 50$			$\Sigma f.dx^2 = 428$	$\Sigma f.dx = 10$	

$$\text{Arithmatic mean } (\bar{x}) = A + \frac{\Sigma f.dx}{n}$$

$$\bar{x} = 8 + \frac{10}{50} = 8 + 0.2 = 8.2$$

$$\text{S.D. or } \sigma = \sqrt{\frac{\Sigma fdx^2}{n} - \left(\frac{\Sigma fdx}{n}\right)^2}$$

$$= \sqrt{\frac{428}{50} - \left(\frac{10}{50}\right)^2} = \sqrt{8.56 - 0.04}$$

$$= \sqrt{8.52} = 2.92$$

$$\text{Variance} = \sigma^2$$

$$= \left(\sqrt{8.52}\right)^2$$

$$\text{Co-efficient of variation} = \frac{\sigma}{\bar{x}} \times 100$$

$$\text{or C.V.} = \frac{2.92}{8.2} \times 100$$

$$\text{or C.V.} = \frac{292}{8.2} = 35.61\%.$$

Heritability:

Heritability**,** amount of phenotypic (observable) variation in a population that is attributable to individual genetic differences. Heritability, in a general sense, is the ratio of variation due to differences between genotypes to the total phenotypic variation for a character or trait in a population. The concept typically is applied in behaviour genetics and quantitative genetics, where heritability estimates are calculated by using either correlation and regression methods or analysis of variance (ANOVA) methods.

Heritability is expressed as $H^2 = Vg/Vp$, where H is the heritability estimate, Vg the variation in genotype, and Vp the variation in phenotype. Heritability estimates range in value from 0 to 1. If $H = 1$, then all variation in a population is due to differences or variation between genotypes (i.e., there is no environmentally caused variation). If $H = 0$, there is no genetic variation; in this case all variation in the population comes from differences in the environments experienced by individuals. Heritability is commonly used in twin studies in the field of behaviour genetics. The methodology is based on the fact that identical twins (monozygotic, or one-egg twins) share 100 percent of their genes in common and nonidentical, or fraternal, twins (dizygotic, or two-egg twins) are similar to other siblings (i.e., brothers and sisters) in that they share 50 percent of their genes in common. The correlation between identical twins is expected to be equal to 1.0 and that of fraternal twins to be 0.50. In the field of quantitative genetics, the concept of heritability is used to partition observable phenotypic variation between individuals into genetic and environmental components.

Drawbacks of Heritability Concept:

There are several drawbacks to the use of heritability estimates. First, heritability is not a measurement of how sensitive a character or trait might be to a change in environment. For example, a trait may have complete heritability ($H = 1$) yet be altered drastically by environmental change. This can be seen in certain genetic disorders of metabolism, such as phenylketonuria and Wilson disease, where heritability of phenotypic outcomes equals 1.0 but effective treatment is possible through dietary interventions. A second problem with heritability estimates is that they measure variation only within populations. In other words, a heritability estimate cannot be used to determine the causes of differences between populations, nor can it be used to determine the extent to which an individual's phenotype is determined by genes versus environment.

Furthermore, the heritability concept is subject to misuse when applied to human population differences for traits such as intelligence. For instance, studies have argued that racial differences in measures of intelligence, academic achievement, and crime rates are due to genetic rather than environmental differences. However, other studies have shown that estimates of heritability for such traits within populations do not provide information about genetic differences between populations.

Quantitative Trait Loci (QTL):

A quantitative trait locus (QTL) is a section of DNA (the locus) which correlates with variation in a phenotype (the quantitative trait). QTLs are mapped by identifying which molecular markers (such as SNPs or AFLPs) correlate with an observed trait. This is often an early step in identifying and sequencing the actual genes that cause the trait variation.

A quantitative trait locus (QTL) is a region of DNA which is associated with a particular phenotypic trait, which varies in degree and which can be attributed to polygenic effects, i.e., the product of two or more genes, and their environment. These QTLs are often found on different chromosomes. The number of QTLs which explain variation in the phenotypic trait indicates the genetic architecture of a trait. It may indicate that plant height is controlled by many genes of small effect, or by a few genes of large effect.

Typically, QTLs underlie continuous traits (those traits which vary continuously, e.g. height) as opposed to discrete traits (traits that have two or several character values, e.g. red hair in humans, a recessive trait, or smooth vs. wrinkled peas used by Mendel in his experiments).

Moreover, a single phenotypic trait is usually determined by many genes. Consequently, many QTLs are associated with a single trait. Another use of QTLs is to identify

candidate genes underlying a trait. Once a region of DNA is identified as contributing to a phenotype, it can be sequenced. The DNA sequence of any genes in this region can then be compared to a database of DNA for genes whose function is already known.

Quantitative traits:

Polygenic inheritance refers to inheritance of a phenotypic characteristic (trait) that is attributable to two or more genes and can be measured quantitatively. Multifactorial inheritance refers to polygenic inheritance that also includes interactions with the environment. Unlike monogenic traits, polygenic traits do not follow patterns of Mendelian inheritance (discrete categories). Instead, their phenotypes typically vary along a continuous gradient depicted by a bell curve.

An example of a polygenic trait is human skin colour variation. Several genes factor into determining a person's natural skin colour, so modifying only one of those genes can change skin colour slightly or in some cases, such as for SLC24A5, moderately. Many disorders with genetic components are polygenic, including autism, cancer, diabetes and numerous others. Most phenotypic characteristics are the result of the interaction of multiple genes. Examples of disease processes generally considered to be results of many contributing factors.

Development of QTL Concept:

Mendelian inheritance was rediscovered at the beginning of the 20th century, and as Mendel's ideas spread geneticists began to connect Mendel's rules of inheritance of single factors to Darwinian evolution. For early geneticists, it was not immediately clear that the smooth variation in traits like body size (i.e., Incomplete Dominance) was caused by the inheritance of single genetic factors. Although Darwin himself observed that inbred features of fancy pigeons were inherited in accordance with Mendel's laws (although Darwin didn't actually know about Mendel's ideas when he made the observation), it was not obvious that these features selected by fancy pigeon breeders can similarly explain quantitative variation in nature.

An early attempt by William Ernest Castle to unify the laws of Mendelian inheritance with Darwin's theory of speciation invoked the idea that species become distinct from one another as one species or the other acquires a novel Mendelian factor. Castle's conclusion was based on the observation that novel traits that could be studied in the lab and that show Mendelian inheritance patterns reflect a large deviation from the wild type, and Castle believed that acquisition of such features is the basis of "discontinuous variation" that characterizes speciation. Darwin discussed the inheritance of similar mutant features but did not invoke them as a requirement of speciation. Instead Darwin used the emergence of such features in breeding populations as evidence that mutation can occur at random within breeding populations, which is a central premise of his model of selection in nature. Later in his career, Castle would refine his model for speciation to allow for small variation to contribute to speciation over time. He also was

able to demonstrate this point by selectively breeding laboratory populations of rats to obtain a hooded phenotype over several generations. Castle's was perhaps the first attempt made in the scientific literature to direct evolution by artificial selection of a trait with continuous underlying variation, however the practice had previously been widely employed in the development of agriculture to obtain livestock or plants with favourable features from populations that show quantitative variation in traits like body size or grain yield.

Castle's work was among the first to attempt to unify the recently rediscovered laws of Mendelian inheritance with Darwin's theory of evolution. Still, it would be almost thirty years until the theoretical framework for evolution of complex traits would be widely formalized. In an early summary of the theory of evolution of continuous variation, Sewall Wright, a graduate student who trained under Castle, summarized contemporary thinking about the genetic basis of quantitative natural variation: "As genetic studies continued, ever smaller differences were found to Mendelize, and any character, sufficiently investigated, turned out to be affected by many factors." Wright and others formalized population genetics theory that had been worked out over the preceding 30 years explaining how such traits can be inherited and create stably breeding populations with unique characteristics. Quantitative trait genetics today leverages Wright's observations about the statistical relationship between genotype and phenotype in families and populations to understand how certain genetic features can affect variation in natural and derived populations.

QTL mapping:

For organisms whose genomes are known, one might now try to exclude genes in the identified region whose function is known with some certainty not to be connected with the trait in question. If the genome is not available, it may be an option to sequence the identified region and determine the putative functions of genes by their similarity to genes with known function, usually in other genomes. This can be done using BLAST, an online tool that allows users to enter a primary sequence and search for similar sequences within the BLAST database of genes from various organisms. It is often not the actual gene underlying the phenotypic trait, but rather a region of DNA that is closely linked with the gene.

Another interest of statistical geneticists using QTL mapping is to determine the complexity of the genetic architecture underlying a phenotypic trait. For example, they may be interested in knowing whether a phenotype is shaped by many independent loci, or by a few loci, and do those loci interact. This can provide information on how the phenotype may be evolving. In a recent development, classical QTL analyses were combined with gene expression profiling i.e. by DNA microarrays. Such expression QTLs (eQTLs) describe cis- and trans-controlling elements for the expression of often disease-associated genes. Observed epistatic effects have been found beneficial to identify the

gene responsible by a cross-validation of genes within the interacting loci with metabolic pathway- and scientific literature databases.

Probable questions:

1. What do you mean by polygenic traits? Describe its characteristics .

2. What are the similarities between polygenic and oligogenic traits?

3. What are the differences between polygenic and oligogenic traits?

4. How polygenic traits are analysed ?

5. Describe three types of polygenic variation .

6. what is additive variance and dominance variance ? What are the differences between these two?

7. What is Pleiotropic traits ? Give examples.

8. Write a note on Threshold Traits with suitable examples .

9. Define variance. How it is calculated ?

10. What is coefficient of variation ?

11. What do you mean by heritability ? hat are the drawbacks of this theory ?

12. Define Quantitative trait and Quantitative trait loci.

13. How QTL mapping is done ?

Suggested Readings:

1. Principles of Genetics. Snustad and Simmons.

2. Genetics . Verma and Agarwal.

3. Principles of Genetics by Tamarin.

4. Biotechnology by V. Kumaresa

UNIT-XVI

Measures of Central Tendency

Objective: In this unit we will discuss different types of central tendency and dispersal methods

Mean:

(A) Arithmetic Mean:

It is most commonly used of all the averages. It is the value which we get by dividing the aggregate of various items of the same series by the total number of observations.

Calculation for Ungrouped Data:

When observations are denoted by x values showing x_1, x_2, x_3... x_n; the total number of observations is calculated by summing up the observations and dividing the sum by the total number of observations (n)

Find out the average pod length of the plant.

$$\bar{x} = \frac{x_1 + x_2 + x_3 + \cdots + x_n}{n}$$

Example 1: The pod length of ten pods of a plant shows following data:

5.2 cm,	5.3 cm,	5.6 cm,	5.7 cm,	5.4 cm,
5.2 cm,	5.3 cm,	5.3 cm,	5.4 cm,	5.2 cm.

Find out the average pod length of the plant.

$$\bar{x} = \frac{\sum x}{n} = \frac{5.2 + 5.3 + 5.6 + 5.7 + 5.4 + 5.2 + 5.3 + 5.3 + 5.4 + 5.2}{10} \text{cm}$$

$$= \frac{53.6}{10} \text{cm} = 5.36 \text{ cm}$$

Calculation for Grouped Data:

When the series is discrete, each value of the variable is multiplied by their respective frequencies, sum of all values is divided by total number of frequencies. Variable x has the values like x_1, x_2, x_3, ..., x_n and their frequencies are f_1 , f_2, f_3, ..., f respectively.

Then Arithmetic Mean:

$$\bar{x} = \frac{f_1 x_1 + f_2 x_2 + f_3 x_3 + \cdots + f_n x_n}{f_1 + f_2 + f_3 + \cdots + f_n} = \frac{\sum fx}{\sum f}$$

When the series is continuous, the arithmetic mean is calculated after taking the midpoint value of class intervals.

$$\bar{x} = \frac{\sum fm}{\sum f}$$

where, $\bar{x}$ = Arithmetic mean

$\sum fm$ = Sum values of midpoint value multiplied by their frequencies

If = Sum of frequencies

m = Mid points of various class intervals.

Example 2:
An observation on 32 Balsam plants shows the following data. Calculate the arithmetic mean.

No. of flowers/plant (x)	4	5	6	7	8	9
No. of plants (f)	3	5	6	9	5	4

No. of flowers / plant (x)	No. of plants (f)	f × x
4	3	12
5	5	25
6	6	36
7	9	63
8	5	40
9	4	6
	$\Sigma f = 32$	$\Sigma fx = 212$

$$\bar{x} = \frac{\sum fx}{\sum f} = \frac{212}{32} = 6.62 \text{ (approx.)}$$

The average number of flowers/plant is 6.62.

No. of pods/plant	Mid points of class (m)	No. of plants frequency (f)	m.f.
15-17	16	5	80
18-20	19	6	114
21-23	22	8	176
24-26	25	12	300
27-29	28	22	616
30-32	31	18	558
33-35	34	15	510
36-38	37	9	333
39-41	40	5	200
		$\Sigma f = 100$	$\Sigma mf = 2,887$

$$\text{Arithmetic Mean} = \bar{x} = \frac{\Sigma mf}{\Sigma f} = \frac{2,887}{100} = 28.87.$$

Merits, Demerits and Uses of Arithmetic Mean:

Merits:

1. It has the simplest formula to calculate and it is easily understood.

2. It is rigidly defined mathematical formula the same result will come on repeated calculations.

3. The calculation is based on all the observations.

4. It is least affected by sampling fluctuation.

5. The arithmetic mean balances the value on either side.

6. It is the best measure to compare two or more series.

7. Arithmetic mean is totally dependent on values not on the position

Demerits:

1. It cannot be calculated if all the values are not known.

2. The extreme values have greater effect on mean.

3. The qualitative data cannot be measured in this way.

Uses:

1. The arithmetic mean is mostly used in practical statistics.

2. Mean helps to calculate many other estimates in statistics.

3. The arithmetic mean is most popular method of any measurement used by common people to get the average of any data.

(b) Geometric Mean:

The geometric mean is defined as the n-th root of the product of n observations.

$$\text{Geometric Mean (GM)} = \sqrt[n]{x_1 \cdot x_2 \cdot x_3 \cdots x_n}$$

Where n = number of observations; x_1, x_2, $x_3 \ldots x_n$ = variable values.
When n is small then the above formula can be applied but in case of large 'n' number the logarithms are used to find out the GM

$$GM = \text{Anti log}\frac{\log x_1 + \log x_2 + \log x_3 + \cdots + \log x_n}{n} = \text{Anti log}\frac{\log x}{n}$$

Example 3:

Find out the geometric mean of the following seeds, x denotes the weight of each seed in mg.

5 mg, 7 mg, 8 mg, 6 mg and 4 mg.

x	log x
5 mg	0.70
7 mg	0.85
8 mg	0.90
6 mg	0.78
4 mg	0.60
	Σ log x = 3.83

$$\frac{\Sigma \log x}{5} = \frac{3.83}{5} = 0.77$$

Antilog of $\frac{\Sigma \log x}{5}$, i.e., antilog of 0.77 = 5.89 mg

So the geometric mean of seed weight = 5.89 mg.

This mean is based on all observations, rigidly defined, less affected by extreme values. This mean is difficult to understand, compute and interpret.

This mean is mostly helpful in averaging ratios, percentage and determining ratio of change. This mean is important in construction of index number.

(c) Harmonic Mean:

When the variables are expressed in ratios or rates, the proper average is to be calculated through harmonic mean. The harmonic mean is defined as the reciprocal of arithmetic mean of the reciprocal of the given values.

The harmonic mean is applicable only in restricted field such as oxygen consumption/hour, calorie requirement/hour, CO_2 evolution/hour, flow of sap/min, etc.

$$\text{Harmonic mean (HM)} = \frac{n}{\frac{1}{x_1}+\frac{1}{x_2}+\frac{1}{x_3}+\cdots+\frac{1}{x_n}} = \frac{n}{\Sigma\left(\frac{1}{x}\right)}$$

Where n = Total number of observation; x_1, x_2, x_3 are the values of variables.

Example 4:

In a particular experiment, 5 different sets of Hydrilla plants showed O_2, evolution/hour, was recorded.

2.5 c.c./hour, 1.8 c.c./hour, 2.0 c.c./hour
2.2 c.c./hour, 2.4 c.c./hour.

$$HM = \frac{5}{\frac{1}{2.5}+\frac{1}{1.8}+\frac{1}{2.0}+\frac{1}{2.2}+\frac{1}{2.4}} = \frac{5}{.4+.55+.5+.45+.41} = \frac{5}{2.3} = 2.17 \text{ c.c./hour.}$$

So, harmonic mean of the observation is 2.17 c.c./hour. This HM determination is based on all the observations of a series. It gives more weightage to the smaller items and also not much affected by sample fluctuation.It is not very easy to calculate and also the positive and negative, both values, cannot be computed.

Average of Position:

From the data of any observation, one can find a peak in the middle with higher and lower values distributed more or Jess symmetrically towards both sides of the peak.

Mode:

Most frequent value in a series. Mode cannot be determined from a series of individual observations unless it is converted into either a discrete or continuous series. In a discrete series the value of the variable against which the frequency is the largest would be the modal value.

For example, 2, 4, 4, 4, 6, 9, 3, 2, 4, 6, 11, 13 mode is 4 as it is occurring maximum.

For example, 5, 3, 6, 3, 5, 10, 7, 2 mode is 3 and 5 such series is known as bi-modal series.

Similarly in a continuous frequency distribution the class interval having the maximum frequency would be the modal class.In a frequency distribution, 'mode' is defined as "the value of the variable for which the frequency is maximum". From the definition it is clear that mode cannot be determined from a series of individual observation, always depends on the frequency of occurrence of any item.

When the concentration of data gives only one peak then the distribution is unimodal, but if the data concentrates at two or more points on a scale of values, then the series is called bimodal or multimodal.

Mode can be determined from grouped data using the following formula:

$$\text{Mode} = l_1 + \frac{\Delta_1}{\Delta_1 + \Delta_2} \times i,$$

where l_1 = lower limit of modal class, Δ_1 = difference of frequencies between modal class and the preceding class, Δ_2 = difference of frequencies between modal class and post modal class and i = class interval.
For example:

Class interval (Marks obtained)	*Class value*	*Frequency*
1–9	5	3
10–20	15	5
21–29	25	12
30–40	35	15
41–49	45	25
50–60	55	40
61–69	65	18
70–80	75	11
81–89	85	3
90–100	95	2

Modal class (*maximum frequency*) is 50–60.

$$\text{Mode} = l_1 + \frac{\Delta_1}{\Delta_1 + \Delta_2} \times i$$

$$l_1 = 50, \Delta_1 = 40 - 25 = 15, \Delta_2 = 40 - 18 = 22, i = 10.$$

$$\text{Mode} = 50 + \frac{15}{15 + 22} \times 10 = 50 + 4.054 = \mathbf{54.05.}$$

In the Example 2, we find the maximum frequency in case of variable value 7. So the mode value of this observation is 7. This type of distribution is called unimodal distribution.The maximum frequency (22) is observed in case of class value 27-29 (Table 9.4), the mid value of this class is 28. So, the mode value of this observation is 28.

Example 5:
In another observation on 30 Balsam plants shows the following data.

No. of flowers/plant (x)	3	4	5	6	7	8	9	10
No. of plants (f)	1	3	2	8	5	8	2	1

Here the mode value cannot be calculated by mere inspection, as the maximum frequency is observed in case of two values of variable 6 and 8. So to determine the modal class, the data is grouped.

If we take 2 values together then the grouped data can be arranged in following ways:

Class value	Mid value (m)	frequency
3-4	3.5	4
5-6	5.5	10
7-8	7.5	13
9-10	9.5	3

Here the modal class is 7-8, where mid value is 7.5, so the mode-value of this distribution is 7.5. This type of distribution is called bimodal distribution.

Merits and Demerits of Mode:

Merits of Mode:

i. It is simple and easily understood.

ii. Mode is not affected by the values of extreme items provided they follow to the natural law relating to extremes.

iii. For determination of mode all values in the series are not considered.

Demerits of Mode:

i. As mode is not based on all observations of a series, therefore, it is not rigidly defined.

ii. Mode is not capable of further mathematical treatment.

iii. Mode may be unrepresentative in many cases and it may be impossible to set a definite value of mode as in a set of observations 2 or 3 or more modal values may occur.

Median:

When the values of all items of a series are arranged in increasing (ascending) or decreasing (descending) order it is usually called an array and the middle item of an array is called median. The median divides the series into two groups; one group in which the values of items are less than the middle value and the other group in which the values of the items are greater than the middle item. Median is denoted by Me or Mdn.

The methods of calculating the median are comparatively simple. The value of median is not affected by change in extreme values. If the number of data in a series is odd, the median is the middle value. But if the number of data in a series is even, the median is the average of the two middle values.The median of a distribution is defined as the value of that variable which divides the total frequency into two equal parts when the series is arranged in ascending or descending order of magnitude. So in a distribution, half of the values remain below median value and half of the values remain above the median value.

Methods of Determining Median:

1. For unclassified and un-tabulated data:

In order to calculate the median, the data are first arranged in increasing or decreasing order and then the following formula is used:

Me = n+1/2, where n = number of items or data.

Example:
The heights (in cm) for 9 plants are given below. Find out the Media Height — 67, 65, 70, 68, 62, 63, 64, 63, 66.

Solution:
The height measurements can be arranged in ascending order as follows:

62, 63, 64, 65, 66, 67, 68, 69, 70.

The number of data in the above array $n = 9$.

$$\text{Median} = \frac{n+1}{2} = \frac{9+1}{2} = 5 \text{ (i.e., value of 5th data in the array)}$$

$$= 66$$

(ii) For even number of data in the series:
The median is calculated as follows:

$$\text{Me} = \frac{\frac{n}{2}\text{th item} + \frac{n}{2}+1}{2}$$

Suppose, for example, there are 10 data in a series.

$$\text{So Me} = \frac{\left(\frac{10}{2}\right)+\left(\frac{10}{2}+1\right)}{2} = \frac{5+(5+1)}{2} = \frac{5+6}{2}$$

$$= \frac{\text{Value of fifth data + Value of sixth data of the array}}{2}.$$

Example:
The number of flowers recorded on 10 plants are:
15,10,8,12,13,7,11,14,9,16. Find out the median value of flowers per plant.

Solution:
The given numbers of flowers on 10 plants can be arranged in ascending order as under:

7, 8, 9, 10, 11, 12, 13, 14, 15, 16.

Total number of data in the above array $n = 10$ (even number).

$$\text{Me} = \frac{\frac{n}{2} + \frac{n}{2} + 1}{2}$$

$$= \frac{\frac{10}{2} + \frac{10}{2} + 1}{2} = \frac{\text{5th value + 5 + 1 value}}{2}$$

$$= \frac{11+12}{2} = \frac{23}{2} = 11.5 \text{ flowers.}$$

Example:

Calculate the median of the following series of data obtained by measuring the heights of 16 plants: 9, 10, 10, 8, 9, 7, 8, 11,7, 12, 14, 12, 11, 14, 15, 13.

Solution:
The given data of plant heights are arranged in ascending order as follows:
7, 7, 8, 8, 9, 9, 10, 10, 11,11, 12, 12, 13, 13, 14, 14, 15

n = 16 (even number)

$$Me = \frac{\frac{n}{2} + \frac{n}{2} + 1}{2} = \frac{\frac{16}{2} + \frac{16}{2} + 1}{2} = \frac{\text{Value of 8th item + Value of 9th item}}{2}$$

$$= \frac{10 + 11}{2} = 10.5$$

So the median height = 10.5 cm

2. For Grouped data:

(i) Discontinuous or Discrete series of data. To calculate the median for discrete grouped data, first of all the cumulative frequency of whole series is obtained. The value of data against n+1/2 the cumulative frequency will be the median for odd number of data and the mean of values against n/2+n/2+1th cumulative frequencies will be median for series containing even number of data.

Example:

Calculate the median of the following data obtained by counting the number of flowers on 19 plants.

Class (No. of flowers/Plant)	1	2	3	4	5
Frequency (No. of plants)	3	4	6	3	3

Calculation.

Class (No. of flowers/Plant)	*Frequency*	*Cumulative frequency*
1	3	3
2	4	7
3	6	13
4	3	16
5	3	19

$n = 19$ (odd number)

Median (Me) = Value of data against $\frac{n+1}{2}$ cumulative frequency

= Value against $\frac{19+1}{2}$ cumulative frequency

$= \frac{19+1}{2} = \frac{20}{20} = 10.$

Since cumulative frequency 10 is included in 13 which represents the class value 3, therefore, for cumulative frequency 10, the class value will be 3 which is the median.

Example:

Calculate the median for the following data recorded for height (in cm) of 80 plants.

Example. Calculate the median for the following data recorded for heights (in cm) of 80 plants.

Class (Plant height)	119	120	121	122	123	124	125
No. of plants	4	9	14	18	15	13	7

Solution:

Class [Plant height (in cm)]	*Frequency (No. of plants)*	*Cumulative frequency (Cf)*
119	4	4
120	9	13
121	14	27
122	18	45
123	15	60
124	13	73
125	7	80

$n = 80$ (even number)

$$\text{Median (Me)} = \frac{\text{Class values against } \frac{n}{2}\text{th} + \frac{n}{2}+1 \text{ th cumulative frequency}}{2}$$

$$= \frac{\frac{80}{2}\text{th} + \frac{80}{2} + 1 \text{ cumulative frequencies}}{2}$$

$$= \frac{\text{class values against } c.f.\ 40 \text{ and } 41}{2}$$

The class values for cumulative frequencies 40 and 41 are included in the class value of cumulative frequency 45 which is 122. Therefore, Median (Me) = 122+122/2 = 122.

(ii) For classified grouped date:

The median is determined in the following way:

(a) First, the cumulative frequency of all the classes are obtained from the given frequencies.

(b) Median class value is determined which is N/2th class.

(c) The n that class is ascertained whose cumulative frequency precedes that of median class (c.f).

(d) The median is calculated by the following formula.

$$\text{Median (Me)} = L + \frac{\frac{N+1}{2} - F}{f} \times i$$

$$= L + \frac{\frac{N}{2} - CF}{f} \times i$$

where L = lower limit of median class

N = sum of all the frequencies ($N = \Sigma f$)

cf = cumulative frequency of class preceding that of median class

f = frequency of median class

i = class interval of the median class (*i.e.*, upper limit – lower limit or $L_2 - L_1$).

Example:

The number of seeds produced by 55 plants of a plot are given in the following table.

Calculate the median seed number of a plant.

Class interval	40 – 50	50 – 60	60 – 70	70 – 80	80 – 90	90 – 100	100 – 110	110 – 120
No. of plants	5	9	9	15	8	4	3	2

Solution:

Class	*Frequency (f)*	*Cumulative frequency (c.f.)*
40 – 50	5	5
50 – 60	9	14
60 – 70	9	23 = *c.f. of the class preceding that of median class*
70 – 80	15 (f)	38
80 – 90	8	46
90 – 100	4	50
100 – 110	3	53
110 - 120	2	55
	$\Sigma f = 55$	

To find out median class we use formula $\frac{n}{2}$

So, Median class $= \frac{55}{2} = 27.5$

Now, the median class value falls within the cumulative frequency of class interval 70 – 80, therefore, the median class is 70 – 80.

The width or class interval (i) $= L_2 - L_1$

$= 80 - 70 = 10$

Now the median is calculated by the following formula

$$\text{Me} = L + \frac{\frac{n}{2} - cf}{f} \times i$$

$$= 70 + \frac{27.5 - 23}{15} \times 10$$

$$= 70 + \frac{4.5}{15} \times 10 = 70 + \frac{45}{15}$$

$$= 70 + 3 = 73.$$

While calculating the median for classified grouped data the following facts must be kept in mind:

(i) Class intervals must be equal for all classes. If not equal, they should be rearranged allowing equal interval as shown below:

(ii)

Class	10 – 15	15 – 17.5	17.5 – 20	20 – 30	30 – 35	35 – 40	40 – 50
Frequency	10	17	19	27	28	30	40

Because the class intervals are not equal in different classes, the above data are rearranged as follows:

Class	10 – 20	20 – 30	30 – 40	40 – 50
Frequency	46	27	58	40

(ii) The classes should be presented by exclusive method (for example 10 – 20,20 – 30, 30 – 40 – —and so on).

If the classes are presented in inclusive manner then they should be changed to exclusive one by subtracting 0.5 from the lower limit and adding 0.5 to the upper limit as exemplified below:

Inclusive presentation of classes:

50 – 54, 55 – 59, 60 – 64, 65 – 69, 70 – 74

↓ ↓ ↓ ↓ ↓

49.5 – 54.5, 54.5 – 59.5, 59.5 – 64.5, 64.5 – 69.5, 69.5 – 74.5 exclusive presentation.

Similarly, if the classes are 0 – 9, 10 – 19, then they will be changed to 0 – 9.5, 9.5 – 19.5.

Merits and Demerits of Median:

Merits of Median:

1. It is calculated easily and located exactly.

2. It is not affected by abnormally large or small values.

3. Its size cannot be changed much by adding a few more items.

4. Median can be used in quantitative measurements.

Demerits of Median:

1. The median of two or more series cannot be calculated by using the median of the component series.

2. It may not be represented in central data.

3. It cannot be used where weight-age is given to some items.

Probable Questions:

1. Define arithmetic mean. What are the merits and demerits of arithmetic mean?
2. What are the uses of arithmetic mean?
3. How geometric mean is calculated?
4. How harmonic mean is calculated?
5. Define mode? How it is calculated?
6. What are the merits and demerits of mode?
7. Define median.
8. How median is determined in unclassified and un-tabulated data?
9. How median is determined in grouped data?
10. What are the merits and demerits of median?

Suggested Readings:

1. Zar, J.H. (2013) Biostatistical Analysis
2. Pagano M., Gauvreau, K, (2000), Principles of Biostatistics.

UNIT-XVII

Measures of dispersion. Concept of Probability and significant test, Probability Distribution (Binomial, Poisson and normal)

Objective: In this unit we will discuss about different ways how can dispersion in statistical data be measured. We will also discuss different types of probability such as Binomial, Poisson and normal. In this unityou will learn about the measures of dispersion which is designed to state numerically the extent to which individual observation vary in the average.

Introduction:

A measure of dispersion **reflects how closely the data clusters around the measure of central tendency**. It represents the deviation of value of individual observations on either side of the central value in a set of data. To judge the reliability of measure of Central tendency.

The following points highlight the five types of measures of variability. The types are: 1. Range 2. Standard Deviation 3. Variance 4. Standard Error 5. Coefficient of Variation

1. Range:

Range is the difference between the lowest and the highest values present in the observations in a sample. It is the difference between the largest and smallest observation.

Range = Maximum value — Minimum value.

If there are 20 observations on seed oil content in cotton, the highest value being 25% and the lowest 15%. The range will be 25-15 = 10. Thus, it is a measure of the spread of variation in a sample.

It is the simplest possible measure of variability and its computation is very easy. However, it is very crude measure of variability. It is not capable of further algebraic treatment and cannot be defined rigidly. It is greatly affected by fluctuation of sampling. It does not indicate as to how the data behave in between the highest and the lowest value. It is commonly used as a measure of variability in plant breeding populations.

Mean Deviation:

Mean deviation is the arithmetic mean of absolute deviation from mean or any other specified value.

Mean deviation = $\sum fd/n = \sum f(x - \bar{x})/n$

x = specified value,

$\bar{x}$ = mean value,

f = frequency,

n = total number of observations

2. Variance:

Variance is defined as the average of the squared deviation from the mean or it is the square of the standard deviation. It is expressed as the sum of squares of the deviations of all observations of a sample from its mean and divided by degree of freedom (N-1). It is an effective measure of variability which permits partition of variation into various components.

It is estimated by the following formula:https://www.biologydiscussion.com/wp-content/uploads/2016/07/image-52.png

$$\text{Variance} = \sqrt{[\Sigma x^2 - \{(\Sigma x)^2/N\}/N - 1]}$$

where, $\sum$, x, x^2 and N = summation, an observation, square of an observation, and number of observations, respectively.

Variance depends on the deviations where the squared deviations are summed up and then divided by the number of observations to get the sample variance. It has a distinct advantage over mean deviation as the squaring is done for the deviated values as a result all values become positive.

$$\textbf{Sample variance } (s^2) = \frac{\Sigma(x - \bar{x})^2}{n} = \frac{\Sigma d^2}{n}$$

where, $\bar{x}$ = Mean of the sample

n = Total no. of observation.

In case of grouped data, due to frequency distribution, the variance

$$s^2 = \frac{\sum f \cdot d^2}{\sum f} \quad .$$

Since the sample is the part of the population with only n number of items, to get the estimate of population variance the formula for sample variance is transformed to —

$$\textbf{Population Variance} = \sigma^2 = \frac{\sum d^2}{n-1} \quad \text{or} \quad \frac{\sum f \cdot d^2}{\sum f - 1}$$

No. of Pods (x)	Frequency (f)	Deviation (|d|)	Deviation² (d²)	f ×d²
15	1	14	196	196
16	2	13	169	338
17	2	12	144	288
18	2	11	121	242
19	2	10	100	200
20	2	9	81	162
21	2	8	64	128
22	3	7	49	147
23	3	6	36	108
24	3	5	25	75
25	4	4	16	64
26	5	3	9	45
27	6	2	4	24
28	7	1	1	7
29	9	0	0	0
30	7	1	1	7
31	5	2	4	20
32	6	3	9	54
33	6	4	16	96
34	5	5	25	125
35	4	6	36	144
36	3	7	49	147
37	3	8	64	192
38	3	9	81	243
39	2	10	100	200
40	2	11	121	242
41	1	12	144	144
	$\Sigma f = 100$			$\Sigma fd^2 = 3,638$

$\bar{x} = 28.9$ or 29; $\Sigma fd^2 = 3,638$

$\Sigma f = 100$; variance, $s^2 = \frac{\Sigma fd^2}{\Sigma f} = \frac{3,638}{100} = 36.38$

Significance:

Variance is a quantitative mathematical expression representing squared units.

It has two major difficulties:

1. When the deviation and number of observation are more, then variance becomes a large number which is difficult to be expressed numerically.

2. The unit in which the variance is expressed is not in the same unit of the observation, such as, if the observations are made in cm, then the variance is expressed in sq. cm.

3. Standard Deviation:

It is the square root of the arithmetic mean of the squares of the deviations measured from the mean. In other words, it is a square root of the variance. It is the best measure of variation in a population. Thus,

$$\text{Standard Deviation} = \sqrt{[\Sigma x^2 - \{(\Sigma x)^2 N\} / N - 1]}$$

or $$\text{Standard Deviation} = \sqrt{\text{variance}}$$

Standard deviation is based on all the observations of a sample and is capable of further algebraic treatment. It is rigidly defined and is less affected by fluctuation of sampling. Its value is always definite. However, it gives more weight to extreme items and less to those which are near to the mean.

Standard deviation expressed as percentage of the mean and is denoted by the formula:

$$CV = \frac{\sigma}{\bar{x}} \times 100.$$

This is most useful method of measurement of dispersion of a series where the values deviated from mean are squared and summed up and then expressed as square root of the summed up value divided by no. of observations. Thus the standard deviation is defined as the square root of the variance.

$$\text{Sample Standard Deviation or } s = \sqrt{s^2} \text{ or } \sqrt{\frac{\Sigma d^2}{n}} \text{ or } \sqrt{\frac{\Sigma f \cdot d^2}{\Sigma f}}$$

where, d = deviation from mean

n = total no. of observations

f = frequency of each class

When the population standard deviation is estimated then the formula is:

$$\text{Population standard deviation or } \sigma = \sqrt{\frac{\Sigma d^2}{n-1}} \text{ or } \sqrt{\frac{\Sigma f \cdot d^2}{\Sigma f - 1}}$$

Why n – 1 is used to calculate the standard deviation when sample size is small?

When the sample size is large enough (e.g., 1,000) to reach the population size, i.e., if 'n' approaches towards V (n = sample size, v = population size), then the value of variance will show negligible difference whether divided by n or n – 1. But when the sample size is small, e.g. 30, then the value of population variance should be obtained by dividing with n – 1, which gives more appropriate estimate of population variance.

n – 1 denotes number of degrees of freedom, i.e., number of comparisons that can be made between any one observation and the rest number of observations taking them in pairs.

Computation of standard deviation needs 7 steps:

Step I : Mid-point calculation of each class (m).

Step II : Calculation of mean of total observations $(\bar{x})$.

Step III : Deviation from mean of each mid value of each class $d = (\bar{x} - m)$.

Step IV : Squaring of each deviation (d^2).

Step V : Multiplication of squared deviation with frequency $(f \cdot d^2)$.

Step VI : Summation of all $f \cdot d^2$ and then division by $(\Sigma f - 1)$.

Step VII : Square root of the total value, $\sqrt{\frac{\Sigma fd^2}{\Sigma f - 1}}$.

Variance (s^2) calculated previously in this example is 36.38.

$\therefore$ Standard deviation or $s = \sqrt{36.38} = 6.03$

No. of Pods/Plant	Mid point (m)	No. of Plants (f)	Deviation (|d|)	d^2	$f \cdot d^2$
15-17	16	5	13	169	845
18-20	19	6	10	100	600
21-23	22	8	7	49	392
24-26	25	12	4	16	192
27-29	28	22	1	1	22
30-32	31	18	2	4	72
33-35	34	15	5	25	375
36-38	37	9	8	64	576
39-41	40	5	11	121	605
		$\Sigma f = 100$			$\Sigma fd^2 = 3,679$

From previous calculation the data shows the mean $(\bar{x}) = 29$.

Deviation is calculated using the formula $d = \bar{x} - m$

Standard deviation or $s = \sqrt{\frac{3,679}{100}} = \sqrt{36.79} = 6.06$.

Merits and Demerits of Standard Deviation:

Merits:

1. The calculation is based on all observations.

2. It is more rigidly defined.

3. Less affected by fluctuations of sampling compared to other measures of dispersion.

4. It summarizes the deviation of large number of observations from mean and is expressed as one unit of variation.

Demerits:

1. It requires a lengthy calculation, i.e., squaring of deviations and then again square root of summed up values.

2. Not very simple to understand.

3. The calculation gives more weightage to extreme values.

Uses of Standard Deviation:

1. It helps in correlating and comparing of different samples.

2. It helps in finding the suitable size of sample for valid conclusion.

3. It helps in finding the standard error which determines whether

the difference between means of two similar samples by chance or real.

4. The value of mean and standard deviation help to comment on the population on the

basis of observation of sample (Fig. 10.3).

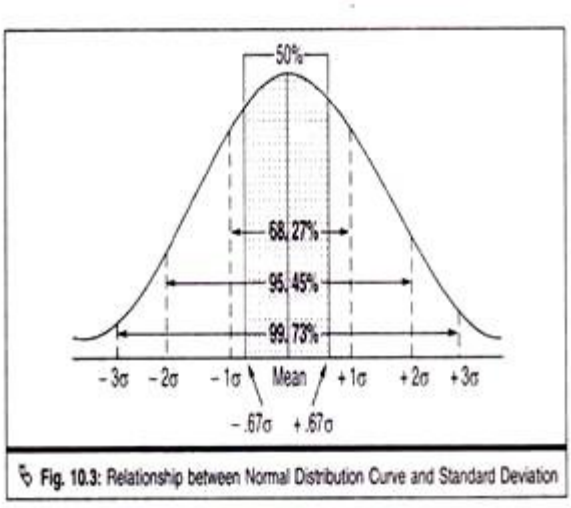


Fig. 10.3: Relationship between Normal Distribution Curve and Standard Deviation

(a) 50% of total observations lie in an area bounded by a distance of 0.6745 σ on both side of the mean.

(b) Mean ± 1σ covers the 68.27% area of the curve.

(c) Mean ± 2σ covers the 95.45% area of the curve.

(d) Mean ± 3a covers the 99.73% area of the curve.

4. Standard Error:

It is the measure of the mean difference between sample estimate of mean (X) and the population parameter (μ), i.e., it is the measure of uncontrolled variation present in a sample. It is the measure of the variation of the means. From standard error we can analyze how the sample mean ($\bar{x}$) is related to the mean of the population (μ) and is given by the formulaSE = σ/√n. It is estimated by dividing the estimates of standard deviation by the square root of number of observations in the sample, and is denoted by SE. Thus,

$$SE = SD/\sqrt{N}$$

where, SD = standard deviation and N = number of observations.

5. Coefficient of Variation:

The standard deviation is an absolute measure of variation and is expressed in terms of the unit of the variable. For example, it would be in rupees for income, in cm for height and in kg or gm. for weight. For the purpose of comparative studies a relative measure of dispersion or variation is required.

Coefficient of variation serves this purpose as it does not have any unit. The ratio of standard deviation of a sample to its mean expressed in percentage is called coefficient of variation. Thus,

Coefficient of Variation (CV) = SD/x X100

This measure was evolved by Karl Pearson. It is very useful for the study of variation in more than one sample or series. A sample in which coefficient of variation is higher would have greater variation than the one in which it is lower. In other words, when the coefficient of variation is high the sample is less consistent or more variable and when it is low the sample is more consistent or less variable.

In plant breeding, phenotypic, genotypic and environmental coefficients of variation are estimated from the corresponding variances, and are used for the assessment of variability. Simple measures of variability can be worked out from both un-replicated and replicated data.

Solved Problem:

Find out mean, standard deviation, mean deviation, coefficient of variation and standard error from the given sample:

Class value (x)	*Frequency* (f)	fx	$(x - \bar{x}) = d$	fd	d^2	fd^2
48	8	384	−4.75	+38.00	22.56	180.50
50	32	1600	−2.75	+88.00	7.56	242.00
52	75	3900	−0.75	+56.25	0.56	42.18
54	52	2808	+1.25	+65.00	1.56	81.25
56	28	1568	+3.25	+91.00	10.56	295.75
58	5	290	+5.25	+26.25	27.56	137.81
	n = 200	Σfx = 10,550		Σfd = 364.50		979.49

$$\text{Mean } \bar{x} = \frac{\Sigma fx}{n} = \frac{10,550}{200} = \mathbf{52.75.}$$

$$\text{Mean deviation} = \frac{\Sigma fd}{n}$$

[Note: The positive signs exactly cancel negative sign of deviations and the summation of deviations gives a value of zero and, therefore, the spread of distribution cannot be shown. In calculation of mean deviation, the algebraic signs may be ignored.]

$$= \frac{364.50}{200} = 1.8225 = \mathbf{1.82.}$$

$$\text{Standard deviation } \sigma = \sqrt{\frac{\Sigma fd^2}{n-1}} = \sqrt{\frac{979.49}{200-1}}$$

$$= \sqrt{4.9221} = 2.2185 = \mathbf{2.22.}$$

$$\text{Coefficient of variation (CV)} = \frac{\sigma}{\bar{x}} \times 100 = \frac{2.22}{52.75} \times 100$$

$$= 4.2085 = \mathbf{4.21\%.}$$

$$\text{Standard error} = \frac{\sigma}{\sqrt{n}} = \frac{2.2}{\sqrt{200}} = 0.1556 = 0.16.$$

Therefore, mean is represented as $52.75 \pm \mathbf{0.16.}$

PROBABILITY

The measurement of relative chance of occurrence of an event from among a set of alternatives can be defined as probability. When in an experiment there are chances of occurrence of many events then the question of probability arises. Such as, if a coin is tossed then either 'head' or 'tail' will happen; if a dice is thrown then there are possibility of getting 1 or 2 or 3 … or 6.

Probability is a number expressed in a quantitative scale. When one event will not occur at all then the probability of that event is 0, and if there is any event which will happen positively without fail then the probability of that event is 1. But in biological science, mostly we find the probability of any event lies between impossibility to certainty i.e., the value ranges from 0 to 1.

Mathematically probability can be explained in the following way:

If an event can happen in 'a' number of ways, and fails to happen in 'b' number of ways, then the probability of its happening 'p' is written as.

$$p = \frac{a}{a+b} \quad \text{or} \quad p = \frac{\text{Number of events occurring}}{\text{Total number of events}}$$

Similarly **probability of failure** of any event is denoted as 'q', where,

$$q = \frac{b}{a+b} \quad \text{or} \quad q = \frac{\text{Number of events not happening}}{\text{Total number of events}}$$

$$\text{Therefore,} \quad p + q = \frac{a}{a+b} + \frac{b}{a+b} = 1$$

So, if the probability of happening any event is 0.7, then the probability of not happening of that event is 0.3.

Events:

The results of any experiment in all possible forms are said to be events of that experiment. Such as, throwing of a dice has 6 possible outcomes, either 1 or 2 or 3 or 4 or 5 or 6. All these six outcomes are called events of that single experiment.

Null Event:

When there is no chance of getting an event is called null or impossible event. It is symbolically denoted by ϕ. Such as, survival of any human being forever is an impossible event or null event.

Sure Event:

If the likelihood of occurrence of any event is sure then the event is called sure event. Such as, the death of a human being is a sure event.

Equally likely events:
If the likelihood of the occurrence of every event in an experiment is same then those are called as equally likely events. Such as, when a dice is thrown, there is no biasness, there are the possibilities of coming any number 1 to 6 in equal probability, so the events are equally likely events.

Mutually Exclusive Events:
If in an experiment the occurrence of one event prevents or rules out the happening of all other events, then these are called as mutually exclusive events. Such as, when a coin is tossed either 'head' or 'tail' will come.

The occurrence of one event affects the occurrence of another event, both events cannot occur together, i.e., occurrence of 'head' rules out getting 'tail' in the same trial. Here the events are connected by the words 'either' or 'or'.

Simple Event and Compound Event:
Any event having only one sample point of a sample space is called simple event and if any event is decomposable into a number of simple events then it is called as compound event.

Such as, if a bag contains 4 white and 6 red balls, and if one ball is drawn then it is simple event, but if two balls are drawn together then the events will be — 'both the balls are white', 'both the balls are red', 'one ball is white and another ball is red' — these are compound events.

The compound events may be of two types:

Independent Event:
Two or more events are said to be independent events when the outcome of one event does not affect or is not affected by the other events. For example, if a coin is tossed twice, the result of second tossing would in no way be affected by the result of first tossing, so these are independent events.

Dependent Event:
The occurrence or non-occurrence of one event in one trail affects the probabilities of other events in other trails are called dependent events. For example, the probability of drawing a queen from a pack of 52 cards is 4/52, but if the card drawn for the first time (queen) is not replaced then the probability of second drawing of a queen is 3/51, as the pack now contains 3 queens and 51 cards.

Addition and Multiplication Rules:

Probability is estimated usually on the basis of following two rules of chances:

1. Addition rule

2. Multiplication rule

Addition Rule:

This rule is applied when two events are mutually exclusive, i.e., both events cannot occur simultaneously. The birth of a male child excludes the birth of a female child in the same trial. The probability that one of several mutually exclusive events will occur is the sum of the probabilities of the individual events.

Mathematically, when two events A and B are mutually exclusive, the chance of occurrence or probability of occurrence of either A or B can be calculated from the following formula:

p (A or B) = p(A) + p(B)

This rule is applicable to any number of mutually exclusive events as follows:

$p(E_1 \text{ or } E_2 \text{ or } E_3 \ldots E_n) = p(E_1) + p(E_2) + p(E_3) + \ldots + p(E_n)$

Example 1:

If a dice is rolled, what is the probability of getting either 3 or 5?

Probability of getting 3 is p(3) = 1/6

Probability of getting 5 is p(5) = 1/6

∴ Probability of getting either 3 or 5 is p(3) + p(5) = 1/6 + 1/6 = 1/3

Example 2:

What is the probability of getting a king or a joker from a pack of 54 cards?

Probability of getting a king is p(K) = 4/54 = 2/27

Probability of getting a joker is P(J) = 2/54 = 1/27

So, the probability of either a king or a joker is

p(K or J) = p(K) + p(J) = 2/27 +1/27 = 3/27 = 1/9

Addition rule changes when the events are not mutually exclusive, i.e., if two events A and B can occur simultaneously in few cases, then the rule becomes modified in the following way:

p(A or B) = p(A) + p(B) – p(A and B)

Example 3:
What is the probability of getting a king or club from a pack of 52 cards?

In this example, getting a king and a club are not mutually exclusive events as there will be one king which is king of club. So the chance or probability of getting that event should be subtracted.

p(King or Club) = p(King) + p(Club) – p(King and Club)

Probability of King = p(King) = 4/52 = 1/13

Probability of Club = p(Club) = 13/52 = 1/4

Probability of King and Club = p (King and Club) = 1/52

So, p(King or Club) = (1/13 + 1/4) – 1/52 = 4/13

Multiplication Rule:

(a) When the Events are Independent:
Probability of two or more independent events occurring together is the product of the probabilities of individual events.

Symbolically, if p(A) and p(B) are the probabilities of two respective events A and B, and the happening of these two events are independent then the probability of happening both the events together can be calculated with the following formula:
p(A and B) = p(A) x p(B)

Thus the rule may be extended to any number of independent events like E_1, E_2, E_3... E_n, and the formula will be as follows:
$p(E_1 \text{ and } E_2 \text{ and } E_3 \ldots \text{and } E_n) = p(E_1) \times p(E_2) \times p(E_3) \times \ldots \times p(E_n)$

Example 4:
If two dice are thrown simultaneously what is the probability of getting 3 in both the dice?

The probability of getting 3 in first dice is p(A) = 1/6

The probability of getting 3 in 2nd dice is p(B) = 1/6

So, the probability of getting 3 in both the dice is

p(A and B) = p(A) × p (B)= 1/6 × 1/6 = 1/36

(b) When the Events are Dependent:
When the probability of happening one event is affected by the occurrence of another event then it is called conditional probability. Such as, conditional probability of happening A, when B has already happened, is denoted as p(A/B); conditional probability of B, and A has already happened, is denoted as p(B/A).

When the two events A and B are occurring simultaneously but any one event has conditional probability then the multiplication rule will be written as:
p(ab) = p(A)p(B/A) or p(B)-p(A/B)

where p(A/B) = Conditional probability of A given that B has happened

p(B/A) = Conditional probability of B given that A has happened

Example 5:
Four cards are drawn consecutively four times from a pack of 52 cards. Find the chances of drawing an ace, a king, a queen and a jack. The cards are not replaced after each withdrawal.

Probability of drawing an ace = p(A) = 4/52

Probability of drawing a king = p(K) = 4/51

Probability of drawing a queen = p(Q) = 4/50

Probability of drawing a jack = p(J) = 4/49

So, the combined probability

p(A and K and Q and J) = p(A) x p(K) x p(Q) x p(J)

= 4/52 x 4/51 x 4/40 x 4/49 = 0.317

Example 6:
Four cards are drawn in four consecutive drawals from a pack of 52 cards without replacing the cards after each drawal. What is the probability of drawing a king in each drawal?

The probability of getting a king in 1st drawal = 4/52

The probability of getting a king in 2nd drawal = 3/51

The probability of getting a king in 3rd drawal = 2/50

The probability of getting a king in 4th drawal = 1/49

So, the combined probability of getting a king in 4 consecutive drawals is

4/52 x 3/51 x 2/50 x 1/49 = 1/270725

Binomial Distribution:

The binomial distribution is one of the most widely used probability distributions of random discrete variates. This process is one where an experiment can result in only one or two mutually exclusive outcome such as success or failure, male or female, dead or alive, etc.

For example, in case of birth of a child there are two possible happenings, the male or female. So, the probability of male child is p = -y and also the probability of female child is q = 1/2.

If the two deliveries of two ladies are considered then there are four possible outcomes.

1st lady	2nd lady	Probability
M	M	$= p \times p = p^2$
F	M	$= q \times p = pq$ } 2 pq
M	F	$= p \times q = pq$ } 2 pq
F	F	$= q \times q = q^2$

We can get this probability distribution through the binomial expansion.

$(p + q)^2 = p^2 + 2pq + q^2$

In case of three births, we may get 3 males, 3 females, 2 males one female, one male two females, etc., which we can get through expansion of $(p + q)^3 = p^3 + 3p^2q + 3pq^2 + q^3$.
Therefore for 'n' number of events, the expected result may be expressed as $(p + q)^n$

Significance in Genetics:

An understanding of the laws of probability is of great importance in genetics. Because it helps in:

(i) Forecasting the chance of obtaining certain results from a cross,

(ii) Elucidating the operation of genetic principles, and

(iii) Assessment of goodness of fit of phenotypic ratio in relation to particular genetic principles.

The principles of probability can be applied to obtain the expected phenotypic ratios from multiple hybrid crosses by avoiding the use of complicated checker boards. However, the actual ratios of offspring obtained in a cross, very rarely, exactly tally with the expected results calculated by the principles of probability.

Within certain limit this deviation may be attributed due to chance; but when the actual results deviate to a great extent, the factor other than chance is responsible. Considering the variability in biological materials, a probability of 0.05 is as significant.

Some problems on Probability:

Example 1:
What is the probability of obtaining head in a single toss of an unbiased coin?

Solution:
When a coin is tossed either head or tail will come but not both. As the events are mutually exclusive and the toss was made in an unbiased coin, the outcome is expected to be equal. Therefore, probability of head = ½.

Example 2:

If two coins are tossed un-biasedly, what is the probability of occurrence of (a) 2 heads, (b) 2 tails, (c) 1 head and 1 tail, (d) at least 1 head?

The outcome of the toss:

Event	*Favourable cases*	*No. of favourable cases*
Both heads	HH	1
1 head and 1 tail	HT, TH	2
2 tails	TT	1
1 head at least	HH, HT, TH	3

Therefore, P(both head) = 1/4 (*4 is the total number of events*)
P(1 H 1 T) = 2/4 = 1/2
P(2 T) = 1/4
P(at least 3 H) = 3/4

Probability of occurrence of either of the events will be:

$$\frac{1}{4}+\frac{1}{2}+\frac{1}{4}+\frac{3}{4} = \frac{1+2+1+3}{4}$$
$$= \frac{7}{4} = 1.75.$$

Example 3:
What will be the probability of getting both heads or both tails when 2 coins are tossed?

Solution:
Out of 4 outcomes, viz., HH, HT, TH, TT, which are mutually exclusive, number of favourable cases of HH is ¼ (4 events) and that of TT is ¼.

Therefore, probability of getting both heads or both tails will be

¼ + ¼ = ½

Example 4:

What is the probability of getting an ace or a joker from a pack of 54 cards?

Solution:
Considering that the events are mutually exclusive:
probability of an ace is 4/54 = 2/27

probability of joker is 2/54 = 1/27.

Therefore, the probability of getting either an ace or a joker is

2/27 + 1/27 = 3/27 = 1/9.

Example 5:
Find out the probability of occurrence of a spade or a king from 52 cards.

Solution:
Probability of occurrence of a spade is 13/52 = 1/4.

Probability of occurrence of a king is 4/52 = 1/13.

As one of the four king is a spade and is included in 13 spade cards, then the probability of getting this card is 1/52.

Therefore, the probability of getting a king or a spade is

¼ + 1/13 – 1/52 = 13 + 4 – 1/52 = 16/52 = 4/13.

Example 6:
A bag contains 6 black balls and 4 white balls. One ball is drawn. What is the probability that the ball is black?

Solution:
Balls are numbered serially as: Black-1, 2, 3, 4, 5, 6 and White-7, 8, 9, 10. Therefore, 10 outcomes as regards the number on the selected ball, because any of the 10 balls could be drawn. As the balls are assumed to be identical except in colour, any of the balls is as likely to appear as any other ball. Of the 10 possible outcomes (mutually exclusive), only 6 cases are favourable to the event black ball. Hence

P = 6/10 = 3/5.

Example 7:
If 2 balls are drawn one after another from a bag containing 3 white and 5 black balls, what is the probability that (i) the first ball is white and the second is black; (ii) one ball is white and the other is black?

Solution:
Serial number in the balls: White-1, 2, 3; Blacks-4, 5, 6, 7, 8. First ball is selected in 8 ways because any of the 8 balls can be drawn; while the 2nd ball may be drawn in 7 ways. Hence, the 2 balls may be drawn in 8 x 7 = 56 possible ways. Since the balls are identical in all respects except in colour, 56 possible ways are mutually exclusive to one another.

(i) First ball can only be white if any of the ball numbered 1, 2, 3 is drawn, i.e., in 3 ways. Second ball will be black if any of the ball numbered 4, 5, 6, 7, 8 is drawn, i.e., 5 ways. 'Hence the number of cases favourable to the event is 3 x 5 = 15. So,the probability (P) = 15/56.

(ii) Number of ways of drawing a white ball and a black ball in the order (white, black) is 15. Similarly, the number of ways of drawing a white ball and a black ball in the order (black, white) is 5 x 3 = 15. Hence, the number of cases favourable to the event one ball is white and the other black irrespective of the order in which they are drawn is 15 + 15 = 30. Therefore,P = 30/56 = 15/28.

2. Multiplication Theory:

The probability of occurrence of the event A as well as B is given by the product of (unconditional) probability of A and conditional probability of B, assuming that A has actually occurred.

Probability of (A and B) = Probability of A x Conditional probability of B, assuming A.

P(AB) = P(A). P(B/A).

This is also known as multiplication theorem.

Proof:
In a random experiment n mutually exclusive events are there among which x cases are favourable to an event A. So, the unconditional probability of A is

P(A) = x/n

of the x cases, let y cases be favourable to another event B also; i.e., the number of cases favourable to A as well as B is y. Hence by definition

P(AB) = y/r

Once A has occurred, the occurrence of B is limited to only y cases out of x (in which A occurs). So, the conditional probability of B, assuming that A has already occurred is

$$P\left(\frac{B}{A}\right) = \frac{y}{x}.$$

We find that

$$\frac{y}{n} = \frac{x}{n} \cdot \frac{y}{m}; \quad P(AB) = P(A) \cdot P(B/A).$$

When the events are independent (A and B), the probability of join occurrence is given by the product of their separate probabilities.

Thus,P(A and B) = p(A) x p(B).

Example 8:

What will be the probability of getting 2 tails when 2 coins are tossed independently?

Solution:
Probability of getting tail in 1st toss (event A) of the coin = ½

Probability of getting tail in 2nd toss (event B) of the coin = ½

Therefore, probability of getting tails with both coins is

P(A and B) = P(A) x P(B) = ½ x ½ = ¼ .

Example 9:
Two cards are drawn from a full pack of 52 cards.

Find out the probability that:
(i) Both are black cards;

(ii) One is a spade and the other is a club.

Solution:
The first card may be drawn in 52 ways and corresponding to each way of drawing the 1st card, the 2nd card may be drawn in 51 ways. Hence, the total number of cases, considering the order, is 52 x 51 = 2652.

(i) Since the 1st black card can be drawn in 26 ways (13 spades + 13 clubs), the number of cases favourable to two black cards is 26 x 25 = 650 (independent events)

P = 650/2652 = 25/102.

(ii) Number of cases favourable to the order spade-club is 13 x 13 = 169; similarly, the number of cases favourable to the order club-spade is also 169. Therefore, the number of cases favourable to one club and one spade is (169 + 169) =338.

P = 338/2652 = 13/102

Example 10:
What is the probability that all 4 children in a family have different birthdays?

Solution:
The 1st child may be born on any of 365 days of the year, 2nd also on any of the 365 days and similarly the 3rd and 4th child. Hence, the total number of possible ways in which the 4 children have birthdays is 365 x 365 x 365 x 365. These cases are mutually exclusive and as regards to the number of favourable cases out of these, it can happen that the 1st child may have any of the 365 days of the year as its birthday.

In order that the 2nd child has a birthday different from that of the 1st, it should have been born on any of the 364 remaining days of the year; similarly 3rd on any 363 and 4th on any 362 remaining days. Hence, the number of cases favourable to the event different birthdays is 365 x 364 x 363 x 362

P = 365 x 364 x 363 x 362/ 365 x 365 x 365 x 365 = 0.984.

Example 11:
Four persons A, B, C, D occupy sets in a row at random. What is the probability that A and B sit next to each other?

Solution:
Four persons arrange themselves in a row, without restriction in 4! = 1 x 2 x 3 x 4 = 24 ways. Considering A and B together, they can arrange themselves in3′ x 2 = (3 x 2 x 1) x 2 = 12 ways; because A may be to the left or to the right of B.

p = 12/24 = ½.

Example 12:
Four cards are in 4 drawals consecutively from a pack of 52 cards without replacing cards after drawal. What is the probability of drawing a queen in each drawal?

Solution:
Probability of a queen in 1st drawal =4/52

Probability of a queen in 2nd drawal = 3/51

Probability of a queen in 3rd drawal = 2/51

Probability of a queen in 4th drawal = 1/50

Therefore, the probability of getting a queen in each drawal is

$$= \frac{4}{52} \times \frac{3}{51} \times \frac{2}{51} \times \frac{1}{50} = \frac{1}{13} \times \frac{1}{17} \times \frac{2}{51} \times \frac{1}{50}$$
$$= \frac{3}{563550} = \frac{1}{187850}.$$

NORMAL DISTRIBUTION, BINOMIAL DISTRIBUTION & POISSON DISTRIBUTION

Normal Distribution or Gaussian Distribution or Bell Curve:

In probability theory, the normal distribution or Gaussian distribution is a very common continuous probability distribution. The normal distribution is sometimes informally called the bell curve.

The probability density of the normal distribution is:

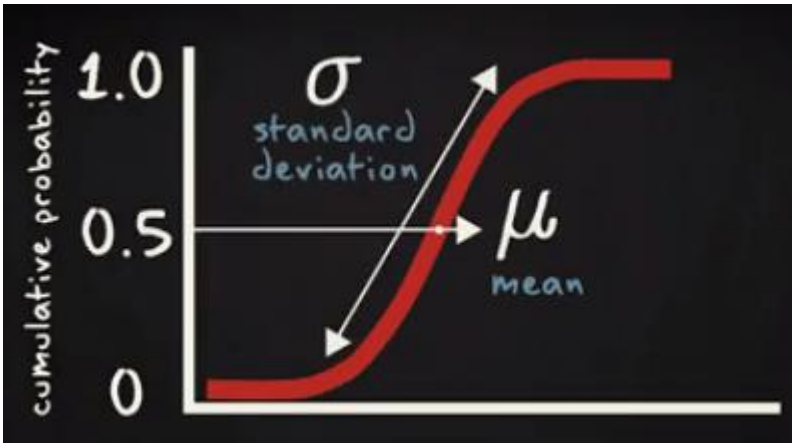
$$P(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-(x-\mu)^2/(2\sigma^2)}$$

μ is mean or expectation of the distribution is the variance

In short hand notation of normal distribution has given below.

$$X \sim N(\mu, \sigma^2)$$

Cumulative normal probability distribution will look like the below diagram.



Properties of a normal distribution:

- The mean, mode and median are all equal.
- The curve is symmetric at the center (i.e. around the mean, μ).
- Exactly half of the values are to the left of center and exactly half the values are to the right.
- The total area under the curve is 1.

Normal Distribution Probability Calculation:

Probability density function or p.d.f. specified the probability per unit of the random variable. Here is an example of a p.d.f. of the daily waiting time by the taxi driver of Uber taxi company. In the X axis, daily waiting time and Y-axis probability per hour has been shown.

If one Uber taxi driver want to know the probability to wait more than 7 hours in a day? Then he will be interested in the yellow surface arear shown above. On basis of this graph you can estimate the area. Same thing you can get form below cumulative probability curve.

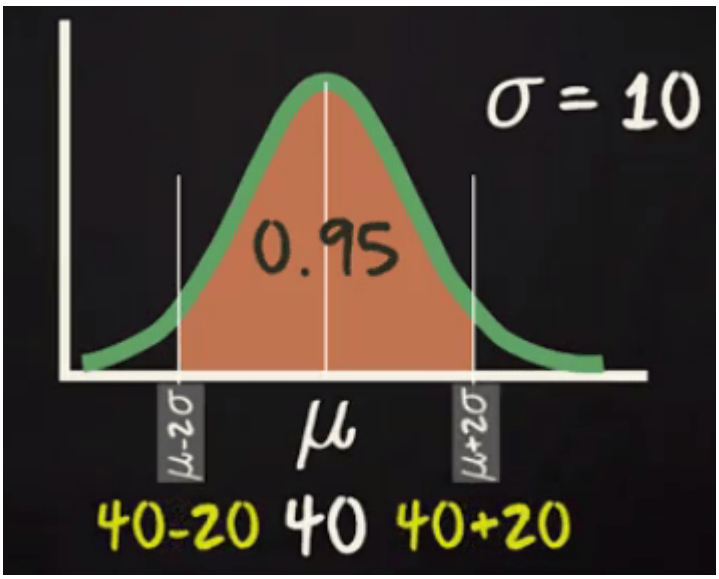
Probability to wait more than 7 hours will be calculated using complementary rule 1- P. Because corresponding to 7 in X axis we marked the probability is P and we are interested in more than 7 hours. So, P should be subtracted from 1 to get desired result.

Bell Shaped Distribution and Empirical Rule:

If distribution is bell shape then it is assumed that about 68% of the elements have a z-score between -1 and 1; about 95% have a z-score between -2 and 2; and about 99% have a z-score between -3 and 3.

Assume the time you spend in week days by travelling has given by a normal distribution with mean= 40 mins and SD= 10 mins.

What will be your range of travel time for 95 % of your week days?

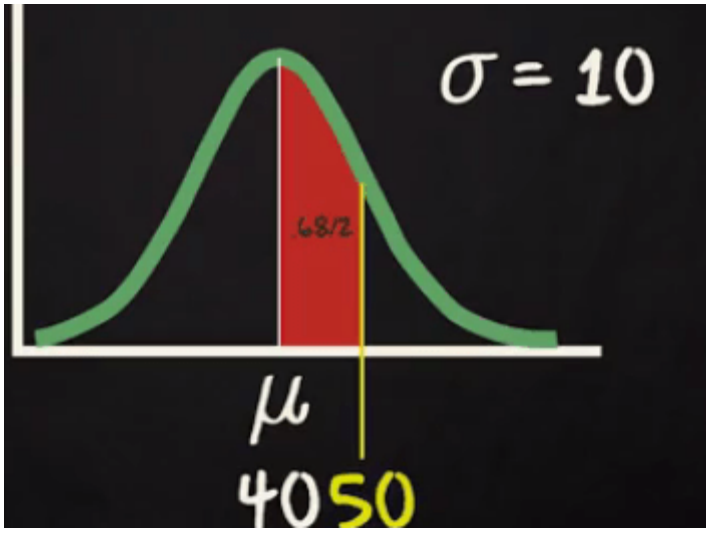


As you know 95 % will come within 2 standard deviation of your mean. So, the range will be (40-20) = 20 to (40+20) =60 mins.Now another question you want to answer that what will be the probability to be travelling more than 50 mins?

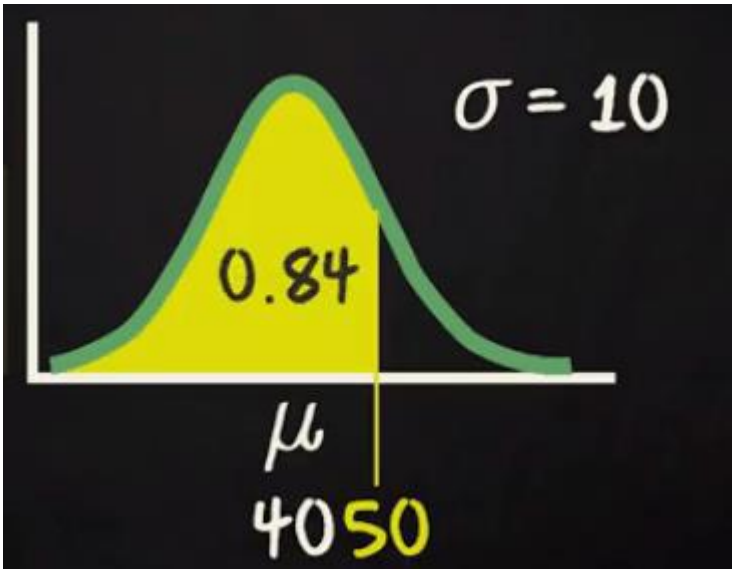
Actually you are interested in the yellow surface given in above diagram. You know that a normal distribution is symmetric. So, half of the probability located one side of the mean and another half located another side of the mean.

As SD =10. So, one standard deviation will be 30 to 50 range.

You already know for left side up 40 the probability is 0.5. Now if you calculate the probability from 40 to 50 range it will be half of 1 Standard deviation i.e. 0.68/2 = 0.34



So the probability to travel less than 50 mins = 0.5 +. 0.34 = 0.84



But you are interested in more than 50 mins traveling time so it will be 1- 0.84 =0.16

Bernoulli trial & Binomial Distribution:

Every random variable has a corresponding probability distribution. The probability distribution applies the theory of probability to describe the behavior of the random variable. A discrete random variable X has a finite number of possible integer values. The probability distribution of X lists the values and their probabilities in a table

Value of X	x_1	x_2	x_3	...	x_k
Probability	p_1	p_2	p_3	...	p_k

- Every probability pi is a number between 0 and 1.
- The sum of the probabilities must be 1.

This properties we have already studied before. Now we will discuss about the most important probability for discrete random variable is Binomial Distribution. Before that it is necessary to know about Bernoulli trial.

Bernoulli trial or Binomial Trial:

Bernoulli trial (or binomial trial) is a random experiment with exactly two possible outcomes, "success" and "failure", in which the probability of success is the same every time the experiment is conducted.

- The event (or trial) results in only one of two mutually exclusive outcomes – success/failure
- Probability of success is known, P(success) = π

Examples:

– A single coin toss (heads or tails), P(heads) = π = 0.5

– Survival of an individual after CABG surgery, P(survival) = π = 0.98

– Pick an individual from the Indian population, P(obese) = π = 0.31

Binomial Distribution:

A distribution is said to be binomial distribution if the following conditions are met.

1. Each trial has a binary outcome (One of the two outcomes is labeled a 'success')
2. The probability of success is known and constant over all trials
3. The number of trials is specified
4. The trials are independent. That is, the outcome from one trial doesn't affect the outcome of successive trials

If all the above conditions met then the binomial distribution describes the probability of X successes in n trials.

A classic example of the binomial distribution is the number of heads (X) in n coin tosses.

The Notation for a binomial distribution is

$X \sim B(n, \pi)$

which is read as 'X is distributed binomial with n trials and probability of success in one trial equal to π '.

Formula for Binomial Distribution:

Using this formula, the probability distribution of a binomial random variable X can be calculated if n and π are known.

n! is called 'n factorial' = n(n-1)(n-2) . . .(1)

P(X) = #of Scenario * Single Scenario

The first factorial terms gives the number of scenario and the second term describes the probability of success to power of number of successes and probability of failure to the power of number of failures.

Example:

What is the probability of 2 heads in 6 coin tosses?

- Success = 'heads'
- n = 6 trials
- π = 0.5
- X = number of heads in 6 tosses which is 2 here.

- X has a binomial distribution with n = 6 and π = 0.5
- X ~ B (6, 0.5)

$$P(X=2)=\frac{6!}{2!(6-2)!}0.5^2(1-0.5)^{6-2}=15*0.5^6=0.234$$

So, probability of getting 2 heads is 0.234.

Consider another example:

In a sample of 8 patients with a heart attack, what is the probability that 2 patients will die if the probability of death from a heart attack = 0.03.

Assume that the probability of death is the same for all patients.

– Death from heart attack is a binary variable (Yes or No)

– 'Success' in this case is defined as death from heart attack

– n = number of 'trials' = 8 patients

– π = 0.03 = probability of success

– X = number of deaths. X =2 here.

X ~ B (8, 0.03)

If you follow the same formula you will get P(x=2) = 0.021

Poisson Distribution:

Another probability distribution for discrete variables is the Poisson distribution. The Poisson distribution is used to determine the probability of the number of events occurring over a specified time or space. This was named for Simeon D. Poisson, 1781 – 1840, French mathematician.

Examples of events over space or time: -number of cells in a specified volume of fluid

-number of calls/hour to a help line

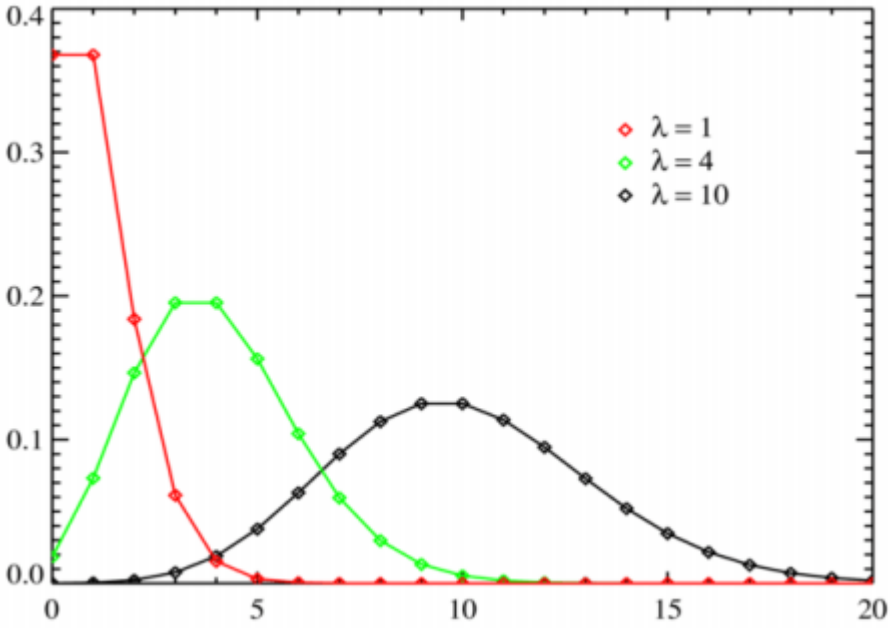
-number of emergency room beds filled/ 24 hours

Like the binomial distribution and the normal distribution, there are many Poisson distributions.

- Each Poisson distribution is specified by the average rate at which the event occurs.

- The rate is notated with λ
- λ = 'lambda', Greek letter 'L' – There is only one parameter for the Poisson distribution

The probability that there are exactly X occurrences in the specified space or time is equal to



The horizontal axis is the index X. The function is defined only at integer values of X. The connecting lines are only guides for the eye and do not indicate continuity. Notice that as λ increases the distribution begins to resemble a normal distribution.

- If λ is 10 or greater, the normal distribution is a reasonable approximation to the Poisson distribution
- The mean and variance for a Poisson distribution are the same and are both equal to λ
- The standard deviation of the Poisson distribution is the square root of λ

Example:

A large urban hospital has, on average, 80 emergency department admits every Monday. What is the probability that there will be more than 100?

If we put λ =80 and x= 100 then we will get the probability value as 0.01316885.

To get the same result we can use normal approximation and then get the probability value.

emergency room admits on a Monday?

- λ is the rate of admits / day on Monday = 80

- we can use the normal approximation since $\lambda > 10$

The normal approximation has mean = 80 and SD = 8.94 (the square root of 80 = 8.94)

Now we can use the same way we calculate p-value for normal distribution. If you do that you will get a value of 0.01263871 which is very near to 0.01316885 what we get directly form Poisson formula. Here main intention is to show you how normal approximation works for Poisson Distribution.

Probable Questions:

1. Define range? How it is calculated?
2. Define variance. How it is calculated?
3. State two difficulties of variance.
4. Describe different steps for calculation of standard deviation.
5. Describe merits and demerits of standard deviation. What are he uses of SD?
6. Differentiate equally likely events, null events, sure events and mutually exclusive events.
7. Describe the addition rule of probability by suitable example.
8. Describe the multiplication rule of probability by suitable example.
9. What is Poisson distribution? Explain it.
10. What is Batesian Distribution? Explain it.
11. What is Normal Distribution? Explain it.
12. What are the properties of normal distribution?

Suggested Readings:

1. Zar, J.H. (2013) Biostatistical Analysis
2. Pagano M., Gauvreau, K, (2000), Principles of Biostatistics.

UNIT-XVIII

Graphical representation of biological data: Box plot analysis, leaf and stem diagram

Objective: In this unit we will discuss about Graphical representation of biological data: Box plot analysis, leaf and stem diagram.

A. Box/Whisker plot analysis

A box and whisker plot, also called a box plot, displays the five-number summary of a set of data. The five-number summary is the minimum, first quartile, median, third quartile, and maximum. Box plots give a good graphical image of the concentration of the data. They also show how far the extreme values are from most of the data.

To construct a box plot, a horizontal or vertical number line and a rectangular box is used. The smallest and largest data values label the endpoints of the axis. The first quartile marks one end of the box and the third quartile marks the other end of the box. Approximately the middle 50 percent of the data fall inside the box. The "whiskers" extend from the ends of the box to the smallest and largest data values. The median or second quartile can be between the first and third quartiles, or it can be one, or the other, or both. The box plot gives a good, quick picture of the data.

In a box plot, a set of data, measured using an interval scale, is summarized. These are maximum used for data analysis. We use these types of graphs or graphical representation to know:

- Distribution Shape
- Central Value of it
- Variability of it

A box plot is a chart that shows data from a five-number summary including one of the measures of central tendency. It does not show the distribution in particular as much as a stem and leaf plot or histogram does. But it is primarily used to indicate a distribution is skewed or not and if there are potential unusual observations (also called outliers) present in the data set. Box plots are also very beneficial when large numbers of data sets are involved or compared.

In simple words, we can define the box plot in terms of descriptive statistics related concepts. That means box or whiskers plot is a method used for depicting groups of numerical data through their quartiles graphically. These may also have some lines extending from the boxes or whiskers which indicates the variability outside the lower and upper quartiles, hence the

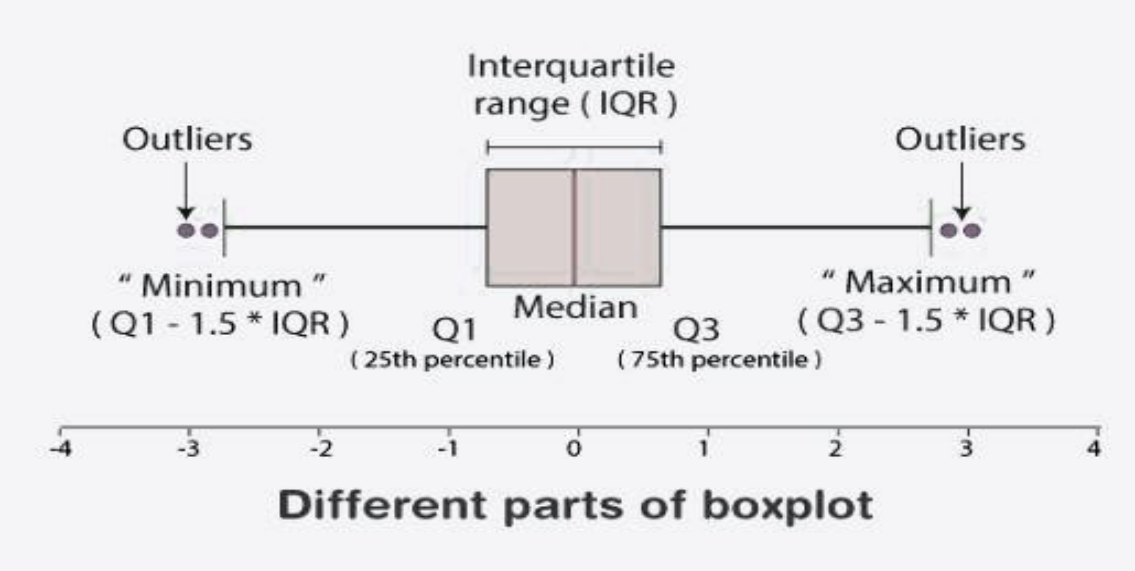
terms box-and-whisker plot and box-and-whisker diagram. Outliers can be indicated as individual points.

It helps to find out how much the data values vary or spread out with the help of graphs. As we need more information than just knowing the measures of central tendency, this is where the box plot helps. This also takes less space. It is also a type of pictorial representation of data.

Since, the centre, spread and overall range are immediately apparent, using these boxplots the distributions can be compared easily.

Parts of Box Plots

The image below shows the minimum, maximum, first quartile, third quartile, median and outliers.



Minimum: The minimum value in the given dataset

First Quartile (Q1): The first quartile is the median of the lower half of the data set.

Median: The median is the middle value of the dataset, which divides the given dataset into two equal parts. The median is considered as the second quartile.

Third Quartile (Q3): The third quartile is the median of the upper half of the data.

Maximum: The maximum value in the given dataset.

Apart from these five terms, the other terms used in the box plot are:

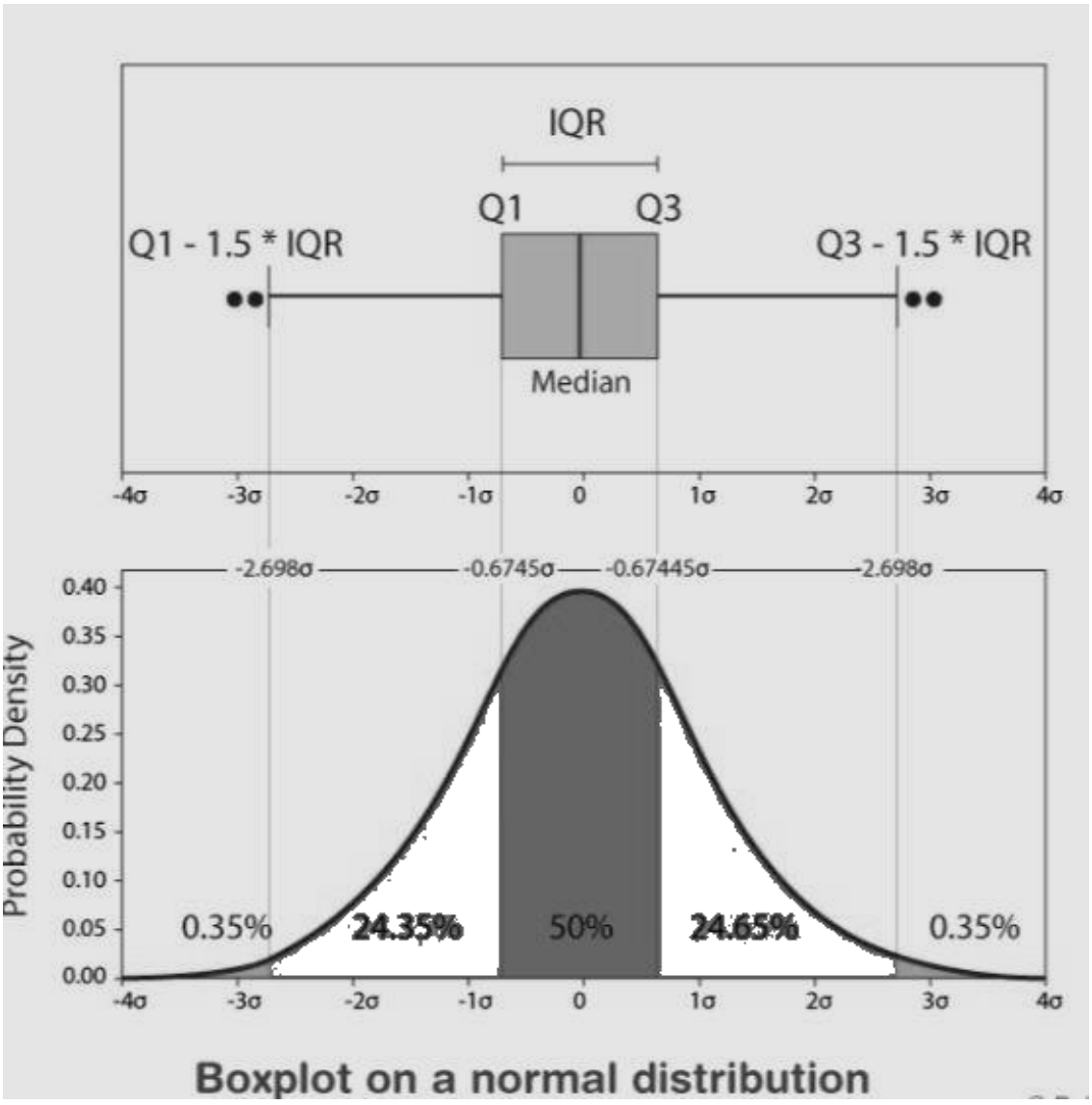
Interquartile Range (IQR): The difference between the third quartile and first quartile is known as the interquartile range. (i.e.) IQR = Q3-Q1

Outlier: The data that falls on the far left or right side of the ordered data is tested to be the outliers. Generally, the outliers fall more than the specified distance from the first and third quartile.

(i.e.) Outliers are greater than Q3+(1.5 . IQR) or less than Q1-(1.5 . IQR).

Box plot Distribution

The box plot distribution will explain how tightly the data is grouped, how the data is skewed, and also about the symmetry of data.



Positively Skewed: If the distance from the median to the maximum is greater than the distance from the median to the minimum, then the box plot is positively skewed.

Negatively Skewed: If the distance from the median to minimum is greater than the distance from the median to the maximum, then the box plot is negatively skewed.

Symmetric: The box plot is said to be symmetric if the median is equidistant from the maximum and minimum values.

Explanation:

- The ends of the box are the upper and lower quartiles so that the box crosses the interquartile range
- A vertical line inside the box marks the median
- The two lines outside the box are the whiskers extending to the highest and lowest observations.

Example: Finding the five-number summary

A sample of 10 boxes of raisins has these weights (in grams):
25, 28, 29, 29, 30, 34, 35, 35, 37, 38

Make a box plot from the provided data.

Solution:
Step 1: Order the data from smallest to largest.
The data is already in order.
25, 28, 29, 29, 30, 34, 35, 35, 37, 38

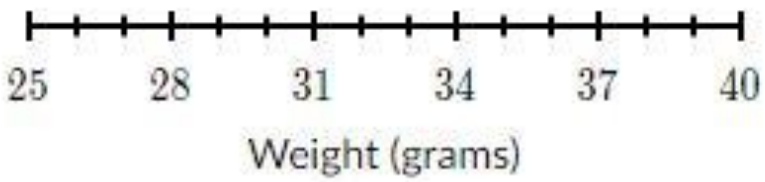
Step 2: Find the median.
The median is the mean of the middle two numbers:
25, 28, 29, 29, **30, 34**, 35, 35, 37, 38
(30+34)/2 = 32
The median is 32.

Step 3: Find the quartiles.
The first quartile is the median of the data points to the *left* of the median.
25, 28, **29**, 29, 30
Q1 = 29
The third quartile is the median of the data points to the *right* of the median.
34, 35, **35**, 37, 38
Q 3 = 35

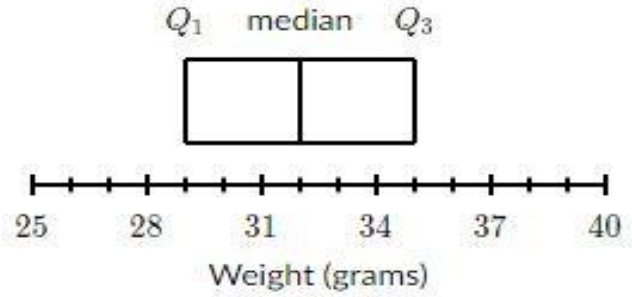
Step 4: Complete the five-number summary by finding the min and the max.
The min is the smallest data point, which is 25.
The max is the largest data point, which is 38.
The five-number summary is 255, 29, 32, 35, 38.

Example 2: Making a box plot
Let's make a box plot for the same dataset from above.

Step 1: Scale and label an axis that fits the five-number summary.

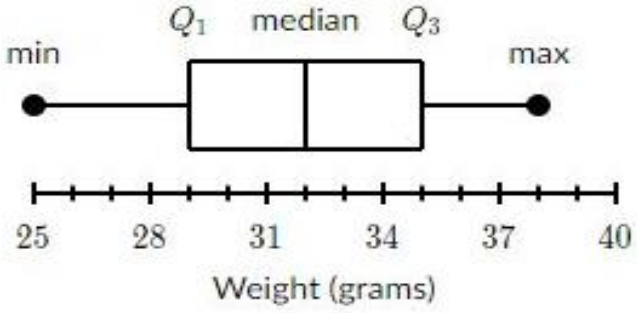


Step 2: Draw a box from Q1 to Q 3 with a vertical line through the median.
Recall that Q1 = 29, the median is 32, and Q 3 = 35.

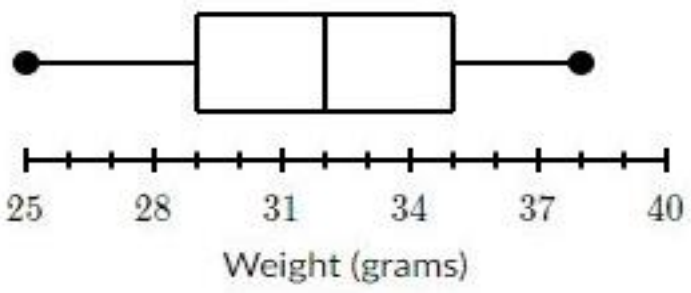


Step 3: Draw a whisker from Q1 to the min and from Q 3 to the max.

Recall that the min is 25 and the max is 38.



We don't need the labels on the final product

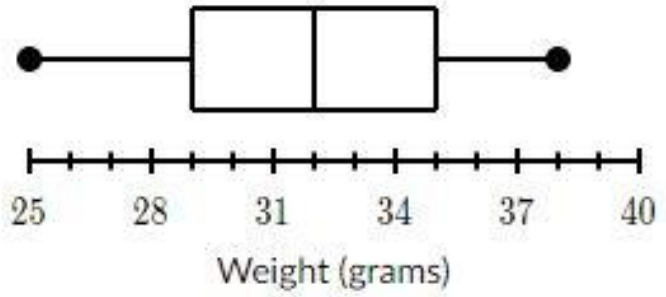


Interpreting quartiles:

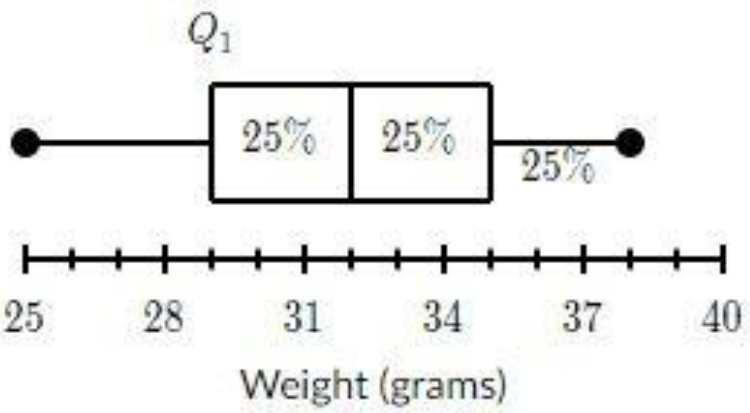
The five-number summary divides the data into sections that each contain approximately 25% of the data in that set.

Example: Interpreting quartiles

About what percent of the boxes of raisins weighed more than 29 grams?



Since Q 1=29, about 25% is lower than 29 and about 75% is above 29.



About 75 % of the boxes of raisins weighed more than 29 grams.

Stem and Leaf plot analysis :

A **stem-and-leaf display** is a way to organize data that, similar to a histogram, presents the data in a visual format. The distribution of the data can be seen with a glance using a stem-and-leaf display, in which each data value is separated into a *stem* and a *leaf*. Other names for this display include stem-and-leaf plot, stem-and-leaf diagram, and stem-and-leaf graph.

A stem and leaf plot is defined as a type of table where the stem (or first column) represents the first place values of numbers and the leaves (or second column) represent the final place value of the numbers. They evolved from Arthur Bowley's work in the early 1900s, and are useful tools in exploratory data analysis. Stem and leaf plots are one such way of representing data in an easier and convenient way. They are similar to a histogram, to assist in visualizing the shape of a distribution. Stem and leaf plots have several advantages that make them very handy for the purpose of analyzing large sets of data easily.

The stem and leaf graph is used to be able to easily see data points such as:

- Range: the maximum and minimum number in the data set
- Outliers: any numbers in the data set that is an abnormal distance from other data points
- Median: the central number when looking at the number chronologically
- Mode: the most common number
- Shape of data distribution: explains if most numbers are grouped around the center, lower range, higher range, or evenly distributed across the graph

How to draw a stem and leaf plot

- On the left hand side of the page, write down the thousands, hundreds or tens (all digits but the last one). These will be your stems.
- Draw a line to the right of these stems.
- On the other side of the line, write down the ones (the last digit of a number). These will be your leaves.

For example, if the observed value is 25, then the stem is 2 and the leaf is the 5. If the observed value is 369, then the stem is 36 and the leaf is 9. Where observations are accurate to one or more decimal places, such as 23.7, the stem is 23 and the leaf is 7. If the range of values is too great, the number 23.7 can be rounded up to 24 to limit the number of stems.

❖ Let us understand with an example:

Consider we have to make a Stem and Leaf plot for the data: 71, 43, 65, 76, 98, 82, 95, 83, 84, 96.

We'll use the tens digits as the stem values and the one's digits as the leaves. For better understanding, let's arrange the list in ascending order, but this is optional:

43, 65, 71, 76, 82, 83, 84, 95, 96, 98.

The stem and leaf plot should look like this:

Stem	Leaf
4	3
5	
6	5
7	1 6
8	2 3 4
9	5 6 8

Key 6 | 5 is equal to 65

We can use stem and leaf plots with decimals the same way we use for whole number. For example, stem and leaf plot key 4 | 2 is 4.2. 4 on the stem and 2 on the leaf read as 4.2. The decimal part will show on the leaf side.

- Rounding may be needed to create a stem-and-leaf display. Based on the following set of data, the stem plot below would be created:

−23.678758, −12.45, −3.4, 4.43, 5.5, 5.678, 16.87, 24.7, 56.8

For negative numbers, a negative is placed in front of the stem unit, which is still the value X / 10. Non-integers are rounded. This allowed the stem and leaf plot to retain its shape, even for more complicated data sets. As in this example below:

Stem	Leaf
−2	4
−1	2
−0	3
0	4 6 6
1	7
2	5
3	
4	
5	7

Key: −2 | 4 = −24

Usage

- Stem-and-leaf displays are useful for displaying the relative density and shape of the data, giving the reader a quick overview of the distribution.
- Useful for highlighting outliers and finding the mode.
- Stem and leaf plots have several uses in real-life situations where one needs to present a set of data graphically. These plots are useful to display a large amount of data easily like, marks of students, rainfall over a period of time, surveys of people's age in the city, heights and weights of a large number of people in a given area, temperature changes over a period and speed of wind over a set no of weeks in an area etc.

Limitations

- Stem and leaf displays are only useful for moderately sized data sets (around 15–150 data points).
- With very small data sets a stem-and-leaf displays can be of little use, as a reasonable number of data points are required to establish definitive distribution properties

- With very large data sets, a stem-and-leaf display will become very cluttered, since each data point must be represented numerically. A box plot or histogram may become more appropriate as the data size increases.

Possible Questions:

1. Describe different parts of a box plot.
2. What do you mean by positive skew and negative skew?
3. A sample of 10 boxes of raisins has these weights (in grams):
   25, 28, 29, 29, 30, 34, 35, 35, 37, 38. Make a box plot from the provided data.
4. What is stem and leaf plot analysis?
5. What are the usage of stem and leaf plot analysis?
6. What are the limitations of stem and leaf plot analysis?

Suggested Readings:

1.Zar, J.H. (2013) Biostatistical Analysis
2.Pagano M., Gauvreau, K, (2000), Principles of Biostatistics.

UNIT-XIX

Test of Hypothesis, Student's t-test and z-test and their application. Analysis of Variance (ANOVA)

Objective:I this unit we will discuss we will discuss Test of Hypothesis, Student's t-test and z-test and their application and also analysis of Variance (ANOVA).

Student's T-Test or T-Test:

It is one of the simplest tests used for drawing conclusions or interpretations for small samples. This test was worked out by W.S. Gosset (pen name **"Student"**), f-test is used to test the significance of means of two samples drawn from a population, as well as the significance of difference between the mean of small sample and hypothetical mean of population (expressed in terms of standard error).

(I) Application of t-test for assessing the significance of difference between the sample mean and population mean:

The computation of t-value involves the following steps:

(i) Null Hypothesis:

First of all, it is presumed that there is no difference between the mean of small sample and the population means (μ) or hypothetical mean. Thus,

Null hypothesis (Ho): sample mean ($\bar{X}$) = population mean (μ) or Ho = $\bar{X}$ = μ

(ii) Test statistics:

T-value is calculated by the following formula:

$$t = \frac{(\bar{X} - \mu)}{S/\sqrt{n}}$$

where, $\bar{X}$ = Sample mean, μ = population mean

n = number of units in the sample

s = standard deviation of sample

For small samples

$$s = \sqrt{\frac{1}{n-1}\sum(X - \bar{X})^2} \quad \text{or} \quad \sqrt{\frac{1}{n-1}\sum d^2}$$

(iii) Degree of freedom:

It is one less than the number of units in the sample.

Degree of freedom (d.f. or v) = No. of units in the sample – 1

= n – 1

(iv) Level of significance:

Any level of significance can be considered to test the hypothesis but generally 1 % (=0.01) or 5% (= 0.05) levels of probability is considered for testing the hypothesis.

(v) Table value of t:

After calculating the value of (= t – cal) with the help of above formula, the value of t is noted from Fishers and Yates Mable at given degree of freedom and 5% level of significance. Then after the calculated value of t is compared with the table value of t.

Table 1. STUDENT'S *t*-DISTRIBUTION

Degrees of freedom	Value of P					
	0.10	*0.05*	*0.02*	*0.01*	*0.002*	*0.001*
1.	6.314	12.71	31.82	63.66	318.3	636.6
2.	2.920	4.303	6.965	9.925	22.33	31.60
3.	2.353	3.182	4.541	5.841	10.21	12.92
4.	2.132	2.776	3.747	4.604	7.173	8.610
5.	2.015	2.571	3.365	4.032	5.893	6.869
6.	1.943	2.447	3.143	3.707	5.208	5.959
7.	1.895	2.365	2.998	3.499	4.785	5.408
8.	1.860	2.306	2.896	3.355	4.501	5.041
9.	1.833	2.262	2.821	3.250	4.297	4.781
10.	1.812	2.228	2.764	3.169	4.144	4.587
11.	1.796	2.201	2.718	3.106	4.025	4.437
12.	1.782	2.179	2.681	3.055	3.930	4.318
13.	1.771	2.160	2.650	3.012	3.852	4.221
14.	1.761	2.145	2.624	2.977	3.787	4.140
15.	1.753	2.131	2.602	2.947	3.733	4.073
16.	1.746	2.120	2.583	2.921	3.686	4.015
17.	1.740	2.110	2.567	2.898	3.646	3.965
18.	1.734	2.101	2.552	2.878	3.610	3.922
19.	1.729	2.093	2.539	2.861	3.579	3.883
20.	1.725	2.086	2.528	2.845	3.552	3.850
21.	1.721	2.080	2.518	2.831	3.527	3.819
22.	1.717	2.074	2.508	2.819	3.505	3.792
23.	1.714	2.069	2.500	2.807	3.485	3.767
24.	1.711	2.064	2.492	2.797	3.467	3.745
25.	1.708	2.060	2.485	2.787	3.450	3.725
26.	1.706	2.056	2.479	2.779	3.435	3.707
27.	1.703	2.052	2.473	2.771	3.421	3.690
28.	1.701	2.048	2.467	2.763	3.408	3.674
29.	1.699	2.045	2.462	2.756	3.396	3.659
30.	1.697	2.042	2.457	2.750	3.385	3.646

(vi) Test criterion: If the calculated value of t (t-cal) irrespective of the positive (+) or negative sign (-) is less than the tabulated value off at respective degree of freedom and at 5% or 0.05 level of probability, then the Null hypothesis is correct, i.e., difference between the sample mean and population mean is insignificant and so the hypothesis is

acceptable.But, if the calculated value of t (- cal) is greater than the tabulated value of t (t-tab) at given degree of freedom and at 5% level of probability, then the observed difference is considered statistically non-significant and so the null hypothesis is incorrect and rejected.

In such a ease the observed data are not according to the expected data or in other words, the sample under test does not represent the population with μ as its mean.

(II) T-test for assessing the significance of the difference between the means of two samples drawn from the same population:

t- test is also applied to test the significance of the difference between the arithmetic means off two samples drawn from the same population.

The procedure of the test is as follows:

(i) Null hypothesis:

In this, first of all it is presumed that there is no difference in the standard deviations of the two samples under test, i.e.

HO = $\mu_1 = \mu_2$

where μ_1 and $\mu_2$are the standard deviations of the sample I and sample II respectively.

(ii) Test statistics:

Next, the value of t is calculated by the following formula:

$$t = \frac{(\bar{X}_1 - \bar{X}_2) - (\mu_1 - \mu_2)}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

where, $\bar{X}_1$ = arithmetic mean of sample I

$\bar{X}_2$ = arithmetic mean of sample II

n_1 = No. of observations in sample I

n_2 = No. of observations in sample II

S_1 = Standard deviation of sample I

S_2 = Standard deviation of sample II

Since according to hypothesis $\mu_1 = \mu_2$

$$\text{therefore, } t = \frac{X_1 - X_2}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} = \frac{\text{difference between the sample means}}{\text{standard error of difference between the means}}$$

$$S = \frac{\sqrt{n_1 S_1^2 - n_2 S_2^2}}{n_1 + n_2 - 2}$$

$$\text{or } \frac{\sqrt{\sum(X_1 - \bar{X}_1) + \sum(X_2 - \bar{X}_2)}}{n_1 + n_2 - 2}$$

(iii) Degree of freedom:

(d.f.) = $n_1 + n_2 - 2$)

(iv) Level of significance:

The level of significance is generally considered at 5% (0.05) level «f probability.

(v) Tabulated value of t:

Value of t is recorded from the Fisher and Yates table at the given degree of freedom and at 5% level of significance.

(vi) Test criterion:

At last, the calculated value of t (|t|- cal) is compared with the table value of t at the given degree of freedom and 5% level of significance.

(a) If the calculated value of r-exceeds the table value, the observed difference is considered statistically significant and hence null hypothesis is rejected.

(b) If the calculated value of (|t|- cal) is less than the table value of t, the differences are not significant. Therefore, Ho hypothesis is accepted and the samples represent the population well.

Example:

Two varieties of maize (variety X and Y) were collected from four different localities. They showed differences in yield in Kg/hectare as mentioned in Table 2.2. With the help of t-test, find out whether yield of variety Y is significantly superior to the yield of variety X.

Table 2.2 Data showing yield of maize varieties, their yield differences and square of differences:

Locality	Maize yield in Kg/hectare		Differences	Square of differences
	Variety x	Variety Y		
1	780	800	20	400
2	800	810	10	100
3	750	770	20	400
4	825	855	30	900
		Sum =	80	1800

Calculations:

The details of Table 2.2 may be calculated as under:

Mean differences = Sum of differences/Number of trials (n) = 80/4 = 20

$$\text{Standard error of differences} = \sqrt{\frac{\text{Sum of square of differences}}{(n-1)n} - \frac{(\text{Sum of differences})^2}{n}}$$

$$= \sqrt{\frac{1800}{(4-1)4} - \frac{(80)^2}{4}}$$

$$= \sqrt{\frac{1800}{(3)4} - \frac{(6400)}{4}}$$

$$= \sqrt{\frac{1800 - 1600}{12}}$$

$$= \sqrt{\frac{200}{12}}$$

$$= \sqrt{16.66}$$

$$= \pm 4.07$$

In case of some measurements, the average is mentioned as ± standard error.

Therefore t = 20/4.07 = 4.93

The t-value is not true to the expectations, if it is more than 6.

Fisher's Z-Test or Z-Test:

Z-test is based on the normal probability distribution and is used for testing the significance of several measures. The relevant test statistics is worked out and compared with its probable value (to be read; table showing the area under normal curve) at a given level of significance in order to judge the significance of measures concerned. Z-test is generally used to compare the mean of large sample; hypothetical mean for population.

A ***Z*-test** is any statistical test for which the distribution of the test statistic under the null hypothesis can be approximated by a normal distribution. Z-tests test the mean of a distribution. For each significance level in the confidence interval, the *Z*-test has a single critical value (for example, 1.96 for 5% two tailed) which makes it more convenient than the Student's *t*-test whose critical values are defined by the sample size (through the corresponding degrees of freedom). Both the Z test and Student's t-test have similarities in that they both help determine the significance of a set of data. However, the z-test is rarely used in practice because the population deviation is difficult to determine.

Applicability:

Because of the central limit theorem, many test statistics are approximately normally distributed for large samples. Therefore, many statistical tests can be conveniently performed as approximate *Z*-tests if the sample size is large or the population variance is known. If the population variance is unknown (and therefore has to be estimated from the sample itself) and the sample size is not large ($n < 30$), the Student's *t*-test may be more appropriate.

Procedure:

How to perform a Z test when T is a statistic that is approximately normally distributed under the null hypothesis is as follows:

First, estimate the expected value μ of T under the null hypothesis, and obtain an estimate s of the standard deviation of T.

Second, determine the properties of T : one tailed or two tailed.

For Null hypothesis $\boldsymbol{H_0}$: $\boldsymbol{\mu \geq \mu_0}$ vs alternative hypothesis $\boldsymbol{H_1}$: $\boldsymbol{\mu < \mu_0}$, it is lower/left-tailed (one tailed).

For Null hypothesis $\boldsymbol{H_0}$: $\boldsymbol{\mu \leq \mu_0}$ vs alternative hypothesis $\boldsymbol{H_1}$: $\boldsymbol{\mu > \mu_0}$, it is upper/right-tailed (one tailed).

For Null hypothesis $\boldsymbol{H_0}$: $\boldsymbol{\mu = \mu_0}$ vs alternative hypothesis $\boldsymbol{H_1}$: $\boldsymbol{\mu \neq \mu_0}$, it is two-tailed.

Third, calculate the standard score:

$$Z= (X -\mu 0) / \sigma$$

which one-tailed and two-tailed *p*-values can be calculated as $\Phi(Z)$(for lower/left-tailed tests), $\Phi(-Z)$ (for upper/right-tailed tests) and $2\Phi(-|Z|)$ (for two-tailed tests) where Φ is the standard normal cumulative distribution function.

Conditions:

For the *Z*-test to be applicable, certain conditions must be met.

Nuisance parameters should be known, or estimated with high accuracy (an example of a nuisance parameter would be the standard deviation in a one-sample location test). *Z*-tests focus on a single parameter, and treat all other unknown parameters as being fixed at their true values. In practice, due to Slutsky's theorem, "plugging in" consistent estimates of nuisance parameters can be justified. However if the sample size is not large enough for these estimates to be reasonably accurate, the *Z*-test may not perform well.

The test statistic should follow a normal distribution. Generally, one appeals to the central limit theorem to justify assuming that a test statistic varies normally. There is a great deal of statistical research on the question of when a test statistic varies approximately normally. If the variation of the test statistic is strongly non-normal, a *Z*-test should not be used.

If estimates of nuisance parameters are plugged in as discussed above, it is important to use estimates appropriate for the way the data were sampled. In the special case of *Z*-tests for the one or two sample location problem, the usual sample standard deviation is only appropriate if the data were collected as an independent sample.

In some situations, it is possible to devise a test that properly accounts for the variation in plug-in estimates of nuisance parameters. In the case of one and two sample location problems, a *t*-test does this.

Example

Suppose that in a particular geographic region, the mean and standard deviation of scores on a reading test are 100 points, and 12 points, respectively. Our interest is in the scores of 55 students in a particular school who received a mean score of 96. We can ask whether this mean score is significantly lower than the regional mean—that is, are the students in this school comparable to a simple random sample of 55 students from the region as a whole, or are their scores surprisingly low?

First calculate the standard error of the mean: $\mathbf{SE = \sigma/\sqrt{n} = 12 / \sqrt{55}}$

$\mathbf{= 12/7.42 = 1.62}$

where $\boldsymbol{\sigma}$is the population standard deviation.

Next calculate the *z*-score, which is the distance from the sample mean to the population mean in units of the standard error:

$$\mathbf{Z= (M-\mu) /SE = (96-100)/1.62 = -2.47}$$

In this example, we treat the population mean and variance as known, which would be appropriate if all students in the region were tested. When population parameters are unknown, a Student's t-test should be conducted instead.

The classroom mean score is 96, which is −2.47 standard error units from the population mean of 100. Looking up the *z*-score in a table of the standard normal distribution cumulative probability, we find that the probability of observing a standard normal value below −2.47 is approximately 0.5 − 0.4932 = 0.0068. This is the one-sided *p*-value for the null hypothesis that the 55 students are comparable to a simple random sample from the population of all test-takers. The two-sided *p*-value is approximately 0.014 (twice the one-sided *p*-value).

Another way of stating things is that with probability 1 − 0.014 = 0.986, a simple random sample of 55 students would have a mean test score within 4 units of the population mean. We could also say that with 98.6% confidence we reject the null hypothesis that the 55 test takers are comparable to a simple random sample from the population of test-takers.

The *Z*-test tells us that the 55 students of interest have an unusually low mean test score compared to most simple random samples of similar size from the population of test-takers. A deficiency of this analysis is that it does not consider whether the effect size of 4 points is meaningful. If instead of a classroom, we considered a subregion containing 900 students whose mean score was 99, nearly the same *z*-score and *p*-value would be observed. This shows that if the sample size is large enough, very small differences from the null value can be highly statistically significant. See statistical hypothesis testing for further discussion of this issue.

ANOVA

The classical technique for comparing the two sample means is based on student's 't' test, but a different statistical technique, analysis of variance, is required when analysis of data of three or more experimental or field samples are involved. For example, a plant physiologist investigating the effect of IBA on emergence of roots from detached leaves.

He used three different concentrations of IBA in five replicates. The aim of the investigator is to find out if there is any differential effect of IBA concentration on root emergence or not. The 't' test may be employed here to compare the means of treatment effects taking two at a time, i.e., comparison can be done between any two concentrations.

For three different concentrations (A, B, C) altogether three tests (A & B, B & C, A & C) are required and which will make the procedure unnecessary lengthy. If in an experiment 7 different effects are to be studied then 21 't'-tests will be required. Also each time 't'-test is concerned with Type-1 error, i.e., the probability of false rejection of null hypothesis may be much higher than it appears to be.

In order to get certain positive results with greater efficiency, a different test is applied involving many samples which is called analysis of variance (ANOVA). Though the name of the test is somewhat misleading as it does not test the variance, rather the test is aimed at comparing means. The analysis is directed to variations between the treatments and variations among the individuals of particular treatment.

F-Statistics:

The analysis of variance is based on F-statistics which is a ratio of variances. The ratio of variances due to treatments and variances due to random differences within the treatment is calculated and the estimate, known as the 'F', is then used for comparison.

To find out the variance ratio 'F', the deviations are dealt in two parts:

(a) Deviation due to treatment effect and

(b) Deviation due to random differences in the individual items within treatments.

For calculation of 'F' value, the deviations due to all observations, deviations due to treatments and deviations due to random differences within treatments are needed.

Steps to be followed for calculation of F-statistics:

1. The data should be arranged in tabular form showing the treatments in columns and replicates in rows.

		Treatments			
		1	2	3	
Replicates	1	x_{11}	x_{21}	x_{31}	$\sum x_{r1}$
• $\sum x_{c1}, \sum x_{c2}, \sum x_{c3}$	2	x_{12}	x_{22}	x_{32}	$\sum x_{r2}$
are the columnwise total	3	x_{13}	x_{23}	x_{33}	$\sum x_{r3}$
• $\sum x_{r1}, \sum x_{r2}, \sum x_{r3}, \sum x_{r4}$	4	x_{14}	x_{24}	x_{34}	$\sum x_{r4}$
are rowwise total.		$\sum x_{c1}$	$\sum x_{c2}$	$\sum x_{c3}$	

2. Column totals are summed up to get the grand total G.

$$G = \sum x_{c1} + \sum x_{c2} + \sum x_{c3}$$

3. The totals of the squares of each observations column wise are summed up to get the value of A.

$$x_{11}^2 + x_{12}^2 + x_{13}^2 + x_{14}^2 = a_1$$

$$x_{21}^2 + x_{22}^2 + x_{23}^2 + x_{24}^2 = a_2$$

$$x_{31}^2 + x_{32}^2 + x_{33}^2 + x_{34}^2 = a_3$$

Therefore, $A = a_1 + a_2 + a_3$

4. The squared column totals divided by the no. of observations are summed up to get the value of B.

$$\frac{(\sum x_{c1})^2}{n_{c1}} = b_1, \quad \frac{(\sum x_{c2})^2}{n_{c2}} = b_2, \quad \frac{(\sum x_{c3})^2}{n_{c3}} = b_3$$

Therefore, $B = b_1 + b_2 + b_3$

5. The squared row totals divided by the no. of observations in row are summed up to get the value of c.

$$\frac{(\sum x_{r1})^2}{n_{r1}} = c_1, \quad \frac{(\sum x_{r2})^2}{n_{r2}} = c_2, \quad \frac{(\sum x_{r3})^2}{n_{r3}} = c_3, \quad \frac{(\sum x_{r4})^2}{n_{r4}} = c_4$$

Therefore, $C = c_1 + c_2 + c_3$

6.The squared grand total (G^2) divided by the no. of observations made which is the value of D.

$$D = \frac{(G^2)}{n} \qquad n = n_{c1} + n_{c2} + n_{c3}$$

7. Tabulation of all Calculations for analysis of variance:

Source of variation	Sum of squares (SS)	Degree of freedom	Mean squares
Between treatments (column)	B – D	Column number - 1 $(u-1)$	$\frac{B-D}{u-1}$
Between replicates (row)	C – D	Row number - 1 $(v-1)$	$\frac{C-D}{v-1}$
Residual	$(A-D)-\{(B-D)+(C-D)\}$	$(u-1)(v-1)$	

8.Calculation of F-value between treatment variation and between replicate variations.

$$F \text{ (between treatments)} = \frac{\text{Mean square of variation between treatments}}{\text{Residual mean square}}$$

$$= \frac{\frac{(A-D)-\{(B-D)+(C-D)\}}{(u-1)(v-1)}}{\frac{(B-D)}{u-1}}$$

$$= \frac{u-1}{\frac{(A-D)-\{(B-D)+(C-D)\}}{(u-1)(v-1)}}$$

$$F \text{ (between replicates)} = \frac{\text{Mean square of variation between replicates}}{\text{Residual mean square}}$$

$$= \frac{\frac{(C-D)}{v-1}}{\frac{(A-D)-\{(B-D)+(C-D)\}}{(u-1)(v-1)}}$$

9. Table for comparison of F-value.

Source of variation	degree of freedom	Calculated F	Tabulated F	Probability	Significance level
Between treatments	$n_1 = u-1$ $n_2 = (u-1)(v-1)$				
Between replicates	$n_1 = v-1$ $n_2 = (u-1)(v-1)$				

10. When the calculated F-value is less than tabulated F-value then the treatment has no significant effect, whereas if calculated F-value is more than tabulated value then the treatment has significant effect.

Practical sheet for analysis of variance (one way classification):

	Treatments				
Replicates	1	2	3	4	**Total**
1	x_{11}	x_{21}	x_{31}	x_{41}	
2	x_{12}	x_{22}	x_{32}	x_{42}	
3	x_{13}	x_{23}	x_{33}	x_{43}	
4	x_{14}	x_{24}	x_{34}	x_{44}	
5	x_{15}	x_{25}	x_{35}	x_{45}	
Total					
$\sum x$	$\sum x_{c_1} + \sum x_{c_2} + \sum x_{c_3} + \sum x_{c_4} = G$ (Grand total)				
$\sum x^2$	$\sum x_{c_1}^2 + \sum x_{c_2}^2 + \sum x_{c_3}^2 + \sum x_{c_4}^2 = A$				
$\frac{(\sum x)^2}{n_c}$	$\frac{(\sum x_1)^2}{n_{c_1}} + \frac{(\sum x_2)^2}{n_{c_2}} + \frac{(\sum x_3)^2}{n_{c_3}} + \frac{(\sum x_4)^2}{n_{c_4}} = B$				
	n_c = number of observations in the columns				
	$\frac{(\sum x)^2}{n}$ or $\frac{G^2}{\text{Total number of observations}} = \frac{G^2}{n} = D$				

where c and r stand for the number of columns and rows, respectively.

Total SS = A – D u (number of columns)

Between treatment SS = B – D v (number of rows)

Residual SS = A – B Analysis of variance table:

Source of variation	Sum of squares (SS)	Degrees of freedom	Mean squares
Between treatments	B – D	$u - 1 = n_1$	$\frac{B-D}{u-1}$
Residual	A–B = (A–D) – (B–D)	$u(v - 1) = n_2$	$\frac{A-B}{u(v-1)}$
Total	A – D	uv – 1	

$$F\,(\text{Between treatments}) = \frac{\text{Between treatment mean square}}{\text{Residual mean square}} = \frac{B-D}{u-1} \Big/ \frac{A-B}{u(v-1)}$$

Inference table:

1	2	3	4	5	6
Source of variation	Degree of freedom	Calculated F	Tabulated F	Probability	Significance level

Practical sheet for analysis of variance (Two way classification)

Test subjects (Replicates)	Treatments 1	2	3	Total
1	x_{11}	x_{21}	x_{31}	$x_{11} + x_{21} + x_{31} = x_{r_1}$
2	x_{12}	x_{22}	x_{32}	$x_{12} + x_{22} + x_{32} = x_{r_2}$
3	x_{13}	x_{23}	x_{33}	$x_{13} + x_{23} + x_{33} = x_{r_3}$
Total u = 3	$x_{11}+x_{12}+x_{13}$	$x_{21}+x_{22}+x_{23}$	$x_{31}+x_{32}+x_{33}$	= Grand total (G)
Σx	$(=\Sigma x_{c_1})$	$(=\Sigma x_{c_2})$	$(=\Sigma x_{c_3})$	
Σx^2	$x_{11}^2 + x_{12}^2 + x_{13}^2$	$x_{21}^2 + x_{22}^2 + x_{23}^2$	$x_{31}^2 + x_{32}^2 + x_{33}^2$	
	$(\Sigma x_{c_1}^2)$	$(\Sigma x_{c_2}^2)$	$(\Sigma x_{c_3}^2)$	
	$= a_1$	$= a_2$	$= a_3$	$(a_1 + a_2 + a_3) = (A)$
$\frac{(\Sigma x)^2}{n_c}$	$\frac{(\Sigma x_{c_1})^2}{n_{c_1}}$	$\frac{(\Sigma x_{c_2})^2}{n_{c_2}}$	$\frac{(\Sigma x_{c_3})^2}{n_{c_3}}$	
	$= b_1$	$= b_2$	$= b_3$	$(b_1 + b_2 + b_3) = (B)$
$\frac{(\Sigma x)^2}{n_r}$	$\frac{(\Sigma x_{r_1})^2}{n_{r_1}}$	$\frac{(\Sigma x_{r_2})^2}{n_{r_2}}$	$\frac{(\Sigma x_{r_3})^2}{n_{r_3}}$	
	$= c_1$	$= c_2$	$= c_3$	$(c_1 + c_2 + c_3) = (C)$
	$\frac{(\Sigma x_{c_1} + \Sigma x_{c_2} + \Sigma x_{c_3})^2}{n_{c_1} + n_{c_2} + n_{c_3}} = \frac{G^2}{n} = D$			(D)

Total sum of squares = A – D

Between treatment sum of squares = B – D

Between rows sum of squares = C – D

Residual sum of squares = (A – D) – {(B – D) + (C – D)}

Table for analysis of variance:

Source of variation	Sum of squares	Degrees of freedom	Mean squares
1. Between treatments	B – D	u – 1	$\frac{B-D}{u-1}$
2. Between rows	C – D	v – 1	$\frac{C-D}{v-1}$
3. Residual	(A–D)–{(B–D)+(C–D)}	(u–1)(v–1)	$\frac{(A-D)-\{(B-D)+(C-D)\}}{(u-1)(v-1)}$
Total	A – D	uv – 1	

$$F\,(\text{Between treatments}) = \frac{\text{Between treatment mean square}}{\text{Residual mean square}} = \frac{\dfrac{B-D}{u-1}}{\dfrac{(A-D)-\{(B-D)+(C-D)\}}{(u-1)(v-1)}}$$

$$F\,(\text{Between replicates}) = \frac{\text{Between replicate mean square}}{\text{Residual mean square}} = \frac{\dfrac{C-D}{v-1}}{\dfrac{(A-D)-\{(B-D)+(C-D)\}}{(u-1)(v-1)}}$$

Inference table:

1	2	3	4	5	6
Source of variation	Degree of freedom	Calculated F	Tabulated F	Probability	Significance level

Example 5: A plant physiologist was investigating the effect of IBA on emergence of roots from detached leaves. He used three different concentrations in five replicates and got the following results. Calculate the 'F' value to study the analysis of variance.

	Conc. of IBA (ppm)				
Replicates	10	20	30		
1	5	7	4	$\Sigma x_{r_1} = 16$	$C_1 = \frac{256}{3} = 85.3$
2	4	9	3	$\Sigma x_{r_2} = 16$	$C_2 = \frac{256}{3} = 85.3$
3	6	8	5	$\Sigma x_{r_3} = 19$	$C_3 = \frac{361}{3} = 120.3$
4	5	10	3	$\Sigma x_{r_4} = 18$	$C_4 = \frac{324}{3} = 108.0$
5	6	9	4	$\Sigma x_{r_5} = 19$	$C_5 = \frac{361}{3} = 120.3$
	$\Sigma x_1 = 26$	$\Sigma x_2 = 43$	$\Sigma x_3 = 19$	G = 88	C = 519.2
	$a_1 = 138$	$a_2 = 375$	$a_3 = 75$	A = 588	$D = \frac{88^2}{15} = 516.3$
	$b_1 = \frac{26^2}{5}$ = 135.2	$b_2 = \frac{43^2}{5}$ = 369.8	$b_3 = \frac{19^2}{5}$ = 72.2	B = 577.2	

A – D = 71.7
B – D = 60.9
C – D = 2.9

Source of Variation	SOS	D.f.	MS	F-Value	Tabulated F-Value		
Between Treatments	B – D = 60.93	u – 1 = 2	B–D/u–1 = 60.93/2 = 30.46	30.46 / .984 = 30.95	p = .05 4.5	p = .01 8.7	p = .001 18.5
Between Replicates	C – D = 2.9	v – 1 = 4	C–D/v–1 = 2.9/4 = 0.725	.725 / .984 = 0.736	3.8	7.0	14.4
Residual	(A – D) – {(B – D) + (C – D)} = 71.7 – (60.93 + 2.9) = 7.87	(u – 1) (v – 1) = 2×4 = 8	7.87 / 8 = .984				

Inference:

The calculated F-value 30.95 for between treatments of three concentrations of IBA is found to be greater than the tabulated F value for $n_1 = 2$, $n_2 = 8$ degrees of freedom at .001 and .01 probability level. Thus the deviation is highly significant. Here the null hypothesis stating that the 3 different concentrations of IBA have the identical effect is rejected at 99.9% level.

The calculated F-value is 0.736 for between the replicates of each treatment is found far less than the tabulated F-value for $n_1 = 4$, $n_2 = 8$ degrees of freedom at 0.001 and 0.01 probability level. Thus the conclusion is there is no significant difference among the replicates. Here the null hypothesis is accepted, i.e., replicates do not have any difference.

Probable Questions:

1. When student's t test is applied?
2. What is the procedure for deduction of t value?
3. What is Z test?
4. What are the applications of Z test?
5. How the Z value is calculated?
6. Describe the significance of ANOVA?
7. Describe different steps of ANOVA analysis?

Suggested readings:

1. Zar, J.H. (2013) Biostatistical Analysis
2. Pagano M., Gauvreau, K, (2000), Principles of Biostatistics.

UNIT-XX

Nonparametric tests: Chi-square test and Wilcoxon sign-rank test. Linear Regression, Correlation analysis and rank Correlation analysis

Objective: In this unit we will discuss about Nonparametric tests such as Chi-square test and Wilcoxon sign-rank test. We will also discuss about Linear Regression, Correlation analysis and rank Correlation analysis.

Introduction:

In biological experiments and field surveys, apart from quantitative data we get the qualitative data which is genetical character such as tall and short, colour of flower, seed coat character which do not have any numerical values. But the number of flowers or seeds having a particular colour falls under any category can be counted numerically.

This type of observation requires the calculation of the expected number of individuals under any category. Thus it becomes necessary to know whether there is any deviation in between the observed and expected frequencies. The measurement of this deviation is done with the help of a particular test which is called Chi-square (X^2) test.

The formula for Chi-square test is:

$$X^2 = \sum(O-E)^2/E$$

where O = Observed value,

E = Expected value.

Application of X^2-Test:

It is an alternative test to find significance of difference in two or more than two proportions:

(a) It can compare the values of two binomial samples when they are small.

(b) It can compare the frequencies of two multinomial samples.

(c) Chi-square measures the probability of association between two discrete attributes.

(d) The Chi-square test is applied as a test for goodness of fit which reveals the closeliness of observed frequency with those of the expected frequency. Thus it helps to answer whether physical or chemical factors did or did not have an effect.

(e) Occasionally it is desirable to compare one set of observations taken under particular conditions to those of a similar nature taken under different conditions. In this case there are no definite expected values, only the question is whether the results are dependent (contingent upon) or independent of conditions. Then the X^2-test is called as test for independence or contingency test.

Limitations of X^2 Test:

(i) It is used only on the numerical data, not on the percentages or ratios.

(ii) It cannot properly be used when expected frequency within any phenotypic class is less than 5.

Correction for Small Sample:
Yates' correction as given below is applied:

$$X^2 \text{ (corrected)} = \sum \frac{(|O - E| - 0.5)^2}{E}$$

Degrees of Freedom:

The number of degrees of freedom is calculated as the number of classes whose value is required to describe the outcome from all classes. The concept of degrees of freedom is important in experiments and genetic ratios because one must consider the total number of observed individuals in the experiment as a fixed or given quantity. This fixed quantity is composed of one or more classes some of which are variable.

In the experiment between tall and dwarf pea plants there are only two classes, tall and dwarf. As soon as the number of one class is set, the other can be determined. Thus when two classes are scored, there is one degree of freedom.

In an experiment where three classes are scored, there are two degrees of freedom, and so on. The rule states that for the kind of genetic experiments described, the degrees of freedom are equal to one less than the number of classes.

Level of Significance:

In the experiment described the actual ratio departs from that which is expected. We must now determine how significant is this discrepancy so that we can decide to accept or reject the results.

Small discrepancies are not significant; large discrepancies are significant and lead to rejection of a result or hypothesis. Therefore values are assigned to these two kinds of discrepancies—the large discrepancies are the largest 5% and small discrepancies are remaining 95%.

On this basis if the discrepancy lies in the large class it is significant and the result may be discarded. The 5% frequency value that enables us to reject the result is called the 5% level of significance. The level of significance can be changed.If 5% is too high we can decide on a low level of significance say 1%. In this case it is not so easy to reject a result. Contrarily, if we decide on a high level of significance say 10%, it is easier to reject a result. Usually the accepted level of significance is between the two extremes, that is 5%.

After determining the degrees of freedom in an experiment and deciding on the level of significance, the actual size of the discrepancy between expected and observed is found by chi-square.Statisticians have prepared tables that relate the number of degrees of freedom with the probability that particular groups of chi-square values will be found (Table 2). For a more detailed table refer to Table IV in Fisher and Yates, 1963.

We can now examine the results of the experiment described in Table 1. The chi-square values of the first two crosses are 1.33 and 0.133. Both are acceptable discrepancies because these values are smaller than the chi-square value for one degree of freedom given as 3.84 in Table 2.The results of the first two crosses therefore may be considered to be consistent with Mendel's hypo thesis, the difference between expected and observed being due to chance.

TABLE 2. Values of chi-square corresponding to 5% and 1% levels of significance and up to 10 degrees of freedom

Degrees of freedom	*Level of significance*	
	5%	*1%*
1	3.84	6.64
2	5.99	9.21
3	7.82	11.34
4	9.49	13.28
5	11.07	15.09
6	12.59	16.81
7	14.07	18.48
8	15.51	20.09
9	16.92	21.67
10	18.31	23.21

X^2-Test For Goodness of Fit:

X^2-test is applied to a wide range of studies relating to experimental biology and field studies. The aim of this test is to test the closeliness of observed frequencies with those of the expected, i.e., how well the observed frequency curve fits into theoretical curve.
If both the observed and expected frequency distribution are in complete agreement with each other then the X^2-value will be zero. But in experimental observations there is always some degree of deviation. The critical X^2-value will be exceeded due to sampling fluctuations.
For example, if a crossing experiment gives two different sizes of seeds in F_2 progeny then these two types seeds may segregate according to 3:1 (Mendelian Monohybrid), 1:1 (Monohybrid test cross), 9:7 (Complementary factor interaction), 13:3 (Inhibitory factor) or 15:1 (Duplicate gene interaction) ratio, etc.

Again if crossing experiment results in three types of seeds then these may be due to incomplete dominance (1:2:1), supplementary factor (9:3:4) or due to dominant epistasis (12:3:1) interaction, etc.Likely, 4 types of seeds with 4 different combinations of two different characters may either follow the 9:3:3:1 (Mendelian dihybrid) or 1:1:1:1 (Dihybrid test cross) ratio for segregation.

Test for the goodness of fit is required in these above cases for studying the closeliness of observed data of the experiments with those of expected frequencies.

Steps to be followed to test the Goodness of Fit:

1. Deviation between the observed and the expected results should be calculated.

2. Comparing the minimum deviation the null hypothesis should be selected for X^2-test.
3. X^2-value should be determined.
4. Comparing the calculated X^2-value with tabulated X^2-value the conclusion has to be made.

Example `1 → (3:1):

Following number of seeds with the associated character is observed. Test the goodness of fit and comment.

Yellow seed: 428

Green seed: 152

Step I: Calculation of expected value for each ratio:

(i) **3:1 ratio**

Total no. of samples = 428 + 152 = 580

Expected yellow seeds = $\frac{3}{4} \times 580 = 435$

Expected green seeds = $\frac{1}{4} \times 580 = 145$

(ii) **1:1 ratio**

Expected yellow seeds and green seeds = $\frac{1}{2} \times 580 = 290$

(iii) **9:7 ratio**

Expected yellow seeds = $\frac{9}{16} \times 580 = 326.25$

Expected green seeds = $\frac{7}{16} \times 580 = 253.75$

(iv) **13:3 ratio**

Expected yellow seeds = $\frac{13}{16} \times 580 = 471.25$

Expected green seeds = $\frac{3}{16} \times 580 = 108.75$

(v) **15:1 ratio**

Expected yellow seeds = $\frac{15}{16} \times 580 = 543.75$

Expected green seeds = $\frac{1}{16} \times 580 = 36.25$

Step II: Determination of expected segregation ratio:

According to table, the deviation is minimum in 3:1 ratio, so the observed sample should fit the 3:1 ratio.

Table for calculation of minimum deviation

	Expected segregation ratios									
Observed value	3:1		1:1		9:7		13:3		15:1	
	Expected	Deviation	Expected	Deviation	Expected	Deviation	Expected	Deviation	Expected	Deviation
Yellow 428	435	7	290	138	326.25	101.75	471.25	43.25	543.75	115.75
Green 152	145	7	290	138	253.75	101.75	108.75	43.25	36.25	115.75
Total deviation		14		276		203.50		86.50		231.50

Step-III Calculation of X^2-Value:

No. of classes	Character	Observed value (O)	Expected value (E)	(O – E)	$(O - E)^2$	$\frac{(O - E)^2}{E}$
1	Yellow	428	435	– 7	49	0.113
2	Green	152	145	+ 7	49	0.338

$$\chi^2 = \Sigma\frac{(O-E)^2}{E} = 0.113 + 0.338 = 0.451$$

Since, in this experiment the samples are of two classes, so degree of freedom = 2-1 = 1.

Step IV: Conclusion:

The calculated Chi-square (X^2) value is 0.451. The tabulated chi-square at 0.05 probability level with 1 degree of freedom is 3.841.

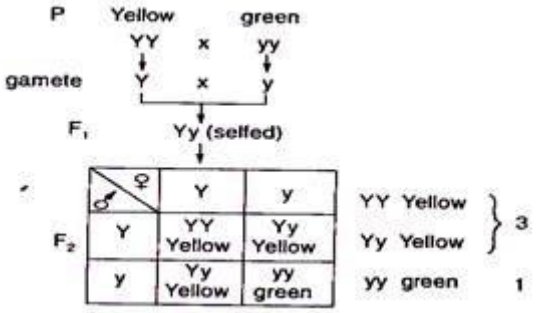
The calculated value is much less than the table value, so the deviation is insignificant, the observed deviation is due to chance factor only. It lies in the probability range 50-70%.The observed result is in good fit with Mendelian Monohybrid ratio, i.e., 3:1.

Step V: Comment:

Comment:

It is concluded that the experimental result shows Mendelian monohybrid ratio 3:1.

So the assumed genotypes of parents are:



So the phenotypic ratio is 3:1.

Example 2 → (9:3:3:1):

In an experiment on garden pea, we count 4 different kinds of plants,

Yellow cotyledon and inflated pod	—	555
Yellow cotyledon and constricted pod	—	185
Green cotyledon and inflated pod	—	195
Green cotyledon and constricted pod	—	65
Total		1000

Test the goodness of fit for this data and comment.

Step I: Calculation of expected value of assumed ratio:

(i) **9:3:3:1 ratio**

Total number of plants = 1000

Yellow cotyledon and inflated pod = $\frac{9}{16} \times 1000 = 562.5$

Yellow cotyledon and constricted pod = $\frac{3}{16} \times 1000 = 187.5$

Green cotyledon and inflated pod = $\frac{3}{16} \times 1000 = 187.5$

Green cotyledon and constricted pod = $\frac{1}{16} \times 1000 = 62.5$

(ii) **1:1:1:1 ratio**

Yellow cotyledon and inflated pod = $\frac{1}{4} \times 1000 = 250$

Yellow cotyledon and constricted pod = $\frac{1}{4} \times 1000 = 250$

Green cotyledon and inflated pod = $\frac{1}{4} \times 1000 = 250$

Green cotyledon and constricted pod = $\frac{1}{4} \times 1000 = 250$

Step II: Determination of expected segregation ratio

	9:3:3:1		1:1:1:1		According to the table, we find that the deviation is minimum in case of 9:3:3:1 ratio. So it is assumed that the observed segregation pattern will be in good fit with this **9:3:3:1** ratio.
Observed value	Expected value	Deviation |d|	Expected value	Deviation |d|	
Yellow cotyledon inflated pod 555	562.5	7.5	250	305	
Yellow cotyledon constricted pod 185	187.5	2.5	250	65	
Green cotyledon inflated pod 195	187.5	7.5	250	55	
Green cotyledon constricted pod 65	62.5	2.5	250	185	
Total deviation		20		610	

Step III: Determination of Chi-square value

No. of classes	Sample characters	Observed value (O)	Expected value (E)	(O–E)	$(O-E)^2$	$\frac{(O-E)^2}{E}$
1	Yellow, inflated	555	562.5	– 7.5	56.25	$\frac{56.25}{562.5}=0.1$
2	Yellow, constricted	185	187.5	– 2.5	6.25	$\frac{6.25}{187.5}=0.03$
3	Green, inflated	195	187.5	+ 7.5	56.25	$\frac{56.25}{187.5}=0.3$
4	Green, constricted	65	62.5	+ 2.5	6.25	$\frac{6.25}{62.5}=0.1$

$$\chi^2=\Sigma\frac{(O-E)^2}{E}=0.53$$

Since, in this experiment the samples are of 4 classes, so degrees of freedom = 4-1

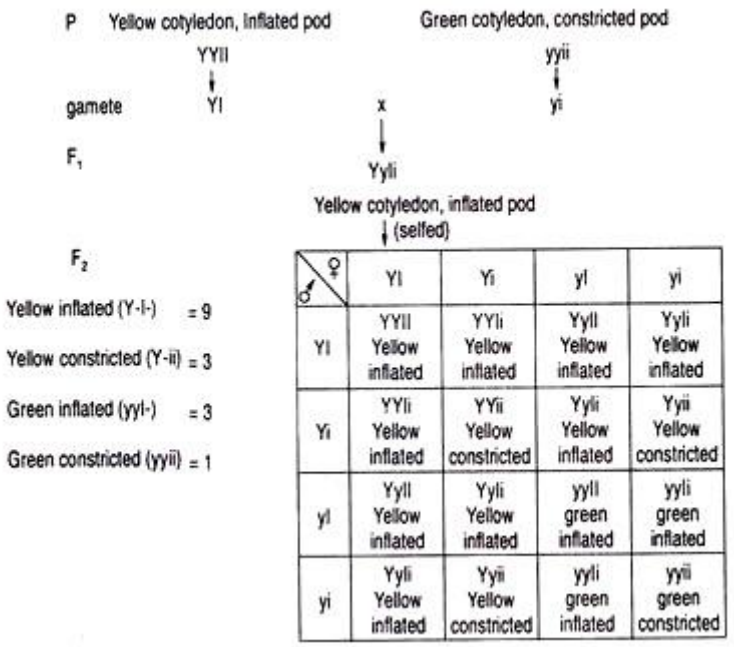
Step IV: Conclusion:

The tabulated X^2 value at 0.05 probability level for 3 degree of freedom is 7.81 which is more than the calculated value and it lies at 90-95% probability level. So the deviation found in the experiment is insignificant.

We can conclude that the observation is in good fit with Mendelian Dihybrid ratio 9:3:3:1.

Step V: Comment:

As the observation shows good fit with Mendelian dihybrid ratio, so the two genes for cotyledon colour and pod shape can independently assort.



Example 3 → (1:1):

One yellow seeded pea plant when crossed with a green seeded pea plant, produced 50 yellow seeds and 46 green seeds in F_2. Write down your comment with the help of x^2-analysis.

Sample character and sample size

No. of classes	Sample character	Sample size
1	Yellow seed	50
2	Green seed	46
	Total	96

Determination of expected segregation ratio (By estimating magnitude of deviation)

	3:1		1:1		9:7		13:3		15:1	
Observed value	Exp	|d|	Exp	|d|	Exp	|d|	Exp	|d|	Exp	|d|
Yellow seed 50	72	22	48	2	56	6	78	28	90	40
Green seed 46	24	22	48	2	42	6	18	28	6	40
Total deviation		44		4		12		56		80

According to the table, we find that the minimum deviation is in case of 1:1 ratio. So it is assumed that the observed sample should fit well with this ratio.

Determination of Chi-squre value:

No. of classes	Sample characters	Observed value (O)	Expected value (E)	(O–E)	$(O-E)^2$	$\frac{(O-E)^2}{E}$
1	Yellow	50	48	2	4	0.083
2	Green	46	48	2	4	0.083

$$\chi^2 = \Sigma\frac{(O-E)^2}{E} = 0.083 + 0.083 = 0.166$$

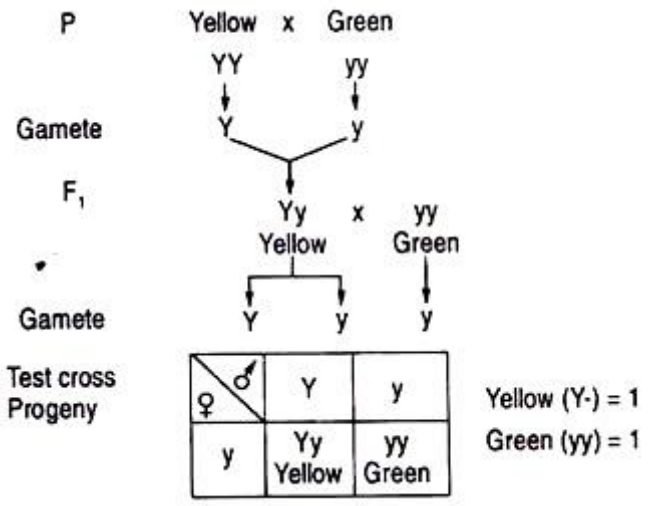
Conclusion:

Since there are 2 classes, so the degree of freedom = 2-1 = 1. The calculated X^2-value is 0.166 which is much less than the table value 3.841 for 1 degree of freedom at 0.05 probability levels.

The X^2 value 0.166 lies between 80-90% probability level. Therefore the deviation which is observed in the sample from the expected value is highly insignificant, and the observed ratio has a very good fit with the expected ratio, i.e., 1:1.

Comment:

As the experimental result shows good fit with 1:1 ratio, i.e., Mendelian monohybrid test cross ratio, so the assumed genotypes of the parents and the offsprings are as follows:



Example 4→(9:7):

Selfing of a hybrid plant produced a population with 120 pink flowers and 88 white flowers. Explain with X^2-analysis, what does the result show?

Sample characters and sample size:

No. of classes	Sample character	Sample size
1	Pink flower	120
2	White flower	88

Total no. of samples = 208

Determination of deviation in different segregation ratio

	3:1		1:1		9:7		13:3		15:1	
Observed value	Exp	|d|	Exp	|d|	Exp	|d|	Exp	|d|	Exp	|d|
Pink 120	156	36	104	16	117	3	169	49	195	75
White 88	52	36	104	16	91	3	39	49	13	75
Total deviation		72		32		6		98		150

According to this table, we find that the deviation is minimum in case of 9:7 ratio. So, it is assumed that the observation should fit well with this ratio.

Determination of Chi-square value

No. of classes	Sample characters	Observed value (O)	Expected value (E)	Deviation (O–E)	$(O-E)^2$	$\frac{(O-E)^2}{E}$
1	Pink flower	120	117	3	9	0.076
2	White flower	88	91	3	9	0.098

$$\chi^2 = \Sigma\frac{(O-E)^2}{E} = .076 + 0.098 = 0.175$$

Conclusion:

Since in the observation there are two classes, so the degree of freedom =2 – 1 = 1

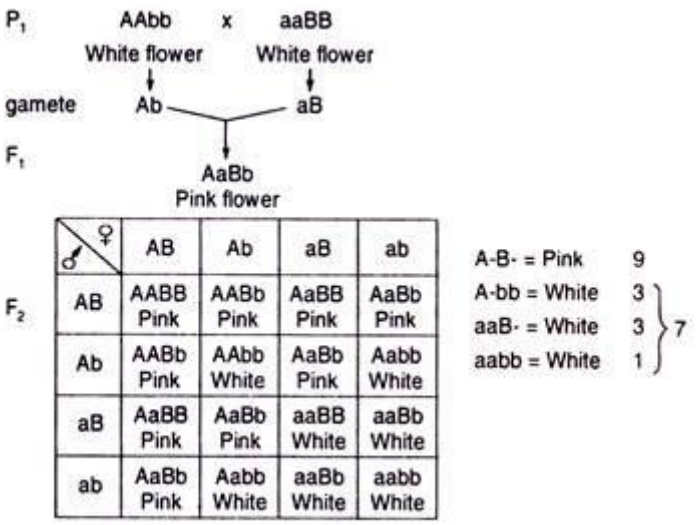
The calculated X^2 value is 0.175, which is much less than the table value 3.84 for 1 degree of freedom at 0.05 probability level.

The X^2 value 0.175 lies between 50-70% probability range. Therefore this deviation from the expected value is insignificant and the observed ratio is in good fit with 9:7 ratio.

Comment:

It is concluded that the experimental result shows the characters with complementary factor interaction in F_2 generation.

As the observed samples are assumed to show complementary factor interaction, so the assumed genotypes are:



Here, the analysis shows that 2 pairs of factors control the same character and two dominant genes A and B are complementary to each other. Each of which has no effect on expression of character, but when in combination shows their effect, i.e., pink colour.

Absence of any one of the them (A or B) leads to absence of pink colour and it can be concluded very easily that the colour character is controlled by two pairs of factors which are complementary to each other.

Example 5 → (13:3):

From a plant after selfing total 96 seeds are harvested of which yellow seeds 79, brown seeds 17. Explain the result with X^2-analysis.

Sample character and sample size

Sample character	Sample size
Yellow seed	79
Brown seed	17
Total	96

Determination of expected segregation ratio:

(By estimating magnitude of deviation)

	3:1		1:1		9:7		13:3		15:1	
Observed value	Exp	|d|	Exp	|d|	Exp	|d|	Exp	|d|	Exp	|d|
Yellow seed 79	72	7	48	31	56	23	78	1	90	11
Brown seed 17	24	7	48	31	42	23	18	1	6	11
Total deviation		14		62		46		2		22

According to the table, we find that the minimum deviation is in case of 13:3 ratio. So it is assumed that the observed sample should fit well with this ratio.

Determination of Chi-Square value

No. of classes	Sample characters	Observed value (O)	Expected value (E)	Deviation (O–E)	$(O-E)^2$	$\frac{(O-E)^2}{E}$
1	Yellow	79	78	1	1	0.0126
2	Brown	17	18	1	1	0.055

$$\chi^2 = \Sigma \frac{(O-E)^2}{E} = 0.0126 + 0.055 = 0.068$$

Conclusion:

Since there are two classes, degree of freedom = 2-1 = 1. The calculated X^2 value 0.068 is much less than the table value 3.841 for 1 degree of freedom at 0.05 probability level.
The X^2-value 0.068 lies between 90-95% probability level. Therefore, this deviation from the expected value is highly insignificant and the observed ratio has a very good fit with the expected ratio.

Comment:

As the experimental result shows good fit with 13:3 ratio. So it is assumed that the brown colour of seed coat is controlled by gene C, but the presence of another dominant gene D inhibits the effect of C, and the seed coat colour becomes yellow.

The dominant gene D does not have its own expression but it inhibits the effect of another dominant gene C. So the effect of C is only expressed when there is recessive gene d. This phenomenon is called Inhibitory Factor.

P_1 Brown seed CCdd × Yellow seed ccDD

gamete Cd × cD

F_1 CcDd Yellow seeds

F_2

♀ / ♂	CD	Cd	cD	cd
CD	CCDD Yellow	CCDd Yellow	CcDD Yellow	CcDd Yellow
Cd	CCDd Yellow	CCdd Brown	CcDd Yellow	Ccdd Brown
cD	CcDD Yellow	CcDd Yellow	ccDD Yellow	ccDd Yellow
cd	CcDd Yellow	Ccdd Brown	ccDd Yellow	ccdd Yellow

C-D- = Yellow = 9
ccD- = Yellow = 3
C-dd = Brown = 3
ccdd = Yellow = 1

So, the phenotypic ratio of Yellow seed: Brown seed = 13: 3.

Example 6 → 15:1):

In an experiment, the hybrid plant yielded 193 brown coloured seeds and 15 white seeds. Comment on the observed result with X^2-analysis.

Sample character and sample size:

No. of classes	Sample characters	Sample size
1	Brown seed	193
2	White seed	15
	Total	208

Determination o expected segregation ration:

(By estimating magnitude of deviation)

	3:1		1:1		9:7		13:3		15:1	
Observed value	Exp	|d|	Exp	|d|	Exp	|d|	Exp	|d|	Exp	|d|
Brown seed 193	156	37	104	89	117	76	169	24	195	2
White seed 15	52	37	104	89	91	76	39	24	13	2
Total deviation		74		178		152		48		4

According to the table, we find that the deviation is minimum in case of 15:1 ratio. So, it is assumed that the observed sample should fit with this ratio.

Determination of Chi-square value

No. of classes	Sample characters	Observed value (O)	Expected value (E)	Deviation (O–E)	$(O-E)^2$	$\frac{(O-E)^2}{E}$
1	Brown	193	195	2	4	0.0205
2	White	15	13	2	4	0.307

$$\chi^2 = \sum \frac{(O-E)^2}{E} = 0.0205 + .307 = 0.332$$

Conclusion:

Since there are two classes, degree of freedom = 2 – 1 = 1. The calculated X^2 value 0.332 is less than the table value 3.841 for 1 degree of freedom at 0.05 probability level.

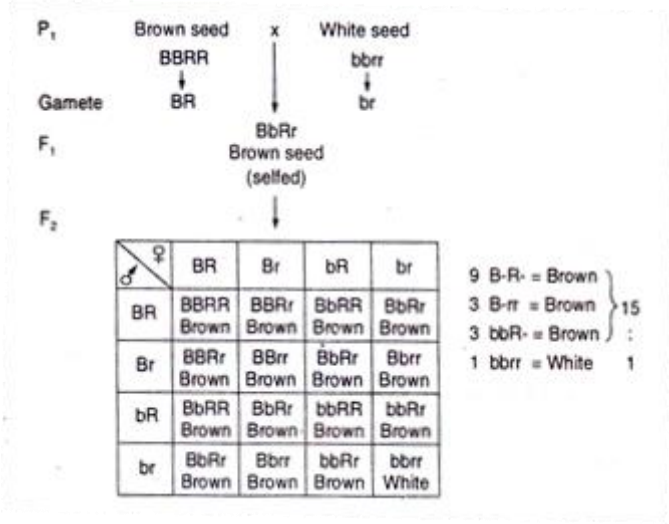
The X^2 value 0.332 lies between 75-90% probability levels. Therefore this deviation from the expected value is insignificant and the observed ratio has good fit with the expected ratio.

Comment:

As the experimental result shows good fit with 15:1 ratio, so it is assumed that the brown colour of seed is controlled by two pairs of factors or alleles. Presence of any one

of them will give them brown seed colour. Whereas the recessive condition of both will give the white colour.

This case may be represented as follows:



So the observed result shows the duplicate factor interaction in F_2 ratio.

Example 7 → (1:1:1:1):

Test the goodness of fit in the following sample from an experiment with garden pea plant.

Round, yellow 31

Round, green 26

Wrinkled, yellow 27

Wrinkled, green 26

No. of classes	Sample character	Observed no.
1	Round, yellow	31
2	Round, green	26
3	Wrinkled, yellow	27
4	Wrinkled, green	26

Determination of expected segregation ratio:

	9:3:3:1		1:1:1:1	
Observed value	Expect.	|d|	Expect.	|d|
Round, yellow 31	61.875	30.875	27.5	3.5
Round, green 26	20.625	5.375	27.5	1.5
Wrinkled, yellow 27	20.625	6.375	27.5	0.5
Wrinkled, green 26	6.875	19.125	27.5	1.5
Total		61.75		7.0

According to the table, we find that the deviation is minimum in case of 1:1:1:1 ratio. So, it is assumed that the observed sample should fit with this ratio.

Determination of Chi-square value

No. of classes	Sample characters	Observed no. (O)	Expected no. (E)	(O–E)	$(O-E)^2$	$\frac{(O-E)^2}{E}$
1	Round, yellow	31	27.5	3.5	12.25	$\frac{12.25}{27.5}=0.445$
2	Round, green	26	27.5	– 1.5	2.25	$\frac{2.25}{27.5}=0.082$
3	Wrinkled, yellow	27	27.5	– 0.5	0.25	$\frac{0.25}{27.5}=0.009$
4	Wrinkled, green	26	27.5	– 1.5	2.25	$\frac{2.25}{27.5}=0.082$

$$\chi^2=\Sigma\frac{(O-E)^2}{E}=0.618$$

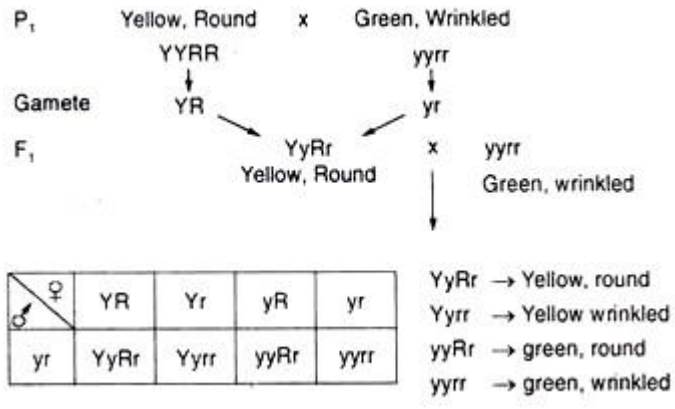
Conclusion:

Since there are 4 classes, degrees of freedom = 4-1=3. The calculated X^2 value is 0.618, which is much less than the tabulated X^2 value for 3 degree of freedom at 0.05 probability level.

The calculated value lies between 70-90% probability level. Therefore the deviation from the expected value is insignificant and the observed ratio has a good fit with the expected ratio.

Comment:
As the experimental result shows good fit with 1:1:1:1 ratio, so it is assumed that the result is obtained from a dihybrid test cross. The dominant characters are yellow and round.

Parental genotypes are assumed as:



Chi-Square Test for Association Of Attributes:

By using 2×2 contingency table the X^2 analysis is applied to test whether there is any association between two or more classifications, i.e., to test for independence of the two attributes.

Steps to be followed to calculate Chi-Square:

1. Null hypothesis should be set up, which is no association exists between the attributes.

H_0: No association exists between the attributes.
H_1 : Association exists between the attributes.

2. Expected frequency (E) is calculated corresponding to each category by the formula.

$E_{ij} = R_i \times C_j / n$
R_i = Sum total of row in which E_{ij} is present.
C_j = Sum total of column in which E_{ij} is present,
n = Total sample.

3. The Chi-square value is calculated according to formula

$$\text{Chi} - \text{square}\left(\chi^2\right) = \Sigma \frac{(O-E)^2}{E}$$

E = Expected value,
O = Observed value.

Degrees of freedom = (R – 1) (C – 1)

R = No. of rows,

C = No. of columns.

4. Table value is found out for a particular level of significance and for the calculated degree of freedom.

5. The calculated value and table value are compared, if the calculated value of X^2 < the table value then the null hypothesis is accepted. But if the X^2 value is larger than the tabulated value then null hypothesis is rejected.

Example 8:
An experiment was carried out to see the effect of an organo-mercuric compound on the survival of seedling. Two different concentrations are applied to test whether the percentage of death in higher concentration is significantly different from that of lower concentration or both are independent.

	Conc. of Fungicide		Total
	10 ppm	30 ppm	
Dead	23	35	58
Alive	62	55	117
Total	85	90	175

In this case the null hypothesis will be – there is no significant difference, i.e., both the effects are independent.

H_0: Both the effects are independent.
H_1: Both the effects are significantly different.

Table showing expected results and deviation

	Low Conc.			High Conc.			
	Observed	Expected	(O–E)=d	Observed	Expected	(O–E)=d	Total
Dead	23	28.2	– 5.2	35	29.8	+ 5.2	58
Alive	62	56.8	+ 5.2	55	60.2	– 5.2	117
Total	85			90			175

$$\text{The Chi - square value } (\chi^2) = \Sigma \frac{(O-E)^2}{E}$$

In this example, as the (O-E) is always 5.2, so we can compute the value in following way.

$$\chi^2 = (\pm 5.2)^2\left(\frac{1}{28.2}+\frac{1}{29.8}+\frac{1}{56.8}+\frac{1}{60.2}\right)$$
$$= 27.04 \times (0.035 + .034 + 0.018 + 0.017)$$
$$= 2.812$$

The degree of freedom for this example is

(C-1XR-1) = (2-1) x (2-1) = 1

The tabulated X^2 value at degree of freedom 1, and at p = 0.05 level is 3.84.
As the calculated X^2 value is much less than the tabulated X^2 value so the null hypothesis is accepted, i.e., both the effects are independent, there is no relation of death percentage with low and high concentration of fungicide.

Yates Correction:

Yates correction is applied to increase the precision of X^2 test, only when the degree of freedom is 1 and the expected classes are small (less than 30). In case of 1 degree of freedom, there is possibility of underestimating the probabilities listed in the table. This can be adjusted by subtracting the correction value from the deviation value.

For goodness of fit, Chi-square formula using Yates correction (½ or 0.5) will be:

$$\chi^2 = \Sigma \frac{\left\{|(O-E)| - \frac{1}{2}\right\}^2}{E}$$

In Example 5,

$$\chi^2 = \frac{(2-0.5)^2}{195} + \frac{(2-0.5)^2}{13} = 0.012 + 0.173 = 0.185.$$

In case of contingency Chi-square, using Yates correction the Chi-square value is calculated as follows:

			Total
a	b		R_1
c	d		R_2
Total	C_1	C_2	N

$$\chi^2 = \frac{N\left\{|ad-bc| - \frac{N}{2}\right\}^2}{R_1 \times R_2 \times C_1 \times C_2}$$

where $\frac{N}{2}$ is the factor of Yates correction.

If we apply this formula in the Example 15, then

$$\chi^2 = \frac{175\left\{\left|(1265-2170)\right|-\frac{175}{2}\right\}^2}{58\times117\times85\times90}$$

$$= \frac{175(905-875)^2}{51912900} = 2.253$$

where $ad = 23 \times 55 = 1265$

$bc = 35 \times 62 = 2170$

Correlation and Regression

Correlation:

In Biostatistics, sometimes we study two characters or variables on the same sample and try to find out the existence of any kind of relationship between these two characters. For example, different concentrations of pesticide and their effect on germination, panicle length and number of grains.

Definition:

Correlation is the relationship which can reveal whether the change in one variable would cause change in the other or not. Such relationship between the two sets of characters or variables can be expressed quantitatively by the degree of relationship, called Correlation Coefficient.

Kinds of Correlation:

There are 3 different kinds of correlations:

Positive, Zero and Negative correlations.

(a) Positive Correlation:

When the values of two variables change together in the same direction then the relationship is called positive correlation.

This type of correlation may be perfect positive or moderately positive:

(i) Perfect Positive Correlation:

When both the variables increase and decrease in the same proportion then it is perfect positive correlation.

(ii) Moderately Positive Correlation:

In this case, two variables are positively correlated but the changes do not occur in the same proportion. The coefficient value lies between + 1 and 0.

(b) Negative Correlation:

If one variable increases (or decreases) and the other decreases (or increases) then the relationship is called negative correlation.

Such as size and number of fruits/plant are negatively correlated.

This negative relation may also be of two kinds:

(i) Perfect Negative Correlation:

This kind of relationship is really very rare in case of biological situation, such as increase in temperature decreases the lipid content of the cell.

(ii) Moderately Negative Correlation:

In this relationship, the variables are negatively correlated but not very perfectly, such as increase in post harvesting period decreases the viability of seeds. Here also the coefficient value lies in between 0 and – 1.

(c) Zero Correlation:

If the two variables have no correlation, i.e., there is no consistency on value of observation, in such cases the two values of variables are called with zero correlation.

Coefficient of Correlation:

When the two variables have any direct relationship then the degree of relationship between these two variables is expressed by quantitative expression which is called Coefficient of Correlation. This quantitative measure expresses the degree of closeness of the linear relationship between the two variables.

The correlation coefficient is designated by the letter 'r' and it is also called as Karl Pearson's Coefficient of Correlation which is calculated by the following formula:

$$r_{x,y} = \frac{\sum dx \cdot dy}{\sqrt{\sum dx^2 \cdot \sum dy^2}} \quad \text{or} \quad \frac{\sum dx \cdot dy}{n \cdot \delta x \cdot \delta y}$$

where

$dx = x_1 - \bar{x}$ [deviation of x variable]

$dy = y_1 - \bar{y}$ [deviation of y variable]

δx = S.D. of x variable

δy = S.D. of y variable.

n = total no. of observation.

Steps to calculate the value of 'r':

(a) Two series are made by x and y variable.

(b) Mean of both the series are calculated, x and y.

(c) The deviation of each observation is calculated as dx and dy.

(d) Squaring of the deviations are noted.

(e) The deviations of both the variables are multiplied.

(f) All the data are summed up according to formula to calculate 'r'.

Properties of Correlation Coefficient (Fig. 13.1):

1. Correlation coefficient lies between – 1 and + 1, i.e., $-1 \leq r \leq +1$

2. If r = + 1, the correlation is perfect and positive, if it is less than + 1 then moderately positive.

3. If r = – 1, the correlation is perfect and negative, if it is higher than – 1 then moderately negative.

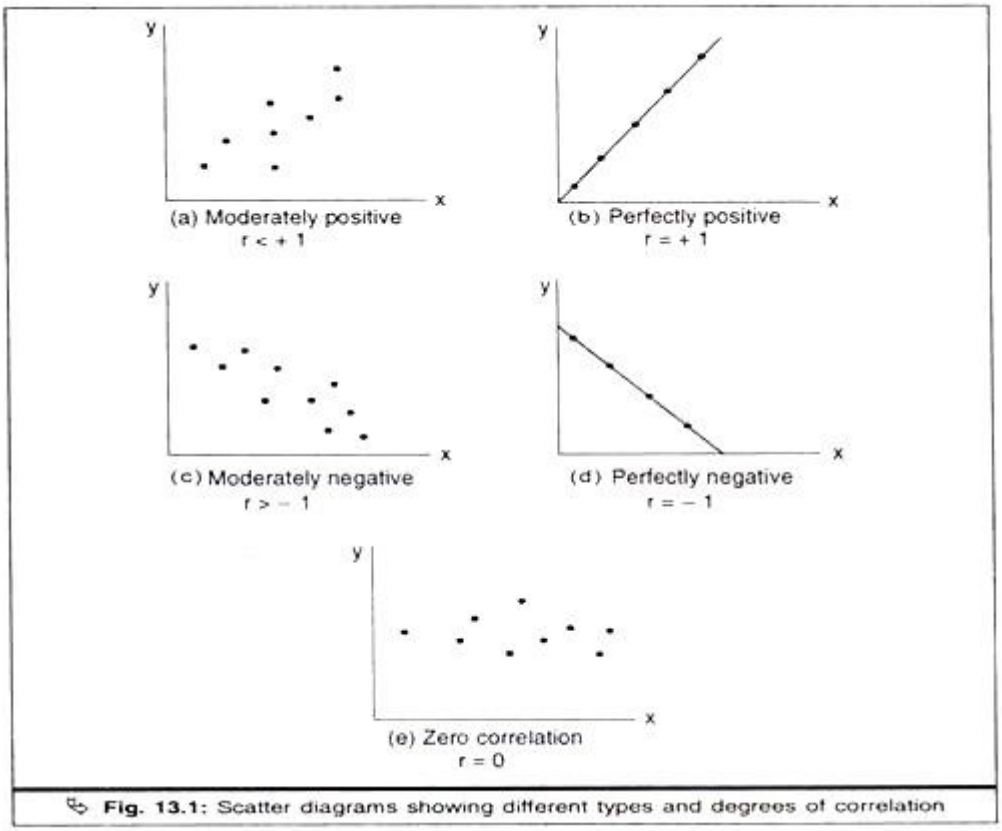


Fig. 13.1: Scatter diagrams showing different types and degrees of correlation

4. If r = 0, there is no correlation between the variables.

5. The coefficient of correlation is not affected by change and scale of origin.

Example 1:
Find out the correlation coefficient between the two attributes of 5 plants.

Height of the plant (x)	5 cm	9 cm	13 cm	17 cm	21 cm
Number of leaves per plant (y)	12	20	25	33	35

The data is arranged in the following table to calculate 'r':

x	dx	dx^2	y	dy	dy^2	dx.dy
5	5 – 13 = – 8	64	12	12 – 25 = – 13	169	104
9	9 – 13 = – 4	16	20	20 – 25 = – 5	25	20
13	13 – 13 = 0	0	25	25 – 25 = 0	0	0
17	17 – 13 = + 4	16	33	33 – 25 = + 8	64	32
21	21 – 13 = + 8	64	35	35 – 25 = + 10	100	80
Σx = 65 $\bar{x} = \frac{65}{5}$ =13		Σdx^2 = 160	Σy = 125 $\bar{y} = \frac{125}{5}$ = 25		Σdy^2 = 358	$\Sigma dx.dy$ = 236

Now,

$$r = \frac{\Sigma dx \cdot dy}{\sqrt{\Sigma dx^2 \cdot \Sigma dy^2}} = \frac{236}{\sqrt{160 \times 358}} = \frac{236}{\sqrt{57280}} = \frac{236}{239.33} = 0.986 \text{ (approx.)}$$

The value of r = 0.986 gives us the view that the two attributes like height of the plant and number of leaves are positively correlated and the value is as near about 1, so it can be concluded that the relationship is towards perfect positive relation.

Example 2:

The effect of pesticide, 'Nuvan' is tested on germination of Phaseolus seeds. Find out the correlation coefficient.

The data is arranged in the following table to calculate 'r':

x	dx	dx^2	y	dy	dy^2	dx.dy
0	– 8.16	66.58	90	+ 44.45	1975.8	– 362.71
1	– 7.16	51.26	81	+ 35.45	1256.7	– 253.82
2.5	– 5.66	32.03	65	+ 19.45	378.3	– 110.08
5	– 3.16	9.98	52	+ 6.45	41.6	– 20.38
7.5	– 0.66	0.436	39	– 6.55	42.9	+ 4.32
10	+ 1.84	3.38	32	– 13.55	183.6	– 24.93
12.5	+ 4.34	18.83	28	– 17.55	308.0	– 76.16
15	+ 6.84	46.78	17	– 28.55	815.1	– 195.28
20	+ 11.84	140.18	06	– 39.55	1564.2	– 468.27
$\Sigma x =$ 73.5 $\bar{x} = \frac{73.5}{9}$ = 8.16		$\Sigma dx^2 =$ 369.46	$\Sigma y =$ 410 $\bar{y} = \frac{410}{9}$ = 45.55		$\Sigma dy^2 =$ 6566.2	$\Sigma dx.dy =$ – 1507.31

$$r = \frac{\sum dx \cdot dy}{\sqrt{\sum dx^2 \cdot \sum dy^2}} = \frac{-1507.31}{\sqrt{369.46 \times 6566.2}} = \frac{-1507.31}{1557.55} = -0.9677$$

The value of r = – 0.9677 denotes that the two variables, i.e., pesticide concentration and germination percentage are negatively correlated, though it is not perfectly negative but approaching towards perfect negative correlation.

Significance of Correlation Coefficient:

The calculated correlation coefficient should be checked from the correlation coefficient (r) table for the degree of freedom (number of pairs of observation minus one), i.e., (n – 1) at 0.05 to 0.001 probability level.

In Example 1, the 'r' value obtained is 0.986, the table r value at (10 – 1) = 9 degree of freedom at 0.001 probability level is 0.847. As the observed value is higher than the table value, so the 'r' value is highly significant.

In Example 2, the 'r' value is – 0.9677, the table 'r' value at (18 – 1) = 17 degree of freedom at 0.001 probability level is 0.693. As the observed value is much higher than the table value so, the 'r' value or the correlation is highly significant.

Methods of Determining Correlation:

The following methods are generally used to determine simple correlation:

a. Graphic method.

b. Scatter diagram or Dotogram method.

c. Karl Pearson's method

d. Spearman's ranking method.

e. Coefficient of correlation by concurrent deviation.

a. Graphic Method:

When the values of dependent series are plotted on O –X axis and independent series are plotted on O-Y axis of graph paper, a linear or non-linear graph will be obtained which will simply indicate the direction of correlation and not the numerical magnitude.

If the graph lines of two independent series move in upward direction from left to right, the correlation is positive, but if the graph line of one series moves upward from left to right and that of the other independent series moves downward from left to right, they show negative correlations.

If the values of two data series do not show either positive or negative trend then it should be inferred that there is no correlation.

b. Scatter Diagram or Dotogram Method:

This method is more or less similar to graphic method. In this method, the values of independent data series are plotted on O – X axis and those of dependent series on 0-Y axis and then the pairs of values are plotted on the graph paper.

In this ways, graphs of dots are obtained. These dots are scattered in different forms. Therefore, the graphs are called scatter diagrams or dotograms. The patterns of scatter diagrams indicate the Erection and magnitude of correlation.

The scatter diagram may indicate the following conditions:

(i) If the dots of the two series are advancing in a definite direction like a current, this condition indicates that the data series are definitely correlated.

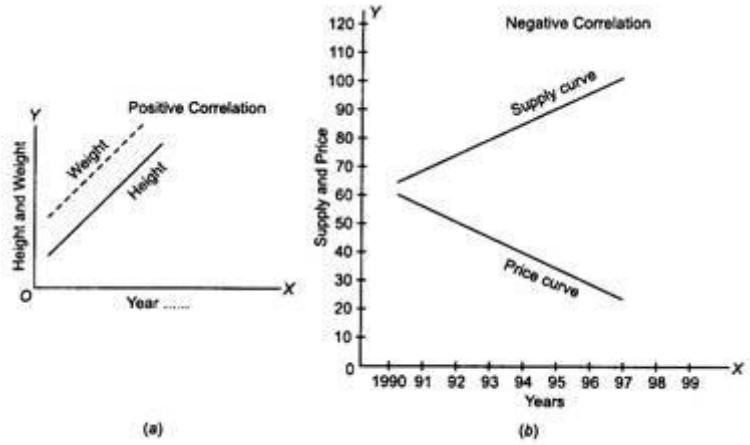


Fig. 34.1 (a) Positive correlation (b) negative correlation.

(ii) When the arrays of dots advance from left to right in upward direction, the correlation is definitely positive [Fig. 34.2 (c)].

(iii) When the scatter diagram advances from left to right in downward direction, the correlation' is negative [Fig. 34.2 (a)].

(iv) When the dots are not in definite arrays and are scattered haphazardly, this condition indicates that there is no correlation between the data series [Fig. 34.2 (b)].

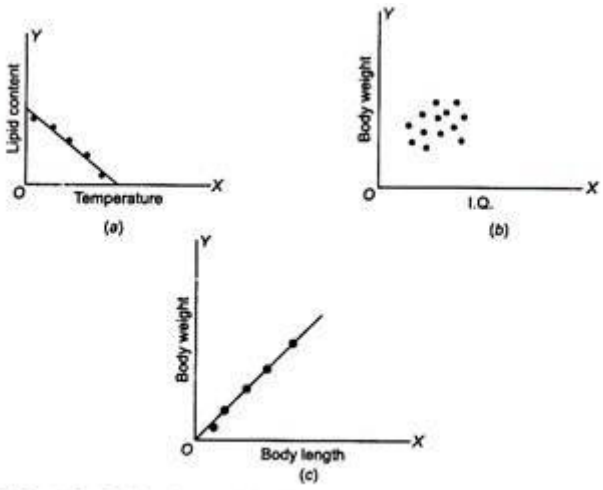


Fig. 34.2 (a) Perfect Negative correlation (b) No correlation (c) Perfect positive correlation

(v) When the dots appear to be situated on a line which advances upward at 45° angle from the O-X axis, this condition indicates perfect positive correlation among the data series.

(vi) If the dots appear to be situated on a line which moves from left to right in downward direction at 45° angle from 0-X axis, this condition is indicative of perfect negative correlation.

c. Karl Pearson's Coefficient of Correlation Method:

This is the best mathematical method of determining the correlation. Coefficient of correlation (r) is obtained by dividing the product of values of covariance of the two series by the product of their standard deviations.

$$r = \frac{Cov.(X.Y)}{\sigma_X . \sigma_Y}$$

Where σ_X and σ_Y are the standard deviation of variables of data series, X and Y. Covariance of tow series is obtained by dividing the sum of the products of deviations of two series and the arithmetic means by the number of observations

$$\text{Cov.}(X.Y) = \frac{\Sigma(X-\bar{X})(Y-\bar{Y})}{N}$$

$$\text{So,} \quad r = \frac{\Sigma(X-\bar{X})(Y-\bar{Y})}{N.\sigma_X.\sigma_Y}$$

Where $\bar{X}$ = The arithmetic mean of series X

$\bar{Y}$ = Arithmetic mean of series Y

σ_X = Standard deviation of series X

σ_Y = Standard deviation of series Y

N = Number of observations

If $X-\bar{X}=x$ and $Y-\bar{Y}=y$

$$r = \frac{\Sigma xy}{n\sigma x\,\sigma y}$$

$$\text{Since } \sigma x = \frac{\sqrt{\Sigma x^2}}{N} \text{ and } \sigma y = \frac{\sqrt{\Sigma y^2}}{N}$$

$$\text{Therefore } r = \frac{\Sigma xy}{N\left(\frac{\sqrt{\Sigma x^2}}{N}.\frac{\sqrt{\Sigma y^2}}{N}\right)}$$

$$\text{or } r = \frac{\Sigma xy}{\sqrt{\Sigma x^2}\times\sqrt{\Sigma y^2}}$$

If the numbers of observations are small, Pearson's coeffcient of correlation is calculated by the following formula:

$$r = \frac{N\Sigma xy - \Sigma x.\Sigma y}{\sqrt{N\Sigma x^2-(\Sigma x)^2}\sqrt{N\Sigma y^2-(\Sigma y)^2}}$$

If the data in two series are classified, Pearson's coefficient of correlation is calculated by the following formula:

$$r = \frac{\Sigma dxdy - N(X - A_1)(Y - A_2)}{N \sigma x \sigma y}$$

Where, A_1 = assumed mean of series X

A_2 = assumed mean of series Y

dx = deviation from assumed mean of series – X or $(X - A)$

dy = deviation from assumed mean of series Y or $(Y - A_2)$

σx = standard deviation of series X

σy = standard deviation of series Y

The above formula can also be expressed as follows:

$$r = \frac{N \Sigma dxdy - \Sigma dx \Sigma dy}{\sqrt{N \Sigma dx^2 - (\Sigma dx)^2} \sqrt{N \Sigma dy^2 - (\Sigma dy)^2}}.$$

Example. Calculate Pearson's coefficient of correlation from the following data:

Series X—18, 20, 22, 27, 27, 26, 27, 29, 28, 26.

Series Y—23, 27, 28, 28, 29, 30, 31, 33, 35, 36.

Solution.

S. No.	Series X			Series Y			
	Observation (X)	Deviation $dx = X - \bar{X}$	dx^2	Observation (Y)	Deviation $dy = Y - \bar{Y}$	dy^2	$dx\,dy$
1	18	18 – 25 = – 7	49	23	23 – 30 = – 7	49	49
2	20	20 – 25 = – 5	25	27	27 – 30 = – 3	9	15
3	22	22 – 25 = – 3	9	28	28 – 30 = – 2	4	6
4	27	27 – 25 = 2	4	28	28 – 30 = – 2	4	– 4
5	27	27 – 25 = 2	4	29	29 – 30 = – 1	1	– 2
6	26	26 – 25 = 1	1	31	30 – 30 = 0	0	0
7	27	27 – 25 = 2	4	30	31 – 30 = 1	1	2
8	29	29 – 25 = 4	16	33	33 – 30 = 3	9	12
9	28	28 – 25 = 3	9	35	35 – 30 = 5	25	15
10	26	26 – 25 = 1	1	36	36 – 30 = 6	36	6
10	$\Sigma X = 250$		$\Sigma dx^2 = 122$	$\Sigma Y = 300$		Σdy^2	$\Sigma dx\,dy$
Mean	$\bar{X} = \frac{250}{10} = 25$			Mean $\bar{Y} = \frac{300}{10} = 30$		=138	=105 – 6 =99

$\bar{X} = 25$

$\bar{Y} = 30$

$$\sigma_X = \frac{\sqrt{\Sigma dx^2}}{n} = \frac{\sqrt{122}}{10} = 3.49$$

$$\sigma_Y = \frac{\sqrt{\Sigma dy^2}}{n} = \frac{\sqrt{138}}{10} = 3.71.$$

Pearson's coefficient of correlation:

$$r = \frac{\Sigma dx\, dy}{n\sigma_X \sigma_Y}$$

$$= \frac{99}{10 \times 3.49 \times 3.71} = \frac{99}{129.479}$$

$r = 0.76.$

Example. Calculate Pearson's coefficient of correlation from the following data series:

Series X	63	66	67	67	71	69	68	59
Series Y	66	64	65	66	67	68	70	71

Solution. Suppose that the assumed mean for series $X(A_1) = 68$ and the assumed mean for series $Y(A_2) = 67$.

S. No.	Series X			Series Y			
	Observation (X)	$dx = X - A_1$	dx^2	Observation (Y)	$dy = Y - A_2$	dy^2	$dx\, dy$
1	63	$63 - 68 = -5$	25	66	$66 - 67 = -1$	1	5
2	66	$66 - 68 = -2$	4	64	$64 - 67 = -3$	9	6
3	67	$67 - 68 = -1$	1	65	$65 - 67 = -2$	4	2
4	67	$67 - 68 = -1$	1	66	$66 - 67 = -1$	1	1
5	71	$71 - 68 = 3$	9	$67 = A_2$	$67 - 67 = 0$	0	0
6	69	$69 - 68 = 1$	1	68	$68 - 67 = 1$	1	1
7	$68 = A_1$	$68 - 68 = 0$	0	70	$70 - 67 = 3$	9	0
8	59	$59 - 68 = -9$	81	71	$71 - 67 = 4$	16	-36
$n = 8$		$\Sigma dx = -14$	$\Sigma dx^2 = 122$		$\Sigma dy = 1$	$\Sigma dy^2 = 41$	$\Sigma dx\, dy = 15 - 36 = -21$

Coefficient of correlation

$$r = \frac{n\Sigma dx\, dy - \Sigma dx\, dy}{\sqrt{n\Sigma dx^2 - (\Sigma dx)^2}\sqrt{n\Sigma dy^2 - (\Sigma dy)^2}}$$

$$r = \frac{8 \times -21 - (1 \times -14)}{\sqrt{8 \times 122 - (-14)^2}\sqrt{8 \times 41 - (1)^2}}$$

$$= \frac{-168 + 14}{\sqrt{976 - 196}\sqrt{328 - 1}} = \frac{-154}{\sqrt{780} \times \sqrt{327}}$$

$$= \frac{-154}{\sqrt{255060}} = \frac{-154}{505.03}$$

$r = -0.305.$

d. Spearman's Ranking Method:

Professor Charls Spearman worked out a method for determining correlation in which the values of all data of a series are assigned ranks in decreasing or increasing (ascending) order. In this ranking process, the highest value is given rank 1 and the next higher value is given rank 2 and so on. In some series the values of two or more data are similar.

In that case, the mean of the ranks will be equally shared by those data, as for example in one series there are two observations; one at S. No. 3 and the other at S. No. 10 of 67 each. In ranking process 67 at S. No. 3 and 67 at S. No. 10 instead of being ranked 6 and 7 respectively are ranked at 6.5 (mean of rank 6 and rank 7).

In the same way if there are three or more data in a series as have got same value, all those data will share the rank which will be the mean of their ranks. The number or frequency of the data with similar value is indicated by m.

In the next step, the difference between the ranks (D) of respective data of the two series arc obtained ($D = R_1 - R_2$) which may be positive or negative figures. Then after, the values of D^2 and sum of D^2 ($= \sum D^2$) are determined.

For two such series as are taking in data with similar values, the following formula is used to determine the coefficient of correlation by ranking method (Symbolized by Rho = p):

$$\rho = 1 - \frac{6\Sigma D^2}{N(N^2-1)}$$

For determining correlation coefficient by ranking method of two such series as have got 2 or more data of similar values, the following Spearman's formula is used:

$$\rho = \frac{1 - 6[\Sigma D^2 + \frac{1}{12}(m_1^3 - m_1) + \frac{1}{12}(m_2^3 - m_2) + \frac{1}{12}(m_3^3 + m_3)...]}{N(N^2-1)}$$

Where D = difference between the ranks of respective data $(R_1 - R_2)$

N = total number of data in a series

m = frequencies of different data of same value in the series.

Example. Calculate coefficient of correlation for the following series by Spearman's ranking method:

Series X	64	65	67	63	68	62	68	70	66	67	69	71
Series Y	65	68	68	66	69	66	71	68	65	67	68	70

Solution.

S. No.	*Series X*	*Series Y*	*Ranks of series X (R_1)*	*Ranks of series Y (R_2)*	*$D = R_1 - R_2$*	*D^2*
1	64	65	10	11.5 m_5	– 1.5	2.25
2	65	68	9	5.5 m_3	3.5	12.25
3	67	68	6.5 m_2	5.5 m_3	1.0	1.00
4	63	66	11	9.5 m_4	1.5	2.25
5	68	69	4.5 m_1	3	1.5	2.25
6	62	66	12	9.5 m_4	2.5	6.25
7	68	71	4.5 m_1	1	3.5	12.25
8	70	68	2	5.5 m_3	– 3.5	12.25
9	66	65	8	11.5 m_5	– 3.5	12.25
10	67	67	6.5 m_2	8	– 1.5	2.25
11	69	68	3	5.5 m_3	– 2.5	6.25
12	71	70	1	2	– 1.0	1.00
$n = 12$						$\Sigma D^2 = 72.5$

$$\rho = \frac{1 - 6[\Sigma D^2 + \frac{1}{12}(m_1^3 - m_1) + \frac{1}{12}(m_2^3 - m_2) + \frac{1}{12}(m_3^3 - m_3) + \frac{1}{12}(m_4^3 - m_4) + \frac{1}{12}(m_5^3 - m_5)]}{N(N^2-1)}$$

Here the frequencies of m_1 data is 2, that of m_2 is 2, that of m_3 is 4, $m_4 = 2$ and $m_5 = 2$

$$\therefore P = 1 - \frac{6\left[72.50 + \frac{1}{12}(2^3-2) + \frac{1}{12}(2^3-2) + \frac{1}{12}(4^3-4) + \frac{1}{12}(2^3-2) + \frac{1}{12}(2^3-2)\right]}{12(144-1)}$$

$$= 1 - \frac{72.50 + \frac{1}{12}(6) + \frac{1}{12}(6) + \frac{1}{12}(60) + \frac{1}{12}(6) + \frac{1}{12}(6)}{2(144-1)}$$

$$= 1 - \frac{72.50 + 0.5 + 0.5 + 5.0 + 0.5 + 0.5}{2(143)}$$

$$= 1 - \frac{79.50}{286}$$

P = 1-0.278 = 0.722 (coefficient of correlation), i.e., the correlation between the two data series is moderate.

Example:

Calculate the coefficient of correlation by Spearman's ranking method and indicate the degree of correlation in the following two data series:

Series X	19	28	26	23	27	13	26	31	21	22
Series Y	21	28	23	25	31	17	22	29	27	20

Solution.

S. No.	Series X	Series Y	Rank of data in series X (R_1)	Rank of data in series Y (R_2)	$D = R_1 - R_2$	D^2
1	19	21	9	8	+1	1
2	28	28	2	3	-1	1
3	26	23	4.5	6	-1.5	2.25
4	23	25	6	5	+1	1
5	27	31	3	1	+2	4
6	13	17	10	10	0	0
7	26	22	4.5	7	-2.5	6.25
8	31	29	1	2	-1	1
9	21	27	8	4	4	16
10	22	20	7	9	-2	4
$n = 10$						$\Sigma D^2 = 36.50$

$$\text{Coefficient of correlation } (P) = 1 - \frac{6\Sigma D^2}{N(N^2-1)}$$

$$P = 1 - \frac{6 \times 36.50}{10(100-1)}$$

$$= 1 - \frac{6 \times 36.50}{990}$$

$$= 1 - \frac{7.3}{33} \text{ or } 1 - 0.22$$

$$= +0.78.$$

The value of coefficient of correlation + 0.78 indicates high degree of positive correlation.

e. Correlation Coefficient by Concurrent Deviation:

This method is used to indicate whether the correlation is in positive or negative direction especially in the data series characterized by short-term fluctuations of data.

Correlation coefficient by concurrent deviation is calculated as follows:

1. First of all, the direction of deviation [positive (+) or negative (-)] of each observation in respect of preceding data are marked for different series in separate columns. If the value of data is greater than that of the preceding data of the series, the direction of deviation is marked + and if it is less, then the direction of deviation will be marked

2. Next, the deviation signs of respective data of the two series are multiplied (+ x + = +, + X – = – and – X – = +) and the products are recorded in a separate column.

3. The total number of positive signs in the column for product of deviation signs is recorded which is called concurrent deviation (= C)

4. The coefficient of correlation (RC) by concurrent deviation is determined by the following formula:

$$RC = \pm\frac{\sqrt{\pm(2C-N)}}{N}$$

Where, C = total number of + signs in the column for products of two deviations

N = number of observations in a series.

The following example will illustrate the process:

Example:

Calculate the co-efficient of correlation of the following two data series by concurrent deviation method:

Series X	50	51	53	51	50	54	55	56	61	62	63
Series Y	73	71	75	76	79	80	83	84	86	85	91

Solution.

S. No.	*Series X*	*Deviation from preceding data*	*Series Y*	*Deviation from preceding data*	*Product of deviation signs of respective data in two series*
1	50		73		
2	51	+	71	-	-
3	53	+	75	+	+
4	51	-	76	+	-
5	50	-	79	+	-
6	54	+	80	+	+
7	55	+	83	+	+
8	56	+	84	+	+
9	61	+	86	+	+
10	62	+	85	-	-
11	63	+	91	+	+
	$N = 10$		$N = 10$		$C = 6$

$$RC = \pm\frac{\sqrt{\pm(2C-N)}}{N}$$

$$= \pm\frac{\sqrt{\pm 2\times6-10}}{10}$$

$$= \pm\sqrt{\pm\frac{12-10}{10}} = \sqrt{\frac{2}{10}}$$

$RC = +0.477.$

Therefore, the series X and series Y show positive correlation.

Regression:

Regression is used to denote the estimation and prediction of the average value of one variable for a specified value of the other variable. This estimation is done by deriving a suitable equation on the basis of available bivariate data. This equation is called Regression equation and its geometrical representation is called Regression curve.

The regression equation requires the Regression coefficient. The regression coefficient (b) is calculated in two different ways.

The regression coefficient of y on x is:

and the regression coefficient of x on y is:

δy = standard deviation of y

$$b_{yx} = r\frac{\delta y}{\delta x}$$

δx = standard deviation of x

$$b_{xy} = r\frac{\delta x}{\delta y}$$

Regression Line:

When the bivariate data are plotted on graph paper, the concentration of points shows certain pattern showing the relationship. When the trend points are found to be linear then by least square method we can obtain the regression line.

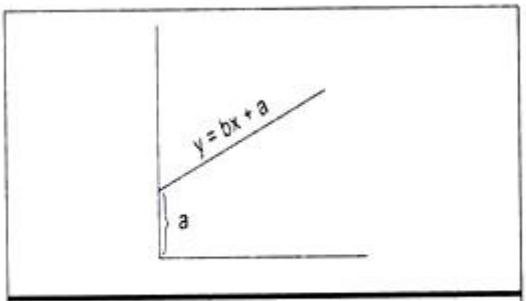


Fig. 13.2: Regression line showing the slope and constant

If two variables are linearly related then the relation can be expressed as y = bx + a, where 'b' is the slope of the line and 'a' is the intercept of that line.

If we put the values on regression equation then we can get the regression line equation easily. With the help of Example 1, we can discuss it.

The regression equation of x on y is:

$$x - \bar{x} = b_{xy}(y - \bar{y})$$

where x = Value of x; y = Value of y;
$\bar{x}$ = Mean of x; $\bar{y}$ = Mean of y;

$$b_{xy} = r\frac{\delta x}{\delta y}$$

In example 1,

$$\bar{x} = 13, \quad \bar{y} = 25$$

$$\delta x = \sqrt{\frac{160}{5}} = \sqrt{32} = 5.65$$

$$\delta y = \sqrt{\frac{358}{5}} = \sqrt{71.6} = 8.46$$

$$b_{xy} = .986 \times \frac{5.65}{8.46} = .986 \times .66 = 0.658$$

So, the equation of x on y will be

$$x - 13 = 0.658(y - 25)$$

$$\text{or} \quad x = 0.658y - 16.46 + 13$$

$$\text{or} \quad x = 0.658y - 3.46$$

$$\text{or} \quad y = \frac{x}{0.658} - \frac{3.46}{0.658} = 1.5x - 5.25$$

$$\therefore \quad y = 1.5x - 5.25$$

The regression equation of y on x is:

$$y - \bar{y} = b_{yx}(x - \bar{x})$$

$$\text{or} \quad y - 25 = 1.476(x - 13)$$

$$\text{or} \quad y = 1.476x - 19.19 + 25$$

$$\text{or} \quad y = 1.476x + 5.8$$

$$b_{yx} = .986 \times \frac{8.46}{5.65}$$

$$= .986 \times 1.49$$

$$= 1.476$$

Significance:

This equation will help us to get the estimate of one variable when the other is given or else we can predict the values of one variable when the other one is also assumed, i.e., extrapolation is possible.

Meaning of Regression Coefficient:

Regression coefficient is a statistical measure of the average functional relationship between two or more variables. In regression analysis, one variable is considered as dependent and other(s) as independent. Thus, it measures the degree of dependence of one variable on the other(s). Regression coefficient was first used for estimating the relationship between the heights of fathers and their sons.

Properties of Regression Coefficient:

The important properties of regression coefficient are given below:

1. It is denoted by b.

2. It is expressed in terms of original unit of data.

3. Between two variables (say x and y), two values of regression coefficient can be obtained. One will be obtained when we consider x as independent and y as dependent and the other when we consider y as independent and x as dependent. The regression coefficient of y on x is represented as byx and that of x on y as bxy.

4. Both regression coefficients must have the same sign. If byx is positive, bxy will also be positive and vice versa.

5. If one regression coefficient is greater than unity, then the other regression coefficient must be lesser than unity.

6. The geometric mean between two regression coefficients is equal to the coefficient of correlation, r = $\sqrt{byx.bxy}$

7. Arithmetic mean of both regression coefficients is equal to or greater than coefficient of correlation.

(byx + bxy)/2 = equal or greater than r

Regression coefficients are classified as:

(1) Simple, partial and multiple

(2) Positive and negative and

(3) Linear and non-linear.

Computation of Regression Coefficient:

Regression coefficient can be worked out from both un-replicated and replicated data. For calculation of regression coefficient from un-replicated data three estimates, viz.,

(1) sum of all observations on x and y ($\sum x$, $\sum y$) variables, (2) their sum of squares ($\sum x^2$ and $\sum y^2$) and (3) sum of products of all observations on x and y variables ($\sum xy$).

Then regression coefficient can be worked out as follows:

$byx = \sum xy - (\sum x.\sum y) / \sum y^2 - (\sum y)^2$
$bxy = \sum xy - (\sum x.\sum y) / \sum x^2 - (\sum x)^2$

In case of replicated data, first analysis of variances and co-variances is performed and then regression coefficient is worked out as given below:

byx = Cov. (xy)/Vx, and bxy = Cov. (xy) /Vy,

where Cov = co-variance between x and y, Vx = variance of x, and Vy = variance of y.

The significance of regression coefficient is generally tested with the help of t-test.

First t is worked out as given below:

t = byx /SE (b)

The calculated value of t is compared with the table value of t at desired level of significance and appropriate degrees of freedom. If the calculated value of t is greater than table value, it is considered significant and vice versa.

The value of dependent variable can be predicated with the value of independent variable. By substituting the value of dependent variable we can get value of independent variable.

Applications of Regression Coefficient in Genetics:

Regression analysis has wide applications in the field of genetics and breeding as given below:

1. It helps in finding out a cause and effect relationship between two or more plant characters.

2. It is useful in determining the important yield contributing characters.

3. It helps in the selection of elite genotypes by indirect selection for yield through independent characters.

4. It also helps in predicting the performance of selected plants in the next generation.

Linear Regression Analysis:

The statistical analysis employed to find out the exact position of the straight line is known as Linear regression analysis. From simple correlation analysis if there exist relationship between independent variable x and dependent variable y then the relationship can be expressed in a mathematical form known as Regression equation.

From regression equation we can work out the actual value of y variable (dependent) based on X variable (independent) and such values plotted graphically will give precise nature of the straight line (point of interception to y-axis can be noted).

Simple regression equation Yx = a + bx, where a and b are constant which minimize the residual error of Y. Y is the dependent variable.

The constants a and b can be obtained from the formula:

$$b = \frac{\Sigma dxdy}{\Sigma dx^2}, \quad \text{where } \Sigma dxdy = \Sigma xy - \frac{\Sigma x \Sigma y}{n};$$

$$\Sigma dx^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{n}.$$

$$a = \bar{y} - b\bar{x}.$$

Example 2:

From the data find out the regression equation and draw a regression line on the graph paper.

No. of Branches (x)	*No. of Capsules* (y)	x^2	xy
2	4	4	8
5	10	25	50
8	15	64	120
10	20	100	200
15	25	225	375
20	30	400	600
25	40	625	1000
$\Sigma x = 85$	$\Sigma y = 144$	$\Sigma x^2 = 1443$	$\Sigma xy = 2353$

Solution:

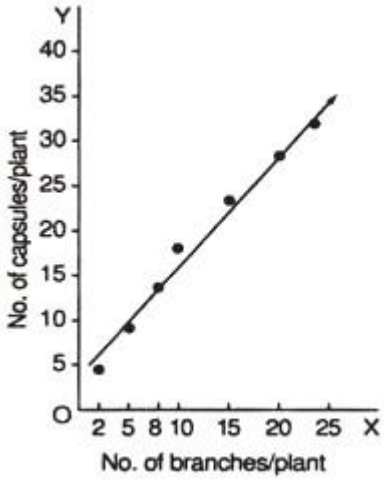
$$\bar{x} = \frac{85}{7} = 12.14; \quad \bar{y} = \frac{144}{7} = 20.57.$$

$$b = \frac{\Sigma xy - \frac{\Sigma x \Sigma y}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}} = \frac{2353 - \frac{85 \times 144}{7}}{1443 - \frac{(85)^2}{7}} = 1.48.$$

$$a = \bar{y} - b\bar{x} = 20.57 - (1.48)(12.14) = 2.6.$$

Therefore, the regression equation $y_x = a + bx$, $y_x = 2.6 + 1.48x$.

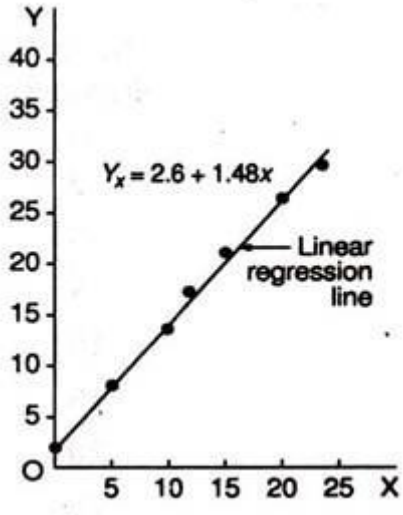
Using the regression equation $y_x = 2.6+1.48x$ the actual values of dependent variable can be worked out.



If $x = 0, \quad y = 2.60$
$x = 2, \quad y = 5.56$
$x = 5, \quad y = 10.00$
$x = 8, \quad y = 14.44$
$x = 10, \quad y = 17.40$
$x = 15, \quad y = 24.80$
$x = 20, \quad y = 32.20$
$x = 25, \quad y = 39.60$

Using data of the given example the straight line is drawn but the point of interception to y-axis is lacking and, therefore, precise nature of the straight line is not understood. However, from the straight line it is evident that the variables were significantly and positively correlated between themselves.

These set of values plotted graphically will give a straight and the precise nature of the straight line can be obtained from x = 0, y = 2.6 (point of interception to y- axis can be found out).



Multiple Regression:

Example 3:

The following data giving mean yield (grain), mean ear number per plant and mean grain number per acre of 10 wheat varieties were obtained in low soil condition moisture plots in the experiment conducted at IARI during 2000-01 to study the influence of soil drought on the relation between yield and ear character.

Sl. No.	Mean grain yield in gm (y)	Mean ear no. per plant (x_1)	Mean grain no. per ear (x_2)
1	42.8	3.14	29.4
2.	40.8	3.04	24.2
3.	34.9	3.92	21.6
4.	34.3	3.36	27.5
5.	30.8	3.47	24.9
6.	29.6	3.40	22.5
7.	24.2	3.12	20.5
8.	18.0	4.28	16.1
9.	16.6	3.02	19.7
10.	12.4	3.16	16.3

Fit a multiple regression equation giving mean grain yield in terms of mean ear no. per plant and mean grain no. per ear.

Solution: Total of $y = 284.4$; $\bar{y} = 28.44$.
Total of $x_1 = 33.91$; $\bar{x}_1 = 3.391$.
Total of $x_2 = 222.7$; $\bar{x}_2 = 22.27$.

$$S_y^2 = \Sigma y^2 - \frac{(\Sigma y)^2}{n} = 9054.74 - \frac{(284.4)^2}{10} = 966.40$$

$$S_{x_1}^2 = \Sigma x_1^2 - \frac{(\Sigma x_1)^2}{n} = 116.52 - \frac{(33.91)^2}{10} = 1.53$$

$$S_{x_2}^2 = \Sigma x_2^2 - \frac{(\Sigma x_2)^2}{n} = 5132.31 - \frac{(222.7)^2}{10} = 172.78$$

$$S_{yx_1} = \Sigma yx_1 - \frac{\Sigma y \Sigma x_1}{n} = 959.86 - \frac{284.4 \times 33.91}{10} = -4.54$$

$$S_{yx_2} = \Sigma yx_2 - \frac{\Sigma y \Sigma x_2}{n} = 6690.73 - \frac{284.4 \times 222.7}{10} = 357.14$$

$$S_{x_1x_2} = \Sigma x_1x_2 - \frac{\Sigma x_1 \Sigma x_2}{n} = 740.73 - \frac{33.91 \times 222.7}{10} = -5.45.$$

Multiple Regression Equation

$$Syx_1 = b_1Sx_1^2 + b_2Sx_1x_2 \quad (1)$$
$$Syx_2 = b_1Sx_1Sx_2 + b_2Sx_2^2 \quad (2)$$

or, $b_1Sx_1^2 + b_2Sx_1x_2 = Syx_1$

$b_1Sx_1x_2 + b_2Sx_2^2 = Syx_2$

$$\text{or,} \quad \left.\begin{aligned} b_1 \times 1.53 + b_2 \times (-5.45) &= -4.54 \\ b_1 \times (-5.45) + b_2 \times (172.78) &= 357.14 \end{aligned}\right\} \begin{aligned} &\times 5.45 \\ &\times 1.53 \end{aligned}$$

$$\text{or,} \quad 8.3385b_1 - 29.7025b_2 = -24.7430$$
$$-8.3385b_1 + 264.3534b_2 = 546.4242$$
$$\text{By adding} \quad 234.6509b_2 = 521.6812$$

$$b_2 = \frac{521.6812}{234.6509} = 2.22$$

Again,

$$1.53b_1 - 5.45 \times 2.22 = -4.54$$
$$1.53b_1 = 12.10 - 4.54 = 7.56.$$

$$\therefore\ b_1 = \frac{7.56}{1.53} = 4.94.$$

Now $b_0 = \bar{y} - b_1\bar{x}_1 - b_2\bar{x}_2 = 28.44 - 4.94 \times 3.391 - 2.22 \times 22.27$

$= 28.44 - 16.75 - 49.44 = -37.75.$

Multiple Regression Equation $Y = b_0 + b_1x_1 + b_2x_2$.

$$\therefore\ Y = -37.75 + 4.94x_1 + 2.22x_2.$$

$$\therefore\ \text{SS Regression } (SR^2) = b_1S_yx_1 + b_2Syx_2$$
$$= 4.94 \times (-4.54) + 2.22 \times 357.14$$
$$= 22.43 + 792.85 = 770.42.$$

Anova Table

Source of Variation	df	SS	MS	F
Regression	2	$SR^2 = 770.42$	385.21	13.76
Deviation from regression	7	195.98	28.00	
Total	9	$Sy^2 = 966.40$		

$$\left.\begin{aligned} F_{0.05:2,7} &= 4.75 \\ F_{0.01:2,7} &= 9.55 \end{aligned}\right\} \text{Table values.}$$

Since the calculated value of F in respect of regression is greater than the table value both at 5% and 1% level of significance, the regression is highly significant. Thus, mean grain yield is significantly related to ear characters.

Probable Questions:

1. Why we use chi square test? What is degree of freedom?
2. What are the applications of chi square test?
3. What are the limitations of chi square test?
4. What do you mean by level of significance?
5. What is correlation? Discuss different types of correlation with suitable graph.
6. What is coefficient of correlation? How it is determined?
7. What are the properties of coefficient of correlation?
8. State the significance of coefficient of correlation.
9. Discuss graphic method for determination of coefficient of correlation.
10. Discuss dotogram method for determination of coefficient of correlation.
11. Discuss Karl Pearson's Coefficient of Correlation Method for determination of coefficient of correlation.
12. What is regression? How regression coefficient is calculated?
13. What is regression line?
14. What is linear regression ?
15. What is multiple regression?

Suggested readings:

1.Zar, J.H. (2013) Biostatistical Analysis
2.Pagano M., Gauvreau, K, (2000), Principles of Biostatistics.

Disclaimer :

The study materials of this book have been collected from books, various e- books, journals and other e-sources.

Post-Graduate Degree Programme (CBCS)

in

ZOOLOGY

(M.Sc. Programme)

SEMESTER-II

APPLIED ZOOLGY

ZGECT-201

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

Kalyani, Nadia

West Bengal, India

CONTENT WRITER:

Sl. No.	Name of Content Writer	Designation	Unit
1.	Dr. Sudeshna Banerjee	Assistant Professor of Zoology, Directorate of Open and Distance Learning, KU	Unit V,X-XII,XIV,XV
2.	Dr. Subhabrata Ghosh	Assistant Professor of Zoology, Directorate of Open and Distance Learning, KU	Unit I-IV, VI-IX,XIII

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Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2020 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Manas Kumar Sanyal, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every member of PGBOS (DODL), University of Kalyani. Heartfelt thanks are also due to the Course Writers-faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and coordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

All the Self Learning Materials are self writing and collected from e-book, journals and websites.

Director
Directorate of Open and Distance Learning
University of Kalyani

List of PGBOS members

1	Dr. Subhankar Kumar Sarkar, Associate Professor and Head, Dept. of Zoology, University of Kalyani	Chairperson
2	Prof. Banabehari Jana , Retd Professor, Dept of Zoology, University of Kalyani	External Expert
3	Prof. Kausik Mondal, Professor, Dept. of Zoology, University of Kalyani	Member
4	Dr. Kakali Bhadra, Associate Professor, Dept. Of Zoology, University of Kalyani	Member
5	Dr. Subhabrata Ghosh, Assistant Professor of Zoology, DODL, University of Kalyani	Member
6	Dr. Sudeshna Banerjee, Assistant Professor of Zoology, DODL, University of Kalyani	Member
7	Director, DODL, University of Kalyani	Convener

HARD CORE THEORY PAPER (ZGECT- 201)

APPLIED ZOOLOGY

Module	Unit	Content	Credit	Pg. No.
ZGECT - 201 (APPLIED ZOOLOGY)	I	Categories of wild life	4	
	II	Wild life and wild life habitat in India: Wild life wealth of India		
	III	Wild life management: Distribution, status, habitat utilization pattern, threats and survival of – Royal Bengal Tiger, *Rhinoceros*, Olive Ridley Turtle		
	IV	National and International efforts for conservation: CITES, IUCN, CBD, Protected area concept		
	V	Conservation of natural enemies of insect pest		
	VI	Concept of habitat and niche		
	VII	Ecological principles		
	VIII	Community ecology: nature of communities; levels of species diversity and its measurements		
	IX	Biogeographical zones of India		
	X	Environmental management: Solid waste management; Bioremediation; Bioreactors in Environment monitoring.		
	XI	Organic farming and vermicomposting		
	XII	Insect pollinators: Types and role in agriculture		
	XIII	Genetics of Human Diseases. Nutrigenomics, Pharmacogenomics and their applications.		
	XIV	Preliminary knowledge on zoonotic diseases		
	XV	Immunodiagnostics: Concepts of Innate and Humoral Immunity, Antigen Presentation, Antigen-antibody interactions, its application in medical Diagnosis (Western Blot, ELISA, RIA, Elispot, FACS, Immunofluorescence		

Total Counselling Session 12 hrs

Unit-I

Categoriesofwildlife

Objective: In this unit you will learn about categories of wild life.

Introduction:

The International Union for Conservation of Nature (IUCN), established in 1948, is an international organization comprised of over 1,200 government and non-government members. Its objective is to promote nature conservation and sustainable use of natural resources throughout the world. This organization also focusses on issues such as poverty, gender equality, and sustainable business practices in order to address its core objective. To achieve its goal, the organization engages in field-work, data collection and analysis, lobbying, and public education outreach. IUCN has organized animals under threat on the basis of their past and present distribution, abundance, rate of decline of population, quality of natural habitats and ecological importance. Priority of conservation of a species depends on the estimation of the level of threat and its probability of its extinction over a give period of time. IUCN developed the Red Data Book to fix the priority for conservation and identify those that are in immediate need of protection. IUCN red list of threatened species is an inventory of the conservation status of the species that are at high risk of extinction on a global scale. In 2003, IUCN developed a set of transparent , quantitative criteria to assess the conservation status of species t the regional and national level. IUCN along with species survival commission assess the status of species based on the observable decline in population, total number of living species and presence of the number of breeding pair. The current and projected trend of decline and probability of extinction in certain number of years or generation are also measured.

Reasons for species extinction

Main reasons for extinction are either natural or manmade. Through evolution, new species arise through the process of speciation and species become extinct when they are no longer able to survive in changing conditions or against superior competition. A typical species becomes extinct within 10 million years of its first appearance although some species, called living fossils, survive virtually unchanged for hundreds of millions of years. Extinction, though, is usually a natural phenomenon; it is estimated that 99.9% of all species that have ever lived are now extinct.

Various anthropogenic activities causing extinction are manmade reasons. Only recently scientists have become alarmed at the high rates of recent extinctions due to various

anthropogenic activities. Some of these anthropogenic activities include intentional or accidental introduction of invasive alien species, over exploitation and unscientific collection of Non-Timber Forest Produce (NTFPs) including medicinal plant, climate change, unsustainable tourism, habitat destruction, encroachment etc.

The IUCN Red List :

The IUCN is perhaps best known for its Red List of Threatened Species (also known as the Red List). The Red List, established in 1964, provides the conservation status of plant and animal species around the world. IUCN members determine the risk of a species' extinction by utilizing criteria such as population size, subpopulations, the number of mature individuals, generation, the decline in population size, extreme fluctuations in population size, fragmented populations and habitats, habitat area size, and distribution of the population.

The Red List provides scientifically based information about species' survival, promotes public education about biodiversity, influences governmental policies, and offers advice about conservation efforts. The category assigned to each species is reassessed every 5 to 10 years by the IUCN Species Survival Commission Specialist Groups. This list is generally accepted as the most comprehensive information on the health and conservation of the world's species.

Objectives of IUCN

The International Union for Conservation of Nature works to achieve the following goals:

1. To provide scientific data on the status of species and subspecies at a global level.
2. To address the factors of concern and spread awareness regarding the species and biodiversity extinction.
3. To plan a layout for the conservation of biodiversity.

IUCN Red List Users

The IUCN Red List provides accurate data on the status of different species on the Earth. This information is used by various departments, institutes, and organizations.

The users of the IUCN Red List are given below:

- Government agencies (National & International)
- Wildlife organizations and departments
- Conservation-related NGOs
- Natural resource planners

- Educational organizations
- Zoos and aquariums
- Media
- Business communities

Purpose of the IUCN Red List Data

The information cited in the IUCN Red List is used by various organizations in the following ways:

- International Agreements such as CITES, Ramsar Convention use the Red List data to make important decisions in sync with the status of nature as and when required.
- World Bank Group performance standard uses the IUCN Red List data to evaluate the risk of damage to biodiversity due to large-scale infrastructures and global projects.
- Zoos and National parks use this information to upgrade important policies like parks regulations from time to time.

Following are the 9 categories in the IUCN red list:

IUCN Conservation Plan

The strategy for the conservation of nature by IUCN is as follows:

1. **Assess –** Focus on monitoring species and informing the world about the status and trends of biodiversity, thus providing measures for the protection of our biosphere.
2. **Plan –** Aims to enhance collaborative and science-based strategies to ensure the most effective species conservation actions.
3. **Act –** Improve the status of biodiversity, by mobilizing actions involving governments, educational institutions, civil society, and the private sector.
4. **Communicate –** The effectiveness of IUCN's species conservation work is enhanced through strategic and targeted communications.

IUCN Categories: The IUCN Red List assigns a specific category to each of the evaluated species. The major IUCN threatened categories (IUCN Red List Categories, 1995) currently recognised, together with their definitions are:

(i) Extinct (EX):

Species not definitely located in the wild during the past fifty years but which may survive in cultivation (e.g. Franklinia aloetamha). Some authors suggest that 'Extinct' should denote those taxa that have been totally lost and that the terminology **'Extinct in the wild'** should be used to refer to species lost in the wild, while living under cultivation (dubbed by IUCN as EW).

(ii) Endangered (EN):

Species in danger of extinction (within a few decades) and whose survival is unlikely if the causal factors continue to operate (*Areca concinna, Euphorbia obdelkuri*). In this category are included those taxa whose numbers have been reduced to a critical level or whose habitats have been so drastically reduced that they are deemed to be in immediate danger of extinction. Also included are taxa that may now be extinct even though seen in the wild in the past 50 years. The other criteria are 50% decline in the last 10 years; <5,000 km^2 area of occupancy or <500 km^2 in fragmented areas; 2,500 individuals or subpopulation of 250 mature individuals.

The category Critically Endangered (CR) includes species that face an extremely high risk of extinction in the wild in the immediate future. These are characterized by 80% decline in the last 10 years, 100 km^2 occupancy or 10 km^2 in fragmented areas.

(iii) Vulnerable (VU):

Taxa likely to move into the endangered category in near future if the causal factors continue to operate. Included in this category are taxa in which most or all populations decrease in size because of overexploitation, extensive destruction of habitat or other environmental disturbances. Also included are taxa with populations that are still abundant but under threat from severe adverse factors throughout their distribution range. The other criteria include 50% decline in last 20 years; < 20,000 km^2 occupancy or < 2,000 km^2 in fragmented populations; 10,000 individuals or subpopulation of 1,000 mature individuals.

(iv) Rare (R):

Taxa with small populations that are not endangered or vulnerable at present but are at risk are included under this category (*Lactuca saligna, Salvia saxicola*). A species may be rare because of restricted geographical range, high habitat specificity and small local population size, or thinly scattered over a more extensive range, or due to a

combination of two or more of these characteristics. Rare species have a population of less than 20,000 individuals. Some species are naturally rare and have never occurred in greater numbers, yet they are able to maintain these numbers. Other species become rare through man's action or other natural forces.

(v) Indeterminate (I):

Species considered definitely to be endangered, vulnerable or rare but for which information is insufficient to categorically assign them to any of these three categories.

(vi) Near Threatned (NT)

A taxon is Near Threatened when it has been evaluated against the criteria but does not qualify for Critically Endangered, Endangered or Vulnerable now, but is close to qualifying for or is likely to qualify for a threatened category in the near future.

(vii) Least Concern (LC) :

A taxon is Least Concern when it has been evaluated against the criteria and does not qualify for Critically Endangered, Endangered, Vulnerable or Near Threatened.

(viii) Data Deficient (DD):

A taxon is Data Deficient when there is inadequate information to make a direct, or indirect, assessment of its risk of extinction based on its distribution and/or population status.

(ix) Not Evaluated (NE):

A taxon is Not Evaluated when it is has not yet been evaluated against the criteria.

(x) Out of danger : The taxon formerly included in any one of the extinction prone categories (critically endangered, endangered, vulnerable or near threatened) but which are now considered as relatively secured because of the effective conservation measures or the previous threat to their survival has been removed.

(xi) Endemic: Taxon with restricted geographical distribution are called endemic taxon. Due to such restricted distribution with small population size, they are vulnerable to both natural and anthropological threats of extinction.

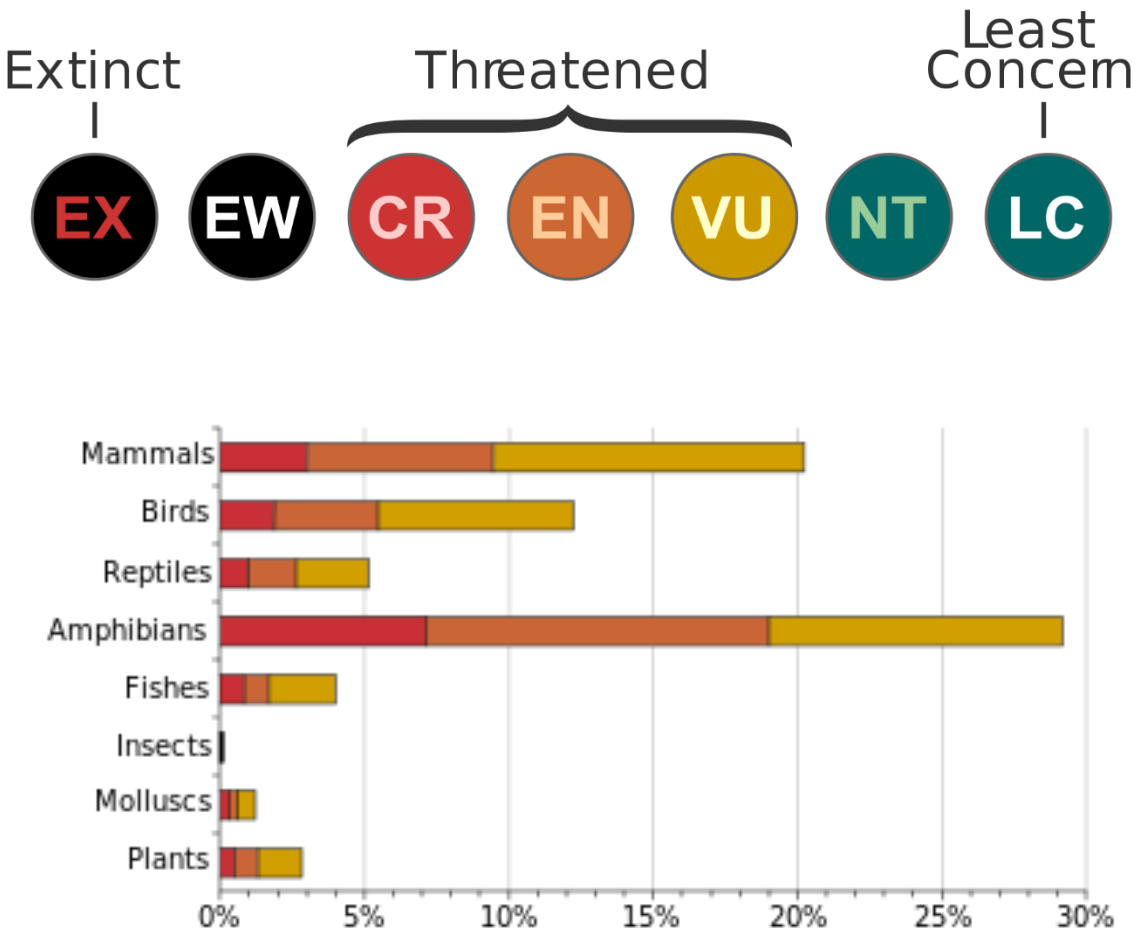
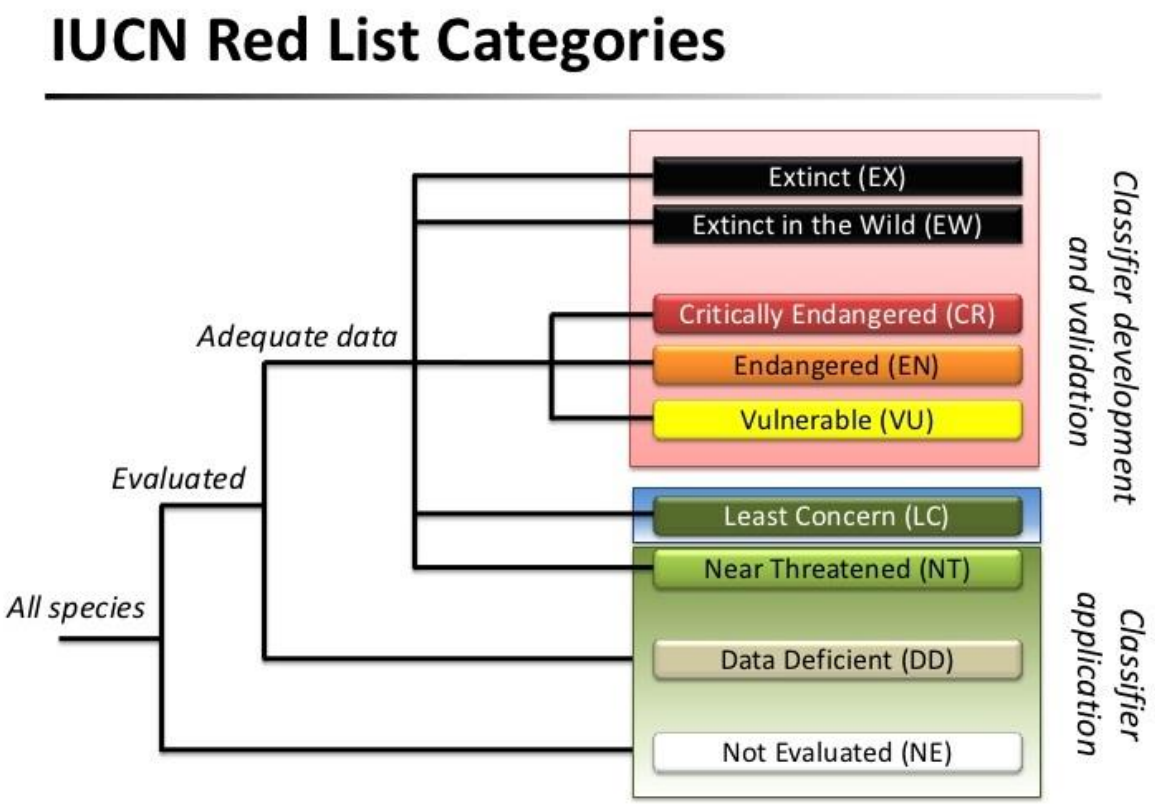


Figure: The percentage of species in several groups which are listed as critically endangered, endangered, or vulnerable on the 2007 IUCN Red List



IUCN Categories, and Some Animals in those Categories, Include:

a. **Extinct:** Examples: Atlas bear, Aurochs, Bali Tiger, Blackfin Cisco, Caribbean Monk Seal, Carolina Parakeet, Caspian Tiger, Dinosaurs, Dodo, Dusky Seaside Sparrow, Elephant Bird, Golden Toad, Great Auk, Haast's Eagle, Japanese Sea Lion, Javan Tiger, Labrador Duck, Moa, Passenger Pigeon, Pterosaurs, Saber-toothed cat, Schomburgk's deer, Short-faced bear, Steller's Sea Cow, Thylacine, Toolache Wallaby, Western Black Rhinoceros, Woolly Mammoth, Woolly Rhinoceros.

b. **Extinct in the Wild:** captive individuals survive, but there is no free-living, natural population. Examples: Barbary Lion (maybe extinct), Catarina Pupfish, Hawaiian Crow, Northern White Rhinoceros, Scimitar Oryx, Socorro Dove, Wyoming Toad.

c. **Critically Endangered:** It faces an extremely high risk of extinction in the immediate future. Examples: Addax, African Wild Ass, Alabama Cavefish, Amur Leopard, Arakan Forest Turtle, Asiatic Cheetah, Axolotl, Bactrian Camel, Brazilian Merganser, Brown Spider Monkey, California Condor, Chinese Alligator, Chinese

Giant Salamander, Gharial, Hawaiian Monk Seal, Iberian Lynx, Island Fox, Javan Rhino, Kakapo, Leatherback Sea Turtle, Mediterranean Monk Seal, Mexican Wolf, Mountain Gorilla, Philippine Eagle, Red Wolf, Saiga, Siamese Crocodile, Spix's Macaw, Southern bluefin tuna, Sumatran Orangutan, Sumatran Rhinoceros, Vaquita, Yangtze River Dolphin.

d. **Endangered:** It faces a very high risk of extinction in the near future. Examples: African Penguin, African Wild Dog, Asian Elephant, Asiatic Lion, Blue Whale, Bonobo, Bornean Orangutan, Chimpanzees, Dhole, Ethiopian Wolf, Hispid Hare, Giant Otter, Giant Panda, Goliath Frog, Gorillas, Green Sea Turtle, Grevy's Zebra, Hyacinth Macaw, Japanese Crane, Lear's Macaw, Malayan Tapir, Markhor, Persian Leopard, Proboscis Monkey, Pygmy Hippopotamus, Red-breasted Goose, Rothschild Giraffe, Snow Leopard, Steller's Sea Lion, Scopas tang, Takhi, Tiger, Vietnamese Pheasant, Volcano Rabbit, Wild Water Buffalo.

e. **Vulnerable:** It faces a high risk of extinction in the medium-term. Examples: African Elephant, American paddlefish, Clouded Leopard, Cheetah, Dugong, Far Eastern Curlew, Fossa, Galapagos Tortoise, Gaur, Blue-eyed cockatoo, Golden Hamster, Whale Shark, Crowned Crane, Hippopotamus, Humboldt Penguin, Indian Rhinoceros, Komodo Dragon, Lesser White-fronted Goose, Lion, Mandrill, Maned Sloth, Mountain Zebra, Polar Bear, Red Panda, Sloth Bear, Takin, Yak.

f. **Near Threatened:** These species may be considered threatened in the near future. Examples: African Grey Parrot, American Bison, starry blenny, Asian Golden Cat, Blue-billed Duck, Emperor Goose, Emperor Penguin, Eurasian Curlew, Jaguar, Leopard, Magellanic Penguin, Maned Wolf, Narwhal, Okapi, Solitary Eagle, Southern White Rhinoceros, Striped Hyena, Tiger Shark, White Eared Pheasant.

g. **Least Concern:** There is no immediate threat to the survival of the species. Examples: American Alligator, American Crow, Indian Peafowl, Baboon, Bald Eagle, Brown Bear, Brown Rat, Brown-throated sloth, Canada Goose, Cane Toad, Common Wood Pigeon, Cougar, Common Frog, Orca, Giraffe, Grey Wolf, House Mouse,[5] Human, Palm cockatoo, cowfish, Mallard, Meerkat, Mute Swan, Platypus, Red-billed Quelea, Red-tailed Hawk, Rock Pigeon, Scarlet Macaw, Southern Elephant Seal, Milk shark, Red howler monkey.

Probable Questions:

1. What is IUCN Red list? What is its objectives?
2. State the purpose of Red list data of IUCN?
3. Describe different categories of IUCN.

Suggested Readings :

1. Akçakaya, H.R. and Ferson, S. 2001. RAMAS® Red List: Threatened Species Classifications under Uncertainty. Version 2.0. Applied Biomathematics, New York.

2. Akçakaya, H.R., Ferson, S., Burgman, M.A., Keith, D.A., Mace, G.M. and Todd, C.A. 2000. Making consistent IUCN classifications under uncertainty. Conservation Biology 14: 1001-1013.

3. Baillie, J. and Groombridge, B. (eds). 1996. 1996 IUCN Red List of Threatened Animals. IUCN, Gland, Switzerland.

4. Burgman, M.A., Keith, D.A. and Walshe, T.V. 1999. Uncertainty in comparative risk analysis of threatened Australian plant species. Risk Analysis 19: 585-598.

5. Fitter, R. and Fitter, M. (eds). 1987. The Road to Extinction. IUCN, Gland, Switzerland.

6. Gärdenfors, U., Hilton-Taylor, C., Mace, G. and Rodríguez, J.P. 2001. The application of IUCN Red List Criteria at regional levels. Conservation Biology 15: 1206-1212.

UNIT-II
Wild Life and Wild Life Habitat in India: Wild life wealth of India

Objective: In this unit you will learn about wild life and wild life habitat in India.

Introduction:

India is home to a variety of animals. Apart from a handful of domesticated animals, such as cows, buffaloes, goats, chickens, and both Bactrian and Dromedary camels, India has a wide variety of animals native to the country. It is home to Bengal and Indochinese tigers, Asiatic lions, Indian and Indochinese leopards, snow leopards, clouded leopards, various species of Deer, including Chital, Hangul, Barasingha; the Indian Elephant, the Great Indian Rhinoceros, and many others. The region's diverse is preserved in more than 120 national parks, 18 Bio-reserves and more than 500 wildlife sanctuaries across the country. India has some of the most biodiverse regions of the world and contains four of the world's 36 biodiversity hotspots – the Western Ghats, the Eastern Himalayas, Indo-Burma and Sunda Land. Wildlife management is essential to preserve the rare and endangered endemic species. India is one of the seventeen megadiverse countries. According to one study, India along with the other 16 megadiverse countries is home to about 60-70% of the world's biodiversity India, lying within the Indomalaya ecozone, is home to about 7.6% of all mammalian, 12.6% of avian (bird), 6.2% of reptilian, and 6.0% of flowering plant species. Many Indian species are descendants of taxa originating in Gondwana, of which India originally was a part. Peninsular India's subsequent movement towards, and collision with, the Laurasian landmass set off a mass exchange of species. However, volcanism and climatic change 20 million years ago caused the extinction of many endemic Indian forms. Soon thereafter, mammals entered India from Asia through two zoogeographical passes on either side of the emerging Himalaya. As a result, among Indian species, only 12.6% of mammals and 4.5% of birds are endemic, contrasting with 45.8% of reptiles and 55.8% of amphibians.[9] Notable endemics are the Nilgiri leaf monkey and the brown and carmine Beddome's toad of the Western Ghats. India contains 172, or 2.9%, of IUCN-designated threatened species. These include the Asian elephant, the Asiatic lion, Bengal tiger, Indian rhinoceros, mugger crocodile, and Indian white-rumped vulture, which suffered a near-extinction from ingesting the carrion of diclofenac-treated cattle. In recent decades, human encroachment has posed a threat to India's wildlife; in response, the system of national parks and protected areas, first established in 1935, was substantially expanded. In 1972, India enacted the Wildlife Protection Act and Project Tiger to safeguard crucial habitat; further federal protections were promulgated in the 1980s. Along with over 515 wildlife sanctuaries, India now hosts 18 biosphere reserves, 10 of which are part of the World Network of Biosphere Reserves; 26 wetlands are registered

under the Ramsar Convention. The peepul tree, shown on the seals of Mohenjo-Daro, shaded Gautama Buddha as he sought enlightenment. The varied and rich wildlife of India has had a profound impact on the region's popular culture. The wildlife has also been made famous in *The Jungle Book* by Rudyard Kipling. India's wildlife has been the subject of numerous other tales and fables such as the *Panchatantra*.
India is rich in various biogeographical provinces, ranging from the cold deserts of Ladakh and Spiti to the hot deserts of Thar, temperate forests in the Himalayas to the lush green tropical rain forests of the low lands. India has also large freshwater bodies such as Wular and Manasbal lakes in Kashmir, Chilka lake in Orissa and Kolleru lake in Andhra Pradesh and the rugged and rich coastline and coral reefs of Deccan.

Protected Areas are ecological/biogeographical areas where wildlife is conserved. Their habitats and natural resources are conserved and poaching is prevented. They are delimited to protect biological diversity. They are cold desert (Ladakh and Spiti), hot desert (Thar), wetland (Assam and N.E. States), saline swampy areas (Sunderbans, Rann of Kutch), mangroves, temperate forests, subtropical forests, tropical forests, tropical wet evergreen forests, tropical moist deciduous forests, tropical deciduous forests, tropical thorn, coral reef, etc. Protected Areas include national parks, sanctuaries and biosphere reserves.

Wildlife Organizations in India:

The following three organizations are dedicated for the preservation of Indian wildlife.

1. IBWL (Indian Board for Wildlife): It is an advisory body on country's wildlife constituted by Government of India in 1952.

2. BHNS (Bombay Natural- History Society): It is a non-governmental organization founded in 1881 to the cause of wildlife conservation in the country. The society conducts research and educational activities and brings out a journal on the wildlife of India.

3. WPSI (Wildlife Preservation Society of India): It is also a non-governmental body founded in 1958 at Dehra Dun. The society conducts tours of students and members to sanctuaries and parks, carries out research on vanishing flora and fauna, organizes a Corbett Memorial Essay competition for school students and brings forth a bilingual quarterly journal called "Cheetal".

Indian Wildlife Habitats:

A. Biosphere Reserve of India:

A biosphere reserve is a protected area in which multiple use of land is permitted by dividing it into zones, each for a particular activity.This area is meant for preserving genetic diversity in representative ecosystems (of natural biomes and biological

communities) by protecting wild populations, traditional life style of tribals and domesticated plant/animal genetic resources.Under the programme MAB (Mand and Biosphere) of UNESCO creation of biosphere reserve was initiated in the year 1975.

The number of biosphere reserves established in 94 countries till May 2002 was 408. In India, 18 biosphere reserves have been set up by now (Fig. 8.1). and four of those are recognized as world heritage sites. Nanda Devi, Sunder bans, Nilgiri and Gulf of Manar.

Fig. 8.1. Biosphere reserves of India

List of Biosphere Reserves in India

	Name of the Biosphere Reserve	State	Area in km^2
1.	Great Rann of kutch	Gujarat	12,454
2.	Gulf of Mannar	Tamil Nadu	10,500
3.	Sunderbanas	West Bengal	9,630
4.	Cold Desert	Himachal Pradesh	7,770
5.	Nandadevi	Uttarakhand	5,860
6.	Nilgiri	T.N., Kerala, Karnatak	5,520
7.	Dehang-Dibang	Arunachal Pradesh	5,112
8.	Pachmarhi	Madhya Pradesh	4981.72
9.	Seshachalam Hills	Andhra Pradesh	4755
10.	Similipal	Odisha	4374
11.	Achanakamar-Amarkantak	Madhya Pradesh, Chattisgarh	3835
12.	Manas	Assam	2837
13.	Khangechendzonga	Sikkim	2620
14.	Agasthyamalai	Kerala, T.N.	1828
15.	Great Nicobar	Andaman & Nicobar Islands	885
16.	Nokrek	Meghalaya	820
17.	Dibru-Saikhowa	Assam	765
18.	Panna	Madhya Pradesh	

A biosphere reserve is divided into three zones: Core, buffer and manipulation.

(i) Core Zone:

In core or natural zone human activity is not allowed. This area is legally protected and undisturbed ecosystem.

(ii) Buffer zone:

The immediate surrounding area of core zone is buffer zone. Here limited human activities live like research, education and research strategy is permitted.

(iii) Manipulation zone:

Manipulation or transition zone is the outermost or peripheral area of biosphere reserve. With the cooperation of reserve management and local people several human activities like settlements, cropping, recreation, and forestry are carried out without disturbing the environment. Buffer zone has different parts like forestry, agriculture, tourism and restoration regions. Through the restoration activities selected degraded area called restoration region is restored to natural form.

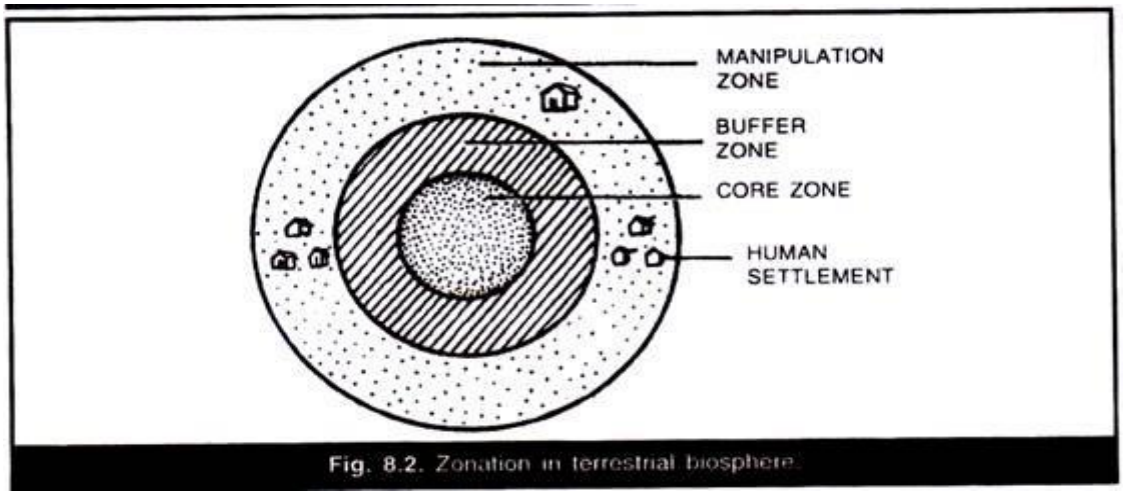


Fig. 8.2. Zonation in terrestrial biosphere

Importance of Biosphere Reserve:

1. Conservation:

Biosphere reserves conserve genetic resources, species, ecosystems and landscapes without uprooting inhabitants. Rather the traditional life style and traditional resources of the local people are maintained.

2. Development:

A biosphere reserve is a protected area in which multiple use of land is permitted by dividing it into zones, each for a particular activity.This area is meant for preserving genetic diversity in representative ecosystems (of natural biomes and biological communities) by protecting wild populations, traditional life style of tribals and domesticated plant/animal genetic resources.

3. Restoration:

Biosphere reserve helps to rebuild any damage caused to ecosystems and habitats.

4. Education and Research:

Biosphere reserve provides a lot of scientific information for specific scientific studies and research. Exchange of information on restoration, conservation and development of biosphere can be made at national and international levels.

B. Sanctuaries of India:

A sanctuary is a protected place or area with Natural environment having optimum conditions and protection for wild animals. Shooting and hunting are prohibited in a sanctuary. But the degree of protection is lower than a national park. Manipulation up to an extent is allowed with permission of competent authority which does not harm wild life. Operations such as harvesting of timber, collection of minor forest products

and private ownership rights are allowed so long as they do not affect the animals adversely. A sanctuary is established by notification of the state Forest Department and can be abolished by similar procedure. The main purpose is to provide protection to wild and indigenous animals.

Some examples of sanctuaries of India are given below:

1. Annamalai Sanctuary:

This sanctuary is situated in the southern part of Coimbatore District in Tamil Nadu and extends in a vast area of 958sq km. This sanctuary has rich fauna of animals like elephant, gaur, sambhar, spotted deer, barking deer, Nilgiritahr, lion-tailed monkeys, tiger, panther, sloth bear, langur, porcupine and pangolin.

2. Kaziranga Wildlife Sanctuary:

This sanctuary is situated on the south bank of the river Brahmaputra in Sibsagar district of Jorhat subdivision of Assam. It consists of 430sq km of forest grasslands and swamps and supports a fauna of nearly 700 rhinoceros.Besides, it also has elephant, wild buffalo, bison, tiger, leopard, sloth bear, sambhar, swamp deer, hog deer, barking deer, wild boar, gibbon, and birds like pelican, stork and ring-tailed fishing eagle. This is, in fact, a Rhino Sanctuary.

3. Jaldapara Wildlife Sanctuary:

This sanctuary is situated in Jalpaiguri district of West Bengal and is extended in a 65 sq km stretch of grassland. It is known for rhinoceros and, hence, referred to as a Rhino Sanctuary. Besides, it also has gaur, elephant, tiger, leopard, deer, and a variety of birds and reptilian fauna.

4. Manas Wildlife Sanctuary and Tiger Reserve:

This sanctuary is situated in Kamrup district in Assam and is extended in an area of 540 sq km. River Manas passes through it. It contains the following wild animals: tiger, panther, wild dog, wild boar, rhinoceros, gaur, wild buffalo, sambhar, swamp deer and golden langur.

5. Periyar Wildlife Sanctuary:

This sanctuary is situated in Kerala and has an area of 777 sq km. It has the fauna of wild elephants, gaur, leopards, sloth bear, sambhar, barking deer, wild dogs, wild boars, black Nilgiri Langur and some birds.

6. Mudumalai Wildlife Sanctuary:

This sanctuary is situated in north-western part of Nilgiris in Tamil Nadu and extends in an area of 22 sq km. It has diverse fauna of wild elephant, gaur, sambhar, chital, barking deer, mouse deer, four-horned antelope, tiger, panther, bonnet monkey, langur, giant

squirrel, flying squirrel, wild dog, jackal, wild cat, sloth bear, porcupine, pangolin, flying lizard, monitor lizard, rat snake, python and various birds.

7. Bandipur Wildlife Sanctuary:

This sanctuary is situated in south of Mysore city of Karnataka state and extends in an area of 874sq km. Its fauna includes elephant, leopard, sloth bear, wild dog, chital, panther, barking deer, porcupine and langur.

8. SesanGir Sanctuary:

This sanctuary is situated near Ahmedabad in Gujarat state and extends in a vast area of 1295sq km. It is the largest sanctuary of our country. It is known for Asiatic lions and other fauna includes spotted dear, blue bull, four-horned antelope, chinkara, striped hyaena, wild boar, porcupine, langur, python, crocodiles and some birds.

9. Dachigam Wildlife Sanctuary:

This sanctuary is situated in Kashmir and extends in an area of 89sq km. Its fauna includes Kashmir stag or hungal, musk deer, leopard, black bear, brown bear and baboon.

10. Bharatpur Bird Sanctuary:

It is also named as Koeladeo Ghana Sanctuary. Situated in Eastern Rajasthan, this park is known as Keoladeo Ghana National Park and is one of the world's most important heronries. It became a Sanctuary in the mid-1950's and about a third of 'its 28-sq. kms comprise a shallow, fresh-water, marsh, known as the wetlands. The dry areas are mostly scrub, thorn and mixed deciduous forest though famous for its water birds, many ", mammal species can be seen which include Sambar, blackbuck, chital, nilgai and many others, Access to Bharatpur is by a short drive from Agra, or a longer drive from Delhi or Jaipur. Should "accommodation in Bharatpur be full, Agra can be; used as a base. The sanctuary is open all year and The best times to visit are from August to March.

11. Point Calimera Wildlife Sanctuary in Tamil Nadu and includes the fauna of Chital, wild boar and black buck.

12.Sariska Sanctuary near Alwar in Rajasthan and includes tiger, leopard, spotted deer, four-horned antelope, etc.

13. Chilka Lake Sanctuary in Orissa and has variety of water birds.

14. Shivpuri Sanctuary in Madhya Pradesh and is known for its tiger fauna.

15. Gautam Buddha Sanctuary in Gaya of Bihar state and has tiger, leopard, chital, sambhar, etc.

16. Bhimbadh Sanctuary of Monghyr district in Bihar state and has wolf, tiger, leopard, etc.

17. Valmikinagar Sanctuary in Champaran of Bihar state and has tiger, leopard, sambhar, chital, etc.

C. National Parks of India:

A national park is a reserve of land, usually owned by a national government. It is a tract of land, which is declared public property to preserve and develop for the purpose of recreation and culture. It is protected from human development activities and pollution. National parks are protected areas of IUCN category II. There are 10 existing national parks in India covering an area of 38,024.10 km^2, which is 1.16% of the geographical area of the country. Yellowstone National Park in California was established as the world's first protected area. The first national park in India was Hailey National Park, now known as Jim Corbett National Park, established in the year 1935.

Some examples of National Parks in India are given below:

a. Bandhavgarh National Park

Situated 'in the heart of India and the far north of Madhya Pradesh, Bandhavgarh National Park was established in 1968. Originally the private hunting preserve of the Maharaja of Rewa, it covers 453sq km of undulating terrain. The vegetation comprises mixed forest with bamboo breaks and to the south, where village land has been reclaimed, there is open grassland. The park offers varied habitats to a large amount of \animals and excellent game viewing. Tiger sightings are common. Access to Bandhavgarh is by road from Khajuraho (reached by air from Delhi, Varanasi or Agra) or from Kanha which is a fairly long drive. The park is open from 1st November to early July.

b. Corbett National Park

Established in 1936, following the advice and guidelines laid down by the great hunter turned naturalist Jim Corbett, this park is located in Uttar Pradesh. Set in the undulating Siwalik Foothills of the Himalayas, the park covers 518sq km of stunning countryside. The diversity of wildlife has made it ii the most famous park in the region. Thick forest and pools of the Ramganga River are home to many animal and fish species including crocodile and Mahseer. Tiger sightings are common. Access to Corbett is by road from

Delhi or a short journey through the hills from Nainital. The park is open from early November to 15 June.

c. Gir National Park

Situated in Gujarat, Gir National Park covers a core area of 259 sq. km and was established in 1965. Originally protected by Junagadh State, the area comprises mixed deciduous forest and grassland with low, rolling hills and meandering rivers. The monsoon brings little rain and much of the area is dry, making for excellent gameviewing. It remains the only home of the Asiatic Lion, slightly smaller than its African cousin and a highly protected species. There is an abundance of other wildlife to be seen and lion sightings are possible. Access to Gir is by road from Bhavnagar, Gondal, Jasdan and many other areas of Gujarat. The park is open from end October to June.

d. Kanha National Park

Situated in Madhya Pradesh and declared a national park in 1955, Kanha now covers 648sq kms and comes under the auspices of Project Tiger. The vegetation comprises forest with bamboo breaks, grassy plateaus and meadows in the valleys. The Barasingha (swamp deer) are one of the park's success stories with its population having increased seven-fold in the past 35 years. It is also home to Tara, Mark Shand's famous elephant. Tiger sightings are common. Access to Kanha is by road from Nagpur (reached by flight from Mumbai, Calcutta or Delhi) or by a longer journey from Bandhavgarh. The park is open from 1 November to 30 June.

Difference between National Parks, Sanctuaries and Biosphere Reserves:

a. Wildlife Sanctuary:

It is a consecrated place where sacred species are kept. It is not open for general public, unlike zoo. In other words, we say, it tries not to allow any activity that would place the animals in an unduly stressful situation. India has 543 wildlife sanctuaries. In them protection is given only to the fauna (animals) and harvesting of timber, collection of MFP and private ownership rights are permitted, but interference with the well-being of animals is not allowed. Here wild animals can take refuge without being hunted. Here collection of forest products, harvesting of timber, private ownership of land, tilling of land, etc., are allowed. Sanctuary is declared by the State Government under Section 18(1) of Wildlife (Protection) Act, 1972, whereas National Park is declared under Section 35(1) of the Act. In sanctuary the boundary is demarcated at the time of declaration. In national park boundary is well-defined and accurate.

Characteristics of Wildlife Sanctuary:

1. It is natural area which is reserve by a governmental or private agency for the protection of particular species.

2. Area is designated for the protection of wild animals.

3. Only animals are conserved, Could be private property also, outside activities allowed.

4. It came under the category called "**Protected Areas**". The Protected Areas are declared under **Wildlife (Protection) Act, 1972**.

5. **International Union for Conservation of Nature (IUCN)** has defined its Category IV type of protected areas

b. National Park :

It is a home to many species of birds and animals which is established by central and state government for the conservation.They are strictly reserved areas meant for the betterment of the wildlife. They are reserved for improvement of wildlife. In them cultivation, grazing, forestry operation and habitat manipulation is prohibited.

Characteristics of National Park:

1. Reserve area of land, owned by the government.

2. Area is protected from human exploitation, industrialization and pollution.

3. No cutting, Grazing allowed, Outside Species Allowed

4. It came under the category called "**Protected Areas**". The Protected Areas are declared under **Wildlife (Protection) Act, 1972**.

5. Conservation of 'wild nature' for posterity and as a symbol of national pride.

6. **International Union for Conservation of Nature (IUCN)**, and its **World Commission on Protected Areas**, has defined its Category II type of protected areas.

c. Biosphere Reserves:

The International Co-ordinating Council (ICC) of UNESCO designated of 'Biosphere reserve' for natural areas from November, 1971. There are 18 Biosphere Reserves in India. Man and Biosphere Programme (MAB) of the UNESCO evolved the concept of Biosphere Reserves. In biosphere reserve, multiple land use is permitted designating various zones.

(i) Core zone in which human activity is not permitted. All forestry and harvesting operations are prohibited and even entry is restricted. Only population studies and scientific investigations are allowed.

(ii) Buffer zone in which limited human activity is permitted. Here no shooting is allowed, but no professional graziers are allowed to establish cattle pens. Camping for tourists are allowed.

(iii) Manipulation zone in which large number of human activities is allowed, but ecology is not permitted to be disturbed.

In a biosphere reserve, wild population, traditional tribals and varied domesticated plant and animal genetic resources are protected. India has identified 14 areas as Biosphere Reserves. Nilgiri Biosphere Reserve includes parts of Karnataka, Kerala and Tamil Nadu. It was declared in 1986.

(iv) Restoration zone is a degraded area for restoration to near natural form.

Characteristics of Biosphere Reserve:

1. Notified areas which cover a larger area of land which may cover multiple National Parks, Sanctuaries and reserves as well.

2. Areas are meant for conservation of biodiversity of a specific area.

3. Three areas: **Core, Buffer & Marginal**. No outside Species allowed Conservation & research purpose.

4. It is internationally recognized within the framework of **UNESCO's Man and Biosphere (MAB) programme** and nominated by national governments.

5. The Ministry of Environment and Forest provides financial assistance to the respective State governments for conservation of landscape and biological diversity and cultural heritage.

Conservation Strategies:

For wildlife conservation and its propagation, proper management techniques should be employed. Sanctuaries, national parks, biosphere reserves, projects, etc., have been created for exclusively protecting the wild flora and fauna in India as well as in other countries of the world. Scientists of 100 countries of the world have evolved comprehensive "World Conservation Strategies" for the judicious use of resources.

To save the existing species of wildlife they proposed some steps which are as follows:

1. Efforts should be made to preserve the endangered species. Species that are sole representative of their family or genus should receive special attention. Endangered species should be given priority over a vulnerable one, a vulnerable species over a rare one and a rare species over other categories.

All the threatened species should be protected. Priority be given belonging to monotypic genera, endangered over-vulnerable, vulnerable over rare and rare over other species.

2. Wildlife should be protected in their natural habitat in situ and in zoo and botanical gardens (ex situ). The threatened species should be conserved in situ as well as in ex situ.

3. Identify the habitats of wild relatives of the economically valuable and useful plants and animals and preserve them in protected areas like sanctuaries, national parks and biosphere reserves.

4. The critical habitats of the species like feeding, breeding, nursery and resting areas should be protected (safeguarded).

5. In case of migratory or wide ranging animals, protected areas should be established to preserve their habitats.

6. For migratory or wide ranging animals, pollution and exploitation of the environment along their migration routes should be controlled.

7. Unique ecosystem (national parks and biospheres) should be protected as a matter of priority.

The national protection programmes have to be coordinated with international programmes like biosphere reserve programme of UNESCO. Man and Biosphere Project and National Parks and Protected Areas of International Union for Conservation of Nature and Natural Resources (IUCN).National Wildlife (Protection) Act was enacted in

India in 1972. Wildlife protection strategies were formulated in India in 1983. Biosphere reserves have also been put into practice since 1986. Wildlife Institute of India is located at Dehradun (Uttaranchal). Indian Board for Wildlife (IBWL) was established in 1952.

8. The productive capacities of exploited species and ecosystems have to be determined and their utilisation should not exceed from those capacities.

9. International trade in wild plants and animals has to be regulated by appropriate legislative and administrative measures.India is a signatory to the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES).

Smt. Indira Gandhi (Late Prime Minister of India) addressed in her inaugural speech of World Conservation Strategies in 1980. She said that Indian tradition teaches us that all forms of life – human, animal and plant – are so closely interlinked that disturbance in one gives rise to imbalance in the other … Nature is beautifully balanced. Any disturbance creates a chain reaction which may not be visible for some time.

Important Indian Wildlife:

The science of zoogeography has both ecological and historical aspects and the two are intimately interwoven. Animals and plants are living indicators of the characteristics of their environment. Their ranges mark the places in which environmental conditions are the same or similar. The evolution and distribution of species throws light upon the geological evolution of various parts of earth and upon the course of global changes in climate and vegetation.

Based mainly on historical-cum-geographical factors, Philip Lutley Schaler (1825-1913) and Alfred Russel Wallace (1823-1913) have divided the world into six zoo-geographical regions, namely- Neoarctic, Palaearctic, Ethiopian, Oriental, Australian and Neotropical. India is of recent origin and it is a part of Oriental region. North Indian fauna during tertiary period were mastodons, eleven species of elephants, Siwalik bison, buffalo, ox, tamarau as well as the recent African animals like hippopotamus, giraffe, chimpanzee, rhinoceros and four-horned ruminant Sivatherium.Area was covered with savannah and woodlands. Asiatic lion, striped hyaena and antelopes can be the relics of the past. The dhole, most endangered top predator, is on the edge of extinction. Less than 2500 members of species are alive in the world. From Siwalik were discovered fragments of jaw of Ramapitheus (primitive hominid ape).

India has three sub-regions on the basis of physiography and climate:

a. Himalayan mountain systems which has three distinct sub-zones:

(i) Himalayan foothills (from eastern frontiers of Kashmir to Assam),

(ii) High altitudes in the Western Himalayas (from Kashmir including Ladakh to Kumaon), and

(iii) Eastern Himalayan sub-region.

b. Peninsular-Indian sub-region which shares the animals of North Africa, such as lion, cheetah, leopard, hyaena, jackal and antelopes.

c. Indo-Malayan sub-region (Tropical evergreen forests) has similarities with Indian, Malayan and Indo-Chinese fauna. This sub-region contains red panda, gibbon, tree shrew, tapir, giant squirrel, and flying lemur.

1. Lion (*Panthera Leo persica*):

It is gregarious carnivorous animal. It prefers open scrub forest mixed with thorny deciduous forest. The temperature of its habitat should not be more than 45°C in summer and not below 5°C in winter. It is found only in Gir forest of Gujarat State as well as in the whole Asian continent. Its habitat should be improved to raise the carrying-capacity for the prey species to meet the full demand of lion.

For herbivorous prey species, there should be total control in grazing of the livestock. Moghul emperor Jahangir was fond of hunting male lions and tigers. Col. Smith during Sepoy Mutiny in India in 1857 killed 300 lions of which 50 were from Delhi alone. By 20th century, the lions population was only 15. The last lion was killed at Anadra and Jaswantpura (Rajasthan) in 1876. Gir Lion Sanctuary Project was started in 1972. Due to conservation measures, lion population increased gradually to 250 in 1977.

2. Indian Tiger (*Panthera tigris tigris*):

Tiger is a solitary carnivorous animal having apparent territory. It is nocturnal predatory inhabiting dense forest such as thorny forest, dry and moist deciduous forest, evergreen and semi-evergreen forest. For raising its population, pasture lands should be improved for raising the carrying-capacity of the habitat for herbivorous preys.

Since 1972, tiger has been declared India's National Animal. In the beginning of 20th century tiger population was about 40,000. In early 1970s their population was reduced to about 1800 due to unrestricted killing for skin, flesh and fat, etc. Indian tiger census conducted in 2008 showed their existence of only 1411 tigers. To save them from

extinction, Project Tiger was started on April 1, 1973, by the Government of India with the help of W.W.F. (Worldwide Fund for Nature). Today there are 39 Project Tiger wildlife reserves in India covering an area of 37,761 sq. km.

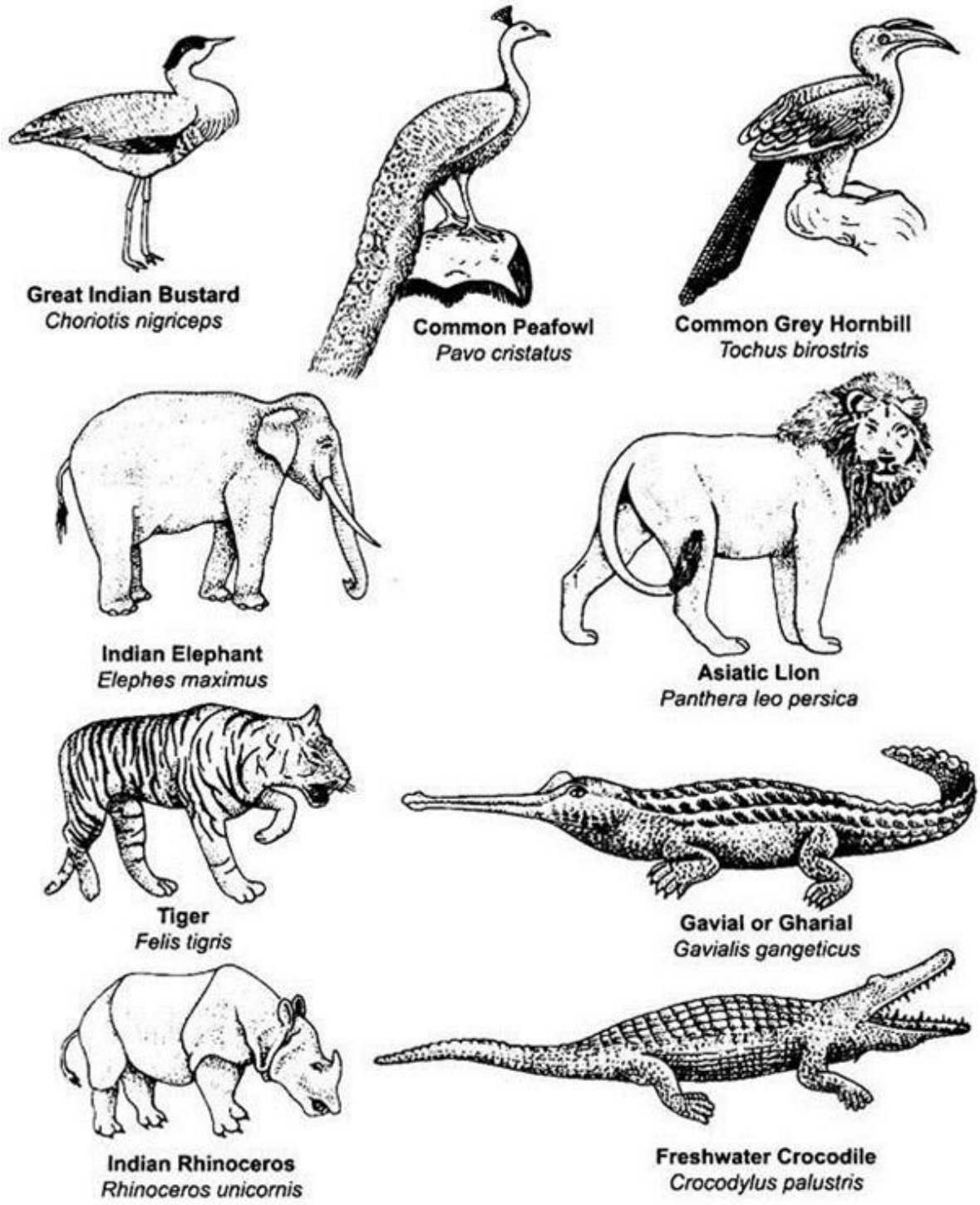


Fig. 51.2. Important wildlife of India.

3. Elephant (*Elephas maximns*):

It is found in plains and hilly forest up to 1500 metre elevation. It needs a lot of water for drinking and bathing, so there must be perennial river, lake, etc., in the habitat. Its food is bamboo and grass, which should be in sufficient quantity. An adult elephant needs about three quintals green fodder daily. It is distributed throughout India except Madhya Pradesh, Andhra and Maharashtra. Generally only the males have large tusks, which are the extension of second pair of incisors. Canines and all incisors have lost.

Lengthened nose and upper lip forms the trunk. In some males, tusks are no longer than females (a few inches long) and called tuskless or Makhana. Elephants have very poor sight, but smell and hearing are acute. Limbs are pillar-like. Toes are embedded in a common mass of foot and encased in a common skin. Their position is indicated externally by broad flat nails which may be fewer than the number of toes. Project Elephant started in 1992 which works for elephant protection. Elephants in India are trained for hunting, transportation, processions, travelling, visiting wildlife parks and sanctuaries, etc. Periyar wildlife sanctuary situated in Kerala having an area about 777 sq. km., was established in 1940 for elephant and other wildlife.

4. Indian Rhinoceros (*Rhinoceros unicornis*):

It inhabits forest having marshy land and tall grasses. It feeds on grasses and also water-hyacinth. In 1904, only about twelve rhinos were left in Assam and fewer in North Bengal. In the past they were found extensively in river Indus plain and Ganges in northern India. Temur killed several of them on the frontiers of Kashmir. Babar also hunted rhinos in different parts of northern India.

Wanton hunting and decrease of natural habitat eliminated them in western part of India and now they are restricted in Nepal, Assam, etc. Its flesh and blood are offered as libation in Nepal. Urine is supposed to be antiseptic and is hung in a vessel at the main door as a charm against ghosts, evil spirits and diseases. Rhinos are poached and killed for their horn which is a strong aphrodisiac and an antidote for poison. In Kaziranga National Park, Sibsagar/Nowgong district (Assam) about 1,654 rhinos is found. They are also transferred in Dudhwa Sanctuary in Kheri district of Uttar Pradesh. The species stands on the verge of extinction and needs strict protection.

5. Gaur or Indian Bison (*Bos gaurus*):

It is gregarious, shy and largest animal of the family Bovidae. It lives in dense forest having meadows. It is also found in hilly areas below 1500 to 1800 metre height. It also needs sufficient water in its habitat. Bison is confined in India, Myanmar, and Malaya. In Mandla district of Madhya Pradesh they are found in jungles north of river Narmada. Few bisons are also found in Bandipur (Karnataka).

Both sexes of bison possess horns. They feed on coarse grasses, leaves and bark of certain trees. Bisons live in herds and breed in cold weather. Gaur is distributed in western ghats southwards from South Maharashtra, hill-forests of central and south-eastern Peninsula and West Bengal, Myanmar and Malay Peninsula. Gaur has the habit of visiting "salt-licks" spots where the ground is impregnated with salts and other minerals.

6. Wild Buffalo (*Bubalus bubalis*):

It is distributed from east of Assam (plane of Brahmaputra), eastern portion of Tara, Midnapur and Orissa. They are also found in forests of Bastar, Balaghat, Mandla and Raipur districts. They like large grass plains and plenty of water. Wild buffalo is larger in size than the tamed buffalo and black in colour. Its horns are black, triangular and large. Adult buffalo weighs about 800 kg. They live in herds of 8 to 15 animals. Breeding starts in autumn.

7. Bear:

There are three important species of bear- Sloth bear (Melursus ursinus), Himalayan black bear (Selenarctos thibetanus) and brown bear (Ursus arctos).

Sloth bear is widely distributed in India, found in the forests from Himalayan foothills to Sri Lanka and Assam. Its long hairy coat is black or black-brown in colour. There is a white V-shaped breast mark. Nails are white. It is nocturnal in habit and feeds on honey, insects and fruits. At the time of hunger, it takes carrion. Sloth bear shares habitat of tiger and elephant.Himalayan black bear is found in forests of Himalayas up to 3,000 metre height. Its hairy black coat is shorter and smoother than the sloth bear. V-shaped breast mark is white or yellowish. Nails are black. It is carnivorous and kills sheep, goat, fowl and even cattle, though its main food is honey and fruit. It is nocturnal in habit and climbs the trees. It hibernates in winter.

Brown bear is found in Himalayas above the tree line (treeless mountain areas). It is large and heavy having hair coat of reddish brown colour. It mainly feeds on grass, roots, insects, fruits and grain but also eats meat of goat, sheep, cattle and fowl.

8. Black Buck (*Antilope cervicapra*):

It is called Indian antelope, lives outside the forest in herds. It is gregarious and male has its territory. It is found in 13 states of India but the largest number is found in Rajasthan. About 10,000 are found in Jodhpur district. In Thar desert in Taal Chhaper Sanctuary in Churu about 1400 live in 7 sq. km. Black buck is found in 8 wildlife sanctuaries and nearly 14 other areas in Rajasthan. Black buck prefers open grassy fields and is dependent on water. It is not found in arid areas. Black buck is not found in

Jaisalmer, parts of Bikaner and parts of Jodhpur with less than 200 mm annual rainfall. It feeds on small grasses.

9. Cheetal (*Axis axis*):

Sambhar (Cervus unicolor), Four-horned antelope (Tetracerus quadricornis). Cheetal lives in moist deciduous forest, evergreen and thorny forests. It feeds on grasses, leaves, flowers and fruits.Sambhar lives in open dry deciduous forest, and also found in dry and moist deciduous forest and evergreen forest. It is the largest among all deer species. It lives in small herds.

Four-horned antelope prefers dry and bushy savannah but also lives in open grassy field near the forest. It is solitary animal and generally lives in pair.Cheetal and sambhar both are prey of top carnivore.

10. Swamp Deer/Barasingha (*Cervus duvauceli*):

It is found in open grassy lands with marshy lands. Its habitat should have sufficient water. It lives in marshy tracts of Tarai and Duars from northern parts of upper Gangetic plains east to Assam. Its other subspecies (C. d. branderi) thrives on the open grassy land of Madhya Pradesh. They are now mostly confined to the Kanha National Park in Madhya Pradesh.

11. Musk Deer (*Moschus moschiferous*):

Himalayan musk deer is a small primitive deer, was widespread throughout Himalayas from Pakistan, through India, Nepal, Bhutan, Myanmar, Tibet and South-west China. Its head is dark grey with slight orange-brown patches above and below the eyes with white tipped ears. Throat is whitish around a central grey oval patch. Along the back, pelage is dark grey or brown, becoming orange-brown around the anal region. Male deer lacks antlers but possess tusks (canines of upper jaw) for fighting. A unique feature of this is presence of musk gland in male, which is chiefly responsible for its decline.

The gelatinous, brown musk secreted by preputial gland has been used in medicines and cosmetics for centuries. Its cost now is 40,000 to 59,000 U.S. Dollar per kg., in the international market. Its habitat destruction due to destruction of forest for livestock, timber and fuelwood is the main cause for the decline of species. The species was listed as vulnerable in Red Data Book. IUCN and WWF Project was launched with the cooperation of India to conserve the musk deer. The project is based in Kedarnath Sanctuary, Uttar Pradesh.

12. Indian Crocodiles:

In India, there are three species of crocodilians such as:

(i) The Mugger or Freshwater Swamp Crocodile (*Crocodylus palustris*). It has average adult size of 3.5 metres, inhabiting rivers, pools, ponds, village tanks, lakes, swamps and reservoirs.

(ii) The Saltwater or Estuarine Crocodile (*Crocodylus porosus*). It grows more than 7 metres and is restricted to the coastal mangrove area in the saltwater (sea).

(iii) The Gharial (*Gavialis gangeticus*). It is the sole living member of the family Gavialidae. It has unique long snout. It has large size of more than 7 metres. It is a fish-eating, riverine species found in large rivers such as Ganga, Brahmaputra, Mahanadi, Kosi, Gandak, etc., of North Indian Himalayan-fed river system.Once abundant in all the major rivers and even ponds, they are among threatened animals today. Their population declined because of uncontrolled and all-season hunting for skin, flesh and sport. Loss of habitat due to construction of dams, diversion of rivers and human interference were other factors. In the wake of declining population, 'Save the Crocodile' projects were launched in 1974 under the guidance of Dr. H.R. Bustard as the chief technical adviser.

13. Great Indian Bustard (*Aredotis nigricaps*):

It is one of the rarest birds of the world. In mid-1980s, bustard population was estimated to be between 500 and 1500, of which half of the birds surviving in Rajasthan. Bustard survived in nearly 200,000 sq. km. of the Thar desert. Surveys of 1993 and 1994 indicate that bustard numbers have almost half in the whole Rajasthan. In mid-1980s survey of bustard indicated that it is found in Jaisalmer, Barmer, Jodhpur, Bikaner, Pali, Jalore, Ajmer, Bhilwara, Tonk, Kota and Sawai Madhopur (11 districts). Bustard is distributed from central Punjab to central Tamil Nadu, western Orissa into eastern Pakistan.

Great Indian Bustard is under Schedule 1 of Wildlife (Protection) Act, 1972. Rajasthan Government has declared it as State Bird.Bustard population decreased due to the development of 649-km Indira Gandhi Nahar Project (IGNP). It has resulted in expansion of agriculture, land colonisation, development of new towns and their expansion and change in natural vegetation due to extensive plantation of exotic trees, Shooting also played a major role in decimation of bustards of the Thar desert.

Bustards live in flocks. It was listed as globally threatened in 1966. During last 10 to 12 years the bustard population has crashed in many areas and now the total population could be as low as 500. It is extinct in Karera and Sorsan bustard areas.

Bustard is about one metre tall with long sturdy yellow legs without hind toe. Its plumage is dull brown above and white below. One male lives with 3 to 5 hens. It feeds on arthropods, lizards, snakes, mice and also grains and young shoots of plants. Their breeding season is from July to October and lay one or two eggs.

14. Common Peafowl (*Pavo cristatus*):

It is also called peacock and in Hindi "Mor" or "Mayur". It is found throughout India up to 1650 metres in Himalayas. It displays sexual dimorphism, male has a gorgeous occellated tail feathers, which are not found in female bird. The bird is not threatened but it is the National Bird of India.

15. Green Peafowl (Pavo muticus):

It was distributed widely from north-east India to southern China, Myanmar, Thailand, Laos, Vietnam, Cambodia and Indonesia. It is not found in Malaysia and Bangladesh. It has declined rapidly and now occurs in fragmented, greatly reduced populations. In India it is restricted to far north-eastern states in a very reduced number. Its number is declining due to hunting for meat, exploitation for trade and persecution by farmers, habitat conversion to farmland. It is a vulnerable species and receives legal protection in India and also in other countries.

15. Hornbills:

Rufous-Necked Hornbill (Aceros ripalensis) is found in mountainous regions between eastern Nepal and Vietnam. It is now absent from or very rare in this range, but still occurs in southern China, north-eastern India, Myanmar, Thailand, Laos and Vietnam. In India, it has been recorded in West Bengal, Arunachal Pradesh, Assam, Meghalaya, Manipur, Mizoram, Nagaland and Sikkim. Recently it has been scarce in West Bengal and Assam and perhaps disappeared from its previous range. It is threatened by the combination of habitat loss and hunting.

16. Narcondam Hornbill (*Aceros narcondami*):

It is found in Narcondam, a small area of the Andaman Islands in the Bay of Bengal. In 1972 their number was 400, while in 1998 their number reduced to 295 to 320 birds. Its population is susceptible to climatic disasters and disease. The primary threats arise from establishment of police outpost on the island manned by 17 persons in 1969. In 1976 police introduced pairs of goats which increased to 130-150 in 1998 and further increased to 250 due to which natural woodland regeneration is reduced.

Each year at least 10-12 live standing trees are cut down for fuelwood and for poles to make fences to check goats not to enter the vegetable plots. Hunting for meat was also a

threat to these birds. It is also listed in Schedule 1 of Wildlife (Protection) Act, 1972. Narcondam Island has been protected since February 1977 as a wildlife sanctuary.

TABLE 51.4. ENDANGERED SPECIES OF INDIAN HORNBILLS.

S.No.	Common name	Zoological name	Distribution
1.	Common grey hornbill "Dhanesh" in Hindi	*Tochus birostris*	In semi-open forests of North India.
2.	Malabar grey hornbill	*Tochus griseus*	Western Ghats
3.	White-throated brown hornbill	*Ptilolaemus tickelli*	Evergreen forests of Eastern India from 300–5000 metre.
4.	Rufous-necked hornbill	*Aceros ripalensis*	Deciduous evergreen forests of Sikkim, Arunachal Pradesh, Manipur, and Mizoram.
5.	Wreathed hornbill	*Rhyticeros undulatus*	Evergreen forests of Himalayan foothills south of Brahmaputra.
6.	Narcondam hornbill	*Rhyticeros (undulatus) narcondami*	Narcondam Island of Andaman Islands.
7.	Giant hornbill	*Buceros bicornis homri*	Heavy forests of Terai up to 2000 metre height. Western Ghats.
8.	Indian pied hornbill	*Anthracoceros malabaricus*	Terai and lower foothill forests.
9.	Malabar pied hornbill	*Anthracoceros coronatus*	*Plains to 300 metre altitude forests of South India.*

Project Tiger:

Tiger is our National Animal. It is found in diverse habitats and in different parts of the country. Tiger is at the apex position as top carnivore of the complex food-chain in most of our forest ecosystems. Over the years, the over-exploitation of the forest areas, merciless hunting, unscientific management, etc., reduced the habitat of tiger as well as leading to a rapid decline has, therefore, been in India not only as effort to save an endangered species but also with equal importance as a means of preserving biotopes.

Project Tiger has been inspired by such an approach. The Indian Board for Wildlife (IBWL) set up a Task Force for studying the condition of tiger population and its status. On the recommendation of this Task Force, Project Tiger was initiated a Central Sector Scheme in 1973 with 9 Tiger Reserves (total area: 13,017 sq. km.) located in different habitat types in 9 different states, but two more reserves have since been subsequently

added to it constituting 11 Tiger Reserves in 10 different states of the country (total area: 15,800 sq. km.). But this number is not last and increasing always.The main aim of the project was to conserve and improve the natural habitat of the tiger under different habitat types. The management practices and strategies in the reserves are controlled in such a way that all the limiting factors of habitats are removed. The steps to be taken in this direction are intensive anti-poaching drive, fire prevention, elimination of cattle-grazing, soil conservation, water management, eradication of weeds, non-interference by human activities, relocation of human habitation and so on.The project started initially as a Central Sector Scheme and expenditure incurred by the States was provided by the Central Government till 1979-80. After this, the project had been given the status of Centrally Sponsored Scheme and the Centre and States are sharing cost on a 50:50 basis. The Worldwide Fund for Nature and Natural Resources (WWF) is also extending financial and technical help.

Project Lion (Gir Lion Project):

The lion stands as top carnivore in the food-chain of the ecosystem. The Indian race of lion (Panthera leo persica) is found only in the Gir Forest of Junagarh district in the Saurashtra peninsula of Gujarat State in whole Asian continent and, hence, also called "Asiatic Lion". Another race of lion (*Panthera leo*) is found in Africa and called "African Lion".Today the Asiatic lion is restricted only in the Gir Forest of Gujarat State. Even in this very forest; due to merciless hunting, conversion of forest into agricultural land, uncontrolled cattle-grazing and spreading of infectious diseases through them, pressure of maldharis inside the forest, etc., hampered the habitat of this magnificent creature too much and subsequently declined its population reaching to the endangered stage.

Observing the situation of Gir Forest, it was discussed at the technical session of International Union for Conservation of Nature and Natural Resources (IUCN) in New Delhi in November, 1969 towards the conservation of Asiatic Lion and its habitat. A number of wildlifers and ecologists all over the world were consulted in 1972. The State Government gave guidelines to Forest Department for the management of this project.The guidelines of the project were implemented in the same year. The area of sanctuary was increased from 1265.1 to 1412.12 sq. km., in 1974. The central core-zone of the sanctuary covering area of 258.71 sq. km., was declared as National Park. Many other fruitful and effective practices were done. Through the practical practices, approaches, marvelous results were achieved and the population of lion began to increase year after year.

Project Elephant:

The project was started officially in 1991-92 but launched in 1993 by the Central Government to afford protection to the elephant. The census of elephant stated in 1993.

Now-a-days, two species of elephants are found:

(i) Indian Elephant (*Elephas maximus*) and

(ii) African Elephant (*Loxodonta africana*).

Indian elephant is comparatively smaller than the African and its pinna is also smaller. Indian elephants are distributed in Uttar Pradesh, Uttaranchal, Bihar, Jharkhand, West Bengal, Assam, Arunachal Pradesh, Meghalaya, Mizoram, Orissa, Tamil Nadu, Kerala and Karnataka.Elephants were extensively used as beast of burden, as transportation vehicle, and so on. In spite of these, they have been hunted for their tusks as ivory materials and export also. Hence, elephant catching was popular and an economic operation. As a result, its population declined very sharply.Protection of the elephant began with the Elephant Preservation Act of 1987. To make more effective, Elephant Project was started for protection and propagation of the animal.

Through the project, habitat should be managed in such an effective manner that the elephant may take shelter peacefully inside the jungle which is its natural habitat, and in this way, we can check their hindrance, invasion, migration, etc., towards the village and agricultural-fields. By managing the habitat in good and scientific way providing the sufficient facilities, the animal will be bound to live properly in their natural-home without harming and killing the man and destroying the crops, which is happening now-a-days often giving much tension and worry to our society and the government.

Crocodile Breeding Project:

In India, there are three species of crocodilians such as:

(i) The Mugger or Freshwater Swamp Crocodile (*Crocodylus palustris*). It has average adult size of 3.5 metres, inhabiting rivers, pools, ponds, village tanks, lakes, swamps and reservoirs.

(ii) The Saltwater or Estuarine Crocodile (*Crocodylus porosus*). It grows more than 7 metres and is restricted to the coastal mangrove areas in the saltwater (sea).

(iii) The Gharial (*Gavialis gangeticus*). It is the sole living member of the family Gavialidae. It has unique long snout. It has large size of more than 7 metres. It is a fish eating riverine species found in large rivers such as Ganga, Brahmaputra, Mahanadi, Kosi, Gandak, etc., of the North Indian Himalayan-fed river systems.

Crocodiles have catastrophically declined worldwide in the post-war period largely as a result of sophisticated hunting methods for their hides by the luxury leather market. The problem continued around poaching and destruction of the remaining crocodile resource by fishery activities either direct or indirect, lethal effect of set nylon nets

being used in fishing, disappearance of habitat of the sand-banks (which is essential for gharial nesting) replaced by concrete embankments, loss of riverine-habitats by dam-construction for irrigation or hydroelectric schemes. As a result, these crocodilian species came on the verge of extinction.

Crocodile hunting is now legally banned in India. The Wildlife (Protection) Act, 1972 lists both species of crocodile and gharial under Schedule I which affords total protection at all times. Similarly, Export I Instruction No 46/73 forbids the export of crocodiles and gharials, their hides or products therefrom.

Project Crocodile Breeding and Management was started as the report given by FAO expert, Dr. H.R. Bustard in 1974, stating "only management will restore the crocodile quickly and it appears that without management, the gharial will become extinct". The actual project was started on April 1, 1975 in Orissa. Gharial eggs were collected and hatched for the first time in captivity anywhere in the world at Tikerpada, District Dhenkanal, Orissa, in June 1975. A small batch was also hatched at Kukrail near Lucknow the same year.

The Tikerpada hatched gharials were successfully reared for subsequent release back into the wild. At the same time, a Saltwater Crocodile Project was initiated in the tidal mangrove forests at Bhitar Kanika, District Cuttack, Orissa, and a Mugger Project and a Captive Breeding Project were also initiated, the latter for capitive-breeding of all three species was located at Nandankanan Biological Park, Orissa. All these projects were started by the Government of India under the help and guidance of FAO and UNDP. The Head Office of the Project is at Hyderabad.

The project has main functions such as:

(i) Conservation and Management of Crocodiles and Development of Sanctuary;

(ii) Rehabilitation of Crocodiles.

Conservation and Management of Crocodiles and Development of Sanctuary:

For the conservation of crocodile/mugger, their eggs are collected and hatched and reared in sanctuaries and released in rivers after attaining proper length of 1.2 metre. With the development of husbandry centres, steps have been taken to gazette and manage sanctuaries in ideal habitat areas for all three crocodilian species into which individuals were reared in. The various husbandry centres could be released when they attain a length of 1.2 metres.

The first sanctuaries to be gazetted in the country were Satkoshia Gorge Sanctuary and Bhitar Kanika Sanctuary, both in Orissa, Tristate Chambal Sanctuary of Madhya Pradesh-Rajasthan-Uttar Pradesh and the Katerniaghat Sanctuary in northern Uttar

Pradesh (Uttaranchal). With the exception of Bhitar Kanika, declared for the saltwater crocodile, these sanctuaries were all for gharial, which due to its critically endangered status, was given prime attention during the early stages of the project.

The management of sanctuaries is, of course, a long-term task, but immediate steps were taken to try to cut down disturbances which resulted in the loss of many animals (for instance, fishing with nylon gill-nets was immediately banned in all four sanctuaries mentioned above), to implement protection and to make a start on implementing management plans (the first of which was ready in December, 1980).

Release:

Young crocodiles of size 1.2 metre are released into ideal areas of the natural habitat in small batches in early spring. Selected areas are specifically managed in the sanctuaries. This includes following cares- (a) Location of release-ideal habitat areas should be located where they will be free from disturbances, (b) Timing of release-it is important that the release be carried out at a time when water levels are low so that the young crocodiles can gain an intimate knowledge of their future home-range prior to the onset of the monsoon floods. The ideal time in South India is early February, this may be delayed by 4-6 weeks in the extreme North of the country.

Rehabilitation of Crocodiles:

Crocodiles breed in the end of winter. In the end of March or in the first week of April, the pregnant female makes 10-15 circular ditches of radius 30 – 50 cm in the sand of the river's bank. Out of these 10-15 ditches, she lays whole of its eggs in one ditch and covers all the ditches by sands. The aim to construct more than one ditch is to protect the eggs from predators like jackals, etc. At a time, the female lays up to 100 eggs. Male and female crocodiles also watch the ditch in the night. Under the effects of heat and moisture of the Sand, the embryos develop and hatch in 60-70 days (incubation period) duration.The timing of hatching of youngs from the eggs is acknowleded by the female by hearing special sound emitted by them. On hearing such sound, the female takes out the youngs by removing sand of the ditch. The young are removed by the female from the unhatched eggs also by breaking it. After this, the female brings the youngs into water keeping them on its back and looks after them till the next breeding season. But even after taking so much care, large number of youngs are eaten by fishes, jackals, birds and crocodiles themselves and very less number of youngs survive in nature.

(i) Gharial:

Gharial rehabilitation started in 1977 with release of 26 individuals into Mahanadi river, Orissa. By January, 1980, 107 individuals had been released into Mahanadi where the wild population had been reduced to 5 individuals. 3 individuals were also released

in Rajasthan in a trial release in October, 1977. Large scale releases by Uttar Pradesh (into the National Chambal Sanctuary) commenced in May, 1979, and between then and late March, 1980, 185 were released. The total number of gharial released by the end of March, 1980, totalled 324.

(ii) Saltwater Crocodile:

The first saltwater crocodile release occurred in Bhitar Kanika Wildlife Sanctuary, also in Orissa, on April 27, 1977. By January, 1980, a total of 125 had been introduced into this one sanctuary. The total release up to May, 1980, including 40 released in West Bengal and 3 in Andhra Pradesh, was 168.

(iii) Mugger:

The first release of Indian mugger took place on February 7, 1977, with 4 individuals being released at Ethipothalla, Andhra Pradesh. This group has now been strengthened to 8 individuals and a further 33 have been released into Kinnersani Wildlife Sanctuary of Andhra Pradesh (March, 1980). On March 9-10, 1979, 130 were released in Tamil Nadu followed by 47 on May 29, 1979, hence, a total of 218 individuals have been introduced.

Probable Questions:

1. Write a note on wildlife management organizations in India.

2. What is Biosphere reserve. Describe different zones of an ideal biosphere reserve.

3. State the importance of Biosphere reserve.

4. What is Sanctuaries. Describe any two sanctuaries situated in India.

5. What is National park. Describe any three national parks in India.

6. State the characteristics of National Parks.

7. State the characteristics of wildlife sanctuaries.

8. State the characteristics of Biosphere reserves.

9. What are the differences between National Parks, Wildlife Sanctuaries and Biosphere reserves ?

Suggested readings:

1.https://nt.gov.au/environment/animals/classification-of-wildlife
2. Biodiversity by Maity and Maity
3. Ecology and Environmental science by Rana

Unit-III

Wildlife management: Distribution, status, habitat utilization pattern,threats and survival of–Royal Bengal Tiger,Rhinoceros, Olive Ridley turtles

Objective: In this Unit you will learn distribution, habitat utilization pattern, threats and survival of Royal Bengal Tiger, Rhinoceros, Olive Ridley turtles.

Introduction:

The management of human use of the biosphere so that it may yield the greatest sustainable benefit to present generation and to maintain its potential to meet the needs and aspirations of future generations is called the conservation. It is scientific management of wildlife to maintain it at its optimum level. The conservation of wildlife is directly related to healthy and better forests. Wildlife conservation includes protection, preservation, and perpetuation of rare species of plants and animals in their natural habitats.

A. Royal Bengal Tiger

The **Bengal tiger** is a *Panthera tigristigris* population in the Indian subcontinent. It is listed as Endangered on the IUCN Red List since 2008, and was estimated at comprising fewer than 2,500 individuals by 2011. It is threatened by poaching, loss, and fragmentation of habitat. None of the Tiger Conservation Landscapes within its range is considered large enough to support an effective population of more than 250 adult individuals. India's tiger population was estimated at 1,706–1,909 individuals in 2010. By 2014, the population had reputedly increased to an estimated 2,226 individuals. Around 440 tigers are estimated in Bangladesh, 163–253 tigers in Nepal and 103 tigers in Bhutan. The tiger is estimated to be present in the Indian subcontinent since the Late Pleistocene, for about 12,000 to 16,500 years.

The Bengal tiger ranks among the biggest wild cats alive today. It is considered to belong to the world's charismatic megafauna.It is the national animal of both India and Bangladesh. It is also known as the Royal Bengal tiger.

Taxonomic Position

Kingdom: **Animalia**

Phylum: **Chordata**

Class: **Mammalia**

Order: **Carnivora**

Suborder: **Feliformia**

Family: **Felidae**

Subfamily: **Pantherinae**

Genus: ***Panthera***

Species: ***tigris***

a. Distribution and Habit:

Tigers appear to have arrived in Sri Lanka during a pluvial period, during which sea levels were depressed, evidently prior to the last glacial maximum about 20,000 years ago. In 1929, the British taxonomist Pocock assumed that tigers arrived in southern India too late to colonize Sri Lanka, which earlier had been connected to India by a land bridge. Results of a phylogeographic study using 134 samples from tigers across the global range suggest that the historical north-eastern distribution limit of the Bengal

tiger is the region in the Chittagong Hills and Brahmaputra River basin, bordering the historical range of the Indochinese tiger.

In the Indian subcontinent, tigers inhabit tropical moist evergreen forests, tropical dry forests, tropical and subtropical moist deciduous forests, mangroves, subtropical and temperate upland forests, and alluvial grasslands. Latter habitat once covered a huge swath of grassland, riverine and moist semi-deciduous forests along the major river system of the Gangetic and Brahmaputra plains, but has now been largely converted to agricultural land or severely degraded. Today, the best examples of this habitat type are limited to a few blocks at the base of the outer foothills of the Himalayas including the *Tiger Conservation Units* (TCUs) Rajaji-Corbett, Bardia-Banke, and the transboundary TCUs Chitwan-Parsa-Valmiki, Dudhwa-Kailali and Shuklaphanta-Kishanpur. Tiger densities in these TCUs are high, in part because of the extraordinary biomass of ungulate prey.The tigers in the Sundarbans in India and Bangladesh are the only ones in the world inhabiting mangrove forests. The population in the Indian Sundarbans is estimated as 70 tigers in total.

b. Ecology and Behavior:

The basic social unit of the tiger is the elemental one of mother and offspring. Adult animals congregate only on an *ad hoc* and transitory basis when special conditions permit, such as plentiful supply of food. Otherwise they lead solitary lives, hunting individually for the dispersed forest and tall grassland animals, upon which they prey. They establish and maintain home ranges. Resident adults of either sex tend to confine their movements to a definite area of habitat within which they satisfy their needs, and in the case of tigresses, those of their growing cubs. Besides providing the requirements of an adequate food supply, sufficient water and shelter, and a modicum of peace and seclusion, this location must make it possible for the resident to maintain contact with other tigers, especially those of the opposite sex. Those sharing the same ground are well aware of each other's movements and activities.

In the Panna Tiger Reserve an adult radio-collared male tiger moved 1.7 to 10.5 km (1.1 to 6.5 mi) between locations on successive days in winter, and 1 to 13.9 km (0.62 to 8.64 mi) in summer. His home range was about 200 km^2 (77sq mi) in summer and 110 km^2 (42 sq mi) in winter. Included in his home range were the much smaller home ranges of two females, a tigress with cubs and a sub-adult tigress. They occupied home ranges of 16 to 31 km^2 (6.2 to 12.0 sq mi).

The home ranges occupied by adult male residents tend to be mutually exclusive, even though one of these residents may tolerate a transient or sub-adult male at least for a time. A male tiger keeps a large territory in order to include the home ranges of several females within its bounds, so that he may maintain mating rights with them. Spacing among females is less complete. Typically there is partial overlap with neighboring female residents. They tend to have core areas, which are more exclusive, at least for most of the time. Home ranges of both males and females are not stable. The shift or alteration of a home range by one animal is correlated with a shift of another. Shifts from less suitable habitat to better ones are made by animals that are already resident.

New animals become residents only as vacancies occur when a former resident moves out or dies. There are more places for resident females than for resident males. During seven years of camera trapping, tracking, and observational data in Chitwan National Park, 6 to 9 breeding tigers, 2 to 16 non-breeding tigers, and 6 to 20 young tigers of less than one year of age were detected in the study area of 100 km^2 (39 sq mi). One of the resident females left her territory to one of her female offspring and took over an adjoining area by displacing another female; and a displaced female managed to re-establish herself in a neighboring territory made vacant by the death of the resident. Of 11 resident females, 7 were still alive at the end of the study period, 2 disappeared after losing their territories to rivals, and 2 died. The initial loss of two resident males and subsequent take over of their home ranges by new males caused social instability for two years. Of 4 resident males, 1 was still alive and 3 were displaced by rivals. Five litters of cubs were killed by infanticide, 2 litters died because they were too young to fend for themselves when their mothers died. One juvenile tiger was presumed dead after being photographed with severe injuries from a deer snare. The remaining young lived long enough to reach dispersal age, 2 of them becoming residents in the study area.

c. Threat:

Over the past century tiger numbers have fallen dramatically, with a decreasing population trend. None of the Tiger Conservation Landscapes within the Bengal tiger range is large enough to support an effective population size of 250 individuals. Habitat losses and the extremely large-scale incidences of poaching are serious threats to the species' survival.

The challenge in the Western Ghats forest complex in western South India, an area of 14,400 square miles (37,000 km^2) stretching across several protected areas is that people live within its borders. The Save the Tiger Fund Council estimates that 7,500 landless people live illegally inside the boundaries of the 386-square-mile (1,000 km^2) Nagarhole National Park in southwestern India. A voluntary if controversial resettlement is underway with the aid of the Karnataka Tiger Conservation Project led by K. UllasKaranth of the Wildlife Conservation Society.A 2007 report by UNESCO, "Case Studies on Climate Change and World Heritage" has stated that an anthropogenic 45-cm rise in sea level, likely by the end of the 21st century, according to the Intergovernmental Panel on Climate Change, combined with other forms of anthropogenic stress on the Sundarbans, could lead to the destruction of 75% of the Sundarbans mangroves. The Forest Rights Act passed by the Indian government in 2006 grants some of India's most impoverished communities the right to own and live in the forests, which likely brings them into conflict with wildlife and under-resourced, under-trained, ill-equipped forest department staff. In the past, evidence showed that humans and tigers cannot co-exist.

Poaching

The most significant immediate threat to the existence of wild tiger populations is the illegal trade in poached skins and body parts between India, Nepal and China. The governments of these countries have failed to implement adequate enforcement response, and wildlife crime remained a low priority in terms of political commitment and investment for years. There are well-organised gangs of professional poachers, who move from place to place and set up camp in vulnerable areas. Skins are rough-cured in the field and handed over to dealers, who send them for further treatment to Indian tanning centres. Buyers choose the skins from dealers or tanneries and smuggle them through a complex interlinking network to markets outside India, mainly in China. Other factors contributing to their loss are urbanisation and revenge killing. Farmers blame tigers for killing cattle and shoot them. Their skins and body parts may however become a part of the illegal trade. In Bangladesh, tigers are killed by professional poachers, local hunters, trappers, pirates and villagers. Each group of people has different motives for killing tigers, ranging from profit, excitement to safety concerns. All groups have access to the commercial trade in body parts.

The illicit demand for bones and body parts from wild tigers for use in Traditional Chinese medicine is the reason for the unrelenting poaching pressure on tigers on the Indian subcontinent. For at least a thousand years, tiger bones have been an ingredient in traditional medicines that are prescribed as a muscle strengthener and treatment for rheumatism and body pain. Between 1994 and 2009, the Wildlife Protection Society of India has documented 893 cases of tigers killed in India, which is just a fraction of the actual poaching and trade in tiger parts during those years. In 2006, India's Sariska Tiger Reserve lost all of its 26 tigers, mostly to poaching. In 2007, police in Allahabad raided a meeting of suspected poachers, traders and couriers. One of the arrested persons was the biggest buyer of tiger parts in India who used to sell them off to the Chinese traditional medicinal market, using women from a nomadic tribe as couriers. In 2009, none of the 24 tigers residing in the Panna Tiger Reserve were left because of excessive poaching. In November 2011, two tigers were found dead in Maharashtra: a male tiger was trapped and killed in a wire snare; a tigress died of electrocution after chewing at an electric cable supplying power to a water pump; another tigress was found dead in Kanha Tiger Reserve landscape — poisoning is suspected to be the cause of her death

d. Conservation:

An area of special interest lies in the "Terai Arc Landscape" in the Himalayan foothills of northern India and southern Nepal, where 11 protected areas composed of dry forest foothills and tall-grass savannas harbor tigers in a 49,000 square kilometres (19,000 sq mi) landscape. The goals are to manage tigers as a single metapopulation, the dispersal of which between core refuges can help maintain genetic, demographic, and

ecological integrity, and to ensure that species and habitat conservation becomes mainstreamed into the rural development agenda. In Nepal a community-based tourism model has been developed with a strong emphasis on sharing benefits with local people and on the regeneration of degraded forests. The approach has been successful in reducing poaching, restoring habitats, and creating a local constituency for conservation.WWF partnered with Leonardo DiCaprio to form a global campaign, "Save Tigers Now", with the ambitious goal of building political, financial and public support to double the wild tiger population by 2022. *Save Tigers Now* started its campaign in 12 different WWF Tiger priority landscapes, since May 2010.

In India

In 1973, Project Tiger was launched aiming at ensuring a viable tiger population in the country and preserving areas of biological importance as a natural heritage for the people. The project's task force visualised these tiger reserves as breeding nuclei, from which surplus animals would disperse to adjacent forests. The selection of areas for the reserves represented as close as possible the diversity of ecosystems across the tiger's distribution in the country. Funds and commitment were mustered to support the intensive program of habitat protection and rehabilitation under the project. By the late 1980s, the initial nine reserves covering an area of 9,115 square kilometres (3,519 sq mi) had been increased to 15 reserves covering an area of 24,700 square kilometres (9,500 sq mi). More than 1100 tigers were estimated to inhabit the reserves by 1984.

Through this initiative the population decline was reversed initially, but has resumed in recent years; India's tiger population decreased from 3,642 in the 1990s to just over 1,400 from 2002 to 2008. The Indian Wildlife Protection Act of 1972 enables government agencies to take strict measures so as to ensure the conservation of the Bengal tigers. The Wildlife Institute of India estimates showed that tiger numbers had fallen in Madhya Pradesh by 61%, Maharashtra by 57%, and Rajasthan by 40%. The government's first tiger census, conducted under the Project Tiger initiative begun in 1973, counted 1,827 tigers in the country that year. Using that methodology, the government observed a steady population increase, reaching 3,700 tigers in 2002. However, the use of more reliable and independent censusing technology (including camera traps) for the 2007–2008 all-India census has shown that the numbers were in fact less than half than originally claimed by the Forest Department.[99]

Following the revelation that only 1,411 Bengal tigers existed in the wild in India, down from 3,600 in 2003, the Indian government set up eight new tiger reserves.[100] Because of dwindling tiger numbers, the Indian government has pledged US$153 million to further fund the Project Tiger initiative, set up a Tiger Protection Force to combat poachers, and fund the relocation of up to 200,000 villagers to minimize human-tiger interaction.[101] Tiger scientists in India, such as Raghu Chundawat and UllasKaranth have called for use of technology in the conservation efforts.

In January 2008, the Government of India launched a dedicated anti-poaching force composed of experts from Indian police, forest officials and various other environmental agencies. Indian officials successfully started a project to reintroduce the tigers into the Sariska reserve. The Ranthambore National Park is often cited as a major success by Indian officials against poaching. The population increased to 1,706 in 2011 and 2,226 in 2014.[107] There are 48[108] tiger reserves in. Kuno-Palpur in Madhya Pradesh was supposed receive Asiatic lions from Gujarat. Since no lion has been transferred from Gujarat to Madhya Pradesh so far, it may be used as a sanctuary for the tiger instead.

Bengal tigers have been captive bred since 1880 and widely crossed with other tiger subspecies. Indian zoos have bred tigers for the first time at the Alipore Zoo in Kolkata. The 1997 International Tiger Studbook lists the global captive population of Bengal tigers at 210 individuals that are all kept in Indian zoos, except for one female in North America. Completion of the Indian Bengal Tiger Studbook is a necessary prerequisite to establishing a captive management program for tigers in India.

B. Rhinoceros

The **Indian rhinoceros** (*Rhinoceros unicornis*), also called the **greater one-horned rhinoceros** and **great Indian rhinoceros**, is a rhinoceros native to the Indian subcontinent. It is listed as Vulnerable on the IUCN Red List, as populations are fragmented and restricted to less than 20,000 km^2 (7,700 sq mi). Moreover, the extent and quality of the rhino's most important habitat, alluvial grassland and riverine forest, is considered to be in decline due to human and livestock encroachment. As of 2008, a total of 2,575 mature individuals were estimated to live in the wild.

The Indian rhinoceros once ranged throughout the entire stretch of the Indo-Gangetic Plain, but excessive hunting and agricultural development reduced their range drastically to 11 sites in northern India and southern Nepal. In the early 1990s, between 1,870 and 1,895 rhinos were estimated to have been alive.

Taxonomic position:

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order:Perissodactyla

Family: Rhinocerotidae

Genus:*Rhinoceros*

Species: *unicornis*

a. Distribution and Habit:

The one-horned rhinoceros once ranged across the entire northern part of the Indian Subcontinent, along the Indus, Gangesand Brahmaputra River basins, from Pakistan to the Indian-Myanmar border, including Bangladesh and the southern parts of Nepal and Bhutan. It may have also occurred in Myanmar, southern China and Indochina. It inhabits the alluvial grasslands of the Terai and the Brahmaputra basin. As a result of habitat destruction and climatic changes its range has gradually been reduced so that by the 19th century, it only survived in the Terai grasslands of southern Nepal, northern Uttar Pradesh, northern Bihar, northern West Bengal, and in the Brahmaputra Valley of Assam.The species was present in northern Bihar and Oudh at least until 1770 as indicated in maps produced by Colonel Gentil.On the former abundance of the species, Thomas C. Jerdon wrote in 1867: This huge rhinoceros is found in the Terai at the foot of the Himalayas, from Bhutan to Nepal. It is more common in the eastern portion of the Terai than the west, and is most abundant in Assam and the Bhutan Dooars.

b. Ecology and behavior :

Adult male Indian rhinos are usually solitary. Groups consist of females with calves, or of up to six subadults. Such groups congregate at wallows and grazing areas. They are foremost active in early mornings, late afternoons and at night, but rest during hot days. They are excellent swimmers and can run at speeds of up to 55 km/h (34 mph) for short periods. They have excellent senses of hearing and smell, but relatively poor eyesight. Over 10 distinct vocalizations have been recorded. Males have home ranges of around 2 to 8 km^2 (0.77 to 3.09 sq mi) that overlap each other. Dominant males tolerate males passing through their territories except when they are in mating season, when dangerous fights break out Indian rhinos bathe regularly. The folds in their skin trap water and hold it even when they come back on land.

Indian rhinos have few natural enemies, except for tigers, which sometimes kill unguarded calves, but adult rhinos are less vulnerable due to their size. Mynahs and egrets both eat invertebrates from the rhino's skin and around its feet. *Tabanus* flies, a type of horse-fly, are known to bite rhinos. The rhinos are also vulnerable to diseases spread by parasites such as leeches, ticks, and nematodes. Anthrax and the blood-disease septicemia are known to occur. In March 2017, of a group of four tigers consisting of an adult male, female and two cubs killed a 20-year-old male rhino in the Dudhwa Tiger Reserve.

c. Threat:

Sport hunting became common in the late 1800s and early 1900s.[1] Indian rhinos were hunted relentlessly and persistently. Reports from the middle of the 19th century claim that some British military officers in Assam individually shot more than 200 rhinos. By 1908, the population in Kaziranga had decreased to around 12 individuals.[10] In the

early 1900s, the species had declined to near extinction.Poaching for rhinoceros horn became the single most important reason for the decline of the Indian rhino after conservation
measures were put in place from the beginning of the 20th century, when legal hunting ended. From 1980 to 1993, 692 rhinos were poached in India. In India's Laokhowa Wildlife Sanctuary, 41 rhinos were killed in 1983, virtually the entire population of the sanctuary. By the mid-1990s, poaching had rendered the species extinct there.
In 1950, Chitwan's forest and grasslands extended over more than 2,600 km^2(1,000 sq mi) and were home to about 800 rhinos. When poor farmers from the mid-hills moved to the Chitwan Valley in search of arable land, the area was subsequently opened for settlement, and poaching of wildlife became rampant. The Chitwan population has repeatedly been jeopardized by poaching; in 2002 alone, poachers killed 37 animals to saw off and sell their valuable horns.

Six methods of killing rhinos have been recorded:

- Shooting is by far the most common method used; rhino horn traders hire sharpshooters and often supply them with rifles and ammunition.
- Trapping in a pit depends largely on the terrain and availability of grass to cover it; pits are dug out in such a way that a fallen animal has little room to manoeuvre with its head slightly above the pit, so that it is easy to saw off the horn.
- Electrocution is used where high voltage powerlines pass through or near a protected area, to which poachers hook a long, insulated rod connected to a wire, which is suspended above a rhino path.
- Poisoning by smearing zinc phosphide rat poison or pesticides on salt licks frequently used by rhinos is sometimes used.
- Spearing has only been recorded in Chitwan National Park.
- A noose, which cuts through the rhino's skin, kills it by strangulation.

Poaching, mainly for the use of the horn in traditional Chinese medicine, has remained a constant and has led to decreases in several important populations. Apart from this, serious declines in quality of habitat have occurred in some areas, due to:

- severe invasion by alien plants into grasslands affecting some populations;
- demonstrated reductions in the extent of grasslands and wetland habitats due to woodland encroachment and silting up of beels;
- grazing by domestic livestock.

The species is inherently at risk because over 70% of its population occurs at a single site, Kaziranga National Park. Any catastrophic event such as disease, civil disorder, poaching, or habitat loss would have a devastating impact on the Indian rhino's status. However, small population of rhinos may be prone to inbreeding depression.

d. Conservation:

Rhinoceros unicornis has been listed in CITES Appendix I since 1975. The Indian and Nepalese governments have taken major steps towards Indian rhinoceros conservation, especially with the help of the World Wide Fund for Nature (WWF) and other non-governmental organizations. In the early 1980s, a rhino translocation scheme was initiated. The first pair of rhinos was reintroduced from Nepal's Terai to Pakistan's Lal Suhanra National Park in Punjab in 1982. In 1910, all rhino hunting in India became prohibited. In 1984, five rhinos were relocated to Dudhwa National Park — four from the fields outside the Pobitora Wildlife Sanctuary and one from Goalpara.

The Indian rhinoceros was initially difficult to breed in captivity. The first recorded captive birth of a rhinoceros was in Kathmandu in 1826, but another successful birth did not occur for nearly 100 years. In 1925, a rhino was born in Kolkata. No rhinoceros was successfully bred in Europe until 1956. On September 14, 1956, Rudra was born in Zoo Basel, Switzerland. In the second half of the 20th century, zoos became adept at breeding Indian rhinoceros. By 1983, nearly 40 babies had been born in captivity.[9] As of 2012, 33 Indian rhinos were born at Zoo Basel,[32] which means that most animals kept in a zoo are somehow related to the population in the zoo of Basel, Switzerland. Due to the success of Zoo Basel's breeding program, the International Studbook for the species has been kept there since 1972. Since 1990, the Indian rhino European Endangered Species Programme is being coordinated there, as well, which ensures that the captive global Indian rhinoceros population stays genetically as healthy as possible.[33] As of 2010, 174 rhinos are kept in zoos worldwide.

In June 2009, an Indian rhino was artificially inseminated using sperm collected four years previously and cryopreserved at the Cincinnati Zoo's CryoBioBank before being thawed and used. She gave birth to a male calf in October 2010. The calf died 12 hours after birth.In June 2014, the first "successful" live-birth from an artificially inseminated rhino took place at the Buffalo Zoo in New York. As in Cincinnati, cryopreserved sperm was used to produce the female calf, Monica

C. Olive Ridley Turtles

The **olive ridley sea turtle** (*Lepidochelysolivacea*), also known as the **Pacific ridley sea turtle**, are the second smallestand most abundant of all sea turtles found in the world; this species of sea turtle is found in warm and tropical waters, primarily in the Pacific and Indian Oceans. They can also be found in the warm waters of the Atlantic Ocean.

These turtles, along with the related Kemps ridley turtle, are best known for their unique mass nesting called *arribada*, where thousands of females come together on the same beach to lay eggs

Taxonomic Position

Kingdom: **Animalia**
Phylum: **Chordata**
Class: Reptilia
Order: **Testudines**
Suborder: **Cryptodira**
Family: **Cheloniidae**
Genus: ***Lepidochelys***
Species: ***olivacea***

a. Distribution:

The olive ridley turtle has a circumtropical distribution, living in tropical and warm waters of the Pacific and Indian Oceans from India, Arabia, Japan, and Micronesia south to southern Africa, Australia, and New Zealand. In the Atlantic Ocean, it has been observed off the western coast of Africa and the coasts of northern Brazil, Suriname, Guyana, French Guiana, and Venezuela. Additionally, the olive ridley has been recorded in the Caribbean Sea as far north as Puerto Rico. A female individual was found alive on an Irish Sea beach on the Isle of Anglesey, British Isles in November 2016, giving this species its northernmost appearance. It was taken in by the

nearby Anglesey Sea Zoo while its health was being assessed. The olive ridley is also found in the eastern Pacific Ocean from the Galapagos Islands and Chile north to the Gulf of California, and along the Pacific coast to at least Oregon Migratory movements have been studied less intensely in olive ridleys than other species of marine turtles, but they are believed to use the coastal waters of over 80 countries. Historically, this species has been widely regarded as the most abundant sea turtle in the world. More than one million olive ridleys were commercially harvested off the coasts of Mexico in 1968 alone.

The population of Pacific Mexico was estimated to be at least 10 million prior to the era of mass exploitation. More recently, the global population of annual nesting females has been reduced to about two million by 2004, and was further reduced to 852,550 by 2008. This indicated a dramatic decrease of 28 to 32% in the global population within only one generation (i.e., 20 years).

The olive ridley sea turtles are considered the most abundant, yet globally they have declined by more than 30% from historic levels. These turtles are considered endangered because of their few remaining nesting sites in the world. The eastern Pacific turtles have been found to range from Baja California, Mexico, to Chile. Pacific olive ridleys nest around Costa Rica, Mexico, Nicaragua, and the northern Indian Ocean; the breeding colony in Mexico was listed as endangered in the US on July 28, 1978.

b. Habitat and ecology:

Most observations are typically within 15 km of mainland shores in protected, relatively shallow marine waters (22–55 m deep). Olive ridleys are occasionally found in open waters. The multiple habitats and geographical localities used by this species vary throughout its life cycle. More research is needed to acquire data on and use of pelagic habitats.

Known predators of olive ridley eggs include raccoons, coyotes, feral dogs and pigs, opossums, coatimundi, caimans, ghost crabs, and the sunbeam snake.[10] Hatchlings are preyed upon as they travel across the beach to the water by vultures, frigate birds, crabs, raccoons, coyotes, iguanas, and snakes. In the water, hatchling predators most likely include oceanic fishes, sharks, and crocodiles. Adults have relatively few known predators, other than sharks, and killer whales are responsible for occasional attacks. On land, nesting females may be attacked by jaguars. It is notable that the jaguar is the only cat with a strong enough bite to penetrate a sea turtle's shell, thought to be an evolutionary adaption from the Holocene extinction event. In observations of jaguar attacks, it was noted that the cats consumed the neck muscles of the turtle and occasionally the flippers, but left the remainder of the turtle carcass for scavengers as most likely, despite the strength of its jaws, a jaguar still cannot easily penetrate an adult turtle's shell to reach the internal organs or other muscles. In recent years, increased predation on turtles by jaguars has been noted, perhaps due to habitat loss and fewer alternative food sources. Sea turtles are comparatively defenseless in this situation as they cannot pull their head into their shell

like freshwater and terrestrial turtles. Females are often plagued by mosquitos during nesting. Humans are still listed as the leading threat to *L. olivacea*, responsible for unsustainable egg collection, slaughtering nesting females on the beach, and direct harvesting adults at sea for commercial sale of both the meat and hides.

Other major threats include mortality associated with boat collisions, and incidental takes in fisheries. Trawling, gill nets, ghost nests, longline fishing, and pot fishing, have significantly affected olive ridley populations, as well as other species of marine turtles. Between 1993 and 2003, more than 100,000 olive ridley turtles were reported dead in Odisha, India from fishery-related practices. In addition, entanglement and ingestion of marine debris is listed as a major threat for this species. Coastal development, natural disasters, climate change, and other sources of beach erosion have also been cited as potential threats to nesting grounds. Additionally, coastal development also threatens newly hatched turtles through the effects of light pollution. Hatchlings which use light cues to orient themselves to the sea are now misled into moving towards land, and die from dehydration or exhaustion, or are killed on roads.

However, the greatest single cause of olive ridley egg loss results from *arribadas*, in which the density of nesting females is so high, previously laid nests are inadvertently dug up and destroyed by other nesting females. In some cases, nests become cross-contaminated by bacteria or pathogens of rotting nests. For example, in Playa Nancite, Costa Rica, only 0.2% of the 11.5 million eggs produced in a single *arribada* successfully hatched. Although some of this loss resulted from predation and high tides, the majority was attributed to conspecifics unintentionally destroying existing nests. The extent to which *arribadas*contribute to the population status of olive ridleys has created debate among scientists. Many believe the massive reproductive output of these nesting events is critical to maintaining populations, while others maintain the traditional *arribada* beaches fall far short of their reproductive potential and are most likely not sustaining population levels.[6] In some areas, this debate eventually led to legalizing egg collection.

Threats:

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organs or other muscles. In recent years, increased predation on turtles by jaguars has been noted, perhaps due to habitat loss and fewer alternative food sources. Sea turtles are comparatively defenceless in this situation, as they cannot pull their heads into their shells like freshwater and terrestrial turtles. Females are often plagued by mosquitos during nesting. Humans are still listed as the leading threat to *L. olivacea*, responsible for unsustainable egg collection, slaughtering nesting females on the beach, and direct harvesting adults at sea for commercial sale of both the meat and hides.

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Conservation status:

The olive ridley is classified as vulnerable according to the International Union for Conservation of Nature and Natural Resources (IUCN), and is listed in Appendix I of CITES. These listings were largely responsible for halting the large-scale commercial exploitation and trade of olive ridley skins.The Convention on Migratory Species and the Inter-American Convention for the Protection and Conservation of Sea Turtles have also provided olive ridleys with protection, leading to increased conservation and management for this marine turtle. National listings for this species range

from endangered to threatened, yet enforcing these sanctions on a global scale has been unsuccessful for the most part. Conservation successes for the olive ridley have relied on well-coordinated national programs in combination with local communities and nongovernment organizations, which focused primarily on public outreach and education. *Arribada* management has also played a critical role in conserving olive ridleys. Lastly, enforcing the use of turtle excluder devices in the shrimp-trawling industry has also proved effective in some areas. Globally, the olive ridley continues to receive less conservation attention than its close relative, the Kemp's ridley (*L. kempii*). Also, many schools arrange trips for students to carry out the conservation project, especially in India.Several projects worldwide seek to preserve the olive ridley sea turtle population. For example, in Nuevo Vallarta, Mexico, when the turtles come to the beach to lay their eggs, some of them are relocated to a hatchery, where they have a much better chance to survive. If the eggs were left on the beach, they would face many threats such as getting washed away with the tide or getting poached. Once the eggs hatch, the baby turtles are carried to the beach and released.Another major project, in India involved in preserving the olive ridley sea turtle population was carried out in Chennai, where the Chennai wildlife team collected close to 10,000 eggs along the Marina coast, of which 8,834 hatchlings were successfully released into the sea in a phased manner.

Probable Questions:

1. What is the taxonomic position of Royal Bengal tiger?
2. Describe the habitat of Royal Bengal Tiger.
3. What types of threats are faced by Royal Bengal Tiger ?
4. State the conservation measures taken for Royal Bengal Tiger ?
5. What is the taxonomic position of Rhinoceros ?
6. Describe the habitat of Rhinoceros
7. What types of threats are faced by Rhinoceros ?
8. State the conservation measures taken for Rhinoceros.
9. What is the taxonomic position of olive ridley sea turtle ?
10. Describe the habitat of olive ridley sea turtle .
11. What types of threats are faced by olive ridley sea turtle.
12. State the conservation measures taken for olive ridley sea turtle.

Suggested Readings:

1.https://nt.gov.au/environment/animals/classification-of-wildlife
2. Biodiversity by Maity and Maity
3. Ecology and Environmental science by Rana

UNIT- IV

National and International efforts for conservation

Objectives: In this Unit you will learn about NationalandInternationaleffortsforconservation:CITES,CBD, IUCN,Protected areaconcept.

Introduction:

India is one of 12 mega-diversity countries of the world. The innumerable life forms harboured by the forests, deserts, mountains, other land, air and oceans provide food, fodder, fuel, medicine, textiles, etc.There are innumerable species, the potential of which is not as yet known. It would therefore be prudent to not only conserve the species we already have information about, but also species we have not get identified and described from economic point of view.

Taxus baccata, a tree found in the sub-Himalayan regions, once believed to be of no value is now considered to be effective in the treatment of certain types of cancer. The diversity of genes, species and ecosystem is a valuable resource that can be tapped as human needs and demands change, the still more basic reasons for conservation are the moral, cultural and religious values.

Major problems with biodiversity conservation in India are:

(i) Low priority for conservation of living natural resources.

(ii) Exploitation of living natural resources for monetary gain.

(iii) Values and knowledge about the species and ecosystem inadequately known,

(iv) Unplanned urbanisation and uncontrolled industrialisation.

Biodiversity conservation in India is also impeded by a lack of knowledge of the magnitude, patterns, causes and rates of deforestation and biodiversity loss at the ecosystem and landscape level.

Poaching and trade in wildlife species are among the most important concerns in the management of protected areas today but information on poaching, trade and trade routes is sketchy and current wildlife protection and law enforcement measures are inadequate and inefficient.

Existing Policy of Biodiversity Conservation:

a. A protected area network of National Parks and Wildlife Sanctuaries have been created.

b. The Indian Council of Forestry Research and Education (ICFRE) has identified forest preservation plots of representative forest types for conservation of viable and representative areas of biodiversity.

c. A programme entitled 'eco-development' for in situ conservation of biological diversity involving local communities has been initiated.

d. To conserve the respective ecosystems, a Biosphere Reserve Programme is being implemented.

e. Programmes have also been launched for scientific management and wise use of fragile ecosystem,

f. Specific programmes for management and conservation of wetlands, mangroves and coral reef systems are also being implemented.

g. Six internationally significant wetlands of India have been declared as 'Ramsar Sites' under the Ramsar Convention.

h. Wildlife Protection Act is in the final stage of revision and provisions have been made for conservation reserves and community reserves to allow restrictive use to make it more people oriented. There will also be State Biodiversity Boards to control access to domestic consumers.

i. Under the World Heritage Convention, five natural sites in India, have been declared as World Heritage Sites.

j. Project Tiger and Project Elephant have been launched to protect the wildlife. Rhinos have been given special attention in selected sanctuaries and national parks.

k. The Ministry of Environment and Forests (MOEF) constituted the National Afforestation and Eco-development Board (NAEB) in August 1992, which has evolved specific schemes for promoting afforestation and management strategies and eco-development packages for promoting biomass production through a participatory planning process of Joint Forest Management (JFM) and micro-planning.

International Efforts for Conserving Biodiversity:

It is apparent that action plans and strategies, when designed appropriately and implemented, can make important contributions to conservation.

A collaborate effort of the World Resources Institute, IUCN (International Union for Conservation of Nature and Natural Resources), and UNEP (United Nations Environment Programme), working with other institutions, is leading to the

preparation of a Global Strategy for the Conservation of Biodiversity, as a companion to the new version of the World Conservation Strategy now being prepared.

The aim of the Strategy is to provide a comprehensive framework to stimulate urgent, positive, innovative and coordinated action to stem the loss and degradation of the world's biological resources and enhance the contribution of these resources to human well-being.The strategy will be developed by and for national governments, NGOs (Non-government organisations), resource managers, scientists, international institutions, multilateral banks, and bilateral aid agencies.

The development of the Global Strategy will be centered around a series of regional workshops in Asia, Africa, Europe, Latin America and North America. The Global strategy will include considerations of a variety of factors influencing biological resource conservation, such as international financing, international cooperation, research, education, training, public awareness, and ecological restoration.The Earth Summit held in 1992 at Rio de Janeiro, Brazil resulted into a Convention on Biodiversity, which came into force on December, 29, 1993.

The Convention has three main objectives:

(i) Conservation of biological diversity

(ii) Sustainable use of biodiversity, and

(iii) Fair and equitable sharing of benefits that arise by the utilization of genetic resources.

The World Conservation Strategy (WTS) and the World Wide Fund for Nature (WWF) support projects worldwide to promote conservation of biological diversity.

A. Convention on International Trade in Endangered Species(CITES) :

CITES is the Convention on International Trade in Endangered Species of Wild Fauna and Flora. CITES was signed on 3rd March 1973 and entered into force on 1st July 1975. This has been in operation for almost 40 years.

a. Purpose of CITES:

The purpose of CITES is to ensure that wild fauna and flora in international trade are not exploited unsustainably.

- CITES is an international convention that combines wildlife and trade themes with a legally binding instrument for achieving conservation and sustainable use objectives
- The Convention establishes an international legal framework together with common procedural mechanisms for the strictest control of international

commercial trade in species threatened with extinction, and for an effective regulation of international trade in others

- This framework and common procedural mechanism are now used by 180countries to regulate and monitor international trade in listed species

b. Basic Provisions of the Convention:

The text of the Convention outlines the basic provisions for trade and obligations of each Party such as:

- Trade procedures and requirements
- Enforcement measures
- Trade facilitation
- Exemptions and special procedures
- Marking
- Confiscations
- Reporting
- Trade with non-Parties
- Amendment of the Appendices

c. Resolutions and Decisions taken in the Conference of the Parties:

- The Conference of the Parties adopts Resolutions to guide the interpretation and implementation of the Convention, and Decisions to provide specific short-term time-bound instructions.
- Till date 89 Resolutions and 196 Decisions are in effect.

d. CITES Appendices:

Species subject to CITES regulation are divided amongst three Appendices: I, II & III.

1. Appendix I -

- ❖ Species threatened with extinction, which are or may be affected by trade.
- ❖ International (commercial) trade in wild-taken specimens is generally prohibited.
- ❖ 625 animal species and 301 plant species.

2. Appendix II -

- ❖ Species not necessarily threatened with extinction, but for which trade must be controlled to avoid their becoming so, and species that resemble species already included in Appendix II.
- ❖ International trade is permitted but regulated.
- ❖ 4685 animal species and 29105 plant species (97% of all listings).

3. **Appendix III -**
    - ❖ Species for which a country is asking Parties to help with its protection.
    - ❖ International trade is permitted but regulated (less restrictive than Appendix II).
    - ❖ 147 animal species and 119 plant species.

e. Species in trade:

- ➢ Not all listed species appear in trade.
    - ❖ Of the 34,782 listed species, some 3,680 animal species and 9,577 plant species have appeared in trade during 2004-2008.
    - ❖ Of these, 11,076 species appeared in 100 or fewer shipments worldwide during this period.
    - ❖ Overall, 157 animal species and 1,878 plant species accounted for 90% of CITES transactions during this period

The Appendices require careful interpretation.

- Species listings can be annotated to specify:
    - ❖ The inclusion or exclusion of designated geographically separate populations, subspecies, species, groups of species, or higher taxa, which may include export quotas.
    - ❖ The types of specimens or export quotas.

f. CITES Permits and Certificates:

- CITES regulates the export, re-export, import and introduction from the sea of live and dead animals and plants andtheir parts and derivatives (listed species only) through a system of permits and certificates
- These permits or certificates may only be issued if certain conditions are met and which must be presented when leaving or entering a country
- For Appendix I and II species, the most important conditions are legal acquisition and that international trade must not be detrimental to their survival in the wild

g. Collaboration and cooperation:

Collaboration and cooperation at the national level are essential for CITES implementation

- CITES Authorities
- Resource sectors
- Customs
- Police
- Judiciary
- Affected stakeholders, including the private sector
-

h. Trade with non-Parties:

- Where export or re-export is to, or import is from, a non-Party, comparable documentation issued by the competent authorities which substantially conforms with CITES requirements for permits and certificates may be accepted.
- Parties accept documentation from States not party to the Convention only if the details of the competent authorities and scientific institutions of such States are included in the online CITES Directory.
- This also applies to specimens in transit destined for or coming from non-Parties.

Summary:

- CITES regulates international trade in wild fauna and flora listed in its Appendices on the basis of a system of permits and certificates which are issued when certain conditions are met, and which must be presented when leaving and entering a country.
- For Appendix-II and –III species, international trade is permitted but regulated, and for Appendix-I species, international (commercial) trade in wild-taken specimens is generally prohibited.
- The Conference of the Parties is the ultimate decision-making body in CITES.

B. CONVENTION ON BIOLOGICAL DIVERSITY (CBD):

Conscious of the intrinsic value of biological diversity and of the ecological, genetic, social, economic, scientific, educational, cultural, recreational and aesthetic values of biological diversity and its components, Conscious also of the importance of biological diversity for evolution and for maintaining life sustaining systems of the biosphere, Affirming that the conservation of biological diversity is a common concern of humankind, Reaffirming that States have sovereign rights over their own biological resources, Reaffirming also that States are responsible for conserving their biological diversity and for using their biological resources in a sustainable manner, Concerned that biological diversity is being significantly reduced by certain human activities, Aware of the general lack of information and knowledge regarding biological diversity and of the urgent need to develop scientific, technical and institutional capacities to provide the basic understanding upon which to plan and implement appropriate measures, Noting that it is vital to anticipate, prevent and attack the causes of significant reduction or loss of biological diversity at source, Noting also that where there is a threat of significant reduction or loss of biological diversity, lack of full scientific certainty should not be used as a reason for postponing measures to avoid or minimize such a threat, Noting further that the fundamental requirement for the conservation of biological diversity is the in-situ conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural

surroundings, Noting further that ex-situ measures, preferably in the country of origin, also have an important role to play, Recognizing the close and traditional dependence of many indigenous and local communities embodying traditional lifestyles on biological resources, and the desire- —3 Convention On Biological Diversity 5 JUNE 1992 ability of sharing equitably benefits arising from the use of traditional knowledge, innovations and practices relevant to the conservation of biological diversity and the sustainable use of its components, Recognizing also the vital role that women play in the conservation and sustainable use of biological diversity and affirming the need for the full participation of women at all levels of policy-making and implementation for biological diversity conservation, Stressing the importance of, and the need to promote, international, regional and global cooperation among States and intergovernmental organizations and the nongovernmental sector for the conservation of biological diversity and the sustainable use of its components, Acknowledging that the provision of new and additional financial resources and appropriate access to relevant technologies can be expected to make a substantial difference in the world's ability to address the loss of biological diversity, Acknowledging further that special provision is required to meet the needs of developing countries, including the provision of new and additional financial resources and appropriate access to relevant technologies, Noting in this regard the special conditions of the least developed countries and small island States, Acknowledging that substantial investments are required to conserve biological diversity and that there is the expectation of a broad range of environmental, economic and social benefits from those investments, Recognizing that economic and social development and poverty eradication are the first and overriding priorities of developing countries, Aware that conservation and sustainable use of biological diversity is of critical importance for meeting the food, health and other needs of the growing world population, for which purpose access to and sharing of both genetic resources and technologies are essential, Noting that, ultimately, the conservation and sustainable use of biological diversity will strengthen friendly relations among States and contribute to peace for humankind, Desiring to enhance and complement existing international arrangements for the conservation of biological diversity and sustainable use of its components, and Determined to conserve and sustainably use biological diversity for the benefit of present and future generations.

The objectives of this Convention, to be pursued in accordance with its relevant provisions, are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to technologies, and by appropriate funding.

Sustainable Use of Components of Biological Diversity:

(a) Integrate consideration of the conservation and sustainable use of biological resources into national decision-making;

(b) Adopt measures relating to the use of biological resources to avoid or minimize adverse impacts on biological diversity;

(c) Protect and encourage customary use of biological resources in accordance with traditional cultural practices that are compatible with conservation or sustainable use requirements;

(d) Support local populations to develop and implement remedial action in degraded areas where biological diversity has been reduced; and

(e) Encourage cooperation between its governmental authorities and its private sector in developing methods for sustainable use of biological resources.

Access to Genetic Resources :

1. Recognizing the sovereign rights of States over their natural resources, the authority to determine access to genetic resources rests with the national governments and is subject to national legislation.

2. Each Contracting Party shall endeavor to create conditions to facilitate access to genetic resources for environmentally sound uses by other Contracting Parties and not to impose restrictions that run counter to the objectives of this Convention.

3. For the purpose of this Convention, the genetic resources being provided by a Contracting Party, as referred to in this Article and Articles 16 and 19, are only those that are provided by Contracting Parties that are countries of origin of such resources or by the Parties that have acquired the genetic resources in accordance with this Convention.

4. Access, where granted, shall be on mutually agreed terms and subject to the provisions of this Article.

5. Access to genetic resources shall be subject to prior informed consent of the Contracting Party providing such resources, unless otherwise determined by that Party.

6. Each Contracting Party shall Endeavour to develop and carry out scientific research based on genetic resources provided by other Contracting Parties with the full participation of, and where possible in, such Contracting Parties.

7. Each Contracting Party shall take legislative, administrative or policy measures, as appropriate, and in accordance with Articles 16 and 19 and, where necessary, through the financial mechanism established by Articles 20 and 21 with the aim of sharing in a fair and equitable way the results of research and development and the benefits arising from the commercial and other utilization of genetic resources with the Contracting Party providing such resources. Such sharing shall be upon mutually agreed terms.

Entry Into Force:

1. This Convention shall enter into force on the ninetieth day after the date of deposit of the thirtieth instrument of ratification, acceptance, approval or accession.

2. Any protocol shall enter into force on the ninetieth day after the date of deposit of the number of instruments of ratification, acceptance, approval or accession, specified in that protocol, has been deposited.

3. For each Contracting Party which ratifies, accepts or approves this Convention or accedes thereto after the deposit of the thirtieth instrument of ratification, acceptance, approval or accession, it shall enter into force on the ninetieth day after the date of deposit by such Contracting Party of its instrument of ratification, acceptance, approval or accession.

4. Any protocol, except as otherwise provided in such protocol, shall enter into force for a Contracting Party that ratifies, accepts or approves that protocol or accedes thereto after its entry into force pursuant to paragraph 2 above, on the ninetieth day after the date on which that Contracting Party deposits its instrument of ratification, acceptance, approval or accession, or on the date on which this Convention enters into force for that Contracting Party, whichever shall be the later.

5. For the purposes of paragraphs 1 and 2 above, any instrument deposited by a regional economic integration organization shall not be counted as additional to those deposited by member States of such organization.

CARTAGENA PROTOCOL ON BIOSAFETY TO THE CONVENTION ON BIOLOGICAL DIVERSITY:

In accordance with the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development, the objective of this Protocol is to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements.

For the purposes of this Protocol:

(a) “Conference of the Parties” means the Conference of the Parties to the Convention;

(b) “Contained use” means any operation, undertaken within a facility, installation or other physical structure, which involves living modified organisms that are controlled

by specific measures that effectively limit their contact with, and their impact on, the external environment; (c) “Export” means intentional transboundary movement from one Party to another Party;

(d) “Exporter” means any legal or natural person, under the jurisdiction of the Party of export, who arranges for a living modified organism to be exported;

(e) “Import” means intentional transboundary movement into one Party from another Party;

(f) “Importer” means any legal or natural person, under the jurisdiction of the Party of import, who arranges for a living modified organism to be imported;

(g) “Living modified organism” means any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology;

(h) “Living organism” means any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids;

(i) “Modern biotechnology” means the application of:

(a) In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or

(b) Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection;

(j) “Regional economic integration organization” means an organization constituted by sovereign States of a given region, to which its member States have transferred competence in respect of matters governed by this Protocol and which has been duly authorized, in accordance with its internal procedures, to sign, ratify, accept, approve or accede to it;

(k) “Transboundary movement” means the movement of a living modified organism from one Party to another Party, save that for the purposes of Articles 17 and 24 transboundary movement extends to movement between Parties and non-Parties.

This Protocol shall apply to the transboundary movement, transit, handling and use of all living modified organisms that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health.

Public Awareness and Participation:

1. The Parties shall: (a) Promote and facilitate public awareness, education and participation concerning the safe transfer, handling and use of living modified organisms in relation to the conservation and sustainable use of biological diversity, taking also into account risks to human health. In doing so, the Parties shall cooperate,

as appropriate, with other States and international bodies; (b) Endeavour to ensure that public awareness and education encompass access to information on living modified organisms identified in accordance with this Protocol that may be imported.

2. The Parties shall, in accordance with their respective laws and regulations, consult the public in the decision-making process regarding living modified organisms and shall make the results of such decisions available to the public, while respecting confidential information in accordance with Article 21. 3. Each Party shall endeavor to inform its public about the means of public access to the Biosafety Clearing-House.

Socio-Economic Considerations :

1. The Parties, in reaching a decision on import under this Protocol or under its domestic measures implementing the Protocol, may take into account, consistent with their international obligations, socio-economic considerations arising from the impact of living modified organisms on the conservation and sustainable use of biological diversity, especially with regard to the value of biological diversity to indigenous and local communities.

2. The Parties are encouraged to cooperate on research and information exchange on any socio-economic impacts of living modified organisms, especially on indigenous and local communities.

Liability and Redress :

The Conference of the Parties serving as the meeting of the Parties to this Protocol shall, at its first meeting, adopt a process with respect to the appropriate elaboration of international rules and procedures in the field of liability and redress for damage resulting from transboundary movements of living modified organisms, analyzing and taking due account of the ongoing processes in international law on these matters, and shall endeavor to complete this process within four years.

Financial Mechanism and Resources:

1. In considering financial resources for the implementation of this Protocol, the Parties shall take into account the provisions of Article 20 of the Convention.
2. The financial mechanism established in Article 21 of the Convention shall, through the institutional structure entrusted with its operation, be the financial mechanism for this Protocol.
3. Regarding the capacity-building referred to in Article 22 of this Protocol, the Conference of the Parties serving as the meeting of the Parties to this Protocol, in providing guidance with respect to the financial mechanism referred to in paragraph

2 above, for consideration by the Conference of the Parties, shall take into account the need for financial resources by developing country Parties, in particular the least developed and the small island developing States among them.

4. In the context of paragraph 1 above, the Parties shall also take into account the needs of the developing country Parties, in particular the least developed and the small island developing States among them, and of the Parties with economies in transition, in their efforts to identify and implement their capacity-building requirements for the purposes of the implementation of this Protocol.
5. The guidance to the financial mechanism of the Convention in relevant decisions of the Conference of the Parties, including those agreed before the adoption of this Protocol, shall apply, mutatis mutandis, to the provisions of this Article.
6. The developed country Parties may also provide, and the developing country Parties and the Parties with economies in transition avail themselves of, financial and technological resources for the implementation of the provisions of this Protocol through bilateral, regional and multilateral channels.

Monitoring and Reporting:

Each Party shall monitor the implementation of its obligations under this Protocol, and shall, at intervals to be determined by the Conference of the Parties serving as the meeting of the Parties to this Protocol, report to the Conference of the Parties serving as the meeting of the Parties to this Protocol on measures that it has taken to implement the Protocol.

Compliance:

The Conference of the Parties serving as the meeting of the Parties to this Protocol shall, at its first meeting, consider and approve cooperative procedures and institutional mechanisms to promote compliance with the provisions of this Protocol and to address cases of non-compliance. These procedures and mechanisms shall include provisions to offer advice or assistance, where appropriate. They shall be separate from, and without prejudice to, the dispute settlement procedures and mechanisms established by Article 27 of the Convention.

Assessment and Review:

The Conference of the Parties serving as the meeting of the Parties to this Protocol shall undertake, five years after the entry into force of this Protocol and at least every five years thereafter, an evaluation of the effectiveness of the Protocol, including an assessment of its procedures and annexes.

Entry Into Force:

1. This Protocol shall enter into force on the ninetieth day after the date of deposit of the fiftieth instrument of ratification, acceptance, approval or accession by States or regional economic integration organizations that are Parties to the Convention.

2. This Protocol shall enter into force for a State or regional economic integration organization that ratifies, accepts or approves this Protocol or accedes thereto after its entry into force pursuant to paragraph 1 above, on the ninetieth day after the date on which that State or regional economic integration organization deposits its instrument of ratification, acceptance, approval or accession, or on the date on which the Convention enters into force for that State or regional economic integration organization, whichever shall be the later.

3. For the purposes of paragraphs 1 and 2 above, any instrument deposited by a regional economic integration organization shall not be counted as additional to those deposited by member States of such organization.

C. INTERNATIONAL UNION FOR CONSERVATION OF NATURE AND NATURAL RESOURCES (IUCN)

It is a membership Union uniquely composed of both government and civil society organizations in the field of nature conservation and sustainable use of natural resources. IUCN is best known for compiling and publishing red list: access the conservation status of species worldwide. Created in 1948, IUCN has evolved into the world's largest and most diverse environmental network.

Mission: Influence, encourage and assist societies throughout the world to conserve the integrity and diversity of nature and to ensure that any use of natural resources is equitable and ecologically sustainable.

Milestones:

- 1947-International Conference on Protection of Nature was held in Brunnen.
- 1948-IUPN (International Conference on Protection of Nature)was formed.
- 1949-IUPN and UNESCO jointly organized conference on protection of nature.
- 1956-IUPN renamed as IUCN (INTERNATIONAL UNION FOR CONSERVATION OF NATURE AND NATURAL RESOURCES).
- 1961-IUCN took part in setting up World Wild Life Fund (WWF).
- 1964-Red data book on the conservation status of species was first published.
- 1990-Began using World Conservation Union as official name.
- 2008-The name was revert back to IUCN.
- 2012-IUCN published the list of world's 100 most threatened species.

Present status:

Since 1948, IUCN has evolved into the world's largest and most diverse environmental network. It harnesses the experience, resources and reach of its 1,300 Member organizations and the input of some 16151 experts. IUCN is the global authority on the status of the natural world and the measures needed to safeguard it. IUCN experts are organized into six commissions dedicated to species survival, environmental law, protected areas, social and economic policy, ecosystem management, and education and communication. IUCN headquarter is now situated in Gland, Switzerland. It includes 161 countries, 217 states and Govt. agencies. There are also 1066 NGOs currently works with IUCN.

IUCN-Management and Governing body:

The IUCN Council is the principal governing body of IUCN, International Union for Conservation of Nature, in between sessions of the World Conservation Congress - the general assembly of the Union's members.Thisorganization is managed by IUCN global secretariat led by Director general Inger Andersen.
The Council is composed of:

- The President;
- Four Vice Presidents (elected by Council from among its members)
- The Treasurer;
- The Chairs of IUCN's six Commissions;
- Twenty-eight Regional Councilors;
- A Councilor from the State in which IUCN has its seat (Switzerland);
- One additional appointed Councilor.

The president of 2012-16 council is ZHANG Xinsheng (China).

IUCN council 202-16 priorities:

- Valuing and conserving nature.
- Deploying nature based solutions to climate, food and development.
- Effective and equitable governance of nature's use.

IUCN themes:

IUCN work across various themes related to conservation, environmental and ecological issues like-

- Business and biodiversity
- Climate change
- Economics
- Ecosystem management
- Environmental law
- Forests
- gender
- global policy
- marine and polar protected areas
- science and knowledge
- social policy
- species
- water
- world heritage

Regions of action:

- Asia
- Central and West Africa
- Eastern and Southern Africa
- Eastern Europe and Central Asia
- Europe
- Mediterranean
- Mexico, Central America and the Caribbean
- Oceania
- South America
- Washington D.C. Office
- West Asia

IUCN India:

India, a mega diverse country with only 2.4% of the world's land area, accounts for 7-8% of all recorded species, including over 45,000 species of plants and 91,000 species of animals. The country's diverse physical features and climatic conditions have resulted in a variety of ecosystems such as forests, wetlands, grasslands, desert, coastal and marine ecosystems which harbour and sustain high biodiversity and contribute to human well-being. Four of 34 globally identified biodiversity hotspots: The Himalayas, the Western Ghats, the North-East, and the Nicobar Islands, can be found in India.
India became a State Member of IUCN in 1969, through the Ministry of Environment, Forest and Climate Change (MoEFCC).
The IUCN India Country Office was established in 2007 in New Delhi.

IUCN Red list:

First published in 1964,The IUCN Global Species Programme working with the IUCN Species Survival Commission (SSC) has been assessing the conservation status of species, subspecies, varieties, and even selected subpopulations on a global scale for the past 50 years in order to highlight taxa threatened with extinction, and thereby promote their conservation. This system is designed to determine the relative risk of extinction, and the main purpose of the IUCN Red List is to catalogue and highlight those plants, fungi and animals that are facing a higher risk of global extinction.

Red list categories and criteria:

- **Extinct-**
  A taxon is extinct when there is no reasonable doubt that the last individual has died.
- **Extinct in the Wild**
  A taxon is extinct in the wild when it is known only to survive incultivation, In captivity or as a naturalized population well outside the past range
- **Near Threatened(NT)**

A taxon is NT when it has been evaluated against the criteria but does not qualify for CR, EN or VU now, but is close to qualifying for or is likely to qualify for a threatened category in the near future

- **Least Concern(LC)**
  A taxon is LC when it has been evaluated against the criteria and does not qualify for CR, EN, VU, or NT. Widespread and abundant taxa are included in this category
- **Data Deficient(DD)**
  A taxon is DD when there is inadequate information to make direct or indirect assessment of its risk of extinction based on its distribution and /or population status
- **Not Evaluated(NE)**
  A taxon is not evaluated when it has not yet been evaluated against the criteria

D. PROTECTED AREA CONCEPT:

India is one of the 17 mega diverse countries of the world. With only 2.4% of the world's land area, 16.7% of the world's human population and 18% livestock, it contributes about 8% of the known global biodiversity, however, putting enormous demands on our natural resources. India is home to world's largest wild tigers population and has got unique assemblage of globally important endangered species like Asiatic lion, Asian Elephant, One-horned Rhinoceros, Gangetic River Dolphin,Snow Leopard, Kashmir Stag, Dugong, Gharial, Great Indian Bustard, Lion Tailed Macaque etc.

Protected Area Network in India:

A National Board for Wildlife (NBWL), chaired by the Prime Minister of India provides for policy framework for wildlife conservation in the country. The National Wildlife Action Plan (2002-2016) was adopted in 2002, emphasizing the people's participation and their support for wildlife conservation. India's conservation planning is based on the philosophy of identifying and protecting representative wild habitats across all the ecosystems. The Indian Constitution entails the subject of forests and wildlife in the Concurrent list. The Federal Ministry acts as a guiding torch dealing with the policies and planning on wildlife conservation, while the provincial Forest Departments are vested with the responsibility of implementation of national policies and plans.

A network of 668 Protected Areas (PAs) has been established, extending over 1, 61,221.57 sq. kms. (4.90% of total geographic area), comprising 102 National Parks, 515 Wildlife Sanctuaries, 47 Conservation Reserves and 4 Community Reserves. 39 Tiger Reserves and 28 Elephant Reserves have been designated for species specific management of tiger and elephant habitats. UNESCO has designated 5 Protected Areas as World Heritage Sites.

As the ecosystems and species do not recognize political borders, the concept of Transboundary Protected Areas has been initiated for coordinated conservation of

ecological units and corridors with bilateral and/or multilateral cooperation of the neighboring nations. There are 4 categories of the Protected Areas viz, National Parks, Sanctuaries, Conservation Reserves and Community Reserves.

1. Sanctuary:

It is an area which is of adequate ecological, faunal, floral, geomorphological, natural or zoological significance. The Sanctuary is declared for the purpose of protecting, propagating or developing wildlife or its environment. Certain rights of people living inside the Sanctuary could be permitted. Further, during the settlement of claims, before finally notifying the Sanctuary, the Collector may, in consultation with the Chief Wildlife Warden, allow the continuation of any right of any person in or over any land within the limits of the Sanctuary.

2. National Park:

It is an area having adequate ecological, faunal, floral, geomorphological, natural or zoological significance. The National Park is declared for the purpose of protecting, propagating or developing wildlife or its environment, like that of a Sanctuary. The difference between a Sanctuary and a National Park mainly lies in the vesting of rights of people living inside. Unlike a Sanctuary, where certain rights can be allowed, in a National Park, no rights are allowed. No grazing of any livestock shall also be permitted inside a National Park while in a Sanctuary, the Chief Wildlife Warden may regulate, control or prohibit it. In addition, while any removal or exploitation of wildlife or forest produce from a Sanctuary requires the recommendation of the State Board for Wildlife, removal etc., from a National Park requires recommendation of the National Board for Wildlife (However, as per orders of Hon'ble Supreme Court dated 9th May 2002 in Writ Petition (Civil) No. 337 of 1995, such removal/ exploitation from a Sanctuary also requires recommendation of the Standing Committee of National Board for Wildlife).

3. Conservation Reserves:

This can be declared by the State Governments in any area owned by the Government, particularly the areas adjacent to National Parks and Sanctuaries and those areas which link one Protected Area with another. Such declaration should be made after having consultations with the local communities. Conservation Reserves are declared for the purpose of protecting landscapes, seascapes, flora and fauna and their habitat. The rights of people living inside a Conservation Reserve are not affected.

4. Community Reserves:

It can be declared by the State Government in any private or community land, not comprised within a National Park, Sanctuary or a Conservation Reserve, where an individual or a community has volunteered to conserve wildlife and its habitat. Community Reserves are declared for the purpose of protecting fauna, flora and traditional or cultural conservation values and practices. As in the case of a

Conservation Reserve, the rights of people living inside a Community Reserve are not affected.

Regulations/ laws relating to Protected Areas (PAs):

The PAs are constituted and governed under the provisions of the Wild Life (Protection) Act, 1972, which has been amended from time to time, with the changing ground realities concerning wildlife crime control and PAs management. Implementation of this Act is further complemented by other Acts viz. Indian Forest Act, 1927, Forest (Conservation) Act, 1980, Environment (Protection) Act, 1986 and Biological Diversity Act, 2002 and the Scheduled Tribes and Other Traditional Forest Dwellers (Recognition of Forest Rights) Act, 2006. The Wildlife Crime Control Bureau of the Central Government supplements the efforts of provincial governments in wildlife crime control through enforcement of CITES and control of wildlife crimes having cross-border, interstate and international ramifications. In order to strengthen and synergize global wildlife conservation efforts, India is a party to major international conventions viz. Convention on International Trade in Endangered Species of wild fauna and flora (CITES), International Union for Conservation of Nature (IUCN), International Convention for the Regulation of Whaling, UNESCO-World Heritage Committee and Convention on Migratory Species (CMS).

Main issues concerning the management of Protected Areas:

Wildlife conservation and management in India is currently facing a myriad of complex challenges that are both ecological and social in nature. Issues such as habitat loss/fragmentation, overuse of biomass resources in the context of biotic pressures, increasing human-wildlife conflicts, livelihood dependence on forests and wildlife resources, poaching and illegal trade in wildlife parts and products, need for maintaining a broad base of public support for wildlife conservation exemplify and characterize the contemporary wildlife conservation scenario in India. The government and the civil society are taking several measures to address these issues.

Probable Questions:

1. What are the major problems in conservation of Biodiversity in India?

2. State the existing policy of Biodiversity conservation.

3. Describe the international efforts for conserving biodiversity ?

4. Write down the purpose of CITES.

5. Write a short note on CBD.

6. What is IUCN Red List?

7. Write about protected area network in India.

8. Describe the regulations/ laws relating to Protected Areas (PAs).

Suggested Readings:

1.https://nt.gov.au/environment/animals/classification-of-wildlife

2. Biodiversity by Maity and Maity

3. Ecology and Environmental science by Rana

4.http://www.envfor.nic.in/downloads/public-information/protected-area-network.pdf

UNIT V

Conservation of natural enemies of insect pest

Objective:

In this unit you will learn about Conservation of natural enemies of insect pest.

Introduction:

Biological control is the beneficial action of parasites, pathogens, and predators in managing pests and their damage. Biocontrol provided by these living organisms, collectively called "natural enemies," is especially important for reducing the numbers of pest insects and mites. Use of natural enemies for biological control of rangeland and wildland weeds (e.g., Klamath weed, St. Johnswort) is also effective. Plant pathogens, nematodes, and vertebrates also have many natural enemies, but this biological control is often harder to recognize, less well understood, and/or more difficult to manage. Conservation, augmentation, and classical biological control are tactics for harnessing natural enemies' benefits.

- **TYPES OF NATURAL ENEMIES**

Parasites, pathogens, and predators are the primary groups used in biological control of insects and mites (Table 1). Most parasites and pathogens, and many predators, are highly specialized and attack a limited number of closely related pest species.

i. Parasites

A parasite is an organism that lives and feeds in or on a host. Insect parasites can develop on the inside or outside of the host's body. Often only the immature stage of the parasite feeds on the host. However, adult females of certain parasites (such as many wasps that attack scales and whiteflies) feed on and kill their hosts, providing an easily overlooked but important source of biological control in addition to the host mortality caused by parasitism.

Although the term "parasite" is used here, true parasites (e.g., fleas and ticks) do not typically kill their hosts. Species useful in biological control, and discussed here, kill their hosts; they are more precisely called "parasitoids."

Most parasitic insects are either flies (Order Diptera) or wasps (Order Hymenoptera). Parasitic wasps occur in over three dozen Hymenoptera families. For example, Aphidiinae (a subfamily of Braconidae) attack aphids. Trichogrammatidae parasitize insect eggs. Aphelinidae, Encyrtidae, Eulophidae, and Ichneumonidae are other groups that parasitize insect pests. It's important to note that these tiny to medium-sized wasps are incapable of stinging people. The most common parasitic flies are the typically

hairy Tachinidae. Adult tachinids often resemble house flies. Their larvae are maggots that feed inside the host.

ii. Pathogens

Natural enemy pathogens are microorganisms including certain bacteria, fungi, nematodes, protozoa, and viruses that can infect and kill the host. Populations of some aphids, caterpillars, mites, and other invertebrates are sometimes drastically reduced by naturally occurring pathogens, usually under conditions such as prolonged high humidity or dense pest populations. In addition to a naturally occurring disease outbreak (epizootic), some beneficial pathogens are commercially available as biological or microbial pesticides. These include *Bacillus thuringiensis* or Bt, entomopathogenic nematodes, and granulosis viruses. Additionally, some microorganism by-products, such as avermectins and spinosyns are used in certain insecticides; but applying these products is not considered to be biological control.

iii. Predators

Predators kill and feed on several too many individual preys during their lifetimes. Many species of amphibians, birds, mammals, and reptiles prey extensively on insects. Predatory beetles, flies, lacewings, true bugs (Order Hemiptera), and wasps feed on various pest insects or mites. Most spiders feed entirely on insects. Predatory mites that feed primarily on pest spider mites include *Amblyseius* spp., *Neoseiulus* spp., and the western predatory mite, *Galendromus occidentalis*.

Table 1. Some pests and their common natural enemies.

	NATURAL ENEMIES					
PESTS	**Lacewings**	**Ladybeetles**	**Parasitic flies**	**Parasitic wasps**	**Predatory mites**	**Other Groups and Examples**
aphids	X	X		X		entomopathogenic fungi, soldier beetles, syrphid fly larvae
carpenterworm, clearwing moth larvae				X		entomopathogenic nematodes
caterpillars (e.g., California oakworm)	X		X	X		*Bacillus thuringiensis,* birds, entomopathogenic fungi and viruses, predaceous bugs and wasps, *Trichogramma* spp. (egg parasitic wasps), spiders

cottony cushion scale		X	X			*Cryptochaetum iceryae* (parasitic fly), vedalia beetle
elm leaf beetle			X	X		*Erynniopsis antennata* (parasitic fly), *Oomyzus* (=*Tetrastichus*) spp. (parasitic wasps)
eucalyptus longhorned borers				X		*Avetianella longoi* (egg parasitic wasp)
eucalyptus redgum lerp psyllid				X		*Psyllaephagus bliteus* (parasitic wasp)
giant whitefly	X	X		X		*Encarsia hispida, Encarsia noyesi, Entedononecremnus krauteri*, and *Idioporus affinis* (parasitic wasp), syrphid fly larvae
glassy-winged sharpshooter	X			X		assassin bugs, *Gonatocerus* spp. (egg parasitic wasps), spiders
lace bugs	X	X		X		assassin bugs and pirate bugs, spiders
mealybugs	X	X		X		mealybug destroyer lady beetle
mosquitoes						*Bacillus thuringiensis* spp. *israelensis*, mosquito-eating fish
psyllids	X	X		X		pirate bugs
scales	X	X		X	X	*Aphytis, Coccophagus, Encarsia,* and *Metaphycus* spp. parasitic wasps
slugs, snails			X			*Rumina decollata* (predatory snail), predaceous ground beetles, birds, snakes, toads, and other vertebrates
spider mites	X	X			X	bigeyed bugs and minute pirate bugs, *Feltiella* spp. (predatory cecidomyiid fly larvae), sixspotted thrips, *Stethorus picipes* (spider mite

						destroyer lady beetle)
thrips	X			X	X	minute pirate bugs, predatory thrips
weevils, root or soil-dwelling				X		*Steinernema carpocapsae* and *Heteror habditis bacteriophora* (entomopa thogenic nematodes)
whiteflies	X	X		X		bigeyed bugs and minute pirate bugs, *Cales, Encarsia,* and *Eretmoceru s* spp. parasitic wasps, spiders

- **RECOGNIZING THE ROLE OF NATURAL ENEMIES OF PEST INSECTS**

Pests are those species that attack some resource we human beings want to protect, and do it successfully enough to become either economically important or just a major annoyance. They are only a tiny fraction of the insect species around us. Even many of the species we would recognize as important pests only occasionally do significant damage to us or our resources.

Natural enemies play an important role in limiting the densities of potential pests. This has been demonstrated repeatedly when pesticides have devastated the natural enemies of potential pests. Insects which were previously of little economic importance often become damaging pests when released from the control of their natural enemies. Conversely, when a non-toxic method is found to control a key pest, the reduced use of pesticides and increased survival of natural enemies frequently reduces the numbers and damage of formerly important secondary pest species.

- The three categories of natural enemies of insect pests are: predators, parasitoids, and pathogens.

***Predators*:** Many different kinds of predators feed on insects. Insects are an important part of the diet of many vertebrates, including birds, amphibians, reptiles, fish, and mammals. These insectivorous vertebrates usually feed on many insect species, and rarely focus on pests unless they are very abundant. Insect and other arthropod predators are more often used in biological control because they feed on a smaller range of prey species, and because arthropod predators, with their shorter life cycles, may fluctuate in population density in response to changes in the density of their prey. Important insect predators include lady beetles, ground beetles, rove beetles, flower bugs and other predatory true bugs, lacewings, and hover flies. Spiders and some families of mites are also predators of insects, pest species of mites, and other arthropods.

***Parasitoids*:** Parasitoids are insects with an immature stage that develops on or in a single insect host, and ultimately kills the host. The adults are typically free-living, and may be predators. They may also feed on other resources, such as honeydew, plant nectar or pollen. Because parasitoids must be adapted to the life cycle, physiology and defenses of their hosts, they are limited in their host range, and many are highly specialized. Thus, accurate identification of the host and parasitoid species is critically important in using parasitoids for biological control.

***Pathogens*:** Insects, like other animals and plants, are infected by bacteria, fungi, protozoans and viruses that cause disease. These diseases may reduce the rate of feeding and growth of insect pests, slow or prevent their reproduction, or kill them. In addition, insects are also attacked by some species of nematodes that, with their bacterial symbionts, cause disease or death. Under certain environmental conditions, diseases can multiply and spread naturally through an insect population, particularly when the density of the insects is high.

An example of an established population of an insect pathogen which has been successfully controlling its host is the fungus *Entomophaga maimaiga*, a pathogen of the gypsy moth. This fungus is believed to have been introduced about 1911, but was not discovered in forests until 1989, when it was widespread and abundant in New England. It has continued to control gypsy moth populations here for several years. It overwinters in leaf litter as resting spores, which germinate when gypsy moth larvae are present. First-instar caterpillars are dispersed by wind, and those that fall to the forest floor are probably infected while crawling to a tree. While these larvae are feeding in the tree canopy, if there is adequate rainfall, the fungus in their bodies produces spores that spread to other caterpillars. If conditions are suitable, this infection cycle will occur again during the larval stage. Large caterpillars rest during the day in forest litter, where they are also susceptible to infection by germinating resting spores. In late June, as infected caterpillars die in large numbers, new resting spores are produced to survive the next winter. This biological control agent is dependent on rain at appropriate times during the season to be successful.

- **USING BIOLOGICAL CONTROL IN THE FIELD**

There are three primary methods of using biological control in the field:

1) Conservation of existing natural enemies,

2) Introducing new natural enemies and establishing a permanent population (called "classical biological control"), and

3) Mass rearing and periodic release, either on a seasonal basis or inundatively.

1. Conservation of existing natural enemies

Reducing pesticide use: Most natural enemies are highly susceptible to pesticides, and pesticide use is a major limitation to their effectiveness in the field. The original idea that inspired integrated pest management (IPM) was to combine biological and chemical control by reducing pesticide use to the minimum required for economic production, and applying the required pesticides in a manner that is least disruptive to biological control agents. The need for pesticides can be reduced by use of resistant varieties, cultural methods that reduce pest abundance or damage, methods of manipulating pest mating or host-finding behavior, and, in some cases, physical methods of control. Many IPM programs, however, have not been able to move beyond the first stage of developing sampling methods and economic thresholds for pesticide application.

Several USDA and EPA surveys of pesticide use in major crops indicate that the quantity of pesticides used in the U.S. has been stable or increasing since the late 1980's. Although there are variations by crop and class of pesticide, the overall trend is that previous reductions, due to the substitution of economic thresholds for calendar spraying and the use of pesticides effective at lower dosages, are being reversed by increases in acreage treated and number of treatments per season. This stagnation of pest management has resulted in calls for IPM to be re-focused toward preventing pest problems by greater understanding of pest ecology, enhancing the ability of plants and animals to defend themselves against pests, and building populations of beneficial organisms. This strategy is sometimes called "biointensive IPM."

Selecting and using pesticides to minimize the effect on natural enemies

The effect of a pesticide on natural enemy populations depends on the physiological effect of the chemical and on how the pesticide is used -- how and when it is applied, for example. While insecticides and acaricides are most likely to be toxic to insect and mite natural enemies, herbicides and fungicides are sometimes toxic as well. A database has been compiled on the effects of pesticides on beneficial insects, spiders and mites (summarized in Croft 1990 and Benbrook 1996). This database compares the toxicity of different pesticides and the "selectivity ratio" -- the dose required to kill 50% of the target pest divided by the dose that kills 50% of the affected natural enemy species. Among the insecticides, synthetic pyrethroids are among the most toxic to beneficials, while *Bacillus thuringiensis* and insect growth regulators were among the least toxic. In general, systemic insecticides, which require consuming plant material for exposure, and insecticides that must be ingested for toxicity affect natural enemies much less than pests.

Pesticides may also have more subtle effects on the physiology of natural enemies than direct toxicity. Several fungicides, such as benomyl, thiophanate-methyl, and carbendazim, inhibit oviposition by predacious phytoseiid mites. Certain herbicides (diquat and paraquat) make the treated soil in vineyards repellent to predacious mites.

The impact of pesticides on natural enemies can be reduced by careful timing and placement of applications to minimize contact between the beneficial organism and the pesticide. Less persistent pesticides reduce contact, especially if used with knowledge of the biology of the natural enemy to avoid susceptible life stages. Spot applications in the areas of high pest density or treatment of alternating strips within a field may leave natural enemies in adjacent areas unaffected. The effectivenss of limiting the areas treated may depend on the mobility of the natural enemy and the pest.

Providing habitat and resources for natural enemies

Natural enemies are generally not active during the winter in the Northeast, and thus, unless they are re-released each year, must have a suitable environment for overwintering. Some parasitoids and pathogens overwinter in the bodies of their hosts (which may then have overwintering requirements of their own), but others may pass the winter in crop residues, other vegetation, or in soil. A classic example is the overwintering of predacious mites in fruit orchards. Ground cover in these orchards provides shelter over the winter, refuge from pesticides used on the fruit trees, and a source of pollen and alternate prey.

The adults of many predators and parasitoids may require or benefit from pollen, nectar or honeydew (produced by aphids) during the summer. Many crop plants flower uniformly for only a short time, so flowering plants along the edges of the field or within the field may be needed as supplemental sources of pollen and nectar. However, diversification of plants within the field can also interfere with the efficiency of host-finding, particularly for specialist parasitoids. Populations of generalist predators may be stabilized by the availability of pollen and alternative prey, but the effectiveness of the predators still depends on whether they respond quickly enough, either by aggregation or multiplication, to outbreaks of the target pest. Thus, diversification of plants or other methods of supplementing the nutrition of natural enemies must be done with knowledge of the behavior and biology of the natural enemy and pest.

For example, the native lady beetle *Coleomegilla maculata* is a potentially important predator of the eggs and early instar larvae of Colorado potato beetle. The population feeding on the potato beetle depends on the availability of aphid prey in surrounding fields, including crops of alfalfa, brassicas, cucurbits, and corn, and on the availability of pollen from corn and several weeds, such as dandelion and yellow rocket. Although this predator does not currently control Colorado potato beetle on its own, more knowledge about managing *C. maculata* populations in the agricultural landscape could make it more effective.

2. Introducing new natural enemies and establishing a permanent population

This is a process which requires extensive research into the biology of the pest, potential natural enemies and their biology, and the possibility of unintended consequences (e.g. negative effects on native species which are not pests or on other

natural enemies of the pest). After suitable natural enemies are found, studied, and collected, they must undergo quarantine to eliminate any pathogens or parasites on the natural enemy population. Then, the natural enemies are carefully released, with attention to proper timing in the enemy and pest life cycles, in a site where the target pest is abundant, and where disturbance of the newly released enemies is minimized. Although this process is long and complex, when it is successful, the results can be impressive and permanent, as long as care is taken in production practices to minimize negative effects on the natural enemy.

One of many examples of a pest controlled by successful introduction of new natural enemies is the alfalfa weevil. The alfalfa weevil is native to Europe, and was first reported in the US in 1904. It appeared in the eastern US about 1951, and by the 1970's was a major pest across the country. Larval densities were high enough to require most growers to spray one or more times per year. Several parasitoids were introduced from Europe against this pest. The most successful introductions include two species of parasitoids attacking the larvae, one attacking the adult, and a parasitoid and predator attacking the eggs. A program to collect the most effective natural enemies, rear them in large numbers, and release the progeny assisted in the spread of some of these species. These natural enemies, plus a fungal disease that infects larvae and pupae, have kept the densities of alfalfa weevil far below the economic injury level in most years in the Northeast. The success of this biological control has been enhanced by cultural methods, such as timing cuttings to reduce weevil populations and avoid disruption of natural enemies. The introduction of additional natural enemies against other alfalfa pests and the use of pest-resistant alfalfa varieties have mimimized the insecticide use against alfalfa blotch leafminer and aphids, thus avoiding disruption of the natural enemies of alfalfa weevil.

3. Mass culture and periodic release of natural enemies

a. Seasonal inoculative release

In some cases, a natural enemy is not able to overwinter successfully here in the Northeast, due to the weather or the lack of suitable hosts or prey. In other cases, such as in greenhouses, all possible habitat for the natural enemy is removed at the end of the season or production cycle. Thus, particularly in annual crops, or in other highly disturbed systems, the natural enemy may need to be reintroduced regularly in order to maintain control of the pest.

Seasonal inoculative release of insect parasitoids and predators has been a highly successful strategy for biological control in greenhouses in Europe. This strategy was adopted by growers because of the prevalence of resistance to insecticides in many greenhouse pests, and the rising costs of chemical control. The program was originally built around use of the parasitoid *Encarsia formosa* against the greenhouse whitefly and the predacious mite *Phytoseiulus persimilis* against the two-spotted spider mite. Over the years, additional natural enemies have been added to control other pests, such as thrips, leafminers, aphids, caterpillars, and additional species of whiteflies, as needed.

The costs of using biological control are now much lower in Europe than using chemical control for insect pests. Growers are informed about the details of implementation of the program, new developments, and new natural enemies through a network of extension advisers, specialized journals and grower study groups.

Two examples of seasonal inoculative release in the field are the use of the parasitic wasp, *Pediobius foveolatus,* against Mexican bean beetles, and the parasitic wasp, *Edovum puttleri*, against the Colorado potato beetle. Neither of these parasitoids can survive the winter in the Northeastern U.S. However, methods have been developed for rearing them in the laboratory and releasing them annually, and they multiply in the field, killing their hosts through the season. *P. foveolatus* is commercially available, and *E. puttleri* is being reared and released by the New Jersey Department of Agriculture for IPM of eggplant.

b. Biological insecticides or inundative release

These two approaches are fundamentally different from all the other approaches to biological control because they do not aim to establish a population of natural enemies that multiplies to a level where it reaches a long-term balance with the population of its hosts or prey. Instead, the idea is to use biological agents like an pesticide -- to release them in quantities that will knock down the pest population. Most commercially available formulations of insect pathogens are used inundatively.

Products based on the bacteria *Bacillus thuringiensis* are the best known example of a biological insecticide. A Bt spray is essentially an insecticide which works by paralyzing the gut of the insect (depending on the strain used, either caterpillars, Colorado or elm leaf beetle larvae, or mosquito or fungus gnat larvae). A protein produced by the bacterium is the active ingredient which paralyzes the gut, and in many products, there are no viable bacterial spores present, just a formulation of the active protein. Thus, the disease does not continue to spread in the insect population.

Beneficial nematodes are an example of live natural enemies that are inundatively released. These nematodes travel either through the soil or on the soil surface, and actively attack their insect hosts. Once inside, they release symbiotic bacteria, which multiply and kill the host. The nematodes feed on the bacteria and insect tissue, then mate and reproduce. After one to two weeks, new young nematodes emerge from the insect cadaver to seek new hosts. Nematodes are highly susceptible to desiccation, exposure to ultraviolet light, and extremes of temperature. They are most useful against insects living on or in the soil, or in other protected environments (such as tunneling inside plants). Adequate moisture and temperatures from about 53 to 86 degrees F. are critical to success.

Inundative release of insect and mite natural enemies in the field is still rather expensive, due to the costs of mass rearing, storage, and transportation of live organisms in the numbers required. However, research into artificial diets for natural enemies and other aspects of commercial production continues to bring down the cost.

- *CONSERVATION: PROTECT NATURAL ENEMIES*

Preserve existing natural enemies by choosing cultural, mechanical, or selective chemical controls that do not harm beneficial species. Remember, only about 1% of all insects and mites are harmful. Most pests are attacked by multiple species of natural enemies (Table 1), and their conservation is the primary way to successfully use biological control. Judicious (e.g., selective, timing) pesticide use, ant control, and habitat manipulation are key conservation strategies.

Pesticide Management

Biological control's importance often becomes apparent when broad-spectrum, residual pesticides (those that persist for days or weeks) cause secondary pest outbreaks or pest resurgence. An example is the dramatic increase in spider mite populations (flaring) that sometimes results after applying a carbamate (e.g., carbaryl* or Sevin) or organophosphate (malathion) to control caterpillars or other insects.

Eliminate or reduce the use of broad-spectrum, persistent pesticides whenever possible. Carbamates, organophosphates, and pyrethroids kill natural enemies that are present at the time of spraying and for days or weeks afterwards their residues kill predators and parasites that migrate in after spraying. Neonicotinoids (e.g., dinetofuran, imidacloprid) and other systemic insecticides that translocate (move) into blossoms can poison natural enemies and honey bees that feed on nectar and pollen. Even if beneficials survive an application, low levels of pesticide residues can interfere with natural enemies' reproduction and their ability to locate and kill pests.

When pesticides are used, apply them in a selective manner. Treat only heavily infested areas with "spot" applications instead of entire plants. Choose insecticides that are more specific in the types of invertebrates they kill, such as *Bacillus thuringiensis* (Bt) that kills only caterpillars that consume treated foliage.

For most other types of exposed-feeding insects, rely on contact insecticides with little or no persistence, including azadirachtin, insecticidal soap, narrow-range oil (horticultural oil), neem oil, and pyrethrins, which are often combined with the synergist piperonyl butoxide.

In situations where you wish to foster biological control, use of nonpersistent pesticides can provide better long-term control of the pest because they do less harm to natural enemies that migrate in after the application. To obtain adequate control, thoroughly wet the infested plant parts with spray beginning in spring when pests become abundant. To provide sustained control, repeated application may be needed.

For certain harder-to-control pests where contact-only insecticides are inadequate, other choices include spinosad, a fermentation product of a naturally occurring bacterium. This insecticide persists about 1 week and it has translaminar activity (is absorbed short distances into plant tissue). Spinosad can be toxic to certain natural enemies (e.g., predatory mites, *Trichogramma* wasps, and syrphid fly larvae) and bees

when sprayed and for about 1–4 days afterwards; do not apply spinosad to plants that are flowering.

Ant Control and Honeydew Producers

The Argentine ant and certain other ant species are considered pests primarily because they feed on honeydew produced by insects that suck phloem sap, such as aphids, mealybugs, soft scales, psyllids, and whiteflies. Ants protect honeydew producers from predators and parasites that might otherwise control them. Ants sometimes move these honeydew-producing insects from plant to plant (called "farming"). Where natural enemies are present, if ants are controlled, populations of many pests will gradually (over several generations of pests) be reduced as natural enemies become more abundant. Control methods include cultivating soil around ant nests, encircling trunks with ant barriers of sticky material, and applying insecticide baits near plants.

Habitat Manipulation

Plant a variety of species that flower at different times to provide natural enemies with nectar, pollen, and shelter throughout the growing season. The adult stage of many insects with predaceous larvae (such as green lacewings and syrphid flies) and many adult parasites feed only on pollen and nectar. Even if pests are abundant for the predaceous and parasitic stages, many beneficials will do poorly unless flowering and nectar-producing plants are available to supplement their diet. To retain predators and parasites, grow diverse plant species well adapted to the local conditions and that tolerate low populations of plant-feeding insects and mites so that some food is always available.

Other cultural controls that can help natural enemies include reducing dust and properly fertilizing and irrigating. Dust can interfere with natural enemies and may cause outbreaks of pests such as spider mites. Reduce dust by planting ground covers and windbreaks and hosing off small plants that become excessively covered with dust. Avoid excess fertilization and irrigation, which can cause phloem-feeding pests, such as aphids, to reproduce more rapidly than natural enemies can provide control.

AUGMENTATION

When resident natural enemies are insufficient, their populations can sometimes be increased (augmented) through the purchase and release of commercially available beneficial species. However, there has been relatively little research on releasing natural enemies in gardens and landscapes. Releases are unlikely to provide satisfactory pest control in most situations. Some marketed natural enemies are not effective. Many natural enemies are generalist predators and are cannibalistic and feed indiscriminately on pest and beneficial species, thereby reducing their effectiveness.

Only a few natural enemies can be effectively augmented in gardens and landscapes. For example, entomopathogenic nematodes can be applied to control certain tree-boring and lawn-feeding insects. Convergent lady beetles (*Hippodamia convergens*) purchased in bulk through mail order, stored in a refrigerator, and released in very large numbers

at intervals can temporarily control aphids; however, lady beetles purchased through retail outlets are unlikely to be sufficient in numbers and quality to provide control.

Successful augmentation generally requires advanced planning, biological expertise, careful monitoring, optimal release timing, patience, and situations where certain levels of pests and damage can be tolerated. Situations where pests or damage are already abundant are not good opportunities for augmentation.

Probable Questions:

1. What do you mean by Biological pest control?
2. Discuss the role of parasite in biological control.
3. Discuss the role of natural enemies of pest insects.
4. What do you mean by parasitoid?
5. Describe the role of natural enemies in biological control in the field.
6. Discuss about introducing new natural enemies and establishing a permanent population during pest control.
7. What do you mean by Pesticide Management?
8. What is augmentation?

Suggested Readings / References

- DeBach, P. 1991. Biological control by natural enemies. 2nd edition. Cambridge University Press.
- US Congress, Office of Technology Assessment. 1995. Biologically based technologies for pest control. OTA-ENV-636. US Government Printing Office.
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UNIT-VI

Concept of Habitat and Niche

Objective: In this unit you will learn about concept of habitat and niche.

Introduction:

Habitat refers to a specific place where a species normally lives. For example, habitat of a tiger is the forest, of a shark is the sea, and of Plasmodium are the red blood cells. More than one animal or plant may live in the same habitat. For example, tiger, deer, wolf, fox, lion, etc. may be found in the same forest. Animals exhibit habitat specificity and require specific environmental conditions to live. For example, a fish lives in an aquatic habitat, but a river fish can live only in freshwater, while a sea fish can live only in a marine habitat. Some organisms are more tolerant than the other.

A habitat can be subdivided into regions with different environmental conditions. These subdivisions are called microhabitat. For example, in a pond, some organisms are surface dwellers while some others are bottom dwellers. For a species to maintain its population, its individuals must survive and reproduce. Certain combinations of environmental conditions are necessary for individuals of each species to tolerate the physical environment, obtain energy and nutrients and avoid predators.

The total requirements of a species, i.e. resources and physical conditions determine where it can live and how abundant it can be at any one place within its range. These requirements are termed abstractly as the ecological niche. In other words, niche is a term used to indicate not only the habitat but also the role played by the organisms in the environment. G.E. Hutchinson (1958) suggested that the niche could be modelled as an imaginary space with many dimensions, in which each dimension or axis represents the range of specific environmental condition or resource that is required by the species. Thus, the niche of a plant might include the range of temperatures that it can tolerate, the intensity of light required for photosynthesis, specific humidity regimes and minimum quantities of essential soil nutrients for uptake.

A useful extension of the niche concept is the distinction between fundamental and realised niches (Fig. 2). The fundamental niche of a species includes the total range of environmental conditions that are suitable for existence without the influence of interspecific competition or predation from other species. The realised niche describes that part of the fundamental niche was actually occupied by the species.

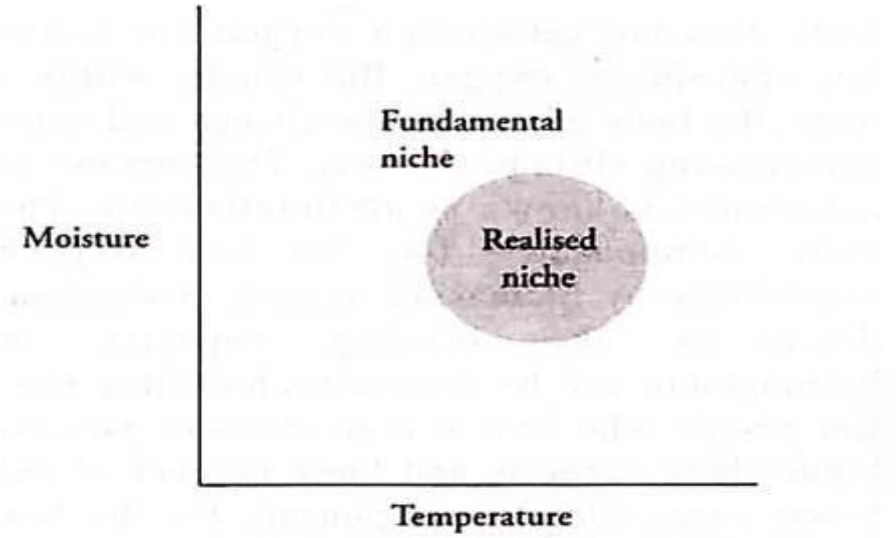


Fig. 2 A hypothetical situation where species distribution is controlled by just two environmental variables: temperature and moisture. The dotted area depicts the fundamental niche that indicates the combination of temperature and moisture condition required by the species for survival and reproduction in its habitat. The non-dotted area depicts the realised niche that is actually utilised by the organism for its survival.

A habitat possesses many niches and supports many species. An organism changes its niches as they develop. For example, the common toad, *Bufobufo* occupies the aquatic environment when it is a tadpole and feeds on algae and detritus. But after it metamorphosis's into an adult it becomes terrestrial and becomes insectivorous. According to Odum, while the habitat is the organism's 'address', its ecological niche is its 'profession'. Two organisms may be found in the same habitat, but do not occupy the same ecological niche. Each plays a different role in its habitat. Different animals that occupy similar ecological niche in different geographical regions are called 'ecological equivalents'.

Aspects of Ecological Niche:

The three aspects of ecological niche are designated as:

(i) Spatial or habitat niche,

(ii) Trophic niche, and

(iii) Multidimensional or hyper volume niche.

(i) Spatial or habitat niche:

Joseph Grinnell's (1928) thought of a niche in terms of the microhabitat that a species occupies— it is now called spatial niche. Spatial or habitat niche represents the physical

space occupied by an organism. Thus, in a habitat occupied by many species, each species is confined to a microhabitat. No two species can occupy the same habitat.

An interesting example of habitat niche was provided by O'Neill (1967). He identified seven species of millipedes living in the forest floor of a maple-oak forest. All the seven species occur in the same basic trophic level as all are detritus feeder.

The log of maple-oak has several gradients in decomposition stage from the centre to the position underneath the leaf litter. These gradients constitute distinct microhabitats and each specie:; of millipede occupies a specific different microhabitat.

(ii) Trophic niche:

Charles Elton (1927) was the first who used the term niche as the "functional status of an organism in its community". He thus emphasised the importance of energy relations and, thus, the concept is designated as trophic niche. Trophic niche is concerned with the trophic position of an organism.

Example of trophic niche is provided by weaver birds present in the vicinity of Lake Mweru in Africa. All the three weaver birds present had different choice of food, thus occupying different trophic niches. Two of them, *Ploceus collaris* and *P. melanocephalus*, live in the same nest but one feeds on seeds and the other on insects.

(iii) Multidimensional or hyper-volume niche:

In 1957, G. E. Hutchinson suggested that niche could be visualised as a multidimensional space or hyper-volume. Within this, the environment permits an individual species to survive indefinitely. This niche concept of Hutchinson is designated as multidimensional or hyper-volume niche. In this case the niche can be measured, mathematically manipulated and represented in climo-graphs.

Concept of Ecological Niche:

The ecological niche concept was first formally defined by Hutchinson in 1957. According to him, the activity range of any species could be described along all the dimensions (parameters) of the environment.

These dimensions include physical and chemical parameters such as temperature, humidity, salinity, oxygen concentration etc. and biological factors such as prey species, resting backgrounds against which an individual may escape detection by predators and so on.Each of these dimensions are represented as dimension in space. Supposing there are 'n' number of dimensions, then the niche would be described in n-dimensional space. As it is not possible to visualize a space of more than three dimensions, the multidimensional aspect could only be represented mathematically and statistically, and then depicted by their essence physically or graphically (by set of independent axis).

A graphical representation of a biological activity to a single environmental gradient representing the distribution of a species activity along one niche dimension is given in Fig. 4.50a. It depicts the degree to which the environment can support that species in relation to a particular parameter.

Fig. 4.50b shows two dimensions where the species niche may be depicted as a hill, with contours representing the various levels of biological activity. A three dimensional aspect can be visualised as a cloud in space whose density conveys niche utilisation (Fig. 4.50c).

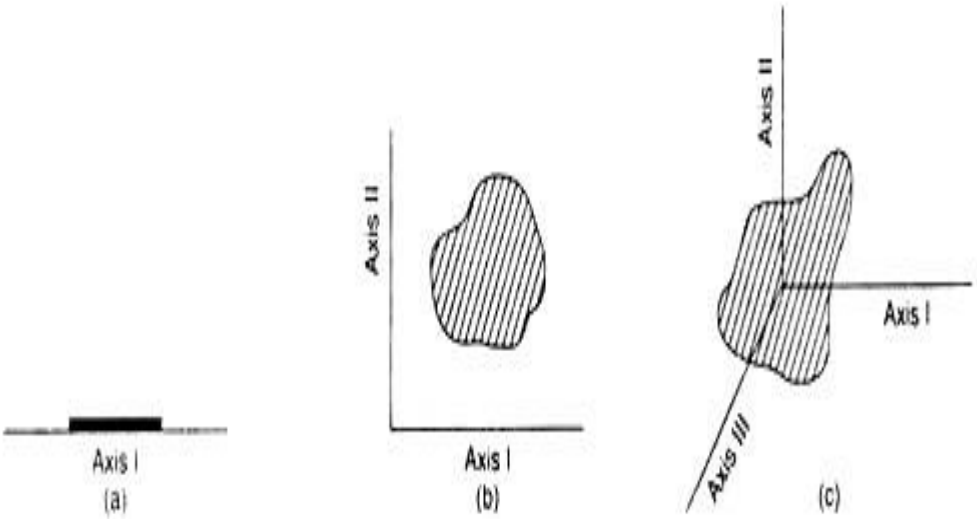


Fig. 4.50 : The pictorial representation of niche dimensions along (a) a single axis, (b) two axes and (c) three axes

As an example of the above, the blue-grey gnatcatcher's (Polioptila caerulea) feeding niche is represented on a two-dimensional graph (4.51a). The length of its prey is represented along the horizontal axis and the height above the ground at which the blue-grey gnatcatcher feed is shown along the vertical axis.

The contour lines on the figure represent the frequency with which the birds fed at a particular height and on a particular length of the prey. The data of the Fig. 4.51a is represented as a three-dimensional volume in Fig. 4.51b. Here, the feeding niche of the birds is represented as a hill. The peak of the hill shows where the birds are most likely to be found.As such, other axes such as ambient temperature, risk from predation at different times of the year and so on, could be added to the above figure. It almost becomes impossible to represent graphically the niches where more than two or three environmental variables are involved. However, computers can easily hold and analyse the data.

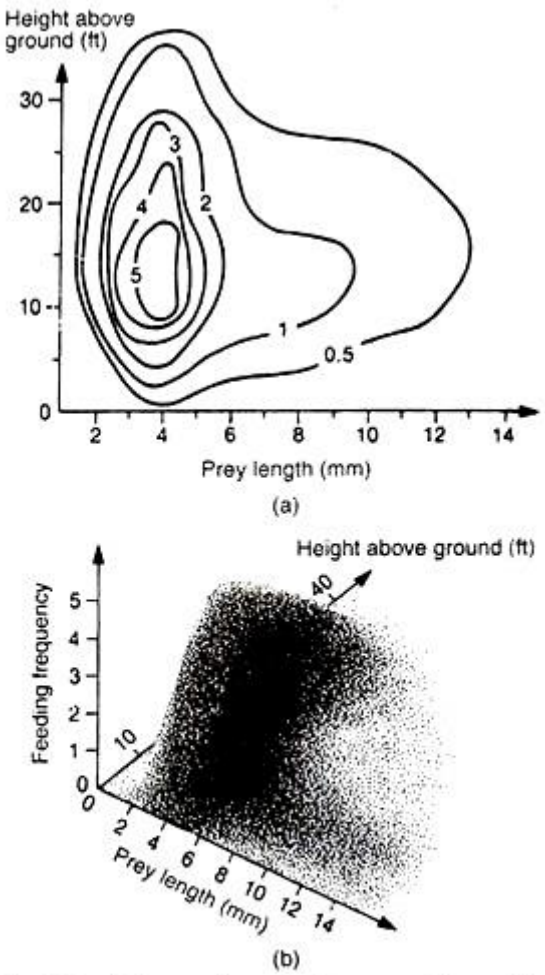


Fig. 4.51 : (a) A two-dimensional representation of the feeding niche of the blue grey gnatcatcher. (b) The data of Fig. (a) is plotted as a three-dimensional hill

The total resource space or niche space available to a community is represented as the n-dimensional volume, where the niche of all the species fit or where the niche of each species occupies a part. All the n-dimensions of a species niche is difficult to identify and measure.However, niche relationship within biological communities can be characterised by observing the patterns of resource utilisation and micro-habitat preferences on one or a few niche dimensions. When the preferences of individuals are plotted on a line representing the resource (or condition), a frequency distribution of utilisation (or tolerance) is obtained for each species (Fig. 4.52).

In this figure the utilisation of resource by individuals of two species m and n are plotted. Some individuals of both the species m and n utilise much more of the resource or some that utilise less. The distribution of each species is characterised by the location of its peak, its breadth and its light.The extent of the variety of resources used (or the extent of conditions tolerated) by the individuals in the population is represented by the width in the figure and is referred to as the niche breadth. Sometimes the term niche width, used in place of niche breadth, is used to represent the standard deviation of the distribution of the resource used.

Some individuals of species m, showing a higher utilisation of the resource than the average for their population, overlaps in their utilisation of resource with some individuals of species n having lower than average utilisation (represented in the Fig. 4.52 by the shaded region). Such overlap (or similarity) of utilisation of resources (or tolerance of conditions) is called niche overlap.

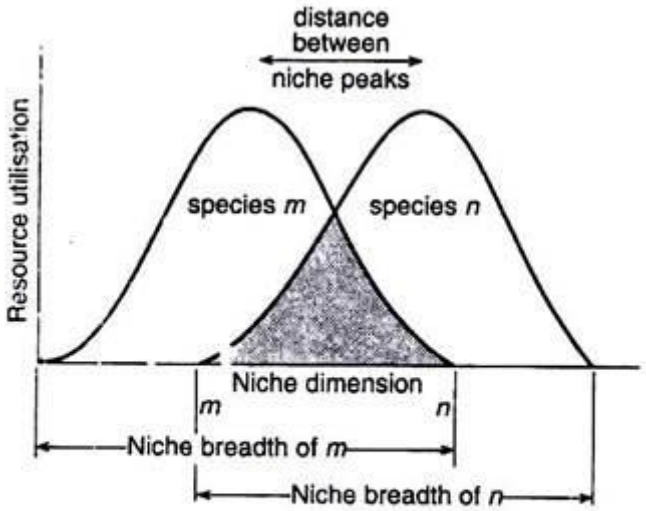


Fig. 4.52. Positions of two species (m and n) along a single resource dimension. The shaded area represents niche overlap, which is a portion of the resource that is used by both the species

Such niche overlaps indicate the intensity of competition between species in the community for a particular resource. If the two peaks (Fig. 4.52) of the distribution of species m and n are moved closer together, the niche overlap will increase.

When the curves of m and n totally overlap or when m = n, the utilisation of the resources by the two species (m and n) is said to be identical. When such a situation does occur, one would expect that competitive exclusion would eliminate one of the species from the system.If the two species—in spite of the similarity in their use of resource—still coexist, then it is called limiting similarity. Sometimes species may be too similar to coexist on one axis, they then differ in their resource utilisation. Such a situation is referred to as niche complementarity.

Species which occupy similar niches in different geographical regions are called ecological equivalent species. They tend to be closely related taxonomically in contiguous regions but often differ in noncontiguous regions. In different floral and faunal regions, the species composition of communities differs widely.Whenever physical conditions are similar (regardless of geographical locations), similar ecosystems develop equivalent functional niches. A grassland ecosystem thus develops whenever there is a grassland climate, but the species of grass and grazers may be quite different. For example, the kangaroos of the Australian grassland are the ecological equivalents of the bison and pronghorn of the North American grassland.

Fundamental and realised niches:

In 1958, Hutchinson introduced the terms fundamental niche and realised niche. Fundamental niche is the niche that an organism would occupy in the absence of competitors and predators. When the fundamental niches of two species overlap (Fig. 4.52), then the two species are said to be competing with one another.

However, in nature, two species do not overlap or compete for food, even if they eat the items of the same size, as they can look for them in different places. Most well-integrated communities like coral reef, climax forest etc. are made up of species with non-overlapping niches.Realised niche is referred to as the role an organism actually plays in the community. Hutchinson viewed that the outcome of inter-specific competition would lead either to extinction or the development of differences allowing coexistence. In nature, the realised niche of an organism is smaller than its fundamental niche.

Niche and species diversity:

In the species concept discussed above, the discussion included two species only. If the concept is expanded by taking more than two species (which occurs in a natural community) then some fundamental ideas about the mechanisms regulating diversity in the community can be uncovered.

Addition of species increases the species richness and the community accommodates them in three possible ways:

1. The original species and the added ones could maintain the same niche widths and overlaps and in such case the total niche space has to be increased. Fig. 4.53B depicts the increase by extending the niche axis line which implies the addition of new resource types. This could increase the biodiversity through increased resource diversity.

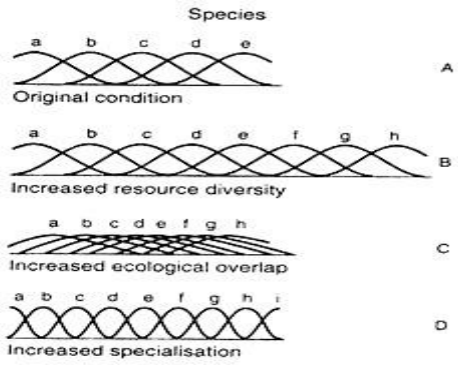


Fig. 4.53 : Diagram showing accommodation of more species through alternation of resource utilisation along a single niche axis : A. The original community. B. Increasing resource diversity (shown by extending the axis). C. Increasing ecological overlaps. D. Increased specialisation through decreased niche breadth of the species. Both C and D are forms of species packing

2. Without increasing the resource diversity the added species could be accommodated by increasing the niche overlaps (Fig. 4.53C). This would lead to a decrease of the average productivity of each species, due to increased sharing of resources.

3. Without increasing niche overlap the added species could be accommodated by increasing species specialisation within a community's niche space (Fig. 4.53D). Here also the average productivity would decrease as each species would have access to a narrower range of resource. Increase in species richness without a change in resource diversity is called species packing.

Species adaptation and diversity:

Discussion of ecological niche is not limited to resources and physical conditions only. Predation avoidance is also important to population processes. Niche discussion does constitute areas of predator escape along which species may escape from getting noticed. Such niche space that is defined by adaptations (including behaviour) of prey organisms and helps them to avoid predation is called escape space.

When many prey species using the same mechanism to escape predation and occupying portions of densely populated niche space, they become very much susceptible to predators having appropriate learning behaviour.This would result in increased mortality. However, those organisms having unusual adaptation for predator escape would be strongly selected. Thus, predation pressure would diversify prey and make them uniformly distributed within available escape space.

Advantages of Ecological Niche:

1. Animals can escape competition by occupying different ecological niches.

2. The ecological niche occupied by a species is favourable to it as it furnishes a suitable substratum and microclimate.

3. Segregation of organisms into niches avoids confusion of activities in the community and gives a more orderly and efficient life pattern for each species.

4. Segregation of different species in a particular niche results in full exploitation of all available resources.

Examples of Ecological Niches

a. Kirtland's Warbler

Kirtland's warbler is a rare bird that lives in small areas in Michigan's northern Lower and Upper Peninsulas. The niche of Kirtland's warbler is the jack pine forest, and the forest must have very specific conditions. Jack pine forests with areas of over 80 acres

are ideal for this species. Specifically, these forests must have dense clumps of trees with small areas of grass, ferns and small shrubs in between. Kirtland's warbler nests on the ground beneath the branches when the tree is about 5 feet tall, or around 5-8 years old. When the tree reaches about 16-20 feet tall, the lower branches start to die, and the bird will no longer nest beneath the tree branches.

Jack pine forests remained virtually undisturbed during Michigan's lumber boom in the early 1800s because white pine was a much more valuable. The consistent availability of young jack pines for nesting was generated by naturally occurring wildfires in this habitat. When the lumber boom ended in the late 1800s, the wildfires continued and allowed the jack pine to spread and create more habitat for Kirtland's warbler. The species population reached its peak from 1885-1900. Humans began to alter this niche by fighting and putting out forest fires. Over time, this severely affected the Kirtland's warbler population. Large areas of jack pine forest were designated for habitat management via logging, burning, seeding and replanting in the 1970s, and the species recovered.

The image above shows a female Kirtland's Warbler, *Dendroicakirtlandii*.

Dung Beetle

As the name implies, dung beetles eat dung, both as adults and as larvae. They live on all continents except Antarctica. Dung is plentiful throughout the world, and over time, the dung beetle has learned to exploit it as a resource, and create its own niche. Dung beetles are known for the way in which they roll dung into a ball before transporting it. These balls area buried in an underground burrow to either be stored as food or used as brooding balls. The female lays eggs in the brooding ball and the larvae hatch inside. When they reach adult size, the beetles dig out of the ball and work their way to the soil surface. The actions of dung beetles serve several important functions in their habitat. Digging burrows and tunnels turns over and aerates the soil. The buried dung releases nutrients into the soil that benefits other organisms. In addition, the beetle's use of dung leaves less available for flies to breed on, thus controlling some of the fly population.

The image above shows *Khepernigro aeneus*, the Large Copper Dung Beetle, on a ball of dung

Xerophytic Plants

Xerophytic plants have developed several adaptations to living in dry ecological niches. The adaptations evolved to help save water stored in the plant and to prevent water loss. Examples of xerophytes are cacti and aloe vera, also called succulents. These plants have thick fleshy leaves that store water, and long roots to reach water deep underground. Other adaptations that xerophytic plants use include the ability to move or fold up their leaves, dropping their leaves during dry periods, a waxy coating to prevent evaporation (called the cuticle) and thick hairy leaf coverings. The surface of plant leaves features stomata, which are tiny mouth-like structures that take in carbon dioxide and release oxygen and water. Plants usually open their stomata during the day and close them at night. Succulents do the opposite in order to reduce water loss during the heat of the day.

There are mainly three concepts about niches which are described below:

a. Grinnellian niche

The ecological meaning of niche comes from the meaning of niche as a recess in a wall for a statue, which itself is probably derived from the Middle French word *nicher*, meaning *to nest*.[1][8] The term was coined by the naturalist Roswell Hill Johnson but Joseph Grinnell was probably the first to use it in a research program in 1917, in his paper "The niche relationships of the California Thrasher".

The Grinnellian niche concept embodies the idea that the niche of a species is determined by the habitat in which it lives and its accompanying behavioural adaptations. In other words, the niche is the sum of the habitat requirements and behaviours that allow a species to persist and produce offspring. For example, the

behaviour of the California thrasher is consistent with the chaparral habitat it lives in—it breeds and feeds in the underbrush and escapes from its predators by shuffling from underbrush to underbrush. Its 'niche' is defined by the felicitous complementing of the thrasher's behaviour and physical traits (camouflaging color, short wings, strong legs) with this habitat.

This perspective of niche allows for the existence of both ecological equivalents and empty niches. An ecological equivalent to an organism is an organism from a different taxonomic group exhibiting similar adaptations in a similar habitat, an example being the different succulents found in American and African deserts, cactus and euphorbia, respectively. As another example, the anole lizards of the Greater Antilles are a rare example of convergent evolution, adaptive radiation, and the existence of ecological equivalents: the anole lizards evolved in similar microhabitats independently of each other and resulted in the same ecomorphs across all four islands.

b. Eltonian niche:

In 1927 Charles Sutherland Elton, a British ecologist, defined a niche as follows: "The 'niche' of an animal means its place in the biotic environment, *its relations to food and enemies*."

Elton classified niches according to foraging activities ("food habits"). For instance there is the niche that is filled by birds of prey which eat small animals such as shrews and mice. In an oak wood this niche is filled by tawny owls, while in the open grassland it is occupied by kestrels. The existence of this carnivore niche is dependent on the further fact that mice form a definite herbivore niche in many different associations, although the actual species of mice may be quite different.

Conceptually, the Eltonian niche introduces the idea of a species' response to and effect on the environment. Unlike other niche concepts, it emphasizes that a species not only grows in and responds to an environment based on available resources, predators, and climatic conditions, but also changes the availability and behaviour of those factors as it grows. In an extreme example, beavers require certain resources in order to survive and reproduce, but also construct dams that alter water flow in the river where the beaver lives. Thus, the beaver affects the biotic and abiotic conditions of other species that live in and near the watershed. In a more subtle case, competitors that consume resources at different rates can lead to cycles in resource density that differs between species. Not only do species grow differently with respect to resource density, but their own population growth can affect resource density over time.

c. Hutchinsonian niche

The Hutchinsonian niche is an "n-dimensional hypervolume", where the dimensions are environmental conditions and resources, that define the requirements of an individual or a species to practice "its" way of life, more particularly, for its population to

persist. The "hypervolume" defines the multi-dimensional space of resources (e.g., light, nutrients, structure, etc.) available to (and specifically used by) organisms, and "all species other than those under consideration are regarded as part of the coordinate system."

The niche concept was popularized by the zoologist G. Evelyn Hutchinson in 1957. Hutchinson inquired into the question of why there are so many types of organisms in any one habitat. His work inspired many others to develop models to explain how many and how similar coexisting species could be within a given community, and led to the concepts of 'niche breadth' (the variety of resources or habitats used by a given species), 'niche partitioning' (resource differentiation by coexisting species), and 'niche overlap' (overlap of resource use by different species).

Statistics were introduced into the Hutchinson niche by Robert MacArthur and Richard Levins using the 'resource-utilization' niche employing histograms to describe the 'frequency of occurrence' as a function of a Hutchinson coordinate. So, for instance, a Gaussian might describe the frequency with which a species ate prey of a certain size, giving a more detailed niche description than simply specifying some median or average prey size. For such a bell-shaped distribution,the position, width and form of the niche correspond to the mean, standard deviation and the actual distribution itself.[19] One advantage in using statistics is illustrated in the figure, where it is clear that for the narrower distributions (top) there is no competition for prey between the extreme left and extreme right species, while for the broader distribution (bottom), niche overlap indicates competition can occur between all species. The resource-utilization approach consists in postulating that not only competition *can* occur, but also that it *does* occur, and that overlap in resource utilization directly enables the estimation of the competition coefficients. This postulate, however, can be misguided, as it ignores the impacts that the resources of each category have on the organism and the impacts that the organism has on the resources of each category. For instance, the resource in the overlap region can be non-limiting, in which case there is no competition for this resource despite niche overlap.

An organism free of interference from other species could use the full range of conditions (biotic and abiotic) and resources in which it could survive and reproduce which is called its fundamental niche. However, as a result of pressure from, and interactions with, other organisms (i.e. inter-specific competition) species are usually forced to occupy a niche that is narrower than this, and to which they are mostly highly adapted; this is termed the realized niche. Hutchinson used the idea of competition for resources as the primary mechanism driving ecology, but overemphasis upon this focus has proved to be a handicap for the niche concept. In particular, overemphasis upon a species' dependence upon resources has led to too little emphasis upon the effects of organisms on their environment, for instance, colonization and invasions.

The term "adaptive zone" was coined by the palaeontologist George Gaylord Simpson to explain how a population could jump from one niche to another that suited it, jump to an 'adaptive zone', made available by virtue of some modification, or possibly a change in the food chain, that made the adaptive zone available to it without a discontinuity in its way of life because the group was 'pre-adapted' to the new ecological opportunity.

Hutchinson's "niche" (a description of the ecological space occupied by a species) is subtly different from the "niche" as defined by Grinnell.

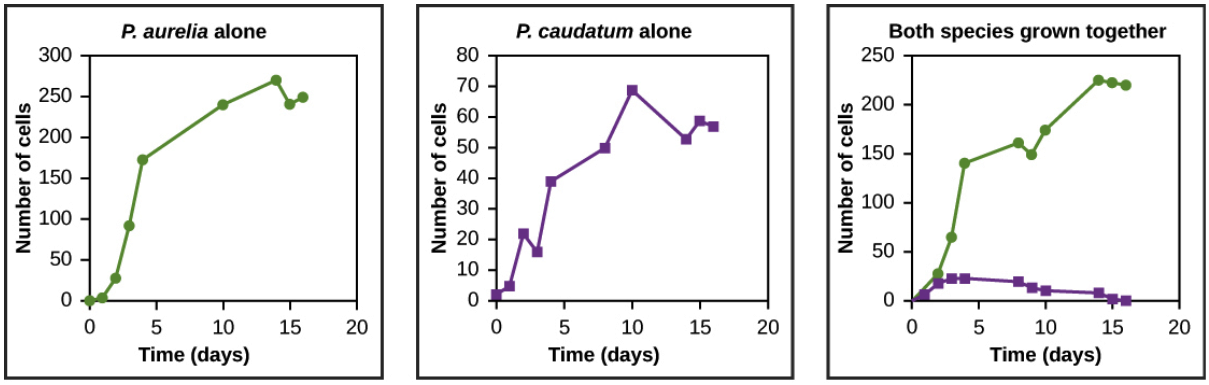
A niche is a very specific segment of ecospace occupied by a single species. On the presumption that no two species are identical in all respects (called Hardin's 'axiom of inequality') and the competitive exclusion principle, *some* resource or adaptive dimension will provide a niche specific to each species. Species can however share a 'mode of life' or 'autecological strategy' which are broader definitions of ecospace. For example, Australian grasslands species, though different from those of the Great Plains grasslands, exhibit similar modes of life.

Once a niche is left vacant, other organisms can fill that position. For example, the niche that was left vacant by the extinction of the tarpan has been filled by other animals (in particular a small horse breed, the konik). Also, when plants and animals are introduced into a new environment, they have the potential to occupy or invade the niche or niches of native organisms, often outcompeting the indigenous species. Introduction of non-indigenous species to non-native habitats by humans often results in biological pollution by the exotic or invasive species.

Competitive exclusion principle

The **competitive exclusion principle** tells us that two species can't have exactly the same niche in a habitat and stably coexist. That's because species with identical niches also have identical needs, which means they would compete for precisely the same resources.

A famous example of the competitive exclusion principle is shown in the figure below, which features two types of single-celled microorganisms, *Paramecium aurelia* and *Paramecium caudatum*. When grown individually in the lab, both species thrive. But when they are grown in the same test tube (habitat) with a fixed amount of nutrients, both grow more poorly and *P. aurelia* eventually outcompetes *P. caudatum* for food, leading to *P. caudatum*'s extinction.



Graphs a, b, and c all plot number of cells versus time in days. In Graph (a), P. aurelia is grown alone. In graph (b), *P. caudatum* is grown alone. In graph (c), both species are grown together. When grown separately, the two species both exhibit logistic growth and grow to a relatively high cell density. When the two species are grown together, *P. aurelia* shows logistic growth to nearly the same cell density as it exhibited when grown alone, but *P. caudatum* hardly grows at all, and eventually its population drops to zero.In nature, it's rarely the case that two species occupy exactly identical niches. However, the greater the extent to which two species' niches overlap, the stronger the competition between them.

Resource partitioning

Competitive exclusion may be avoided if one or both of the competing species evolves to use a different resource, occupy a different area of the habitat, or feed during a different time of day. The result of this kind of evolution is that two similar species use largely non-overlapping resources and thus have different niches. This is called **resource partitioning**, and it helps the species coexist because there is less direct competition between them.

The anole lizards found on the island of Puerto Rico are a good example of resource partitioning. In this group, natural selection has led to the evolution of different species that make use of different resources. The figure below shows resource partitioning among 111111 species of anole lizards. Each species lives in its own preferred habitat, which is defined by type and height of vegetation (trees, shrubs, cactus, etc.), sunlight, and moisture, among other factors.

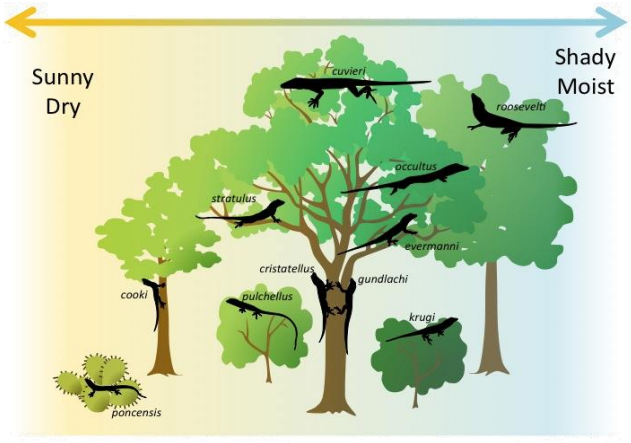


Fig: Resource partitioning

Probable Questions:

1. Define habitat and niche. What is the difference between them?
2. What is fundamental niche and realized niche ? Give examples.
3. Write notes on Grinnellian niche.
4. What is hypervolume niche.
5. Describe Eltonian niche.
6. Describe Hutchinsonian niche.
7. State competitive exclusion principle with suitable examples.
8. Describe resource partitioning with suitable examples.

Suggested readings:

1. Thomas M. Smith and Robert Leo Smith. Elements of ECOLOGY. *Eighth Edition*. ISBN 978-0-321-73607-9
2. Chaki, K. K., Kundu, G., and Sarker, S. (2012) Introduction to General Zoology. Vol II. ISBN: 81-7381-568-2, New Central Book Agency.
3. Ghosh, A. (2018) Notes on Habitat and Niche. Environment (http://www.yourarticlelibrary.com/ecology/notes-on-habitat-and-niche-environment /90914 2/5)

UNIT-VII

PRINCIPLES OF ECOLOGY

Objective: In this unit we will discuss different principles of ecology

Definition of Ecosystem:

The term Ecology (Greek Oikos-house, logos-study) was coined by German biologist Ernst Haeckel in 1869.Ecology deals with the study of interactions between living organisms and their physical environment.

Now ecology is defined as the study of ecosystems. The term ecosystem was proposed by A.C. Tansley in 1935 where eco implies the environment and system denotes an interacting, interdependent, integrated complex.Ecosystem may be defined as the system resulting from the integration of all living and non-living factors of the environment. Thus any structural and functional unit of biosphere where the organisms interact with the physical environment so that a flow of energy leads to clearly defined trophic structure, biotic diversity and material cycle (i.e., exchange of materials between living and non-living components) within the system is known as an ecological system or ecosystem.

Earth is a giant ecosystem where abiotic and biotic components are constantly acting and reacting with each other bringing structural and functional changes in it. This vast ecosystem-biosphere is subdivided into units of smaller ecosystems such as terrestrial and aquatic ecosystems.These systems may be freely exchanging energy and matter from outside—an open ecosystem or may be isolated from outside—a closed ecosystem.An ecosystem is normally an open system with a continuous but variable influx and loss of material and energy. It is a basic, functional unit with no limits of boundaries.Thus an ecosystem represents the highest level of ecological integration which is energy based and this functional unit is capable of energy transformation, accumulation and circulation. Its main function in ecological sense is to emphasize obligatory relationships, interdependence and casual relations.

Classification of Ecosystems

1. Natural Ecosystems (Self-operating):

These systems operate by themselves under natural conditions without any major interference by man.

These are further divided into following ecosystems:

(i) Terrestrial ecosystem includes forests, grasslands and deserts etc.

(ii) Aquatic ecosystem may be further distinguished as

(a) Fresh water which may be lotic (running water as springs, streams or rivers) or lentic (standing water as lakes, ponds, pools, ditches, puddles, swamps etc.).

(b) Marine water such as oceans (deep bodies) or seas or estuaries (shallow ones).

2. Artificial (Man-engineered) Ecosystems:

These are maintained artificially by man where, by addition of energy and planned manipulations, natural balance is disturbed regularly. Crop, urban, industrial, space and control of biotic community as well as the physico-chemical environment are man-engineered ecosystems.

3. Space Ecosystem is also recognised as one of ecosystems and play a very important role in human life.

The common features of all ecosystems — terrestrial, aquatic and agricultural are the interactions of the autotrophic and heterotrophic components.

Components of Ecosystem:

An ecosystem has two major components—biotic and abiotic.

(A) Biotic (Living) Components:

Plants, animals and micro-organisms having different nutritional behaviour constitute the biotic components of an ecosystem.

1. Producers (or Autotrophs-Self nourishing):

Producers are mainly chlorophyll bearing green plants (photo autotrophs) which can synthesize their food in presence of sunlight making use of CO_2 and water through the process of photosynthesis. Since plants convert solar energy into chemical energy so they must be better called converters or transducers. Chemosynthetic organisms or chemo-autotrophs can also synthesize some organic matter by the oxidation of certain chemicals in absence of sunlight.

2. Consumers (or Heterotrophs or Phagotrophs):

Consumers consume the matter built up by the producers. They utilise, rearrange and decompose complex materials.

[Note: The major autotrophic metabolism occurs in the upper green belt stratum where solar energy is available while the intense heterotrophic metabolism occurs in the lower brown belt where organic matter accumulates in soil and sediments.

Consumers are of the following types:

(i) Herbivores:

They feed directly on producers and hence are known as primary consumers, e.g., rabbit, deer, cattle, insects etc. Elton (1927) called herbivores as key industry animals because they convert plants into animal materials.

(ii) Carnivores (Meat eaters):

They feed on other consumers. If they feed on herbivores, they are called secondary consumers (e.g., frog, birds, cat) and if they prey on other carnivores (snake, peacock), they are known as tertiary carnivores/consumers. Lion, tiger etc. which cannot be preyed are called top carnivores since they occupy top position in the food chain.

(iii) Omnivores:

They feed both on plants and animals, e.g., rat, fox, birds and man.

(iv) Detritivores (Detritus feeders or saprotrophs):

They feed on partially decomposed matter such as termites, ants, crabs, earthworms etc.

3. Decomposers (or Micro-consumers):

Decomposers are saprophytic (osmotrophs) micro-organisms such as bacteria, actinomycetes and fungi. They derive their nutrition by breaking down complex organic compounds and release inorganic nutrients into environment, making them available again to producers.The biotic components of any ecosystem may be thought of as the functional kingdom of nature, since they are based on the type of nutrition and the energy source used. The entire earth is considered as an ecosystem which is referred to as biosphere or ecosphere.

(B) Abiotic (Non-living) Components:

Structurally abiotic components include:

1. Climatic regime:

Precipitation, temperature, sunlight, intensity of solar flux, wind etc. have a strong influence on the ecosystem.

2. Inorganic substances:

These are C, N, H, O, P, S involved in material cycles. The amount of these substances present in an ecosystem is known as standing state or standing quality.

3. Organic Substances:
Carbohydrates, proteins, lipids and humic substances link the abiotic components with the biotic components. All the biotic and abiotic components of an ecosystem are influenced by each other and are linked together through energy flow and matter cycling.

Structure of an Ecosystem:

The structure of an ecosystem is characterised by the composition and organisation of biotic communities and abiotic components.

The major structural features of an ecosystem are:

1. Species Composition:
Every ecosystem has its own type of species composition which differs from other ecosystems.

2. Stratification:
The organisms in each ecosystem form one or more layers or strata each comprising the population of particular kind of species. In some ecosystems like tropical rain forests, the crown of trees, bushes and ground vegetation form different strata and are occupied by different species. On the other hand, desert ecosystem shows a low discontinuous herbal layer consisting of extensive bare patches of soil.The quantity and distribution of non-living materials such as nutrients and water etc.The range or gradient of conditions of existence such as temperature and light etc.

Functions of an Ecosystem:

Every ecosystem performs under natural conditions in a delicately balanced and systematic controlled manner. Functionally, the biotic and abiotic components of ecosystem are so interwoven into the fabric of nature that their separation from each other is practically very difficult.

The producers, green plants, fix radiant energy and with the help of minerals (C, H, O, N, P, K, Ca, Mg, Zn, Fe etc.) taken from the soil and aerial environment (nutrient pool) they build up complex organic matter (carbohydrates, fats, proteins, nucleic acids etc.). Herbivores feed on plants and in turn serve as food for carnivores. Decomposers breakdown complex organic materials into simple inorganic products which can be used by the producers.The two ecological processes of energy flow and nutrient cycling, involving interaction between the physico-chemical environment and the biotic communities constitute the heart of the ecosystem dynamics (Fig. 1).

The major functional features of an ecosystem are as follows:

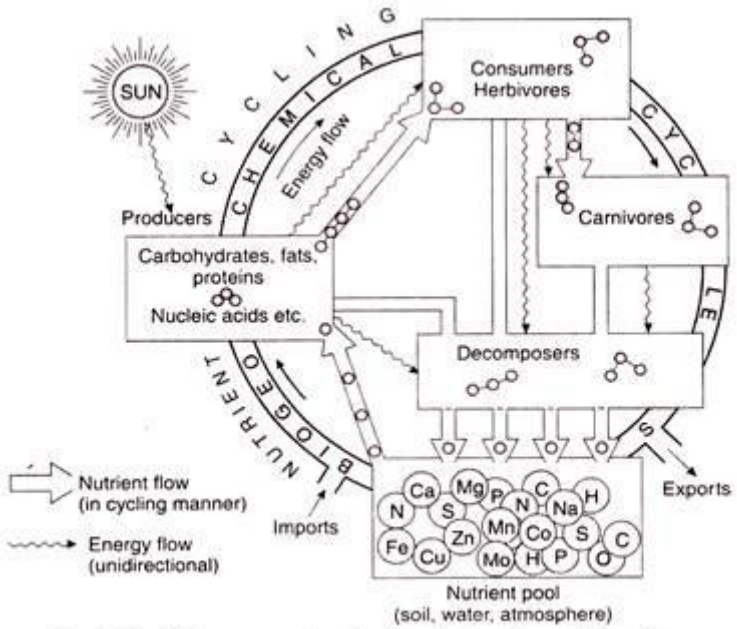


Fig. 1. Model of an ecosystem to show it's structure and function. Nutrient cycling and energy flow are mediated through food chain.

A. Trophic Structure:

The trophic structure of an ecosystem is a kind of producer-consumer arrangement and their interaction with population size. Each food level is known as trophic level and the amount of living matter at each trophic level at a given time is known as standing crop or standing biomass. In the ecosystem various trophic levels are connected through food chain.

(i) Food Chain:

The transfer of food energy from the producers, through a series of organisms (herbivores to carnivores to decomposers) with repeated eating and being eaten, is known as food chain. All organisms, living or dead, are potential food for some other organisms, hence there is no waste in the functioning of a natural ecosystem.

Some examples of simple food chain are:

Grass → Grass hopper → Frog → Snake → Hawk

(Grassland ecosystem)

Phytoplanktons → Water fleas → Small fish → Large fish → Tuna

(Pond ecosystem)

Types of Food Chains:

(a) Grazing food chain:

It starts from green plants (primary producers), goes to grazing herbivores and culminates to carnivores (Fig. 2). The chain thus depends on autotrophic energy capture and movement of this captured energy to carnivores. Examples constitute sequence of

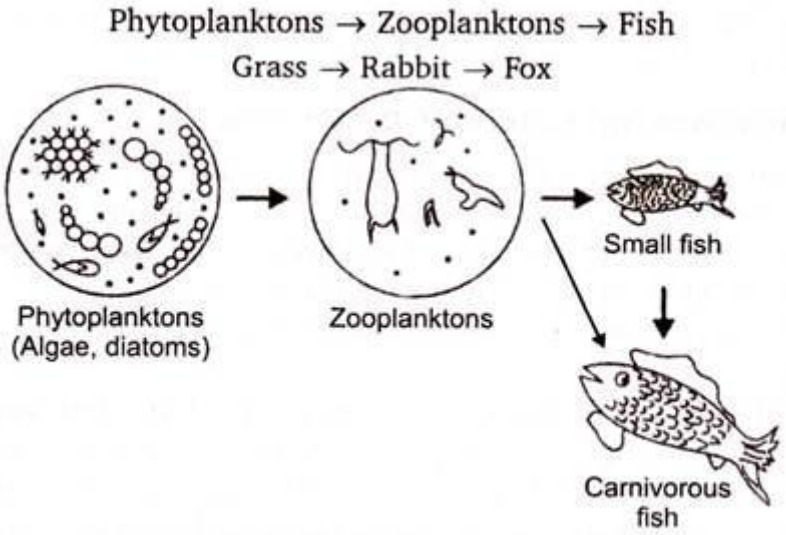


Fig. 2. A grazing food chain in a pond ecosystem.

(b) Detritus food chain:

It starts from dead organic matter and passes through micro-organisms to detritivores (organisms feeding on detritus), their predators and decomposers. The ecosystems exhibiting detritus food chain are less dependent on direct solar energy. These depend chiefly on the influx of organic matter produced in another ecosystem. Such type of food chain operates in the decomposing accumulated litter in a temperate forest.A good example of detritus food chain (Fig. 3) is seen in a Mangrove (estuary). Mangrove leaf fragments acted on by saprotrophs (fungi, bacteria), colonized by algae are eaten by detritus consumers (crabs, shrimps, nematodes, molluscs etc.). These are, in turn, eaten by minnows and small carnivorous fish which serve as the food for large game fish and birds.

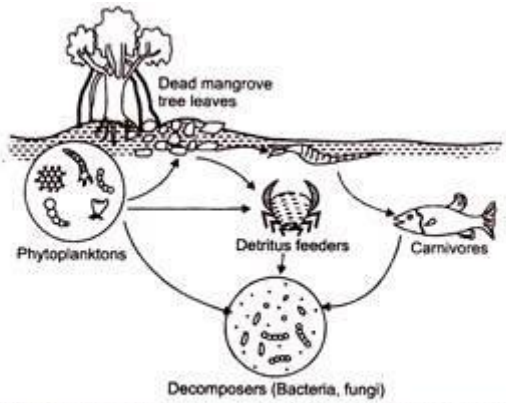


Fig. 3. A detritus food chain in an estuary based on dead leaves of mangrove trees.

Thus the grazing food chain derives its energy from plants while in detritus food chain energy is obtained primarily from plant biomass, secondarily from microbial biomass and tertiary from carnivores. Both the food chains occur together in natural ecosystems but the grazing food chain usually predominates.

(ii) Food Web-Interlocking Pattern of Organisms:

Food chains in ecosystems are rarely found to operate in isolated linear sequence. Rather, they are interconnected with several linkages forming a complex network of interlocking pattern which is referred to as food web. Thus, food web is a network of food chains where different types of organisms are interconnected with each other at different trophic levels so that there are a number of options of eating and being eaten at each trophic level.

An example of food web is illustrated by the unique Antarctic ecosystem (Fig. 4). It represents the total ecosystem including the Antarctic sea and the continental land. The land does not show any higher life forms of plants. The only species are those of some algae, lichens and mosses. The animals include snow petrel and penguins which depend on the aquatic food chain. In a tropical region, on the other hand, the ecosystems have a rich species diversity and therefore, the food webs are much more complex.

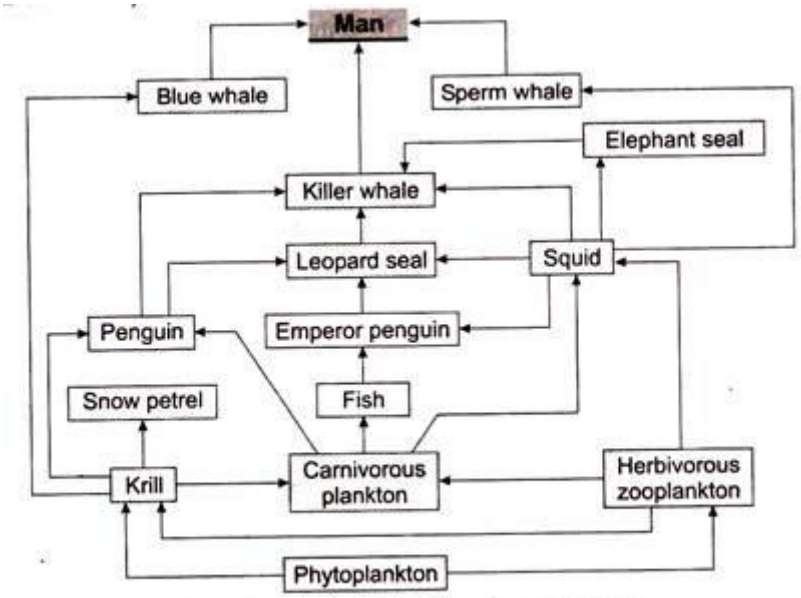


Fig. 4. Food web in Antarctic ecosystem.

Why has nature evolved food webs in ecosystem instead of simple linear food chains? This is because food webs give greater stability to the ecosystem. In a linear food chain, if one species becomes extinct then the species in the subsequent trophic levels are also affected. Just consider the simple food chains of Arctic Tundra ecosystem.

$$\text{Cladonia} \rightarrow \text{Reindeer} \rightarrow \text{Man}$$

$$\text{Grass} \rightarrow \text{Caribou} \rightarrow \text{Wolf}$$

If due to some stress, the population of reindeer or caribou falls, it will leave little option for man or wolf to feed from the ecosystem. Had there been more biodiversity, it would have led to complex food web giving the ecosystem more stability. In a food web, there are a number of options available to each trophic level.

So, if one species is affected, it does not alter other trophic levels so seriously. For instance, in grazing food chain of a grassland, in the absence of rabbit, grass may be eaten by mouse, which in turn, may be eaten by hawk or snake (Fig 5.)

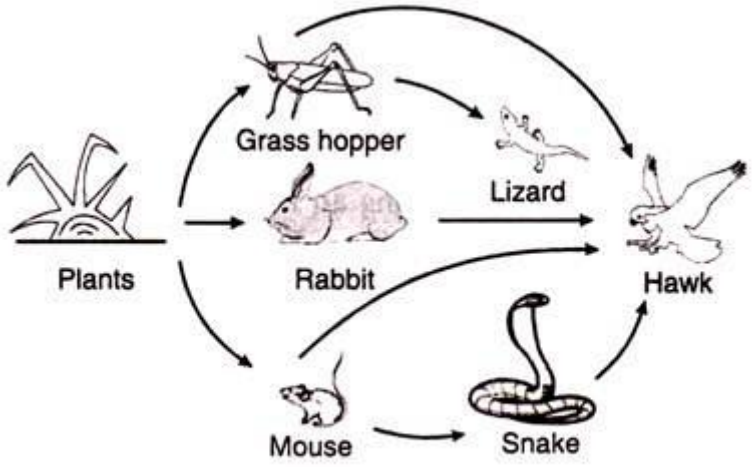


Fig. 5. Food web in a grassland ecosystem.

Besides those shown in Fig. 5, there may also be present some other consumers as vultures, fox and man in grasslands, and if so, the food web may be even more complex than shown here. In fact, real food webs usually have hundreds of species interlinked according to their feeding habits.

Note:

The complexity of any food web depends upon the diversity of organisms in the system.

It would accordingly depend upon:

1. Length of the food chain:
More diverse the organisms in food habits, longer would be the food chain.

2. Alternatives at different levels of consumers in the chain:
More the alternatives, more would be the interlocking pattern.

Significance of Food Chains and Food Webs:

1. Food chains and food webs play a very significant role in the ecosystem because the most important functions of energy flow and nutrient cycles take place through them.

2. Food chains help in maintaining and regulating the ecological balance.

3. Food chains show a unique property of biological magnification of several pesticides and heavy metals which are non-biodegradable in nature. Such chemicals increase in concentration at each successive trophic level.

B. Ecological Pyramids:

Graphic representation of trophic structure and functions of an ecosystem, starting with producers at the base and successive trophic levels (herbivores -> carnivores) forming the apex is known as ecological pyramid. These were first devised by British ecologist Charles Elton (1927) and so are also known as Eltonion pyramids.

Ecological pyramids are of three types:

1. Pyramid of Numbers:

It represents the number of individual organisms at each trophic level. There may be upright or inverted pyramid of numbers depending upon the type of ecosystem and food chain as shown in Fig. 6. A grassland ecosystem [Fig. 6(a)] and a pond ecosystem [Fig. 6(b)] shows an upright pyramid of numbers. In grassland, the producers (grasses) are very large in number and form a broad base.

The primary consumers (herbivores like rabbit, mice), secondary consumers (snakes, lizards etc.) and tertiary consumers (hawks or other birds) gradually decrease in number, hence the pyramid apex becomes narrower forming an upright pyramid. Similar is the case with pond ecosystem. Here the producers, mainly phytoplanktons such as algae and bacteria, are maximum in number. The carnivores (small fish, beetles etc.) and top carnivores (large fish) decrease in number at higher trophic levels forming an upright pyramid of numbers.In a forest ecosystem, the producers are big trees which are less in number and hence form a narrow base. A large number of herbivores including birds, insects and several species of animals feed upon trees and form a much broad middle level. The secondary consumers like fox, snakes, lizards etc. are less in number than herbivores while top carnivores such as lion, tiger are still less in number. So the pyramid is spindle-shaped, i.e., narrow on both sides and broader in the middle [Fig. 6(c)].

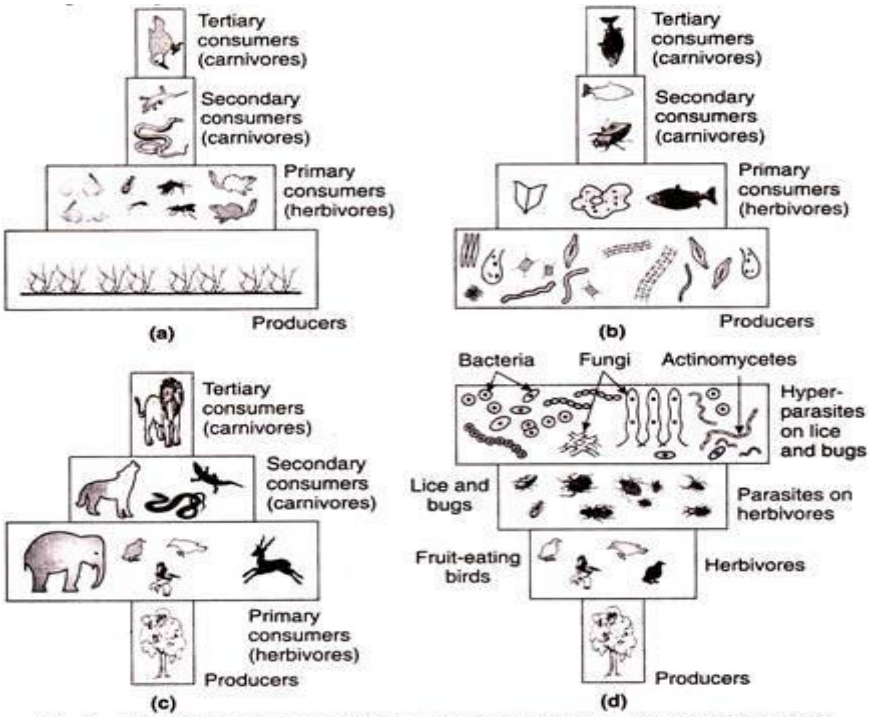


Fig. 6. Pyramid of numbers (a) Grassland ecosystem, (b) Pond ecosystem, (c) Forest ecosystem and (d) Parasitic food chain.

Parasitic food chain shows an inverted pyramid of numbers. The producers like a few big trees harbour fruit eating birds acting as herbivores which are larger in number. A much higher number of lice, bugs etc. grow as ectoparasites on these birds while a still greater number of hyperparasites such as bugs, fleas and microbes feed upon them, thus making an inverted pyramid [Fig. 6(d)],

Note that the pyramids of numbers do not reflect a true picture of the food chain as they are not very functional. They do not indicate the relative effects of the geometry, food chain and size factors of the organisms. They vary with different communities with different types of food chains in the same environment.

2. Pyramid of Biomass:

These are comparatively more fundamental since instead of geometric factor, they show quantitative relationship of the standing crops. Pyramid of biomass is based upon the total biomass (dry matter per unit area) at each trophic level in a food chain. In a forest, the pyramid of biomass is upright in contrast to its pyramid of numbers.

This is because the producers (trees) accumulate a huge biomass while the consumers total biomass feeding on them declines at higher trophic levels resulting in broad base and narrowing top [Fig. 7(a)], In a pond ecosystem, the total biomass of producers (phytoplanktons) is much less as compared to herbivores (zooplanktons, insects), carnivores (small fish) or tertiary carnivores (large fish). Thus the pyramid takes an inverted shape with narrow base and broad apex [Fig. 7(b)].

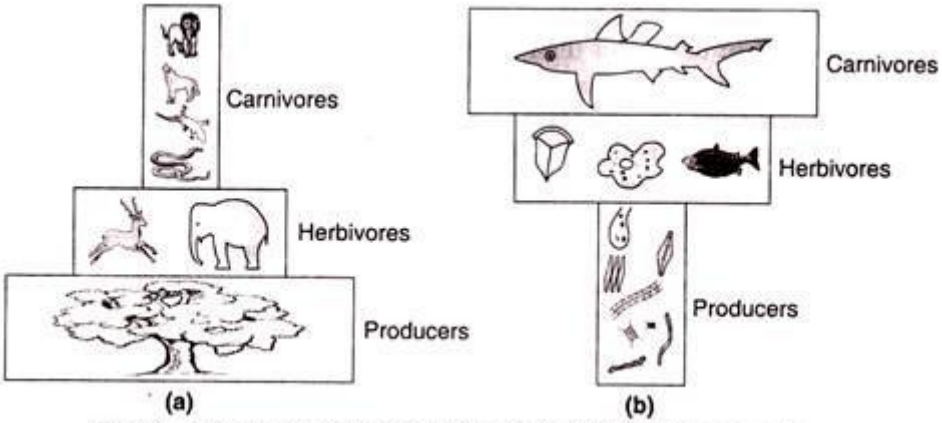


Fig. 7. Pyramid of biomass in (a) Forest and (b) Pond ecosystems.

3. Pyramid of Energy:

Pyramid of energy is based on the amount of energy trapped per unit time and area in different trophic levels of a food chain. It gives the best representation of the trophic relationships and is always upright (Fig. 8). The energy content is generally expressed as $kJ/m^2/yr$. At each successive trophic level, there is sharp decline in energy (about 90% in the form of heat and respiration) as we move from producers to top carnivores.

Thus only 10% of the energy passes on at each next higher level forming an upright pyramid.

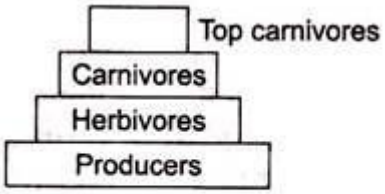


Fig. 8. Pyramid of energy in any ecosystem.

C. Energy Flow in an Ecosystem:

The functioning of ecosystem depends on the flow of energy through matter. The most important feature of energy flow is that it is unidirectional or one way flow. The energy captured by autotrophs does not revert back to solar input.

Unlike nutrients (like C, N, P) which move in a cyclic manner and are reused by the producers after flowing through the food chain, energy is not reused in the food chain. Also the flow of energy follows the two laws of thermodynamics.

First law of thermodynamics states that energy can neither be created nor destroyed but it can be transformed from one form into another. The solar energy captured by the green plants (producers) gets converted into biochemical energy of plants and later into that of consumers.

Second law of thermodynamics states that every transformation or transfer of energy is accompanied by its dispersion. As energy flows through the food chain, there occurs dissipation of energy at every trophic level. The loss of energy takes place through respiration or other metabolic activities. At every trophic level there is about 90% loss of energy and the energy transferred from one trophic level to the other is only 10%.

Types of Major Ecosystems:

Various types of ecosystems operate as self-sufficient interacting systems in the biosphere. They have, more or less, similar fundamental plan of their gross structure and function but they differ in respect of their species composition and rates in production etc.

1. Land-Based Ecosystem:

Land (terrestrial) ecosystem depends largely on the climate and soil. Higher plants (seed plants) and animals (vertebrates, insects, micro-organisms) dominate on land. The major terrestrial communities consist of herbaceous plants, shrubs, grass, trees besides numerous insects, arthropods, birds etc. Forest ecosystem regulates exchange of atmospheric gases and trace elements.

2. Fresh Water Ecosystem:

Fresh water bodies (lakes, ponds, rivers, springs) are rich in nutrients (nitrates, phosphates) and provide good habitat for zooplanktons, phytoplanktons, aquatic plants and fishes.

3. Marine Ecosystem:

Oceans occupy 70% of the earth surface, offering habitat to numerous plants (mainly algae), animals like zooplanktons, fishes, reptiles, birds and mammals (whales and seals). They serve as the sink for a large quantity of run-off and wastes from land. Marine water has a high salt content and poor fertility due to lack of nitrates and phosphates as compared to fresh water.

4. Wet Land Ecosystem:

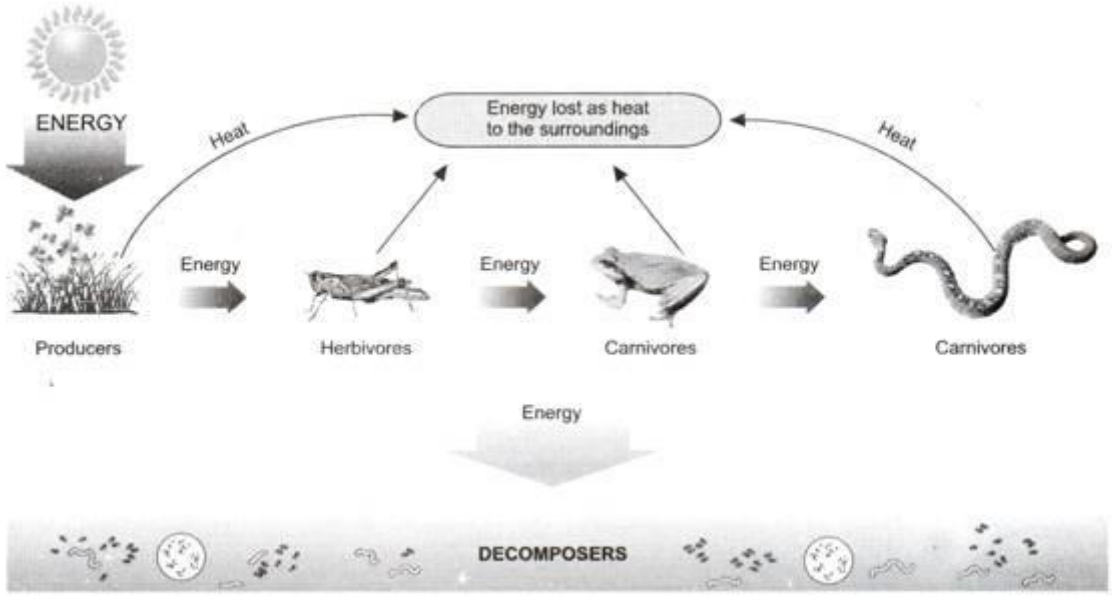
Wet lands are transitional lands between terrestrial and aquatic ecosystems where water stands at 3 to 300 cm. These include valuable natural ecosystem harbouring a variety of plants, animals, fishes and micro-organisms. At present, they are in danger due to increasing urbanization as in the case of eastern part of Kolkata.

5. Mangroves (Forests between Land and Sea):

Mangroves are important forests in tidal zones or equatorial and tropical coasts. Sunderbans in the Gangetic estuarine delta near the Bay of Bengal offer valuable mangroves having several plant species and wild animals including Royal Bengal Tiger.

Energy Flow:

We know that there is a flow of energy in the form of food within an ecosystem.This energy cannot flow back because a higher-level consumer such as a snake cannot be food for a lower-level consumer such as a rabbit. Let us now look at the flow of energy a bit more closely.



Flow of energy in an ecosystem

The flow of this energy is unidirectional, i.e., it flows in one direction—from the producers to the consumers at successively higher trophic levels.Green plants absorb a very small fraction (about 1%) of the solar energy reaching the outer part of the atmosphere. Through photosynthesis they convert this energy into chemical energy, which is stored as food (carbohydrates).

A part of the trapped energy is used by plants in metabolic activities like the growth of new tissues, and a part of it is lost into the surroundings as heat. The remaining energy is available as food to primary consumers. Thus we see that only a fraction of the energy absorbed by plants is finally available to the next trophic level.When primary consumers like deer eat plants, they get the available energy in plants. Some of this energy is used for activities like moving, digesting, etc., and some of it is lost as heat. Only about 10% of the available energy in the food gets transformed into new tissues (flesh) of the deer.

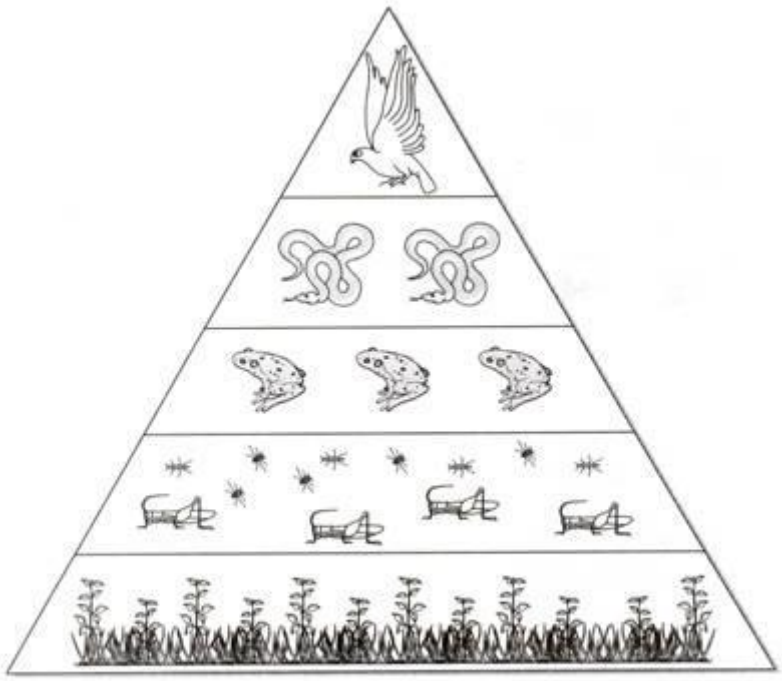
This is available to the carnivores (secondary consumers) at the next trophic level. At this level too, the usage, loss and storage of energy follow the same pattern. And this continues at every trophic level. Apart from this, energy from dead plants and animals is transferred to the decomposers.We find that when energy flows from the producers to the consumers at different levels, there is a loss of energy at each trophic level. It has been found that only about 10% of the energy available to a trophic level is transferred to the next higher level. This is called the ten per cent law.

Let us look at an example. If 10,000 kilocalories of energy are available to grass (producers), 1,000 kilocalories of energy would be available to grasshoppers (primary

consumers), 100 kilocalories would be available to frogs (secondary consumers) and only 10 kilocalories would be available to snakes (consumers of the third order). After this, very little energy would be left for the next level. So, food chains generally have up to three or four trophic levels.

Ten per cent law of transfer of energy

Now, the organisms at a trophic level are food for the organisms at the next higher trophic level. But there is a loss of energy as one goes from a lower to a higher trophic level. Therefore, the organisms at the higher level need to eat a large amount of food to fulfill their requirement of energy.So, the number of organisms at a lower trophic level is usually more than that at the next higher trophic level. If the numbers of organisms at different trophic levels are represented graphically, a pyramid is formed, which is called the pyramid of numbers.



Pyramid of numbers

The interdependence of the flora and fauna of an ecosystem is evident from the fact that some species met their energy (both catabolic and anabolic) need by consuming some other species. The basic source of energy for all living beings on earth is solar energy. Utilizing this energy, the primary producers (autotrophs) synthesize food.On land these are the various types of vegetation, while in water they are the algae, phytoplankton and

various other aquatic plants. Some species (the herbivores) partake of these autotrophs as their food. These herbivores are the primary consumers, which are consumed by the secondary consumers (the carnivores). Some bigger carnivores (the tertiary consumers) live on the smaller ones. This energy flow pattern, from species to species, is schematically shown in Fig. 1.2.

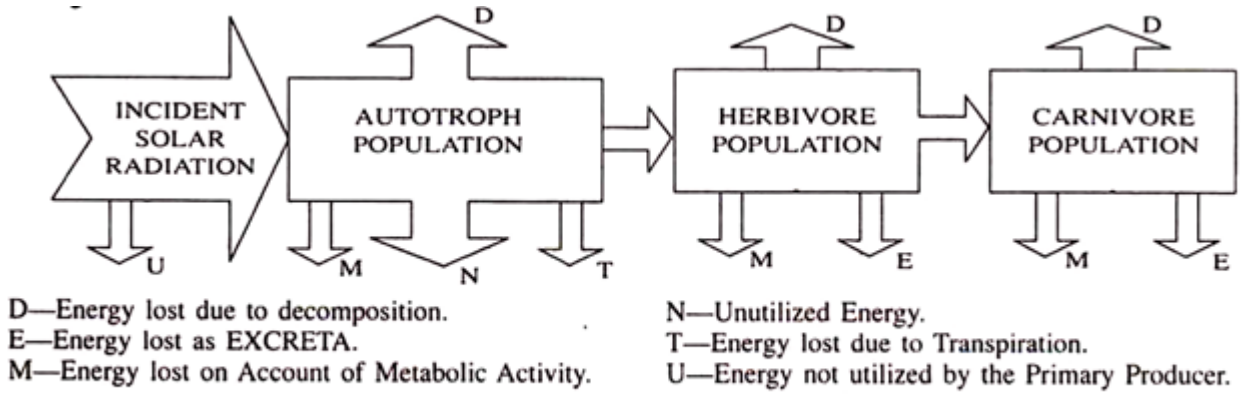


Fig. 1.2 : Energy Flow and Food Chain.

In a simplified way, the sequential dependence of a species of a higher trophic level on another of a lower trophic level for food is termed as the "food chain". However, the dependence pattern is a complex one and a better term to represent the situation is "food web", as many species take in, as food, species of different trophic levels.

The efficiency of assimilation of energy is higher at a lower trophic level and lower at a higher trophic level. Because of this, the population and the total mass of a species of lower trophic level are much larger than that of the immediate higher trophic level. The population structure is termed as "population pyramid", as shown in Fig. 1.3.

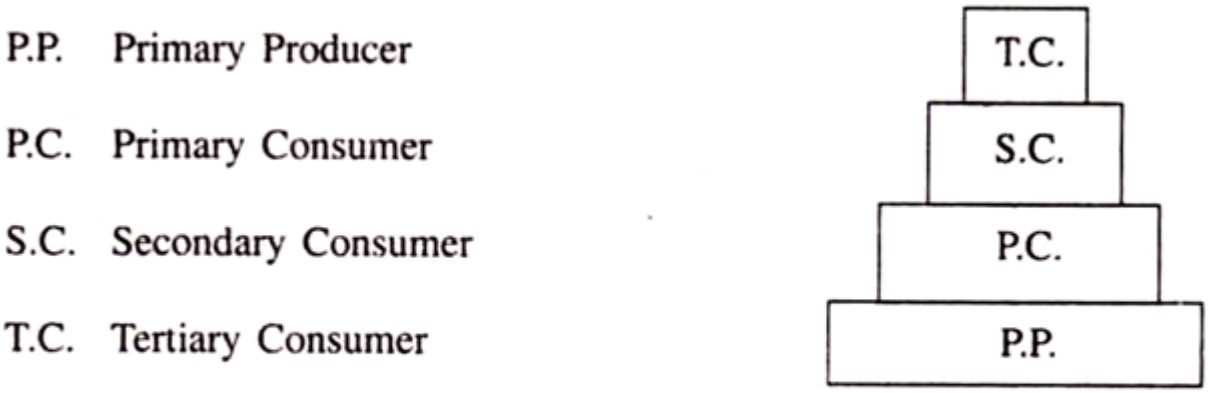


Fig. 1.3 : Population Pyramid.

The Food Chain:

The food chain is an idealized concept of trophic dependence of one species on another. Classically, it is considered that, the producer, i.e., the species belonging to the plant

kingdom (termed as autotroph) synthesize food to meet their nutrition requirement by utilizing carbon dioxide, water and sunlight.All other species, directly or indirectly, live on the autotrophs. The herbivores (animals belonging to the next higher trophic level) live on autotrophs. Similarly, the carnivores belonging to the third trophic level live on the herbivores.

The Food Web:

In reality, a species of a specific trophic level is dependent on several species belonging to different trophic levels for its nourishment. Because of such food habits, a complex relation exists between the different species as consumer and consumed.The actual complex food habit of different species in an ecosystem is termed as the food web. As a consequence of food chain /food web, once a pollutant gets absorbed in a species, it is transmitted to the other species of the higher trophic levels.

Probable Questions:

1. Describe different biotic components of ecosystem.
2. Describe different types of consumers.
3. Describe different abiotic components of ecosystem.
4. Describe different types of food chain.
5. What is food web, Give examples.
6. What is ecological pyramids? Describe different types of ecological pyramids.
7. How energy is flowed in ecosystem?

Suggested Readings:

1. Robert E. Ricklefs and Gary L. Miller. ECOLOGY. *Fourth Edition*.
2. Thomas M. Smith and Robert Leo Smith. Elements of ECOLOGY. *Eighth Edition*. ISBN 978-0-321-73607-9

Unit-VIII
Communityecology:natureofcommunities;levelsofspeciesdiversityandits Measurements

Objectives: In this unit you will earn about Community ecology and nature of communities, levels of species diversity and its Measurements.

Introduction:

By definition, community represents the population of all species living and interacting in an area at a particular time. Population can, within limits, adapt to changes in environmental conditions. The major driving force of adaptation to environmental changes is believed by most biologists to be biological evolution, the change in a population's genetic makeup through successive generation.

Concept of Community:

A group of organisms constitute population. Each population has characteristics like natality, mor-tality, age structure, growth dynamics and so on. But when several populations share a common habitat and its resources, they interact among them-selves and develop into a biotic community or sim-ply, a community.

Microorganisms, plants and animals populations sharing a common habitat and interacting among themselves develop into biotic communities. The composition of a biotic community in any habitat is dependent upon the prevalence of environmental conditions in that habitat and the ecological amplitude of species populations.Thus the climate and other abiotic as well as biotic conditions of a habitat determine the type of community which survives and develops. The organisms of a community usually exhibit trophic (feeding) relationships among themselves. They also interact in sharing the space and there may be interactions at a reproductive and behavioural level.

Each biotic community exhibits a number of characteristics, such as diversity, density, dominance, composition and stratification. Each community has its special limit. Sometimes the boundary between two communities may be very sharp or gradual.The transitional zone or junction between two or more diverse communities is called "eco-tone". The eco-tone harbours a community termed eco-tonal community with organisms of overlapping communities and some of unique types.

Origin of Biotic Community:

All the living things make the biotic system of the earth. Neither organisms nor species populations exist by themselves in nature, but are always part of an assemblage of different species populations living together in the same area. Any assemblage of populations of living organisms in the prescribed area or habitat is termed biotic community.

For example, the different species of organisms occurring in a pond constitute the pond community. Some other communities such as swamps, deserts, large lakes, grass lands and many others of different dimensions.Similarly, the interdependent species in any environment, such as a forest, a lawn or a desert track, constitute biotic communities. A community may be composed primarily of animals, or primarily of plants, but most communities consist of both animals and plants.

The factors that influence the living organisms are known as biotic factors. In the natural world there is interdependence of one form of life on another. A population of single species is not a viable entity by itself. It will not be able to survive for long.For example, the sparrows require worms, insects or seeds or some other kind of food. Thus the most appropriate definition of biotic community— **"a biotic community is a naturally occurring assemblage of plants and animals that live in the same environment, are mutually sustaining and interdependent, and are constantly fixing and dissipating energy."**

However, the different species in a biotic community share a common environment and their relationships are based on direct or indirect functional interactions. The nature of relationship is determined by the requirements of the members of the community.Biotic community organisation results from interdependence and interaction among populations of different species in a habitat. Large number of biotic communities found in nature due to two specific reasons.

They are as follows:

(i) Existence of diverse habitats with characteristic environmental conditions and

(ii) Co-occurrence of different species whose tolerance ranges overlap with the environmental condition obtained in that habitat. When similar habitat conditions are repeated at another location, the same biotic community gets established there.

Structure of Community:

Communities may be small, consisting of few species populations in a small space, or large, comprising several species populations in a large area. The community structures, composition and other characteristics can be readily described by visual observation without actual measurement.This is a qualitative approach which is easier than the quantitative population analysis where measurements are actually made. Communities usually categories by the ecologists in various ways primarily based of habitat features like water availability, high exposure, or other habitat features.

For instance, depending on the amount of water availability, plant communities may be hydrophytic (aquatic habitats), mesophytic (moderately moist soil habitat) and xerophytic (dry or arid habitat).Similarly communities growing on conditions of abundant light are called heliophytic and those growing in shade sciophytic. Identically communities growing on various habitats designated as desert communities, mountain communities and estuarine communities and so on.

In general, a community is dynamic since it changes over time. This dynamic nature is reflected in the succession of organisms in a habitat. A series of changes results in the development of a relatively stable community, which maintains its structure and influences the climate of the area.Such a stable and mature community is called a climax community, while communities of successional stages are called seral communities.

Community Dynamics:

Communities are dynamic systems constantly interacting with another system, the environment, which is equally dynamic. The community charges are gradual and imperceptible at any time but easily recognisable if observed at regular intervals over a long period of time. Seasonal changes in plant communities always occur at every place, particularly in areas where temperature variation is significant.

However, in course of very long period of time at many places the communities have reached a peak stage and attained a dynamic balance with the environmental changes. The process of change in communities and their environment at one place in the course of time is called "ecological succession".

Characteristics of Biotic Community:

Each biotic community consists of very diverse organisms belonging to different kingdoms of living things. The number of species and abundance of population in communities also vary greatly. The organisms in a community depend upon each other as well as upon the non-living environment for food, shelter and reproduction.

A stable and self-sufficient community has certain specific characteristics:

(a) Dominance of Species:

Each community has one or more dominant species. These could be Pine, Oak, Sal or Teak depending on the area and climatic conditions in that biotic community. The dominant species are the most numerous plants. They are generally large in size and have the greatest biomass. They influence the local environment.

The species may be fewer in number in temperate forests 90% of trees may be pines and oaks of a biotic community. The species may be more in tropical rain forests. Andaman group of islands where there are about 12 dominant species.

(b) Habitat:

Biotic community occupies particular area with specific physical environment like temperature, humidity, soil, availability of water etc., which acts as a limiting factor and regulate the population size of various species within the community.

(c) Self-Sufficiency:

On the bases of nutrition, each community comprises autotrophic and heterotrophic as perfectly balanced. Plants are autotrophic and referred as producer; animals are heterotrophic organisms and referred as consumers. The remains and dead bodies of these producers and consumers are decomposed by the decomposers (bacteria and fungi) and help in the recycling of materials.

(d) Structure and Stratification:

A stable community comprises of various strata, each community consists of the population of particular kind of species. Their growth forms help to establishment the structure of a community and on their arrangement; community shows either horizontal layering called zonation or vertical layering called stratification.

(e) Species Diversity:

Community is formed of a number of different populations. The number of species and population abundance in a community also vary greatly. Species diversity depends upon size of the area, diversity of habitats in that area, temperature, humidity, availability of water soil type, altitude etc.

(f) Dominance and Succession:

Each community has different types of population only few species especially one of them is expressed in majority and called dominants. Each community develops as a result of a directional change called succession.

Community Stratification:

Every biotic community has a vertical layering or stratification of organisms or environmental conditions. A number of examples can be cited to support the concept of community stratification from different habitats.

In grassland community three strata, namely:

(a) Subterranean,

(b) Floor and

(c) Herbaceous

may be recognized.

The subterranean stratum contains the roots of the principal vegetation and provides permanent shelter to bacteria, fungi, protozoans, nematodes, earthworms, ringworms and several other invertebrates. The floor stratum consists of basal parts of the vegetation, including the rhizomes of grass plants.

In this stratum, generally the insects, spiders, reptiles and rodents are present. The herbaceous stratum of grassland community is represented by upper parts of grasses and herbs. Several types of insects, birds and grazing mammals are included in this stratum.

In a forest community, five vertical strata are present. They are:
(a) Overstorey stratum,

(b) Understorey stratum,

(c) Transgressive stratum,

(d) Seedling stratum, and

(e) Subterranean stratum.

A typical forest may have an overstorey stratum comprised of trees that are more than forty feet in height. These trees make a canopy. Just beneath this canopy there is an understory stratum that extends from twenty feet in height to a short distance below the overstorey. A transgressive stratum extends from four feet to twenty feet or more and comprises of shorter shade loving species.

The seedling stratum begins at the soil level and extends to the lower limit of the transgressive stratum. The subterranean stratum in the forest community is moist and contains a large amount of humus. It is very prominent up-to a depth of two to three meters. Each stratum has its own group of animals.

However, most animals can move from one stratum to another in search of food or in response to many biotic factors, such as insects, snails, birds and squirrels.The animals found above the soil include turtles, snakes, some birds, insects and a variety of mammals, such as rabbits, deer, wolves and foxes. The animals living in humus soil are beetles, fly larvae, spiders, annelids, antipodes, mites, protozoans, nematodes and some springtails.

In pond community, vertical stratification is very little. However, in deep ponds and lakes three strata:

(a) Littoral zone,

(b) Limnetic zone and

(c) Profundal zone

can be recognized.

The littoral zone comprises shallow water region and is occupied by rooted plants. The limnetic zone occupies the depth up-to which the light penetrates and inhabited by planktons, nektons andneustons. In profundal zone, there is no penetration of light, and therefore, it has no photosynthetic organisms.

Ecotone and Edge Effect:

The zone where two or more different communities meet and integrate, is called transition zone or ecotone. This zone of integration may be narrow or wide, local (e.g., a zone between field and a forest) or regional (e.g., the transition between forest and grass land). Ecotone contains few species from both communities. The total number of species is often greater in the ecotone than in the adjoining communities.

The ecotone or transition zone exhibits a shift in dominance of the conspicuous species of both sides. It may also include a number of highly adaptable species that tend to colonize such transitional areas. Because of this, the variety (i.e., species diversity) and density of life is often greatest in such areas.This potential for the ecotone to act as a habitat for species found in neither major community is called edge effect. Thus the tendency of increased variety and density of some organisms at the community border is known as edge effect.

The organisms that occur primarily, or most abundantly, or spend the greatest amount of their time in junctions between communities are called edge species. A common example of the edge effect in action can be seen in those species of owl that live in or near ecotones between forests and grasslands. They depend on forest trees for nesting and do their hunting in the grassland, where they depend on field rodents for food.

In man-made communities such as agricultural fields, the ecotone between the field and the forest act as refuge for species formerly found in the ploughed area, as well as for other plants such as weeds. Ecotones of this type are also the prime habitat of may species of insects, game birds, and mammals.

Keystone Species:

The species, which have much greater influence on community characteristics, relative to their low abundance or biomass, are known as keystone species. Such species play a vital role in controlling the relative abundance of other species.

When keystone species is removed, it causes serious disruption in the functioning of the community. For example, in the tropical rain forests, the different species of figs are the keystone species as they produce large quantity of fruits. During the time of food scarcity, these fruits are consumed by monkeys, bats, birds, etc. Thus, by protecting the fig trees, the animals dependent on them are also conserved.

Link Species:

However, only a few species act as keystone species, while others act as link species. For example, mycorrhizal fungi in soil are critical link species as they establish essential links in the absorption of nutrients from the soil and other organic substances.

Some critical link species are responsible for providing food to the network species, while others act as pollinators of flowers, and some act as agents for dispersal of seeds and fruits. Tropical rain forests are rich in critical link species due to high degree of animal dependent pollination and dispersal.

Composition of Biotic Community:

Biotic communities consist of living as well as non-living component. Non-living organisms are called as abiotic and include water, sunlight, air etc. while living organisms are referred as biotic.

There are three types of living organisms inhabit a biotic community:

The classification of biotic organisms is as follows:

(i) Producers:

Producers or autotrophs include plants, algae and bacteria that survive via converting solar energy into food. These are mainly green plants. They synthesize organic food from simple inorganic compounds, namely carbon dioxide and water, with the help of chlorophyll and sunlight, hence the name as producer. The process is called photosynthesis.

It may be briefly represented as under:

$6CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6O_2$

The producers are very important for biotic community because they:

(i) Provide food and oxygen to the animals

(ii) Reduce CO_2 and H_2O contents from the environment.

Thus, they influence animals as well the environments.

(ii) Consumers:

Consumers or heterotrophs eat plants and animals to survive. These are mainly the animals. They are unable to synthesize food for themselves. Therefore, they take other organisms or their parts, hence their name. The consumers are called herbivores when they feed on plants and carnivores if they take other animals. Grasshopper, rat, rabbit, goat and cattle are common herbivores.

Frog, wolves, tiger and lions are familiar carnivores. Cat, dog, bear and man take both plant and animal food. They are termed omnivores. The consumers also use O_2 of the environment and add to it CO_2, nitrogenous waste matter and faeces. They, thus; also influence the environment as well as the organisms. Consumers include herbivores (known as primary

consumers), which eat only plants, carnivores (known as secondary consumers), which eat other animals, and omnivores, which eat both plants and animals.
Subcategories of consumers are detritivores, or detritus feeders. These creatures eat plants and animals that are already dead. Their diet consists of dead organisms, as well as organic waste. Crabs, vultures and termites are well known example of detritivores, or detritus feeders.

(iii) Decomposers:

The final type of living organism is decomposers. They change plants that have died into nutrients that allow them to survive. Animals that live in the water or soil often feed off dead decomposers. These are mainly bacteria and fungi of decay. They are also named as reducers. They obtain food from the dead producers (plants) and consumers (animals) and latter's waste products.

They decompose these materials into:

(i) Small organic molecules which they utilize themselves.

(ii) Inorganic compounds that are released into the environment for reuse as raw materials by the producers.

The decomposers not only return chemical nutrients to the environment, but also make space available for new producers. Without this, all life will ultimately cease to exit. Thus, the decomposers have a role in the environment. They are found in the soil, and the bottom of ponds, lakes and oceans.

Biotic Stability:

A biotic community is a naturally occurring group of plants and animals living in the same environment. They all interact to make the community stable. It has been seen that more the number of species, more stable is the community. A biotic community containing a large population of Eucalyptus or any other plant may be totally wiped out by a fungal disease or insect infection.But if a biotic community contains many species and different kinds of plants, only one would wipe out at a time and the rest would survive. Since a biotic community is formed of a large number of diverse type of populations of plants and animals and microbes, all these populations are essential for biotic stability. Each species eats on a different kind of grass or shrubs. Some that feed on the same species while few feed at different stages of grass. This makes the biotic community rich and stable.

Interaction among Biotic Community:

When various species (community) live together in a biotic community a numbers of interactions take place according to specific needs of food, shelter and habits.

Following interactions have been observed:

(i) Predation:

Predation is a direct and often complex interaction of two species in a biotic community. The stronger animal called predator that captures and feeds on the weak animal called prey. The decrease in number of predators leads to an increase in number of the prey.

An increase in the predator population leads to decrease in the prey population in a biotic community. These fluctuations play an important part in regulating natural population. Tiger feeds on deer, owls on rats etc. In a common biotic community an animal could be a predator as well as a preyat different times.

(ii) Scavenging:

Scavenging is a direct food relationship where animals feed on other dead animals called scavengers where as another animal in a biotic community, which either died naturally or have been killed. They play an important role in a biotic community as food is not wasted and also is disposed off. Vulture feeds on dead bodies. Hyenas and jackals feed on left over killed animals by lion. They may feed on big animals like zebra and giraffe killed by other animals.

(iii) Parasitism:

Parasitism is a negative interaction in a biotic community where one not only derives nourishment but also lives a part or the whole of life on another organism. The parasite is an organism that lives on another organism, the host from which it obtains food as well as shelter in a biotic community. It may be between animals between plants or between animals and plants in a biotic community.In the host parasite relationship the weak attacks on the stronger. It is beneficial to parasite and harmful to host. Generally a parasite may cause an illness or disease but not kill the host except in few specific cases. There may be number of parasites on one host.

(iv) Commensalism:

In Commensalism one organism or a population is benefited while the other is neither benefited nor harmed. In some cases the host may be able to derive some minor benefit. There is no physiological exchange of any kind. The association may be temporary or permanent in a biotic community. Remora is a small fish attaches itself to the lower side of a shark. Remora feeds on scraps of shark’s food and is not harmed.

(v) Symbiosis:

Symbiosis is an association of two populations in a biotic community where both the populations are benefited. There is often a close or permanent association. In some cases both are so inter-dependent that neither can live alone in that biotic community. Lichens are the best example of symbiotic association.

(vi) Competition:

The two species in a biotic community interact in such a way that it affects their growth and survival. Both the species share the same resources like water, nutrients, space, sunlight, food, etc. of a biotic community. Members of both species compete to survive in that respective biotic community. There is a direct inhibition of one by another. There is no set pattern as anyone can succeed. Carnivorous animals like tiger and lion compete for the prey. Trees, herbs and shrubs compete for sunlight, water and nutrients in a biotic community.

(vii) Some Passive Interactions:

There are many interactions that exist in nature of a biotic community in between the animals, or between animals and the surrounding, which also help an organism to survive in that biotic community. The blending of an animal with its surrounding is called camouflaging. It is also known as protective coloration.

They camouflage their body shape and colour to suit the environment. Stick insect *Carausiusmorosus* resembles a thin dry branch. Dead Leaf butterfly *Kalimaparolecta* resembles a dry leaf. Praying mantis *Mantis religiosa* resembles the green foliage. Mimicry is also a type of protective resemblance in a biotic community. In this an animal mimics another animal so as to avoid predation.

(viii) Other Interactions:

There may be many other interactions have been observed. The birds/animals are associated to bringing seed and fruit dispersal and pollination. The birds feed on ticks; they are parasites on the body of cattle. The Rufus woodpecker makes a nest in a hole in the ball shaped nest of the ants. The ferocious ants do not harm the eggs or young ones but the birds feed on these ants and keep a check on their population to continue their generation in a biotic community.

Ecological Succession or Changes in a Biotic Community:

Ecological succession is the process of change in the species structure of an ecological community over time. The community begins with relatively few pioneering plants and develops through increasing complexity until it becomes stable community. It is a phenomenon or process by which an ecological community undergoes more or less orderly and predictable changes following disturbance or initial colonization of new habitat.

A community is built up over a period of time. As the time passes, communities change. In a biotic community there are interactions among the organisms and between the biotic and abiotic factors like climate, light, soil, etc. All these bring about changes in a biotic community.A biotic community is a dynamic unit where tropic levels exist; there is a flow of energy and cycling of nutrients. It is a living part of an ecosystem. The wind, fire, volcanic activity or any other event in nature or man may destroy the organisms living in a biotic community.

Now if this area is left alone, a succession would start and ultimately a permanent biotic community would take shape. This process from the beginning to the climax may take thousands of years.During this time there will be an orderly and progressive replacement of one biotic community by another till a relatively stable biotic community is established. This is called as ecological succession. A complete succession is called as Sere. A Sere is made up of a number of seral stages. A climax community is die final or the seral stage.

Ecological succession may be expressed as follows:

1. The species living in a particular place gradually change over time as does the physical and chemical environment within that area.

2. Succession takes place because through the processes of living, growing and reproducing, organisms interact with and affect the environment within an area, gradually changing it.

3. Each species is adapted to thrive and compete best against other species under a very specific set of environmental conditions. If these conditions change, then the existing species will be outcompeted by a different set of species which are better adapted to the new conditions.

4. The most often quoted examples of succession deal with plant succession. It is worth remembering that as plant community's change, so will the associated micro-organism, and fungus and animal species. Succession involves the whole community, not just the plants.

5. Change in the plant species present in an area is one of the driving forces behind changes in animal species. This is because each plant species will have associated animal species which feed on it. The presence of these herbivore species will then dictate which particular carnivores are present.

6. The structure or 'architecture' of the plant communities will also influence the animal species which can live in the microhabitats provided by the plants.

7. Changes in plant species also alter the fungal species present because many fungi are associated with particular plants.

8. Succession is directional. Different stages in a particular habitat succession can usually be accurately predicted.

9. These stages, characterized by the presence of different communities, are known as 'seres'.

10. Communities change gradually from one sere to another. The seres are not totally distinct from each other and one will tend to merge gradually into another, finally ending up with a 'climax' community.

11. Succession will not go any further than the climax community. This is the final stage.

"The developmental study of vegetation necessarily rests upon the assumption that the unit or climax formation is an organic entity. As an organism the formation arises, grows, matures, and dies… Furthermore, each climax formation is able to reproduce itself, repeating with essential fidelity its development."

Causes of Plant Succession:

Climatic factors may be very important, but on a much longer time-scale than any other. Changes in temperature and rainfall patterns will promote changes in communities. As the climate warmed great successional changes took place. The tundra vegetation and bare glacial till deposits underwent succession to mixed deciduous forest.

The greenhouse effect resulting in increase in temperature is likely to bring community changes. Geological and climatic catastrophes such as volcanic eruptions, earthquakes, avalanches, meteors, floods, fires, and high wind also bring allogenic changes. Allogenic succession is caused by external environmental influences and not by the vegetation.

Soil changes due to erosion, leaching or the deposition of silt and clays can alter the nutrient content and water relationships in the ecosystems. Autogenic succession can be brought by changes in the soil caused by the organisms there. These changes include accumulation of organic matter in litter or humic layer, alteration of soil nutrients, change in pH of soil by plants growing there.The structure of the plants themselves can also alter the community. When larger species like trees mature, they produce shade on to the developing forest floor that tends to exclude light-requiring species. Animals also play an important role in allogenic changes as they are pollinators, seed dispersers and herbivores. They can also increase nutrient content of the soil in certain areas creating patches in the habitat. This may create regeneration sites that favour certain species.

Mechanisms of Succession:

Frederic Clements in 1916, proposed the theory of ecological succession. According to this theory succession had a powerful influence on ecological community. Clements' concept is usually termed classical ecological theory.

According to Clements, succession is a process involving several phases:

(i) Nudation:

It is the development of a bare site uninhabited by any organisms. The process is usually caused by disturbances Succession begins with the development of a bare site, called Nudation. These factors can be either topographic like soil erosion, wind action etc., climatic like hails, storm, glaciations, fire etc.; or biotic. The area thus formed can sustain only autotrophic organisms which can utilize inorganic substrates. The environmental conditions are set up for the inhabitation of new species.

(ii) Migration:

The process of migration helps the arrival of seeds, spores or other reproductive propagules for establishment of species. The other species are non-native organisms which can spread widely in a community. These are usually threatening the normal ecosystem and causative agents for community disturbance.

However, in succession process, they help to alter the soil texture and function. R-selected species are often the first colonizers due to their high reproductive rates and better dispersal mechanisms.

(iii) Ecesis:

This is the initial establishment of plant community. It involves establishment and initial growth of vegetation. This is dependent on the soil structure. The stage is also called as 'colonization'. In this stage, the early colonizing species proliferate abundantly through germination, growth, and reproduction.

Ecesis is due to allogenic mechanisms. This is the stage at which the pioneer species survive through the dispersal mechanisms. The different pioneer species can have different maturation rates which allow this process to be longer and gradually allowing replacement of some species by others. The process also makes the soil structure suitable for those species.

(iv) Competition:

Once the few initial species have become established the intra as well as interspecific competition among the species starts. As vegetation became well established, grew, and spread; various species began to compete for space, light and nutrients.

This stage is called competition. The competition is usually for resources such as food, water etc. Competition is found in both plants as well as animal species. The process leads to sharing of resources (resource partitioning) or competitive exclusion.

(v) Reaction:

The environmental conditions get modified by the action of species occupying the habitat. These changes subsequently trigger the displacement and replacement of one species by another. During this phase autogenic changes affect the habitat resulting in replacement of one plant community by another.

The existing community will be unable to support itself due to the harsh conditions. The major underlying mechanism is autogenic succession in which the plants themselves alter the environmental conditions.

(vi) Stabilization:

Stabilization is the process by which the climax community gets established. A climax community is mature, self-sustaining, and stable and is the final stage of succession. The

physical and chemical conditions are altered and stabilized to such levels that it supports the entire community.

The climax communities are best adapted to the regions of succession and the community structure is likely to continue until another disturbance steps in. This represents a steady state of ecological equilibrium with specific composition, structure and energy flow. Reaction phase leads to development of a climax community.

(vii) Aggregation:

Aggregation is the increase in population of the species which has become established in the area. The shrubs replace the small herbs in most successions. This also proves as a source of food for future inhabitants

a. Hydrosere:

Hydrosere is the primary succession sequence which develops in aquatic environments such as lakes and ponds. It results in conversion of water body and its community into a land community. A hydrosere is a plant succession which occurs in an area of fresh water. Hydrosere is simply a succession which starts in water. Hydrosere, also called hydrarch involves the ecological succession in the newly formed pond or lake.

A wetland is a transitional area. Freshwater provides a good and an excellent place to observe several stages of a hydrosere at the same time. In time, an area of open freshwater such as a lake will naturally dry out, ultimately becoming woodland.During this process, a range of different habitats such as swamp and marsh will succeed each other. This succession from open water to climax woodland is likely to take at least two hundred or more years. Some intermediate stages will last a shorter time than others.

Fresh Water:

Fresh water lack soil, minerals etc., which support the growth of vegetation. Deep freshwater will not support rooted, submerged plants because there is not enough light for photosynthesis in the depths. There will be micro-organisms and plankton floating in the water.

Ecological succession is of great importance as:

(i) It provides information, which help to have control on the growth rate of one or more species in a given geographical area.

(ii) It helps in reforestation and forest management programs.

b. Xerosere/ Lithosere:

A xerosere may include lithoseres (on rock) and psammoseres (on sand). A lithosere is a sere (succession) on rock. Lithosere is a plant succession starts on a newly exposed rock surface. Xerosere is a plant succession which is limited by water availability. It includes the different

stages in a xerarch succession. Xerarch succession of ecological communities originated in extremely dry situation such as sand deserts, sand dunes, salt deserts, rock deserts etc.

Pioneer species are the first organisms that colonies an area, of which lithoseres are an example. They will typically be very hard i.e., they will be xerophytes, wind-resistant or cold-resistant. In the case of a lithosere the pioneer species will be lichens, cyanobacteria and algae, which create their own food and water.They are autotrophic and so do not require any external nutrition except sunlight. Other examples of lithoseres include communities of mosses and lichens, as they are extremely resilient and are capable of surviving in areas without soil. Xerosere or lithosere is a gradual change in community due to change in climate, nutrition etc.

Key points of Lithosere /Xerosere:

(i) Bare rock colonized, by pioneer community, for example, lichens, mosses, bacteria, that can survive in hardy conditions, and need few nutrients.

(ii) Rock slowly weathered creating thin soil.

(iii) Plants die, creating humus, leading to a more fertile soil; grasses replace the mosses and lichens as the dominant species.

(iv) Grasses decrease in number; quick-growing shrubs become dominant.

(v) Fast growing trees dominate.

(vi) Over time slower growing trees such as oak become dominant and form the climatic climax community.

Varieties of Ecological Diversity:

a. Species Diversity:

The number of species that occurs in a particular area is called its species richness (Donovan and Welden, 2002). It means the species richness in any habitat and is common currency of the study of biodiversity. Species richness index is essentially a measure of the number of species in a defined sampling unit.

The biotic community is a natural assemblage of a large number of plant and animal species in an area. However, in any particular habitat there is no considerable variation in environmental condition, the plants growing together in a community show unique uniformity in their behaviour.Vegetation, therefore, is reflection of a climate and, in general, widely separated areas having similar climate have similar aspects of landscape.Some communities, for example, tropical rain forest and coral reef community, show high species

diversity with many different kinds of species living at each trophic level.Some community areas have limits but more often the community boundaries are hard to define. A clearly distinguished area or a type of area with uniform habitat conditions and supporting characteristic type of vegetation is termed biotype.

Species richness is a function of sample size. However, it should not be confused with species abundance. Each natural habitat has a variety of species, which differ in their relative abundance. No community consists of species of equal abundance; some species are rare, others are common and still others may be abundant.Species diversity measures are often more informative than species counts alone. According to Harper, (1977), there is "importance of taking an organism's eye view of community diversity". This comment is relevant to structural diversity as it is to species composition.

b. Resource Diversity:

It means the diversity of resources that an organism (species) utilizes. For example, some fish species in the hill-streams have a wide trophic niche and depend on zooplankton, insects, and algae and diatoms for their food (Singh and Bahuguna 1983). In many cases food resources consumed by an organism differ during different stages of the life cycle, such as fry, fingerling and adult stages in case of fish. Thus, niche width is the measure of the diversity of resources utilized by a species.

The usual approach is to use the Simpson index or the Shannon index to calculate the niche width. The number of resource types observed (e.g., types of food items eaten, varieties of habitat utilized, kinds of behaviour employed) replace number of species in the equation. A separate value must be calculated for each type of resource and measures of abundance will depend on the way in which the index is being used.

If the niche width of a particular species is under consideration then abundance may be measured as the number of individuals eating each type of food, living in each sort of habitat, or adopting each kind of behaviour. However, if we wish to measure the niche width of an individual, then abundance can be taken as the amount of each food type eaten, the time spent in each habitat or the frequency with which each behaviour is performed.

c. Habitat Diversity:

It is the number of habitat types in a defined geographical area. This is an index, which measures the structural complexity of the habitat. This structural complexity of environment, in turn, is responsible for the presence of a wide variety of spatial and trophic niches. This means that if any habitat supports more microhabitats its biological diversity will be more as compared to a habitat which has less number of microhabitats. More studies on habitat diversity have been made for terrestrial environments.

The number of substrate types has been related to species diversity for aquatic insects, molluscs and benthic macro-invertebrates. Gorman and Karr (1978) have taken bottom type, depth and current into account to investigate the link between habitat diversity in streams and

fish species diversity. The author found that habitat diversity was more in small hill streams and some tributaries than large, snow-fed rivers in the Garhwal Himalayas.

d. Differentiation Diversity:

It is also called beta diversity. It means degree of change in species composition between sites or communities or along gradients. A number of studies on faunal diversity of fish and insects have clearly indicated that their distribution and abundance is governed by gradient and altitude, among other factors (Singh et al, 1994, Singh and Nautiyal, 1990). For example, stoneflies and rheophilic fish species in the rhithron parts of hill-streams are characteristic and are absent from their potamon parts.

One study indicated that there is zonation of animals within a river; *Simuliummonticole* occurs from source to 12 km, *Simuliumvariegatum* from 12 to 35 km., and S*imuliumequinum* from 20 to 50 km. All these studies indicate that greater diversity of species and habitats means greater ecological quality.

Measurement of Species Diversity:

Any measure of species diversity, by itself, does not convey much information; we appreciate its significance only when we compare with any other measure.Measures of species diversity can be divided into three categories.

These are:
(i) Species richness indices,

(ii) Species abundance models,

(iii) Species proportional abundance based indices

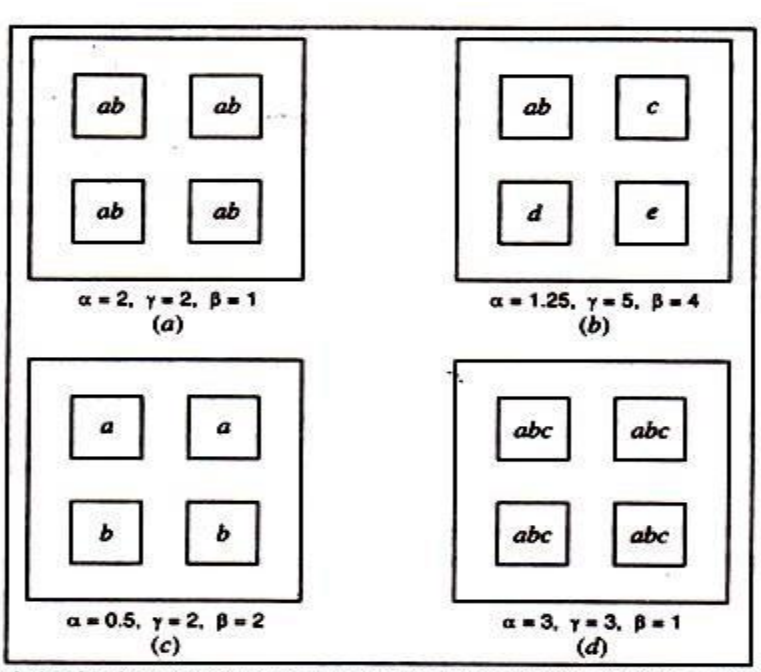


Fig. 7.1. Relationship between alpha, gamma and beta diversity (after Ricklefs and Miller, 2002). Each large box (region) has four small boxes (habitats). In (*a*), the diversity of each habitat (alpha diversity) is the smae for all 4 habitats, each contains species a and b, species richness of 2. The regional diversity (gamma) is 2. The beta diversity is gamma/alpha, 2/2 = 1. In (*b*), alpha diversity is 2 for one habitat (species a and b) and 1 for the other three (species c, d and e occur alone in a habitat), yielding an average a diversity of 1.25; gamma diversity is 5 (No. of habitats × average alpha diversity), so beta diversity is gamma/alpha, 5/1.25 = 4. In (*c*) average alpha diversity is 2/4 = 0.5, gamma diversity 2, beta diversity 2/1 = 2. In (*d*) alpha diversity is 12/4 = 3, gamma diversity 3, beta diversity is gamma/alpha, 3/3 = 1. Regions (*a*) and (*d*) have different gamma diversities but the same beta diversity, indicating little species turnover in those areas.

a. Species Richness Indices:

Species richness, as measure of diversity, has been used by ecologists. Species density or the number of species per m^2 is most commonly used to measure species richness. However, species richness increases with sample size. The smallest sample size may be 1 km^ and the largest may be the entire region or country.

Rarefaction:

As the sample sizes are always unequal, Sanders technique called Rarefaction is used to cope with this difficulty

Sanders's formula, as modified by Hurlbert (1971) is as follows:

$$E(S) = \sum\left\{1-\left[\binom{N-N_i}{n}\Big/\binom{N}{n}\right]\right\}$$

where $E(S)$ = expected number of species in the rarefied sample
n = standardized sample size
N = the total number of individuals recorded in the sample to be rarefied
N_i = the number of individuals in the *ith* species in the sample to be rarefied

The simplest approach is to take the number of individuals in the smallest sample as the standardized sample size.

This may be explained with the help of the following example:

If in one catch of fish we obtain 9 species with 23 individuals, and in another catch from the same area made for the same duration we obtained only 13 individuals belonging to 6 species, Hurlberts' formula may be used to find out the number of species we would have expected in the first catch if it too had only 13 individuals. Thus, expected number of species for the first catch x is 6.6 species (Table 7.4).

Table 7.4. Rarefaction with the help of Hurlbert's formula (see text for details)

Species	*Catch X*	*Catch Y*
A	9	1
B	3	0
C	0	1
D	4	0
E	2	0
F	1	0
G	1	1
H	0	2
I	1	0
J	0	5
K	1	3
L	1	0
Total No. of species (S)	9	6
Total No. individuals (N)	23	13

1. The term $\binom{x}{y}$ is a 'combination' which is calculated as follows.

$$\binom{x}{y} = \frac{x!}{y!(x-y)!}$$

x ! is a factorial. For example $5! = 5 \times 4 \times 3 \times 2 \times 1 = 120$

With these points in mind the computations can proceed

2.The first step is to take each species abundance from catch X and insert it in the formula

$$\left\{1 - \left[\binom{N - N_i}{n} \Big/ \binom{N}{n}\right]\right\}$$

Thus, for the species A which was represented by 9 individuals, the calculations are

$$\left\{1 - \left[\left(\frac{14!}{13! \times 1!}\right) \Big/ \left(\frac{23!}{13! \times 10!}\right)\right]\right\} = \{1 - [14/1144066]\}$$

$$= 1 - 0.00 = 1.00$$

The result for each species is listed and summed to give the expected species number for catch X

Ni	
9	1.00
3	0.93
4	0.98
2	0.82
1	0.57
1	0.57
1	0.57
1	0.57
1	0.57
Expected No. of Species for catch X	E(S) = 6.58 or 6.6

Menhinick's Index (I M_n):

This index is based on the ratio of number of species (S) and the square root of the total number of individuals (N).

$I_{Mn}=S/\sqrt{N}$ or $D_{Mn} = S/\sqrt{N}$

It is claimed that this index may be used to compare samples of different sizes and that the effect of the number of individuals is reduced. However, some authors have shown that this index is not independent of sample size.

Using the data given in Table 7.4, the value of IM_n for catch x and catch y will be 1.88 and 1.66 respectively.

Margalefs index (I_{Mg}):

This index also relates the number of species to the number of individuals.

$I_{Mg} = S - 1/\log_e N$ or $D_{Mg}= (S - 1)/\ln N$

The index is influenced by sample size. However, some authors have demonstrated that both this and Manhinick's index are insensitive to changes in community structure.

Using the data given in Table 7.4, the value of for sample x and sample y will be 2.55 and 1.95 respectively.

b. Species Abundance Models:

No community has species of equal abundance. Some species are very abundant, others may have medium abundance and still others may be rare or represented by only a few individuals. This observation led to the development of species abundance models.

Species diversity data is frequently described by one or more patterns of distribution (Piclou, 1975), diversity is usually examined in relation to the following four models:

(a) The geometric series

(b) The log normal distribution

(c) The logarithmic series

(d) The broken stick model (the random niche boundary hypothesis)

When plotted on a rank abundance graph, the four models represent a progression ranging from the geometric series where a few species are dominant with the remaining fairly uncommon, through the log series and log normal distributions where species of intermediate abundance become more common and ending in the conditions represented by the broken stick model in which species are equally abundant as may be hardly observed.

c. Species Proportional Abundance Based Indices:

These indices provide an alternative approach to the measurement of diversity. These indices are called heterogeneity indices (Peet 1974) as they take both species richness and evenness into consideration. South wood (1978) called them nonparametric indices in view of the fact that no assumptions are made about the shape of the underlying species abundance distribution. The following indices are used.

Simpson's Index:

This index relates the contribution made by each species to the total number of individuals present.

$I = \Sigma p_i^2$

Where pi is the proportion of individuals in the ith species. The equation given by Wilhm (1967) is the following:

$$I = \Sigma (ni(ni-1)/N(N-1)$$

Where pi = the number of individuals in the ith species and N= the total number of individuals. The values of Simpson's index range from zero to 1 (unity) and are inversely proportional to the wealth of species (As I increases, diversity decreases). Pielou (1969) has given the following form of equation.

$$I =1- \Sigma (ni(ni-1)/N(N-1)$$

Therefore, index is usually expressed as 1 – I or l/I. The reciprocal form of Simpson's index ensures that the value of the index increases with diversity.

Shannon Index:

The index independently derived by Shannon and Wiener from the application of information theory is known as the Sharmon index of diversity. It is sometimes incorrectly referred to as the Shannon – weaver index (Krebs, 1985).

The index assumes that:

(a) All species are represented in the sample, and

(b) Individuals are randomly sampled from an 'indefinitely large' population (Pielou, 1975).

It is calculated from the equation:

$H' = -\Sigma pi \ In \ pi$

Where pi is the proportion of individuals found in the ith species. It is estimated as (ni/N). N is total number of individuals in S species. The value of Shannon index usually varies between 1.5 and 3.5 and rarely exceeds 4.5. The value of H' is related to species richness but is also influenced by the underlying species abundance distribution. May (1975) has shown that if the underlying distribution is log normal, 10 species will be required to give a value of $H' < 5.0$. Log_2 is often used to calculate Shannon index. Usually the index is obtained from the series.

$$H' = -\Sigma pi \ In \ pi - S-1/N+1-\Sigma pi^{-1}/12N^{2} + \Sigma(pi^{-1} - pi^{-2})/12N^{3}$$

Types of Diversity Indices of Biodiversity

The two types are: (1) Dominance Indices, and (2) Information-Statistic Indices.

1. Dominance Indices:

Dominance indices are weighted toward the abundance of the commonest species. A widely used dominance index is Simpson's diversity index. It takes into account both richness and evenness.

Simpson's Diversity Indices:

The term "Simpson's diversity index" can actually refer to any one of 3 closely related indices.

Simpson's Index (D):

Simpson's index measures the probability that any two individuals drawn at random from an infinitely large community will belong to same species. There are two versions of the formula for calculating D.

Either is Acceptable but is to be Consistent:

$$D = \sum (n/N)^2 \qquad D = \frac{\sum n(n-1)}{N(N-1)}$$

where, n = the total number of individuals of each species, N = the total number of organisms of all species.

The value of D ranges between 0 and 1.

With this index, 0 represents infinite diversity and 1, no diversity. That is, the bigger the value of D, the lower the diversity. This does not sound logical, so to get over this problem, D is often subtracted from 1 or the reciprocal of the index is taken.

Simpson's Index of Diversity 1-D:

This index represents the probability that two individuals randomly selected from a community will belong to different species. The value of this index also ranges between 0 and 1, but here, the greater the value, the greater the diversity.

Simpson's Reciprocal Index 1/D:

The value of this index starts with 1 as the lowest possible figure. This figure would represent a community containing only one species. The higher the value, the greater would be the diversity. The maximum value is the number of species in the sample. For example, if there are five species in the sample, then maximum value is 5.

The name Simpson's diversity index is often very loosely applied and all three related indices described above (Simpson's index, Simpson's index of diversity and Simpson's reciprocal index) have been quoted under term, depending on authors.

As an example, let us consider the following table:

Species	Number(n)	n(n – 1)
A	2	2
B	8	56
C	1	0
D	1	0
E	3	6
Total (N)	15	64

Putting the values into the formula for Simpson's index:

$$D = \frac{\sum n(n-1)}{N(N-1)} = \frac{64}{15 \times 14} = 0.3 \text{ (Simpson's index)}$$

Then, Simpson's index of diversity 1 – D = 0.7 and Simpson's reciprocal index 1/D = 3.3.

All these three values represent the same biodiversity. It is, therefore, important to ascertain which index has actually been used in any comparative studies of biodiversity. The disadvantage of Simpson's index is that it is heavily weighed toward the most abundant species, as are in all dominance indices.

The addition of rare species with one individual will fail to change the index. As a result, Simpson's index is of limited value in conservation biology if an area has many rare species with just one individual.

2. Information-Statistic Indices:

Information-statistic indices can take into account rare species in a community. Information-statistic indices are based on the rationale that diversity in a natural system can be measured in a way that is similar to the way information contained in a code or message is measured.

By analogy, if we know how to calculate the uncertainty of the next letter in a coded message, then we can use the same technique to calculate the uncertainty of the next species to be found in a community.

Shannon Index:

A widely used diversity index is Shannon index.

The Index is given by:

$$H_s = \sum_{i=1}^{s} p_i \ln p_i$$

where, p_i is the proportion of individuals found in the i^{th} species and In denotes natural logarithm.

The following table gives an example:

	Species	Abundance	p_i	$p_i \ln p_i$
	A	50	0.5	– 0.347
	B	30	0.3	– 0.361
	C	10	0.1	– 0.230
	D	9	0.09	– 0.217
	E	1	0.01	– 0.046
Total	5	100	1.00	– 1.201

Putting the values into the formula for Shannon index, $H_s = 1.201$

Even the rare species with one individual (species E) contributes some value to the Shannon index, so if an area has many rare species, their contributions would accommodate. Shannon index has a minus sign in the calculation, so the index actually becomes 1.201, not-1.201. Values of Shannon index for real communities are often found to fall between 1.5 and 3.5. The value obtained from a sample is in itself of no significance. The index becomes useful only while comparing two or more sites.

Brillouin Index:

A second information-statistic index, designed to reflect species abundance.

The Brillouin index and is given by:

$$H_B = \frac{\ln(N!) - \sum \ln(ni!)}{N}$$

where, N is the total number of individuals in the community, ni is the number of individuals in the i^{th} species.

The following table gives an example:

Species	No. of individuals	$\ln(n_i!)$
A	5	4.79
B	5	4.79
C	5	4.79
D	5	4.79
E	5	4.79
	N = 25	Σ ln(n!) = 23.95

Putting the values into the formula for Brillouin index, we get

$$H_B = \frac{1n\,(25!) - 23.95}{25} = \frac{58 - 23.95}{25} = 1.362$$

This index describes a known population. There is no room for uncertainty while using this index. It places more emphasis on species richness and is moderately sensitive to sample size.

Probable questions:

1. Define ecological community.

2. Describe the structure of a community.

3. Describe the components of a community.

4. Describe stratification of a grassland.

5. describe stratification of a forest.

6. Describe stratification of a pond.

7. What is ecotone and edge effect.

8. What is keystone species and link species? Give suitable examples of each.

9. Describe different types of biotic interaction with suitable examples.

10. What do you mean by ecological succession. What do you mean by climax community ?

11. What are species diversity, resource diversity and habitat diversity ?

12. How species diversity is measured ?

Suggested Readings:

1. Robert E. Ricklefs and Gary L. Miller. ECOLOGY. *Fourth Edition.*
2. Thomas M. Smith and Robert Leo Smith. Elements of ECOLOGY. *Eighth Edition.* ISBN 978-0-321-73607-9

UNIT- IX

Biogeographical Zones of India

Objective: In this unit you will know about different Biogeographical zones of India

Introduction:

Biogeography is the study of the distribution of species and ecosystems in geographic space and through geological time. Organisms and biological communities often vary in a regular fashion along geographic gradients of latitude, elevation, isolation and habitat area.Phytogeography is the branch of biogeography that studies the distribution of plants. Zoogeography is the branch that studies distribution of animals.

India has only 2.4% of the world's land area, but contributes about 8.1% to global species diversity. This makes India one of the 12 mega biodiversity countries in the world. The country is divided into 10 biogeographic regions. The diverse ecological habitats such as forests, grasslands, wetlands, coastal and marine ecosystems and desert ecosystems have helped to harbor and sustain immense biodiversity in the country. The Indian subcontinent is rich in biodiversity with a good percentage of endemic flora and. fauna. This richness in biodiversity is due to variety of climate, topography and varied ecological habitats. These vary from the humid tropical Western Ghats to the hot deserts of Rajasthan, from the cold desert of Ladakh and snowcapped mountains of Himalayas to the warm coasts of peninsular India.

There are over 45,000 species of plants and 75,000 species of animals identified. There are more than 100,000 plant species and more than 300,000 animal species yet to be discovered. About 4,900 species of flowering plants are endemic to the country.These are concentrated in the floristically rich areas of North-East India, the Western Ghats, Northwest Himalayas and the Andaman and Nicobar Islands. It is estimated that 62% of the known amphibian species are endemic to India, of which a majority is found in Western Ghats. The Western Ghats in peninsular India and the North-East region are the treasure house of species diversity.The coastline of India is about 7,000 kms long where there is an abundance of seaweeds, fish, crustaceans, molluscs, corals, reptiles and mammals.

The conservation and sustainable use of biological resources in India is based on local knowledge systems and practices ingrained in Indian ethos. The country has a number .of alternative medicines, like Ayurveda, Unani, Siddha and Homeopathic systems, which are predominantly based on plant-based raw materials. Plant-based raw materials are also used in various herbal preparations in. the pharmaceutical and cosmetic industry.However, India as a country has several challenges such as overpopulation, growing demand for land, energy and water supply. Due to overexploitation there is shortage of various materials and many animal species face the danger of extinction.

India has different climate and topography in different parts and hence is termed as a mega diversity country. India occupies 10th place among plant rich countries of the world. It is essential to acquire knowledge about the distribution and environmental interaction of flora and fauna of India. With only 2.4 per cent of the total land area of the world, the known biological diversity of India contributes 8 per cent to the known global biological diversity. Biogeographic classification of India is the division of India according to biogeographic characteristics. There are ten biogeographic zones in India:

1. **Trans Himalayan zone.**
2. **Himalayan zone**
3. **Desert zone.**
4. **Semiarid zone.**
5. **Western Ghat zone.**
6. **Deccan plateau zone.**
7. **Gangetic plain zone.**
8. **North east zone.**
9. **Coastal zone**
10. **Island present near the shoreline**

Biogeographic Region	%*
Andaman & Nicobar Island	0.3
Coastal region	2.5
North East Region	5.2
Gangetic Plains	10.8
Deccan Plateau	42
Western Ghats	4
Semi Arid Region	16.6
Indian Desert Zone	6.6
Himalayan Zone	6.4
Transhimalayan Region	5.6
Total	100
*Of total geographic area	

Table 1: Total Geographical areas of biogeographical zones

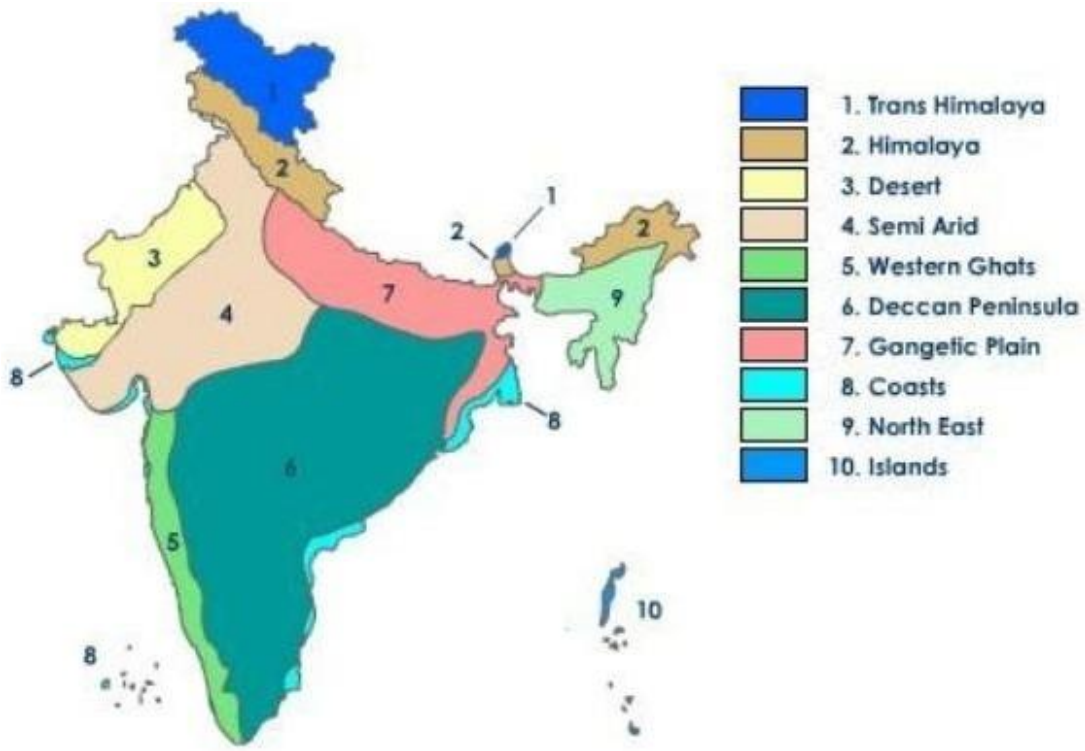


Figure-1. Map Indicating locations of various Biogeographical zones of India

1. Trans Himalayan zone:

Trans-Himalayas, eastward continuation of the most northerly ranges of the Himalayas in the southern part of the Tibet Autonomous Region of China. It consists of an ill-defined mountain area about 600 miles (1,000 km) long and 140 miles (225 km) wide in the centre, narrowing to a 20-mile (32-km) width at the eastern and western ends. The Trans-Himalayas, mainly composed of granites and volcanic rocks of Neogene and Paleogene age (i.e., about 2.6 to 65 million years old), are bounded by the Kailas (southwest), Nganglong Kangri (north), and Nyainqêntanglha (southeast) mountain ranges and by the Brahmaputra (YarlungZangbo) River (south). Trans Himalayas are divided into following ranges:

1**. Ladakh Range**
2. Zaskar Range
3. Karakoram Range
4. Kailash Range

Unlike the main Himalayas, the mountains are not divided by deep river gorges and lack a definite alignment. Passes average 17,500 feet (5,330 meters) in height, with the highest being Chargoding Pass (19,308 feet ,885 meters). The first recorded European sighting of the mountains was that of the Swedish explorer Sven Anders Hedin in 1906.

Fig 2: Kailash Range

It constitutes 5.6 per cent of the total geographical area, includes the high altitude, cold and arid mountain areas of Ladakh, Jammu & Kashmir, North Sikkim, Lahaul and Spiti areas of Himachal Pradesh. This zone has sparse alpine steppe vegetation that harbours several endemic species and is a favourable habitat for the *biggest populations of wild sheep and goat in the world* and other rare fauna that *includes* **Snow Leopard** *and the migratory Black necked Crane (Grus nigricollis).* The cold dry desert of this zone represents an extremely fragile ecosystem.

Fig 3: Wild goat (left) and sheep (right)

Fig 4: Snow leopardFig 5: *Black necked Crane*

2. Himalayan zone:

The Himalayas consist of the youngest and loftiest mountain chains in the world. The Himalayas have attained a unique personality owing to their high altitude, steep gradient and rich temperate flora. The Himalayas are the northern boundaries of India. The entire mountain chain is running from Kashmir in the North-west to Assam in the north-east. The Himalayas comprise of a diverse range of biotic provinces and biomes.

The alpine and sub-alpine forests, grassy meadows and moist mixed deciduous forests . Oak, chestnut, conifer, ash, pine, deodar are abundant in Himalayas. There is no vegetation above the snowline. It provides diverse habitat for endangered species of bovids such as Bharal (*Pseudoisnayaur*), Ibex (*Capra ibex*), Markhor (*Capra falconeri*), Himalayan Tahr (*Hemitragusjemlabicus*), and Takin (*Budoreastaxicolor*). Other rare and endangered species restricted to this zone include Hangul (*Cervus eldieldi*) and Musk Deer (*Moschus moschiferus*). Panda and snow leopard are also found here.

Fig 6: Alpine IbexFig 7: Musk deer

3. Desert zone

Indian desert zone, an extremely arid area west of the Aravalli hill range constituting 6.6 per cent of the total geographical area, includes the Thar and the Kutch deserts (both the salty desert of Gujarat and the sand desert of Rajasthan). Climate is characterized by very hot in dry summer and cold in winter. It has large expanses of grassland that supports several endangered species of mammals such as Wolf (*Canis lupus*), Caracal (*Felis caracal*), Desert Cat (*Felislibyca*) and birds of conservation interest viz., Houbara Bustard (*Chamydotis undulate*) and the Great Indian Bustard (*Ardeotisnigriceps*). Camel, wild asses, foxes and snakes are also found in hot arid deserts. The plants are mostly xerophytic, babul, kikar, wild palm grows in areas of moderate rainfall.


Fig 8: CaracalFig 9: Great Indian bustard

4. Semiarid zone

Semi-arid Region, constituting 16.6 per cent of the total geographical area, is a transition zone between the desert and the dense forests of Western Ghats. Peninsular India has two large regions, which are climatically semi-arid. This region is characterized by discontinuous vegetation cover with open areas of bare soil and soil-water deficit throughout the year. *This semi-arid region also has several artificial and natural lakes and marshy lands.*

The *dominant grass and palatable shrub layer in this zone supports the highest wildlife biomass*. Thorny shrubs, grasses, and some bamboos are present in some region. The cervid species of **Sambar** (*Cervus unicolor*) and **Chital** (*Axis axis*) are restricted to the better wooded hills and moister valley areas respectively. The Lion (*Leo persica*), an endangered carnivore species (restricted to a small area in Gujarat, specifically Gir National Park), Caracal (*Felis caracal*), Jackal (*Canis aureus*) and Wolf (*Canis lupus*) are some of the endangered species that are characteristic of this region.


Fig 10: Sambar deerFig 11: Cheetal deer

Fig 12: Lion of Gir national park

5. Western Ghats zone:

The mountains along the west coast of peninsular India are the Western Ghats, which constitute one of the unique biological regions of the world. The Western Ghats extend from the southern tip of the peninsula (8°N) northwards (Kanyakumari) about 1600 km to the mouth of the river Tapti (21°N). They constitutes 4.0 per cent of the total geographical area. It is one of the major tropical evergreen forest regions in India and represents one of the two biodiversity 'hot spots'. Western Ghats are home to viable populations of most of the vertebrate species found in peninsular India, besides an endemic faunal element of its own.

The mountains rise to average altitudes between 900 and 1500 m above sea level, intercepting monsoon winds from the southwest and creating a rain shadow in the region to their East. The varied climate and diverse topography create a wide array of habitats that support unique sets of plant and animal species. Apart from biological diversity, the region boasts of high levels of cultural diversity, as many indigenous people inhabit its forests.The Western Ghats are amongst the 25 biodiversity hot-spots recognized globally. These hills are known for their high levels of endemism expressed at both higher and lower taxonomic levels. Most of the Western Ghat endemic plants are associated with evergreen forests.The region also shares several plant species with Sri Lanka. The higher altitude forests were, if at all, sparsely populated with tribal people. Rice cultivation in the fertile valley proceeded gardens of early commercial crops like areca nut and pepper. The original vegetation of the ill-drained valley bottoms with sluggish streams in elevations below 100m would be often a special formation, the Myristica swamp.

Expansion of traditional agriculture and the spread of particularly rubber, tea, coffee and forest tree plantations would have wiped out large pockets of primary forests in valleys. The Western Ghats are well known for harboring 14 endemic species of caecilians (i.e., legless amphibians) out of 15 recorded from the region so far. This zone have large population of Nilgiri langurs, tiger, leopard and Indian elephants. Other species which are endemic in this region are **Lion Tailed Macaque** *(Macacasilenus),* **Grizzled Giant Squirrel** *(Ratufa macroura),* **Malabar Civet** *(Viverriculamegaspila),* **NilgiriTahr** *(Hemitragusbylocrius)* **and Malabar Grey Hornbill** *(Ocycerous griseus).*


Fig 13: Legless caecilians Fig 14: Nilgiri langurs

Fig 15: Malabar grey hornbill

6. Deccan Plateau

Deccan Plateau is India's largest biogeographic region making 42 per cent of the total geographical area. It's a semi-arid region that falls in the rain shadow area of the Western Ghats. This bio-geographic zone of peninsular India is by far the most extensive zone, covering India's finest forests, particularly in the States of Madhya Pradesh, Maharashtra and Odisha. *Majority of the forests are deciduous in nature* but there are regions of greater biological diversity in the hill ranges. The zone comprising of deciduous forests, thorn forests and degraded scrubland support diverse wildlife species.

The Deccan plateau includes the region lying south of the Satpura range. It extends up to the southern tip of peninsular India. Anaimudi is the highest peak of this region. The Deccan plateau is surrounded by the western and the eastern ghats. These ghats meet each other at the Nilgiri hills. The western ghats includes the Sahyadri, Nilgiris, Anamalai, and cardamom hills. Many rivers such as Mahanadi, Godavari, Krishna, and Kaveri originates from western ghats and flow toward the east. The eastern ghats are broken into small hill ranges by river coming from the western ghats. Most of these rivers fall into the bay of Bengal. The Godavari is the longest river in the Deccan plateau .the Narmada and the Tapi flow westwards and fall into the Arabian seaSpecies found in this region are **Chital** *(Axis axis),* **Sambar** *(Cervus unicolor),* **Nilgai** *(Boselapbustragocamelus)* **andChousingha** *(Tetracerusquadricornis),* **Bar king deer** (*Muntiacusmuntjak*) and Gaur (*Antilope cervicapra*), Elephant (*Elephas maximus*) in Bihar-Orissa and Karnataka-Tamil Nadu belts, Wild Buffalo (*Bubalusbubalis*) in a small area at the junction of Orissa, Madhya Pradesh and Maharashtra and the hard ground Swamp Deer (*Cervus duvauceli*), now restricted to a single locality in Madhya Pradesh.


Fig 16: NilgaiFig 17: Indian elephants

7. Gangetic plain zone

In the North is the Gangetic plain extending up to the Himalayan foothills. This is the largest unit of the Great Plain of India. Ganga is the main river after whose name this plain is named. Gangetic plain constitutes around 10.8 per cent of the total geographical area. The Gangetic plain is topographically homogenous for hundreds of kilometers. This one of the most fertile area. Three zone can be divided Upper plain of ganga (from Delhi to Allahabad), Middle plain of ganga (from Allahabad to Bihar) and lower plain of ganga (in West Bengal).
The thickness in the alluvial sediments varies considerably with its maximum in the Ganga plains. The physio geographic scenery varies greatly from arid and semi-arid landscapes of the Rajasthan Plains to the humid and per-humid landscapes of the Delta and Assam valley in the east. Topographic uniformity, except in the arid Western Rajasthan is a common feature throughout these plains. The plain supports some of the highest population densities depending upon purely agro-based economy in some of these areas. The trees belonging to these forests are teak, sal, shisham, mahua, khair etc. The characteristic fauna of this region include Rhino (*Rhinoceros unicornis*), Elephant (*Elephas maximus*), Buffalo (*Bubalusbubalis*), Swamp Deer (*Cervus duvauceli*), Hog-Deer (*Axis porcinus*) and Hispid Hare (*Carprolagusbispidus*).


Fig 18: Indian hog deer **Fig 19: Hispid hare**

8. North East zone

North East Region constitutes 5.2 per cent of the total geographical area. This region represents the transition zone between the Indian, Indo-Malayan and Indo-Chinese bio-geographical regions as well as being a meeting point of the Himalayan mountains and peninsular India. The North-East is thus the biogeographical 'gateway' for much of India's fauna and flora and also a biodiversity hotspot (Eastern Himalaya). Many of the species contributing to this biological diversity are either restricted to the region itself, or to the smaller localized areas of the Khasi Hills. It has several species of orchids, bamboos, ferns and other plants. Here the wild relatives of cultivated plants such as banana, mango, citrus and pepper can be grown.

9. Coastal zone

India has coastline of 7, 516.5 km. It extends from Rann of Kutch to Bangladesh. The west coast is narrow and extends from gulf of Cambay to south. Coastal region constitutes 2.5 per cent of the total geographical area with sandy beaches, mangroves, mud flats, coral reefs and marine angiosperm pastures make them the wealth and health zones of India. The coastline from Gujarat to Sunderban is estimated to be 5,423 km long. A total of 25 islets constitute the Lakshadweep, which are of coral origin, and have a typical reef lagoon system, rich in biodiversity. However, the densely populated Lakshadweep islands virtually have no natural vegetation.

The backwaters are the characteristic features of this coast. The east coast plains, in contrast are broader due to depositional activities of the east-flowing rivers owing to the change in their base levels. Extensive deltas of the Godavari, Krishna and Kaveri are the characteristic features of this coast. Mangrove vegetation is characteristic of estuarine tracts along the coast for instance, at Ratnagiri in Maharashtra. Larger parts of the coastal plains are covered by fertile soils on which different crops are grown. Rice is the main crop of these areas. Coconut trees grow all along the coast.

10. Islands

This constitutes 0.3 per cent of the total geographical area are one of the three tropical moist evergreen forests zones in India. The two groups of islands, i.e., the Arabian Sea islands and Bay Islands differ significantly in origin and physical characteristics. The Arabian Sea Islands (Laccadive, Minicoy, etc.) are the foundered remnants of the old land mass and subsequent coral formations. On the other hand, the Bay Islands lay only about 220 km. Away from the nearest point on the main land mass and extend about 590 km. With a maximum width of 58 km the island forests of Lakshadweep in the Arabian Sea have some of the best-preserved evergreen forests of India. Some of the islands are fringed with coral reefs. Many of them are covered with thick forests and some are highly dissected. *The islands house an array of flora and fauna not found elsewhere.*These islands are centres of high endemism and contain some of India's finest evergreen forests and support a wide diversity of corals. In India, *endemic island biodiversity is found only in the Andaman and Nicobar Islands.* Some of the endemic fauna of Andaman & Nicobar islands include *Narcondam hornbill*, South Andaman krait etc.

Fig 20: ***Narcondam hornbill*****Fig 21: South Andaman krait**

Biodiversity Hotspots in India

Of the twenty-five hotspots of biodiversity, recognized in the world, two are found in India, which extend into the neighbouring countries:

(i) The Indo-Burma region covering the Eastern Himalayas and

(ii) The Western Ghats – Sri Lankan region.

The hotspots are rich in floral wealth, reptiles, amphibians, mammals and also in their endemism.

The botanical hotspots of India include:
(1) Western Ghats,

(2) North-East India,

(3) Himalayas and

(4) Andaman and Nicobar Islands.

Eastern Himalayas:

As compared to the Western Himalayas that are colder and drier, the Eastern Himalayan ranges are much wetter with suitable climatic conditions. This supports endemism and biodiversity. The Eastern Himalayas comprise of .parts of Nepal, Bhutan, Sikkim, Arunanchal Pradesh and extends up to Burma. The forest vegetation ranges from tropical rain forests to temperate alpine forests. Rhododendron is the dominant tree of this region.

The topography in the Eastern Himalayan region is quite varied that helps biodiversity and endemism. The rugged mountains and valleys support the virgin forests which are rich in

many endemic plant species. In the Indian part of the Eastern Himalayan hotspot, about 5800 plant species are found of which around 2000 are endemic. Sikkim is one of the most blessed Indian states so far as endemism is concede; as of the 4250 plant species found there, 60% are endemic to the region.Palaeobotanists consider the Indo-Burma region to be one of the centres of origin for the flowering plants (angiosperms). Many primitive angiosperm families occurring there, such as Magnoliaceae, Winteraceae, etc. support this contention. The Eastern Himalayas are home to around 8000 species of angiosperms of which nearly 40% are endemic. The characteristic floras are Rhododendron, Alnus, Betula, Magnolia, etc.

The animals include members of the goat family, antelopes, musk deer, snow leopard, brown bear, black bear and the red panda. Due to continuous habitat destruction and hunting, many of these species are highly endangered. Andaman and Nicobar Islands are rich in littoral and Island type floras. About 2500 species of flowering plants have been recorded from there, of which 250 species are endemic. The important hotspots of Andaman & Nicobar Islands are North Andaman, spike Island, Table Island. South reef Island, Little and Great Nicobars.Western Himalayas are well-known for Alpine flora which abounds in Gymnosperms. Some 5000 species of flowering plants are known to occur in this region of which 800 species are endemic. The valley of flowers, Pithoragarh, Gori Valley, Mandal Chopta Valley, Karakoram and Laddakh are some of the major hotspots of this region.

Western Ghats:

The Western Ghats run parallel to the west coast of India and constitute more than 16,000 km strip of forests in the states of Maharashtra, Goa, Karnataka, Tamil Nadu and Kerala. Locally they are also known as the Sahyadris. The Western Ghats are characterised by low hills; however, it achieves the highest elevation of 2675 m at Annamalai up to an elevation of 500 m the forests are evergreen in nature, while the forests occurring between 500-1500 m altitude are of semi-evergreen type.

The Western Ghats by virtue of having a humid tropical climate and geological stability supports one of the most biodiversity rich areas of the country. According to an estimate, of the 17,000 flowering plants species reported from India, more than 4,500 occur in the Western Ghats region. The Botanical Survey of India has listed 518 endangered species endemic to Peninsular India, the majority of which occur in the Western Ghats.The dominating plant families are Acanthaceae, Graminae, Orchidaceae, Rubiaceae, Labiatae, Compositeae and Leguminoseae. More than 200 species of rare orchids, many of them are endemic, are found in Western Ghats. Many economically important plants such as banana, rice, black pepper, ginger, etc. have spread to other parts of the country from here.

The Western Ghats are home to a rich variety of fauna which are unique and many are endemic to this region. Nilgiri Langur, Lion-tailed Macaque, tiger, leopard, elephant, rare species of tortoise and other amphibians represent the faunal wealth. Many varieties of birds and fishes are also found here.

Sacred Forests:

These real hotspots of biodiversity are protected and worshiped by tribals due to religious sanctity. These are known as Devaskadu in Karnataka Devarahati in Maharashtra and Lakyntok in Meghalaya. These forests contain many rare endangered and endemic species.

Mangroves:

Mangroves are salt tolerant forest ecosystems found in Saline habitat, tropical and subtropical intertidal zones near estuaries. The total area under mangrove vegetation in India is estimated to be 6740 km and of this 4200 km^2 is covered by mangrove forest of sundarban alone and the second being in the Andaman & Nicobar Islands.

Probable Questions:

1. Define biogeography. How many biogeographic zones are there in India?

2. State the characteristics and faunal diversity in Himalayan region.

3. State the characteristics and faunal diversity in desert zone.

4. State the characteristics and faunal diversity in Gangetic plain zone.

5. State the characteristics and faunal diversity in semi arid zone of India

6. State the characteristics and faunal diversity in coastal zone of India

7 Write about biodiversity hotspots in India with special emphasis on Western Ghat region and Eastern Himalayan region.

8. Write a short note on mangrove islands of India.

Suggested Readings:

1. Animal Ecology by Veerbala Rastogi

2. Biodiversity by Maity and Maity

3. Ecology and Environmental science by Rana

UNIT X

Environmental management: Solid waste management; Bioremediation; Bioreactors in Environment monitoring

Objective:

In this unit you will learn about Environmental management: Solid waste management; Bioremediation; Bioreactors in Environment monitoring.

Introduction:

Environmental management (EM) is perceived as a framework or a set of practices and processes that enable any organization, whether private or public, to reduce its environmental impacts while aiming at increasing its operating productivity. Environmental management is therefore concerned with the description and monitoring of environmental changes, with predicting future changes and with attempts to maximise human benefit and to minimise environmental degradation due to human activities.

A. Solid waste management

Solid waste is commonly known as everything that goes out in trash.

Solid wastes are any discarded or abandoned materials that can be solid, liquid, semi-solid or containerized gaseous material discarded by the human society. These include urban wastes, agricultural wastes, biomedical wastes and radioactive wastes. The term refuse is also used for solid wastes.

Examples of solid wastes include waste tires, septage, scrap metal, latex paints, furniture and toys, garbage, appliances and vehicles, oil and anti-freeze, empty aerosol cans, paint cans and compressed gas cylinders, construction and demolition debris, asbestos, plastics, styrofoam containers, bottles etc.

The term solid waste management mainly refers to the complete process of collecting, treating and disposing of solid wastes.

Sources of Solid Wastes

- Solid domestic garbage.
- Solid waste material from various industries.
- Solid agricultural waste.
- Plastics, glass, metals, e-waste, etc.
- Medical waste.
- Construction waste, sewage sludge

Classification of solid wastes

It is mandatory to classify solid wastes into groups that pose similar risks to the environment and human health for safe disposal. According to the modern systems of waste management, solid wastes are classified based on their source, type, properties and its effect on human health and environment.

I. Source based classification

Wastes are produced from different sources and are categorized as follows

i. Municipal solid waste (MSW)

Municipal solid waste commonly referred to as trash, garbage or refuse comprises of street wastes, dead animals, market wastes, abandoned vehicles, household garbage, rubbish, construction and demolition debris, sanitation residue, packaging materials, trade refuges etc. They are collected from residential houses, markets, streets and other places mostly from urban areas and disposed of by municipal bodies. The proportion of different constituents of municipal wastes varies from place to place and season to season depending on the food habits, life style, standard of living and extent of commercial and industrial activities in the area. Municipal wastes their contents and source are illustrated in table given below. Municipal solid wastes are further categorized based on their physical, chemical and biological properties.

ii. Industrial wastes

Wastes generated during industrial activities such as manufacturing and processing involved in chemical plants, paint industry, cement factories, metallurgical plants, thermal power plants, petroleum, coal, gas, sanitary, textile, food processing and paper industry are referred to as industrial wastes. Some examples of industrial wastes are chemical solvents, paints, sandpaper, paper products, industrial by-products, metals, and radioactive wastes. Industrial solid wastes are further classified as hazardous and nonhazardous wastes.

iii. Institutional/ Commercial wastes

Solid wastes originating from administrative, educational and public buildings such as offices, schools, colleges, hospitals, government centres, prisons and other commercial establishments like wholesale and retail stores, restaurants, hotels, markets, warehouses. Paper, cardboard, plastics, wood, food wastes, glass, metals, special wastes, hazardous wastes are the examples of industrial and commercial wastes.

iv. Agricultural wastes

Agriculture wastes include both natural (organic) and non-natural wastes generated through farming activities. These activities include but are not limited to dairy farming, horticulture, seed growing, livestock breeding, grazing land, market gardens, nursery plots, and even woodlands. Some of agricultural wastes include spoiled food grains, vegetables, animal and plant wastes, litter, pesticides fertilizers etc. Other agricultural wastes are produced from agricultural products processing industries like sugarcane

factories, tobacco processing units, slaughter houses, livestock, poultry etc. Agricultural wastes are mostly biodegradable but few wastes like pesticide and fertilizers are toxic. When discharged to the environment, agricultural wastes can be both beneficial and detrimental to living matter.

v. Biomedical wastes

Wastes produced from hospitals, medical centres and nursing homes are called biomedical wastes. Hospital wastes are generated during diagnosis, treatment or immunisation of human beings/animals or in research activities in these fields or in the production/testing of biologicals. These wastes are highly infectious and may pose severe threat if not managed properly. Biomedical wastes may be solid or liquid wastes that includes discarded blood, sharps, soiled wastes, disposables, anatomical wastes, cultures, discarded medicines, chemical wastes etc.

II. Type based classification

i. Garbage

Garbage wastes mean and include animal and vegetable wastes generated from kitchen, cooking, serving of foods, slaughter houses, market refuse. These wastes contain putrescible organic matter which produces strong odour. They attract rats, vermins, flies and other insects and so they requires immediate attention in handling and disposal.

ii. Rubbish

Solid wastes arising as a result of households, commercial and institutional activities excluding garbage and ashes are termed as rubbish. They are categorized into combustible and non-combustible wastes. Combustible wastes consists of all rubbish and refuse that can be incinerated to flames at 1400-1500◦ F which includes leaves, plants, clothes, paper, leather, rubber, grasses. Non-combustible wastes are characterized as wastes that cannot be incinerated to flames at 1400-1500◦ F and that includes glass, metals, plastic materials, stones, auto parts etc.

iii. Bulk waste

Bulky household wastes consists of household furniture; appliances such as stoves, washing machines and refrigerators; mattresses and springs, rugs, TV sets, water heaters, tires, lawn mowers, auto parts, tree and brush debris etc.

Commercial bulky wastes include packaging and containers such as cardboard, wood boxes, fiber, plastic and steel drums, loose and bundled paper, bundles of textiles and plastics, wires, furniture and equipment etc.

Industrial bulky waste includes crates, cartons; steel, fiber and plastic drums; bales and rolls of paper, plastics, and textiles; miscellaneous metal items etc.

iv. Ashes

Ashes are defined as fine powdery residues, cinders and clinkers arising from the burning of wood, coal, charcoal, coke and other combustible materials during cooking and heating in houses, institutions and outer industrial establishments.

v. Street wastes

Wastes comprising of leaves, dirt, dust litter, paper, plastics and other vegetable matter collected from streets, walkways, alleys, parks, beaches and vacant lots are termed as street wastes.

vi. Dead animals

Animals that die naturally or accidentally comprises of dead animal wastes. They are categorized as small animals such as cats, dogs, rats, rabbits, poultry and large animals like horses, cows, goats, Sheep etc. However, animal carcasses and animal parts from slaughterhouses are excluded from dead animal wastes and considered as industrial wastes. These dead animal wastes are putrescible and attract flies and other vermin posing severe health risk to the environment. Hence they must be collected and disposed off promptly.

vii. Construction and demolition wastes

Construction and demolition wastes are the waste materials generated in large amounts during the construction, refurbishment, repair and demolition of houses, commercial buildings, roads and other structures. They consists of earth, stones, concrete, bricks, lumber, steel, roofing materials, plumbing materials, heating systems and electrical wires.

viii. Sewage wastes/sludge

Settled solid components, residual or semi-solid materials that are discharged from sewage treatment plants and septic tanks are classified as sewage waste. The raw and the treated sewage consist of organic and inorganic fraction. The sewage wastes contains putrescible organic matter which may contain pathogens and so must be disposed off without delay.

ix. Plastics

Plastic due to their versatile property of being light, durable, easy to mould and economical has invaded almost all sectors of the economy. Likewise, they are generated as wastes from almost all sectors that includes agriculture, construction, consumer goods, household, health care, hotel and catering, packaging, telecommunications, air and travel industries. Some of the plastic wastes include carry bags, bottles, plates, spoons, glasses, gloves, boxes, syringes, catheter tubes, surgical items etc. Plastics due to its non-biodegradable nature are now considered a serious threat to the environmental and health.

x. Mining wastes

Mining wastes are generated from extractive operations of mineral resources. They include materials such as topsoil, overburden and waste rock that must be removed to gain access to the mineral resource. Also, other waste material like slags, mine water, mine tailings, water treatment sludge and gaseous wastes etc are released during or after processing of mineral ores. Some of these wastes are inert and are not considered as threat to the environment. However, other fractions, generated by the non-ferrous metal mining industry contain large quantities of dangerous substances such as heavy metals. These metals and metal compounds after extraction and subsequent mineral processing, tend to become chemically more available resulting in the generation of acid or alkaline drainage. Therefore, mine wastes requires to be carefully characterized to prevent and minimize air, water, and soil contamination.

xi. Radioactive wastes

Radioactive wastes are hazardous, by-products of nuclear reactions. They pose severe threat to human life and environment. Radioactive wastes decays over time ranging from a few days for highly radioactive isotopes to millions of years for slightly radioactive ones. Hence, these wastes have to be isolated and confined at appropriate disposal facilities for it to completely decay. The sources of radioactive wastes are from mining of radioactive substances, atomic explosion, nuclear fuel cycle, nuclear weapons reprocessing, medical and industrial wastes etc.

III. Property based classification

Solid wastes are also classified based on their biological and chemical property.

i. Biodegradable / Organic wastes

Biodegradable wastes are those that can be decomposed by the natural processes such as composting, aerobic/ anaerobic digestion and converted into the elemental form like carbon dioxide, methane, water or simple organic molecules. Some of the biodegradable wastes include municipal solid wastes (green waste, food waste, paper waste, biodegradable plastics, human and animal wastes, sewage, sludge, slaughter house wastes etc).

ii. Non-biodegradable /inorganic wastes

Non-biodegradable wastes are those that cannot be decomposed and remain as such in the environment indefinitely. They are persistent and threaten to overwhelm landfills and create disposal problems creating environmental concern. As non-biodegradable wastes cannot be decomposed, recycling is the ideal option for managing it. Example of non-biodegradable wastes includes plastics, nuclear wastes, glass, rubber tyres, styrofoam, fiberglass and metals.

iii. Hazardous wastes

Hazardous waste is defined as chemical material that can no longer be used for its intended purpose and is known to be harmful or potentially harmful to plants, animals and human health or to the environment. Hazardous wastes may be in the form of

solids, liquids, sludge's or gases. In some cases, although the active agents may be liquid or gaseous, they are classified as solid waste because they are confined in solid containers. They are generated primarily by chemical production, manufacturing and other industrial activities. The hazardous waste materials may be toxic, reactive, ignitable, explosive, corrosive, infectious or radioactive. If improperly handled, they can cause substantial harm to human health and to the environment. So good management practice should ensure that hazardous wastes are collected, stored, transported and disposed off separately, to render them innocuous. Some of the important hazardous wastes are lead, mercury, cadmium, chromium, many drugs, leather, pesticides, dye, rubber, solvents, paints and effluents from different industries.

iv. Non-hazardous wastes

Non-hazardous wastes are defined as substances safe to use commercially, industrially, agriculturally or economically. Some of the non-hazardous wastes produced are from the food processing plants, cotton mills, paper mills, textile mills and sugarcane industries. Other non-hazardous wastes includes paint, oil, antifreeze, buffers, salts etc.

• Methods of Solid Waste Management & Treatment of Solid Waste

There are various methods of solid waste management. The most recognized ones include:

- **Solid Waste Collection:** Collection refers to the method in which solid wastes are collected for transportation to final disposal. A collection system should be planned keeping in mind that there is no overload of storage systems and the process of collection also takes place periodically. These days in the country solid waste collection is done on a daily basis by the municipal corporations where the wastes are then transported to the disposal site.
- **Separation:** It is the manual sorting of solid waste before disposing it. For e.g. sorting out the dry solid waste from the wet solid wastes or sorting out between biodegradable wastes and non-biodegradable wastes.
- **Sanitary Landfill:** This is the most common and popular way of carrying out the disposal of solid waste today. The trash that is collected by the municipal workers is transported to huge areas of land that are dug deep. In these are as, the garbage is spread out and once the land is full, it is topped with layers of soil, sand, and gravels top revent any water seepage. These days layers of plastic and sand are used to line landfills with an impervious liner to prevent any percolation and contamination of groundwater.
- **Incineration:** In simple terms, this method is the burning of solid wastes at a very high temperature until the waste turns to ashes. Incinerators today are made in a way that does not give off a large amount of heat energy. Recycling incinerators have also come up that transforms the heat energy from the furnace into the boiler. These are called waste-to-energy plants. These are very

expensive in nature. Although incineration helps in reducing thevolume of the solid waste to 20-30% of the original volume, it emits gaseous pollutants through the smoke and can also cause a fire.

- **Recycling:** Recycling is the conversion of discarded solid waste into new products. Recycling reduces solid waste and helps in recovering the material to make new products for reuse. Hence, the three Rs – Recycle, Reduce and Reuse. The waste collected is segregated and then re-processed to create new ones. For example, collecting plastics to create new plastic materials. Other examples are – glass, metal, paper, e-waste.
- **Composting:** Composting is a biological process in which biodegradable solid wastes are allowed to decompose in the presence of a microorganism like fungi & bacteria which turns this degradable waste into the organic matter. The decomposed matter is high in carbon and nitrogen and therefore, functions well as eco-friendly manure to be used in agricultural practices for growing plants and trees.
- **Pyrolysis:** Pyrolysis is a chemical decomposition process whereby solid wastes are subjected to heat up to 430degrees Celsius at a specific pressure and in presence of oxygen. The waste gets converted to solid residue of ash, carbon and some liquid. The method may be effective in managing solid waste but it can cause incomplete combustion which can produce toxic residue which will again require proper treatment.

• Importance of Solid Waste Management

Solid waste management is an essential service in this modern society. Solid waste management is important because it is very important to manage and handle solid waste according to prescribed law to ensure that the environment is not littered and polluted as it can not only be very harmful for the environment at large, but also the people who are a part of the environment. Environmental hygiene and public health are the biggest reasons as to why solid management is so necessary today. Other objectives include reduction and elimination of waste materials for better quality of life and economic development in the society.

- **Littered environment:** If there is no proper waste disposal system, then the solid wastes will be thrown in heaps in roads and the environment will become more and more polluted. People will clean their homes, but their surroundings will be littered and because of that air, water, and land pollution will increase.
- **Affect on human health:** Improper disposal of solid waste can lead to pollution of air, water and land that can in turn cause various health risks. The polluted air can lead to bronchitis, asthma, lung cancer; water containing lead etc can lead to growth problems and even reproductive issues etc.

- **Emission of toxic gases:** If there is poor solid waste management in function then toxic substances will not be disposed properly and then these substances will release toxic fumes which can be fatal to our health.
- **Affect on land and marine creatures:** Since water, land, and air will pollute high levels of poisonous substances like plastic, metals, chemicals etc consumed by animals on land and marine animals can lead to their death.

B. Bioremediation

Bioremediation is the process of removing or utilizing the pollutants from a particularly polluted area (like soil, municipal water tanks or sewage water, oil spills in water, or land) with the help of microorganisms like bacteria, fungi and also plants. It is a type of biotechnical waste management method which uses no harmful chemicals and, in order, protects the Earth and promotes a sustainable environment.

Principle of Bioremediation

- Bioremediation relies on stimulating the growth of certain microbes that use contaminants like oil, solvents, and pesticides as a source of food and energy.
- These microbes consume the contaminants, converting them into small amounts of water and harmless gases like carbon dioxide.
- Effective bioremediation needs a combination of the right temperature, nutrients, and food; otherwise, it may take much longer for the cleanup of contaminants.
- If conditions are not favorable for bioremediation, they can be improved by adding "amendments" to the environment, such as molasses, vegetable oil or simply air.
- These amendments create optimum conditions for microbes to flourish and complete the bioremediation process.
- The process of bioremediation can take anywhere from a few months to several years.
- The amount of time required depends on variables such as the size of the contaminated area, the concentration of contaminants, conditions such as temperature and soil density, and whether bioremediation will take place in situ or ex-situ.

Types of Bioremediation

Bioremediation is of three types –

1) Biostimulation

As the name suggests, the bacteria is stimulated to initiate the process. The contaminated soil is first mixed with special nutrients substances including other vital components either in the form of liquid or gas. It stimulates the growth of microbes thus resulting in efficient and quick removal of contaminants by microbes and other bacterias.

2) Bioaugmentation

At times, there are certain sites where microorganisms are required to extract the contaminants. For example – municipal wastewater. In these special cases, the process of bioaugmentation is used. There's only one major drawback in this process. It almost becomes impossible to control the growth of microorganisms in the process of removing the particular contaminant.

3) Intrinsic Bioremediation

The process of intrinsic bioremediation is most effective in the soil and water because of these two biomes which always have a high probability of being full of contaminants and toxins. The process of intrinsic bioremediation is mostly used in underground places like underground petroleum tanks. In such place, it is difficult to detect a leakage and contaminants and toxins can find their way to enter through these leaks and contaminate the petrol. Thus, only microorganisms can remove the toxins and clean the tanks.

Other methods of Waste Management

- *Incineration*

This is a process where wastes and other unwanted substances are burnt. During combustion, the organic waste turns into ash, flue gas, and heat. The inorganic constituents of the waste remain in the form of an ash. It is also termed as thermal treatment.

- *Phytoremediation*

In this scenario, plants are directly used to clean up or contain contaminants in the soil. This method of bioremediation will help mitigate the environmental problem without the need to excavate the contaminant material and dispose of it elsewhere.

Applications of Bioremediation

- Bioremediation is used for the remediation of metals, radionuclides, pesticides, explosives, fuels, volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs).

- Research is underway to understand the role of phytoremediation to remediate perchlorate, a contaminant that has been shown to be persistent in surface and groundwater systems.
- It may be used to clean up contaminants found in soil and groundwater.
- For radioactive substances, chelating agents are sometimes used to make the contaminants amenable to plant uptake.

Advantages of bioremediation

Bioremediation has a number of advantages over other cleanup methods.

- As it only uses natural processes, it is a relatively green method that causes less damage to ecosystems.
- It often takes place underground, as amendments and microbes can be pumped underground to clean up contaminants in groundwater and soil; therefore, it does not cause much disruption to nearby communities.
- The process of bioremediation creates few harmful byproducts since contaminants and pollutants are converted into water and harmless gases like carbon dioxide.
- Bioremediations is cheaper than most cleanup methods, as it does not require a great deal of equipment or labor.
- Bioremediation can be tailored to the needs of the polluted site in question and the specific microbes needed to break down the pollutant are encouraged by selecting the limiting factor needed to promote their growth.
- The toxicity and bioavailability of biodegradation products are not always known.
- Degradation by-products may be mobilized in groundwater or bio-accumulated in animals.
- Additional research is needed to determine the fate of various compounds in the plant metabolic cycle to ensure that plant droppings and products do not contribute to toxic or harmful chemicals into the food chain.

Limitations and concerns of Bioremediation

- Scientists need to establish whether contaminants that collect in the leaves and wood of trees are released when the leaves fall in the autumn or when firewood or mulch from the trees is used.
- Disposal of harvested plants can be a problem if they contain high levels of heavy metals.
- The depth of the contaminants limits treatment. In most cases, it is limited to shallow soils, streams, and groundwater.

- Generally, the use of phytoremediation is limited to sites with lower contaminant concentrations and contamination in shallow soils, streams, and groundwater.
- The success of phytoremediation may be seasonal, depending on location. Other climatic factors will also influence its effectiveness.
- The success of remediation depends on establishing a selected plant community. Introducing new plant species can have widespread ecological ramifications. It should be studied beforehand and monitored
- If contaminant concentrations are too high, plants may die.
- Some phytoremediation transfers contamination across media, (e.g., from soil to air).
- Phytoremediation is not effective for strongly sorbed contaminants such as polychlorinated biphenyls (PCBs).
- Phytoremediation requires a large surface area of land for remediation.

C. Bioreactors in Environment monitoring

A bioreactor is a type of fermentation vessel that is used for the production of various chemicals and biological reactions. It is a closed container with adequate arrangement for aeration, agitation, temperature and pH control, and drain or overflow vent to remove the waste biomass of cultured microorganisms along with their products.

A bioreactor should provide for the following:

1. Agitation (for mixing of cells and medium),
2. Aeration (aerobic fermentors); for O2 supply,
3. Regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, and liquid leveled.
4. Sterilization and maintenance of sterility, and
5. Withdrawal of cells/medium

Bioreactors are used for the production of biomass, metabolites, and antibiotics.

Bioreactor Designing

- The design and mode of operation of a bioreactor are based on the production of an organism, optimum conditions required for desired product formation, product value, and its scale of production.
- A good bioreactor design will help to improve productivity and provide higher quality products at lower prices.

- A bioreactor is a device that consists of various features such as an agitator system, an oxygen delivery system, a foam control system, and a variety of other systems such as temperature & pH control system, sampling ports, cleaning, and sterilization system, and lines for charging & emptying the reactor.
- The material used for the construction of a bioreactor must have the following important properties:
- It should not be corrosive.
- It should not add any toxic substances to the fermentation media.
- It should tolerate the steam sterilization process.
- It should be able to tolerate high pressure and resist pH changes.
- The sizes of the bioreactor vary widely depending on the application.
- Some bioreactors are designed for small scale fermenters and some for large scale industrial applications from the microbial cell (few mm3) to shake flask (100-1000 ml) to the laboratory-scale fermenter (1 – 50 L) to pilot level (0.3 – 10 m3) to plant scale (2 – 500 m3) for large volume).

Bioreactor principle

- The bioreactor is the heart of any biochemical process as it provides an environment for microorganisms to obtain optimal growth and produce metabolites for the biotransformation and bioconversion of substrates into desirable products.
- The reactors can be engineered or manufactured based on the growth requirements of the organisms used.
- Reactors are machines that can be made to transform biological-based materials into desirable products.
- They can be used for the production of various enzymes and other bio-catalysis processes.

Parts of Bioreactor

- These reactors have been designed to maintain certain parameters like flow rates, aeration, temperature, pH, foam control, and agitation rate.
- The number of parameters that can be monitored and controlled is limited by the number of sensors and control elements incorporated into a given bioreactor
- Other factors should be kept in mind before designing a fermenter as described below and demonstrated in the figure below.

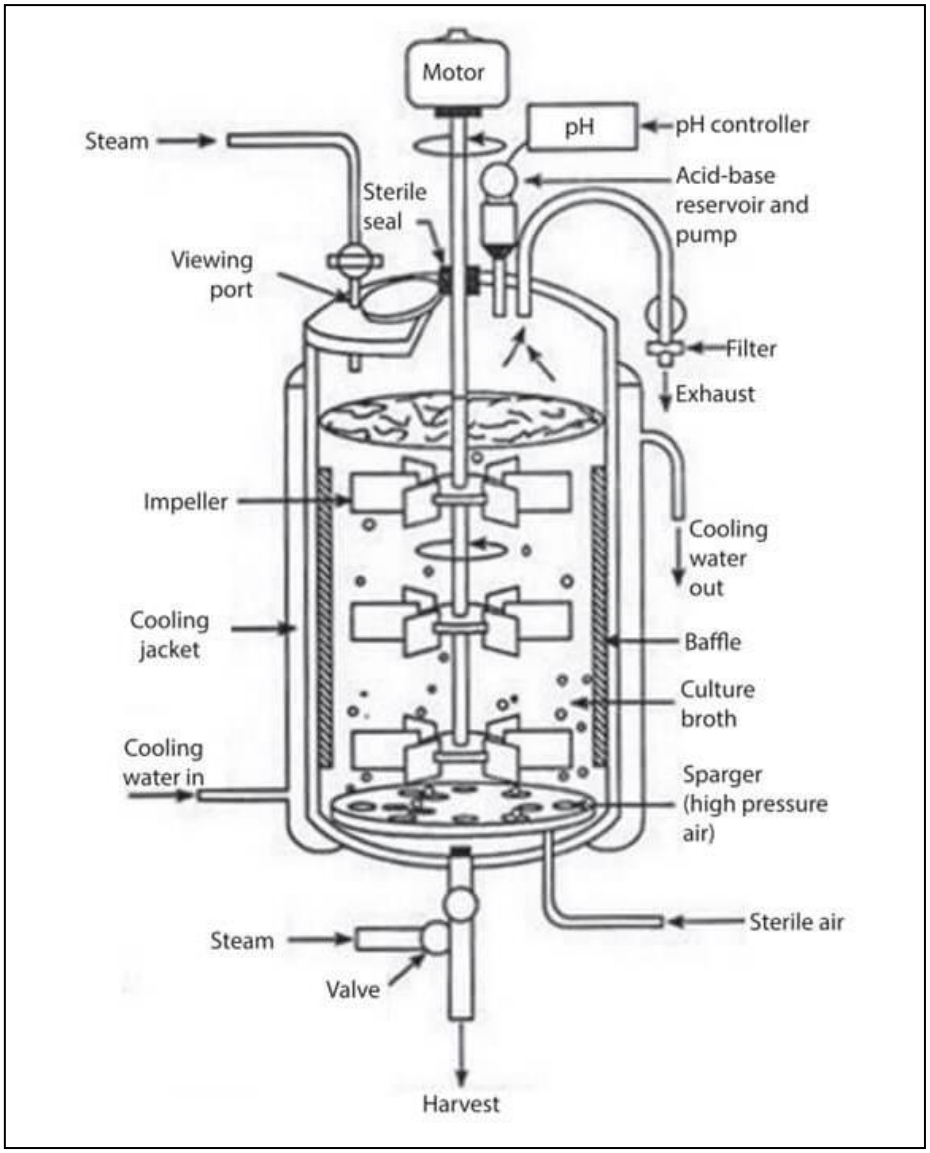


Figure: Bioreactor. Image Source: Kuila, A., & Sharma, V. (2018). Principles and applications of fermentation technology. John Wiley & Sons, Inc.

1. Fermenter Vessel

- A fermenter is a large cylinder closed at the top and bottom connected with various pipes and valves.
- The vessel is designed in such a way that it allows to work under controlled conditions.
- Glass and stainless steels are two types of fermenter vessels used.
- The glass vessel is usually used in small-scale industries. It is non-toxic and corrosion-proof.
- Stainless steel vessel is used in large scale industries. It can resist pressure and corrosion.

2. Heating and Cooling Apparatus

- The fermentor vessel's exterior is fitted with a cooling jacket that seals the vessel and provides cooling water.
- Thermostatically controlled baths or internal coils are generally used to provide heat while silicone jackets are used to remove excess heat.
- A cooling jacket is necessary for sterilization of the nutrient medium and removal of the heat generated during fermentation in the fermentor.

3. Aeration System

- An aeration system is one of the very important parts of a fermentor.
- It is important to choose a good aeration system to ensure proper aeration and oxygen availability throughout the culture.
- It contains two separate aeration devices (sparger and impeller) to ensure proper aeration in a fermentor.
- The stirring accomplishes two things:
- It helps to mix the gas bubbles through the liquid culture medium and
- It helps to mix the microbial cells through the liquid culture medium which ensures the uniform access of microbial cells to the nutrients.

4. Sealing Assembly

- The sealing assembly is used for the sealing of the stirrer shaft to offer proper agitation.
- There are three types of sealing assembly in the fermenter:
- Packed gland seal
- Mechanical seal
- Magnetic drives

5. Baffles

- The baffles are incorporated into fermenters to prevent a vortex improve aeration in the fermenters.
- It consists of metal strips attached radially to the wall.

6. Impeller

- Impellers are used to provide uniform suspension of microbial cells in different nutrient mediums.
- They are made up of impeller blades attached to a motor on the lid.
- Impeller blades play an important role in reducing the size of air bubbles and distribute them uniformly into the fermentation media.

- Variable impellers are used in the fermenters and are classified as follows.
- Disc turbines
- Variable pitch open turbine

7. Sparger

- A sparger is a system used for introducing sterile air to a fermentation vessel. It helps in providing proper aeration to the vessel.
- The sparger pipes contain small holes of about 5-10 mm, through which pressurized air is released.
- Three types of sparger are used
- Porous sparger
- Nozzle sparger
- Combined sparger–agitator

8. Feed Ports

- They are used to add nutrients and acid/alkali to the fermentor.
- Feed ports are tubes made up of silicone.
- In-situ sterilization is performed before the removal or addition of the products.

9. Foam-Control

- The level of foam in the vessel must be minimized to avoid contamination, this is an important aspect of the fermentor.
- Foam is controlled by two units, foam sensing, and a control unit.
- A foam-controlling device is mounted on top of the fermentor, with an inlet into the fermentor.

10. Valves

- Valves are used in the fermentor to control the movement of liquid in the vessel.
- There are around five types of valves are used, that is,
- globe valve,
- butterfly valve,
- a ball valve, and
- diaphragm valve.
- A safety valve is built-in in the air and pipe layout to operate under pressure

11. Controlling Devices for Environmental Factors

- A variety of devices are utilized to control environmental elements like temperature, oxygen concentration, pH, cell mass, essential nutrient levels, and product concentration.

12. Use of Computer in Fermenter

- For an efficient process, monitoring, and data collecting, fermentors are generally coupled with modern automated and semi-automated computers and databases.

There are so many types of bioreactors. Photo bioreactor is used to Wastewater treatment, water quality management, remediation of contaminated soil i,e. environment monitoring. So it is discussed here elaborately. Other types of bioreactors are namely, Stirred tank fermenter, Bubble column fermentor, Airlift fermentor, Fluid bed fermentor, Packed bed fermentor, Membrane bioreactor.

Photobioreactor

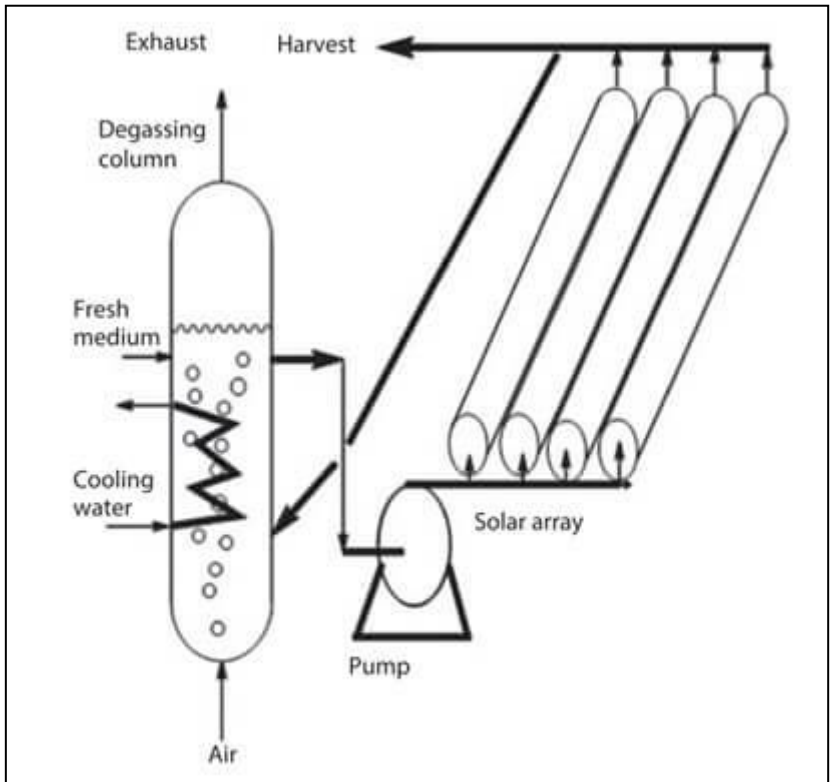


Figure: Photobioreactor. Image Source: Singh, J., Kaushik, N., & Biswas, S. (2014). Bioreactors – Technology & Design Analysis. April 2016.

- A photobioreactor is a specialized unit for fermentation that is either illuminated by direct sunlight or artificially illuminated

- They are made up of glass or more commonly transparent plastic and the tubes or flat panels is consist of light receiving systems.
- In this bioreactor, centrifugal pumps or airlift pumps can be used to circulate the medium through solar receivers.
- Photo-bioreactors are usually operated in a continuous mode at a temperature in the range of 25–40 °C.
- Photobioreactors are used for the photosynthetic culture of microalgae and cyanobacteria to produce products such as astaxanthin and β-carotene.

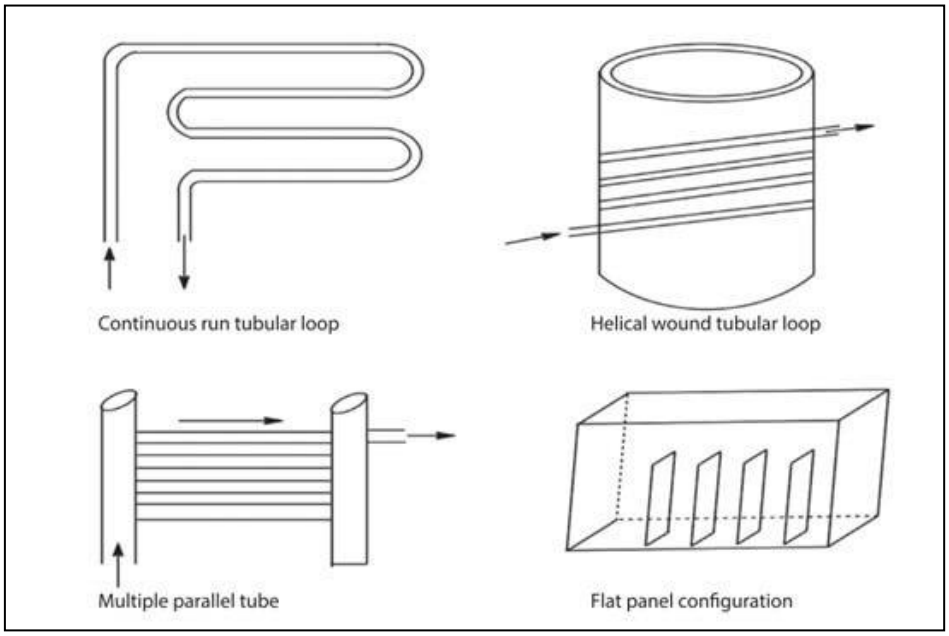


Figure: Types of photobioreacto

Limitation of photobioreactor

i. Salability problems
ii. Require temperature maintenance as they lack evaporative cooling
iii. Periodic cleaning due to light exposure
iv. Need maximum light exposure

UNIT XI

Organic farming and vermicomposting

Objective:

In this unit you will learn about Organic farming and vermicomposting.

Introduction:

Organic farming can be defined as an agricultural process that uses biological fertilisers and pest control acquired from animal or plant waste. Organic farming was actually initiated as an answer to the environmental sufferings caused by the use of chemical pesticides and synthetic fertilisers. In other words, organic farming is a new system of farming or agriculture that repairs, maintains, and improves the ecological balance.

Vermicomposting is a process in which earthworms are used to convert organic materials into humus-like material known as vermicompost. A number of researchers throughout the world have found that the nutrient profile in vermicompost is generally higher than traditional compost.

Vermicompost can be used in organic farming and small scale sustainable farming. Vermicompost has several excellent properties and has many advantages when applied to the soil. Vermicompost is an excellent nutrient-rich organic fertiliser, which helps plants to grow well and give better yields.

- **Organic farming**

The introduction of chemicals in farming got many delirious at the sight of what they could accomplish. Yields exploded. At the start, the soil was healthy. Any damage brought about by chemical fertilizers was hardly noticeable. Pests had not developed resistance to the chemicals. The technology spread across the world as it was considered the revolution in agriculture.

Flash forward to today and many people are marveling at organic farming again. This is after learning that conventional farming methods come with a host of problems including health-related diseases like cancer, pollution, degradation of soil and water, and impact on domestic animals.

Organic farming is a technique, which involves the cultivation of plants and rearing of animals in natural ways. This process involves the use of biological materials, avoiding synthetic substances to maintain soil fertility and ecological balance thereby minimizing pollution and wastage.

In other words, organic farming is a farming method that involves growing and nurturing crops without the use of synthetic based fertilizers and pesticides. Also, no genetically modified organisms are permitted.

Types of Organic Farming

Organic farming is divided into two types, namely:

1. Integrated organic farming
2. Pure organic farming

Pure organic farming means avoiding all unnatural chemicals. In this process of farming, all the fertilisers and pesticides are obtained from natural sources such as bone meal or blood meal.

Integrated organic farming includes the integration of pest management and nutrients management to achieve ecological requirements and demands.

Reasons for Organic Farming

The population of the planet is skyrocketing and providing food for the world is becoming extremely difficult. The need of the hour is sustainable cultivation and production of food for all.

The Green Revolution and its chemical-based technology are losing its appeal as dividends are falling and returns are unsustainable. Pollution and climate change are other negative externalities caused by the use of fossil fuel based chemicals.

In spite of our diet choices, organic food is the best choice you'll ever make, and this means embracing organic farming methods.

Four Principles of Organic Farming

1. Principle of Health

Organic agriculture must contribute to the health and well being of soil, plants, animals, humans and the earth. It is the sustenance of mental, physical, ecological and social well being. For instance, it provides pollution and chemical-free, nutritious food items for humans.

2. Principle of Fairness

Fairness is evident in maintaining equity and justice of the shared planet both among humans and other living beings. Organic farming provides good quality of life and helps in reducing poverty. Natural resources must be judiciously used and preserved for future generations.

3. Principle of Ecological Balance

Organic farming must be modeled on living ecological systems. Organic farming methods must fit the ecological balances and cycles in nature.

4. Principle of Care

Organic agriculture should be practiced in a careful and responsible manner to benefit the present and future generations and the environment.

As opposed to modern and conventional agricultural methods, organic farming does not depend on synthetic chemicals. It utilizes natural, biological methods to build up soil fertility such as microbial activity boosting plant nutrition

Secondly, multiple cropping practiced in organic farming boosts biodiversity which enhances productivity and resilience and contributes to a healthy farming system. Conventional farming systems use mono-cropping that destroys soil fertility.

✓ Here are the reasons why we need to take up organic farming methods:

1. To Accrue the Benefits of Nutrients

Foods from organic farms are loaded with nutrients such as vitamins, enzymes, minerals and other micro-nutrients compared to those from conventional farms. This is because organic farms are managed and nourished using sustainable practices. In fact, some past researchers collected and tested vegetables, fruits, and grains from both organic farms and conventional farms.

The conclusion was that food items from organic farms had way more nutrients than those sourced from commercial or conventional farms. The study went further to substantiate that five servings of these fruits and vegetables from organic farms offered sufficient allowance of vitamin C. However, the same quantity of fruits and vegetables did not offer the same sufficient allowance.

2. Stay Away From GMOs

Statistics show that genetically modified foods (GMOs) are contaminating natural foods sources at real scary pace, manifesting grave effects beyond our comprehension. What makes them a great threat is they are not even labeled. So, sticking to organic foods sourced from veritable sources is the only way to mitigate these grave effects of GMOs.

3. Natural and Better Taste

Those that have tasted organically farmed foods would attest to the fact that they have a natural and better taste. The natural and superior taste stems from the well balanced and nourished soil. Organic farmers always prioritize quality over quantity.

4. Direct Support to Farming

Purchasing food items from organic farmers is a surefire investment in a cost-effective future. Conventional farming methods have enjoyed great subsidies and tax cuts from most governments over the past years. This has led to the proliferation of commercially produced foods that have increased dangerous diseases like cancer.

It's time governments invested in organic farming technologies to mitigates these problems and secure the future. It all starts with you buying food items from known organic sources.

5. To Conserve Agricultural Diversity

These days, it normal to hear news about extinct species and this should be a major concern. In the last century alone, it is approximated that 75 percent of the agricultural diversity of crops has been wiped out. Slanting towards one form of farming is a recipe for disaster in the future. A classic example is a potato. There were different varieties available in the marketplace. Today, only one species of potato dominate.

This is a dangerous situation because if pests knock out the remaining potato specie available today, we will not have potatoes anymore. This is why we need organic farming methods that produce disease and pest-resistant crops to guarantee a sustainable future.

6. To Prevent Antibiotics, Drugs, and Hormones in Animal Products

Commercial dairy and meat are highly susceptible to contamination by dangerous substances. A statistic in an American journal revealed that over 90% of chemicals the population consumes emanate from meat tissue and dairy products.

According to a report by Environmental Protection Agency (EPA), a vast majority of pesticides are consumed by the population stem from poultry, meat, eggs, fish and dairy product since animals and birds that produce these products sit on top of the food chain.

This means they are fed foods loaded with chemicals and toxins. Drugs, antibiotics, and growth hormones are also injected into these animals and so, are directly transferred to meat and dairy products. Hormone supplementation fed to farmed fish, beef and dairy products contributes mightily to the ingestion of chemicals. These chemicals only come with a lot of complications like genetic problems, cancer risks, growth of tumor and other complications at the outset of puberty.

Why is Modern Farming Unsustainable?

1. Loss of soil fertility due to excessive use of chemical fertilizers and lack of crop rotation.
2. Nitrate runoff during rains contaminates water resources.
3. Soil erosion due to deep ploughing and heavy rains.
4. More requirements of fuel for cultivation.

5. Use of poisonous bio-cide sprays to curb pest and weeds.
6. Cruelty to animals in their housing, feeding, breeding and slaughtering.
7. Loss of biodiversity due to monoculture.
8. Native animals and plants lose space to exotic species and hybrids.

- **Differences Between Organic and Conventional Farming Methods**

In the conventional farming methods, before seeds are sown, the farmer will have to treat or fumigate his farm using harsh chemicals to exterminate any naturally existing fungicides. He will fertilize the soil using petroleum-based fertilizers. On the flip side, the organic farmer will prepare and enrich his land before sowing by sprinkling natural-based fertilizers such as manure, bone meal or shellfish fertilizer.

Before planting seeds, the organic farmer will soak the seeds in fungicides and pesticides to keep insects and pests at bay. Chemical are also incorporated in the irrigation water to prevent insects from stealing the planted seeds.

On the other hand, the organic farmer will not soak his seeds in any chemical solution nor irrigate the newly planted seeds using water with added chemicals. In fact, he will not even irrigate with council water, which is normally chlorinated to kill any bacteria. He will depend on natural rain or harvest and stored rainwater to use during dry months.

When the seeds have sprung up, and it's time to get rid of weeds, the conventional farmer will use weedicide to exterminate weeds. The organic farmer will not use such chemicals to get rid of the weed problem. Instead, he will physically weed out the farm, although it's very labor-intensive. Better still, the organic farmer can use a flame weeder to exterminate weeds or use animals to eat away the weeds.

When it comes to consumption, it's a no-brainer that anyone consuming products from the conventional farmer will absorb the pesticide and weedicide residues into the body, which could lead to developing dangerous diseases like cancer. People understand that health is important to them and that's why they are going organic in record numbers today.

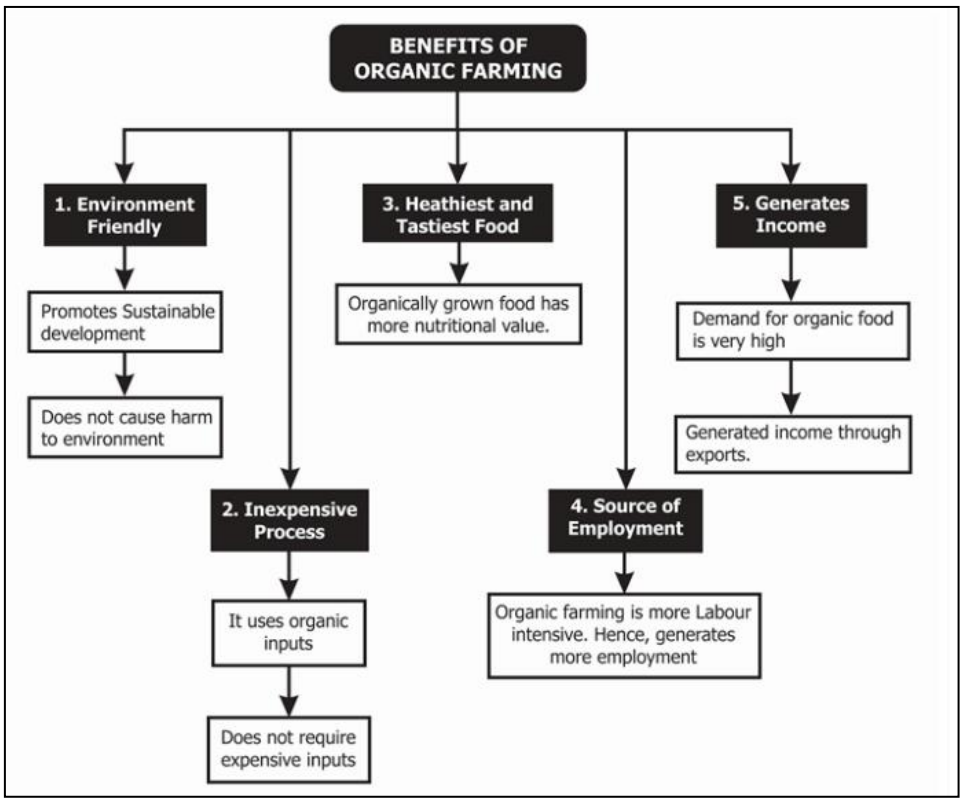
- ✓ **Advantages of Organic Farming:**

i. Environment-friendly.
ii. Promotes sustainable development.
iii. Healthy and tasty food.
iv. Inexpensive process.
v. It uses organic inputs.
vi. Generates income.

vii. Generates income through exports.

viii. Source of employment.

ix. Organic farming is more labour intensive. Hence, it generates more employment.



✓ Disadvantages of Organic Farming

Incompetent: The major issue of organic farming is the lack of inadequate infrastructure and marketing of the product.

Less production: The products obtained through organic farming are less in the initial years as compared to that in chemical products. So, farmers find it difficult to accommodate large-scale production.

Shorter shelf life: Organic products have more flaws and a shorter shelf life than that of chemical products.

Limited production: Off-season crops are limited and have fewer options in organic farming

• Benefits of Organically Grown Food Items and Agricultural Produce

1. Better Nutrition

As compared to a longer time conventionally grown food, organic food is much richer in nutrients. The nutritional value of a food item is determined by its mineral and vitamin

content. Organic farming enhances the nutrients of the soil which is passed on to the plants and animals.

2. Helps us Stay Healthy

Organic foods do not contain any chemicals. This is because organic farmers don't use chemicals at any stage of the food-growing process like their commercial counterparts. Organic farmers use natural farming techniques that don't harm humans and the environment. These foods keep dangerous diseases like cancer and diabetes at bay.

3. Free of Poison

Organic farming does not make use of poisonous chemicals, pesticides and weedicides. Studies reveal that a large section of the population fed on toxic substances used in conventional agriculture have fallen prey to diseases like cancer. As organic farming avoids these toxins, it reduces the sickness and diseases due to them.

4. Organic Foods Are Highly Authenticated

For any product to qualify as organic food, it must undergo quality checks and the creation process rigorously investigated. The same rule applies to international markets. This is a great victory for consumers because they are getting real organic foods. These quality checks and investigations weed out quacks who want to benefit from the organic food label by delivering commercially produced foods instead.

5. Lower Prices

There is a big misconception that organic foods are relatively expensive. The truth is they are actually cheaper because they don't require the application of expensive pesticides, insecticides, and weedicides. In fact, you can get organic foods direct from the source at really reasonable prices.

6. Enhanced Taste

The quality of food is also determined by its taste. Organic food often tastes better than other food. The sugar content in organically grown fruits and vegetables provides them with extra taste. The quality of fruits and vegetables can be measured using Brix analysis.

7. Organic Farming Methods are Eco-friendly

In commercial farms, the chemicals applied to infiltrate into the soil and severely contaminate it and nearby water sources. Plant life, animals, and humans are all impacted by this phenomenon. Organic farming does not utilize these harsh chemicals so; the environment remains protected.

8. Longer Shelf–life

Organic plants have greater metabolic and structural integrity in their cellular structure than conventional crops. This enables the storage of organic food for a longer time.

Organic farming is preferred as it battles pests and weeds in a non-toxic manner, involves fewer input costs for cultivation and preserves the ecological balance while promoting biological diversity and protection of the environment.

- **Vermicomposting**

Vermicomposting is the scientific method of making compost, by using earthworms. They are commonly found living in soil, feeding on biomass and excreting it in a digested form.

Vermiculture means "worm-farming". Earthworms feed on the organic waste materials and give out excreta in the form of "vermicasts" that are rich in nitrates and minerals such as phosphorus, magnesium, calcium and potassium. These are used as fertilizers and enhance soil quality.

Vermicomposting and vermiculture are two interlinked and interdependent processes, and vermiculture can be done in the presence of decomposable waste organic matter.

Vermicomposting comprises two methods:

- **Bed Method:** This is an easy method in which beds of organic matter are prepared.
- **Pit Method:** In this method, the organic matter is collected in cemented pits. However, this method is not prominent as it involves problems of poor aeration and water logging.

➢ Participating Organisms of the Vermicomposting:

Bacteria, micro-organisms and earthworms participate in the vermicomposting process, of which bacteria and micro-organisms are used as food for worms.

Throughout the world, 3 or 4 species are used in vermiculture but two species are used extensively in India. *Eisenia foetida* and *Eudrilus eugeniae* are used throughout India for vermiculture. A list of some earthworm species is given below with some features which are used in different parts of the country.

A. Family Lumbricidae:

I. *Eisenia foetida*:

Distribution:

They are found throughout the country in muddy areas, sewage damps and tanks. The species is used in vermiculture throughout the world.

Features:

The colour of the body is red or brown or purple. The dorsal surface bears coloured bands often two per segment and the ventral surface is paler in colour.

The clitellum occurs over 7-9 segments which include 24, 25 or 26-32 body segments. The body weight is about 1.5 gm/matured worm. Maturity attains in about 50-55 days and during adult stage the body wall becomes ridged. The species is generally called red worm or tiger worm.

II. *Bimastos parvus*:

Distribution:

They occur in Kashmir, Himachal Pradesh, Punjab, Rajasthan and Uttar Pradesh

Features:

The body colour is brownish red. The clitellum is saddle-shaped and occurs over 6 or more segments which include 24 or 25-30. The body segments are usually 90.

B. Family Eudrilidae:

III. *Eudrilus eugeniae*:

Distribution:

Though the species was first recorded in South-West India but at present it is widely used in South India in vermiculture. The species was originally distributed in equatorial West Africa but recently it is found in most areas of the world. It is called 'night crawler' in U.S.A.

Features:

The colour of the body is brown or red dark violet. The clitellum occurs over 5-6 segments and include 13 or 14-18. Female gonopores are on the 14th segment and male gonopore, a few segments behind them.

The body segments usually range 145 to 196, and length of the body varies from 32 to 140 mm and diameter is about 5 to 8 mm. Maturity is attained within 40 days. The weight of the mature worms is about 4.3 mg per individual. The higher temperature tolerance is higher than *E. foetida*.

C. Family Megascolecidae:

IV. *Lampito mauritii* (*Megascoles mauritii*):

Distribution:

They are found in Punjab, South Rajasthan, Gujarat, Maharashtra, Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Kerala, Lakshadweep Islands and Andaman Island.

Features:

The body colour is dark yellowish except the anterior end which bears purplish tinge. The clitellum is ring-shaped and spreads over 4 segments that include 14- 17 body segments. The length of the body ranges from 80-210 mm, with diameter 3.5 to 5 mm. The total body segments vary 166-190.

V. *Metaphire anomala* (= *Pheretima anomala*)

Distribution:

The species has recorded in Kokata (W. Bengal), Odisha, Bihar, Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Karnataka and Kerala.

Features:

Total length of the body ranges from 80 to 90 mm, with diameter varies from 5 to 5.5 mm. The clitellum is ring-shaped which spreads over 3 body segments that include 13 to 16.

VI. *Pheretima posthuma*:

Distribution:

It is the most common species in India and South-East Asia including Japan and Australia. They prefer to live the damp soil in the gardens and also in the dead organic matter.

Features:

Coloration of the dorsal side is brown and the ventral side is brightly coloured. The whole length of the full grown worm measures about 200 mm and 3 to 5 mm in diameter. The body segments about 100-120. The clitellum covers 3 segments on XIV to XVI. The use of the species in vermitechnology is not well studied.

D. Family Octochaetidae

VII. *Octochaetus surensis*:

Distribution:

The species was recorded in Burkul and Sur Lake in Odisha, and has also been recorded across other parts of India.

Features:

Colouration of the body is greyish with dark tinge at the dorsal part of the anterior region. The total length of the body measures 75 mm and diameter is about 2 to 2.5 mm. The clitellum is ring-shaped and occurs over 5 segments which include 13^{th} to 18^{th}.

VIII. *Dichogaster affinis*:

Distribution:

The species is found in Gujarat, Maharastra and South India. Outside India it is recorded in Sri Lanka, East Africa, and Madagascar etc.

Features:

The colour of the body is pinkish brown. The worm measures 30-32 mm in length with diameter is about 1.2 to 1.5 mm. The total body bears 140 segments. The clitellum is saddle-shaped and 8-10 segments, namely 13^{th} or 14^{th} to 21^{th} or 22^{th}.

IX. *Oenerodrilus occidentalis*:

Distribution:

They are found in Rajasthan, Maharashtra, Karnataka and Andaman Islands.

Features:

The total body length measures 15 to 30 mm and diameter is about 1 mm. The body segments are 70. The clitellum is ring-shaped and contains 6-8 segments, namely 13th or 14th to 19th or 20th.

E. Family Moniligastridae:

X. *Moniligaster perrieri*:

Distribution:

South India.

Features:

The body colour is generally blackish grey which is deeper on the dorsal side than the ventral side. The body length is about 210 mm and diameter is 5 mm. The total body segments are 175. The clitellum is ring-shaped and occurs over 5 segments from 9th to 14th segments.

• Principle of vermicomposting:

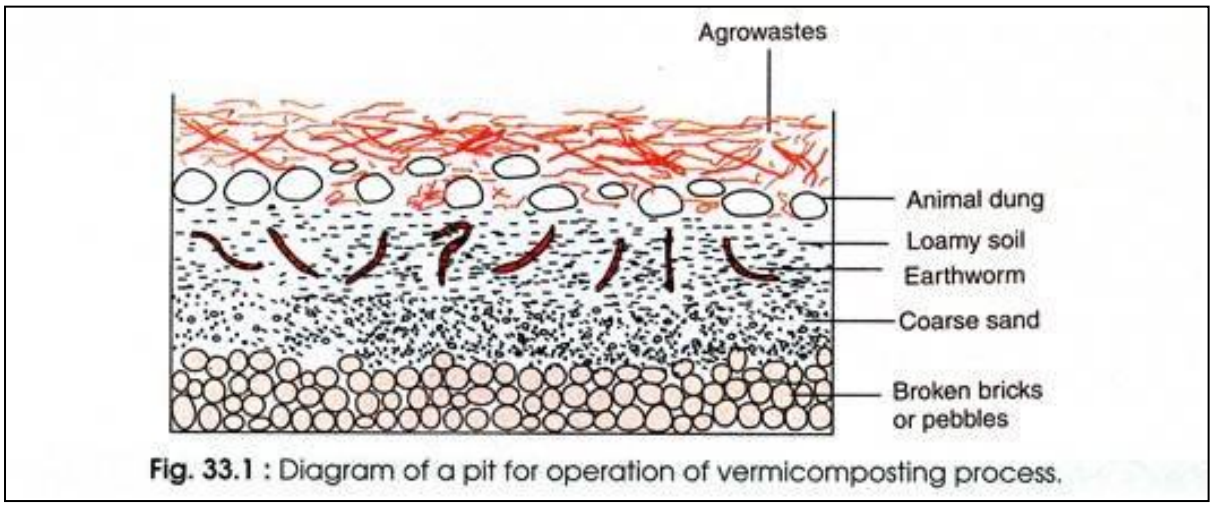


Fig. 33.1 : Diagram of a pit for operation of vermicomposting process.

1. Feeding materials:

- Worms can eat dung from animals, agricultural waste, residues from vegetables, waste from the food market, waste from the flower market, agro-industrial waste, waste from the fruit market and all other bio-degradable waste.
- Before being used for vermicompost production, cattle dung should be dried in open sunlight.

- Depending on the feedstock being used, temperature, moisture levels and the density of the worm population, the exact loading rate (at which raw feedstock will be applied to a worm bed) can differ.
- Proper loading rates require no inclusion of new feedstock until the bulk of the feedstock previously introduced has been decomposed.
- A high protein feedstock such as grains, mash, or cottonseed meal is added if worms are not growing.

2. Bedding materials:

- As bedding products, certain agricultural residues may be used, such as plant waste and solid composted manure.
- In general, because of bedding content's effect on increasing soil pH, which is harmful to worms, the bedding content should maintain moisture, stay loose and aerated, and be low in protein and nitrogen.
- The bedding content should be varied to provide the earthworms with a variety of nutrients and to create richer compost.
- **Suitable bedding materials include:**
    - 1. coir waste
    - 2. cardboard
    - 3. shredded fall leaves
    - 4. sawdust
    - 5. chopped straw
    - 6. mulched paper such as newspaper
    - 7. semi-composted solid manure
- **Sieving and shredding:**
    - By shredding raw materials into small pieces, decomposition can be accelerated.

3. Blending:

- To achieve a near optimal C/N ratio of 30:1-40:1, carbonaceous substances such as sawdust, paper and straw can be combined with nitrogen-rich products such as sewage sludge, biogas slurry, and fish scraps.
- Good quality compost, rich in main and micro nutrients, is produced by a varied mixture of substances.

4. Pre-composting/Half digestion:

- In order to avoid worm systems from feeling so much sun, manure feedstocks and bedding should be pre-composted.

- When introduced into the worm systems, fresh manures produce a lot of energy that transfers into additional heat.
- Strong heat in the beds of worms can be deadly.
- The bedding and feeding materials are then combined, watered and allowed to ferment for approximately two to three weeks.
- It is necessary to hold the raw materials in piles to allow the temperature to exceed 50-55^{o} C.
- The substance is overturned 3 to 4 times during this phase to get the temperature down and to aid in uniform decomposition.
- It is passed to the vermicompost production method as the material becomes very fragile, and worms are inserted into it ranging from a few days to a few weeks old.

5. Moisture, temperature and pH:

- 50-60 percent is the optimal moisture level for maintaining aerobic conditions.
- The temperature should be within 25-30^{o} C of the stacks.
- The activity of microflora and earthworms can be decreased by higher or lower temperatures.
- The height of bed can help to regulate the increase in temperature.
- The raw material's pH should not be greater than 6.5 to 7.

Methods of vermicomposting:

- A 1 meter by 1 meter by 0.3 meter container carries about 30-40 kg of bedding and feeding materials.
- It is possible to prepare a vermiculture bed or worm bed (3 cm) by putting dust or husk or coir waste or sugar cane garbage in the bottom of the tub/container.
- The culture bed can be spread with a sheet of fine sand (3 cm) followed by a layer of garden soil (3 cm).
- A15-20 cm sheet of organic waste material (pre-composted/half digested) can be spread on the worm bed.
- It is sprinkled with rock phosphate powder (to increase the content of phosphate) if required.
- Soil or cow dung is used to cover the organic layer with (sprinkle cow dung slurry).
- The selected earthworms are released through the cracks created (1000-1500).
- In order to prevent birds from eating the earthworms, cover the ring with wire mesh or gunny sack.

- Water is sprinkled to maintain adequate humidity and temperature regularly/daily.

Harvesting of vermicompost:

- In about 3 months, the vermicompost is ready (may vary depending on organic waste used as substrate).
- It will be black, granular, lightweight and humus-rich.
- Vermicompost harvesting requires manual isolation of worms from the castings
- Watering is stopped for two to three days before emptying the beds to facilitate the removal of the worms from the compost.
- The worms will be pushed to the bottom of the bed by this.
- For new culture beds, the worms are collected.
- To retrieve the cocoons, young worms, and unconsumed organic waste, the gathered vermicompost is dried and passed through a 3 mm sieve.
- For seeding the new culture beds, cocoons and young worms are used.

Storage and packing of vermicompost:

- The harvested vermicompost should be stored in dark, cool location.
- It should have moisture of at least 40 percent.
- Sunlight should not fall on the content being composted.
- At the point of sale, packaging can be done.
- Periodic sprinkling of water can be done to retain the level of moisture and also to maintain a beneficial microbial population if it is kept in an open location.
- Vermicompost may be preserved for a duration of one year without loss of quality if the moisture level is kept at 40%.

Natural enemies of earthworms?

Birds, frogs, toads, snakes and rats are some of the common enemies of earthworms. Apart from these, earthworms are also subject to attack by a variety of pests e.g. springtails (small, oblong, white to gray insects that jump when disturbed), centipedes, slugs, mites, beetles etc. Earthworms also have a large number of internal parasites including numerousprotozoa, some nematodes and also larvae of some flies. The arthropods e.g. red ants, are of major concern. Their populations can be checked by introduction of neem leaves @ 5% along with the waste material being vermicomposted.

➢ What are the precautions to be taken while preparing vermicompost?

- In vermicompost preparation, only plant-based materials such as hay, leaves or vegetable peelings can be used.
- Animal products such as eggshells, beef, bone, chicken droppings, etc, are not appropriate for vermicompost preparation.
- For the rearing of earthworms, toxic plant species like tobacco are not appropriate.

- It is necessary to protect earthworms from birds, termites, ants and rats.
- During the process, sufficient moisture should be maintained.
- The earthworms could die by either stagnant water or lack of moisture.
- The vermicompost should be withdrawn from the bed at periodic intervals after completion of the process and replaced by fresh waste materials.

Significance of Vermiculture

Vermiculture is the culture of earthworms. It is a beneficial way of improving the fertility of the plant and soil. Vermiculture mainly focuses on the breeding of worms so as to increase their population. Vermicomposting is then prepared to promote the growth and development of crops. It also causes disease in plants along with increasing water retention and the porosity of the soil. This greatly reduces the need for chemical fertilizers and encourages organic matter.

Advantages of Vermicomposting

The major benefits of vermicomposting are:

1. Develops roots of the plants.
2. Improves the physical structure of the soil.
3. Vermicomposting increases the fertility and water-resistance of the soil.
4. Helps in germination, plant growth, and crop yield.
5. Nurtures soil with plant growth hormones such as auxins, gibberellic acid, etc.

Disadvantages of Vermicomposting

Following are the important disadvantages of vermicomposting:

1. It is a time-consuming process and takes as long as six months to convert the organic matter into usable forms.
2. It releases a very foul odour.
3. Vermicomposting is high maintenance. The feed has to be added periodically and care should be taken that the worms are not flooded with too much to eat.
4. The bin should not be too dry or too wet. The moisture levels need to be monitored periodically.
5. They nurture the growth of pests and pathogens such as fruit flies, centipede and flies.

Probable Questions:

1. What is organic farming?
2. What is Integrated organic farming?
3. What are the reasons for Organic Farming?
4. Write down the principles of Organic Farming?
5. Why we need to take up organic farming methods?
6. Discuss about the benefits of organically grown Food Items and Agricultural Produce.
7. Describe the advantages and disadvantages of organic farming.
8. What is vermicomposting? Is it different from vermiculture?:
9. What is pit method of Vermicomposting?
10. What is the principle of vermicomposting?
11. Which worms are used for vermicomposting?
12. Can any earthworm type be used for vermicomposting?
13. What are the conditions favourable for earthworms?
14. *What type of container should I use for vermicomposting?*
15. What is the Colour of vermicompost?
16. Describe the methods of vermicomposting.
17. What is the significance of vermiculture?
18. Describe the advantages and disadvantages of Vermicomposting.

Suggested Reading:

1. Odum, E. P. and Barret, G. W. (2005). *Fundamentals of Ecology*. 5th ed. Thompson Brooks/Cole.
2. Santra, S. (2005). E*nvironmental Science*. New Central Book Agency (P) Ltd.

UNIT XII

Insect pollinators: Types and role in agriculture

Objective:

In this unit you will learn about insect pollinators: types and role in agriculture.

Introduction:

Insect pollinators include beetles, flies, ants, moths, butterflies, bumble bees, honey bees, solitary bees, and wasps. Butterflies and moths (Lepidopterans) are important pollinators of flowering plants in wild ecosystems and managed systems such as parks and yards.

Entomophily or insect pollination is a form of pollination whereby pollen of plants, especially but not only of flowering plants, is distributed by insects.

1. Bees

Bees are perhaps the most important pollinator of many garden plants and most commercial fruit trees. The most common species of bees are bumblebees and honeybees. Since bees cannot see the color red, bee-pollinated flowers usually have shades of blue, yellow, or other colors. Bees collect energy -rich pollen or nectar for their survival and energy needs. They visit flowers that are open during the day, are brightly colored, have a strong aroma or scent, and have a tubular shape, typically with the presence of a nectar guide. A nectar guide includes regions on the flower petals that are visible only to bees, which help guide bees to the center of the flower, thus making the pollination process more efficient. The pollen sticks to the bees' fuzzy hair; when the bee visits another flower, some of the pollen is transferred to the second flower.

Recently, there have been many reports about the declining population of honeybees. Many flowers will remain unpollinated, failing to bear seeds if honeybees disappear. The impact on commercial fruit growers could be devastating.

2. Bumble Bees

Bumble bees are important pollinators of wild flowering plants and agricultural crops. They are able to fly in cooler temperatures and lower light levels than many other bees, making them excellent pollinators—especially at higher elevations and latitudes. They are characterized by their rounded, fuzzy bodies and their ability to perform "buzz pollination," which involves grasping a flower in their jaws and vibrating their wing muscles to dislodge the pollen. Many plants—including a number of wildflowers and crops like tomatoes, peppers, and cranberries—benefit from buzz pollination.

Because they are essential pollinators, the loss of bumble bees can have far-ranging ecological consequences.

3. Flies

With over eighty-five thousand species worldwide, flies form one of the most diverse orders of insects, Diptera. Although a number of these species are reviled as crop pests and carriers of disease, many are beneficial—from the aquatic midges that serve as an abundant food source for migratory birds to the fly pollinators of apples, peppers, mangoes and cashews.

Many flies are attracted to flowers that have a decaying smell or an odor of rotting flesh. These flowers, which produce nectar, usually have dull colors, such as brown or purple. They are found on the corpse flower or voodoo lily (*Amorphophallus*), dragon arum (*Dracunculus*), and carrion flower *(Stapleia, Rafflesia*). The nectar provides energy while the pollen provides protein. Wasps are also important insect pollinators, pollinating many species of figs. Because flies are generalist foragers, with no nests to provision and sometimes sparsely haired bodies, they don't get much credit as significant pollinators. However, they can be important pollinators for specific plants.

4. Butterflies and Moths

Butterflies, such as the monarch, pollinate many garden flowers and wildflowers, which are usually found in clusters. These flowers are brightly colored, have a strong fragrance, are open during the day, and have nectar guides. The pollen is picked up and carried on the butterfly's limbs. Moths, on the other hand, pollinate flowers during the late afternoon and night. The flowers pollinated by moths are pale or white and are flat, enabling the moths to land. One well-studied example of a moth-pollinated plant is the yucca plant, which is pollinated by the yucca moth. The shape of the flower and moth have adapted in a way to allow successful pollination. The moth deposits pollen on the sticky stigma for fertilization to occur later. The female moth also deposits eggs into the ovary. As the eggs develop into larvae, they obtain food from the flower and developing seeds. Thus, both the insect and flower benefit from each other in this symbiotic relationship. The corn earworm moth and *Gaura* plant have a similar relationship.

5. Wasps

Wasps are wrongly maligned due to a reputation for being aggressive. In reality, they are adept hunters that we wouldn't want to live without. They are doing us all a great service in keeping insect populations in balance and managing pests—they are counted among the many beneficial insects that can be utilized for conservation biological control. From an evolutionary standpoint, bees are simply wasps that have adopted a vegetarian diet!

Many wasps are smooth-bodied and do not actively collect pollen. Those with hairs lack the branched, pollen-trapping hairs found on most bees, making them relatively minor pollinators of most plants. Nonetheless, they do provide some incidental pollination, carrying and dropping some pollen grains as they move among flowers.

6. Beetles

Beetles are the most diverse group of organisms in the world. In fact, approximately one of every four species of plant, animal, bacterium, or fungus that has been described is a type of beetle. As might be expected in such a large group, beetles are quite diverse in color, shape, and ecological role.

Fossil records suggest that beetles, along with flies, were probably the first insect pollinators of prehistoric flowering plants in the late Jurassic era, around 150 million years ago. Beetles are still pollinators of many flowers such as magnolias and water lilies—whose flowers harken back to more ancient forms.

7. Ants

Pollination by ants is relatively rare, but it does occur. Most ant pollinators can fly, enabling them to distribute pollen grains over a wider area, and thus promote genetic diversity among the plants they visit. Since ants walk from flower to flower, any pollen exchange conducted by ants will be limited to a small population of plants.

Formica argentea worker ants have been observed carrying pollen grains between flowers of cascade knotweed, also known as *Polygonum cascadense*. Other species of Formica ants distribute pollen among the flowers of elf orpine, a compact herb that grows on granite outcrops. In Australia, ants pollinate several orchids and lilies effectively.

Overall, as a family of insects, ants may not be the best pollinators. Ants produce an antibiotic called myrmicacin, which is thought to reduce the viability of the pollen grains they carry.

Pollinators are in trouble

You may have heard that bees are disappearing and bats are dying. These and other animal pollinators face many challenges in the modern world. Habitat loss, disease, parasites, and environmental contaminants have all contributed to the decline of many species of pollinators.

- **Importance of Pollination to Agriculture:**

Insects in their pollination activities have a direct impact on the evolution of flora and fauna. It is believed that angiosperm plants and the more highly evolved insects evolved together. Primitive flowering plants are all insect pollinated. Therefore, grasses and all other angiosperms arose from plants dependent upon insects. Some beetles, most Hymenoptera, many Diptera and almost all Lepidoptera are dependent upon materials provided by flowers. Without angiosperms the evolution of mammals would certainly have been different. Rodents, herbivores and primates are especially dependent upon the products of flowering plants. Thus, angiosperms were a required forerunner to the stocks, which gave rise to humans, and insect pollination was necessary to the development of angiosperms.

There would be grave consequences for the flora and fauna were pollinating insects to disappear or cease pollinating. Many types of plants would most likely perish eventually because in time they would be dependent on insect pollination for competitive reproduction. These would embrace by far most of the angiosperms. Certain elements of flora would rapidly perish. Plants that are usually propagated by seed are dependent upon insects for adequate pollination. Included here would probably be over half of the existing species. Plants that usually propagate asexually could probably survive for many seasons or generations. But asexual propagants are very limited in powers of dissemination and those species would have a fixed genetics incapable of adjusting to changes, which would be expected to be rapid under such conditions. Self-fertile plants those are capable of auto-self pollination might be able to persist longer. However, most of these are dependent upon occasional crossing in order to retain vigor. All would require some crossing in order to retain the genetic plasticity necessary to adjust to changing environmental conditions.

Some plants might survive indefinitely without insect pollinators and some might increase in the absence of normal competition. These include many nut-bearing trees,

grasses, all conifers, and various other wind pollinated plants such as poplars, birches, elms, alders, etc. Even so, many grasses and other plants most certainly depend upon the surrounding flora for their survival. Those plants that are produced as crops by humans and propagated by asexual means might also be unaffected. Breeding for disease resistance, for example, could be done with hand pollination. Nevertheless, there are many consequences of a drastic reduction and elimination of most floras. These include the loss of plants with nitrifying bacteria, soil erosion, a drastic curtailment of the human diet, loss in forage values for livestock, loss of many kinds of animals, loss of most kinds of wild flowers, and a general upset in the balance of nature, with unpredictable results.

Advanced agriculture manages the production of products that require pollination, which are primarily fruits and seeds. Seeds are used for general plant propagation and for bedded plants. Some plants like papaya require occasional seeding; alfalfa is seeded every few years and spinach is seeded annually. Alfalfa and forage grasses often require a large amount of seed, while tomatoes and melons need little seeding. Plant breeding by crossing, selfing and selecting is done with pollination and planting with seeds. Plant products that are consumed directly include cereals, beans, nuts, oils, fruits, preserves and many vegetables. Seeds such as grains, oilcake and peanuts are also used for livestock feed. Many seeds are used as medicines, spices and flavourings. Seeds, fruit oils and seed fibers are deployed in industry for soaps, paints, plastics, explosives, alcohol and textiles.

Probable Questions:

1. What is pollination?
2. Discuss about the different types of insect pollinators.
3. Discuss the role of bumble bee in pollination.
4. Briefly discuss the importance of pollination to agriculture.

Suggested Reading:

1. Odum, E. P. and Barret, G. W. (2005). *Fundamentals of Ecology*. 5th ed. Thompson Brooks/Cole.
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UNIT-XIII

Genetics of Human Diseases: Nutrigenomics, Pharmacogenomics and their applications

Objective: In this nit we will discuss different aspects of Nutrigenomics and Pharmacogenomics. We will also discuss their applications.

Meaning of Nutrigenomics:

Nutrigenomics may be defined as the application of genomic tools to study the integrated effects of nutrients on gene regulation. However, it holds great promise in increasing the understanding of how nutrients affect molecular events in an organism for development and progression of various diseases.

The working definition of Nutrigenomic is that it provides a genetic and molecular understanding for how common dietary chemicals (i.e., nutrients) affect the balance between health and disease by altering the expression and/or structure of an individual's genetic make-up.

The new branch of genomic and nutritional research can finely be summarized with the following five points:

a. Common dietary chemicals and nutrients directly or indirectly act on the human genome to alter gene expression or structure.

b. Under certain circumstances and in some individuals, diet can be a serious risk factor for a number of diseases.

c. Some diet-regulated genes are susceptible genes and likely to play a role in the onset, incidence, progression, and/or severity of chronic diseases.

d. The degree to which diet influences the balance between healthy and disease states may depend on an individual's genetic make-up (e.g., efficient genetic polymorphism and nutrient metabolism).

e. Dietary process based on knowledge of nutritional requirements, nutrition states, and genotype (i.e., "individualized nutrition") can be used to prevent, mitigate, or cure chronic disease.

Many chemicals in foods are nutrients, i.e., these are metabolized to energy or involved in key metabolic reactions (e.g., vitamins). But some naturally occurring chemicals in foods are ligands for transcription factors and directly alter gene expression. Other dietary chemicals alter signal transduction pathways and chromatin structure to indirectly affect gene expression.

Studies have shown that intake of different diets are associated with the incidence and severity of chronic diseases. Overconsumption of proteins, fat or carbohydrates, or lack of key micronutrients is associated with obesity, cardiovascular diseases, certain cancers, developmental defects, and neurological diseases such as Alzheimer's.

At the cellular level nutrient may:
a. Act as a ligand for transcription factor receptor.

b. Be metabolized by primary, secondary pathways, thereby altering concentration of substrate or intermediates.

c. Positively or negatively affects signal pathway.

Dietary chemical may interact with one or more variants to increase or decrease disease risk.

Table 58.1 : Nutrients deficiency and DNA damage

Nutrients	DNA damage	Health effect
Folic acid	Chromosome breaks and hampers DNA repair	Colon cancer, heart disease, brain dysfunction
Vitamin B_{12}	Unknown	Same as folic acid, memory loss.
Vitamin B_6	Unknown	Same as folic acid
Niacin	Hampers DNA repair	Nerve problem, memory loss
Vitamin C	Mimics radiation damage	Cataract, cancer
Vitamin E	Mimics radiation damage	Colon cancer, heart disease, immune dysfunction.
Vitamin D	Prevent gene variation	Colon, breast, prostate cancer.
Zinc	Chromosome breaks	Brain and immune dysfunction.

Micronutrients, Macronutrients: Effects on Gene:

- Human diets almost require 40 micronutrients. The specific micronutrients are associated with CVD (B Vitamin, carotinoids, Vitamin E), cancer (folate, carotinoids), neural tube defect (folate) and bone mass.

- The deficiency of vitamin B_{12}/B_6/folic acid/niacin/, vitamin C and E or iron and zinc appear to mimic radiation in damaging DNA by causing single and double strand breaks, oxidative lesion or both. A good number of other degenerative diseases of aging are also being associated with low fruits and vegetables intake.

- The specific mechanisms are being determined for the role of certain minerals (calcium, magnesium, manganese, copper, and selenium) and vitamins in heart disease from work in humans in cell culture systems.

Unbalanced intake of any of the three major macronutrients, carbohydrates, fats, proteins causes the initiation, progression, and severity of chronic disease. The single macronutrient or micronutrient is unable to prevent chronic diseases. Dietary imbalances and dietary supplements from micronutrients deficiencies to overconsumption of macronutrients of dietary supplements are the modifiers of metabolism and potentiates of chronic diseases.

Specific subgroups are already targeted with "subgroup nutrition" (e.g., cholesterol-lowering margarines) without stressing the genetic background of variation of nutritional response. A big challenge is in front of us in validating the combined action of these minor-impact polymorphisms and their practical effect on the relation between nutrition and health on the basis of scientific point of view (Fig. 56.1).

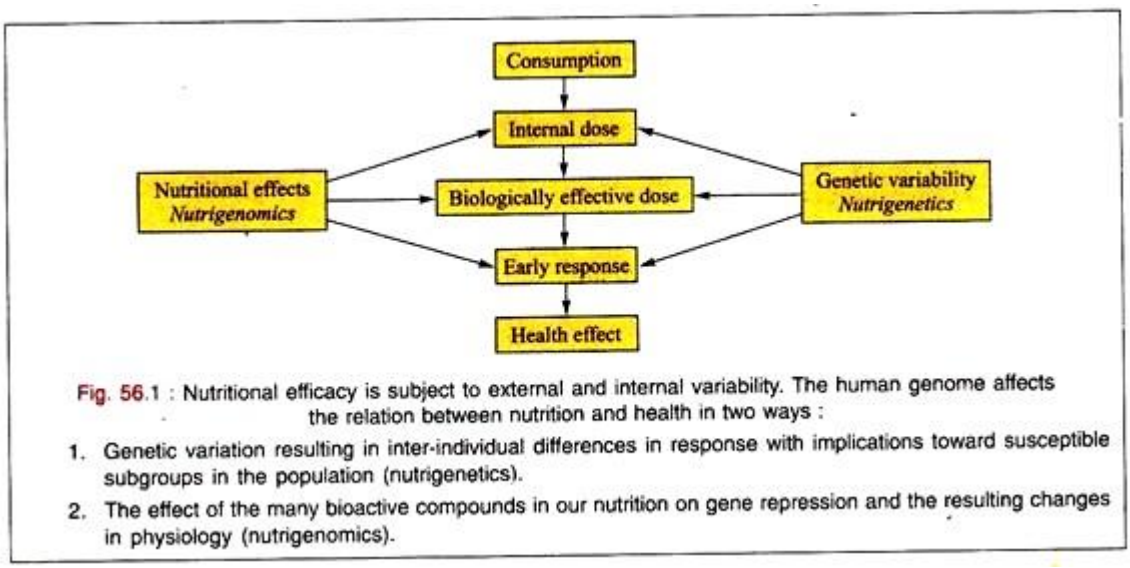


Fig. 56.1 : Nutritional efficacy is subject to external and internal variability. The human genome affects the relation between nutrition and health in two ways :

1. Genetic variation resulting in inter-individual differences in response with implications toward susceptible subgroups in the population (nutrigenetics).
2. The effect of the many bioactive compounds in our nutrition on gene repression and the resulting changes in physiology (nutrigenomics).

Our diet is a complex mixture of many possible bioactive chemical compounds, chronically administered in different compositions, and with a multitude of biological effects.

The majority of these biological responses are mediated through effector genes, effects on enzyme concentration or activity, and changes in metabolite concentration.

Each of our genes contains ten deviations in its code from the "standard gene". But not all of these polymorphisms have a functional impact.

A small number of these polymorphism have serious health implications and may even be lethal. This is the domain of clinical genetics. Many polymorphisms have only a mild

effect on the functionality of the resulting protein. A large variety in response to nutrition has been observed within certain limits of "health". Many examples have been set up in which nutritional compounds directly cause DNA damage or modulate susceptibility against DNA damage through regulation of specific pathways in many processes involved in these events.

Role of Folic Acid in Nutri-genomics:

Up to this date 1,000 human diseases gene are being identified. 97 per cent of which cause monogenic diseases. Most of the chronic diseases (obesity, diabetes, cardiovascular diseases, cancer) are due to complex interaction between several genes and environmental factors.

More complete single nucleotide polymorphism (SNP) and halo type maps are helpful in identifying the genes involved in the disease. Deficiency of folic acid and other macro and micronutrients appear to mimic radiation in damaging DNA by causing single and double strand breaks, oxidative lesion or both. Nutrient deficiencies are orders of magnitude more important than radiation because of constancy of exposure to milieu promoting DNA damage. Folate deficiency breaks chromosome due to substantial incorporation of uracil in human DNA.

Single strand break in DNA are formed during base excision repair, with two nearby single-strand breaks on opposite DNA strands leading to chromosomal fragmentation. In humans, folate level and variation of different genes that code the folate-dependent enzymes are linked to many diseases like cancer, vascular diseases, birth defects and complications of pregnancy. The genomic machinery is very much sensitive to folate and vitamin B status and responsible to interaction between folate nutrition and folate-dependent enzyme polymorphism (folate Nutri-genomics).

Mechanisms that may affect:

a. Maintenance of genomic CpG methylation pattern (which regulate gene expression).

b. Synthesis of nucleotide to prevent DNA damage.

c. Influence plasma homocysteine status, thus risk of vascular diseases.

Currently, worldwide interest on folate research is due to discovery of several single nucleotide polymorphisms (SNP) which modulate risk of several diseases (Table 56.2).
Currently, worldwide interest on folate research is due to discovery of several single nucleotide polymorphisms (SNP) which modulate risk of several diseases (Table 56.2).

Table 56.2 : Some important single nucleotide polymorphism (SNP) of B-vitamins genes associated with various clinical conditions	
C677T variant of 5, 10 methylenetetrahydrofolate reductase gene	• Colon cancer. • Spina bifida. • Down syndrome. • Leukaemia. • Oral cleft. • Risk of vascular diseases due to elevated homocysteine. • Complication of pregnancy (pre-eclampsia, recurrent pregnancy loss, fetal growth retardation).
A1298C variant of 5, 10 methylenetetrahydrofolate reductase gene	• Spina bifida. • Leukaemia.
A2756G variant of methionine synthase gene	• Thromboembolic diseases.
A66G variant of methionine synthase gene	• Spina bifida. • Down syndrome
C1420T variant of serine hydroxymethylenetransferase gene	• Leukaemia
2R3R variant thymidylate synthetase gene	• Leukaemia
C1561T variant of glutamate carboxy peptidase gene	• May affect cardiovascular diseases.

Table 56.2 : Shows the consequence of SNP of 5, 10 methylenetetrahydrofolate reductase in terms of dTMP nucleotide biosynthesis, DNA methylation, homocysteine metabolism. All of these are related to pathology of cancer, vascular and developmental diseases.

Dietary folate interacts with proteins that are encoded by various genes and reduces the risk to development of various diseases and gives protection over the diseases.

Direct Biochemical Effects:

Folate stabilizes the polymorphic enzyme encoded by $C_{677}T$ variant gene by preventing it from relinquishing its flavin cofactors. Since 5, 10 methylene-tetrahydrofolate reductase is a flavin protein people with TT recessive genotype may respond more rapidly to riboflavin supplements as well as folate to lower homocysteine.

Nucleotide Biosynthesis:

dTMP synthesized by the thymidylate synthetase from dUMP and requires the one carbon unit of 5, 10 methylene-tetrahydrofolate. dTMP is used by DNA. If there is low level of folate, uracil misincorporation occurs leading to breakage of DNA strand which

predisposes to cancer. The polymorphic enzyme coded by $C_{677}T$ variant genes can enhance the synthesis of dTMP nucleotide if folate status is good, and this is thought to afford protection against colon cancer and leukaemia.

Polymorphism in Gene for MTHFR:

A common functional polymorphism in the gene of methylene-tetrahydrofolate reductase (MTHFR, a major enzyme involved in folate metabolism) is associated with an increased risk of colorectal cancer. Dietary folate and methionine intake modify colorectal cancer risk in people with MTHFR polymorphism. Nurses' health study showed that folate in women who used alcohol had a 25 per cent reduction in breast cancer risk. Recently a team of American and Chinese researchers showed that folic acid have protective effect against breast cancer. The effect of it had been pronounced when taken with other vitamins especially B_6, B_{12}, and methionine.

Researchers believed that folic acid exerts its protective effect by preventing errors in DNA replication and by helping to regenerate methionine, a vital component of DNA synthesis, vitamin B_6, B_{12} and act as cofactors required for folic acid to "do its job".
If folate status is poor, single nucleotide polymorphism may confer risk rather than protection.

Biological Methylation:

Since dietary methionine cannot provide all methyl groups for cellular methylation reaction there is requirement of de novo synthesis of methionine from folate one carbon pool. S-adenosylmethionine regulates protein, biogenic amine, lipid, and DNA methylation. The S-adenosylmethionine dependent DNA methylation of specific CpG site regulates gene expression and play a critical role in the developmental process.

Methylation of cluster of CpG sites associated with promoter regions tends to silence gene expression. A deficiency of methyl group may alter the normal control of proto-oncogene expression. The polymorphic enzyme encoded C677T variant gene may reduce availability of de novo methyl groups for this important reaction. Since folate is necessary in embryogenesis its supplementation reduces the risk of neural tube defects. Various studies show that folate supplementation decreases the risk of first occurrence of neural tube defect and recurrent defects in women with previously affected pregnancy.

Homocysteine Metabolism:

Polymorphic 5, 10 methylene-tetrahydrofolate reductase reduces one carbon flux to methyl-folate, the donor molecule for conversion of homocysteine into methionine. This single nucleotide polymorphism may thus elevate homocysteine which is the independent risk factor for the cardiovascular diseases.

Homocysteine is atherogenic and undergoes redox cycling in the presence of transition metal ions, forming radical that causes oxidative damage to low density lipoprotein. It also reacts with cysteine SH groups and modifies apolipoprotein. It is also a hypertensive compound. It also reacts with endothelium—derived relaxation factor to form S-nitrosohomocysteine and superoxide. This leads to loss of vasodilatation action. Since homocysteines promote atherosclerosis through oxidative stress hyperhomocysteinemia is being associated with coronary artery diseases. Recent researchers have studied that folic acid supplementations reduce the risk of CAD (coronary artery diseases).

It also inhibits and down regulates anticoagulants including prostacycline synthesis, activation Protein C, thrombomodulin expression, heparin sulphate expression and fibrinolysis. People with inflammatory bowel diseases (ulcerative colitis, Crohn's disease) have high risk of thromboembolic events, such as stroke and peripheral venous thrombosis. It is also observed that patients with Crohn's disease may be benefited from supplementation of folic acid. It also activates factor V and tissue clotting factor. The final effects of it are to chelate copper and inhibit lysyl oxidase which impairs crosslinking of collagen and elastin and leads to connective tissue abnormalities.

Conclusion :

The new science of nutritional systems biology is emerging, taking up the challenge of exploiting all available data generated by genomics technology in a complete description of a biological system.

This new method is ideally fit for the evaluation of many vital changes in biological activity as propagated by nutrition. A good number of bioactive compounds act simultaneously and chronically in constantly changing combinations. The recent unrevealing of human genomic and the coinciding technological developments, genotyping, transcriptomics, proteomics, and metabolomics are now available to nutritional research. In future we are likely to see new screening tools for the selection of bioactive nutrients, new biomarkers for the in vivo efficacy of nutrients, and better insight into the influence of genetic polymorphisms on nutrient metabolism.

Pharmacogenomics:

Pharmacogenomics is the study of the role of the genome in drug response. Its name (*pharmaco-* + *genomics*) reflects its combining of pharmacology and genomics. Pharmacogenomics analyzes how the genetic makeup of an individual affects their response to drugs. It deals with the influence of acquired and inherited genetic variation on drug response in patients by correlating gene expression or single-nucleotide polymorphisms with pharmacokinetics (drug absorption, distribution, metabolism, and elimination) and pharmacodynamics (effects mediated through a drug's biological

targets). The term *pharmacogenomics* is often used interchangeably with *pharmacogenetics*. Although both terms relate to drug response based on genetic influences, pharmacogenetics focuses on single drug-gene interactions, while pharmacogenomics encompasses a more genome-wide association approach, incorporating genomics and epigenetics while dealing with the effects of multiple genes on drug response.

Pharmacogenomics aims to develop rational means to optimize drug therapy, with respect to the patients' genotype, to ensure maximum efficiency with minimal adverse effects. Through the utilization of pharmacogenomics, it is hoped that pharmaceutical drug treatments can deviate from what is dubbed as the "one-dose-fits-all" approach. Pharmacogenomics also attempts to eliminate the trial-and-error method of prescribing, allowing physicians to take into consideration their patient's genes, the functionality of these genes, and how this may affect the efficacy of the patient's current or future treatments (and where applicable, provide an explanation for the failure of past treatments). Such approaches promise the advent of precision medicine and even personalized medicine, in which drugs and drug combinations are optimized for narrow subsets of patients or even for each individual's unique genetic makeup. Whether used to explain a patient's response or lack thereof to a treatment, or act as a predictive tool, it hopes to achieve better treatment outcomes, greater efficacy, minimization of the occurrence of drug toxicities and adverse drug reactions (ADRs). For patients who have lack of therapeutic response to a treatment, alternative therapies can be prescribed that would best suit their requirements. In order to provide pharmacogenomic recommendations for a given drug, two possible types of input can be used: genotyping or exome or whole genome sequencing. Sequencing provides many more data points, including detection of mutations that prematurely terminate the synthesized protein (early stop codon).

History:

Pharmacogenomics was first recognized by Pythagoras around 510 BC when he made a connection between the dangers of fava bean ingestion with hemolytic anemia and oxidative stress. This identification was later validated and attributed to deficiency of G6PD in the 1950s and called favism.[13][14] Although the first official publication dates back to 1961, circa 1950s marked the unofficial beginnings of this science. Reports of prolonged paralysis and fatal reactions linked to genetic variants in patients who lacked butyryl-cholinesterase ('pseudocholinesterase') following administration of succinylcholine injection during anesthesia were first reported in 1956. The term pharmacogenetic was first coined in 1959 by Friedrich Vogel of Heidelberg, Germany (although some papers suggest it was 1957 or 1958). In the late 1960s, twin studies supported the inference of genetic involvement in drug metabolism, with identical

twins sharing remarkable similarities to drug response compared to fraternal twins.[18] The term pharmacogenomics first began appearing around the 1990s. The first FDA approval of a pharmacogenetic test was in 2005[11] (for alleles in CYP2D6 and CYP2C19).

Drug-metabolizing enzymes:

There are several known genes which are largely responsible for variances in drug metabolism and response. The focus of this article will remain on the genes that are more widely accepted and utilized clinically for brevity.

- Cytochrome P450s
- VKORC1
- TPMT

a. Cytochrome P450:

The most prevalent drug-metabolizing enzymes (DME) are the Cytochrome P450 (CYP) enzymes. These enzymes introduce reactive or polar groups into xenobiotics such as drugs. The term Cytochrome P450 was coined by Omura and Sato in 1962 to describe the membrane-bound, heme-containing protein characterized by 450 nm spectral peak when complexed with carbon monoxide. The human CYP family consists of 57 genes, with 18 families and 44 subfamilies. CYP proteins are conveniently arranged into these families and subfamilies on the basis of similarities identified between the amino acid sequences. Enzymes that share 35-40% identity are assigned to the same family by an Arabic numeral, and those that share 55-70% make up a particular subfamily with a designated letter. For example, CYP2D6 refers to family 2, subfamily D, and gene number 6.

From a clinical perspective, the most commonly tested CYPs include: CYP2D6, CYP2C19, CYP2C9, CYP3A4 and CYP3A5. These genes account for the metabolism of approximately 70-90% of currently available prescription drugs. The table below provides a summary for some of the medications that take these pathways.

CYP2B6:

CYP2B6 plays an important role in the metabolism of drugs including the anti-HIV drug efavirenz, the anti-malarial artemisinin, the antidepressants bupropion and ketamine, the anticancer drug cyclophosphamide, and the opioid methadone. This is a highly polymorphic enzyme with the variant CYP2B6*6

having special importance, as it leads to errors in RNA processing and reduced enzyme levels. A second important variant CYP2B6*18 also fails to produce functional protein. The CYP2B6*6 variant occurs with prevalences of 15% to 60% in various populations worldwide, while the CYP2B68*18 is found predominantly in Africans. The higher prevalence of central nervous system side effects in African as compared to American and European patients treated with efavirenz has been attributed to the higher frequency of the CYP2B6 slow metabolizer phenotype in sub-Saharan African populations.

CYP2D6:

Also known as debrisoquine hydroxylase (named after the drug that led to its discovery), CYP2D6 is the most well-known and extensively studied CYP gene. It is a gene of great interest also due to its highly polymorphic nature, and involvement in a high number of medication metabolisms (both as a major and minor pathway). More than 100 CYP2D6 genetic variants have been identified. Both polymorphisms in the CYP2D6 gene (leading to versions of the enzyme having differing levels of metabolic activity) and copy number variants are known. For certain drugs predominantly metabolized by CYP2D6, these variations can lead to unusually high or low drug concentrations in serum (Referred to as poor metabolizer and ultra metabolizer phenotypes, respectively), thus leading to increased side effects or reduced efficacy. Commonly affected drugs include tramadol, venlafaxine, morphine, mirtazapine, and metoprolol. The frequency of CYP2D6 varies geographically, with the highest prevalence of slow metabolizers found in east Asia and the lowest prevalence in the Americas.

CYP2C19:

Discovered in the early 1980s, CYP2C19 is the second most extensively studied and well understood gene in pharmacogenomics. Over 28 genetic variants have been identified for CYP2C19, of which affects the metabolism of several classes of drugs, such as antidepressants and proton pump inhibitors.

CYP2C9:

CYP2C9 constitutes the majority of the CYP2C subfamily, representing approximately 20% of the liver content. It is involved in the metabolism of approximately 10% of all drugs, which include medications with narrow therapeutic windows such as warfarin and tolbutamide. There are approximately 57 genetic variants associated with CYP2C9.

CYP3A4 and CYP3A5:

The CYP3A family is the most abundantly found in the liver, with CYP3A4 accounting for 29% of the liver content. These enzymes also cover between 40-50% of the current prescription drugs, with the CYP3A4 accounting for 40-45% of these medications. CYP3A5 has over 11 genetic variants identified at the time of this publication.

VKORC1:

The vitamin K epoxide reductase complex subunit 1 (VKORC1) is responsible for the pharmacodynamics of warfarin. VKORC1 along with CYP2C9 are useful for identifying the risk of bleeding during warfarin administration. Warfarin works by inhibiting VKOR, which is encoded by the VKORC1 gene. Individuals with polymorphism in this have an affected response to warfarin treatment.

TPMT:

Thiopurine methyltransferase (TPMT) catalyzes the S-methylation of thiopurines, thereby regulating the balance between cytotoxic thioguanine nucleotide and inactive metabolites in hematopoietic cells. TPMT is highly involved in 6-MP metabolism and TMPT activity and TPMT genotype is known to affect the risk of toxicity. Excessive levels of 6-MP can cause myelosuppression and myelotoxicity.[36] Related patent litigation arose in Mayo Collaborative Services v. Prometheus Laboratories, Inc., in which the Supreme Court of the United States found that patent around measuring doses of the drug was patent-eligible. Codeine, clopidogrel, tamoxifen, and warfarin a few examples of medications that follow the above metabolic pathways.

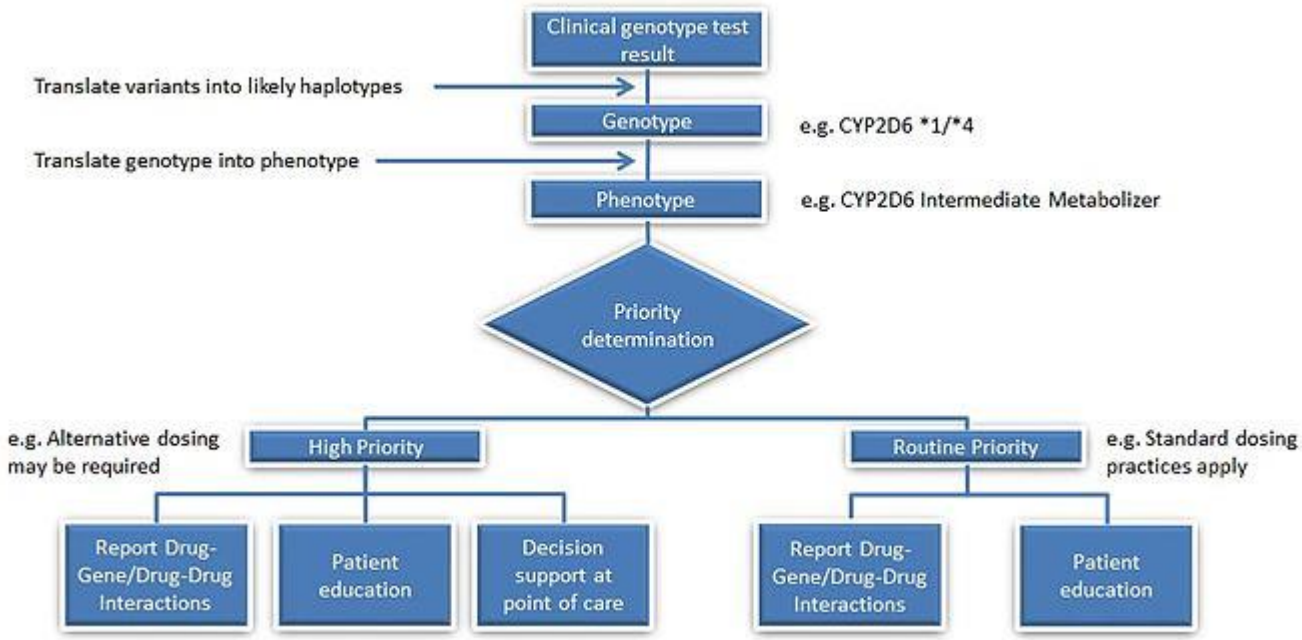
Predictive prescribing:

Patient genotypes are usually categorized into the following predicted phenotypes:

- UM: Ultra-rapid metabolizer: patients with substantially increased metabolic activity;
- EM: Extensive metabolizer: normal metabolic activity;
- IM: Intermediate metabolizer: patients with reduced metabolic activity; and
- PM: Poor metabolizer: patients with little to no functional metabolic activity.

The two extremes of this spectrum are the poor metabolizers and ultra-rapid metabolizers. Efficacy of a medication is not only based on the above metabolic statuses,

but also the type of drug consumed. Drugs can be classified into two main groups: active drugs and prodrugs. Active drugs refer to drugs that are inactivated during metabolism, and prodrugs are inactive until they are metabolized.



An overall process of how pharmacogenomics functions in a clinical practice. From the raw genotype results, this is then translated to the physical trait, the phenotype. Based on these observations, optimal dosing is evaluated.

For example, we have two patients who are taking codeine for pain relief. Codeine is a prodrug, so it requires conversion from its inactive form to its active form. The active form of codeine is morphine, which provides the therapeutic effect of pain relief. If person A receives one *1 allele each from mother and father to code for the CYP2D6 gene, then that person is considered to have an extensive metabolizer (EM) phenotype, as allele *1 is considered to have a normal-function (this would be represented as CYP2D6 *1/*1). If person B on the other hand had received one *1 allele from the mother and a *4 allele from the father, that individual would be an Intermediate Metabolizer (IM) (the genotype would be CYP2D6 *1/*4). Although both individuals are taking the same dose of codeine, person B could potentially lack the therapeutic benefits of codeine due to the decreased conversion rate of codeine to its active counterpart morphine.

Each phenotype is based upon the allelic variation within the individual genotype. However, several genetic events can influence a same phenotypic trait, and establishing genotype-to-phenotype relationships can thus be far from consensual with many

enzymatic patterns. For instance, the influence of the CYP2D6*1/*4 allelic variant on the clinical outcome in patients treated with Tamoxifen remains debated today. In oncology, genes coding for DPD, UGT1A1, TPMT, CDA involved in the pharmacokinetics of 5-FU/capecitabine, irinotecan, 6-mercaptopurine and gemcitabine/cytarabine,
respectively, have all been described as being highly polymorphic. A strong body of evidence suggests that patients affected by these genetic polymorphisms will experience severe/lethal toxicities upon drug intake, and that pre-therapeutic screening does help to reduce the risk of treatment-related toxicities through adaptive dosing strategies.

Applications:

The list below provides a few more commonly known applications of pharmacogenomics:

- Improve drug safety, and reduce ADRs;
- Tailor treatments to meet patients' unique genetic pre-disposition, identifying optimal dosing;
- Improve drug discovery targeted to human disease; and
- Improve proof of principle for efficacy trials.

Pharmacogenomics may be applied to several areas of medicine, including pain management, cardiology, oncology, and psychiatry. A place may also exist in forensic pathology, in which pharmacogenomics can be used to determine the cause of death in drug-related deaths where no findings emerge using autopsy.

In cancer treatment, pharmacogenomics tests are used to identify which patients are most likely to respond to certain cancer drugs. In behavioral health, pharmacogenomic tests provide tools for physicians and care givers to better manage medication selection and side effect amelioration. Pharmacogenomics is also known as companion diagnostics, meaning tests being bundled with drugs. Examples include KRAS test with cetuximab and EGFR test with gefitinib. Beside efficacy, germline pharmacogenetics can help to identify patients likely to undergo severe toxicities when given cytotoxics showing impaired detoxification in relation with genetic polymorphism, such as canonical 5-FU. In particular, genetic deregulations affecting genes coding for DPD, UGT1A1, TPMT, CDA and CYP2D6 are now considered as critical issues for patients treated with 5-FU/capecitabine, irinotecan, mercaptopurine/azathioprine, gemcitabine/capecitabine/AraC and tamoxifen, respectively.

In cardiovascular disorders, the main concern is response to drugs including warfarin, clopidogrel, beta blockers, and statins.[12] In patients with CYP2C19, who take clopidogrel, cardiovascular risk is elevated, leading to medication package insert updates by regulators. In patients with type 2 diabetes, haptoglobin (Hp) genotyping shows an effect on cardiovascular disease, with Hp2-2 at higher risk and supplemental vitamin E reducing risk by affecting HDL. In psychiatry, as of 2010, research has focused particularly on 5-HTTLPR and DRD2.

Clinical implementation:

Initiatives to spur adoption by clinicians include the Ubiquitous Pharmacogenomics program in Europe and the Clinical Pharmacogenetics Implementation Consortium (CPIC) in the United States. In a 2017 survey of European clinicians, in the prior year two-thirds had not ordered a pharmacogenetic test. In 2010, Valderbilt University Medical Center launched Pharmacogenomic Resource for Enhanced Decisions in Care and Treatment (PREDICT); in 2015 survey, two-thirds of the clinicians had ordered a pharmacogenetic test. In the United States, the FDA has updated medication package inserts based on genomic evidence.

In 2019, the largest private health insurer, United Healthcare, announced that it would pay for genetic testing to predict response to psychiatric drugs; as of 2019, it is the only private insurer to offer such coverage. In 2020, Canada's 4th largest health and dental insurer, Green Shield Canada, announced that it would pay for pharmacogenetic testing and its associated clinical decision support software to optimize and personalize mental health prescriptions.

Polypharmacy:

A potential role pharmacogenomics may play would be to reduce the occurrence of polypharmacy. It is theorized that with tailored drug treatments, patients will not have the need to take several medications that are intended to treat the same condition. In doing so, they could potentially minimize the occurrence of ADRs, have improved treatment outcomes, and can save costs by avoiding purchasing extraneous medications. An example of this can be found in psychiatry, where patients tend to be receiving more medications than even age-matched non-psychiatric patients. This has been associated with an increased risk of inappropriate prescribing.

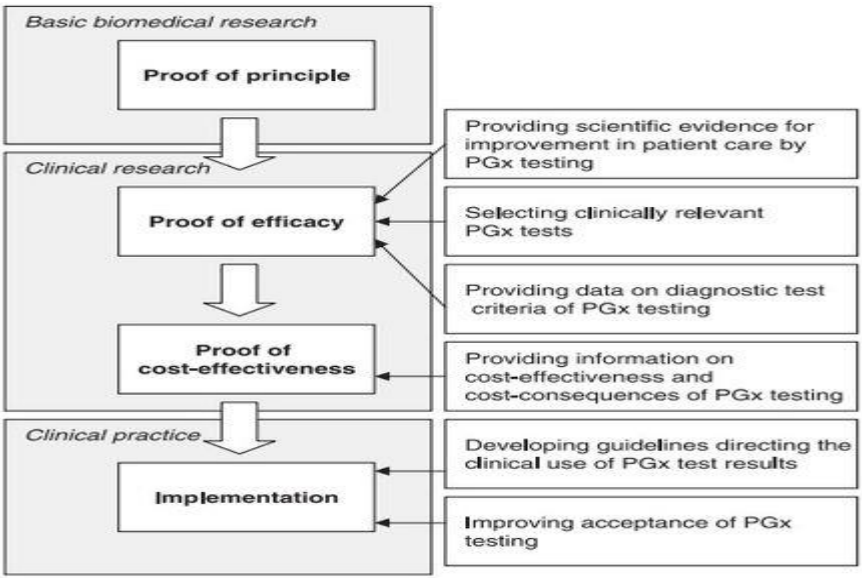
The need for pharmacogenomics tailored drug therapies may be most evident in a survey conducted by the Slone Epidemiology Center at Boston University from February 1998 to April 2007. The study elucidated that an average of 82% of adults in the United

States are taking at least one medication (prescription or nonprescription drug, vitamin/mineral, herbal/natural supplement), and 29% are taking five or more. The study suggested that those aged 65 years or older continue to be the biggest consumers of medications, with 17-19 % in this age group taking at least ten medications in a given week. Polypharmacy has also shown to have increased since 2000 from 23% to 29%.

Drug labeling

The U.S. Food and Drug Administration (FDA) appears to be very invested in the science of pharmacogenomics as is demonstrated through the 120 and more FDA-approved drugs that include pharmacogenomic biomarkers in their labels. This number increased varies over the years. A study of the labels of FDA-approved drugs as of 20 June 2014 found that there were 140 different drugs with a pharmacogenomic biomarker in their label. Because a drug can have different biomarkers, this corresponded to 158 drug–biomarker pairs. Only 29% stated a requirement or recommendation for genetic biomarker testing but this was higher for oncology drugs (62%). On May 22, 2005, the FDA issued its first *Guidance for Industry: Pharmacogenomic Data Submissions*, which clarified the type of pharmacogenomic data required to be submitted to the FDA and when. Experts recognized the importance of the FDA's acknowledgement that pharmacogenomics experiments will not bring negative regulatory consequences. The FDA had released its latest guide *Clinical Pharmacogenomics (PGx): Premarket Evaluation in Early-Phase Clinical Studies and Recommendations for Labeling* in January, 2013. The guide is intended to address the use of genomic information during drug development and regulatory review processes.

Challenges:



Challenges in Pharmacogenomics: Although there appears to be a general acceptance of the basic tenet of pharmacogenomics amongst physicians and healthcare professionals, several challenges exist that slow the uptake, implementation, and standardization of pharmacogenomics. Some of the concerns raised by physicians include:

- Limitation on how to apply the test into clinical practices and treatment;
- A general feeling of lack of availability of the test;
- The understanding and interpretation of evidence-based research;
- Combining test results with other patient data for prescription optimization; and
- Ethical, legal and social issues.

Issues surrounding the availability of the test include:

- *The lack of availability of scientific data*: Although there are considerable number of DME involved in the metabolic pathways of drugs, only a fraction have sufficient scientific data to validate their use within a clinical setting; and
- *Demonstrating the cost-effectiveness of pharmacogenomics*: Publications for the pharmacoeconomics of pharmacogenomics are scarce, therefore sufficient evidence does not at this time exist to validate the cost-effectiveness and cost-consequences of the test.

Although other factors contribute to the slow progression of pharmacogenomics (such as developing guidelines for clinical use), the above factors appear to be the most prevalent. Increasingly substantial evidence and industry body guidelines for clinical use of pharmacogenetics have made it a population wide approach to precision medicine. Cost, reimbursement, education, and easy use at the point of care remain significant barriers to widescale adoption.

Future prospect:

Computational advances have enabled cheaper and faster sequencing. Research has focused on combinatorial chemistry, genomic mining, omic technologies and high throughput screening. As the cost per genetic test decreases, the development of personalized drug therapies will increase. Technology now allows for genetic analysis of hundreds of target genes involved in medication metabolism and response in less than 24 hours for under $1,000. This is a huge step towards bringing pharmacogenetic technology into everyday medical decisions. Likewise, companies like deCODE genetics, MD Labs Pharmacogenetics, Navigenics and 23andMe offer genome scans. The companies use the same genotyping chips that are used in GWAS studies and provide

customers with a write-up of individual risk for various traits and diseases and testing for 500,000 known SNPs. Costs range from $995 to $2500 and include updates with new data from studies as they become available. The more expensive packages even included a telephone session with a genetics counselor to discuss the results.

Ethics:

Pharmacogenetics has become a controversial issue in the area of bioethics. Privacy and confidentiality are major concerns. The evidence of benefit or risk from a genetic test may only be suggestive, which could cause dilemmas for providers. Drug development may be affected, with rare genetic variants possibly receiving less research. Access and patient autonomy are also open to discussion.

Probable Questions:

1. What is nutrigenomics? How gene regulation is controlled by micronutrients and macronutrients?
2. Discuss the role of folic acid in nutrigenomics.
3. Discuss different drug metabolizing enzymes in pharmacogenomics.
4. What are the implications of pharmacogenomics?
5. Discuss clinical implementation of pharmacogenomics.
6. What are the future prospects of pharmacogenomics?

Suggested Reading:

1. *Katsnelson A (August 2005). "A Drug to Call One's Own: Will medicine finally get personal?". Scientific American.*
2. *Karczewski KJ, Daneshjou R, Altman RB (2012). "Chapter 7: Pharmacogenomics". PLOS Computational Biology. **8** (12):*

UNIT XIV

Preliminary knowledge on zoonotic diseases

Objective:

In this unit you will learn about preliminary knowledge on zoonotic diseases.

Introduction:

A zoonosis is any disease or infection that is naturally transmissible from vertebrate animals to humans. Animals thus play an essential role in maintaining zoonotic infections in nature. Zoonosis may be bacterial, viral, or parasitic, or may involve unconventional agents. As well as being a public health problem, many of the major zoonotic diseases prevent the efficient production of food of animal origin and create obstacles to international trade in animal products.

Animals provide many benefits to people. Many people interact with animals in their daily lives, both at home and away from home. Pets offer companionship and entertainment, with millions of households having one or more pets. We might come into close contact with animals at a county fair or petting zoo, or encounter wildlife while enjoying outdoor activities. Also, animals are an important food source and provide meat, dairy, and eggs.

Zoonosis refers to diseases that can be passed from animals to humans. They are sometimes called zoonotic diseases.

Animals can carry harmful germs, such as bacteria, fungi, parasites, and viruses. These are then shared with humans and cause illness. Zoonotic diseases range from mild to severe, and some can even be fatal.

Zoonotic diseases are widespread both in the U.S. and worldwide. The World Health Organization (WHO) estimates that 61 percent of all human diseases are zoonotic in origin, while 75 percent of new diseases discovered in the last decade are zoonotic. Before the introduction of new hygiene regulations around 100 years ago, zoonotic diseases such as bovine tuberculosis, bubonic plague caused millions of deaths. They are still a major problem in developing countries.

However, some animals can carry harmful germs that can be shared with people and cause illness – these are known as zoonotic diseases or zoonosis. Zoonotic diseases are caused by harmful germs like viruses, bacteria, parasites, and fungi. These germs can cause many different types of illnesses in people and animals ranging from mild to serious illness and even death. Some animals can appear healthy even when they are carrying germs that can make people sick.

Zoonotic diseases are very common, both in the United States and around the world. Scientists estimate that more than 6 out of every 10 known infectious diseases in people

are spread from animals, and 3 out of every 4 new or emerging infectious diseases in people are spread from animals. Every year, tens of thousands of Americans will get sick from harmful germs spread between animals and people. Because of this, CDC works 24/7 to protectpeople from zoonotic diseases.

How do germs spread between animals and people?

Because of the close connection between people and animals, it's important to be aware of the common ways people can get infected with germs that can cause zoonotic diseases. Thesecan include:

* **Direct contact**: Coming into contact with the saliva, blood, urine, mucous, feces, or other body fluids of an infected animal. Examples include petting or touching animals, andbites or scratches.
* **Indirect contact**: Coming into contact with areas where animals live and roam, or objects or surfaces that have been contaminated with germs. Examples include aquarium tank water, pet habitats, chicken coops, plants, and soil, as well as pet food and water dishes.
* **Vector-borne**: Being bitten by a tick, or an insect like a mosquito or a flea.
* **Foodborne**: Each year, 1 in 6 Americans get sick from eating contaminated food. Eating or drinking something unsafe (such as unpasteurized milk, undercooked meat or eggs, or raw fruits and vegetables that are contaminated with feces from an infected animal).

Types

Common zoonotic illnesses include:

1. **Rabies**

   Rabies is a disease that affects the nervous system of mammals. It is usually caused by a virus and is transmitted if an infected animal bites a person or other animal.

   Rabies is almost always fatal once symptoms appear. However, rabies vaccines existand are commonly available.

2. **Lyme disease and Rocky Mountain spotted fever**

   Lyme disease is transmitted through tick bites. Symptoms can range from mild to severe; but it can be treated using antibiotics.

3. **Dengue, malaria, and chikungunya**

   These are mosquito-borne diseases and are more common in certain areas, such as the Caribbean. Symptoms include fever, vomiting, and headaches. It is vital to treat these conditions as soon as possible, as they can be fatal.

4. ***Salmonella* infection**

Salmonella is often caused by handling reptiles or amphibians that carry *Salmonella*, or by handling baby chicks or ducks.

The illness usually lasts for between 4 and 7 days, and symptoms include diarrhoea, fever, and abdominal cramps. People can usually recover without medical treatment, although conservative measures are recommended.

5. *E. coli* infection

This infection is often caused by touching infected animals or handling contaminatedfood. Cows also have *E. coli* germs on their udders.

Often associated with food poisoning, salmonella can cause vomiting, abdominal cramps, anddiarrhoea. It is essential that infected people rest and drink plenty of fluids.

6. Psittacosis

Also known as ornithosis or parrot fever, psittacosis is a bacterial disease that most often affects birds. Humans can get it from feathers, secretions, and droppings. Symptoms include fever, headache, and dry cough. In serious cases, it may cause pneumonia and require a hospital visit.

7. Other types

There are hundreds of zoonotic diseases, but many are rare. Other well-known typesinclude:

- Anthrax
- avian influenza or bird flu
- bovine tuberculosis
- brucellosis
- cat scratch fever
- Ebola
- West Nile virus
- leprosy
- Zika fever
- trichinosis
- swine influenza
- histoplasmosis

Populations at increased risk

Any person who comes into contact with an infected animal, vector, or contaminated area can become infected with a zoonotic disease. However, the risk of acquiring disease, the clinical signs of disease, and the risk of death are not uniformly distributed

across individuals. The proportion of people who remain asymptomatic and the case fatality rate (proportion of ill persons who die) vary with certain risk factors. For example, age often is associated with disease severity. Of those infected with *Escherichia coli* O157:H7 from contact with animals or their environment, very young children and the elderly are more likely to develop potentially fatal hemolytic uremic syndrome (HUS) than are older children and healthy adults. By contrast, Hantavirus appears to be especially deadly among fit young adults and middle-aged individuals, possibly owing to the increased likelihood of those individuals' coming into contact with the infectious agent. The risk of becoming infected with a zoonotic disease is increased in persons affected by immunosuppression from a preexisting disease or medication. For example, cryptosporidiosis caused by *Cryptosporidium parvum*, which is transmitted to humans following contact with calves, their manure, or manure-contaminated objects or food, can occur as a coinfection with acquired immunodeficiency syndrome (AIDS). Normally a self-limiting disease, in those with AIDS cryptosporidiosis can cause serious illness, sometimes ending in death. Persons without a functioning spleen have an increased risk of illness and death from *Capnocytophaga canimorsus* infection, which can be acquired through contact with cats or dogs (particularly through dog bites). Persons who take chloroquine for malaria prophylaxis concurrently with rabies preexposure immunizations are less likely to develop a sufficient immunologic response to survive a rabies exposure. Other populations at risk include those who are cognitively impaired; such individuals, for example, may not be able to recognize or report bites from rabid bats. Pregnant women are at risk of fetal congenital malformations with lymphocytic choriomeningitis virus (LCMV) infection. Solid-organ transplant recipients have died from rabies and LCMV infections transmitted from donors.

Zoonotic disease control

Zoonotic diseases are difficult to control, particularly because of their animal reservoirs. Indeed, unlike diseases such as smallpox and polio, most zoonotic diseases cannot be eradicated through intensive human vaccination campaigns. Their successful control relies instead on strategies aimed at reducing the burden of disease among wild animals. In the case of rabies, for example, the distribution of baits containing oral rabies vaccine has led to the near-elimination or eradication of variant rabies (e.g., the Arctic fox and red fox variants) from regional wildlife reservoirs (e.g., foxes and raccoons).

Zoonotic disease risk is increased when humans live in close proximity to domestic animals such as poultry and livestock. Although the practice allows for the efficient use of limited land resources and constant care and protection of the animals, it also

increases the risk of humans' becoming infected with disease agents such as HPAI (highly pathogenic avian influenza—e.g., H5N1 virus). Pets, which often live in human homes, are common sources of zoonotic disease. For example, *Salmonella* infections (sometimes with multidrug-resistant strains) can occur as a result of contact with pet

reptiles and amphibians (e.g., turtles, iguanas, and snakes), exotic pets (e.g., hedgehogs and sugar gliders), pocket pets (e.g., hamsters, mice, and rats), pet birds (e.g., chicks and ducklings), and dogs and cats. Pet treats and other pet-associated environmental factors may also be sources of *Salmonella*.

Limiting contact between humans and wild animals is critical to reducing the risk of zoonotic disease transmission. Many human rabies deaths are due to bites from bats, frequently in home settings. Although the human immunodeficiency virus (HIV), which causes AIDS, is not zoonotic, it is thought to have evolved from similar monkey viruses that jumped to humans through the practice of hunting and consuming bush meat (monkeys). Contact with rodent feces is associated with hantavirus infection, and plague infection is associated with activities that bring people into contact with wild rodents and their fleas. The risk of zoonotic disease in humans can be further reducedby limiting contact between wild and domestic animals.

Because zoonotic disease agents can be found in humans, animals, the environment, and vectors, management requires the collaboration of many types of health and disease-control specialists. Disease control may include vector-control programs for ticks, fleas, or mosquitoes, and environmental cleanup or protection may be required to address disease agents that remain viable from days to years on surfaces, in soils, or in the water. In most state health agencies, public health veterinarians are available to assist in disease-control coordination.

Zoonotic diseases continue to be of significant concern for public health. In the early 21st century, an estimated 60 percent of novel human pathogens were zoonotic in origin, and increasing numbers of zoonotic diseases were spreading into areas where they previously had not occurred. In addition, several zoonotic disease agents were identified as candidates for use in bioterrorism attacks.

Probable Questions:

1. What is Zoonosis? Give example.
2. How do germs spread between animals and people in Zoonotic disease?
3. How Zoonotic disease can be controlled?
4. Write short notes about Rabies and Dengue.

Suggested Reading:

1. Cheng, T. C. (1986). General Parasitology. 2nd ed. Academic Press, Inc. Orlando.U.S.A.
2. Noble, E. R. and Noble G. A. (1989). Parasitology. The Biology of animal Parasites.6th ed.
3. Roberts, L. S., Janovy, J. and Nadler S. (2013) Gerald D. Schmidt &Lary S. Roberts'Foundation of Parasitology. 9th ed. McGraw-Hill International.

UNIT XV

Immunodiagnostics: Concepts of Innate and Humoral Immunity, Antigen Presentation, Antigen-antibody interactions, its application in medical Diagnosis (Western Blot, ELISA, RIA, Elispot, FACS, Immunofluorescence

Objective:

In this unit you will learn about Immunodiagnostics: Concepts of Innate and Humoral Immunity, Antigen Presentation, Antigen-antibody interactions, its application in medical Diagnosis (Western Blot, ELISA, RIA, Elispot, FACS, Immunofluorescence.

Introduction:

Antigen-antibody interaction, or antigen-antibody reaction, is a specific chemical interaction between antibodies produced by B cells of the white blood cells and antigens during immune reaction. The antigens and antibodies combine by a process called agglutination. It is the fundamental reaction in the body by which the body is protected from complex foreign molecules, such as pathogens and their chemical toxins. In the blood, the antigens are specifically and with high affinity bound by antibodies to form an antigen-antibody complex. The immune complex is then transported to cellular systems where it can be destroyed or deactivated. The types of antigen - antibody reactions are: Precipitation Reaction, Agglutination Reaction, and Complement Fixation.

- ## Innate immunity

This type of immunity is present in an organism by birth.

This is activated immediately when the pathogen attacks. Innate immunity includes certain barriers and defence mechanisms that keep foreign particles out of the body.

Innate immunity refers to the body's defence system.

This immunity helps us by providing the natural resistance components including salivary enzymes, natural killer cells, intact skin and neutrophils, etc. which produce an initial response against the infections at birth prior to exposure to a pathogen or antigens.

It is a long-term immunity in which our body produces the antibodies on its own. Our body has few natural barriers to prevent the entry of pathogens.

Types of Barriers

The four types of barriers are:

i. Physical barrier

These include the skin, body hair, cilia, eyelashes, the respiratory tract, and the gastrointestinal tract. These form the first line of defence.

The skin does more than providing us with fair or dark complexions. Our skin acts as a physical barrier to the entry of pathogens. The mucus coating in our nose and ear is a protective barrier which traps the pathogen before it gets inside.

ii. Physiological barriers

We know that our stomach uses hydrochloric acid to break down the food molecules. Due to such a strongly acidic environment, most of the germs that enter our body along with the food are killed before the further process is carried on.

Saliva in our mouth and tears in our eyes also have the antibiotic property that does not allow the growth of pathogens even though they are exposed all day.

iii. Cellular barriers

In spite of the physical and physiological barriers, certain pathogens manage to enter our body. The cells involved in this barrier are leukocytes (WBC), neutrophils, lymphocytes, basophil, eosinophil, and monocytes. All these cells are all present in the blood and tissues.

iv. Cytokine barriers

The cells in our body are smarter than we give them credit for. For instance, in case a cell in our body experiences a virus invasion, it automatically secretes proteins called interferons which forms a coating around the infected cell and prevents the cells around it from further infections.

Cells Involved In Innate Immunity

- **Phagocytes**: These circulate through the body and look for any foreign substance. They engulf and destroy it defending the body against that pathogen.
- **Macrophages**: These have the ability to move across the walls of the circulatory system. They release certain signals as cytokines to recruit other cells at the site of infections.
- **Mast Cells**: These are important for healing wounds and defence against infections.
- **Neutrophils**: These contain granules that are toxic in nature and kill any pathogen that comes in contact.

- **Eosinophils**: These contain highly toxic proteins that kill any bacteria or parasite in contact.
- **Basophils**: These attack multicellular parasites. Like the mast cells, these release histamine.
- **Natural Killer Cells**: These stop the spread of infections by destroying the infected host cells.
- **Dendritic Cells**: These are located in the tissues that are the points for initial infections. These cells sense the infection and send the message to the rest of the immune system by antigen presentation.

• Humoral immunity

Humoral immunity is also called antibody-mediated immunity. This physiological mechanism protects the body from pathogens and foreign substances in extracellular fluids and is part of both the innate and adaptive immune systems. It involves a humoral immune response that occurs in two stages: primary and secondary. The primary phase is set into motion upon the body's first contact with an antigen (surface protein found on pathogen membranes); the secondary phase describes the immune-system reaction to subsequent contact with the same antigen.

Humoral Immune Responses:

Most defenses that are mediated by antibody present in the plasma, lymph and tissue fluids are called humoral immune responses. It protects against extra-cellular bacteria and foreign macromolecules. Transfer of antibodies confers this type of immunity on the recipient. Humoral immune responses have an activation phase and an effector phase.

These phases occur as follows (Fig. 10.1):

1. The antigen is taken up by phagocytosis and degraded in a lysosome in an APC, such as a macrophage.

2. A T-cell receptor recognizes processed antigen bound to a class II MHC protein on the macrophage.

3. Cytokines released by the T_H cell and IL-1 released by macrophage stimulate the T_H cell to produce a clone of differentiated cells capable of interacting with B-cells.

Activation phase occurs in lymphatic tissue.

4. B-cells are also antigen presenting cells. Binding of antigen to a specific IgM receptor triggers receptor mediated endocytosis, degradation and display of the processed antigen on class II MHC proteins.

5. When a T_H cell receptor binds to the displayed antigen—MHC II complex on the B cell, it releases cytokines.

6. These cytokines cause the B-cell to produce a clone of B-cells.

7. Now, these B-cells produce antibody secreting plasma cells.

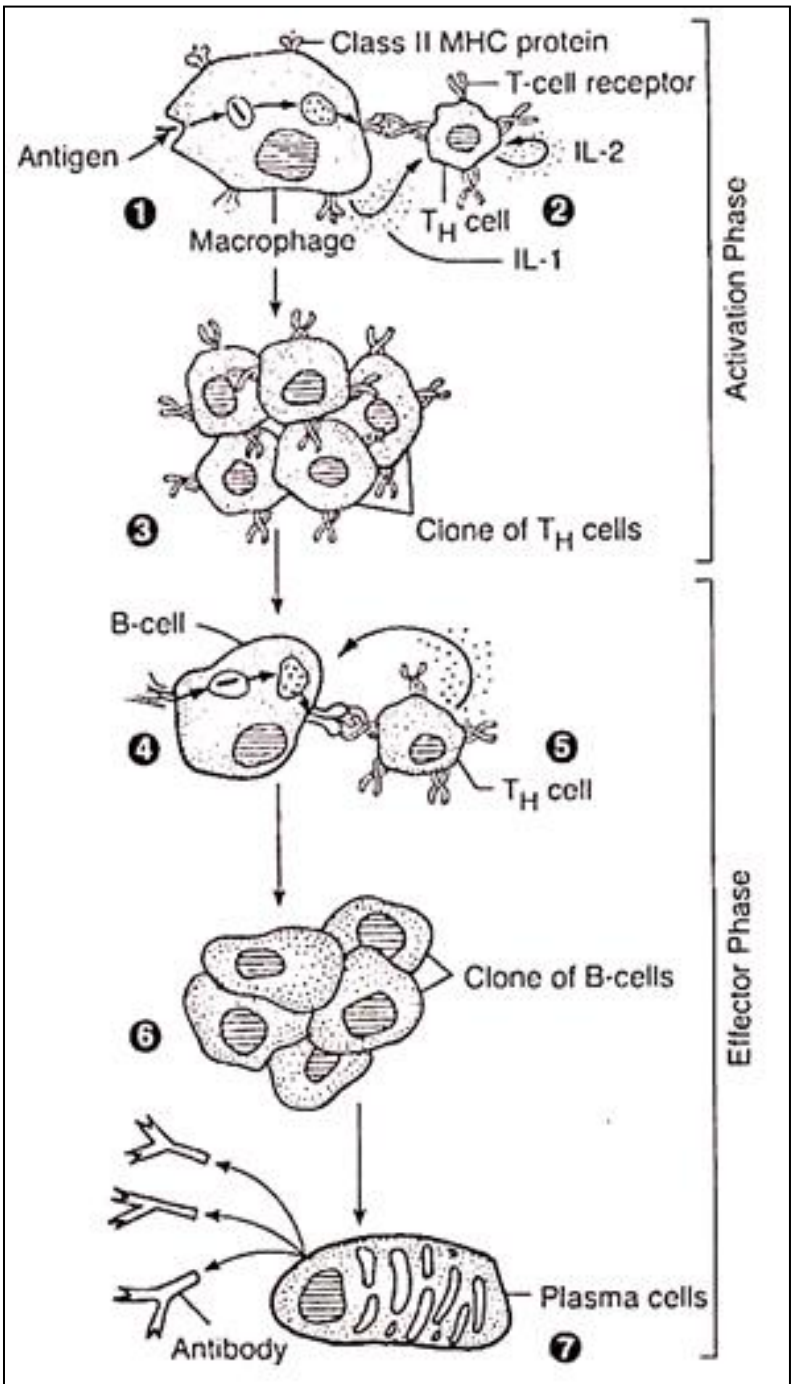


Fig. 10.1: Humoral immune responses—generation of plasma cells

- **Antigen presentation**

Antigen presentation is the expression of antigen molecules on the surface of a macrophage or other antigen-presenting cell in association with MHC class II molecules when the antigen is being presented to a $CD4^+$ helper T cell or in association with MHC class I molecules when presentation is to $CD8^+$ cytotoxic T cells.

Process

On the surface of a single cell, MHC class I molecules provide a readout of the expression level of up to 10,000 proteins. This array is interpreted by cytotoxic T lymphocytes and Natural Killer cells, allowing them to monitor the events inside the cell and detect infection and tumorigenesis.

MHC class I complexes at the cell surface may dissociate as time passes and the heavy chain can be internalised. When MHC class I molecules are internalised into the endosome, they enter the MHC class-II presentation pathway. Some of the MHC class I molecules can be recycled and present endosomal peptides as a part of a process which is called **cross-presentation**.

The usual process of antigen presentation through the MHC I molecule is based on an interaction between the **T-cell receptor** and a peptide bound to the MHC class I molecule. There is also an interaction between the CD8+ molecule on the surface of the T cell and non-peptide binding regions on the MHC class I molecule. Thus, peptide presented in complex with MHC class I can only be recognised by CD8+ T cells. This interaction is a part of so-called 'three-signal activation model', and actually represents the first signal. The next signal is the interaction between CD80/86 on the APC and CD28 on the surface of the T cell, followed by a third signal – the production of cytokines by the APC which fully activates the T cell to provide a specific response.

- **Immunoassay**

An immunoassay is a biochemical test used to identify the presence or amount of a particular molecule referred to as an "analyte", in a solution by combining it with an antibody or an antigen. The principal of immunoassays is formation of an immune complex involving the recognition and binding of an antibody to a specific molecule among a mixture of molecules. A key feature of all immunoassays is generation of a measurable signal in response to the binding. Immunoassays utilize a wide range of labels; some emit radiation, result in a visible colour change, fluoresce under light, or could be induced to emit light.

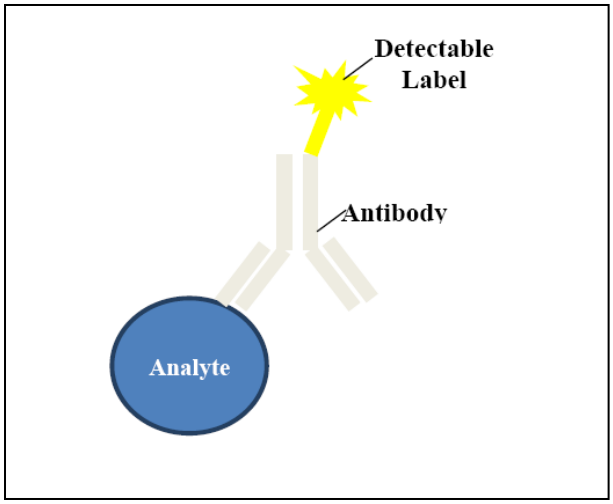


Figure 1: Basic components of an Immunoassay. The analyte specifically binds to the antibody labeled with detectable label

❖ **Examples of the application of immunoassay include:**

i. Drug testing
ii. Hormone testing (insulin in diabetic patients)
iii. Bacterial or viral testing (AIDS, hepatitis)
iv. Environmental testing (herbicides, pesticides)

❖ **Advantages of immunoassays are:**

i. Inexpensive
ii. Highly selective
iii. Low limits of detection
iv. High-throughput usually
v. Applicable to the determination of a wide-range of compounds

❖ *Categories of Immunoassays*

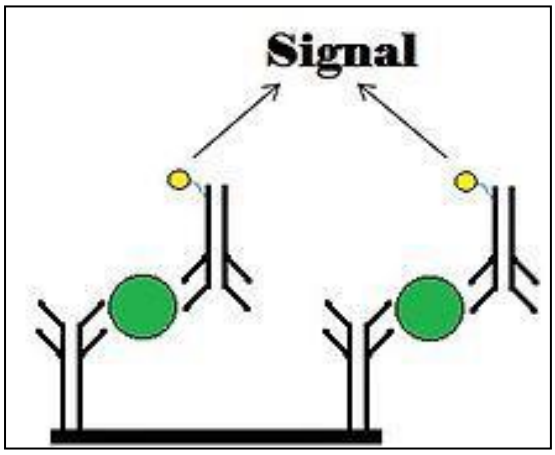
Competitive –A competitive assay or limited reagent assay involves measurement of an unlabeled analyte or antigen by its ability to compete with the labelled antigen in the immunoassay. The assay mixture consists of antibodies saturated by labelled antibodies, hence higher the reduction in label at the end of assay, greater is the amount of antigen in the test sample.

Non-Competitive

One site Non-competitive - The unknown analyte in the sample are allowed to react with labelled antibodies. After the binding reaction is complete, unbound antibodies are washed

away, and the bound labelled antibodies are measured as signals for the complexes formed. Therefore, intensity of the signal is directly proportional to the concentration of unknown antigen.

Two site Non-competitive - An antibody adsorbed on the solid phase surface is allowed to interact with the test sample. The labeled antibodies are in excess in this system and bind specifically to their respective analyte. Subsequently, a second labeled antibody is added causing sandwiching of the target analyte. The quantitation of the labelled antibody helps in determining the concentration of the antigen since the two are directly proportional. The technique is also known as sandwich assay because the analyte is "sandwiched" between two antibodies.



❖ Enzyme-Linked Immunosorbent Assay Enzyme-linked immunosorbent assay (ELISA)

Enzyme-Linked Immunosorbent Assay Enzyme-linked immunosorbent assay, commonly known as **ELISA** or EIA was first developed by Avramais (1966, 1969) and Pierce (1967).In this assay, an antibody coupled enzyme reacts with a colorless substrate called a **chromogenic substrate** to generate a visible coloured reaction product. The enzymes commonly employed for ELISA, include alkaline phosphatase, horseradish peroxidase, and galactosidase. These assays possess high sensitivity and are safe and cost effective. The result generated from an ELISA assay could be qualitative identifying the presence or absence of a particular antigen molecule; semi-quantitative, analyzing relative antigen amounts in assay samples or quantitative, defining precise antigen concentrations with respect to a standard curve. ***There are Numerous Variants of ELISA.***

ELISA assays can be performed in a variety of ways which allow both qualitative and quantitative analysis of either antigen or the antibody. ELISA can be used to identify the presence of antibody or antigen qualitatively. The unknown concentration of a sample can

alternatively be determined by a curve based on known concentrations of antibody or antigen. The assay variants are described below –

I. ***Indirect ELISA*** Indirect ELISA is used for both qualitative and quantitative measurements of antibodies. The procedure includes addition of the sample solution containing primary antibody (Ab1) to a microtiter well pre-coated with the antigen, such that the antibody would react with this well bound antigen. Unbound antibody is washed away and this is followed by detection of the antibody bound to the antigen with the help of an enzyme-conjugated secondary anti-isotype antibody (Ab2) which specifically binds to the primary antibody Ab1.Unbound secondary antibody is also washed away followed by addition of substrate for the enzyme. The coloured reaction product is analyzed spectrophotometrically by plate readers.

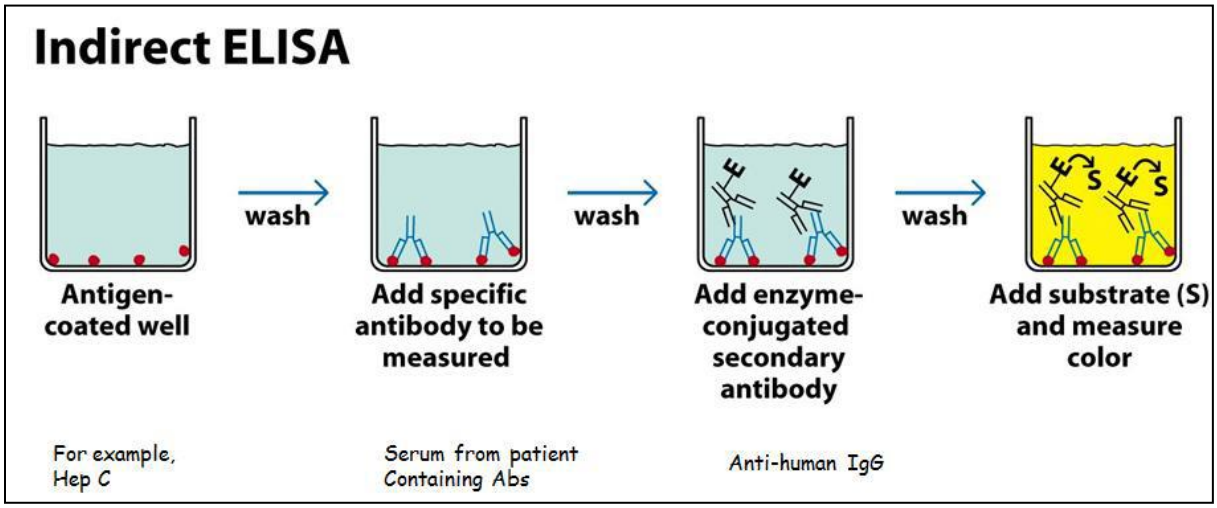


Figure 3: Indirect ELISA - Antigen is immobilized on the surface and sample is added, if antibodies specific to the antigen of interest is present binding would occur and visualized with the help of enzyme conjugated secondary antibody. Immunology, Sixth edition, Kuby, 2007, WH Freeman and company.

Indirect ELISA is preferred for detecting the presence of serum antibodies against human immunodeficiency virus (HIV), the causative agent of AIDS. The recombinantenvelope and core proteins of HIV are used as antigens plated on to microtiter wells.

II. ***Sandwich ELISA*** Sandwich ELISA is used for quantitative or qualitative analysis of antigens. The basis of this technique remains the antigen-antibody interaction however;the antibody instead of the antigen is immobilized on the microtiter well. An antigen sample is then added to the well pre-coated with theimmobilized antibody. The excess or unbound antigen is washed off using buffers, followed by addition of a second enzyme-linked

antibody specific to a second epitope on thebound antigen. Unbound secondary antibody is then washed off and a substrate corresponding to the enzyme on the secondary antibody this added, and the coloured reaction product analyzed spectrophotometrically.

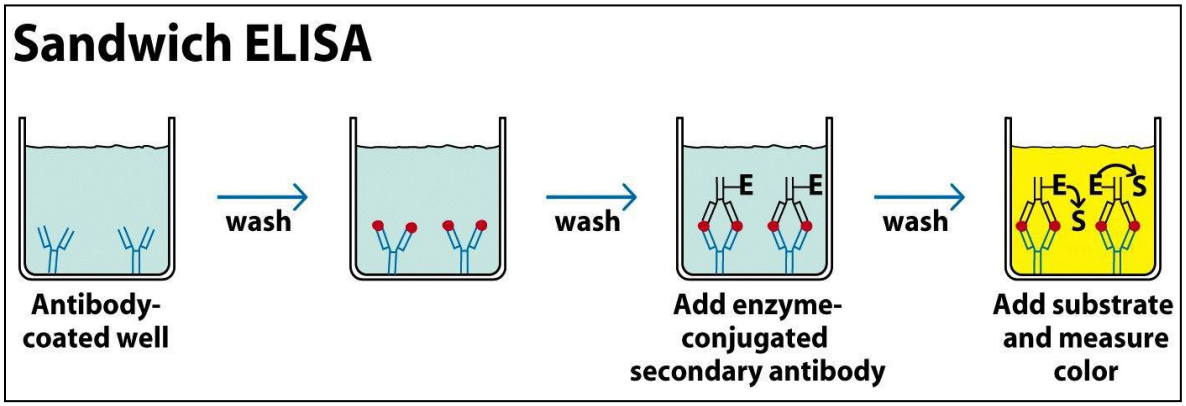


Figure 4: Sandwich ELISA – the antigen of interest is sandwiched between primary antibody immobilized on solid surface and enzyme conjugated secondary antibody. Immunology, Sixth edition, Kuby, 2007, WH Freeman and company.

III. ***Competitive ELISA*** Competitive ELISA is also a variant technique for the quantitation of antigen. The procedure consists of a pretreatment step where the antibody isincubated in solution with a sample containing the antigen. A microtiter plate coated with the same antigen is then incubated with the previously procured antigen-antibody mixture. Greater the amount of antigen in the sample, lesser would be the amount of free antibody available to bind to the antigen-coated well. On addition of an enzyme-conjugated secondary antibody (Ab2) specific for the isotype of the primary antibody, the amount of primary antibody immobilized on the well can be determined like in an indirect ELISA. This competitive interaction suggests that higher the amount of antigen in the original sample, the lower would be the value of absorbance.

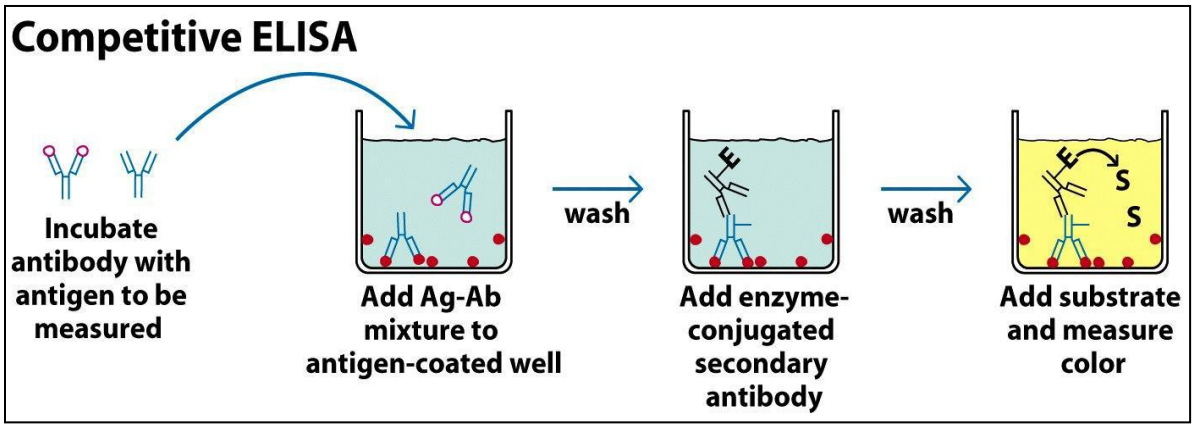


Figure 5: Competitive ELISA - Antigen-antibody mixture is added in addition to the free antibodies and incubated with antigen coated wells. Enzyme conjugated secondary antibodies when allowed to react with substrate generate coloured product which is quantitated by measuring absorbance.Immunology, Sixth edition, Kuby, 2007, WH Freeman and company.

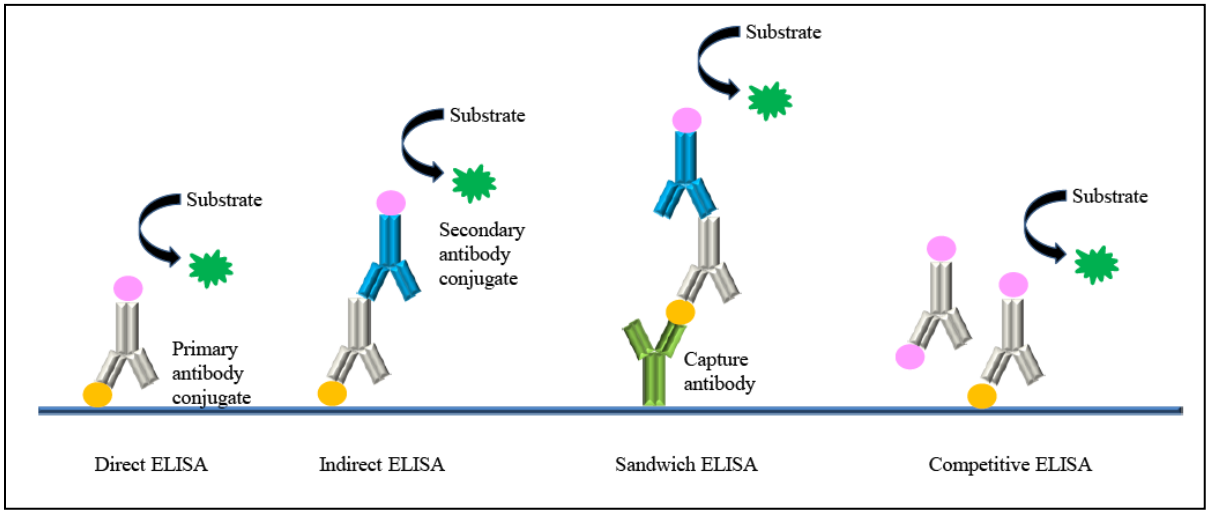


Figure 6: Comparison between different types of ELISA. This overview helps to visualize the differences between different ELISA variants. A particular type of assay can be selected depending upon specific interests.

❖ ELISPOT Assay:

A modification of the ELISA assay called the ELISPOT assay allows the quantitative determination of the number of cells in a population that are producing antibodies specific for a given antigen or an antigen for which one has a specific antibody. In this approach, the plates are coated with the antigen (capture antigen) recognized by the antibody of interest or with the antibody (capture antibody) specific for the antigen whose production is being assayed.

A suspension of the cell population under investigation is then added to the coated plates and incubated. The cells settle onto the surface of the plate, and secreted molecules reactive with the capture molecules are bound by the capture molecules in the vicinity of the secreting cells, producing a ring of antigen-antibody complexes around each cell that is producing the molecule of interest.

The plate is then washed and an enzyme-linked antibody specific for the secreted antigen or specific for the species (e.g., goat anti-rabbit) of the secreted antibody is added and allowed to bind. Subsequent development of the assay by addition of a suitable

chromogenic or chemiluminescence-producing substrate reveals the position of each antibody- or antigen-producing cell as a point of colour or light.

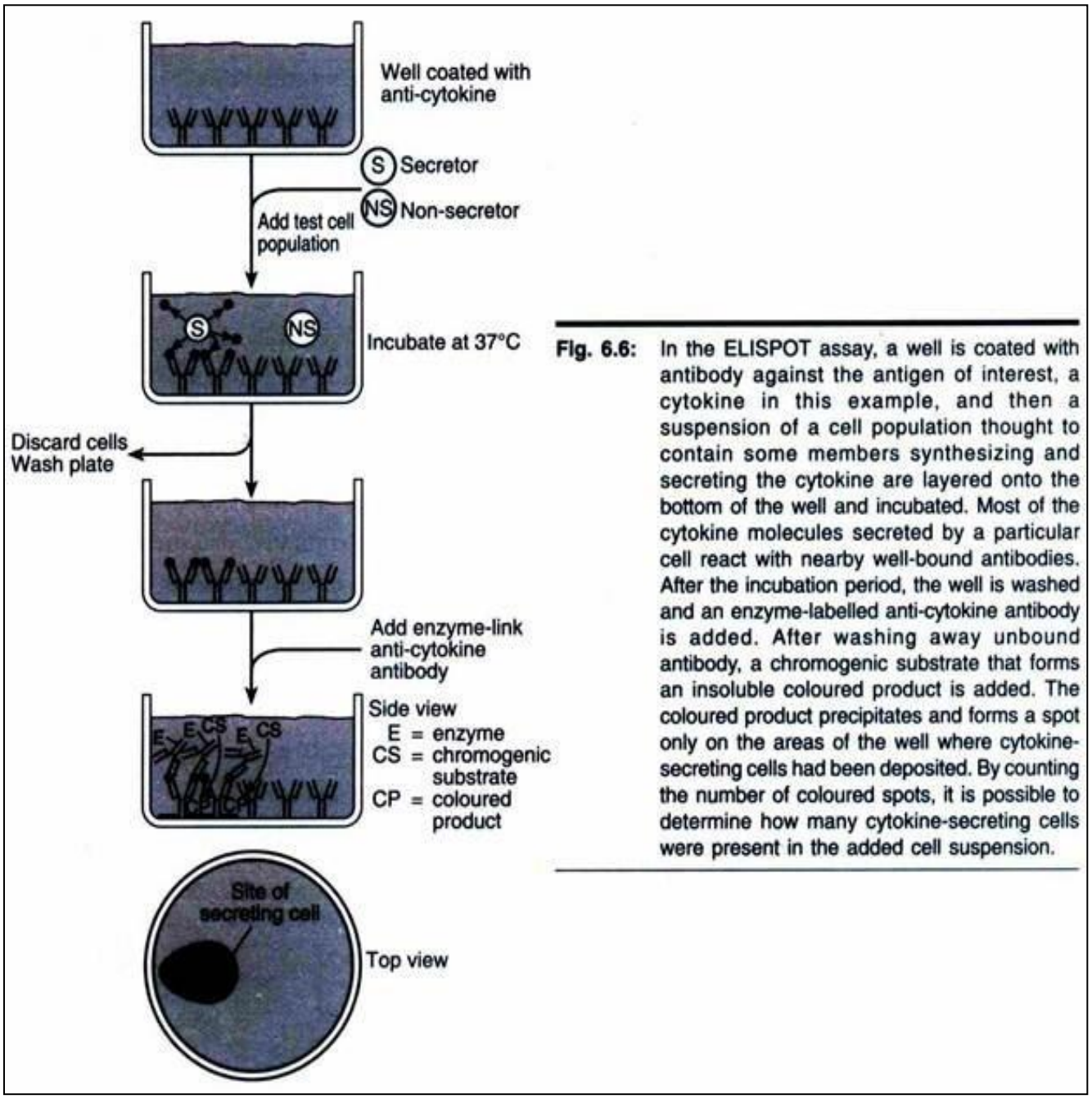


Fig. 6.6: In the ELISPOT assay, a well is coated with antibody against the antigen of interest, a cytokine in this example, and then a suspension of a cell population thought to contain some members synthesizing and secreting the cytokine are layered onto the bottom of the well and incubated. Most of the cytokine molecules secreted by a particular cell react with nearby well-bound antibodies. After the incubation period, the well is washed and an enzyme-labelled anti-cytokine antibody is added. After washing away unbound antibody, a chromogenic substrate that forms an insoluble coloured product is added. The coloured product precipitates and forms a spot only on the areas of the well where cytokine-secreting cells had been deposited. By counting the number of coloured spots, it is possible to determine how many cytokine-secreting cells were present in the added cell suspension.

❖ *Radioimmunoassay – RIA*

Radioimmunoassay (RIA) one of the most sensitive techniques for detecting antigen orantibody was first reported by S. A. Berson and Rosalyn Yalow in 1960, in order to analyze the levels of insulin - anti-insulin complexes in diabetics. This was the first attempt

for detection of blood hormones by an *in-vitro* assay. The technique demonstrates high sensitivity and is capable of quantitating hormones, serum proteins, drugs, and vitamins at concentrations as low as 0.001 *micrograms* per milliliter. The basic principle of this technique is competitive binding between the radiolabeled and unlabeled antigen to a high-affinity antibody. First the antibody is allowed to interact with the radio labeled antigen saturating the antigen-binding sites of the antibody. This is followed by addition of large amounts of sample containing unknown amount of unlabeled antigen. The available binding sites on the antibodies are available to both the labeled and unlabeled antigens as the antibody is unable to distinguish between the two. The labeled antigen is progressively displaced from the antibody binding sites with increasing amount of the unlabeled antigen.

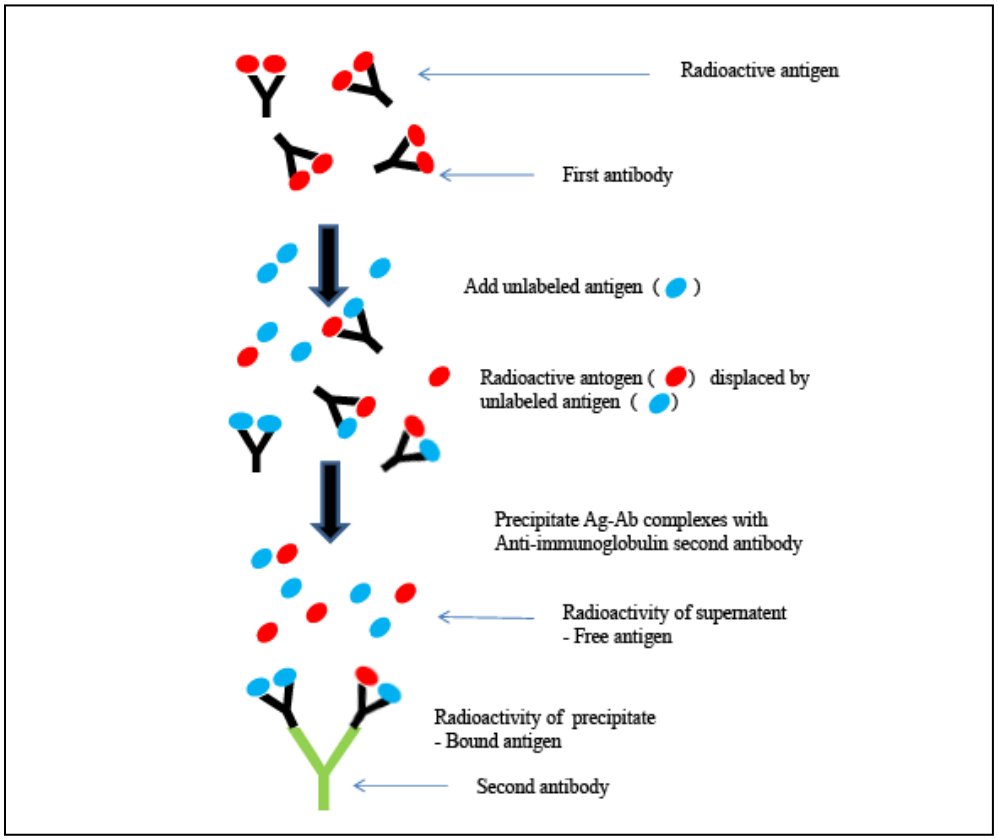


Figure 9: Radioimmunoassay (RIA): Based on competitive binding of radiolabeled and unlabeled antigen to a high-affinity antibody

This reduction in the amount of radio labeled antigen bound to the specific antibody on increasing antigen concentration in the unknown sample is measured in order to quantitate antigen concentrations in the test sample. The primary step for this assay is to ascertain the amount of antibody needed to saturate 50% - 70% of a specific quantity of radioactive antigen in the test mixture. The antibody to antigen ratio is taken such that the labeled antigen displays more number of epitopes than the total number of antibody binding sites. This ensures competitive binding between unlabeled antigen added to the

mixture and the radio labeled antigen against the limited supply of antibody. The bound labeled antigen is quantitated by precipitating the Ag-Ab complex and segregating it from free antigen; and eventually the radio activity of the precipitate is measured.

❖ *Immunofluorescence*

Albert Coons first demonstrated the labeling of antibodies with fluorescent molecules in 1944. These molecules possess the inherent property of absorbing light of a particular wavelength (excitation) and emitting light of another wavelength. Antibodies tagged with a fluorescent dye, or fluorochrome, can be identified by emission of colored light when excited by light of a specific wavelength**,** when these are a part of immune complexes. This technique also allows detection of antibodies bound to antigen epitopes in cell cultures or tissue sections. Molecules with luminescent properties emit light of a different wavelength on absorbing light of a particular wavelength. Fluorescent materials give off light very promptly due to their atomic structure. The light emitted from luminescent objects can be visualized using a fluorescence microscope equipped with a UV light source. These fluorescent probes are conjugated to the Fc arm of an antibody molecule ensuring that its specificity is not affected. Commonly used fluorochromes are:

i. **Fluorescein** is the most frequently used organic label dye for immunofluorescence. It absorbs blue light (490 nm) and emits a higher wavelength intense yellow-green fluorescence (517 nm).

ii. **Rhodamine** is another organic dye, absorbing light in the yellow-green range (515 nm) and emitting a deep red fluorescence (546 nm). Two-color immunofluorescence assays can be performed using a combination of these two dyes simultaneously as rhodamine emits fluorescence at a longer wavelength than fluorescein. Spatial distribution and comparative assays for two antigens are performed in a single experiment where an antibody specific to one determinant is tagged with fluorescein, and a second antibody recognizing another antigen is labeled with rhodamine. The co-localization of the fluorescein-tagged antibody visualized by its yellowgreencolor, is discretely distinguishable from the red color emitted where the rhodamine-tagged antibody is bound.

iii. **Phycoerythrin** an efficient absorber of light (~30-fold greater than fluorescein) and a brilliant emitter of red fluorescence is also widely used as an immunofluorescence label.

Applications of immunofluorescence can be wide range, starting with identification of a number of subpopulations of cells in culture, identifying bacterial species, detecting Ag-Ab complexes in disease conditions, detection of complement components, as well as localizing and staining of hormones and other subcellular molecules in situ. It also finds use in

analysis of cells in suspension, cultured cells, tissue, beads and microarrays for the detection of specific proteins. A very important application of immunofluorescence is tissue or cell specific antigen localization. The target antigens can be localized in cells or tissues andvisualized by fluorescence microscopy thus, making it a potent tool for associating the molecular architecture of tissues and organs to gross anatomy and physiology.

❖ Western blot

Principle:

- Western blotting technique is used for identification of particular protein from the mixture of protein.
- In this method labelled antibody against particular protein is used identify the desired protein, so it is a specific test. Western blotting is also known as immunoblotting because it uses antibodies to detect the protein.

Procedure/Steps:

1. Extraction of protein
2. Gel electrophoresis: SDS PAGE
3. Blotting: electrical or capillary blotting
4. Blocking: BSA
5. Treatment with primary antibody
6. Treatment with secondary antibody(enzyme labelled anti Ab)
7. Treatment with specific substrate; if enzyme is alkaline phosphatase, substrate is p-nitro phenyl phosphate which give color.

Step I: Extraction of Protein

- Cell lysate is most common sample for western blotting.
- Protein is extracted from cell by mechanical or chemical lysis of cell. This step is also known as tissue preparation.
- To prevent denaturing of protein protease inhibitor is used.
- The concentration of protein is determined by spectroscopy.
- When sufficient amount of protein sample is obtained, it is diluted in loading buffer containing glycerol which helps to sink the sample in well.
- Tracking dye (bromothymol blue) is also added in sample to monitor the movement of proteins.

Step II: Gel electrophoresis

- The sample is loaded in well of SDS-PAGE Sodium dodecyl sulfate- poly-acrylamide gel electrophoresis.
- The proteins are separated on the basis of electric charge, isoelectric point, molecular weight, or combination of these all.
- The small size protein moves faster than large size protein.
- Protein are negatively charged, so they move toward positive (anode) pole as electric current is applied.

Step III: Blotting

- The nitrocellulose membrane is placed on the gel. The separated protein from gel get transferred to nitrocellulose paper by capillary action. This type of blotting is time consuming and may take 1-2 days
- For fast and more efficient transfer of desired protein from the gel to nitrocellulose paper electro-blotting can be used.
- In electro-blotting nitrocellulose membrane is sandwich between gel and cassette of filter paper and then electric current is passed through the gel causing transfer of protein to the membrane.

Step IV: Blocking

- Blocking is very important step in western blotting.
- Antibodies are also protein so they are likely to bind the nitrocellulose paper. So before adding the primary antibody the membrane is non-specifically saturated or masked by using casein or Bovine serum albumin (BSA).

Step V: Treatment with Primary Antibody

- The primary antibody (1° Ab) is specific to desired protein so it form Ag-Ab complex

Step VI: Treatment with secondary antibody

- The secondary antibody is enzyme labelled. For eg. alkaline phosphatase or Horseradish peroxidase (HRP) is labelled with secondary antibody.
- Secondary antibody (2° Ab) is antibody against primary antibody (anti-antibody) so it can bind with Ag-Ab complex.

Step VII: Treatment with suitable substrate

- To visualize the enzyme action, the reaction mixture is incubated with specific substrate.

- The enzyme convert the substrate to give visible colored product, so band of color can be visualized in the membrane.
- Western blotting is also a quantitative test to determine the amount of protein in sample.

❖ FACS (fluorescence-activated single cell sorting)

FACS is an abbreviation for **fluorescence-activated single cell sorting**, which is a flow cytometry technique that further adds a degree of functionality.

Flow cytometry principle

The basic principle of flow cytometry is based on the measurement of light scattered by particles, and the fluorescence observed when these particles are passed in a stream through a laser beam.

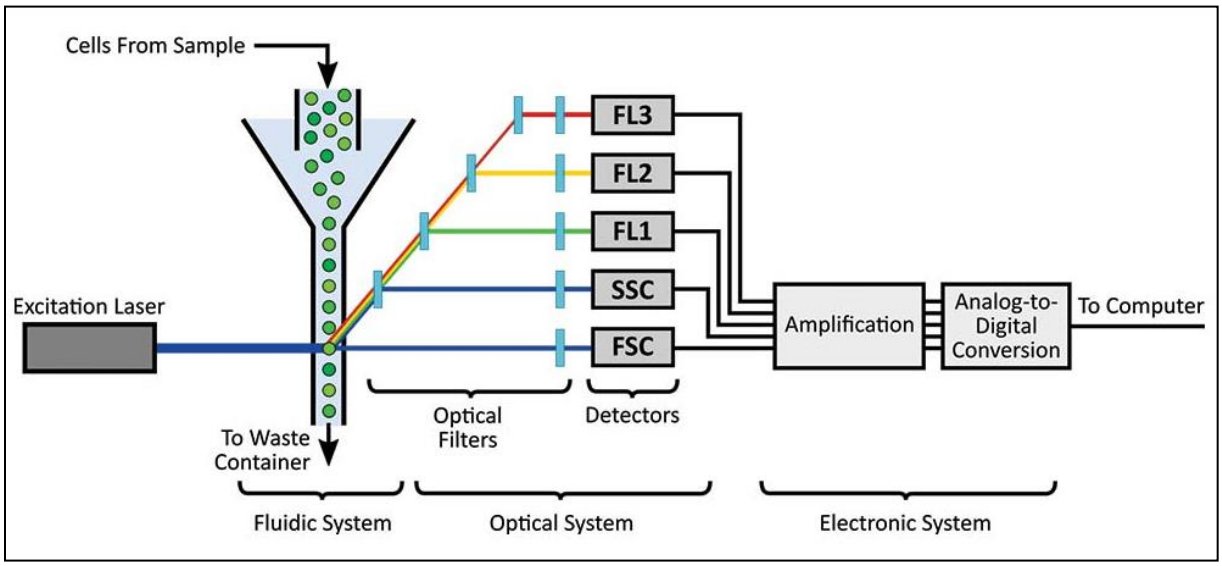


Fig: Flow cytometry

Figure: Schematic of a common flow cytometer, illustrating the fluidic, optical, and electronic systems.

Light Scattering

- Light scattering results when a particle deflects incident laser light. The extent to which this happens depends on the physical properties of a particle, namely its size and internal complexity.
- Forward-scattered light (FSC) is proportional to the cell-surface area or size of the cell. It is a measurement of mostly diffracted light and detects rays that are just off the axis of the incident laser beam dispersed in the forward direction by a photodiode.

- Side-scattered light (SSC) indicates the cell granularity or internal complexity of the cells. SSC is a measurement of mostly refracted and reflected light that occurs at any interface within the cell where there is a change in the refractive index.
- The measurements of FSC and SSC are used for the differentiation of cell types in a heterogeneous cell population.

Fluorescence

- Fluorescent markers used to detect the expression of cellular molecules such as proteins or nucleic acids in a system.
- The fluorescent compound absorbs light energy over a range of wavelengths that is characteristic of that compound.
- This absorption of light causes an electron in the fluorescent compound to be raised to a higher energy level.
- The excited electron quickly decays to its ground state, emitting the excess energy in the form of fluorescence which is then collected by detectors.
- In a mixed population of cells, different fluorochromes can be used to distinguish separate subpopulations.
- The fluorescence pattern of each subpopulation, combined with FSC and SSC data, can be used to identify which cells are present in a sample and to count their relative percentages.
- The electronics system then converts the detected light signals into electronic signals that can be processed by the computer.

Protocol/Procedure/Process/Steps of Flow Cytometry

The process of flow cytometry consists of the following:

Sample Preparation

- Before running in the flow cytometers, the cells under analysis must be in a single-cell suspension.
- Clumped cultured cells or cells present in solid organs should first be converted into a single cell suspension before the analysis by using enzymatic digestion or mechanical dissociation of the tissue, respectively.
- It is then followed by mechanical filtration should to avoid unwanted instrument clogs and obtain higher quality flow data.

- The resulting cells are then incubated in test tubes or microtiter plates with unlabeled or fluorescently conjugated antibodies and analyzed through the flow cytometer machine.

Antibody Staining

- Once the sample is prepared, the cells are coated with fluorochrome-conjugated antibodies specific for the surface markers present on different cells. This can be done either by direct, indirect, or intracellular staining.
- Indirect staining, cells are incubated with an antibody directly conjugated to a fluorophore.
- In indirect staining, the fluorophore-conjugated secondary antibody detects the primary antibody
- The intracellular staining procedure allows direct measurement of antigens presents inside the cell cytoplasm or nucleus. For this, the cells are first made permeable and then are stained with antibodies in the permeabilization buffer.

Running Samples

- At first, control samples are run to adjust the voltages in the detectors.
- The flow rates in the cytometer are set and the sample is run.

Applications/Uses

Flow Cytometry is used in several fields including molecular biology, pathology, immunology, virology, plant biology, and marine biology. Some of the common application includes:

- It is used in clinical labs for the detection of malignancy in bodily fluids like leukemia.
- Cytometers like cell sorters can be used to separate the cells of interest in separate collection tubes physically.
- It can be used for the detection of the content of DNA by using fluorescent markers.
- Flow cytometers allow the analysis of replication cells by using fluorescent dye for four different stages of the cell cycle.
- Acoustic flow cytometers are used in the study of multi-drug resistant bacteria in the blood and other samples.
- The different stages of cell death, apoptosis, and necrosis can be detected by flow cytometers based on the differences in the morphological and biochemical changes

Probable Questions:

1. What is Antigen-antibody interaction?
2. What do you mean by innate immunity?
3. Discuss different type of barriers in innate immunity.
4. Discuss about different types of cells involved in innate immunity.
5. Define humoral immunity. Write down the phases of humoral immunity.
6. Elaborate the process of antigen presentation.
7. Describe sandwich ELISA?
8. Describe sandwich ELISA with diagram.
9. Describe the process of Radioimmunoassay (RIA) with diagram.
10. What do you mean by Immunofluorescence? Which flurochromes are used in this technique?
11. Write down the procedure of western blot technique.
12. Write down the applications of FACS.

Suggested readings/ references:

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