Post-Graduate Degree Programme (CBCS)
in
ZOOLOGY

SEMESTER-III

HARDCORE THEORY PAPER

Environmental Toxicology and Endocrinology

SELF LEARNING MATERIAL

DIRECTORATE OFOPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
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Director's Message

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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, 2017 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 co-ordinated 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

ZCORT-310: ENVIRONMENTAL TOXICOLOGY AND ENDOCRINOOGY

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UNIT-I

Toxicology: Scope, division, toxicants and toxicity, LD50, LC 50 and ED50, Dose-response relationship.

Objective: In this unit you will learn about basic concept of toxicology. You will also learn about scope and division of toxicology and dose response relationship. In this unit you will also learn about different toxicity testing procedures. You will also know about LC50, LD50, ED50, synergism, antagonism and additive Effect.

Introduction to Toxicology:

Science of Toxicology, deals with the study of interactions between chemicals and biological systems in order to quantitatively determine the adverse effects in living organisms and investigate the nature, incidence, mechanism of production, factors influencing their development and reversibility of such adverse effects. In formal terms, it appears to be a young science, however, it was conceptualized by a physician known as Paracelsus. Borzellaca (2000) honoured Paracelsus as herald of modern toxicology. Paracelsus discounted the humoral theory of Galen who postulated that balance amongst four humors in the body (blood, phlegm, yellow and black bile) is essential for health. Paracelsus believed in three humors- salt (representing stability), sulfur (representing combustibility) and mercury (representing liquidity).

He defined disease as a separation of one humor from the other two. He propounded the principle of simplitude meaning that "a poison in the body will be cured by a similar poison". He introduced chemistry into medicine. He extended his interest in chemistry and biology to what we now consider toxicology. Basic tenets of Paracelsianism were summed up by Temkin and Coworkers (1996).Since then, toxicological developments have witnessed new heights. War and prospects of war played a great role in the development of toxicology. In "world war I", a variety of chemicals were used in the battlefields of France. Occupational toxicology originated in 19^{th} century as a product of industrial revolution.

Development of chemical and pharmaceutical industries in 19^{th} and 20^{th} century gave birth to regulatory toxicology. Increasing concerns for consumer and environmental health during last three decades brought toxicology to the age of Science. World War II offered stimulus to the evolution of Environmental Toxicology.

Definition of Toxicology:

Toxicology can be defined quite simply as the branch of science dealing with poisons. Broadly speaking, a poison is any substance causing harmful effects in an organism to which it is administered, either deliberately or by accident. Clearly, this effect is closely related since any substance, at a low enough dose, is without effect, while many, if not most, substances have deleterious effects at some higher dose.

Much of toxicology deals with compounds exogenous to the normal metabolism of the organism. Such compounds are referred to as foreign compounds or more recently, as xenobiotics. However, many compounds endogenous to the organism e.g., metabolic intermediates such as glutamate, and hormones such as thyroxine, are toxic when administered in unnaturally high doses.

Similarly, trace elements such as selenium, which are essential in the diet in low concentrations, are frequently toxic at higher levels. Whether the harmful effects of physical phenomena such as irradiation, sound, temperature, and humidity are included in toxicology, appear to be largely dependent on the preference of the writer, it is convenient, however, to include them under the broad definition of toxicology. The method of assessing toxic effects is another parameter of considerable complexity. Acute toxicity, usually measured as mortality and expressed as the LD_{50}– the dose required to kill 50% of a population of the organism in question under specified conditions- is probably the simplest measure of toxicity.

Even so, reproducibility of LD_{50} values is highly dependent upon the extent to which many variables are controlled. These include the age, sex, and physiological condition of the animals, their diet, the environmental temperature and humidity, and the method of administering the toxicant.

Chronic toxicity may be manifested in a variety of ways – carcinomas, cataracts, peptic ulcers, and reproductive effects, to name only a few. Furthermore, compounds may have different effects at different doses. Vinyl chloride, a potent hepatotoxic at high doses, is a carcinogen with a very long latent period at low doses. Most drugs have therapeutic effects at low doses but are toxic at higher levels. The relatively nontoxic acetylsalicylic acid (aspirin) is a useful analgesic at low doses, is toxic at high doses, and may cause peptic ulcers with chronic use. Considerable variation also exists in the toxic effects of the same compound administered to different animals, or even to the same animal by different routes. The insecticide Malathion has a low toxicity to mammals, whereas it is toxic enough to insects to be a widely used commercial insecticide. The route of entry of toxicants into the animal body is frequently oral, in the food or drinking water in the case of many chronic environmental contaminants such as lead or insecticide residues, or directly as in the case of accidental or deliberate acute poisoning.Other routes for non-experimental poisoning include dermal absorption and pulmonary absorption. The above routes of administration are all used experimentally, and in addition, several types of injection are also common -intravenous, intraperitoneal, intramuscular, and subcutaneous. The toxicity of many compounds varies tenfold or greater depending upon the method of administration.

Earlier Developments in Toxicology:

Soon the nature and magnitude of toxic effects were studied. Factors viz.- physicochemical properties of the substance, its bioconversion, the conditions of exposure, and the presence of bio-protective mechanisms subsequently dominated the scene.

Morphological and biochemical injury produced by a toxin was classified as inflammation, necrosis, enzyme inhibition, biochemical uncoupling, lethal synthesis, lipid peroxidation, covalent binding, receptive interaction, immune mediated hypersensitive reactions, genotoxicity, developmental and reproductive toxicity and pharmacological effects. In 1848, Blake in the United States published his opinion that the biological activity of a salt was due to its basic or its acidic component and not to be whole salt; as with lead nitrate it was the lead moiety and not the acetate or nitrate part. This was, for 1st time, a daring thought, because it was not until 1884 Arrhenius introduced his theory of electrolytic dissociation.

The Scottish authors Crum Brown and Fraser (1869) made a major discovery. They wrote, "there can be no reasonable doubt that a relation exists between the physiological action of a substance and its chemical composition and constitution", understanding by the latter term, the mutual relations of the atoms in the substance. This discovery was the first to show structure-action relationship at the turn of present century. Ernest Overton and Hans Meyer independently put forward a, "Lipoid theory of cellular Depression". This stated that chemically inert substances exert depressant properties on cells (particularly those of central nervous system) that are rich in lipids and that higher the partition coefficients, the greater the depressant action.

The idea that drugs act upon receptors began with John Langley in (1878) in Cambridge. Later, Langley coined the term 'receptive substances'. Paul Ehrlich was already using the term receptor in Germany. In his Noble prize address Ehrlich outlined the receptor as a small chemically defined area, which was normally occupied with the cell's nutrition and metabolism but which could take up specific antigens or drugs instead.

First the idea of receptor was received with skepticism because of repeated failure to isolate any such substance. However, the idea of receptors became more firmly established by the work of Alfred Clark who showed that combination of drug with a receptor quantitatively followed the law of mass action. He summed up his work in a monograph, a few years before his death in 1941.The period of Second World War (1939-1944) was a turning point in the study of structure action relationship.
There was a period when dose response relationships were highly predominant. Development of physiology and biochemistry also influenced the growth of toxicology. Metabolism of substances was conveniently classified as phase – I and phase – II reactions.

Concept of QSAR:

The concept of QSAR (quantitative structure activity relationships) was applied to study the toxicity of inorganic cations. While molecules of organic compounds reflect their properties as a whole, the inorganic compounds dissociate in various degrees and properties have thus to be attributed to anions, cations or un-dissociated molecules.

Inorganic cations can form complexes with inorganic or organic ligands contributing new properties to the complex. Components of this system (cations, anions, un-dissociated molecules) could mutually influence each other depending upon the ratio amongst components. Quantitative relationships between a chemical structure of the complex and the biological activity formed a new line of action in toxicology.QSAR studies generated the concept of molecular connectivity in order to characterize the organic biologically effective substances. The index of connectivity is deduced from the numeric evaluation of the extent of branching of chemical bonds in the section of the molecule. There exists a correlation between the connectivity index and toxicity of cations.

Relationship of Toxicology to other Sciences:

Toxicology is frequently said to be a branch of pharmacology, a science that deals primarily with the therapeutic effects of exogenous substances and with all the chemical and biochemical ramifications involved in those effects. Since the therapeutic dose range of pharmacological compounds is usually quite small, and most of these compounds are toxic at higher doses, it may be more appropriate to consider pharmacology a branch of toxicology.
Toxicology is clearly related to the two applied biology-medicine and agriculture. In the former, clinical diagnosis and treatment of poisoning as well as the management of toxic side effects are areas of significance, while in the latter the development of agricultural biocides such as insecticides, herbicides, nematicides, and fungicides is of great importance.

The detection and management of the off-target effects of such compounds is also an area of increasing importance that is essential to their continued use. Toxicology may also be considered an area of fundamental biology since the adaptation of organisms to toxic environments has important implications for ecology and evolution. The tools of chemistry and chemical biology since the adaptation of organisms to and progress in toxicology are closely related to the development of new methodology. Those of chemistry provide analytical methods for toxic compounds, particularly for forensic toxicology and residue analysis, and those of biochemistry provide the techniques to investigate the metabolism and mode of action of toxic compounds.

On the other hand, studies of the chemistry of toxic compounds have contributed to fundamental organic chemistry, and studies of the enzymes involved in detoxication and toxic action have contributed to our basic knowledge of biochemistry.

Scope of Toxicology:

Toxicology in the most general sense may be one of the oldest practised sciences. From his earliest beginnings, man must have been aware of numerous toxins such as snake venoms and those of poisonous plants. From the earliest written records it is clear that the ancients had considerable knowledge of poisons.

The Greeks made use of hemlock as a method of execution, more particularly, the Romans made much use of poisons for political and other assassinations. Indeed, it was Dioscorides, a Greek at the court of Nero, who made the earliest known attempt to classify poisons. Although poisoning has enjoyed a considerable vogue at many times and places, the scientific study of toxicology can probably be dated from Paracelsus, who in the sixteenth century, put forward the necessity for experimentation and included much in his range of interests that would today be classified as toxicology.

The modem study of toxicology is usually dated from the Spaniard, Orfila (1787- 1853), who, at the University of Paris, identified toxicology as a separate science. Among his many contributions, he devised chemical methods for the detection of poisons and stressed the value of chemical analysis to provide legal evidence. He was also the author, in 1815, of the first book devoted entirely to the toxic effects of chemicals.

Toxicology can be subdivided in a variety of ways. Loomis refers to the three “basic” subdivisions as environmental, economic, and forensic. Environmental toxicology is further divided into such areas as pollution, residues, and industrial hygiene; economic toxicology is said to be devoted to the development of drugs, food additives, and pesticides; and forensic toxicology is concerned with diagnosis, therapy, and medicolegal considerations. Clearly, these categories are not mutually exclusive; for example, the off target effects of pesticides are considered to be environmental, while the development of pesticides is economic.

Environmental Toxicology:

Environmental toxicology is the most rapidly growing branch of science. Public concern over environmental pollutants and their possible chronic effects, particularly carcinogenicity, has given rise, in the United States, to new research and regulatory agencies and recently to the Toxic Substances Control Act. Similar developments are also taking place in many other countries. The range of environmental-pollutants is enormous, including industrial and domestic effluents, combustion products of fossil fuels, agricultural chemicals, and many other compounds that may be found in food, air, and water. Such compounds as food additives and cosmetics are also being subjected to the same scrutiny. Other sub-specialties are frequently mentioned that do not fit into the above divisions. Behavioural toxicology, an area of increasing importance, could be involved in any of these and is usually treated as a separate sub-speciality. Analytical toxicology provides the methods used in essentially every branch of the subject, while biochemical toxicology, provides the fundamental basis for all branches of toxicology.

Language of Toxicology:

Like any other specialized field, environmental health has its own language. Some of the terms may need a few words of introduction. Toxic, a central concept simply means capable of causing illness. The types of illnesses caused by environmental toxins are conventionally divided into acute and chronic. Acute illness are those which appear soon after exposure to a toxic compound, last for a relatively short time, and then resolve themselves, even if the resolution is in death. The term sub-acute is also occasionally used to describe disorders with subtle symptoms that are not immediately obvious without special tests. Lead workers, for example, often appear to be much healthier than a thorough medical examination reveals them to be Chronic illnesses, by contrast, that may appear years or even decades after exposure, and which may "remain", unresolved, for the victim's lifetime.

There are three special kinds of toxic hazards that have special relevance to environmental health: carcinogens, mutagens and teratogens. As most of us know, a carcinogenic substance is one that causes cancer. A mutagenic substance is one that causes changes in the genetic material of a cell. Spontaneous, natural mutations occur in our body cells all the time; the vast majority of them cause no damage, and even when they do it is usually limited to the lifetime of the cell they occur in.But in rare cases the cell may continue to grow and divide after a mutagen has altered its basic genetic structure, and if this mutation is passed on to succeeding generations via egg or sperm, it may cause birth defects, inherited diseases, mental deficiency, increased susceptibility to disease, and a host of other abnormalities and disorders. If the mutated genes are recessive, it may take more than one generation for these effects to show up.

Mutagenicity and carcinogenicity are related in some way, but were not yet sure just how. Radiation is probably the best-known example of a mutagenic environmental hazard. Contamination is measured in terms of the concentration of a substance in the environment, and there are a number of different conventions governing the measurement of concentration. The most common system makes use of metric units, particularly the milligram (one-thousandth of a gram, abbreviated mg) and the microgram (one million of a gram, abbreviated Ì g). Occasionally, in very refined, ultrasensitive measurements, nanograms (one-billionth of a gram), picograms (one-trillionth of a gram), and even smaller units may be used.

Obviously, a contaminant concentration of one milligram per kilogram is the same as one part of contaminant per million parts of non-contaminant, or 1 ppm, and this alternative method of indicating relative concentrations is also widely used, particularly for air pollutants, food additives, and pesticides residues. The terms ppm and ppb (parts per billion) are common- ppt (parts per trillion) appears only rarely.Exposure is a way of saying that the contamination in the environment has passed into an organism; a human being is exposed to a toxic compound if some amount of it has entered his or her body. Exposure does not mean that a person has merely been in the proximity of a toxic substance.

For example, if you walk past a drum bearing a warning label and containing a toxin, you are not necessarily exposed to whatever it contains. But if the drum leaks its contents into the air or soil, and pollutes the air you breathe or the water you drink, you probably will be exposed to its contents. Like illnesses, exposures may also be subdivided into acute and chronic types. Acute exposures are those that occur over short periods of time, often to high concentrations of a

hazardous substance. Chronic exposures, which are much more common among the general public, involve longer periods of time and for the most part, lower concentrations. Dose is the term for measuring exposures. Basically, the dose a person exposed to a toxic substance receives is dependent on its concentration in the immediate environment and the duration of the exposure. However, because the interaction of human beings and the environment is a complex, constantly changing process, numerous other factors may also play a part in determining dose.

Dose can be a function of weather conditions, the persistence and solubility of the toxic substance in the biosphere, the size of its molecules or particulates, the presence of other compounds in the environment that it may react with, the age and overall health of the exposed individual, whether the substance is inhaled, swallowed, or absorbed through the skin, and the effectiveness of the body's natural defenses in detoxifying the substance and eliminating it from the body. Some substances, like asbestos, become virtually permanent contaminants in the body once they have penetrated far enough into the lungs or other organs. Others, such as methanol, are metabolized and excreted from the body in a matter of hours at most.

The tendency for some substances to collect in the tissues of a living organism and stay there is known as bioaccumulation. Their tendency to move up the food chain as one species consumes another, becoming ever more concentrated as they go, is called biomagnification.

Traditionally a threshold was a measurable level of exposure to a toxic substance below which there would probably be no adverse health effect and above which there probably would be. The setting of safety standards for the work site as well as the general environment often involves the assumption that approximate thresholds can be determined, monitored and enforced for the toxin in question. But this assumption has been subjected to various criticisms in the past few decades. First, it is often pointed out that, whether we can measure it or not, it is entirely possible that every molecule of every substance we take into our bodies has some effect on us. It may not be a detectable effect and it may not be harmful or long lasting, but it is an effect.

Thus, this argument goes, the concept of a specific cutoff point below which a substance is treated as though it didn't exist and above which it is considered harmful is misleading. Far more appropriate, proponents of this view argue, is the assumption that these substances have a range of effects, beginning at once end with those that are imperceptibly molecular and extending to the catastrophically toxic, ultimately fatal effect at the other end of the spectrum. An LD_{50} is customarily expressed in terms of milligrams per kilogram of body weight. Thus, a substance whose LD_{50} is 2 mg/kg is five times as toxic as one whose LD_{50} is 10 mg/kg. In general, substances with LD_{50} values below 50 mg/kg are considered highly toxic. Those with values between 50 and 500 mg/kg are considered moderately toxic, and those with values above 500 mg/kg are regarded as less toxic.

Distribution of a Toxin:

Various factors responsible for the distribution of toxicants throughout the body need discussion. This is primarily concerned with the binding of toxicants to blood proteins particularly lipoproteins. Lipoproteins are an important class of protein, particularly in the vascular fluids.

They vary in molecular weight from 200,000 to 10,000,00 and the lipid content varies from 4% to 95%, being composed of triglycerides, phospholipids, and free and esterified cholesterol. Although

they are classified into groups based on their flotation constants, each group is, in fact, a mixture of many similar lipoproteins. The nature and importance of various types of ligand-protein interactions are assessed, including covalent binding, ionic binding, hydrogen bonding, Vander Waals forces, and hydrophobic interactions. Many of the same binding forces are also important in toxicant receptor interactions. It deals with the mathematical approach to the distribution of toxicants, or toxicokinetics. It provides a simplified, but still mathematically rigorous, treatment of distribution data, including both analysis and the formulation of mathematical models.

Metabolism of a Toxin:

The majority of xenobiotics that enter the body do so because they are lipophilic. The metabolism of xenobiotics, which is carried out by a wide range of relatively nonspecific enzymes, serves to increase their water solubility and make possible their elimination from the body. This process consists of two phases. In phase I, a reactive polar group is introduced into the molecule, rendering it a suitable substrate for phase II reactions.

Phase I reactions include the well-known cytochrome P_{450} -dependent mixed-function oxidations as well as reductions, hydrolyses, etc. Phase II reactions include all the conjugation reactions in which a polar group on the toxicant is combined with an endogenous compound such as glucuronic acid, glutathione etc. to form a highly water- soluble conjugate that can be eliminated from the body. It should be pointed out at this early stage that these metabolic reactions are not all detoxications since many foreign compounds are metabolized to highly reactive products that are responsible for their toxic effect. These include the activation of carcinogens and hepatotoxic cants. Although the liver is the most studied organ with regard to xenobiotic metabolism, several other organs are known to be active in this respect, although neither the specific activity nor the range of substrates metabolized is as large as in the liver.These organs include the lungs and the gastrointestinal tract, as one might expect of organs that are important site for the entry of xenobiotic into the body, and to a lesser extent, the other important portal of entry, the skin. Other organs, such as the kidney, may also be important sites for xenobiotic metabolism.

Because toxicants are both activated and inactivated metabolically, physiological factors affecting metabolic rates can have dramatic effects on the expression of toxicity. These effects, including age, sex, pregnancy, and diet need to be considered. Comparative toxicology is of considerable importance from the point of view of selectivity, resistance to toxic action, and environmental studies of toxicants, as well as of some academic importance from the evolutionary viewpoint. Although only a few generalizations can be made on the basis of phylogenetic relationships, there have been many comparisons between species of toxicological interest. Foreign compounds can be substrates, inhibitors, inducers of the enzymes that metabolize them and, not infrequently, serve in more than one of these roles. Since the enzymes in question are nonspecific, numerous interactions between foreign compounds are possible. These may be synergistic or antagonistic and may have a profound effect on the expression of toxicity. Depending upon the compounds and the enzymes involved in a particular interaction, the effect can be an increase or a decrease in either acute or chronic toxicity. The basic principles of such interactions are summarized.

The cell type that has been studied most intensively in biochemical toxicology is the hepatocyte, the cell that forms the bulk of the liver. These cells are highly active metabolically, both in normal

intermediary metabolism and in reactions involving xenobiotics. The principal cell organelles shown in the diagram play of important sole in biochemical toxicology. The nucleus, the chromosomes that contain DNA responsible for most of the proteins synthesized in the cell, is the site for the primary reaction of carcinogenesis, since carcinogens react with DNA. Depending upon the toxicant, the organ, and the cell type involved, similar reactions are involved in mutagenesis and teratogenesis. The nuclear envelope has recently been shown to have an active aryl hydrocarbon hydroxylase system. Mitochondria are the site of electron transport and oxidative phosphorylation pathways that provide sites for the action of many acute toxicants.

The endoplasmic reticulum exists in two forms- rough, which is associated with protein synthesis, and while both rough and smooth are active in the oxidation of xenobiotics, the latter usually has the highest specific activity. After, disruption of the cells, followed by differential centrifugation, the two types are isolated as rough and smooth microsomes.

Sites of Action of a Toxin:

Compounds of intrinsic toxicity and active metabolites produced in the body ultimately arrive either at a site of action or an excretory organ. Although almost any organ can show the effects of toxicity, some are more easily affected than others by particular classes of toxicants, and some have been studied in greater detail than others. In all cases, however, toxicant-receptor interactions are important. Acute toxicants tend to affect either oxidative metabolism, the synapses of the nervous system, or the neuromuscular junction. Toxic effects on the central nervous have been widely studied.

The commonest modes of chronic toxicity involve interaction with nucleic acids, causing carcinogenesis or reproductive effects. Although specific organ damage is known for several toxicants, the central role of the liver in studies of toxic action is acknowledged. While toxicants can be classified in many ways, based either on natural distribution, commercial use patterns, or chemistry, only two such groups viz. metals and pesticides.

Many metabolic pathways are affected by toxicants. They include glycolysis, the tricarboxylic acid cycle, the pentose cycle, the electron transport system and oxidative phosphorylation, nucleic acid synthesis, protein synthesis, and many others, as well as such specialized systems as photosynthesis in plants. In vivo testing for chronic toxicity in animals and short-term mutagenicity tests are both somewhat remote from a strictly biochemical treatment of the mechanisms involved in toxicology.

Excretion of Toxin:

Either the un-metabolized toxicants or their metabolic products are ultimately excreted, the latter usually as conjugated products resulting from phase II reactions. The two primary routes of excretion (the urinary system and the biliary system), minor routes also (such as the lungs, sweat glands, sebaceous glands, hair, feathers, and nails) and sex related routes (such as milk, eggs, and foetus) constitute the routes of excretion.

Nature of Toxic Effects:

The nature and magnitude of a toxic effect depend on many factors, amongst which are the physicochemical properties of the substance, its bioconversion, the conditions of exposure, and the

presence of bio-protective mechanisms. The last factor includes physiological mechanisms such as adaptive enzyme – induction, DNA repair mechanisms and phagocytosis. Some of the frequently encountered types of morphological and biochemical injury constituting a toxic response is listed below. They may take the form of tissue pathology, aberrant growth processes, altered or aberrant biochemical pathways or extreme physiological responses.

Inflammation is a frequent local response to irritant chemicals or may be a component of systemic tissue injury. The inflammatory response may be acute with irritant or tissue damaging materials, or chronic with repetitive exposure to irritants or the presence of insoluble particulate material. Fibrosis may occur as a consequence of the inflammatory process.

Necrosis, used to describe circumscribed death of tissues or cells, may result from a variety of pathological processes induced by chemical injury, e.g. corrosion, severe hypoxia, membrane damage, reactive metabolite binding, inhibition of protein synthesis and chromosome injury. With certain substances, differing patterns of zonal necrosis may be seen. In the liver, for example, galactosamine produces diffuse necrosis of the lobules, acetominophen (paracetamol) mainly centrilobular necrosis and certain organic, arsenicals peripheral lobular necrosis.

Enzyme Inhibition by chemicals may inhibit biologically vital pathways, producing impairment of normal function. The induction of toxicity may be due to accumulation of substrate or to deficiency of product or function.For example, organophosphate anticholinesterases produce toxicity by – accumulation of acetylcholine at cholinergic synapses and neuromuscular junctions. Cyanide inhibits cytochrome oxidase and interferes with mitochondrial oxygen transport, producing cytotoxic hypoxia.

Biochemical uncoupling agents interfere with the synthesis of high-enephosphate molecules, but electron transport continues resulting in excess liberation of energy a heat. Thus, uncoupling produces increased oxygen consumption and hyperthermia. Examples of uncoupling agents are dinitrophenol and pentachlorophenol.

Lethal synthesis occurs when foreign substances of close structural similarity to normal biological substrates become incorporated into biochemical pathways and are then metabolized to a toxic product. A classical example is fluoroacetate, which becomes incorporated in the Kreb's cycle as fluoroacetyl coenzyme A, which combines with oxaloacetate to form fluorocitrate. The latter inhibits aconitase, blocking the tricarboxylic acid various system toxicity.

Lipid peroxidation in biological membranes by free radicals starts a chain of events causing cellular dysfunction and death. The complex series of events includes oxidation of fatty acids to lipid hydro-peroxides which undergo degradation to various products, including toxic aldehydes. The generation of organic radicals during peroxidation results in a self-propagating reaction.

Carbontetrachloride, for example, is activated by a hepatic cytochrome P_{450}-dependent mono-oxygenase system to the trichloromethyl and trichloromethyl peroxy radicals; that covalently bind with macromolcules and the latter initiates the process of lipid peroxidation leading to hepatic centrilobular necrosis. The zonal necrosis is possibly related to high cytochrome P_{450} activity in centrilobular hepatocytes.

Covalent binding of electrophilic reactive metabolites to nucleophilic macromolecules may have a role in certain genotoxic, carcinogenic, teratogenic and immunosuppressive events. Important

cellular defense mechanisms exist to moderate these reactions, and toxicity may not be initiated. Receptor interaction at a cellular or macromolecular level, with specific chemical structures may modify the normal biological effect mediated by the receptor, these may be excitatory or inhibitory. An important example is effects on Ca channels. Immune-mediated hypersensitivity reactions by antigenic materials are particularly important considerations for skin and lung resulting in allergic contact dermatitis and asthma, respectively.

Immunosuppression by xenobiotics may have important repercussions in increased susceptibility to infective agents and certain aspects of tumorigenesis. Neoplasia, resulting from aberration of tissue growth and control mechanisms of cell division, and resulting in abnormal proliferation and growth, is a major consideration in repeated exposure to xenobiotics.

The terms tumorigenesis and oncogenesis are general words used to describe the development of neoplasms; the word carcinogenesis should be restricted specifically to malignant neoplasms. In experimental and epidemiological situations, oncogenesis may be exhibited as an increase in specific types of neoplasm, the occurrence of rare, or unique neoplasms or a decreased latency to detection of neoplasm. The preceding description of the nature and scope of biochemical toxicology should make it clear that the biochemistry of toxic action is a many-faceted subject, covering, all aspects from the initial environmental contact with a toxicant to its ultimate excretion back into the environment.

Modern Toxicology:

In recent years, toxicology has developed from an activity relying principally on the tools of classical pathology to observe and classify harmful effects so as to become a discipline of increasing ability to explain the effects of toxic compounds in molecular and mechanistic terms. Over the last several years, it has become apparent that many environmental toxicants exert their effects by the action or disruption of specific signalling pathways, ultimately resulting in alterations in gene expression. With the completion of the human genome project and the advent of many powerful new technologies, there has been a revolution in our understanding of these mechanisms at molecular level.

Toxicant-induced alterations in gene expression depend on receptors. Four receptors namely the Ah receptor (AhR, the constitutive Androstone Receptor (CAR), the Pregnane X Receptor (PXR), and the peroxisome Proliferator Activated Receptor (PPAR) mediate the toxicity of four broad classes of chemicals. In contrast to these specific receptor mechanisms, metals exert their toxicity through both stress response pathways and specific metal responsive transcription factors. Role of tissue selective transcription factors on the expression of xenobiotic metabolizing enzymes is now being investigated in several laboratories.

Recent developments show that toxicology is not merely a study of the effects of a variety of poisons in animals, plants and man but a multidisciplinary science embracing pathology, pharmacology, cell biology, biochemistry and public health.While dwelling with the subject for about three decades, I could witness the sustained progress made by science of toxicology. Earlier developments got smoothly integrated into modern concepts of toxicology. This communication is an attempt to review the present status of toxicology.

i. Toxicogenomics:

It is broadly defined as gene and protein expression technology that addresses pertinent issues of toxicology. The term genome has been traditionally used to define the haploid set of chromosomes in the nuclei of multicellular organisms. The study of genome is referred as genomics. The patterns by which genes and their protein products act in concert to affect function is known as functional genomics. Certain environmental stimuli will perturb the normal cellular functions of proteins and cause changes in gene expression. These kinds of environmental factors can also lead to the pathology of disease. Often the development of disease will be the result of complex mix of factors including inherent genetic susceptibility and a series of environmental changes or challenges. The term proteome was coined in 1994 by Mark Wilkins. It refers to total protein repertoire able to be expressed from a given genome. A proteome of a cell, tissue, or organ is not only different, it can express differently under particular set of conditions. Thus toxicogenomics appear challenging.

ii. Metabonomic Technology: Toxicants disrupt the normal composition and flux of endogenous biochemical in, or through, key intermediate cellular metabolic pathways. These disruptions, either directly or indirectly, alter the blood that percolates through the target tissues. The diagnostic utility of any one trace biomolecule is limited by the number of variables affecting its concentration in situ and by the common biochemical processes disrupted by toxicants. However, if a significant member of trace molecules is monitored, the overall pattern or "fingerprint" produced may be more consistent and protective than any other marker. This comprehensive information can be obtained from high field nuclear magnetic resonance (NMR) spectroscopy coupled with pattern recognition technology. Magic angle spinning NMR technology enables similar information to be garnered from tissues as well. Temporal evaluation of metabolic consequences of toxicity, coupled with genomic and proteomic technologies and metabolomics permit complete assessment of toxicity from genotype through phenotype.

iii. Pharmacogenetics: Pharmacogenetics, a term originally coined in the 1950s may now be viewed as the study of correlations between an individual's genotype and the same individuals' ability to metabolize an administered drug or compound. Genotypic variations, often in the form of single nucleotide polymorphism (SNPs), exist for many of the enzymes that metabolize drugs/chemicals. Extensive metabolism of a drug is a general characteristic of the normal population.

Poor metabolism which typically is associated with excessive accumulation of specific drugs or active metabolic products is a recessive trait requiring a functional change, such as frame shift or splicing defect in both copies of the relevant gene. Ultra-extensive metabolism which may have the effect of diminishing a drug, apparent efficacy in an individual, is generally an autosomal dominant state derived from a gene duplication or amplification. For example in cancer chemotherapy, several common drugs show wide polymorphism related metabolic variations with 30 fold or greater inter-individual variability.

iv. Molecular Toxicology: Apoptosis is a natural consequence in vivo and there is now substantial evidence that apoptosis plays an important role in the toxic effects of a number of drugs and chemicals. Numerous coherent pathways regulate cell suicidal process. Target organ toxicities, target organ apoptogenic drugs and chemicals, regulation of apoptosis at organs, cellular and subcellular and molecular levels emerges a new discipline. Since oxidative stress, caspases, caspase activated DNase, reactive oxygen species, mitochondrial and cell cycle related events are known to modulate this process, their respective roles are under investigation in several laboratories.

In-excitable and non-excitable tissues are the direct and indirect targets of many xenobiotics that produce apoptotic and necrotic cell death. Determination of the temporal and sequential relationships between the opening of the mitochondrial permeability transition (PT) pore, mitochondrial depolarization and swelling, cytochrome C release and caspase activation during cell death are critically important.

In toxicology, understanding the role and molecular mechanisms of PT pore opening will allow the development of pharmacological and genetic strategies to prevent inappropriate apoptosis as well as to initiate and control the apoptotic process for therapeutic purposes. The current evidence suggests that the PT pore is a complex of the voltage dependent anion channel (VDAC), adenine nucleotide translocase (ANT) and cyclophilin-D (CyP-D), formed at connect sites between the inner and outer mitochondrial membranes.

v. Concept of Biomarkers:

The emergence of specific biomarkers offer the promise of being able to measure signals and/or events that reflected more accurately the biology associated with exposure, effects, and susceptibility. The 1983 NRC publication formalized human health risk assessment into a four component process namely exposure, assessment, hazard identification, dose response assessment and risk characterization.

vi. Chronotoxicology:

Biological rhythms are toxicologically important because they have a positive or negative effect on all measures of normal physiological functions, and health of the individual. Circadian patterns affect the absorption of drug/toxin. Once the drug/toxin has been absorbed, it is transported to its tissue/target sites and its elimination sites. Large circadian variations have been shown in humans and rats in plasma proteins binding of a variety of drugs. In addition, drug/toxin transport can occur by their binding to red blood cells. In general, lipophilic materials pass into blood cells more rapidly than hydrophilic materials. Circadian variation with respect to drug permeability of the blood-brain barrier is of interest. Circadian rhythms in heavy metal toxicology have been described for mercury and cadmium. The level of liver microsomal benzene hydroxylase activity is highest at a particular time of the day. Carcinogenicity and teratogenicity of chemicals have also been found to be affected by circadian rhythms.

Circadian time structure is not routinely considered in toxicity testing in human or preclinical (rodent) models. Actually, they are not the only rhythms which modulate the outcome following drug or chemical exposure. Other cycles, such as fertility cycle and seasonal cycles markedly and reproducibly alter toxicity profiles. In summary, considering toxicology in the absence of these three factors within the biological time structure of living animals seems to be uneconomical, misleading and unwise.

Superinteractions:

It is now well recognized that human environmental exposures are not to single chemical. Rather humans are exposed concurrently or sequentially to multiple chemicals, by various routes of exposure and from a variety of sources. The process of carcinogenesis can be modified significantly by other chemicals. The term co-carcinogenesis was initially defined as the enhancement of neoplasm induction brought about by new carcinogenic factors, which act in conjunction with an initiating carcinogen.

Whereas the additive or synergistic effects of two or more carcinogens in neoplasm production has been defined as syn-carcinogenesis. When the toxic responses grossly exceed the expected response after an ideal substantial, the process is called as superinteraction. An important example of an environmental 'superinteraction' is that of chlordecone (CD) and CCl_4.

In this case a prior 15 days exposure to CD enhanced the acute toxicity of CCl_4 in male rats by 67-fold. This physiological/biochemical framework within which extremely potent interactions could occur is important in planning screening programmes or to predict superinteractions in toxicology/pharmacology. A brief review of studies made by the science of Toxicology from the times of its founder Paracelsus to modern times as presented in this communication might attract young workers to this wonderful discipline of science.

Toxicity Testing:

The purpose of toxicity testing is to generate information about a substance's toxic properties so that the health and environmental risks it poses can be adequately evaluated. Federal agencies use information from toxicity testing to establish acceptable concentrations of environmental agents in drinking water, to set permissible exposure limits for workers, to establish tolerances for pesticide residues on food, to register and re-register pesticides, and ultimately to protect public health and the environment. As reflected in new directives and initiatives for toxicity testing in the United States and Europe, the demand for toxicity information to provide a rational basis for regulating environmental agents has increased. At the same time, testing technologies and methods have continued to emerge. Thus, the U.S. Environmental Protection Agency (EPA) recognized the need for a comprehensive review of established and emerging toxicity-testing methods and strategies and asked the National Research Council (NRC) to conduct such a review and to develop a long-range vision and strategy for toxicity testing. In response to EPA's request, the NRC convened the Committee on Toxicity Testing and Assessment of Environmental Agents, which prepared this report.

Toxicity tests:

Toxicity tests have taken on increased importance after scientists realized that many substances are toxic to living things at levels below chemical detection limits and that there are no methods to analyze for many toxic substances. Toxicity of chemicals is determined in the laboratory. The normal procedure is to expose the test animals to the concerned chemical and measure the effect.

Route of exposure:

By ingestion (oral), application to the skin (dermal), by inhalation, gavage, or some other method which introduces the material into the body orby placing the test material in the water or air of the test animals 'environment.

Duration of exposure:

Acute: short-term exposure (hours or days) of higher doses of toxicant in a single event or in multiple events over the time period and usually produce immediate effects, depending on absorption time of the toxicant. These tests are generally conducted on organisms during a specific time period of the organism's life cycle, and are considered partial life cycle tests. Acute tests are not valid if mortality in the control sample is greater than 10%. Generally it use lethal endpoints and results are reported inEC50.

Chronic: long-term exposure (weeks, months years) of low, continuous doses of a toxicant, relative to the test organism's life span (>10% of life span), and generally use sub-lethal endpoints. Usually, slowly effects are developed in test organism. Chronic tests are generally considered full life cycle tests and cover an entire generation time or reproductive life cycle ("egg to egg"). Chronic tests are not considered valid if mortality in the control sample is greater than 20%. These results are generally reported in NOECs (No observed effects level) and LOECs (Lowest observed effects level).

Sub-chronic: chronic exposure during early, sensitive life stages of an organism that arelessthanacompletereproductivelifecycle.Itisalsocalledasearlylifestage tests, critical life stage, embryo-larval, or egg-fry tests. Early life stage tests are not considered valid if mortality in the control sample is greater than 30%.

Toxicity Endpoints: Toxicity is measured as clinical "endpoints" which include behavioral, physiological, biochemical, histological changes, as follows,

a. Mortality(death)

b. Teratogenicity (ability to cause birth defects)

c. Carcinogenicity (ability to cause cancer), and

d. Mutagenicity or Genotoxicity (ability to cause heritable change in the DNA).

Measures of Toxicity:

Median Lethal Concentration (LC50): The concentration of a chemical in an environment (generally air or water) which produces death in 50% of an exposed population of test animals in a specified time frame. It is normally expressed as milligrams of substance per litter of air or water (mg/L) or as ppm.

- **The Median Lethal Dose (LD50):** The concentration of a chemical that is expected to kill 50% of a group of organisms to which it is administered by any of a variety of methods. It is normally expressed as milligrams of substance per kilogram of animal body weight (mg/kg). One of the more commonly used measures of toxicity is the LD50. Example: LD_{50} of sugar and ethanol are 30,000 mg/kg and 13,700mg/kg.

- **Median Effective Concentration (EC_{50})**: The concentration of a chemical that is expected to have one or more specified effects in 50% of a group of organisms after a specified exposure time.

- **Median Effective Dose (ED_{50}):** The dose level at which 50 percent of the test organism have turned over is known as the ED_{50}, which means effective dose for 50 percent of the organism tested. The ED_{50} of any toxicant varies depending on the effect measured. In general, the less severe the effect measured, the lower the ED_{50} for that particulareffect.

- **Lowest Observed Effect Concentration (LOEC)**: The lowest test concentration that has a statistically significant effect over a specified exposure time.

- **No Observed Effect Concentration (NOEC)**: The highest test concentration for which no effect is observed relative to a control over a specified exposure time. In toxicology, residue tolerance levels of poisons that are permitted in food or in drinking water, for instance, are usually set from 100 to 1,000 times less than the NOEL to provide a wide margin of safety for humans.

- **Maximum Acceptable Toxicant Concentration (MATC)**: An estimated value that represents the highest “no-effect” concentration of a specific substance within the range including the NOEC and LOEC.

- **Application Factor (AF)**: An empirically derived “safe” concentration of a chemical.

- **TLV (threshold limit value)**: The TLV for a chemical is the airborne concentration of the chemical (expressed in ppm) that produces no adverse effects in organism exposed for eight hours per day to five days per week. The TLV is usually set to prevent minor toxic effects like skin or eye irritation.

- **Model animals:** Obviously toxicity is not tested in humans. Instead, animals are used to predict the toxicity that may occur in humans. Common standard aquatic test species are the fathead minnow (*Pimephalespromelas*), daphnids (*Daphnia magna*, *D. pulex*, *D. pulicaria*, *Ceriodaphniadubia*), midge (*Chironomus tentans*, C. *ruparius*), rainbow trout (*Oncorhynchus mykiss*), sheepshead minnow (*Cyprinodonvariegatu*), mysids (Mysidopsis), oyster (Crassotreas), scud (*Hyalalla Azteca*), grass shrimp (*Palaemonetes pugio*), mussels *(Mytilus*). Common standard mammalian test species include rat and dog. These species are routinely selected on the basis of availability, commercial, recreational, and ecological importance, past successful use, and regulatory use.

- **Factors:** Toxicity assessment is quite complex, many factors can affect the results of toxicity tests. Some of these factors include variables like temperature, food, light, and stressful environmental conditions. Other factors related to the animal itself include age, sex, health, and hormonalstatus.

- **Application:** Toxicity tests are used to,

    i. provide qualitative and quantitative data on adverse (deleterious) effects on organisms from a toxicant, and
    ii. assess the potential for damage and the risk associated with in a situation for a specific toxicant.

Bioassay:

The foundation of bioassays was laid down by a German physician, Paul Ehrlich. His bioassay on diphtheria antitoxin was the first bioassay to receive recognition.

Definition: A bioassay is an analytical method to determine concentration or potency of a substance by its effect on living cells or tissues.

Principle: Bioassay is a biochemical test to estimate the relative potency of a sample compound to a standard compound. Typical bioassay involves a *stimulus* (ex. drugs) applied to a *subject* (ex. animals, tissues, plants) and a *response* (ex. death) of the subject is triggered and measured. The intensity of stimulus is varied by doses and depending on this intensity of stimulus, a change/response will be followed by a subject.

Classifications:

I. ***In vivo* bioassay**: if assays are used to estimate the potency of agents by observing their effects on living animals, it is called in vivo bioassay. In vivo studies are very important both in the field and laboratory (for validation). They are based on a wide variety of end points, including cell differentiation and enzyme activities. However, it is not possible to use *in vivo* methods for routine or monitoring studies due to ethical problems, expensive, time consuming, and big installations (aquariums etc.) are needed.

II. ***In vitro* bioassays:** if assays are used to estimate the potency of agents by observing their effects on tissues (in vitro), it is called in vivo bioassay. It can be performed more quickly, and much more cost-effectives than *in vivo* assays. However, *in vitro* assays are not able to explain all the mechanisms.

III. **Direct assay:** The stimulus/standard sufficiently produces measurable and specific response. The response must be clear, easily recognized, and directly measured.

IV. **Qualitative bioassay:** If the measured response is binary, the assay is qualitative, if not, it is quantitative.

V. **Indirect assay based on quantitative response**: The relationship between the dose and the response is first ascertained. Then the dose corresponding to a given response is obtained from the relation for each preparation separately.

VI. **Indirect assay based on quantal response:** The assay involves 'all or none' response (ex. life or death). The response is produced by threshold effect.

Examples:

1. **Plant and algae bioassay:** Test species, such as marine unicellular algae *Selenastrum capricornutum* or *Dunaliella tertiolecta* are used as indicator species. Inhibition of algal growth is used as the indicator of toxicity. The main disadvantage of algal methods is a lack of reproducibility between consecutive assays.

2. **Invertebrate bioassays:** Chronic toxicity test using macro invertebrates have been extensively used in aquatic risks assessment studies. The parameters measured are mortality or reproduction. One of the most common invertebrate toxicity tests uses *Daphnia* and *Ceriodaphnia*, both freshwater species pertaining to *Cladocera*. Tests are carried out by exposing the test organisms to toxic substances under control conditions. Acute lethality tests with Daphnia conducted for 21 days are well established and standardized.

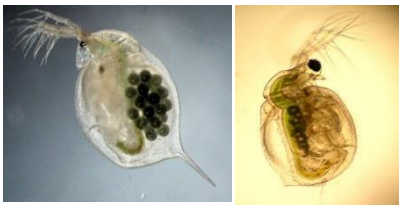


Fig: *Daphnia magna* and *Ceriodaphnia* sp. from left to right.

3. **"In vivo" Fish toxicity bioassays:** Zebra fish, medka, rainbow trout and fathead minnow are generally used in toxicological study. End Points of test includes, mortality (routinely used, 96 hr exposure), larval growth, larval survival, and reproduction. In vivo assays for estrogenicity are widely used. They are based on a wide variety of end points, including cell differentiation and enzyme activities. Vitellogenin (VTG) analysis is done by means of Immunoassay or any other analytical approach.

4. **"In vitro" Recombinant yeast assay:** This assay is based on the evaluation of the potential of a compound to interact with oestrogen receptor and activate hormone-regulated gene promoters. Yeast reporter assay is based on a two-hybrid system. Beta-galactosidase, has been used as the most common reporter enzyme. Novel yeast reporter assay are more suitable for high-throughput analysis, employing in the reporter assay luciferase, named CLuc, as a reporter enzyme.

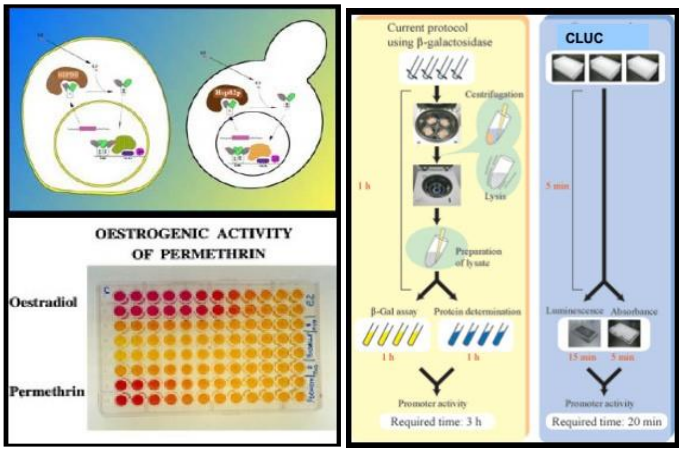


Fig: "In vitro" Recombinant yeast assay.

5. **Bacterial toxicity assays:**

a) Bioluminescence inhibition: The more widely used bioassays in routine laboratories for evaluating toxicity of wastewater effluents and industrial discharges are based on inhibition of the bioluminescence of marine bacteria. The better-known species of luminescent marine bacteria are *Vibrio fischeri* and *Photobacterium phosphoreum*, which naturally emit light due to an enzyme, the bacterial luciferase. This technique allows the easy screening of large numbers of aqueous samples in a quick, reliable, and inexpensive way.

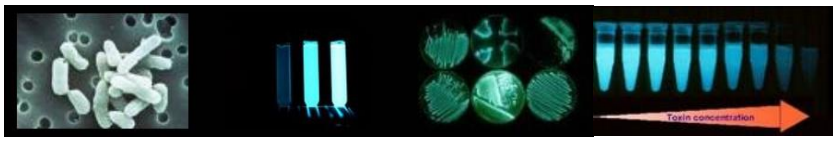


Fig: Bacterial toxicity assays: Bioluminescence inhibition

b) Genotoxicity Ames Test and umu test*:* Genotoxicity is associated with different structures, such as phenols, chlorophenols, polychlorinated biphenyls (PCBs), or polyaromatic hydrocarbons (PAHs), and constitutes an early screening for possible cancer inducing activity of pollution. The most widespread is the Ames test that is based on the retromutation of *Salmonella typhimurium* TA98 (histidine dependent). The umu test is also based on genetically engineered bacteria *Salmonella thyphimurium* TA 1535 pSK1002 (gram negative, facultative anaerobic enterobacteriaceae) and the genotoxicity is detected measuring the activation of the bacterial SOS repair response of genetic damage in the bacterium, through measuring b-galactosidaseactivity.

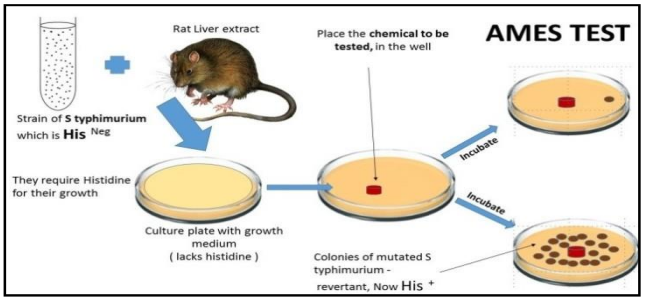


Fig: Bacterial toxicity assays: Genotoxicity Ames Test.

6. **ELISA (Enzyme-linked immunosorbent assay)**: quantitative analytical method that measures absorbance of colour change from antigen-antibody reaction (ex. Direct,indirect, sandwich, competitive). ELISA is used to measure variety of substances in human body from cortisol levels for stress to glucose level fordiabetes.

Uses: Bioassay is used for

1. To test carcinogenicity of chemicals.
2. To test toxicity and safety of drugs, food additives and pesticides.
3. To detect biological hazards.
4. Give a quality assessment of a mixture.
5. To monitor water quality and also sewage discharge and its impact on surrounding.
6. To assess the environmental impact and safety of new technologies and facilities.

Reliance on bioassay increased as the public concern for occupational and environmental hazards increased.

Determination of LC_{50}:

Different procedures are adopted depending on the size of the organisms subjected to a toxicant.

Mosquito Larvae (Test Animal):

Four 1000 ml glass beakers are half-filled with tap water/tank water. A measured amount of toxicant-liquid/solid—is thoroughly mixed with water in three beakers—1, 2, 3 to prepare an aquatic medium of toxicant of three known concentrations.The concentration of the toxicant in the three beakers should be in a gradually increasing order from a lower to higher level. The fourth beaker containing only water is used as a control. Properly label the beakers with necessary information.

A batch of 10 mosquito larvae of same age and size are released in each beaker.

Observe the behaviour—movement, irritability, etc. of the larvae both in test and control beakers and record those with reference to time of exposure.

Continue observation, count the dead larvae in each beaker and record the time of death till the mortality reaches 50%.

The period of 50% mortality is dependent on the concentration of the toxicant.

A dose-response curve for each dose may be prepared by plotting the period of exposure on x-axis (abscissa) and the number of dead larvae on the y-axis (ordinate).A dose — 50% mortality time curve may be prepared by plotting dose (concentration of toxicant) on x-axis and time for 50% mortality on y-axis.

Major Carp Fingerlings (Test Animal):

Four aquaria measuring 75 x 30 x 50 cm each are filled with tank water, preferably from a fish fingerling culture pond up to a height of 45 cm. A measured quantity of toxicant—liquid/ solid is thoroughly mixed with water in three aquaria — 1, 2, 3 to prepare an aquatic medium of toxicant of known concentration.
The concentration of the toxicant in the aquaria should be in a gradually increasing order from a lower to higher level. The fourth aquarium containing only water is used as a control. Properly label the aquaria with necessary information. All the aquaria are covered with mosquito net to prevent jumping out of fingerlings.

A batch of 10 healthy fingerlings — size 75 mm — are released in each aquarium. Regular food supply and aeration of aquarium water is maintained.

Observe the behaviour—movements irritability, loss of balance, etc. of the fingerlings — both in test and control aquaria and record these with reference to the time of exposure.

Continue observation, count the dead fingerlings in each aquarium and record the time of death till the mortality reaches 50%.

The period of 50% mortality is dependent on the concentration of the toxicant.

A dose-response curve for each dose may be prepared by plotting the period of exposure on x-axis and number of dead fingerlings on y-axis.

Determination of LD_{50}:

Albino Mice (Test Animal):

Four batches of healthy albino mice, each consists of six individuals of almost same age group and weight are taken. The toxicant may be used in solid/liquid state. The doses are prepared by mixing the toxicant with food at the ratio mg (toxicant)/kg (weight of mice) — 1 mg/kg, 2 mg/kg, 3 mg/kg.

The total amount of food for each concentration of toxicant should be such that it can be consumed by 6 mice of a batch in one meal. Six baits are prepared for each concentration. The total number of baits will be 3 x 6 = 18. Six baits are prepared without toxicant for the control batch.

The animals are not given food for about 12 hours prior to commencement of the experiment. The control group is treated in the same way.

The fasting animals are given the bait—one for each mice and water. The control batch is given bait without toxicant and water.

Observe the behaviour—movement, irritability, lack of activity, balance, etc. in both the test and control mices and record those with reference to the period of exposure.

Continue observations, count the dead mices in each batch and record the time of death, till the mortality reaches 50%.

The period of 50% mortality is dependent on concentration of the toxicant.

A dose-response curve for each dose may be prepared by plotting the period of exposure on x-axis and the number of dead mices in y-axis[Dose-50% mortality time curve]

Types of Interactions among toxicants:

When toxic chemicals and substances come in contact with each other, chemical reactions occur. These reactions can be divided into one of four categories: additive, synergistic, antagonistic, and potentiating.

I. Additive effects: The sum of the effects of the chemicals involved in the reaction. This usually occurs with chemicals that are similar in structure, so they work well as a team. The sum of the additive effects is sum of the effects exposed to each chemical individually.

Example: If you take aspirin and acetaminophen both together, you get the total effect of both pain-killing drugs on your body. Aspirin and acetaminophen, are the active ingredient in drugs like Tylenol.

II. Synergistic effects are when the sum of the effects is more than each chemical individually. This can create dangerous situations because each chemical is designed to work well on its own.

Example: Alcohol and acetaminophen are a dangerous combination for your body. This is because both are processed in your liver, and each puts a lot of strain on this small but powerful organ. If you put both drugs into your body at the same time, it can overwhelm the liver, sending it into failure.

III. Antagonistic effects are when the net effect of the chemical reaction is zero. If one is positive and another is negative, both neutralize each other's effect. Antagonistic effects are important because this is where we get antidotes for poisons.

Example: Anti-venom for snakebites and combination of caffeine and alcohol show antagonistic effect.

IV. Potentiating effects: This is when one chemical enhances the effect of another chemical. Some chemicals are not toxic on their own, but when they are in the presence of some other chemicals, they become toxic. This is one more less-common type of interaction.

Table 1. Types of interactions between toxic chemicals and substances.

additivity	a combination of two or more chemicals is the sum of the expected individual responses
antagonism	exposure to one chemical results in a reduction in the effect of the other chemical
potentiation	exposure to one chemical results in the other chemical producing an effect greater than if given alone
synergism	exposure to one chemical causes a dramatic increase in the effect of another chemical

Other Types of Toxicity Tests:

1. **Bioaccumulation tests** are toxicity tests that can be used for hydrophobic chemicals that may accumulate in the fatty tissue of organisms. Toxicants with low solubility in water generally can be stored in the fatty tissue due to the high lipid content in this tissue. The

storage of these toxicants within the organism may lead to cumulative toxicity. Bioaccumulation tests use bioconcentration factors (BCF) to predict concentrations of hydrophobic contaminants in organisms. The BCF is the ratio of the average concentration of test chemical accumulated in the tissue of the test organism (under steady state conditions) to the average measured concentration in the water.

2. **Effluent toxicity tests** are conducted under the Clean Water Act, National Pollutant Discharge Elimination System (NPDES) permit program and are used by wastewater dischargers. Acute Effluent Toxicity Tests are used to monitor the quality of industrial effluent monthly using acute toxicity tests. Effluent is used to perform static-acute multi concentration toxicity tests with *Ceriodaphniadubia* and *Pimephalespromelas*. The test organisms are exposed for 48 hours under static conditions with five concentrations of the effluent. Short-term Chronic Effluent Toxicity Tests are used to monitor the quality of municipal waste water treatment plants effluent quarterly using short-term chronic toxicity tests. It lasts for seven days. The goal of this test is to ensure that the wastewater is not chronically toxic. Short term sublethal tests are used to evaluate the toxicity of effluents to aquatic organisms. These methods are developed by the EPA (Environmental Protection Agency), and only focus on the most sensitive life stages. Endpoints for these tests include changes in growth, reproduction and survival and results are reported in NOECs, LOECs and EC50s.

Probable Questions:

1. Define toxicology. How toxicology was developed.
2. State interrelationship between toxicology and other science disciplines.
3. What are scopes of toxicology.
4. How toxins are metabolized?
5. Briefly state the nature of toxic effects.
6. What is toxico-genomics?
7. What is pharmacogenetics?
8. Define molecular toxicology.
9. What is chronotoxicology?
10. How toxic products are eliminated?
11. What is acute, chronic and sub chronic toxicity?
12. How toxicity is measured?
13. Define bioassay? State the classification of bioassay.
14. How bacterial toxicity is assessed?
15. What is in vitro recombinant yeast assay?
16. What is in vivo fish toxicity bioassay?
17. What are the uses of bioassay?
18. State an experiment on fish by which you can determine LC_{50}
19. State an experiment on fish by which you can determine LD_{50}
20. What is additive effect of toxicity? Give examples.
21. What is synergistic effect of toxicity? Give examples.
22. What is antagonistic effect of toxicity? Give examples.
23. What is Potentiating effects of toxicity? Give examples.
24. What is bioaccumulation test?
25. What is Effluent toxicity tests?

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett & Doull's Toxicology: The Basic Science by Curtis D. Klaassen

UNIT-II

Carcinogenic, Mutagenic and Teratogenic effects, Method of testing chemicals on insect and evaluation of toxicity

Objective: In this unit we will learn about Carcinogenic, Mutagenic and Teratogenic effects of different chemicals.

Carcinogen:

Carcinogen definition can be given as the substances, radionuclide, or the radiations, which are involved directly in the formation of cancer, are known as carcinogenic substances, otherwise, as a carcinogen, and this entire process is known to be carcinogenicity. These carcinogenic substances can damage the genome or even disrupt the cells involved in the metabolism process. Various radioactive substances are considered carcinogenic, but these substances' carcinogenic behavior is caused by the radiation they emit. Alpha particles and Gamma rays are the carcinogens examples or examples of carcinogenic substances. Also, we have non-radioactive carcinogens such as certain dioxins, tobacco smoke, and the inhaled asbestos. Note: Tobacco smoke produces harmful gases such as carbon monoxide, cancer-causing substances. Often, carcinogenic substances are thought of as synthetic chemicals or chemical carcinogens, but they can be synthetic and natural in reality. These substances need not be toxic immediately, as they are insidious. Carcinogenic Substances Cancer is a disease group that causes abnormal cell growth to spread to other parts of the human body. It is a disease where the body cells get damaged. In general, carcinogenic substances increase cancer risk because they damage the body's metabolic cells. Also, they damage the cell's DNA component, which is associated directly with various biological processes in the body, leading to tumors.

Aflatoxin B1, which is produced by a fungus, grows on the surface of peanut butter, grains, and many nuts. It is also a microbial carcinogenic substance that occurs naturally. Also, the virus hepatitis B and human papilloma can cause cancer to the person infected by them. Besides the virus, radiations, and fungus, there are various carcinogenic substances. The substances like polynuclear hydrocarbons and benzene, which have more than two benzene rings fused together, also contain carcinogenic effects. These polynuclear hydrocarbons form when incomplete combustion of organic material like coal, tobacco, and petroleum occurs. These substances undergo biochemical reactions by entering into the human body, which damages the DNA cells and causes cancer, further leading to death. Toxicity is the degree to which a chemical can damage the body's human cells. We have seen the carcinogenicity effect and the substances associated with it. These are highly toxic substances in nature, and their use should be avoided to sustain a healthy body.

Identifying Chemicals that Cause Cancer:

We seem to be surrounded by a sea of chemical carcinogens. They are found in the air we breathe, the food we eat, the water and beverages we drink, the medications we take, the places where we work, and the homes in which we live. However, this assessment—while technically correct—

conveys the misimpression that we are faced with severe hazards everywhere we look and that these dangers cannot be avoided.

In fact, many of the carcinogens we normally encounter are only weakly carcinogenic, and most of the more potent ones can be easily avoided by the general public. So rather than lumping all chemical carcinogens together, we need to consider them as individual molecules and make informed judgments about the dangers posed by each one.

Discovery of Chemical Carcinogens:

The first indication that chemicals might cause cancer came from the observations of doctors who, in their struggle to understand the nature of the disease, asked cancer patients a variety of questions about their backgrounds, experiences, and habits. This allowed physicians to gain some impressions, if not firm evidence, about the possible causes of cancer.

Such an approach led a London doctor, John Hill, to point to chemicals as a probable cause of cancer more than two hundred years ago. In 1761, Hill reported that people who routinely use snuff— a powdered form of tobacco that is inhaled—suffered an abnormally high incidence of nasal cancer, suggesting the existence of one or more cancer-causing chemicals in tobacco. Several years later Percival Pott, another British physician, reported an unusual prevalence of oozing sores on the scrotums of men coming to his medical practice in London. While a less astute observer might have thought it was just one of the venereal diseases that were widespread at the time, Pott's close examination of the sores revealed that they were actually a form of skin cancer.

Careful questioning revealed that the men with this condition shared something in common. They had all served as chimney sweepers in their youth. It was common practice at the time to employ young boys to clean chimneys because they fit into narrow spaces more readily than adults. Pott therefore speculated that chimney soot chemicals had become dissolved in the natural oils of the scrotum, irritating the skin and eventually triggering the development of cancer. These ideas led to the first successful public health campaign for preventing a particular type of cancer- Scrotal cancer was virtually eliminated by promoting the use of protective clothing and regular bathing practices among chimney sweeps.

In the years since these pioneering observations, it has become increasingly evident that certain kinds of chemicals can cause cancer. Unfortunately, the ability of a particular chemical to cause cancer has often become apparent only after large numbers of cancers arise in people exposed to that chemical on a regular basis. For example, in the early 1900s elevated rates of skin cancer were noted among workers in the coal tar industry, and an increased incidence of bladder cancer was seen in factories that produced aniline dyes. The experience in the aniline dye industry was especially dramatic and led to the discovery of several basic principles of chemical carcinogenesis, as will now be described.

Workers who developed the First Cancer:

The late 1800s witnessed the birth of a series of new chemical industries that for the first time exposed large numbers of workers to high concentrations of toxic substances. A prominent example involved the industrial production of dyes used to color clothing and other fabrics.

Prior to the mid-1800s, most dyes were natural substances extracted from vegetable or animal sources. An accidental discovery made in 1856 by William Perkin, however, led to the birth of the synthetic dye industry and the first mass exposure of workers to potent carcinogenic chemicals. Perkin was attempting to synthesize quinine, a drug for treating malaria, by carrying out chemical reactions on substances present in coal tar (a thick, black liquid formed during the distillation of coal). In one experiment, he extracted aniline from coal tar and oxidized it with potassium dichromate.

The result was a dark brown precipitate. Most nineteenth-century chemists would have discarded any such dark masses of material because scientists were generally interested in clear, crystalline products. But Perkin was instinctively curious and decided to investigate further. To his pleasant surprise, dissolving the dark sludge in alcohol yielded an intense purple solution that exhibited strong dying properties. Perkin had discovered aniline purple, the first synthetic dye. Within a few years, coal tar had yielded several other dyes and the aniline dye industry was born. Chemists quickly discovered that a compound related to aniline called 2-naphthylamine is an ideal starting material for the synthesis of many dyes, and large-scale production began in Germany around 1890. Unfortunately, factory employees working with 2-naphthylamine soon began developing bladder cancer in alarming numbers (Figure 1).

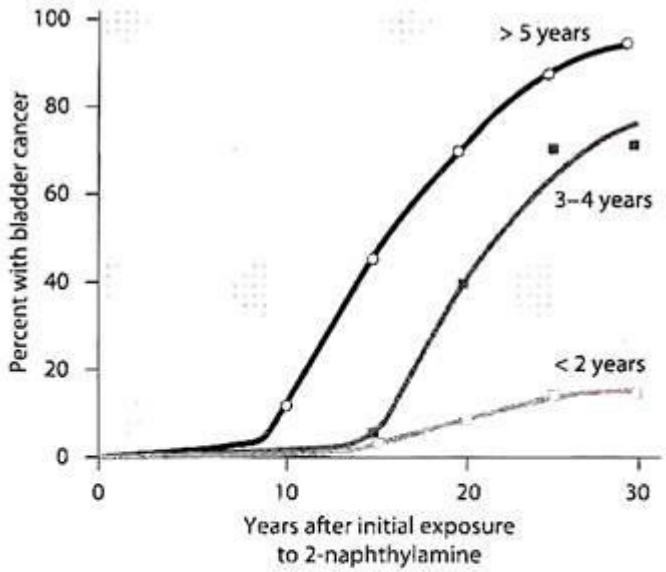


Figure 1 Relationship Between 2-Naphthylamine Exposure and Bladder Cancer in Factory Workers. These data show bladder cancer rates in three groups of men exposed for varying amounts of time to 2-naphthylamine in the workplace. Note that in the group of men with the longest exposure (more than 5 years), almost every person eventually developed bladder cancer. [Data from J. Cairns, *Cancer: Science and Society* (San Francisco: Freeman, 1978), p. 56.]

In one small factory, all 15 workers developed the disease. A vigorous and protracted debate ensued as to whether 2-naphthylamine was actually responsible because bladder cancer also occurs among the general public and there was little precedence for using epidemiological data to infer cause and effect. Eventually the cancer-causing ability of 2-naphthylamine was demonstrated in convincing fashion, using both epidemiological and animal data, but it took almost 50 years before the large-scale production of this highly potent carcinogen was stopped.
Bladder cancer triggered by occupational exposure to 2-naphthylamine was the first example of a human cancer known to be caused by a specific chemical compound. The introduction of 2-naphthylamine into the workplace was also a key event because it marked the beginning of a massive increase in industrial chemical production and illustrated some important principles that are widely relevant to chemical carcinogenesis.

One of these principles involves the long delay that is typically observed between exposure to a chemical carcinogen and the onset of cancer. Few cases of bladder cancer were seen in factory workers until 10 years after initial exposure to 2-naphthylamine, and most cases took between 15 and 30 years to appear. Such a long delay is typical of chemical carcinogenesis and reflects the multiple events that take place on the road to developing cancer. Another principle illustrated by the experience with 2-naphthylamine is dose dependence- Workers who were exposed to the chemical over a longer period of time, and hence had a larger total exposure, exhibited higher bladder cancer rates than workers with shorter exposures.

Figure 1 reveals that almost every worker in the group with the longest exposure to 2-naphthylamine eventually developed bladder cancer. If virtually everyone develops cancer under such conditions, it means that hereditary differences did not play a significant role in determining risk.Finally, the experience with 2-naphthylamine illustrated the organ specificity that is a common feature of chemical carcinogenesis. Instead of causing cancer in general, chemical carcinogens tend to preferentially cause a few particular types of cancer. In the case of 2-naphthylamine, the bladder is the prime target. We have already encountered other examples of organ specificity and will encounter additional examples later. Organ specificity is generally caused by the selective ways in which chemicals make contact with, or accumulate in, certain body tissues. For example, chemicals that become concentrated in the urine are likely to produce bladder cancer, and carcinogens that are inhaled tend to cause lung cancer. The ability of cigarette smoke to cause many kinds of cancer in addition to lung cancer may seem to violate this principle; tobacco smoke, however, contains more than 40 different carcinogens, and some of these accumulate in tissues other than the lung.

Asbestos as a Cause of Cancer Deaths:

The natural mineral asbestos is a particularly striking example of an organ-specific carcinogen. Commercial use of asbestos began in the late 1800s, when large deposits of asbestos rock were discovered in Canada and shipped to the United States and to the newly industrializing countries in Europe. Crushing the rock yields a mixture of fine fibers that can be woven into materials that exhibit excellent insulating and fire-retarding properties.

The most commonly used form of asbestos has the formula $(Mg,Fe)_3Si_2O_5(OH)_4$, but many chemical variations exist. Numerous fireproof products, ranging from oven mittens and fireproof clothing to various kinds of construction materials, have been manufactured with asbestos. Unfortunately, the widespread use of asbestos has had severe health consequences. Asbestos readily breaks down into a fine dust containing numerous sharp, needle-like fibers that are so tiny that they can only be seen with an electron microscope. These "needles of death" are easily inhaled and become lodged in the lung, where they cause scarring that kills people through suffocation.
Shortly after this disease, called asbestosis, was first recognized among asbestos workers in the 1920s, the same workers began to develop lung cancer. Because cigarette smoking was not yet popular, lung cancer was still rare, and the connection between the cancer outbreak and exposure to asbestos was therefore easy to detect.

Scientists eventually found that asbestos and cigarette smoke interact synergistically in causing lung cancer. As a result, smokers who have been heavily exposed to asbestos exhibit lung cancer rates that are 50 times higher than is observed in people who do not smoke or have significant exposure to asbestos. An unusual property of asbestos is its ability to cause mesothelioma, a rare form of cancer derived from the mesothelial cells that cover the interior surfaces of the chest and abdominal cavities. This type of cancer was uncommon prior to the 1950s, when the first mesothelioma epidemic was reported in and around a group of asbestos mines in South Africa.

Mesotheliomas were subsequently detected in many locations around the world; virtually everywhere that asbestos is used. An increased risk for mesothelioma is exhibited mainly by asbestos workers and by individuals who experience significant exposure to asbestos either by living in neighborhoods surrounding asbestos factories or by working or living in asbestos-insulated buildings. At present, asbestos is the only clearly established cause of mesothelioma. Microscopic examination of lung tissue obtained from asbestos workers has revealed that mesothelioma is caused by a rather unusual mechanism. Tiny, microscopic fibers of inhaled asbestos become embedded in the lung and gradually penetrate completely through the lung tissue, emerging into the chest cavity.

Here the asbestos fibers trigger a chronic irritation and inflammation that promotes the development of cancer in the mesothelial cells that cover the lungs and line the interior chest wall. In a similar fashion, asbestos fibers that have been inadvertently ingested can penetrate through the walls of the stomach and intestines, emerging into the abdominal cavity and triggering the development of abdominal mesotheliomas.When the fatalities caused by asbestos- induced mesotheliomas and lung cancers are combined, asbestos ranks as the second most lethal commercial product (after tobacco) in terms of the number of cancer deaths caused. Governmental actions to regulate the production and use of asbestos began in earnest in the 1960s and have become progressively more restrictive in many countries, so the incidence of asbestos-induced cancers should eventually begin to decline.Nonetheless, mesothelioma deaths are still rising (Figure 2) and will probably continue to do so for several decades because a lag period of 30 or more years can intervene between asbestos exposure and developing cancer. Moreover, countries that formerly used asbestos still contain vast reservoirs of the carcinogen in existing buildings.

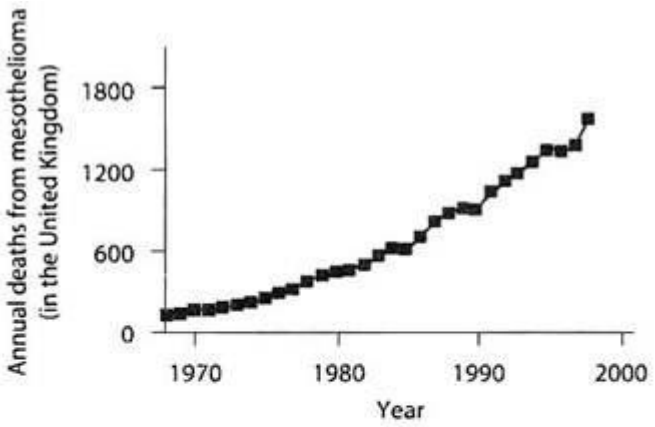


Figure 2 Death Rate Trends for Mesothelioma.
Despite increasing restrictions on the production and use of asbestos, deaths from mesothelioma (a cancer induced almost solely by asbestos exposure) continue to rise. The most likely reasons are that a long delay can intervene between asbestos exposure and developing cancer and that vast reservoirs of asbestos remain behind in existing buildings. Data are for the United Kingdom. [Data from G. Tweedale, *Nature Reviews Cancer* 2 (2002): 311 (Figure 2).]

Workplace Exposure to Chemical Carcinogens as a Cause of Cancer:

As in the case of 2-naphthylamine and asbestos, carcinogenic chemicals are often identified only after a particular type of cancer starts to appear in people exposed to a specific substance in high doses. Once such observations point to a particular chemical as a potential carcinogen, follow-up animal testing is carried out to determine whether the substance really causes cancer.

Beginning around 1900 with 2-naphthylamine in the aniline dye industry, the list of known chemical carcinogens grew progressively as the Industrial Revolution proceeded throughout the twentieth century and unusual cancer patterns began to emerge among workers in various industries. Table 1 lists some of the main occupational carcinogens that were eventually discovered, including examples from the rubber, chemical, plastic, mining, fuel, and dye industries. Workplace exposure to occupational carcinogens was substantial in the first half of the twentieth century before the cancer risks from such agents came to be fully appreciated. In a few extreme cases, all the workers exposed to the chemicals present in certain factories eventually developed cancer.

However, most of the currently known occupational carcinogens were identified by the 1970s and relatively few new ones have been identified since then. In 1970, an act of the United States Congress created the Occupational Safety and Health Administration (OSHA) to formulate regulations designed to protect the safety and health of workers.OSHA has worked to eliminate the most dangerous chemicals from the workplace and to limit worker exposure to other chemicals. As a result, many occupationally induced cancers that were once prevalent in the United States have declined in frequency, and workplace exposure to carcinogens now accounts for less than 5% of all cancer deaths.

While a similar pattern is evident in many other industrialized nations, progress has been far from uniform. To illustrate some of the disparities, the use of asbestos in Nordic countries has decreased dramatically in recent decades, falling to a negligible value of 4 grams per person in 1996; in that same year, asbestos use in the former Soviet Union was 600 times higher at a value of 2400 grams per person. In general, exposure to industrial carcinogens is a greater problem in developing countries, where less progress has been made in regulating the workplace use of toxic chemicals.

Environmental Pollution not a Major Source of Cancer Risk:

Cancers arising in the workplace are usually triggered by sustained high-dose exposures to specific chemical carcinogens. Small amounts of the same chemicals are also released inadvertently into the environment, where they contaminate the air we breathe, the water we drink, and the food we eat. It has therefore become fashionable to blame industrial pollution for creating a growing cancer threat of epidemic proportions.

However, there are reasons to question such a far-reaching conclusion. First, cancer risk is related to carcinogen dose, and the doses of industrial carcinogens to which the public is exposed are generally orders of magnitude lower than is encountered in the workplace. For example, consider the pesticide ethylene dibromide (EDB), which is designated by the U.S. government as a probable human carcinogen based on its ability to cause cancer in animals.

Workers who have experienced high-dose exposures to this suspected carcinogen encountered about 150 milligrams (mg) per day, whereas EDB residues in food (before EDB was banned in 1984) exposed the average person to a daily dose of 0.00042 mg, which is 300,000 times less than the workers' daily intake. A similar situation exists for most of the other chemical contaminants in our food, air, or water, which do not represent a major cancer threat because they are present in concentrations thousands of times lower than typical industrial exposures.

Another reason to question the assumption that pollution represents a major cancer hazard is based on historical trends. If industrial pollution were a major cancer threat to the general public, one would have expected a significant increase in overall cancer rates during the twentieth century in response to the explosive growth in the use of industrial chemicals.

For example, the yearly production rates of plastics, pesticides, and synthetic rubber in the United States increased more than 100-fold between the 1940s and the 1970s. Any impact the chemicals used in these industries might have had in causing a cancer epidemic through environmental pollution should have been apparent by now. In fact, when the data are adjusted for the increasing average age of the population, it is clear that a significant growth in age-adjusted cancer rates has not occurred (Figure 3). Most of the cancers that are common today were also common one hundred years ago, and the main exception, lung cancer, is triggered by cigarette smoke and has little to do with industrial pollution.

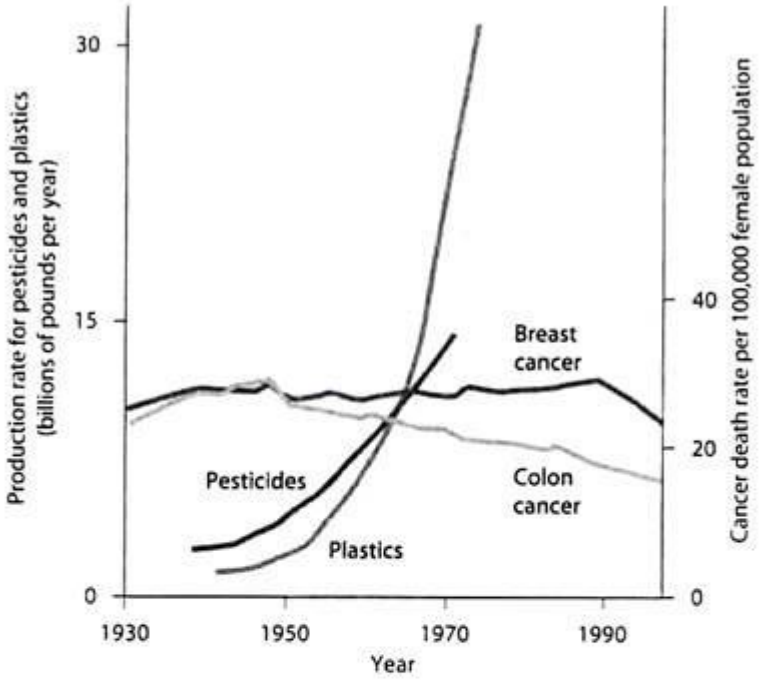


Figure 3 Patterns of Industrial Pollution and Cancer Death Rates in the United States. Industrial use of chemical carcinogens, as occurs in the plastics and pesticide industries, increased dramatically between the 1940s and 1970s. For most types of cancer, there was no corresponding increase in cancer incidence during the following decades. If industrial pollution had a major effect on cancer rates, it should have been evident by now. Mortality rates for breast and colon cancer in women are shown, but many cancers exhibit a similar pattern. The only cancer that has shown a major increase in incidence is lung cancer, which is caused mainly by tobacco smoke rather than industrial pollution. [Based on data from *Cancer Facts & Figures 2002* (Atlanta, GA: American Cancer Society, 2002), p. 3; and R. H. Harris et al. in *Origins of Human Cancers* (H. H. Hiatt et al., eds., Cold Spring Harbor, NY: CSHL Press, 1977), pp. 309–330 (Figure 2).]

Risks from Low-Dose Exposures to Chemical Carcinogens as a Cause of Cancer:

The preceding arguments suggest that for most people other than those who receive high-dose exposures by working with or living near a concentrated source of toxic chemicals, the cancer risks posed by environmental chemicals are relatively small. This does not mean, however, that the risk for the average citizen is zero.

If the public were being routinely exposed to low carcinogen doses that cause a tiny fraction of the population to develop cancer, such small effects would be difficult to detect using traditional epidemiological methods. In fact, a weak carcinogen present in the environment could theoretically cause hundreds or even thousands of cancer cases each year in a country of several hundred million people without being noticed. Assessing the actual risk, if any, from low-dose exposures to known or suspected carcinogens is a difficult task. To illustrate, let us briefly consider the dioxins, a family of chlorinated chemicals produced as a by-product during the burning of municipal wastes, the bleaching of paper, and the production of herbicides. Several epidemiological studies have demonstrated increased rates of cancer in workers exposed to large concentrations of dioxin, and high-dose animal studies have also shown increased cancer rates.

Although the quantities released into the environment are quite small, dioxins are stable molecules that tend to persist for long periods of time, accumulating in the food chain. For humans the main source of exposure is through the food we eat especially fatty meats. The ingested dioxin molecules are fat soluble and are metabolized slowly, so they tend to accumulate in our body fat. The net result is that even tiny exposures to dioxin can lead to significant concentrations inside the body.

The large unanswered question is whether this accumulated dioxin represents a significant cancer risk. The most conservative approach has been to assume that even one molecule of a carcinogen can cause cancer—in other words, that there is no safe dose of dioxin. However, testing in rats has revealed that while high doses of dioxin cause cancer, low doses can sometimes decrease cancer rates compared to those observed in control animals. Such data indicate that it is possible for low-dose carcinogen exposures to be safe, and perhaps even beneficial. Unfortunately, differences between humans and rodents in mode of exposure, metabolism, and genetics make it difficult to extrapolate such results to humans exposed to tiny amounts of dioxin. In other words, we don't really know whether the tiny amount of dioxin that we typically encounter is a small cancer risk, poses no cancer risk, or perhaps even decreases our risk of developing cancer.

Another family of environmental contaminants that might represent a cancer hazard are the organochlorine pesticides, a group that includes the now-banned insecticide dichlorodiphenyltrichloroethane (DDT). Compounds of this type can mimic the action of estrogen, which is known to promote the development of breast cancer. Animals exposed to organochlorines exhibit increased cancer rates, and several widely quoted epidemiological studies have detected a correlation between exposure to organochlorine pesticides and breast cancer rates in women. However, most epidemiological studies have failed to detect such a relationship. Some especially interesting data emerged from a study of several thousand women in Long Island, an area in which organochlorines were extensively used and in which breast cancer rates are higher than the national average. To precisely quantify exposure to organochlorine pesticides, blood samples were obtained from breast cancer patients and from a group of comparable women without the disease. Measurements of the concentration of organochlorines in the blood failed to reveal any relationship between exposure to organochlorine compounds and the development of breast cancer.

Pollution of Outdoor and Indoor Air Creates Small Cancer Risks:

The general topic of air pollution provides yet another example of the difficulties encountered when trying to assess environmental cancer hazards involving low-dose exposures. In many cities, both large and small, the air is contaminated with fine particles of airborne soot emitted by cars and trucks, power plants, and factories.

A recent epidemiological study of 500,000 adults living in dozens of cities across the United States revealed that people located in cities with the largest amounts of this fine-particle soot have lung cancer death rates roughly 10% higher than in cities with minimal pollution.Of course, a 10% increase is not very much; for comparison purposes, cigarette smokers increase their risk of developing lung cancer by 2500% or more, a value that is 250 times higher than the small increase in lung cancer risk that might be associated with air pollution.

Although discussions of air pollution usually focus on outdoor air, our main exposure to polluted air may be indoors. In studies involving more than a dozen different cities, researchers equipped people with air-quality monitoring devices that were small enough to carry around as people performed their normal daily activities. For the average citizen, the greatest exposure to toxic airborne chemicals turned out to occur inside their homes (Figure 4). The sources of this indoor air pollution included ordinary consumer products such as cleaning compounds, paints, carpeting, gasoline, air fresheners, dry cleaning, and disinfectants.

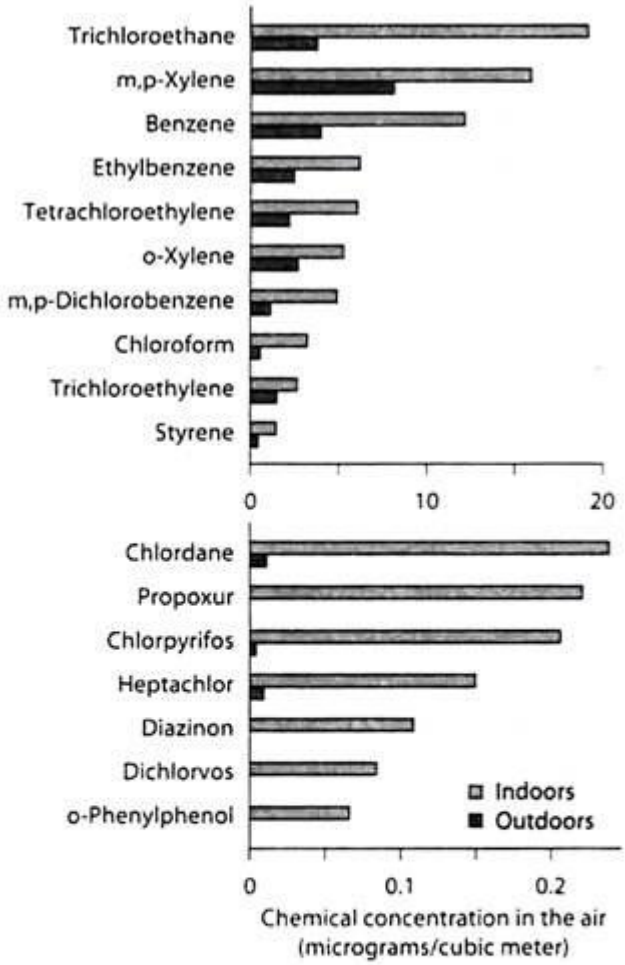


Figure 4 Comparison of Indoor and Outdoor Air Pollution. Pollution data were obtained from people equipped with portable air-quality monitoring devices designed to measure the concentration of toxic volatile organic chemicals (*top*) and pesticides (*bottom*) in indoor and outdoor air. Results from studies involving more than a dozen cities in the United States, including cities with chemical processing plants, have revealed that the air inside a person's home usually contains higher concentrations of potential carcinogens than are present in outdoor air. [Data from W. R. Ott and J. W. Roberts, *Sci. Amer.* 278 (February 1998): 88.]

Even in cities where the outdoor air was polluted by emissions from chemical processing plants, the concentration of many airborne carcinogens was higher inside homes than outdoors. However, these indoor concentrations were still much lower than those typically encountered in industrial workplaces, and it is difficult to know whether such low-dose exposures pose any cancer risks

Thresholds can Cause Animal Studies to Overestimate Human Cancer Risks:

The difficulty in assessing the hazards of low-dose chemical exposures arises from limitations that are inherent to epidemiological and animal testing. The main problem with the epidemiological approach is that it is not sensitive enough to reliably detect small increases (less than a doubling) in cancer incidence, which is the type of effect that might be expected from low-dose carcinogen exposures. As a consequence, scientists often turn to animal testing. Animal testing also has shortcomings that limit the ability to assess risks from low-dose carcinogen exposures. One problem is the need to obtain a sufficient number of cancer cases to generate statistically reliable results.

For this reason, animals are often exposed for their entire lifetime to the maximum tolerated dose (MTD) of a suspected carcinogen, which is defined as the highest dose that can be administered without causing serious weight loss or signs of immediate life-threatening toxicity. At these high doses, many chemicals cause tissue destruction and cell death. The remaining cells proliferate to replace those cells that have been destroyed, and this enhanced cell proliferation creates conditions that are favorable for the development of cancer. If the ability of a given chemical to cause cancer stems from this capacity to cause cell death at high doses, lower doses that do not kill cells may not cause cancer. The dose- response curve for such a carcinogen would exhibit a threshold—that is, a dose that must be exceeded before cancer rates begin to rise. Doses below the threshold would be safe in terms of cancer risk. Figure 5 shows how the existence of a threshold can cause the cancer risk of low-dose exposures to be overestimated.

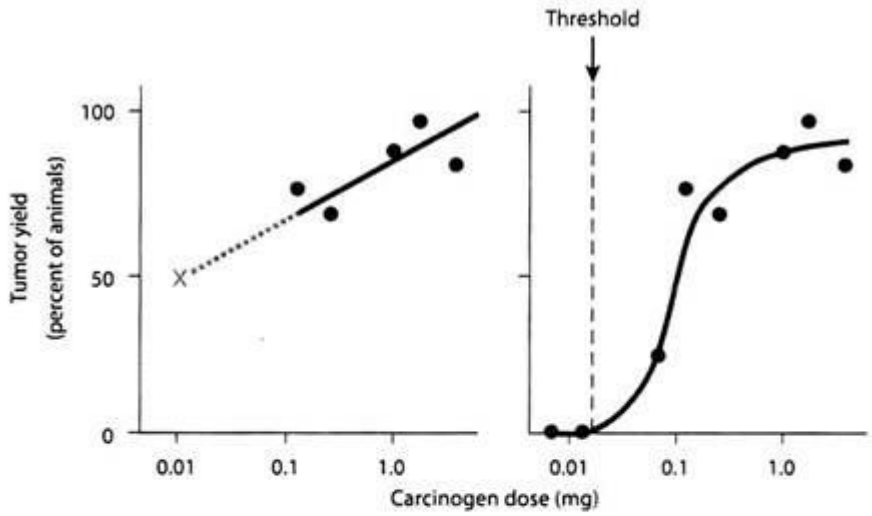


Figure 5 Possibility of Overestimating Cancer Risk When Extrapolating from High-Dose Data. Dose-response curves are illustrated for sarcomas arising in mice after a single injection of benzo[*a*]pyrene. (*Left*) The graph on the left is restricted to high doses of carcinogen (> 0.1 mg). The results appear to be roughly linear, and a straight line can be drawn through the data points to estimate the cancer risk for a lower dose (0.01 mg) of benzo[*a*]pyrene. This estimated cancer risk is indicated by the "X". (*Right*) When actual experiments are carried out that include lower doses of benzo[*a*]pyrene, the shape of the overall curve is seen to exhibit a threshold. Note that the actual cancer risk associated with a 0.01 mg dose of benzo[*a*]pyrene shown in the graph on the right is much lower than the risk estimated by the linear extrapolation derived from the high-dose data shown in the graph on the left. [Based on data from W. R. Bryan and M. B. Shimkin, *J. Natl. Cancer Inst.* 3 (1943): 503.]

The data in Figure 5 are for benzo[a]pyrene, a carcinogen present in gasoline exhaust fumes and in smoke generated by burning organic matter, including tobacco smoke. The graph on the left shows cancer rates for animals exposed to high doses of benzo[a]pyrene. If this data were the only information available, a straight line could be drawn through the data points and extrapolated to lower doses to estimate cancer risk for low-dose exposures to benzo[a]pyrene.

The graph on the right, however, shows what happens when actual experiments are performed using lower doses of benzo[a]pyrene. The shape of the overall curve exhibits a threshold, and the actual cancer risk associated with low-dose benzo[a]pyrene exposure is much lower than predicted. The preceding example highlights that cancer biologists have traditionally had two ways of viewing the relationship between high-dose and low-dose cancer risks: the linear model and the threshold model. As shown in Figure 6, the linear model assumes a linear dose-response relationship with no threshold, whereas the threshold model assumes no cancer risk at lower doses followed by a linear dose-response relationship at higher doses.

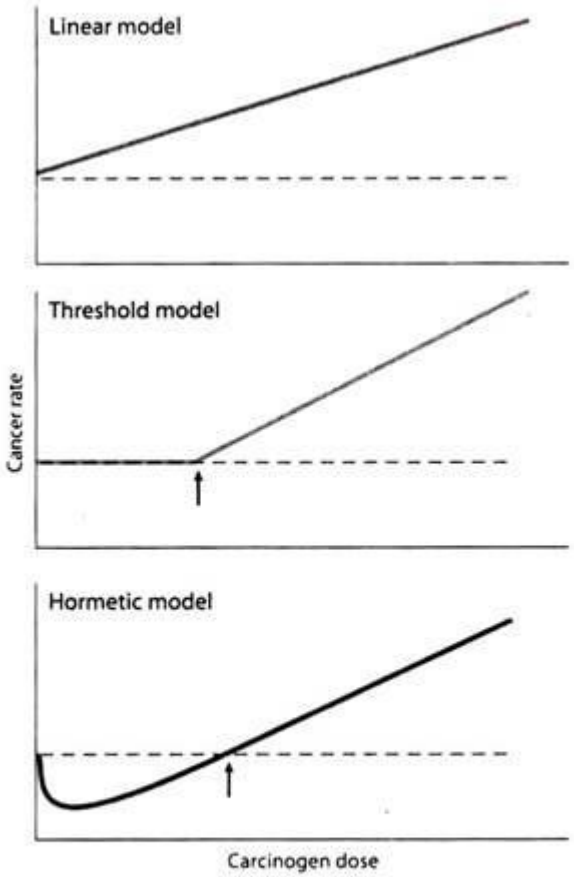


Figure 6 Three Models for the Relationship Between Carcinogen Dose and Cancer Risk. The dashed lines represent the background cancer rate in the absence of carcinogen. Note that the threshold and hormetic models both involve a threshold dose (*arrow*) that must be exceeded before cancer rates begin to rise. [Adapted from E. J. Calabrese, *Mutation Res.* 511 (2002): 181 (Figure 1).]

A third possibility, called the hormetic model, has also begun to receive some attention. The hormetic model proposes that dose-response curves can also be U-shaped. The U-shape, known as hormesis, reflects a situation in which cancer rates actually decline at very low doses of carcinogen and then begin to go up as the dose is further increased.

While it is not clear how widely this model might apply to cancer risk, several carcinogenic agents have been reported to reduce cancer rates when administered to animals at low doses. The hormetic and threshold models both include the concept of a "threshold"—that is, a dose that must be exceeded before cancer rates begin to rise. One possible explanation for the existence of thresholds is the ability of high-dose exposures to cause tissue destruction and cell death, which creates a unique set of conditions that do not exist at lower doses.

Another possible reason for thresholds is that carcinogens often act by causing DNA damage. The presence of damaged DNA triggers a group of repair mechanisms that attempt to correct the problem. Such repair mechanisms might be able to fix small amounts of DNA damage caused by low doses of carcinogens and may even help prevent cancer from arising in response to subsequent exposures to carcinogenic agents. According to this view, carcinogen-induced mutations only begin to accumulate and initiate the development of cancer after a threshold dose has been exceeded and the capacity of these DNA repair pathways is overcome by massive DNA damage.

Humans and Animals Differ in their Susceptibilities to some Carcinogens:

Another problem that can complicate the extrapolation of animal data to humans is that animals often differ from one another, as well as from humans, in their susceptibility to different carcinogens. Consider the behavior of 2-acetylaminofluorene (AAF), which is a potent carcinogen in rats but does not cause cancer in guinea pigs. Based on this information alone, it would be difficult to predict whether or not AAF is likely to be carcinogenic in humans.

The reason for the differing behavior of AAF in rats and guinea pigs became apparent when it was discovered that AAF is actually a "precarcinogen" that needs to be metabolically activated before it can cause cancer. Rats, but not guinea pigs, contain the enzyme that catalyzes this metabolic activation. Biochemical analysis of human tissues has revealed that we also contain the activating enzyme, so it is likely that AAF is carcinogenic in humans just as it is in rats. Of course, if AAF had only been tested in guinea pigs, it never would have been suspected of being a carcinogen in the first place.

The artificial sweetener saccharin provides another illustration of the difficulties that can arise when extrapolating data from animal studies to humans. At the peak of its use in the 1970s, Americans consumed more than five million pounds of saccharin per year in artificially sweetened foods and beverages. In 1981, the U.S. government labelled saccharin as a suspected human carcinogen and attempted to ban its use as a food additive because saccharin causes bladder cancer when fed to rats. Subsequent investigations, however, have revealed that saccharin causes bladder cancer in rats for reasons that do not apply to humans.

When rats ingest large amounts of any sodium salt, including sodium saccharin, a crystalline precipitate forms in the bladder that irritates the bladder lining, triggering cell proliferation and increasing the risk of developing cancer. But the precipitate only forms when there are large amounts of protein in the urine, and the urine protein concentration in rats is 100 to 1000 times greater than in humans. Subsequent studies have shown that other laboratory animals, such as

hamsters, guinea pigs, and mice, do not develop bladder cancer when fed saccharin. As a consequence, saccharin was recently taken off the government's list of suspected human carcinogens. Because of the uncertainties involved in applying animal data to humans, caution is needed in labelling substances as human carcinogens when the information has been derived largely from animal studies.

The U.S. government therefore publishes a list that subdivides carcinogens into two distinct categories:

(1) Known to be human carcinogens and

(2) Reasonably anticipated to be human carcinogens.

The list of "known" human carcinogens contains several dozen chemicals for which the data from animal studies have been supplemented with enough human data to clearly establish a cancer risk for humans. The list of "anticipated" human carcinogens includes more than 100 additional chemicals for which the potential cancer hazard has been extrapolated largely from animal studies.

While many of these substances will almost certainly turn out to be human carcinogens, mistakes are possible because of the heavy reliance on animal data. For example, saccharin was listed as an "anticipated" human carcinogen for about 20 years before eventually being removed from the list.

Medications and Hormones can Cause Cancer:

We have now seen how difficult it can be to measure cancer risks associated with chemicals to which our exposures are small. Of course, this means that the hazards of such low-dose exposures must be rather small (or nonexistent) because larger risks would be readily detectable through epidemiological and animal testing.

The greatest cancer hazards are posed by chemical carcinogens that we encounter in high concentrations. Included in this category are several situations, including occupational exposure to industrial chemicals and inhalation of the carcinogenic chemicals present in tobacco smoke. Another type of high-dose exposure to specific chemicals comes from the use of prescription drugs for treating certain illnesses. Prescription drugs are often taken for prolonged periods, so it is important to know whether the resulting high-dose chemical exposures can cause cancer.

One tragic example involves diethylstilbestrol (DES), a synthetic estrogen that was prescribed to pregnant women starting in the 1940s as a way of preventing miscarriages. Several decades later, women whose mothers had taken DES during pregnancy began developing vaginal cancer at alarmingly high rates. By that time, roughly five million women in the United States had already taken DES. This episode illustrates the difficulty in establishing the risks associated with ingesting any new chemical compound, even when it appears to be safe and is prescribed for a specific medical purpose. In the case of DES, the drug's ability to cause cancer did not become apparent until several decades after women had used DES, and the cancer did not affect the person who took the drug, but rather her children.

Although DES has now been banned as a prescription drug, a number of other medications can also cause cancer (Table 2). Most are prescribed for serious medical problems where the potential benefits of the drug in question are thought to outweigh the risk that cancer might arise.

Table 2 Some Medications That Can Cause Cancer

Medication	Type of Cancer Caused
Analgesic:	
Phenacetin	Kidney
Cancer chemotherapy:	
Chlorambucil	Leukemia
Cyclophosphamide	Bladder, leukemia
Melphalan	Leukemia
Thiotepa	Leukemia
Hormones:	
Estrogens	Breast, uterus, vagina
Oxymetholone	Liver
Immunosuppressive drugs:	
Azathioprine	Lymphoma, skin, liver
Cyclosporin	Lymphoma, skin
Skin treatments:	
Arsenic compounds	Skin, liver, lung
Methoxypsoralen	Skin

For example, some drugs used for slowing or stopping tumor growth in cancer patients can themselves trigger development of a new cancer as a side effect. In a person who already has cancer, the small risk of causing another cancer (often many years in the future) is far outweighed by the possible benefits to be gained from a drug that might cure an existing cancer. A similar situation exists with immunosuppressive drugs, which inhibit immune function and are given to organ transplant patients to prevent rejection of a transplanted organ, such as a heart or kidney. Two of the most commonly used immunosuppressive drugs, azathioprine and cyclosporin, increase the risk of developing cancer, but organ transplant patients depend on the transplanted organ for survival and the benefits of these drugs are thought to outweigh the risk of developing cancer.

Nonetheless, cancer has turned out to be a major cause of death in organ transplant patients and a need therefore exists for better immunosuppressive drugs that do not increase cancer risk. One drug under current investigation is rapamycin (also called sirolimus), an antibiotic with immunosuppressive activity. Animal studies have shown that besides suppressing immune function, rapamycin inhibits tumor growth under conditions in which another immunosuppressive drug, cyclosporin, stimulates tumor growth. A possible explanation for the antitumor effect of rapamycin has come from the discovery that it inhibits angiogenesis, both by depressing the production of VEGF and by inhibiting the ability of endothelial cells to respond to VEGF. Suppression of angiogenesis by rapamycin may therefore limit the ability of newly forming tumors to obtain the blood supply they require for growth beyond a tiny size.

Mechanisms of Chemical Carcinogenesis:

As the list of substances known to cause cancer has grown over the years, it has become increasingly apparent that carcinogens exhibit wide variations in structure and potency. At first this variability complicated our thinking about the origins of cancer because it was difficult to envision how such a diverse array of chemical substances could cause the same disease. Through an extensive series of studies, however, a common set of mechanisms and principles has begun to emerge that helps explain how the various kinds of carcinogens work.

Chemical Carcinogens can be grouped into Several Distinct Categories:

Despite their structural diversity, chemical carcinogens can be grouped into a relatively small number of categories (Figure 7). Most are natural or synthetic organic chemicals—that is, carbon-containing compounds. They range from small organic molecules containing only a few carbon atoms to large, complex molecules constructed from multiple carbon-containing rings.

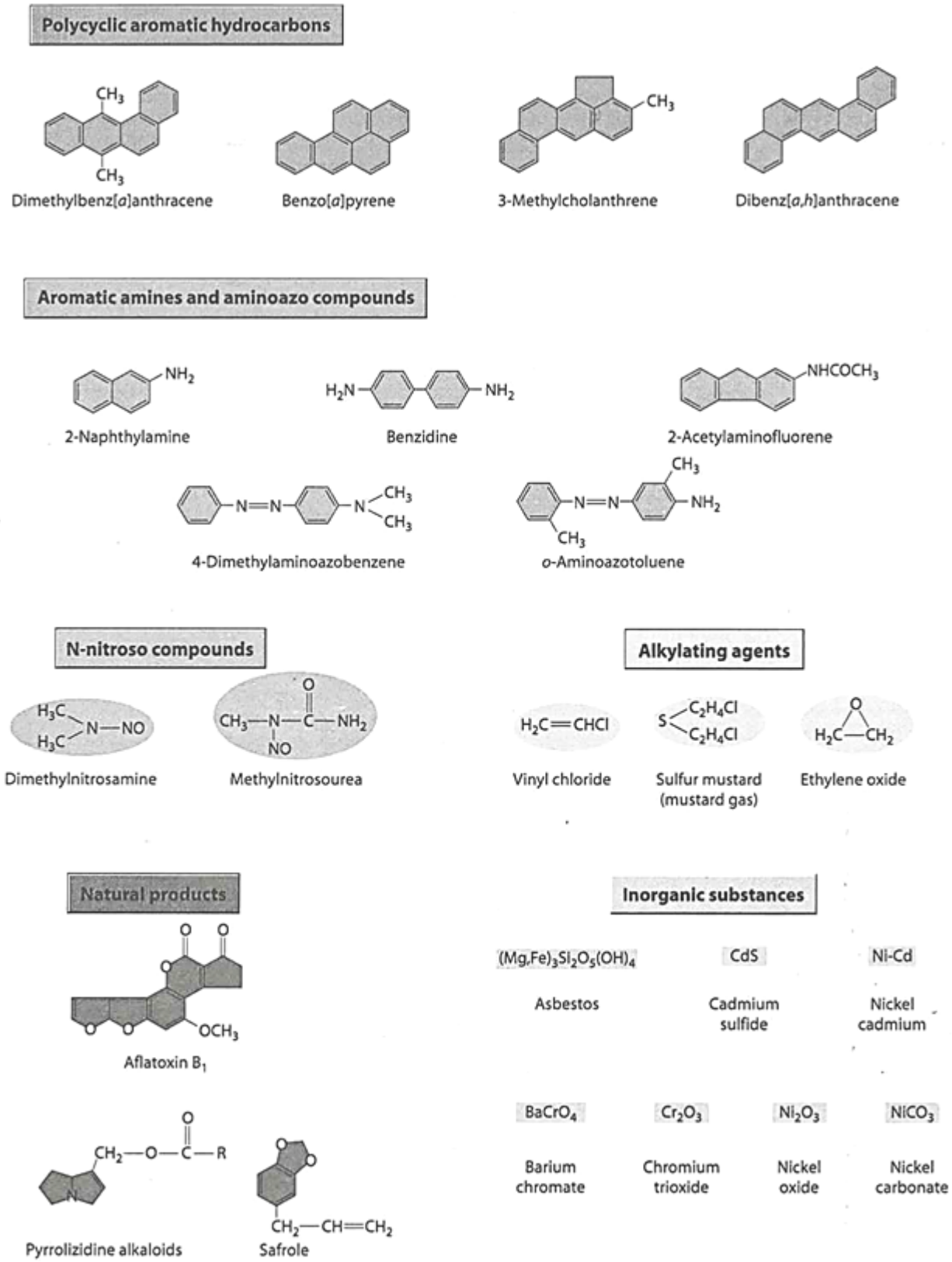


Figure 7 Main Classes of Carcinogenic Chemicals. Selected examples are illustrated for each of the main classes of cancer-causing chemicals. Some of these molecules are precarcinogens that need to be metabolically activated before they can cause cancer, whereas others are direct-acting carcinogens that do not require metabolic activation.

The vast majority fall into one of the following five categories:

1. Carcinogenic polycyclic aromatic hydrocarbons (or simply polycyclic hydrocarbons) are a diverse group of compounds constructed from multiple, fused benzene rings. Polycyclic hydrocarbons are natural components of coal tars, soots, and oils, and are also produced during the incomplete combustion of coal, oil, tobacco, meat, and just about any other organic material that can be burned.

The carcinogenic potency of polycyclic hydrocarbons varies widely, from weak or noncarcinogenic molecules to very potent carcinogens. The polycyclic hydrocarbons benzo[a]pyrene and dibenz[a,h]anthracene, isolated from coal tar in the 1930s, were the first purified chemical carcinogens of any kind to be identified.

2. Carcinogenic aromatic amines are organic molecules that possess an amino group (—NH_2) attached to a carbon backbone containing one or more benzene rings. Some aromatic amines are aminoazo compounds, which means that they contain an azo group (N=N) as well as an amino group. Among the carcinogens in these categories are the aromatic amines benzidine, 2-naphthylamine, 2-acetylaminofluorene, and 4-aminobiphenyl, and the aminoazo dyes 4-dimethylaminoazobenzene and o-aminoazotoluene.
Many of these compounds were once employed in the manufacturing of dyes, although most are no longer used in significant quantities because of their toxicity. Some aromatic amines, such as 2-naphthylamine and 4-aminobiphenyl, are components of tobacco smoke. As in the case of polycyclic hydrocarbons, the carcinogenic potency of aromatic amines and aminoazo dyes varies from substances that are strongly carcinogenic to substances that are not carcinogenic at all.

3. Carcinogenic N-nitroso compounds are organic chemicals that contain a nitroso group (N=O) joined to a nitrogen atom. Members of this group include the nitrosamines and nitrosoureas, which are potent carcinogens when tested in animals. Most of these compounds are industrial or research chemicals encountered mainly in the workplace, although a few are present in cigarette smoke. Nitrates and nitrites used in the curing of meats, which are not carcinogenic in themselves, can be converted in the stomach into nitrosamines, but no consistent relationship between these compounds and human cancer has been established.

4. Carcinogenic alkylating agents are molecules that readily undergo reactions in which they attach a carbon-containing chemical group to some other molecule. Unlike the three preceding groups of carcinogens, which are defined by their chemical structures (i.e., the presence of multiple benzene rings, amino groups, or nitroso groups), alkylating agents are defined not by their structural features but by their chemical reactivity—that is, their ability to join a chemical group to another molecule. The N-nitroso compounds, discussed in the preceding paragraph, are examples of carcinogens that function as alkylating agents. Other examples include vinyl chloride (used in the production of plastics) and ethylene oxide (used in the production of antifreeze and other chemicals). Vinyl chloride and ethylene oxide are among the highest-volume chemicals produced in the United States. Other carcinogenic alkylating agents include sulfur mustard (a chemical warfare agent) and several drugs used in cancer chemotherapy.

5. Carcinogenic natural products are a structurally diverse group of cancer-causing molecules produced by biological organisms, mainly microorganisms and plants. Included in this category is aflatoxin, a carcinogenic chemical made by the mold Aspergillus. One of the most potent carcinogens known, aflatoxin sometimes contaminates grains and nuts that have been stored under humid conditions. Other carcinogenic natural products include plant-derived molecules such as safrole, a major component of sassafras root bark, and pyrrolizidine alkaloids, produced by a variety of different plants.

In addition to the preceding five classes of organic molecules, a small number of inorganic substances (compounds without carbon and hydrogen) are carcinogenic. Included in this group are compounds containing the metals cadmium, chromium, and nickel. Some inorganic substances appear to be carcinogenic in the absence of chemical reactivity. For example, asbestos is a mineral composed of silicon, oxygen, magnesium, and iron, but its ability to cause cancer is related to the crystal structure and size of the microscopic fibers it forms rather than their precise chemical makeup.

Some Carcinogens need to be cctivated by Metabolic Reactions Occurring in the Liver:
The chemicals illustrated in Figure 7 are considered to be "carcinogens" because humans or animals develop cancer when exposed to them. This designation does not mean, however, that every carcinogen triggers cancer directly. For example, consider the behaviour of 2-naphthylamine, whose ability to cause bladder cancer in industrial workers.

As might be expected, feeding 2-naphthylamine to laboratory animals induces a high incidence of bladder cancer, but cancer rarely arises when 2-naphthylamine is directly inserted into an animal's bladder. The reason for this discrepancy is that when 2-naphthylamine is ingested (by animals) or inhaled (by humans), it first passes through the liver and is metabolically converted into chemical compounds that are the actual causes of cancer. Inserting 2-naphthylamine directly into the bladder bypasses the liver and the molecule is never activated, so cancer does not arise.

Many carcinogens share a similar need for metabolic activation before they can cause cancer. Carcinogens exhibiting such behaviour are more accurately called precarcinogens, a term referring to any substance that is capable of causing cancer only after it has been metabolically activated. The activation of precarcinogens is generally carried out by liver proteins that are members of the cytochrome P450 enzyme family. One function of these liver enzymes is to catalyze the oxidation of ingested foreign chemicals, such as drugs and pollutants, with the aim of making molecules less toxic and easier to excrete from the body.The hydroxylation reaction illustrated in Figure 8 is one of several ways in which cytochrome P450 oxidizes foreign chemicals to make them more water soluble, thereby facilitating their excretion in the urine. Occasionally, however, oxidation reactions catalyzed by cytochrome P450 accidentally convert substances into carcinogens, a phenomenon known as carcinogen activation.

Evidence that cytochrome P450 is involved in carcinogen activation has come from numerous animal studies. One set of experiments involved a mutant strain of mice that produce abnormally large amounts of cytochrome P450 1A1, a form of cytochrome P450 that oxidizes polycyclic

hydrocarbons. As would be expected if cytochrome P450 1A1 were involved in carcinogen activation, cancer rates are elevated in the mutant mice that produce large amounts of this enzyme. Cancer rates can be reduced in these same animals by using inhibitors that block the action of cytochrome P450 1A1.

Elevated amounts of cytochrome P450 1A1 are found in the livers of people who smoke cigarettes, apparently because tobacco smoke stimulates production of the enzyme by the liver. This means that in addition to containing dozens of chemicals that cause cancer, cigarette smoke also induces the production of liver enzymes that make the situation worse by activating carcinogenic activity in chemicals that might not otherwise cause cancer. About one person in ten inherits a form of cytochrome P450 1A1 that is produced in especially large amounts in response to tobacco smoke. If such a person smokes cigarettes, he or she has an even higher risk of developing lung cancer than other smokers. The role played by liver enzymes in carcinogen activation explains why chemicals being assayed for mutagenic activity in the Ames test are first incubated with a liver homogenate to mimic any reactions that might take place in the body. The requirement for metabolic activation also helps explain why some chemicals only cause cancer in certain organisms.For example, 2-acetylaminofluorene (AAF) is carcinogenic in rats but not in guinea pigs because guinea pigs lack the enzyme needed to convert AAF into an active carcinogen. Because of such differences in liver enzymes between organisms, it is important that suspected carcinogens be tested in more than one animal species.

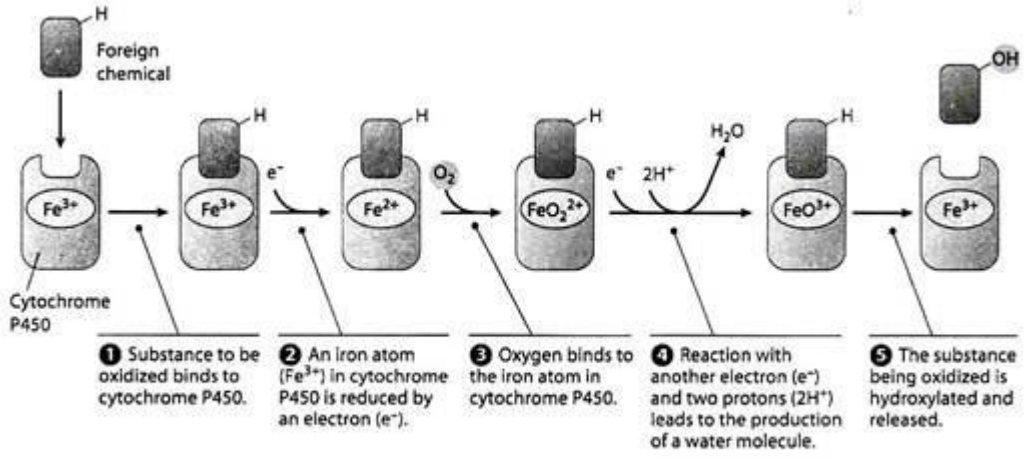


Figure 8 Hydroxylation Reaction Catalyzed by Cytochrome P450. Cytochrome P450 oxidizes chemicals by linking them to a hydroxyl group in a five-step oxidation reaction.

Many Carcinogens are Electrophilic Molecules that React Directly with DNA:

Despite the variations in molecular structure exhibited by the carcinogens illustrated in Figure 7, many share the same property: When metabolized in the liver, they are converted into highly unstable compounds with electron-deficient atoms. Such molecules are said to be electrophilic ("electron-loving") because they readily react with substances possessing atoms that are rich in electrons. DNA, RNA, and proteins all have electron-rich atoms, making each a potential target for electrophilic carcinogens. Of the three, DNA is the prime candidate because the Ames test has shown that most carcinogens cause DNA mutations. An experiment designed to determine whether DNA is in fact the direct target of chemical carcinogens is summarized in Figure 9.

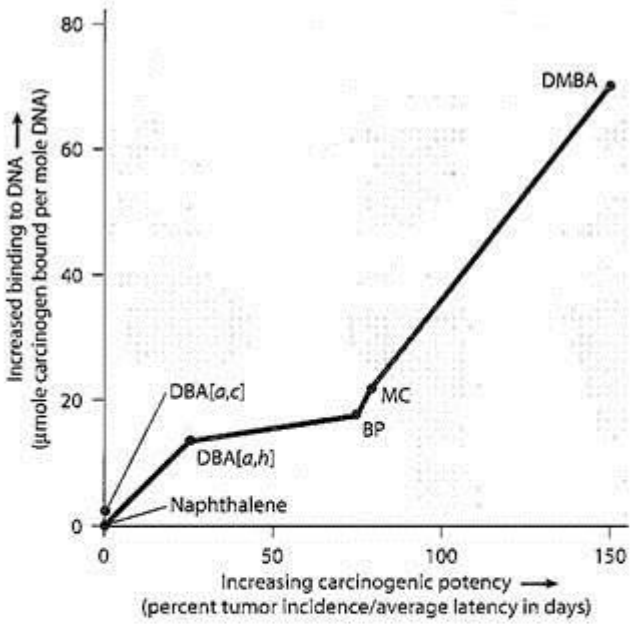


Figure 9 Relationship Between Carcinogenic Potency and DNA-Binding Ability. Six polycyclic hydrocarbons of varying carcinogenic potency were injected into animals and measurements were then made to identify the intracellular molecules to which they had become bound. The data show that the more potent the carcinogen, the more extensively it binds to DNA. *Abbreviations*: DBA[*a*,*c*] = dibenz[*a*,*c*]anthracene, DBA[*a*,*h*] = dibenz[*a*,*h*]anthracene, BP = benzo[*a*]pyrene, MC = 3-methylcholanthrene, DMBA = dimethylbenz[*a*]anthracene. [Data from P. Brookes and P. D. Lawley, *Nature* 202 (1964): 781 (Figure 5).]

In this study, animals were injected with various polycyclic hydrocarbons that differed in carcinogenic potency. Cells were then isolated from the treated animals and measurements were made to determine which intracellular molecules (if any) had become bound to the polycyclic hydrocarbons.

The data revealed a direct relationship between the carcinogenic potency of different polycyclic hydrocarbons and their ability to become covalently linked to DNA; in other words, those polycyclic hydrocarbons that became extensively bound to DNA were the most effective at causing cancer. Before a polycyclic hydrocarbon can interact with DNA, it must be activated. For example, consider the behaviour of benzo[a]pyrene, which is normally a nonreactive, non-mutagenic compound. After entering the body, metabolic reactions catalyzed by cytochrome P450 in the liver convert benzo[a]pyrene into activated derivatives containing an epoxide group (Figure 10).

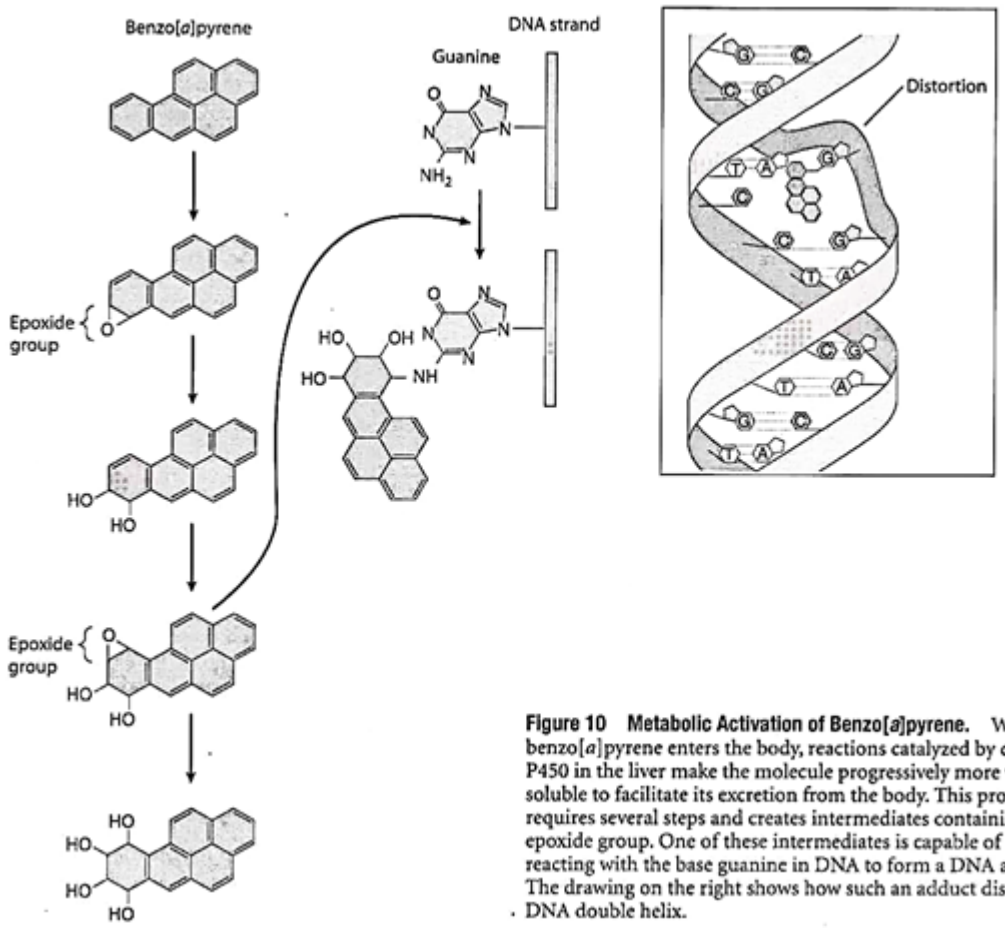
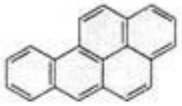
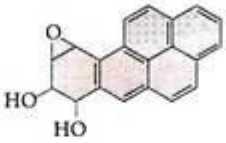
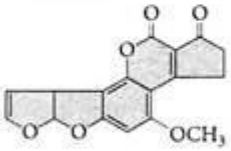
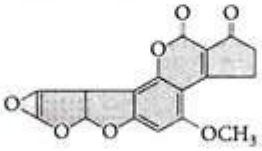
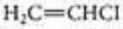
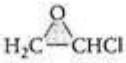


Figure 10 Metabolic Activation of Benzo[*a*]pyrene. When benzo[*a*]pyrene enters the body, reactions catalyzed by cytochrome P450 in the liver make the molecule progressively more water soluble to facilitate its excretion from the body. This process requires several steps and creates intermediates containing an epoxide group. One of these intermediates is capable of reacting with the base guanine in DNA to form a DNA adduct. The drawing on the right shows how such an adduct distorts the DNA double helix.

An epoxide is a three-membered ring containing an oxygen atom covalently bonded to two carbon atoms; these two carbons are electron deficient and therefore tend to react with atoms that are electron rich, such as the amino nitrogen found in the DNA base guanine. Reaction of the epoxide group with guanine causes the benzo[a]pyrene to become covalently bonded to DNA, thereby forming a DNA-carcinogen complex called a DNA adduct. The presence of the bound carcinogen distorts the DNA double helix and thereby causes errors in base sequence (mutations) to arise during DNA replication.

Epoxide formation is also involved in the activation of other classes of chemical carcinogens. For example, aflatoxin and vinyl chloride, which differ significantly from polycyclic hydrocarbons in chemical structure, are both oxidized by cytochrome P450 into epoxides that, like benzo[a]pyrene, react with DNA bases to form DNA adducts (Table 3).

Table 3 Examples of Several Carcinogens Activated by Epoxide Formation

Carcinogen	Major Active Metabolite*	Site of DNA Modification**
Benzo[*a*]pyrene (BP) 	BP 7,8-diol 9,10-epoxide 	N2 of guanine N6 of adenine
Aflatoxin B_1 	Aflatoxin B_1 8,9-epoxide 	N7 of guanine
Vinyl chloride 	Chloroethylene oxide 	N3 and N4 of cytosine*** N1 and N6 of adenine N2 and N3 of guanine

*Green shading is used to highlight the epoxide group.
**The numbers in the third column refer to the numbered positions of nitrogen atoms illustrated in Figure 11.
***Vinyl chloride simultaneously attacks two positions on the same base, forming a cyclic adduct.

However, the various epoxides do not all react with the same DNA bases. In fact, depending on the carcinogen involved, almost every electron-rich site in the various DNA bases can serve as a target for carcinogen attachment (Figure 11). And epoxides are not the only electrophilic groups that react with DNA.

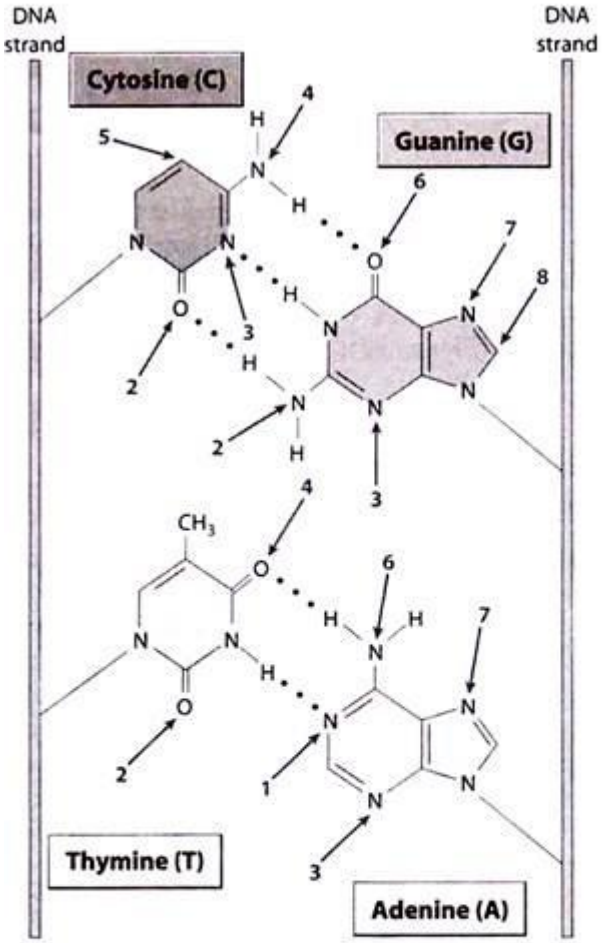


Figure 11 Sites of Carcinogen Attack in DNA Bases. The red arrows indicate the main sites in the four DNA bases that serve as targets for carcinogen attachment. The red numbers refer to the numbering system used to identify the atoms located at different positions within each base. The dotted blue lines represent the hydrogen bonds that normally hold the complementary base pairs together in the DNA double helix. Attachment of carcinogen molecules to the bases tends to distort the double helix and interfere with this hydrogen bonding, thereby leading to errors in DNA replication.

Some carcinogens are activated by reactions that create other types of electrophilic groups, such as positively charged nitrogen atoms (nitrenium ions) or carbon atoms (carbonium ions), or compounds containing an unpaired electron (free radicals). Like epoxides, these electrophilic groups also attack electron-rich atoms in DNA.

The preceding mechanisms illustrate that, despite their chemical diversity, many carcinogens share the property of being converted into electrophilic molecules that in turn become linked to DNA. This ability to form DNA adducts is one of the best predictors of a molecule's capacity to cause cancer. In addition, carcinogens can inflict DNA damage in several other ways; for example, they may generate crosslinks between the two strands of the double helix, create chemical linkages between adjacent bases, hydroxylate or remove individual DNA bases, or cause breaks in one or both DNA strands (Figure 12).

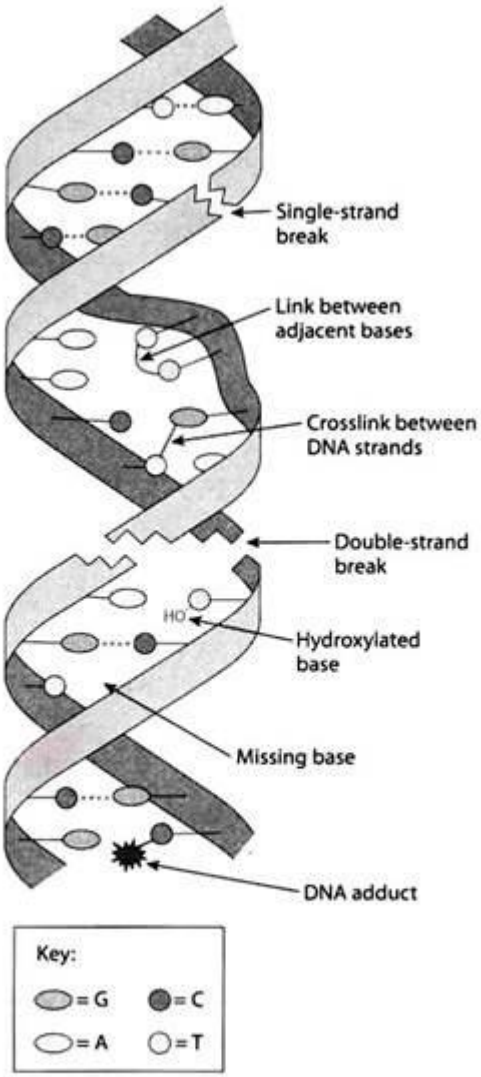


Figure 12 Summary of DNA Damage Caused by Chemical Carcinogens. Chemical carcinogens inflict DNA damage in a variety of ways, altering or removing individual bases and triggering breaks in one or both DNA strands.

Chemical Carcinogenesis is a Multistep Process:

An attack on DNA by an activated carcinogen is just the first of several steps involved in creating a cancer cell. The idea that cancer arises through a multistep process was first proposed in the early 1940s by Peyton Rous to explain a phenomenon he encountered when studying the ability of coal tar to cause cancer in rabbits.

Rous had observed that repeated application of coal tar to rabbit skin caused tumors to develop, but the tumors disappeared when application of the coal tar was stopped. Subsequent application of an irritant such as turpentine, which does not induce cancer by itself, caused the tumors to reappear.

This pattern suggested to Rous that coal tar and turpentine play two different roles, which he called initiation and promotion. According to his theory, initiation converts normal cells to a precancerous state and promotion then stimulates the precancerous cells to divide and form

tumors. Because coal tar is a mixture of various chemicals, clarification of the initiation/promotion hypothesis required the isolation and study of individual coal tar components. One such chemical is the polycyclic hydrocarbon dimethylbenz[a]anthracene (DMBA). DMBA is a potent carcinogen, but feeding mice a single dose rarely causes tumors to arise. However, if the skin of a mouse fed a single dose of DMBA is later painted with a substance that causes skin irritation, cancer develops in the treated area (Figure 13).

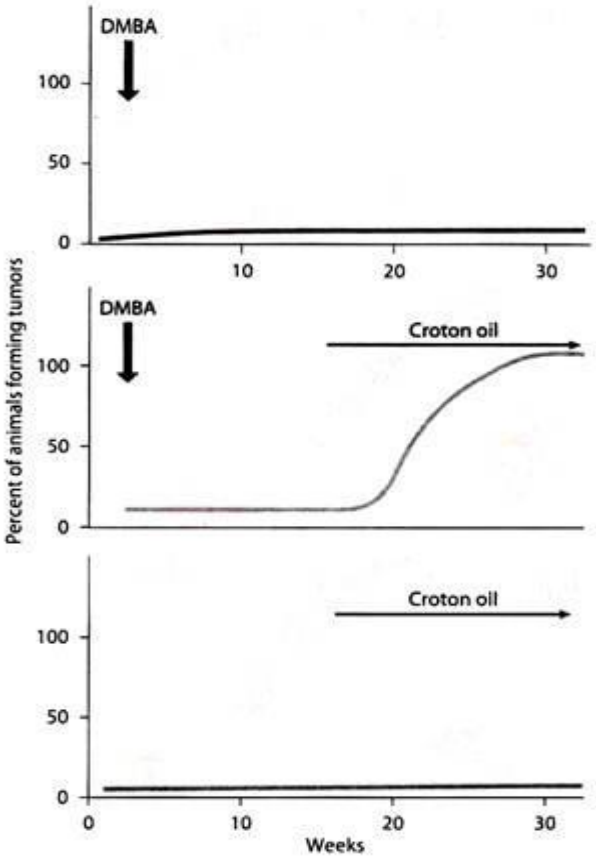


Figure 13 Evidence for the Existence of Initiation and Promotion Stages in Chemical Carcinogenesis. (*Top*) Mice treated with a single dose of DMBA (dimethylbenz[*a*]anthracene) do not form tumors. (*Middle*) Painting the skin of such animals twice a week with croton oil after the DMBA treatment leads to the appearance of skin tumors. If the croton oil application is stopped a few weeks into the treatment (data not shown), the tumors will regress. (*Bottom*) Croton oil alone does not produce skin tumors. These data are consistent with the conclusion that DMBA is an initiator and croton oil is a promoter. [Adapted from R. K. Boutwell, *Prog. Exp. Tumor Res.* 4 (1963): 207.]

Besides turpentine, the irritant most commonly used for triggering tumor formation in such experiments is croton oil, a substance derived from seeds of the tropical plant Croton tiglium. Croton oil does not cause cancer in the absence of prior exposure to a carcinogen such as DMBA, nor will cancer arise if DMBA is administered after the croton oil. These observations support the concept that chemical carcinogenesis is a multistep process in which an initiator (in this case, DMBA) first creates an altered, precancerous state and then a promoting agent (in this case, croton oil) stimulates the development of tumors.

The Initiation Stage of Carcinogenesis is based on DNA Mutation:

A year or more can transpire after feeding animals a single dose of DMBA and yet tumors will still arise if an animal's skin is then irritated with croton oil. Thus a single DMBA treatment creates a permanently altered, initiated state in cells located throughout the body, and a promoting agent (croton oil) can then act on these altered cells to promote tumor development.

Because the carcinogenic potency of most chemicals correlates with their ability to bind to DNA and cause mutations (see Figure 9), the permanent alteration is thought to be a mutation. Carcinogens that act in this way are said to be genotoxic because they cause gene damage. The ability to cause gene mutations explains how a single exposure to an initiating carcinogen can create a permanent, inheritable change in a cell's properties.

Referring to carcinogen-induced mutations as "permanent," however, implies that DNA damage cannot be repaired, which seems to contradict what we know about the existence of DNA repair mechanisms. Such mechanisms are in fact capable of repairing mutations created by initiating carcinogens as long as the damage is repaired in a timely fashion. Once a damaged DNA molecule has been replicated, as occurs each time a cell divides, mutations become very difficult, if not impossible, to repair and the initiated state therefore becomes permanent. Figure 14 illustrates why this is the case, using the carcinogen methylnitrosourea as an example. Methylnitrosourea attacks the base guanine (G) in DNA, creating a methylated guanine derivative. If the cell's DNA is replicated before repair mechanisms correct the defect, the methylated guanine tends to form an incorrect base pair with thymine (T) during DNA replication rather than pairing with its correct partner, cytosine (C).

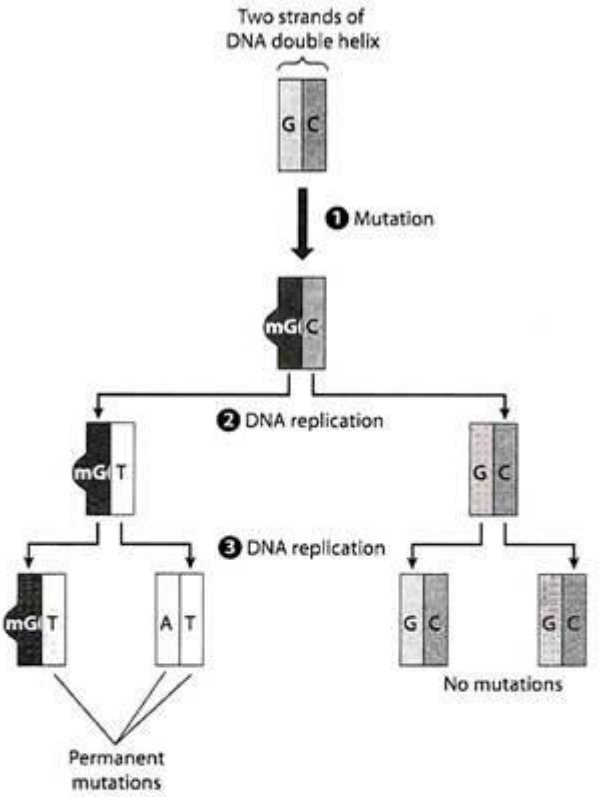


Figure 14 Process by Which a DNA Mutation Becomes Permanent. This hypothetical illustration involves a mutation triggered by exposure to the carcinogen *methylnitrosourea*, but a similar principle applies to many kinds of base mutations caused by chemical carcinogens. ① Methylnitrosourea causes a mutation by adding a methyl group to the base G (the methylated base is designated *mG*). ② If the DNA is replicated before the mG mutation can be repaired, the mG will form an incorrect base pair with T during DNA replication. ③ When the DNA is again replicated, the T forms a normal base pair with A. Hence, the DNA molecule now contains an AT base pair where a GC base pair had originally been located. Because the cell would not recognize anything abnormal about an AT base pair, the mutation is permanent.

During the next round of DNA replication, the incorrectly inserted T will form a base pair with its normal complementary base, adenine (A), creating an AT base pair. The DNA molecule now contains an AT base pair where a GC base pair had originally existed. Since DNA repair mechanisms would not recognize anything abnormal about an AT base pair, the error will persist.

The preceding scenario demonstrates an important principle that applies to many mutations- If DNA replication occurs before mutations are repaired, base-pair alterations tend to arise during replication that cannot be subsequently detected as mutations by cellular repair mechanisms. For this reason it is crucial that mutations be repaired swiftly, before subsequent rounds of DNA replication create a permanent mutation.

Tumor Promotion involves a Prolonged Period of Cell Proliferation:

In contrast to initiation, which requires only a single exposure to an initiating carcinogen, promotion is a gradual process that depends on prolonged or repeated exposure to a promoting agent. If the promoting agent is removed during the early stages of tumor formation, tumors stop growing and may even disappear.

How do we explain the ability of promoting agents to trigger an event that is potentially reversible, at least in its early stages? Studies of a wide variety of promoting agents have revealed that their main shared property is the ability to stimulate cell proliferation. When an initiated cell is exposed to a promoting agent, the cell starts dividing and the number of initiated cells increases.

In the early stages of this process, cell proliferation depends on the presence of the promoting agent, and the cells will stop dividing if the agent is removed. As cell division continues, however, natural selection favors those newly forming cells whose proliferation is faster and autonomous, eventually leading to the formation of a malignant tumor whose growth no longer depends on external promoting agents. The time required for promotion contributes to the long delay that often transpires between exposure to an initiating carcinogen and the development of cancer.The way in which specific promoting agents stimulate cell proliferation was first established for phorbol esters, the class of tumor promoters found in croton oil. In terms of its tumor promoting activity, the most potent phorbol ester is tetradecanoyl phorbol acetate (TPA). TPA binds to and activates an enzyme called protein kinase C, which plays a key role in one of the cell's normal pathways for controlling cell proliferation (Figure 15). In the normal operation of this pathway, external signaling molecules bind to cell surface receptors whose activation leads to the production of an intracellular signaling molecule called diacylglycerol (DAG).

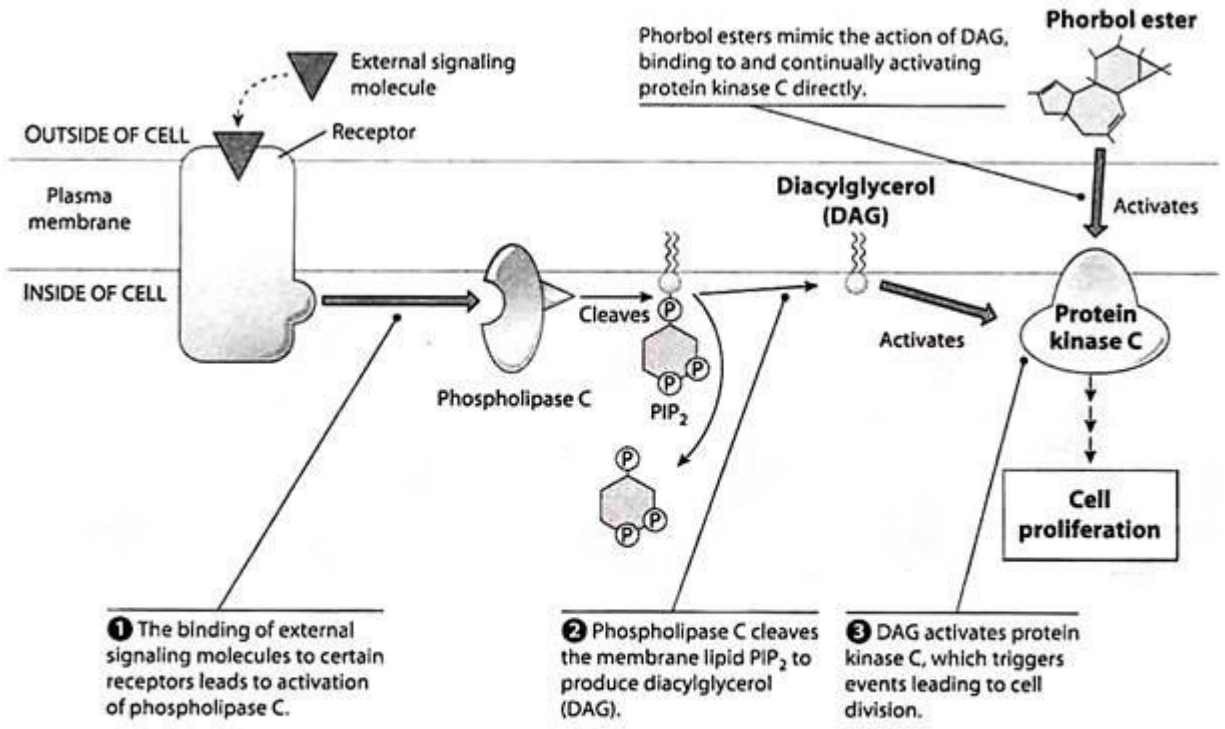


Figure 15 Mechanism of Action of Phorbol Esters. Phorbol esters activate protein kinase C, a component of a signaling pathway that stimulates cell proliferation. In the normal operation of this pathway, external signaling molecules bind to cell surface receptors whose activation leads to the production of diacylglycerol (DAG). The DAG then activates protein kinase C, which triggers events leading to cell division. Phorbol esters mimic the action of DAG, binding to and activating protein kinase C directly. (Green arrows represent activation reactions.)

DAG in turn activates protein kinase C, which triggers events leading to cell division. Phorbol esters mimic the action of DAG, binding to and activating protein kinase C. Unlike DAG, however, which is converted to inactive forms, phorbol esters continually activate the protein kinase C

molecule. Activation of protein kinase C by TPA is a highly selective interaction; small changes in the chemical structure of TPA yield derivatives that exhibit diminished ability to bind to protein kinase C and, as a result, decreased ability to function as tumor promoters (Figure 16).

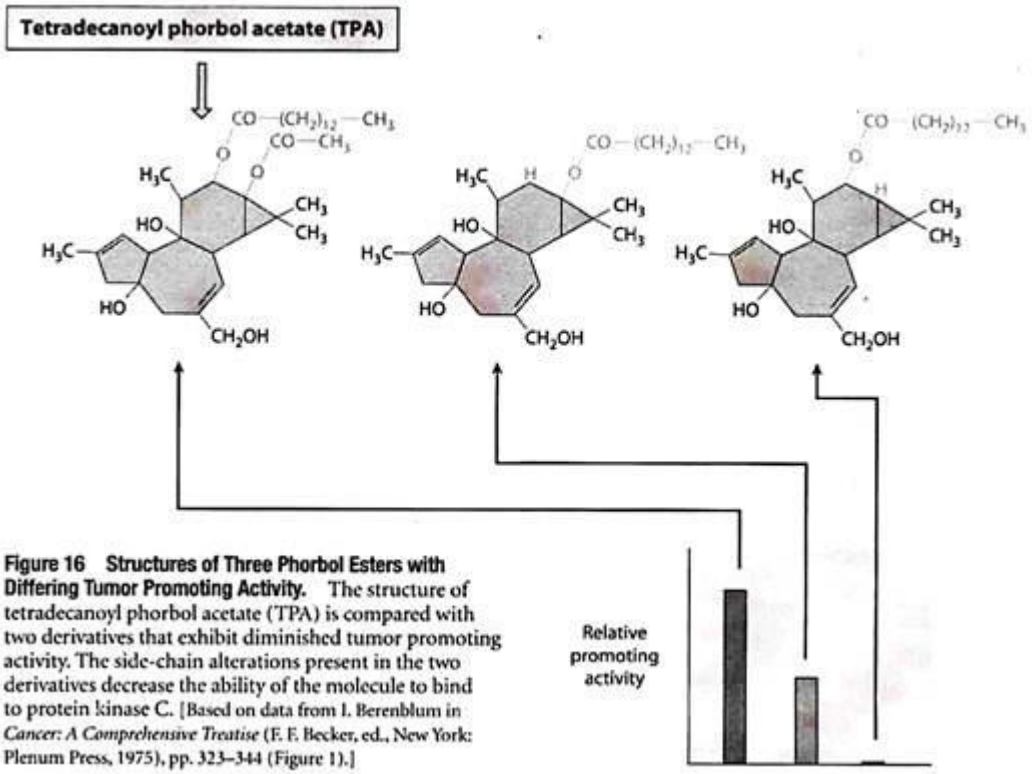


Figure 16 Structures of Three Phorbol Esters with Differing Tumor Promoting Activity. The structure of tetradecanoyl phorbol acetate (TPA) is compared with two derivatives that exhibit diminished tumor promoting activity. The side-chain alterations present in the two derivatives decrease the ability of the molecule to bind to protein kinase C. [Based on data from I. Berenblum in *Cancer: A Comprehensive Treatise* (F. F. Becker, ed., New York: Plenum Press, 1975), pp. 323–344 (Figure 1).]

In addition to phorbol esters, a variety of other foreign substances stimulates cell proliferation and thereby acts as promoting agents. Some of these molecules resemble phorbol esters in being able to interact with protein kinase C. The fungal toxin teleocidin and the algal toxin aplysiatoxin are two such agents that function by activating protein kinase C, even though their chemical structures differ significantly from those of phorbol esters.

Other promoting agents stimulate cell proliferation indirectly, causing tissue damage and cell destruction that make it necessary for the remaining cells of the affected tissue to proliferate to replace the cells that have been damaged and destroyed. Asbestos and alcohol are two previously discussed substances that function in this way.Not all tumor promoters are foreign substances. Because cell proliferation occurs in normal cells as well as in tumor cells, molecules produced by the body for the purpose of stimulating normal cell division may also function inadvertently as tumor promoters. For example, estrogen is a naturally produced steroid hormone that can contribute to the development of breast and ovarian cancer by stimulating the proliferation of cells in these tissues. The hormone testosterone stimulates the proliferation of cells in the prostate gland and plays a comparable role in promoting the development of prostate cancer. Of course, the intended function of estrogen and testosterone is to stimulate the growth and division of normal cells, not cancer cells. But if a breast or prostate cell has acquired an initiating mutation caused by

a carcinogen or by an error in DNA replication, any normal hormone or growth factor that stimulates the proliferation of the mutant cell will act inadvertently as a tumor promoter.

In addition to foreign chemicals and natural hormones, certain components of the diet may also act as promoting agents—that is, agents that increase cancer risk by stimulating cell proliferation rather than by creating mutations. Dietary fat and alcohol are two examples that fit this category. In general, any chemical associated with an increased cancer risk that is found not to be genotoxic can be suspected of acting as a promoting agent.

Tumor Progression involves Repeated Cycles of Selection for Rapid Growth and Other Advantageous Properties:

When Rous first proposed that chemical carcinogenesis is a multistep process, he identified only two stages- initiation and promotion. It has gradually become apparent that a third stage, known as tumor progression, follows initiation and promotion (Figure 17). The concept of tumor progression refers to the gradual changes in the properties of proliferating tumor cell populations that occur over time as cells acquire more aberrant traits and become increasingly aggressive.

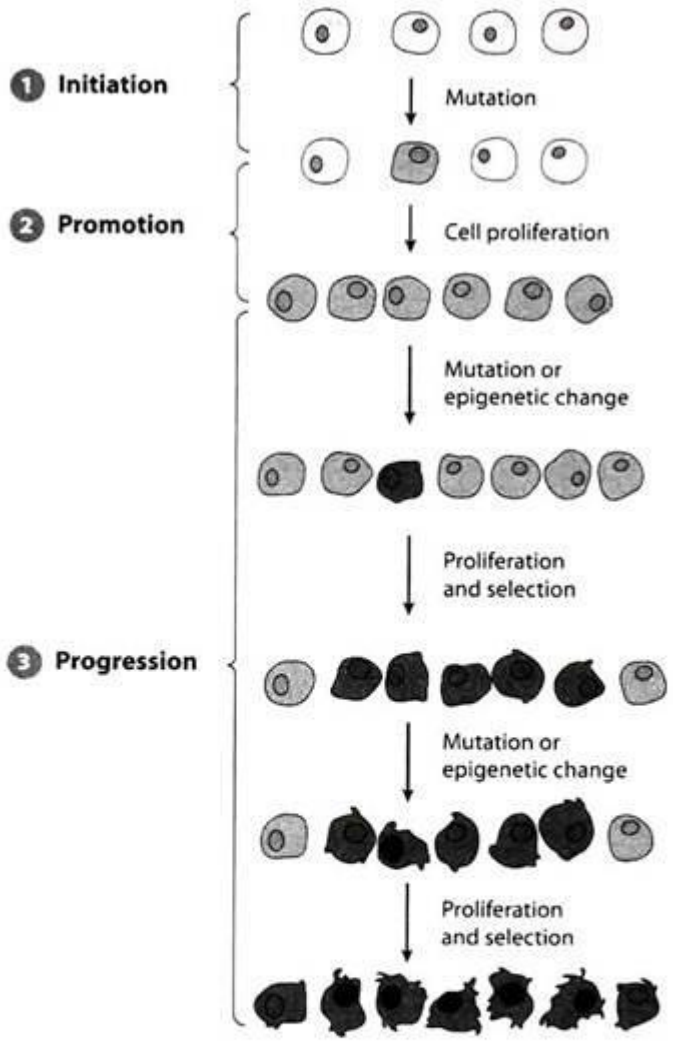


Figure 17 Main Stages of Carcinogenesis. Cancer arises by a complex process involving three main stages. ① The first stage, initiation, is based on DNA mutation. Initiation is followed by a ② promotion stage in which the initiated cell is stimulated to proliferate. ③ During tumor progression, further mutations and epigenetic changes in gene expression create variant cells exhibiting enhanced growth rates or other aggressive properties that give certain cells a selective advantage. Such cells tend to outgrow their companions and become the predominant cell population in the tumor. Repeated cycles of this process, called clonal selection, create a population of cells whose properties change over time.

The underlying explanation for tumor progression is that cells exhibiting traits that confer a selective advantage—for example, increased growth rate, increased invasiveness, ability to survive in the bloodstream, resistance to immune attack, ability to grow in other organs, resistance to drugs, and evasion of death-triggering mechanisms (apoptosis)—will be more successful than cells lacking these traits and will gradually come to predominate.

While it is easy to see why cells exhibiting such traits tend to prevail through natural selection, that does not explain how the aberrant traits originate in the first place. One way of creating new traits is through additional mutations. If a particular mutation causes a cell to divide more rapidly, cells

produced by the proliferation of this mutant cell will outgrow their companions and become the predominant cell population in the tumor.

Such a process is called clonal selection because the cells that predominate represent a clone—that is, a population of cells derived from a single initial cell by successive rounds of cell division.One member of a clonal population may acquire another mutation that makes it grow even faster and the process repeats again, generating an even faster growing clone of cells. Multiple cycles of mutation and selection can occur in succession, each creating a population with enhanced growth rate or some other advantageous property. Although mutations play a central role in tumor progression, they are not the whole story. Cancer cell properties are also influenced by alterations in the expression of normal genes. The term epigenetic change is used to refer to any such alteration in gene expression that does not involve mutating the structure of a gene itself.

Cells possess a variety of mechanisms for altering gene expression. Among them, activating or inhibiting the transcription of individual genes into messenger RNA is especially important in cancer cells. For example, many of the traits required for cancer cell invasion and metastasis are produced by epigenetically turning on or turning off the transcription of normal genes rather than by gene mutation. Because the DNA base sequence is not being altered, epigenetic changes are easier to reverse than mutations. The question therefore arises as to whether a cancer cell can be epigenetically reprogrammed to reverse some of the changes responsible for malignant behavior. One way of addressing this question experimentally is to transfer the nucleus of a cancer cell into a different cytoplasmic environment to see if its gene expression patterns can be converted to a more normal state.

When nuclei are taken from mouse cancer cells and transplanted into mouse eggs whose own nuclei have been removed, the eggs divide and proceed through the early stages of embryonic development, even though the cells possess cancer cell nuclei. Especially striking results have been reported when mouse melanoma cells (a cancer of pigment cells) are used as a source of nuclei for transplantation. Eggs receiving melanoma nuclei divide and produce embryonic cells that give rise to normal-appearing cells and tissues of adult mice (Figure 18). Nonetheless, mice containing such cells are not completely normal; the mice still exhibit an increased susceptibility to developing cancer.

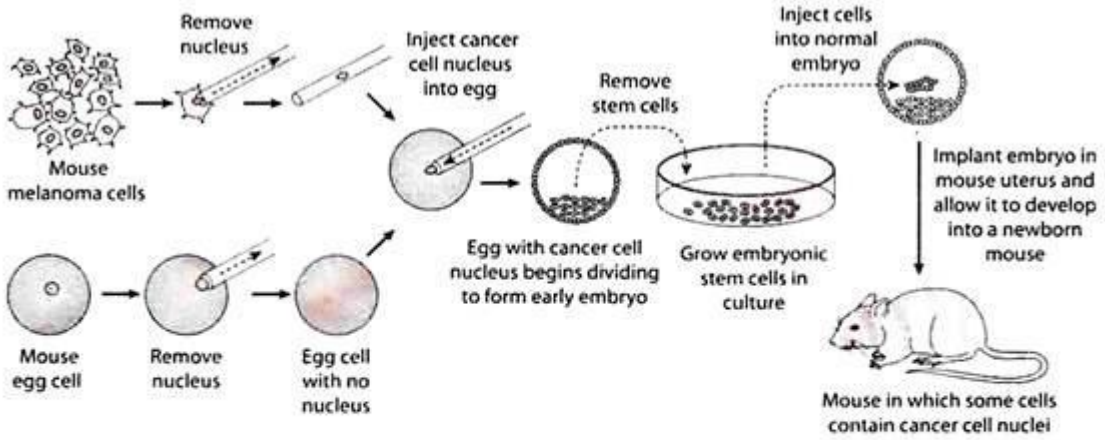


Figure 18 Transplanting a Cancer Cell Nucleus into an Egg Cell. When the nucleus of a mouse melanoma cell is transferred into a mouse egg whose own nucleus has been removed, the egg divides and proceeds through the early stages of embryonic development, although it cannot complete the process to form a new mouse. If *stem cells* (undifferentiated cells whose division gives rise to specialized cells) are removed at this stage and injected into a normal early embryo, these embryonic stem cells, with their cancer cell nuclei, will participate in the development of normal-appearing cells and tissues of a new mouse. Nonetheless, mice containing such cells are not completely normal; they still exhibit an increased susceptibility to developing cancer. [Based on experiments of K. Hochedlinger et al., *Genes Dev.* 18 (2004): 1875.]

Such results indicate that the DNA of a cancer cell nucleus can be reprogrammed to a more normal state, but a propensity for cancer to arise still remains. In other words, epigenetic and genetic changes both play important roles in tumor development. To sum up, tumor progression is a phase of carcinogenesis that involves the gradual acquisition of DNA mutations and epigenetic changes in gene expression, accompanied by natural selection of cells that have acquired advantageous properties generated by these mechanisms.

The net result is a population of cells whose properties, including growth rate and the ability to invade and metastasize, slowly change over time. The time required for tumor progression contributes to the lengthy delay commonly observed between exposure to carcinogenic chemicals and the development of cancer. These principles, derived largely from studies of chemical carcinogenesis, apply to cancers triggered by other cancer-causing agents as well.

Carcinogenesis is a Probabilistic Event that Depends on Carcinogen Dose and Potency:

The realization that chemical carcinogenesis is a multistep process involving several distinct stages and mechanisms can cause some confusion about the meaning of the term carcinogen. In common usage, any agent that increases the risk of developing cancer in animals or humans is considered to be a carcinogen. In this sense, either an initiating or a promoting agent would qualify as a carcinogen.

For clarification, the term incomplete carcinogen is sometimes employed when referring to a chemical that exerts only one of these two actions. Some chemicals possess both initiating and promoting activities, and can therefore cause cancer by themselves; such chemicals are called complete carcinogens.

The ability to function as a complete carcinogen may be dose dependent. For example, certain polycyclic hydrocarbons act as initiating agents at lower doses but are complete carcinogens at

higher doses. In normal human experience, people are exposed to chemical mixtures, such as tobacco smoke or coal tar that contain both initiating and promoting carcinogens. In such cases, the mixture acts as a complete carcinogen.The multistep nature of chemical carcinogenesis also complicates the question of what scientists mean when they say that something “causes” cancer. For example, exposure to an incomplete carcinogen (i.e., an initiating or promoting agent) will not, by itself, cause cancer. Even a complete carcinogen rarely causes cancer in every exposed person or animal.

When it is stated that a particular carcinogen causes cancer, what is really meant is that the agent in question increases the probability that cancer will arise. The magnitude of the increased risk depends on several factors, including the dose and potency of the agent involved and the issue of whether it is acting as an incomplete or complete carcinogen (complete carcinogens obviously carry a greater risk).The reason for carcinogen dose dependence should now be more apparent. As the dose of an initiating carcinogen is increased, more DNA adducts and other types of DNA damage accumulate. To initiate the development of cancer, this damage must affect certain critical genes. The probability that one of these cancer-related genes will happen to undergo mutation is quite small because carcinogens trigger random DNA damage and the critical genes constitute only a tiny fraction of the total DNA. The higher the dose of carcinogen, however, the greater the overall DNA damage and hence the greater the chance that a critical gene will be affected by a random mutation. The likelihood that a particular carcinogen will cause cancer also depends on its potency. Carcinogen potency is generally assessed in animals by determining how large a dose is needed to cause cancer in 50% of the animals tested. Such testing has revealed that a ten-million-fold difference in strength separates the strongest carcinogens from the weakest (Figure 19).

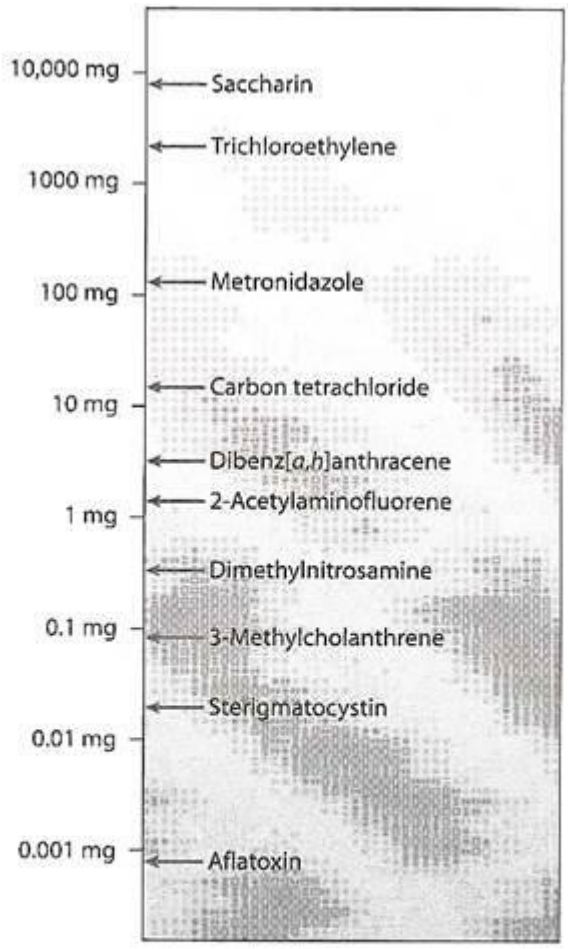


Figure 19 Differences in Carcinogenic Potency. The relative potency of several carcinogenic chemicals is compared on a scale that indicates how large a dose is needed to cause cancer in 50% of the animals tested. Note that the required dose of the weakest carcinogen shown (saccharin) is about ten-million-fold higher than the dose of the strongest carcinogen shown (aflatoxin). [Data from T. H. Maugh, *Science* 202 (1978): 38.]

Two properties are especially important in explaining this enormous variation in potency. The first involves the activation reactions catalyzed by cytochrome P450, which are more effective in converting certain types of chemicals into active carcinogens than they are for other chemicals. The second factor is related to the electrophilic strength of different carcinogens. Some substances are strongly electrophilic and are highly reactive with DNA, whereas others are weaker electrophiles and are less reactive with DNA. The probability that a carcinogen will happen to mutate a critical gene randomly is much greater for carcinogens that are stronger electrophiles because they trigger more mutations.

The random nature of mutation helps explain why everyone who is exposed to carcinogens does not develop cancer. For many years, tobacco companies tried to cast doubt on the relationship between cigarette smoking and lung cancer by pointing out that some cigarette smokers live long lives without ever developing cancer. The ability of carcinogens to trigger random DNA mutations provides a simple explanation for such observations: It is largely a matter of chance. Tobacco smoke contains numerous carcinogens that cause random DNA damage, but for cancer to develop, a mutation must arise in a critical cancer-related gene. An apt metaphor is the game of Russian

roulette, in which a single bullet is placed in a gun containing six chambers and the cylinder is then spun. When the trigger is pulled, the probability of firing a bullet that can kill is 1 in 6.

A similar principle applies to smoking cigarettes, a potentially lethal practice governed by the laws of probability. Each cigarette has a small but finite probability of randomly creating a mutation that can cause cancer. Like Russian roulette, the more the game is played, the greater the chance that lethal damage will occur. So when it is stated that smoking cigarettes (or exposure to any other carcinogen) "causes" cancer, it simply means that a person's risk of developing cancer is increased. For this reason, agents exhibiting the potential to cause cancer are sometimes referred to as cancer risk factors.

In concluding, brief mention should be made of the fact that immunosuppressive drugs increase cancer risk in a fundamentally different way from the chemical carcinogens we have been discussing. Immunosuppressive drugs are given to organ transplant patients to inhibit the immune system and thereby minimize the possibility that a transplanted organ will be rejected. Because they inhibit immune function, some immunosuppressive drugs increase cancer risk by diminishing the likelihood that immune surveillance will destroy newly forming cancer cells. These immunosuppressive drugs differ from typical carcinogens in that they increase cancer risk indirectly, targeting the immune system rather than acting directly on the cells destined to become cancerous.

Teratogen:

A teratogen is any agent that causes an abnormality following fetal exposure during pregnancy. Teratogens are usually discovered after an increased prevalence of a particular birth defect. For example, in the early 1960's, a drug known as thalidomide was used to treat morning sickness. Exposure of the fetus during this early stage of development resulted in cases of phocomelia, a congenital malformation in which the hands and feet are attached to abbreviated arms and legs.

Teratogens can also be found at home or the workplace. The effect is related to type of agent, dose and duration and time of exposure. The first half of pregnancy is the most vulnerable. Teratogenic agents include infectious agents (rubella, cytomegalovirus, varicella, herpes simplex, toxoplasma, syphilis, etc.); physical agents (ionizing agents, hyperthermia); maternal health factors (diabetes, maternal PKU); environmental chemicals (organic mercury compounds, polychlorinated biphenyl or PCB, herbicides and industrial solvents); and drugs (prescription, over- the-counter, or recreational). In general, if medication is required, the lowest dose possible should be used and combination drug therapies and first trimester exposures should be avoided.

Causes of Teratogenicity:

The toxicants which cause teratogenesis are known as teratogenic agents. A gestating-embryo exhibits great dynamicity of the living cells. The embryonic cells multiply and differentiate at a tremendous rate making the embryo more susceptible to the drugs.

Stage Sensitivity for Teratogenicity:

i. Pre-Differentiation Stage:
During this stage the embryo is not susceptible to teratogenic agents. These agents either cause death to the embryo by killing all or most of the cells, or have no apparent effect on the embryo. Even when some widely harmful effects have been produced, the surviving cells can compensate and form a normal embryo. This resistant stage varies from 5-9 days depending on the species.

ii. Embryonic Stage:
In fact this is the period when the cells undergo intensive differentiation, mobilization and organization. It is during this period that most of the organogenesis takes place. As a result, the embryo becomes most susceptible to the effects of various teratogens.
This period generally ends sometimes from the 10th-14th day in rodents and in the 14th week of the gestation period in humans. All organs are, however, not susceptible in the same period of the pregnancy. Rat embryo is most susceptible between days 8 and 12 for most organs, but the palate and urinogenital organs are more susceptible at a later stage for teratogens.

J. G. Wilson (1965) observed teratogenic treatment on the 10th day of gestation which resulted in the following incidences of malformations in rat:

Brain defects – 35%
Eye defects – 33%

Heart defects – 24%
Skeletal defects – 18%
Urinogential defects – 6%

iii. Fetal Stage:
This stage is characterized by growth and functional maturation. Teratogens are thus unlikely to cause morphological defects during this stage, but they may induce functional abnormalities. Whereas, morphologic defects are, in general, readily detected at birth or shortly thereafter functional abnormalities, viz., CNS impairment, may not be diagnosed for some time even after birth.

Mode of Action of Teratogens:

Various mechanisms are involved in teratogenic effects:

i. Interference with Nucleic Acids:
Various teratogenic agents interfere with nucleic acid replication, transcription, or RNA translation. These include alkylating agents, antimetabolites, intercalating agents and amino acid antagonists.

ii. Inhibition of Enzymes:

Inhibitors of enzymes, e.g. 5-flourouracil, may induce malformation through interference with differentiation or growth by inhibiting thymidylate synthatase. Other examples include 6-aminonicotinamide, which inhibits glucose-6-phosphate dehydrogenase, and folate antagonists which inhibit dihydrofolate reductase.

iii. Deficiency of Energy Supply and Osmolarity:

Certain teratogens can affect the energy supply for the metabolism by restricting the availability of substrates either directly (e.g., dietary deficiencies) or through the presence of analogs for antagonists of vitamins, essential amino acids, and others.

In addition, hypoxia and agents i.e., CO and CO_2, can be teratogenic by depriving the metabolic process of the required O_2 and probably also by the production of osmolar imbalances. These can induce edema, which, in turn, cause mechanical distortion and tissue ischemia. Physical agents that can cause malformations include radiation, hypothermia, hyperthermia and mechanical trauma. It shall not be out of place to mention that the mode of action of many teratogens is yet uncertain. Furthermore, a potential teratogen may or may not exert teratogenic effects depending on such factors as bio-activating mechanism, stability and detoxifying capability of the embryonic tissues. Appropriate experimental testing for the teratogenicity of toxicants is, therefore, essential.

Testing Procedures:

Animals:

For teratogenic tests, the animals should be young, mature and healthy. Usually, Prima gravida females are preferred. Rats, rabbits and hamsters are the commonly used animals, because of their ready availability, easy handling, little size and short gestational period. Pigs, are sometimes also used because they are phylogenetically more similar to humans. WHO (1967) suggested the use of nonhuman primates because of their phylogenetic proximity to humans. Other animals such as dogs and cats have also been used by some investigators.

The timing of administering the substance is of great importance. For routine teratologic studies, it is customary to administer the substance during the entire period of organogenesis when the embryo is most susceptible. This period varies from one species to another.

Observations:

The Pregnant Animals:

The animals should be examined daily for gross signs of toxicity and many females that show signs of impending abortion or premature delivery (e.g., vaginal bleeding) should be examined.

The Fetuses:

Fetuses are usually surgically removed from the mother about one day prior to the expected delivery. This procedure is intended to avoid cannibalism and permit counting of resorption sites and dead fetuses.

Following observations are to be made and recorded:

i. Number of corpora lutea
ii. Number and position of implantations
iii. Number and position of resorptions
iv. Number and position of dead fetuses
v. Number and position of live fetuses
vi. Sex of each live fetus
vii. Weight of each live fetus
viii. Length of each live fetus, and
ix. Abnormalities of each fetus.

Detailed Examinations:

To determine the different types of abnormalities, each fetus is examined for external defects. In addition, about 2/3rd of random sampled fetusesare closely examined for skeletal abnormalities after staining with Alizarin Red. The remaining one-third of the fetuses are examined for visceral defects after fixations in Bowin's fluid and sectioned by microtome. With larger animals, e.g., dogs, pigs, and non-human primates, the skeletal structure is generally examined with X-ray instead of staining.

Delayed Effects:

With toxicants that are suspected of having effects on the central nervous system or genitourinary system, a sufficient number of pregnant females are allowed to deliver their pups. These pups are nursed either by their biological mothers — thus possibly being exposed to the toxicants via the milk — or by foster mothers. In the latter case, the potential effects of postnatal exposure are eliminated.

Neuromotor and behavioural tests may be applied to detect CNS effects. These include posture, mother activity, coordination, endurance, vision, hearing, learning ability, response to foreign environment, mating behaviour and maternal behaviour.

Evaluation of Teratogenic Effects:

Categories and Relative Significance:

Aberrations:

In addition to functional abnormalities, morphologic defects may involve external/or internal structure. Not all types of aberrations have the same significance. For example, supernumerary ribs decrease, or abnormal sternal ossification might have little or no visible effect on external morphology, functional activity, or survival of the fetus. These have been considered as deviations.

Malformations of doubtful significance include curly tail, straight legs, malrotated limbs and paws, wrist drop, protruding tongue, enlarged atria and/or ventricles, abnormal renal pelvic development, and translucent skin. In general, these have been characterized as minor anomalies.

There are, at the other extreme, major malformations that are incompatible with survival, growth, development, fertility, and longevity, e.g., Spina bifda, hydrocephalus.

In practice, the distinction between these categories is not always clear cut. It is then necessary to take other factors into consideration:

(i) Resorption:

This is a manifestation of death of the conceptus. Although the site of resorption can be readily identified with a close examination of the uterus; the number of resorptions is more reliably determined by subtracting the total near term offsprings from the total implantations, as indicated by the number of corpora lutea.

If there is an appreciable increase in the number of resorptions in the treated group, it may be necessary to alter the testing procedure to differentiated embryotoxicity from teratogenicity, e.g. by lowering the dose used to reduce the toxicity or shorten the exposure period.

(ii) Fetal Toxicity:

This may manifest as reduced body weight on non-viable fetus. This type of data is often useful in assessing the teratogenicity of the toxicant in question. With rabbits, the viability of fetus, if in question, may be determined by incubating it for 24 hours.

Sources of Error:

i. The animals used may exhibit an excessive number of spontaneous malformation or may be resistant to teratogenic effects. These errors can usually be assessed by the response of the animals to the negative and positive control agents.

ii. Poor animal husbandry and mishandling of the animals may also result in an increased incidence of malformations.

iii. The food consumption can.be affected by the toxicant used. This fact may then alter the body weight of the mothers and indirectly affect the fetuses.

iv. Excessively large doses can result in many resorptions but few or no malformations. On the other hand, if the doses are too small, there may not be any evidence of teratogenicity.

Analysis of the Results:

In comparing the treated and control groups, the proper experimental unit is the litter rather than the individual fetuses. The number of litters with malformed fetuses, resorptions, or dead fetuses is the parameters to be used in statistical analysis. However, an increase in the average number of fetuses with defects per litter may provide evidence of teratogenicity. If the results indicate a relationship between the doses and the response (incidence of malformation), it is generally justifiable to conclude that the agent is teratogenic under the specific experimental conditions. When the incidence of malformation does not provide a definite conclusion, an analysis of the data from the historical controls may be valuable. Furthermore, a close examination of the data on other parameters of the fetus and on the mother is sometimes useful.

Extrapolation to Humans:

The results obtained in teratogenesis studies in animals cannot be readily extrapolated to humans. The lack of a suitable animal model is evidenced by the fact that the most potent human teratogen, thalidomide, which is effective at a dose of 0.5 -1.0 mg/kg, has no teratogenic effect in rats and mice at 4,000 mg/kg. Only moderate embyropathy is noted in rabbits. On the other hand, acetylsalicylic acid has a long history of safe use in human pregnancy but is a potent teratogen in rat, mice and hamsters. The mechanism of teratogenesis and the differences in response among various species of animals are poorly understood. The cause of spontaneous congenital malformations in humans are unknown. More basic animal studies and prospective epidemiologic studies are essentially required.

Nevertheless, since all chemicals that are teratogenic in humans were found to be active in certain animals as well, it is, therefore, prudent to carry out appropriate animal tests on all chemicals to which females of child-bearing age are usually exposed. If positive results are achieved with a substance — especially this is so in more than one species of animal — exposure of females of childbearing age to this substance should be avoided, if possible. In assessing the teratogenic effects of a chemical, not only the incidence but also the severity of the aberrations should be taken into account.

In Vitro Tests:

Actually, these tests are not in routine use as yet, though they may show the mode of actions of teratogens. Some of these tests are — cell culture, organ culture, etc.

Mutagen:

A mutagen is a substance or agent that causes DNA impairment that results in the alteration of the DNA sequence. This alteration of the DNA sequence is known as mutation. Any agent causing mutation is called mutagen. Mutagens can be physical mutagens, chemical mutagens, or biological mutagens.

1. Chemical Mutagens:

Singer and Kusmierek (1982) have published an excellent review on chemical mutagenesis.

Some of the chemical mutagens and mutagenesis are given in Table 9.3, and described below:

Table 9.3 : Different types of chemical mutagens

Class of Chemical	*Chemical Mutagens*
Acridines	Ethyleneimine (EI)
Mustard	Nitrogen mustard
	Sulphur mustard
Nitrosamines	Diethylnitrosamine (DMN)
	Diethylsulphonate (DES)
	Nitrosomethylurea (NMU)
Epoxide	Ethyleneoxide (EO)
	Diepoxybutane (DEB)
Alkyl sulphonates	Diethylsulphonate (DES)
	Methylmethanesulphonate (MMS)
	Ethylmethanesulphonate (EMS)
Others	Nitrous acid
	Maleic hydrazide
	Hydroxylamine

i. Base Analogues:

A base analogue is a chemical compound similar to one of the four bases of DNA. It can be incorporated into a growing polynucleotide chain when normal process of replication occurs.' These compounds have base pairing properties different from the bases. They replace the bases and cause stable mutation.

A very common and widely used base analogue is 5-bromouracil (5-BU) which is an analogue of thymine. The 5-BU functions like thymine and pairs with adenine (Fig. 9.6A).

The 5-BU undergoes tautomeric shift from keto form to enol form caused by bromine atom. The enol form can exist for a long time for 5-BU than for thymine (Fig. 9.6B). If 5-BU replaces a thymine, it generates a guanine during replication which in turn specifies cytosine causing G: C pair (Fig. 9.6A).

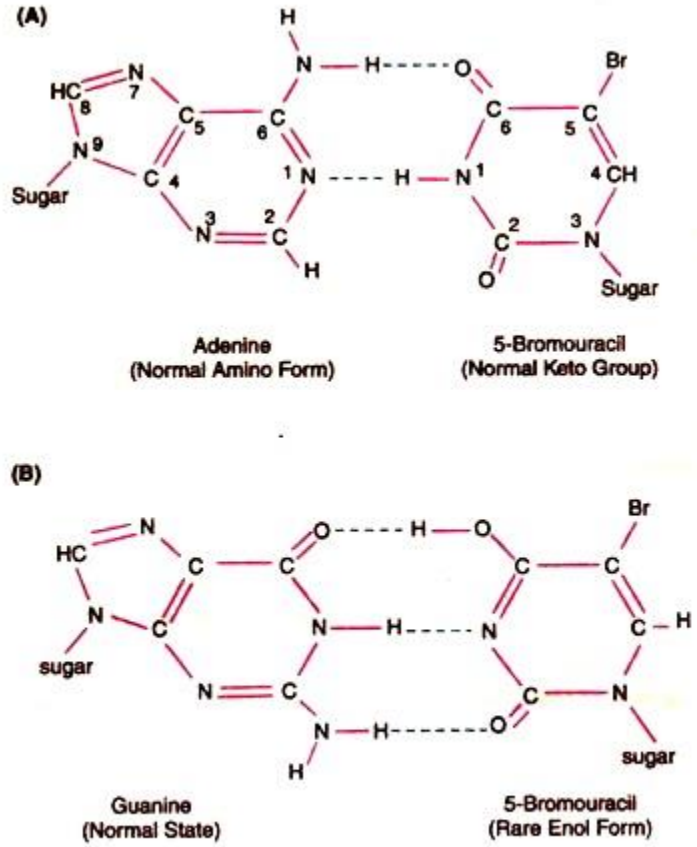


Fig. 9.6 : Mutagenesis by base analogue 5-bromouracil. A, the keto form of 5-BU pairs with adenine; B, 5-BU is tautomarised to enol form and pairs with guanine rather than adenine.

During the replication, keto form of 5-BU substitutes for T and the replication of an initial AT pair becomes an A: BU pair (Fig. 9.7A). The rare enol form of 5-BU that pairs with G is the first mutagenic step of replication. In the next round of replication G pairs with C. Thus, the transition is completed from AT→GC pair.

The 5-BU can also induce the conversion of GC to AT. The enol form infrequently acts as an analogue of cytosine rather than thymine. Due to error, GC pair is converted into a G: BU pair which in turn becomes an AT pair (Fig. 9.7B). Due to such pairing properties 5-BU is used in chemotherapy of viruses and cancer. Because of pairing with guanine it disturbs the normal replication process in microorganisms.

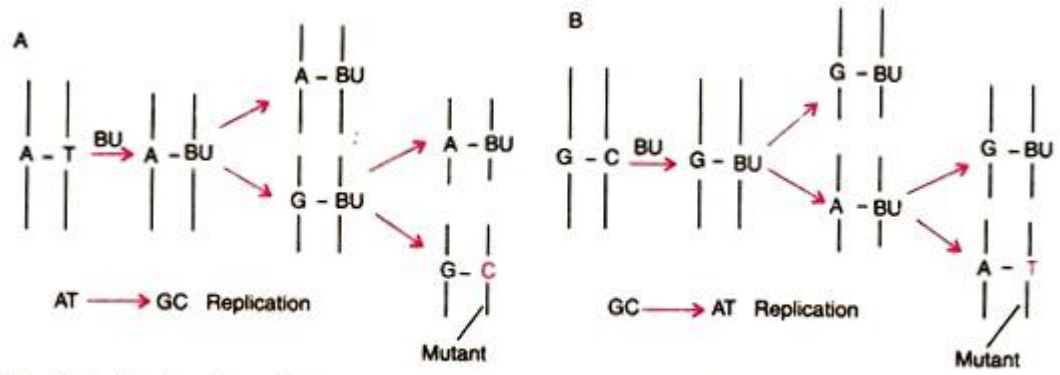


Fig. 9.7 : Mechanism of 5-bromouracil (BU)-induced mutagenesis. A, AT→GC replication; B, GC→AT replication.

The 5-bromodeoxyuridine (5-BDU) can replace thymidine in DNA molecule. The 2-amino-purine (2-AP) and 2, 6-di-amino-purine (2, 6-DAP) are the purine analogues. The 2-AP normally pairs with thymine but it is able to form a single hydrogen bond with cytosine resulting in transition of AT to GC. The 2-AP and 2, 6-DAP are not as effective as 5-BU and 5-BDU.

ii. Chemicals Changing the Specificity of Hydrogen Bonding:

There are many chemicals that after incorporation into DNA change the specificity of hydrogen - bonding. Those which are used as mutagens are nitrous oxide (HNO_2), hydroxylamine (HA) and ethyl-methane-sulphonate (EMS).

(a) Nitrous Oxide (HNO_2):

Nitrous oxide converts the amino group of bases into keto group through oxidative deamination. The order of frequency of deamination (removal of amino group) is adenine > cytosine > guanine.

(b) Deamination of Adenine:

Deamination of adenine results in formation of hypoxanthine, the pairing behaviour of which is like guanine. Hence, it pairs with cytosine instead of thymine replacing AT pairing by GC pairing (Fig. 9.8A).

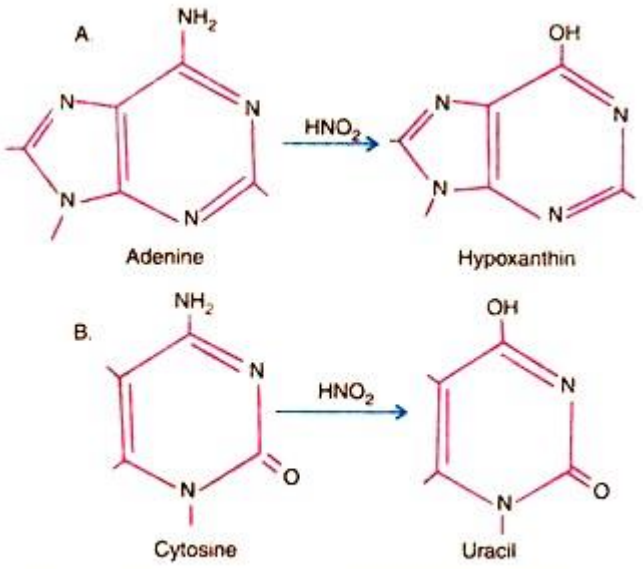


Fig. 9.8 : Deamination by nitrous oxide of adenine into hypoxanthin (A), and cytosine into uracil (B).

(c) Deamination of Cytosine:

Deamination of cytosine results in formation of uracil by replacing – NH_2 group with -OH group. The affinity for hydrogen bonding of uracil is like thymine; therefore, C-G pairing is replaced by U-A pairing (Fig. 9.8B).

(d) Deamination of Guanine:

Deamination of guanine results in formation of xanthine, the later is not mutagenic. Xanthine behaves like guanine because there is no change in pairing behaviour. Xanthine pairs with cytosine. Therefore, G-C pairing is replaced by X-C pairing.

(e) Hydroxylamine (NH_2OH):

It hydroxylates the C_4 nitrogen of cytosine and converts into a modified base via deamination which causes to base pairs like thyamine. Therefore, GC pairs are changed into AT pairs.

iii. Alkylating Agents:

Addition of an alkyl group to the hydrogen bonding oxygen of guanine (N_7 position) and adenine (at N_3 position) residues of DNA is done by alkylating agents. As a result of alkylation, possibility of ionization is increased with the introduction of pairing errors. Hydrolysis of linkage of base-sugar occurs resulting in gap in one chain.This phenomenon of loss of alkylated base from the DNA molecule (by breakage of bond joining the nitrogen of purine and deoxyribose) is called depurination. Depurination is not always mutagenic. The gap created by loss of a purine can effectively be repaired.

Following are some of the important widely used alkylating agents:

(a) Dimethyl sulphate (DMS)

(b) Ethyl methane sulphonate (EMS) -$CH_3CH_2SO_3CH_3$

(c) Ethyl ethane sulphonate (EES) -$CH_3CH_2SO_3CH_2CH_3$

EMS has the specificity to remove guanine and cytosine from the chain and results in gap formation. Any base (A,T,G,C) may be inserted in the gap. During replication chain without gap will result in normal DNA. In the second round of replication gap is filled by suitable base.

If the correct base is inserted, normal DNA sequence will be produced. Insertion of incorrect bases results in transversion or transition mutation. Another example is methyl nitrosoguanidine that adds methyl group to guanine causing it to mispair with thyamine. After subsequent replication, GC is converted into AT transition.

iv. Intercalating Agents:

There are certain dyes such as acridine orange, proflavine and acriflavin which are three ringed molecules of similar dimensions as those of purine pyrimidine pairs (Fig. 9.9). In aqueous solution these dyes can insert themselves in DNA (i.e. intercalate the DNA) between the bases in adjacent pairs by a process called intercalation.

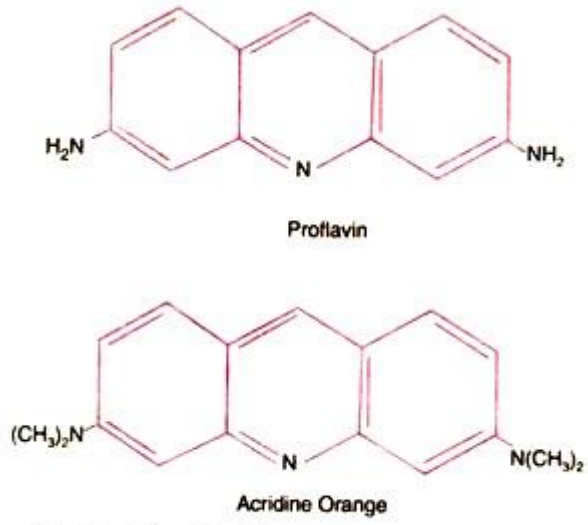


Fig. 9.9 : Chemical structure of two mutagenic acridine derivatives.

Therefore, the dyes are called intercalating agents. The acridines are planer (flat) molecules which can be intercalated between the base pairs of DNA; distort the DNA and results deletion or insertion after replication of DNA molecule. Due to deletion or insertion of intercalating agents, there occur frameshift mutations (Fig. 9.10).

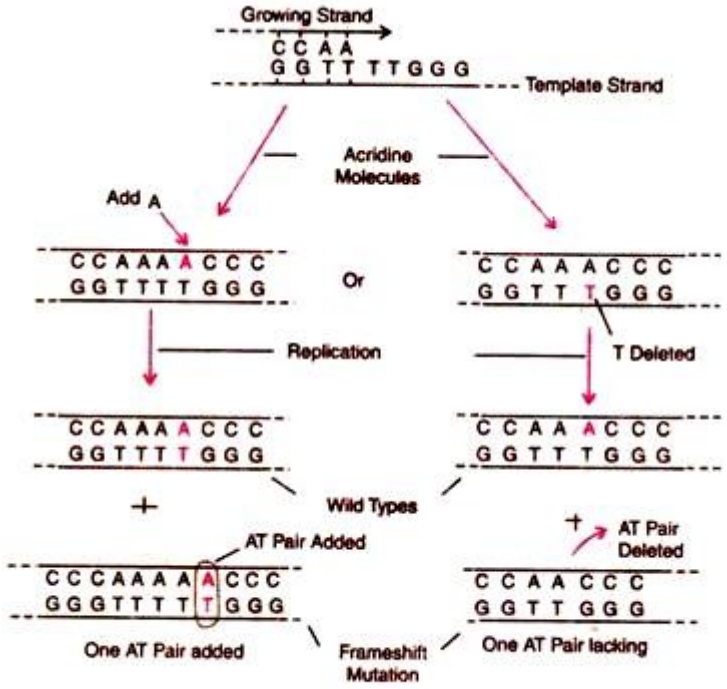


Fig. 9.10 : Mechanism of intercalation of an acridine molecule in the replication fork.

3. Physical Mutagens:

i. Radiations as Mutagens:

Radiation is the most important among the physical mutagens. Radiations damaging the DNA molecules fall in the wavelength range below 340 nm and photon energy above 1 electro-volt (eV). The destructive radiation consists of ultraviolet (UV) rays, X-rays, γ-rays, alpha (α) rays, beta (β) rays, cosmic rays, neutrons, etc. (Fig. 9.11).

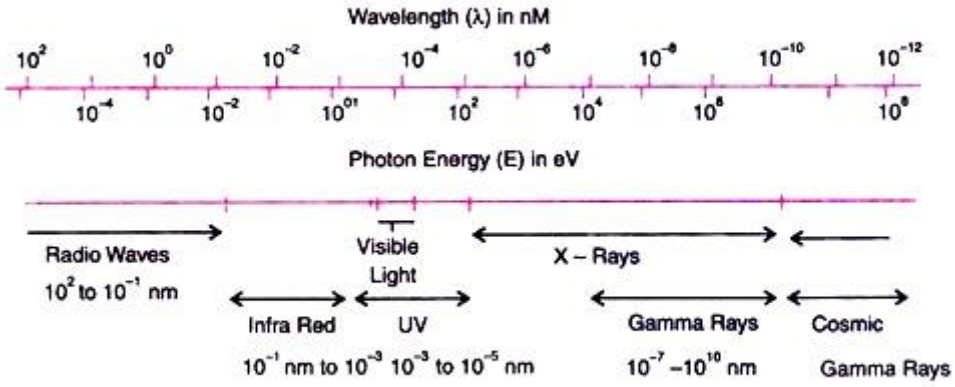


Fig. 9.11 : Wavelengths and photon energy of various radiations.

Radiation induced damage can be categorized into the three broad types: lethal damage (killing the organisms), potentially lethal damage (can be lethal under certain ordinary conditions) and sub-lethal damage (cells do not die unless radiation reaches to a certain threshold value). The effect of damage is at molecular level.

In a live cell radiation damage to proteins, lipoproteins, DNA, carbohydrates, etc. is caused directly by ionization/excitation, or indirectly through highly reactive free radicals produced by radiolysis

of cellular water. DNA stores genetic information's so a damage to it assumes great dimension. It can perpetuate genetic effects and, therefore, the cellular repair system is largely devoted to its welfare.

When the bacteria are exposed to radiation they gradually lose the ability to develop colonies. This gradual loss of viability can be expressed graphically by plotting the surviving colonies against the gradually increasing exposure time. This dose-response graph is called survival curve. The survival curve of bacteria is given in Fig. 9.12. The survival curve is analysed by a simple mathematical theory called hit theory.

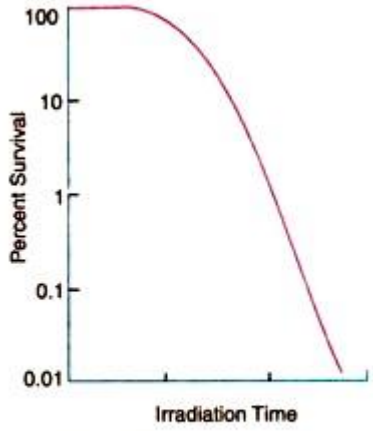


Fig. 9.12 : A typical ultra violet light survival curve for a bacterium.

Hit Theory:

Each organism possesses at least one sensitive site which is known as target site. Radiation photons (particles of light) damage or hit the target site and inactivate the organisms. One can derive the equation based on this theory.

The equations help to calculate the survival curve for many kinds of populations of N identical organisms exposed to dose D of radiation causing damage. The number dN damaged by a dose dD is proportional to the initial population that has not received radiation; hence $dN = KN$

where,

K is the constant which measures the effectiveness of dose.

Integrating this equation from N = No at D = O we get

$N = No^{e-KD}$ ...(1)
The surviving fraction S = N/No is

$S = N/NO = e^{-KD}$ ...(2)

A plot of S virus D gives a straight line with a slope of -K (Fig. 9.12). This type of curves are called exponential or single hit curve. The exponential curve is obtained when the phages are irradiated with X-rays.

If there is a population of different organisms, and each organism consists of at least n sites, each site must be hit to inactivate an organism. Therefore, each organism is hit by n times. The probability of one unit being hit by a dose D is, $P = 1-e^{-KD}$), so the probability of Pn will be $Pn = (1-e^{-KD})^n$
The surviving fraction S of the population is 1-pn or $S = 1-(1-e^{-KD})n$ ...(3)

This equation can be expanded as:
$S = 1- (1-ne^{-KD} + e^{-nKD})$
At the large value of D, the higher order terms become negligible as compared to Therefore, at high dose D,

$S = ne^{-KD}$ or
In S = In n – KD ...(4)

When the equation 3 is plotted for K – 1, various values of n reveals that for small valuesof D, In S gradually changes (Fig. 9.13). At large value of D, equation 4 dominates and curve becomes linear.

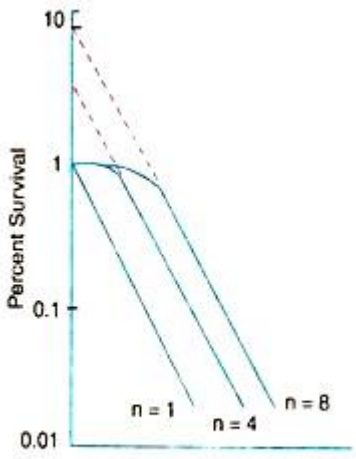


Fig. 9.13 : Survival curve for different values of n (hits' time).

ii. Ultraviolet (UV) Radiation:

UV radiation causes damage in the DNA duplex of the bacteria and phages. The UV rays are absorbed and cause excitation of macromolecules. The absorption maxima of nucleic acid = (280 nm) and protein (260 nm) are more or less similar. The DNA molecule is the target molecule for UV rays but not the proteins. However, absorption spectrum of RNA is quite similar to that of DNA.

The excited DNA leads to cross-linking, single strand breaks and base damage as minor lesion and generation of nucleotide dimer as a major one. Purines are generally more radio – resistant than the pyrimidine of the latter, thymine is more reactive than cytosine.

Hence, the ratio of thymine-thymine (TT), thymine-cytosine (TC), cytosine-cytosine (CC) dimer (Fig. 9.14) is 10:3:3, respectively. A few dimers of TU and UU also appear. The initial step in pyrimidine dimerization is known to be hydration of their 4: 5 bonds.

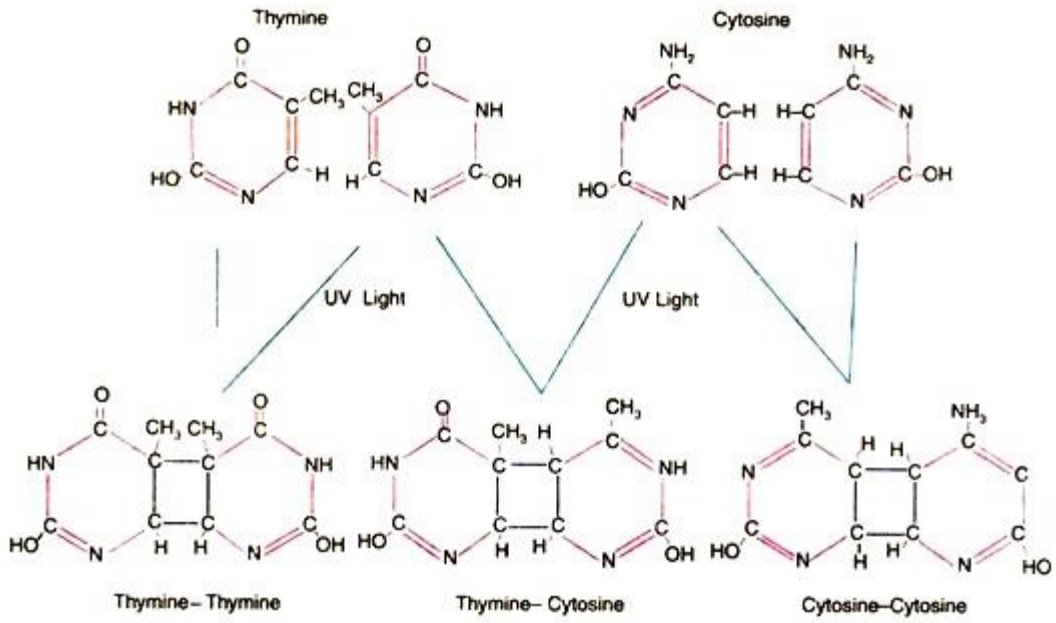


Fig. 9.14 : Formation of pyrimidine dimer induced by UV radiation.

Formation of thymine-thymine (TT) dimer causes distortion of DNA helix because the thymines are pulled towards one another. The distortion results in weakening of hydrogen-bonding to adenines in the opposing strand. This structural distortion inhibits the advance of replication fork.

iii. The X-Rays:

The X-rays cause breaking of phosphate ester linkages in the DNA. This breakage occurs at one or more points. Consequently, a large number of bases are deleted or rearranged in the DNA molecule.

The X-rays may break the DNA either in one or both strands. If breaks occur in both strands, it becomes lethal. The DNA segment between the two breaks is removed resulting in deletion. Since both the X-rays and UV rays bring about damage in DNA molecule, they are used in sterilization of bacteria and viruses.

Radiation Exposure:

High energy radiation or ionising radiation produces a genetic alteration or mutation at a very low dose also. It has been shown experimentally that a low dose of X-ray (100R) will destroy a large part of the spermatogonia in male mammals resulting in sterility. Similarly, there is a high level of risk if fertilization occurs within the first few weeks after radiation exposure in human male.

In case of acute irradiation, generally two types of danger could occur:

(a) The immediate damage to the exposed person, which may be indicated by burns or other direct or secondary effects on the body tissues.

(b) The more insidious damage to the DNA in his/her reproductive cells which would affect the future generations.

Of the above two types of danger, the first one cannot be detected if the doses are on the magnitude of 50 mR (milliroentgens) but it may produce the second danger.

In this regard one important thing is to be remembered: the human female is more sensitive than the male in consequence with the effect of irradiation in the germ cells, because it has been shown experimentally that mature oocytes (about the time of fertilization) is particularly vulnerable to radiation. The effect of dose rate of ionising radiation is also a very important one. A dose of radiation that is given over a longer period of time at a lower rate induces only 1/4th the number of recessive point mutations in oocytes and spermatogonia as the same dose given all at once; this is probably due to action of repair enzymes.

Therefore, it should be remembered that extended exposure at a lower dose rate is considerably less dangerous to human beings than a brief exposure with a high dose rate. Actually, from the genetical point of view, there is no safe dose of ionising radiation or, in other words, there is no such dose which can produce a threshold effect. Another most important factor regarding the effects of ionising radiation on the rate of mutation is the oxygen tension and the temperature change. These two factors can enhance the effect of radiation-induced mutation frequency.

It has been generally found that low oxygen tension decreases the rate of mutations, or, in other words, oxygen can magnify the effect of radiation if it is present during the time of irradiation. Oxygen has less effect with intense conditions than with moderate conditions of ionization. It is interesting to note that environmental agents that protect germ cells from radiation damage by lowering the oxygen concentration of the tissues.

Major Consequences with the Radiation Exposure:

1. Radiation damages the spermatogonia and the damaged germ cells could occur for a very long time, perhaps a lifetime.

2. Radiation also induces recessive and dominant point mutations.

3. Sometimes gross chromosomal damage may also occur.

4. Majority of the mutations after radiation exposure will be of recessive type and, therefore, not affect the phenotype in the first generation.

5. Mature oocytes are more susceptible regarding the radiation induced mutation than spermatogonia.

6. If conception has taken place shortly after radiation exposure it will be more dangerous.

Radiation Dose which will Induce the Mutations:

Following are the doubling dose (a doubling dose is the intensity of radiation necessary to double the normal spontaneous mutation rate in spermatogonia) for mice in pre- meiotic germ cell stage and may serve as a frame of reference to humans:

1. Dominant morphological mutations: 16- 26 R

2. Recessive mutations: 32 R

3. Autosomal recessive lethals: 51 R

4. Structural chromosomal aberrations: 31 R

Probable Questions:

1. Define carcinogen. How workplace exposure can cause cancer?
2. How asbestos can cause cancer?
3. Can low dose exposure of carcinogen can cause cancer? Discuss.
4. Medications and hormones can cause cancer, discuss.
5. State five categories of chemical carcinogens.
6. How liver can transform compounds more carcinogenic?
7. How electrophile DNA can cause cancer?
8. Discuss different steps of chemical carcinogenesis in brief.
9. How dose and potency of carcinogen chemicals affect carcinogenesis.
10. Define teratogen? Discuss different stages of teratogenicity.
11. Discuss mode of action of teratogens.
12. Define mutagen. How base analogues cause DNA mutation?
13. How intercalating agents can cause DNA mutation?
14. How ionizing radiation can cause DNA mutation?
15. How UV ray can cause DNA mutation?
16. How X ray can cause DNA mutation?

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett&Doull's Toxicology: The Basic Science by Curtis D. Klaassen

UNIT-III

Properties of few individual insecticides i.e. DDT, HCH(BHC), Lindane, Endosulfan, Parathion, Malathion, Carbaryl, Cypermethrin.

Objective: In this unit we will discuss properties and toxicity of some selected insecticides such as DDT, HCH(BHC), Lindane, Endosulfan, Parathion, Malathion, Carbaryl, Cypermethrin.

Insecticides:

Substances which are used to kill insects are called insecticides. Insecticides have a wide application in the field of medicine, agriculture, and industry. They have the potential to alter ecosystem components majorly and are toxic to animals as well as humans. Some insecticides become concentrated as they spread in the food chain.

Insecticide is defined as any toxic substance that is used to kill insects. Such substances are used primarily to control pests that infest cultivated plants or to eliminate disease-carrying insects in specific areas.

Insecticides can be classified in any of several ways, on the basis of their chemistry, their toxicological action, or their mode of penetration. In the latter scheme, they are classified according to whether they take effect upon ingestion (stomach poisons), inhalation (fumigants), or upon penetration of the body covering (contact poisons). Most synthetic insecticides penetrate by all three of these pathways, however, and hence are better distinguished from each other by their basic chemistry. Besides the synthetics, some organic compounds occurring naturally in plants are useful insecticides, as are some inorganic compounds; some of these are permitted in organic farming applications. Most insecticides are sprayed or dusted onto plants and other surfaces traversed or fed upon by insects.

Classification of insecticide

- Based on chemical composition, it is classified as organic and inorganic.
- Based on the mode of entry in the insects, it is classified as contact poisons, fumigants poisons, stomach poisons, and systemic poisons.
- Based on the mode of action, it is classified as physical poisons, nerve poisons, respiratory poisons, protoplasmic poisons, general poisons, and chitin inhibitors.
- Based on toxicity, it is classified into four types:

1. **Extremely toxic** – Colour: red, symbol: skull and poison, oral LD50: 1-50
2. **Moderately toxic –** Colour: blue, symbol: danger, oral LD50: 501 – 5000
3. **Highly toxic** – Colour: yellow, symbol: poison, oral LD50: 51 – 500
4. **Less toxic** – Colour: green, symbol: caution, oral LD50: >5000

- Based on the stage of specificity, it is classified as ovicides, pupicides, larvicides, and adulticides.

Modes of penetration:

Stomach poisons are toxic only if ingested through the mouth and are most useful against those insects that have biting or chewing mouth parts, such as caterpillars, beetles, and grasshoppers. The chief stomach poisons are the arsenicals—e.g., Paris green (copper acetoarsenite), lead arsenate, and calcium arsenate; and the fluorine compounds, among them sodium fluoride and cryolite. They are applied as sprays or dusts onto the leaves and stems of plants eaten by the target insects. Stomach poisons have gradually been replaced by synthetic insecticides, which are less dangerous to humans and other mammals.

Contact poisons penetrate the skin of the pest and are used against those arthropods, such as aphids, that pierce the surface of a plant and suck out the juices. The contact insecticides can be divided into two main groups: naturally occurring compounds and synthetic organic ones. The naturally occurring contact insecticides include nicotine, developed from tobacco; pyrethrum, obtained from flowers of *Chrysanthemum cinerariae folium* and *Tanacetum coccineum*; rotenone, from the roots of *Derris* species and related plants; and oils, from petroleum. Though these compounds were originally derived mainly from plant extracts, the toxic agents of some of them (e.g., pyrethrins) have been synthesized. Natural insecticides are usually short-lived on plants and cannot provide protection against prolonged invasions. Except for pyrethrum, they have largely been replaced by newer synthetic organic insecticides.

Fumigants are toxic compounds that enter the respiratory system of the insect through its spiracles, or breathing openings. They include such chemicals as hydrogen cyanide, naphthalene, nicotine, and methyl bromide and are used mainly for killing insect pests of stored products or for fumigating nursery stock.

Systemic insecticides are water-soluble and are taken up by a plant and transported throughout its body. The chemicals can thus be found in every part of the plant, including the leaves, roots, stems, fruits, flowers, and even the pollen and nectar. They can kill insects directly on contact or through the ingestion of treated plant tissue. Systemic protection is longer-lasting than contact sprays, and it is particularly useful against root-feeding insects and boring insects, such as the emerald ash borer, that typically evade foliar pesticides.

Types of insecticides:

There are three different types of insecticides. They are

1. **Systemic –** This type of insecticide is introduced into the soil for it to get absorbed by the plant roots. Once the insecticide enters the roots, it moves to external areas such as leaves, fruits, twigs, and branches. It forms a layer on the plant surface area and acts as a poison to any insect that comes to chew the plant.
2. **Ingested** – Some examples of ingested pesticides are rats and roaches.

3. **Contact** – These types of insecticides act like bullets that aim only at a particular target to kill insects by its application. Usually, household insect spray works like contact insecticides as it must directly hit the insect.

Classification of insecticides based on chemical nature

Based on their chemical nature, insecticides are classified into four groups

1. Organic insecticides
2. Synthetic insecticides
3. Inorganic insecticides
4. Miscellaneous compounds

Disadvantages of insecticides:

1. **Non-target organisms –** Insecticides can kill more than intended organisms and are risky to humans. Also, when insecticides mix with water sources through leaching, drift, or run off, they harm aquatic wildlife. When birds drink such contaminated water and eat affected insects, they die. Some examples of insecticides, like DDT, were banned in the US as it affects the reproductive abilities of predatory birds.
2. **Resistance** – Insects when repeatedly exposed to insecticides build up resistance until finally, they have little or no effect at all. The reproduction in insects is so quick that they produce a new generation every three to four weeks. Therefore, the resistance builds up rapidly.

DDT:

DDT, or dichlorodiphenyltrichloroethane, is a chemical compound with the formula $C_{14}H_9Cl_5$. Under standard conditions for temperature and pressure (STP), this chemical compound exists as a colourless and tasteless crystalline solid. Some important properties and uses of DDT are listed in this article along with the hazards this compound poses to human health.

Properties of DDT:

- The chemical formula of dichlorodiphenyltrichloroethane is $C_{14}H_9Cl_5$.
- The molar mass of this chemical compound corresponds to 354.48 grams per mole.
- Under standard conditions, the density of this compound is roughly equal to 1 gram per cubic centimetre.
- The melting point of this chemical compound is approximately equal to 108.5 degrees Celsius (or 381.6 Kelvin).

- The boiling point of this chemical compound is approximately equal to 260 degrees Celsius (or 533 Kelvin). However, it is important to note that DDT undergoes decomposition when it is heated to this temperature range.
- DDT is very poorly soluble in water. For all practical purposes, this compound is insoluble in water. The solubility of dichlorodiphenyltrichloroethane in water corresponds to 25 micrograms per litre (at a temperature of 25 degrees Celsius).

Uses of DDT:

Between the 1950s and the 1980s, DDT was widely used in the agricultural industry as an insecticide. The use of DDT to control diseases like typhus and malaria was not uncommon in the early 1940s.

DDT acts upon the sodium ion channels in the neurons of insects, making them fire in a spontaneous manner. This causes the insects to undergo spasms and eventually die. However, certain mutations in insects can make them resistant to DDT. The primary application of this compound was, therefore, as an insecticide for the control of dangerous diseases like malaria. However, due to concerns over its negative impacts on the environment and human health, the use of this compound has been banned in several countries.

Health Hazards Associated with DDT:

- DDT is known to act as an endocrine disruptor. Therefore, exposure to this compound can result in interference with the endocrine system.
- This compound is also suspected to be a carcinogen to human beings. However, it can be noted that many studies suggest that this compound is not genotoxic.
- It can also be noted that DDT is classified as a moderately toxic substance by the US NTP (national toxicology program). Indirect exposure to this chemical compound is believed to be non-toxic to humans.
- DDT is also believed to interfere with the regular thyroid function in pregnant women.
- This compound has also been linked to a higher risk of developing autism in children.

It can also be noted that DDT is classified as a persistent organic pollutant. This compound can penetrate soils and remain there for up to 30 years. To learn more about DDT and other pesticides, register with BYJU'S and download the mobile application on your smartphone.

Benzene hexachloride (BHC), any of several stereoisomers of 1,2,3,4,5,6-hexachlorocyclohexane formed by the light-induced addition of chlorine to benzene. One of these isomers is an insecticide called lindane, or Gammexane.

Benzene hexachloride was first prepared in 1825; the insecticidal properties were identified in 1944 with the γ-isomer (gamma-isomer), which is about 1,000 times more toxic than any of the

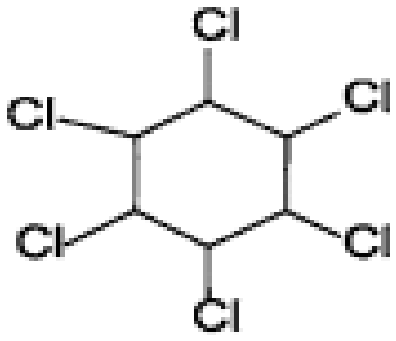
other diastereomers formed in the reaction. The structural differences between these individuals are in the orientations of the chlorine atoms with respect to the ring of carbon atoms.

The chemical addition of chlorine to benzene produces a mixture of several stereoisomers of 1,2,3,4,5,6-hexachlorocyclohexane. The γ-isomer, which makes up 20–25 percent of this mixture, is more soluble than the other isomers in certain solvents and can be separated from them. More volatile than DDT, BHC has a faster but less protracted action upon insects.

Lindane has been shown to accumulate in the food chain. This occurs because animals, including humans, eat foods grown in lindane-contaminated soils, and fishes and other marine life are exposed to lindane-contaminated waters. In fishes and mammals, exposure to high levels of lindane may cause acute poisoning, which is evidenced by nervous system dysfunction. Chronic exposure may adversely affect liver function in humans. Lindane's use indoors in smoke fumigators is no longer permitted, and its use as an insecticide has been banned in many countries. Topical use in lotions to combat lice is permitted.

Lindane, also known as gamma-hexachlorocyclohexane, gammaxene, Gammallin, and benzene hexachloride, is an organochlorine useful as an agricultural insecticide and as a pharmaceutical agent to eliminate lice and scabies (Ahmed et al., 2008). Lindane is a neurotoxin that interferes with GABA neurotransmitter function by interacting with the GABAA receptor–chloride channel complex at the picrotoxin binding site. In humans, lindane affects the nervous system, liver, and kidneys and may be a carcinogen (Ahmed et al., 2008). Preclinical studies have shown that ginger possesses protective effects against the hepatotoxicity induced by lindane. Oral administration of lindane (30 mg kg^{-1} b.wt) for 4 weeks enhanced lipid peroxidation and depleted the antioxidant defenses in rats, while the concomitant feeding of ginger (1%, w/w) attenuated lindane-induced lipid peroxidation and enhanced the levels of ROS-scavenging enzymes (GPx, GR, GST) and GSH (Ahmed et al., 2008).

Lindane is used for control of ectoparasites on cattle and for control of insects on a variety of commercial crops. A 20% formulation used for control of borers and leaf miners is labeled as a skin irritant. It is also a sensitizer in the Buehler assay. The handler database contained two cases of contact dermatitis following direct accidental exposure to lindane, both consistent with irritant reaction. Dermatitis has also been reported among workers in lindane manufacturing operations, but the reported cases were possibly attributable to precursors and by-products not typically found in commercial formulations of lindane (Smith, 1991a). Although the agricultural products may contain as much as 40% lindane, post-treatment dermatitis has also occasionally occurred in patients treated for scabies with 1% formulations of lindane. The extensive series reported by Farkas also contained cases reacting to a 20% scabicidal formulation of sulfur (Farkas, 1983).



Lindane

Lindane is absorbed from the gastrointestinal tract, the respiratory tract, and skin. The metabolism of lindane is complex and involves a number of pathways depending on which isomer of hexachlorocyclohexane (HCH) is involved (lindane is the gamma (γ) isomer). It is nonetheless rapid. Lindane is metabolized in the liver by microsomal enzymes. The main pathways include stepwise elimination of chlorines to form tri- and tetrachlorophenols and conjugation with sulfates or glucuronides and subsequent elimination. Other metabolic pathways involve the production of mercapturates. These water-soluble products are eliminated in the urine. Lindane is bound by serum proteins in the blood. Storage is in adipose tissue and other fat-containing tissues. The γ isomer is stored in fat at a much higher rate than the other isomers.

Lindane is a pesticide that is widely used in the treatment of scabies and pediculosis, usually in a 1% lotion. It is the gamma-isomer of 1,2,3,4,5,6-hexachlorocyclohexane. The hazards of excessive industrial exposure and accidental ingestion have been well documented.
Some feel that 1% lindane is safe when used properly, but precautionary recommendations have been made:

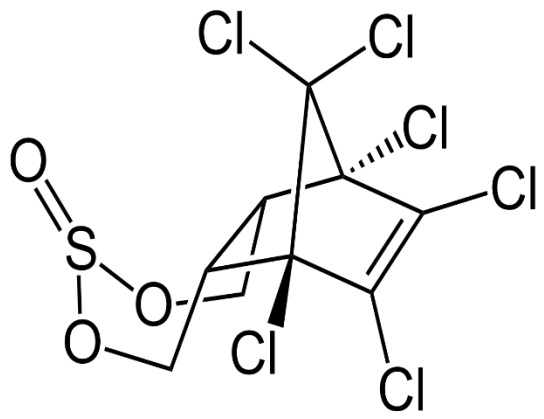
a. **a hot soapy bath before treatment is not necessary;**
b. **application for 24 hours may be too long, 6 hours having a cure rate of 96%;**
c. **a concentration weaker than 1% may be adequate;**
d. **lindane should not be repeated within 8 days, and then only if active parasites can still be demonstrated;**

lindane 1% should be used with extreme caution, if at all, in pregnant women, very small infants, and people with massively excoriated skin.There has been concern from animal experiments that lindane may be mutagenic, carcinogenic, and teratogenic; in therapeutic use, however, such hazards are highly unlikely

Endosulfan:

Endosulfan is an off-patent organochlorine insecticide and acaricide that is being phased out globally. It became a highly controversial agrichemical due to its acute toxicity, potential for bioaccumulation, and role as an endocrine disruptor. Because of its threats to human health and the environment, a global ban on the manufacture and use of endosulfan was negotiated under the Stockholm Convention in April 2011. The ban has taken effect in mid-2012, with certain uses exempted for five additional years. More than 80 countries,[4] including the European Union, Australia, New Zealand, several West African nations,[5] the United States, Brazil, and Canada[9] had

already banned it or announced phase-outs by the time the Stockholm Convention ban was agreed upon. It is still used extensively in India and China despite laws against its use. It is also used in a few other countries. It is produced by the Israeli firm Makhteshim Agan and several manufacturers in India and China. On 13.05.2011, the India Supreme Court ordered a ban on the production and sale of endosulfan in India, pending further notice. Endosulfan is an organochlorine insecticide and acaricide that is being phased out globally due to its acute toxicity. In 2011, the Stockholm Convention initiated a global ban on the manufacture and use of this chemical because of its threats to the environment and human health. It is highly toxic and has a large potential for bioaccumulation. It is also an endocrine disruptor. In India, a 2011 Supreme Court order has banned its production and sale until further notice.



Endosulfan Uses:

Endosulfan is primarily used as an insecticide in agriculture and it is also used as a wood preservative. It is used primarily on food crops like tea, fruits, vegetables and on grains.. It is used against cabbage worms, whiteflies, leafhoppers, aphids and Colorado potato beetles. Due to its unique mode of action, it is useful in resistance management; however, as it is not specific, it can negatively impact populations of beneficial insects. It is, however, considered to be moderately toxic to honey bees, and it is less toxic to bees than organophosphate insecticides.

How is Endosulfan produced?

Endosulfan is a derivative of hexachlorocyclopentadiene, and is chemically similar to aldrin, chlordane, and heptachlor. Specifically, it is produced by the Diels-Alder reaction of hexachlorocyclopentadiene with cis-butene-1,4-diol and subsequent reaction of the adduct with thionyl chloride. Technical endosulfan is a 7:3 mixture of stereoisomers, designated α and β. α- and β-Endosulfan are configurational isomers arising from the pyramidal stereochemistry of the teravalentsulfur.

The World Health Organization estimated, that before the substance was banned worldwide, annual production was about 9,000 metric tonnes (t) in the early 1980s. From 1980 to 1989, worldwide consumption averaged 10,500 tonnes per year, and for the 1990s use increased to 12,800 tonnes per year.

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Endosulfan negative health effects

1. **Toxicity:** It is highly neurotoxic to both insects and mammals. Acute endosulfan poisoning causes symptoms like hyperactivity, convulsions, tremors, staggering, lack of coordination, nausea, vomiting, breathing difficulty, diarrhea and also unconsciousness. Some low doses can also cause death in humans, or permanent brain damage.
2. **Endocrine disruption:** Endosulfan is believed to be an endocrine disruptor. Studies have shown that it causes hormone disruption, and reproductive and developmental toxicity.
3. **Reproductive and developmental effects:** Endosulfan adversely affects human development. Many villages in Kasargod District of Kerala have been affected severely because of the exclusive use of endosulfan as a pesticide for a period of almost 20 years. Endosulfan was applied to cashew plantations in these villages. Researchers found that exposed boys from the villages had high levels of endosulfan in their bodies, lower levels of testosterone and have delayed sexual maturity. Studies in California have found that pregnant women exposed to this pesticide are more likely to give birth to autistic children.
4. **Cancer:** Although not conclusive based on any study, it is believed that endosulfan can promote proliferation of human breast cancer cells.

Why was Endosulfan banned in India?

India was one of the biggest producers and consumers of endosulfan. After the toxicity of the pesticide came into limelight because of the precipitating health issues in Kasargod District, Kerala banned it in 2001. Later, the Supreme Court banned the manufacture, storage and sale of endosulfan temporarily in May 2011. And, by the end of the year, endosulfan was banned permanently.

Endosulfan as a pesticide has been in the news for more than a decade now because of the horrid effects it has had on people, especially in villages in northern Kerala. Many children have been born with extreme birth defects and are living miserable lives. It is important to understand the topic from both an environment and a current affairs point-of-view. This is also a potential area from where essays can be asked in the UPSC exam.

Health effect:

Endosulfan is alleged to be responsible for many fatal pesticide poisoning incidents around the world by NGOs opposing pesticide usage. Endosulfan is also a xenoestrogen—a synthetic substance that imitates or enhances the effect of estrogens—and it can act as an endocrine disruptor, causing reproductive and developmental damage in both animals and humans. It has also been found to act as an aromatase inhibitor. Whether endosulfan can cause cancer is debated. With regard to consumers' intake of endosulfan from residues on food, the Food and Agriculture Organization of United Nations has concluded that long-term exposure from food is unlikely to present a public health concern, but short-term exposure can exceed acute reference doses.

Toxicity:

Endosulfan is acutely neurotoxic to both insects and mammals, including humans. The US EPA classifies it as Category I: "Highly Acutely Toxic" based on a LD_{50} value of 30 mg/kg for female rats, while the World Health Organization classifies it as Class II "Moderately Hazardous" based on a rat LD_{50} of 80 mg/kg. It is a GABA-gated chloride channel antagonist, and a Ca^{2+}, Mg^{2+} ATPase inhibitor. Both of these enzymes are involved in the transfer of nerve impulses. Symptoms of acute poisoning include hyperactivity, tremors, convulsions, lack of coordination, staggering, difficulty breathing, nausea and vomiting, diarrhea, and in severe cases, unconsciousness. Doses as low as 35 mg/kg have been documented to cause death in humans, and many cases of sublethal poisoning have resulted in permanent brain damage. Farm workers with chronic endosulfan exposure are at risk of rashes and skin irritation.

EPA's acute reference dose for dietary exposure to endosulfan is 0.015 mg/kg for adults and 0.0015 mg/kg for children. For chronic dietary expsoure, the EPA references doses are 0.006 mg/(kg·day) and 0.0006 mg/(kg·day) for adults and children, respectively.

Endocrine disruption:

Theo Colborn, an expert on endocrine disruption, lists endosulfan as a known endocrine disruptor, and both the EPA and the Agency for Toxic Substances and Disease Registry consider endosulfan to be a potential endocrine disruptor. Numerous *in vitro* studies have documented its potential to disrupt hormones and animal studies have demonstrated its reproductive and developmental toxicity, especially among males. A number of studies have documented that it acts as an antiandrogen in animals. Endosulfan has shown to affect crustacean molt cycles, which are important biological and endocrine-controlled physiological processes essential for the crustacean growth and reproduction. Environmentally relevant doses of endosulfan equal to the EPA's safe dose of 0.006 mg/kg/day have been found to affect gene expression in female rats similarly to the effects of estrogen. It is not known whether endosulfan is a human teratogen (an agent that causes birth defects), though it has significant teratogenic effects in laboratory rats. A 2009 assessment concluded the endocrine disruption in rats occurs only at endosulfan doses that cause neurotoxicity.

Reproductive and developmental effects:

Some studies have documented that endosulfan can also affect human development. Researchers studying children from many villages in Kasargod District, Kerala, India, have linked endosulfan exposure to delays in sexual maturity among boys. Endosulfan was the only pesticide applied to cashew plantations in the villages for 20 years, and had contaminated the village environment. The researchers compared the villagers to a control group of boys from a demographically similar village that lacked a history of endosulfan pollution. Relative to the control group, the exposed boys had high levels of endosulfan in their bodies, lower levels of testosterone, and delays in reaching sexual maturity. Birth defects of the male reproductive system, including cryptorchidism, were also more prevalent in the study group. The researchers concluded, "our study results suggest that endosulfan exposure in male children may delay sexual maturity and interfere with sex hormone synthesis." Increased incidences of cryptorchidism have been observed in other studies of endosulfan exposed populations.

A 2007 study by the California Department of Public Health found that women who lived near farm fields sprayed with endosulfan and the related organochloride pesticide dicofol during the first eight weeks of pregnancy are several times more likely to give birth to children with autism. However a 2009 assessment concluded that epidemiology and rodent studies that suggest male reproductive and autism effects are open to other interpretations, and that developmental or reproductive toxicity in rats occurs only at endosulfan doses that cause neurotoxicity.

Cancer:

Endosulfan is not listed as known, probable, or possible carcinogen by the EPA, IARC, or other agencies. No epidemiological studies link exposure to endosulfan specifically to cancer in humans, but *in vitro* assays have shown that endosulfan can promote proliferation of human breast cancer cells. Evidence of carcinogenicity in animals is mixed.

In a 2016 study by the Department of Biochemistry, Indian Institute of Science, Bangalore published in *Carcinogenesis*, endosulfan was found to induce reactive oxygen species (ROS) in a concentration and time-dependent manner leading to double-stranded breaks in the DNA and also found to favour subsequent erroneous DNA repair.

Parathion:

Parathion, also called **parathion-ethyl** or **diethyl parathion** and locally known as "**Folidol**", is an organophosphate insecticide and acaricide. It was originally developed by IG Farben in the 1940s. It is highly toxic to non-target organisms, including humans, so its use has been banned or restricted in most countries. The basic structure is shared by parathion methyl

History:

Parathion was developed by Gerhard Schrader for the German trust IG Farben in the 1940s. After World War II and the collapse of IG Farben due to the war crime trials, the Western allies seized the patent, and parathion was marketed worldwide by different companies and under different brand names. The most common German brand was **E605** (banned in Germany after 2002); this was not a food-additive "E number" as used in the EU today. "E" stands for *Entwicklungsnummer* (German for "development number"). It is an irreversible acetylcholinesterase inhibitor.

Safety concerns have later led to the development of parathion methyl, which is somewhat less toxic. In the EU, Parathion was banned after 2001. In Switzerland, the substance is no longer approved as a pesticide.

Handling properties

Pure parathion is a white crystalline solid. It is commonly distributed as a brown liquid that smells of rotting eggs or garlic. The insecticide is somewhat stable, although it darkens when exposed to sunlight.

Industrial synthesis:

Parathion is synthesized from diethyl dithiophosphoric acid $(C_2H_5O)_2PS_2H$ by chlorination to generate diethylthiophosphoryl chloride ($(C_2H_5O)_2P(S)Cl$), and then the chloride is treated with sodium 4-nitrophenolate (the sodium salt of 4-nitrophenol).

$$2\ (C_2H_5O)_2P(S)SH + 3\ Cl_2 \rightarrow 2\ (C_2H_5O)_2P(S)Cl + S_2Cl_2 + 2\ HCl$$

$$(C_2H_5O)_2P(S)Cl + NaOC_6H_4NO_2 \rightarrow (C_2H_5O)_2P(S)OC_6H_4NO_2 + NaCl$$

Applications:

As a pesticide, parathion is generally applied by spraying. It is often applied to cotton, rice and fruit trees. The usual concentrations of ready-to-use solutions are 0.05 to 0.1%. The chemical is banned for use on many food crops.

Insecticidal activity:

Parathion acts on the enzyme acetylcholinesterase indirectly. After an insect (or a human) ingests parathion, an oxidase replaces the double bonded sulfur with oxygen to give paraoxon.

$$(C_2H_5O)_2P(S)OC_6H_4NO_2 + 1/2\ O_2 \rightarrow (C_2H_5O)_2P(O)OC_6H_4NO_2 + S$$

The phosphate ester is more reactive in organisms than the phosphorothiolate ester, as the phosphorus atoms become much more electropositive.Parathion resistance is a special case of acetylcholinesterase inhibitor resistance.

Degradation:

Degradation of parathion leads to more water-soluble products. Hydrolysis, which deactivates the molecule, occurs at the aryl ester bond resulting in diethyl thiophosphate and 4-nitrophenol.

$$(C_2H_5O)_2P(S)OC_6H_4NO_2 + H_2O \rightarrow HOC_6H_4NO_2 + (C_2H_5O)_2P(S)OH$$

Degradation proceeds differently under anaerobic conditions: the nitro group on parathion is reduced to the amine.

$$(C_2H_5O)_2P(S)OC_6H_4NO_2 + 6\ H \rightarrow (C_2H_5O)_2P(S)OC_6H_4NH_2 + 2\ H_2O$$

Safety:

Parathion is a cholinesterase inhibitor. It generally disrupts the nervous system by inhibiting acetylcholinesterase. It is absorbed via skin, mucous membranes, and orally. Absorbed parathion is rapidly metabolized to paraoxon, as described in Insecticidal activity. Paraoxon exposure can result in headaches, convulsions, poor vision, vomiting, abdominal pain, severe diarrhea, unconsciousness, tremor, dyspnea, and finally pulmonary edema as well as respiratory arrest. Symptoms of poisoning are known to last for extended periods, sometimes months. The most common and very specific antidote is atropine, in doses of up to 100 mg daily. Because atropine may also be toxic, it is recommended that small frequently repeated doses be used in treatment. If human poisoning is detected early and the treatment is prompt (atropine and artificial respiration), fatalities are infrequent. Insufficient oxygen will lead to cerebral hypoxia and permanent brain damage. Peripheral neuropathy including paralysis is noticed as late sequelae after recovery from acute intoxication. Parathion and related organophosphorus pesticides are used in hundreds of thousands of poisonings annually, especially suicides. It is known as *Schwiegermuttergift* (mother-in-law poison) in Germany. For this reason, most formulations contain a blue dye providing warning.

Parathion was used as a chemical warfare agent, most notably by an element of the British South Africa Police (BSAP) attached to the Selous Scouts during the Rhodesian Bush War. They used it to poison clothing that was then supplied to anti-government guerrillas. When the enemy soldiers put on the clothes, they were poisoned by absorption through the skin.

Based on animal studies, parathion is considered by the U.S. Environmental Protection Agency to be a possible human carcinogen. Studies show that parathion is toxic to fetuses, but does not cause birth defects.It is classified by the United Nations Environment Programme (UNEP) as a persistent organic pollutant and by the World Health Organization (WHO) as Toxicity Class Ia (extremely hazardous).Parathion is toxic to bees, fish, birds, and other forms of wildlife.

Protection against poisoning:

To provide the end user with a minimum standard of protection, suitable protective gloves, clothing, and a respirator with organic-vapour cartridges is normally worn. Industrial safety during the production process requires special ventilation and continuous measurement of air contamination in order not to exceed PEL levels, as well as careful attention to personal

hygiene. Frequent analysis of workers' serum acetylcholinesterase activity is also helpful with regards to occupational safety, because the action of parathion is cumulative. Also, atropine has been used as a specific antidote.

Use in suicides:

A chemist swallowed .00424 ounces (0.120 g) of parathion to find the most lethal means of exposure to humans, intending to take an antidote afterwards, but was paralyzed and so died before he could reach it.Parathion was commonly used for suicides in the 1950s and 1960s.

Malathion:

Malathion is an insecticide in the chemical family known as organophosphates. Products containing malathion are used outdoors to control a wide variety of insects in agricultural settings and around people's homes. Malathion has also been used in public health mosquito control and fruit fly eradication programs. Malathion may also be found in some special shampoos for treating lice. Malathion was first registered for use in the United States in 1956.

Some products that contain malathion:

Products containing malathion may be liquids, dusts, wettable powders, or emulsions. There are thousands of products containing malathion registered for use in the United States.

Always follow label instructions and take steps to avoid exposure. If any exposures occur, be sure to follow the First Aid instructions on the product label carefully. For additional treatment advice, contact the Poison Control Center.

How does malathion work?

Malathion kills insects by preventing their nervous system from working properly. When healthy nerves send signals to each other, a special chemical messenger travels from one nerve to another to continue the message. The nerve signal stops when an enzyme is released into the space between the nerves. Malathion binds to the enzyme and prevents the nerve signal from stopping. This causes the nerves to signal each other without stopping. The constant nerve signals make it so the insects can't move or breathe normally and they die.

People, pets and other animals can be affected the same way as insects if they are exposed to enough malathion. About the same amount of malathion will be taken into the body whether you breathe it in or you swallow it. Malathion is also readily taken into the body through skin, though the amount absorbed will depend on where the exposure occurs on the body. Malathion can become more toxic if it has been sitting for a long time, especially in a hot place.

How might I be exposed to malathion?

You could be exposed to malathion if you get it on your skin or breathe it in, or if you use a product and eat, drink, or smoke afterwards without washing your hands. People who apply products containing malathion may be exposed if they do not wear the proper protective equipment. You could also be exposed to residues of malathion if you ate food that had been treated with this pesticide.

What are some signs and symptoms from a brief exposure to malathion?

People who were exposed to enough malathion to become sick felt nauseated or vomited, had muscle tremors, cramps, weakness, shortness of breath, a slowed heart rate, headache, abdominal pain and diarrhea.

Pets could be exposed to malathion if they get into a product by accident, or touch or eat plants that have just been sprayed. Pets will be affected by malathion like other animals. The nervous system is very similar in people and other animals, so animals poisoned by malathion may show signs similar to those observed in people.

What happens to malathion when it enters the body?

In both humans and animals, malathion travels to the liver and kidneys and affects the nervous system. Generally, the body can break down malathion and remove it quickly. Studies in rats showed that most malathion was gone from their bodies within a day of exposure.

Is malathion likely to contribute to the development of cancer?

Researchers fed malathion to rats for up to two years and to mice for a year and a half. They found no evidence of increased cancer in the treated animals. Other studies using higher doses of malathion in rats and mice found that they developed liver cancer. The United States Environmental Protection Agency (U.S. EPA) has determined that there is "suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential by all routes of exposure," for malathion.

Has anyone studied non-cancer effects from long-term exposure to malathion?

Rats fed malathion when they were pregnant had lower levels of the target enzyme than other rats. The fetuses also had less of the target enzyme. Rabbits were more likely to resorb their fetuses if they were fed malathion when pregnant. Rats that were fed malathion for three weeks had less thyroid activity than other rats.

Are children more sensitive to malathion than adults?

While **children may be especially sensitive to pesticides** compared to adults, there are currently no data showing that children have increased sensitivity specifically to malathion.

What happens to malathion in the environment?

Bacteria in the soil may break down malathion and sunlight can break down malathion in the air. Malathion will mix with water and can move quickly through soil. Because of these properties, malathion can be found in surface waters such as streams, and sometimes it is found in well water. The time it takes for malathion to break down to half of the original amount in soil is about 17 days, depending on the soil type. This length of time is known as the **half-life**. In water, malathion has a half-life between 2 and 18 days, depending on conditions like temperature and pH. Malathion vapor may also move long distances in air or fog.

Can malathion affect birds, fish, or other wildlife?

Malathion is highly toxic to bees and other beneficial insects, some fish, and other aquatic life. Malathion is moderately toxic to other fish and birds, and is considered low in toxicity to mammals.

Carbaryl:

Carbaryl is a man-made pesticide that is toxic to insects. It is commonly used to control **aphids**, **fire ants**, **fleas**, **ticks**, **spiders**, and many other outdoor pests. It is also used in some orchards to thin out blossoms on fruit trees.

Carbaryl has been registered for use in pesticide products since 1959. No carbaryl products are currently registered for use inside homes or on pets.

Some products that contain carbaryl:

Currently, there are over 190 registered **pesticide products** that contain carbaryl. These include sprays, dusts, granules, and water soluble packages. Many of these products can be used on agricultural crops, home gardens, lawns, and other ornamental plants. Others are used around the outside of homes and on anthills.

Always **follow label instructions** and take steps to avoid exposure. If any exposures occur, be sure to follow the First Aid instructions on the product label carefully. For additional treatment advice, contact the Poison Control Center.

How does carbaryl work?

When insects eat or touch carbaryl, it over stimulates their nervous systems. Nerves pass along signals to other nerves using the signaling chemical, acetylcholine. When it reaches its target, it has a stimulating effect on these nerves. Normally, an enzyme then quickly breaks down this signaling chemical. This allows nerves to return to rest. Carbaryl prevents this enzyme from working properly. This keeps affected nerves stimulated continuously, resulting in the inability to contract breathing muscles, ultimately causing the death of insect pests.

Carbaryl also acts as a plant growth regulator, but the way it works is not fully known. However, it is similar to certain plant hormones. It also breaks down into another chemical which is a known plant hormone.

How might anyone be exposed to carbaryl?

People are most commonly exposed to very low levels of carbaryl through their diet. Exposure can also occur if you breathe it in or get it on your skin or in your eyes. For example, exposure can occur while applying sprays or dusts during windy conditions. People may also be exposed if they eat, drink, or smoke if they don't wash their hands after using a product. You can **limit exposure** to pesticide products by carefully **following label instructions**.

What are some signs and symptoms from a brief exposure to carbaryl?

Soon after exposure, weakness, dizziness, and sweating are commonly reported. Pinpoint pupils, lack of coordination, muscle twitching, and slurred speech have also been reported. People may also experience headaches, nausea, vomiting, stomach cramps, diarrhea, or drooling. The severity of these effects can depend on the dose and the person.

In cases of severe poisoning, high blood pressure, decreased muscle tone, and seizures have been reported. Other serious signs include difficulty breathing, constriction of the airways, mucous production, fluid buildup in the lungs, and reduced heart and lung function.

What happens to carbaryl when it enters the body?

When eaten, carbaryl is absorbed into the body. However, skin absorption is slower. In an animal study, peak blood levels of carbaryl were found 15 to 30 minutes after it was eaten but 4 to 12 hours after skin contact.

Once inside, it moves in the blood stream to many tissues. Carbaryl works on nerves by binding to certain enzymes. However, this is not permanent. In an animal study, half of the carbaryl became unbound in less than two hours.

Carbaryl is then broken down into inactive products and is removed from the body. In one study rats were fed a single dose of carbaryl. Less than half of the carbaryl remained after two hours; about 97% percent left the body in urine and feces within seven days.

Is carbaryl likely to contribute to the development of cancer?

In studies, mice were fed high daily doses of carbaryl for 2 years. Male mice had an increased number of blood vessel tumors at all dose levels. At the highest dose, both male and female mice had an increased number of kidney and liver cancers. Based on these studies, the Environmental Protection Agency (EPA) has classified carbaryl as 'likely to cause cancer.' In 2007, the EPA estimated the lifetime cancer risk from eating foods with carbaryl residues to be less than 1 in 30 million.

Has anyone studied non-cancer effects from long-term exposure to carbaryl?

Scientists have also tested whether carbaryl causes developmental or reproductive effects in rats and rabbits. In these studies, animals were fed low to moderate daily doses of carbaryl throughout their lives or during their pregnancies. Developing rats weighed less and some of their bones did not fully form. After birth, fewer young rats survived than normal. Also, changes in the length of parts of the brain were observed in adults and their young. At moderate doses with rabbits, their young had lower body weights. No reproductive effects were observed in test animals. Carbaryl is not likely to act as an endocrine disruptor. It does not interact with the estrogen, androgen, or thyroid pathways.

In a two years study, rats were fed moderate to high daily doses of carbaryl. At the highest dose, rats developed cataracts, lung inflammation, and damage to certain muscles and nerves. They also showed effects to their liver, kidneys, and thyroid. At the highest two doses, carbaryl blocked an important enzyme in blood that prevents the overstimulation of nerves. Vomiting, tearing, drooling, and tremors have also been reported in long-term feeding studies with dogs.

Are children more sensitive to carbaryl than adults?

Children may be especially sensitive to pesticides compared to adults. In a study with rats, carbaryl's effect on the brain of young and adult rats was compared. Young rats were found to be 80% more sensitive than adults.

Young children may also act in ways that put them at greater risk of being exposed. For example, they may spend more time near the ground. They may also be more likely to place their hands in their mouths after touching treated surfaces.

What happens to carbaryl in the environment?

At soil and water surfaces, microbes break carbaryl down quickly. **Half-lives** are 4 days in water and 16 days on soil surfaces. Sunlight can also break carbaryl down. When carbaryl was not broken down by water but was still exposed to sunlight, a half-life of 21 days was reported. Carbaryl has a moderate ability to dissolve in water and migrate through soil toward ground water. Deep down in soil, where oxygen is absent, carbaryl breaks down more slowly. A half-life of 72 days has been reported. In water and soil, carbaryl has a low potential to make vapors into the air.

When carbaryl gets on leaf surfaces, very little is absorbed into the leaf. However, carbaryl is more readily taken up by the roots and moves to areas of active growth. On leaf surfaces, a half-life of 3.7 days has been reported.

Can carbaryl affect birds, fish, or other wildlife?

Carbaryl is practically non-toxic or slightly toxic to birds, and slightly to moderately toxic to mammals. However, it is moderately to highly toxic to fish and highly toxic to earthworms and honey bees. Carbaryl is very highly toxic to shrimp, waterfleas, and stoneflies. The main breakdown product of carbaryl is also highly toxic to some fish.In long-term studies, birds and mammals were fed low doses of carbaryl. There were decreases in the number of eggs laid and young that survived, respectively. There is some evidence that carbaryl can affect hormone systems in fish at low doses.

Cypermethrin:

Cypermethrin is a synthetic pyrethroid used as an insecticide on a large scale. Cypermethrin is highly toxic to fish, bees, and aquatic insects, according to the National Pesticides Telecommunications Network. It is found in many household ants and cockroach killers, including Raid and ant chalk. Cypermethrin 10% emulsifiable concentrate (EC) when sprayed at the concentration of 100 g ai ha^{-1} showed a residue level of 0.71 mg kg^{-1} at 0 day followed by 0.52 mg kg^{-1} at 1 day in dry season, which were higher than the recommended MRL of 0.5 mg kg^{-1} set for beans with pods. The average residue level of any insecticides depends primarily on the quantity of its active ingredient. A preharvest interval of 3 days might be considered for dry season. But in case of early wet season where crop canopy was smaller allowing higher deposition of cypermethrin residues on pods, it showed a preharvest interval of 10 days for the safe use (Nurika et al., 2022). Therefore, vegetable soybean should be harvested after 10 days of cypermethrin application to avoid any health risk of consumers.

Cypermethrin is moderately persistent in soils. Cypermethrin degrades more rapidly in sandy compared to clay soils, and in soils with low organic content. Under aerobic conditions, the half-life is 0.5–8 weeks. Cypermethrin is more persistent under anaerobic conditions. Cypermethrin is subject to photodegradation and microbial degradation under aerobic conditions. Cypermethrin binds strongly to soil particles and poses minimal leaching concerns.

Cypermethrin hydrolyzes slowly under acidic or neutral conditions but more rapidly under alkaline conditions. Concentrations decrease rapidly due by adsorption to sediment, particles, and plants.

Probable Questions:

1. Define insecticide. How insecticide can be classified?
2. Classify insecticide on the basis of penetration.
3. Classify insecticide on the basis of chemical structure.
4. What are the disadvantages if insecticides?
5. Discuss properties and uses of DDT?
6. Discuss properties and uses of BHC?
7. Discuss properties and uses of Endosulfan?
8. What are the negative effects of Endosulfan?
9. Discuss properties and uses of Malathion?
10. How malathion causes environmental pollution?
11. How does carbaryl work?
12. What happens to carbaryl when it enters the body?

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett & Doull's Toxicology: The Basic Science by Curtis D. Klaassen

UNIT-IV

Toxico-kinetics and toxico-dynamics: Absorption, distribution, Metabolism, elimination, organ toxicity

Objective: In this unit we will learn about Toxico-kinetics and toxico-dynamics. We will discuss about how a toxin is absorbed, distributed and how its metabolism and elimination occur. We will also learn about organ toxicity.

Introduction:

The sequence between exposure to a chemical and the generation of an adverse effect can be divided into two aspects (Figure 3.1); toxicokinetics or the delivery of the compound to its site of action and toxicodynamics or the response at the site of action. This subdivision is particularly useful in risk assessment.

Toxicokinetics is the study of the movement of chemicals around the body. It includes absorption (transfer from the site of administration into the general circulation), distribution (*via* the general circulation into and out of the tissues), and elimination (from the general circulation by metabolism or excretion). The term toxicokinetics has useful connotations with respect to the high doses used in toxicity studies, but it may be misleading if interpreted as the 'movement of toxicants around the body' since, as all toxicologists agree, 'all things are toxic and it is only the dose which renders a compound toxic'. Toxicodynamics relates to the processes and changes that occur in the target tissue, such as metabolic bioactivation and covalent binding, and result in an adverse effect.

Useful toxicokinetic data may be derived using a radiolabelled dose of the chemical, *i.e.*in which a proton in the molecule is replaced by a tritium atom or a carbon or sulfur atom is replaced by the radioactive equivalent (^{14}C or ^{35}S). Such studies are invaluable in following the fate of the chemical skeleton as it is transferred from the site of administration into the blood, is distributed to the tissues, and is eliminated as carbon dioxide or more likely as metabolites in air, urine, or bile. The advantage of using the radiolabelled chemical is that measured radioactivity reflects both the chemical and its metabolites, and this allows quantitative balance studies to be performed, *e.g.*to determine how much of the dose is absorbed, which organs accumulate the compound, and the pathways of metabolism. However, such simple radioactive absorption, distribution, metabolism, and excretion (ADME) studies provide only a part of the total picture, because the lack of chemical specificity in the methods does not allow an assessment of how much of the chemical is absorbed intact and how much is distributed around the body as the parent chemical. A further advantage of radiolabelling studies is that radiochromatographic methods can be invaluable in the separation and identification of metabolites, which is an important aspect of the fate of the chemical in the body. Thus, initial ADME studies define the overall fate of the chemical in the body and recognize the main chemical species (parent compound and/or metabolites) that are present in the circulation and in the urine and faeces following metabolism and excretion.

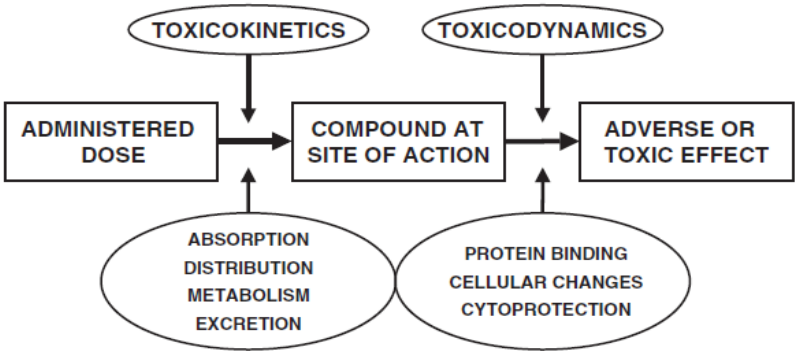

Figure 3.1 *The relationship between delivery of the administered dose to the target site and the generation of the adverse or toxic response*

In recent years, it has been recognized that measurement of the circulating concentrationsofthechemicaland/oritsmetabolitescanprovideusefulinformation on both the magnitude and the duration of exposure of targets for toxicity. The term toxicokinetics is sometimes restricted to studies based on measurements of blood or plasma concentrations, since these provide a vital link between the dosing of experimental animals and the amounts of the chemical in the general circulation (Figure 3.2). Such information can be of great value in the interpretation of species differences in toxic response, and in estimating the possible risk to humans of hazards identified in animal experiments. Toxicokinetic data are also useful in extrapolating across different routes of exposure or administration, as well as from single doses to chronic administration. Chemical-specific toxicokinetic measurements are essential if the results of *in vitro* toxicity tests are to be interpretedlogically.

The ever increasing sensitivity of modern analytical techniques should allow the measurements of 'toxicokinetics' in humans receiving the compound at safe expo- sure levels. Thus, toxicokinetic differences between test animals and humans are open to direct measurement, and such data should increase confidence in the extrapolation process. In contrast, it is unethical intentionally to generate potentially adverse effects in humans and therefore data on inter-species differences in toxicodynamics are limited to observations following accidental poisonings, mild and reversible biomarkers of the potential adverse effect, and *in vitro* studies related to the mode of action of the chemical in animals. The toxicokinetics of a chemical are determined by measuring the concentrations of the chemical in plasma (usually)or blood at various times following a single dose. The fundamental parameters that define the rates and extents of distribution and elimination are derived from data following an intravenous dose (Figure 3.2). The parameters relating to absorption from an extravascular site of administration, such as gut, lungs, *etc.*, are derived from comparisons of data following an extravascular dose with an intravenous dose. Additional useful information can be obtained from measurements of the concentrations in plasma (or blood) over a period of 24 h in animals treated chronically with the chemical since the area under the plasma concentration–time curve often referred to as 'area under the curve' (AUC) is the best indication of exposure.

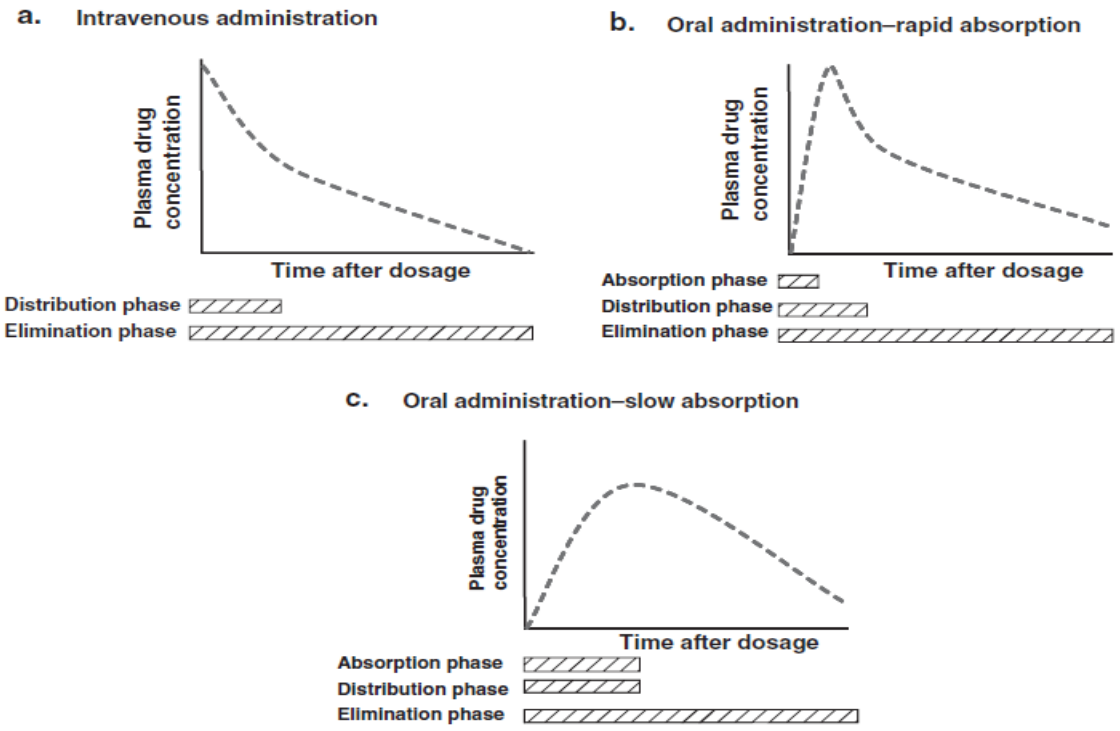

Figure 3.2 *The plasma concentration–time profiles of a chemical following intravenous and oral dosage*

The interpretation of toxicokinetic data requires an understanding of both the biological basis of the processes of absorption, distribution, and elimination and the way that simple measurements of plasma or blood concentrations can be converted into useful quantitative kinetic parameters that describe these processes. The mathematics used to define and describe the movement of a chemical around the body can display various levels of sophistication and complexity. Compartmental analysis (Figure3.3) allows the derivation of a mathematical equation which fits the data and allows the prediction of plasma concentrations at time points that were not measured directly and also outside the confines of the period of experimental observations. Physiologically based pharmacokinetic (PBPK) modelling (Figure 3.4) allows a greater interpretation of the data in biologically relevant terms but requires a sophisticated database to produce valid results. PBPK models (see below) can be used to bridge the gap between species, based on physiological differences and*in vitro* metabolic data, and extended to a biologically based dose–response model by the incorporation of *in vitro* response data.

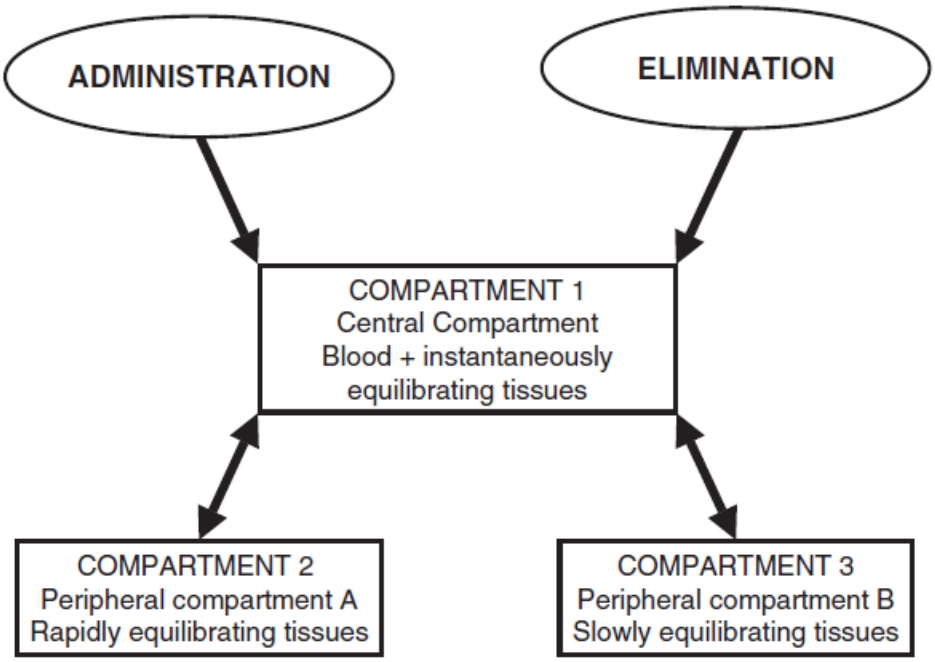

Figure 3.3 *Compartmental analysis. In the example shown, the body is considered to consist of two peripheral compartments that equilibrate with the central compartment. Strictly speaking the only property that links tissues that are part of the same "compartment" is the rate of transfer into and out of the tissue. The central compartment usually comprises blood and well-perfused tissues and equilibrates instantaneously. In the example shown, the compound is eliminated from the central compartment, for example by extraction by the liver or kidneys. The number of compartments necessary in the mathematical model fitted to the data depends on the number of exponential terms necessary to describe the plasma concentration–time curve. The mathematical model can be used to estimate the concentration in plasma or blood at any time after dosage*

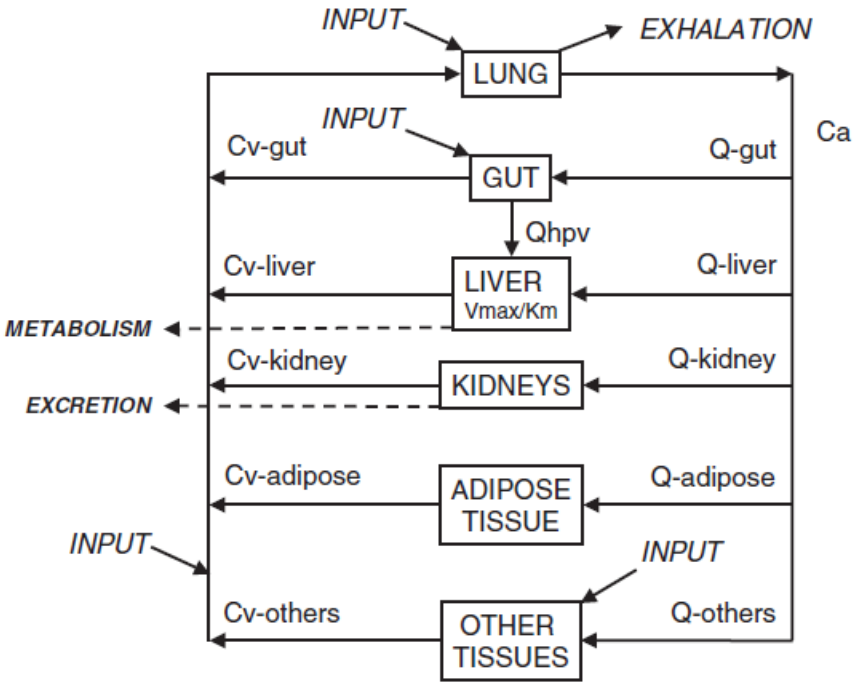

Figure 3.4 *Physiologically based pharmacokinetic model (PBPK). The PBPK model is derived from known rates of organ blood flow, the partition coefficient of the chemical between blood and the tissue, and the rates of the process of elimination, such as V_{max} and K_m for enzymes. PBPK modelling represents a powerful technique for estimating the dose delivered to specific tissues and can facilitate inter-species extrapolation by replacing animal blood flows and enzyme kinetic constants with human data. Removal across an organ equals (C_a–C_v) times the organ blood flow (Q)*

I. ABSORPTION:

The term absorption describes the process of the transfer of the parent chemical from the site of administration into the general circulation, and applies whenever the chemical is administered *via* an extravascular route (*i.e.* not by direct intravascular injection). The term 'absorption' is also used to describe the extent to which the radioactivity from a radiolabelled chemical is transferred from the site of administration into the excreta and/or expired air. However, many chemicals will be metabolized or transformed during their passage from the site of administration into the general circulation, so that little parent chemical may reach the general circulation, despite the fact that all of the radiolabel may leave the site of administration and be eliminated in the urine. This raises the possibility of confusion in discussing the 'extent of absorption' depending on whether the data refer to the parent chemical *per se*, or to radiolabel (which will include the chemical plus metabolites). This confusion is resolved by the proper use of the term bioavailability given below to describe the extent of absorption.

Rate of Absorption :

The rate of absorption may be of toxicological importance because it is a major determinant of the peak plasma concentration and, therefore, the likelihood of acute toxic effects. Transfer of chemicals from the gut lumen, lungs, or skin into the general circulation involves movement across cell membranes, and simple passive diffusion of the unionized molecule down a concentration gradient is the most important mechanism. Lipid-soluble molecules tend to cross cell membranes easily and are absorbed more rapidly than water-soluble ones. The gut wall and lungs provide a large and permeable surface area and allow rapid absorption; in contrast the skin is relatively impermeable and even highly lipid-soluble chemicals can enter only slowly. The lipid solubility and rate of absorption depend on the extent of ionization of the chemical. Compounds are most absorbed from regions of the gastrointestinal tract at which they are least ionized. Weak bases are not absorbed from the stomach, but are absorbed from the duodenum which has a higher luminal pH, whereas weak acids are absorbed from the stomach. The rate of absorption can be affected by the vehicle in which the compound is given, because rapid absorption requires the establishment of a molecular solution of the chemical in the gut lumen. Extremely lipid-soluble compounds, such as dioxins, may be only partially absorbed, because they do not form a molecular solution in the aqueous phase of the intestinal contents. There are few membrane barriers to absorption following subcutaneous or intra-muscular dosage, and the absorption rate may be limited by the water solubility of the injected materials; slow absorption occurs with lipid-soluble compounds injected in an oily vehicle (which contrasts with the rapid absorption possible if such a dose is given *via* the gastrointestinal tract).Irrespective of the route of administration,

the rate of absorption is determined from the early time points after dosing (Figure3.5), and is usually described by an absorption rate constant or absorption half-life.

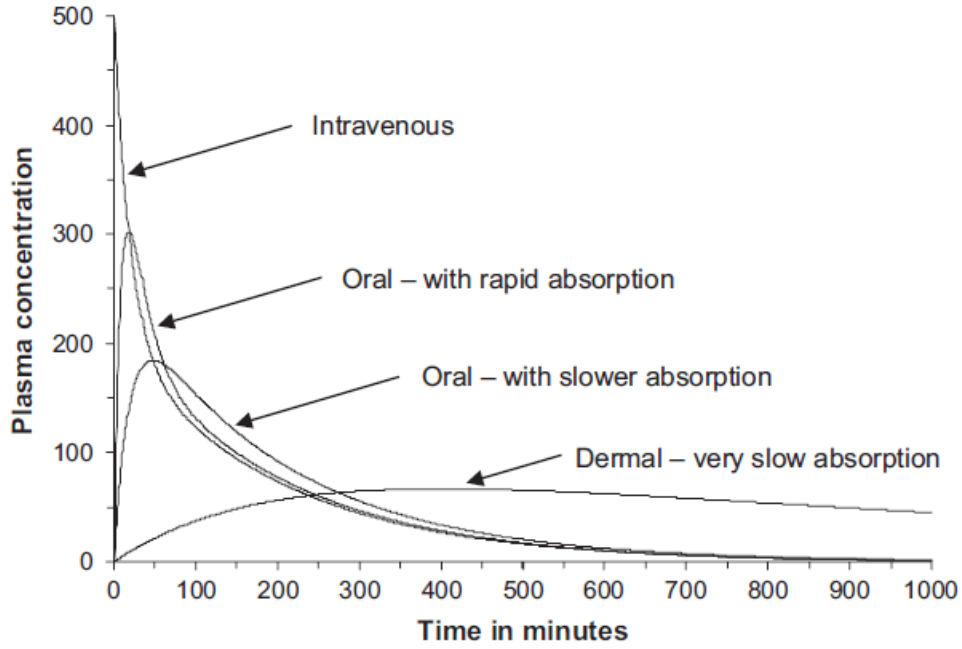

Figure 3.5 *The influence of the rate of absorption of a chemical on the plasma concentration–time curve. A relatively flat low profile is obtained when the rate of absorption is less than the rate of elimination, and this pattern is normally seen with transdermal absorption*

Extent of Absorption

The extent of absorption is important in determining the total body exposure or internal dose, and therefore is an important variable during chronic toxicity studies and/or chronic human exposure. The extent of absorption depends on the extent to which the chemical is transferred from the site of administration, such as the gut lumen, into the local tissue, and the extent to which it is metabolized or broken down by local tissues prior to reaching the general circulation. An additional variable affecting the extent of absorption is the rate of removal from the site of administration by other processes compared with the rate of absorption.

Chemicals given *via* the gastrointestinal tract may be subject to a wide range of pH values and metabolizing enzymes in the gut lumen, gut wall, and liver before they reach the general circulation. The initial loss of chemical prior to it ever entering the blood is termed first-pass metabolism or pre-systemic metabolism; it may in some cases remove up to 100% of the administered dose so that none of the parent chemical reaches the general circulation. The intestinal lumen contains a range of hydrolytic enzymes involved in the digestion of nutrients. The gut wall can perform similar hydrolytic reactions and contains enzymes involved in oxidation, such as cytochrome P450 3A4, and conjugation of foreign chemicals. Enterocytes contain P-glycoprotein (PGP) which transports a range of absorbed complex foreign chemicals from the cytosol back into the gut lumen, which can increase the likelihood of first-pass metabolism in the gut lumen or gut wall, or incomplete absorption from the gut lumen. The portal

circulation drains into the hepatic portal vein which carries compounds absorbed across the gut wall to the liver, which is the main site of foreign compound metabolism, and is responsible for most first-pass metabolism. The other main reason for incomplete absorption of the parent chemical occurs when the rate of absorption is so slow that the chemical is lost from the body before absorption is complete. Examples of this include incomplete absorption of very water-soluble chemical from the gut and their loss in the faeces, or incomplete dermal absorption, before the chemical is removed from the skin by washing.

Irrespective of the reason that is responsible for the incomplete absorption of the chemical as the parent compound, it is essential that there is a parameter which defines the extent of transfer of the intact chemical from the site of administration into the general circulation. This parameter is the bioavailability, which is simply the fraction of the dose administered that reaches the general circulation as the parent compound. (The term bioavailability is perhaps the most misused of all kinetic parametersandissometimesusedincorrectlyinageneralsenseastheamountavailable specifically to the site of toxicity).The fraction absorbed or bioavailability (F) is determined by comparison with intravenous (i.v.) dosing (where $F = 1$ by definition). The bio-availability can be determined from the area under the plasma concentration–time curve (AUC) of the parent compound(seeFigure3.6),or the percentage dose excreted in urine as the parent compound, *i.e.* for an oral dose:

$$F = \frac{\text{AUC oral}}{\text{AUC}} \times \frac{\text{dose i.v.}}{\text{dose oral}}$$

$$F = \frac{\%\text{ in urine as parent compound after oral dosing}}{\%\text{ in urine as parent compound after intravenous dosing}}$$

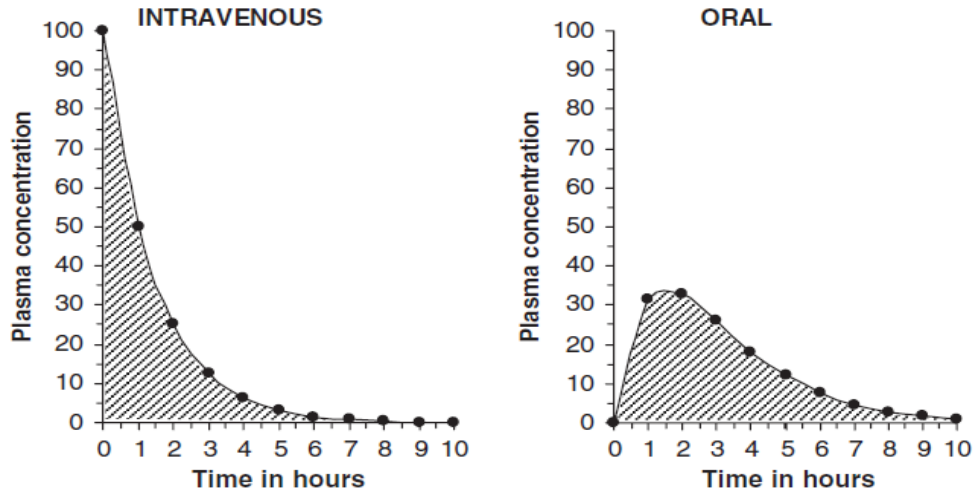

Figure 3.6 *The relationship between the area under the plasma concentration–time curve (AUC) and bioavailability. By definition, the bioavailability (fraction absorbed as the parent compound) is 1 for an intravenous dose. For other routes the bioavailability is given by the AUC for that route divided by the AUC after an intravenous dose (normalized to the same dose in mg kg^{-1})*

II. DISTRIBUTION:

Distributionisthereversibletransferofthechemicalbetweenthegeneralcirculationand the tissues. Irreversible processes such as excretion, metabolism, or covalent binding are part of elimination and do not contribute to distribution parameters. The important distribution parameters relate to the rate and extent of distribution.

Rate of Distribution

The rate at which a chemical may enter or leave a tissue may be limited by two factors:

(i) the ability of the compound to cross cell membranes and
(ii) the blood flow to the tissues in which the chemical accumulates.

The rate of distribution of highly water-soluble compounds may be slow due to their slow transfer from plasma into body tissues such as liver and muscle; water-soluble compounds do not accumulate in adipose tissue. In contrast, very lipid-soluble chemicals may rapidly cross cell membranes but the rate of distribution may be slow because they accumulate in adipose tissue, and their overall distribution rate may be limited by blood flow to adipose tissue.
Highly lipid-soluble chemicals may show two distribution phases: a rapid initial equilibration between blood and well perfused tissues, and a slower equilibration between blood and poorly perfused tissues (Figure 3.7). The rate of distribution is indicated by the distribution rate constant(s), which is(are) determined from the decrease in plasma concentrations in early time points after an intra venous dose. The rate constants refer to a mean rate of removal from the circulation and may not correlate with uptake into a specific tissue (for which the PBPK approach is more appropriate; see Figure 3.4). Once an equilibrium has been reached between the general circulation and a tissue, any process which lowers the blood (plasma) concentration will cause a parallel decrease in the tissue concentration (Figure 3.8). Thus the elimination half-life measured from plasma or blood samples is also the elimination half-life from tissues.

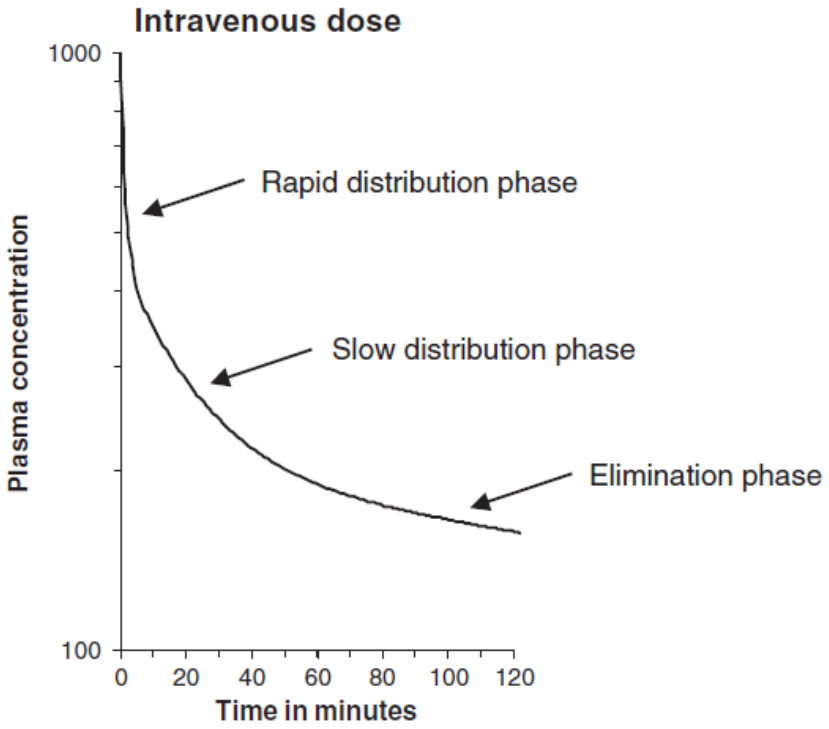

Figure 3.7 *The plasma concentration–time curve for a chemical that requires a three-compartment model (see Figure 3.3)*

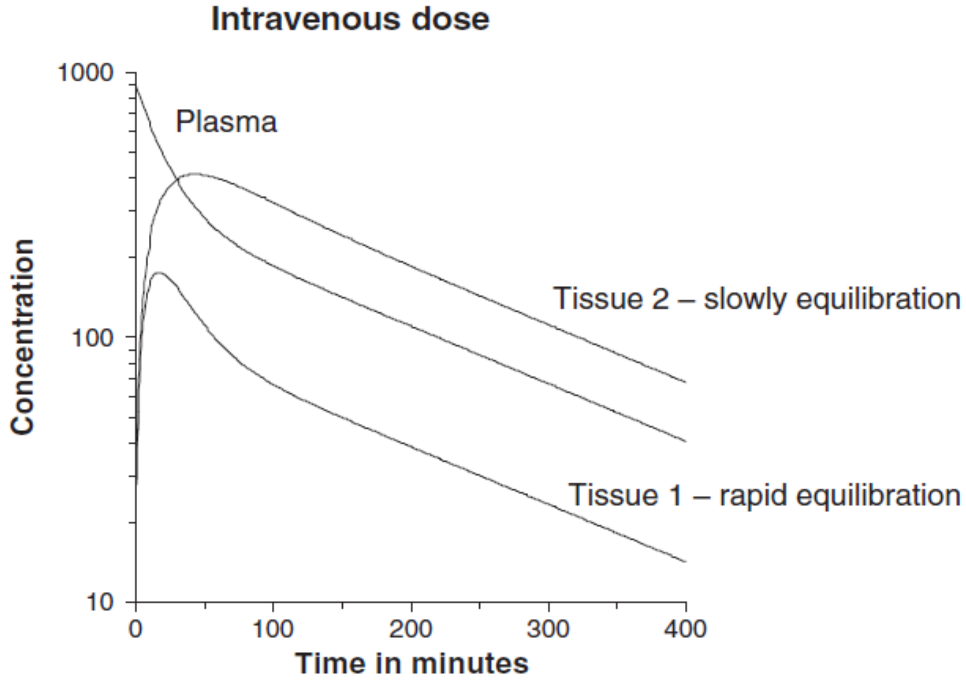

Figure 3.8 *Tissue distribution of a chemical after an intravenous bolus dose. Tissue 1 shows a greater rate of uptake and reaches equilibrium before tissue 2. Tissue 1 shows a lower affinity than tissue 2, so that the concentrations are lower. The concentrations measured in toxicokinetic studies are usually the total concentration (free + bound to proteins or present in cellular lipids) and tissue 2 may show greater tissue binding than tissue 1. The concentrations in all tissues decrease in parallel once all tissues have reached equilibrium with plasma*

Extent of Distribution :

The extent of tissue distribution of a chemical depends on the relative affinity of the blood or plasma compared with the tissues. Highly water-soluble compounds that are unable to cross cell membranes readily (*e.g.* tubocurarine) are largely restricted to extracellular fluid (about 13 L per 70 kg body weight). Water-soluble compounds capable of crossing cell membranes (*e.g.* caffeine, ethanol) are largely present in total body water (about 41Lper 70kg body weight).When one or more body tissues has an affinity for the chemical, such as reversible tissue binding, then the blood (plasma) concentration will be lower than if the compound was evenly distributed through body water. Lipid-soluble compounds frequently show extensive uptake into tissues and may be present in the lipids of cell membranes, adipocytes, central nervous system (CNS), *etc.*; the partitioning between circulating lipoproteins and tissue constituents is complex and may result in extremely low plasma concentrations. A factor which may further complicate the plasma/tissue partitioning is that some chemicals bind reversibly to circulating proteins such as albumin (for acid molecules) and a1-acid glycoprotein (for basic molecules).

The internal environment of the brain is controlled by the endothelial cells of the blood capillaries to the brain which have tight junctions between adjacent cells, fewer and smaller pores, little endocytosis, and the presence of transporters such as PGP which can extrude chemicals that diffuse across the blood brain barrier. In consequence, water-soluble molecules cannot 'leak' into the brain between endothelial cells (as could happen, for example, in muscle capillaries) and are excluded from the brain. The endothelial membranes have specific transporters for the uptake of essential water-soluble nutrients and some ions and also for the exclusion of organic acids. This so- called blood–brain barrier serves to exclude most water-soluble compounds, so that CNS toxicity may be limited. In contrast, lipid-soluble chemicals readily cross the blood–brain barrier and the CNS is a common site for toxicity (*e.g.*organic solvents). Similar permeability barriers are present in the choroid plexus, retina, and testes. The extent and pattern of tissue distribution can be investigated by direct measurement of tissue concentrations in animals. Tissue concentrations cannot be measured in human studies and, therefore, the extent of distribution in humans has to be determined based solely on the concentrations remaining in plasma or blood after distribution is complete. The parameter used to reflect the extent of distribution is the apparent volume of distribution (V), which relates the total amount of the chemical in the body (Ab) to the circulating concentration (C) at any time after distribution is complete:

$$V = \frac{Ab}{C}$$

V may be regarded as the volume of plasma in which the body load appears to have been dissolved and simply represents a dilution factor. The volumes of distribution of tubocurarine and caffeine are about 13 and 41 L per 70 kg because of their restricted distribution (see above). However, when a chemical shows a more extensive reversible uptake into one or more tissues the plasma concentration will be lowered and the value of V will increase. For highly lipid-soluble chemicals, such as organochlorine pesticides, which accumulate in adipose tissue, the plasma

concentration may be so low that the value of *V* may be many litres for each kilogram of body weight. This is not are volume of plasma and therefore is called the apparent volume of distribution. It is an important parameter because extensive reversible distribution into tissues, which will give a high value of *V*, is associated with a low elimination rate and a long half-life (see below). It must be emphasized that the apparent volume of distribution simply reflects the extent to which the chemical has moved out of the site of measurement (the general circulation) into tissues, and it does not reflect uptake into any specific tissue(s).

Information on the uptake into specific tissues requires sampling of hat specific tissue, although PBPK modelling can provide useful estimates of tissue concentrates based on *in vitro* partition coefficients and organ blood flows. Once equilibrium has been reached for a tissue, the tissue/plasma ratio will remain constant, so that as the chemical is eliminated from the plasma, the chemical will leave the tissue, maintaining the same ratio (Figure3.8).

III. ELIMINATION

The parameter most commonly used to describe the rate of elimination of a chemical is the half-life (Figure3.9). Most toxicokinetic processes are first-order reactions, *i.e.*the rate at which the process occurs is proportional to the amount of chemical present. High rates (expressed as mass/time) occur at high concentrations and the rate decreases as the concentration decreases; in consequence the decrease is an exponential curve. The usual way to analyze exponential changes is to use logarithmically transformed data which converts an exponential into a straight line. The slope of the line is the rate constant (*k*)for the process and the half-life for the process is calculated as 0.693/*k*. Rate constants and half-lives can be determined for absorption, distribution, and elimination processes.

There are two important biological variables that determine the rate at which a chemical can be eliminated from the body: (i) the functional capacity/ability of the organs of elimination to remove the chemical from the body (the clearance) and (ii) the extent of distribution of the chemical from the general circulation into tissues. The clearance of a chemical is determined by the ability of the organs of elimination (*e.g.* the liver ,kidney, or lungs) to extract the chemical from the plasma or blood and permanently remove it by metabolism or excretion. (Note that this is different from distribution in which the chemical is free to leave the tissue and re-enter the blood when the concentration in the general circulation decreases.)

The mechanisms of elimination depend on the chemical characteristics of the compound:

- volatile chemicals are exhaled,
- water-soluble chemicals are eliminated in the urine and/or bile and
- lipid-soluble chemicals are eliminated by metabolism to more water-soluble molecules, which are then eliminated in the urine and/or bile.

Foreign compound metabolism is an enormous subject and involves a wide range of enzyme systems. Foreign chemicals (xenobiotics) may be metabolized by the enzymes of normal intermediary metabolism, *e.g.*esterases will hydrolyse ester groups. Alternatively, chemicals may be metabolized by enzymes such as cytochrome P450, a primary function of which is xenobiotic metabolism. Species differences in metabolism can be a major source of differences in toxic response. The usual consequence of metabolism is the formation of an inactive excretory

products of that species with low metabolizing ability will be likely to show greater toxicity. However, for many com- pounds, metabolism is a critical step in the generation of a toxic or reactive chemical entity(bioactivation) ,and for such compounds high rates of metabolism will be linked with greater toxicity. If a chemical undergoes metabolic activation then toxicokinetic studies should measure both the parent chemical and the active metabolite. If the metabolite is so reactive that it does not leave the tissue in which it is produced (*e.g.*alkylating metabolites of chemical carcinogens), then toxicokinetic studies should define the delivery of the parent chemical to the tissues, and the process of local activation should be regarded as part of tissue sensitivity (toxicodynamics) because it is not strictly speaking part of toxicokinetics, *i.e.* the movement of the chemical and/or metabolites around the body. The best measure of the ability of the organs of elimination to remove the compound from the body is the clearance (*CL*):

$$CL = \frac{\text{rate of elimination}}{\text{plasma concentration}}$$

Because the rate of elimination is proportional to the concentration (see Figure 3.9), clearance is a constant for first-order processes and is independent of dose. It can be regarded as the volume of plasma (or blood) cleared of compound within a unit of time (*e.g.*mL min^{-1}).

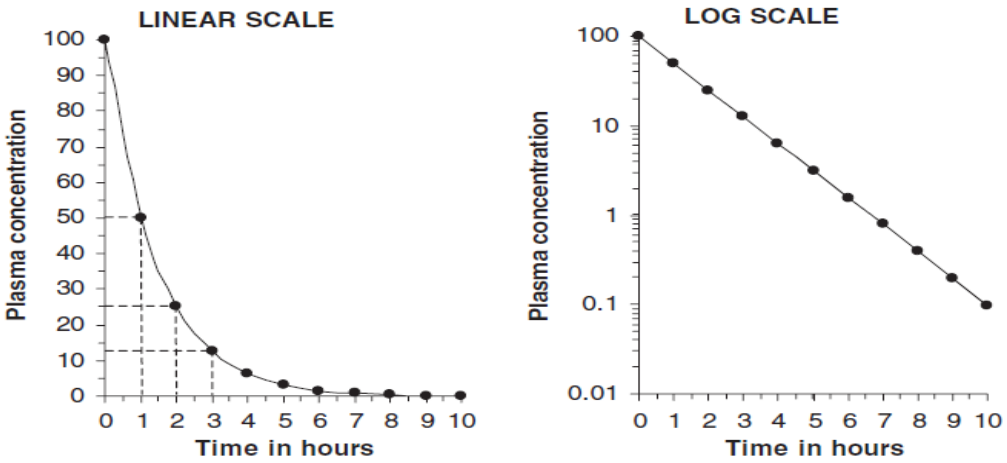

Figure 3.9 *The half-life of a chemical and its determination from plasma data. In the example in this figure the half-life is 1 h. Logarithmic conversion allows the concentration data to be fitted by linear regression analysis; the half-life is calculated as 0.693/slope. Plasma kinetic data are usually fitted by a non-linear least-squares method and there are various programmes available, such as Win-Nonlin*

Renal clearance depends on the extent of protein binding, tubular secretion and passive reabsorption in the renal tubule; it can be measured directly from the concentrations present in plasma and urine:

The total clearance or plasma clearance (which is the sum of all elimination processes, *i.e.*renal + metabolic, *etc.*) is possibly the most important toxicokinetic parameter. It is measured from the total amount of compound available for removal (*i.e.*an intravenous dose) and the total area under the plasma concentration–time curve (AUC) extrapolated to infinity.

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$$CL = \frac{\text{Dose i.v.}}{\text{AUC i.v.}}$$

Plasma clearance reflects the overall ability of the body to remove permanently the chemical from the plasma. Plasma clearance is the parameter that is altered by factors such as enzyme induction, liver disease, kidney disease, inter-individual or inter-species differences in hepatic enzymes or in some cases organ blood flow. Once the chemical is in the general circulation, the same volume of plasma will be cleared of chemical per minute (*i.e.*the clearance value) applies irrespective of the route of delivery of chemical into the circulation. However, the bioavailability (*F*) will determine the proportion of the dose reaching the general circulation. Therefore, bioavailability has to be taken into account if clearance is calculated from data from a non-intravenous route (*e.g.*oral).

$$CL = \frac{\text{dose oral} \times F}{\text{AUC oral}}$$

Measurement of dose/AUC for an oral dose determines *CL/F*, which contains two potentially independent variables – the amount of chemical delivered to the blood from the site of administration and the clearance of chemical present in theblood.

The overall rate of elimination, as indicated by the terminal half-life (t^{-1}), is dependent on two physiologically related and independent variables:

$$t_{\frac{1}{2}} = \frac{0.693V}{CL}$$

where *CL* is the ability to extract and remove irreversibly the compound from the general circulation, and *V* the extent to which the compound has left the general circulation in a reversible equilibrium with tissues. Therefore, a chemical may have along half-life because the organs of elimination have a low ability to remove it from plasma and/or because it is extensively distributed to body tissues and only a small proportion of the total body burden remains in the plasma and is available for elimination. Chemicals that are extremely lipid-soluble and are sequestered in adipose tissue are eliminated slowly. Lipid- soluble organochlorine compounds, which are not substrates for P450 oxidation, due to the blocking of possible sites of oxidation by chloro-substituents, are eliminated extremely slowly: for example, the half-life of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) is about 8 years in humans.

Organ toxicity: Organ toxicity means the capacity of substances (xenobiotics) to damage various organs such as kidneys, liver, heart, lungs, nerves etc.

Hepatotoxicity

Hepatotoxicity: It is a toxicants-induced damage of liver, bile duct and gall bladder. The liver is exposed to high very high amount of xenobiotics or its metabolites because of extensive

blood supply for the metabolic process of xenobiotics and other substances. The liver is plays a central role in transforming and clearing xenobiotics from the body. The over use of some medicinal drugs or other substances (e.g., natural, agricultural, industrial, herbal, chemical etc.) can induce hepatotoxicity.

Forms of hepatotoxicity: The various forms of hepatotoxicity are-

1. **Steatosis (Fatty liver):** Lipid accumulation in the adipocytes.
2. **Necrosis:** Death of hepatocytes.
3. **Cirrhosis:** Chronic fibrosis due to alcohol intake.
4. **Cholestasis:** Backup of bile salts into the liver.
5. **Hypersensitivity:** Hepatic necrosis due to immune response.
6. **Cancer:** Liver cancer.

Hepatotoxins:

The substances that cause liver injury is called hepatotoxins. There are more than 900 hepatotoxins causing liver damage. Some most familiar hepatotoxins are-

1. **Antipyretic-analgesics (Paracetamol or Acetaminophen):** The overdose of paracetamol causes acute liver failure worldwide. Actually damage to the liver is not due to paracetamol but to its metabolites (NAPQI = N-acetyl benzoquinone imine) of paracetamol produced by the action of cytochrome p450 enzyme in the liver. Normally NAPQI is detoxified in conjugation with glutathione, but its overuse produces a large amount of NAPQI that inhibits the detoxification process and causes liver damage.

2. **NSAIDs (Non steroidal anti-inflammatory drugs):** The NSAIDs (e.g., Aspirin, Ibuprofen, diclofenac, Aceclofenac, nimesulide, piroxicam etc.) are nonnarcotic, nonopoidanalgesic, antipyretic and anti-inflammatory drugs. The individual analgesic rarely induces liver damage. But, worldwide over use of these drugs is showing hepatotoxicity. It is actually dose-dependent hepatotoxic drugs.

3. **Glucocorticoids (corticosterone, cortisone, cortisol):** They are so named due to their effect on carbohydrate metabolism. They cause enlargement of liver due to storage of glycogen that may causes side effect in children. But the prolong use of these drugs causes fattyliver.

4. **Ioniazid (Anti-tuberculosis drugs):** It is associated with upto 20% elevation of liver enzymes (SGOT & SGPT) and severe hepatotoxicity to 1-2% of patients.

5. **Natural products:** The natural hepatotoxins are amanita mushrooms, aflatoxins, alkaloids & green tea extract.

6. **Industrial toxins (Arsenic, CCl_4, vinyl chloride):** They may cause liver damage.

Nephrotoxicity

The kidneys are the highly susceptible to the toxicants because a high volume of blood with toxicants flows through these organs for the filtration of all kind of toxins from the blood. The toxicants (especially lipid soluble) are concentrated within the cell during this process that can cause nephrotoxicity.

Forms of nephrotoxicity:-

1. Decrease rate of excretion of body waste.
2. Inability to maintain body fluid &electrolytes.
3. Decrease synthesis of essential hormone like erythropoietin (Function: It promotes the production of blood cells).

Nephrotoxic agents & their function:

i) **Cardiovascular drugs as nephrotoxins:** Diuretics (thiazides, furosemide), vasodilators, beta blockers, ACE inhibitors etc.
ii) **NSAIDs :** Aspirin, ibuprofen, diclofenacetc.
iii) **Antibiotics:** Gentamicin, amphotericin B, cisplatin, ciprofloxacin, rifampicinetc.
iv) **Antacids (PPI & H_2 antagonists):** Ranitidine, cemetidineetc.
v) **Heavy metals:** Lead, mercury &cadmium
vi) **Others:** Lithium salt, gold salt, Heroin, fluorideetc.

Respiratory toxicity

Respiratory toxicity: The toxicants-induced damage of respiratory system (Upper respiratory system e.g., nose, pharynx, larynx & trachea & lower respiratory system e.g., bronchi, bronchioles & lungs &alveoli).

Forms of respiratory toxicity:

1. Asthma
2. Bronchitis
3. Emphysema

4. Laryngitis /pharyngitis
5. Allergic reactions
6. Pneumoconiosis
7. Lung cancer

Respiratory toxicants: There are numerous respiratory toxicants in environment-

i. SO_x, CO_x, NO_x , Ammonia, Chlorine, Fluorine, bromine.

ii. Arsenic & arsenic compounds, cadmium, lead, mercury, nickel, pyrethrum

iii. Aldrin, Dieldrin, Endrin, formaldehyde, kerosene, methane, ethanol, methanol, phenol, xylene, benzene, caffeine, colchicines, DDT

iv. Hydrogen, hydrogen peroxide, HCN, ozone

Reproductive toxicity

The toxicants which are involved in the damage of male or female reproductive systems is called reproductive toxicity.

Forms of toxic effects:

1. Infertility
2. Impotence
3. Interruption of pregnancy: Abortion, fetal death, premature delivery etc.
4. Infant death
5. Altered sexratio
6. Chromosomal abnormalities & birth defects
7. Childhood cancer

Reproductive toxicants:

i. Steroids

ii. Colchicine

iii. DDT

iv. Etodolac

v. Gemfibrozil

vi. Lead & its compounds, cadmium,benzene

vii. Levonorgerserol

viii. Nifedipine, Rifampicin,streptozocin

ix. Cocaine

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Probable Questions:

1. Define toxicokinetics. Which parameters affect it?
2. How a toxic chemical is absorbed. Give suitable examples.
3. How a toxic chemical is eliminated. Give suitable examples.
4. How a toxic chemical is distributed. Give suitable examples.
5. What is organ toxicity?
6. Define hepatotoxicity? Describe different forms of hepatotoxicity.
7. Describe different kinds of hepatotoxins.
8. What is nephrotoxicity?
9. Discuss about Nephrotoxic agents & their functions.
10. Define Respiratory toxicity? Describe different forms of respiratory toxicity.
11. Describe different kinds of respiratory toxicants.
12. What is reproductive toxicity? What are toxic effects of toxins on reproductive system?
13. Name some chemicals which act as toxins to reproductive system.

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett& Doull's Toxicology: The Basic Science by Curtis D. Klaassen.

Unit-V

Toxicants of public health hazards: Pesticides, Heavy Metals, Radiation, Food and Additives

Objective: In this unit you will learn about toxicants related to public health hazards such as pesticides, heavy metals, radiation, food and additives.

Introduction:

Environmental toxicology is a multidisciplinary field of science concerned with the study of the harmful effects of various chemical, biological and physical agents on living organisms. Ecotoxicology is a sub discipline of environmental toxicology concerned with studying the harmful effects of toxicants at the population and ecosystem levels. Rachel Carson is considered the mother of environmental toxicology, as she made it a distinct field within toxicology in 1962 with the publication of her book *Silent Spring*, which covered the effects of uncontrolled pesticide use. Carson's book was based extensively on a series of reports by Lucille Farrier Stickel on the ecological effects of the pesticide DDT.

Organisms can be exposed to various kinds of toxicants at any life cycle stage, some of which are more sensitive than others. Toxicity can also vary with the organism's placement within its food web. Bioaccumulation occurs when an organism stores toxicants in fatty tissues, which may eventually establish a trophic cascade and the biomagnification of specific toxicants. Biodegradation releases carbon dioxide and water as by-products into the environment. This process is typically limited in areas affected by environmental toxicants.

I. PESTICIDES:

According to World Health Organization (WHO), Pesticides are chemical compounds that are used to kill pests, including insects, rodents, fungi and unwanted plants (weeds). Pesticides are used in public health to kill vectors of disease, such as mosquitoes, and in agriculture, to kill pests that damage crops. By their nature, pesticides are potentially toxic to other organisms, including humans, and need to be used safely and disposed of properly. In general, a pesticide is a chemical or biological agent (such as a virus, bacterium, or fungus) that deters, incapacitates, kills, or otherwise discourages pests. Target pests can include insects, plant pathogens, weeds, molluscs, birds, mammals, fish, nematodes (roundworms), and microbes that destroy property, cause nuisance, or spread disease, or are disease vectors. Although pesticides have benefits, some also have drawbacks, such as potential toxicity to humans and other species.

A. Classification of pesticides by target organisms:

There are many different types of pesticides, each is meant to be effective against specific pests.

The term "**-**cide" comes from the Latin word "to kill."

a. Algaecides are used for killing and/or slowing the growth of algae.

b. Antimicrobials control germs and microbes such as bacteria and viruses.

c. Acaricides are used against mites and ticks, members of Acaridae.

d. Herbicides kill or inhibit the growth of unwanted plants, aka weeds.

e. Fungicides are used to control fungal problems like molds, mildew, and rust.

f. *Insecticides* are used to control insects.

g. Molluscicides are designed to control slugs, snails and other mollusks.

h. Rodenticides are used to kills rodents like mice, rats, and gophers.

i. Ovicides are used to control eggs of insects and mites.

j. Pisicidesused to reduce the population of rough fish in a water body.

k. Nematicides are used against nematodes.
l. Mothballs are insecticides used to kill fabric pests by fumigation in sealed containers.

B. Classification of pesticides by mode of action:

Pesticides can be classify on the basis of the following ways-

a. **Cell toxicants *:*** inhibit different important steps of cell metabolism.

b. **Neurotoxicants or nerve Poison *:*** interferes with nervous system function.

c. **Chemosterilants*:*** sterilize males of insects or pest vertebrates, classical mode of biological pest control.
d. **Disinfectant (Eradicant**): effective against pathogen that has already infected the crop.

e. **Defoliants*:*** removes the leaves of plants.

f. Germination Inhibitor: inhibits germination of weed seeds, fungus spores and bacterial spores.

g. **Nonselective:** kills broad range of pests and/or crop plants, usually used in reference

to herbicides.

h. Protectants: protects crop if applied before pathogens infect the crop.

i. **Repellents:** deters or repels pest from crop or interferes with pest's ability to locate crop.

j*. Systemic pesticides*: absorbed and translocated throughout the plant to provide protection.

k. Stomach Poison: kills after ingestion by an animal.

l. **Pheromones:** are biologically active chemicals used to attract insects or disrupt their mating behavior.

Benefits of Pesticides:

Pesticides are used for the following purposes:

1. In agriculture, the protection of crops from variouspests.
2. In public health programmes for the control of vectors of variousdiseases.
3. To control household and garden pests.
4. In control of ectoparasites of domestic animals and even the humanbeings.
5. In industry and commercial establishment.
6. In weed control.
7. Invasive species control.

Effects of Pesticides:

a. Health Effects:

Pesticides may cause acute and delayed health effects in people who are exposed. Pesticide exposure can cause a variety of adverse health effects, ranging from simple irritation of the skin and eyes to more severe effects such as affecting the nervous system, mimicking hormones causing reproductive problems, and also causing cancer.

A 2007 systematic review found that "most studies on non-Hodgkin lymphoma and leukemia showed positive associations with pesticide exposure" and thus concluded that cosmetic use of pesticides should be decreased. There is substantial evidence of associations between organophosphate insecticide exposures and neurobehavioral alterations. Limited evidence also exists for other negative outcomes from pesticide exposure including neurological, birth defects, and fetal death.

b. Environmental Effects:

Pesticide use raises a number of environmental concerns. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water and soil. Pesticide drift occurs when pesticides suspended in the air as particles are carried by wind to other areas, potentially contaminating them. Pesticides are one of the causes of water pollution, and some pesticides are persistent organic pollutants and contribute to soil contamination.

In addition, pesticide use reduces biodiversity, contributes to pollinator decline, destroys habitat (especially for birds), and threatens endangered species. Pests can develop a resistance to the pesticide (pesticide resistance), necessitating a new pesticide. Alternatively a greater dose of the pesticide can be used to counteract the resistance, although this will cause a worsening of the ambient pollution problem.

c. Economic aspects:

In one study, the human health and environmental costs due to pesticides in the United States was estimated to be $9.6 billion: offset by about $40 billion in increased agricultural production.

Additional costs include the registration process and the cost of purchasing pesticides: which are typically borne by agrichemical companies and farmers respectively. The registration process can take several years to complete (there are 70 different types of field test) and can cost $50–70 million for a single pesticide. At the beginning of the 21st century, the United States spent approximately $10 billion on pesticides annually.

Control of Pesticide Pollution:

1. The non-selective persistent pesticides such as DDT must be phased out of use.
2. Only selective pesticides must be used.
3. Measurement of pesticides to be applied is so important.
4. Repeated pesticides application should be stopped.
5. Proper knowledge about pesticides should be given to public and farmers.
6. Research on pesticides should progress.

2. HEAVY METALS

Metals are natural constituents that exist in the ecosystem. They are substances with high electrical conductivity which voluntarily lose their electrons to form cations. Metals are found all over the earth including the atmosphere, earth crust, water bodies, and can also accumulate in biological organisms including plants and animals. Among the 35 natural existing metals, 23 possess high specific density above 5 g/cm3 with atomic weight greater than 40.04 and are generally termed heavy metals. Theses metals generally termed heavy metals include: antimony,

tellurium, bismuth, tin, thallium, gold, arsenic, cerium, gallium, cadmium, chromium, cobalt, copper, iron, lead, mercury, manganese, nickel, platinum, silver, uranium, vanadium, and zinc. This category of metals termed heavy metals have not only been known for their high density but most importantly for their adverse effects to the ecosystem and living organisms. Some of these heavy metals such as cobalt, chromium, copper, magnesium, iron, molybdenum, manganese, selenium, nickel and zinc are essential nutrients that are required for various physiological and biochemical functions in the body and may result to deficiency diseases or syndromes if not in adequate amounts but in large doses they may cause acute or chronic toxicities.

These heavy metals are distributed in the environment through several natural processes such as volcanic eruptions, spring waters, erosion, and bacterial activity, and through anthropogenic activities which include fossil fuel combustion, industrial processes, agricultural activities as well as feeding. These heavy metals do bioaccumulate in living organisms and the human body through various processes causing adverse effects. In the human body, these heavy metals are transported and compartmentalized into body cells and tissues binding to proteins, nucleic acids destroying these macromolecules and disrupting their cellular functions. As such, heavy metal toxicity can have several consequences in the human body. It can affect the central nervous function leading to mental disorder, damage the blood constituents and may damage the lungs, liver, kidneys and other vital organs promoting several disease conditions. Also, long term accumulation of heavy metals in the body may result in slowing the progression of physical, muscular and neurological degenerative processes that mimic certain diseases such as Parkinson's disease and Alzheimer's disease. More so, repeated long-term contact with some heavy metals or their compounds may even damage nucleic acids, cause mutation, mimic hormones thereby disrupting the endocrine and reproductive system and eventually lead to cancer.

This chapter will highlight on the various sources of heavy metals and the processes that promote their exposure and bioaccumulation in the human body. More focus will be laid on the various mechanisms that lead to heavy metal toxicity with emphasis on macromolecule and cellular damages, carcinogenesis, neurotoxicity and the molecular basis for their noxious effects.

Sources of heavy metal exposure to humans

Heavy metals are naturally present in our environment. They are present in the atmosphere, lithosphere, hydrosphere and biosphere. Although these heavy metals are present in the ecosystem, their exposure to humans is through various anthropogenic activities of man. In the earth crust, these heavy metals are present in ores which are recovered during mining activities as minerals. In most ores heavy metals such as arsenic, iron, lead, zinc, gold, nickel, silver and cobalt exist as sulfides while others such as manganese, aluminum, selenium gold, and antimony exist as oxides. Certain heavy metals such as copper, iron and cobalt can exist both as sulfide and oxide ores. Some sulfides may contain two or more heavy metals together such as chalcopyrite, ($CuFeS_2$) which contains both copper and iron. During these mining activities, heavy metals are released from the ore and scattered in open in the environment; left in the soil, transported by air and water to other areas. Furthermore, when these heavy metals are used in the industries for various

industrial purposes, some of these elements are released into the air during combustion or into the soil or water bodies as effluents. More so, the industrial products such as paints, cosmetics, pesticides, and herbicides also serve as sources of heavy metals. Heavy metals may be transported through erosion, run-off or acid rain to different locations on soils and water bodies. As reviewed from, the sources of specific heavy metals are described below.

a. Arsenic:

Arsenic is the 20th most abundant element on earth and the 33rd on the periodic table. The inorganic forms such as arsenite and arsenate compounds are lethal to humans and other organisms in the environment. Humans get in contact with arsenic through several means which include industrial sources such as smelting and microelectronic industries. Drinking water may be contaminated with arsenic which is present in wood preservatives, herbicides, pesticides, fungicides and paints.

b. Lead:

Lead is a slightly bluish, bright silvery metal in a dry atmosphere. The main sources of lead exposure include drinking water, food, cigarette, industrial processes and domestic sources. The industrial sources of lead include gasoline, house paint, plumbing pipes, lead bullets, storage batteries, pewter pitchers, toys and faucets. Lead is released into the atmosphere from industrial processes as well as from vehicle exhausts. Therefore, it may get into the soil and flow into water bodies which can be taken up by plants and hence human exposure of lead may also be through food or drinking water.

c. Mercury:

The metallic mercury is a shiny silver-white, odorless liquid metal which becomes colorless and odorless gas upon heating. Mercury is used in producing dental amalgams, thermometers and some batteries. Also, it can be found in some chemical, electrical-equipment, automotive, metal-processing, and building industries. Mercury can exist in a gaseous form thus it can be inhaled. Other forms of mercury contamination in humans may be through anthropogenic activities such as municipal wastewater discharges, agriculture, incineration, mining, and discharges of industrial wastewater.

d. Cadmium:

This metal is mostly used in industries for the production of paints, pigments alloys, coatings, batteries as well as plastics. Majority of cadmium, about three-fourths is used as electrode component in producing alkaline batteries. Cadmium is emitted through industrial processes and from cadmium smelters into sewage sludge, fertilizers, and groundwater which can remain in soils and sediments for several decades and taken up by plants. Therefore, significant human exposure to cadmium can be by the ingestion of contaminated foodstuffs especially cereals, grains, fruits and leafy vegetables as well as contaminated beverages. Also, humans may get exposed to cadmium by inhalation through incineration of municipal waste.

e. Chromium:

Chromium is a metal that is present in petroleum and coal, chromium steel, pigment oxidants, fertilizers, catalyst, oil well drilling and metal plating tanneries. Chromium is extensively used in industries such as wood preservation, electroplating, metallurgy, production of paints and pigments, chemical production, tanning, and pulp and paper production. These industries play a major role in chromium pollution with an adverse effect on biological and ecological species. Following the anthropogenic activities by humans, disposal of sewage and use of fertilizers may lead to the release of chromium into the environment. Therefore, these industrial and agricultural practices increase the environmental contamination of chromium. Environmental pollution by chromium has been mostly by the hexavalent chromium in recent years.

f. Copper:

This is a heavy metal which is used in industries to produce copper pipes, cables, wires, copper cookware, etc. It is also used to make copper intrauterine devices and birth control pills. Copper in the form of copper sulfate is added to drinking water and swimming pools. Due to man's anthropogenic and industrial activities, it can accumulate in the soil and up taken by plants. As such, copper is present in some nuts, avocado, wheat germ and bran etc.

g. Manganese:

This metal is added to gasoline as methyl cyclopentadienyl manganese tricarbonyl (MMT) and thus, gasoline fumes contain a very toxic form of manganese.

h. Nickel:

It is used in the production of batteries, nickel-plated jewelry, machine parts, nickel plating on metallic objects, manufacture of steel, cigarette smoking, wire, electrical parts, etc. Also, it can be found in food stuff such as imitation whip cream, unrefined grains and cereals, commercial peanut butter, hydrogenated vegetable oils, as well as contaminated alcoholic beverages [19]. The various sources of heavy metals are summarized in Figure 1.

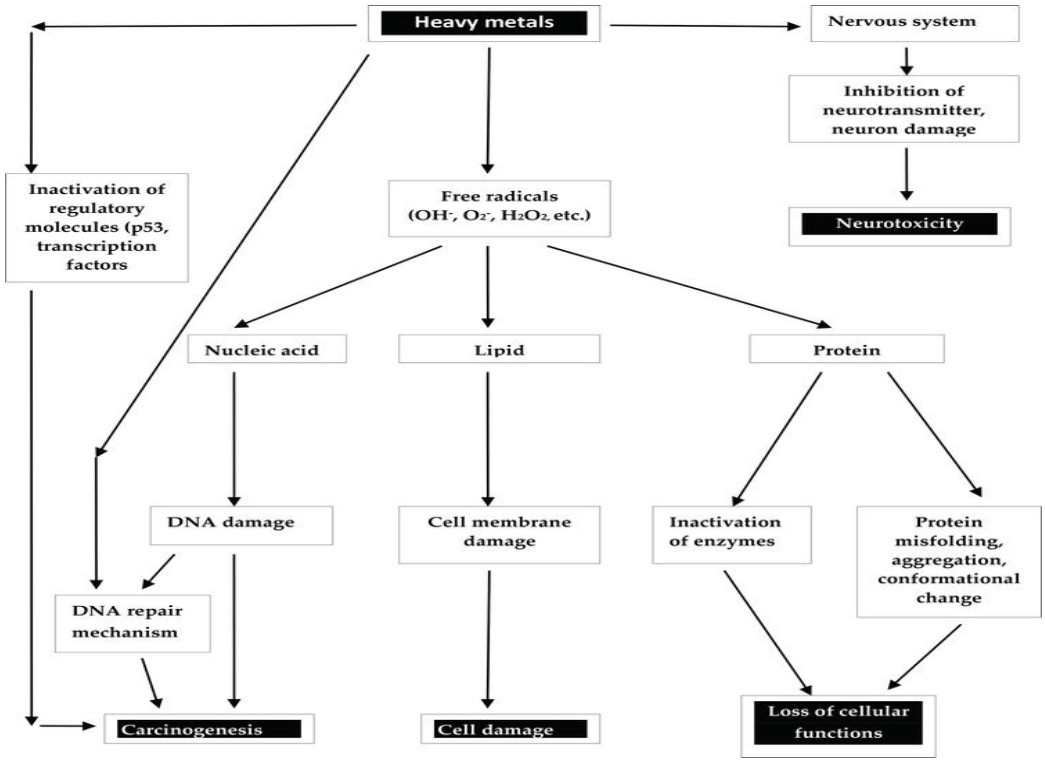


Figure 1. Pathway of heavy metals sources and exposure to human

Heavy Metals	Acute exposure	Chronic exposure
Cadmium	Pneumonitis (lung inflammation)	Lung cancer; Osteomalacia (bones dysfunction); Proteinuria (excess protein in urine).
Mercury	Diarrhea; Fever; Vomiting.	Stomatitis (inflammation of gums and mouth); Nausea; Nephritic syndrome (nonspecific kidney disorder); Paraguesia (metallic disorder); Neurasthenia (neurotic disorder); Pink disease (pink coloration and pain of hands and feet).
Lead	Encephalopathy(brain dysfunction); Nausea; Vomiting.	Anemia; Foot drop/wrist drop (palsy); Neuropathy (kidney disease).
Chromium	Gastrointestinal hemorrhage; Hemolysis; renal failure.	Pulmonary fibrosis; Lung cancer.

Arsenic	**Arrhythmia; Nausea; Vomiting; painful neuropathy.**	**Diabetes; Hypopigmentation; Cancer.**

3. Route of exposure, bio-uptake and bioaccumulation of heavy metals in humans:

Humans may directly get in contact with heavy metals by consuming contaminated food stuffs, sea animals, and drinking of water, through inhalation of polluted air as dust fumes, or through occupational exposure at workplace. The contamination chain of heavy metals almost usually follows this cyclic order: from industry, to the atmosphere, soil, water and foods then human. These heavy metals can be taken up through several routes. Some heavy metals such as lead, cadmium, manganese, arsenic can enter the body through the gastrointestinal route; that is, through the mouth when eating food, fruits, vegetables or drinking water or other beverages. Others can enter the body by inhalation while others such as lead can be absorbed through the skin.

Most heavy metals are distributed in the body through blood to tissues. Lead is carried by red blood cells to the liver and kidney and subsequently redistributed to the teeth, bone and hair mostly as phosphate salt. Cadmium initially binds to blood cells and albumin, and subsequently binds to metallothionein in kidney and liver tissue. Following its distribution from blood to the lungs, manganese vapor diffuses across the lung membrane to the Central nervous system (CNS). Organic salts of manganese which are lipid soluble are distributed in the intestine for fecal elimination while inorganic manganese salts which are water soluble are distributed in plasma and kidney for renal elimination. Arsenic is distributed in blood and accumulates in heart, lung, liver, kidney, muscle and neural tissues and also in the skin, nails and hair.

A. Mechanism of heavy metal toxicity:

a. Iron: Iron is a useful heavy metal in the human body as it is a constituent of certain biological molecules like the hemoglobin and involved in various physiological activities. However, in its free state, iron is one of the heavy metals generally known to generate hydroxyl radical (OH•) as shown below by the Fenton reaction.

$Fe3++O2-\rightarrow Fe2++O2$
$Fe2++H2O2\rightarrow Fe3++OH\cdot+OH-$(Fenton reaction)

Net reaction (Haber-Weiss reaction):

$O2-+H2O2\rightarrow OH-+OH\cdot+O2$

In addition to the above reactions, the following reactions below can also occur:

$OH\cdot H_2O_2 \rightarrow H_2O + H^+ + O_2^{\cdot -}$
$OH\cdot + Fe^{2+} \rightarrow Fe^{3+} + OH^-$
$LOOH + Fe^{2+} \rightarrow Fe^{3+} + LO^- + OH\cdot$

Hydroxyl radical (OH•) is the most common free radical generated by the oxidation of iron. OH• is capable of reacting with biological molecules such as proteins, lipids and DNA damaging them. When OH• reacts with guanine, a nitrogenous base of nucleic acids, it leads to the generation of 8-oxo-7,8-dihydro-20-deoxyguanosine (8-oxo-dG) and 2,6-diamino-5-formamido-4-hydroxypyrimidine (FAPy-G), in which the former is a good marker for oxidative damage.

It is well documented that metal-induced generation of oxygen reactive species can attack polyunsaturated fatty acid such as phospholipids. The first of such observation was first presented by Bucher et al. who showed that iron-generated OH• can oxidize lipid membranes through a process known as lipid peroxidation. Following his experimental observations, he proposed the following mechanism:

Steps of lipid peroxidation:

$\text{Initiation: Lipid} + R\cdot / OH\cdot \rightarrow \text{Lipid}\cdot$
$\text{Propagation: Lipid}\cdot + O_2 \rightarrow \text{Lipid} - OO\cdot$
$\text{Lipid} - OO + \text{Lipid}\cdot \rightarrow \text{Lipid} - OOH + \text{Lipid}\cdot$
$\text{Termination: Lipid}\cdot + \text{Lipid}\cdot \rightarrow \text{Lipid} - \text{Lipid}$
$\text{Lipid} - OO\cdot + \text{Lipid}\cdot \rightarrow \text{Lipid} - OO - \text{Lipid}$

At the initiation stage, the radical (R•)/OH• attacks the lipid membrane to form a radial lipid. This radical lipid further propagates the formation of peroxyl lipid radical by reacting with dioxygen molecule or with a lipid. This reaction further promotes damage of the lipid molecule. At the termination stage, two radical lipid molecules and/or with a peroxyl lipid radical reacts to form a stable lipid molecule. The major aldehyde product of lipid peroxidation is malondialdehyde and it serves as a marker for lipid peroxidation.

Generally, proteins are not easily damaged by H_2O_2 and other simple oxidants unless transition metals are present. Thus, protein damaged are usually metal-catalyzed and involves oxidative scission, bityrosine cross links, loss of histidine residues, the introduction of carbonyl groups, and the formation of protein-centered alkyl (R•), alkoxyl (RO•) and alkylperoxyl (ROO•) radicals.

b. Copper :

Copper ions have been identified to participate in the formation of reactive oxygen species (ROS) as cupric (Cu^{2+}) and cuprous (Cu^{1+}) which can participate in oxidation and reduction reactions. The Cu^{2+} in the presence of biological reductants such as glutathione (GSH) or ascorbic acid can be

reduced to Cu+ which is capable of catalyzing the decomposition of H2O2 to form OH• via the Fenton reaction as shown below.

$$Cu^{+}+H_2O_2 \rightarrow Cu^{2+}+OH\cdot+OH^{-}$$

The OH• radical formed is capable of reacting with several biomolecules. Experimental studies confirmed that copper is also capable of inducing DNA strand breaks and oxidation of bases via oxygen free radicals. Though in vivo studies have not revealed copper-induced oxidation of low density lipoprotein (LDL), in vitro studies clearly demonstrated LDL oxidation induced by copper.

c. Chromium :

Chromium (Cr), particularly Cr4+ has been shown in in vitro studies to generate free radicals from H2O2. Also, in vivo studies were able to show the detection of free radicals due to chromium in the liver and blood of animals. It was observed that Cr5+ intermediates were generated as a result of one-electron reduction.

d. Cobalt :

Cobalt (Co), particularly Co2+ has been shown to generate superoxide (•O2−) from the decomposition of H2O2.

$$Co^{2+}+O_2 \rightarrow Co^{+}+O_2\cdot^{-} \rightarrow Co^{+}-OO\cdot$$

e. Vanadium :

Vanadium is a heavy metal that occurs in various oxidative states and has been shown to generate free radical. In the plasma, vanadium (V) is rapidly reduced to vanadium (IV) by NADPH and ascorbic acid antioxidants which bind to plasma proteins for transportation.

$$V^{5+}+NADPH \rightarrow V^{4+}+NADP^{+}+H^{+}$$
$$V^{4+}+O_2 \rightarrow V^{5+}+O_2\cdot^{-}$$
$$V^{5+}+O_2\cdot^{-} \rightarrow [V^{5+}-OO\cdot]$$

More so under physiological conditions at approximately pH of 7, V(IV) can generate OH• from the decomposition of H2O2 according to the Fenton reaction.

$$V^{4+}+H_2O_2 \rightarrow V^{5+}+OH^{-}+\cdot OH$$

f. Arsenic:

Arsenic has also been shown to generate free radicals such as superoxide (O2•−), singlet oxygen (1O2), nitric oxide (NO•), hydrogen peroxide (H2O2), the peroxyl radical (ROO•), dimethylarsinic peroxyl radicals ((CH3)2AsOO•) and also the dimethylarsinic radical ((CH3)2As•) in some studies though the mechanism for the generation of all these reactive species remains unclear.

B. Heavy metal-induced carcinogenesis:

Some heavy metals are known to have carcinogenic effect. Several signaling proteins or cellular regulatory proteins that participate in apoptosis, cell cycle regulation, DNA repair, DNA methylation, cell growth and differentiation are targets of heavy metals. Thus, heavy metals may induce carcinogenic effect by targeting a number of these proteins. More so, the carcinogenic effects of certain heavy metals have been related to the activation of redox-sensitive transcription factors such as AP-1, NF-κB and p53 through the recycling of electrons by antioxidant network. These transcription factors control the expression of protective genes that induce apoptosis, arrest the proliferation of damaged cells, repair damaged DNA and power the immune system. Metal signalization of transcription factor AP-1 and NF-κB has been observed in the mitogen-activated protein (MAP) kinase pathways where the nuclear transcription factor NF-κB, is involved in controlling inflammatory responses while AP-1 is involved in cell growth and differentiation . The p53 protein is an important protein in cell division as it guards a cell-cycle checkpoint and control cell division. Inactivation of p53 allows uncontrolled cell division and thus p53 gene disruption has been associated with most human cancers. Also, AP-1 and NF-κB family of transcription factors are involved in both cell proliferation and apoptosis, and also regulate p53. Heavy metals generated free radicals inside the cell selectively activates these transcription factors and thus, may suggest that cell proliferation or cell death may be related to the exposure to carcinogenic metals. There exist various mechanisms of heavy metal-induced carcinogenesis.

a. Arsenic:

Arsenic-induced carcinogenic mechanisms include epigenetic alterations, damage to the dynamic DNA maintenance system and generation of ROS. Alterations of histones, DNA methylation, and miRNA are the key epigenetic changes induced by arsenic which have shown to possess potentials to cause malignant growth. In vitro studies have shown arsenic to alter the expression of p53 protein which also led to decreased expression of p21, one downstream target. Arsenic compounds have been shown in an in vitro cell line study to promote genotoxicity in humans and mice leucocytes. Also, a methylated form of arsenic was shown to inhibit DNA repair processes and also generate ROS in liver and spleen as metabolic products. Arsenic can bind DNA-binding proteins and disrupt the DNA repair processes thereby increasing the risk of carcinogenesis. For example, the tumor suppressor gene-coded DNA was suppressed when arsenic was bound to methyl-transferase. Also, cancers of the liver, skin, prostate and Kupffer cell were associated with Arsenic poisoning.

b. Lead:

The mechanism of lead-induced carcinogenic process is postulated to induce DNA damage, disrupt DNA repair system and cellular tumor regulatory genes through the generation of ROS. Studies have supported with evidence that ROS generation by lead is key in altering chromosomal structure and sequence. Lead can disrupt transcription processes by replacing zinc in certain regulatory proteins.

c. Mercury:

Little is known on the potential of mercury to act as a mutagen or carcinogen. However, the proposed mechanism of mercury-induced cancer is through the generation of free radicals inducing oxidative stress thereby damaging biomolecules. Mercury has been shown to induce malignant growth through the generation of free radicals as well as disruption of DNA molecular structure, the repair and maintenance system.

d. Nickel:

Nickel has an extensive range of carcinogenic mechanisms which include regulation of transcription factors, controlled expression of certain genes and generation of free radicals. Nickel has been shown to be implicated in regulating the expression of specific long non-coding RNAs, certain mRNAs and microRNAs. Nickel can promote methylation of promoter and induce the down regulation of maternally expressed gene 3 (MEG3) thereby upregulating hypoxia-inducible factor-1α, two proteins which are known to be implicated in carcinogenesis. It has also been demonstrated that nickel can generate free radicals, which contributes to carcinogenic processes.

e. Cadmium:

Cadmium has been implicated in promoting apoptosis, oxidative stress, DNA methylation and DNA damage.

f. Iron :

The main cause of cancer due to iron intoxication is through the generation of free radicals. A school of thought produced a mechanism for iron-induced cancer whereby bile acids (deoxycholic acid), iron(II) complexes, vitamins K and oxygen interact to generate free radicals which induced oncogenic effect in the colon.

C. Heavy metal-induced neurotoxicity:

Some heavy metals such as lead and manganese may affect the brain and cause neurological toxicity as reviewed from.

a. Lead:

Lead toxicity is targeted towards the memory and learning processes of the brain and can be mediated through three processes. Lead can impair learning and memory in the brain by inhibiting the N-methyl-d-aspartate receptor (NMDAR) and can block neurotransmission by inhibit neurotransmitter release, block the neuronal voltage-gated calcium (Ca2+) channels (VGCCs) and reduce the expression of brain-derived neurotrophic factor (BDNF).

b. Manganese:

Manganese is known to accumulate in the mitochondria of neurons, astrocytes and oligodendrocytes cells and disrupts ATP synthesis by inhibiting the F1/F0 ATP synthase or complex 1 (NADH dehydrogenase) of the mitochondrial respiration chain. More so, it has recently been shown that manganese inhibits ATP synthesis at two sites in the brain mitochondria which

are either the glutamate/aspartate exchanger or the complex II (succinate dehydrogenase) depending on the mitochondrial energy source. The disruption of ATP synthesis by manganese leads to decreased intracellular ATP levels and generation of free radicals thereby increasing oxidative stress which may contribute to manganese cellular toxicity. Furthermore, manganese can oxidize dopamine (DA) to react with quinone species thereby disrupting the dopaminergic system. This has been shown in animal studies were manganese exposure has led to specific deficits in the dopaminergic system. The DA reactive species are taken up by the dopamine transporter (DAT1) thus causing dopaminergic neurotoxicity.

Biochemical mechanism of heavy metal toxicity:

When heavy metals are ingested through food or water into the body, they are acidified by the acid medium of the stomach. In this acidic medium, they are oxidized to their various oxidative states (Zn2+, Cd2+, Pb2+, As2+, As3+, Ag+, Hg2+, etc.) which can readily bind to biological molecules such as proteins and enzymes to form stable and strong bonds. The most common functional group that heavy metals bind is the thio groups (SH group of cysteine and SCH3 group of methionine). Cadmium has been shown to inhibit human thiol transferases such as thioredoxin reductase, glutathione reductase, thioredoxin in vitro by binding to cysteine residues in their active sites. The equations of these reactions are shown below (Figure 2).

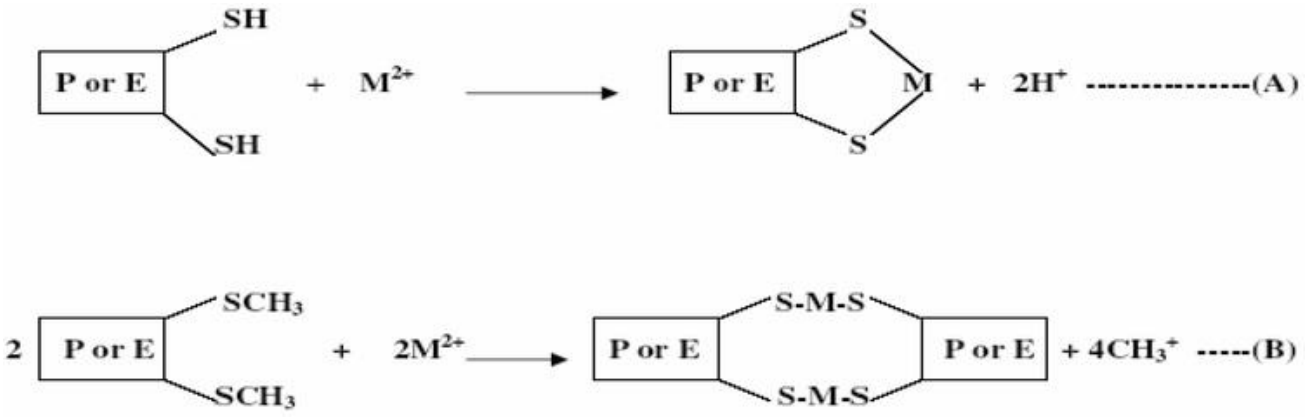


Figure 2.Reactions of Heavy metals with sulphydryl groups of proteins or enzymes (A) = Intramolecular bonding; (B) = Intermolecular bonding; P = Protein; E = Enzyme; M = Metal.

In the above reaction, the oxidized heavy metal replaces the hydrogen of the SH group and the methyl of the SCH3 group thereby inhibiting the function of the protein or activity of the enzyme. For example, methylmercury (MeHg) strongly inhibits the activity of l-glutamine d-fructose-6-phosphate amidotransferase in yeast.

Heavy metal-bound proteins may be a substrate for certain enzymes. In such situations, the heavy metal-bound protein fits into an enzyme in a highly specific pattern to form an enzyme-substrate complex and thus cannot accommodate any other substrate until it is freed. As such, the product of

the substrate is not formed as the enzyme is blocked and therefore, the heavy metal remains embedded in the tissue leading to dysfunctions, abnormalities and damages in the body. Inhibition of thiol transferases lead to increased oxidative stress and cell damage. For example, toxic arsenic present in fungicides, herbicides and insecticides can attack –SH groups in enzymes to inhibit their catalytic activities as shown in Figure 3.

Figure 3.Reaction of arsenic with the thio group of enzymes.

Also, heavy metal toxicity may be induced by the replacement of a metallo-enzyme by another metal ion of similar size. Cadmium displaces zinc and calcium ions from zinc finger proteins and metalloproteins. For instance, cadmium can replace zinc in certain dehydrogenating enzymes, leading to cadmium toxicity. Such replacement can convert the enzyme structurally to an inactive form and completely alter its activity. These heavy metals in their ionic species such as Pb2+, Cd2+, Ag+ Hg2+ and As3+ form very stable biotoxic compounds with proteins and enzymes and are difficult to be dissociated.

Heavy metals may also inhibit protein folding. This was first observed when heavy metals such as cadmium, lead, mercury and arsenite were shown to effectively interfere with the refolding of chemically denatured proteins. It was also observed that when protein misfolded in the presence of heavy metals, the misfolded protein could not be rescued in the presence of reduced glutathione or EDTA chelator. The order of heavy metal in terms of their efficacy in folding inhibition is mercury > cadmium > lead and correlates with the relative stability of their monodentate complexes with imidazole, thiol and carboxylate groups in proteins.

Heavy metal may cause proteins to aggregate as arsenite-induced protein aggregation was observed and shown to be concentration-dependent. Also, the aggregates contained a wide variety of proteins enriched in functions related to metabolism, protein folding, protein synthesis and stabilization. Saccharomyces cerevisiae (budding yeast) cells was shown to accumulate aggregated proteins after it was exposed to equi-toxic concentrations of cadmium, arsenite and chromium (Cr(VI)) and the effect of protein aggregation was influenced by heavy metals in this order: arsenic > cadmium > chromium [80]. The in vivo potency of these agents to trigger protein aggregation probably depends on the efficiency of their cellular uptake/export and on their distinct modes of biological action. Summarized in Figure 4 is the various mechanisms of heavy metal intoxication.

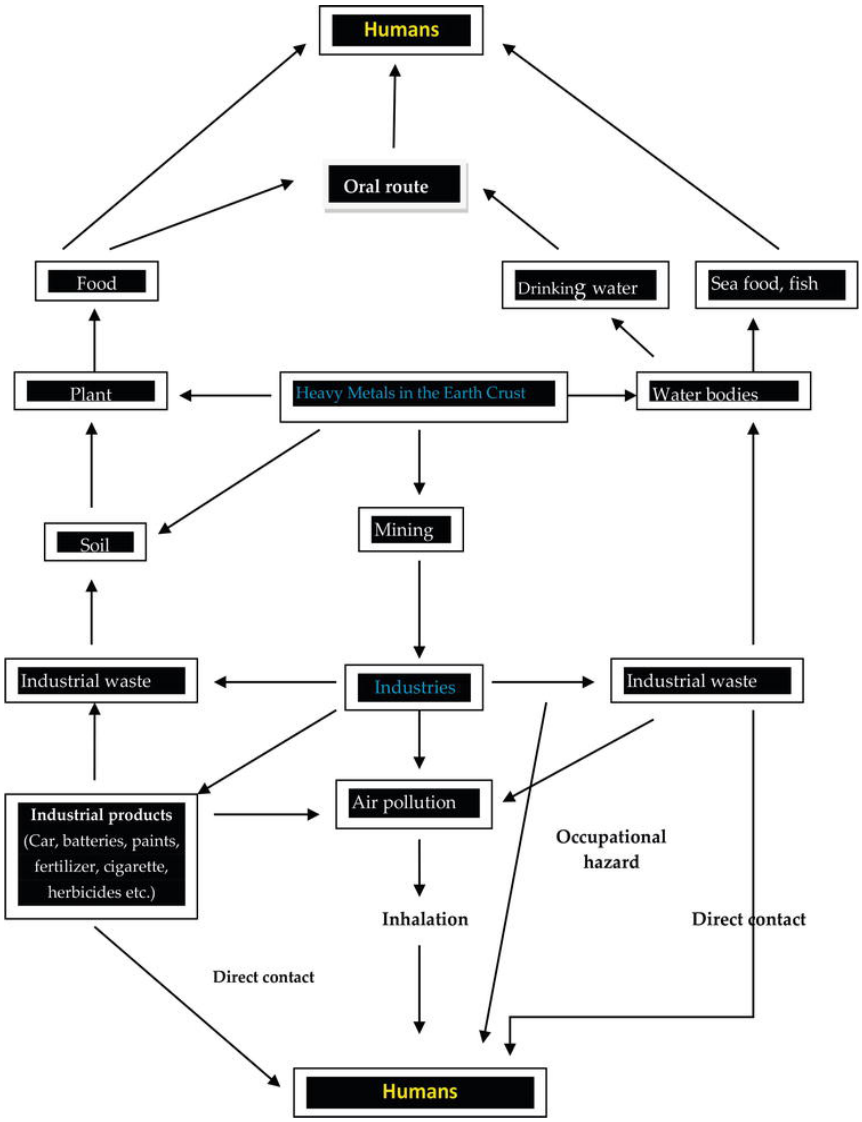


Figure 4. Mechanisms of heavy metal intoxication in humans.

Health effects of heavy metal toxicity in humans:

Heavy metal toxicity can have several health effects in the body. Heavy metals can damage and alter the functioning of organs such as the brain, kidney, lungs, liver, and blood. Heavy metal toxicity can either be acute or chronic effects. Long-term exposure of the body to heavy metal can progressively lead to muscular, physical and neurological degenerative processes that are similar to diseases such as Parkinson's disease, multiple sclerosis, muscular dystrophy and Alzheimer's disease. Also, chronic long-term exposure of some heavy metals may cause cancer. The various health effects of some heavy metals will be highlighted belo

a. Arsenic :

Arsenic exposure can lead to either acute or chronic toxicity. Acute arsenic poisoning can lead to the destruction of blood vessels, gastrointestinal tissue and can affect the heart and brain. Chronic arsenic toxicity which is termed arsenicosis usually focus on skin manifestations such as

pigmentation and keratosis. Lower level exposure to arsenic can cause nausea and vomiting, reduced production of erythrocytes and leukocytes and damage blood vessels, cause abnormal heart beat and pricking sensation in hands and legs. Long-term exposure can lead to the formation of skin lesions, pulmonary disease, neurological problems, peripheral vascular disease, diabetes mellitus, hypertension and cardiovascular disease. Chronic arsenicosis may results to irreversible changes in the vital organs and possibly lead to death. Also, chronic arsenic exposure can promote the development of a number of cancers which include skin cancer, cancers of the bladder, lung, liver (angiosarcoma), and possibly the colon and kidney cancers. Recently in the United States, the tolerable amount of arsenic in drinking water is 50 μg/liter but there is much concern of lowering this standard dose of population exposures to arsenic as the present dose is believed to increase the risk for cancer. Most environmental scientists studying this problem are of the view that the current tolerable limit of arsenic in drinking water or food be reduced.

b. Lead:

Toxicity due to lead exposure is called lead poisoning. Lead poisoning is mostly related to the gastrointestinal tract and central nervous system in children and adults. Lead poisoning can be either acute or chronic. Acute exposure of lead can cause headache, loss of appetite, abdominal pain, fatigue, sleeplessness, hallucinations, vertigo, renal dysfunction, hypertension and arthritis while chronic exposure can result in birth defects, mental retardation, autism, psychosis, allergies, paralysis, weight loss, dyslexia, hyperactivity, muscular weakness, kidney damage, brain damage, coma and may even cause death. Although lead poisoning is preventable, it still remains a dangerous disease as it can affect most of the organs of the body. Exposure to elevated levels of lead can cause the plasma membrane of the blood brain barrier to move into the interstitial spaces leading to edema. Also, lead exposure can disrupt the intracellular second messenger systems and alter the functioning of the central nervous system. Developing fetuses and children are most vulnerable to neurotoxic effects due to lead exposure. A number of prospective epidemiologic studies in children less than 5 years of age have shown that low-level of lead exposure (5–25 μg/dL in blood) resulted to the impairment of intellectual development which was manifested by the lost of intelligence quotient points [85]. As such, the Centers for Disease Control (CDC) in the United States has reduced the tolerable amount of lead in children's blood from 25 to 10 μg/dL and recommended universal screening of blood lead for all children.

c. Mercury:

Mercury is an element that can easily combine with other elements to form inorganic and organic mercury. Exposure to elevated levels of metallic, inorganic and organic mercury can damage the kidney, brain and developing fetus while methyl mercury is highly carcinogenic. Organic mercury is lipophilic in nature and thus can easily penetrate cell membranes. Mercury and its compound affects the nervous system and thus increased exposure of mercury can alter brain functions and lead to tremors, shyness, irritability, memory problems and changes in hearing or vision. Short-term exposure to metallic mercury vapors at higher levels can lead to vomiting, nausea, skin rashes, diarrhea, lung damage, high blood pressure, etc. while short-term exposure to organic mercury poisoning can lead to depression, tremors, headache, fatigue, memory problems, hair loss, etc. Since these symptoms are also common in other illness or disease conditions, diagnosis of

mercury poisoning may be difficult in such cases. Chronic levels of mercury exposure can lead to erethism, a disease condition characterized by excitability, tremor of the hands, memory loss, timidity, and insomnia. Also, occupational exposure to mercury as observed by researchers has been associated with measurable declines in performance on neurobehavioral tests of motor speed, visual scanning, visuomotor coordination, verbal and visual memory. Dimethylmercury is a very toxic compound that can penetrate the skin through latex gloves and its exposure at very low dose can cause the degeneration of the central nervous system and death. Mercury exposure to pregnant women can affect the fetus and offspring may suffer from mental retardation, cerebellar symptoms, retention of primitive reflexes, malformation and other abnormalities. This has been confirmed in recent studies in which pregnant women exposed to mercury through dietary intake of whale meat and fish showed reduce motor neuron function, loss of memory, impaired speech and neural transmission in their offspring.

d. Cadmium:

Cadmium and its compounds have several health effects in humans. The health effects of cadmium exposure are exacerbated due to the inability of the human body to excrete cadmium. In fact, cadmium is re-absorbed by the kidney thereby limiting its excretion. Short-term exposure to inhalation of cadmium can cause severe damages to the lungs and respiratory irritation while its ingestion in higher dose can cause stomach irritation resulting to vomiting and diarrhea. Long-term exposure to cadmium leads to its deposition in bones and lungs. As such, cadmium exposure can cause bone and lung damage. Cadmium can cause bone mineralization as studies on animals and humans have revealed osteoporosis (skeletal damage) due to cadmium. It has been observed that “Itai-itai” disease, an epidemic of bone fractures in Japan is due to cadmium contamination. Increased cadmium toxicity in this population was found to be associated with increased risk of bone fractures in women, as well as decreased bone density and height loss in males and females. Cadmium is highly toxic to the kidney and it accumulates in the proximal tubular cells in higher concentrations. Thus, cadmium exposure can cause renal dysfunction and kidney disease. Also, cadmium exposure can cause disturbances in calcium metabolism, formation of renal stones and hypercalciuria. Cadmium is also classified as group 1 carcinogens for humans by the International Agency for Research on Cancer. Tobacco is the main source of cadmium uptake in smokers and thus, smokers are more susceptible to cadmium intoxication than non-smokers. Also, cadmium can cause testicular degeneration and a potential risk factor for prostate cancer.

e. Chromium:

Chromium, in its hexavalent form, is the most toxic species of chromium though some other species such as Chromium (III) compounds are much less toxic and cause little or no health problems. Chromium (VI) has the tendency to be corrosive and also to cause allergic reactions to the body. Therefore, breathing high levels of chromium (VI) can cause irritation to the lining of the nose and nose ulcers. It can also cause anemia, irritations and ulcers in the small intestine and stomach, damage sperm and male reproductive system. The allergic reactions due to chromium include severe redness and swelling of the skin. Exposure of extremely high doses of chromium (VI) compounds to humans can result in severe cardiovascular, respiratory, hematological, gastrointestinal, renal, hepatic, and neurological effects and possibly death. Exposure to chromium

compounds can result in the formation of ulcers such as nasal septum ulcer which are very common in chromate workers. Exposure to higher amounts of chromium compounds in humans can lead to the inhibition of erythrocyte glutathione reductase, which in turn lowers the capacity to reduce methemoglobin to hemoglobin. In vivo and in vitro experiments have shown chromate compounds to induce DNA damage in many different ways and can lead to the formation of DNA adducts, chromosomal aberrations, alterations in replication sister chromatid exchanges, and transcription of DNA. Thus, there are substantial evidence of chromium to promote carcinogenicity of humans as increase stomach tumors have been observed in animals and humans who were exposed to chromium(VI) in drinking water.

f. Iron :

Iron salts such as iron sulfate, iron sulfate heptahydrate and iron sulfate monohydrate are of low acute toxicity when exposure is through dermal, oral and inhalation routes. However, other forms of iron are of serious health problems. Iron toxicity occurs in four stages. The first stage which commences 6 h after iron overdose is marked by gastrointestinal effects such as vomiting, diarrhea and gastro-intestinal bleeding. The progression to the second stage occurs 6–24 h after an overdose and it is considered as a latent period of apparent medical recovery. The third stage commences between 12 and 96 h after the onset of clinical symptoms and is characterized by hypotension, shocks, lethargy, hepatic necrosis, tachycardia, metabolic acidosis and may sometimes lead to death. The fourth and final stage usually occurs within 2–6 weeks of iron overdose. This stage is marked by the development of strictures and formation of gastrointestinal ulcerations. Meat is rich in iron and thus meat eating countries are at risk of cancer as excess iron uptake increases the risk of cancer. Asbestos contains about 30% of iron and thus workers who are highly exposed to asbestos are at high risk of asbestosis, a condition which is known to cause lung cancer. Iron is known to generate free radicals which are suggested to be responsible for asbestos related cancer. Iron-induced free radicals can initiate cancer by the oxidation of DNA leading to DNA damage.

g. Manganese :

Although manganese is an essential metal for the body, it recently became a metal of global concern when methylcyclopentadienyl manganese tricarbonyl (MMT), which was known to be toxic was introduced as a gasoline additive. MMT has been claimed to be an occupational manganese hazard and linked with the development of Parkinson’s disease-like syndrome of tremour, gait disorder, postural instability, and cognitive disorder. Exposure to elevated levels of manganese can result in neurotoxicity. Manganism is a neurological disease due to manganese characterized by rigidity, action tremour, a mask-like expression, gait disturbances, bradykinesia, micrographia, memory and cognitive dysfunction, and mood disorder. The symptoms of manganism are very similar to that of Parkinson disease. However, the main differences between manganism and Parkinson disease is the insensitivity of manganism to levodopa (l-DOPA) administration and also the differences in the symptoms and progression of the disease.

Remediation

In humans, heavy metal poisoning is generally treated by the administration of chelating agents. These are chemical compounds, such as CaNa2 EDTA (calcium disodium ethylene diamine tetra acetate) that convert heavy metals to chemically inert forms that can be excreted without further interaction with the body. Chelates are not without side effects and can also remove beneficial metals from the body. Vitamin and mineral supplements are sometimes co-administered for this reason.

Soils contaminated by heavy metals can be remediated by one or more of the following technologies: isolation; immobilization; toxicity reduction; physical separation; or extraction. *Isolation* involves the use of caps, membranes or below-ground barriers in an attempt to quarantine the contaminated soil. *Immobilization* aims to alter the properties of the soil so as to hinder the mobility of the heavy contaminants. *Toxicity reduction* attempts to oxidize or reduce the toxic heavy metal ions, via chemical or biological means into less toxic or mobile forms. *Physical separation* involves the removal of the contaminated soil and the separation of the metal contaminants by mechanical means. *Extraction* is an on or off-site process that uses chemicals, high-temperature volatization, or electrolysis to extract contaminants from soils. The process or processes used will vary according to contaminant and the characteristics of the site.

III. RADIATION HAZARD:

The term 'radiation' can refer to a wide variety of forms of energy moving around as waves or particles. It can mean x-rays, or it can mean microwaves. It can also refer to infrared light and even visible light. But when we say 'radioactive pollution,' we're being more specific. **Radioactive pollution** refers to the release of ionizing radiation into the environment as a result of human activity.

Type of radiation

1. Ionizing radiation:

It is the form of radiation that has a short wavelength and a high frequency. In short, it's the form of radiation that's commonly thought of as being high energy and thus harmful to living things. Ionizing radiation includes x-rays and gamma rays. Typical ionizing subatomic particles from radioactivity include alpha particles, beta particles and neutrons. Almost all products of radioactive decay are ionizing because the energy of radioactive decay is typically far higher than that required to ionize. Other subatomic ionizing particles which occur naturally are mesons, positrons, and other particles that constitute the secondary cosmic rays that are produced after primary cosmic rays interact with Earth's atmosphere. Cosmic rays are generated by stars and certain celestial events such as supernova explosions. Cosmic rays may also produce radioisotopes on Earth (for example, carbon-14), which in turn decay and produce ionizing radiation. Cosmic rays and the decay of radioactive isotopes are the primary sources of natural ionizing radiation on Earth referred to as background radiation. Ionizing radiation can also be generated artificially by X-ray tubes, particle accelerators, and any of the various methods that produce radioisotopes artificially. The ionizing radiations are further categorized into two types such as electromagnetic radiation and particulate radiation.

Measuring Ionising Radiation:

Radiation is measured in terms of an ionisation unit called roentgen or r unit, one r being equal to 1.8×10^9 ion pairs per cubic cm of air. In tissue which is ten times as dense as air, a high energy radiation produces about 1000 times the number of ion pairs per cubic cm as it does in air. Another unit called rad measures the total amount of radiant energy absorbed by the medium. One rad equals 100 ergs per gram of tissue. Another unit called gray is equivalent to 100 rads.

In the case of X-rays about 90 % of the energy left in the tissue is used to produce ions, the rest produces heat and excitation. Ultraviolet (UV) is a non-ionising type of radiation and is measured in rads instead of r units. When ionisation is caused by subatomic particles, the doses are measured in different units called rem and sievert. One rem is defined as the amount of any radiation that produces a biological effect equivalent to that resulting from one rad of gamma rays. A sievert is equal to 100 rems. For detecting radiation the Geiger-Muller tube is used. The tube contains a gas which is ionized by radiation. The amount of radiation is gauged from suitable amplifiers and counters.

2. Non-ionic radiation:

Non-ionizing radiation refers to any type of electromagnetic radiation that does not carry enough energy per quantum (photon energy) to ionize atoms or molecules—that is, to completely remove an electron from an atom or molecule. Instead of producing charged ions when passing through matter, non-ionizing electromagnetic radiation has sufficient energy only for excitation, the movement of an electron to a higher energy state. *Ionizing radiation* which has a higher frequency and shorter wavelength than nonionizing radiation, has many uses but can be a health hazard; exposure to it can cause burns, radiation sickness, cancer, and genetic damage. Using ionizing radiation requires elaborate radiological protection measures which in general are not required with nonionizing radiation.

Alpha (α), beta (β), and gamma (γ) radiations are mainly responsible for radiation pollution. Alpha radiation contains energetic alpha particles. Each alpha particle carries two units of positive charges and interacts strongly with living tissues. Beta, radiation is made up of energetics electrons. Each beta particle carries one unit of negative charge and interacts strongly with matter. Gamma radiations are made up of high energy photons. Photons bring about strong electro-magnetic interaction with matter.

Sources of Radiation Pollution:

a. Natural sources of radiation:

1. Radioactive minerals:

The minerals containing Uranium- 235 (U^{235}), Uranium-238 (U^{238}), Thorium-232 (Th^{232}), Plutonium- 239 (Pu^{239}) etc. are capable of emitting energetic radiations causing pollution.

2. Cosmic rays:

The cosmic rays containing highly energetic particles reach the surface of the earth causing pollution. The intensity of cosmic rays depends on latitudes and altitude of the place. The intensity is maximum at the poles and minimum at the equator.

3. Radionuclides:

The unstable radio-nuclides in the atmosphere can be splitted up into smaller parts emitting energetic radiation. The smaller radio-nuclides enter into the body of organism along with air during respiration.

b. Anthropogenic or Man-made radiation:

1. Nuclear power plants:

Nuclear power plants emit radiation to a very smaller extent except accidental leaks (Chernobyl accident of undivided USSR).

2. Radio-active Wastes:

The nuclear power plants produce a lot of nuclear radio-active wastes. The disposal of these wastes has become a global problem. Some countries producing large quantity of nuclear wastes dump them in ocean near other countries.

3. Nuclear Explosion:

During nuclear explosion, a large number of radio-nuclides are generated in the atmosphere. The radio nuclides settle down with rain contaminating the soil and water bodies. Finally, these enter into food chain causing serious problem to the living organisms.

4. Radio-isotopes:

Radio-isotopes are also prepared artificially either by nuclear fusion or by nuclear fission. If these radio-isotopes are not properly handled, these emit radiations causing pollution.

5. Television Set:

Television sets produce radiations which can also cause cancer.

Mechanism of Radiation Toxicity:

Ionizing radiation comprising alpha and beta particles and gamma rays loses energy when passing through organic matter by releasing ion pairs of an electron and a positively charged atoms. Ionization can break the bonds in DNA and subsequently damage DNA.

These ion pairs rapidly interact with organic molecules in the tissue and produce free highly reactive oxidative species (ROS) radicals by forming super oxide O_2" anion which subsequently converted to a strong oxidizing agent hydrogen peroxide.

Free radicals or H_2O_2 cause cellular damage by interacting and disrupting structure and function of proteins, amino acids, carbohydrates, nucleic acids, lipids, thiols etc. Damage to DNA results in mutation, chromosomal aberrations and loss of genes and subsequently leading to cell death. The extent and rate of chromosomal aberrations is directly related to radiation dose.

Radiation Toxicity:

There is a variation in susceptibility among different species of organism and also their organs to radiation toxicity as donkeys, rabbits, and poultry are less susceptible than man, dogs, pigs and goats and the organs with fast proliferating cells like skin, gastrointestinal tract and haematopoietic system are most affected with the exception of human lymphocytes. Young animals and foetus are more radiosensitive than adults. Depending upon dose and exposure of duration radiation may cause acute, sub-acute and chronic toxicity in man and animals.

a. Acute radiation toxicity:

Exposure to high doses of irradiation results in acute toxicity and is characterized by severe irritation of GIT resulting in intense and refractory diarrhoea, dehydration, redness of skin, thirst, weakness, recumbency, rapid respiration, panting, profuse and blood stained nasal discharge.

If animal survive, there may be severe depression of bone marrow manifested by anaemia, lymphopenia, agranulocytosis, thrombocytopenia, impaired blood clotting and antibody production, and necrosis of mucosa of GIT, loss of hair and ulceration of skin followed by secondary infections, degenerative changes in lens of eye (cataract), high rate of mutations, tumours mostly of haemopoietic system, particularly leukaemia may be observed.Death may occur due to dehydration and salt depletion few days or weeks post exposure. Most of the deaths in acute and sub-acute cases occur in 1-4 weeks of irradiation.

b. Sub-acute Radiation Toxicity:

Sub-acute toxicity occurs as result of low level radiation continuously for few weeks and is characterized by anorexia, vomiting, depression and weakness followed by fever, knuckling at the fetlock, swelling of legs, diarrhoea, dysentery, polydipsia, recumbency and hyperirritability and severe anaemia and septicaemia in terminal stages leading to death 3-4 weeks post exposure.

c. Chronic Radiation Toxicity:

Prolong exposure of animals as result of ingestion of contaminated pasture may result in chronic toxicity. Consumption of milk, vegetables food grains etc. contaminated with radioactive material may be the source of chronic toxicity and is manifested by retarded growth alopecia, sterility, mutational changes, cancer of blood (leukaemia), thyroid, breast, lungs, colon, stomach, liver, urinary bladder and other tissues and teratogenesis.

d. Pathological Lesions:

Oedema of the dermis, swelling and ulceration of mucosa of gastrointestinal tract, severe congestion and fibrosis in lungs, hypertrophy of adrenals, atrophy and degenerative changes in bone marrow, lymphoid organ, testicles and hepatomegaly, ascites and jaundice are the major pathological lesion.

Diagnosis: Diagnosis is made on the basis of history, clinical signs and pathological lesions.

Effect of Radiation Pollution:

When radiation passes through different living organisms the following disorders takes place:

1. Radiation splits the molecules of the tissues into ions and free radicals and causes mutation by breaking DNA (Deoxy ribonucleic acid) molecules in the nucleus.

2. Radiation in bone marrow may cause leukaemia.

3. Radiation may cause skin burns which may lead to skin cancer.

4. Radiation at pelvic regions of pregnant ladies, cause damage to the foetus.

Effects of Ionising Radiation on DNA:

Zirkle in 1930 showed that in plants the nucleus is more sensitive to ionising radiation than the cytoplasm. It is now known with certainty that many molecules including DNA are affected by ionising radiation. The purines are less sensitive to radiation than pyrimidines. Out of the pyrimidines, thymine is most sensitive. Large doses of ionising radiation destroy thymine, uracil and cytosine in aqueous solutions. By depolymerizing DNA, ionising radiations prevent DNA replication and stop cell division.

Several mechanisms have been proposed to explain the effects of X-rays and gamma rays. They can break different kinds of chemical linkages and damage genetic material in a variety of ways. Figure 20.2 shows that the effect may be direct or indirect. When a hydrogen atom consisting of one proton and one electron is ionised, the free electron may directly interact with DNA. Or the electron may interact with a molecule of water to produce OH, a free radical which can cause damage to DNA in the same way as the free electron. The following types of destruction of DNA are possible; hydrogen bonds may break between chains; a base may be changed or deleted; a single

or double chain fracture may occur; cross linking might take place within the double helix; a deoxyribose may become oxidised.

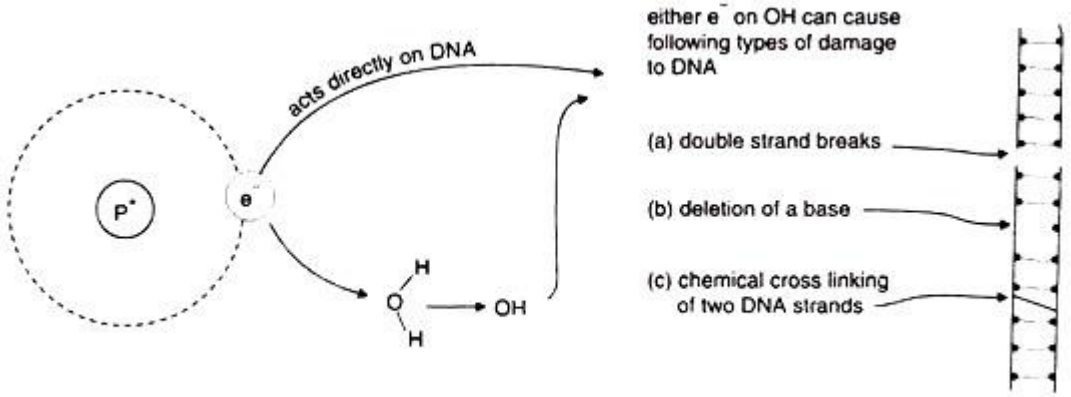


Fig. 20.2 Diagram showing types of damage to DNA by ionising radiation.

If a cell is irradiated in the S phase, DNA replication is inhibited resulting in failure of cell division and cell death. But if the cell is irradiated during mitosis or in G1, in that case DNA replicates normally but mitosis is delayed. Ionising radiation causes breakage and rearrangements in chromosomes which may interfere with normal segregation of chromosomes during cell division. When breaks in two different chromosomes in a cell occur close together in time and space they can join to produce chromosomal aberrations such as inversions, translocations and deletions.

Micro-organisms are more resistant to ionising radiation than higher organisms. It is found that D_{37} dose, that is the radiation dose to a cell population with 37% survival is about 2000 to 30000 rads in bacteria. In human cells D_{37} is about 120 rads. Some chemicals have a protective effect on the cell in reducing the effect of a radiation dose. Aminothiols which have an – SH and – NH_2 group separated by two carbon atoms are most powerful in reducing the effect. The protective effect is expressed as dose reduction factor (DRF).DRF is the ratio of $LD_{50(30)}$ for protected animals to $LD_{50(30)}$ for unprotected animals. LD is the lethal dose or the amount of radiation that kills all individuals in a large group of organisms. $LD_{50(30)}$ is the dose which kills 50 % of organisms within 30 days of exposure. LD_{50} for dog is estimated to be 350 rads, for mouse 550, goldfish 2300.Whether the natural background radiation, though small in amount is dangerous for human beings or not has been questioned. The background radiation consists mainly of cosmic rays, emissions from radioactive elements in the earth such as uranium, radium and thorium, as well as emissions from radioactive isotopes (carbon 14, potassium 40) occurring naturally in the body.

People living at sea level receive an average dose of about 0.8 millisievert of radiation per year. A study of the coastal area of Kerala in South India, a region having high background radiation, has revealed a high incidence of Down's syndrome in the population. Radiation-induced genetic and chromosomal anomalies were also observed.

Control of Radiation Pollution:

Radiation pollution can be controlled in the following ways:

1. Care should be taken to check manmade radiation pollution at source.

2. Nuclear reactor should be perfectly maintained to avoid accidental leakage.

3. Nuclear tests should be banned.

IV. FOOD AND ADDITIVES

Substances that are added to food to maintain or improve the safety, freshness, taste, texture, or appearance of food are known as food additives. Some food additives have been in use for centuries for preservation – such as salt (in meats such as bacon or dried fish), sugar (in marmalade), or sulfur dioxide (in wine).Many different food additives have been developed over time to meet the needs of food production, as making food on a large scale is very different from making them on a small scale at home. Additives are needed to ensure processed food remains safe and in good condition throughout its journey from factories or industrial kitchens, during transportation to warehouses and shops, and finally to consumers.

The use of food additives is only justified when their use has a technological need, does not mislead consumers, and serves a well-defined technological function, such as to preserve the nutritional quality of the food or enhance the stability of the food.Food additives can be derived from plants, animals, or minerals, or they can be synthetic. They are added intentionally to food to perform certain technological purposes which consumers often take for granted. There are several thousand food additives used, all of which are designed to do a specific job in making food safer or more appealing. WHO, together with FAO, groups food additives into 3 broad categories based on their function.

Types of Food Additives

Food additives can be divided into several groups, although there is some overlap because some additives exert more than one effect. For example, salt is both a preservative as well as a flavor.

1. **Acidulants:** Acidulants confer sour or acid taste. Common acidulants include vinegar, citric acid, tartaric acid, malic acid, fumaric acid, and lactic acid.

2. **Acidity regulators :**Acidity regulators are used for controlling the pH of foods for stability or to affect activity of enzymes.

3. **Anticaking agents:** Anticaking agents keep powders such as milk powder from caking or sticking.

4. **Antifoaming and foaming agents:** Antifoaming agents reduce or prevent foaming in foods. Foaming agents do the reverse.

5. **Antioxidants:** Antioxidants such as vitamin C are preservatives by inhibiting the degradation of food by oxygen.
6. **Bulking agents**: Bulking agents such as starch are additives that increase the bulk of a food without affecting its taste.
7. **Food colouring agents** : Colourings are added to food to replace colors lost during preparation or to make food look more attractive.
8. **Fortifying agents** :Vitamins, minerals, and dietary supplements to increase the nutritional value
9. **Colour retention agents**: In contrast to colourings, colour retention agents are used to preserve a food's existing colour.
10. **Emulsifiers :**Emulsifiers allow water and oils to remain mixed together in an emulsion, as in mayonnaise, ice cream, and homogenized milk.
11. **Flavours** : Flavours are additives that give food a particular taste or smell, and may be derived from natural ingredients or created artificially.
12. **Flavour enhancers**: Flavour enhancers enhance a food's existing flavors. A popular example is monosodium glutamate. Some flavor enhancers have their own flavors that are independent of the food.
13. **Flour treatment agents :**Flour treatment agents are added to flour to improve its color or its use in baking.
14. **Glazing agents:** Glazing agents provide a shiny appearance or protective coating to foods.
15. **Humectants**: Humectants prevent foods from drying out.
16. **Tracer gas :**Tracer gas allow for package integrity testing to prevent foods from being exposed to atmosphere, thus guaranteeing shelf life.
17. **Preservatives :**Preservatives prevent or inhibit spoilage of food due to fungi, bacteria and other microorganisms.
18. **Stabilizers**: Stabilizers, thickeners and gelling agents, like agar or pectin (used in jam for example) give food a firmer texture. While they are not true emulsifiers, they help to stabilize emulsions.
19. **Sweeteners**: Sweeteners are added to foods for flavouring. Sweeteners other than sugar are added to keep the food energy (calories) low, or because they have beneficial effects regarding diabetes mellitus, tooth decay, or diarrhoea.
20. **Thickeners** : Thickening agents are substances which, when added to the mixture, increase its viscosity without substantially modifying its other properties.

With the increasing use of processed foods since the 19th century, food additives are more widely used. Many countries regulate their use. For example, boric acid was widely used as a food preservative from the 1870s to the 1920s but was banned after World War I due to its toxicity, as demonstrated in animal and human studies. During World War II, the urgent need for cheap, available food preservatives led to it being used again, but it was finally banned in the 1950s. Such cases led to a general mistrust of food additives, and an application of the precautionary principle led to the conclusion that only additives that are known to be safe should be used in foods. In the United States, this led to the adoption of the Delaney clause, an amendment to the Federal Food, Drug, and Cosmetic Act of 1938, stating that no carcinogenic substances may be used as food additives. However, after the banning of cyclamates in the United States and Britain in 1969, saccharin, the only remaining legal artificial sweetener at the time, was found to cause cancer in rats. Widespread public outcry in the United States, partly communicated to Congress by postage-paid postcards supplied in the packaging of sweetened soft drinks, led to the retention of saccharin, despite its violation of the Delaney clause However, in 2000, saccharin was found to be carcinogenic in rats due only to their unique urine chemistry.

Probable Questions:

1. What is environmental toxicology?
2. Classify pesticides on the basis of target organisms.
3. Classify pesticides on the basis of mode of action.
4. What are the benefits of pesticides?
5. Write down the effects of pesticides on health, environment and economy.
6. How pesticide toxicity can be controlled?
7. What are the sources of heavy metal poisoning?
8. Write down Mechanism of heavy metal toxicity of any 3 metals.
9. What is the biochemical mechanism of heavy metal toxicity?
10. What are the effects of heavy metal on human health?
11. Write down the remediation method of heavy metal toxicity.
12. What is ionizing radiation? How it is measured?
13. What are the sources of radiation pollution?
14. What is ionizing radiation?
15. Describe different types of radiation toxicity.
16. what are the effects of radiation pollution?
17. What are the effects of ionizing radiation on DNA?
18. Describe the health hazards caused by food additives.
19. Describe different types of food additives with examples.
20. How radiation pollution can be controlled?

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett& Doull's Toxicology: The Basic Science by Curtis D. Klaassen

UNIT-VI

Plant Allelochemicals: Types and its role in insect-plant interaction. Plant secondary metabolites in insect response

Objective: In this unit you will learn about plant allelochemicals. Its types and its role in insect - plant interaction. We will also learn about role of plant secondary metabolites in insect response.

Introduction:

Allelopathy is a biological phenomenon by which an organism produces one or more biochemicals that influence the germination, growth, survival, and reproduction of other organisms. These biochemicals are known as allelochemicals and can have beneficial (positive allelopathy) or detrimental (negative allelopathy) effects on the target organisms and the community. Allelochemicals are a subset of secondary metabolites, which are not required for metabolism (i.e. growth, development and reproduction) of the allelopathic organism. Allelochemicals with negative allelopathic effects are an important part of plant defence against herbivory. The production of allelochemicals is affected by biotic factors such as nutrients available, and abiotic factors such as temperature and pH.

Allelopathy is characteristic of certain plants, algae, bacteria, coral, and fungi. Allelopathic interactions are an important factor in determining species distribution and abundance within plant communities, and are also thought to be important in the success of many invasive plants. For specific examples, see black crowberry (*Empetrumhermaphroditum*), spotted knapweed (*Centaurea maculosa*), garlic mustard (*Alliariapetiolata*), *Casuarina/Allocasuarina* spp, and nutsedge. The process by which a plant acquires more of the available resources (such as nutrients, water or light) from the environment without any chemical action on the surrounding plants is called resource competition. This process is not negative allelopathy, although both processes can act together to enhance the survival rate of the plant species.

Allelopathy describes those situations and events where chemicals produced by higher plants, algae, fungi, or microorganisms cause some effect, either inhibitory or stimulatory, on other members of the plant or microbial community . Unlike competition for a resource, the central principle in allelopathy arises from the fact that plants and microorganisms collectively produce thousands of chemicals, and many of these chemicals are released from the producing organism by leaching, exudation , volatilization , or decomposition processes. Subsequently, some of these compounds (known as allelochemicals) alter the growth or physiological functions of organisms that encounter them during growth. For example, almost pure droplets of sorgoleone (a quinone) are exuded from the roots of *Sorghum* species, and sorgoleone inhibits growth in plants that contact it by blocking photosynthesis and respiration. While the word "allelopathy" was first used in the 1930s, the phenomenon that it describes was suggested by natural philosophers more than two thousand years ago as they observed that some plants did not grow well near other kinds

of plants.

Research conducted in the last half of the twentieth century demonstrated cases of growth inhibition by allelochemicals that influenced vegetational patterns, rate and sequences in plant succession, weed abundance, crop productivity, and problems in replanting fruit and other crops. Investigators have focused on identifying the producing plants and the chemicals they give off, the physiological effects on receiving species, and how climatic and soil conditions change the action of allelochemicals. Cinnamic and benzoic acids, flavonoids , and various terpenes are the most commonly found allelochemicals, but several hundred chemicals have been identified, including many other classes of secondary plant compounds. A few allelochemicals have been developed as herbicides and pesticides, and it may be possible to genetically engineer a crop to produce its own herbicides.

Allelochemicals:

A substance (semiochemical) produced by members of one species that influences the behaviour or growth of members of another species. Allelochemicals can be divided into several categories. *Kairomones* benefit the receiving organism but cause disadvantage to the producer. For example, many plants (e.g. cabbages) release aromatic chemicals that attract insect predators, while parasites often exploit the pheromones released by their hosts to locate a suitable host; certain insect predators detect their prey in a similar way. *Allomones* benefit the producer but have no effect on the receiver. For example, many members of the beetle family Lycidae emit pungent chemicals that warn potential predators of their distasteful nature. Hence they are protected from predation, while the impact on the potential predator is neutral. The flowers of certain orchids emit allomones that mimic the sex pheromones of their bee or wasp pollinator. Males of the respective insect species attempt to copulate with the orchid flower, and pollinate it in the process, thus benefiting the orchid, while the cost to the deceived male insect is minimal. *Synomones* are beneficial to both producer and recipient. For example, pine trees damaged by beetles often emit terpenes that attract parasitoid insects that parasitize the pest beetles. Hence the parasitoid finds a suitable host, and the tree's pests are controlled.

History:

The term allelopathy from the Greek-derived compounds *allelo-* and *-pathy* (meaning "mutual harm" or "suffering"), was first used in 1937 by the Austrian professor Hans Molisch in the book *Der EinflusseinerPflanze auf die andere - Allelopathie* (The Effect of Plants on Each Other - Allelopathy) published in German. He used the term to describe biochemical interactions that inhibit the growth of neighbouring plants, by another plant. In 1971, Whittaker and Feeny published a study in the journal *Science*, which defined allelochemicals as all chemical interactions among organisms. In 1984, Elroy Leon Rice in his monograph on allelopathy enlarged the definition to include all direct positive or negative effects of a plant on another plant or on micro-organisms by the liberation of biochemicals into the natural environment. Over the next ten years, the term was used by other researchers to describe broader chemical interactions between organisms, and by 1996 the International Allelopathy Society (IAS) defined allelopathy as "Any process involving secondary metabolites produced by plants, algae, bacteria and fungi that

influences the growth and development of agriculture and biological systems." In more recent times, plant researchers have begun to switch back to the original definition of substances that are produced by one plant that inhibit another plant. Confusing the issue more, zoologists have borrowed the term to describe chemical interactions between invertebrates like corals and sponges.

Long before the term allelopathy was used, people observed the negative effects that one plant could have on another. Theophrastus, who lived around 300 BC, noticed the inhibitory effects of pigweed on alfalfa. In China around the first century AD, the author of *Shennong Ben Cao Jing* described 267 plants that had pesticidal abilities, including those with allelopathic effects. In 1832, the Swiss botanist De Candolle suggested that crop plant exudates were responsible for an agriculture problem called soil sickness.

Allelopathy is not universally accepted among ecologists and many have argued that its effects cannot be distinguished from the competition which results when two (or more) organisms attempt to use the same limited resource, to the detriment of one or both. Allelopathy is a direct negative effect on one organism resulting from the input of substances into the environment by another. In the 1970s, great effort went into distinguishing competitive and allelopathic effects by some researchers, while in the 1990s others argued that the effects were often interdependent and could not readily be distinguished.

However, by 1994 D. L. Liu and J. V. Lowett at the Department of Agronomy and Soil Science, University of New England in Armidale, NSW, Australia wrote two papers in the *Journal of Chemical Ecology* that developed methods to separate the allelochemical effects from other competitive effects, using barley plants and inventing a process to examine the allelochemicals directly. In 1994, M-C Nilsson at the Swedish University of Agricultural Sciences in Umeå, showed in a field study that allelopathy exerted by *Empetrum hermaphroditum* reduced growth of Scots pine seedlings by c. 40%, and that below-ground resource competition by *E. hermaphroditum* accounted for the remaining growth reduction. For this work she inserted PVC-tubes into the ground to reduce below-ground competition or added charcoal to soil surface to reduce the impact of allelopathy, as well as a treatment combining the two methods.

Application :

The possible application of allelopathy in agriculture is the subject of much research. Current research is focused on the effects of weeds on crops, crops on weeds, and crops on crops. This research furthers the possibility of using allelochemicals as growth regulators and natural herbicides, to promote sustainable agriculture. A number of such allelochemicals are commercially available or in the process of large-scale manufacture. For example, Leptospermone is a purported thermochemical in lemon bottlebrush (*Callistemon citrinus*). Although it was found to be too weak as a commercial herbicide, a chemical analog of it, mesotrione (tradename Callisto), was found to be effective. It is sold to control broadleaf weeds in corn but also seems to be an effective control for crabgrass in lawns. Sheeja (1993) reported the allelopathic interaction of the weeds *Chromolaena odorata* (*Eupatorium odoratum*) and *Lantana camara* on selected major crops.

Many crop cultivars show strong allelopathic properties, of which rice (*Oryza sativa*) has been most studied. Rice allelopathy depends on variety and origin: Japonica rice is more allelopathic than Indica and Japonica-Indica hybrid. More recently, critical review on rice allelopathy and the possibility for weed management reported that allelopathic characteristics in rice are quantitatively inherited and several allelopathy-involved traits have been identified.

Many invasive plant species interfere with native plants through allelopathy. A famous case of purported allelopathy is in desert shrubs. One of the most widely known early examples was *Salvia leucophylla*, because it was on the cover of the journal *Science* in 1964. Bare zones around the shrubs were hypothesized to be caused by volatile terpenes emitted by the shrubs. However, like many allelopathy studies, it was based on artificial lab experiments and unwarranted extrapolations to natural ecosystems. In 1970, *Science* published a study where caging the shrubs to exclude rodents and birds allowed grass to grow in the bare zones. A detailed history of this story can be found in Halsey 2004.

Allelopathy has been shown to play a crucial role in forests, influencing the composition of the vegetation growth, and also provides an explanation for the patterns of forest regeneration. The black walnut *(Juglans nigra)* produces the allelochemical juglone, which affects some species greatly while others not at all. The leaf litter and root exudates of some *Eucalyptus* species are allelopathic for certain soil microbes and plant species. The tree of heaven, *Ailanthus altissima*, produces allelochemicals in its roots that inhibit the growth of many plants. The pace of evaluating allelochemicals released by higher plants in nature has greatly accelerated, with promising results in field screening. Garlic mustard is an invasive plant species in North American temperate forests. Its success may be partly due to its excretion of an unidentified allelochemical that interferes with mutualisms between native tree roots and their mycorrhizal fungi.

A study of *Kochia scoparia* in northern Montana by two high school students showed that when *Kochia* precedes spring wheat (*Triticum aestivum*), it reduces the spring wheat's growth. Effects included delayed emergence, decreased rate of growth, decreased final height and decreased average vegetative dry weight of spring wheat plants. A larger study later showed that *Kochia* seems to exhibit allelopathy on various crops in northern Montana

Effect of some allelochemicals on insects:

a. Phenolics (allomones): non-nitrogen compounds, hydroxyl group attached to the benzene rings; they affect nutritional quality of plants; the major groups are phenylpropanoids, flavonoids, quinones. They have deleterious effects on larval growth of the insects. Compounds harmful to one insect may have little effect on another. For example, proanthocyanins or condensed tanninsare feeding inhibitors, however anthocyanins promote pollinator attraction, rotenone, an isoflavanoid has insecticidal properties. Protein inhibitors in plants are found in seeds, tubers and foliage and inhibitory activity of protein inhibitors is specific to digestive proteinases.

b. Terpenoids: monoterpenes act as attractants/repellents, diterpenes exhibit considerable biological activity in relation to the action of toxins and hormones produced by plants. These

chemicals are non toxic to the plant itself but on being consumed by insects are activated into lethal cytotoxins. Terpene induces cytochrome P-450 in insect to higher activity. Such activity may influence the hormone balance or pheromone products in the insect so that regulation of reproductive processes by these allelochemicals is implicated. Oligosaccharides are also reported to regulate not only activation of defense mechanism but also regulate the various aspects of plant product and morphogenesis. If the plant allelochemicals stimulate mating, dependence of the female on the plant is greater so that the adjustment of fecundity to the carrying capacity of the environment is better. For example, some phytophagous insects do not mate without eating the pollen of particular hosts, thus flower stimulates vitellogenesis and induces the female to a state for oviposition.

Plant secondary metabolites and interaction with insects:

Plants and insects have been living together for more than 350 million years. In co- evolution, both have evolved strategies to avoid each other's defense systems. This evolutionary arms race between plants and insects has resulted in the development of an elegant defense system in plants that has the ability to recognize the nonself molecules or signals from damaged cells, much like the animals, and activates the plant immune response against the herbivores. To counter the herbivore attack, plants produce specialized morphological structures or secondary metabolites and proteins that have toxic, repellent, and/or antinutitional effects on the herbivores. Plants confront the herbivores both directly by affecting host plant preference or survival and reproductive success (direct defense), and indirectly through other species such as natural enemies of the insect pests (indirect defense). Direct defenses are mediated by plant characteristics that affect the herbivore's biology such as mechanical protection on the surface of the plants (e.g., hairs,trichomes,thorns,spines,andthickerleaves)orproductionoftoxicchemicalssuchasterpenoids, alkaloids, anthocyanins, phenols, and quinones) that either kill or retard the development of the herbivores. Indirect defenses against insects are mediated by the release of a blend of volatiles that specifically attract natural enemies of the herbivores and/or by providing food (e.g., extra floral nectar) and housing to enhance the effectiveness of the natural enemies. Understanding the nature of gene expression of the plant defensive traits will have a tremendous application in designing crop plants with better protection against the herbivores. This in turn will reduce the need for use of harmful pesticides for insect control.

Host plant defences against insects:

Plants respond to herbivore attack through an intricate and dynamic defense system that includes structural barriers, toxic chemicals, and attraction of natural enemies of the target pests. Both defense mechanisms (direct and indirect) may be present constitutively or induced after damage by the herbivores. Induced response in plants is one of the important components of pest control in agriculture, and has been exploited for regulation of insect herbivore population. Over the past few decades, considerable progress has been made in studying induced responses in plants against different stresses, and has become an important topic in evolutionary biology and ecology. Although induced responses have some metabolic costs, they are very important when aimed at alleviating the stress of immediate concern, as most of these chemicals are

produced in response to herbivore attack. Induced defenses make the plants phenotypically plastic, and thereby, decrease the chances of the attacking insects to adapt to the induced chemicals.

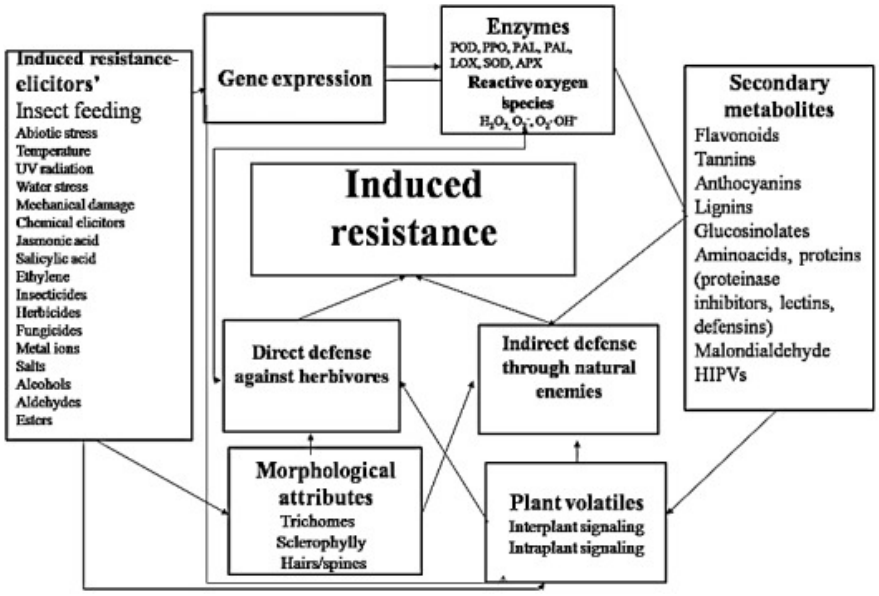


Figure 1. Mechanism of induced resistance in plants POD, peroxidase; PPO, polyphenol oxidase; PAL, phenylalanine ammonia lyase; TAL, tyrosine alanine ammonia lyase; LOX, lipoxygenase; SOD, superoxide dismutase; APX, ascorbate peroxidase; HIPVs, herbivore induced plant volatiles.

Changes in defensive constituents of a plant on account of insect attack develop unpredictability in the plant environment for insect herbivores, which in turn, affects the fitness and behavior of the herbivores. If induced response occurs very early, it is of great benefit to the plant, and reduces the subsequent herbivore and pathogen attack, besides improving overall fitness of the plant. Plants with high variability in defensive chemicals exhibit a better defense compared with those with moderate variability. Progress in insect-plant interactions has improved our understanding of the evolution of defensive approaches deployed by plants against herbivory; however, the underlying mechanisms of defense are less clearlyunderstood.

Direct defenses:

Plant structural traits such as leaf surface wax, thorns or trichomes, and cell wall thickness and lignification form the first physical barrier to feeding by the herbivores, and the secondary metabolites such act as toxins and also affect growth, development, and digestibility reducers form the next barriers that defend the plant from subsequent attack. Moreover, synergistic effect among different defensive components enhances the defensive system of plants against the herbivores invaders. In tomato, alkaloids, phenolics, proteinase inhibitors (PIs), and the oxidative enzymes, when ingested separately result in a reduced affect, but act together in a synergistic manner, affecting the insect during ingestion, digestion and metabolism. In *Nicotiana attenuate* (Torr. ex Watson), trypsin proteinase inhibitors and nicotine expression, contributed synergistically to the defensive response against *Spodopteraexigua*(Hub.). The role of

morphological and biochemical constituents in host plant resistance (HPR), and induced responses to insect damage will be discussed below.

Morphological structures:

Plant structures are the first line of defense against herbivory, and play an important role in HPR to insects. The first line of plant defense against insect pests is the erection of a physical barrier either through the formation of a waxy cuticle, and/or the development of spines, setae, and trichomes. Structural defenses includes morphological and anatomical traits that confer a fitness advantage to the plant by directly deterring the herbivores from feeding, and range from prominent protrubances on a plant to microscopic changes in cell wall thickness as a result of lignification and suberization. Structural traits such as spines and thorns (spinescence), trichomes (pubescence), toughened or hardened leaves (sclerophylly), incorporation of granular minerals into plant tissues, and divaricated branching (shoots with wiry stems produced at wide axillary angles) play a leading role in plant protection against herbivory. Sclerophylly refers to the hardened leaves, and plays an active role in plant defense against herbivores by reducing the palatability and digestibility of the tissues, thereby, reducing the herbivore damage.Spinescence includes plant structures such as spines, thorns and prickles. It has been reported to defend the plants against many insects. Pubescence consists of the layer of hairs trichomes) extending from the epidermis of the above ground plant parts including stem, leaves, and even fruits, and occur in several forms such as straight, spiral, stellate, hooked, and glandular. Chamarthi et al. reported that leaf glossiness, plumule and leaf sheath pigmentation were responsible for shoot fly *Atherigonasoccata* (Rondani) resistance in sorghum *Sorghum bicolor* (L.)(Moench).

Trichomes:

Trichomes play an imperative role in plant defense against many insect pests and involve both toxic and deterrent effects. Trichome density negatively affects the ovipositional behavior, feeding and larval nutrition of insect pests. In addition, dense trichomes affect the herbivory mechanically, and interfere with the movement of insects and other arthropods on the plant surface, thereby, reducing their access to leaf epidermis. These can be, straight, spiral, hooked, branched, or un-branched and can be glandular or nonglandular. Glandular trichomes secrete secondary metabolites including flavonoids, terpenoids, and alkaloids that can be poisonous, repellent, or trap insects and other organisms, thus forming a combination of structural and chemicaldefense.

Secondary metabolites and plant defense:

Secondary metabolites are the compounds that do not affect the normal growth and development of a plant, but reduce the palatability of the plant tissues in which they are produced. The defensive (secondary) metabolites can be either constitutive stored as inactive forms or induced in response to the insect or microbe attack. The former are known as phytoanticipins and the latter as phytoalexins. The phytoanticipins are mainly activated by β-glucosidase during herbivory, which in turn mediate the release of various biocidal aglycone metabolites. The classic examples of phytoanticipins are glucosinolates that are hydrolyzed by

myrosinases (endogenous β-thioglucosidegluco hydrolases) during tissue disruption. Other phytoanticipins include Benzoxazinoids (BXs), which are widely distributed among Poaceae. Hydrolyzation of BX-glucosides by plastid-targeted β-glucosidases during tissue damage leads to the production of biocidal aglycone BXs, which play an important role in plant defense against insects. Phytoalexins include isoflavonoids, terpenoids, alkaloids, etc., that influence the performance and survival of the herbivores. The secondary metabolites not only defend the plants from different stresses, but also increase the fitness of the plants. It has been reported that maize HPR to corn earworm, *Helicoverpazea*(Boddie) is mainly due to the presence of the secondary metabolites C-glycosyl flavone maysin [2"-*O* - a-L-rhamnosyl-6-*C*- (6-deoxy-*xylo*-hexos-4-ulosyl) luteolin] and the phenylpropanoid product, chlorogenic acid. Compound, 4, 4-dimethyl cyclooctene has been found to be responsible for shoot fly *A. soccata*resistance in sorghum *S. bicolor*.

Secondary metabolites have been primarily studied as the mediators of direct defense, however much is to be done to reveal the unidentified or emerging signaling pathways. Mass spectrometry used for the secondary metabolite profiling and gene expression analysis by high-throughput sequencing has made this field more exciting and cost-effective. Study on secondary metabolites could lead to the identification of new signaling molecules involved in plant resistance against herbivores and other stresses. Ultimately genes and enzymes involved in the biosynthesis of these metabolites could be identified. Role of some of the secondary metabolites in plant defense will be discussed below.

Plant phenolics:

Among the secondary metabolites, plant phenols constitute one of the most common and widespread group of defensive compounds, which play a major role in HPR against herbivores, including insects. Phenols act as a defensive mechanism not only against herbivores, but also against microorganisms and competing plants. Qualitative and quantitative alterations in phenols and elevation in activities of oxidative enzyme in response to insect attack is a general phenomenon.

Lignin, a phenolic heteropolymer plays a central role in plant defense against insects and pathogens. It limits the entry of pathogens by blocking physically or increasing the leaf tough-ness that reduces the feeding by herbivores, and also decreases the nutritional content of the leaf. Lignin synthesis has been found to be induced by herbivory or pathogen attack and its rapid deposition reduce further growth of the pathogen or herbivore fecundity. Increase in expression of lignin associated genes (*CAD/ CAD*-like genes) in plants infected with pests and pathogens have been documented.

Oxidation of phenols catalyzed by polyphenol oxidase (PPO) and peroxidase (POD) is a potential defense mechanism in plants against herbivorous insects. Quinones formed by oxidation of phenols bind covalently to leaf proteins, and inhibit the protein digestion in herbivores. In addition, quinones also exhibit direct toxicity to insects. Alkylation of amino acids reduces the nutritional value of plant proteins for insects, which in turn negatively affects the insect growth and development. Phenols also play an important role in cyclic reduction of reactive oxygen species (ROS) such as superoxide anion and hydroxide radicals, H_2O_2, and singlet oxygen, which

in turn activate a cascade of reactions leading to the activation of defensive enzymes. Simple phenolics (salicylates) act as antifeedant to insect herbivores suchas*Operophterabrumata*(L.) in *Salix* leaves, and there is a negative correlation between the salicylate levels and the larval growth, however, salicylic acid (SA) is much more important as phytohormone than as deterrent.

Flavonoids:

Flavonoids play a central role in various facets of plant life especially in plant- environment interactions. These defend plants against various biotic and abiotic stresses including UV radiations, pathogens and insect pests. Flavonoids are cytotoxic and interact with different enzymes through complexation. Both flavonoids and isoflavonoids protect the plant against insect pests by influencing the behavior, and growth and development of insects. In addition, flavonoids scavenge the free radicals including ROS, and reduce their formation by chelating the metals. Flavonoids are divided into various classes that include anthocyanins, flavones, flavonols, flavanones, dihydroflavonols, chalcones, aurones, flavan, and proanthocyanidins. More than 5,000 flavonids have been reported in plants. A number of flavones such as flavonols, flavones, proanthocyanidins, flavan 3-ols, flavonones, flavans, and isoflavonoids have been investigated as feeding deterrents against many insect pests. Flavonoids such as flavones 5-hydroxyisoderricin, 7-methoxy-8- (3-methylbutadienyl)-flavanone and 5- methoxyisoronchocarpin isolated from *Tephrosiavillosa*(L.), *T. purpurea*(L.), and *T. vogelii*Hook, respectively have been found as feeding deterrents against *Spodopteraexempta*(Walk.), and *Spodopteralittoralis* Bios. Over-expressing a transcription factor controlling flavonoid production in Arabidopsis has been reported to confer resistance against *Spodopterafrugi-perda*(J.E. Smith). Angustone A, licoisoflavone B, angustone B, and angustone C. Isoflavones, licoisoflavone A, luteone, licoiso-flavone B, and wighteone have been found to be not only feed- ing deterrents to insects, but also have antifungal activity against the fungi, *Colletotrichum gloeosporiode*(Penz.) and *Cladosporium cladosporioides*(Fres.). Isoflavonoids (judaicin, judaicin-7-*O*-glu-coside, 2-methoxyjudaicin, and maackiain) isolated from the wild relatives of chickpea act as antifeedant against *Helicoverpaarmig-era* (Hubner) at 100 ppm. Judaicin and maackiain were also found to be deterrent to *S. littoralis and S. frugiperda,* respectively. Cyanopropenyl glycoside and alliarinoside strongly inhibit feeding by the native American butterfly, *Pieris napi oleracea* L., while flavone glycoside, isovitexin-6"-D-β-glucopyranoside acts as a direct feeding deterrent to the lateinstars.

Tannins:

Tannins have a strong deleterious effect on phytophagous insects and affect the insect growth and development by binding to the proteins, reduce nutrient absorption efficiency, and cause midgut lesions. Tannins are astringent (mouth puckering) bitter polyphenols and act as feeding deterrents to many insect pests. They precipitate proteins nonspecifically (including the digestive enzymes of herbivores), by hydrogen bonding or covalent bonding of protein-NH_2 groups. In addition, tannins also chelate the metal ions, thereby reducing their bioavailability to herbivores. When ingested, tannins reduce the digestibility of the proteins thereby decrease the nutritive value of plants and plant parts to herbivores. Role of tannins in plant defense against various stresses and their induction in response to insect damage has been studied in many plants. For example, e.g., in *Populus*species and in *Pinus sylvestris*L. However, no effect of

herbivore damage on tannin content was observed in *Quercus serrata* (Thunb.) and *Betula pendula* Roth. Like proteinase inhibitors and oxidative enzymes, tannins have been reported to be systemically induced in neighboring leaves of the damagedplant.

Condensed tannins are oligomeric or polymeric flavonoids, also known as proanthocyanidins. They have diverse structures and functions. They act as feeding deterrents against some insects such as, *Lymantria dispar*(L.), *Euproctischrysorrhoea*(L.) and *O. brumata*. Condensed tannins such as (+) -catechin, (+) - gallocatechin, and vanillin in leaves of *Quercus robur*L. inhibited winter moth larvae, *O. brumata*. Procyanindin polymers have been found as feeding deterrent to *Aphis craccivora*(Koch) in groundnut. Condensed tannins from Alaska paper birch (coated on birch leaves at 3% dry wt.) reduced the pupal mass and survival of *Rheumapterahastata* (L.) larvae. It has been reported that induction of tannins in *Populus tremuloides* Michx. leaves in response to wound- and herbivore occurs by transcriptional activation of the flavonoid pathway. Genes responsible for the production of tannins in response to wounding have been identified and are activated by the expression of a condensed tannins regulatory gene, *PtMYB134*, which is itself induced by damage. Furthermore, induction of tannin is also stimulated by light stress, and exposure to UV light in hybrid poplar. However, some polyphagous insect species have the ability to tolerate gallo tannins, e.g., *Shistocerca gregaria* (Forsk.) tolerates tannins by hydrolyzing them rapidly to avoid any damaging effects by restricting the passage of tannins by adsorbing them on the thick peritrophic membrane, and by inhibiting the tannin protein complex formation by surfactants in the midgut.

Plant defensive proteins:

Ecologically, in insect-plant interaction, interrelationship between two is important for the survival of the both. Insects always look for a true and healthy host plant that can provide them proper food and could be suitable for mating, oviposition and also provides food for the offspring. The nutritional requirements of insects are similar to other animals, and any imbalance in digestion and utilization of plant proteins by the insects' results in drastic effects on insect physiology. Alteration of gene expression under stress including insect attack leads to qualitative and quantitative changes in proteins, which in turn play an important role in signal transduction, and oxidative defense. Many plant proteins ingested by insects are stable, and remain intact in the midgut, and also move across the gut wall into the hemolymph. An alteration in the protein's amino acid content or sequence influences the function of that protein. Likewise, anti-insect activity of a proteolysis-susceptible toxic protein can be improved by administration of protease inhibitors (PIs), which prevent degradation of the toxic proteins, and allows them to exert their defensive function. Better understanding of protein structure and post-translational modifications contributing to stability in the herbivore gut would assist in predicting toxicity and mechanism of plant resistance proteins (PRPs). Recent advances in microarray and proteomic approaches have revealed that a wide spectrum of PRPs is involved in plant defense against herbivores. Due to diverse feeding habits of arthropods, multiple signaling pathways including jasmonic acid (JA), SA and/or ethylene (ET) regulate arthropod-inducible proteins.

Plant lectins: Lectins are carbohydrate-binding (glyco) proteins, ubiquitous in nature, and have protective function against a range of pests. The insecticidal activities of different plant lectins have been utilized as naturally occurring insecticides against insect pests. One of the most important properties of lectins is their survival in the digestive system of herbivores that gives them a strong insecticidal potential. They act as antinutritive and/or toxic substances by binding to membrane glycosyl groups lining the digestive tract, leading to an array of harmful systemic reac-tions. Lectins are stable over a large range of pH and damage the luminal epithelial membranes, thereby interfere with the nutrient digestion and absorption. Disruption of lipid, carbohydrate, and protein metabolism causes enlargement and/or atrophy of key tis-sues, which in turn alters the hormonal and immunological status, threatening the growth and development of insects.Lectins have been found to be promising against homopteran, lepidopteran, and coleopteran insects. Insecticidal properties of *Galanthus nivalis*L. agglutinin (GNA) were the first plant lectin shown to be active against hemipteran insect.Efficacies of carbohydrate binding plant lectins such as GNA, *Phaseolus* haemagglutinin, and wheat germ agglutinin, have been studied in detail against many insect pests. Mannose - binding lectins have been reported to be effective against sucking insects, because of their interaction with a specific carbohydrate residue of the cell membrane. Expression of lectin coding genes in trans-genic plants and their defense against insects has been worked out in many plants, e.g., GNA, PSA (*Pisum sativum* L.; pea), WGA (*Triticum vulgare* Kunth; wheatgerm), ConA (*Canavalia ensiformis* (L.); jack bean), AIA (*Artocarpusintegrifolia*Forst.; jack fruit), OSA (*Oryza sativa* L.; rice), ASAL (*Allium sativum* L.), and UDA (*Urticadioica*L.; stinging nettle). The *Arum maculatum*L. lectin has been found effective against the aphids *Lipaphiserysimi*(Kalt.) and *A. craccivora*when incorporated in an artificialdiet.

Studies on the mechanism of action of the mannose-specific lectin, GNA against brown planthopper (*Nilaparvatalugens*(Stal.) in rice has shown that GNA binds to the luminal surface of the midgut epithelial cells within the planthopper by recognizing the cell surface carbohydrate moieties of glycoproteins and/ or other glycoconjugates in the gut. Immuno- labeling GNA assay has shown its presence in the fat bodies, ovarioles, and hemolymph, indicating the ability of GNA to cross the midgut epithelial barrier and pass into the insect's circulatory system leading to systemic toxic effect. Partial resistance to homopteran insect pests has been reported in transgenic plants expressing snowdrop lectin in tobacco, rice, andwheat.

Plant lectins are induced by elicitors as an induced response to various stresses. JA induced the expression of *NICTABA* lectin in tobacco leaves. Induction of NICTABA by herbivores infestation including *S. littoralis*, *Manduca sexta*L. and *Tetranychusurticae* Koch has been reported in tobacco plants. Expression of a mannose-binding jacalin-like lectin called Hessian fly, *Mayetiola destructor* (Say) responsive protein 1 (HFR1), and two chimerolectin like proteins called HFR2 and HFR3 have been reported to be induced by the larvae of Hessian fly, *M. destructor* in wheat. Differences in feeding behavior of insects results in expression of different lectins, e.g., larvae of the fall armyworm, *S. frugiperda*induced HFR2, but not HFR3 expression while the phloem-feeding bird cherry-oat aphid, *Rhopalosiphumpadi*Koch, induced HFR3 andHFR2, but latter was expressed much later (12 d) than the former (24 h). Several jasmonate- inducible lectins are expressed in leaf tissues of monocots such as rice, bar-ley, wheat, rye, and maize. Advancement of our understanding in induction of plant lectins in response to various stresses, especially

herbivory, and their role in plant defense has the potential for utilization of these entomotoxic lectins in crop protection through genetic engineering. Although, transformation of lectin genes into plants seems to be very attractive and effective, care is needed, because of possible toxicity of some lectins to non-target organisms, including mammals.

Proteinase inhibitors:

Proteinase inhibitors (PIs) cover one of the most abundant defensive classes of proteins in plants. Higher concentration of PIs occurs in storage organs such as seeds and tubers, and 1 to 10% of their total proteins comprise of PIs, which inhibit different types of enzymes and play an important role in plant defense against insect herbivory. PIs bind to the digestive enzymes in insect gut and inhibit their activity, thereby reduce protein digestion, resulting in the short-age of amino acids, and slow development and/or starvation of the insects. The defensive function of many PIs against insect pests, directly or by expression in transgenic plants to improve plant resistance against insects has been studied against many lepidopteran, and hemipteran insects. The success of transgenic crops in expressing PIs against insect pests has accentuated the need to understand the mechanisms, and interactions of multiple PIs with other defenses, and the adaptive responses of the herbivores.

Many classes of PIs are induced in plants in response to stresses. Kunitz proteinase inhibitors (KPIs) are the serine PIs (SPIs), which are among the most strongly upregulated defense genes in response to wounding or herbivore feeding in plants. The SPIs from *Solanum nigrum* L. have been found to adversely affect a number of insect pests. Progress in genome sequencing has resulted in identification of a large number of proteinase inhibitors and other defense components induced in plants on account of herbivore damage. Although most of the KPIs in plants are upregulated in response to insect herbivory, their degree of induction varies as per the insect plant interaction. Various KPIs allow plants to deal with multiple generations of insects by providing a genetic storehouse of varied PIs. However, some insects respond to PIs by constitutive or induced production of PI-insensitive proteases or by inactivation of ingested PIs, thereby, preventing them from binding to sensitive proteases. Such a feeding response by insects negatively affects the PI activity, and may result in even greater damage to the plants. This counter defense by the insects is a major hindrance to manipulation and utilization of PIs for a longer-lasting plant defense, and there is a need to understand the mechanisms by which insects counteract the PI-based plant defense.

Enzymes:

One of the important aspects of HPR against insects is the disruption of insect's nutrition. The enzymes that impair the nutrient uptake by insects through the formation of electrophiles includes peroxidases (PODs), polyphenol oxidases (PPOs), ascorbate peroxidases, and other peroxidases by oxidizing mono- or dihydroxyphenols, that lead to the formation of reactive o-quinones, which in turn polymerize or form covalent adducts with the nucleophilic groups of proteins due to their electrophilic nature (e.g., -SH or e-NH_2 of Lys). Other important antioxidative enzymes include lipoxygenases, phenylalanine ammonia lyase, superoxide

dismutase, etc. Induction of antioxidative enzymes in plants following herbivory has received considerable attention in recent years.

Peroxidases (POD):

Oxidative state of the host plants has been associated with HPR to insects, which results in production of ROS, that are subsequently eliminated by antioxidative enzymes. POD constitutes one such group of enzymes, which scavenges the ROS besides having other defensive roles. PODs are an important component of the immediate response of plants to insect damage. PODs are monomeric hemoproteins distributed as soluble, membrane-bound, and cell wall-bound within the cells, and are widely spread in plants and include several isozymes, whose expression depends on tissue, developmental stage, and environmental stimuli. A number of process are regulated by PODs that have direct or indirect role in plant defense, including lignification, suberization, somatic embryo-genesis, auxin metabolism, and wound healing. Role of PODs in plant resistance to insect pests has been studied in various plant systems. Production of phenoxy and other oxidative radicals by the PODs in association with phenols directly deter the feeding by insects and/or produces toxins that reduce the plant digestibility, which in turn leads to nutrient deficiency in insects with drastic effects on their growth and development. In addition, PODs have been reported to have direct toxicity in guts of herbivores. PODs have been purified and characterized from many plants where they were induced in response to insect attack.

Polyphenol oxidases (PPO):

The PPOs are important enzymes in plants that regulate feeding, growth, and development of insect pests, and play a leading role in plant defense against the biotic and abiotic stresses. PPOs can function in following ways: a) PPO-generated quinones could alkylate essential amino acids, decreasing plant nutritional quality, (b) quinones may produce oxidative stress in the gut lumen through redox cycling, and (c) quinones and ROS produced by phenolic oxidation, could be absorbed and have toxic effects on herbivores. The PPOs are metalloenzymes that catalyze the oxidation of monophenols and *o*-diphenols to quinones, which are highly reactive intermediate compounds that readily polymerize, and react with nucleophilic side chain of amino acids and crosslink proteins, thereby reducing the availability of such proteins, and affect the nutritional quality of the food. Under acidic conditions, quinones form semiquinone radicals that in turn give rise to ROS, while under basic conditions; quinines react with cellular nucleophiles. Quinines are more toxic to plant herbivores than the original phenols. In addition to their role in digestibility and palatability of plant tissues, melanin formation by PPOs increases the cell wall resistance to insects and pathogens. Induction of PPO activity under abiotic and biotic stresses and by treatment with compounds related to the octadecanoid pathway makes it an important tool in plant resistance against different stresses. The PPO genes are differentially induced by signaling molecules and injury due to wounding, and pathogen, or insect infestation. Correlation between induction of PPO activity and insect fitness has been reported in many plants including tomato and lettuce. Although PPOs accumulate in leaves, roots, stems and flowers of the plants, young tissues with greater vulnerability to insect attack exhibit greater induction. The PPOs confer resistance to *Spodopteralitura*(Fab.), *H. armigera, Bemisiatabaci*(Gen.),

Tetranychuscinnabarinus(Boisd.), *Myzuspersicae*(Sulzer), *Empoascafabae*(Harris), *Aphis medicaginis*(Koch), *S. exigua*, and *Agelasticaalni*(L.). However, induced PPO levels had no or limited impact on *L. dispar*, *Orgyialeucostigma*(JE Smith), and *Blissus occiduus* Barber.

Lipoxygenases:

Lipoxygenases (LOXs) are another group of anti-oxidative enzymes involved in plant defense against many stresses through octadecanoid pathway. They catalyze hydro- peroxidation of polyunsaturated fatty acids resulting in the formation of fatty acid hydroperoxides. The latter are enzymatically and/or chemically degraded to unstable and highly reactive aldehydes, γ-ketols, epoxides, and ROS such as hydroxyl radicals, singlet oxygen, superoxide ion and peroxyl, acyl and carbon centered radicals. The unstable reactive products interact with proteins resulting in protein-protein cross linking and amino acid damage that in turn affects the amino acid assimilation. In addition, lipid peroxidation end products also act as insect repellents or antixenosis and are toxic to insect pests (antibiosis). Major substrates of LOX in plants are linoleic and linolenic acids. One of the most important aspects of LOX in plant defense is the oxidation of linolenic acid in JA signaling pathway, which in turn plays a leading role in activation of plant defense, both directly by production of oxidative enzymes and protease inhibitors, and indirectly through the production of volatile organic compounds (VOC) that attract the natural enemies of insect pests. Oxygenation of polyunsaturated fatty acids has been found to be catalyzed by LOX, which results in the production of hydroperoxides that are metabolized to compounds such as JA and traumatin.

Induction of LOX activity in response to herbivory has been studied in many plants such as soybean in response to two-spot-ted spider mite, *T. urticeae*, in tomato in response to aphids, *Macrosiphium euphorbiae* Thom., and *M. persicae*, in *N. attenuate* following infestation by *Myzusnicotianae* Black. and in wheat following *Sitobionavenae*(F.) infestation. The *N. attenuate* plants deficient in LOX are more vulnerable to attack by *M. sexta,* which also attract the new herbivores such as *Empoasca*spp, as compared with the plants where LOX3-mediated defense reduced larval growth, food consumption, and frass production. Maize plants transformed with the wheat oxalate oxidase gene had upregulation of LOX transcripts and elevation of free phenolics (14-fold), which were positively associated with resistance to the European corn borer, *O.nubilalis*.

Indirect defenses:

The defensive response in plants to attract natural enemies of herbivores plays a pivotal role in protecting the plants against herbivore attack. Indirect defenses can be constitutive or induced as a result of combined action of mechanical damage and elicitors from the attacking herbivore. Production of volatiles and the secretion of extra floral nectar (EFN) mediate interactions of plants with natural enemies of the insect pests (i.e., parasitoids or predators), which actively reduce the numbers of feeding herbivores. Induced indirect defenses have received increasing attention recently and have been studied on the genetic, biochemical, physiological, and ecological levels.

Herbivore induced plant volatiles (HIPVs):

Plants indirectly defend themselves from herbivore feeding by emitting a blend of volatiles and non-volatile compounds. Herbivore-induced plant volatiles (HIPVs) play an important role in plant defense by either attracting the natural enemies of the herbivores or by acting as feeding and/or oviposition deterrent. HIPVs are the lipophilic compounds with higher vapor pressure which are released from the leaves, flowers, and fruits into the atmosphere, and into the soil from the roots by plants in response herbivore attack. The HIPV's produced vary according to the plant and herbivore species, the developmental stage and condition of the plants and the herbivores. An optimum quantity of volatile compounds is normally released by the plants into the atmosphere, whereas a different blend of volatiles is produced in response to herbivory. The volatile blend released by plants in response to insect attack is specific for a particular insect-plant system, including natural enemies and the neighboring plants. The HIPVs mediate the interactions between plants and arthropods, microorganisms, undamaged neighbouring plants, or intraplant signalling that warns undamaged sites within the plant. Depending upon the modes of feeding of insect pests, different defense signaling pathways are activated, which induce the production of specific volatile compounds.

The HIPVs include terpenes, green leafy volatiles (GLVs), ethylene, methyl salicylate and other VOCs. The well-studied metabolites of hydroperoxide lyase (HPL) branch of oxylipin-pathway producing stress-inducible compounds are the GLVs. GLVs are reactive electrophile species involved in stress and defense signals. GLVs consist of C6-aldehydes [(Z)-3-hexenal, n-hexanal] and their respective derivatives such as (Z)-3-hexenol, -3-hexen-1-yl acetate, and the corresponding E-isomers. To understand the role of C6-aldehydes and their respective derivatives in plant defense, the GLVs levels have been altered either by application of elicitors, or by manipulating genetically the HPL expression in plants. GLVs play an important role in plant defense by attracting natural enemies. Plant volatiles such as methyl salicylates and the C_{16}-homoterpene 4, 8, 12-trimethyl-1, 3(*E*), 7(*E*), 11-tridecatetraene [(*E, E*)-TMTT] have been found to attract the predatory mites. The most frequent component of the HIPVs is methyl salicylate (MeSA), and has been reported in the headspace of many insect-infested plants including lima bean, and Arabidopsis. MeSA is a ubiquitous component of many leaf and floral blends and MeSA baited sticky cards attract many insect predators including the big- eyed bug, *Geocoris pal-lens* Stal., ladybird beetle, *Stethorus punctum picipes* (Casey), green lacewing *Chrysopanigricornis* Burmeister, and other natural enemies. Ulland et al. reported the inhibition of oviposition of cabbage moths *Mamestrabrassicae*L. by MeSA released during infestation, suggesting that MeSA can also be detected by the attacking herbivores. Methyl benzoate (MeBA), which structurally resembles MeSA, has also been detected from insect-infested plants. *S. frugiperda* infestation in rice induces emission of about 30 volatiles, including MeSA and MeBA, which are highly attractant to the natural enemies of *S. frugiperda*, such as, *Cotesia marginiventris*(Cresson). However, there is an ecological cost of using HIPVs to engineer natural enemies; because HIPVs have the potential of attracting crop pests. For example, Colorado potato beetles, *Leptinotarsa decemlineata* (Say) is attracted to a blend of volatiles consisting of cis-3-hexenyl acetate, linalool, and MeSA.

Compounds such as ester methyl salicylate (MeSA), mono-terpenes myrcene and β-ocimene, homoterpene (*E*, *E*)-4, 8, 12-trimethyltrideca-1, 3, 7, 11-tetraene (TMTT), and sesquiterpene (*E*, *E*)-α-farnesene are emitted hours after infestation. Systemic release of VOCs is one of the best studied responses specific to herbivores. The HIPVs defend the plants either directly by repelling, deterring and toxicity to the herbivore or indirectly by attracting the natural enemies of the attackers, and thus, protect the plants from further damage. Lipoxygenase and Shikimic acid pathway metabolites and terpenoid pathway products (terpenoids) play an important role in plant defense, both directly and indirectly. Period specific volatile emission has been observed in many plants e.g., lima bean leaves attacked by *S. littoralis*, and hybrid poplar (*Populus trichocarpa* Torr. and A. Grey X *deltoides*) leaves infested by forest tent caterpillar, *L. dispar*emitted blend of volatiles containing (*E*)-β-ocimene and other mono-, sesqui- and homoterpenes. Maize plants when exposed to -3-hexanol induced the volatile blend emission that is usually released after caterpillar infestation, and attracts the natural enemies. Priming of the volatile emission signals has been reported in many plants. Engelberth et al. reported that application of GLV compounds such as (Z)-3-hexanal, (Z)-3-hexen-1-ol, and -3-hexenyl acetate individually and blend of volatiles to the maize seedlings enabled the seedlings to respond to wounding and beet armyworm, *S. exigua* caterpillar regurgitate, and resulted in accumulation of JA and sesquiterpenes as compared with the control plants. Similar observations were recorded by Kessler et al. in *N. attenuate* in response to *M. sexta* infestation, where low damage was shown by plants primed with clipped sagebrush-released volatiles. Thus, priming plays an important role in plant defense by incomplete turning on of defense related processes to reduce the biochemical investments until the onset of actual attack. However, there are a few reports where some non-target insect pests were also attracted on account of volatile emission in infested plants, thereby, increasing the insect attack on the plant.

Transgenic Arabidopsis with overexpression of strawberry nerolidol synthase, a terpene synthase (TPS) responsible for the production of sesquiterpene alcohol (3S)-(*E*)-nerolidol has been reported to attract the predatory mite, *P. persimilis*. The parasitic wasp, *Cotesiamarginiventris*(Cresson) was attracted to the lepidopteran larvae infesting transgenic maize plants with over- expression of the corn *TPS10* gene responsible for the formation of (*E*)-β-farnesene, (*E*)-α- bergamotene, and other herbivore induced sesquiterpene hydrocarbons.

In addition to the plant volatiles released from aerial parts of the plant, roots have also been found to release diverse volatiles that defend the plants from belowground insect pests by acting as antimicrobial and antiherbivore, and also by attracting the natural enemies of the root feeding insect pests. Root feeding insect, *Diuraphisnoxia*(Mord.) triggers the emission of 1,8-cineole,a monoterpene volatile, which is toxic and repellent to some insects. Sesquiterpene (*E*)-β-caryophyllene produced by maize roots in response to feeding by the larvae of *Diabrotica virgifera virgifera* LeConte attracts the nematode *Heterorhabditi smegidis* Poinar. However, root emitted volatiles such as 1,8-Cineole inhibits the growth of *Brassica campestris* seedlings due to the inhibition cell proliferation more severely than cell elongation because root growth requires both elongation and proliferation of the constituent cells, and also due to the interference with nuclear as well as organelle DNA synthesis in root apical meristem and alteration in root phospholipids and sterol composition.

Defense elicitors (insect oral secretion):

Plants undergo a dynamic change in transcriptomes, proteomes, and metabolomes in response to herbivore-induced physical and chemical cues such as insect oral secretions (OS) and compounds in the oviposition fluids. It is generally believed that insect-induced plant responses are mediated by oral secretions and regurgitates of the herbivore. The defenses generated by various elicitors differ based on the type of the elicitor and the biological processes involved. A potential elicitor of herbivore-induced plant volatiles from the regurgitate of *Pieris brassicae*L. larvae has been identified as β-glucosidase which results in emission of a volatile blend from mechanically wounded cabbage leaves that attract the parasitic wasp, *Cotesiaglomerata*(L.).Fatty acid-amino acid conjugates (FACs) are the major components in the oral secretions of insects. The first FAC elicitor identified was volicition, N-(17-hydroxylinolenoyl)-L-glutamine (volicitin), detected in the OS of beet armyworm larvae, *S. exigua*. Volicitin when applied on *Zea mays* L. induced the emission of elicitor that attracts the natural enemies of the feeding larvae. N-linolenoyl-glu isolated from regurgitate of tobacco hornworm, *M. sexta*has been found to be a potential elicitor of volatile emissions in tobacco plants. The FACs in OS of insects have been found to activate mitogen-activated pro-tein kinase (MAPK) pathway, that regulate plant growth and development, and play an important role in signaling transduction in responses to various stresses including cold, heat, ROS, UV, drought, pathogen and insect attack. FACs in oral secretions of *M. sexta*, when applied to the wounded leaves have been found to activate signaling processes that lead to the activation of MAPKs, salicylic acid-induced protein kinase (SIPK) and wound-induced protein kinase (WIPK), and bursts of jasmonic acid (JA), JA- isoleucine conjugate (JA-Ile), salicylic acid (SA), and ethylene. In wild rice, *Oryza minuta*Presl., expression of putative MAPK, *OmMKKI*, is induced by brown plant hopper, *N. lugens*feeding. Several other FAC elicitors such as *N*-acyl Gln/Glu have been isolated from regurgitates of various lepidopteran species. The FACs has also been reported to induce accumulation of 7-*epi*-jasmonic acid, an octadecanoid-derived phytohormone, which is a potent elicitor of transcripts of herbivore-responsive genes in tobacco plants. The FACs in lepidopteran OS evokes specific responses such as transcriptomic and proteomic alteration, induction of nicotine, and proteinase inhibitors in *N. attenuate*. Besides FACs, other groups of elicitors identified in insect oral secretions include inceptins, and caeliferins. Inceptins are disulphide-bonded peptides formed by the proteolytic fragmentation of plastidic ATP synthase, γ-subunit, whereas caeliferins are sulfated fatty acids, in the oral secretion of *S. americana*(Stal.), and other grasshopper species. The lipase activity of grasshopper oral secretions evoked an immediate and quick accumulation of various oxylipins, such as, 13-hydroperoxy octadecatrienoic acid, 12-oxo-phytodienoic acid (OPDA), JA, and jasmonic acid-isoleucine in Arabidopsis. Furthermore, there was increase in cytosolic calcium, ethylene emission and activity of MAPKs on treatment with grasshopper oral secretions.

Role of phytohormones in induced resistance in plants:

Plant defense against herbivore attack involves many signal transduction pathways that are mediated by a network of phytohormones. Plant hormones play a critical role in regulating plant growth, development, and defense mechanisms. A number of plant hormones have been implicated in intra- and inter-plant communication in plants damaged by herbivores. Most of the

plant defense responses against insects are activated by signal-transduction pathways mediated by JA, SA, and ethylene. Specific sets of defense related genes are activated by these path-ways upon wounding or by insect feeding. These hormones may act individually, synergistically or antagonistically, depending upon the attacker.

Jasmonic acid:

Although various phytohormones are involved in plant defense against herbivores, JA is the most important phytohormone linked to plant defense against herbivores and activates the expression of both direct and indirect defenses. JA is derived from linolenic acid through octadecanoid pathway and accumulates upon wounding and herbivory in plant tissues. Chewing of plant parts by insects causes the dioxygenation of linoleic acid (18:2) and linolenic acid (18:3) by specific LOXs at C9 or C13 to form (9S)- or (13S)-hydroperoxy- octadecadi(tri)enoic acids, which are converted into 12-oxophytodienoic acid (12-OPDA) by allene oxide synthase and allene oxide cyclase. OPDA is transferred to the peroxisome, where it is reduced by OPDA reductase 3 (OPR3), forming JA. Oxidative burst produces ROS, which convert linolenic acid into phytoprostanes that signal trans-duction pathways. A broad spectrum of defensive responses are induced by jasmonates that include antioxidative enzymes, PIs, VOCs, alkaloid production, trichome formation, and secretion of EFN. A large numbers of genes involved in defense against herbivores are regulated by JA. Concentration of indole glucosinolate, an important defensive compound, is induced by jasmonates. In addition to its role in the production of JA, OPDA signals the defense pathways individually. For example, OPDA signaling regulates the CORONATIN-INSENSITIVE 1 (COI1) -dependent and -independent transcription, alters the intracellular calcium levels and cellular redox status. Jasmonates (most likely the JA-amino acid conjugate jasmonoyl-isoleucine) have been found to interact with the COI1 unit of an E3 ubiquitin ligase complex, termed SCFCOI1 (Skip/Cullin/F-box–COI1), which promotes binding of the COI1-unit to JAZ (jasmonate ZIM-domain) proteins, resulting in degradation of JAZ proteins, which otherwise suppress JA-inducible gene expression. JA has also been reported to affect calcium-dependent protein kinases (CDPK) transcript, and activity in potato plants. CDPKs comprise of a large family of serine/threonine kinases in plants (34 members in Arabidopsis) and play an important role in plant defense against a variety of biotic andabioticstressesthroughsignaltransduction.InadditiontotheroleplayedbyJaindirectresistance against insect pests through the induction of various defensive compounds, its role in indirect resistance has also been well established. For example, EFN produced by JA is used as an alternate food by natural enemies of insect pests. JA also induces the defense enzymes such as POD, and PPO.

Salicylic acid:

Salicylic acid (SA), a benzoic acid derivative, is an important phytohormone involved in regulation of plant defense. It is an important endogenous plant growth regulator that generates a wide range of metabolic and physiological responses in plants involved in defense in addition to their impact on plant growth and development. Responses to SA depend on a regulatory protein called Non-Expressor of Pathogenesis-Related Genes1 (*NPR1*). The *NPR1* gene is activated through redox pathways by SA accumulation and is translocated to the nucleus,

however, it does not bind to DNA directly, but acts through transcription factors. SA induces greater defense against piercing and sucking type of insect pests than the chewing ones. SA signaling molecule is involved in local defense as well as induction of systemic resistance. Production of ROS by SA pathway has been proposed to induce resistance in plants against insect pests, e.g., in tomato plants against *H. armigera*. H_2O_2 induced by SA in plants defends them against various insect pests since H_2O_2 actively damages the digestive sys-tem of insects leading to reduced growth and development. Furthermore, SA signals the release of plant volatiles that attract the natural enemies of insect pests, e.g., Lima bean and tomato plants infested by spider mite attract the natural enemies of spider mite. However, it has been reported that SA and JA act antagonistically, where SA inhibits the activity of JA and vice versa. MeSA serves as a volatile signal to trigger induced defenses in plants, including HIPV emission, and a number of predaceous arthropods are attracted to MeSA under fieldconditions.

Ethylene:

Ethylene is an important phytohormone, which plays an active role in plant defense against many insects. Ethylene signaling pathway plays an important role in induced plant defense against herbivores and pathogens both directly and indirectly, however, there are limited reports on its role in indirect defense through the emission of HIPVs. ET signaling pathway works either synergistically or antagonistically, with JA in expression of plant defense responses against pathogens and herbivorous insects. It has been reported that ET and JA work together in tomato in PIs expression. Infestation by *A. alni* induced the emission of ethylene and release of various valatiles in *Alnusglutinosa*L. leaves in addition to mono-, sesqui and homoterpenes. ETprecursor, 1-amino-cyclopropane-1-carboxylic acid has been reported to enhance the volatile emission from the JA treated detached leaves. Ethylene further induced the emission of volatiles induced by volicitin, JA or (Z)-3-hexen-ol in maize.

Role of Calcium ions (Ca2+) in plant defense:

Plant defense elicitors induced in plants upon herbivory undergo different signal transduction pathways. Ca signaling is one of the early events in insect-plant interaction, where Ca acts as a second messenger, which in turn mediates a number of plant signaling pathways. Herbivore induced signals rapidly spread over the leaf and leads to a strong Ca-dependent trans-membrane potential (Vm) depolarization in the damage zone, and is followed by a transient Vm hyperpolarization in the surrounding area, and a constant depolarization at distances greater than 6–7 mm. Organelle and apoplastic fluid Ca concentration is generally higher (about 10 to 10 times) as compared with that in the cytosol (100 and 200 nM). However, upon insect attack, the cytosolic Ca increases, which in turn activates the calcium-sensing proteins such as calmodulin, calmodulin-binding proteins, and calcium- dependent protein kinases (CDPKs) that promote the signaling events such as, phosphorylation and transcriptional change. However, CDPKs are the important proteins against biotic and abiotic stresses, which form Ca sensors that contain a protein kinase domain and a calmodulin like domain (including an EF-hand calcium-binding site) in a single polypeptide. *NtCDPK2* regulates the activation of stress-induced MAP kinases in tobacco. Involvement of two Arabidopsis CPKs (CPK3 and CPK13) in herbivory-induced signaling

network through *HsfB2a*- mediated regulation of the defense-related transcriptional machinery has been observed in tobacco. Damage by *S. littoralis*larvae on *Phaseolus lunatus*L. induced Ca not only in cells adjacent to the feeding site, but throughout the leaf. Expression of calmodulin binding proteins involved in plant defense signaling increased considerably in wheat damaged by *D. noxia*and Arabidopsis by *M.persicae*.

Role of reactive oxygen species (ROS) in plant defense:

Oxidative state of plants is an important tactic that enables plants to defend against various stresses. Rapid and transient generation of ROS is a common phenomenon in plants on account of oxidative stress due to biotic and abiotic factors. ROS play versatile signaling functions that mediate multiple responses, and can also act directly as toxins. However, production of ROS on account of biotic stress is still debatable. ROS include partially reduced forms of oxygen such as superoxide (O), hydrogen peroxide (H_2O_2), and hydroxyl radicals (HO). Distinct signaling pathways are activated by different types of ROS especially the ones involving MAPKs. Rapid increase in ROS content under stress conditions is referred as "oxidative burst." Following insect attack, ROS accumulate in apoplastic as well as in symplastic regions, besides their main concentration in exo-cellular matrix, peroxisomes/mitochondria, and plasma mem-brane. Apoplastic burst of ROS acts as a first barrier against subsequent attack by the pathogens and herbivores. Being highly reactive, ROS can potentially react with and/or cause damage to proteins, lipids, and nucleic acids. However, to prevent the self-toxicity of ROS, plant cells have developed ROS scavenging systems for removing the excess ROS to maintain a relatively low and constant ROS concentration. Among all the ROS, high stability and freely diffusible H_2O_2 is a central component of induced defense response in plants against different stresses. Although H_2O_2 is produced in various ways, the oxidative burst is supposed to occur through the activation of membrane bound NADPH complex. NADPH oxidase generates superoxide anion at the plasma membrane or in the apoplast extracellularly, which is then converted to H_2O_2 by superoxide dismutase (SOD). Besides having direct effect on the pathogens and herbivores, H_2O_2 stimulates a cascade of reactions that lead to the expression of defense genes, which pre-vent the plants from subsequent attack by pathogens and herbivores. H_2O_2 application in Arabidopsis results in up- and downregulation of many genes (113 and 62 genes, respectively), suggesting that ROS act as secondary messengers to control gene expression. ROS also play an important role in mediating cross-linking of cell wall components by peroxidase, and also for the activation of many defenses related genes. Oxidative changes in plants after insect attack cause oxidative damage to insect mid-gut, mainly due to accumulation of H_2O_2. Many physiological and molecular responses in plants against insect attack are triggered by H_2O_2, and its levels remain elevated as long as the herbivore attack persists. Induction of H_2O_2 has been studied in oat, wheat, barley and groundnut against *D. noxia, R. padi*, *Schizaphis graminum* Rond., *H. armigera*and*S. litura*. Argandona et al. observed induction of H_2O_2 in barley infested with *S. graminum* after 20 min of infestation, indicating that H_2O_2 could be the beginning of a cascade of physiological and molecular events leading to production of further defensive components, and protection of plants from subsequent damage. ROS mediate the defensive gene activation and establish additional defenses by regulating the transcription and/or by interacting with other signal components like phosphorylation in plant systems in response to a variety of stresses.

The basic process of plant defense:

Extensive rearrangements in gene expression occur in plants in response to herbivory with hundreds, and even up to several thousands of genes getting up- or downregulated. Advances in genomics and transcriptomics including availability of whole-genome sequence data, expressed sequence tags (ESTs), and microarrays, has led to better understanding of the changes in gene-expression profiles in response to insect attack DNA microarrays provide a closer and complete view of gene- expression patterns and signaling responses mediated by insect elicitors and plant signals, and has proven to be exceptional tools to monitor the expression of thousands of genes simultaneously. However, with the advent of next-generation sequencing (NGS) technologies, it is anticipated that microarrays will be soon replaced by some new and innovative technologies like RNA-sequencing, RAD-sequencing, and reduced represented sequencing etc., for measuring gene expression directly. Expression quantitative trait loci (eQTL) mapping has revolutionized the area of gene expression. The eQTL mapping is having the advantage of dealing with thousands of traits at a time and has been used in many plants including Arabidopsis and rice. Investigation of inducible defenses in Arabidopsis against *P. rapae*and*Brassica oleracea* var *capitata*L. and *Brassica nigra*L., or the aphid *Brevicorynebrassicae*L. by microarrays has been studied extensively. Responses against feeding of *D. noxia*(Mord.), *S. graminum, M.nicotianae,persicae*and*S. avenae*on foliage of Arabidopsis, celery, sorghum, *Apiumgraveolens*L. cereal, tobacco or wheat plants have been well established.

Change in gene expression profiles after herbivory has shown a substantial reallocation of plant resources to defense. Gene expression levels have also been used to analyze the differences in transcriptional profiles of different genotypes within a plant species. Large numbers of genes (2182) are expressed by the aphid, *M. persicae*as compared with caterpillar, *P. rapae* attack. Lepidopterans usually elicit changes in the expression of genes involved in glucosinolate metabolism in Brassicaceae, detoxification, cell survival, and signal transduction, while the aphids regulate the expression of genes involved in cell wall modifications, oxidative stress, calcium-dependent signaling, and glucosinolate synthesis. Different attackers face different responses in plants based on the feeding behavior and the plant attacked; e.g., transcriptional changes in *Arabidopsis thaliana* (L.) in response to feeding by aphid, *M. persicae* and whitefy, *Bemisiatabaci*(Gen.). Different plants respond differently to the same herbivore, e.g., two white cabbage cultivars differ considerably in gene expression in response to feeding by *P. rapae*. Combination of various technologies such as genetic, genomic tools including microarrays, deep sequencing, and transcriptional profiling tools and proteomics through mass spectrometry will advance our understanding of molecular mechanisms of plant defense against insect herbivores to a greater extent.

Transgenerational induced resistance to herbivores:

Biotic and abiotic stresses in plants have been found to induce resistance not only in the maternal plants, but also in the offspring. This maternally induced resistance (transgenerational immunity) has been found to protect the progeny of plants exposed to herbivory from insect pests, besides producing vigorous seeds and seed-lings. However, there are only few reports on transgenerational immunity of plants against insect pests. Wild radish plants, *R. raphanistrum*

damaged by *P. rapae*or treated with JA produce offspring with high levels of induced resistance to this insect. Arabidopsis plants exposed to stresses such as, cold, heat and flood, resulted in a higher homologous recombination frequency and increased genome methylation, which in turn induced the resistance to stress in the progeny. Maternal plants with low to intermediate levels of herbivore damage could produce the seeds that are more vigorous and seedlings that are resistant to insect pests. However, further studies are required to understand the genetic and molecular mechanisms of such signaling interactions. Furthermore, research on plant-insect interactions should be focused not only to genetic effects, but also toward the epigenetic regulation of plant defense pathways and insect responses, because a substantial body of evidence has been demonstrated for mobile siRNA signals and inheritance of DNA methylation-based changes in gene expression. There is much need for in-depth studies on this subject to exploit it for pest management by manipulating the maternal ecology. An understanding of transgenerational induced resistance might answer some of the intricate questions regarding the ability of plants to withstand herbivore damage.

Probable Questions:

1. What is allelochemicals?
2. Define allelopathy.
3. Discuss about effect of allelochemicals on insects.
4. What are the applications of allelopathy?
5. What are direct responses of plants to herbivores.
6. How secondary metabolites help plants in defence?
7. Describe the role of flavonoids in plant defence.
8. Describe the role tannins in plant defence
9. Describe the role enzymes in plant defence.
10. Describe the role polyphenol oxidases in plant defence.
11. Describe the role Lipoxygenases in plant defence.
12. Describe the role peroxidises in plant defence.
13. Describe the role Proteinase inhibitors in plant defence.
14. Describe the role plant lectins in plant defence.
15. Describe role of phytohormones in induced resistance in plants.
16. Describe the role plant jasmonic acid in plant defence.
17. Describe the role of ROS in plant defence.
18. Describe the role of ethylene in plant defence.
19. Describe the role of salicylic acid in plant defence.

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett & Doull's Toxicology: The Basic Science by Curtis D. Klaassen.

5. Mechanisms of Plant Defense against Insect Herbivores. War *et al.* 2012. Plant Signaling& Behavior 7:10, 1306-1320

UNIT-VII

Group Characteristics and function of pesticides: Organochlorines, Organophosphates insecticides, Carbamates, Pyrethroids, other plant origin bio-insecticides, neonicotinoids and nitrogenous insecticides; fumigants; IGRs, attractants, repellents and anti-feedants

Objective: In this unit we will discuss different types of pesticides. Their action, structure, advantages, disadvantages will be discussed.

Pesticides: In general, a pesticide is a chemical compound (such as carbamate) or a biological agent (such as a virus, bacteria, or fungus) that incapacitates, kills, or otherwise prevents pests. Target pests can include insects, plant parasites, weeds, molluscs, birds, mammals, fish, nematodes (roundworms) and microbes that damage property, cause or transmit disease, or are vectors of disease. In addition to these benefits, pesticides often have disadvantages, such as potentially toxic to humans and other species.

Pesticides are chemical compounds useful in killing pests. Generally, a pesticide is a chemical compound or even a biological agent such as a bacteria, virus, antimicrobial, or disinfectant that prevents, incapacitates, or kills pests. The term pesticide includes all of the following: herbicide, insecticide (which can involve insect growth regulators, termiticides, etc.) nematicide, molluscicide, pesticide, avicide, rodenticide, bactericide, insect repellent, animal repellent, antimicrobial and fungicide. The most popular of these are herbicides, which account for nearly 80% of all pesticide use. Many pesticides serve as plant protection products (also known as crop protection products) that usually protects plants from weeds, fungi or insects. As an example-The fungus, Alternaria is used to battle Aquatic Weed, Salvinia.

A. Brief History

Even before 2000 BC, humans have used pesticides to protect their crops. The first known pesticide was the elemental sulphur dust used by ancient Sumerians around 4,500 years ago in ancient Mesopotamia. Rigveda, about 4,000 years old, refers to the use of poisonous plants to control pests. By the time of the 15th century, toxic chemicals such as arsenic, mercury and lead were useful in destroying the pests in crops. Nicotine sulphate was derived from tobacco leaves in the 17th century for use as an insecticide. The 19th century saw the introduction of two more natural pesticides, pyrethrum, derived from chrysanthemums, and rotenone, derived from the roots of tropical vegetables. Until the 1950s, pesticides based on arsenic were dominant.

Paul Müller found that DDT was a highly effective insecticide. Until 1975 organochlorines such as DDT were dominant, but by the year of 1975 organophosphates and carbamate replaced DDT in the

US. However, 20 years later, because of the biological consequences and human safety concerns, almost 86 counties declared the banning of DDT. Since then, pyrethrin compounds have become the dominant insecticide in the industry. Herbicides became popular in the early 1960s, then came "triazine and other similar nitrogen-based compounds, carboxylic acids including glyphosate and 2,4-dichlorophenoxyacetic acid".

Definitions of Pesticides

The Food and Agriculture Organization (FAO) has defined pesticide as: "any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances that may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies".

Types of Pesticides

Pesticides are also referred to by the type of pest they control. Pesticides can be either biodegradable pesticides, that break down into harmless compounds by bacteria and other living organisms, or persistent/Non-Biodegradable pesticides, which can take months or years to break down.

Classification of pesticides are according to the types of pests they kill

Grouped by the pest types they Kill;

- Insecticides – Insects
- Herbicide – Plants
- Rodenticides – Rodents (rats & mice)
- Bactericides – Bacteria
- Fungicides – Fungicide
- Larvicides – Larvae

In modern agriculture a large number of pesticides and insecticides are used.

They persist in the environment for a long time and accumulate in certain vital tissues of organisms and other items, which are used by humans.

The important categories of pesticides and their toxicity are mentioned below:

1. Organ chlorine insecticides: These are chlorinated ethane derivations, such as DDT and Methoxychlor; cyclodienes such as endrin, aldrin, dieldrin, chlordane, heptachlor, and mirex; and hexachlorocyclohexanes (HCH) such as Lindane. Of these, methoxychlor is less toxic than DDT; endrin is extremely toxic; Lindane is also highly toxic but less cumulative; the remaining are less toxic.

They stimulate the nervous system and induce irritability, disturbed equilibrium, paresthesia, tremor, and convulsions. Some of these chemicals, such as aldrin, dieldrin and lindane affect neurotransmitter activity. DDT may exert its toxic effects in the nervous system by adversely affecting ion transport across the axon membrane (Doherty, 1979; Narahashi, 1980). Some organ chlorine insecticides including DDT are hepato toxic.

2. Organophosphorus insecticide:

These are esters of phosphoric acid (Dichlorvos) and theophosphoric acid (Parathion). Other pesticides in this group are Diazinon, Dimethoate, Malathion, Mevinphos and Dipterex (Trichlorfon). Their toxic effects vary over a wide range. They act by inhibiting acetylcholinesterase (AchE). As a result, the accumulated acetylcholine (Ach) induces tremor, incoordination and convulsion. The accumulation of Ach at the neuromuscular synapse will lead to contraction of the muscles, loss of reflexes and paralysis. Several organophosphorus compounds may cause delayed neurotoxicity.

3. Carbamate Insecticides:

Insecticides of this class include Carbaryl (Sevin), Aldicarb (Temik), Carbofliran Methomyl and Propoxur (Baygon). These are esters of N. methylcarbamic acid. They also act by inhibiting AchE. However, inhibition of AchE by a carbamate is readily reversible.

4. Herbicides:

Several types of herbicides are used. Herbicides like 2, 4-D (2, 4-dichlorophenoxy- acetic acid). Paraquat and Diquat have been widely used. Some herbicides retard the growth of weeds by inhibiting photosynthesis, respiration, cell division or protein or lipid synthesis. Their toxicities in animals are relatively low. Paraquat and Diquat exert their toxicity via the formation of free radicals. Paraquat causes lung damage after inhalation and also after ingestion. It also causes hemorrhage and fibrosis.

5. Rodenticides:

A number of rodenticides including Warfarin, Thioureas, Sodium fluoroacetate and Sodium fluoroacetamide have been used. Warfarin is an antimetabolite to vitamin K, thus acts as an anticoagulant. Thioureas main toxicity is pulmonary edema and pleural effusion. They are highly

toxic to rats but moderately toxic to humans. Sodium fluoroacetate exerts its toxic effect through blockage of the citric acid cycle.

6. Fungicides:

Methyl and ethyl mercury are very effective fungicides. However, permanent neurologic damage and deaths have been reported after their use. Fungicides which have been widely used in agriculture are Dicarboximides but they are reported to have carcinogenic effects.

Depending on how biodegradable they are

Biodegradable Pesticides

Biodegradable pesticides are those that can be broken down into harmless compounds by microbes and other living organisms within less period of time.

Non-Biodegradable Pesticides

Few pesticides are known as non-biodegradable, also called persistent pesticides. The most long-lived pesticide materials include aldrin, parathion, DDT, chlordane, and endrin, they take a long period of time to break down. These pesticides can survive in the soil for over 15 years or more. Another way of thinking about pesticides is considering the chemical pesticides extracted from a common source or some production method.

Chemical pesticides:

Organophosphates: Many organophosphates are insecticides that impact on the nervous system by compromising the enzyme that regulates the neurotransmitter.

Carbamate: Carbamate pesticides affect the nervous system by compromising the enzyme that regulates the neurotransmitter similar to the organophosphates, but carbamate enzyme effects are usually reversible.

Organochlorine Insecticides: This type was common in the early years when pesticides came into the market. Many countries have banned organochlorine insecticides from their markets because of their impacts and persistence on health and the environmental factors (e.g., DDT, chlordane and toxaphene).

Pyrethroid: There are synthetic variants of pyrethrin, a naturally occurring pesticide present in chrysanthemums (Flower). Their development is such a way they can maximize their environmental resilience.

Sulfonylurea herbicides: The commercial production of sulfonylureas herbicides was for weed control like flupyrsulfuron-methyl-sodium, ethoxysulfuron, chlorimuron-ethyl, bensulfuron-methyl, azimsulfuron, and amidosulfuron, rimsulfuron, pyrazosulfuron-ethyl, imazosulfuron, nicosulfuron, oxasulfuron, nicosulfuron, flazasulfuron, primisulfuron-methyl, halosulfuron-methyl, pyrithiobac-sodium, cyclosulfamuron, bispyribac-sodium, terbacil, sulfometuron-methyl Sulfosulfuron.

Biopesticides

The biopesticides are a type of pesticides obtained from natural resources such as animals, plants, bacteria, and certain minerals.

Uses

- Pesticides are useful in controlling organisms that are toxic or harmful to their environment.
- Herbicides are useful in controlling algae and weeds.
- They are useful in grocery stores and food storage facilities to control rats and insects infesting on food.
- They are in use to kill mosquitoes that can spread life-threatening diseases such as the West Nile virus, yellow fever, and malaria.
- Also, they are useful in the agricultural sector to prevent or kill insects and other organisms that feed on crops.

Types of Pesticides:

These are grouped according to the types of pests which they kill:

Grouped by Types of Pests They Kill

1. Insecticides – insects
2. Herbicides – plants
3. Rodenticides – rodents (rats & mice)
4. Bactericides – bacteria
5. Fungicides – fungi
6. Larvicides – larvae

Based on how biodegradable they are:

Pesticides can also be considered as:

- **Biodegradable:** The biodegradable kind is those which can be broken down by microbes and other living beings into harmless compounds.

- **Persistent:** While the persistent ones are those which may take months or years to break down.

Another way to classify these is to consider those that are chemical forms or are derived from a common source or production method.

Chemically-related pesticides:

- **Organophosphate:**

  Most organophosphates are insecticides, they affect the nervous system by disrupting the enzyme that regulates a neurotransmitter.

- **Carbamate:**

  Similar to the organophosphorus pesticides, the carbamate pesticides also affect the nervous system by disrupting an enzyme that regulates the neurotransmitter. However, the enzyme effects are usually reversible.

- **Organochlorine insecticides:**

  They were commonly used earlier, but now many countries have been removed Organochlorine insecticides from their market due to their health and environmental effects and their persistence (e.g., DDT, chlordane, and toxaphene).

- **Pyrethroid:**

  These are a synthetic version of pyrethrin, a naturally occurring pesticide, found in chrysanthemums(Flower). They were developed in such a way as to maximise their stability in the environment.

- **Sulfonylurea herbicides:**

  The sulfonylureas herbicides have been commercialized for weed control such as pyrithiobac-sodium, cyclosulfamuron, bispyribac-sodium, terbacil, sulfometuron-methyl Sulfosulfuron, rimsulfuron, pyrazosulfuron-ethyl, imazosulfuron, nicosulfuron, oxasulfuron, nicosulfuron, flazasulfuron, primisulfuron-methyl, halosulfuron-methyl, flupyrsulfuron-methyl-sodium, ethoxysulfuron, chlorimuron-ethyl, bensulfuron-methyl, azimsulfuron, and amidosulfuron.

Examples of pesticides

Examples of pesticides are fungicides, herbicides, and insecticides. Examples of specific synthetic chemical pesticides are glyphosate, Acephate, Deet, Propoxur, Metaldehyde, Boric Acid, Diazinon, Dursban, DDT, Malathion, etc.

Benefits of Pesticides

The major advantage of pesticides is that they can save farmers. By protecting crops from insects and other pests. However, below are some other primary benefits of it.

- Controlling pests and plant disease vectors.

- Controlling human/livestock disease vectors and nuisance organisms.
- Controlling organisms that harm other human activities and structures.

Effects of Pesticides

- The toxic chemicals in these are designed to deliberately released into the environment. Though each pesticide is meant to kill a certain pest, a very large percentage of pesticides reach a destination other than their target. Instead, they enter the air, water, sediments, and even end up in our food.
- Pesticides have been linked with human health hazards, from short-term impacts such as headaches and nausea to chronic impacts like cancer, reproductive harm.
- The use of these also decreases the general biodiversity in the soil. If there are no chemicals in the soil there is higher soil quality, and this allows for higher water retention, which is necessary for plants to grow.

Bio-pesticides Types: Bio-Herbicides and Bio-Insecticides:

- Bio-pesticides are those biological agents that are used for control of weeds, insects and pathogens.

- The micro-organisms used as bio-pesticides are viruses, bacteria, protozoa, fungi and mites. Some of the bio-pesticides are being used on a commercial scale.

- Most important example is the soil bacterium, Bacillus thuringiensis (Bt). Spores of this bacterium possess the insecticidal Cry protein.
- Therefore, spores of this bacterium kill larvae of certain insects. The commercial preparations of B. thuringiensis contain a mixture of spores, Cry protein and an inert carrier.
- This bacterium was the first bio-pesticide to be used on a commercial scale in the world, and is the first bio-pesticide being produced on a commercial scale in India.

Bio-pesticides are of two types: bio-herbicides and bio-insecticides:

(i) Bio-herbicides: Herbicides are chemicals that are used for inhibiting the growth of plants in unwanted places. Herbicides used for controlling weeds in the cultivated areas are called weedicides. A number of risks are involved in the use of chemical herbicides. This can be avoided if herbicide resistance can be introduced in the crop plants. It is possible through genetic engineering or recombinant DNA technology. Transgenic Tomato and Tobacco plants have been developed which show tolerance to specific herbicides.

Certain crop plants do not allow the weeds to grow nearby. They are called smoother crops, e.g., Barley, Rye, Sorghum, Millet, Sweet clover, Alfalfa, Soybean, Sunflower. Smoother crops eliminate

weeds through chemicals. Crop rotation with these crops will naturally reduce the incidence of weeds.

Another way of weed control is the introduction of specific insects which feed on the weeds. Extensive growth of Opuntia in India and Australia was checked through the introduction of its natural herbivore, cochineal insect (*Cactoblastis cactorum*). Similarly, growth of *Hypericum perforatum* or Klamath weed was checked by U.S.A. through the introduction of Chrysolina beetles. An organism which controls or destroys unwanted plant growth without harming the useful plant is called bioherbicide. The first bioherbicide happened to be mycoherbicide. It was put to use in 1981. The herbicide is *Phytophthora palmivora*. The fungus does not allow the Milkweed Vine to grow in Citrus orchards. Growth of Eichhornia crassipes (Water Hyacinth) is being controlled by *Cercospora rodmanii* in USA and *Alternaria eichhorniae* in India.

Puccinia chondrilla has controlled the growth of skeleton weed, Chondrillajuncea in Australia. Fungal spores are now available to be sprayed over weeds for their elimination. Two of them are 'Devine' and 'Collego'. The spores are ideal for marketing because they can tolerate adverse conditions and can remain viable for long periods.

(ii) Bio-insecticides:

Bio-insecticides are those biological agents that are used to control harmful insects. They include the following.

(a) Predators:

Destructive insects or plant pests can be brought under control through introduction of their natural predators. The predators should be specific and unable to harm the useful insects. Introduction of ladybugs (Lady Bird Beetles) and Praying Mantis has been successful in combating scale insects or aphids which feed on plant sap.

(b) Parasites and Pathogens:

This is alternate biological control of plant pests through the search of their natural parasites and pathogens. They include viruses, bacteria, fungi and insect parasitoids. Parasitoids are organisms that live as parasites for some time (as early or larval stage) and free living at other times, e.g., Trichogramma. Nucleopolyhedrovirus (NPV) are species specific.

For example, Baculovirus heliothis (a virus) can control Cotton bollworm (HeliothisZea). Similarly, Bacillus thuringenesis (a bacterium) is effective against the cabbage looper (Trichoplausiani) and Entomophthoraignobilis (a fungus) the green peach aphid of Potato (Myzuspersicae). In U.S.S.R. the fungus Beauveria bassiana has been successfully employed in controlling Potato beetle and Codling moth.

(c) Natural Insecticides:

They are insecticides and related pesticides which are obtained from microbes and plants. A number of natural insecticides are available. The common ones include (i) Azadirachtin from

Margosa or Neem (Azadirachta indica). It occurs in Margosa extract. Spray of the same keeps away the Japanese beetles and other leaf eating pests because of the antifeedant property of azadirachtin. (ii) Rotenones. They are powerful insecticides which are harmless to warm blooded animals. Chinese are believed to be first to discover their insecticidal properties. Rotenones are obtained from the roots of *Derris elliptica* and *Loncho carpusnicou*. (iii) Squill. The red variety of Sea Onion (Red Squill, *Ureginea maritima*) produces a radicide which does not have any harmful effect on other animals, (iv) Nicotine. It is obtained from Nicotiana species. The purified chemical is highly poisonous. Nicotine sulphate is one of the most toxic insecticides, (v) Pyrethrum.

It is an insecticide which is obtained from the inflorescence of Chrysanthemum cinerarifolium (*Dalmation Pyrethrum), C. coccineum and C. marshallii*. The active compounds are pyrethrin and cinerin. Pyrethrin is also used in fly sprays, aerosols, mosquito coils, etc. (vi) Thurioside. It is a toxin produced by bacterium Bacillus thuringenesis. The toxin is highly effective against different groups of insects like moths, flies, mosquitoes and beetles. It does not cause any adverse environmental pollution or disturbance. Thurioside occurs as crystals in the bacterium. It kills the susceptible insects through inhibiting ion transport in the midgut, formation of pores in gut epithelium, swelling and bursting of cells, (vii) Transgenic Plants. They are crop plants which are modified through genetic engineering to develop natural resistance to insects by inserting cry genes of *Bacillus thuringenesis* into them, e.g., Bt Cotton. Similarly, transgenic Tomato has been developed which is resistant to homworm larvae.

Anti-feedant:

Most researchers define antifeedants as those chemicals that have antifeedant properties at low concentration, and that act on very specific sensory cells (antifeedant receptors) in the pest. The neurons associated with these antifeedant receptors either prevent insect feeding (feeding deterrent effect) or cause cessation or slowing of further feeding (feeding suppressant effect). Another mode of action of some antifeedants is through an apparent ability to block the function of a herbivore's feeding-stimulant receptors, or an ability to bind directly to its normal feeding cues, such as sugars and amino acids. An example of this mechanism is the action of DEET (*N*, *N*-diethyl-*m*-toluamide) in repellent lotions, which deter blood-sucking arthropods by blocking their ability to perceive feeding stimulants. The very potent antifeedant azadirachtin acts in a similar way by reducing the sensitivity of sugar-sensing cells in herbivorous insects and thus causing the insects to incorrectly assess nutritional adequacy of treated host leaves. Only careful electrophysiological studies on the two categories of receptors (feeding deterrent versus feeding stimulant) can determine whether the putative antifeedant has a direct effect on antifeedant receptors, a distinction that is important when asking certain research questions. Another type of “false” antifeedant category is those compounds that act nonspecifically on all gustatory sensilla (e.g., through immediate and general cell toxicity) are also not considered true antifeedants. The finer points of categorizing antifeedants, however, are not usually an issue because data are rarely available on the precise electrophysiological mode of action of antifeedant compounds or extracts.

Although antifeedants can belong to a variety of chemical classes, the majority are alkaloid, flavonoid, and terpene secondary compounds. Some of the more commonly known antifeedants are presented in Table 1. In general, antifeedants are often bitter (when tasted by

humans), although this is certainly not diagnostic of an antifeedant, and they are usually not involved in primary metabolism (there are exceptions, such as some sugars).

Insecticidal and antiparasitic activity:

An antifeedant effect is a main strategy related to the use of quinoline and quinazoline alkaloids against insects. Dictamnine (**25**) was deterrent against three insect pests, including *Sitophilus zeamays, Trilobiumcastaneum*, and *Spodoptera litura*, for plant protection. Evolitrine (**103**) (Figure below) exhibited antifeedant activity against fourth instar larvae of the tobacco caterpillar *S. litura*, and acetylcupreine and 3,4-dihydroxyquinoline-2-carboxylic acid (**104**) (Figure below) presented powerful feeding deterrent effects toward the potato beetle *Leptinotarsa decemlineata* and the fish species *Blennius sphynx,* respectively. In addition, tryptanthrin (**31**) showed antifeedant activity against larvae of the house longhorn beetle *Hylotrupesbajulus* and the termite *Reticulitermissantonensis*.At levels of 0.05 and 0.1%, vasicine (**2**) (Fig. 2), vasicinone (**105**), and vasicinol (**106**) (Figure below) showed feeding deterrence against two beetle species *Aulacophorafoveicollis* and *Epilachna vijintioctopunctata*.

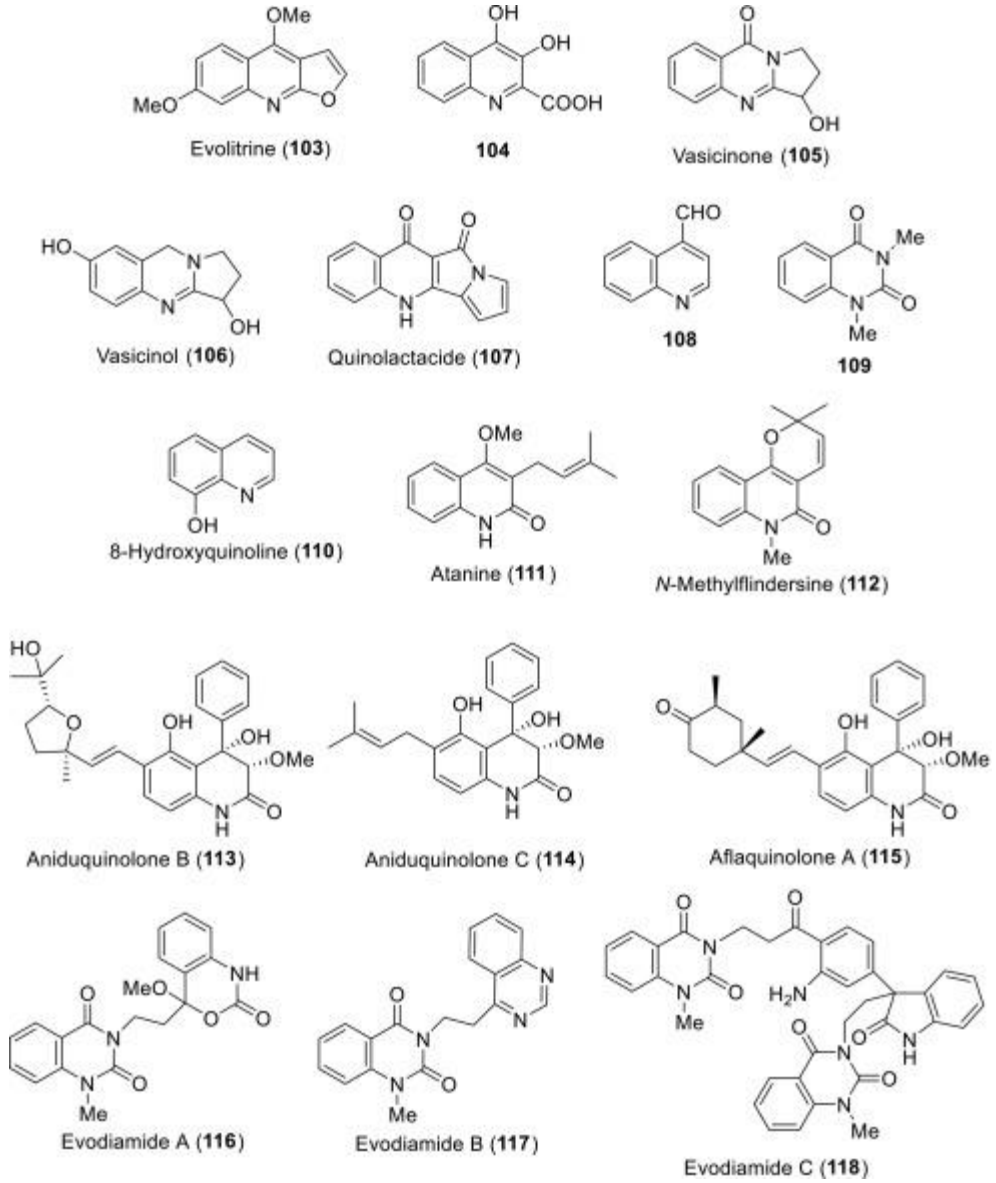


Fig. Chemical structures of some anti-feedant compounds

Furthermore, quinolactacide (**107**)) from the fermentation broth of *Penicillium citrinum* Thom F 1539 showed potent insecticidal activity against the green peach aphid *Myzuspersicae*. Quinoline-4-carbaldehyde (**108**) from *Rutachalepensis* exhibited insecticidal activity against the rice weevil *Sitophilus oryzae* with LD_{50} values of 0.084 mg/cm^2 using the fumigant method and 0.065 mg/cm^2 using the contact method. Changing the position of the aldehyde group on the quinoline skeleton affected the insecticidal activity.Another plant protection strategy is the use of insecticidal sex pheromones. Wojtasek and Leal found that 1,3-dimethylquinazoline-2,4-dione (**109**) has agricultural potential as a sex pheromone of the chafer beetle *Phylloperthadiversa* and is highly specific to males of this species.

Quinoline and quinazoline alkaloids also exhibit potent activity against human and animal, as well as plant, parasites. With an IC_{50} value of 0.213 μM, 8-hydroxyquinoline (**110**) was effective against the growth of *Toxoplasma*, the causative parasite of human toxoplasmosis. Febrifugine (**72**) from *Hydrangea macrophylla* showed anticoccidial activity against *Eimeria* parasites in chickens. Atanine (**111**) from *Evodia rutaecarpa* showed antiparasitic and anthelmintic activity against larvae of the blood fluke *Schistosoma mansoni* and the soil nematode *Caenorhabditis elegans*.

Luo et al. found that *N*-methylflindersine (**112**) from *Micromelumfalcatum* is highly toxic toward brine shrimp (*Artemia salina*) with an LD_{50} value of 1.39 μg/mL. Two 4-phenyl-3,4-dihydroquinolones aniduquinolones B and C (**113** and **114**), as well as aflaquinolone A (**115**) from the endophytic fungus *Aspergillus nidulans* exhibited LD_{50} values of 7.1, 4.5 and 5.5 μM, respectively, against brine shrimp. Three new quinazoline alkaloids, evodiamides A, B, and C (**116–118**), were isolated from *E. rutaecarpa* in 2018; however, they did not show significant pesticidal or antibacterial activity.

Fumigants:

Fumigants are either toxic gases or substances that produce toxic gases that are lethal when inhaled. In vertebrate pest control, fumigants are principally used to control rodents in one of two ways, as a building or transportation vehicle fumigant or as a burrow fumigant. Fumigants have many advantages over other control methods because they do not require any particular behavior or action on the part of the target animal. Fumigation of buildings, rail cars, etc. is often conducted for insect control and, depending on the fumigant, the process can also provide rat and house mouse control. Fumigation of buildings specifically for rodent control is sometimes conducted but it is generally prohibitively expensive. Building fumigation can only be conducted by licensed pest management professionals under a strict set of regulations.

Burrow fumigants are used outdoors against a wide variety of burrowing rodents, including Norway rats, chipmunks, ground squirrels, prairie dogs, woodchucks (marmots), voles, and pocket gophers. Fumigants are also used, to a limited extent, as burrow or den fumigants to control certain carnivorous species such as coyotes, foxes, and skunks. Some are registered for use against moles; however, moles are not easily controlled with burrow fumigants.

There are two fumigants that are commonly used in vertebrate pest control, aluminum phosphide and ignitable gas cartridges. Aluminum phosphide, a “Restricted Use Pesticide” to be

used only by certified applicators, comes in tablet or pellet form. When the prescribed number of tablets or pellets is placed well within the burrow or den, they react with the soil or atmospheric moisture to produce lethal phosphine gas. The burrows or dens are sealed off with soil immediately following treatment to retain as much of the toxic gas as is possible and for as long as possible. If there are multiple entrances to a burrow or den, each entrance must be sealed so the toxic gas remains in the burrow.

The other and more commonly used fumigant is the ignitable gas cartridge, which is sold over-the-counter to the public. There are several manufacturers of these cartridges, but all generally contain two basic ingredients, sodium nitrate and charcoal, combined with smaller amounts of active or inert ingredients. The formulated ingredients are compressed into a cardboard tube with a fuse inserted in one end. When ignited, they produce a toxic suffocating smoke that is lethal to animals in a confined space. To use, the cartridges are placed in the burrow or den entrance and the fuse is lit. Once lit, the cartridge is pushed deep into the burrow or den with a shovel handle and the opening is sealed off with a soil plug and tamped tightly to retain the smoke. When used in accordance with the directions, gas cartridges present little hazard to the user.

Fumigants must remain in the burrow in sufficient concentration and time to be effective. Soil type and moisture level can impact the effectiveness of this control method. Dry and loose, rocky or sandy soil is less likely to maintain the gas concentration needed to be effective. Also, many fumigants, especially the gas cartridge, present a fire hazard and should not be used when dry grass or other flammable materials are present. Since rodent burrows can go beneath buildings, fumigants should not be used in close vicinity to buildings.

There are machines that use a fumigation-type approach in an attempt to control burrowing rodents including pocket gophers and ground squirrels. These are the burrow exploders that inject oxygen and propane into the burrow and ignite the mixture, causing a significant explosion. There is very little reported evidence that these devises are effective in controlling ground squirrels or other burrowing rodents.

Fumigants have little in common either in their chemical structure or in their mode of action. The only common characteristic is that all have a relatively high vapor pressure, and, as a rule, they are highly toxic to both pests and humans. Handling requires great skill, and strict safety rules must be followed, including the wearing of specific personal protective clothing (gas masks with specific cartridges). Many countries have therefore posed restrictions on the availability of these fumigants. To this group belong the frequently used hydrogen cyanide, phosphine, methyl bromide, chloropicrin, and some others.

Types of Fumigants:

a. Inorganic (aluminum phosphide, hydrogen cyanide, carbon disulfide, sulfur dioxide)

b. Organic (methyl bromide, ethylene dibromide, dibromochloropropane).

In agriculture, fumigants are injected into the soil prior to planting or used in structures to protect stored crops. When injected into the soil they kill insects, fungi, weeds, bacteria, and nematodes.

Often the injected area is covered in tarps to prevent dispersion into the air and restricted-entry intervals are in place to keep workers out of the treated area. Fumigation is also used to protect the harvested crops from pest infestations or decay in grain bins and other storage facilities. They are designed to release the active ingredient in the form of a gas or vapor which can easily be inhaled. Many products are restricted use pesticides because of their high toxicity to humans. Several fumigants (methyl bromide, ethylene oxide, carbon disulfide) have been associated with central nervous system effects

Attractants and Repellents pesticides:

Although attract and repel are etymological opposites, meaning to draw to and to drive back, they are not entomological opposites. In the lives of most insects and many other animals there are two essential attractions, to sources of food, and to repositories of the germ cells-males to females, females to egg-laying sites. The range over which these may act is at least metres and may be kilometres. Repulsions, on the other hand, are essential only in the lives of comparatively few animals: skunks, stink bugs, bombardier beetles, and the like. Although a skunk may be detectable from a distance of a kilometre, neither it nor any other animal actually repels or drives away others for a distance of more than a metre or so, and usually the action is restricted to centimetres or even millimetres. The term " repellent " is commonly used for stimuli which merely inhibit or neutralize attraction or which in some other way bar the expression of this. I shall use it in this general sense, but would draw your attention to the plea of Dethier et al. (1960) for a revision of terminology in this field. In this context, reference may be made to the controversy that arose during the Second World War over the effectiveness of pyrethrum as an ingredient of antimalarial " repellent " creams. The question was whether this material could be depended upon to knock down a mosquito before the injection of saliva had begun. The answer was that it could not. But it was surely taking a liberty to refer to this material as a repellent. Attraction is necessarily much more specific than repulsion, both in the kind of animal which it affects and also in the kind of behaviour it elicits. Both attraction and repulsion are most commonly used in relation to chemical stimuli but may also apply to visual and auditory stimuli. Attraction to lights is familiar enough and that to moving objects is well recognized. Certain yellow lamps which are claimed commercially to repel insects in fact merely offer minimal attraction for a given luminosity to man. Attraction to auditory stimuli is familiar in nature, e.g., stridulation as a means of bringing the sexes together. A practical application of this has been attempted in the use of audio-frequency oscillators to attract female mosquitos. Auditory stimuli have also been used to repel, as in the use of klaxon horns to prevent locusts from alighting. This may depend on a resonance effect. Thermal and moisture stimuli can also attract or repel. This paper will be restricted to a consideration of chemical attractants and repellents, but it should be remembered that these other possibilities exist. Some of the principles I propose to enunciate for chemicals also apply to other stimuli.

ATTRACTANT AND REPELLENT MATERIALS AND THEIR ACTION:

In the search for insect attractants, the sources explored have been those which function in nature: components of the preferred food material, sex attractants, and components of oviposition sites. An example of the first of these is the discovery by Brown (1961a, 1961b) of a component of blood, lysine, which is attractive to mosquitos. It is interesting to note that protein hydrolysates have proved to be generally attractive to insects, especially Diptera, and much use has been made of them in agricultural entomology (Steiner, 1952; Lockmiller& Thomas, 1957a, 1957b). So far as I am aware, there has been no successful exploitation, in the field of vector control, of the other two possible natural sources of attractants, but it is interesting to note that moisture, which often functions as an attractant to oviposition sites, also plays a role in attraction to a source of blood. The early repellents for blood-sucking flies were naturally available plant products, such as oil of citronella and oil of camphor, but these have no specific role in the life of blood-sucking flies in nature. Being highly odorous materials to man, they were supposed to act through the olfactory senses of insects, and investigators in search of new repellents were presented with the conflicting requirements of high volatility for olfactory effect and low volatility for prolonged protection. The advent of dimethyl phthalate (Granett, 1942) and many similar materials with low vapour pressure and much less pronounced odour threw some doubt on the validity of this assumption. Although it is now clearly established that these materials produceat least a part of their effect in the vapour phase (Kalmus & Hocking, 1960, and others) it does not follow that the olfactory sense is involved. Two properties are so commonly found in repellent materials that one may suspect they are in some way connected with repellent action: they irritate the mucous membranes of man, and they are plasticizers of many paint films and plastics (Ihndris et al., 1955). Wright (1958) has claimed that there is a correlation between repellency to mosquitos and high infra-red absorption at or near 460 cm-'. Water shows this property and this led him to suggest as an experimentumcrucis that repellents should be attractive at very low humidities. This has not been shown. Wright also advances, as a part of his hypothesis, a theory of olfactory perception based on infra-red absorption and derived from that proposed by Ramsay (1882). More recently, Wright (1962) has suggested that absorption in a broader band extending to a lower frequency, about 200 cm-', is the important property. The correlation between this property and repellency, however, has not been clearly demonstrated, and the theory could not explain attraction from a distance of more than 15-30 cm. A third property of repellents-toxicity to insects-is possessed by many modem materials. Many insecticides, too, are repellent and no firm line can be drawn between the two groups of materials; but caution is necessary in drawing teleological inferences. The observation by Kalmus & Hocking (1960) that the inhibition of flight by tarsal contact was interfered with by mosquito repellents led to a hypothesis that these repellents act in a nonspecific way by simply inactivating receptors with which they come in contact. This is supported by recent work by Khan (unpublished communication, 1962) which shows that repellents interfere with sugar feeding, mating, and oviposition responses as well as with blood feeding. On the other hand, nerve impulses picked up from the ventral nerve cord of a cockroach when the cerci are mechanically stimulated are almost unaffected by painting the cerci with repellent (Reddy, unpublished communication, 1962). Perhaps this is because muscle proprioceptors or some other internal sensilla are involved. A further possibility which, so far as I know, has not been tested is

that repellent substances are impervious to materials such as lysine, alanine, and arginine, and also perhaps, in part, to carbon dioxide, although they apparently do not affect the evolution of moisture (Gouck & Bowman, 1959).

APPLICATIONS OF ATTRACTANTS AND REPELLENTS AND FUTURE PROSPECTS:

Very little use has so far been made of attractants in the direct control of vectors of public health importance, but this possibility is beginning to receive attention. It would be interesting to see how the performance of a moving-stripes light-trap could be improved by adding a supply of lysine to it and reversing the air flow. Doubtless the air speed would have to be reduced. Light-traps of all types are, of course, attractants, but their value has lain more in the estimation of populations than in the control of them. Another possible application for attractants is as spray additives in order to counter the repellent properties which many spray ingredients have been shown to possess (Hocking & Lindsay, 1958; Hocking, 1961). It is possible, too, that economies in insecticides, desirable for many reasons, can be effected by using attractants, or repellents (Pest Control, 1958), to concentrate insect populations. While repellents are most widely applied directly on the skin, many other applications are possible. Space repellents were suggested by Ginsburg as long ago as 1935 but do not seem to have had more than occasional use. Materials that have high repellency but have been rejected for use on the skin because of irritation or other toxic effects may prove of value when used in this way, or as additives to protective air currents (Hocking, 1960). Used on the skin or in the clothing or both, present-day repellents that have been developed on an ad hoc basis are nevertheless extremely valuable. Tremendous improvements can be expected with increased understanding of how these materials work. The lesser attractiveness to mosquitos of newborn infants and of women may be useful clues in the search for a repellent for oral administration. The claim that thiamine taken orally exerts a repellent action (Shannon, 1943) seems to be unfounded where mosquitos are concerned (Wilson et al., 1944; Rahm, 1958b), but in the case of fleas (Eder, 1945) the question is still open. There are three stages of blood feeding where a repellent can usefully act, each calling for different properties. A repulsive odour can interfere with attraction through the sense of smell; an irritant action through chemical perception can prevent landing and probing; a true taste repellent may prevent the consummation of probing as piercing and, if present in the blood, may inhibit actual feeding. Only when these properties can be measured separately will it be possible to seek correlations with chemical structure. This may allow the development of better materials for each stage and their formulation into an ideal repellent. Both attractants and repellents offer a way of partly circumventing the problems of resistance to insecticides. An individual insect, for example, which does not respond to its natural attractant to man when this is used in a bait or a trap is unlikely to feed on man anyhow. If it feeds at all, it is likely to feed on some other host, and the result would thus be selection for zoophily. Similarly, extensively used repellents tend to force insects to select other hosts. Mosquito species, at least, readily develop strains with particular host preferences (Mattingly et al., 1951).

Probable Questions:

1. Classify pesticides according to the types of pests they kill.
2. Differentiate biodegradable and non biodegradable pesticides.
3. What is Biopesticides? What are the uses of it?
4. What are the effects of pesticides?
5. What is anti feedant? How it is used?
6. What is fumigant? How it is used?
7. Discuss Attractants and Repellents pesticides.
8. Discuss different types of fumigants.

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett& Doull's Toxicology: The Basic Science by Curtis D. Klaassen

UNIT-VIII

Safer pesticides: Next generation molecules to be used as pesticides for plant protection and their chemistry

Objective: In this unit we will discuss about next generation pesticides which have less impact on environment and also on human health.

Introduction: Pesticides are widely used, as an example, in 2013, the total quantity of pesticide sales amounted to close to 360 000 tonnes. In the EU, the Member States where the highest quantities of pesticides were sold are Spain (19.5%), France (18.7%), Italy (13.8%), Germany (12.3%), and Poland (6.2%). Altogether, they made up 70.5% of the EU-28's pesticide sales. However, since many years ago, the number of new pesticides bringing to the market has decreased exponentially mostly due to the long and expensive procedure required to put them in the market. The introduction of a new pesticide in the market involves the synthesis or isolation of the active principle and its testing using a series of complex screens to assess their 'biological activity' or potential as a pesticide (including toxicological and environmental assessment).) On average only 1 in 70 000 go forward.

New-generation pesticides are often developed to replace earlier, more toxic chemicals in effort to clean up the environmental and human health impacts of these older agricultural pesticides. However, it is important to continually monitor impacts of all pesticides and indeed, a recent study found that new-generation pesticides are the most prevalent chemicals detected in streams in an intensive agriculture region of California. These chemicals were designed to replace harmful organophosphate pesticides linked to many environmental and human health issues. Using an older, common method as well as a newly developed method aimed at detecting new pesticide formulas, researchers screened samples from 85 streams for 223 different pesticide compounds. 83 streams (98%) tested positive for at least one pesticide, and 81% of the samples tested positive for two or more pesticides. One third of the samples detected 10+ pesticides. Nearly half of the 253 pesticides were detected at least once, indicating that a wide variety of chemicals continues to be used and contaminate nearby waterways.

The most commonly detected pesticide was a new-generation diamide insecticide, and the top six most commonly detected chemicals were new-generation pesticides. When the potential toxicity of the detected pesticides was assesses, the researchers found more potential toxicity to invertebrates than fish or plants, largely because the majority of pesticides detected were insecticides. Because of the common occurrence and potential toxicity of these new-generation pesticides the researchers recommend that monitoring methods be continuously updated and efforts remain vigilant to comprehensively assess pesticide occurrence and impacts on non-crop habitats.

The cost of this research phase averages 90 million € for each new product. After this, the development of the new product also involved their large-scale production, testing in variety of

technical formulations as well as under a wide range of crops, pest and conditions, determining their fate and metabolism in food and the environment and also testing their toxicity and environmental impact. The total time taken from first synthesis to first sale averages about 9 years.(2–4) This high cost justifies the lack of registration of new pesticides and explains why in the third update of this article the groups included as miscellaneous compounds of not so new generation are still macrocyclic lactones, chloronicotinyls, tetranordtriterpenoids, ammonium quaternary salts, dinitroanilines, acetamides, oximes, triazoles, and pyridine-based molecules.

Characteristics:

There are many registered chemicals that can be included under the heading of miscellaneous pesticides and related compounds, which comprise a large group of substances with very different characteristics and applications Their common attribute is that they do not belong to the classical chemical categories of pesticides, such as organochlorines, organophosphates, carbamates, or triazines. To develop the best residue analytical methods with the most appropriate instruments, knowledge of the physical and chemical properties of these pesticides is essential. For example, it is impossible to develop a GC residue method for imidacloprid without derivatization because of the polar and nonvolatile properties displayed by this molecule. Similarly, a direct GC method for diquat (DQ), paraquat (PQ), or azadirachtin is also impossible because of the polarity of these compounds.

The avermectins are macrocyclic lactones produced by the soil microorganisms *Streptomyces avermitilis*. They are probably the antiparasitic agents most widely used in the treatment of food-producing animals, poultry, aquaculture, and crops. Ivermectin was the first macrocyclic lactone product to be licensed for use about 20 years ago.

A number of alternative products, such as abamectin, doramectin, emamectin, eprinomectin, moxidectin, milbemycin, and selamectin, have been marketed since then.(23,24) Avermectin B1, known as abamectin, is widely used as an insecticide and a miticide for agricultural crops.) Abamectin is a mixture of two components. The major one is avermectin B1a, which makes up 80% or more of the mixture, and the minor component is avermectin B1b, which supplies 20% or less; the two components differ in a single methylene group. Ultraviolet (UV) light below 280nm rapidly isomerizes the E (trans) 8,9 and 10,11 double bonds of avermectin to 8,9- and 10,11-Z isomers. However, solutions of avermectin in Pyrex flasks are generally stable because Pyrex excludes most of the UV light below 280 nm. Avermectin residues degrade rapidly by both oxidative and photochemical pathways, forming a variety of products when applied to a number of different crops. Therefore, the only residues of toxicological significance are avermectin and 8,9-Z avermectin. Avermectins are not readily hydrolyzed because they are highly lipophilic substances that dissolve in most organic solvents. Their solubility in water is relatively low at 0.006–0.009 ppm (=mgL−1).

The chloronicotinyl insecticides act on cholinergic receptors and have good contact properties and powerful systemic action after uptake through the root system. Their common names are acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam. Owing to their insecticidal effectiveness at low concentrations, they are one of the most used

family of pesticides. Soil biodegradation studies on the chloronicotinyl imidacloprid have demonstrated that its major metabolite is 6-chloronicotinic acid and that it leaves no soil residue after the first 3 months. Acetamiprid is stable in solution at pH between 4.5 and 7. It is slowly degraded at pH 9 and 45∘C. Azadirachtin is a tetranortriterpenoid (limonoid) present in neem seeds (Azadirachta indica A. Juss). Azadirachtin has gained worldwide attention for its insect antifeedant and ecdysis inhibiting properties. This compound is highly potent at low concentrations against more than 200 agricultural pests and is ecofriendly. It is, therefore, a potentially safe alternative to the toxic synthetic pesticides and a number of commercial formulations have been introduced worldwide. Azadirachtin is nonvolatile and highly polar, soluble in polar organic solvents, and slightly soluble in water. It has UV absorption due to its α, β-unsaturated carbonyl chromophore in the ligate ester and the vinyl ether, but the absorption maximum is at a very short wavelength (UV absorption λmax =217 nm, ε=9000). Azadirachtin hydrolyzes readily at 35∘C, and its disappearance follows simple pseudo-first-order kinetics. Its hydrolysis is faster at a basic than at an acidic pH. Azadirachtin is composed of, at least, nine closely related isomers. The types A and B are dominant, with isomer A accounting for 83% and B 16%.

The bipyridylium herbicides DQ, PQ, and difenzoquat (DF) are important cationic pesticides used in agriculture. They are nonselective contact herbicides that are absorbed by foliage with some translocation though the xylem and are used for the broad-spectrum control of broad-leaved weeds and grasses in fruit orchards, plantation crops, ornamentals, and shrubs. DQ is a highly water soluble, dipyridylium crystalline salt and has a mode of action similar to PQ. They have been applied as defoliants on crops, such as cotton and as desiccants for pineapples, potatoes, sugarcane, and sunflower. Chlormequat (CQ) and mepiquat (MQ) are cationic plant growth regulators, structurally related to the bipyridylium herbicides, which are mainly used to prevent lodging in barley and rye and also to increase the yield of cotton. The PQ salts are hygroscopic, not volatile and very soluble in water. PQ is extremely stable in the presence of acids, but it is destroyed quickly in a basic medium. It is soluble in water and insoluble in organic solvents. DQ is also very thermo-stable and not volatile. The herbicide activity of PQ and DQ is related to the planar structure of their molecules and the 18 possible resonant structures that stabilize the radical formed in reduction reactions in which they are involved. DF occurs as monovalent cation and is also used as a selective herbicide for postemergence control of wild oats in barley and fall-seeded wheat. DF salts are very soluble in water and are stable to hydrolysis. DF is stable at weakly acid pH but is degraded in strong acids and in the presence of oxidizers. The three herbicides present an absorption band in the UV region at 260, 310, and 255 nm, respectively, due to the presence of aromatic rings in their structures. Dinitroanilines are selective pre-emergence herbicides used to control some broad-leaved weeds and the major annual grasses in a wide variety of agronomic crops. The mechanism of action of dinitroanilines is determined by their specific binding to parasite tubulins (the main structural component of microtubules), which causes an antimitotic activity. The chloracetanilide herbicides include benfluralin, butralin, dinitramine, ethalfluralin, fluchloralin, isopropalin, methalpropalin, nitralin, oryzalin, pendimethalin, prodiamine, profluralin, and trifluralin. Although these herbicides are chemically related, they differ in volatility, persistence in soil, and absorption by crops and for this reason may differ in their effects on soil, plants, and air. These compounds are among the least mobile

herbicides and, therefore, runoff is the principal route of the contamination of surface waters. Dinitroaniline herbicides are water insoluble, relatively volatile, and strongly adsorbed to soil colloids. In soil, both chemical reactions and biological processes degrade pendimethalin. In general, dinitroaniline herbicides degradation is more rapid under flooded anaerobic conditions than under aerobic ones. The group of acetamide pesticides (some also known as chloroacetamides) encompasses a considerable number of herbicides and fungicides used to control weeds and fungi in crops, including acetochlor, alachlor, butachlor, metolachlor, and propachlor. The compounds are widely used to control annual grasses and certain broad-leaved weeds in corn, soybeans, and peanuts and to control phytopathogenic fungi (Peronosporales) in potatoes, sugar beets, and other crops. They act by inhibiting protein synthesis by the reaction of the activated Cl atom of the chloroacetyl group with reactive sites in proteins.(37,38) Acetamide herbicides have moderate water solubility and are rapidly absorbed into plants. In susceptible plants, the herbicides act by inhibiting protein synthesis, whereas insensitive plants rapidly inactivate these herbicides via gluthathion conjugation. In sensitive fungi, the structurally related fungicides have effects on ribonucleic acid (RNA) synthesis. As these reactions involve various chiral structures, some stereoselectivity is expected in the activity of these compounds. Oximes are bioactive compounds, originally discovered in insects, that have recently been synthesized, and some are effective herbicides, applied as a good alternative to control glyphosate-resistant pests. Some representatives of this group are alloxydim, butroxydim, clethodim, cloproxydim, cycloxydim, profoxydim, sethoxydim, tepraloxydim, and tralkoxydim. Field trials with these compounds showed that they were not consistently effective, perhaps because of the instability of this active ingredient. Sethoxydim undergoes degradation, including photodegradation. Clethodim is a fatty acid synthesis inhibitor that works via inhibition of acetyl CoA carboxylase and it is degraded in aqueous solution by acid medium and light. Clethodim degradation increases as pH of the solution decreases and photolysis is more rapid and more complete than hydrolysis. Previous study indicated that clethodim is mainly oxidized to clethodim sulfoxide or clethodim sulfone in the field. Triazole derivatives are aromatic heterocycles widely used as weed killers, fungicides, insecticides, plant growth regulators, and antimicrobial agents. They are nonselective systematic herbicides used against a wide variety of plants, including applications for the treatment and protection of cereals, soybeans, and a variety of fruits. It is known that most of triazole fungicides are chiral and their optical isomers exhibit different bioactivity and toxicity. For example, the R-enantiomer of diniconazole and uniconazole shows stronger fungicidal activities, whereas the S-enantiomer has higher plant growth regulating activity. Amitrole belongs to the triazole group and is soluble in water, methanol, ethanol, and chloroform, slightly soluble in ethyl acetate, and insoluble in ether and acetone. Aqueous solutions are neutral. Other examples of these pesticides are cafenstrole, epronaz, and flupoxam. Pyridine-based molecules are a group of substances that include pyridazines, pyridazones, and pyridones. All of them are herbicides, but their applications vary. For example, pyridate is a pyridazine that acts by contact, while pyridazone derivatives, such as norflurazon and cloridazon, are soil-applied herbicides, and fluridone, a pyridone, are an experimental herbicide developed for aquatic plant management systems. Pyridate is a colorless crystalline solid that melts at 27∘C and boils at 220∘C under 10−6 mbar vacuum. Its vapor pressure is 1.3 × 10−9 mbar at 20∘C. It is stable in neutral medium but is hydrolyzed in strong acid and strong alkali media.

Toxicology:

Pesticide residues are regulated at the international and national levels according to the toxicity of the pesticide and the human exposure to a particular substance. The macrocyclic lactones are neurotoxins that manifest their action by disrupting the normal function of γ-aminobutyric acid (GABA), an important neurotransmitter in the central nervous system of vertebrates and in the peripheral nervous system of invertebrates. Because mammals have only GABA ergic synapses in the central nervous system, the mammalian blood–brain barrier ensures a degree of specificity. A notable feature of this group of compounds is their low LD50 (lethal dose 50) values, but they are not usually highly toxic by the dermal route because of their large molecules and poor transdermal absorption. In vitro studies with preparations of rat brain have shown that avermectin B1*a* stimulates presynaptic binding of GABA and also enhances its postsynaptic binding; the action of avermectin B1*a* is antagonized by bicuculline and picrotoxin. Avermectin and ivermectin are metabolized in a qualitatively similar way among different species. The major metabolites of both in cattle, sheep, swine, and rats are either 24-hydroxymethyl or 3′′-*O*-desmethyl derivatives. However, the enzymes responsible for the metabolism have not been identified in any species. These compounds undergo little metabolism and most of the dose given to the animal is excreted relatively unaltered, primarily in the feces. Abamectin induces teratogenic effects such as cleft palate. Although belonging to the biopesticide group, abamectin may be toxic tomammals including human beings. The oral LD50 of abamectin for rats is about 11 mg kg−1, while dogs showed pupillary dilation, weight loss, lethargy, tremors,

and recumbency after exposure to 0.5–1 mg kg day−1 levels. The chloronicotinyl insecticides interfere with neuronal functions as do organophosphate, carbamate, and pyrethroid insecticides. Unlike the latter pesticides, they act on nicotinergic acetylcholine receptors in the postsynaptic membrane of an identified insect motor neuron. They generally have low toxicity to mammals (acute and chronic), birds, and fish. Imidacloprid can persist in soil depending on soil type, pH, use of organic fertilizers, and presence or absence of ground cover. The metabolites of imidacloprid, namely olefin and nitrosimine, have greater insecticidal activity than the parent compound while the guanidine metabolite does not possess insecticidal properties but has a higher mammalian toxicity than the parent compound. After soil application or seed treatment, a quick degradation of the active substance was observed after root uptake of the active substance. It is a systemic broadspectrum insecticide and acts as a contact and stomach poison against sucking and some biting insects (rice hoppers, aphids, thrips, whitefly, termites, etc.). Azadirachtin is nonmutagenic and does not appear to exhibit mammalian toxicity. Insects ingesting azadirachtin and related minor compounds in the seed kernels do not die immediately, but soon stop feeding. This drug interrupts the life cycle of flies by inhibiting the development of the eggs, larvae, or pupae and by blocking the molting of larvae or nymphs, and inhibiting mating and sexual communication. PQ toxicity in both experimental animals and humans targets primarily the lung, whereas DQ does not. Differences between both the compounds are because the lung selectively accumulates PQ and not DQ. PQ-induced pulmonary injury takes place in two phases: destructive and proliferative. It has been suggested that the biochemical reactions that lead to the destructive effect of PQ are analogous to its toxic action on plant cells. NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) is the donor for the single-electron reduction of PQ. Reduced PQ is reoxidized

rapidly by molecular oxygen and superoxide radicals are formed. The superoxide radicals initiate a chain of reactions that produce toxic reactive intermediates, which include hydrogen peroxide, and hydroxy radicals and also produce lipid peroxidation. They are responsible for the disruption of cellular membranes. In addition, PQ competes for and deprives other systems of essential NADPH and compromises their cellular integrity. DQ poisoning differs from that of PQ, in that the renal effects are more prominent and lung changes do not generally occur. An effect of DQ, which has been extensively investigated, is its ability to produce cataracts in experimental animals. PQ produces chronic effects such as costal cartilage malformation in rats when injected during the gestation. Teratogenicity of PQ results from its effect on collagen biosynthesis. CQ and MQ are widely used as plant growth inhibitors. They are usually used together for controlling unwanted longitudinal shoot growth, improving fruit setting and increasing yield of fruit and vegetables. However, toxicological studies showed that CQ and MQ have adverse effects on animal reproduction. The National Institute of Occupational Safety and Health (NIOSH) indicated that CQ has been classified as a suspected endocrine disruptor.(59) CQ is known to be a competitive inhibitor of cholinesterase in animals. This anticholinesterase chemical causes acetylcholine accumulation at cholinergic receptor sites and, thus, is capable of producing effects equivalent to excessive stimulation of cholinergic receptors through the central and peripheral nervous system. MQ chloride is considered by the World Health Organization (WHO) to be slightly hazardous with an LD50 of 1490 mg kg−1 in rats. Due to its toxicity, maximum residue limits (MRLs) ofMQ chloride has been established in different matrices. Substituted anilines have the general property of causing methemoglobinemia, as do many other aniline derivatives. The probable mechanism of methemoglobinemia is the N-hydroxylation to the corresponding hydroxylamine, which then takes part in an intraerythrocytic cycle with the corresponding nitroso derivative generating methemoglobin at the same time. Alachlor is classified as a ‘likely’ human carcinogen at high doses by the US Environmental Protection Agency (EPA) because of its carcinogenic effect on rodents, where it produces posterior nasal and stomach tumors, possibly by a nongenotoxic mechanism.(61) Acetochlor is absorbed through the roots and shoots just above the seed of the target weeds; they act as a growth inhibitor by suppressing synthesis of protein. Triazole fungicides are among the flourishing new generations of pesticides applied to fruits, vegetables, and grain crops. Besides their antifungal activity, they are also of concern as a group of compounds that disturb endocrine activity in human beings. Due to their lipophilic nature, these compounds can be bioaccumulated in various tissues of living organisms and they can be transported between various compartments of ecosystems and contaminate food chains. The triazole antifungals myclobutanil, propiconazole, and triadimefon cause various degrees of hepatic toxicity
and disrupt steroid hormone homeostasis in rodent in vivo models. Modulation of hepatic sterol and steroid metabolism is a plausible mode of action for changes in serum testosterone and adverse reproductive outcomes observed in rat studies and may be relevant to human risk assessment. There is a solid evidence that amitrole produces thyroid tumors in mice and rats by a nongenotoxic mechanism, which involves interference with the functioning of thyroid peroxidase. However, the International Agency for Research on Cancer (IARC), in its last evaluation,(43,65) has changed the amitrole classification from ‘possibly carcinogenic to humans’ to ‘not classifiable as to its carcinogenicity to humans’ because it would not be expected to produce thyroid cancer in humans exposed to concentrations that do not alter thyroid hormone homeostasis. The literature

is scarce on toxicity of the oximes. Clethodim is a cyclohexenone herbicide introduced by Chevron Chemical Co., and has been used as a selective postemergence herbicide to control annual and perennial grasses in a wide variety of broadleaf crops, including soybeans, cotton, flax, peanuts, sunflowers, sugar beet, potatoes, alfalfa, and most. It is also applied on rape to prevent annual gramineae and broadleaf weeds. Clethodim is a fatty acid synthesis inhibitor that works via inhibition of acetyl CoA carboxylase. There are only a few studies demonstrating that sethoxydim produceslesions in bone marrow and the liver of dogs. There are concerns about the possible effects of pyridine-based molecules. Fluoridone produces cytotoxicity, decreasing the number of cells. A perturbing effect of chloridazon is its interference with the phospholipid moiety of the nerve fiber membrane, leading to interference with total ion transport across the nerve skin junction. However, in the last years these compounds have been reduced for use.

Probable Questions:

1. What are the needs of introduction of new generation safer pesticides?
2. What are the characteristics of safer pesticides?
3. What are the toxicological evaluations of safer new generation pesticides?

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett& Doull's Toxicology: The Basic Science by Curtis

UNIT-IX

Metabolism of Pesticides: Phase I and Phase II reactions and metabolism of pesticides

Objective: In this unit we will discuss about how pesticides are metabolized. We will discuss Phase I and Phase II reactions and metabolism of different pesticides.

Introduction: As a result of human activities, currently a large number of pollutants and waste are eliminated to the environment. Worldwide, more than one billion pounds of toxins are released into the air and water. Approximately $6x10^6$ chemical compounds have been produced; annually 1,000 new products are synthetized and between 60,000 and 95,000 chemicals are commercially used. Among these substances are chemical pesticides, which are used extensively in most areas of crop production in order to minimize pest infestations, to protect the crop yield losses and to avoid reducing the product quality.

The pesticides belong to a category of chemicals used worldwide as herbicides, insecticides, fungicides, rodenticides, molluscicides, nematicides, and plant growth regulators in order to control weeds, pests and diseases in crops as well as for health care of humans and animals. The positive aspect of application of pesticides renders enhanced crop/food productivity and drastic reduction of vector-borne diseases. A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (insects, mites, nematodes, weeds, rats, etc., including insecticide, herbicide, fungicide, and various other substances used to control pests . The definition of pesticide varies with times and countries. However, the essence of pesticide remains basically constant: it is a (mixed) substance that is poisonous and efficient to target organisms and is safe to non-target organisms and environments.

Pesticides are by no means a new invention. In fact, intentional pesticide use goes back thousand years when Sumerians, Greeks, and Romans killed pests using various compounds such as sulphur, mercury, arsenic, copper or plant extracts. However, results were frequently poor because of the primitive chemistry and the insufficient application methods. A rapid emergence in pesticide use began mainly after World War II with the introduction of DDT (dichlorodiphenyltrichloroethane), BHC (benzene hexachloride), aldrin, dieldrin, endrin, and 2,4-D (2,4-dichlorophenoxyacetic acid). These new chemicals were effective, easy to use, inexpensive, and thus enormously popular. However, under constant chemical pressure, some pests became genetically resistant to pesticides, non-target organisms were harmed, and pesticide residues often appeared in unexpected places.

Chemical pesticides can be classified in different ways, but one of the most used is according to their chemical composition, which allows to group pesticides in a uniform and scientific way and to establish a correlation between structure, activity, toxicity and degradation mechanisms, among others.

Worldwide approximately 9,000 species of insects and mites; 50,000 species of plant pathogens, and 8,000 species of weeds damage crops. Different pests such as insects and plants causing losses estimated in 14% and 13% respectively. Pesticides are indispensable in agricultural production. About one-third of the agricultural products are produced by using pesticides. Without pesticide application the loss of fruits, vegetables and cereals from pest injury would reach 78%, 54% and 32% respectively. Crop loss from pests declines to 35% to 42% when pesticides are used.

Over 1990s, the global pesticide sales remained relatively constant, between 270 to 300 billion dollars, of which 47% were herbicides, 79% were insecticides, 19% were fungicides/bactericides, and 5% the others. Over the period 2007 to 2008, herbicides ranked the first in three major categories of pesticides (insecticides, fungicides/bactericides, herbicides). Fungicides/bactericides increased rapidly and ranked the second. Europe is now the largest pesticide consumer in the world, followed by Asia. As for countries, China, the United States, France, Brazil and Japan are the largest pesticide producers, consumers or traders in the world. Most of the pesticides worldwide are used to fruit and vegetable crops. In the developed countries pesticides, mainly herbicides are mostly used to maize. Since the 1980s hundreds of thousands of pesticides have been developed, including various biopesticides.

The global agricultural sector is the primary user of pesticides, consuming over 4 million tons of pesticides annually. Pesticides have been extensively used for decades and have substantially increased the food production. However a large amount of applied pesticides often never reach their intended target due to their degradation, volatilization and leaching, leading to serious ecological problems. Under actual agricultural practices, different groups of pesticides are often simultaneously or consecutively applied interacting with each other.

Although pesticides are beneficial in controlling the proliferation of pests, their unregulated and indiscriminate applications for the application of pesticides can cause adverse effects to human health, to different life forms and to the ecosystems, which depend on the degree of sensitivity of organisms and toxicity of pesticides. The continued application of pesticides has increased its concentration in soils and waters, besides; they enter to the food chains. Dispersion mechanisms also have increased the level of environmental risk for the occupationally exposed population and the inhabitants of surrounding villages. Despite ban on application of some of the environmentally persistent and least biodegradable pesticides (like organochlorines), in many countries their use is ever on rise. Pesticides cause serious health hazards to living systems because of their rapid fat solubility and bioaccumulation in non-target organisms. The main forms of pollution are direct applications to agricultural crops, accidental spills during transport and manufacturing, as well as waste from tanks where cattle are treated to ectoparasites control.

The effects of the impacts of pesticides can be analyzed from two different points of view: environmental and public health. The first occurs when pesticides are introduced to food chains, for example: a) producing a change in the decline of populations of phytoplankton and zooplankton (indicators of water pollution); b) producing carcinogenic, neurotoxic, and on fertility and viability (in invertebrates, fish, amphibians, insects and mammals) of their descendants; c) the presence of pesticides in the environment have caused the resistance of organisms considered as

pests and disease vectors (for example malaria, dengue and Chagas disease), and instead other beneficial insect populations are diminished (like pollinators); d) alter biogeochemical cycles by decreasing the macro and microbiota, e) leaching of pesticides pollute water bodies, f) can be adsorbed pesticides when soil particles interact with positively or negatively charged, thus increasing their persistence in the environment (4-26 weeks). From the point of view of public health impact of pesticides is mainly acute intoxications (especially in occupationally exposed populations) or indirect exposure of the general population (through air, water and food contaminated with pesticide residues).

In natural environments, pesticides or their degradation products may be further transformed or degraded by other microorganisms or eventually leading to complete degradation by the microbial consortium. However, persistent xenobiotics like pesticides and metabolic dead-end products will accumulate in the environment, become part of the soil humus, or enter the food chain leading to biomagnification

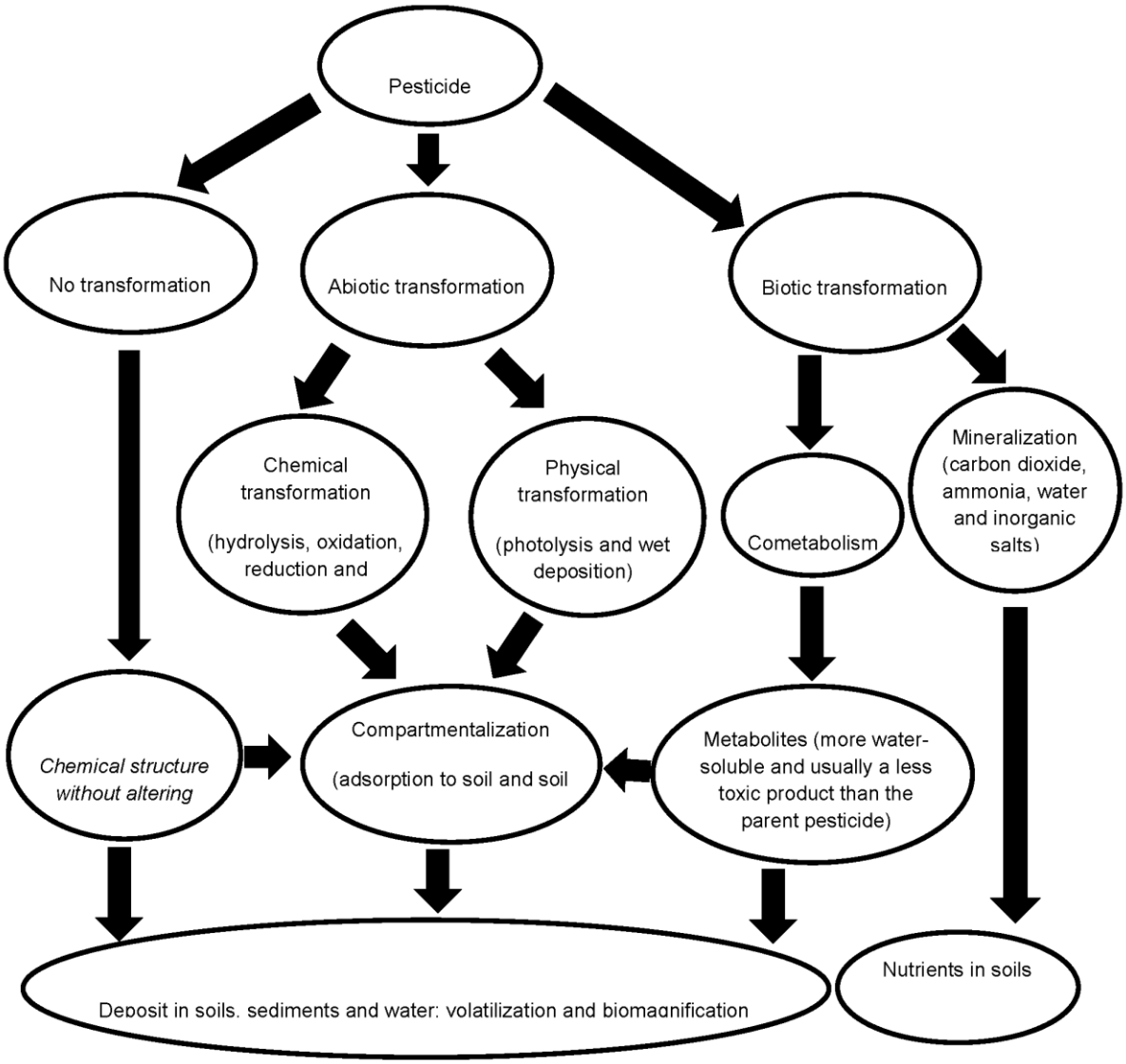


Figure 1. Fate of pesticides in the environment.

The fate of pesticides in the environment is strongly related to the soil sorption rocesses that control not only their transfer but also their bioavailability . Contamination of soil from pesticides as a result of their bulk handling at the farmyard or following application in the field or accidental release may lead occasionally to contamination of surface and ground water. The behaviour of pesticides in soils, the efficiency, persistence and potential as environmental contaminants, depend on their retention and degradation on soil constituents. In soils, several parameters influence the rate of biodegradation processes: environmental factors such as moisture and temperature, physicochemical properties of the soil, presence of other nitrogen sources or carbon, etc. can completely modify the microbial population and therefore the microbial activity.

On the other hand, liquid and solid wastes and obsolete products are stored or disposed in an inappropriate manner, which has favored the appearance of significant amounts of environmental liabilities, which in most cases are not reported to the appropriate authority. There are more than half a million tons of obsolete, unused, forbidden or outdated pesticides, in several developing and transitional countries, which endanger the environment and health of millions of people. In the

absence of a clear obsolete pesticides management strategy, over the years, significant amounts of obsolete pesticides have been stockpiled in developing countries. An obsolete pesticide may be recognized as one that is undesirable or impossible to use and has to be eliminated. Because of their characteristics, obsolete pesticides are hazardous wastes that should be managed as such. Obsolete pesticides have accumulated in almost every developing country or economy in transition over the past several decades . It is estimated that in Africa and Middle East there are more than 100,000 tons of these products, in Asia almost 200,000 and a similar quantity in East Europe and the old Soviet Union. Nowadays the FAO is elaborating the inventories of Latin America. In Mexico, there is knowledge of the existence of obsolete pesticide products, both liquid and solid. A total of 551 records of obsolete pesticide products have been registered, distributed in 29 of 33 states of Mexico, achieving a total of 26,725.02 liters, 147,274 kg and 500 m^3 of highly polluted soils. In addition there are 28 reports of pesticide-contaminated sites in 15 states of the Mexican Republic. Besides, some data indicate that the total of empty pesticide containers can be about 7,000 tons annually.

Microorganisms involved in the biodegradation of pesticides

Different biological systems, as microorganisms, have been used to biotransform pesticides. It has been reported that a fraction of the soil biota can quickly develop the ability to degrade certain pesticides, when they are continuously applied to the soil. These chemicals provide adequate carbon source and electron donors for certain soil microorganisms , establishing a way for the treatment of pesticide-contaminated sites.

Furthermore, the isolated microorganisms capable of degrading pesticides can be used for bioremediation of other chemical compounds to whom any microbial degradation system is known. However, the transformation of such compounds depends not only on the presence of microorganisms with appropriate degrading enzymes, but also a wide range of environmental parameters. Additionally, some physiological, ecological, biochemical and molecular aspects play an important role in the microbial transformation of pollutants .

There are different sources of microorganisms with the ability to degrade pesticides. Because pesticides are mainly applied to agricultural crops, soil is the medium that mostly gets these chemicals, besides pesticide industry's effluent, sewage sludge, activated sludge, wastewater, natural waters, sediments, areas surrounding the manufacture of pesticides, and even some live organisms. In general, microorganisms that have been identified as pesticide degraders have been isolated from a wide variety of sites contaminated with some kind of pesticide. At present, in different laboratories around the world there are collections of microorganisms characterized by their identification, growth and degradation of pesticides. The isolation and characterization of microorganisms that are able to degrade pesticides give the possibility to count with new tools to restore polluted environments or to treat wastes before the final disposition.

Microbial processes that eliminate organic environmental contamination are important. Progress in the biotechnology of biodegradation relies upon the underlying sciences of environmental microbiology and analytical geochemistry. Recent key discoveries advancing knowledge of

biodegradation (in general) and the aromatic-hydrocarbon biodegradation (in particular) have relied upon characterization of microorganisms: pure-culture isolates, laboratory enrichment cultures, and in contaminated field sites. New analytical and molecular tools (ranging from sequencing the DNA of biodegrading microorganisms) have deepened our insights into the mechanisms (how), the occurrence (what), and the identity (who) of active players that effect biodegradation of organic environmental pollutants, (Figure 2).

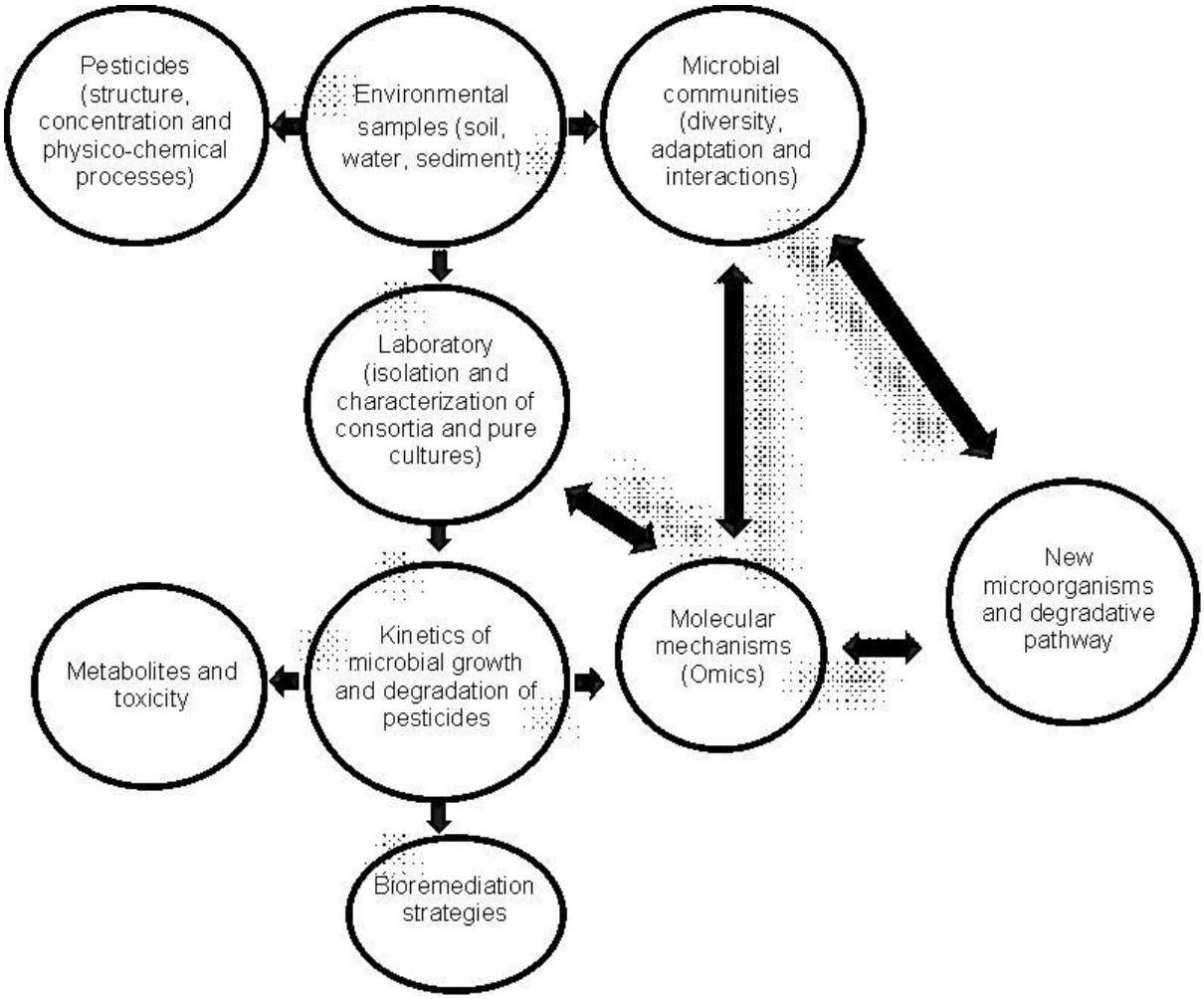


Figure 2. Representation of the relationships between pesticides, microbial communities, and the discovery of new biodegradation processes, Omics = high throughput-based characterization of biomolecules characteristic of bioprocesses; DNA, genomics; mRNA, transcriptomics; protein, proteomics; metabolites, metabolomics.

In the literature there are some examples of microbial pesticide degradation, among them, the following reports deserve mention:

According to , *Pseudomonas*, is the most efficient bacterial genus for the degradation of toxic compounds. The ability of these bacteria to degrade these compounds, is related to the contact time with the compound, the environmental conditions in which they develop and their physiological versatility. In other report, evaluated three *Pseudomonas* species for the biodegradation of the herbicide aroclor 1242, showing that these bacteria have a great ability to degrade it, according to their degradation percentage, 99.8, 89.4 and 98.4 respectively. isolated

various fungi species from Algerian pesticide contaminated soils. Observing that the most frequent species isolated were *Aspergillusfumigatus, A. niger, A. terreus, Absidia and Rhizopusmicrosporusvarcorymberiferamicrosporis.* In this report, 53 of the isolated species were noted for their ability to degrade the herbicide metribuzin in liquid medium. It was demonstrated, at the same time, that the herbicide promoted the *Absidia* and *Fusarium* genera growth; these genera were capable to eliminate the 50% of the compound after 5 days. Moreover, the species *Botrytis cinerea* could eliminate the linuron and metroburon herbicides almost completely, and other 31 isolated species also could eliminate metroburon. Another experiments have been demonstrated the efficiency of the bacterium *Rhodococcus sp.* to degrade triazines to nitrate conducted a test to study the atrazine herbicide transformations resulting from microbial decomposition. After microbial action this compound was transformed into nitrite (30%), nitrous oxide (3.2%), ammonia (10%) and formaldehyde (27%).

Several bacterial genera are adapted to grow in pesticide contaminated soils. These microorganisms have enzymes involved in the hydrolysis of P-O, P-F, P-S and P-C bonds, which are found in a wide variety of organophosphorus pesticides . Some bacteria isolated from the soil are capable of degrading pesticides as ethyl-parathion and methyl-parathion.

Biodegradation mechanisms:

Biodegradation that involves the capabilities of microorganisms in the removal of pollutants is the most promising, relatively efficient and cost-effective technology. Biodegradation is a process that involves the complete rupture of an organic compound in its inorganic constituents. The microbial transformation may be driven by energy needs, or a need to detoxify the pollutants, or may be fortuitous in nature (cometabolism). Because of the ubiquitous nature of microorganisms, their numbers and large biomass relative to other living organisms in the earth, wider diversity and capabilities in their catalytic mechanisms , and their ability to function even in the absence of oxygen and other extreme conditions the search for pollutant-degrading microorganisms, understanding their genetics and biochemistry, and developing methods for their application in the field have become an important human endeavor .

As much as the diversity in sources and chemical complexities in organic pollutants exists, there is probably more diversity in microbial members and their capabilities to synthesize or degrade organic compounds .The microbial populations of soil or aquatic environments are composed of diverse, synergistic or antagonistic communities rather than a single strain. In natural environments, biodegradation involves transferring the substrates and products within a well-coordinated microbial community, a process referred to as metabolic cooperation . Microorganisms have the ability to interact, both chemically and physically, with substances leading to structural changes or complete degradation of the target molecule. Among the microbial communities, bacteria, fungi, and actinomycetes are the main transformers and pesticide degraders. Fungi generally biotransform pesticides and other xenobiotics by introducing minor structural changes to the molecule, rendering it nontoxic. The biotransformed pesticide is released into the environment, where it is susceptible to further degradation by bacteria.

Fungi and bacteria are considered as the extracellular enzyme-producing microorganisms for excellence. White rot fungi have been proposed as promising bioremediation agents, especially for compounds not readily degraded by bacteria. This ability arises from the production of extracellular enzymes that act on a broad array of organic compounds. Some of these extracellular enzymes are involved in lignin degradation, such as lignin peroxidase, manganese peroxidase, laccase and oxidases. Several bacterial that degrade pesticide have been isolated and the list is expanding rapidly. The three main enzyme families implicated in degradation are esterases, glutathione S-transferases (GSTs) and cytochrome P450.

Enzymes are central to the biology of many pesticides . Applying enzymes to transform or degrade pesticides is an innovative treatment technique for removal of these chemicals from polluted environments. Enzyme-catalyzed degradation of a pesticide may be more effective than existing chemical methods. Enzymes are central to the mode of action of many pesticides: some pesticides are activated *in situ* by enzymatic action, and many pesticides function by targeting particular enzymes with essential physiological roles. Enzymes are also involved in the degradation of pesticide compounds, both in the target organism, through intrinsic detoxification mechanisms and evolved metabolic resistance, and in the wider environment, via biodegradation by soil and water microorganisms . suggested that (i) the central metabolism of the global biodegradation networks involves transferases, isomerases, hydrolases and ligases, (ii) linear pathways converging on particular intermediates form a funnel topology, (iii) the novel reactions exist in the exterior part of the network, and (iv) the possible pathway between compounds and the central metabolism can be arrived at by considering all the required enzymes in a given organism and intermediate compounds.

For pesticides degradation, three are mainly enzyme systems involved: hydrolases, esterases (also hydrolases), the mixed function oxidases (MFO), these systems in the first metabolism stage, and the glutathione S-transferases (GST) system, in the second phase. Several enzymes catalyze metabolic reactions including hydrolysis, oxidation, addition of an oxygen to a double bound, oxidation of an amino group (NH_2) to a nitro group, addition of a hydroxyl group to a benzene ring, dehalogenation, reduction of a nitro group (NO_2) to an amino group, replacement of a sulfur with an oxygen, metabolism of side chains, ring cleavage. The process of biodegradation depends on the metabolic potential of microorganisms to detoxify or transform the pollutant molecule, which is dependent on both accessibility and bioavailability.

Metabolism of pesticides may involve a three-phase process. In Phase I metabolism, the initial properties of a parent compound are transformed through oxidation, reduction, or hydrolysis to generally produce a more water-soluble and usually a less toxic product than the parent. The second phase involves conjugation of a pesticide or pesticide metabolite to a sugar or amino acid, which increases the water solubility and reduces toxicity compared with the parent pesticide. The third phase involves conversion of Phase II metabolites into secondary conjugates, which are also non-toxic. In these processes fungi and bacteria are involved producing intracellular or extra cellular enzymes including hydrolytic enzymes, peroxidases, oxygenases, etc.

Due to the diversity of chemistries used in pesticides, the biochemistry of pesticide bioremediation requires a wide range of catalytic mechanisms, and therefore a wide range of enzyme classes.

Generalities of the major enzymatic activities applied for pesticide biodegradation

a. Hydrolases

Hydrolases are a broad group of enzymes involved in pesticide biodegradation. Hydrolases catalyze the hydrolysis of several major biochemical classes of pesticide (esters, peptide bonds, carbon-halide bonds, ureas, thioesters, etc.) and generally operate in the absence of redox cofactors, making them ideal candidates for all of the current bioremediation strategies.

As an example of the catalytic activity of enzymes hydrolases, the degradation pathway of carbofuran, a pesticide the group of carbamates is presented (Figure 3). This pesticide can be transformed in the environment and different metabolites are generated and accumulated in potentially contaminated sites (soil, water and sediments, mainly). Different organisms isolated from contaminated sites that have been identified and characterized as transformers of carbofuran, resulting in different metabolites.

Among the hydrolases involved in the degradation of pesticides are including different types such as:

b. Phosphotriesterases (PTEs)

Among the most studied pesticide degrading enzymes, the PTEs are one of the most important groups . These enzymes have been isolated from different microorganisms that hydrolyze and detoxify organophosphate pesticides (OPs). This reduces OP toxicity by decreasing the ability of OPs to inactivate AchE. The first isolated phosphotriesterase belongs to the *Pseudomonas diminuta* MG species; this enzyme shows a highly catalytic activity towards organophosphate pesticides. The phosphotriesterases are encoded by a gene called *opd* (organophosphate-degrading). *Flavobacterium* ATCC 27551 presents the *opd* gene encoding to a PTE. The gene was cloned and sequenced by . These enzymes specifically hydrolyzephosphoester bonds, such as P–O, P–F, P–NC, and P–S, and the hydrolysis mechanism involves a water molecule at the phosphorus center. Different microbial enzymes with the capacity to hydrolyze MP have been identified, such as organophosphorus hydrolase (OPH; encoded by the *opd* gene), methyl-parathion hydrolase (MPH; encoded by the *mpd* gene), and hydrolysis of coroxon (HOCA; encoded by the *hocA* gene), which were isolated from *Flavobacterium* sp., *Plesimonas* sp. strain M6 and *Pseudomonas moteilli* , respectively.

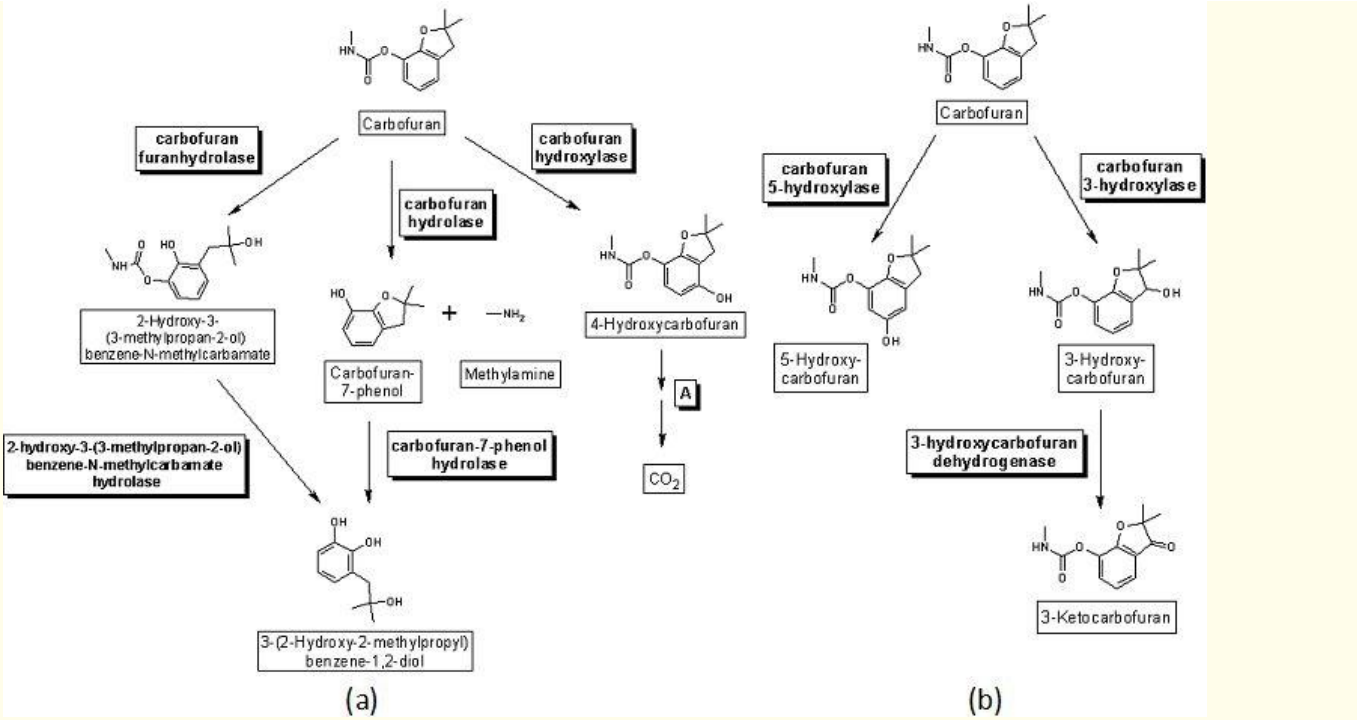


Figure 3.Degradation pathway of carbofuran. In a) several bacteria are involved in the hydrolysis of metabolites and b) fungal degradation of carbofuran may occur via hydroxylation at the three position and oxidation to 3-ketocarbofuran

The phosphotriesterase enzyme is a homo-dimeric protein with a monomeric molecular weight of 36 Kda. As a first step in the PTE organophosphorous pesticide hydrolysis mechanism, the enzymatic active site removes a proton from water, activating this molecule, them, the activated water directly attacks the central phosphorus of the pesticide molecule producing an inversion in its configuration The oxygen is polarized by the active site, with the participation of a zinc atom , (Figure 4). This enzyme has potential use for the cleaning of organophosphorus pesticides contaminated environments

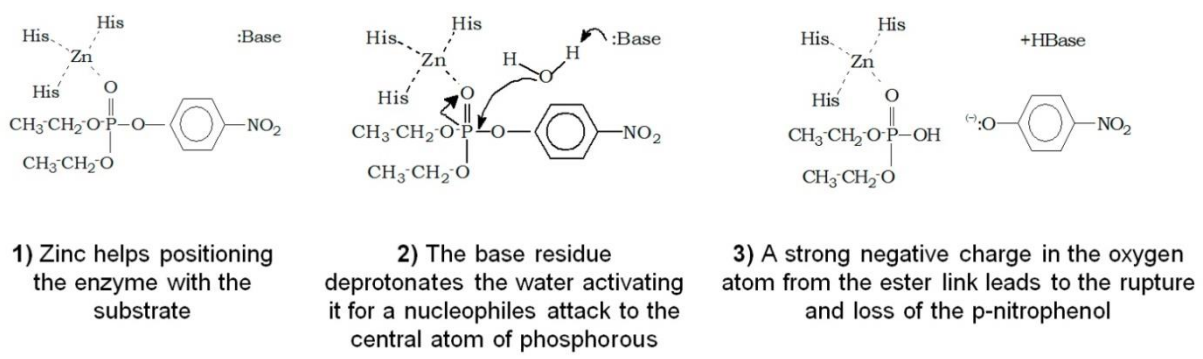


Figure 4.Proposed mechanism for PTE activity. Zinc's active site functions in phosphate polarization, making phosphor more susceptible to the attack. 1) A base subtracts a proton from a water molecule with the subsequent attack of the hydroxyl to the central phosphorous. 2) The intermediary complex originates the products 3) p-nitrophenol and diethyl thiophosphate [6].

c. Esterases

Esterases are enzymes that catalyze hydrolysis reactions over carboxylic esters (carboxiesterases), amides (amidases), phosphate esters (phosphatases), etc. In the reaction catalyzed by esterases, hydrolysis of a wide range of ester substrates occurs in their alcohol and acid components as following:

R = O-OCH3+ H2O↔R = O-OH + CH3OH

Many insecticides (organophosphates, carbamates and pyrethroids) have associated a carboxylic ester, and the enzymes capable of hydrolyze such ester bond are known with the name of carboxylesterases.

At present, multiple classification nomenclature systems are used for these enzymes. According to the International Union of Biochemistry and Molecular Biology (IUBMB) nomenclature, carboxylesterases are located in the group of hydrolases (3), subgroup 1, and within it, in subtype 1 (Enzyme Commission 3.1.1.1, EC 3.1.1.1). Another common classification is the nomenclature divides the esterases into three groups according to the nature of their interactions with organophosphorus insecticides. Carboxylesterases belong, according to this classification, the group of ali-esterases and B-esterases. Esterases are a large family of enzymes in arthropods.

The esterases are a group of enzymes highly variable, which has been recognized as one of the most important in the metabolism of xenobiotics and its mechanism is associated with the mass production of multifunctional hydrolytic enzymes Organophosphate pesticides can be hydrolyzed by such enzymes. There are different types of esterases and with very different distribution in tissues and organisms. The Carboxiesterases (type B esterases) are a group that hydrolyze, additionally to endogenous compounds, xenobiotics with ester, amide, thioester, phosphate esters (parathion, paraoxon) and acid anhydrides (DIPFP=DFP) in mammals.

EsterasesA, contain a Cys residue in the active center and esterases B contain a Ser residue. In esterases A, the organophosphates interact with the functional group-SH forming a bond between P=S, which is easily hydrolyzed by H_2O. In the esterase B, organophosphates interaction with the SER-OH forming a P=O bond that is not hydrolyzed by H_2O. Organophosphates that bind to the esterase B stoichiometrically inhibit its enzymatic activity.Esterases are a diverse group that protects the target site (acetylcholinesterase) by catalyzing the hydrolysis of insecticides, or acting as an alternative blank. Esterases in general have a wide range of substrate specificities; they are capable of binding to phosphate triesters, esters, thioesters, amides and peptides.

d. Oxidoreductases

Oxidoreductases are a broad group of enzymes that catalyze the transfer of electrons from one molecule (the reductant or electron donor) to another (the oxidant, or electron acceptor). Many of these enzymes require additional cofactors, to act as either electron donors, electron acceptors or both. Oxidoreductases have been further sub classified into 22 subclasses (EC 1.1-1.21 and 1.97).

Several of these have applications in bioremediation, albeit their need for cofactors complicates their use in some applications. There are enzymes that catalyze an oxidation/reduction reaction by including the molecular oxygen (O_2) as electron acceptor. In these reactions, oxygen is reduced to water (H_2O) or hydrogen peroxide (H_2O_2). The oxidases are a subclass of the oxidoreductases .

As an example of the many functions of these enzymes in the degradation of pesticides, as an example we present the ensodulfan degradation pathway. In this process not only oxidoreductase enzymes are involved, but different microorganisms and catalytic activities, in combination, can lead to complete mineralization of a pesticide (Figure 5). Endosulfan (*1,2,5,6,7,7-hexachloro-5-norbornene-2,3- dimethanolcyclicsulfite*) is an organochlorine insecticide of the cyclodiene family of pesticides. It is highly toxic and endocrine disruptor, and it is banned in European Union and several countries. Because it has been extensively applied directly to fields, it can be detected a considerable distance away from the original site of application. Contamination of drinking water and food, as well as detrimental effects to wildlife are important concerns. The molecular structure has two stereochemical isomers α and β endosulfan. The end-use product of endosulfan is a mixture of two isomers, typically in a 2:1 ratio.

Microorganisms play a key role in removal of xenobiotics like endosulfan from the contaminated sites because of their dynamic, complex and complicated enzymatic systems which degrade these chemicals by eliminating their functional groups of the parent compound. This pesticide can undergo either oxidation or hydrolysis reactions. Several intensive studies on the degradation of endosulfan have been conducted showing the primary metabolites to normally be endosulfansulfate and endosulfandiol (endodiol). Endosulfan sulphate will be present in the environment as a result of the use of endosulfan as an insecticide. If endosulfan sulphate is released to water, it is expected to absorb to the sediment and may bioconcentrate in aquatic organism. This metabolite has a similar toxicity as endosulfan and has a much longer half-life in the soil compared to endosulfan. Therefore, production of endosulfansulfate by biological systems possesses an ecological hazard in that it contributes to long persistence of endosulfan in soil. Endodiol is much less toxic to fish and other organisms than the parent compound.

Thus, it is important to note that some microbial enzymes are specific to one isomer, or catalyze at different rates for each isomer. For example, a *Mycobacterium tuberculosis* ESD enzyme degrades beta-endosulfan to the monoaldehyde and hydroxyether (depending on the reducing equivalent stoichiometry), but transforms alpha-endosulfan to the more toxic endosulfansulfate. However, oxidation of endosulfan or endosulfansulfate by the monooxygenase encoded by *ese* in *Arthrobacter* sp. KW yields endosulfanmonoalcohol. Both *ese* and *esd* proteins are part of the unique Two Component Flavin Dependent Monooxygenase Family, which require reduced flavin. They are conditionally expressed when no or very little sulfate or sulfite is available, and endosulfan is available to provide sulfur in these starved conditions.

Alternatively, hydrolysis of endosulfan in some bacteria (*Pseudomonas aeruginosa*, *Burkholderiacepaeia*) yields the less toxic metabolite endosulfandiol. Endosulfan can spontanteouslyhydrolyze to the diol in alkaline conditions, so it is difficult to separate bacterial from abiotic hydrolysis. The diol can be converted to endosulfan ether or endosulfanhydroxyether

and then endosulfan lactone. Hydrolysis of endosulfan lactone yields endosulfan hydroxycarboxylate. These various branches of endosulfan degradation all result in desulfurization while leaving the chlorines intact, exhibiting the recalcitrance to bioremediation found in many organohalogen aromatics.

e. Mixed Function Oxidases (MFO)

In the reaction catalyzed by the MFO (EC 1.14.14.1), an atom of one molecule of oxygen is incorporated into the substrate, while the other is reduced to water. For this reason the MFO requires Nicotiamide-adenine dinucleotide phosphate (NADPH) and O_2 for its operation.

It is an enzyme system comprising two enzymes, cytochrome P450 and NADPH-cytochrome P450 reductase, both membrane proteins. They are also known as dependent cytochrome P-450 monooxygenases or P450 system. The genes encoding the different isozymes comprise a superfamily of over 200 genes grouped into 36 families based on their sequence similarity. Cytochrome P450 enzymes are active in the metabolism of wide variety of xenobiotics .

The cytochrome P450 family is a large, well characterized group of monooxygenase enzymes that have long been recognized for their potential in many industrial processes, particularly due to their ability to oxidize or hydroxylate substrates in an enantiospecific manner using molecular oxygen. Many cytochrome P450 enzymes have a broad substrate range and have been shown to catalyse biochemically recalcitrant reactions such as the oxidation or hydroxylation of non-activated carbon atoms. These properties are ideal for the remediation of environmentally persistent pesticide residues. Over 200 subfamilies of P450 enzymes have been found across various prokaryotes and eukaryotes. All contain a catalytic iron-containing porphyrin group that absorbs at 450 nm upon binding of carbon monoxide. In common with many of the other oxidoreductases described before, P450 enzymes require a non-covalently bound cofactor to recycle their redox center (most frequently NAD(P)H is used), which limits their potential for pesticide bioremediation to strategies that employ live organisms.

In insects, MFOs are found in the endoplasmic reticulum and mitochondria, are involved in a large number of processes such as growth, development, reproduction, detoxification, etc. MFO are involved in the metabolism of both endogenous and exogenous substances, for this reason these compounds promote their induction Due to its high inspecificity, the MFOs metabolize a wide range of compounds such as organophosphates, carbamates, pyrethroids, DDT, inhibitors of the chitin synthesis, juvenile hormone mimics, etc.

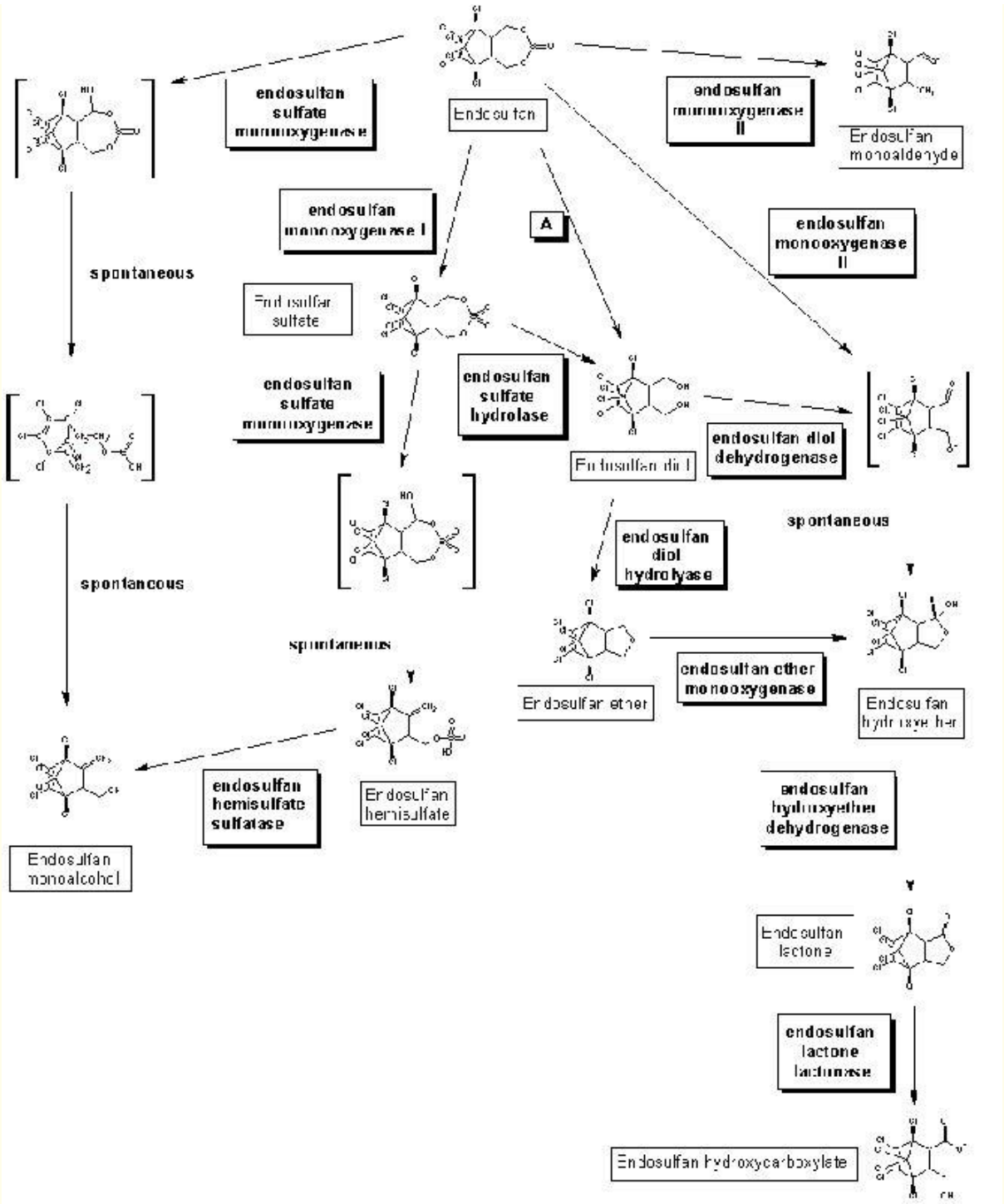


Figure 5. Degradation pathway of endosulfan

f. Glutathione S-Transferase (GST)

The GSTs (EC 2.5.1.18) are a group of enzymes that catalyze the conjugation of hydrophobic components with the tripeptide glutathione (Figure 6). In this reaction, the thiol group of glutathione reacts with an electrophilic place in the target compound to form a conjugate which can be metabolized or excreted, and they are involved in many cellular physiological activities, such as detoxification of endogenous and xenobiotic compounds, intracellular transport, biosynthesis of hormones and protection against oxidative stress.

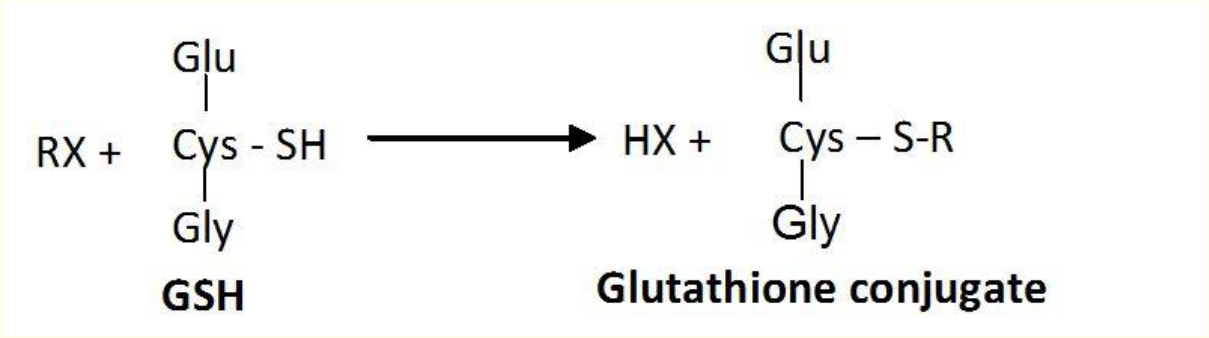


Figure 6 Representation of the conjugation reaction catalyzed by glutathione S-transferase (GST).

Genetics for pesticide degradation

In order to investigate genetic basis of pesticides biodegradation, several works with special emphasis on the role of catabolic genes and the application of recombinant DNA technology, had been reported. Pesticide-degrading genes of only a few microorganisms have been characterized. Most of genes responsible for catabolic degradation are located on the chromosomes, but in a few cases these genes are found in plasmids or transposons. The recent advances in metagenomics and whole genome sequencing have opened up new avenues for searching the novel pollutant degradative genes and their regulatory elements from both culturable and nonculturable microorganisms from the environment. Mobile genetic elements such as plasmids and transposons have been shown to encode enzymes responsible for the degradation of several pesticides. The isolation of pesticide degrading microorganisms and the characterization of genes encoding pesticide degradation enzymes, combined with new techniques for isolating and examining nucleic acids from soil microorganisms, will yield unique insights into the molecular events that lead to the development of enhanced pesticide degradation phenomenon.

An understanding of the genetic basis of the mechanisms of how microorganisms biodegrade pollutants and how they interact with the environment is important for successful implementation of the technology for in situ remediation .

Different microbial enzymes with the capacity to hydrolyze pesticides have been identified, such as **organophosphorus hydrolase** (OPH; encoded by the *opd* gene). This gene has been found in bacterial strains that can use organophosphate pesticides as carbon source; these have been isolated in different geographic regions. These plasmids show considerable genetic diversity, but the region containing the *opd* gene is highly conserved. **Methyl-parathion hydrolase** (MPH; encoded by the *mpd* gene), Are *Pseudaminobacter*, *Achrobacter*, *Brucella* and *Ochrobactrum* genes, they were identified by comparison with the gene *mpd* from *Pleisomonas* sp. M6 strain, the gene for the organophosphorus hydrolase has 996 nucleotides, a typical promoter sequence of the promoter TTGCAA N17 TATACT from *E. coli*.

Genetic engineering

Microorganisms respond differently to various kinds of stresses and gain fitness in the polluted environment. This process can be accelerated by applying genetic engineering techniques. The recombinant DNA and other molecular biological techniques have enabled (i) amplification, disruption, and/or modification of the targeted genes that encode the enzymes in the metabolic pathways, (ii) minimization of pathway bottlenecks, (iii) enhancement of redox and energy generation, and (iv) recruiting heterologous genes to give new characteristics. Various genetic approaches have been developed and used to optimize the enzymes, metabolic pathways and organisms relevant for biodegradation. New information on the metabolic routes and bottlenecks of degradation is still accumulating, requiring the need to reinforce the available molecular toolbox. Nevertheless, the introduced genes or enzymes, even in a single modified organism, need to be integrated within the regulatory and metabolic network for proper expression.

Detoxification of organophosphate pesticides was the first demonstrated by genetically engineered microorganisms and the genes encoding these hydrolases have been cloned and expressed in *P. pseudoalcaligenes*, *Escherichiacoli*, *Streptomyces lividans*, *Yarrowialipolytica* and *Pichiapastoris*.

Another strategy that has been used is phytoremediation, the use of plants to clean-up polluted soil and water resources is recognized as an economically cheaper, aesthetically pleasing, and environmentally friendly 'Green technology. However, the limitation with plants is that they lack the catabolic pathways for complete degradation/mineralization of externally added organic compounds. The potential of plants to degrade organic pollutants can be further enhanced by engineering plants by introduction of efficient heterologous genes that are involved in the degradation of organic pollutants.

Unfortunately, the rates of hydrolysis several enzymes differ dramatically for members of the family of OP compounds, ranging from hydrolysis at the diffusion-controlled limit for paraoxon to several orders of magnitude slower for malathion, chlorpyrifos, and others pesticides. Although site-directed mutagenesis has been used to improve the substrate specificity and stereoselectivity of OPH, the ability to deduce substitutions that are important for substrate specificity is still limited to the active-site residues.

Two interesting papers have shown that an biological solution for efficient decontamination might be to direct evolution. Directed evolution has recently been used to generate OPH variants with up to 25-fold improvements in hydrolysis of methyl parathion , a substrate that is hydrolyzed 30-fold less efficiently than paraoxon, and other report the directed evolution of OPH to improve the hydrolysis of a poorly hydrolyzable substrate, chlorpyrifos (1,200-fold less efficient than paraoxon). Up to 700-fold improvement was obtained, and the best variant hydrolyzeschlorpyrifos at a rate similar to that of the hydrolysis of paraoxon by wild-type OPH.

The application of genomics and functional genomics

Metagenomics

The complexity of microbial diversity results from multiple interacting parameters, which include pH, water content, soil structure, climatic variations and biotic activity. Current estimates indicate that more than 99% of the microorganisms present in many natural environments are not readily culturable and therefore not accessible for biotechnology or basic research . During the last two decades, development of methods to isolate nucleic acids from environmental sources has opened a window to a previously unknown diversity of microorganisms. Analysis of nucleic acids directly extracted from environmental samples allows researchers to study natural microbial communities without the need for cultivation .

Each organism in an environment has a unique set of genes in its genome; the combined genomes of all the community members make up the “metagenome”. Metagenome technology (metagenomics) has led to the accumulation of DNA sequences and these sequences are exploited for novel biotechnological applications. Due to the overwhelming majority of non-culturable microbes in any environment, metagenome searches will always result in identification of hitherto unknown genes and proteins .

Functional genomics

In its broadest definition, functional genomics encompasses many traditional molecular genetics and biological approaches, such as the analysis of phenotypic changes resulting from mutagenesis and gene disruption. Functional genomics has emerged recently as a new discipline employing major innovative technologies for genome-wide analysis supported by bioinformatics. These new techniques include proteomics for protein identification, characterization, expression, interactions and transcriptomic profiling by microarrays and metabolic engineering. The application of proteomics in environmental bioremediation research provides a global view of the protein compositions of the microbial cells and offers a promising approach to address the molecular mechanisms of bioremediation. With the combination of proteomics, functional genomics provide an insight into global metabolic and regulatory networks that can enhance the understanding of gene functions.

The fundamental strategy in a functional genomics approach is to expand the scope of biological investigations from studying a single gene or protein to studying all the genes or proteins

simultaneously in a systematic fashion. The classic approach to assess gene function is to identify which gene is required for a certain biological function at a given condition through gene disruption or complementation. With the combination of technologies, such as transcriptomics and proteomics complementing traditional genetic approaches, the detailed understanding of gene functions becomes feasible .

Metabolic engineering combines systematic analysis of metabolic and other pathways with molecular biological techniques to improve cellular properties by designing and implementing rational genetic modifications . Understanding microbial physiology, will adapt to the host cells to support changes and become more efficient bioremediation processes, events that would be difficult to acquire during evolution.

With these new genomics tools scientists are in a better position to answer questions such as how oxygen stress, nutrient availability, or high contaminant concentrations along differing geochemical gradients or at transitional interfaces impact the organohalide respiring community structure and function. Ultimately, by tracking the overall microbial community structure and function in addition to key functional players, informed decisions can then be made regarding how to best manipulate the field conditions to achieve effective bioremediation of, e.g., pesticides.

Strategies to enhance the efficiency of pesticide degradation: Case cells immobilization

Cell immobilization has been employed for biological removal of pesticides because it confers the possibility of maintaining catalytic activity over long periods of time. Whole-cell immobilization has been shown to have remarkable advantages over conventional biological systems using free cells, such as the possibility of employing a high cell density, the avoidance of cell washout, even at high dilution rates, easy separation of cells from the reaction system, repeated use of cells, and better protection of cells from harsh environments. Previous reports have suggested that this higher productivity results from cellular or genetic modifications induced by immobilization. There is evidence indicating that immobilized cells are much more tolerant to perturbations in the reaction environment and less susceptible to toxic substances, which makes immobilized cell systems particularly attractive for the treatment of toxic substances like pesticides. In addition, the enhanced degradation capacity of immobilized cells is due primarily to the protection of the cells from inhibitory substances present in the environment. The degradation rates for repeated operations were observed to increase for successive batches, indicating that cells became better adapted to the reaction conditions over time .

There are two types of processes for cell immobilization: those based on physical retention (entrapment and inclusion membrane) and those based on chemical bonds, such as biofilm formation. In cell immobilization methods may be used various materials or substrates inorganic (clays, silicates, glass and ceramics) and organic (cellulose, starch, dextran, agarose, alginate, chitin, collagen, keratin, etc.) . Entrapment in polymeric gels natural has become the preferred technique for the immobilization of cells, however, immobilized cell on supports have been used more frequently in xenobiotics biodegradation as pesticides .

In order to degrade pesticides, is important to search for materials with favorable characteristics for the immobilization of cells, including aspects such physical structure, ease of sterilization, the possibility of using it repeatedly, but above all, the support must be cheap than allow in the future apply it for pesticide degradation. Thus, the methods can be grouped in two ways: the active that induce the capture of microorganisms in a matrix, and the passive that uses the tendency of microorganisms to attack surfaces either natural or synthetic, which form biofilms.

By the other hand, a biofilm can be defined as a coherent complex structure of microorganism organized in colony and cell products such as extracellular polymers (exopolymer), which either spontaneously or in forming dense granules, grow attached to a solid surface static (static biofilm) or in a suspension bracket. The biofilm formation process is performed in several steps starting with the attack or recognition to the surface, followed by growth and utilization of various carbon and nitrogen sources for the formation of products with adhesive properties. In parallel a stratified organization dependent on oxygen gradients and other abiotic conditions takes place. This process is known as colonization. Then an intermediate period of maturation of the biofilm is carried out which varies depending on the presence of nutrients from the medium or friction with the surrounding water flow. Finally, a period of aging biofilm where a detachment of cells may occur and colonize other surfaces.

The hydrodynamic plays an important role in the development of biofilm as these organizations develop in a solid-liquid interface, where the flow rate passing through it influences the physical detachment of microorganisms. They possess a system of channels that allow the transport of nutrients and waste; this is vital when modify the environment that deprives microorganisms of molecules necessary for their development. Other biofilm characteristic is its resistance to host defenses and antimicrobial agents. While the microorganism are susceptible to different control factors, the colonies organized and included in a exopolymer form an impermeable layer where only the most superficial microorganisms are affected. Also when released biofilm cells, they can travel and to be deposited on new niche maintaining the same characteristics of a biofilm adhered to a surface. Microorganisms are communicated with each other. This is what has been called quorum sensing and involves regulation and expression of specific genes through signaling molecules that mediate intercellular communication. This characteristic is dependent on cell density; for example, biofilm with a high cell density, it induces expression of resistance genes that provide protection and survival. Similarly, microorganisms can produce substances to promote the propagation of colonies and inhibit the growth of other leaving pathogens microorganisms in a more favorable position within the biofilm . The supports may be of synthetic or natural origin.

A material that has yielded good results in the degradation of mixtures of pesticides is the tezontle (in Nahuatl, tezt means rock and zontli means hair), that is a native volcanic rock of Morelos state (central Mexico). This rock is highly porous, provides a large contact surface, and can also be sterilized and reused. The presence of micropores allows the establishment of bacterial microcolonies. The immobilization method with this material is based on the colonization of the tezontlemicropores through the formation of a biofilm. Subsequently, a current with the pesticides wastes is passed through to allow the contact with the immobilized microorganisms, so this way the biodegradation can be executed. This strategy has been really efficient and is a tool that can be

used for the degradation of pesticides wastes. In our work group, a bacterial consortium was immobilized in a biofilm on tezontle and exhibited a considerable capacity for the removal of a mixture of organophosphate pesticides, which are the pesticides widely used in agriculture and stockbreeding in Mexico. In addition, this material with immobilized cells was packaged in an up-flow reactor, which was obtained the greater viability of the bacteria as more efficient removal of pesticides.

Furthermore, there are several reports that indicate a variety of materials that provide the features necessary to immobilize microorganisms. For example, the use of various plant fibers as support for immobilizing bacterial consortium to degrade xenobiotics has important advantages. The use of the natural structural materials such as petiolar felt-sheath of palm for the cell entrapment has added another dimension to a variety of immobilization matrices. The advantages accruable from such biostructures are reusability, freedom from toxicity problems, mechanical strength for necessary support, and open spaces within the matrix for growing cells thus avoiding rupture and diffusion problems. These have suggested the need to search for other types of biomaterials from diverse plant sources that may be used for cell entrapment.

The loofa sponge (*Luffacylindrica*) was used as carrier material for immobilizing various microorganisms for the purpose of either adsorption or degradation of various xenobiotics. This sponge have been used as natural support to immobilize various organisms such as *Chlorella sorokiniana, Porphyridium cruentrum, Penicillium cyrlopium, Funalia trogii* for nickel and cadmium II treatment, besides dyes and chlorinated substances. Loofa grows well in both tropical and subtropical climates and the sponges are produced in large quantities in Mexico where they are currently used for bathing and dish washing. They are light, cylindrical in shape and made up of an interconnecting void within an open network of matrix support materials. As a result of their random lattice of small cross sections coupled with very high porosity, their potentiality as carriers for cell immobilization is very high. The sponges are strong, chemically stable, and composed of interconnecting voids within an open network of fibers. Because of the random lattices of small cross sections of the sponges coupled with high porosity, the sponges are suitable for cell adhesion. This sponge was used by our work group and we found methyl parathion removal efficiencies of 75%.

Conclusions:

For the biological degradation of pesticides, it is important to understand the molecular mechanisms involved in enzymatic catalysis, which will be possible to design new alternatives and/or efficient tools for the treatment of pesticide residues or for the bioremediation of contaminated sites. This information could be used in the future to treat pesticide residues in the field (such as waste resulting after washing pesticide containers), or the obsolete pesticides. Moreover, in implementing strategies to increase the efficiency of degradation, such as cell immobilization (bacteria or fungi), we may have tools to abate the existence of obsolete pesticides and waste generated, it will reduce the danger of pesticides on the environment and health.

Probable Questions:

1. Briefly discuss how microorganisms help in biodegradation of pesticides.
2. What is the role of hydrolases in degradation of pesticides?
3. What is the role of Phosphotriesterases in degradation of pesticides?
4. What is the role of Esterases in degradation of pesticides?
5. What is the role of Oxidoreductases in degradation of pesticides?
6. What is the role of Mixed Function Oxidases in degradation of pesticides?
7. What is the role of Glutathione S-Transferase in degradation of pesticides?
8. How genetic engineering helps in degradation of pesticides?
9. What is metagenomics? How it is related to metabolism of pesticides?
10. What is functional genomics? How it is related to metabolism of pesticides?
11. Discuss case cell immobilization.

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett & Doull's Toxicology: The Basic Science by Curtis D. Klaassen

UNIT-X

Toxicological symptoms of Organochlorines, Organophosphorus, Carbamates, Pyrethroids, plant origin insecticides and other bioinsecticides

Objective: In this unit we will discuss about toxicological symptoms of various pesticides such as Organochlorines, Organophosphorus, Carbamates, Pyrethroids, plant origin insecticides and other bioinsecticides

Introduction:

Pesticide applicators should understand the hazards and risks associated with the pesticides they use. Pesticides vary greatly in toxicity. Toxicity depends on the chemical and physical properties of a substance and may be defined as the quality of being poisonous or harmful to animals or plants. Pesticides have many different modes of action, but in general cause biochemical changes which interfere with normal cell functions.

The toxicity of any compound is related to the dose. A highly toxic substance causes severe symptoms of poisoning with small doses. A substance with a low toxicity generally requires large doses to produce mild symptoms. Even common substances like coffee or salt become poisons if large amounts are consumed.

Toxicity can be either acute or chronic.

• Acute toxicity is the ability of a substance to cause harmful effects which develop rapidly following exposure, i.e. a few hours or a day.

• Chronic toxicity is the ability of a substance to cause adverse health effects resulting from long-term exposure to a substance.

There is a great range in the toxicity of pesticides to humans. The relative hazard of a pesticide is dependent upon the toxicity of the pesticide, the dose and the length of time exposed. The hazard in using a pesticide is related to the likelihood of exposure to harmful amounts of the pesticide. The toxicity of a pesticide can't be changed but the risk of exposure can be reduced with the use of proper personal protective equipment (PPE), proper handling and application procedures.

Pesticide Toxicity: Some pesticides are dangerous after one large dose (acute toxicity). Others can be dangerous after small, repeated doses (chronic toxicity).

Measuring Acute Toxicity (LD50And LC50 Values) : Acute toxicity of a pesticide refers to the effects from a single dose or repeated exposure over a short time (e.g. one day), such as an accident during mixing or applying pesticides. Acute toxicity is measured by LD50 and LC50 values. The LD50 value is the amount of pesticide (lethal dose) which kills 50% of the test animals. These treatments are through the skin (dermal) or through the mouth (oral). These values are given in milligrams per kilogram of body weight of the animal (mg/kg body wt.). A pesticide with a

lower LD50 is more toxic than a pesticide with a higher number because it takes less of the pesticide to kill half of the test animals. The LC50 value is a measure of the toxicity of a pesticide when test animals breathe air mixed with pesticide dust, vapours or spray mist. The LC50 is the concentration of pesticide which is lethal to 50% of a population of test animals and is usually determined for a specific exposure period (e.g. inhalation for 4 hours). The length of exposure is important because shorter exposure periods generally require higher pesticide concentrations to produce toxic effects. LC50 values for pesticides in air are expressed as the ratio of pesticide to air, in parts per million (ppm) or parts per billion (ppb). LC50 values are also determined for fish and aquatic organisms based on the concentration of pesticide in water.

Important characteristics to note about LD50 and LC50 values:

• they are based on a single dose (LD50) or short exposure (LC50);

• they do not indicate cumulative effects of small doses;

• they are an indicator of the amount of chemical required to kill or severely injure animals, and do not indicate the amount of chemical causing less severe toxic effects.

Chronic Toxicity

Chronic toxicity refers to the effects of long-term or repeated lower level exposures to a toxic substance, such as when a pesticide applicator is frequently wetted with spray during unsafe spray practices. The effects of chronic exposure do not appear immediately after first exposure and may take years to produce symptoms. Pesticides which have a tendency to accumulate, or which break down slowly in body tissues, usually represent the greatest chronic exposure hazard. Someone who is frequently exposed to low doses of such pesticides may develop symptoms of poisoning long after the first exposure. Chronic exposure may include chronic oral, chronic dermal or chronic inhalation poisoning. Very few pesticides now in use are known to cause chronic effects, if used according to label directions. However, a few pesticides are suspected or known to cause chronic illness in test animals or humans when exposure levels are high. The registration of some pesticides has been cancelled because the suspected or identified chronic effects represented a significant health hazard.

Exposure

There are three ways in which pesticides can enter the human body:
1. through the skin or eyes (dermal),
2. through the mouth (oral) and
3. through the lungs (respiratory or inhalation).

Dermal Exposure

In typical work situations, skin absorption is the most common route of pesticide poisoning. Absorption will continue as long as the pesticide remains in contact with the skin. The rate of absorption is different for each part of the body (see diagram). The head (especially the scalp and ear canal) and the genital areas are particularly vulnerable. Absorption may occur as a result of a splash, spill or drift when mixing, loading or applying a pesticide. Applicators may also be exposed to residues on application equipment, protective clothing or treated surfaces after pesticide application. Following exposure, residues can also be transferred from one part of the body to another. A cut or skin abrasion can greatly increase pesticide absorption.

The dermal toxicity of a pesticide depends on the pesticide formulation, the area of the body contaminated and the duration of the exposure. In general, liquids are more easily absorbed through the skin than wettable powders or granules. The hazard from skin absorption increases when workers are mixing pesticides because they are handling concentrated pesticides that contain a high percentage of active ingredient.

Protect yourself from dermal exposure. Follow these guidelines:

1. Wear protective clothing and equipment when using pesticides or repairing contaminated equipment.

2. Spray during periods when there is little or no wind.

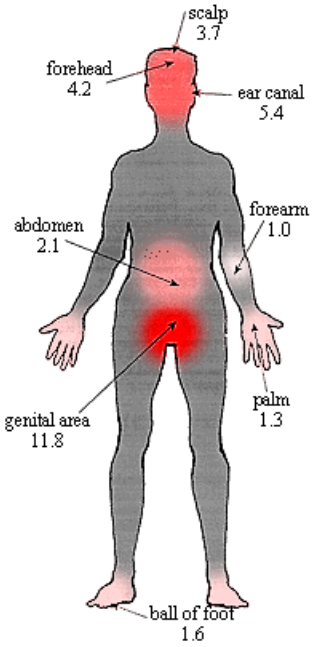
3. Do not re-enter a sprayed field without protective clothing until the re-entry time has elapsed.

4. If your clothes become contaminated, change immediately. Wash affected areas of the skin.

5. Change clothes as part of the clean-up after pesticide use at the end of the day.

6. Wash and shower after using pesticides.

7. Wear clean clothes at the start of each day during pesticide application.



Relative absorption rates, as compared to the forearm (1.0)

Eye Exposure

The tissues of the eyes are particularly absorbent. Enough pesticide can be absorbed through the eyes to result in serious or fatal poisoning. In addition, some pesticides may cause chemical injury to the eye itself. Eye protection is needed when measuring or mixing concentrated or highly toxic pesticides. Protective face shields or goggles should be worn whenever there is a chance that pesticide sprays or dusts may come in contact with the eyes.

Protect yourself from eye exposure. Follow these guidelines:

1. Always wear eye protection when you measure or mix pesticides.

2. Always wear eye protection when pesticide sprays or dusts may contact your eyes.

3. Do Not wipe your eyes with contaminated gloves or hands.

4. Be prepared to respond to accidental eye exposure quickly

Oral Exposure

Pesticides taken through the mouth result in the most severe poisoning, compared to other types of exposure. Pesticides can be ingested by accident, through carelessness, or intentionally. The most frequent cases of accidental oral exposure are those in which pesticides have been stored in an unlabelled bottle or food container. There are many cases where people, especially children, have been poisoned by drinking pesticides from a soft drink bottle. People have also been poisoned by drinking water stored in contaminated containers. Workers handling pesticides or application equipment can also consume excessive levels of pesticides if they do not wash their hands before eating or smoking.

Protect yourself from oral exposure. Follow these guidelines:

1. Always store pesticides in their original labeled containers.

2. Never put pesticides in an unlabelled bottle or food container.

3. Never use your mouth to clear a spray hose or nozzle, or to begin siphoning a pesticide.

4. Always wash after handling pesticides and before eating, drinking, smoking, or using the toilet.

5. Never leave pesticides unattended.

6. Avoid splashes or dusts when mixing pesticides.

7. Label your pesticide measuring containers.

Respiratory Exposure

Certain pesticides may be inhaled in sufficient amounts to cause serious damage to nose, throat and lung tissues, or to be absorbed through the lungs into the bloodstream. Vapours and very small particles pose the most serious risks. The hazard of poisoning from respiratory exposure is great because of the rapid and complete absorption of pesticides through lung tissues.

Lungs may be exposed to pesticides by inhalation of powders, airborne droplets or vapours. Working with wettable powders can be hazardous because the powder may be inhaled during mixing operations and usually contains concentrated pesticide active ingredient. The hazard from inhalation of pesticide spray droplets is fairly low when dilute sprays are being applied with conventional low pressure application equipment. This is because most droplets are too large to remain airborne and be inhaled. However, when high pressures are used or ultra-low volume (ULV) or fogging equipment are used, the potential for respiratory exposure is increased. The droplets produced during these operations are in the mist or fog size-range and can be carried on air currents for a considerable distance.

Many pesticides give off a vapour when exposed to air. As temperatures increase, vapour levels of many pesticides increase. Fumigants are used because their toxic vapours are desirable for pest control. They also have the highest hazard with respect to worker exposure to vapours. Some non-fumigant pesticides are toxic to pests as liquid or solid formulations, but also give off vapours which could be toxic to applicators or bystanders. The hazard is greatest in enclosed spaces where there is little air movement. For example, high vapour levels could result from a spill in an unventilated storage area or application in a confined space such as a greenhouse. Air currents due to wind or ventilation can substantially reduce vapour levels.

Many pesticides that produce vapours provide a warning of their presence by their smell or by causing irritation of the eyes, nose and throat. However, some pesticide vapours have little smell and provide little warning of their presence.

Pesticides with high vapour hazards will have label directions to use respiratory protection equipment. Protect yourself from respiratory exposure. Follow these guidelines:

1. Wear an appropriate and properly fitting respirator: 1. If it is required on the label;

2. If pesticides are used or mixed in poorly ventilated areas;

3. If there is a possibility of inhaling spray droplets, vapour, or powder.

2. Do not re-enter a treated area too soon. Follow the re-entry guidelines on the label.
3. Ventilate greenhouses or enclosed structures after pesticide application, before re-entry.
4. Do not apply pesticides when air temperatures are above 30°C.

Toxicity of pesticides can vary depending on the type of exposure; dermal, oral or respiratory (inhalation), but it is important to remember that, in each case, the danger usually increases as concentration and duration of exposure increases. The longer a pesticide remains on the skin or in eyes, or the longer it is inhaled, the greater the damage that is likely to result.

Pesticide Toxicity

Pesticide toxicity to people can be measured several ways, although it is not easy, since humans cannot be usedas test subjects. Because of this, other animals, such as rats, are used. If a pesticide is poisonous to rats, however, it is not necessarily poisonous to dogs, cows, wildlife, or people.
Toxicity studies are only guidelines: they are used to estimate how poisonous one pesticide is compared with another. Some pesticides are dangerous in one large dose or exposure, which is known as acute toxicity. Others can be dangerous after small, repeated doses, called chronic toxicity.

Measuring toxicity: The LD_{50} (lethal dose, 50 percent) describes the dose of pesticide that will kill half of a group of test animals (rats, mice, or rabbits) from a single exposure or dose by a dermal, oral, or inhalation route. The LD_{50} is thedose per unit of body weight, such as milligrams per kilo- gram (mg/kg). A pesticide with a lower LD_{50} is more toxic than a pesticide with a higher number because it takes less of the pesticide to kill half of the test animals. For example, a pesticide with an LD_{50} of 10 mg/kg is much more toxic than a pesticide with an LD_{50} of 1,000 mg/kg. The toxicity of fumigant pesticides is described in terms of the concentration of the pesticide in the air, LC_{50} (lethal concentration, 50 percent). Researchers use a similar systemto test the potential effects of pesticides on aquatic organismsin water.

Acute toxicity of a pesticide refers to the effects from a single exposure or repeated exposures over a short time, such as an accident when mixing or applying pesticides. Various signs and symptoms are associated with acute poisonings. A pesticide with a high acute toxicity can be deadly even if a small amount is absorbed. Acute toxicity can be measured in terms of oral, dermal, or inhalation.

Chronic toxicity refers to the effects of long-term or repeated low-level exposures to a toxic substance. The effects of chronic exposure do not appear immediately after the first exposure: years may pass before signs and symptoms devel- op. Possible effects of long-term exposure to some pesticidesinclude:

- cancer, either alone or in combination with otherchemicals;
- genetic changes;

- birth defects in offspring following exposure of thepregnant female;
- tumors, not necessarily cancerous;
- liver damage;
- reproductive disorders;
- nerve damage;
- interfering with the endocrine system (hormones and glands that regulate many body functions); and
- sensitivity or allergic reactions such as irritation of theskin and/or respiratory tract.

Signal Words

Nearly all pesticides are toxic at some dose. They differ only in the degree of toxicity. All pesticides are potentially dangerous to people who have had excessive exposure. The label of a pesticide product will have one of three signal words that clearly indicates the degree of toxicity associatedwith that product *(Table I)*. The signal word indicates the degree of risk to a user, not the effectiveness of the product in controlling the target pest. The signal word "Caution" is not required to appear on the label of a relatively nontoxic pesticide, but is required for slightly toxic pesticides.

Recognizing Signs and Symptoms of Poisoning

Anyone who may be exposed to pesticides or is working with someone who may be exposed should be aware of the signs and symptoms of pesticide poisoning. Signs, such as vomiting, sweating, and pinpoint pupils, can be observed
by others. Symptoms are any changes in normal condition that can be described by the victim of poisoning, including nausea, headache, weakness, dizziness, and others. Knowingthese signs and symptoms will allow for prompt treatment and help prevent serious injury. People who are frequently involved with pesticides should become familiar with the following important steps.

1. Recognize the signs and symptoms of pesticide poisoningfor those pesticides commonly used, or to which people may be exposed. Often, pesticide poisoning resembles flusymptoms.
2. If you suspect poisoning due to a pesticide, get immediatehelp from a local hospital, physician, or the nearest PoisonControl Center.
3. Identify the pesticide to which the victim was exposed, giving the chemical name and Environmental Protection Agency (EPA) registration number found on the label, if possible. Provide this information to medical profession-als.
4. Have a copy of the pesticide label available when medicalattention begins. The label provides useful information tothose assisting a victim of pesticide poisoning. The SafetyData Sheet (SDS) has helpful information as well; supply-ing the SDS to medical professionals is required when

theWorker Protection Standard applies.

5. Know emergency measures you can undertake until help arrives or the victim can be taken to the hospital. Both firstaid and medical treatment procedures are listed on the product label.

Recognizing Common Pesticide Poisonings

All pesticides in a given chemical group generally affectthe human body in the same way. Severity of the effects, however, varies depending on the formulation, concentration, toxicity, and route of exposure of the pesticide. Therefore, it isimportant to know both the type of pesticide being used and the signs and symptoms associated with poisoning from it.

Pesticides presenting the greatest potential health risks and those in which the mode of action is better understood are covered in the following sections. Categories of pesticides with similar signs and symptoms are covered together. The listings of pesticides in *Tables III, IV, and V* are not necessarily complete, nor do they guarantee that the product is currently registered. They do, however, represent products that are or have been used in Nebraska. EPA and Nebraska Department of Agriculture maintain registrations for pesticide products. EPA attempts to discontinue use of the most toxic products and replace them with less toxic products. Pesticides mentioned in this publication may not currently be registered for use in Nebraska, but still may be found onsome storage shelves. Therefore, they still present risk, so signs and symptoms are included. Mention of a trade name does not constitute endorsement of a product, nor does omission constitute criticism.

Included are some findings from the Agricultural Health Study (AHS), involving 90,000 applicators and spouses from Iowa and North Carolina. The AHS states that the study "began in 1993 with the goal of answering important questions about how agricultural, lifestyle and genetic factors affect the health of farming populations. The study is a collaborative effort involving investigators from the National Cancer Insti-tute, the National Institute of Environmental Health Sciences, the Environmental Protection Agency, and the National Institute for Occupational Safety and Health." The AHS reliesmainly on participant memory to determine dose-related exposures. Also, keep in mind that an association does not automatically mean there is a cause-and-effect relationship. An association shows that more research is needed.

Some general findings of the AHS are listed below.

- Farmers have lower rates of many diseases compared withthe rest of the population, perhaps because they are less likely to smoke and are more physically active.
- Farmers have a higher risk for developing some cancers,including prostate cancer.
- Gloves matter. Use of chemical-resistant gloves can reducepesticide exposure 50 to 80 percent.
- Accidental high pesticide exposure events may affecthealth later in life.

Insecticides

Insecticides have many different modes of action. Some act on the insect's nervous system. Others slow the production of energy that an insect needs to survive. Another type slows or stops production of chitin, a major component of an insect exoskeleton, so the insect can't molt. Insect growth regulators, another type, also may prevent an insect from molting or keep it from maturing and reproducing. Some insecticides disrupt the water balance in an insect, causing rapid water loss and eventual death. Modes of action involving the nervous system and energy production may affect not only insects, but other animals as well. Insecticides such as insect growth regulators typically are specific to insects. The following is a list of insecticides grouped by their chemical makeup.

Organophosphate and Carbamate Insecticides

Many cases of pesticide poisoning involve organophosphate or carbamate insecticides. Both chemical groups affect humans by inhibiting acetyl cholinesterase, an enzyme essential for proper function of the nervous system. Without acetyl cholinesterase, nerve impulses continue and the victim has uncontrolled twitching. The AHS shows that allergic asthma in men and women may be associated with poisoning caused by these insecticides. EPA registration has been cancelled for some; others are being phased out or are not used as much as other insecticides.

The effects of these materials, particularly organophosphate insecticides, are rapid. Signs and symptoms begin shortly after exposure, and in cases of acute poisonings, during exposure. Exposure to either of these insecticide classes may pose special risks to people with reduced lung function, seizures, or other conditions. In some cases, consuming alcoholic beverages may worsen pesticide effects. The onset of symptoms in milder exposures usually occurs within four hours, but can occur up to 12 hours after exposure. Diagnosis of a suspected poisoning must be rapid. Signs and symptoms associated with mild exposures to organophosphate and carbamate insecticides include headache; fatigue; dizziness; loss of appetite with nausea,

stomach cramps, and diarrhea; blurred vision associated with excessive tearing; contracted pupils; excessive sweating and salivation; slowed heartbeat, often less than 50 beats per minute; and rippling of surface muscles just under the skin. Some symptoms may be mistaken for those of flu, heat stroke, heat exhaustion, or upset stomach. Moderately severe organophosphate and carbamate insecticide poisoning cases exhibit all the signs and symp- toms found in mild poisonings listed previously. In addition, a victim may be unable to walk, complain of chest discomfort and tightness, have marked pinpoint pupils, exhibit muscle twitching, and have involuntary urination and bowel movements. Signs of severe poisonings include incontinence, unconsciousness, and seizures.

The order in which these symptoms appear may vary, depending on how contact is made with the pesticide. If the product is swallowed, stomach and other abdominal manifestations commonly appear first; if it is absorbed through the skin, gastric and respiratory symptoms tend to appear at the same time. Fortunately, antidotes are available for victims of organophosphate or carbamate poisoning at emergency treatment centers, hospitals, and many physicians' offices. As with all pesticide poisonings, prompt assistance is critical. If a pesticide is swallowed, obtain prompt medical treatment. If der- mal exposure has occurred, remove contaminated clothing,

wash exposed skin, and seek medical care.

Organochlorine Insecticides

EPA has sharply curtailed the availability of many organochlorines because they persist in the environment. Organochlorines are formed from carbon and chlorine; ex- amples include DDT, chlordane, dieldrin, aldrin, and lindane. Although few are available for purchase or registered for use, some organochlorine insecticides still may be present in storage areas. In addition, organochlorines such as dioxins and polychlorinated biphenyls (PCBs) are in the environment due to drift from application, spills, leaks, and improper disposal of industrial wastes. Because of the persistence of organochlorines, traces of them still can be found in sediment, water, and living organisms, even though most use wasbanned in the U.S. decades ago.

Organochlorines affect the nervous system as stimulants or convulsants. Nausea and vomiting commonly occur soon after ingesting organochlorines. Other early signs and symptoms include apprehension (feelings of suspicion or fear of the future), excitability, dizziness, headache, disorientation,weakness, a tingling or pricking sensation on the skin, and twitching muscles. Loss of coordination, convulsions (violent seizures with involuntary jerky movements that cause the victim to stop breathing) similar to epileptic seizures, and unconsciousness often follow. When chemicals are absorbed through the skin, the first symptoms may include apprehension, twitching, tremors, confusion, and convulsions. Chronic exposure may lead to cancer, birth defects, and genetic mutations. AHS states that the risk of developing diabetes and thyroid disease may increase for those who use some organochlorine chemicals.

No specific antidotes are available for organochlorine poisoning. People assisting a victim should wear chemical- resistant gloves and be careful to avoid being exposed to the pesticide. Remove contaminated clothing immediately and bathe and shampoo the person vigorously with soap and water to remove pesticides from the skin and hair. If the pesticide has been swallowed, empty the stomach as soon as possible by giving the conscious patient syrup of ipecac and water, or by inserting a clean finger into the throat while thevictim is turned to one side, facing the floor. Never induce vomiting when a victim is unconscious: inhaling vomit maycause suffocation.

Pyrethroid Insecticides:

Pyrethroids are synthetically produced compounds thatmimic the chemical structure of naturally occurring pyrethrins found in a specific type of chrysanthemum plant. As with organophosphates and carbamates, pyrethroids affect the insect's nervous system, but in a different way: they are not cholinesterase inhibitors. In the U.S., pyrethroids have widespread usage as they have replaced many organophosphates. Of all pesticides used,pyrethroid exposures are the most often reported. Risk of pyrethroid poisoning through inhalation and dermal absorption is low. Few poisonings of humans by pyrethroids have been documented, although exposures associated with Total Release Foggers, discussed later in this publication, have caused problems. Dermal contact may result in skin irritation such as stinging, burning, itching, and tingling progressing to numbness. Some people experience a range of allergic reactions from pyrethroids. Repeated exposures may increasethe intensity of the reaction. Although some pyrethroids may be toxic orally, ingestingthis

type of insecticide usually presents relatively little risk. Occasionally, a large dose may cause loss of coordination, tremors, salivation, vomiting, diarrhea, and irritability to sound and touch. Most pyrethroids are promptly excreted bythe kidneys.

Biological Insecticides

Insecticides produced from plant materials or bacteriaare called biological insecticides.
Azadirachtin, derived from the Neem tree, is an insect growth regulator that interferes with the insect molting process. For humans, exposure to azadirachtin causes slight skin and gastrointestinal irritation. Stimulation and depression of the central nervous system also have been reported.

Eugenol is derived from clove oil and is used as both an insect attractant and insecticide. In humans, exposure to skin or eyes can cause irritation and burns. Ingestion of extremely large doses may result in liver problems and coma.

Pyrethrum and pyrethrins. Pyrethrum is found in theflowers of *Chrysanthemum cinerariaefolium*. Crude pyre- thrum is a dermal and respiratory allergen for people. Skinirritation and asthma have occurred following exposures. Refined pyrethrins are less allergenic, but appear to retain some irritant and/or sensitizing properties.

In cases of human exposure to commercial pyrethrum products, realize that other toxicants may be present and listed on the label. Synergists may be added to insecticide products to enhance the killing power of the active ingredi ent. Synergists such as piperonyl butoxide, discussed later, have low toxic potential in humans, but organophosphates or carbamates included in the product may have significant toxicity. Pyrethrins themselves do not inhibit the cholinesterase enzyme.

Rotenone is a naturally occurring substance found in several tropical plants. Until 2011, it was formulated as dusts, powders, and sprays for use in gardens and on food crops. The AHS showed a relationship between exposure to rotenone and the incidence of Parkinson's disease. More research is needed to reach any conclusions on the specifics of that relationship. Rotenone manufacturers have voluntarilystopped producing the pesticide for all uses except to manageundesirable fish species. Rotenone is now a restricted use pesticide.

Antibiotics include abamectin, *Bacillus thuringiensis* (Bt), spinosad, and streptomycin. These compounds are prac tically nontoxic to humans. In studies involving deliberate ingestion by human subjects, slight inflammation of the gut occurred. Antibiotic insecticides in the form of emulsifiable concentrates may cause slight to moderate eye irritation and mild skin irritation due to the solvent carriers. Antibiotic pesticides are different from antibiotics taken by people to cure bacterial infections.

Inorganic Insecticides

Boric acid and borates. Boric acid, derived from borax and usually combined with an anti-caking agent, is commonly used to kill cockroaches. It can be harmful to humans if acci- dentally ingested, especially by children. Avoid inhaling the dust during application. The label may indicate that respiratory protection is required. Inhaled borax dust irritates the respiratory tract and causes shortness of breath. Borax dust is moderately irritating to skin. Infants have developed a red skin rashthat most often affects palms, soles of the feet, buttocks, and scrotum in severe poisonings. The skin developed a "boiled lobster appearance" followed by extensive skin peeling.

Diatomaceous earth (DE) is mined from the fossilized silica shell remains of diatoms, which are microscopic sea animals. Labels may refer to this ingredient as silicon diox- ide, or silicon dioxide from diatomaceous earth. DE is used commercially to control crawling insects, such as cockroaches, ants, and insects that infest grain. It is virtually nontoxic to humans. Avoid inhaling diatomaceous earth, however, as itcan irritate eyes and lungs.

Silica gel is a nonabrasive, chemically inert substance used as a dehydrating agent because the small particles ab- sorb moisture and oils. Avoid inhaling the dust. Some grades of diatomaceous earth contain small amounts of crystalline silica, known to cause a respiratory disease called silicosis, and cancer. The cancer risk depends on the duration and level of exposure. Pesticide-quality diatomaceous earth and silica gel are amorphous (non-crystalline), and do not cause silicosis or cancer.

Sulfur is moderately irritating to skin and has been associated with skin inflammation. Dust is irritating to the eyesand respiratory tract. If swallowed, it acts like a strong laxative.

Other Insecticides

Neonicotinoids were introduced in the 1990s. Chemically similar to nicotine, they have a lower toxicity to humans than do organophosphates and carbamates. Imidacloprid and thiamethoxam are used to control termites, turf insects, and some crop insects. Neonicotinoids are being studied for theirrisk to honeybees and other pollinators. Farm workers reported skin or eye irritation, dizzi- ness, breathlessness, confusion, or vomiting after they were exposed to pesticides containing imidacloprid. Similar symptoms, along with increased heart and breathing rates, also were noted after a victim ingested a product containing imidacloprid; the victim suffered severe cardiac toxicity anddeath 12 hours after oral exposure.

Pyrazoles: Fipronil is a moderately toxic pyrazole thatmay cause mild irritation to the eyes and skin. It is used to control termites (Termidor®, Taurus™), cockroaches (Com- bat®, Maxforce®), certain insect pests of corn, and fleas andticks of cats and dogs (Frontline®, Effipro®, PetArmor™). Lab animals exhibited reduced feeding, reduced urination, increased excitability, and seizures following a toxic oral dose. After ingesting fipronil, humans have reported sweating, nausea, vomiting, headaches, abdominal pain, dizziness, agitation, and weakness. Direct, short-term

contact with skin can result in slight skin irritation. Inhalation or dermal contact while spraying fipronil for five hours may have caused head-ache, nausea, dizziness, and weakness. Symptoms developed two hours after spraying and then disappeared. The National Pesticide Information Center reports that signs and symp- toms from a brief exposure to fipronil generally improve andclear up without treatment.

Pyrroles: Chlorfenapyr (Phantom®, Pylon®) is the only active ingredient in this group. It is formulated to control ants, cockroaches, termites, and some insect and mite pestson fruits and vegetables. It is slightly toxic if swallowed orcontacts skin, and can moderately irritate eyes and skin.

Tetronic acids: Spiromesifen is the sole active ingredient in this group. It is used to control mites and whiteflies on some vegetable crops (Oberon®) and ornamental trees (Forbid™, Judo™, Oberon®). No indication of eye irritation hasbeen reported.

Tetramic acids: Spirotetramat (Kontos®, Movento®) is a systemic insecticide that controls a number of major suckinginsects and mites that are pests of trees, vegetables, potatoes,and other plants. Some products with tetramic acids may cause moderate eye irritation. Prolonged or repeated skin contact may cause allergic reactions in some individuals.

Insect Growth Regulators:

Insect growth regulators (IGR) act on insects in differ- ent ways. Those that mimic juvenile hormones keep insects in immature stages and prevent insect reproduction. Chitin synthesis inhibitors prevent insects from molting and grow- ing into adults. In general, IGRs are very low in toxicity and cause mild skin irritation with limited exposure. No human poisonings or adverse reactions in exposed workers have been reported.

Mosquito Repellents

Diethyltoluamide (DEET) was developed by the U.S. Army in 1946 as an insect repellent and has been available to the general public since 1957. Products containing DEET (Detamide®, OFF!®) have been effective and generally well tolerated when applied to human skin. If left on skin for an extended period, some people have experienced irritation, redness, a rash, and swelling. Tingling and mild irritation have occurred following repeated application. In some cases,DEET has caused skin irritation and worsened preexisting skin disease. It is very irritating to eyes but not corrosive.
When swallowed, it has caused nausea and vomiting.

Serious adverse effects have occurred when DEET was used under hot, humid conditions and not washed off before going to sleep. The skin became red and tender, then blisteredand formed ulcers, leaving painful weeping bare areas that were slow to heal. Permanent scarring resulted from most of these severe reactions. Very rarely, seizures in people have been associated with

exposure to DEET. Most have occurred after drinking products with DEET or using the products inways that do not follow label directions.

Exercise great caution when using DEET on children: only use products containing lower concentrations. The American Academy of Pediatrics (AAP) recommends against using any repellent on infants 2 months of age or younger. The AAP cautions parents not to use DEET on the hands of a child and to avoid applying it to areas around a child's eyes and mouth. Consider applying DEET only to clothing, using as little repellent as possible. If a child experiences a headache or any kind of emotional or behavioral change, immediately discontinue using DEET. Limited information is available on childhood responses to DEET from research or Poison Control Center reports. Most adverse responses were the result ofimproper use or accidents.

Picaridin, a synthetic compound first made in the 1980s, resembles a natural compound found in the group of plants used to produce black pepper. Widely used as an insect repel-lent in Europe and Australia, picaridin has been available in the U.S. only since 2005. Although uncommon, some peoplehave experienced skin irritation. Picaridin also may cause irritation if it gets into a person's eyes. Rats lost weight and their kidneys were affected when fed large doses of picaridin. The material is considered practically nontoxic if inhaled. While children may be especially sensitive to pesticides compared with adults, no data suggest that children have increased sensitivity specifically to picaridin.

Oil of Citronella was registered in 1948 as an insect and animal repellent. It is found in many familiar insect repellentproducts, including candles, lotions, gels, sprays, and towel wipes. These products vary in effectiveness and may repel various insects, such as mosquitoes, biting flies, and fleas. When used according to the label, citronella products are not expected to harm humans, pets, or the environment. The only concern in studies involving laboratory animals is skin irritation. The EPA requires precautionary labeling because some citronella products are applied to human skin. Citronella is not expected to pose health risks to people, including children and other sensitive populations, if used according tolabel instructions.

Fumigants

Fumigants deliver the active ingredient to the target site in the form of a gas. Fumigants can completely fill a space, and many have tremendous penetrating power. They can be used to treat objects such as furniture, structures, grain, and soil for insects and other pests. Fumigants are among the most hazardous pesticide products to use, due to danger of inhalation.

Various fumigants produce differing physiological effects. Headache, dizziness, nausea, and vomiting are common early signs and symptoms of excessive exposure. Prompt medical treatment is critical with fumigant poisoning. After donning appropriate PPE, immediately move a victim of fumigant inhalation to fresh air. Keep the individualquiet in a semi-reclining position even if initial signs and symptoms are mild. If breathing has stopped, give mouth-to-mouth or mouth-to-nose resuscitation. If the victim has no pulse, immediately give cardiopulmonary resuscitation (CPR) using chest compression. Some fumigant products, along with signs and symptoms of poisoning, are listed below.

Chloropicrin causes severe irritation of the upper respiratory tract, eyes, and mucous membranes. Symptoms of exposure include burning eyes, tearing, coughing, difficulty breathing, headaches, nausea, and vomiting. Chloropicrin may be a stand-alone fumigant or may be combined with other fumigants to increase their potency. Chloropicrin can cause eye irritation and tearing in concentrations as low as 0.15 ppm. Some fumigant formulations include small amounts as a warning agent to clear people from an area.

Sulfuryl fluoride (Vikane®) poisoning symptoms include depression, slowed walking pattern, slurred speech, nausea, vomiting, stomach pain, stupor, itching, numbness, twitching, and seizures. Inhalation of high concentrations may irritate the respiratory tract and may be fatal due to respiratory failure. Sulfuryl fluoride almost always is applied with chloropicrin, so the first signs of poisoning are often associated with severe irritation of the eyes and mucous membranes. Skin contact with gaseous sulfuryl fluoride normally poses no hazard, but contact with liquid sulfuryl fluoride can cause pain and frost-bite due to cold temperatures from rapid evaporation.

Phosphine fumigants, such as aluminum and magnesium phosphide (Phostoxin®, PhosFume®, Fumitoxin®, and FumiCel®) affect cell function in the liver and lungs. Mild exposure is signaled by a sensation of cold, chest pains, diarrhea, and vomiting. Exposures that are somewhat more serious will be evidenced by cough, tightness in the chest, difficulty breathing, weakness, thirst, and anxiety. Signs and symptoms of severe exposure include stomach pain, loss of coordination, blue skin color, pain in limbs, enlarged pupils, choking, fluid in the lungs, and stupor. Severe poisonings can lead to seizures, coma, and death.

Methyl bromide (Metabron, Meth-O-Gas®) affects the central nervous system, lungs, heart, and liver. People poisoned by methyl bromide experience the common signs and symptoms of fumigant poisoning along with abdominal pain, weakness, slurred speech, mental confusion, muscle twitching, and convulsions similar to epileptic seizures. Methyl bromide is corrosive to eyes; damage may have a delayed on- set after exposure. Some liquid fumigants cause skin injuries such as redness or blisters that rupture, leaving raw skin or deep ulcers.

Acrolein (Magnacide H®) is an extremely irritating gas used as an aquatic herbicide. Inhaling vapors causes irritation in the upper respiratory tract, which may lead to a buildup of fluids in and narrowing of the air passages. Acrolein is corrosive to the eyes. If ingested, it attacks the stomach lining, resulting in open sores and cell death. Contact with skin may cause blistering.

Dazomet (Basamid® G) is a granular soil fumigant. It is used to sterilize soil to eliminate weeds, nematodes, and soilborne diseases. Dazomet is highly toxic if swallowed and can be fatal. Frequent or prolonged exposure to skin can result in irritation or more serious skin problems for some individuals. Exposure to the eyes can cause irreversible eye damage. Inhalation can cause a variety of acute and chronic lung conditions, including local irritation, inflammation, fluid buildup, and lung disease.

Metam sodium (Vapam®) is a soil fumigant used to kill fungi, bacteria, weed seeds, nematodes, and insects. When combined with water, it produces a gas that is very irritating to respiratory mucous membranes, eyes, and lungs. Inhalation can cause severe respiratory distress, including coughing blood and frothy sputum. It can only be used outdoors, and precautions must be taken to avoid inhaling the gas.

Dichloropropene (Telone®) is very irritating to skin, eyes, and the respiratory tract. Inhalation may cause spasms of the bronchi, where air passes into lungs. Although limited data for humans exist, animals have experienced liver, kidney, and cardiac damage. Most dichloropropene products contain chloropicrin; severe irritation of the eyes and mucous membranes is an early sign of exposure. Apparently, risk for oral toxicity is low for humans unless large quantities of dichloropropene are ingested.

Rodenticides

Pesticides designed to kill rodents pose particular risks to humans. Since they are designed to kill mammals, their mode of action is toxic to humans as well. In addition, rodents often live near humans and other mammals, so accidental exposure to bait is a risk. The active ingredients of rodenticides fall into three categories:

- First-generation anticoagulants,
- Second-generation anticoagulants, and
- Non-anticoagulants.

Anticoagulants slow the blood's ability to clot. Death can result from excessive bleeding. First-generation anticoagulants were developed during World War II, with others appearing before 1970. Rodents die after eating a number of doses, and death usually occurs within five to seven days.

Second-generation anticoagulants were initially developed in the 1970s. They are more hazardous–more likely to kill after a single feeding. Their increased toxicity increases the risk to humans. Also, second-generation anticoagulants remain in body tissues longer than first-generation anticoagulants. Second-generation anticoagulants are designed to poison the rodent as soon as it feeds (one dose), but death may occur after several days. During that time, the rodent can feed many times, meaning that when the rodent finally dies, the residues in its carcass might be much higher than the lethal dose. Predators or scavengers that eat the carcass might consume enough of the poison to suffer harm. This is called secondary poisoning.

Non-anticoagulants affect the nervous system or other body organs. They do not have an effect on clotting of blood. The first non-anticoagulant rodenticides were developed for use against rodents that were resistant to anticoagulants.

First-generation Anticoagulants

Coumarins are anticoagulants: they slow blood's ability to clot, and disrupt capillary and liver function. Examples include warfarin (Kaput® Mole Gel Bait and Mouse Blocks). The main signs and

symptoms are nosebleeds, bleeding gums, blood in the urine, tar-colored feces, and large irregular blue-black to greenish-brown spots on the skin. VitaminK is an antidote.

Indandiones include chlorophacinone (Rozol®) and diphacinone (Ditrac®, d-CON® IX and XI, Kaput Pocket Gopher Bait and Prairie Dog Bait, Ramik®). Main signs and symptoms are similar to coumarin compounds, but some indandiones cause nerve, heart, and blood system damage in laboratory rats, leading to death before hemorrhage occurs. None of these signs and symptoms have been reported in human poisonings. Vitamin K is an antidote.

Second-generation Anticoagulants

Coumarins also may be second-generation anticoagulants, developed with increased toxicity. Examples include brodifacoum (Jaguar®, Talon®, WeatherBlok®), and bromadiolone (Contrac®, Maki®). The main signs and symptoms are nosebleeds, bleeding gums, blood in the urine, tar-colored feces, and large irregular blue-black to greenish-brown spots on the skin. Vitamin K is an antidote.

Non-anticoagulants

Benzenamines: Bromethalin (Tomcat® Mouse Killer), the only chemical in this class of rodenticide, acts on the central nervous system. Possible signs and symptoms of exposure to this compound include skin and eye irritation, headache, confusion, muscle twitching, convulsive seizures, and difficulty breathing. Bromethalin poisoning in dogs usually results in paralysis or convulsions, and sometimes, abdominal swelling or bloating.

Cholecalciferols. (Terad 3 Blox®, d-CON XVI and XVII). This rodenticide is an activated form of vitamin D, and affects the liver and kidneys. It causes elevated levels of calcium in the blood; rodents die due to problems such as blockages in the circulatory system. For humans, signs and symptoms include fatigue, headache, weakness, and nausea. This roden- ticide has poisoned dogs and cats. A high dosage may cause death in humans. Labels caution against direct contact with skin; gloves are required when handling bait or retrieving carcasses.

Strychnine is not easily absorbed through the skin nor does it accumulate in the human body. When ingested, however, it acts on the central nervous system within 10 to 30 minutes. Convulsions also can occur. Treatment of strych- nine poisoning is geared toward eliminating outside stimuli. If strychnine poisoning occurs, place the victim in a warm, dark room to reduce outside stimuli that trigger convulsions. Consequently, in the case of strychnine poisoning, bring medical help to the victim rather than transporting the victim to a medical center, because movement will trigger theconvulsions.

Zinc phosphide causes severe irritation if ingested. It reacts with water and stomach juices to release phosphine gas, which enters the bloodstream and affects lungs, liver, kidneys, heart,

and central nervous system. Zinc phosphide can be absorbed through skin, and inhaled from fumes. With repeated exposure, it accumulates in the body to dangerous levels. Signs and symptoms of mild zinc phosphide poisoning include diarrhea and stomach pains. In more severe cases, nausea, vomiting, chest tightness, coldness, loss of conscious- ness, coma, and death can occur from fluid buildup in lungs, and liver damage. No antidote for zinc phosphide poisoning exists. It is a slow-acting material, which allows time to get the victim medical assistance.

Herbicides

Herbicides kill weeds by affecting metabolic processes in plants. Therefore, risk to humans and other mammals is relatively low. Some herbicides, however, can pose a risk of poisoning if not handled according to label directions. Regardless of their chemical structure, the vast majority of herbicides often affect the human body in a similar way. In general, they can irritate the skin, eyes, and respiratory tract. Always read and follow label recommendations carefully to avoid any of these health risks. Herbicides that present the greatest potential health risks are covered in the next four sections.

Bipyridyl Herbicides

Diquat and **paraquat** are the most common bipyridyl herbicides. Paraquat is more toxic than diquat and produces chronic abnormal cell growth in lungs, cornea and lens of the eyes, nasal mucous membranes, skin, and fingernails. Diquat affects the eye lens and intestinal tract lining but usually does not produce the frequently fatal lung changes characteristic of paraquat. Ingesting diquat or paraquat causes severe irritation to the mucous membranes of the mouth, esophagus, and stomach. Repeated vomiting generally follows. Large doses of diquat also produce restlessness and reduced sensitivity to stimulation. Large doses, and sometimes even small doses, of paraquat initially can affect the kidneys, liver, adrenal glands, and lungs. Potentially fatal fluid accumulation in the lungs can occur in 24 to 72 hours.

Lesser amounts of paraquat will cause decreased urine output because of kidney failure. Yellowing of the skin due to liver damage is sometimes observed. This initial phase is fol-lowed by an inactive period lasting up to two weeks, during which the victim appears to improve. The victim, however, may have permanent and gradually advancing lung damage caused by rapid growth of connective tissue. This prevents proper lung function and eventually leads to death through respiratory failure. Paraquat concentrates in cells in the lungs. AHS states the use of paraquat is linked to an increased risk of developing Parkinson's disease.

Skin exposure to paraquat and diquat concentrates may cause severe skin irritation and burning. Contact with dilute liquids and diquat dusts may cause slight to moderate irrita- tion. Skin absorption of paraquat apparently is slight. Diquat, however, is absorbed and after repeated contact will produce symptoms similar to those following ingestion.

Exposure to paraquat and diquat spray mist may produce skin irritation, nasal bleeding, irritation and inflammation of the mouth and upper respiratory tract, coughing, and chest pain. Exposure to paraquat concentrates may cause nails to blacken and grow abnormally.

No specific antidotes are available to counteract the effects of paraquat, diquat, and other

bipyridyl herbicides once significant exposure and absorption has occurred. Seek medical attention promptly. If ingested, and the victim is conscious, induce vomiting immediately unless a physician advises not to. Flush affected eyes with water, and wash skin with soap and water.

Chlorophenoxy Herbicides

2,4-D and **MCPA** are examples of chlorophenoxy her- bicides. These compounds are moderately irritating to skin and mucous membranes. Inhalation may cause a burning sensation in the nose, sinuses, and chest, which may result in coughing. Prolonged inhalation sometimes causes dizziness.

Stomach irritation usually leads to vomiting soon after ingestion. Victims may experience chest and abdominal pain and diarrhea. Headache, mental confusion, and bizarre behavior are early signs and symptoms of severe poisoning, which may progress to unconsciousness.

Arsenical Herbicides

Ansar®, Montar®, MSMA, and cacodylic acid are examples of arsenical herbicides. Acute arsenic poisoning usually appears within one hour of ingestion. Breath and feces that smell of garlic may help identify the responsible toxicant in severe cases. Effects on the digestive tract include inflammation of the mouth and esophagus, burning abdominal pain, thirst, vomiting, and bloody diarrhea.

Arsenic may affect the central nervous system as well. Effects include headache, dizziness, muscle weakness and spasms, low body temperature, sluggishness, delirium, seizures, and coma. Liver damage may lead to yellowing of the skin. Injury to tissues that form blood may reduce numbers of red and white blood cells and blood platelets. Death usually occurs one to three days after the onset of symptoms,usually the result of circulatory failure.

Chronic arsenic poisoning through skin exposure usually is more of a problem than acute poisoning, characterized by effects in the intestinal tract. Chronic arsenic poisoning may result in cancer. Symptoms of chronic exposure include over- growth of the eye's cornea; scaling off of dead skin; excessive fluids under the skin of the face, eyelids, and ankles; white streaks across the nails; loss of nails or hair; and brick red coloration of visible mucus membranes.

Other Herbicides

Endothall (Aquathol®) is commonly used as an aquat-ic herbicide or algaecide. It is irritating to skin, eyes, and mucous membranes. In one case, a man died after ingesting endothall. In this case, bleeding and swelling were noted inthe gut and the lungs.

Sodium chlorate (Drexel®, Defol®) is used as a defoliant, nonselective herbicide, and soil sterilant. It is irritating to skin, eyes, and stomach. Even though sodium chlorate is poorly absorbed in the digestive tract, ingesting a large dosewill cause severe poisoning. Irritation to the gut causes nau-sea, vomiting, and abdominal pain. Bluish skin sometimes is the only visible sign of poisoning. Dark brown blood andurine can indicate sodium chlorate poisoning.

Fungicides

Fungicides are used extensively in industry, agriculture, and the home and garden. Fungicides vary in their potential to cause adverse effects in humans. According to the EPA manual, *Recognition and Management of Pesticide Poisoning* (Roberts and Reigart, 2013), ". . . most fungicides currently in use are unlikely to cause frequent or severe systemic poisonings for several reasons. First, many have low inherent toxic-ity in mammals and are inefficiently absorbed. Second, many fungicides are formulated as suspensions of wettable powders or granules, from which rapid, efficient absorption is unlikely. And third, methods of application are such that relatively few individuals are intensively exposed." Fungicides probably have caused irritant injuries to skin and mucous membranes,as well as some skin sensitization.

AHS scientists found that applicators with retinal degeneration were twice as likely to have used fungicides. The risk of retinal degeneration increased as the days of fungicide use increased. This trend was noted for five specific fungicides: benomyl, captan, chlorothalonil, maneb, and metalaxyl. In addition, researchers found that applicators reporting retinal degeneration were more likely to raise orchard fruit, where fungicides are commonly used. Those with retinal degeneration were more likely to use hand spray guns, backpack sprayers, and mist blower/foggers. These application methods result in higher exposure to pesticides. As with any pesticide, always read and follow label recommendations carefully to avoid any health risks that a specific fungicide may pose.

Application Method

In some cases, the application method itself is the root cause of increased risk. Using hand spray guns, backpack sprayers, and mist blower/foggers may result in higher pesti-cide exposure. Another example is the Total Release Fogger (TRF). Also known as a bug bomb, TRF is a pesticide prod- uct that uses an aerosol propellant to release an insecticide in an enclosed area. They often are used to control fleas, cock- roaches, and flying insects in homes, offices, etc. Pyrethrins or pyrethroids are common active ingredients found in TRFs.

A 2018 study by the Centers for Disease Control and Prevention (CDC) reported 3,222 exposures to TRFs in 10 states between 2007 and 2015. According to this study, the most commonly reported cause of exposure was failure to leave
the treated premises during the application. The 2018 study stated, "Moderate or high severity illness was more common among males, persons over 60 years of age, those with preexisting asthma, and those who failed to vacate premises during application, or who were exposed to excessive TRFs."

A 2008 CDC study reporting 466 exposures in eight states from 2001–2006 said many exposures resulted from not leaving the enclosed space before the fogger discharged, re-entering the site too soon after the discharge, using too many foggers, or failing to notify others that foggers had been used. According to the 2008 study, the most often reported symptoms involved respiratory problems. Other symptoms dealt with gastrointestinal, neurological, cardiovascular, eye, and skin problems. Although one death was reported, most exposures were not considered severe. For TRF exposures, recommendations are to get the victim(s) to fresh air or administer oxygen if necessary.

Flush the skin and/or eyes with water to wash out chemicals. Because of limited effectiveness and the risks associated with their use, Extension generally does not recommend the use of TRFs.

What if a Pesticide Poisoning Occurs?

The key to surviving and recovering from a pesticide poisoning is rapid treatment. Take emergency action immediate- ly when you suspect a pesticide poisoning has occurred. As time elapses after exposure, the toxic effects are heightened, and the victim may need more time to recover. Immediately dial poison control center whenever you suspect a pesticide poisoning. An advanced life support team will be dispatched to provide assistance. In addition, you may wish to contact the following:

1. The Poison Control Center will provide specific directions on procedures to follow until a life support team arrives.
2. The nearest hospital or a physician. These can benefit by having preliminary information before the patient arrives.
3. Another source of medical and consumer information related to pesticides during non-emergencies is the National Pesticide Information Center.

What a victim might think is a cold or the flu could be a fatal pesticide poisoning. Whenever possible, get answers to the following questions.

1. Has the victim been exposed to a pesticide?
2. If so, which one and how did the exposure occur?
3. What emergency actions are given on the pesticide label?

Many pesticide labels direct that vomiting be induced. You can do this by giving the patient syrup of ipecac and wa- ter or by inserting a clean finger into the throat of the victim. Do not induce vomiting when:

- the label says not to,
- the victim is having or has had seizures accompanied by involuntary jerking movements,
- the victim is unconscious, or
- the pesticide contains petroleum products such as xylene.

Caution: Inhaling vomit can be life-threatening. Timely emergency treatment is vital to survival. After exposure to a pesticide, always wash the victim's exposed skin with soap or detergent and plenty of water, then obtain medical treatment. Skin irritation can result from continuous exposure if not treated. If the victim's clothing has been contaminated by a pesticide that is readily absorbed by the skin, remove the clothing and wash or rinse the victim's skin. Remember to protect yourself as you help the victim. Wear chemical-resistant gloves. If a pesticide spill is involved, move the victim away from the spill. Assist the victim first; take action to clean up the spill after all

first aid has been completed.

Even though most people are careful when working with pesticides, accidents can happen. Be prepared. Keep the telephone number for the Poison Control Center readilyavailable either in or near your phone, or in your telephone directory. Do not hesitate to contact medical authorities if any symptoms of pesticide poisoning occur. It is better to besafe than sorry.

Most pesticides used by Nebraska farmers, ranchers, andpeople with lawns and gardens have lower toxicity levels than many of the pesticides discussed in this publication. When applied properly, with the required protective clothing and equipment, they are unlikely to cause problems for the user. However, any pesticide can cause problems due to exposure or overexposure. Use all pesticides safely.

Probable Questions:

1. Briefly discuss chronic toxicity.
2. What are the guidelines for avoiding dermal exposure to toxic chemicals?
3. What are the guidelines for avoiding oral exposure to toxic chemicals?
4. What are the guidelines for avoiding respiratory exposure to toxic chemicals?
5. What is pesticide toxicity?
6. How toxicity is measured?
7. Discuss Signs and Symptoms of Poisoning.
8. How Organophosphate and Carbamate Insecticides work?
9. How Pyrethroid Insecticides works?
10. What is biological pesticides?
11. Discuss the mechanism of mosquito repellants.
12. How rodenticides are used for pest control?
13. How Fumigants are used in pest control?
14. How Herbicides are used?

Suggested Readings:

1. Principles of Toxicology by Stephen Roberts.
2. Toxicology Handbook by Lindsay Murray
3. Principles of Ecotoxicology by C.H. Walker
4. Casarett & Doull's Toxicology: The Basic Science by Curtis D. Klaassen

UNIT-XI

Classification of hormones; general principles, nature of hormone receptors (cell surface receptors and intracellular receptors)

Objective: In this unit we will discuss about classification oof hormones, hormone receptors types and mode of action.

Definition of Hormones:

Hormones are chemical messengers (may be of proteins, lipids or amines), secreted from special cells of endocrine glands and maintain the physiological activities very specifically on target cells through circulation and disintegrated after action.

Characteristics of Hormones:

The hormones possess the following specific properties:

1. They are chemical entities produced by special cells of endocrine glands.

2. They are transported to the target cells/ tissue/organ via circulation.

3. Their actions are species specific…

4. They are active in very minute quantities.

5. They are mostly water soluble.

6. They are low in molecular weight.

7. They are destroyed after their actions.

8. Chemically they are heterogeneous substances.

9. They cannot be stored for a long time; usually they are synthesized and secreted during the time of requirement.

10. They usually activate target cells by forming hormone receptor complex.

Mechanism of Hormone Action:

1. Enhancement of enzyme synthesis:

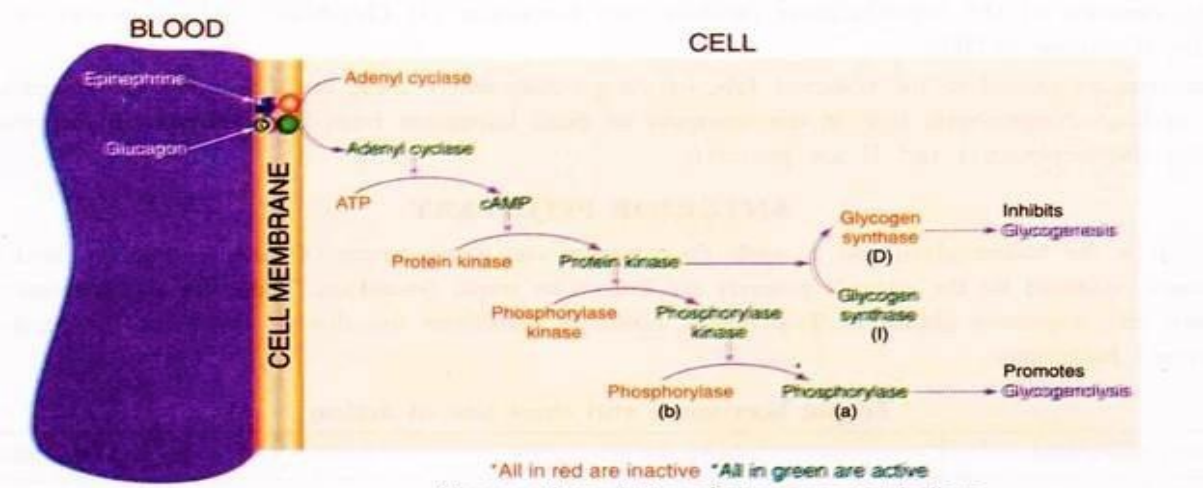
The steroid hormones and the thyroid hormones enter the cell and combine with the specific receptor protein to form 'receptor protein-hormone complex'. This complex will then bind to a specific site on DNA and initiate or enhance the synthesis of mRNA which in turn synthesizes the protein i.e. enzymes. Therefore the cell reactions speed up.

2. Change in cell permeability:

Hormones like insulin binds to a specific receptor on the cell membrane which results in alteration of the permeability of the cell to certain substances like glucose, amino acids and ions. The entry of these substances will bring a change in cell reactions.

3. Action through a second messenger (cAMP):

Hormones like epinephrine, glucagon bind to a regulatory site on the cell membrane. On the inner side of this regulatory site, an enzyme known as adenyl cyclase is present that converts ATP to cAMP which then activates certain protein kinases that in turn will phosphorylate certain enzymes. Some enzymes on phosphorylation become active whereas some other enzymes become inactive. Certain reactions are therefore stimulated while others are inhibited.



Hormonal regulation of glycogen metabolism

There are two mechanism by which hormones exerts its effect:

Mechanism - 1. Mode of Protein Hormone Action through Extracellular Receptors:

(i) Formation of Hormone Receptor Complex:

Every hormone has its own receptor. The number of receptors for each hormone varies. Insulin receptors for most cells is less than 100 but for some liver cells their number may be more than 1,00,000. The molecules of amino acid derivatives, peptides or polypeptide protein hormones bind to specific receptor molecules located on the plasma membrane to form the hormone receptor complex.

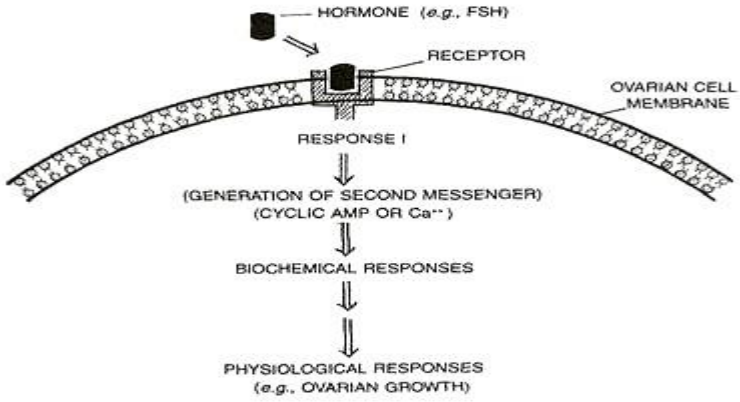


Fig. 22.18. Diagrammatic representation of mechanism of protein hormone action.

(ii) Formation of Secondary Messengers—the Mediators:

The hormone-receptor complex does not directly stimulates adenyl cyclase present in the cell membrane. It is done through a transducer G protein. Alfred Gilmans has shown that the G protein is a peripheral membrane protein consisting of ∝, β and γ subunits (Fig 22.19).It interconverts between a GDP form and GTP form. In muscle or liver cells, the hormones such as adrenaline bind receptor to form the hormone-receptor complex in the plasma membrane.

The hormone- receptor complex induces the release of GDP from the G protein. The α- subunit bearing GTP separates from the combined β and y subunits. The β and y subunits do not separate from each other. The activated β and γ subunits of G protein activate adenyl cyclase. The activated adenyl cyclase catalyses the formation of cyclic adenosine monophosphate (cAMP) from ATP.

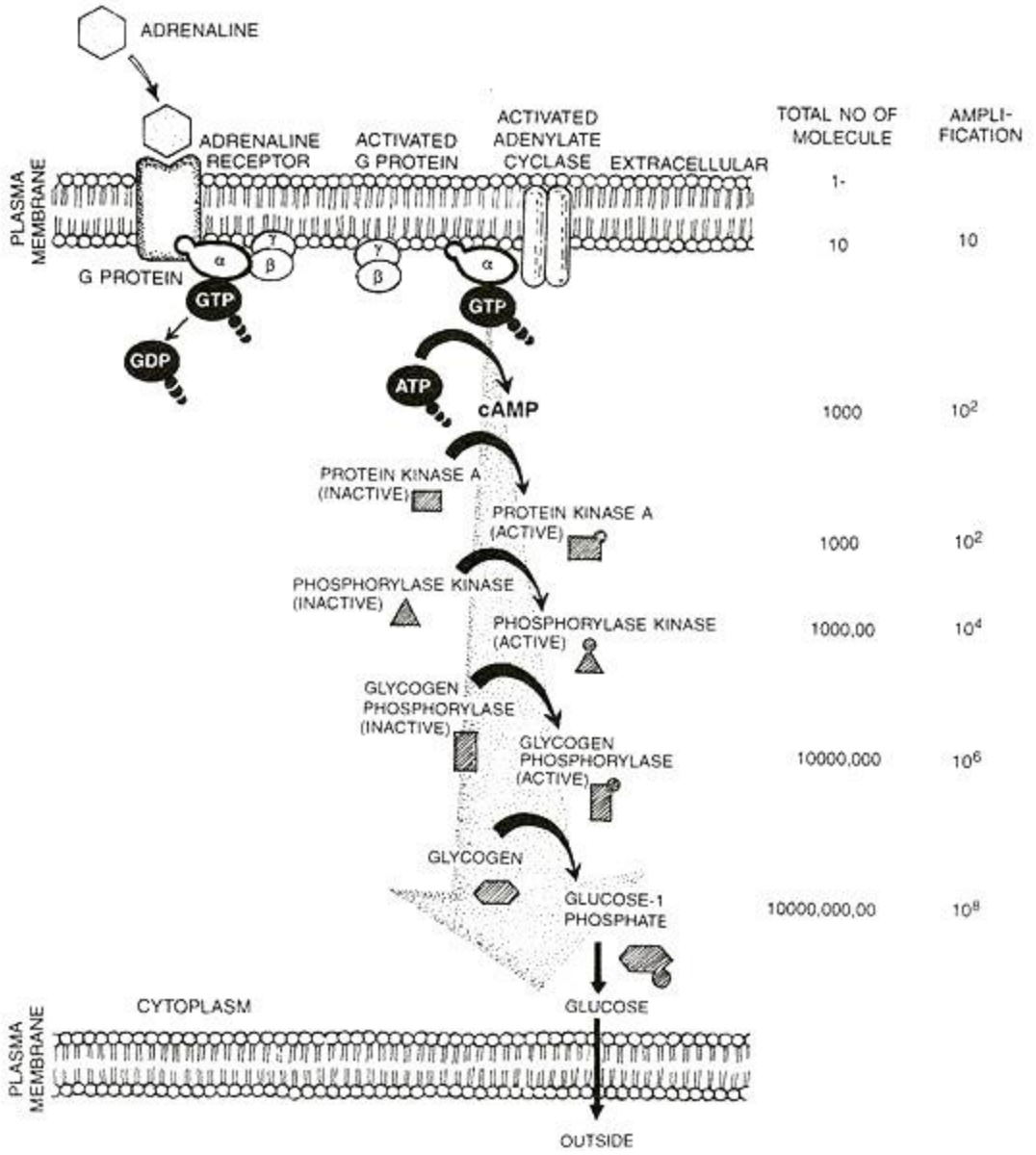


Fig. 22.19. Mode of hormone action through the extracellular receptor and amplification.

The hormone is called the first messenger and cAMP is termed the second messenger.

$$ATP \xrightarrow[\text{adenyl cyclase} + Mg^{++}]{\text{Activated}} cAMP + PPi$$

The hormones which interact with membrane-bound receptors normally do not enter the taget cell, but generate second messengers (e.g., cAMP).

Besides, cAMP, certain other intracellular second messengers are cyclic guanosine monophosphate (cGMP), diacyl-glycerol (DAG), inositol triphosphate (IP_3) and Ca^{++} responsible for amplification of signal. Earl W. Sutherland Jr (1915-1974) discovered cAMP in 1965. He got Nobel prize in physiology of medicine in 1971 for his discovery, "Role of cAMP in hormone action".

(iii) Amplification of Signal:

Single activated molecule of adenyl cyclase can generate about 100 cAMP molecules. Four molecules of cAMP now bind to inactive protein-kinase complex to activate protein-kinase A enzyme. Further steps as shown in involve cascade effect. In cascade effect, every activated molecule in turn activates many molecules of inactive enzyme of next category in the target cell. This process is repeated a number of times.

n the cytoplasm a molecule of protein kinase A activates several molecules of phosphorylase kinase. This enzyme changes inactive form of glycogen phosphorylase into active one. Glycogen phosphorylase converts glycogen into glucose-1 phosphate. The latter changes to glucose. As a result single molecule of ademaline hormone may lead to the release of 100 million glucose molecules within 1 to 2 minutes. This increases the blood glucose level.

(iv) Antagonistic Effect:

The effect of hormones which act against each other are called antagonistic effects. Many body cells use more than one second messenger. In heart cells cAMP acts as a second messenger that increases muscle cell contraction in response to adrenaline, while cGMP acts as another second messenger which decreases muscle contraction in response to acetylcholine.

Thus the sympathetic and parasympathetic nervous systems achieve antagonize effect on heart beat. Another example of antagonistic effect is of insulin and glucagon. Insulin lowers blood sugar level and glucagon raises blood sugar level.

(v) Synergistic Effect:

When two or more hormones complement each other's actions and they are needed for full expression of the hormone effects are called synergistic effects. For example, the production and ejection of milk by mammary glands require the synergistic effects of oestrogens, progesterone, prolactin and oxytocin hormones.

Hormones that Bind to Cell Membrane Receptor mediate their actions through many second messengers, some of which are discussed below:

A. cAMP as the Second Messenger:

i. For the formation of cAMP from ATP needs: Receptor, GS protein, Adenylate cyclase.

ii. cAMP (Cyclic adenosine 3′-5′ monophosphate) is formed from ATP by the action of the enzyme adenylate cyclase and converted to physiologically inactivated 5′- AMP by the action of enzyme phosphodiesterase.

iii. Hormone receptor complex combines with G_s or G_i; (s = stimulatory, i = inhibitory) type of GTP dependent trimeric nucleotide regulatory complex of the cell membrane.

iv. Both G_s or G_1 are made up of 3 subunits. G_s contains $\alpha_s\beta\gamma$ and G_i contains $\alpha_i\beta\gamma$.

v. The α subunit (either G_s or G_i) is bound to GDP. When binding of hormone to R_s or R_i results in a receptor-mediated activation of G, then GDP is exchanged for GTP and the a subunit separates from the combined β and γ subunits.

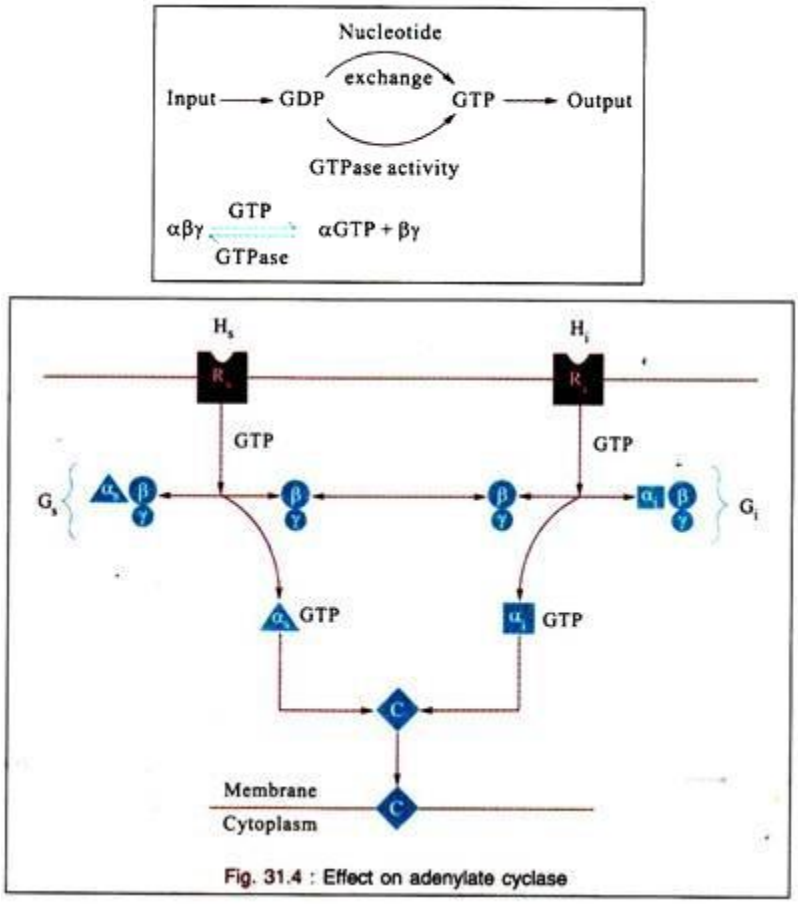


Fig. 31.4 : Effect on adenylate cyclase

vi. This GTP-α_s activates effectors (adenylate cyclase). The intrinsic GTPase activity of the α-subunit then converts GTP and GDP and leads to re-association of the a with βγ subunit.

vii. On the other hand, α_i-GTP inhibits adenylate cyclase by binding with it. This lowers the intracellular concentration of cAMP. Hormones that stimulate adenylate cyclase: ACTH, ADH, FSH, Glucagon. Hormones that inhibit adenylate cyclase: Acetylcholine, Angiotensin II.

viii. cAMP binds to a protein kinase that is a hetero tetrameric molecule consisting of 2 regulatory subunits (R) and 2 catalytic subunits (C). cAMP binding results in the following reaction.

$4cAMP + R_2C_2 \rightleftharpoons R_2 (4cAMP) + 2C$

ix. The R_2C_2 complex has no enzymatic activity but the binding of cAMP by R dissociates R from C, thereby activating protein kinase. This activated protein kinase catalyzes the transfer of the y phosphate of ATP (Mg^{++}) to a serine or threonine residue in a variety of proteins. Thus they regulate the conformational changes of phosphoprotein and physiologic effect occurs.

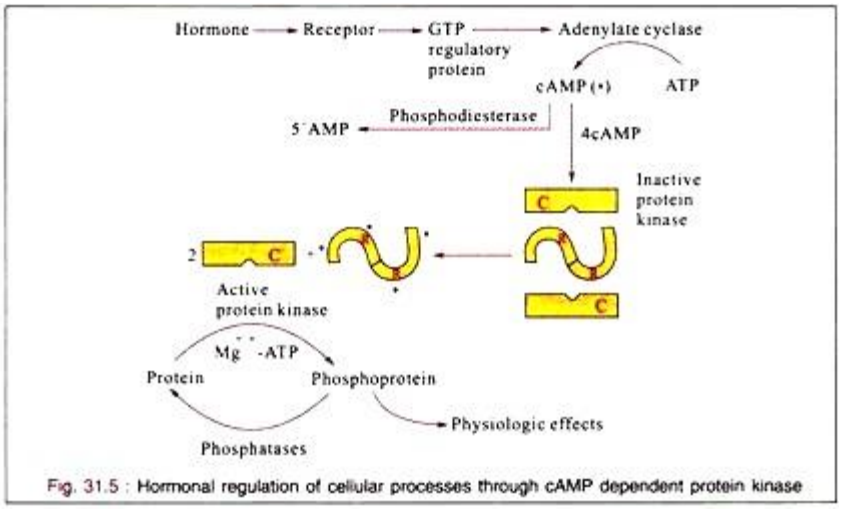


Fig. 31.5 : Hormonal regulation of cellular processes through cAMP dependent protein kinase

B. Role of cGMP in Hormone Action:

i. Hormones such as insulin and growth hormone, affect the guanylate cyclase cGMP system. This will increase the intracellular concentration of cGMP and activate cGMP dependent protein kinases.

ii. The active cGMP protein kinase would in turn bring about phosphorylation of specific cellular proteins to change their activities, leading to relaxation of smooth muscles, vasodilatation and other effects.

iii. The idea of cGMP as second messenger has not been accepted as yet. It is likely that Ca^{++} may act as second messenger to activate guanylate cyclase and thereby increasing the concentration of cGMP inside the cell.

iv. It appears that cGMP has its unique place in hormone action. The atriopeptins, a family of peptides, produced in cardiac atrial tissues cause natriuretic, diuresis, vasodilatation and inhibition of aldosterone secretion.

v. These peptides (e.g., atrial natriuretic factor) bind to and activate the membrane bound form of guanylate cyclase. This results in an increase of cGMP.

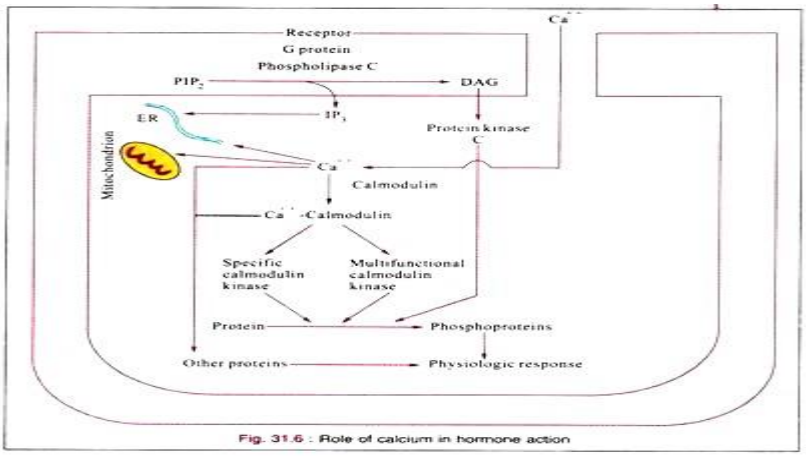


Fig. 31.6 : Role of calcium in hormone action

C. Role of Calcium in Hormone Action:

i. It is suggested that ionized calcium of the cytosol is the important signal for hormone action than cAMP.

ii. The extracellular calcium (Ca^{++}) concentration is about 5 mmol/L, the intracellular concentration of this free ion is much lower 0.1-10 µmol/L.

iii. The hormones that bind cell membrane receptor enhance membrane permeability to Ca^{++} and thereby increase Ca^{++} influx. This is probably accomplished by an Na^{+}/Ca^{++} exchange mechanism that has a high capacity but a low affinity for Ca^{++}. There is a $Ca^{2+}/2H^{+}$-ATPase dependent pump that extrudes Ca^{2+} in exchange for H^{+}. This has a high affinity for Ca^{2+} but a low capacity.

iv. Cell surface receptors such as those for acetylcholine, ADH, when occupied by their respective ligands, potent activators of phospholipase c.

v. Receptor binding and activation of phospholipase c are coupled by a unique G protein.

vi. Phospholipase c catalyses the hydrolysis of phosphatidyl inositol 4, 5-bisphosphate to inositol triphosphate and 1, 2 diacylglcerol.

vii. The diacylglycerol is itself capable of activating protein kinase c, the activity of which also depends upon free ionic calcium.

viii. Inositol triphosphate is an effective releaser of calcium from intracellular storage sites such as endoplasmic reticulum, and mitochondria.

ix. Thus, the hydrolysis of PIP_2 leads to activation of protein kinase c and promotes an increase of cytoplasmic calcium ion.

x. The calcium dependent regulatory protein is now referred to as calmodulin. Calmodulin has $4Ca^{++}$ binding sites. Ca^{++}– calmodulin complex can activate specific kinases. These then modify the conformational changes of phosphoprotein and alters physiologic responses.

xi. The activated protein kinase c can phosphorylate specific substrates and alter physiologic processes.

Mechanism - 2. Mode of Steroid Hormone Action through Intracellular Receptors:

Steroid hormones are lipid-soluble and easily pass through the cell membrane of a target cell into the cytoplasm. In the cytoplasm they bind to specific intracellular receptors (proteins) to form a hormone receptor complex that enters the nucleus.

In the nucleus, hormones which interact with intracellular receptors (e.g., steroid hormones, iodothyromines, etc.) mostly regulate gene expression or chromosome function by the interaction of hormone-receptor complex with the genome. Biochemical actions result in physiological and developmental effects (tissue growth and differentiation, etc.). In-fact the hormone receptor complex binds to a specific regulatory site on the chromosome and activates certain genes (DNA).

The activated gene transcribes mRNA which directs the synthesis of proteins and usually enzymes in the cytoplasm. The enzymes promote the metabolic reactions in the cell. The actions of lipid soluble hormones are slower and last longer than the action of water- soluble hormones.

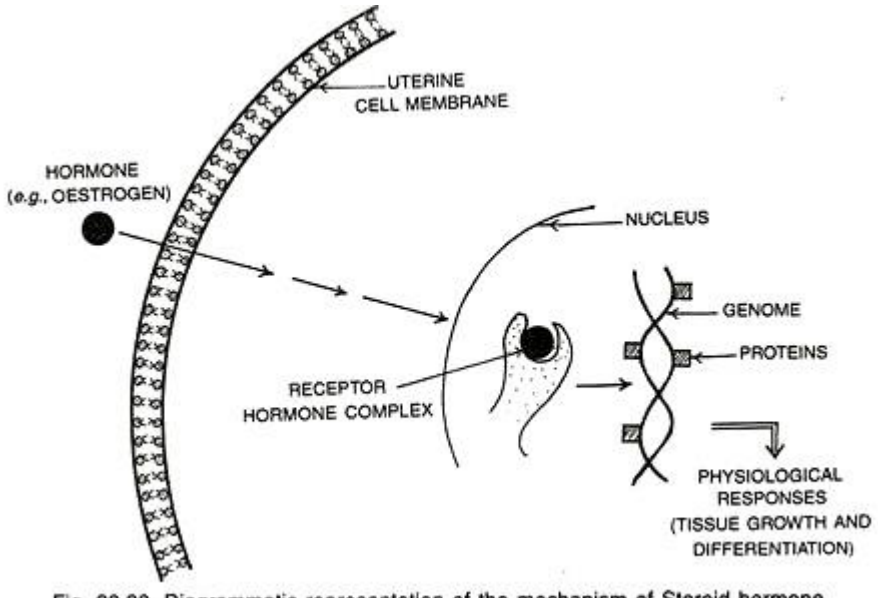


Fig. 22.20. Diagrammatic representation of the mechanism of Steroid hormone.

Role of Hormones as Messengers and Regulators (Role of Hormones in Homeostasis):

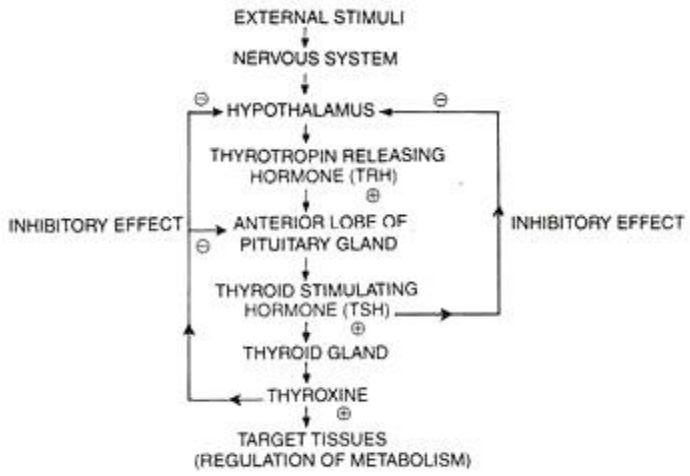


Fig. 22.21. Feed back control involving the hypothalamus, anterior lobe of pituitary gland, thyroid gland and target tissues.

Hormones as Messengers [Hypothalamus-hypophysial (pituitary) Axis]:

Hypothalamus is a part of the fore brain. Its hypothalamic nuclei— masses of grey matter containing neurons, are located in the white matter in the floor of the third ventricle of the brain. The neurons (neurosecretory cells) of hypothalamic nuclei secrete some hormones called neurohormones (releasing factors) into the blood.

The neurohormones are carried to the anterior lobe of the pituitary gland (= hypophysis) by a pair of hypophysial portal veins. In the pituitary gland (hypophysis) the neurohormones stimulate it to release various hormones. Hence the neurohormones are also called “releasing factors”.

Hormones as Regulators (Feed Back Control):

Homeostasis means keeping the internal environment of the body constant. Hormones help in maintaining internal environment of the body. When the secretion of hormones is under the control of factors or other hormones it is called feedback control. The regulation of secretion of thyroxine from the thyroid gland is an example of such feedback control mechanism.

Degradation and excretion of hormones:

All the hormones are degraded and excreted. Peptide hormones are degraded in the liver and/or kidney. The catecholamine’s, steroids and the thyroid hor-mones are inactivated directly by enzymatic modification in the blood and/or in the liver.

Feed back control is of two types:

(i) Negative Feed Back Control:

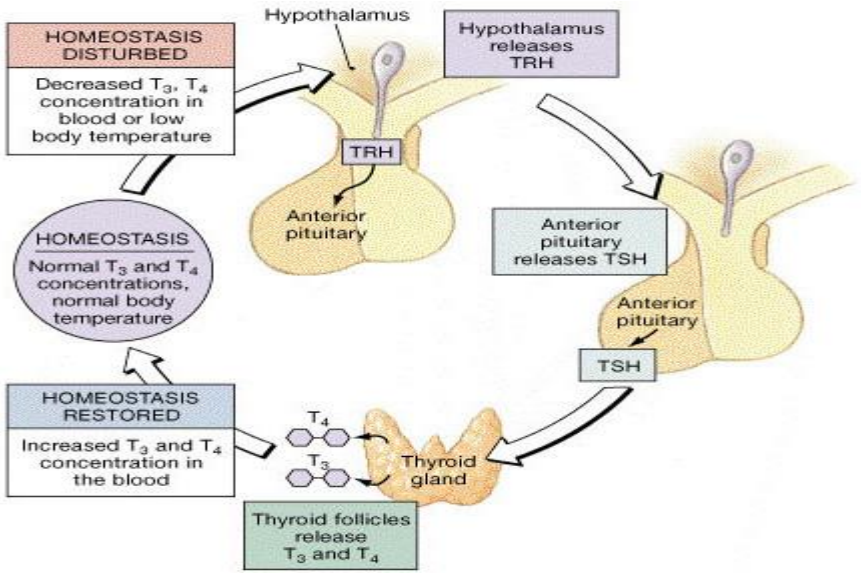
The receptors (sensory cells) present on the body of vertebrates constantly monitors the reference point of internal environment. Any changes in the internal environment can activates the receptor cells, which relay messages to the control centre (Brain or spinal cord). The control centre determines the deviation and activates the effectors. Effectors are generally muscles or glands. The effectors respond to the stimulus and corrects the reference point either by increasing or decreasing the activities. As soon as the system is corrected, the control centre and effectors are turned off by the mechanism called Negative feed-back.

In negative feed-back mechanism, changes occurring in the system automatically activates the corrective mechanism, which reverse the changes and bring back the system to the normal. The principle of thermostat is analog to the Negative feed-back mechanism. In thermostat, when the temperature exceeds the normal ranges, the receptor detects the changes and signals the control center of thermostat to turn off the heating plate, allowing the thermostat to cool down. When the thermostat cool down below the set point, it turn ON the heating plates, so the temperature starts rise again.

The mechanism of Negative feed-back in biological system can be illustrated with the example given below.

Negative feed-back mechanism of thyroid gland

Lower concentration of thyroxine hormone in blood alters the cellular activities ie. Decrease in basic metabolic rates or temperature. Decreases in BMR stimulates neurosecretory cells of hypothalamus to secrete thyrotropin releasing hormone (TRH). The releasing of TRH causes anterior pituitary gland to secrete thyroid stimulating hormone (TSH). This TSH then stimulates the thyroid gland to release thyroxine. Thyroxin causes an increase in the metabolic activity, generating ATP energy and heat and eventually restore homeostasis. Both the raised body temperature and higher thyroxine levels in the body feed-back to inhibit the releasing of TRH and TSH.



(ii) Positive Feed Back Control:

Positive feedback mechanism causes destabilizing effects in the body, so does not results in homeostasis. It is mainly responsible for amplification of the changes caused by the stimulus. Positive feedback is relatively less common than negative feedback, since it leads to unstable condition and extreme state. Most positive feedback mechanisms are harmful and in some cases resulting in death. For example, if a person breathes air that has very high carbon dioxide content. The amount of oxygen in blood decreases while the concentration of carbon-dioxide in blood increases. This is sensed by carbon dioxide receptors, which cause the breathing rate to increase. So the person breathes faster, taking in more carbon dioxide, which stimulates the receptors even more, so they breathe faster and faster which ultimately results in death.

In some cases, the positive feed-back is very useful, such as during blood clotting, fever, child birth, breast feeding etc. Positive feedback also plays a role in the contractions of the uterus during child birth. The contraction of uterine wall is caused by oxytocin hormone. In this case, stretching of the uterus by the fetus stimulates oxytocin release which results in contraction of uterus, and contraction causes further stretching and release of oxytocin; the cycle continues until the fetus is expelled from the uterus.

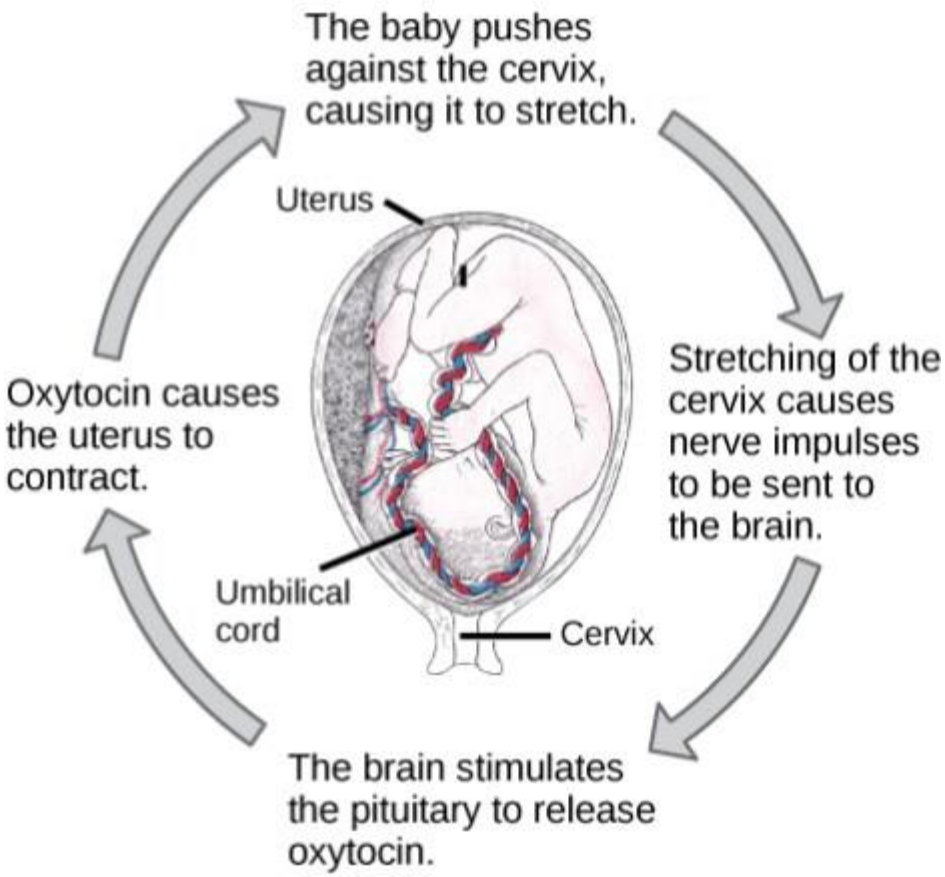


Figure: Regulation of oxytocin hormone; an example of positive feedback mechanism

Hormone Receptors:

Meaning of Hormone Receptors:

A hormone receptor is a receptor protein on the surface of a cell or in its interior that binds to a specific hormone. The hormone causes many changes that take place in the cell. Binding of hormones to hormone receptors often trigger the start of a biophysical signal that can lead to further signal transduction pathways, or trigger the activation or inhibition of genes.

Types of Hormone Receptors

Peptide Hormone Receptors:

Are often trans membrane proteins. They are also called G-protein- coupled receptors, sensory receptors or ionotropic receptors. These receptors generally function via intracellular second messengers, including cyclic AMP (cAMP), inositol 1, 4, 5-triphosphate (IP_3) and the calcium (Ca^{2+})—calmodulin system.

Steroid Hormone Receptors and Related Receptors:

Are generally soluble proteins that function through gene activation. Their response elements are DNA sequences (promoters) that are bound by the complex of the steroid bound to its receptor. The receptors themselves are zinc-finger proteins. These receptors include those for

glucocorticoids, estrogens, androgens, thyroid hormone (T3), calcitriol (the active form of vitamin D), and the retinoids (vitamin A).

Receptors for Peptide Hormones:

With the exception of the thyroid hormone receptor, the receptors for amino acid derived and peptide hormones are located in the plasma membrane. Receptor structure is varied. Some receptors consist of a single polypeptide chain with a domain on either side of the membrane, connected by a membrane-spanning domain. Some receptors are comprised of a single polypeptide chain that is passed back and forth in serpentine fashion across the membrane, giving multiple intracellular, trans membrane, and extracellular domains. Other receptors are composed of multiple polypeptides. Ex. The insulin receptor is a disulfide linked tetramer with the β-subunits spanning the membrane and the α-subunits located on the exterior surface.

Subsequent to hormone binding, a signal is transduced to the interior of the cell, where second messengers and phosphorylated proteins generate appropriate metabolic responses. The main second messengers are cAMP, Ca^{2+}, inositol triphosphate (IP3), and diacylglycerol (DAG).
Proteins are phosphorylated on serine and threonine by cAMP-dependent protein kinase (PKA) and DAG-activated protein kinase C (PKC). Additionally a series of membrane-associated and intracellular tyrosine kinases phosphorylate specific tyrosine residues on target enzymes and other regulatory proteins. The hormone-binding signal of most, but not all, plasma membrane receptors is transduced to the interior of cells by the binding of receptor-ligand complexes to a series of membrane-localized GDP/GTP binding proteins known as G-proteins. The classic interactions between receptors, G-protein transducer, and membrane-localized adenylate cyclase are illustrated using the pancreatic hormone glucagon as an example.

When G-proteins bind to receptors, GTP exchanges with GDP bound to the α-subunit of the G-protein. The G_a-GTP complex binds adenylate cyclase, activating the enzyme. The activation of adenylate cyclase leads to cAMP production in the cytosol and to the activation of PKA, followed by regulatory phosphorylation of numerous enzymes. Stimulatory G-proteins are designated Gs, inhibitory G-proteins are designated Gi. A second class of peptide hormones induces the transduction of 2 second messengers, DAG and IP3. Hormone binding is followed by interaction with a stimulatory G-protein which is followed in turn by G-protein activation of membrane-localized phospholipase C-y, (PLC-y). PLC-y hydrolyzes phosphatidylinositol bisphosphate to produce 2 messengers viz. IP_3, which is soluble in the cytosol, and DAG, which remains in the membrane phase.
Cytosolic IP_3 binds to sites on the endoplasmic reticulum, opening Ca^{2+} channels and allowing stored Ca^{2+} to flood the cytosol. There it activates numerous enzymes, many by activating their calmodulin or calmodulin-like subunits. DAG has 2 roles-it binds and activates PKC, and it opens Ca^{2+} channels in the plasma membrane, reinforcing the effect of IP_3. Like PKA, PKC phosphorylates serine and threonine residues of many proteins, thus modulating their catalytic activity.

Insulin Receptor:

Is a trans membrane receptor that is activated by insulin. It belongs to the large class of tyrosine kinase receptors. Two alpha subunits and two beta subunits make up the insulin receptor. The beta subunits pass through the cellular membrane and are linked by disulfide bonds. The alpha and beta subunits are encoded by a single gene (INSR). The insulin receptor has been designated as CD_{220} (cluster of differentiation 220).

Function of insulin receptor-effect of insulin on glucose uptake and metabolism:

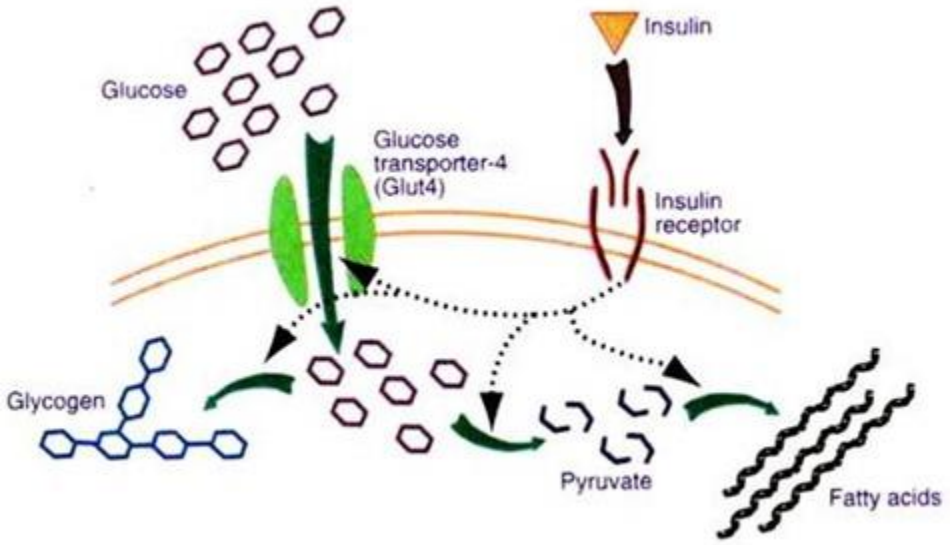
Insulin binds to its receptor which in turn starts many protein activation cascades.

These include—

i. Translocation of Glut-4 transporter to the plasma membrane and influx of glucose

ii. Glycogen synthesis

iii. Glycolysis and fatty acid synthesis



Insulin receptors (a family of tyrosine kinase receptors), mediate their activity by causing the addition of a phosphate group to particular tyrosine's on certain proteins within a cell. The 'substrate' proteins which are phosphorylated by the insulin receptor include a protein called 'IRS-1' for 'Insulin Receptor Substrate-1'.

IRS-1 binding and phosphorylation eventually leads to an increase in the high affinity glucose transporter (Glut4) molecules on the outer membrane of insulin-responsive tissues, including muscle cells and adipose tissue, and therefore to an increase in the uptake of glucose from blood into these tissues. Briefly, the glucose transporter ($Glut_4$) is transported from cellular vesicles to

the cell surface, where it then can mediate the transport of glucose into the cell. Glycogen synthesis is also stimulated by the insulin receptor via IRS-1.

Pathology of insulin receptors:

The main activity of activation of the insulin receptor is inducing glucose uptake. For this reason 'insulin insensitivity', or a decrease in insulin receptor signalling, leads to diabetes mellitus type 2 – the cells are unable to take up glucose, and the result is hyperglycemia (an increase in circulating glucose), and all the sequelae which result from diabetes. Patients with insulin resistance may display acanthosis nigricans.A few patients with homozygous mutations in the INSR gene have been described, which causes Donohue syndrome or Leprechauns. This autosomal recessive disorder results in a totally non-functional insulin receptor. These patients have low set, often protuberant ears, flared nostrils, thickened lips, and severe growth retardation.

In most cases, the outlook for these patients is extremely poor with death occurring within the first year of life. Other mutations of the same gene cause the less severe Rabson-Mendenhall syndrome, in which patients have characteristically abnormal teeth, hypertrophic gingiva (gums) and enlargement of the pineal gland. Both diseases present with fluctuations of the glucose level—after a meal the glucose is initially very high, and then falls rapidly to abnormally low levels.

Degradation of insulin and its receptors:

Once an insulin molecule has docked onto the receptor and effected its action, it may be released back into the extracellular environment or it may be degraded by the cell. Degradation normally involves endocytosis of the insulin-receptor complex followed by the action of insulin degrading enzyme. Most insulin molecules are degraded by liver cells. It has been estimated that a typical insulin molecule is finally degraded about 71 minutes after its initial release into circulation.

It is a 62 kDa peptide that is activated by glucagon and is a member of the G- protein coupled family of receptors, coupled to Gs. Stimulation of the receptor results in activation of adenylate cyclase and increased levels of intracellular cAMP. Glucagon receptors are mainly expressed in liver and in kidney with lesser amounts found in heart, adipose tissue, spleen, thymus, adrenal glands, pancreas, cerebral cortex, and G.I. tract.

Steroid Hormone Receptors:

Are proteins that have a binding site for a particular steroid molecule. Their response elements are DNA sequences that are bound by the complex of the steroid bound to its receptor. The response element is part of the promoter of a gene. Binding by the receptor activates or represses, as the case may be, the gene controlled by that promoter. It is through this mechanism that steroid hormones turn genes on (or off).

The DNA sequence of the glucocorticoid (a protein homodimer) response element is:

5′-AGAACAnnnTGTTCT-3′

3′ TCTT GTnnnACAAGA-5′

where n represents any nucleotide (a palindromic sequence)

The glucocorticoid receptor, like all steroid hormone receptors, is a zinc-finger transcription factor; there are four zinc atoms each attached to four cysteine's.

For a steroid hormone to turn gene transcription on, its receptor must:

(i) Bind to the hormone

(ii) Bind to a second copy of itself to form a homodimer

(iii) Be in the nucleus, moving from the cytosol if necessary

(iv) Bind to its response element

(v) Activate other transcription factors to start transcription

Each of these functions depends upon a particular region of the protein (Ex. The zinc fingers for binding DNA). Mutations in any one region may upset the function of that region without necessarily interfering with other functions of the receptor.

Nuclear Receptor Superfamily:

The zinc-finger proteins that serve as receptors for glucocorticoids and progesterone are members of a large family of similar proteins that serve as receptors for a variety of small, hydrophobic molecules. These include other steroid hormones like the mineralocorticoid-aldosterone, oestrogens, the thyroid hormone (T_3), calcitriol (the active form of vitamin D), vitamin A (retinol) and its relatives-retinal/retinoic acid, bile acids and fatty acids. These bind members of the superfamily called Peroxisome Proliferator Activated Receptors (PPARs). They got their name from their initial discovery as the receptors for drugs that increase the number and size of peroxisomes in cells.

In every case, the receptors consists of at least three functional modules or domains from N-terminal to C-terminal, these are:

i. A domain needed for the receptor to activate the promoters of the genes being controlled

ii. The zinc-finger domain needed for DNA binding (to the response element)

iii. The domain responsible for binding the particular hormone as well as the second unit of the dimer.

Receptors for Thyroid Hormones:

Are members of a large family of nuclear receptors that include those of the steroid hormones. They function as hormone-activated transcription factors and thereby act by modulating gene expression.

Thyroid hormone receptors bind DNA in absence of hormone:

Usually leading to transcriptional repression. Hormone binding is associated with a conformational change in the receptor that causes it to function as a transcriptional activator.

Mammalian thyroid hormone receptors are encoded by two genes, designated alpha and beta. Further, the primary transcript for each gene can be alternatively spliced, generating different alpha and beta receptor isoforms. Currently, four different thyroid hormone receptors are recognized as-(i) α-1 (ii) α-2 (iii) β-1 and (iv) β-2.

Like other members of the nuclear receptor superfamily, thyroid hormone receptors encapsulate three functional domains:

i. A transactivation domain at the amino terminus that interacts with other transcription factors to form complexes that repress or activate transcription. There is considerable divergence in sequence of the transactivation domains of alpha and beta isoforms and between the two beta isoforms of the receptor.

ii. A DNA-binding domain that binds to sequences of promoter DNA known as hormone response elements.

iii. A ligand-binding and dimerization domain at the carboxy-terminus.

Disorders of thyroid hormone receptors:

A number of humans with a syndrome of thyroid hormone resistance have been identified, and found to have mutations in the receptor beta gene which abolish ligand binding. Clinically, such

individuals show a type of hypothyroidism characterized by goiter, elevated serum concentrations of T_3 and thyroxine and normal or elevated serum concentrations of TSH.
More than half of affected children show attention-deficit disorder, which is intriguing considering the role of thyroid hormones in brain development. In most affected families, this disorder is transmitted as a dominant trait, which suggests that the mutant receptors act in a dominant negative manner.

Adrenergic Receptors (or Adrenoceptors):

Are a class of G-protein coupled receptors that are targets of the catecholamine's. Adrenergic receptors specifically bind their endogenous ligands, the catecholamine's adrenaline and noradrenalin (called epinephrine and norepinephrine), and are activated by these.

Many cells possess these receptors, and the binding of an agonist will generally cause a sympathetic response (i.e. the fight-or-flight response) viz. the heart rate will increase and the pupils will dilate, energy will be mobilized, and blood flow diverted from other, non-essential, organs to skeletal muscle. There are several types of adrenergic receptors, but there are two main groups viz. a-adrenergic and P-adrenergic.

α-Adrenergic receptors:

These receptors bind noradrenalin (norepinephrine) and adrenaline (epinephrine). Phenylephrine is a selective agonist of the a-receptor. They exist as α_1-adrenergic receptors and α_2-adrenergic receptors.

β-Adrenergic receptors:

These receptors are linked to Gs proteins, which in turn are linked to adenyl cyclase. Agonist binding thus causes a rise in the intracellular concentration of the second messenger cAMP. Downstream effectors of cAMP include cAMP-dependent protein kinase (PKA), which mediates some of the intracellular events following hormone binding.

Role in circulation:

Epinephrine reacts with both α and β-adrenoreceptors, causing vasoconstricdon and vasodilation, respectively. Although receptors are less sensitive to epinephrine, when activated, they override the vasodilation mediated by β-adrenoreceptors. The result is that high levels of circulating epinephrine cause vasoconstriction. Lower levels of epinephrine dominates β-adrenoreceptor stimulation, producing an overall vasodilation.

The mechanism of adrenergic receptors:

Adrenaline or noradrenalin is receptor ligands to either α_1, α_2 or β-adrenergic receptors, a, couples to Gq, which results in increased intracellular Ca^{2+} which results in smooth muscle contraction. α_2 on the other hand, couples to Gi, which causes a decrease of cAMP activity, resulting in smooth muscle contraction. β receptors couple to Gs, and increase intracellular cAMP activity, resulting in heart muscle contraction, smooth muscle relaxation and glycogenolysis.

Functions of α-receptors:

α-Receptors have several functions in common. They are:

(i) Vasoconstriction of arteries to heart (coronary artery)

(ii) Vasoconstriction of veins

(iii) Decrease motility of smooth muscle in gastrointestinal tract

Alpha-1 adrenergic receptor:

Alpha-1 -adrenergic receptors are members of the G protein-coupled receptor superfamily. Upon activation, a heterotrimeric G-protein, Gq, activates phospholipase C (PLC), which causes an increase in IP_3 and calcium. This triggers all other effects. Specific actions of the β_1 receptor mainly involve smooth muscle contraction.
It causes vasoconstriction in many blood vessels including those of the skin & gastrointestinal system and to kidney (renal artery) and brain. Other areas of smooth muscle contraction are for instance – ureter, vas deferens, hairs (arrector pili muscles), uterus (when pregnant), urethral sphincter, bronchioles (although minor to the relaxing effect of β_2 receptor on bronchioles). Further effects include glycogenolysis and gluconeogenesis from adipose tissue and liver, as well as secretion from sweat glands and Na reabsorption from kidney.

Alpha-2 adrenergic receptor:

There are 3 highly homologous subtypes of α_2 receptors viz. α_2A, α_2B, and α_2C. Specific actions of the a_2-receptor include:

i. Inhibition of insulin release in pancreas

ii. Induction of glucagon release from pancreas

iii. Contraction of sphincters of the gastrointestinal tract

Beta-1 adrenergic receptor:

Specific actions of the β_1 receptor include:

i. Increase cardiac output, both by raising heart rate and increasing the volume expelled with each beat (increased ejection fraction)

ii. Renin release from juxtaglomerular cells

iii. Lipolysis in adipose tissue

Beta-2 adrenergic receptor:

Specific actions of the β_2 receptor include:

i. Smooth muscle relaxation, e.g. in bronchi

ii. Relaxes urinary sphincter and pregnant uterus

iii. Relaxes detrusor urinary muscle of bladder wall

iv. Dilates arteries to skeletal muscle

v. Glycogenolysis and gluconeogenesis

vi. Contract sphincters of GI tract

vii. Thickened secretions from salivary glands

viii. Inhibit histamine-release from mast cells

ix. Increase renin secretion from kidney

Comparison of different adrenergic receptors

Receptor type	Agonist potency order	Selected action of agonist	Mechanism	Agonists	Antagonists
α_1:A, B, D	Adrenaline ≥ Noradrenaline >> Isoprenaline	Smooth muscle contraction	Gq: Phospholipase C (PLC) activated, IP3 and Calcium up	Noradrenaline Phenylephrine Methoxamine Cirazoline	(Alpha blockers) Phenoxy-benzamine Phentolamine Prazosin Tamsulosin Terazosin
α_2: A, B, C	Adrenaline ≥ Noradrenaline >> Isoprenaline	Smooth muscle contraction and neurotransmitter inhibition	Gi: Adenylate cyclase inactivated, cAMP down	Clonidine lofexidine Xylazine Tizanine Guanfacine	(Alpha blockers) Metoprolol atenolol
β_1	Isoprenaline > Adrenaline = Noradrenaline	Heart muscle contraction	Gs: Adenylate cyclase activated, cAMP up	Noradrenaline Isoprenaline Dobutamine	(Beta blockers) Metoprolol atenolol
β_2	Isoprenaline > Adrenaline > > Noradrenaline	Smooth muscle relaxation	Gs: Adenylate cyclase activated, cAMP up	Salbutamol Bitolterol Mesylate Formoterol Isoprenaline Levalbuterol Metaproterenol Salmeterol Terbutaline Ritodrine	(Beta blockers) Butoxamine propranolol
β_3	Isoprenaline = Noradrenaline > Adrenaline	Enhance lipolysis	Gs: Adenylate cyclase activated, cAMP up	L-796568	

Probable Questions:

1. Define hormone. What are the main characteristics of a hormone?
2. How hormones exert their effect through extracellular receptors?
3. How steroid hormones exert their effect?
4. What is antagonistic effect and what is synergistic effect of hormone action?
5. State the role of cAMP as hormone second messenger.
6. State the role of cGMP as hormone second messenger.
7. State the role of Calcium ion as hormone second messenger.
8. Discuss about positive feed back of hormone action with suitable example.
9. Discuss about negative feed back of hormone action with suitable example.
10. What is hormone receptor? Describe its importance.
11. How insulin receptor exerts its effect in cell? What happen when there is defect in the receptor?
12. What are the characteristics of steroid receptors?
13. Discuss the role and types of adrenergic receptors.
14. Compare different types of adrenergic receptors.

Suggested Readings:

1. General Endocrinology. Turner and Bagnara. Sixth Edition.
2. Williams Textbook of Endocrinology. Tenth Edition.
3. Introduction to Endocrinology. Chandra S Negi. Second Edition
4. Endocrinology. Hadley and Levine. Sixth Edition

UNIT-XII

Biosynthesis, secretion and regulation of hormones: biosynthesis of protein and peptide hormones (Growth Hormone and Insulin) including their post-translational event and release

Objective: In this unit you will learn about biosynthesis release and regulations of different hormones such as growth hormone, insulin, thyroxine and steroid hormones.

Meaning of Growth Hormones:

Growth hormone (GH) also known as somatotropic hormone and is a peptide hormone secreted by acidophils of the anterior pituitary gland. GH is stored in large, dense granules present in acidophil cells. It is a single chain polypeptide with molecular weight of 22,000 having 191 amino acids and two disulphide bridges. As the name indicates, its action is on the growth of the body. It stimulates somatic growth and development and helps to maintain lean body mass and bone mass in adults.

Growth hormone (**GH**) or **somatotropin**, also known as **human growth hormone** (**hGH** or **HGH**) in its human form, is a peptide hormone that stimulates growth, cell reproduction, and cell regeneration in humans and other animals. It is thus important in human development. GH also stimulates production of IGF-1 and raises the concentration of glucose and free fatty acids. It is a type of mitogen which is specific only to the receptors on certain types of cells. GH is a 191-amino acid, single-chain polypeptide that is synthesized, stored and secreted by somatotropic cells within the lateral wings of the anterior pituitary gland.

A recombinant form of hGH called somatreopleopin (INN) is used as a prescription drug to treat children's growth disorders and adult growth hormone deficiency. In the United States, it is only available legally from pharmacies by prescription from a licensed health care provider. In recent years in the United States, some health care providers are prescribing growth hormone in the elderly to increase vitality. While legal, the efficacy and safety of this use for HGH has not been tested in a clinical trial. Many of the functions of hGH remain unknown.

In its role as an anabolic agent, HGH has been used by competitors in sports since at least 1982, and has been banned by the IOC and NCAA. Traditional urine analysis does not detect doping with HGH, so the ban was not enforced until the early 2000s, when blood tests that could distinguish between natural and artificial HGH were starting to be developed. Blood tests conducted by WADA at the 2004 Olympic Games in Athens, Greece targeted primarily HGH. Use of the drug for performance enhancement is not currently approved by the FDA.

GH has been studied for use in raising livestock more efficiently in industrial agriculture and several efforts have been made to obtain governmental approval to use GH in livestock production. These uses have been controversial. In the United States, the only FDA-approved use of GH for livestock is the use of a cow-specific form of GH called bovine somatotropin for increasing milk production in dairy cows. Retailers are permitted to label containers of milk as produced with or without bovine somatotropin.

The mammalian *GH* gene (also called *GH-normal* or *GH-N*) belongs to a gene cluster that includes the genes for prolactin and some placental lactogens, and is primarily expressed in the somatotroph cells of the anterior pituitary gland. GH secretion occurs in a pulsatile fashion owing to the action of two hypothalamic factors, growth hormone releasing hormone (GHRH) which stimulates GH secretion, and somatostatin which inhibits GH secretion. GH secretion is also stimulated by ghrelin, an endogenous GH secretagogue that is primarily secreted by the gastrointestinal tract. In the circulation, GH is bound to the growth hormone binding protein (GHBP) which is a soluble truncated form of the growth hormone receptor (GHR). GHBP is generated either as an alternative splice form of the GHR transcript (in rodents) or by limited proteolysis of the GHR protein (in humans). Thus, GH in the circulation exists as bound and free forms, the predominance of each being dependent on the pulsatile pattern of its secretion.

GH secretion exhibits sexual dimorphism; it is secreted more frequently in females than in males. While this could reflect the differential effects of sex steroids on GH secretion and action, recent data suggest the existence of sex-specific differences in the GH/IGF-1 axis at birth. Moreover, inter-species differences in circulating GH profiles have been observed in mammals. In males, GH secretion occurs nocturnally in humans and in 3-4 hour intervals in rodents; while in females, rats have residual GH levels between periods of GH secretion which are absent in humans and mice.

Nomenclature:

The names *somatotropin* (*STH*) or *somatotropic hormone* refers to the growth hormone produced naturally in animals and extracted from carcasses. Hormone extracted from human cadavers is abbreviated *hGH*. The main growth hormone produced by recombinant DNA technology has the approved generic name (INN) *somatropin* and the brand name *Humatrope*, and is properly abbreviated rhGH in the scientific literature. Since its introduction in 1992 Humatrope has been a banned sports doping agent, and in this context is referred to as HGH.

Structure:

The major isoform of the human growth hormone is a protein of 191 amino acids and a molecular weight of 22,124 daltons. The structure includes four helices necessary for functional interaction with the GH receptor. It appears that, in structure, GH is evolutionarily homologous to prolactin and chorionic somatomammotropin. Despite marked structural similarities between growth hormone from different species, only human and Old World monkey growth hormones have significant effects on the human growth hormone receptor.

Several molecular isoforms of GH exist in the pituitary gland and are released to blood. In particular, a variant of approximately 20 kDa originated by an alternative splicing is present in a rather constant 1:9 ratio, while recently an additional variant of ~ 23-24 kDa has also been reported in post-exercise states at higher proportions. This variant has not been identified, but it has been suggested to coincide with a 22 kDa glycosylated variant of 23 kDa identified in the pituitary gland. Furthermore, these variants circulate partially bound to a protein (growth hormone-binding protein, GHBP), which is the truncated part of the growth hormone receptor, and an acid-labile subunit (ALS).

Regulation of Growth hormone secretion:

Secretion of growth hormone (GH) in the pituitary is regulated by the neurosecretory nuclei of the hypothalamus. These cells release the peptides growth hormone-releasing hormone (GHRH or *somatocrinin*) and growth hormone-inhibiting hormone (GHIH or *somatostatin*) into the hypophyseal portal venous blood surrounding the pituitary. GH release in the pituitary is primarily determined by the balance of these two peptides, which in turn is affected by many physiological stimulators (e.g., exercise, nutrition, sleep) and inhibitors (e.g., free fatty acids) of GH secretion.

Somatotropic cells in the anterior pituitary gland then synthesize and secrete GH in a pulsatile manner, in response to these stimuli by the hypothalamus. The largest and most predictable of these GH peaks occurs about an hour after onset of sleep with plasma levels. Otherwise there is wide variation between days and individuals. Nearly fifty percent of GH secretion occurs during the third and fourth NREM sleep stages. Surges of secretion during the day occur at 3- to 5-hour intervals.[3] The plasma concentration of GH during these peaks may range from 5 to even 45 ng/mL. Between the peaks, basal GH levels are low, usually less than 5 ng/mL for most of the day and night. Additional analysis of the pulsatile profile of GH described in all cases less than 1 ng/ml for basal levels while maximum peaks were situated around 10-20 ng/mL.

A number of factors are known to affect GH secretion, such as age, sex, diet, exercise, stress, and other hormones. Young adolescents secrete GH at the rate of about 700 μg/day, while healthy adults secrete GH at the rate of about 400 μg/day. Sleep deprivation generally suppresses GH release, particularly after early adulthood.

Mechanism of Action of Growth Hormones:

i. Receptors for growth hormone are present on the plasma membrane of cells.

ii. Belong to cytokine family of receptors.

iii. Presence of excess of GH down regulates the synthesis of its receptors.

iv. Many hours must elapse after administration of GH before anabolic and growth-promoting actions of the hormones to become evident.

v. Most of the actions of GH require the production of GH induced somatomedin C or insulin-like growth factor (IGF).

vi. The plasma half-life of IGF is much longer than that of GH.

Actions of the hormone can be broadly classified into two types:

a. Indirect growth promoting action

b. Direct anti-insulin action.

1. Indirect growth promoting action (Figs 6.9 and 6.10) is due to the action of growth hormone on liver. When the hormone acts on liver, liver secretes somatomedin C or insulin-like growth factor (IGF- I). This substance acts on skeletal and extraskeletal compartments.

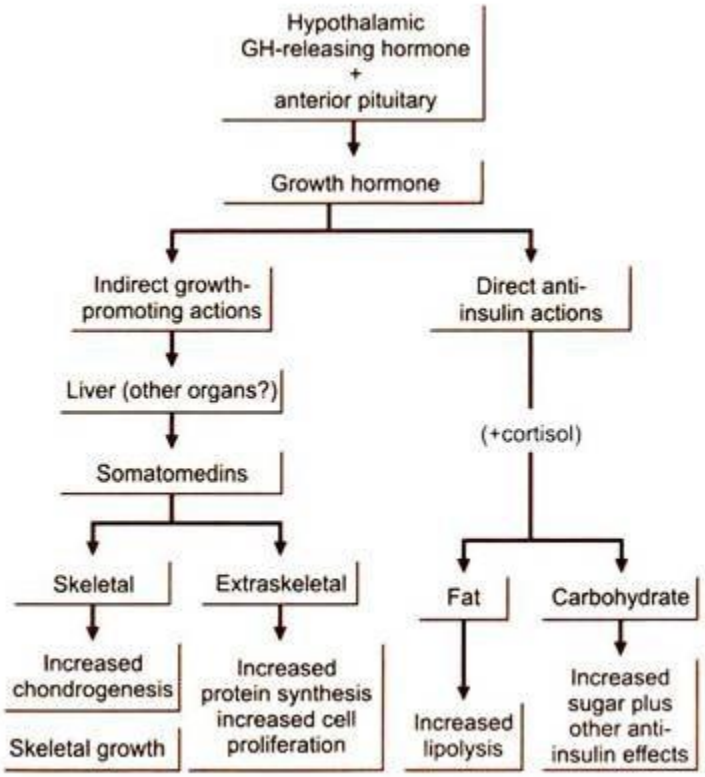


Fig. 6.9: Composite diagram showing actions of GH

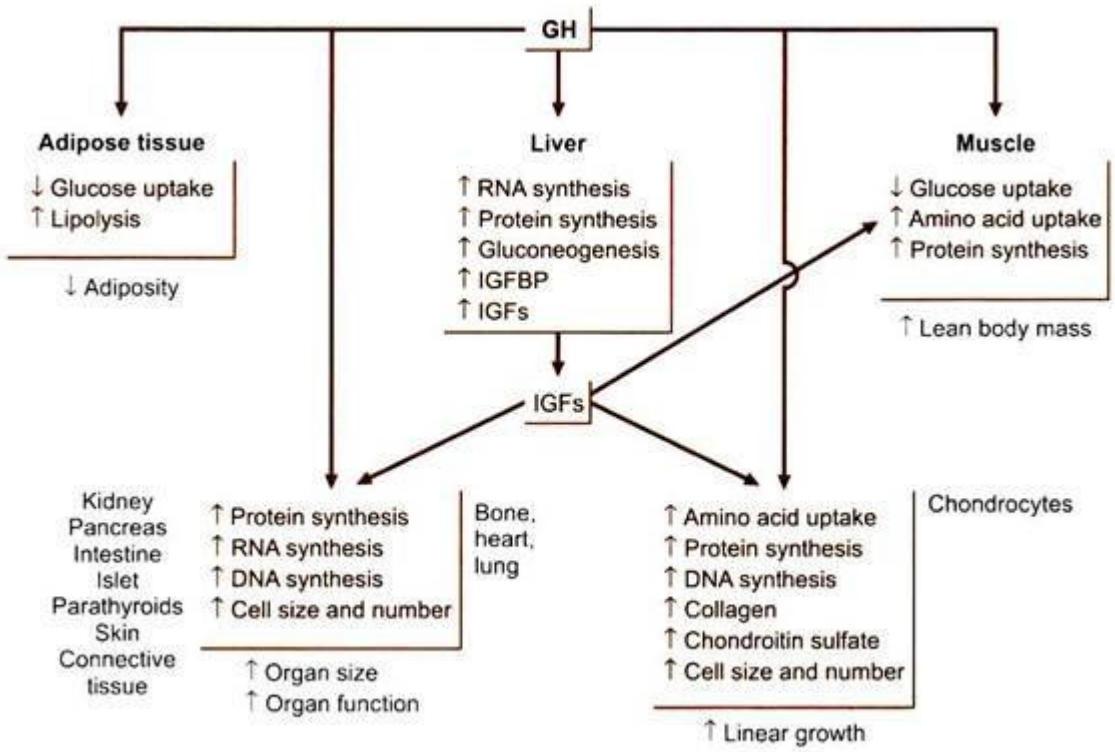


Fig. 6.10: Highlighting various intracellular actions of GH in the body

i. Skeletal compartment:

When somatomedin acts on epiphyseal plate present between the long bones, the epiphyseal plate gets widened. This gives space for the chondrogenesis of the long bones. The long bones grow linearly. Hence, the height of the person increases. The long bones can grow only up to the age of about 18-20 years beyond which the epiphyseal plates get fused with long bones and there can be no more linear growth of body.

ii. Extra-skeletal compartment:

This in general refers to the growth of organ and tissues. The growth is brought about by hyperplasia (stimulating mitotic cell division and hence increase in cell number) and hypertrophy (increase cell size). The various tissues in the body grow. There will be increased protein synthesis because of which it brings about positive nitrogen balance. The proteins synthesized are incorporated for the growth of the organs.

The various parts of the body do not grow in equal proportion at the same time. The growth of the different parts of the body based on chronological age has been shown in Fig. 6.11.

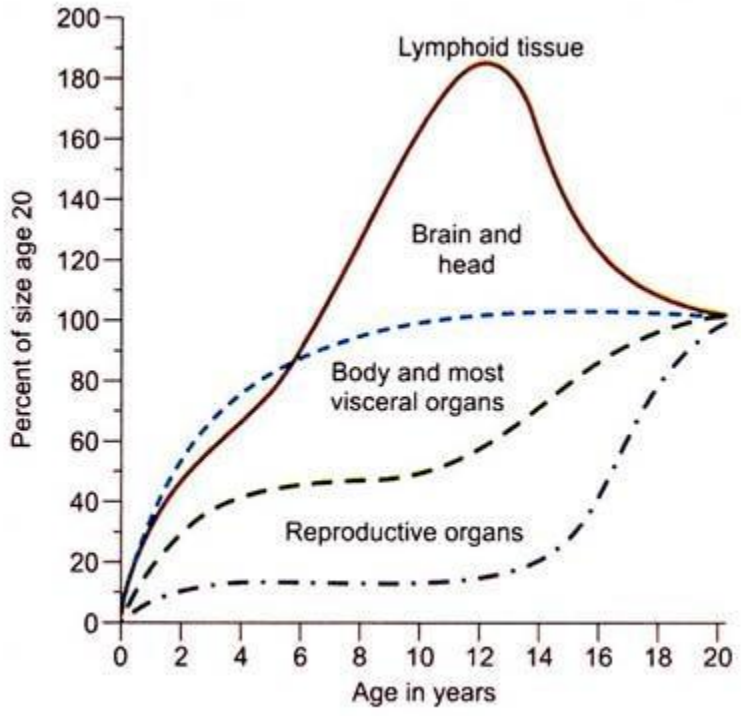


Fig. 6.11: Extent of growth of various tissues at different ages

2. Direct anti-insulin action:

This can be brought about in the target organs in presence of Cortisol (permissive action of Cortisol is required.

i. On carbohydrate metabolism:

It is a hyperglycemic agent. Increases the blood glucose level by:

a. Decreasing the peripheral utilization of glucose.

b. Increased gluconeogensis in liver.

Metahypophyseal diabetes:

Uncontrolled secretion of GH for a long time brings about increase in blood glucose level. This leads to increase stimulation of beta cells of islets of Langerhans to secrete insulin. After sometime, due to constant stimulation, the beta cells get exhausted and lead to development of diabetes mellitus.

ii. Fat metabolism:

Acts on the adipose tissue. Neutral fats and triglycerides are broken down to release the free fatty acids. They are utilized for energy supply to the tissues.

This can lead to increased production of keto acids. Growth hormone also promotes the retention of sodium, potassium, calcium and phosphate since these substances are required for the growth of the body.

Regulation of Secretion of Growth Hormones:

It is mainly by the negative feedback control by the free form of the hormone level in circulation.

Growth hormone releasing hormone (GRH) secreted from the hypothalamus acts on anterior pituitary gland and stimulates the secretion of growth hormone, which in turn increases insulin-like growth factor (IGF) I or somatomedin C secretion from liver. When IGF I level in circulation increases, it acts on hypothalamus to stimulate the secretion of somatostatin (SS). SS on reaching anterior pituitary decreases the secretion of growth hormone (Fig. 6.12).

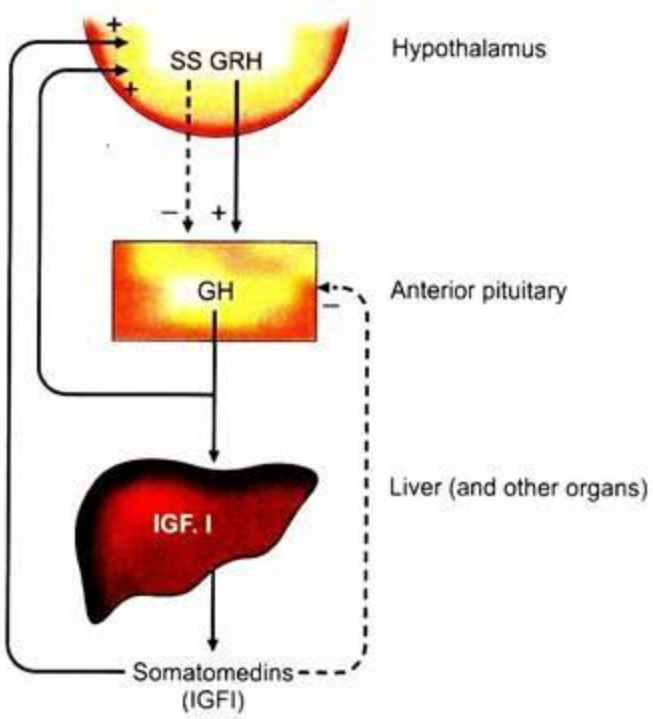


Fig. 6.12: Regulation of secretion of GH by feedback mechanism

IGF I also acts directly on anterior pituitary and exerts inhibitory influence on the secretion of growth hormone.GH secreted by the anterior pituitary gland is able to reach the hypothalamus through circulation and on reaching hypothalamus it stimulates the secretion of somatostatin. Somatostatin on reaching anterior pituitary inhibits further secretion of growth hormone.

Some of the other factors that increase the secretion of growth hormone are:

i. Increase in amino acids in circulation

ii. Hypoglycemia

iii. Free fatty acid decrease

iv. Exercise

v. At puberty

vi. Stage IV sleep.

The factors which inhibit the GH secretion are:

i. Dreaming or rapid eye movement (REM) sleep.

ii. Glucose increase.

iii. Cortisol.

iv. Obesity.

Applied Aspects of Growth Hormones:

Deficiency of GH in children:

i. Hypothalamic dysfunction

ii. Pituitary destruction

iii. Defective GHRH receptor

iv. Biologically incompetent GH or GH receptor

v. Failure to produce IGF

vi. GH receptor deficiency

vii. GH receptor unresponsiveness: Laron dwarfism

Dwarfism:

i. It's because of hyposecretion of GH from childhood.

ii. Person will have short stature. There will be a generalized stunted growth of the body.

iii. The person will have normal reproductive development.

iv. There will not be any mental abnormality and will have normal intelligent quotient (IQ).

v. Facial changes correspond with chronological age.

Achondroplasia is the most common form of dwarfism. The characteristic feature will be short limbs and normal trunk.

Laron dwarf:

i. It will be due to insensitivity of the tissues to GH.

ii. The receptors are non-responsive to GH.

iii. There can be normal or elevated level of GH in circulation.

Progeria:

Deficiency of growth hormone in adult. The person appears older at an younger age.

Dwarfism could also be due to:

i. Cretinism—thyroxine deficiency

ii. Gonadal dysgenesis

iii. Kaspar Hauser syndrome—psychosocial dwarfism

iv. Achondroplasia—child born to aged father

Frolich dwarf:

Destructive disease of part of anterior pituitary. At times may include post-pituitary and hypothalamus.

i. Stunted growth.

ii. Obesity

iii. Decreased sexual development

iv. Somnolence

v. Mentally subnormal

Deficiency of GH in adult:

i. Decreased muscle

ii. Decreased muscle strength and exercise performance

iii. Decreased lean body mass

iv. Decreased bone density

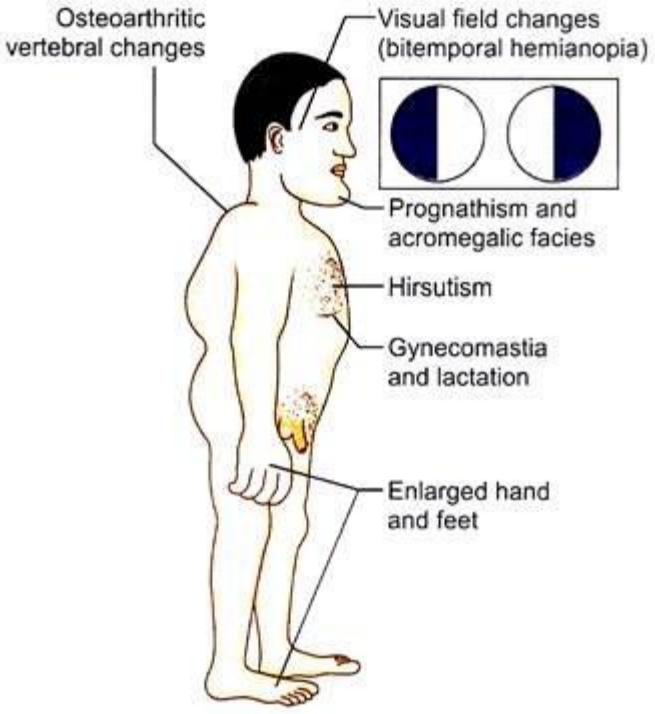


Fig. 6.13: Some of the important features of acromegaly

Acromegaly:

i. Hypersecretion of growth hormone after the puberty.

ii. Enlargement of hand and feet (acral parts of the body only can grow because of the ossification of the long bones).

iii. There will also be enlargement of mandible which results in prognatism. There will also be enlargement and protrusion of frontal bone. Because of this, the person may have gorilla-like appearance.

iv. Certain osteoarthritic changes are also observed leading to kyphosis.

v. There can be enlargement of viscera especially that of heart and may lead to cardiomegaly.

vi. There can be hirsutism (increased hair growth on anterior part of trunk) and gynecomastia (enlargement of breasts even in males) and lactation (secretion of milk).

vii. The person may suffer from bitemporal hemianopia (a type of visual field defect) due to the compressing on the medial part of optic chiasma by enlarged pituitary gland.

Gigantism:

i. Hypersecretion of hormone from childhood.

ii. Size of the person is pathologically big, but the person will be weak. Hence, the person is known as weak giant. There will not be proportionate growth of the contractile proteins in the muscles. Hence muscles are weak.

iii. The person is prone to develop early diabetes. This is because since growth hormone has hyperglycemic action, the sustained increase in blood glucose level may lead to exhaustion of beta cells of islets of Langerhans. So the person develops diabetes.

iv. The longevity of these people is restricted and die early.

Sheehan's syndrome:

i. Observed in female. Due to postpartum hemorrhage, there can be ischemic necrosis of pituitary gland.

ii. The pituitary gland secretion in general gets decreased.

iii. Symptoms include lethargy, sexually inactive, unable to withstand stress. Growth is inhibited and thyroid function is depressed.

iv. There can be atrophy of gonads. The menstrual cycle stops.

v. When there is general deficiency of all the hormones of anterior pituitary gland, this condition is known as panhypopituitarism.

Hyperprolactinemia:

It could be due to administration of dopamine antagonist/prolactin secreting adenomas.

Features:

a. Amenorrhea , b. Galactorrhea, c. Decreased libido, d. Impotence, e. Hypogonadism, f. Testosterone level low

Insulin:

Insulin is a type of protein hormone, which is synthesized in the β-cells of islets of Langerhans. The term insulin is derived from Latin word "Insula" means island. Banting and Best (1916) observed the role of insulin in glucose metabolism.

Structure of Insulin:

Insulin is a peptide hormone and its molecular weight is 5.7 Kdt. It is made up of two polypeptide chains α and β. Insulin is constituted by 51 amino acids, of which a-chain contains 21 amino acids and β-chain contains 30 amino acid residues. Besides the primary peptide bonds, the polypeptide chains are strengthened by disulphide bonds (-S-S-).

One intra -S-S- bond occurs in the a-chain in between 6 and 11 positions of cystine. Two inter -S-S- bonds are found in between a and p chain one in between 7th position of both the chains and other in between 20th position of α-chain and 19th position of β-chain (Fig. 6.1).

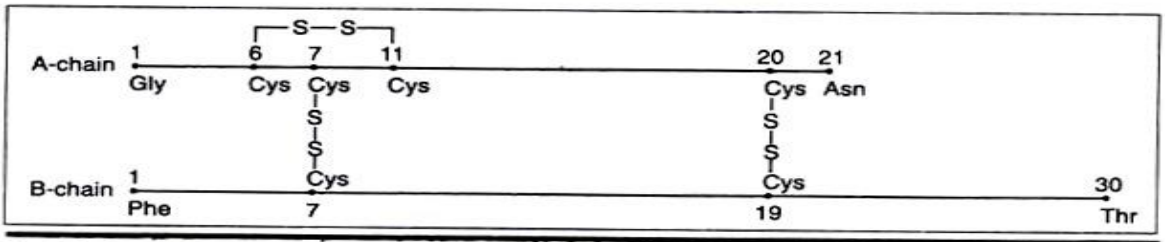


Fig. 6.1: Schematic representation of human insulin molecule

In different species of vertebrates, structure of insulin varies according to variation of amino acid residues. Variations occur at 8th, 9th and 10th position of a-chain and 30th position of β-chain.

Species	α-chain			β-chain
	8th	9th	10th	30th
Horse	Thr	Gly	Ile	Ala
Goat & Cattle	Ala	Ser	Val	Ala
Man	Thr	Ser	Ile	Thr
Rabbit	Thr	Ser	Ila	Ser

Biosynthesis of Insulin:

The synthesis of insulin takes place in p-cells of islets of Langerhans.

It is a complex phenomenon and it occurs in following ways:

1. Transcription of code:

Genes on chromosome 11 coding for insulin and are transcribed to mRNA in the nucleus.

2. Translation of the code:

After moving to the cytoplasm, mRNA is translated by the polysome attach to GER. Polypeptide synthesis is initiated with the formation of N-terminal signal peptide (leading sequence) which penetrates through the membrane of GER.

3. Synthesis of preproinsulin:

Further elongation directs the polypeptide chain into the lumen of GER, resulting in the formation of preproinsulin. It is constituted by 109 amino acid residues and mol. wt. is 11.5 kdt.

4. Separation of signal sequence:

In the lumen of GER, N-terminal signal peptide is hydrolysed away by signal peptidase. Thus signal peptide is cleaved and pro- insulin is formed in the cysternal space of GER. Pro-insulin consists of 86 amino acid residues and its mol. wt. is about 9 kd. Pro-insulin has disulphide bonds.

5. Transfer of pro-insulin:

Pro-insulin is transported from GER to the Golgi complex

6. Splitting of pro-insulin:

In Golgi cisternae pro-insulin is hydrolysed by trypsin like peptidase to yield a 53 amino acid insulin precursor and pro-c-peptide has 33 amino acids.

Under condition of excessive stimulation pro-insulin is secreted by vesicular exocytosis along with the insulin from p-cells (Fig. 6.2).

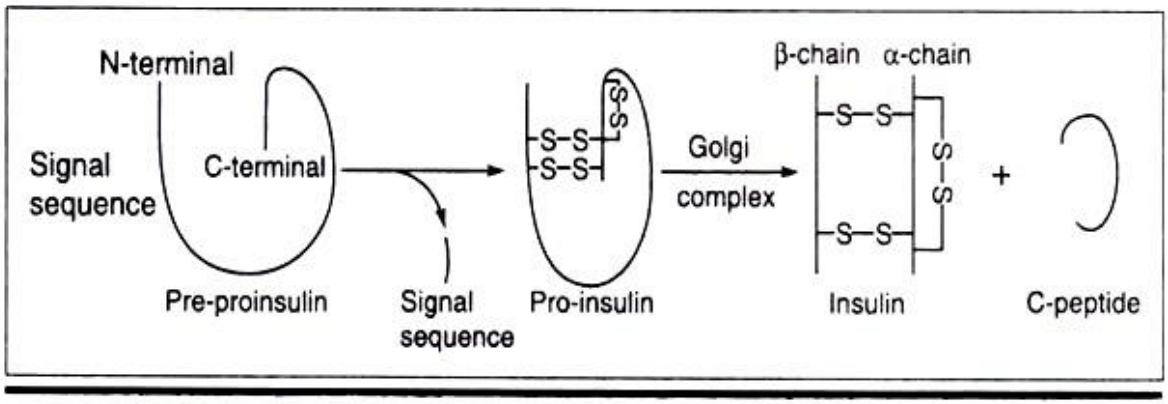


Fig. 6.2: Formation of insulin

7. Formation of insulin:

In the Golgi complex about 95% of the pro-insulin is converted to active insulin. Enzyme carboxylase peptidase hydropses c-terminal peptide bonds in the pro-c- peptide and the insulin precursor to release 2-c-terminal basic amino acids from each. Two molecules of Arg. are driven out from the insulin precursor and lead to the formation of active insulin (consists of 51 amino acids).From pro-c-peptide two amino acids Lys and Arg are separate out and leads to the formation of connective peptide or c-pep- tide (consists of 31 amino acids). Insulin and c-peptide are present in secretory granules of Golgi complex. In some species, insulin is combined with Zn within P-cells. After stimulation insulin is secreted by exocytosis (Fig. 6.3).

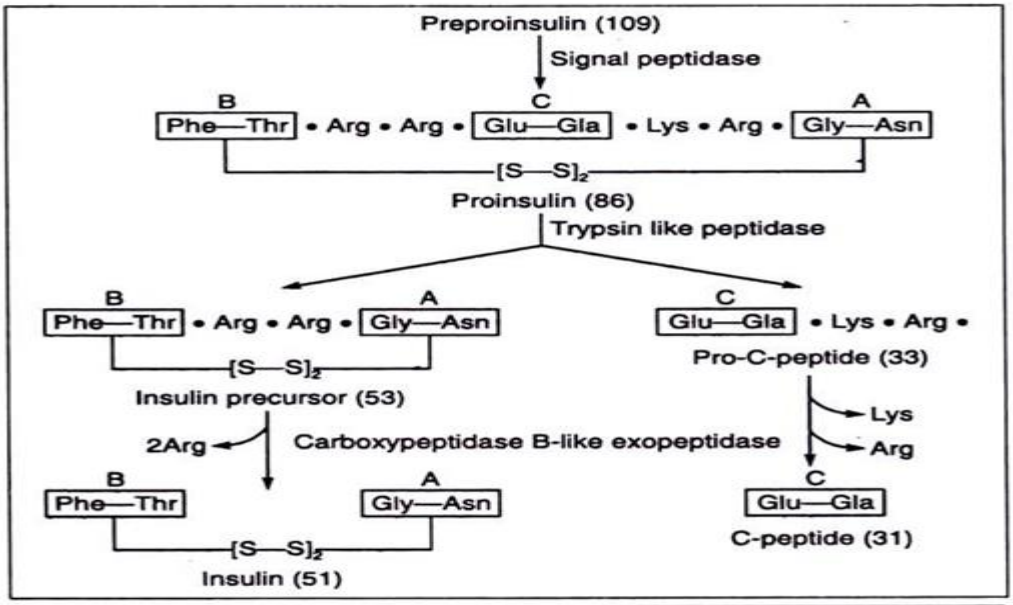


Fig. 6.3: Schematic representation of insulin synthesis

Transport of insulin:

Insulin is directly diffused within the blood sinusoids of the islets and is transported to the target organ.

Catabolism of insulin:

After biochemical reaction, insulin is degraded within the liver, kidney, skeletal muscles and placenta in presence of enzyme insulinase.

Control of secretion:

Insulin synthesis and secretion is controlled by following factors:

1. Carbohydrate meal:

Intake of carbohydrate rich food leads to raise the blood glucose which is signal for increased insulin secretion.

2. Amino acids:

Ingestion of protein causes an increase in plasma amino acids level. Elevated plasma arginine is particularly potent stimulus for insulin secretion.

3. Gastrointestinal hormone:

Intestinal hormones (GIP & VIP) secretion stimulates the insulin synthesis and secretion.

4. Epinephrine:

The synthesis and release of insulin are degraded by negative feedback mechanism of epinephrine in stress condition.

5. Glucagon:

Low blood sugar level stimulates the secretion of glucagon for glycogenesis, which in-turn inhibits the synthesis of insulin (Fig. 6.4).

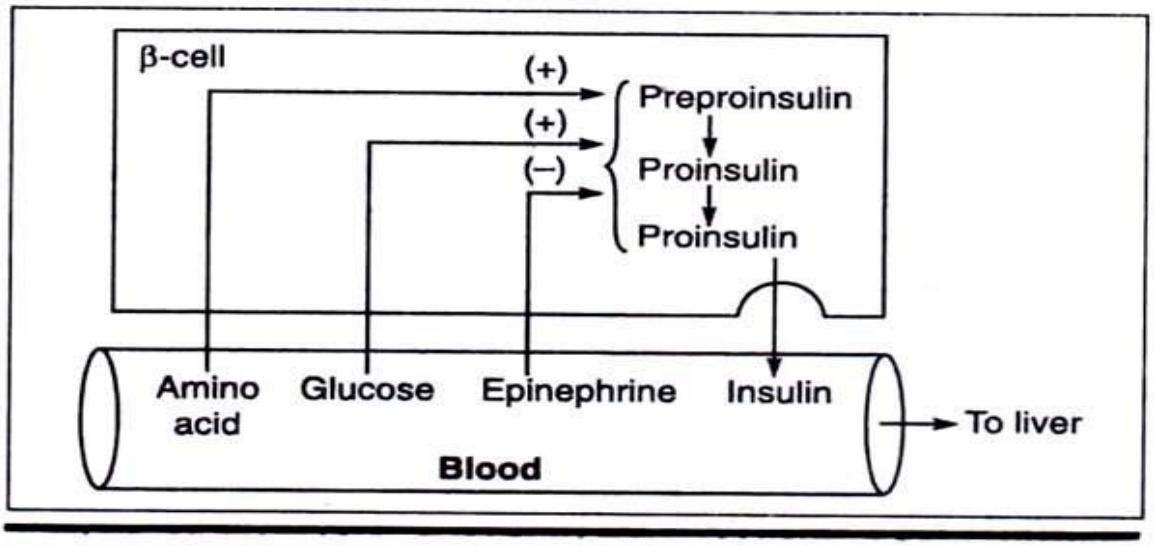


Fig. 6.4: Regulation of insulin release from β-cell

6. Somatostatin:

This hormone is secreted from D-cells of pancreatic islets and regulates the secretion α and β-cells.

Insulin Receptor:

a. Insulin acts on target tissues by binding to specific insulin receptors which are glycoproteins.

b. The human insulin receptor gene is found on chromosome 19. The insulin receptors are being constantly synthesized and degraded. Their half-life is 6 to 12 hours only.

c. It is synthesized as a single chain polypeptide, pro-receptor in the rough endoplasmic reticulum and is rapidly glycosylated in Golgi region.

d. The pro-receptor is cleaved to form mature α and β subunits ($\alpha_2\beta_2$) which is heterodimer, linked by S- S bonds.

e. Both subunits are extensively glycosylated and removal of sialic acid and galactose decreases insulin binding and insulin action.

f. Insulin receptors are found in target cell membrane.

g. Though insulin receptor is a heterodimer consisting of 2 subunits, designated α and β ($\alpha_2\beta_2$) linked by disulphide bonds.

h. The α subunit is entirely extracellular and it binds insulin, probably via a cystinerich domain.

i. The β subunit is a trans-membrane protein that performs the second major function of a receptor, i.e. signal transduction and insulin action.

j. Binding of insulin to the receptor stimulates its tyrosine kinase activity. Tyrosine kinase enzyme phosphorylates the phenolic -OH group of tyrosine residues in specific protein including that of a tyrosine in the chain of insulin receptor itself to modulate their activities, ATP + tyrosine protein → ADP + phosphotyrosine protein.

The cytoplasmic portion of p subunit has tyrosine kinase activity and an auto-phosphorylation site.

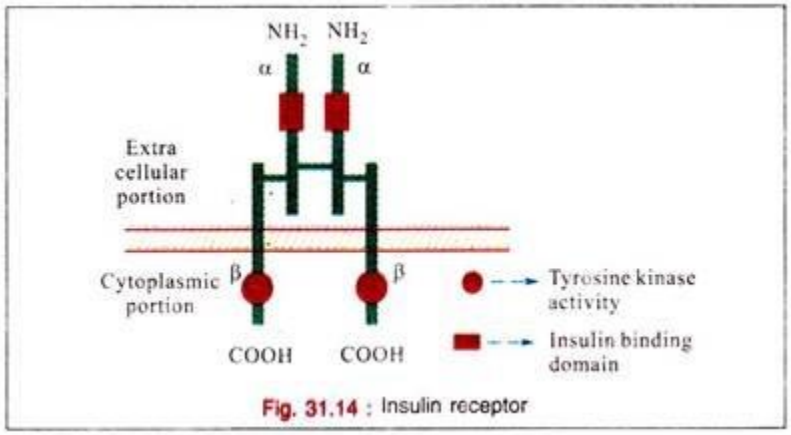


Fig. 31.14 : Insulin receptor

Insulin Secretion:

About 50 units of insulin are required per day. The human pancreas stores about 250 units. Normal concentration of insulin (fasting) in plasma: 6-126 μU/ml.

Factors Stimulating Insulin Secretion:

a. Increased blood glucose level causes an increase in insulin secretion and decreased blood glucose level depresses insulin secretion.

b. The hyperglycemia produced by glucagon enhances insulin production.

c. Since the growth hormone and glucocorticoids cause hyperglycemia they also stimulate insulin secretion.

d. Sugars which are readily metabolized— e.g., mannose and fructose—can stimulate insulin release. But non-metabolised sugars such as glactose, L-arabinose and xylose do not stimulate.

e. Many agents, such as amino acids, fatty acids and some gastro—intestinal products can stimulate insulin release only in presence of glucose.

f. Insulin secretion is enhanced by cAMP, ACTH and thyrotropin.

g. Amino acids particularly leucine and arginine can stimulate pancreas to produce insulin in both vivo and vitro. Proteins like casein also increases secretion of insulin.

h. Central nervous system indirectly influences the release of insulin. Vagal stimulation causes an increase in insulin secretion.

i. Sulfonylureas, the hypoglycemic agent, may act on insulin secretion by a different mechanism than that of glucose.

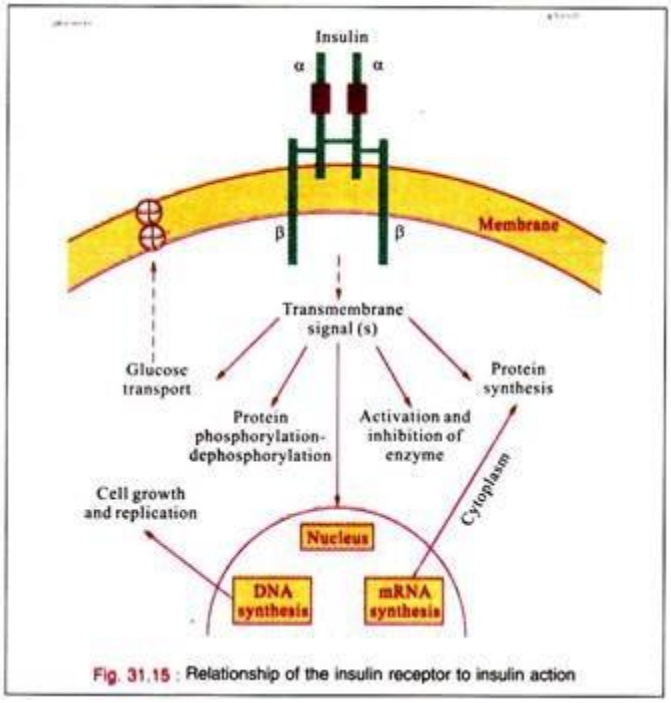


Fig. 31.15 : Relationship of the insulin receptor to insulin action

Factors Inhibiting Insulin Secretion:

a. Epinephrine is the highly effective inhibitor of insulin secretion.

b. Starvation reduces insulin secretion.

c. Magnesium also inhibits insulin secretion.

d. Vagotomy reduces insulin secretion.

Metabolism of Insulin:

a. Insulin is degraded in liver and kidney by the enzyme glutathinone insulin trans-hydrogenase which brings about reductive cleavage of the S-S bonds that connect A and B chains of the insulin molecule. Reduced glutathione acts as a coenzyme.

b. The A and B chains are further degraded by proteolysis. But when insulin is bound to antibody, it is much less sensitive to enzymic degradation.

Functions of Insulin:

a. Insulin is firmly bound to the highly specific receptor site present in the cell membrane. The receptor may probably be a glycoprotein. The biologic activities of insulin's are proportionate to their binding affinities. Insulin, thus, may carry out most of its function without entering the cell. The number of receptors declines where insulin levels are high.

b. Insulin exhibits transport at the membrane site, RNA synthesis at the nuclear site, translation at the ribosome for protein synthesis, and influence on tissue levels of cAMP. It is active in skeletal and heart muscle, adipose tissue, liver, the lens of the eye and leukocytes. It is inactive in renal tissue, red blood cells and gastrointestinal tract. The most metabolic function is centered in the muscle, adipose tissue and liver.

c. It facilitates the transport of glucose and related monosaccharides, amino acids, potassium ion, nucleosides, inorganic phosphate, and calcium ion in muscle and adipose tissue.

d. In muscle for adipose tissue, insulin increases the entry of glucose and thus leads to increased glycogen deposition, stimulation of HMP shunt resulting in increased production of NADPH, increased glycolysis, increased oxidation (Increase in oxygen uptake and CO_2 production), and increased fatty acid synthesis.

e. In adipose tissue, it increases lipid synthesis by means of fatty acid synthesis and glycerophosphate for triacylglycerol synthesis.

f. Insulin increases intracellular concentration of non-metabolized sugars such as galactose, L-arabinose, and xylose. The hormone facilitates the entry of those sugars having the same configuration at carbons, 1, 2, and 3 as D-glucose. Since fructose having a ketone group at position 2 is not transported by insulin. Intracellular transport of glucose is enhanced by anoxia indicating that glucose transport requires energy.

g. It also increases the uptake of nonmetabolizable amino acids such as alpha-aminoisobutyrate. It maintains muscle protein by decreasing protein degradation.

h. In adipose tissue, it quickly depresses the liberation of fatty acids caused by epinephrine or glucagon.

i. Insulin directly increases protein synthesis as the hormone promotes the incorporation of labelled intracellular amino acids into protein. At the ribosomal level, it increases the capacity of this organelle to translate information from messenger RNA to the protein-synthesizing machinery.

j. In the liver, it stimulates glycolysis by increasing the synthesis of glucokinase, phosphofructokinase. and pyruvate kinase. It also depresses the enzymes controlling gluconeogenesis such as pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1, 6-di-phosphatase, and glucose-6-phosphatase. Enzymes which are unimportant in the control of gluconeogenesis as well as glycolysis are not affected by insulin.

Pathophysiology of Insulin:

a. About 90% of persons with diabetes have non-insulin dependent (Type II) diabetes mellitus (NIDDM). Such patients are usually obese, have elevated plasma insulin levels.

b. The other 10% have insulin dependent (Type 1) diabetes mellitus (IDDM).

c. A few individuals produce antibodies directed against their insulin receptors. These antibodies prevent insulin from binding to the receptor so that such persons develop a syndrome of severe insulin resistance.

d. Tumors of β-cell origin cause hyperinsulinism thereby hypoglycemia. Leprechaunism is caused by the role of insulin in organogenesis. The syndrome is characterized by low birth weight, decreased muscle mass, decreased subcutaneous fat, and early death.

Abnormal Metabolism in Diabetic States:

a. In diabetes, hyperglycemia occurs due to the impaired transport and uptake of glucose into muscle and adipose tissue. Transport and uptake of amino acids are also depressed causing the raised level of amino acids into the blood, particularly, alanine, which supply fuel for gluconeo-genesis in the liver. The amino acid breakdown during gluconeogenesis increases the production of urea nitrogen.

b. Lipid and fatty acid synthesis is decreased due to the decrease in acetyl-CoA, ATP, NADPH and glycerophosphate in all tissues. Stored lipids are hydrolysed by increased lipolysis and the liberated fatty acids interfere the carbohydrate phosphorylation in muscle and liver developing hyperglycemia.

c. Fatty acids in high concentration reaching the liver inhibit fatty acid synthesis by a feedback inhibition at the acetyl-CoA carboxylase step. Increased acetyl-CoA from fatty acids activates pyruvate carboxylase, stimulating gluconeogenic pathway for the conversion of amino acid carbon skeletons to glucose. Fatty acids also stimulate gluconeogenesis by entering the citric acid cycle and increasing production of citrate which is an inhibitor of glycolysis (at phosphofructokinase).Thus, the fatty acid cycle at the level of citrate synthetase and pyruvate and isocitrate dehydrogenases. The acetyl CoA, which cannot enter the citric acid cycle or cannot be used for fatty acids synthesis, is utilized in the synthesis of cholesterol or ketones or both. The rise in ketone bodies concentration in body fluids and tissues leads to acidosis.

d. Glycogen synthesis is diminished due to decreased glycogen synthetase activity, increased phosphorylase activity and increased ADP: ATP ratio. The phosphorylase activity is stimulated by epinephrine or glucagon.

e. The insulin deficiency causes hormonal imbalance and favours the action of corticosteroids, growth hormone and glucagon which enhance gluconeogenesis, lipolysis, and decreased intracellular metabolism of glucose. The excess glucose in the urine requires water to be excreted out causing dehydration.

f. In the degradation in insulin, both liver and kidney are required. Therefore, in renal or hepatic disease, insulin requirement is decreased. This is observed in some diabetics with associated kidney or liver disease.

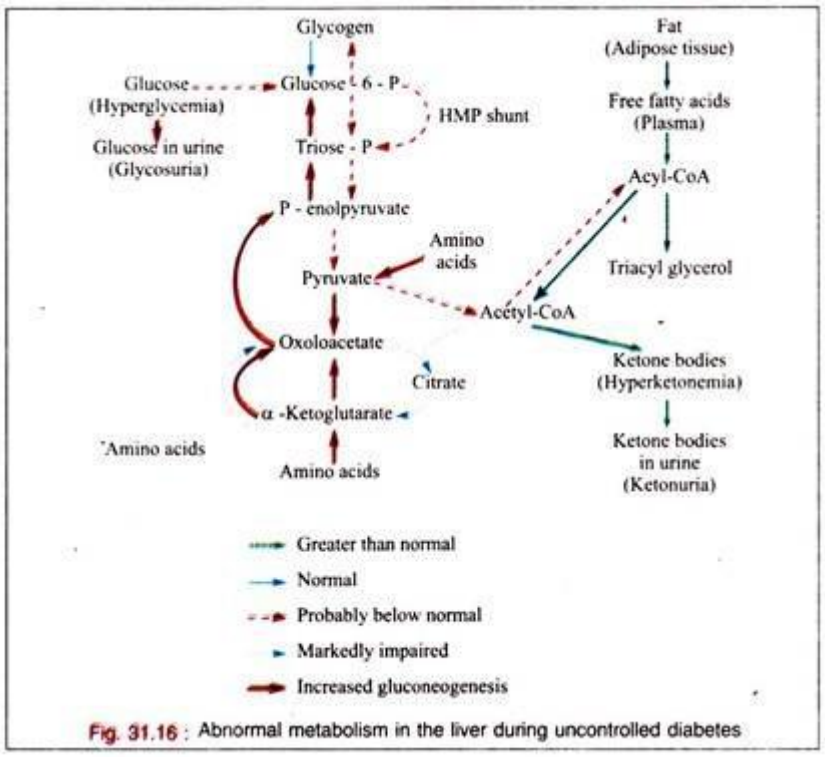


Fig. 31.16 : Abnormal metabolism in the liver during uncontrolled diabetes

Antibodies in Insulin:

a. The repeated injection of insulin produces low levels of an antibody to insulin in all subjects after 2 or 3 months of treatment.

b. The antibodies can produce lesions in the islet cells and severe diabetes.

c. Antibody-bound insulin is only slowly degraded; thus much of the insulin is actually wasted.

Experimental Diabetes:

a. Experimental diabetes can be produced by total pancreatectomy or by a single injection of alloxan, a substance related to the pyrimidine's or with streptozocin, an N-nitroso derivative of glucosamine.

b. Diabetes can also be produced by injection of diazoxide, a sulfonamide derivative which inhibits insulin secretion.

c. The injection of large amounts of antibodies to insulin is also considered to produce experimental diabetes.

d. Phlorhizin diabetes can be produced by the injection of the drug phlorhizin. This is actually a renal diabetes in which glycosuria is only produced by the failure of the reabsorption of glucose by the renal tubules.

Regulation of Insulin Secretion:

40 to 50 units of insulin is daily secreted from human pancreas. This represents about 15 to 20 per cent of the hormone stored in the gland. Insulin secretion is an energy-requiring process. Different factors are involved in insulin release.

a. Glucose:

(a) The increased concentration of glucose is the best regulator of insulin secretion.

(b) Among two ideas, one idea suggests that glucose combines "with a receptor which" is located on the B cell membrane that activates the release mechanism. The second idea suggests that intracellular metabolites pass through a pathway .such as the HMP shunt, the TCA cycle, etc.

b. Hormonal Factors:

(i) Epinephrine inhibits insulin release.

(ii) Beta adrenergic agonists stimulate insulin release by increasing intracellular cAMP.

(iii) Cortisol, estrogens, and progestin's also increase insulin secretion. Hence, insulin secretion is markedly increased during the later stages of pregnancy.

c. Pharmacologic Agents:

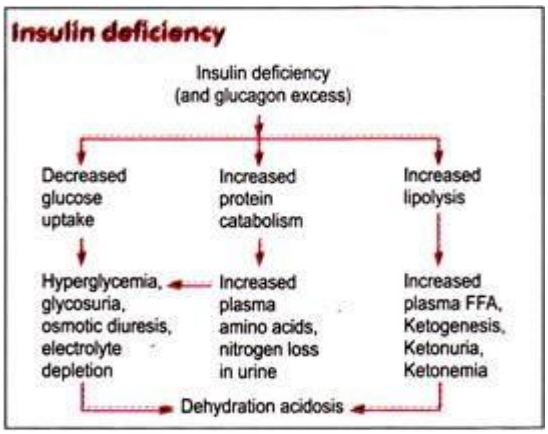
(i) Many drugs stimulate insulin secretion, but the sulfonyl urea compounds are used for therapy in humans.

(ii) Drugs such as tolbutamide stimulate insulin release and effectively used in the treatment of type 11 (non-insulin-dependent) diabetes mellitus. This class of drug is binded by a receptor which has been derived from the pancreatic P cells.

Effect of Insulin on Gene Expression:

(i) The actions of insulin are found to occur at the plasma membrane level or in the cytoplasm.

(ii) The synthesis of phosphoenolpyruvate carboxykinase (PEPCK) which catalyses a rate-limiting step in gluconeogenesis is decreased by insulin and hence gluconeogenesis is decreased.



(iii) Transcription is decreased due to the decreased amount of the primary transcript and of mature mRNA PEPCK which in turn is directly related to the decreased rate of PEPCK synthesis.

(iv) More than 100 specific mRNAs are affected by insulin, and a number of mRNAs in liver, adipose tissue, skeletal muscle, and cardiac muscle.

Probable Questions:

1. Write down the structure of Growth hormone.
2. What are the main functions of GH?
3. How hGH synthesis is regulated?
4. What is the mechanism of action of hGH?
5. state the effect of high and low secretion of hGH?
6. Write down the structure of insulin.
7. How biosynthesis is occurred of Insulin.
8. State the factors which stimulates insulin secretion.
9. State the factors which inhibits insulin secretion.
10. What are the functions of Insulin.
11. Describe the pathophysiology of insulin.

Suggested Readings:

1. General Endocrinology. Turner and Bagnara. Sixth Edition.
2. Williams Textbook of Endocrinology. Tenth Edition.
3. Introduction to Endocrinology. Chandra S Negi. Second Edition
4. Endocrinology. Hadley and Levine. Sixth Edition

UNIT-XIII

Biosynthesis and function of steroid and thyroid hormones (T3 and T4) and their regulations

Objective: In this unit we will discuss about various aspects of steroid and thyroid hormones.

Steroid hormones:

Steroid, any of a class of natural or synthetic organic compounds characterized by a molecular structure of 17 carbon atoms arranged in four rings. Steroids are important in biology, chemistry, and medicine. The steroid group includes all the sex hormones, adrenal cortical hormones, bile acids, and sterols of vertebrates, as well as the moulting hormones of insects and many other physiologically active substances of animals and plants. Among the synthetic steroids of therapeutic value are a large number of anti-inflammatory agents, anabolic (growth-stimulating) agents, and oral contraceptives.

Different categories of steroids are frequently distinguished from each other by names that relate to their biological source—e.g., phytosterols (found in plants), adrenal steroids, and bile acids—or to some important physiological function—e.g., progesterones (promoting gestation), androgens (favouring development of masculine characteristics), and cardiotonic steroids (facilitating proper heart function).Steroids vary from one another in the nature of attached groups, the position of the groups, and the configuration of the steroid nucleus (or gonane). Small modifications in the molecular structures of steroids can produce remarkable differences in their biological activities.

Transport of Steroid Hormones:

Steroid hormones are transported through the blood by being bound to carrier proteins—serum proteins that bind them and increase the hormones' solubility in water. Some examples are sex hormone-binding globulin (SHBG), corticosteroid-binding globulin, and albumin. Most studies say that hormones can only affect cells when they are not bound by serum proteins. In order to be active, steroid hormones must free themselves from their blood-solubilizing proteins and either bind to extracellular receptors, or passively cross the cell membrane and bind to nuclear receptors. This idea is known as the free hormone hypothesis. This idea is shown in Figure 1 to the right.

One study has found that these steroid-carrier complexes are bound by megalin, a membrane receptor, and are then taken into cells via endocytosis. One possible pathway is that once inside the cell these complexes are taken to the lysosome, where the carrier protein is degraded and the steroid hormone is released into the cytoplasm of the target cell. The hormone then follows a genomic pathway of action. This process is shown in Figure 2 to the right. The role of endocytosis in steroid hormone transport is not well understood and is under further investigation.

In order for steroid hormones to cross the lipid bilayer of cells they must overcome energetic barriers that would prevent their entering or exiting the membrane. Gibbs free energy is an

important concept here. These hormones, which are all derived from cholesterol, have hydrophilic functional groups at either end and hydrophobic carbon backbones. When steroid hormones are entering membranes free energy barriers exist when the functional groups are entering the hydrophobic interior of membrane, but it is energetically favourable for the hydrophobic core of these hormones to enter lipid bilayers. These energy barriers and wells are reversed for hormones exiting membranes. Steroid hormones easily enter and exit the membrane at physiologic conditions. They have been shown experimentally to cross membranes near a rate of 20 μm/s, depending on the hormone.

Though it is energetically more favourable for hormones to be in the membrane than in the ECF or ICF, they do in fact leave the membrane once they have entered it. This is an important consideration because cholesterol—the precursor to all steroid hormones—does not leave the membrane once it has embedded itself inside. The difference between cholesterol and these hormones is that cholesterol is in a much larger negative Gibb's free energy well once inside the membrane, as compared to these hormones. This is because the aliphatic tail on cholesterol has a very favourable interaction with the interior of lipid bilayers.

Mechanisms of action and effects:

There are many different mechanisms through which steroid hormones affect their target cells. All of these different pathways can be classified as having either a genomic effect, or a non-genomic effect. Genomic pathways are slow and result in altering transcription levels of certain proteins in the cell; non-genomic pathways are much faster.

a. Genomic pathways

The first identified mechanisms of steroid hormone action were the genomic effects. In this pathway, the free hormones first pass through the cell membrane because they are fat soluble. In the cytoplasm, the steroid may or may not undergo an enzyme-mediated alteration such as reduction, hydroxylation, or aromatization. Then the steroid binds to a specific steroid hormone receptor, also known as a nuclear receptor, which is a large metalloprotein. Upon steroid binding, many kinds of steroid receptors dimerize: two receptor subunits join together to form one functional DNA-binding unit that can enter the cell nucleus. Once in the nucleus, the steroid-receptor ligand complex binds to specific DNA sequences and induces transcription of its target genes.

b. Non-genomic pathways

Because non-genomic pathways include any mechanism that is not a genomic effect, there are various non-genomic pathways. However, all of these pathways are mediated by some type of steroid hormone receptor found at the plasma membrane. Ion channels, transporters, G-protein coupled receptors (GPCR), and membrane fluidity have all been shown to be affected by steroid hormones. Of these, GPCR linked proteins are the most common. For more information on these proteins and pathways, visit the steroid hormone receptor page.

Biosynthesis of Cholesterol:

The main steps of the biosynthesis of cholesterol are diagrammatically represented in figure 5-22. The first reaction consists of the condensation of 2 molecules of acetyl-coA. It is the reverse of the reaction which takes place during the last turn of the helix in β-oxidation.

Then a third molecule of acetyl-coA binds to the acetoacetyl-coenzyme A thus formed which gives β-hydroxy-β-methyl-glutaryl coenzyme A (HMG coA). This binding of an acetyl-coenzyme A to a carbonyl group is similar to the reaction permitting the entry of acetyl-coenzyme A in the Krebs cycle by condensation on oxaloacetic acid. The reduction of the acid group (engaged in a thioester linkage) to alcohol, catalyzed by HMG coA reductase, gives mevalonic acid. It must be noted that all the carbon atoms of cholesterol originate from acetyl- coenzyme A.

A pyrophosphate group will then bind to the primary alcohol group of mevalonic acid. Mevalonyl-pyrophosphate will react with a third molecule of ATP; this reaction gives an unstable compound which decomposes spontaneously, losing the tertiary alcohol group and the free carbonyl group. An isoprene derivative with 5 carbon atoms is formed, isopentenyl-pyrophosphate which can be isomerized to dimethyl-allyl-pyrophosphale. The condensation of 2 fragments in C_5 gives geranyl-pyrophosphate (C_{10}), and after the binding of a third fragment in C_5, farnesyl-pyrophosphate (C_{15}) is obtained. The dimerization of the latter leads to squalene (C_{30}).

In vertebrates, the cyclization of squalene by squalene-oxidocyclase takes place by a series of reactions requiring molecular oxygen and a reducing coenzyme like NADPH, and leads to lanosterol. The passage from lanosterol to cholesterol lakes place through several parallel pathways. The most important intermediates are desmosterol and 7-dehydrocholesterol, immediate precursors of cholesterol. The isoprene derivatives with 5 carbon atoms are the precursors of dolichols, of the side chain of ubiquinone and vitamin K, of the isopentenyl group of some tRNAs.

In vertebrates, the biosynthesis of cholesterol is microsomal. It is regulated by a feedback inhibition mechanism by a metabolite of cholesterol (most probably the 25 hydroxycholesterol) acting on the HMG coA reductase. This feedback inhibition is never total to always permit the synthesis of polyisoprenoids important for other metabolisms, like the dolichols. The liver is one of the principal sites of synthesis. Cholesterol is then carried to other organs in the form of lipoproteins. It enters the cells by binding of the lipoprotein to a specific receptor. In physiological conditions the exogenous input of hepatic cholesterol to various tissues is sufficient to inhibit endogenous synthesis in these tissues.

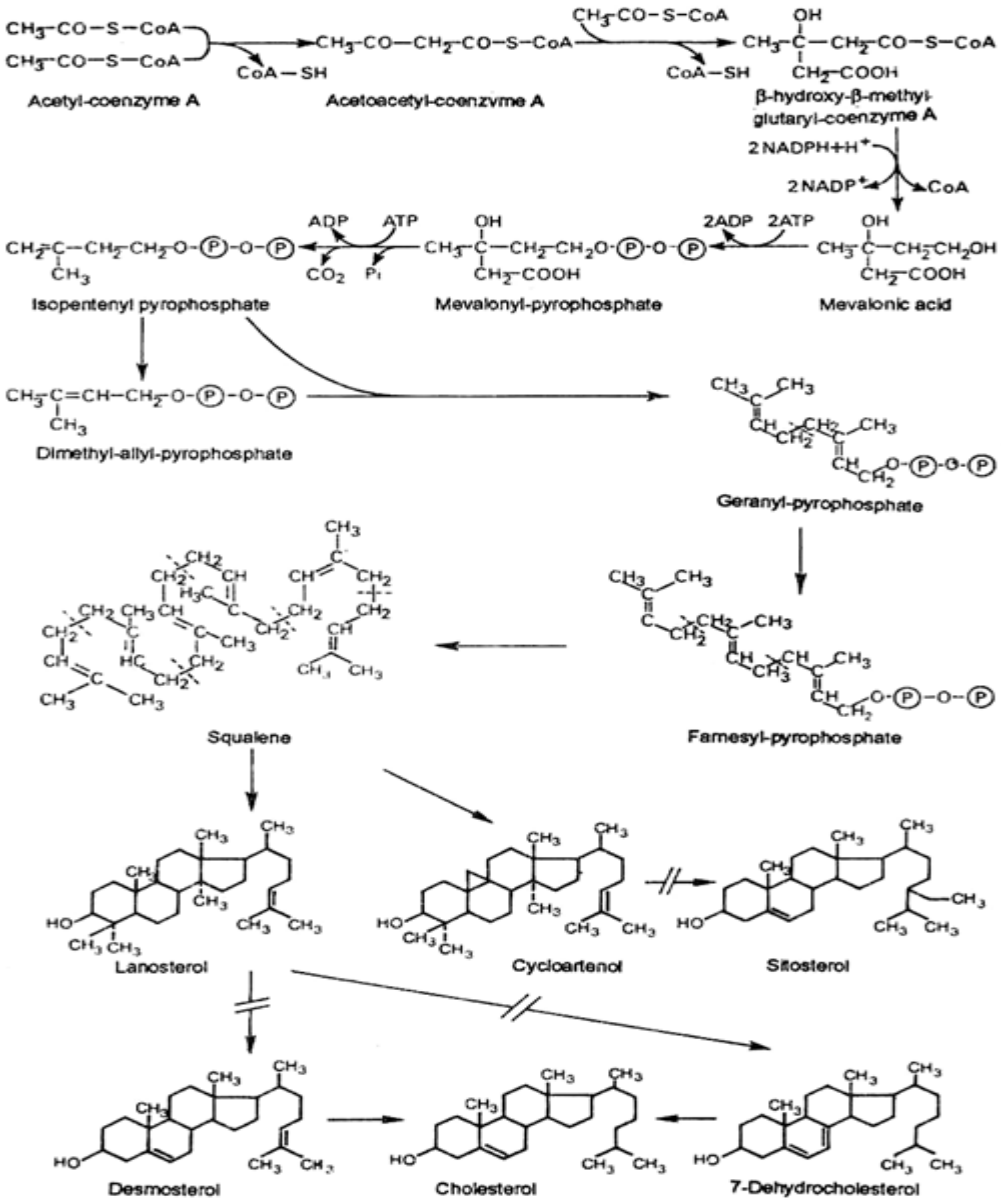


FIG. 5-22. — The principal steps of the biosynthesis of cholesterol.

Insects are capable of synthesizing squalene, but they cannot cyclize it. They use the sterols (animal or plant) present in their food and are capable of metabolizing them to cholesterol, precursor of hormonal derivatives like ecdysone.

Prokaryotes also synthesize squalene. Squalcne-hopene cyclase which carries out the cyclization is an enzyme which does not require oxygen.

Formation of Other Steroids:

Cholesterol is the starting point of the synthesis of various steroids:

1. Progesterone is secreted by the corpus luteum, the placenta and the cortex of the adrenal gland, and acts mainly in the uterus to permit implantation and gestation;

2. Aldosterone, a hormone secreted by the adrenal cortex, which permits the reabsorption of sodium (and secondarily of chlorine and water) in the kidney, hence its name, mineralocorticosteroid;

3. Cortisol and cortisone, also secreted by the adrenal cortex, sometimes called glucocorticosteroids, because they stimulate protein catabolism and neoglucogenesis in the liver (they are therefore hyperglycemic). They also act on conjunctive and limphoid tissues by depressing membrane permeability and opposing the inflammatory processes (which explains their use in therapeutics).

The synthesis of cortisol (and therefore of cortisone) is stimulated by the corticotropic hormone of the anterior lobe of the pituitary gland or ACTH (Adreno Cortico Tropic Hormone):

1. Testosterone, secreted mainly by the testicles, is responsible for the various male sexual characters;

2. Estrogenic hormones (estradiol and estrone), responsible for the various female sexual characters, synthesized mainly in the ovary and placenta, and characterized — from the structural point of view — by a phenolic ring.

It may be observed that it is relatively easy to pass (in few steps) from progesterone to other hormones having very different physiological properties; in other words, in this family of steroid hormones, small structures modifications correspond to large differences in biological activity.

We mentioned that the synthesis of Cortisol and cortisone by the adrenal glands is influenced by ACTH, a hormone of anterior lobe of the pituitary gland; we must indicate that the secretory activities of ovaries and testicles are also controlled by the hormones of the anterior lobe of the pituitary gland called gonadotropins, like FSH (Follicule Stimulating Hormone) and LH (Luteinizing Hormone), for example.

Moreover, this anterior lobe also secretes other stimulines, like the growth hormone or somatotropic hormone, and thyrotropic hormone (TSH), which stimulates the synthesis of thyroid hormones by the thyroid gland.

Chemists sometimes classify the steroid hormones according to the number of carbon atoms contained in their molecules and thus distinguish:

1. C_{18} hormones (estradiol, estrone);
2. C_{19} hormones (testosterone);
3. C_{21} hormones (progesterone and most of the hormones secreted by the cortical part of adrenal glands, the mineralocorticoids like aldosterone as well as the glucocorticoids like Cortisol and cortisone).

Vitamins D are formed by the opening of the B cycle due to ultraviolet light, either from ergosterol (vitamin D_2), or from 7-dehydro-cholesterol (which gives vitamin D_3).

Mode of Steroid Hormone Action through Intracellular Receptors :

Steroid hormones are lipid-soluble and easily pass through the cell membrane of a target cell into the cytoplasm. In the cytoplasm they bind to specific intracellular receptors (proteins) to form a hormone receptor complex that enters the nucleus.

In the nucleus, hormones which interact with intracellular receptors (e.g., steroid hormones, iodothyromines, etc.) mostly regulate gene expression or chromosome function by the interaction of hormone-receptor complex with the genome.

Biochemical actions result in physiological and developmental effects (tissue growth and differentiation, etc.). In-fact the hormone receptor complex binds to a specific regulatory site on the chromosome and activates certain genes (DNA).

The activated gene transcribes mRNA which directs the synthesis of proteins and usually enzymes in the cytoplasm. The enzymes promote the metabolic reactions in the cell. The actions of lipid soluble hormones are slower and last longer than the action of water- soluble hormones.

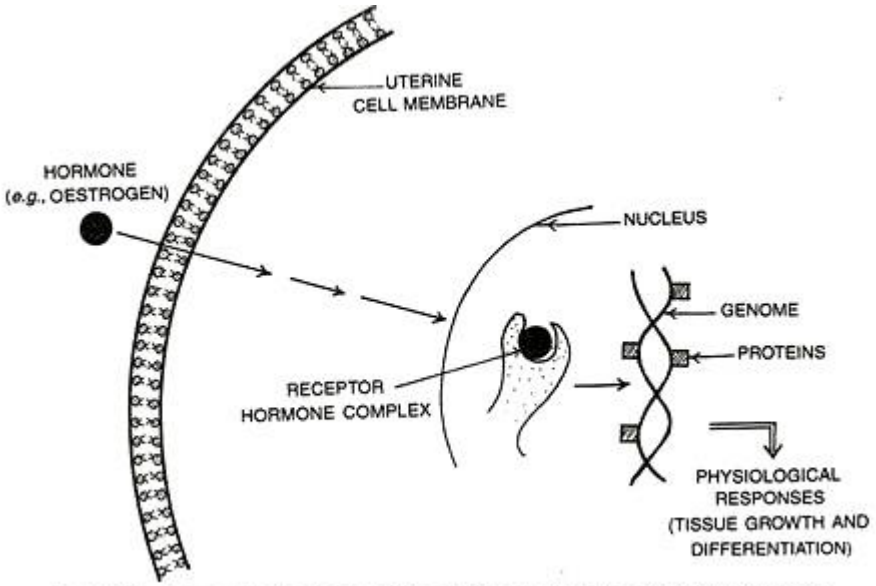


Fig. 22.20. Diagrammatic representation of the mechanism of Steroid hormone.

Thyroid Hormones:

Location and Structure of Thyroid Gland:

The thyroid gland is the largest endocrine gland located anterior to the thyroid cartilage of the larynx in the neck. The gland is well supplied with blood vessels. It is bilobed organ. The two lobes are connected by a narrow structure called the isthmus. The microscopic structure of the thyroid

gland shows thyroid follicles composed of cubical epithelium and filled with a homogenous material called colloid.

Small amount of loose connective tissue forms stroma of the gland. Besides containing blood capillaries, the stroma contains small clusters of specialized Para follicular cells or 'C' cells. The thyroid gland is the only gland that stores hormones in large quantities for about two months.

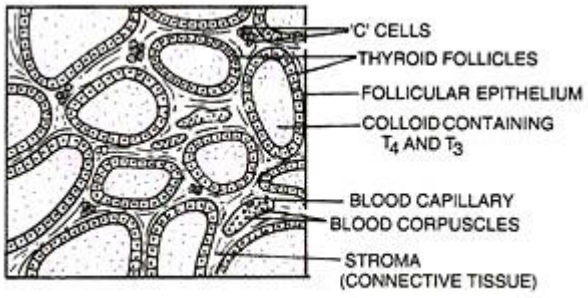


Fig. 22.2. T.S. Thyroid gland.

Hormones of Thyroid Gland:

The thyroid gland secretes three hormones. Thyroxine (tetraiodothyronine or T_4), and tri-iodothyronine or T_3 are secreted by the thyroid follicular cells. Thyrocalcitonin is secreted by the C-cells of the thyroid gland. This gland is stimulated to secrete its hormones by thyroid stimulating hormone (also called thyrotropin) secreted by the anterior lobe of pituitary gland.

(I) Thyroxine (T_4) and Triiodothyronine (T_3):

T_4 and T_3 contain four and three atoms of iodine respectively, therefore, they are named so. T_3 is secreted in smaller amounts but it is more active and several times more potent than T_4. T_4 is converted to T_3 by removal of one iodine in the liver, kidneys and some other tissues. Since both T_4 and T_3 have similar effects on the target cells, they are generally considered together under the name, thyroid hormone (TH).

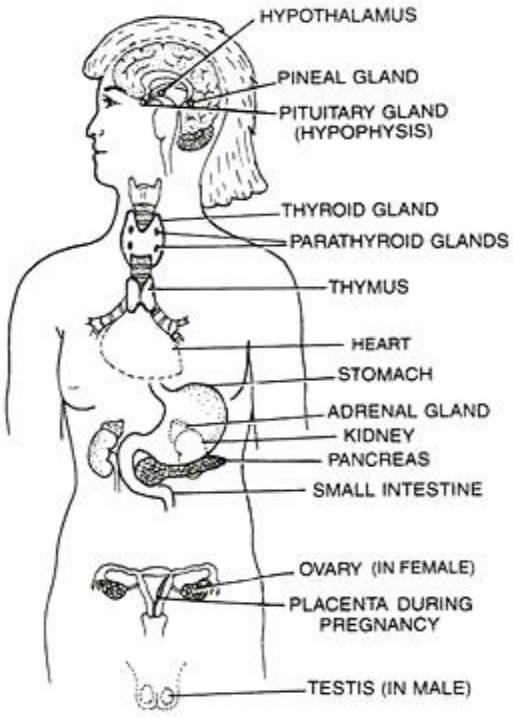


Fig. 22.3. Human endocrine glands.

The thyroid gland is the only gland that stores its hormones in large quantity. T_4 and T_3 are synthesised by attaching iodine to tyrosine amino acid.

The steps in the biosynthesis of the hormone are (Fig. 6.33):

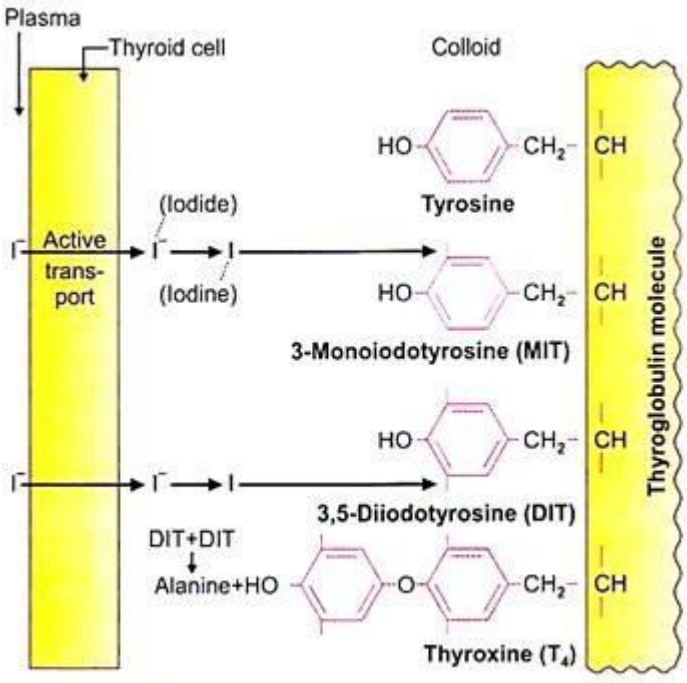


Fig. 6.33: Steps in the biosynthesis of thyroxine

i. Iodide trapping that is the uptake of iodide by the follicular cells from the plasma against the electrochemical gradient. The hormone TSH secreted by the anterior pituitary gland affects this step. Substances, like thiocyanate, pertechnetate and perchlorate that are examples of antithyroid drugs can inhibit iodide trapping.

ii. Oxidation of iodine: It occurs inside the follicular cells by the action of the enzyme peroxidase. Drugs like thiouracil and carbimazole can inhibit this step and act as antithyroid drugs.

iii. Organification: Iodine gets incorporated to tyrosine amino acid present in the colloid and leads to the formation of MIT (Monoiodotyrosine). On further iodination of MIT, there is formation of DIT (Di- iodotyrosine).

iv. Coupling: Coupling of 2 DIT will lead to the formation of T_4 and 1 MIT with 1 DIT will results in T_3. After the synthesis, the hormone with thyroglobulin is stored in the colloid. There are many substances which have the ability to decrease the amount of thyroxin secreted by the gland. These drugs will be of choice when there is a necessity to decrease the amount of thyroxine secretion in certain pathological situations.

Steps involved in hormonopoiesis of thyroxine:

1. Iodide trapping (active process)

2. Conversion of iodide to molecular iodine. Peroxidase is the enzyme involved.

3. Organification of tyrosine to form MIT and DIT—iodinase.

4. Oxidative coupling of

MIT + DIT—to form T_3
DIT + DIT—to form T_4

5. Proteolytic separation of T_3 and T_4 from thyroglobulin— deiodinase

Table 6.6: Transport of thyroxine in plasma

Proteins available	*Quantity of proteins (mg/dl)*	*Affinity*	*Transported bound %*	
			T_4	T_3
TBG	2	+++++	67	46
TBPA	15	++	20	1
Albumin	3500	+	13	53

At the time of release of the hormones into circulation, the acinar cells will engulf the thyro-globulin along with the hormones by endocytosis.In the cells, the hormone will be separated by

proteolysis and released into the circulation and thyroglobulin will be retained for further use.Most of the hormone in circulation is in protein bound form along with thyroid binding globulin (TBG), albumin (TBA), thyroid binding pre-albumin (TBPA) (Table 6.6).

Protein Bound Iodine (PBI) in Blood:

The term PBI in blood represents iodine present in thyroid hormones. The PBI values for normal adults are 3.5-7.5 mg/100 ml of plasma. PBI is a reliable measure of thyroxine content of plasma

The values for PBI in hypo- and hyperthyroidism are given below:

Myxoedema (Hypothyroidism)	0.2-2.5 mg/100 ml
Grave's disease (Hyperthyroidism)	8-18 mg/100 ml

The functions of thyroxine (T_4) and tri-iodothyronine (T_3) are as follows.

(a) They regulate the metabolic rate of the body and thus maintain basal metabolic rate (BMR).

(b) They stimulate protein synthesis and, therefore, promote growth of the body tissues.

(c) They regulate the development of mental faculties.

(d) As they increase heat production, thus they maintain body temperature.

(e) They help in metamorphosis of tadpole into adult frog. If thyroid gland of the tadpole (larva) is removed, the larva fails to change into an adult.

(f) They increase action of neurotransmitters like adrenaline and noradrenaline.

(II) Thyrocalcitonin (TCT):

It is secreted when calcium level is high in the blood. It then lowers the calcium level by suppressing release of calcium ions from the bones. Thus calciton has an action opposite to that of the parathyroid hormone on calcium metabolism. Calcitonin is a peptide which contains 32 amino acids.

(f) They increase action of neurotransmitters like adrenaline and noradrenaline.

(III) Thyrocalcitonin (TCT):

It is secreted when calcium level is high in the blood. It then lowers the calcium level by suppressing release of calcium ions from the bones. Thus calciton has an action opposite to that of

the parathyroid hormone on calcium metabolism. Calcitonin is a peptide which contains 32 amino acids.

Regulation of Thyroid Hormone:

It is brought about by the negative feedback mechanism. There is involvement of hypothalamo-pituitary-thyroid axis (Fig. 6.35).

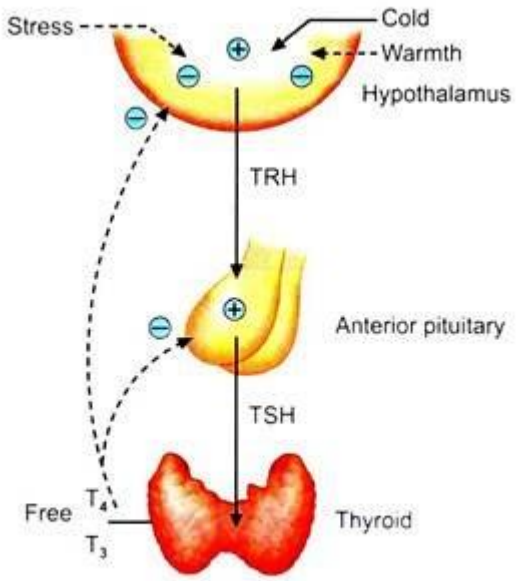


Fig. 6.35: Regulation of secretion of thyroxine (by negative feedback mechanism)

Increase in free form of hormone in circulation acts on hypothalamus and anterior pituitary gland. Acting on hypothalamus, it decreases the secretion of thyrotropin-releasing hormone (TRF/TRH) and this acts on anterior pituitary decreases secretion of TSH.Net effect will be decreased TSH from anterior pituitary gland. This decreases the secretion of thyroid hormones from the gland.

Many of the other chemical influences acting on TRH-TSH-Thyroxine (hypothalamo-pituitary-thyroid axis) secretions have been shown in Table 6.7.

Table 6.7: Thyroid hormone feedback

Hypothalamus	*Stimulatory*	*Inhibitory*
Decreased TRH	Alpha adrenergic agonists	Alpha adrenergic blockers
		Tumors
Anterior pituitary	*TRH*	*Somatostatin*
Decreased TSH	Estrogen	Dopamine
		Glucocorticoids
		Chronic illness
Thyroid gland	*TSH*	*TSH receptor blocking antibody*
Decreased T_3 and T_4	TSH receptor stimulating antibody	Iodine, lithium

Alteration in the temperature can directly act on the hypothalamus to alter the secretion of the hormone

Disorders related to thyroid Hormones:

(A) Hyperthyroidism (Hyper secretion of thyroid hormone).

a. Exophthalmic goitre or Graves' disease or Basedow's disease or Parry's disease:

It is a thyroid enlargement (goitre) in which the thyroid secretes excessive amount of thyroid hormone. It is characterised by exophthalmia (protrusion of eye balls because of fluid accumulation behind them), loss of weight, slightly rise in the body temperature, excitability, rapid heartbeat, nervousness and restlessness.

Fig. 22.4. Graves' disease.

(B) Hypothyroidism (Hypo secretion of thyroid hormone):

(a) Cretinism:

This disorder is caused by deficiency of thyroid hormone in infants. A cretin has slow body growth and mental development of reduced metabolic rate.

Other symptoms of this disorder are slow heart beat, lower blood pressure, decrease in temperature, stunted growth, pot-belly, pigeon chest and protruding tongue and retarded sexual development. This disease can be treated by an early administration of thyroid hormones.

Fig. 22.5. Cretinism.

(b) Myxoedema or Gull's disease:

It is caused by deficiency of thyroid hormone in adults. This disease is characterized by puffy appearance due to accumulation of fat in the subcutaneous tissue because of low metabolic rate. The patient lacks alertness, intelligence and initiative. He also suffers from slow heart beat, low body temperature and regarded sexual development. This disease can be treated by administration of thyroid hormones.

Fig. 22.6. Myxoedema.

(c) Simple Goitre:

It is caused by deficiency of iodine in diet because iodine is needed for the synthesis of thyroid hormone. It causes thyroid enlargement. It may lead to cretinism or myxoedema. This disease is common in hilly areas. Addition of iodine to the table salt prevents this disease.

Fig. 22.7. Simple goitre.

(d) Hashimoto's disease:

In this disease all the aspects of thyroid function are impaired. It is an autoimmune disease in which the thyroid gland is destroyed by autoimmunity.

Probable Questions:

1. Describe the steps of cholesterol biosynthesis.
2. How steroid hormones are transported?
3. What are the mechanism of action of steroid hormones?
4. How secretion of steroid hormones are regulated?
5. Briefly describe the location and structure of thyroid gland.
6. How T3 and T4 hormones are synthesised in thyroid gland?
7. How thyroid hormone secretions are regulated?
8. What are the effects of thyroxine hypo secretion?
9. What are the effects of thyroxine hyper secretion?

Suggested Readings:

1. General Endocrinology. Turner and Bagnara. Sixth Edition.
2. Williams Textbook of Endocrinology. Tenth Edition.
3. Introduction to Endocrinology. Chandra S Negi. Second Edition
4. Endocrinology. Hadley and Levine. Sixth Edition

UNIT-XIV

Physiological role of hormones: hormonal regulation of mineral metabolism and fluid volume

Objective: In this unit you will learn about role of hormones in mineral metabolism and fluid volume regulation.

Water Metabolism:

Distribution of water in the body:

Water is the major constituent of human body. The average body water is 50-70% of the body weight. Females have little less water than males.

1. The water content of intracellular fluid is 50% of total body weight.

2. The water content of extracellular fluid is 20% of the body weight, which is distributed as follows:

Plasma	4.5%
Interstitial and lymph fluid	8%
Dense connective tissue, cartilage and bone	6%
Transcellular fluids(found in salivary glands, liver, pancreas, thyroid gland, gonads, skin, mucous membranes of the respiratory and gastrointestinal tracts, kidneys, fluid spaces in eye, CSF etc)	1.5%

Factors influencing the distribution of body water:

The distribution of water is continuously changing. Osmotic forces are the principal factors for controlling the amount of fluid in various compartments of the body. These are maintained by the solutes of the body. Solutes are of three types.

1. Organic molecules of small molecular size (glucose, urea, amino acids etc.):

Since these diffuse freely across the cell membrane, they are not important in the distribution of water. If they are present in large quantities, they can help retaining water.

2. Organic substances of large molecular size {proteins):

These substances can throw effect in the transport of fluids from one compartment to the other.

These inorganic electrolytes are the most important both in the distribution and in the retention of body water.

Intake and Loss of Body Water:

A. Water intake:

Water is supplied to the body by the following processes:

1. Water taken orally.

2. Along with food.

3. Oxidation of food stuffs i.e. fats, proteins and carbohydrates yield water after combustion.

B. Water loss:

Water is lost from the body by 4 routes

1. Evaporation from lungs.

2. Kidneys eliminate water as urine.

3. The intestines excrete in the feces.

4. Perspiration.

C. Additional water loss in diseases:

1. Water loss is more in diarrhea and vomiting. These losses can be fatal in infants.

2. In kidney disease, renal water loss is more.

3. In fever, insensible losses may rise much higher than normal.

4. Patients in high environmental temperatures sustain extremely high external water loss.

Water Balance:

An equilibrium persists between the intake and output of water in the body. In addition to other factors, certain hormones such as ADH, vasopressin, oxytocin and aldosterone influence the regulatory mechanism.

Balance sheet of water

Water intake (ml/day)		Water loss (ml/day)	
Drinks	1350	Urine	1500
Solid food	900	Lungs	500
Oxidation of food	450	Skin	600
		Feces	100
TOTAL	2700	TOTAL	2700

There is a continuous excretion of water in the form of digestive juices from the body into the alimentary canal. This water (except 100 ml) is reabsorbed with the water of the food and drinks. The amount of this internal secretion is 7 to 10 liters/day.

Physiological Functions of Water:

1. Specific heat:

Heat is required to raise the temperature of 1 gm. of water through one degree Celsius is more than for almost any other solid or liquid. The high specific heat of water helps in minimizing the rise in body temperature due to the heat emitted out of chemical reactions.

2. Latent heat of evaporation:

Water has the highest latent heat of evaporation than any other liquid. A certain amount of water can cause maximum cooling by evaporation, so that body temperature does not rise.

3. Solvent power:

Water forms true solutions as well as colloidal solutions. Even water insoluble substances are made water soluble by the hydrotropic action. Therefore, it is the most suitable solvent for cellular components; water thus brings various substances in contact for chemical reactions to proceed.

4. Dielectric constant:

Oppositely charge particles can coexist in water. Therefore, it is a good ionizing medium. This stimulates the chemical reactions.

5. Catalytic action:

A large number of chemical reactions in the body are accelerated by water due to its ionizing power. All chemical reactions in the body proceed in presence of water only.

6. Lubricating action:

Water acts as a lubricant in the body to prevent friction in joints, pleura, conjunctiva and peritoneum.

Regulation of Passage of Water:

1. If capillary pressure is increased, more water will flow into the tissues.

2. A fall in blood pressure helps in passage of water form the tissues to the blood.

3. If the plasma proteins are decreased, water will flow into the tissues.

4. Dilution of blood by excessive ingestion of water can lower the osmotic pressure of the plasma proteins and thus may increase capillary pressure.

Dehydration:

When the loss of water exceeds the intake, the body's water content is reduced. This means that the body is in negative water balance and the condition is known as dehydration.

Causes:

1. Primary dehydration:

(a) Deprivation of water during desert travel, extreme weakness and mental patients refraining to drinking water causes dehydration. Occurs more quickly in fever and in high environmental temperatures.

(b) Excessive water loss due to vomiting, prolonged diarrhoea, excretion of large quantities of urine and sweat. In water depletion, the concentration of extracellular fluid increases. Water is drawn from the cells and both extracellular and intracellular compartments shrink. Extreme thirst results; individual complains of hot and dry body. The tongue becomes dry.

2. Secondary dehydration:

Concentration of electrolytes of the body fluids is maintained constant through the elimination or retention of water. The reduction or increase in the total electrolytes which affects, chiefly the basic radical Na (extracellular) or K (intracellular) and the acid radicals HCO_3 and CI is accompanied by a corresponding increase or decrease in the volume of body water. This causes intracellular edema, slowing of circulation and impairment of urinal function. The individual becomes weak.

3. Dehydration due to injection of hypertonic solution:

When a highly concentrated sugar or salt solution is injected into the body, the osmotic pressure of blood will increase. This results in the flow of fluid from the tissues into the blood until equilibrium sets in. Consequently, the blood volume increases. This increased blood volume soon returns to normal by the loss of excess material through urination. This causes a net loss of body water producing dehydration.

Effects of dehydration:

1. Loss of weight due to the reduction in tissue water.

2. Disturbances in acid-base balance.

3. Rise in the non-protein nitrogen of blood.

4. Rise in the plasma protein concentration and of chloride.

5. Rise in body temperature due to reduction in circulating fluid.

6. Increased pulse rate and reduced cardiac output.

7. Dryness, wrinkling and looseness of skin.

8. Exhaustion and collapse.

Correction of dehydration:

1. Ordinary NaCl solution may be given parenterally to repair the losses.

2. In case of excretion of fluid high in Na and HCO_3 resulting in fluid and electrolyte loss, a mixture of 2/3 isotonic saline and 1/3 Na lactate should be administered intravenously.
3. Dehydration in diabetes mellitus, Addison's disease, uremia, extensive burns and shock cannot be corrected by the above methods.

Water intoxication:

Caused by excessive water retention due to renal failure, hyper secretion of ADH, excessive administration of fluids parenterally

Symptoms:

Headache, nausea and muscular weakness

Hormonal Control of water volume:

While the kidneys operate to maintain osmotic balance and blood pressure in the body, they also act in concert with hormones. Hormones are small molecules that act as messengers within the body. Hormones are typically secreted from one cell and travel in the bloodstream to affect a target cell in another portion of the body. Different regions of the nephron bear specialized cells that have receptors to respond to chemical messengers and hormones. Table 22.1 summarizes the hormones that control the osmoregulatory functions.

Table 22.1.Hormones That Affect Osmoregulation

Hormone	Where produced	Function
Epinephrine and Norepinephrine	Adrenal medulla	Can decrease kidney function temporarily by vasoconstriction
Renin	Kidney nephrons	Increases blood pressure by acting on angiotensinogen
Angiotensin	Liver	Angiotensin II affects multiple processes and increases blood pressure
Aldosterone	Adrenal cortex	Prevents loss of sodium and water
Anti-diuretic hormone (vasopressin)	Hypothalamus (stored in the posterior pituitary)	Prevents water loss
Atrial natriuretic peptide	Heart atrium	Decreases blood pressure by acting as a vasodilator and increasing glomerular filtration rate; decreases sodium reabsorption in kidneys

Epinephrine and Norepinephrine

Epinephrine and norepinephrine are released by the adrenal medulla and nervous system respectively. They are the flight/fight hormones that are released when the body is under extreme stress. During stress, much of the body's energy is used to combat imminent danger. Kidney function is halted temporarily by epinephrine and norepinephrine. These hormones function by acting directly on the smooth muscles of blood vessels to constrict them. Once the afferent arterioles are constricted, blood flow into the nephrons stops. These hormones go one step further and trigger the **renin-angiotensin-aldosterone** system.

Renin-Angiotensin-Aldosterone

The renin-angiotensin-aldosterone system, illustrated in Figure 22.15 proceeds through several steps to produce **angiotensin II**, which acts to stabilize blood pressure and volume. Renin (secreted by a part of the juxtaglomerular complex) is produced by the granular cells of the afferent and efferent arterioles. Thus, the kidneys control blood pressure and volume directly. Renin acts on angiotensinogen, which is made in the liver and converts it to **angiotensin I**. **Angiotensin converting enzyme (ACE)** converts angiotensin I to angiotensin II. Angiotensin II raises blood pressure by constricting blood vessels. It also triggers the release of the mineralocorticoid aldosterone from the adrenal cortex, which in turn stimulates the renal tubules to reabsorb more sodium. Angiotensin II also triggers the release of **anti-diuretic hormone (ADH)** from the hypothalamus, leading to water retention in the kidneys. It acts directly on the nephrons and decreases glomerular filtration rate. Medically, blood pressure can be controlled by drugs that inhibit ACE (called ACE inhibitors).

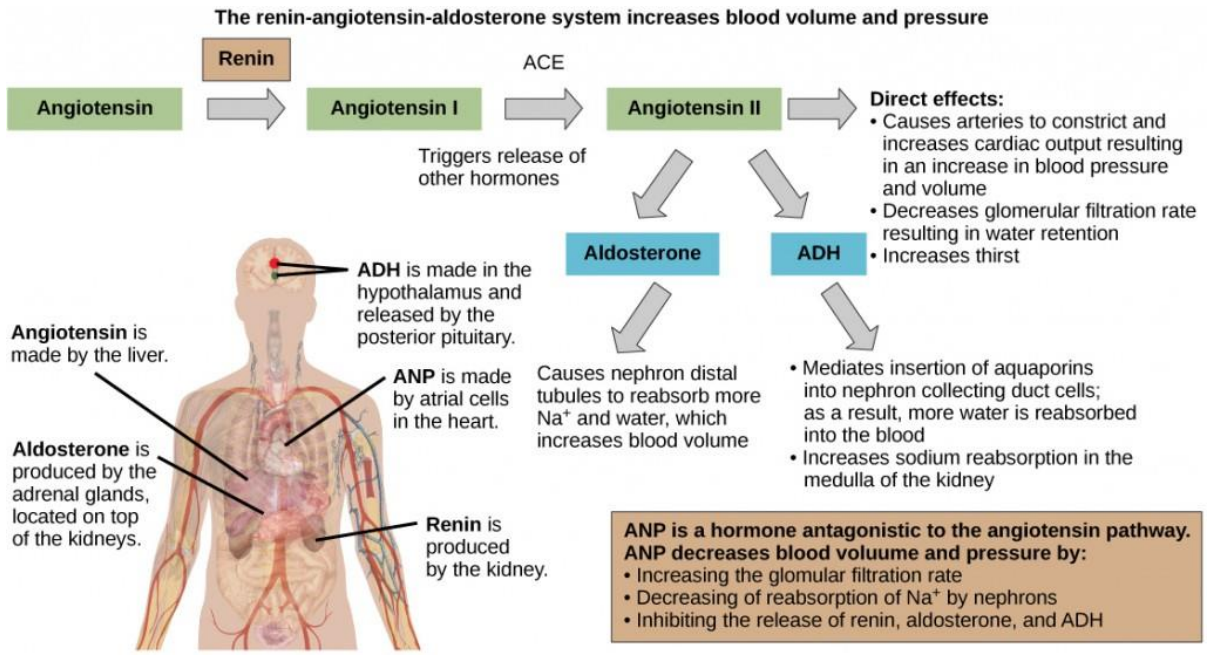


Figure 22.15. The renin-angiotensin-aldosterone system increases blood pressure and volume. The hormone ANP has antagonistic effects. (credit: modification of work by Mikael Häggström)

Mineralocorticoids

Mineralocorticoids are hormones synthesized by the adrenal cortex that affect osmotic balance. Aldosterone is a mineralocorticoid that regulates sodium levels in the blood. Almost all of the sodium in the blood is reclaimed by the renal tubules under the influence of aldosterone. Because sodium is always reabsorbed by active transport and water follows sodium to maintain osmotic balance, aldosterone manages not only sodium levels but also the water levels in body fluids. In contrast, the aldosterone also stimulates potassium secretion concurrently with sodium

reabsorption. In contrast, absence of aldosterone means that no sodium gets reabsorbed in the renal tubules and all of it gets excreted in the urine. In addition, the daily dietary potassium load is not secreted and the retention of K^+ can cause a dangerous increase in plasma K^+ concentration. Patients who have Addison's disease have a failing adrenal cortex and cannot produce aldosterone. They lose sodium in their urine constantly, and if the supply is not replenished, the consequences can be fatal.

Antidiurectic Hormone:

As previously discussed, antidiuretic hormone or ADH (also called **vasopressin**), as the name suggests, helps the body conserve water when body fluid volume, especially that of blood, is low. It is formed by the hypothalamus and is stored and released from the posterior pituitary. It acts by inserting aquaporins in the collecting ducts and promotes reabsorption of water. ADH also acts as a vasoconstrictor and increases blood pressure during hemorrhaging.

Atrial Natriuretic Peptide Hormone:

The atrial natriuretic peptide (ANP) lowers blood pressure by acting as a **vasodilator**. It is released by cells in the atrium of the heart in response to high blood pressure and in patients with sleep apnea. ANP affects salt release, and because water passively follows salt to maintain osmotic balance, it also has a diuretic effect. ANP also prevents sodium reabsorption by the renal tubules, decreasing water reabsorption (thus acting as a diuretic) and lowering blood pressure. Its actions suppress the actions of aldosterone, ADH, and renin.

Mineral Metabolism

Living beings have organic and inorganic types of chemical constituents. The organic constituents i.e. proteins, carbohydrates, fats etc. are made up of C, H, O and N. The inorganic constituents described as 'minerals' comprise of the elements present in the body other than C, H, O and N. Although they constitute a relatively small amount of the total body tissues, they are essential for many vital processes.

There are 31 elements present in the body.

They are divided into two classes:

(1) Essential elements and

(2) Non-essential elements.

Essential elements:

Those which are essential to maintain the normal living state of a tissue.

They are again divided into two sub groups:

Macro elements:

They are required to be present in the diet, more than 1 mg.

Ex. Ca, P, Mg, Na, K, CI and S.

Micro elements:

They are 8 in number and utilized in trace quantities (in microgram or Nano-gram). Hence they are called trace elements. These are Fe, Cu, Zn, Co, Mo, F, I and Mn.

Non-essential elements:

They are 8 in number. They are present in tissues but their functions if any are not clearly defined. They include Al, B, Se, Cr, Br, As, Ti and Pb. Four additional elements, Ni, Tin, Vanadium and Silicon have been suggested as essential trace elements in nutrition but their implications for human nutrition are unknown.

The mineral elements present in the body are supplied in the diet. In poor diets consumed by a large majority of people, calcium and iron deficiency occur commonly. Iodine deficiency occurs in people living in certain hilly tracts, where the soil and water are deficient in iodine. In tropical countries, addition of sodium chloride in the diet is of great importance, because of the loss of NaCl in sweat. The deficiencies of other minerals do not occur normally in average diets.

i. Sodium, potassium and chlorine are involved mainly in the maintenance of acid-base balance and osmotic control of water metabolism.

ii. Calcium, phosphorus and magnesium are constituents of bone and teeth.

iii. Phosphorus is the constituent of body cells of the tissues, such as muscle, liver etc.

iv. Sulphur is present in cysteine, methionine, thiamine, biotin, lipoic acid and coenzyme A.

I. Calcium:

Source:

Milk (0.2 gm./100 ml) and cheese are important dietary sources. Other sources-are egg yolk, lentils, nuts, cabbage, cauliflower and asparagus, etc.

Requirement:

(1) Men and women after 18 years of age require 800 mg/day.

(2) During lactation and in pregnancy of 2^{nd} and 3^{rd} term 1.2 gm./day is required.
(3) Infants under 1 year require-360-540 mg/day.

(4) Children of 1-18 years need 800-1200 mg/day.

Absorption:

Calcium is taken in the diet as calcium phosphate, carbonate, tartarate and oxalate. Calcium is absorbed actively in the upper small intestine. The active process is regulated by 1,25 dihyrocholecalciferol, a metabolite of vitamin D which is produced in the kidney in response to low plasma Ca^{++} concentrations. Absorption of calcium by the intestine is never complete. Ca is absorbed by an active transport process occurring mainly in the upper small intestine.

Calcium absorption is influenced by the following factors:

1. Vitamin D promotes absorption of Ca.

2. Acidic pH favous calcium absorption because Ca salts (phosphate and carbonates) are quite soluble in acid solution arid are relatively insoluble in alkaline solutions. Hence an increase in acidophilic flora, e.g. lactobacilli is recommended to lower pH which favours the absorption of Calcium.

3. Organic acids, lactose and basic amino acids in the diet favour calcium absorption.

4. Higher levels of proteins in the diet help to increase the absorption of calcium. On a high protein diet, about 15% of the dietary calcium is absorbed, compared with 5% absorption on a low protein diet. Certain calcium salts are much more soluble in aqueous solution of amino acids than in water and thus absorption of calcium is increased in presence of amino acids.

5. If calcium: phosphorus ratio is much high, $Ca_3(PO_4)_2$ will be formed and absorption of calcium is reduced. The optimal ratio for both elements is about 1:1 (1:2 to 2:1) and with ratios outside these limits, absorption is decreased. This is because of formation of insoluble calcium phosphate.

6. When fat absorption is impaired much free fatty acids are formed due to hydrolysis. These fatty acids react with free calcium to form insoluble calcium soap and then Ca is lost in faeces.

7. Absorption of calcium is inhibited by a number of dietary factors that cause formation of insoluble calcium salts, i.e. phytate (cereal grain), oxalate, phosphate and iron, etc.

8. High concentration of Mg in the diet decreases absorption of Ca.

9. Presence of excess fibre in the diet interferes with the absorption of Ca.

10. Percentage of calcium absorption decreases as its intake increases.

11. Parathyroid hormone increases the intestinal absorption of calcium.

12. Adrenal glucocorticoids diminish intestinal transport of Ca.

13. After the age of 55 to 60 there is gradual diminution of intestinal transport of calcium. During menopause many women develop negative calcium-phosphorus balance leading to a type of osteoporosis. This is usually accompanied by pain and fractures. The negative balance of calcium and phosphorous are markedly improved by administration of estrogen or by androgens such as testosterone. A combination of estrogen and androgen is more effective.

14. Kidney threshold regulates the blood calcium level. In a normal adult any extra calcium absorbed from the intestine is readily excreted in the urine. In hypocalcaemia kidney threshold also becomes abnormal.

15. Excess of iron also dis-favours absorption of calcium and phosphorus, as ferric phosphate is highly insoluble. The net result is an upset in the Ca:P ratio.

16. Oxalate in certain foods precipitate calcium in the intestine as insoluble calcium oxalate. The phytic acids of food form insoluble salt with calcium and reduce calcium absorption.

17. Vitamin D increases calcium and phosphorus absorption from the intestine. Vitamin D promotes synthesis of specific calcium binding protein which participates in the active transport of calcium across the small intestinal mucosa. Lack of vitamin D, excess of phytates, low Ca/P ratio in diet, increased pH of upper intestine and malabsorption syndromes influence the amount of calcium absorption adversely.

Biological role:

Calcium is involved in the following biological processes:

1. Constituent of bones and teeth:

Calcium along with phosphate constitutes the mineral part of the skeleton and teeth where it is present to the extent of 99% of the total calcium present in the body. It is primarily in the form of crystals of hydroxyapatite, while some is in combination with phosphate (calcium phosphate) in the form of amorphous crystals.

2. Neuromuscular functions:

This involves excitability of nerve function, neural transmission, and contractility of cardiac and skeletal muscle. Normal concentration of calcium ions is required for the normal excitability of heart muscle.

3. Blood coagulation:

It plays a vital role in blood clotting process since it activates the enzymic conversion of prothrombin into thrombin and production of thromboplastin. The removal of calcium from the

blood can prevent blood coagulation and because of this reason EDTA, oxalates, citrates are used as anticoagulant because these ions can precipitate calcium into the respective insoluble salts.

4. Membrane function:

It controls the permeability of all membranes and is often bound by lecithine in the membrane, i.e. it decreases the permeability and balances the opposite action of sodium and potassium capillary permeability. This involves transfer of inorganic ions across cell membranes and release of neurotransmitters at synaptic junction.

5. Selected enzymatic reactions:

Calcium acts as activator for number of en2ymes like ATPase, succinic dehydrogenase, lipase, etc. It also antagonizes the effect of magnesium on many enzymes. It releases cellular enzymes such as amylase from the parotid and increases the level of activity of intracellular enzymes such as—Isocitric dehydrogenase, phosphorylase and phosphofructokinase.

6. Regulation of secretion of certain peptide hormones:

Pituitary hormones, parathyroid hormone, calcitonin and vasopressin are regulated through calcium ionic concentration. Calcium along with zinc plays a vital role in release of insulin from pancreas. Calcium homeostasis: Normal blood values are 9.5-10.5 mg/100 ml. 35-45% of this is bound to proteins, mostly to the albumin fraction. In the extracellular fluid nearly all the calcium is in ionized form (55-65%). 0.5 (5-10%) mg is complexed to organic acids, phosphate, citrate, etc., while in renal failure, it may be complexed to other organic ions as well.

The skeleton is in a dynamic state of equilibrium to maintain calcium homeostasis. 4-8 gm. of calcium in bone is rapidly exchangeable with that in plasma and is present on the surface of the bone crystals—labile calcium storage pool. The remaining 99% of bone calcium is more firmly fixed in bone tissue and exchanges at a very slow rate.

Metabolism:

The blood cells contain very little amount of calcium, most of the blood calcium is therefore, in the plasma, where it is present in 3 fractions:

(1) Ionized about 2 mg/100 ml.

(2) Non-diffusible (protein bound) above 3.5 mg/100 ml.

(3) A small amount as calcium complex of citrate and phosphate.

All these forms of calcium in the serum are in equilibrium with one another. A decrease in ionized calcium in the serum causes tetany. This may be due to an increase in the pH of blood or lack of calcium because of poor absorption from the intestine, decreased dietary intake, increased renal excretion as in nephritis or parathyroid deficiency.

Factors influencing blood calcium level:

1. Parathyroid hormone:

In fasting condition or state there is no absorption from the intestine, the normal plasma Ca concentration is maintained by its rate of excretion and its mobilization from bones through the action of the parathyroid hormone.

2. Vitamin D:

It enhances absorption of Ca from the intestine and thus maintains normal Ca concentration.

3. Plasma proteins:

Half of the blood Ca (non-diffusible) is bound to plasma proteins and thus any decrease in these proteins will be accompanied by a decrease in the total calcium level.

4. Plasma phosphate:

A reciprocal relationship exists between the concentration of Ca and phosphate ions in plasma. The marked increase in serum phosphate causes a fall in serum calcium concentration.

5. Calcitonin:

An increase in the ionized Ca levels in the plasma is the stimulus for the production of calcitonin which then causes a deposition of Ca in bone.

Excretion:

Calcium is excreted in the urine, bile and digestive secretion. About 75% of dietary calcium is absorbed and rest is excreted as fecal calcium. Nearly 10 g of Ca is filtered by the renal glomeruli in 24 hours. But only 200 mg appear in the urine, which is in the ionic state as well as in the complexes with citrate and other organic anions. A very small amount of Ca is excreted into the intestine after absorption. About 15 mg of Ca is excreted in the sweat. Vigorous physical exercise increases the loss of Ca by way of sweat.

Disease state:

Calcium metabolism is highly influenced by parathyroid hormones. In hyperparathyroidism serum calcium rises (12-22 mg/100 ml) (hypercalcaemia), phosphatase activity is increased, urinary calcium is decreased and phosphorus rises in serum. The calcium, phosphorus ratio is important in ossification. In the serum the product of calcium and phosphorus (in mg/100 ml) is normally 50 in children and may be below 30 during rickets.

The following are the diseases related to calcium in the body:

(a) Effects of parathyroid:

1. In hyperparathyroidism, the following changes occur:

(i) Hypercalcemia (12-22 mg/dl).

(ii) Decrease in serum phosphate.

(iii) Diminished renal tubular reabsorption of phosphate.

(iv) Increased phosphatase activity.

(v) Renal urinary Ca and phosphorus found from bone decalcification and dehydration.

(vi) Extra Ca and P are lost from soft tissue and bones by increased bone destroying activity.

2. In hypoparathyroidism, the following changes occur:

(i) The concentration of serum Ca may drop below 7 mg/100 ml.

(ii) Increased serum phosphate and decreased urinary excretion of calcium and phosphorus.

(iii) Normal or occasionally raised serum phosphatase activity.

(iv) Normal acid-base equilibrium.

(v) Probably increased bone density.

(b) Tetany:

Decreased ionized fraction of serum Ca causes tetany.

This may be due to:

1. Increase in the pH of blood.

2. Poor absorption of Ca from the intestine.

3. Decreased dietary intake of Ca.

4. Increased excretion of Ca as in hepatitis.

5. Parathyroid deficiency.

6. Increased retention of phosphorus as in renal tubular disease.

Symptoms:

Muscles lose tone and become flabby.

Affects the face, hands and feet.

(c) Rickets:

This is characterized by faulty calcification of bones in children showing serum phosphate values of 1 to 2 mg/100 ml.

This may be due to:

1. Vitamin D deficiency.

2. A deficiency of Ca and P in the diet or a combination of both.

3. Poor absorption of Ca from the intestine.

4. Parathyroid deficiency.

5. Increased alkaline phosphatase activity.

(d) Osteoporosis:

This disease occurs in adults due to the following causes:

1. Decalcification of bones as a result of Ca deficiency in the diet.

2. Hypoparathyroidism.

3. Low vitamin D content of the body.

Symptoms:

Fractures of the brittle bones occur even after minor accidents.

Pain due to fracture of vertebrae (may radiate round the trunk, to the buttocks or down the legs).

Renal rickets:

It is a hereditary disease. It is called familial hypophosphatemia rickets. Affected persons show severe rickets with hypophosphatemia.

The causes are:

(i) Defective transport of phosphate by the intestine and the renal tubules

(ii) Lowered serum phosphorus and hyperphosphaturia

(iii) Reduced intestinal absorption of calcium and phosphorus. Vitamin D in ordinary doses does not relieve the disease. Hence, it is referred to as vitamin D resistant rickets.

II. Phosphorus:

Source:

Phosphorus is present in nearly all foods therefore a dietary deficiency is not known to occur in man. Dairy products, cereals, egg yolk, meat, beans and nuts are usually rich sources. The daily average intake is 800-1000 mg and is about twice that of calcium.

Absorption:

Like calcium, phosphorus is also absorbed by upper small intestine and factors influencing the absorption are also similar. The normal range for plasma inorganic phosphorus is 3.0-4.5 mg/dl. In children values are higher (5-6 mg/dl) and remain so up-till puberty.

Distribution:

Phosphorus is distributed more widely than calcium. 15% is found in muscle and other soft tissues and 85% in the inorganic mineral phase of bone. It is an integral part of many macromolecules. Ex. Phospholipids, phosphoproteins and nucleic acids.

Functions:

It has no physiological effects comparable to that of calcium but it has many other functions which are as follows:

1. Formation of bone and teeth.

2. Formation of phospholipids essential to every cell.

3. Formation of nucleic acids and derivatives.

Ex. Adenylic acid and is thus significant in (RNA and DNA) protein synthesis and from genetics point of view.

4. Formation of organic phosphates as intermediate in metabolic processes.

Ex. In glycolysis, Glucose + ATP → G-6-P + ADP.

5. Formation of energy rich phosphate compounds.

Ex. ATP (energy currency of the cell).

6. Both inorganic and organic phosphates can take part in buffering the cell.

Ex, Sodium-potassium-phosphates.

7. Formation of coenzymes.

Ex. TPP, NADP.

8. Formation of phosphoprotein.

Ex. Casein.

Excretion:

Urinary excretion is equivalent to dietary phosphate intake. It varies diurnally, more being excreted at night. The usual daily loss is 600-800 mg, tubular resorption being 85-95%. Renal loss of phosphate can be of significant magnitude to lower serum phosphorus values and enhance osteoid demineralization.

Homeostasis:

There is a greater fluctuation observed in blood phosphate values due to easy shift between extracellular fluid and intracellular compartments. Thus it is quite dependent on dietary phosphorus. Inorganic phosphate affects the net movement of calcium into and out of bone.

Raised phosphate will lead to depression of the solubility of the calcium of bone crystals and thus shift equilibrium towards bone. In this manner it opposes the effect of the parathyroids. Ingestion of heavy dose of phosphate can lower serum calcium and increase excretion of calcium in urine. Lowered phosphorus on the other hand will make parathyroid activity more apparent.

Hormonal factors are not directly linked. However renal phosphate clearance is very vital in homeostasis and seems to be secondarily involved in certain endocrinopathies, e.g. involving parathormone, growth hormone and corticosteroids.

Disease state:

The following are the disease states of phosphorus in the body:

1. In rickets, serum phosphate is as low as 1-2 mg/100 ml (There is a temporary decrease in serum P during absorption of carbohydrates and some fats).

2. Organic P content is low but inorganic content is high in the serum in diabetes.

3. P retention causes acidosis in severe renal diseases. This results in increase of serum P.

4. Serum P levels are increased in hypoparathyroidism and decreased in hyperparathyroidism and celiac disease.

5. In renal rickets, blood P is very low with an increased alkaline phosphatase activity.

6. The deficiency of vitamin D is the cause of low serum P and the defects in the calcification of bones (referred to as vitamin D resistant rickets).

III. Magnesium:

Source:

Magnesium is present in milk, egg, cabbage, cauliflower etc.

Daily requirement:

Infants—100-150 mg; Children—150-200 mg and Adults—200-300 mg.

Absorption:

A greater part of the daily ingested Mg is not absorbed. A very high intake of fat, phosphate, calcium and alkalies diminish its absorption. Parathyroid hormone increases its absorption.

Distribution:

Whole blood it is 2-4 mg/dl, CSF it is 3 mg/100 ml and muscle it is 2 mg/100 ml.

Functions:

1. 70% of the total magnesium content (21g) of the body is combined with calcium and phosphorus in the complex salts of bone. The remainder is in the soft tissues and body fluids. It is the principal cation of the soft tissue.

2. Magnesium ions act as activators for many of the phosphate group transfer enzymes.

3. It is found in certain enzymes, such as co-carboxylase.

4. It functions as a cofactor for oxidative phosphorylation.

Disease state:

The following are the disease states of magnesium in the body:

1. Magnesium deficiency causes depression, muscular weakness and liability to convulsions. Its deficiency has also been observed in chronic alcoholics with low serum mg and muscular weakness.

2. Low in Kwashiorkor, causing weakness.

Low levels of Mg are reported in uremia, normal and abnormal pregnancy, rickets, growth hormone treatment, hypercalcemia and recovery phase of diabetic coma.

IV. Sodium, Potassium, Chloride:

Substances whose solutions conduct an electric current are called 'electrolytes'. They are about 11 in general. Na, K, Ca and Mg are cations whereas CI, HCO_3, HPO_4, SO_4, organic acids and proteins are anions. Among these sodium, potassium and chloride are important in the distribution and the retention of body water, thus have close relationship among them. Hence these three elements appear as a single question in the university exams.

Source:

The most important source of Na and CI in the diet is common table salt (NaCl). The good source of K are chicken, calf flesh, beef liver, dried apricot, dried peaches, bananas, the juice of orange and pineapple, potatoes etc.

Absorption:

Normally Na, K and CI are completely absorbed from the gastro-intestinal tract. About 95% of sodium which leaves the body is excreted in the urine.

Distribution:

In the tissues both Na and K occur in a relatively large amount as compared to chloride and other inorganic salts as well as protein and organic salts. Sodium is present in extra cellular fluid and in a very low concentration inside the cells whereas potassium is mainly found inside the cells and in a very low concentration in the extracellular fluid.

Functions of sodium and potassium:

These electrolytes maintain normal osmotic pressure in the body and protect the body against excessive loss of fluid.

1. They maintain the acid base balance in the body. Sodium bicarbonate, sodium phosphate, potassium phosphate form the buffer system of extracellular and intracellular fluids.

2. They maintain normal water balance.

3. Na also functions in the preservation of normal excitability of muscle and the permeability of the cells. K inhibits 'muscular contraction' in general.

4. High intracellular potassium concentrations are essential for several important metabolic functions, including protein biosynthesis by ribosomes.

5. Sodium and Potassium chlorides maintain the viscosity of blood. A number of enzymes including glycolytic enzymes, such as pyruvate kinase, require K^+ for maximal activity.
6. Na helps in the formation of the gastric juice. NaCl takes part in the series of reactions as a result of which HC1 is manufactured by the stomach.

7. K of KHb in the red cells helps in carbon dioxide transport.

8. K ions inhibit cardiac contraction and prolong relaxation.

9. K ions exert important effect on the function of nervous system.

Functions of chloride:

1. It provides 2/3rd of the anion of plasma and is the main factor for regulating body reactions.
2. NaCl and KCl are important agents in regulation of osmotic pressure in the body.

3. HCl of gastric juice is ultimately derived from the blood chlorides.

4. Chloride ions are essential for the action of ptyalin and pancreatic amylase.

5. It is essential in acid-base regulation. Chloride plays a role in the body by chloride shift mechanism.

Metabolism:

The metabolism of these elements is influenced by the following factors:

Hormones:

Mainly adrenocortical steroids and some of the sex hormones facilitate the retention of sodium and chloride in the body and excretion of potassium by kidneys in the urine. In adrenocortical deficiency, serum sodium decreases because excretion increases.

Temperature:

When atmospheric temperature is high as in summer, large amounts of sodium and chloride are lost in perspiration (sweating) and this loss may be checked when temperature is low (in winter).

Renal function:

In renal disease, with acidosis, Na and CI ion excretion in urine is increased due to poor tubular reabsorption of sodium whereas that of K ion is decreased leading to hyponatraemia and

hypochloraemia but hyperkalaemia. Average requirement of Na and K in human body is 5-15 and 4 gm. per day, respectively.

Disorders:

Hyponatraemia:

On sodium deficient diet, young ones grow slowly, lack fat deposit, there is muscle and testicular atrophy, lung infection and deficiency of osteoid tissues. There will also be loss of water, which will be evident by rapid weight loss.

Hypokalaemia:

Extreme potassium depletion in circulating blood causes hypokalemia in young one, they grow slowly and both sexes become sterile. The heart rate is slow, muscle weakness, irritability and paralysis are seen. Bone growth is retarded and in becomes excessively fragile and kidney hypertrophy is exhibited.

Hyperkalemia:

Hyperkalemia paralysis occurs due to excessive amount of potassium in blood. The disease is characterized by periodical attacks of weakness or paralysis. The symptoms of hyperkalaemia are chiefly cardiac and central nervous system depression. They are related to the elevated plasma potassium level and not to increase in intracellular potassium levels.

A dietary chlorine deficiency produces no symptom except a subnormal growth rate. Under normal dietary condition human beings are not subject to a deficiency of sodium, potassium or chlorine. However excessive diarrhoea, vomiting or extreme sweating over long period may bring about a NaCl deficiency. Sometimes the metabolism of individual minerals is asked as a separate question in the university exams. Hence each one is described separately in detail, hereunder.

V. Sodium:

Physiological functions:

1. Major component of extracellular fluids and exists in the body in association with anions chloride, bicarbonate, phosphate and lactate.

2. In association with chloride and bicarbonate it plays a role in acid base equilibrium.

3. Maintains osmotic pressure of the body fluids and thus protects the body against excessive fluid loss.

4. Plays an important role in the absorption of glucose and galactose from small intestine.

5. Maintains normal water balance and distribution.

6. Maintains the normal neuromuscular function.

7. Functions in permeability of cells.

Distribution:

About 1 /3rd of the total sodium content of the body is present in the inorganic portion of the skeleton. Most of the sodium is present in the extracellular fluid.
Plasma — 330 mg/100 ml

Muscles — 60 to 160 mg/100 gm.

Cells — 85 mg/100 gm.

Nerve — 312 mg/100 gm.

Daily requirement:

Adults require 5-15 gms/day. In temperate region, NaCl intake is less. In tropical region, NaCl intake is more. Hypertension patients should not take more than 1 gm. of Na per day.

Absorption:

Normally, Na is completely absorbed from gastro-intestinal tract. Less than 2% is eliminated in feces. In persons suffering from diarrhoea, large amounts are lost in feces.

Excretion:

Urine — 5-35 gm.

Skin — 25-50 mg

Stool — 10-125 mg

Excessive loss of Na by sweating causes heat arrays.

Disease state:

1. Adrenal cortical steroids regulate the metabolism of Na. Insufficiency of adrenal cortical steroids decreases serum Na level with an increase in sodium excretion.

2. In chronic renal disease when acidosis exists, Na depletion occurs due to poor tubular reabsorption of Na as well as to the loss of Na in the buffering acids.

3. In persons not adapted to high environmental temperature large amount of Na is lost in the sweat, developing muscular cramps of extremities, oedema, headache, nausea and diarrhoea.

4. Hyponatremia causes dehydration and reduced blood pressure, decreased blood volume and circulatory failure.

This may be due to:

(a) Prolonged vomiting and diarrhoea resulting in excessive loss of digestive fluid.

(b) Chronic renal disease with acidosis due to poor tubular reabsorption of Na.

(c) Adrenocortical insufficiency.

(d) Loss of weight due to loss of water.

5. In Hypernatremia, serum Na is high.

This occurs in:

(a) Hyperactivity of adrenal cortex as in Cushing's syndrome.

(b) Prolonged treatment with cortisone and ACTH as well as sex hormones, this results in—

(i) Increased retention of water in the body.

(ii) Increase in blood volume,

(iii) Increase in blood pressure.

6. Steroid hormones cause retention of Na and water in pregnancy.

VI. Potassium:

Physiological junctions:

1. Potassium is largely present in intracellular fluid and it is also present in small amounts in the extra cellular fluid because it influences the cardiac muscle activity.

2. It plays an important role in the regulation of acid-base balance in the cell.

3. It maintains osmotic pressure.

4. It functions in water retention.

5. It is essential for protein biosynthesis by ribosomes.

6. The glycolytic enzyme pyruvate kinase requires K^+ for maximal activity.

Sources:

High content of potassium is found in chicken, beef, liver, bananas, orange juice, pineapple, yam, potatoes etc.

Distribution:

Plasma — 20 mg/100 ml

Cells — 440 mg/100 gm.

Muscles — 250-400 mg/100g

Nerves — 530 mg/100g.

Daily requirement:

Normal intake of K^+ in food is about 4 gm. It is so widely distributed that its deficiency is rare except in pathological condition.

Blood potassium:

Normal level of serum K is 14-20 mg/100 ml. Erythrocytes contain large amounts of K which avoids hemolysis. Serum K decreases during increased carbohydrate utilization following glucose or insulin administration. Aldosterone decreases serum K.

Absorption:

Normally, K is practically completely absorbed from gastrointestinal tract and less than 10% of K is eliminated in the feces. In subjects with diarrhea large amounts are lost in feces.

Excretion:

K is normally eliminated almost entirely in urine and a small amount in the feces. Aldosterone exerts an influence on potassium excretion. In normal kidney function; K is very promptly and efficiently removed from the blood.

Disease state:

1. K is not only filtered by the kidney but is also secreted by the renal tubules. Excretion of K is greatly influenced by changes in acid-base balance and also by adrenal cortex. The capacity of kidney to excrete K is very great and therefore hyperkalaemia does not occur even after ingestion of K, if kidney function is impaired K should not be given intravenously unless, circulatory collapse and dehydration are corrected.

2. Hyperkalaemia occurs in patients in the following conditions.

(a) Renal failure

(b) Severe dehydration

(c) Addison's disease due to decreased excretion of K by the kidney

K deficiency occurs in chronic wasting diseases like malnutrition, prolonged negative nitrogen balance, gastrointestinal losses and metabolic alkalosis.

VII. Chlorine:

Physiological functions:

1. As a component of sodium chloride, chloride ion is essential in acid-base balance.

2. As Cl^- it is also essential in water balance and osmotic pressure regulation.
3. It is also important in the production of HCl in the gastric juice.

4. Cl^- ion is an activator of amylase.

Sources:

Mainly as NaCl salt (table salt).

Distribution:

Plasma — 365 mg/100ml

Cells — 190 mg/ 100mg

CSF — 440 mg/100ml

Muscle — 40 mg/100g

Nerve — 171 mg/100g

Daily requirement:

5-20 gms. Excess consumption of NaCl increases blood pressure in hypertensive patients. Causes edema in protein deficiency.

Absorption:

Normally CI is practically completely absorbed from the GI tract.

Excretion:

CI is chiefly eliminated in the urine, also in sweat. Its concentration in sweat is increased in hot climates and decreased by aldosterone.

Diseases state:

1. CI deficit also occurs when losses of Na are excessive in diarrhoea, sweating and certain endocrine disturbances.

2. Loss of CI due to loss of gastric juice by vomiting or pyloric or duodenal obstruction.

3. Hypochloremia alkalosis may develop in Cushing's syndrome or after administration of ACTH or cortisone.

VIII. Sulphur:

Sources:

Sulphur is taken mainly as cysteine and methionine present in proteins. Other compounds in the diet contribute small amounts of sulphur.

Absorption:

Inorganic sulphate is absorbed as such from intestine into the portal circulation. Small amount of sulphide may be formed in the bowel by the action of bacteria, but if absorbed into the blood stream, it is rapidly oxidized to sulphate.

Sulphur in blood (serum):

Inorganic — 0.5-1.1 mg/100 ml

Ethereal sulphate — 0.1-1.0 mg/100 ml

Neutral sulphur — 1.7-3.5 mg/100 ml

Physiological functions:

1. Sulphur is present primarily in the cell protein in the form of cysteine and methionine.

2. Cysteine plays important part in the protein structure and enzyme activity.

3. Methionine is the principal methyl group donor in the body. The 'activated' form of methionine, s-adenosyl methionine is the precursor in the synthesis of a large number of methylated compounds which are involved in intermediary metabolism and detoxification mechanism.

4. Sulphur is a constituent of coenzyme A and lipoic acid which are utilized in the synthesis of acetyl-CoA, malonyl CoA, Acyl-CoA and S-acetyl lipoate (involved in fatty acid oxidation and synthesis).

5. It is a component of a number of other organic compounds such as heparin, glutathione, thiamine, pantothenic acid, biotin, ergothionine, taurocholic acids, sulphocyamides, indoxyl sulphate, chondroitin sulphate, insulin, penicillin, anterior pituitary hormones and melanin.

Excretion:

Excreted in urine in 3 forms. Total sulphate excretion may be diminished in renal function impairment and is increased in condition accompanied by excessive tissue breakdown as in high fever and increased metabolism.

Disease state:

Serum sulphate is increased in renal function impairment, pyloric and intestinal obstruction and leukemia. Marked sulphate retention in advanced glomerulo-nephritis causes the development of acidosis. Increase in blood indica (indoxyl potassium sulphate) may occur in uremia.

IX. Iron:

Iron is present in all organisms and in all the cells. It does not exist in the free state, instead is always present in organic combination, usually with proteins. It exists in two forms i.e. Fe^{2+} (ferrous) and Fe^{3+} (ferric). It serves as an oxygen and electron carrier and is incorporated into redox enzymes and substances which carry out the function of oxygen transport such as haemoglobin and cytochromes.

Total iron content in normal adult is 4 to 5 grams. 60-70% is present in hemoglobin, 3% in myoglobin and 0.1% in plasma combined with β-globulin transport protein transferrin. Hemoprotein and flavoprotein make up to less than 1% of total iron. Rest is stored as ferritin.

Source:

Rich – Liver, heart, kidney, spleen.

Good – Egg yolk, fish, nuts, dates, beans, spinach, molasses, apples, bananas, etc.

Poor — Milk, wheat flour, polished rice, potatoes etc.

Daily requirement:

Only about 10% of ingested iron is absorbed.

i. Infants – 10-15 mg..

ii. Children – 1-3 years 15 mg.

iii. 4-10 years – 10 mg.

iv. Older children and adults of 11 to 18 years — 18 mg.

v. 19 years and above — 10 mg.

vi. Females between 11 and 50 years of age and during pregnancy or lactation – 18 mg.

vii. After 51 years of age — 10 mg.

viii. In adult women the average loss of iron with blood during menstrual period is 16-32 mg per month or an additional loss of 0.5 to 1.0 mg per day. This amount is easily obtained from diet.

ix. In excessive menstrual blood loss and in chronic iron-deficiency anemia, a supplement of 100 mg of iron per day is sufficient to replenish.

x. During growth, pregnancy and lactation iron demand is more.

xi. In healthy adult male or post menopause women dietary iron requirement is negligible unless any deficiency or loss of iron occurs.

xii. Iron deficiency occurs as a result of malabsorption from gastro-intestinal tract.

xiii. A defect in hemoglobin synthesis in anemia is commonly found in copper deficiency.

Biologically active compounds that contain iron:

1. Haemic compounds:

In these compounds the protoporphyrin is combined with iron to form haem (divalent iron) and haematin.

Ex. Hemoglobin, myoglobin, cytochromes, catalases and peroxidases.

2. Non-haemic compounds:

These include Transferrin (siderophilin) to transport iron, ferritin and haemosiderin which are the stored forms of iron and miscellaneous compounds like enzymes.

Absorption:

Very little (less than 10%) of dietary iron is absorbed. Excretion in the urine is minimal. Infants and children absorb more iron as compared to adults. Iron deficiency in infants is due to dietary deficiency. Iron deficient children absorb approximately twice as much as normal children do. Absorption mainly occurs in the duodenum and the proximal jejunum.

(a) Most of the iron in food occurs in the ferric form (Fe^{3+}), ex. either as ferric hydroxide or as ferric organic compounds. Acidic pH of the gastrointestinal tract favours the absorption whereas alkaline pH decreases it. In an acid medium, these compounds are broken down into free ferric ions or loosely bound organic iron, reducing substances such as —SH groups ex. cysteine and ascorbic acid which convert ferric iron into the reduced (ferrous) state, in this form iron is more soluble and should therefore be more readily absorbed.

(b) A diet high in phosphate, phytic acid and oxalic acid decreases iron absorption since these substances form the insoluble compounds with iron. Conversely, a diet very low in phosphate markedly increases iron absorption.

(c) The extent of absorption depends on the degree of saturation of the tissue, ex. anemic individuals absorb more than normal individuals.

(d) Iron absorption is enhanced by protein, possibly as a result of the formation of low molecular weight digestive products (peptides, amino acids) which can form soluble iron chelates.

(e) It is also increased in pernicious anaemia and in hypo plastic anaemia.

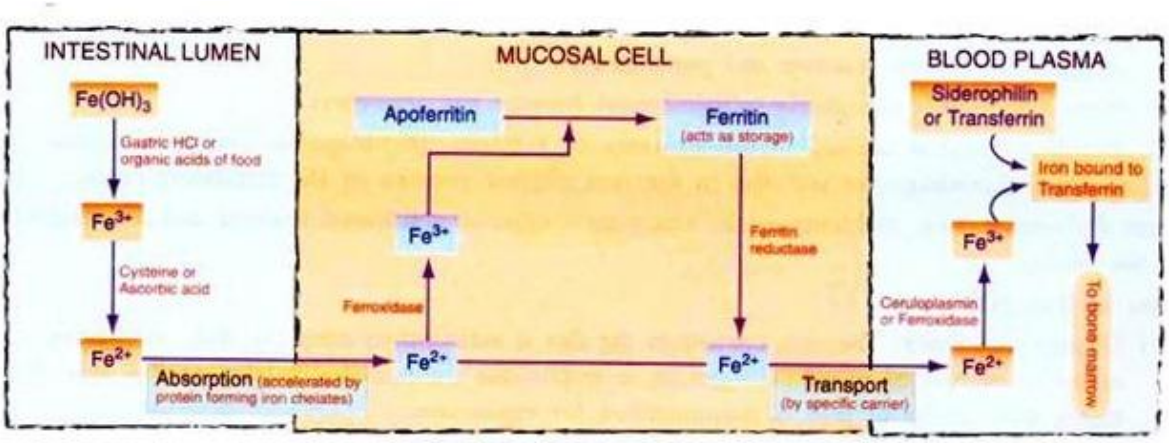
(f) Impaired absorption takes place in patients who have total removal of stomach or a removal of considerable amount of the intestine.

(g) Achlorhydria, administration of alkali, copper deficiency decrease iron absorption.

(h) Alcohol ingestion favours iron absorption.

Mechanism of Iron Absorption:

Ferrous ion on entering the mucosal cells is oxidized to ferric state and then combines with apoferritin forming ferritin which contains 23% of iron by weight. When apoferritin gets saturated with iron no further iron can be taken up by the mucosal cells to store it in the form of ferritin. Heme enters the mucosal cells without being released from the porphyrin ring. Heme is broken down in the mucosa and iron appears in the plasma transferrin.



Mechanism of iron absorption

Transport:

In the plasma, the iron is bound to transferrin which is only partially saturated. Plasma iron is also in exchange with interstitial and intra-cellular compartments. The iron in these compartments is generally referred to as 'labile iron pool' and is estimated to be in the order of 80 to 90 mg. Here the iron may stay briefly on the cell membrane before its incorporation into haem or storage compounds. Nearly all the iron released from the mucosal cell enter the portal blood mostly in the ferrous state (Fe^{2+}). In the plasma, Fe^{2+} is oxidized rapidly to the ferric state (Fe^{3+}) and then incorporated into a specific protein.

Storage:

Stores of iron are maintained chiefly in the liver, spleen and bone marrow in the form of ferritin and haemosiderin. Women have lower stores than men and therefore, develop anaemia much more frequently than men. Iron stores are increased in haemochromatosis, severe haemolytic anaemias, aplastic anaemia and in persons receiving multiple blood transfusions, prolonged oral or parenteral iron therapy.The normal content of protein bound iron (FBI) in plasma of males is

120-140 jig/100 ml; in females it is 90-120 pg/100ml. However, the total iron binding capacity (TIBC) is about the same in both sexes i.e. 300-360 pg/100 ml.

Excretion:

Physiological excretion of iron is minimal. The normal routes of excretion are urine, bile, faeces, cellular desquamation, and sweat. Daily excretion in an adult male is estimated to be about 1 mg. In women of reproductive age, additional loss through menstruation averages to 1 mg per day.

Abnormal iron metabolism:

Ferritin and hemosiderin, the storage forms of iron act as internal iron reserve to protect against sudden loss of iron by bleeding. Ferritin is present not only in the intestine but also in liver (about 700 mg) spleen and bone marrow. If excess iron is administered parenterally exceeding the capacity of the body to store it as ferritin, it accumulates in the liver as hemosiderin, a form of colloidal iron oxide in association with protein.

Iron metabolism is disturbed mainly by the following causes:

(a) Decreased formation of hemoglobin.

(b) Decrease in circulating hemoglobin.

(c) Abnormalities in the serum iron concentration

(d) Abnormal deposition of iron-combining pigments in the tissues.

Physiological functions:

1. Iron functions mainly in the transport of oxygen to the tissues.

2. Involved in the process of cellular respiration.

3. Essential component of hemoglobin, myoglobin, cytochromes and the respiratory enzyme systems (cytochrome oxidase, catalase and peroxidase).

4. Non-heme iron is completely protein-bound (storage and transport).

5. Non-heme iron is utilized in the structure of xanthine dehydrogenase (xanthine oxidase) and succinate dehydrogenase and also in the iron sulphur proteins of the respiratory chain.

Iron deficiency:

Iron deficiency is the commonest cause of nutritional anaemia and is prevalent all over the world. Causes of iron deficiency:

(1) Dietary deficiency:

The iron content in the diet is sufficient to meet the daily requirements, but excessive amount of phytates in cereals, is responsible for non-absorbability of this iron. Hence higher daily intake of iron is recommended for vegetarians.

(2) Lack of absorption:

This may be seen in malabsorptive syndromes.

(3) Increased demand:

This occurs during rapid growth in infancy and pregnancy.

(4) Poor stores at birth:

These are found in premature birth and twin pregnancy.

(5) Pathological blood loss:

With loss of 1g of haemoglobin 3.4 mg of iron is lost. Hook-worm infestation is the most important factor responsible for blood loss. Other sources of blood loss are bleeding piles, peptic ulcer, hiatus hernia, cancer of gastrointestinal tract, chronic aspirin ingestion, and oesophageal varies.

(6) Iron deficiency anemia:

Iron deficiency anemia is widely prevalent among children, adolescent girls and nursing mothers. The hemoglobin content of the blood during iron deficiency anemia is 5 to 9 g/100 ml.

(a) Women of child bearing age:

The clinical symptoms are breathlessness on exertion, giddiness and pallor of the skin. In severe cases, there may be edema of the ankles.

(b) Weaned infants and young children:

The hemoglobin level is 5 to 9 g/100 ml of blood. The children are dull and inactive and show pallor of the skin. The appetite is poor and growth and development are retarded.

Treatment of iron deficiency anaemia:

Anaemia responds to oral iron therapy. The commonly used preparations are ferrous sulphate, ferrous fumarate and ferrous gluconate. Iron dextran can be administered both intramuscularly and intravenously, iron sorbitex is given intramuscularly, and saccharide iron oxide is given intravenously. Anemic women should take ferrous sulphate tablet. For a child below 12 months, a mixture of ferrous ammonium citrate sweetened with glycerine and for children of 1 to 5 years ferrous ammonium citrate mixture should be given for curing.

Iron overload:

Hypersiderosis may occur as a primary disorder (Idiopathic haemochromatosis) or secondary with excessive entry of exogenous, iron into the body.

1. Siderosis:

When excessive amounts of iron are released in or introduced into the body beyond the capacity for its utilization, the excess is deposited in various tissues, mainly in the liver. This may occur due to repeated blood transfusions, excessive breakdown of erythrocytes in hemolytic types of anaemia and inadequate synthesis of haemoglobin as in pernicious anaemia.

2. Nutritional siderosis:

This disorder is found among Bantus in South Africa. Bantus cook their food in large iron pots and consume iron-rich food. The absorption of iron appears to be high, leading to the development of nutritional siderosis. Livers of the Bantus contain large amounts of iron.

Hemochromatosis:

Hemochromatosis is a rare disease in which large amounts of iron are deposited in the tissues, especially the liver, pancreas, spleen and skin producing various disorders. Accumulation of iron in the liver, pancreas and skin produces hepatic cirrhosis, bronze diabetes and bronze-state pigment respectively.

X. Copper:

Source:

Richest sources:

Liver, kidney, other meats, shell fish, nuts and dried legumes.

Poor sources:

Milk and milk products. The concentration of copper in the fetal liver is 5-10 times higher than that in liver of an adult.

Daily requirements:

Infants and children – 0.05 mg/kg body weight

Adults – 2.5 mg

A nutritional deficiency of copper has never been demonstrated in man, although it has been suspected in case of nephrosis.

Absorption:

About 30% of the normal daily diet of copper is absorbed in the duodenum.

Blood copper:

The normal concentration of copper in serum is 90 µg/100 ml. Both RBC and serum contain copper. 80% of RBC copper is present as superoxide dismutase (erythrocuperin), Plasma copper occurs as firmly bound form and loosely bound forms. The firmly bound copper consists of ceruloplas-min. Loosely bound copper is called ‘directly reacting copper’ and is bound to serum

albumin. The plasma copper levels increase in pregnancy because of their estrogen content. Oral contraceptives have a similar effect

Physiological functions:

1. It has important role in hemoglobin synthesis.

2. It is required for melanin formation, phospholipids synthesis and collagen synthesis.

3. It has a role in bone formation and in maintenance of the integrity of myelin sheath.

4. It is a constituent of several enzymes such as tyrosinase, cytochrome oxidase, ascorbic acid oxidase, uricase, ferroxidase I (ceruloplasmin), ferroxidase II, superoxide dismutase, amino oxidase and dopamine hydroxylase.

5. Three copper containing proteins namely cerebrocuperin, erythrocuperin and hepatocuperin are present in brain, RBC and liver respectively.

Excretion:

Only 10 to 60 mg of copper is excreted in the urine. 0.5 to 1.3 mg is excreted through bile and 0.1 to 0.3 mg is excreted by intestinal mucosa into the bowel lumen.

Effects of copper deficiency:

1. Although iron absorption is not disturbed but the release of iron into the plasma is prevented due to the decreased synthesis of ceruloplasmin. As a result, hypoferremia occurs which leads to the depressed synthesis of heme developing anemia in severe deficiency of copper.

2. The experimental animals on a copper deficient diet lose weight and die.

3. In copper deficient lambs, low cytochrome oxidase activity results in neonatal ataxia.

4. Copper deficiency produces marked skeletal changes, osteoporosis and spontaneous fractures.

5. Elastin formation is impaired in the deficiency of copper. Because a copper containing enzyme plays an important role in the connective tissue metabolism, especially in the oxidation of lysine into aldehyde group which is necessary for cross linkage of the polypeptide chains of elastin and collagen.

6. Copper deficiency results in myocardial fibrosis in cows. It is suggested that reduction in cyto-chrome oxidase activity may lead to cardiac hypertrophy.

Disorders of copper metabolism:

Wilson's disease (hepatoreticular degeneration):

Wilson's disease is a rare hereditary disorder of copper metabolism.

The following disorders have been observed in this disease:

(a) The absorption of copper from the intestine is very high (about 50 percent); whereas 2 to 5 percent copper is absorbed in normal subjects.

(b) Ceruloplasmin formation is very less. Hence a greater part of serum copper remains loosely bound to serum protein-notably albumin and therefore, copper can be transported to the tissues, such as brain and liver or to the urine.

(c) Excessive deposition of copper in the liver and the kidney causes hepatic cirrhosis and renal tubular damage respectively. The renal tubular damage results in the increased urinary excretion of amino acids, peptides and glucose.

XI. Iodine:

Source:

Rich sources are sea water, marine vegetation and vegetables as well as fruits grown on the sea board. Plants grown at high altitudes are deficient in iodine because of its low concentration in the water. In such regions, iodide is commonly added to the drinking water or table salt in concentrations of 1:5000 to 1:200000.

Daily requirement:

Adults – 100 to 150 μg

In adolescence and in pregnancy – 200 μg

Distribution:

Normal iodine content of body is 10 to 20 mg. 70 to 80% of this is present in thyroid gland. Muscles contain large amount of iodine. The concentration of iodine in the salivary glands, ovaries, pituitary gland, brain and bile is greater than that in muscle. Iodine in saliva is inorganic iodide, while most of the iodine present in tissue is in the organic form.

Blood Iodine:

Practically all the iodine in the blood is in the plasma. The normal concentration in plasma or serum is 4 to 10 μg/100 ml. 0.06 to 0.08 μg/100 ml is in inorganic form, 4 to 8 μg/100 ml is in the organic form bound to protein, precipitated by protein precipitating agents. 90% of the organic form consists of thyroxine and the remainder tri and di-iodothyronine. About 0.05% of thyroxine is in the free state. RBC contains no organic iodine.

Absorption:

Iodine and iodide are absorbed most readily from the small intestine. Organic iodide compounds (di-iodothyronine and thyroxine) are partly absorbed as such and a part is broken down in the stomach and intestines with the formation of iodides. Absorption also takes place from outer mucus membrane and skin.

Storage:

90% of the iodine of the thyroid gland is in organic combination and stored in the follicular colloid as 'thyroglobulin' a glycoprotein containing thyroxine, di-iodothyronine and smaller amounts of triiodothyronine.

On demand these substances are mobilized and thyroxine as well as triiodothyronine is passed into the systemic circulation. They undergo metabolic degradation in the liver.

Excretion:

1. Inorganic iodine is mostly excreted by the kidney, liver, skin, lungs and intestine and in milk.

2. About 10% of circulating organic iodine is excreted in feces. This is entirely unabsorbed food iodine.

3. 40 to 80 % is usually excreted in the urine, 20 to 70 μg daily in adults, 20 to 35 μg in children. The urinary elimination is largest when the intake is lowest.

4. Urinary iodine is increased by exercise and other metabolic factors.

Physiological functions:

Iodine is required for the formation of thyroxine and triiodothyronine hormones of the thyroid gland. These thyroid hormones are involved in cellular oxidation, growth, reproduction and the activity of the central and autonomic nervous systems. Triiodothyronine is more active than thyroxine in many respects.

Iodine deficiency:

1. In adults the thyroid gland is enlarged producing goiter. If treatment is started very early, the thyroid becomes normal. If treatment is delayed, the enlargement persists.

2. In children, severe iodine deficiency results in the extreme retardation of growth causing cretinism.

Prevention of goiter:

Goiter can be prevented by the regular use of iodized salt or iodine added to the drinking water.

Goitrogenic substances in foods:

Cabbage, cauliflower and radish contain substance like vinyl-2- thiooxazolidone which makes iodide present in the food unavailable by reacting with it. Such substances are called 'goitrogenic' substances.

Selenium:

i. Good dietary sources are kidney cortex, pancreas, pituitary and liver.

ii. It is rapidly absorbed mainly in duodenum.

iii. It is distributed in liver 0.44 μg/gm in skin 0.27 μg/gm and in muscle 0.37 μg/gm.

iv. In the cells it is present as selenocystinenadselenomethionine.

v. Selenium along with Vitamin E plays an important role in tissue respiration.

vi. Selenium is involved in biosynthesis of coenzyme Q (ubiquinone), which is involved in respiratory chain.

vii. Selenium acts as an antioxidant providing protection against peroxidation in tissues and membrane.

viii. It is an essential component of glutathione peroxidase, an enzyme which catalyzes the conversion of reduced glutathione to its oxidized form.

ix. Selenium is excreted in faeces, urine and via exhalation.

x. It causes toxic effect called selenosis.

Probable Questions:

1. State the distribution of water in the body. What factors affect distribution of water?
2. state the procedure of water intake and water loss from the body.
3. What are the physiological functions of water?
4. What is primary and secondary dehydration?
5. Write down the effect of dehydration.
6. How hormones regulate water balance in the body.
7. What is macro elements and micro elements. Give examples.
8. How calcium balance is regulated by hormones.
9. What factors control calcium absorption?
10. What factor control calcium level in blood.
11. State the diseases associated with problems in calcium metabolism.
12. Write down the physiological role of Phosphorous in the body.
13. How hormone controls phosphorous metabolism.
14. How iron get absorbed in the body.

Suggested Readings:

1. General Endocrinology. Turner and Bagnara. Sixth Edition.
2. Williams Textbook of Endocrinology. Tenth Edition.
3. Introduction to Endocrinology. Chandra S Negi. Second Edition
4. Endocrinology. Hadley and Levine. Sixth Edition

Unit-XV

GI tract hormone source, composition and function

Objective: In this unit you will know about source, composition and function of different gastro intestinal hormones.

Introduction:

The gastrointestinal hormones (or gut hormones) constitute a group of hormones secreted by enteroendocrine cells in the stomach, pancreas, and small intestine that control various functions of the digestive organs. Later studies showed that most of the gut peptides, such as secretin, cholecystokinin or substance P, were found to play a role of neurotransmitters and neuromodulators in the central and peripheral nervous systems.

Enteroendocrine cells do not form glands but are spread throughout the digestive tract. They exert their autocrine and paracrine actions that integrate gastrointestinal function.

The primary function of the gastrointestinal tract is to supply nutrients to our bodies via the processes of ingestion, motility, secretion, digestion, and absorption; this occurs through complex coordination of digestive processes that are regulated by intrinsic endocrine and nervous systems. Although the nervous system exerts influence on many digestive processes, the GI tract is the largest endocrine organ in the human body and produces numerous mediators that play an integral role in regulating functions of the GI tract.

Types of GI Hormones:

I. 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.

II. 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.

III. 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.

IV. 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.

V. Duocrmin:

It is secreted by the duodenal epithelium and stimulates the Brunner's glands to release mucus and enzymes into the intestinal juice.

VI. Enterocrinin:

It is secreted by the epithelium of entire small intestine. It stimulates the crypts of Lieberkuhn to release enzymes into the intestinal juice.

VII. 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.

VIII. Villikinin:

It is secreted by the epithelium of entire small intestine. It accelerates movement of villi.

IX. 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.

X. 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.

Bio synthesis of GI Hormones:

The GI hormones classify as endocrines, paracrine, or neurocrine based on the method by which the molecule gets delivered to its target cell(s). Endocrine hormones are secreted from enteroendocrine cells directly into the bloodstream, passing from the portal circulation to the systemic circulation, before being delivered to target cells with receptor-specificity for the hormone. The five GI hormones that qualify as endocrines are **gastrin, cholecystokinin (CCK), secretin, glucose-dependent insulinotropic peptide (GIP), and motilin**. Enteroendocrine cells also secrete paracrine hormones, but they diffuse through the extracellular space to act locally on target tissues and do not enter the systemic circulation. Two examples of paracrine hormones are somatostatin and histamine. Additionally, some hormones may operate via a combination of endocrine and paracrine mechanisms. These "candidate" hormones are glucagon-like peptide-1 (GLP-1), pancreatic polypeptide, and peptide YY. Lastly, neurocrine hormones get secreted by postganglionic non-cholinergic neurons of the enteric nervous system. Three neurocrine hormones with significant physiologic functions in the gut are vasoactive intestinal peptide (VIP), gastrin release peptide (GRP), and enkephalins.

Gastrointestinal hormones undergo synthesis in specialized cells of the GI tract mucosa known as enteroendocrine cells. Enteroendocrine cells are specialized endoderm-derived epithelial cells that originate from stem cells located at the base of intestinal crypts. These cells are dispersed throughout the GI mucosa, sprinkled in between epithelial cells from the stomach all the way through to the colon. Also, these enteroendocrine cells possess hormone-containing granules concentrated at the basolateral membrane, adjacent to capillaries, that secrete their hormones via exocytosis in response to a wide range of stimuli related to food intake. These stimuli include small peptides, amino acids, fatty acids, oral glucose, distension of an organ, and vagal stimulation.

G cells secrete gastrin in the antrum of the stomach and the duodenum in response to the presence of breakdown products of protein digestion (such as amino acids and small peptides), distention by food, and vagal nerve stimulation via GRP. More specifically, phenylalanine and tryptophan are the most potent stimulators of gastrin secretion among the protein digestion products. The vagal nerve stimulation of gastrin secretion is unique because gastrin and motilin are the only hormones released directly by neural stimulation.

CCK is secreted from I cells in the duodenum and jejunum in response to acids and monoglycerides (but not triglycerides), as well as the presence of protein digestion products.Secretin is secreted from S cells in the duodenum in response to H+ and fatty acids in the lumen. Specifically, a pH less than 4.5 signals arrival of gastric contents, which initiates the release of secretin.

GIP is secreted by K cells in the duodenum and jejunum in response to glucose, amino acids, and fatty acids. GIP is the only GI hormone with a response to all three macronutrient types, and newer studies suggest that changes in intraluminal osmolarity may be what stimulates GIP secretion.GLP-1 is also produced in the small intestine and secreted from L cells. The presence of hexose and fat stimulate its release. Pancreatic polypeptide and peptide YY are secreted by protein and fat, respectively, although their functions are still relatively unknown.

Organ Systems Involved:

The digestive system is the primary site of action for most GI hormones and related polypeptides. The stomach is the primary site of gastrin production with some D-cells also populating the duodenum. Somatostatin and histamine are also produced in the stomach by enterochromaffin-like (ECL) cells, which is an enteroendocrine cell subtype. The small intestines, namely the duodenum and jejunum handle secretion of CCK, secretin, GIP, and motilin.

Function

The two gastrointestinal hormone families discussed above are responsible for most of the regulation of gastrointestinal function. The main actions of the gastrin-CCK family and the secretin family of hormones are listed below.

a. Gastrin

- Stimulates H+ (acid) secretion by parietal cells in the stomach
- Trophic (growth) effects on the mucosa of the small intestine, colon, and stomach

- Inhibits the actions of Secretin and GIP
- Inhibited by H+

b. CCK

- Contraction of the gallbladder with simultaneous relaxation of the sphincter of Oddi
- Inhibits gastric emptying
- Stimulates secretion of pancreatic enzymes: lipases, amylase, and proteases
- Secretion of bicarbonate from the pancreas
- Trophic effects on the exocrine pancreas and gallbladder

c. Secretin

- Inhibits gastrin, H+ secretion, and growth of stomach mucosa
- Stimulates biliary secretion of bicarbonate and fluid
- Secretion of bicarbonate from the pancreas
- Trophic effect on the exocrine pancreas

d. GIP

- Stimulation of insulin secretion
- Induces satiety
- In large doses, decreases gastric acid secretion
- In large doses, decreases the motor activity of the stomach and therefore slows gastric emptying when the upper small intestine is already full of food products.
- Stimulates the activity of lipoprotein lipase in adipocytes
- Protects beta-cells of the pancreas from destruction by apoptosis

e. GLP-1

- Decreases gastric emptying
- Induces satiety
- Increases sensitivity of pancreatic beta-cells to glucose.

f. Motilin

- Increases gastrointestinal motility by stimulating the “migrating motility” or “myoelectric complex” that moves through the fasting stomach and small intestines every 90 minutes. This cyclical release and action get inhibited by the ingestion of food. Not much is known about this peptide, except for this essential function.

Mechanism of action of GI hormones:

The release of GI hormones is in response to input from G-protein-coupled receptors that detect changes in luminal contents. Some of these receptors only respond to selective luminal substances and subsequently release GI hormones from their respective enteroendocrine cells through unknown mechanisms. Overall, gastrointestinal hormones manage a diverse set of actions in the body including:

- Contraction and relaxation of smooth muscle wall and sphincters
- Secretion of enzymes for digestion
- Secretion of fluid and electrolytes
- Trophic (growth) effects on tissues of GI tract
- Regulating secretion of other GI peptides (i.e., somatostatin inhibits secretion of all GI hormones)

To better understand how these actions are carried out by GI hormones, it is best to use gastrin's functions as an example. Gastrin is an interesting hormone because it acts through two mechanisms that ultimately increase the secretion of gastric acid (hydrogen ions) into the stomach. The first mechanism involves gastrin binding to CCK-2 receptors on parietal cells, causing increased expression of K/H ATPase enzymes that are directly responsible for increased hydrogen ion secretion into the stomach. The second mechanism is mediated by enterochromaffin-like cells, which secrete histamine in response to activation by gastrin. Histamine then binds H2 receptors on nearby parietal cells, which further stimulates secretion of hydrogen ions. In addition to stimulating ECL cells to produce acid, gastrin also stimulates these parietal cells and ECL cells to proliferate.

Hormone	Major Activities	Stimuli for Release
Gastrin	Stimulates gastric acid secretion and proliferation of gastric epithelium	Presence of peptides and amino acids in gastric lumen
Cholecystokinin	Stimulates secretion of pancreatic enzymes, and contraction and emptying of the gall bladder	Presence of fatty acids and amino acids in the small intestine
Secretin	Stimulates secretion of water and bicarbonate from the pancreas and bile ducts	Acidic pH in the lumen of the small intestine
Ghrelin	Appears to be a strong stimulant for appetite and feeding; also a potent	Not clear, but secretion peaks

	stimulator of growth hormone secretion.	prior to feeding and diminishes with gastric filling
Motilin	Apparently involved in stimulating housekeeping patterns of motility in the stomach and small intestine	Not clear, but secretion is associated with fasting
Gastric Inhibitory Peptide	Inhibits gastric secretion and motility and potentiates release of insulin from beta cells in response to elevated blood glucose concentration	Presence of fat and glucose in the small intestine

Possible Questions:

1. Describe source and function of any five GI hormones.
2. How GI hormones are synthesized in the body.
3. State the mechanism of actions of GI hormones.
4. Describe stimuli of release of any five GI hormones.

Suggested readings:

1. General Endocrinology. Turner and Bagnara. Sixth Edition.
2. Williams Textbook of Endocrinology. Tenth Edition.
3. Introduction to Endocrinology. Chandra S Negi. Second Edition
4. Endocrinology. Hadley and Levine. Sixth Edition.

Unit-XVI

Neuroendocrine system and neurosecretion: neural control of glandular secretion; hypothalamic pituitary unit, neuroendocrine feedback

Objective: In this unit you will learn about Neuroendocrine system and neurosecretion: neural control of glandular secretion; hypothalamic pituitary unit, neuroendocrine feedback

Neuroendocrine system and neurosecretion:

Neuroendocrine system: The nervous system in association with endocrine system that serves as the primary control centre of the body is called neuroendocrine system.

Example: The hypothalamus (releasing factors) stimulates the pituitary gland to release various hormones that control various metabolic activities of the body.

Neurohormone: Any hormone that is produced by a specialized nerve cell (but not by endocrine gland) and is secreted from the nerve endings into the blood stream or tissues to exert its function is called neurohormone.

Example: ADH, noradrenaline, ecdyson, juvenile hormone etc.

Neurosecretion: The synthesis, storage & secretion of (hormones) neurohormones by neurosecretory cells (possess both nerve & endocrine functions) is called neurosecretion.

Example: In the hypothalamus, neurosecretory cells receive nerve impulses from the other parts of the brain or body which signal is transmitted to the pituitary gland by means of neurohormones.

Neurotransmitters: The chemicals that mediate the transmission of nerve impulse across a synapse or neuromuscular junction.
Example: Acetylcholine, adrenaline, noradrenaline, dopamine, serotonin, GABA etc.

Hypothalamus: It is a part of vertebrate forebrain situated below the thalamus & cerebrum that mainly regulates body temperature & neuroendocrine functions.

The neuroendocrine system:

The neuroendocrine system is made up of special cells called neuroendocrine cells. They are spread throughout the body. Neuroendocrine cells are like nerve cells (neurons), but they also make hormones like cells of the endocrine system (endocrine cells). They receive messages (signals) from the nervous system and respond by making and releasing hormones. These hormones control many body functions.

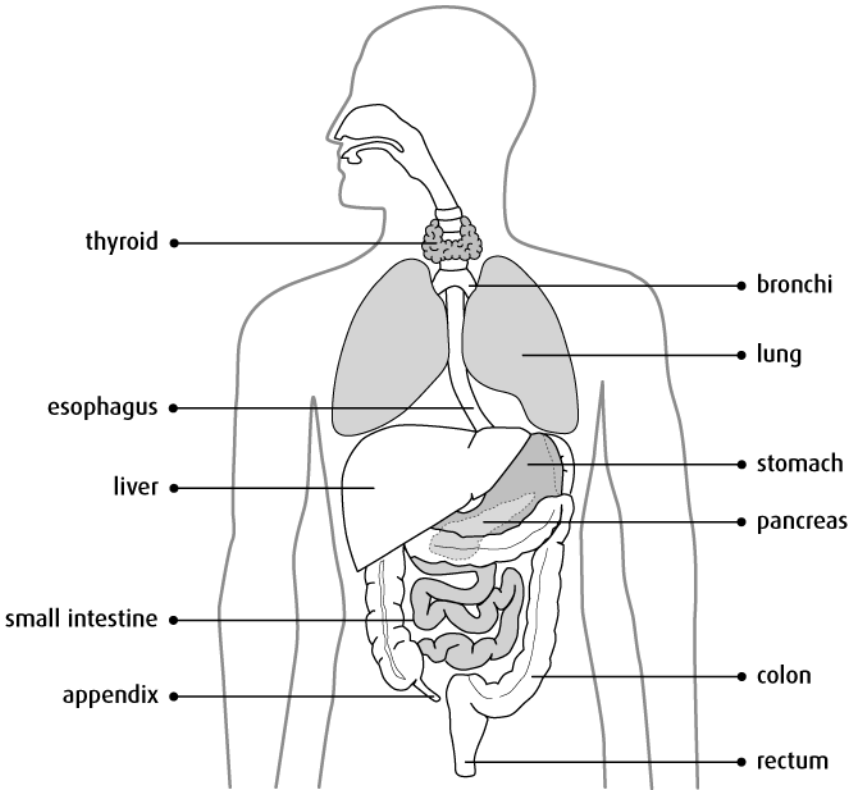
Location of neuroendocrine cells:

Neuroendocrine cells are found in almost every organ of the body. They are mainly found scattered in the gastrointestinal (GI) tract (including the small intestine, rectum, stomach, colon, esophagus and appendix), the gallbladder, the pancreas (islet cells) and the thyroid (C cells). Neuroendocrine cells are also commonly found in the lungs or airways into the lungs (bronchi), as well as the respiratory tract of the head and neck. The neuroendocrine cells scattered throughout these organs are often referred to as the diffuse neuroendocrine system.

The pituitary gland, the parathyroid glands and the inner layer of the adrenal gland (adrenal medulla) are almost all made up of neuroendocrine cells.

Other sites of neuroendocrine cells include the thymus, kidneys, liver, prostate, skin, cervix, ovaries and testicles.

Part of the Neuroendocrine System



Function of neuroendocrine cells :

Neuroendocrine cells make and release hormones and similar substances (peptides) in response to neurological or chemical signals. The hormones then enter the blood and travel throughout the body to other cells (target cells). The hormones attach to specific receptors on target cells, which cause changes in the cells and what they do.

Neuroendocrine cells have many functions, which include controlling:

- the release of digestive enzymes to break down food
- how fast food moves through the GI tract
- air and blood flow through the lungs
- blood pressure and heart rate
- the amount of sugar (glucose) in the blood
- bone and muscle growth and development

The following are examples of hormones or peptides released by neuroendocrine cells and what they do.

- Serotonin (5-HT or 5-hydroxytryptamine) is a chemical released by nerve cells (neurotransmitter) that helps with digestion. A lot of the body's serotonin is found and made in the neuroendocrine cells of the GI tract where it controls the movement of food through the GI tract.
- Gastrin tells the stomach to release acid and enzymes to help with digestion.
- Insulin is made by pancreatic islet cells. It lowers the level of sugar (glucose) in the blood when it's high. It controls when cells absorb (take up) sugar for energy.
- Epinephrine (adrenaline) is made by neuroendocrine cells of the adrenal gland. It is released during times of stress, like when you feel fear, and increases heart rate and blood pressure.
- Growth hormone is made in the pituitary gland. It promotes the growth and development of bones and muscles.

Major Neuroendocrine Systems:

Various endocrine glands are intimately associated with hypothalamus and pituitary to controlthe various physiological function of the body by means of various axes which are:

1. Hypothalamic-pituitary-thyroid (HPT) axis
2. Hypothalamic-pituitary-gonodal (HPG) axis
3. Hypothalamic-pituitary-adrenal (HPA) axis
4. Hypothalamic-neurohypophyseal axis.

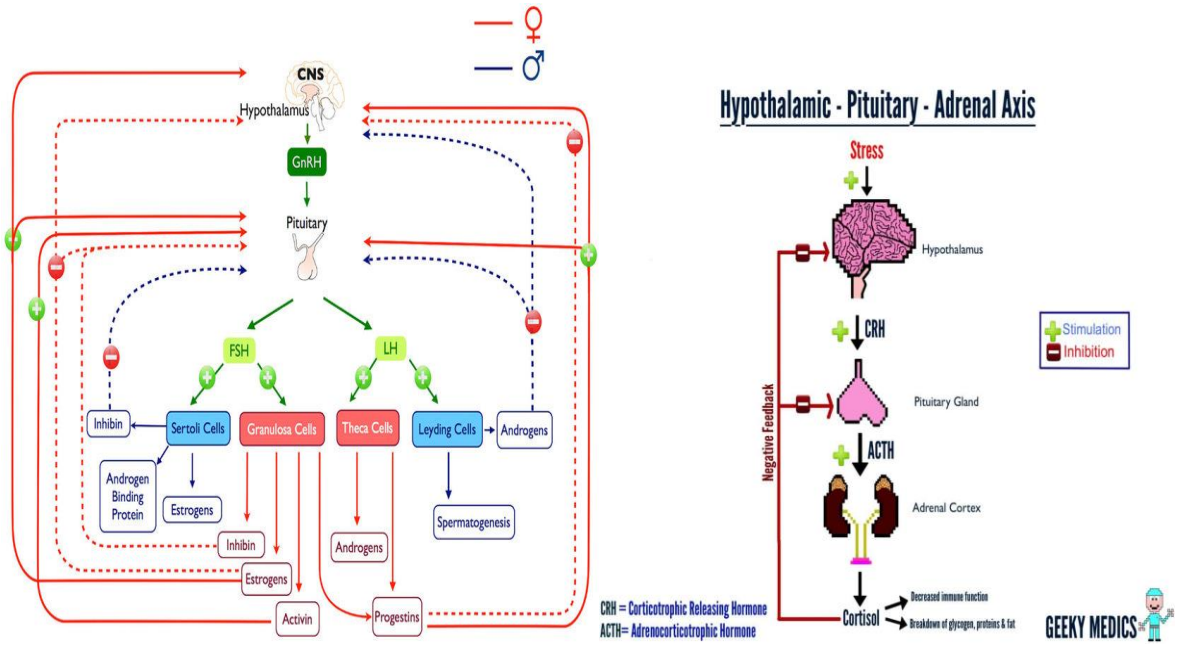
Any of the systems of dual control of certain activities in the body of some higher animals by nervous and hormonal stimulation is the neuroendocrine system. For example, the posterior pituitary gland and the medulla of the adrenal gland receive direct nervous stimulation to secrete their hormones, whereas the anterior pituitary gland is stimulated by releasing hormones from the hypothalamus.

A substantial volume of scientific evidence has been accumulated demonstrating that biological aging is associated with functional deficits at the cellular, tissue, organ, and system levels. Although several theories have been proposed to explain these changes, as well as the increased risk of disease with age, no single explanation has adequately accounted for the diversity of physiological

changes associated with age.

The concept that deficiencies in the neuroendocrine system contribute to aging evolved from **studies indicating that-**
(1) the endocrine system has an important role in developmental processes,
(2) hormones have an important trophic & integrative role in maintaining tissue function, and
(3) hormone deficiency results in deterioration of tissue function.

The neuroendocrine system is composed of the hypothalamus and pituitary gland and is under the influence of neurotransmitters and neuropeptides that regulate hypothalamic releasing and hypothalamic release inhibiting hormones secreted into the blood vessels that connect the hypothalamus and pituitary gland. The release of these hypothalamic hormones influences the secretion of anterior pituitary hormones that subsequently regulate tissue function. The hypothalamus and pituitary gland have the capacity to detect humoral secretions (hormones secreted) from target tissues and adjust hormone production to maintain an optimal internal "milieu" appropriate for normal function.



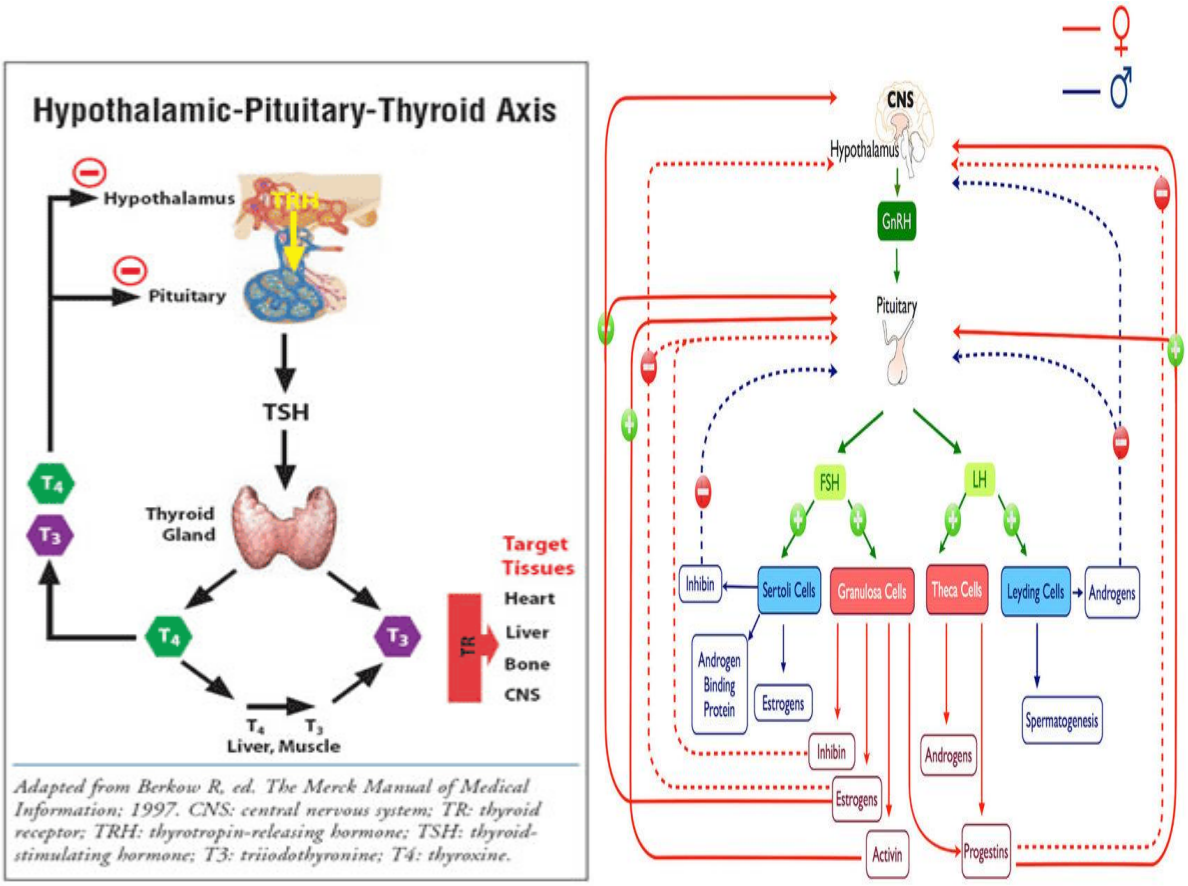


Figure: Various hypothalamic-hypophyseal axes

It is well-established that the neuroendocrine system has a critical role in integrating biological responses and influencing:

(1) cellular protein synthesis and general metabolism through the release of growth hormone andthyroid-stimulating hormone (TSH), respectively,

(2) reproductive function through the release of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, and oxytocin, and

(3) plasma electrolytes and responses to stress through regulation of the hormones vasopressin (antidiuretic hormone, or ADH) and adrenocorticotropin (ACTH).

In addition, the hypothalamus also has an important role in the integration of parasympathetic and sympathetic nervous system activity, and can thereby influence a wide variety of functions, including heart rate, blood pressure, vascular responses, and glucose metabolism. The hypothalamus has been implicated in the regulation of biological rhythms by its interactions with hypothalamic nuclei. More recently, the regulation of fat metabolism and food intake has been shown to be regulated through the hypothalamus by its response to the protein, leptin, and its synthesis of neuropeptide Y. It should be noted that the classification of hormones and their primary function presented here is an overly simplistic view of the neuroendocrine system, since critical interactions occur among these hormones that contribute to the coordinated regulation of

cellular and tissue function.

Although the specific etiology of age-related changes in the neuroendocrine system is unknown, it has been proposed that cellular and molecular alterations in specific subpopulations of neurons within the hypothalamus and pituitary, and/or supporting structures within the brain, contribute to the decrease in tissue function. Some of the alterations may be related to loss of neurons or synapses, genetic errors, and/ or the production of free radicals, all of which lead to progressive aberrations in neurons and contribute to neuroendocrine aging. As a result, the neuroendocrine theory of aging is unique when compared to other theories of aging in that the neuroendocrine alterations are, in many cases, not considered the primary causative factors of biological aging, but rather are considered to be mediators of aging that are initiated by cellular changes in specific subpopulations of neurons or systems that closely interact with hypothalamic neurons. Three classic examples of age-associated changes in neuroendocrine regulation, and the resulting consequences for tissue function, help emphasize the importance of this system in the development of the aging phenotype.

First, with increasing age there is a decline in growth-hormone secretion that results in a decrease in insulin-like growth factor-1 (IGF-1) production in the liver and other tissues. The loss of these anabolic hormones contributes to the general decline in cellular protein synthesis, skeletal muscle mass, immune function, and cognitive ability in rodents, nonhuman primates, and humans. The decrease in growth-hormone release from the pituitary gland results from impaired release of growth-hormone-releasing hormone and increased release of somatostatin (an inhibitor of growth hormone) from hypothalamic neurons.

Second, decreased secretion of gonadotropin-releasing hormone (GnRH) from hypothalamic neurons results in a decline in luteinizing hormone. This is the primary factor in the loss of reproductive cycles in the female rodent, and, in conjunction with the loss of ovarian follicles, contributes to the decline in oestrogen levels in women. These latter changes result in atrophy of secondary reproductive tissues and have been implicated in the post-menopausal loss of bone and cognitive function. Decreased GnRH secretion in the male also contributes to a decrease in LH and androgen levels and to the corresponding loss of skeletal muscle mass and reproductive function.

Finally, increased secretion of ACTH and the adrenal hormone, cortisol, in response to stress have been reported to contribute to atrophy and/or loss of neurons, as well as age-related decline in cognitive function. These latter findings have contributed to the hypothesis that increased levels of glucocorticoids contribute to brain aging.

Although other mechanisms are possible, the alterations in the secretion of hypothalamic hormones with age have been traced to deficiencies in the secretion of brain neurotransmitters. For example, the activity of dopamine and norepinephrine decreases with age, and both acute and chronic procedures used to increase levels of these neurotransmitters in aged animals have been shown to restore some aspects of neuroendocrine function. Studies have shown an increase in growth hormone release and a restoration of some aspects of reproductive function in older

animals in response to the L-Dopa, dopamine and norepinephrine precursor. These findings have led investigators to conclude that a decline in neurotransmitter activity is a contributing factor in the neuroendocrine decline that accompanies aging. Nevertheless, the possibility that interactions with other hypothalamic peptides, the loss of neurons, or intracellular changes within hypothalamic neurons contribute to the loss of function cannot be excluded.

In fact, the inability of hypothalamic neurons to compensate for the age-related alterations in circulating levels of hormones supports the concept that the normal feedback mechanisms that occur within the hypothalamus are impaired in aged animals. Whether these altered feedback mechanisms are related to the deficiencies in neurotransmitters or result from other aberrations within the aging neuroendocrine system remain to be established. Nevertheless, deficits in the regulation of these critical hormonal systems contribute to deterioration of tissue function and undoubtedly are an important factor in age-related disease and disability.

Probable Questions:

1. Define neurohormone. Give examples.
2. Define neuroendocrine system. State the location of neuroendocrine cells.
3. What are the functions of neuroendocrine cells?
4. Name four major neuroendocrine system.

Suggested readings:

1. General Endocrinology. Turner and Bagnara. Sixth Edition.
2. Williams Textbook of Endocrinology. Tenth Edition.
3. Introduction to Endocrinology. Chandra S Negi. Second Edition
4. Endocrinology. Hadley and Levine. Sixth Edition.

UNIT-XVII

Molecular basis of endocrinopathies: thyrotoxicosis, hypothyroidism, Hashimoto's thyroiditis.

Objective: In this unit we will discuss different types of endocrinopathies such as thyrotoxicosis, hypothyroidism, Hashimoto's thyroiditis.

Introduction: The endocrine system is a network of glands that produce and release hormones that help control many important body functions, including the body's ability to change calories into energy that powers cells and organs. The endocrine system influences how your heart beats, how your bones and tissues grow, even your ability to make a baby. It plays a vital role in whether or not you develop diabetes, thyroid disease, growth disorders, sexual dysfunction, and a host of other hormone-related disorders.

Glands of the Endocrine System

Each gland of the endocrine system releases specific hormones into your bloodstream. These hormones travel through your blood to other cells and help control or coordinate many body processes.

Endocrine glands include:

- **Adrenal glands:** Two glands that sit on top of the kidneys that release the hormone cortisol.
- **Hypothalamus:** A part of the lower middle brain that tells the pituitary gland when to release hormones.
- **Ovaries:** The female reproductive organs that release eggs and produce sex hormones.
- **Islet cells in the pancreas:** Cells in the pancreas control the release of the hormones insulin and glucagon.
- **Parathyroid:** Four tiny glands in the neck that play a role in bone development.
- **Pineal gland:** A gland found near the center of the brain that may be linked to sleep patterns.
- **Pituitary gland:** A gland found at the base of brain behind the sinuses. It is often called the "master gland" because it influences many other glands, especially the thyroid. Problems with the pituitary gland can affect bone growth, a woman's menstrual cycles, and the release of breast milk.
- **Testes:** The male reproductive glands that produce sperm and sex hormones.
- **Thymus:** A gland in the upper chest that helps develop the body's immune system early in life.
- Thyroid**:** A butterfly-shaped gland in the front of the neck that controls metabolism.

Even the slightest hiccup with the function of one or more of these glands can throw off the delicate balance of hormones in your body and lead to an endocrine disorder, or endocrine disease.

Causes of Endocrine Disorders

Endocrine disorders are typically grouped into two categories:

- Endocrine disease that results when a gland produces too much or too little of an endocrine hormone, called a hormone imbalance.

- Endocrine disease due to the development of lesions (such as nodules or tumors) in the endocrine system, which may or may not affect hormone levels.

The endocrine's feedback system helps control the balance of hormones in the bloodstream. If your body has too much or too little of a certain hormone, the feedback system signals the proper gland or glands to correct the problem. A hormone imbalance may occur if this feedback system has trouble keeping the right level of hormones in the bloodstream, or if your body doesn't clear them out of the bloodstream properly.

Increased or decreased levels of endocrine hormone may be caused by:

- A problem with the endocrine feedback system
- Disease
- Failure of a gland to stimulate another gland to release hormones (for example, a problem with the hypothalamus can disrupt hormone production in the pituitary gland)
- A genetic disorder, such as multiple endocrine neoplasia (MEN) or congenital hypothyroidism
- Infection
- Injury to an endocrine gland
- Tumor of an endocrine gland

Most endocrine tumors and nodules (lumps) are noncancerous. They usually do not spread to other parts of the body. However, a tumor or nodule on the gland may interfere with the gland's hormone production.

Types of Endocrine Disorders

There are many different types of endocrine disorders. Diabetes is the most common endocrine disorder diagnosed are as follows:

- **Adrenal insufficiency.** The adrenal gland releases too little of the hormone cortisol and sometimes, aldosterone. Symptoms include fatigue, stomach upset, dehydration, and skin changes. Addison's disease is a type of adrenal insufficiency.

- **Cushing's disease.** Overproduction of a pituitary gland hormone leads to an overactive adrenal gland. A similar condition called Cushing's syndrome may occur in people, particularly children, who take high doses of corticosteroid medications.
- **Gigantism (**acromegaly**) and other growth hormone problems.** If the pituitary gland produces too much growth hormone, a child's bones and body parts may grow abnormally fast. If growth hormone levels are too low, a child can stop growing in height.
- **Hyperthyroidism.** The thyroid gland produces too much thyroid hormone, leading to weight loss, fast heart rate, sweating, and nervousness. The most common cause for an overactive thyroid is an autoimmune disorder called Grave's disease.
- Hypothyroidism**.** The thyroid gland does not produce enough thyroid hormone, leading to fatigue, constipation, dry skin, and depression. The underactive gland can cause slowed development in children. Some types of hypothyroidism are present at birth.
- **Hypopituitarism.** The pituitary gland releases little or no hormones. It may be caused by a number of different diseases. Women with this condition may stop getting their periods.
- **Multiple endocrine neoplasia I and II (MEN I and MEN II).** These rare, genetic conditions are passed down through families. They cause tumors of the parathyroid, adrenal, and thyroid glands, leading to overproduction of hormones.
- **Polycystic ovary syndrome (PCOS).** Overproduction of androgens interfere with the development of eggs and their release from the female ovaries. PCOS is a leading cause of infertility.
- Precocious puberty**.** Abnormally early puberty that occurs when glands tell the body to release sex hormones too soon in life.

Thyrotoxicosis: Thyrotoxicosis is the clinical state associated with excess thyroid hormone activity, usually due to inappropriately high-circulating thyroid hormones. The clinical presentation varies, ranging from asymptomatic to life-threatening thyroid storm. Symptoms are due to the hypermetabolic state induced by excess thyroid hormones and include weight loss, heat intolerance, and palpitations. There are many different causes of thyrotoxicosis. It is important to determine the cause since treatment is based on the underlying etiology. Thyrotoxicosis can lead to serious complications when not diagnosed and treated appropriately, including delirium, altered mental status, osteoporosis, muscle weakness, atrial fibrillation, congestive heart failure, thromboembolic disease, cardiovascular collapse, and death. This activity reviews the evaluation and management of thyrotoxicosis and highlights the role of interprofessional team members in collaborating to provide well-coordinated care and enhance outcomes for affected patients.

Thyrotoxicosis is a clinical state of inappropriately high levels of circulating thyroid hormones (T3 and/or T4) in the body from any cause. It is often incorrectly used interchangeably with hyperthyroidism, which is a form of thyrotoxicosis caused by excessive endogenous thyroid hormone production.

The clinical presentation varies, ranging from asymptomatic or subclinical, to life-threatening thyroid storm. Typical symptoms are due to the hypermetabolic state induced by excess thyroid

hormones and include weight loss, heat intolerance, and palpitations. The differential for thyrotoxicosis is broad and will need a combination of a thorough physical exam, laboratory studies, and imaging to determine the underlying etiology for appropriate treatment. If not adequately treated, thyrotoxicosis can lead to serious complications including delirium, altered mental status, osteoporosis, muscle weakness, atrial fibrillation, congestive heart failure (CHF), thromboembolic disease, cardiovascular collapse, and death.

Etiology

The etiology of thyrotoxicosis can be divided into an endogenous or exogenous source of TSH.

Increased endogenous secretion of thyroid hormone

- Grave's disease
- Toxic multinodular goiter
- Toxic adenoma
- TSH-producing adenoma or pituitary adenoma
- HCG-mediated hyperthyroidism
- Thyroiditis
- Drug-induced

Increased exogenous secretion of thyroid hormone

- Factitious hyperthyroidism
- Excessive replacement therapy with levothyroxine

The most common cause of thyrotoxicosis is Graves' disease, followed by toxic multinodular goiter (TMNG) and toxic adenoma (TA)[7]. Other causes include thyroiditis, subacute thyroiditis, painless thyroiditis, and gestational hyperthyroidism. Drug-induced thyrotoxicosis has been associated with amiodarone and iodinated contrast. Rare causes of thyrotoxicosis include TSH-producing adenomas, struma ovarii, gestational trophoblastic neoplasia, thyrotoxicosis factitia, activation mutations of the TSH receptor, and functional thyroid cancer metastases.

Epidemiology:

The prevalence of thyrotoxicosis in the United States is 1.2%, including 0.5% overt thyrotoxicosis and 0.7% subclinical. The incidence of thyrotoxicosis peaks between ages 20 and 50 years. Graves' disease is the most common cause with an incidence of 20 to 50 cases per 100,000 persons followed by toxic multinodular goiter and toxic adenoma. Graves' disease most commonly affects women aged 30 to 50 with a male to female ratio of 5 to 1 but can occur at any age in both genders. Toxic nodular goiter increases with age and in iodine-deficient regions. Thyroiditis accounts for 10% of cases. One percent to 2% of patients with thyrotoxicosis go on to develop the serious complication of thyroid storm.

Pathophysiology:

Thyrotoxicosis results from thyroid hormone excess either from endogenous over-secretion of T3 and T4 or from exogenous ingestion of synthetic thyroid hormone. Thyroid hormone affects almost every tissue and organ system in the body by increasing basal metabolic rate and tissue

thermogenesis by upregulating alpha-adrenergic receptors leading to an increase in sympathetic activity. Thyroid hormone causes increased expression of myocardial sarcoplasmic reticulum calcium-dependent ATP, increasing heart rate and myocardial contractility with the net effect of increased cardiac output. Decreased systemic vascular resistance (SVR) and decreased afterload results from arterial smooth muscle relaxation by metabolic end products, such as lactic acid, produced with increased consumption of oxygen. Decreased SVR leads to activation of the renin-angiotensin system, increasing reabsorption of sodium and expanding blood volume to increase preload. If left untreated, this may lead to left ventricular hypertrophy and congestive heart failure.

Graves' disease is an autoimmune disease comprised of antibodies that stimulate TSH receptors to cause excess secretion of thyroid hormones via a type II hypersensitivity reaction. This results in hyperplasia of thyroid follicular cells causing a diffuse goiter. The cause of Graves' disease is not known, but genetic and environmental factors, such as smoking, stress, and dietary iodine play a role. The thyroid-stimulating immunoglobulin (TSI) triggers the hyperthyroidism.

In toxic multinodular goiter and toxic adenoma, autonomously functioning nodules over-secrete thyroid hormone independently without stimulation from TSH. Rarely, these Nnontoxic adenomas or goiter can convert to toxic adenomas after exposure to iodinated contrast, such as from a cardiac catheterization or undergoing a CT study with contrast.

In thyroiditis, thyrotoxicosis is caused by the release of preformed thyroid hormone into the circulation as inflammation destroys thyroid follicles. This causes transient thyrotoxicosis that most often self-resolves. Inflammation can be precipitated by a variety of insults to the thyroid gland, including autoimmune, infectious, chemical, or mechanical insults.

Gestational hyperthyroidism generally occurs in the first trimester of pregnancy, due to increased stimulation of the thyroid gland by excess human chorionic gonadotropin (HCG), which is similar in structure to TSH and binds the TSH receptor.

Histopathology:

Toxic multinodular goiter and toxic adenoma are follicular adenomas, which are usually a non-malignant proliferation of follicles encased in a fibrous capsule. It is usually a benign, nonfunctional adenoma that usually does not secrete thyroid hormone. [2]

Symptoms :

Patients with thyrotoxicosis most commonly present with signs and symptoms related to excess thyroid hormone including: weight loss with a normal or increased appetite, heat intolerance with increased sweating, palpitations, tremor, anxiety, proximal muscle weakness, alopecia and increased fatigability. Sinus tachycardia is the most common cardiac rhythm problem, but atrial fibrillation may occur and is frequently seen with advanced patient age, valvular disease, and coronary artery disease. Women may present with amenorrhea or oligomenorrhea. Men may present with gynecomastia rarely. It is common for older patients to manifest fewer of the typical clinical manifestations and instead present with depression, fatigue, and weight loss, also known as apathetic thyrotoxicosis.

On physical exam, patients are often cachectic, hyperthermic, diaphoretic, and anxious appearing. They may have goiter, tachycardia or atrial fibrillation, dyspnea, abdominal tenderness, hyperreflexia, proximal muscle weakness, tremor, and gynecomastia. Patients with Graves' disease present with pretibial myxoedema, thyroid acropachy, and onycholysis. In rare cases, patients present in thyroid storm with tachycardia, fever, altered mental status, agitation, features of cardiac failure, and impaired liver function.

Findings specific to Graves' disease include ophthalmopathy resulting in proptosis, chemosis, conjunctival injection and lid lag, exposure keratitis and extra-ocular muscle dysfunction, pretibial myxedema and thyroid acropachy (clubbing). In subacute thyroiditis, patients have a history of recent upper respiratory illness and generally present with fever, neck pain and swelling with a firm and tender thyroid gland. Painless thyroiditis often presents in the postpartum period, and patients frequently have a personal or family history of autoimmune or thyroid disease. Suppurative thyroiditis presents with a tender, erythematous mass in the anterior neck and patients often complain of fever, dysphagia, and dysphonia. Occasionally, patients may present with acute muscle paralysis and severe hypokalemia, termed thyrotoxic periodic paralysis.

Evaluation:

Low serum TSH (less than 0.01 mU/L) has high sensitivity and specificity for the diagnosis of thyroid disorders. If TSH is low, elevated serum free thyroxine (T4) and triiodothyronine (T3) levels can distinguish between overt and subclinical hyperthyroidism. Usually, the increase in T3 precedes the increase in T4. Pituitary-dependent causes of hyperthyroidism may have normal or increased TSH levels and increased T4 and T3 levels with an increase in free-alpha subunit concentrations. Serum levels of antibodies to the TSH receptor diagnose Graves' disease. Levels are 98% sensitive and 99% specific. Thyroid peroxidase antibodies are only present in about 75% of cases of Graves' disease.

Radioactive iodine uptake studies or thyroid scans may be used to distinguish between causes of thyrotoxicosis besides Graves' disease. It is recommended in all thyrotoxic patients without the clinical picture of Graves' disease. In Graves' disease, radioactive iodine uptake is diffuse, unless the patient also has nodules or fibrosis. In single toxic adenoma, there will be a focal uptake in the adenoma, with suppressed uptake in the surrounding thyroid tissue. Toxic multinodular goiter will show multiple areas of focal increased uptake and suppressed uptake in surrounding tissue. Radioactive iodine uptake will be near zero in patients with painless, postpartum, or subacute thyroiditis, as well as patients with ingestion of thyroid hormone or recent excess iodine exposure.

In subacute thyroiditis, inflammatory markers such as erythrocyte sedimentation rate and C-reactive protein are frequently increased. In pregnancy, either free T3 and T4, or the total T3 and T4 with adjusted reference range 1.5 times the nonpregnant range should be used for diagnosis in addition to serum TSH levels. During the first half of pregnancy, the serum TSH levels may be lower than the nonpregnant reference range, but free T4 values should be normal. In suspected factitious hyperthyroidism, thyroglobulin levels are decreased and radioactive uptake studies due to the suppression by exogenous thyroid hormone ingestion.

Treatment / Management:

The recommended treatment of thyrotoxicosis is dependent on the underlying cause. Beta-blocker therapy such as propranolol, is used to reduce adrenergic features such as sweating, anxiety, and tachycardia. There are 3 mainstays of treatment: thionamide drugs, radioiodine, and thyroid surgery.

Thionamide drugs include propylthiouracil (PTU) and methimazole and reduce the production of thyroid hormone by acting as preferential substrates for thyroid peroxidase. In high doses, PTU also decreases peripheral conversion of T4 to T3.

In the treatment of Graves' disease, methimazole is used at a dose of 15 mg to 30 mg per day for 4 to 8 weeks, after which most patients become euthyroid. After patients become euthyroid, there are 2 approaches to treatment. First, in the block-replace method, the same dose of thionamide is continued to block thyroid hormone production, and levothyroxine is added to maintain euthyroidism. Alternatively, the thionamide dose may be titrated progressively to allow endogenous synthesis of thyroid hormone, maintaining a euthyroid state.

In Graves' disease, long-term remission is achieved in about 50% of patients with thionamide drugs. A disadvantage of thionamides is the uncertainty of relapse after treatment is stopped. No advantage has been shown in remission rates with prolonged treatment beyond 18 months. Methimazole has been shown to have better efficacy and has a longer half-life, which allows once-daily dosing. Additionally, there is a higher risk of hepatotoxicity with PTU. Agranulocytosis occurs in 1 in 300 patients treated with thionamides and presents as a sore throat, mouth ulcers and high fever. It is recommended to obtain a differential white blood cell count during febrile illness and pharyngitis in all patients on thionamides. Minor side effects include pruritis, arthralgia, and gastrointestinal upset.

Radioiodine therapy is the most common therapy used for adults with Graves' disease in the United States. It can also be used for toxic nodules and TMNGs. Radioactive iodine is given in one oral dose. It is absorbed by the thyroid gland inducing tissue-specific inflammation that leads to thyroid fibrosis and destruction of thyroid tissue over the next several months. Hypothyroidism usually occurs within 6 to 12 months. Most patients go on to require lifelong levothyroxine treatment. There is a small risk of thyrotoxicosis exacerbation in the month after treatment due to the release of the preformed hormone. Patients with large goiters, severe thyrotoxicosis, ischemic heart disease, heart failure or arrhythmia are recommended to use thionamide pretreatment until they are euthyroid before radioiodine therapy. Radioiodine therapy is contraindicated in pregnancy and lactation and relatively contraindicated in active inflammatory Graves' ophthalmopathy.

Total or partial thyroidectomy is a rapid and effective method of treating thyrotoxicosis. However, it is invasive and expensive, and causes permanent hypothyroidism, requiring levothyroxine treatment. It is recommended that patients be pretreated for euthyroidism before surgery to reduce the risk of worsening thyrotoxicosis and thyroid storm. Complications include hypocalcemia due to hypoparathyroidism, which is usually transient, and vocal cord paresis due to damage to the recurrent laryngeal nerve.

The treatment for thyroiditis differs in that antithyroid drugs are ineffective, since patients usually have low production of new thyroid hormone. It is usually transient, but treatment is aimed at symptom control with beta blockers. In subacute thyroiditis, non-steroidal anti-inflammatory drugs and occasionally systemic glucocorticoids may be used to help with pain and inflammation. Beta blockers are recommended for any elderly patients with symptomatic thyrotoxicosis, and any thyrotoxic patients with resting a heart rate greater than 90 bpm or cardiovascular disease.

Children with thyrotoxicosis may be treated with methimazole, radioiodine therapy or thyroidectomy. Methimazole therapy for 1 to 2 years is the first line therapy for Graves' disease in children since some children will go into remission. Radioiodine therapy is not recommended for children younger than 5 years old. PTU should also be avoided in children due to the risk of hepatotoxicity.

During pregnancy, it is recommended to treat with thionamide drugs in a titrated dose regimen. Block-replace regimens increase the risk of fetal hypothyroidism and goiter. PTU is recommended during the first trimester of pregnancy. PTU is preferred during the first trimester of pregnancy due to the risk of teratogenicity associated with methimazole, including aplasia cutis and choanal or esophageal atresia.

Hypothyroidism:

Hypothyroidism happens when the thyroid gland doesn't make enough thyroid hormone. This condition also is called underactive thyroid. Hypothyroidism may not cause noticeable symptoms in its early stages. Over time, hypothyroidism that isn't treated can lead to other health problems, such as high cholesterol and heart problems. Blood tests are used to diagnose hypothyroidism. Treatment with thyroid hormone medicine usually is simple, safe and effective once you and your health care provider find the right dosage for you.

Symptoms

The symptoms of hypothyroidism depend on the severity of the condition. Problems tend to develop slowly, often over several years. At first, you may barely notice the symptoms of hypothyroidism, such as fatigue and weight gain. Or you may think they are just part of getting older. But as your metabolism continues to slow, you may develop more-obvious problems.

How does thyroid work?

The thyroid gland is a small, butterfly-shaped organ located in the front of your neck just under the voice box (larynx). Picture the middle of the butterfly's body centered on your neck, with the wings hugging around your windpipe (trachea). The main job of the thyroid is to control your metabolism. Metabolism is the process that your body uses to transform food to energy your body uses to function. The thyroid creates the hormones T4 and T3 to control your metabolism. These hormones work throughout the body to tell the body's cells how much energy to use. They control your body temperature and heart rate.

When your thyroid works correctly, it's constantly making hormones, releasing them and then making new hormones to replace what's been used. This keeps your metabolism functioning and all of your body's systems in check. The amount of thyroid hormones in the bloodstream is controlled by the pituitary gland, which is located in the center of the skull below the brain. When the pituitary gland senses either a lack of thyroid hormone or too much, it adjusts its own hormone (thyroid stimulating hormone, or TSH) and sends it to the thyroid to balance out the amounts. If the amount of thyroid hormones is too high (hyperthyroidism) or too low (hypothyroidism), the entire body is impacted.

Who is affected by hypothyroidism?

Hypothyroidism can affect people of all ages, genders and ethnicities. It's a common condition, particularly among women over age 60. Women are generally more likely to develop hypothyroidism after menopause than earlier in life.

Difference between hypothyroidism and hyperthyroidism?

In hypothyroidism, the thyroid doesn't make enough thyroid hormone. The difference between hypothyroidism and hyperthyroidism is quantity. In hypothyroidism, the thyroid makes very little thyroid hormone. On the flip side, someone with hyperthyroidism has a thyroid that makes too much thyroid hormone. Hyperthyroidism involves higher levels of thyroid hormones, which makes your metabolism speed up. If you have hypothyroidism, your metabolism slows down.

Many things are the opposite between these two conditions. If you have hypothyroidism, you may have a difficult time dealing with the cold. If you have hyperthyroidism, you may not handle the heat. They are opposite extremes of thyroid function. Ideally, you should be in the middle. Treatments for both of these conditions work to get your thyroid function as close to that middle ground as possible.

SYMPTOMS AND CAUSES OF HYPOTHYROIDISM:

Hypothyroidism symptoms may include:

- Tiredness.
- More sensitivity to cold.
- Constipation.
- Dry skin.
- Weight gain.
- Puffy face.
- Hoarse voice.
- Coarse hair and skin.

- Muscle weakness.
- Muscle aches, tenderness and stiffness.
- Menstrual cycles that are heavier than usual or irregular.
- Thinning hair.
- Slowed heart rate, also called bradycardia.
- Depression.
- Memory problems

Hypothyroidism in infants

Anyone can get hypothyroidism, including infants. Most babies born without a thyroid gland or with a gland that doesn't work correctly don't have symptoms right away. But if hypothyroidism isn't diagnosed and treated, symptoms start to appear. They may include:

- Feeding problems.
- Poor growth.
- Poor weight gain.
- Yellowing of the skin and the whites of the eyes, a condition called jaundice.
- Constipation.
- Poor muscle tone.
- Dry skin.
- Hoarse crying.
- Enlarged tongue.
- A soft swelling or bulge near the belly button, a condition called umbilical hernia.

When hypothyroidism in infants isn't treated, even mild cases can lead to severe physical and mental development problems.

Hypothyroidism in children and teens

In general, children and teens with hypothyroidism have symptoms similar to those in adults. But they also may have:

- Poor growth that leads to short stature.

- Delayed development of permanent teeth.
- Delayed puberty.
- Poor mental development.

Causes:

The thyroid is a small, butterfly-shaped gland located at the base of the neck, just below the Adam's apple. The thyroid gland makes two main hormones: thyroxine (T-4) and triiodothyronine (T-3). These hormones affect every cell in the body. They support the rate at which the body uses fats and carbohydrates. They help control body temperature. They have an effect on heart rate. And they help control how much protein the body makes.

Hypothyroidism happens when the thyroid gland doesn't make enough hormones. Conditions or problems that can lead to hypothyroidism include:

- **Autoimmune disease.** The most common cause of hypothyroidism is an autoimmune disease called Hashimoto's disease. Autoimmune diseases happen when the immune system makes antibodies that attack healthy tissues. Sometimes that process involves the thyroid gland and affects its ability to make hormones.
- **Thyroid surgery.** Surgery to remove all or part of the thyroid gland can lower the gland's ability to make thyroid hormones or stop it completely.
- **Radiation therapy.** Radiation used to treat cancers of the head and neck can affect the thyroid gland and lead to hypothyroidism.
- **Thyroiditis.** Thyroiditis happens when the thyroid gland becomes inflamed. This may be due to an infection. Or it can result from an autoimmune disorder or another medical condition affecting the thyroid. Thyroiditis can trigger the thyroid to release all of its stored thyroid hormone at once. That causes a spike in thyroid activity, a condition called hyperthyroidism. Afterward, the thyroid becomes underactive.
- **Medicine.** A number of medicines may lead to hypothyroidism. One such medicine is lithium, which is used to treat some psychiatric disorders. If you're taking medicine, ask your heath care provider about its effect on the thyroid gland.

Less often, hypothyroidism may be caused by:

- **Problems present at birth.** Some babies are born with a thyroid gland that doesn't work correctly. Others are born with no thyroid gland. In most cases, the reason the thyroid gland didn't develop properly is not clear. But some children have an inherited form of a thyroid disorder. Often, infants born with hypothyroidism don't have noticeable symptoms at first. That's one reason why most states require newborn thyroid screening.

- **Pituitary disorder.** A relatively rare cause of hypothyroidism is the failure of the pituitary gland to make enough thyroid-stimulating hormone (TSH). This is usually because of a noncancerous tumor of the pituitary gland.
- **Pregnancy.** Some people develop hypothyroidism during or after pregnancy. If hypothyroidism happens during pregnancy and isn't treated, it raises the risk of pregnancy loss, premature delivery and preeclampsia. Preeclampsia causes a significant rise in blood pressure during the last three months of pregnancy. Hypothyroidism also can seriously affect the developing fetus.
- **Not enough iodine.** The thyroid gland needs the mineral iodine to make thyroid hormones. Iodine is found mainly in seafood, seaweed, plants grown in iodine-rich soil and iodized salt. Too little iodine can lead to hypothyroidism. Too much iodine can make hypothyroidism worse in people who already have the condition. In some parts of the world, it's common for people not to get enough iodine in their diets. The addition of iodine to table salt has almost eliminated this problem in the United States.

Risk factors

Although anyone can develop hypothyroidism, you're at an increased risk if you:

- Are a woman.
- Have a family history of thyroid disease.
- Have an autoimmune disease, such as type 1 diabetes or celiac disease.
- Have received treatment for hyperthyroidism.
- Received radiation to your neck or upper chest.
- Have had thyroid surgery.

Complications:

Hypothyroidism that isn't treated can lead to other health problems, including:

- **Goiter.** Hypothyroidism may cause the thyroid gland to become larger. This condition is called a goiter. A large goiter may cause problems with swallowing or breathing.
- **Heart problems.** Hypothyroidism can lead to a higher risk of heart disease and heart failure. That's mainly because people with an underactive thyroid tend to develop high levels of low-density lipoprotein (LDL) cholesterol — the "bad" cholesterol.

- **Peripheral neuropathy.** Hypothyroidism that goes without treatment for a long time can damage the peripheral nerves. These are the nerves that carry information from the brain and spinal cord to the rest of the body. Peripheral neuropathy may cause pain, numbness and tingling in the arms and legs.
- **Infertility.** Low levels of thyroid hormone can interfere with ovulation, which can limit fertility. Some of the causes of hypothyroidism, such as autoimmune disorders, also can harm fertility.
- **Birth defects.** Babies born to people with untreated thyroid disease may have a higher risk of birth defects compared with babies born to mothers who do not have thyroid disease.

  Infants with hypothyroidism present at birth that goes untreated are at risk of serious physical and mental development problems. But if the condition is diagnosed within the first few months of life, the chances of typical development are excellent.

- **Myxedema coma.** This rare, life-threatening condition can happen when hypothyroidism goes without treatment for a long time. A myxedema coma may be triggered by sedatives, infection or other stress on the body. Its symptoms include intense cold intolerance and drowsiness, followed by an extreme lack of energy and then unconsciousness. Myxedema coma requires emergency medical treatment.

Treatment of hypothyroidism:

In most cases, hypothyroidism is treated by replacing the amount of hormone that your thyroid is no longer making. This is typically done with a medication. One medication that is commonly used is called levothyroxine. Taken orally, this medication increases the amount of thyroid hormone your body produces, evening out your levels.

Hypothyroidism is a manageable disease. However, you will need to continuously take medication to normalize the amount of hormones in your body for the rest of your life. With careful management, and follow-up appointments with your healthcare provider to make sure your treatment is working properly, you can lead a normal and healthy life.

What happens if hypothyroidism is not treated?

Hypothyroidism can become a serious and life-threatening medical condition if you do not get treatment from a healthcare provider. If you are not treated, your symptoms can become more severe and can include:

- Developing mental health problems.
- Having trouble breathing.
- Not being able to maintain a normal body temperature.
- Having heart problems.
- Developing a goiter (enlargement of the thyroid gland).

You can also develop a serious medical condition called myxedema coma. This can happen when hypothyroidism isn't treated.

Prevention of hypothyroidism:

Hypothyroidism cannot be prevented. The best way to prevent developing a serious form of the condition or having the symptoms impact your life in a serious way is to watch for signs of hypothyroidism. If you experience any of the symptoms of hypothyroidism, the best thing to do is talk to your healthcare provider. Hypothyroidism is very manageable if you catch it early and begin treatment.

Foods related to hypothyroidism?

Most foods in western diets contain iodine, so you do not have to worry about your diet. Iodine is a mineral that helps your thyroid produce hormones. One idea is that if you have low levels of thyroid hormone, eating foods rich in iodine could help increase your hormone levels. The most reliable way to increase your hormone levels is with a prescription medication from your healthcare provider. Do not try any new diets without talking to your provider first. It's important to always have a conversation before starting a new diet, especially if you have a medical condition like hypothyroidism.

Foods that are high in iodine include:

- Eggs.
- Dairy products.
- Meat, poultry and seafood.
- Edible seaweed.
- Iodized salt.

Work with your healthcare provider or a nutritionist (a healthcare provider who specializes in food) to craft a meal plan. Your food is your fuel. Making sure you are eating foods that will help your body, along with taking your medications as instructed by your healthcare provider, can keep you healthy over time. People with thyroid condition should not consume large amounts of iodine because the effect may be paradoxical (self-contradictory).

Can hypothyroidism go away on its own?

In some mild cases, you may not have symptoms of hypothyroidism or the symptoms may fade over time. In other cases, the symptoms of hypothyroidism will go away shortly after you start treatment. For those with particularly low levels of thyroid hormones, hypothyroidism is a life-long condition that will need to be managed with medication on a regular schedule.

Hashimoto's Disease:

Hashimoto's disease is an autoimmune disorder that can cause hypothyroidism, or underactive thyroid. Rarely, the disease can cause hyperthyroidism, or overactive thyroid.

The thyroid is a small, butterfly-shaped gland in the front of your neck. In people with Hashimoto's disease

- the immune system makes antibodies that attack the thyroid gland
- large numbers of white blood cells, which are part of the immune system, build up in the thyroid
- the thyroid becomes damaged and can't make enough thyroid hormones

Thyroid hormones control how your body uses energy, so they affect nearly every organ in your body—even the way your heart beats. Hashimoto's disease is also called Hashimoto's thyroiditis, chronic lymphocytic thyroiditis, or autoimmune thyroiditis.

How common is Hashimoto's disease?

The number of people who have Hashimoto's disease in the United States is unknown. However, the disease is the most common cause of hypothyroidism, which affects about 5 in 100 Americans.

Who is more likely to have Hashimoto's disease?

Hashimoto's disease is 4 to 10 times more common in women than men. Although the disease may occur in teens or young women, it more often develops in women ages 30 to 50. Your chance of developing Hashimoto's disease increases if other family members have the disease.

Persons are more likely to develop Hashimoto's disease if they have other autoimmune disorders, including

- celiac disease, a digestive disorder that damages the small intestine
- lupus , a chronic, or long-term, disorder that can affect many parts of the body
- rheumatoid arthritis , a disorder that affects the joints
- Sjögren's syndrome , a disease that causes dry eyes and mouth
- type 1 diabetes, a disease that occurs when your blood glucose, also called blood sugar, is too high.

What are the complications of Hashimoto's disease?

Many people with Hashimoto's disease develop hypothyroidism. Untreated, hypothyroidism can lead to several health problems, including

- high cholesterol
- heart disease and heart failure *NIH external link*
- high blood pressure
- myxedema *NIH external link*, a rare condition in which the body's functions slow down to the point that it can threaten your life

What are the symptoms of Hashimoto's disease?

Many people with Hashimoto's disease have no symptoms at first. As the disease progresses, you may have one or more of the symptoms of hypothyroidism.

Some common symptoms of hypothyroidism include

- fatigue
- weight gain
- trouble tolerating cold
- joint and muscle pain
- constipation
- dry skin or dry, thinning hair
- heavy or irregular menstrual periods or fertility problems
- slowed heart rate

Hashimoto's disease causes your thyroid to become damaged. Most people with Hashimoto's disease develop hypothyroidism. Rarely, early in the course of the disease, thyroid damage may lead to the release of too much thyroid hormone into your blood, causing symptoms of hyperthyroidism. Thyroid may get larger and cause the front of the neck to look swollen. The enlarged thyroid, called a goiter, may create a feeling of fullness in your throat, though it is usually not painful. After many years, or even decades, damage to the thyroid may cause the gland to shrink and the goiter to disappear.

Causes of Hashimoto's disease:

Researchers don't know why some people develop Hashimoto's disease, but a family history of thyroid disease is common. Several factors may play a role, including[2]

- genes
- viruses, such as hepatitis C

Hypothyroidism can also be caused by

- some medicines used to treat bipolar disorder or other mental health problems
- iodine-containing medicines used to treat abnormal heart rhythm
- exposure to toxins, such as nuclear radiation

Diagnosis of Hashimoto's disease:

- **medical history and physical exam**. Your doctor will start by taking a medical history and performing a physical exam. In addition to asking about symptoms, the doctor will check your neck for a goiter, which some people with Hashimoto's disease can develop.
- **blood tests**. Your doctor will order one or more blood tests to check for hypothyroidism and its causes. Examples include tests for
  - the thyroid hormones T4 (thyroxine) and T3 (triiodothyronine)
  - thyroid-stimulating hormone, or TSH
  - thyroid peroxidase antibodies (TPO), a type of thyroid antibody that is present in most people with Hashimoto's disease

You probably won't need other tests to confirm you have Hashimoto's disease. However, if your doctor suspects Hashimoto's disease but you don't have antithyroid antibodies in your blood, you may have an ultrasound *NIH external link* of your thyroid. The ultrasound images can show the size of your thyroid and other features of Hashimoto's disease. The ultrasound also can rule out other causes of an enlarged thyroid, such as thyroid nodules—small lumps in the thyroid gland.

Treatment of Hashimoto's disease:

How doctors treat Hashimoto's disease usually depends on whether the thyroid is damaged enough to cause hypothyroidism. If you don't have hypothyroidism, your doctor may choose to simply check your symptoms and thyroid hormone levels regularly.

The medicine levothyroxine which is identical to the natural thyroid hormone thyroxine (T4), is the recommended way to treat hypothyroidism. Prescribed in pill form for many years, this medicine is now also available as a liquid and in a soft gel capsule.[2] These newer formulas may be helpful to people with digestive problems that affect how the thyroid hormone pill is absorbed.

Some foods and supplements can affect how well your body absorbs levothyroxine. Examples include grapefruit juice, espresso coffee, soy, and multivitamins that contain iron or calcium. Taking the medicine on an empty stomach can prevent this from happening. Your doctor may ask you to take the levothyroxine in the morning, 30 to 60 minutes before you eat your first meal.

Your doctor will give you a blood test about 6 to 8 weeks after you begin taking the medicine and adjust your dose if needed. Each time you change your dose, you'll have another blood test. Once you've reached a dose that's working for you, your doctor will likely repeat the blood test in 6

months and then once a year. Never stop taking your medicine or take a higher dose without talking with your doctor first. Taking too much thyroid hormone medicine can cause serious problems, such as atrial fibrillation or osteoporosis. Hypothyroidism can be well-controlled with thyroid hormone medicine, as long as you take the medicine as instructed by your doctor and have regular follow-up blood tests.

How does eating, diet, and nutrition affect Hashimoto's disease?

The thyroid uses iodine, a mineral in some foods, to make thyroid hormones. However, if you have Hashimoto's disease or other types of autoimmune thyroid disorders, you may be sensitive to harmful side effects from io iodine. Eating foods that have large amounts of iodine—such as kelp, dulse, or other kinds of seaweed, and certain iodine-rich medicines—may cause hypothyroidism or make it worse. Taking iodine supplements can have the same effect.

Talk with members of your health care team about

- what foods and beverages to limit or avoid
- whether you take iodine supplements
- any cough syrups you take that may contain iodine

However, if you are pregnant, you need to take enough iodine because the baby gets iodine from your diet. Too much iodine can cause problems as well, such as a goiter in the baby. If you are pregnant, talk with your doctor about how much iodine you need.

Researchers are looking at other ways in which diet and supplements—such as vitamin D and selenium may affect Hashimoto's disease. However, no specific guidance is currently available.

Probable Questions:

1. What are the causes of endocrine disorders?
2. Discuss different types of endocrine disorders?
3. Discuss symptoms of thyrotoxicosis.
4. How thyrotoxicosis is treated?
5. What are the symptoms of hypothyroidism?
6. State difference between hypothyroidism and hyperthyroidism.
7. Discuss symptoms and causes of hypothyroidism.
8. Discuss complications of hypothyroidism.
9. How hypothyroidism is treated?
10. Discuss food related to hypothyroidism.
11. Who are more prone to Hashimoto's disease?
12. State the complications of Hashimoto's disease.
13. What are the symptoms of Hashimoto's disease?
14. How Hashimoto's disease is detected and treated?

Suggested readings:

1. General Endocrinology. Turner and Bagnara. Sixth Edition.
2. Williams Textbook of Endocrinology. Tenth Edition.
3. Introduction to Endocrinology. Chandra S Negi. Second Edition
4. Endocrinology. Hadley and Levine. Sixth Edition.

UNIT-XVIII

Molecular basis of endocrinopathies: Addison's diseases, Cushing syndrome, androgen deficiency syndromes-testicular neoplasm

Objective: In this unit we will discuss different kinds of endocrinopathies such as Cushing syndrome, Addison's diseases, androgen deficiency syndromes-testicular neoplasm.

Addison's disease:

Thomas Addison (1855) first described this disease. It occurs due to hypo secretion of cortical glucocorticoids. It may be due to bilateral tubercular destruction or due to tubercular infection or due to autoimmune diseases. The clinical features are as follows:

Anatomical features:

1. There is increased melanin pigment synthesis in skin and shows bronze in colour.

2. Diseased person shows muscular weakness.

3. Oedema occurs due to loss of water from the capillaries.

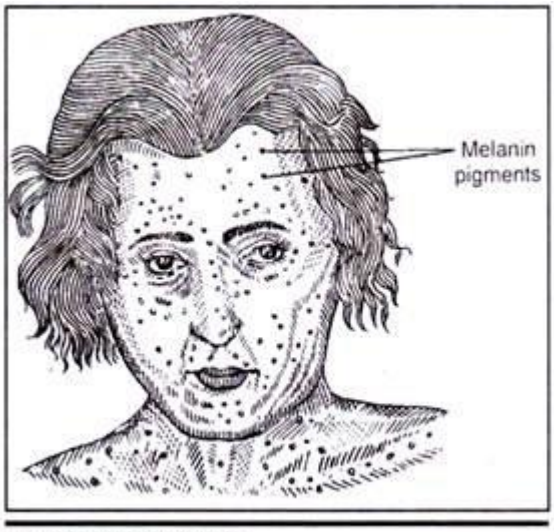


Fig. 12.6: Addison's disease

Physiological features:

1. Gastro-intestinal disturbances and vomiting occurs.

2. There will be fall of blood pressure.

3. Blood pressure becomes lowered.

4. Metabolic rate is reduced.

5. Subnormal body temperature (below 96°C).

6. Depressed glycogenesis and gluconeogenesis.

7. NaCI is excreted in greater quantity.

8. Potassium is retained.

9. Disturbances is ionic balance.

Psychological features:

1. Ionic disturbances may cause lack of mental concentration.

2. Patient shows restlessness.

3. Shows insomnia.

Sexological features:

1. Sexual activities are reduced.

2. Both primary and secondary sex characters are ill developed.

Cushing's syndrome: H. Cushing (1932) first described this disease; Excess glucocorticoids secretion takes place due to adrenocortical tumours. Pituitary tumour may cause over secretion of ACTH which in-turn causes hyperplasia of cortex.

The clinical features are as follows:

Anatomical features :

1. Excessive body hair growth (hirsutism)

2. Osteoporosis occurs due to decalcification of bones.

3. Face becomes round due to accumulation of fat.

4. Presence of buffalo hump on the back of neck.

5. Skin shows purple striate over the abdomen.

6. Obesity takes place.

7. Slow wound healing

8. Wasting of muscles occurs in the limbs.

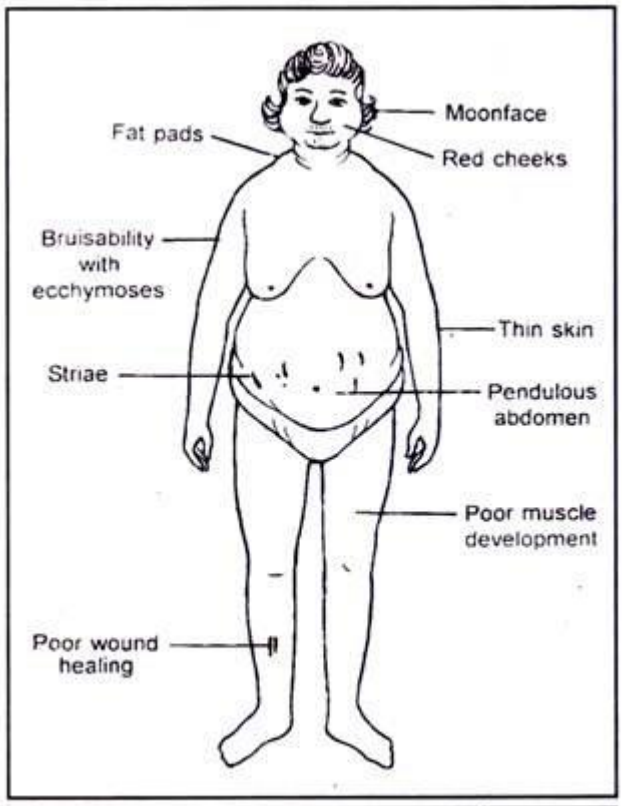


Fig. 12.7: Outstanding signs of Cushing's syndrome

Physiological features:

1. Blood sodium level becomes increased but potassium level decreases.

2. Polyuria and polydipsia occur.

3. Hyperglycemia occurs.

4. Loss of protein takes place from bony matrix.

5. Lipogenic effect occurs.

Psychological features:

1. Patients are mentally deranged.

2. Hypertension takes place.

Sexological features:

1. Female shows masculinization with the development of male secondary sex characters, like growth of beard, mustaches, under developed mammary glands etc.

2. Males suffer from impotence and atrophy of testes.

Androgen Deficiency Syndrome:

Androgen deficiency is when the body has lower levels of male sex hormones, particularly testosterone, than is needed for good health. This deficiency may be caused by problems in the areas of the brain that control the function of the testes (the pituitary gland and the hypothalamus), or by problems in the testes themselves. Treatment involves testosterone replacement therapy.

The term 'male menopause' is meaningless as it doesn't exist: there is no sudden, severe or inevitable drop in sex hormone production in men as experienced by women.

A modest and gradual drop in sex hormone levels is seen across male populations from the age of about 30 but this fall is not seen in all men. In most cases the drop in testosterone appears to be caused by them developing other illnesses along the way.

Androgens are sex hormones

Hormones can be thought of as chemical messengers. They communicate with tissues in the body to bring about many different changes. Hormones are needed for different processes like growth, reproduction and well-being.

Androgens are the group of sex hormones that give men their 'male' characteristics (collectively called virilisation). The major sex hormone in men is testosterone, which is produced mainly in the testes. The testes are controlled by a small gland in the brain called the pituitary gland, which in turn is controlled by an area of the brain called the hypothalamus.

Androgens are crucial for male sexual and reproductive function. They are also responsible for the development of secondary sexual characteristics in men, including facial and body hair growth and voice change. Androgens also affect bone and muscle development and metabolism.

The term androgen deficiency means your body is not making enough androgens, particularly testosterone, for full health. The effects of this depend on how severe the deficiency is, its cause and the age at which the deficiency begins.

Testosterone

The major sex hormone in men is testosterone. Some of the functions of testosterone in the male body include:

- starting and completing the process of puberty
- bone and muscle development
- growth of body hair, including facial hair
- change of vocal cords to produce the adult male voice
- sex drive (libido) and sexual function

- prostate gland growth and function
- sperm production.

Symptoms of androgen deficiency

When there is not enough testosterone circulating in the body, it can cause a wide range of symptoms. However, a number of these symptoms may be non-specific and can mimic the symptoms of other diseases and conditions.

Some of the symptoms of androgen deficiency include:

- reduced sexual desire
- hot flushes and sweating
- breast development (gynaecomastia)
- lethargy and fatigue
- depression
- reduced muscle mass and strength
- increased body fat, particularly around the abdomen
- weaker erections and orgasms
- reduced amount of ejaculate
- loss of body hair
- reduced bone mass, therefore increased risk of osteoporosis.

Androgen deficiency in older men

If testosterone levels decline with age, a number of factors may be causing it. In particular, any cause of poor general health, including obesity, will lower testosterone. Recent research shows that testosterone levels do not drop significantly in healthy older men.

The impact of the fall in testosterone levels in older men is still not completely understood. There has been much media coverage of 'andropause' or 'male menopause', suggesting that many older men would benefit from testosterone treatment (testosterone replacement therapy). However, there is limited evidence to suggest benefit, and the risks are not clear.

A recent **study on the effects of testosterone treatment in older men** showed a small increase in sexual function with testosterone treatment (in some cases for less than 12 months), but no significant improvement in mood, vitality or physical function.

Do not start any testosterone treatment without careful diagnosis of androgen deficiency. Make sure you have a full health assessment, and that your testosterone levels have clearly been shown to be consistently low. Often, there are other health problems at play (such as obesity and diabetes) that should be treated first, which may make testosterone replacement therapy unnecessary.

The effect of lower testosterone levels with increasing age and the effects of testosterone replacement therapy in men are currently being studied. Of concern are some studies suggesting a rise in cardiovascular disease after starting testosterone therapy in older men, but this remains controversial.

Androgen deficiency in boys

Boys who have not completed puberty should only be treated by paediatric hormone specialists (paediatric endocrinologists).

Causes of androgen deficiency

Some of the causes of androgen deficiency include conditions affecting the:

- **testes** – medical problems that affect the testes can stop them from making enough testosterone. Some of these conditions are present from birth (for example, Klinefelter's syndrome – a genetic disorder where there is an extra sex chromosome in the body's cells). Other conditions may occur at various stages of a boy's or a man's life, such as:
    - undescended testes
    - loss of testes due to trauma or 'twisting off' of the blood supply (torsion)
    - complications following mumps
    - side effects of chemotherapy or radiotherapy
- **pituitary gland** – the most common condition that affects the pituitary gland and leads to low testosterone levels is the presence of a benign tumour (adenoma). The tumour may interfere with the function of the pituitary gland, or it may produce the hormone prolactin, which stops the production of the gonadotrophins, which are the hormones needed to signal the testes to produce testosterone
- **hypothalamus** – particular conditions, such as tumours or a genetic disorder (Kallmann's syndrome), can prevent the hypothalamus from prompting the pituitary gland to release hormones. This will inhibit testosterone production by the testes. This is a rare cause of androgen deficiency.

Diagnosis of androgen deficiency

Androgen deficiency is diagnosed using a number of assessments, including:

- **medical history** – a full history is taken, including details about fertility, sexual function, symptoms of androgen deficiency, other medical problems, occupation, medication and drug use (prescribed and non-prescribed)
- **physical examination** – a thorough general examination is performed, including measuring the size of the testicles and checking for breast development
- **blood tests** – are taken to determine the level of testosterone in the blood. Ideally, a fasting blood test should be taken in the morning to detect the body's peak release of testosterone.

Testosterone levels should be measured on two separate mornings. The pituitary hormone levels should also be measured

- **other tests** – may be required to determine if testosterone deficiency is due to another underlying medical condition. These may include blood tests to check for iron levels, genetic tests (to diagnose an underlying genetic condition, such as Klinefelter's syndrome), or MRI scans of the brain (to examine the pituitary gland). Semen analysis will help to determine the potential fertility of men with androgen deficiency.

Treatment of androgen deficiency

Treatment for proven androgen deficiency is based on testosterone replacement therapy. Testosterone is best administered by skin gels creams, or by injection (short- or long-acting).

If your testosterone deficiency is caused by your pituitary gland and you are also wishing to father a child, your doctor will probably recommend gonadotrophin injections, several times a week for many months, to stimulate both testosterone and sperm production.

Testosterone treatment is not recommended for men trying to have a child as it acts as a powerful contraceptive by suppressing the pituitary hormones that drive sperm production. If you are androgen deficient and you and your partner are trying to have a baby, see a fertility specialist.

If you are having testosterone replacement therapy you will have regular reviews with your doctor. How often you have these will depend on your age and other risk factors for prostate cancer.

Older men need to be checked for prostate cancer before testosterone replacement therapy can be started, because increased levels of testosterone could make unrecognised prostate cancer grow. However, testosterone replacement therapy is not thought to increase the risk of a new prostate cancer above that of the general population.

Other diseases associated with sex hormone abnormalities:

True hermaphrodites are individuals who have both ovarian and testicular tissue. This occurs extremely rarely. Conditions involving hormonal abnormalities that result in ambiguities of the genitalia are more common, although their precise frequency is unknown.

A generous estimate in North America and Europe would probably be that somewhere in the region of 1 in 5000 to 1 in 10,000 births involves an intersex condition characterized by dramatic abnormality of the external genitalia. If less dramatic abnormalities, such as hypospadias or gynecomastia are included, estimates could reach as high as 1 in 100 individuals.

Most of our information on the human consequences of early hormonal perturbations has come from the more dramatic, but rarer, causes of genital ambiguity, since some of these are known to involve hormonal abnormalities of prenatal onset.

The primary sources of information have included:
(1) XX individuals exposed to high levels of androgens prenatally because of congenital adrenal hyperplasia (CAH);

(2) XY individuals exposed to lower than normal levels of androgens prenatally because their cells have deficient or defective androgen receptors (androgen insensitivity syndrome-AIS);

(3) XY individuals exposed to reduced androgens prenatally because they are deficient in enzymes needed to produce particular androgens from precursor hormones.

Some other conditions that involve prenatal hormonal abnormality, usually without ambiguity of the external genitalia at birth, have also been studied.

These include:
(4) XY individuals with idiopathic hypogonadotrophic hypogonadism (IHH), a syndrome involving deficiency in the hypothalamic hormones that promotes the production of testicular hormones.

(5) XX or XO individuals exposed to lower than normal levels of ovarian hormones prenatally, because their second X chromosome is absent or imperfect, resulting in- ovarian regression (Turner syndrome).

A third set of conditions involves XY individuals who are reassigned to the female sex early in life, because of problems with the appearance of their external genitalia. Thus, their prenatal hormone environment was that of a normal male but contrasts with their female sex of rearing.

These conditions include:
(1) Cloacal exstrophy;

(2) Penile agenesis (aphallia); and

(3) Ablatio penis.

i. Congenital Adrenal Hyperplasia:

Congenital adrenal hyperplasia (CAH) is an autosomal, recessive disorder that results in overproduction of androgen, beginning prenatally. The underlying problem is a deficiency in enzymes needed to produce adrenal steroids. In 90% of cases the deficient enzyme is 21-hydroxylase (21-OH).

In this and most other forms of CAH, the negative feedback system detects the low levels of Cortisol and the adrenal attempts to compensate by producing additional metabolic precursors to it. Because of the blockage in Cortisol production, however, these precursors are shunted into the androgen pathway, resulting in an overproduction of adrenal androgens, as well as progesterone and 17-hydroxyprogesterone.

Androgen levels in female fetuses with CAH are in the range of normal males and girls with the disorder are typically born with some degree of genital virilization. In some cases the virilization is so severe that the girls are mistaken for boys at birth and reared as such. Typically, however, the girls are diagnosed with CAH near the time of birth based on genital ambiguity, and they are assigned and reared in the female sex.

They are treated with hormones to regulate the postnatal hormone milieu, and their genitalia are feminized surgically. The incidence of CAH caused by 21-OH deficiency in Europe and the United States has been estimated at between 1 in 5000 and 1 in 15,000 births, occurring in both girls and boys.

Boys appear to have normal levels of androgens prenatally, and are not born with genital ambiguity. As a consequence, their condition is usually detected because of salt-losing crises caused by aldosterone deficiency. This typically occurs within a few weeks of birth, but in some cases affected boys are not identified until the elevated adrenal androgens induce precocious puberty in early childhood.

ii. Androgen Insensitivity Syndrome:

Androgen insensitivity refers to a deficiency in the ability of androgen receptors to respond to the hormones, testosterone and DHT. This insensitivity can be complete (CAIS) or partial (PAIS). Both disorders are transmitted as X-linked, recessive traits, and thus occur predominantly in genetic males.

Individuals with CAIS appear female at birth, despite an XY chromosome complement, and typically are raised as girls with no suspicion of the underlying disorder. At puberty the breasts develop under the influence of estrogen derived from testicular androgen. Typically the disorder is detected when menstruation fails to occur, because of the lack of feminine internal reproductive structures.

Physical appearance in PAIS varies enormously, ranging from essentially that of a CAIS individual to uncomplicated hypospadias, infertility, or even gynecomastia in an otherwise healthy-appearing male. Estimates of the incidence of CAIS vary enormously, although it appears to be rarer than CAH. The incidence of PAIS is not known, perhaps because its milder manifestations go undetected.

iii. Deficiencies in Enzymes Needed to Produce Androgens:

These deficiencies are transmitted as autosomal, recessive traits. They are rare in the general population, but can occur frequently in populations where inbreeding is common. In one area of the Dominican Republic, the incidence of 5αR has been estimated at 1 in 90 males. The enzyme 5αR converts T to DHT, and patients deficient in the enzyme have low levels of DHT but normal to high levels of T.

Because DHT is needed for normal virilization of the external genitalia prenatally, 5αR deficiency results in female-appearing or ambiguous genitalia at birth, and individuals with the disorder

usually are assigned and reared as girls. High levels of testosterone and DHT derived from it at puberty however, cause virilization, including growth of the phallus and scrotum, deepening of the voice and development of male-typical musculature.

The enzyme 17βHSD is needed to produce T from its immediate precursor, androstenedione. Patients deficient in this enzyme have low levels of T and DHT, but elevated levels of androstenedione. The natural history of 17PHSD is similar to that of 5aR deficiency. The genital appearance at birth is feminine or ambiguous, but physical virilization occurs at puberty. In populations where these disorders are common they sometimes have descriptive names, such as guevedoce, geuvote (penis at 12 years of age), or machihembra (first woman, then man) or Turnim Man.

iv. Idiopathic Hypogonadotrophic Hypogonadism:

Individuals with IHH have low levels of pituitary gonadotropins or their hypothalamic releasing factor. As a consequence, their gonads lack sufficient stimulation to produce normal levels of hormones. The disorder can occur after puberty, or congenitally. If the disorder is congenital, it is usually detected when the child does not undergo normal puberty.

Males with congenital IHH usually have normal appearing genitalia at birth, perhaps because maternal gonadotropins stimulated their testes to produce hormones prenatally. Thus, it cannot be assumed that their hormone levels are lower than normal before birth. However, beginning at birth, and perhaps to some extent before, their levels of testicular hormones would be lower than in normal males.

v. Turner Syndrome:

Turner syndrome (TS) results from an absent or imperfect second X chromosome, and appears to involve a random genetic error. In 50 to 60% of cases the second X chromosome is entirely missing. Other cases involve mosaicisms or abnormalities of the second sex chromosome.

TS occur in approximately 1 in 2000 to 1 in 5000 live female births in North American and Western Europe. The external genitalia are female, but in the majority of TS girls, the ovaries regress sometime after the third month of gestation, impairing or eliminating their ability to produce hormones.

The syndrome has several stigmata, including short stature, skeletal growth disturbances, cardiovascular and renal abnormalities, otitis media, primary gonadal failure, absence of secondary sexual characteristics, and infertility. Short stature is universal in TS, and over 90% of affected females experience primary gonadal failure and infertility, but other stigmata vary dramatically from individual to individual.

vi. Cloacal Exstrophy:

Cloacal exstrophy is a severe defect of the ventral abdominal wall. It involves abnormalities and insufficiencies in the urinary and bowel systems that until 1960 were universally fatal. Now, many

children born with this syndrome survive. Cloacal exstrophy occurs in approximately 1 in 200,000 to 1 in 400,000 births, and is more common in XY than XX individuals.

In XY individuals, the testes appear histologically normal but are typically undescended, and the penis is usually either absent or represented as two separate and incomplete structures. Even when present as a single structure, the penis typically is small and poorly formed. In XX individuals there are also abnormalities of the genitalia.

Because of this, most XX and XY patients with cloacal exstrophy are surgically feminized and assigned and raised as females. Those who are XY were exposed to male-typical levels of testicular hormones prenatally and neonatally, until surgical removal of the testes.

vii. Penile Agenesis (Aphallia):

In penile agenesis (aphallia), an XY individual is born without a penis, despite the presence of a normal scrotum and functioning testes. The causes of the condition are unknown, although it is usually associated with abnormalities of the urinary and gastrointestinal tracts.

Estimates of its incidence range from 1 in 50,000 to 1 in 10- 30 million, and mortality is high. As a result there are very few individuals with aphallia. However, those who do survive are often surgically feminized and reared as girls. Like XY individuals with cloacal exstrophy, their prenatal and early neonatal hormonal milieu would resemble that of healthy male fetuses.

viii. Ablatio Penis:

In rare instances accidents can cause severe damage or even complete ablation of the penis in an otherwise healthy infant. In some such cases, XY infants have been reassigned as female, surgically feminized, and reared as girls. They would have been exposed to normal male levels of testicular hormones prenatally and postnatally until the time when the testes were removed.

Testicular Neoplasm:

Testosterone controls the development of the reproductive organs and other male physical characteristics. In the United States, around 1 in 250 males develop testicular cancer during their lifetime. In 2019, experts predict that 9,560 males will receive a diagnosis of testicular cancer. The average age at diagnosis is 33 years; the condition mostly affects young and middle aged men. In very rare cases, it can happen before puberty. Only 8% of cases occur after the age of 55.

Early signs:

Symptoms of testicular cancer often appear at an early stage, but sometimes, they do not appear until much later.

The individual may notice a change, or a doctor will find it during a routine physical exam. A common early symptom is a painless lump or swelling in a testicle. Changes occur in the testicles

for many reasons. A lump does not always mean cancer, but anyone who notices a change should see a doctor.

There may also be:

- a sharp pain in the testicle or scrotum
- a heavy feeling in the scrotum
- a difference in size between the testicles

In some cases, hormonal changes will cause the breasts to grow and become sore.

Other symptoms

In the later stages, as cancer spreads to other organs, a person may notice:

- lower back pain, if cancer spreads to the lymph nodes
- difficulty breathing, if it affects the lungs
- abdominal pain, if it affects the liver
- headaches and confusion, if it reaches the brain

Causes:

Most testicular cancers start in the germ cells. These are the cells in the testicles that produce immature sperm.

Doctors do not know why testicular cells become cancerous, but some genetic factors may increase the risk.

Testicular cancer is more likely to occur in people with the following risk factors :

- cryptorchidism, or an undescended testiclea family history of testicular cancer
- being white, rather than black or Asian

Having HIV might increase the risk. Having a vasectomy does not increase the risk.

It is not possible to prevent testicular cancer, as doctors do not know what causes it, and because genetic factors may play a role. A person cannot change these factors.

Treatment:

Testicular cancer is highly treatable, especially in the early stages. Most males with a diagnosis of testicular cancer will live at least another 5 years following diagnosis.

Treatment will usually involve a combination of the following:

- surgery
- radiation therapy
- chemotherapy
- stem cell treatment
- surveillance

Surgery:

A surgeon will remove one or both testicles to prevent the tumor from spreading. The person will receive a general anesthetic. The surgeon will then make a small incision in the groin and remove the testicle through the incision. Removing one testicle does not usually affect the person's sex life or fertility, but removing both testicles means that the male will not be able to conceive naturally. However, other fertility options are available. For example, the doctor might suggest banking sperm for future use, if necessary.

Other effects of removing the testicles may include:

- a loss of sex drive
- difficulty achieving an erection
- fatigue
- hot flashes
- loss of muscle mass

A doctor may prescribe testosterone supplements — as a gel, a patch, or an injection — to help with these issues.

It is also possible to restore the appearance of testicles by having a prosthesis. A surgeon will implant this in the scrotum. It is filled with salt water. A person who has surgery in the early stages may not need any further treatment.

Lymph node surgery

If cancer has reached the lymph nodes, usually those around the large blood vessels at the back of the abdomen, a surgeon will need to remove these. A surgeon can do this as open or laparoscopic surgery.

This procedure will not impact fertility directly, but any nerve damage may affect ejaculation. This may mean that sperm does not come out through the urethra but goes to the bladder instead. This is not dangerous, but a lower sperm count can affect fertility.

Radiation therapy

Radiation therapy damages the DNA inside the tumor cells and destroys their ability to reproduce. In this way, it can remove cancer and may prevent it from spreading or coming back. A person who has surgery may need radiation therapy to ensure that treatment removes any remaining cancer cells. If cancer has spread to the lymph nodes, a doctor may also recommend radiation therapy.

The following temporary side effects may occur:

- tiredness
- rashes
- muscle and joint stiffness
- loss of appetite
- nausea

These symptoms should pass once the treatment is over.

Chemotherapy

Chemotherapy uses medication to destroy cancer cells and stop them from dividing and growing. A doctor may recommend chemotherapy if a person has testicular cancer that has spread to other parts of the body. A doctor will give the treatment either orally or as an injection. Chemotherapy attacks healthy cells as well as cancerous ones, which may lead to the following side effects:

- nausea and vomiting
- hair loss
- mouth sores
- tiredness and a general feeling of being unwell

These symptoms usually resolve after treatment finishes.

Stem cell treatment:

In some cases, stem cell therapy can enable a person to receive higher doses of chemotherapy that would otherwise be too dangerous to administer. During the weeks before treatment, a special machine will harvest stem cells from the person's blood. Healthcare professionals will freeze and store these cells. The person receives a high dose of chemotherapy, and they will then receive the stem cells into a vein as in a transfusion. These cells establish themselves in the bone marrow and start making new blood cells. This enables the person's body to recover from higher doses of chemotherapy.

Disadvantages of this type of therapy include:

- Due to the high dose of chemotherapy, it is risky and may involve life threatening adverse effects.
- It can involve a long stay in the hospital.
- It can be expensive, and medical insurance may not cover it.

Surveillance:

A doctor will carry out surveillance after a person has had treatment for testicular cancer, to check for any signs that the cancer has come back.

Surveillance does not involve active treatment, but the individual will attend regular appointments and undergo tests.

Diagnosis:

To diagnose testicular cancer, a doctor will recommend:

Blood tests: These can measure levels of alpha-fetoprotein, human chorionic gonadotrophin, and lactate dehydrogenase. These are substances that may suggest the presence of a tumor.

Ultrasound: This can reveal the presence and size of a tumor.

Biopsy: The doctor takes a small tissue sample from the testicle for investigation using a microscope. A biopsy can determine whether cancer is present or not.

Types of testicular cancer

If tests show that testicular cancer is present, a doctor will also need to know what type of cancer it is and what stage it is at before discussing a treatment plan with the individual.

There are two main types **of testicular cancer:**

Seminoma: This type grows slowly and contains only seminoma cells. There are two subtypes: classic and spermatocytic.

Nonseminoma: This can involve various kinds of cancer cell. There are several subtypes, including embryonal carcinoma, yolk sac carcinoma, chiorcarcinoma, and teratoma.

Other tumors that are not cancerous include stromal tumors, Leydig cell tumors, and Sertoli cell tumors.

Staging the cancer

The stage of the cancer will also affect treatment options:

Localized: The cancer is only in the testis and has not spread.

Regional: The cancer has reached the lymph nodes in the abdomen.

Distant: The cancer has spread to other parts of the body, such as the lungs, liver, brain, and bones.

Self-examination:

The best time to check for testicular cancer is when the scrotal skin is relaxed, usually after a warm shower or bath.

To perform a self-exam:

1. Gently hold the scrotum in the palms of both hands. Stand in front of a mirror and look for any swelling on the skin of the scrotum.

2. Feel the size and weight of the testicles first.

3. Press around the testicles with the fingers and thumbs, and be aware of any lumps or unusual swellings.

4. Feel each testicle individually. Place the index and middle fingers under one testicle with the thumbs on the top. Gently roll the testicle between the thumbs and the fingers. It should be smooth, oval shaped, and somewhat firm, with no lumps or swellings. The top and back of each testicle should have a tube-like section, called the epididymis, where sperm is stored.

Repeat this process once each month, checking for changes in the size, weight, or feel of the testicles.

Many males have one testicle that hangs lower than the other or one testicle that is bigger than the other, but as long as these proportions do not change over time, it is not a cause for concern.

It is not currently possible to prevent testicular cancer, because there are no known lifestyle risk factors. However, if there is a family history, genetic testing may help detect iyt early, if it happens. Regular self-exams may also lead to an early diagnosis.

The outlook for someone with early stage testicular cancer is excellent, with 95% of people surviving at least another 5 years after diagnosis.

Around 11% of people receive a diagnosis after the cancer has spread to other organs. According to the American Society of Clinical Oncology, 74% of these people will live at least another 5 years.

Being aware of any changes can make it easier to spot testicular cancer in the early stages. With prompt treatment, there is an excellent outlook for this type of cancer.

Probable Questions:

1. What are the anatomical features of Addison's disease?
2. What are physiological features of Addison's disease?
3. What are the psychological features of Addison's disease?
4. What are the sexological features of Addison's disease?
5. What are the anatomical features of Cushing's syndrome?
6. What are physiological features of Cushing's syndrome?
7. What are the psychological features of Cushing's syndrome?
8. What are the sexological features of Cushing's syndrome?
9. What is androgen deficiency syndrome?
10. What are the symptoms of androgen deficiency?
11. How androgen deficiency is diagnosed?
12. How androgen deficiency is treated?
13. Discuss Other diseases associated with sex hormone abnormalities.
14. Discuss Congenital Adrenal Hyperplasia.
15. What is Androgen Insensitivity Syndrome?
16. What is Idiopathic Hypogonadotrophic Hypogonadism
17. Discuss Cloacal Exstrophy.
18. What is Penile Agenesis?
19. What are the symptoms of testicular neoplasm?
20. Discuss different causes of testicular neoplasm
21. Discuss treatment procedures of testicular neoplasm.
22. Discuss different types of testicular neoplasm.
23. How self examination can prevent progression of testicular neoplasm?
24. Discuss diagnosis of testicular neoplasm.

Suggested readings:

1. General Endocrinology. Turner and Bagnara. Sixth Edition.
2. Williams Textbook of Endocrinology. Tenth Edition.
3. Introduction to Endocrinology. Chandra S Negi. Second Edition
4. Endocrinology. Hadley and Levine. Sixth Edition.

UNIT-XIX
Endocrine Cancer

Objective: In this unit we will discuss about various types of endocrine cancer

Introduction:

Endocrine tumors develop when abnormal cells in an endocrine gland or organ grow and multiply uncontrollably. Over time, the new cells can develop into a solid mass of tissue known as a tumor.
In most cases, endocrine tumors are benign (noncancerous). In cases where they are malignant (cancerous), the cells that make up the tumor are capable of invading nearby tissues and spreading to other parts of the body, where they can form new tumors.
While many kinds of endocrine cancers are rare, some are common. For example, thyroid cancer, a type of endocrine cancer, is the seventh most commonly diagnosed cancer in women and the tenth most commonly diagnosed cancer in men in the United States. Other endocrine cancers include cancers of the adrenal glands, parathyroid glands, pituitary gland, hypothalamus, and pancreas. Fortunately, effective treatments, including surgery, chemotherapy, radiation therapy, and medications, among other therapies, are available for endocrine cancers.
The endocrine system is a group of glands and organs that produces and secretes hormones. Hormones are chemicals that travel through the bloodstream to tissues around the body, where they regulate and coordinate many important processes in the body, including metabolism, growth, development, sexual function, reproduction, and mood. An endocrine tumor can form when abnormal cells in an endocrine gland or organ arise and grow uncontrollably. There are two broad categories of endocrine tumors:

Functioning tumors, which produce and secrete hormones. When a tumor arises from a hormone-producing cell, the cells that make up the tumor also produce and secrete excessive levels of the hormones made by the initial cell, which can cause a range of symptoms and problems in the body, depending on which hormone is involved.

Nonfunctioning tumors which do not produce or secrete hormones.

Both functioning and nonfunctioning tumors can develop into large masses that press against nearby tissues and organs, impairing their ability to work properly.

Types of endocrine cancer

Cancer can develop in any endocrine gland or tissue in the body. Common types include the following:

- **Thyroid cancer**: The thyroid is a small, butterfly-shaped organ located at the base of the neck. It produces and secretes thyroid hormones that help regulate metabolism.

- **Pituitary tumors**: A pea-sized organ located just below the brain, the pituitary gland produces several different hormones that regulate the function of other endocrine glands, including the adrenal and thyroid glands, as well as the gonads (the ovaries and testicles). The pituitary helps control important functions of the body, including growth, blood pressure, metabolism, and sperm and egg production, among many others. Pituitary tumors are almost always benign. According to the National Cancer Institute, only around 0.1% to 0.2% of pituitary tumors are cancerous.

- **Adrenal cancers**: The two adrenal glands, located just above the kidneys, produce several different hormones that play an important role in regulating a number of essential bodily processes, including metabolism, the stress response, inflammation, blood pressure, and sexual development. Adrenal hormones include cortisol, aldosterone, and adrenaline (also known as epinephrine). A few types of cancer can occur in the adrenal gland, including adrenocortical carcinoma and pheochromocytoma, though the latter tumor is usually benign.

- **Pancreatic cancers**: Though the pancreas plays an active role in the digestive system, it's also part of the endocrine system. Specialized neuroendocrine cells in the pancreas produce and secrete hormones, including insulin and glucagon, to help regulate blood sugar levels. Neuroendocrine cells have features of both nerve cells and hormone-producing endocrine cells. Cancerous tumors that develop from these neuroendocrine cells are called pancreatic neuroendocrine tumors (pancreatic NETs or pNETs) or islet cell tumors. Pancreatic NETs are rare and, according to the ACS, account for just under 2% of all pancreatic cancers. (Because neuroendocrine cells are found in various tissues throughout the body, including the gastrointestinal tract and lungs, NETs can also occur in those locations.)

- **Parathyroid cancers**: The four pea-sized parathyroid glands, located behind the thyroid gland in the neck, produce parathyroid hormone (PTH). PTH helps regulate blood calcium levels. When blood calcium levels are low, the parathyroid glands secrete PTH, which stimulates the release of calcium from bones into the blood, increases the absorption of calcium from food by the intestine, and prevents the kidney from excreting too much calcium in the urine. Together, these actions increase blood calcium levels. People with parathyroid cancer have severe hypercalcemia (high blood calcium levels). Parathyroid cancer is very rare.

- **Hypothalamic endocrine tumors**: The hypothalamus is a small part of the brain connected to the pituitary gland. It produces and secretes hormones that regulate the activity of the pituitary gland (which in turn secretes hormones that regulate the activity of

several other endocrine glands). Cancer of hormone-secreting cells in the hypothalamus is rare.

Causes of endocrine cancer: The cause of most endocrine cancers is usually unclear.

Normally, the growth and production of new cells are tightly regulated to ensure that only healthy cells are produced and survive. In cancer, genetic changes—or mutations—in a single cell interfere with the careful regulation of cell growth and production. As a result, the affected cell grows and multiplies uncontrollably and can invade nearby tissues and spread to other parts of the body. The genetic changes that trigger cancer may be inherited from one or both parents or may occur sporadically, meaning they are acquired during a person's life. Sporadic mutations can occur randomly or due to environmental exposures, such as smoking tobacco products or radiation exposure.

Symptoms of endocrine cancer:

Endocrine cancer symptoms vary greatly, depending on which part of the endocrine system is affected, whether the tumor has grown large enough to press against nearby tissues, and which hormones, if any, are produced and secreted in excessively high levels.

Risk factors for endocrine tumors may include:

Thyroid cancer:

- Being female
- Being between 25 and 80 years of age
- Radiation exposure, such as head or neck radiation treatments in childhood
- Personal history of goiter, thyroid nodules, or a previous thyroid cancer diagnosis
- Family history of thyroid cancer or thyroid disease
- Certain genetic conditions including multiple endocrine neoplasia type 2A (MEN2A) and type 2B (MEN2B)
- Certain inherited conditions, including familial medullary thyroid cancer (FMTC), Cowden disease, Carney complex type 1, and familial adenomatous polyposis (FAP)

Pituitary Tumors:

- Certain inherited conditions, including multiple endocrine neoplasia type 1 (MEN1) and type 4 (MEN4), Carney complex, McCune-Albright syndrome, familial isolated pituitary adenoma (FIPA)

Adrenal cancer:

- Certain inherited and genetic conditions, including Li-Fraumeni syndrome, Beckwith-Wiedemann syndrome, Carney complex, MEN1, familial adenomatous polyposis (FAP), Lynch syndrome, MEN2A, MEN2B, von Hippel-Lindau (VHL) syndrome, neurofibromatosis type 1 (NF1), hereditary paraganglioma syndrome, Carney-Stratakis dyad, Carney triad

Pancreatic neuroendocrine tumors:

- Smoking
- Alcohol consumption
- Family history of pancreatic NETs
- Certain inherited genetic syndromes, including NF1, MEN1, VHL syndrome
- Diabetes
- Chronic Pancreatitis

Parathyroid Cancer:

- Radiation exposure, such as from previous treatment with radiation therapy to the head or neck
- Certain inherited conditions, including familial isolated hyperparathyroidism (FIHP), hyperparathyroidism-Jaw tumor syndrome (HPT-JT), MEN1, MEN2A

Hypothalamic endocrine tumors:

- Neurofibromatosis

Diagnosis of Endocrine tumor:

Doctors can perform a series of steps to check for endocrine cancer, including:

- A medical history to determine whether the patient has any risk factors for endocrine cancer, such as a family history of certain conditions or a medical condition associated with endocrine cancer
- A physical exam to evaluate symptoms and signs that could be caused by an endocrine cancer
- Lab tests to check for abnormal levels of hormones and/or other markers in the blood or urine
- Imaging studies, such as a computed tomography (CT) scan, magnetic resonance imaging (MRI) scan, ultrasound, or other imaging tests to look for evidence of abnormal tissue
- A biopsy to obtain a tissue sample for analysis that can check for the presence of cancer cells
- Genetic testing to determine the genetic makeup of tumor cells. In many cases, knowing a tumor's genetic makeup helps health care providers choose the best treatment options.

Treatment:

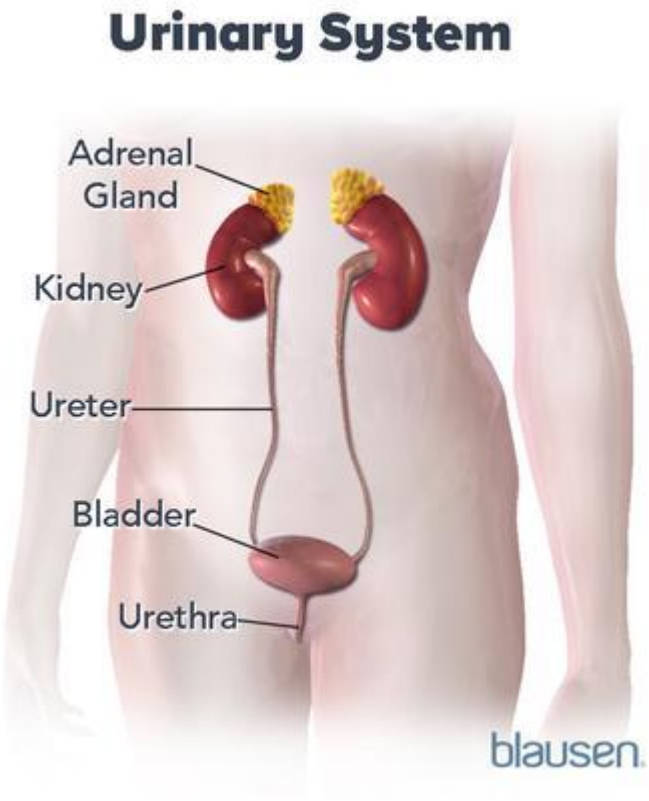
Various treatment options are available for endocrine cancers. The treatment varies based on the type of cancer involved, how far it has progressed, and the patient's overall health, among many other factors.

Endocrine cancer treatment may involve:

- **Surgery** to remove the cancerous tissue. In some cases, the entire affected organ may be removed. For example, adrenalectomy—the surgical removal of an adrenal gland—is frequently used to treat adrenal cancer.
- **Radiation therapy**, also known as radiotherapy, kills cancer cells by exposing them to high doses of radiation. It is often used after surgery to kill any remaining cancer cells.
- **Chemotherapy** involves the use of drugs to kill cancer cells. It may be given before surgery to reduce tumor size or after surgery to eliminate any cancer cells that remain.
- **Targeted drug therapy** uses drugs that target specific genes or proteins found in cancer cells.
- **Ablation** may be used to destroy tumors. During an ablation procedure, a probe is used to deliver heat, cold, alcohol, or other forms of energy to the tumor, thereby destroying the cancerous tissue.
- **Embolization** is a technique to cut off blood flow to—and kill—cancer cells. In an embolization procedure, substances are injected into a blood vessel to block blood flow to a tumor. Sometimes, beads that contain chemotherapy drugs (known as chemoembolization) or a radioactive isotope (known as radioembolization) are also injected into the blood vessel to deliver chemotherapy or radiation, respectively, to the tumor.
- **Immunotherapy** involves the use of drugs to enhance the ability of the patient's immune system to fight against cancer.
- **Hormone therapy** may be used to suppress the production of—or block the effects of—excess hormones made by endocrine tumors. In other cases, cancer or cancer treatments such as surgery, chemotherapy, or radiation therapy can impair the ability of an endocrine gland to produce hormones. In these cases, patients may need to take hormones to replace those that the affected gland can no longer produce.
- **Observation** may be appropriate for select small thyroid cancers that are well differentiated and considered low risk. In such circumstances, patients may choose observation rather than immediate surgery.

1. Adrenal tumors:

The adrenal glands are the pair of small endocrine glands located above the kidneys. They respond to signals from the nervous system and secrete hormones that regulate stress. The adrenal glands also produce hormones that help maintain metabolism as well as distinguish male and female physical and sexual characteristics.



There are two varieties of tumors that can proliferate on the adrenal glands:

- Most growths are **benign** and symptoms are treatable
- **Malignant** adrenal tumors are rare, and generally grow as a result of metastasizing cancer that originated in a different organ

Risk factors:

People with certain genetic conditions are at a higher risk for developing adrenal tumors. These include:

- Li-Fraumeni syndrome
- Carney Complex
- Family history of adrenal tumors

Symptoms:

Adrenal gland tumors do not always present the same group of symptoms and may also not cause any symptoms at all. Nevertheless, the following symptoms may be warning signs:

- Headaches
- Unusual weight changes
- Unusual anxiety
- Heart palpitations or elevated blood pressure
- Unusual hair growth
- Disproportionate acne
- Diminished sex drive
- Muscle weakness
- Easy bleeding or bruising

Diagnosis and treatment

Doctor can detect the presence of adrenal tumors in the following ways:

- **Biopsy:** This is the most definitive diagnostic method. Doctors examine tissue samples for evidence of cancer.

- **Blood and Urine Tests:** The levels of hormones produced under certain circumstances are present in blood and urine and are indicative of possible tumors

- **Imaging:** With either a CT scan or MRI, your physician can verify the existence of an adrenal tumor, as well as determine its exact size and placement

- **Metaiodobenzylguanidine scan (MIBG):** This is a special test administered during the course of two days. It is designed to show adrenal tumors that are not evident on other scans. On the first day, a patient gets an injection followed by a scan with a special camera. The next day, the scan is repeated

After examining the results of one or more of these tests, your doctor may inform you that you have an adrenal tumor. Treatment is based on tumor size, location, and whether it is metastasizing.

The primary types of treatment for adrenal tumors include:

- Surgery
- Radiation Treatment
- Chemotherapy

2. Neuroendocrine tumors

The brain and the nervous system provide the signals to the endocrine system to produce hormones that regulate bodily functions. Since these two systems are so interdependent, they are often referred to as the neuroendocrine system. Tumors that affect the functioning of cells within this system are collectively called neuroendocrine tumors.

The primary types of tumors are:

- **Pheochromocytoma**, which affects production of adrenaline and often presents in the adrenal glands
- **Neuroendocrine tumors**, which is a generic term for tumors that affect hormones in major organs (such as the pancreas)

Risk factors

People are at a higher risk for developing these tumors because of certain factors that include:

- Gender: men are more likely than women to develop Pheochromocytoma
- Age: Pheochromocytoma patients are generally between 40-60 years old
- Genetics

Symptoms

Each variation of neuroendocrine tumor presents specific symptoms.

- Pheochromocytoma
- Elevated blood pressure
- Damp and sticky skin
- Unusual anxiety
- Heart palpitations
- Nausea, headaches, fever
- Neuroendocrine Carcinoma
- Hyper or hypoglycemia
- Unusual weight changes
- Unusual anxiety
- Unexplained lumps
- Jaundice
- Unexplained bleeding
- Unusual bowel or bladder changes
- Ongoing night sweats

Diagnosis and treatment

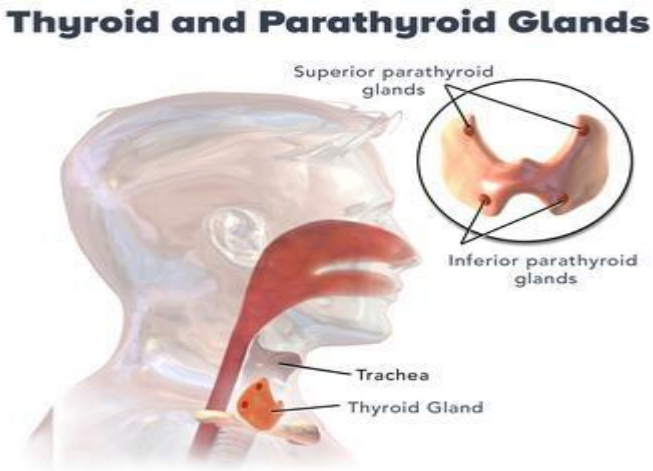
Doctors can detect the presence of the tumors in the following ways:

- **Biopsy:** This is the most definitive diagnostic method. Doctors examine tissue samples for evidence of a tumor
- **Blood and Urine Tests:** The levels of hormones produced under certain circumstances are present in blood and urine and are indicative of possible tumors
- **Imaging:** With a CT scan, MRI, or X-ray, the doctor can usually verify the existence of a neuroendocrine tumor, as well as determine its exact size and placement

  After examining the results of one or more of these tests, your doctor may inform you that you have a neuroendocrine tumor. Treatment is based on where the tumor is located, how big it is, whether it is metastasizing, and the patient's general health. The primary types of treatment include:
- Surgery
- Radiation
- Chemotherapy

3. Parathyroid tumor:

The parathyroid glands are four pea-sized glands located in the neck near the thyroid. They secrete parathyroid hormone (PTH), which regulates calcium levels throughout the body. Tumors can form within the tissues of the parathyroid and tend to grow very slowly, impacting the body with over-production of PTH, also called hyperparathyroidism. The vast majority of parathyroid tumors are benign (not cancerous). In fact, parathyroid cancer has only been diagnosed in a few hundred cases.



Risk Factors

Besides genetics, there are no common characteristics that put people at higher risk for developing parathyroid cancer. Some patients with parathyroid cancer have already been suffering from parathyroid adenomas or hyperplasia.

Symptoms

The following symptoms may indicate the presence of a parathyroid tumor and the resulting hyperparathyroidism:

- Lump or nodule in the neck
- Pain in the bones or in the upper back
- Fractures
- Kidney stones
- Pancreatitis
- Muscle weakness
- Trouble speaking
- Vomiting
- Fatigue
- Weight loss
- Constipation
- Frequent urination
- Extreme thirst

Diagnosis and treatment

Cancer of the parathyroid can be difficult to detect, since symptoms are similar to those of simple hyperparathyroidism. At this time, there are no specific tests for these tumors, but an official diagnosis can emerge with the following:

- **Symptoms:** A patient's symptoms strongly indicate the presence of a parathyroid tumor. The doctor surgically identifies and removes it
- **During hyperparathyroidism surgery:** During surgery to remove various non-cancerous lesions or growths from a patient with hyperparathyroidism, the surgeon may discover cancerous lesions
- **After hyperparathyroidism surgery:** Upon removal and examination of a seemingly non-cancerous lesion or growth from a patient with hyperparathyroidism, the doctor discovers it is indeed cancerous
- **Symptoms after surgery:** If a patient with hyperparathyroidism undergoes surgery but still experiences symptoms additional imaging tests can help verify the diagnosis. Tests include:

Scintigraphy and ultrasound for neck tumors

- CT scan and MR scan

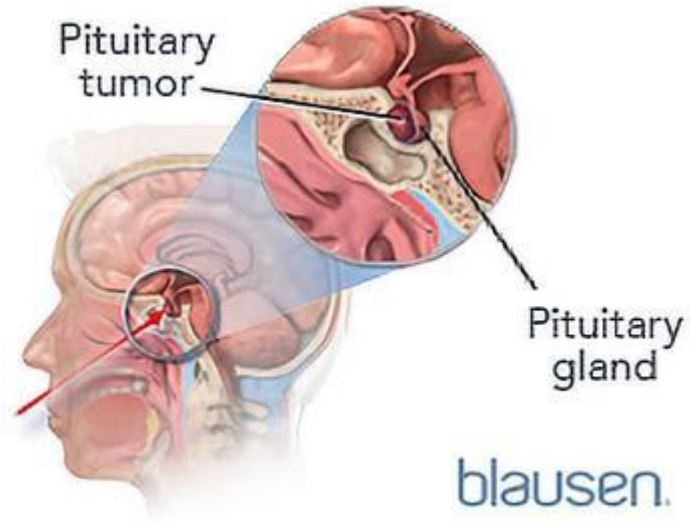
Surgery is the primary treatment for parathyroid tumors. Removal of nearby thyroid gland and lymph nodes may sometimes be performed. When cancer has metastasized, additional methods and drugs are necessary to help the body excrete excess calcium. These include:

- Intravenous saline
- Diuretics
- Bisphosphonates
- Gallium nitrate
- Cinacalcet

4. Pituitary tumors

As tiny as a pea, and located towards the bottom center of the brain, the pituitary gland secretes hormones that stimulate other endocrine glands to function properly. The pituitary gland helps regulate metabolic functions, as well as growth, reproduction, and blood pressure levels.

Pituitary tumors are growths on the gland. Pituitary tumors can cause either too much or too little hormone production. In most cases, these tumors do not spread and are not considered cancerous.



Risk factors

People are at a higher risk for developing pituitary tumors due to particular factors. These may include:

- Age: patients are generally older
- Family history
-

Symptoms

Since the pituitary gland regulates many other hormone-producing organs, symptoms can vary, depending on the affected area. Sometimes pituitary tumors themselves secrete hormones, causing biochemical symptoms.

Three or more of the following symptoms are generally present because of pituitary tumors:

- Sexual dysfunction
- Growth of jaw, hands, and feet
- Breast secretion
- Depression
- Infertility
- Growth issues
- Osteoporosis
- Joint pain
- Excessive bruising
- High or low blood pressure
- Obesity
- Cessation of menstrual periods
- Nausea and vomiting
- Seizures
- Fatigue and weakness
- Headaches or difficulty seeing
- Unusual weight changes

Hormone-producing pituitary tumors can result in the following symptoms:

- Weight gain in the upper back and gut
- Development of a hump on the upper back
- Unusual facial roundness or hardening facial features
- Unusual growth in hands and feet
- Leaking milky liquid from the breasts (women)
- Breast growth (men)
- Loss of muscle and body hair (men)
- Irregular heartbeat
- Unusual jittery or ill-tempered moods

Diagnosis and treatment:

Doctor can detect the presence of a pituitary tumor in the following ways:

- **Blood and Urine Samples:** Abnormal hormone levels can be detected through the blood and urine
- **Imaging:** With either a CT scan or MRI, your physician can verify the existence of a pituitary tumor, as well as determine its exact size and placement
- **Vision Tests:** With an eye test, your doctor will be able to determine whether the pituitary tumor has grown large enough to significantly affect your vision

  After examining the results of one or more of these tests, your doctor may inform you that you have a pituitary tumor. Since the gland affects so many different bodily functions, the specific diagnosis is based on where the tumor is causing the majority of symptoms.

Treatment varies according to the size of the tumor, what structure it is affecting and how deeply embedded in the brain. With early detection and treatment, the prognosis for recovery is generally excellent.

- **Surgery:** This is the most common option, especially in cases where the tumor is putting pressure on the optic nerve and causing vision problems
- **Radiation:** This option can be used along with surgery or by itself. The two types of radiation therapy used are external beam radiation and gamma-knife radiosurgery
- **Medication:** Certain drugs can suppress overproduction of hormones and help reduce tumor size

Some Rare Endocrine Cancer:

1. Anaplastic Thyroid Cancer (ATC)

What is anaplastic thyroid cancer?

Anaplastic thyroid cancer, or ATC, is a type of thyroid cancer. The thyroid is a gland located in the front of your neck, just below the Adam's apple. It is responsible for sending out hormones to the rest of your body. ATC is different than other types of thyroid cancers because ATC invades other parts of the body very quickly. This type of cancer usually affects people over the age of 60. ATC can also be called anaplastic thyroid carcinoma.

How common is anaplastic thyroid cancer?

ATC is a rare type of thyroid cancer, making up 1% to 2% of thyroid cancer cases. ATC affects one to two people per one million per year in the US.

How is anaplastic thyroid cancer diagnosed?

ATC can start as a bump in the throat area. The tumor growing on the thyroid can make your voice hoarse by blocking your vocal chords, or it can make it difficult to breathe by blocking your windpipe. Sometimes people can have ATC for a while and not notice it because the tumor remains small.

Imaging: If you have symptoms of ATC, your doctor will use imaging scans such as ultrasound, CT, and MRI to look at the size of the tumor. They will also check for signs that the tumor has spread to other parts of the body.

Biopsy: To check if the tumor is ATC, your doctor will perform a biopsy, taking a small sample from the tumor with a needle. A pathologist will study cells from the sample under the microscope to see what kind of tumor it is.

How is anaplastic thyroid cancer treated?

Surgery: Once ATC is diagnosed, you may have surgery to remove the thyroid. This surgery is a called a thyroidectomy. If a thyroidectomy is not an option, your doctor will discuss other options with you.

Radiation therapy and chemotherapy: A thyroidectomy is often combined with radiation and chemotherapy treatments. Doctors and scientists are looking for ways to improve radiation therapy. For example, new ways to give radiation therapy have been developed that allow higher radiation doses over less time with more precision. The hope is to target the tumor without injuring the healthy nearby muscle and tissue.

ATC is a difficult disease to treat because of its ability to spread to the rest of the body. Research is being conducted on the different types of treatment options, and support networks are available for people with ATC.

Does anaplastic thyroid cancer run in families?

No, ATC does not run in families.

How does anaplastic thyroid cancer form?

Scientists are always working to understand how cancer forms, but it can be hard to prove. ATC often starts in a thyroid that is already unhealthy. It can form within a goiter or it can arise from another thyroid cancer. Scientists have found many different changes in ATC cells, which tells them that there are likely many ways that ATC can start. This makes it very hard to develop a single treatment that can work for all ATC patients.

What is the prognosis for someone with anaplastic thyroid cancer?

The estimate of how a disease will affect you long-term is called prognosis. Every person is different and prognosis will depend on many factors, such as:

- Where the tumor is in your body
- If the cancer has spread to other parts of your body
- How much of the tumor was taken out during surgery

Doctors estimate ATC survival rates by how groups of people with ATC have done in the past. Because there are so few ATC patients, these rates may not be very accurate. They also don't consider newer treatments being developed. ATC is one of the fastest growing cancers, with only half of people with ATC surviving 6 months after diagnosis. It is very important to work with a team of experts as soon as possible after diagnosis to improve your chances of survival. You can contact MyPART for help connecting with experts in ATC.

2. Neuroendocrine Tumor (NET)

What is neuroendocrine tumor (NET)?

Carcinoid tumor is a type of neuroendocrine tumor that grows from neuroendocrine cells. Neuroendocrine cells receive and send messages through hormones to help the body function. Neuroendocrine cells are found in organs throughout the body.

Carcinoid tumors often grow very slowly. In children and young adults, carcinoid tumors are most often found in the appendix, called appendiceal carcinoid tumors, or in the lungs, called bronchial tumors. In adults, carcinoid tumors are most often found in the digestive tract. This tumor may spread to other parts of the body but does so more often in adults than children.

How common is NET?

Carcinoid tumor is rare in children and more common in adults. Experts think that carcinoid tumor affects 4 in 100,000 adults. Carcinoid tumor in children and young adults is so rare that there is little data on how many young people have it.

How is NET diagnosed?

Some people with carcinoid tumors have symptoms, but others don't. The symptoms of carcinoid tumor depend on where the tumor is inside the body.

Patients with carcinoid tumor of the appendix usually have symptoms of appendicitis, such as pain in the abdomen. They may be diagnosed later with carcinoid tumor if the doctor removes the appendix and finds a tumor. Patients with carcinoid tumor in other parts of the digestive tract may have symptoms such as:

- Pain in the abdomen
- Nausea or vomiting
- Diarrhea

Patients with carcinoid tumor in the lungs may have symptoms such as:

- Trouble breathing
- Chest pain
- Wheezing
- Coughing up blood

Sometimes these symptoms are diagnosed as pneumonia by mistake.

In rare cases, patients with carcinoid tumor may develop carcinoid syndrome. Carcinoid syndrome is a problem that develops from the tumors making hormones. Symptoms include:

- Feeling flushed
- Nausea and vomiting
- Diarrhea

Lab Tests: If you have symptoms of carcinoid tumor, your doctor will order lab tests of your urine or blood to check your hormone levels.

Imaging: Your doctor will use scans such as CT and MRI to see where the tumor is and how big it is. Different types of PET scans can also help find more fast-growing neuroendocrine cancer cells.

Biopsy: To check if the tumor is carcinoid tumor your doctor will do a biopsy, taking a small sample from the tumor with a needle. An expert, called a pathologist, will study cells from the sample under the microscope and run other tests to see what kind of tumor it is.

How is NET treated?

Treatment for each person will be unique. You should go to an expert in neuroendocrine tumor treatment to decide the best approach for your tumor. You can contact MyPART for help finding experts near you.

Surgery: If you have a carcinoid tumor, you may have surgery to remove the tumor and some surrounding tissue. Surgery is the best option for treating carcinoid tumor and preventing it from spreading.

When the carcinoid tumor is large or the cancer cells have spread to other parts of the body other treatments may include:

Somatostatin analogs: Somatostatin analogs are a type of treatment that may stop your body from making too many hormones. This may slow down the growth of the tumor when cancer cells have spread to other part of the body

Targeted therapy: Targeted therapy is a type of treatment that uses drugs that target certain genes or proteins to kill cancer cells. Neuroendocrine tumor cells have receptors on the surface of the cells called somatostatin. A type of targeted therapy called peptide receptor radionuclide therapy (PRRT) can target these cells.

Chemotherapy: is a type of treatment that uses stronger drugs to kill fasting growing cells.

Does NET run in families?

NET does not seem to run in families. But people with a genetic condition that can run in families called multiple endocrine neoplasia type 1 (MEN1) do have a higher risk of getting carcinoid tumor.

How does NET form?

We do not know what causes NET to form. Scientists are always working to understand how cancer starts, but it can be hard to prove. We know that patients with a condition called multiple endocrine neoplasia type 1 (MEN1) with changes in the gene called *MEN1* have a higher chance of developing bronchial and intestinal carcinoid tumor. So this gene may play a role in NETs of the lung and digestive tract.

What is the prognosis for someone with carcinoid tumor?

The estimate of how a disease will affect you long-term is called prognosis. Each person is different and prognosis will depend on many factors, such as:

- Where the tumor is in your body
- If the cancer has spread to other parts of your body
- How much of the tumor was taken out during surgery

Doctors estimate NET survival rates by how groups of people with NET have done in the past. In children, because there are so few cases of NET, these rates may not be very accurate.

The prognosis for children and young adults who have surgery to remove the tumor have a very good prognosis. Some studies show the 5-year survival rate for children and young adults with bronchial NET that has been removed is over 90%. Prognosis for people whose carcinoid tumor has spread to other parts of the body may be lower.

3. Paraganglioma

What is paraganglioma?

Paraganglioma is a type of neuroendocrine tumor that forms near certain blood vessels and nerves outside of the adrenal glands. The adrenal glands are important for making hormones that control many functions in the body and are located on top of the kidneys. The nerve cells involved in

paraganglioma are part of the peripheral nervous system, meaning the part of the nervous system outside of the brain and spinal cord. These tumors can also be called extra-adrenal pheochromocytomas. Approximately 35-50% of paragangliomas may spread to other parts of the body.

How common is paraganglioma?

Paraganglioma is rare and it is estimated that only 2 people out of every 1 million people have paraganglioma. It is most often found in people aged 30 to 50 years old.

How is paraganglioma diagnosed?

Some people with paraganglioma have symptoms, but others don't. Symptoms can include:

- High blood pressure
- Fast heartbeat
- Sweating
- Headache
- Shaking or tremors

Lab Tests: If you have symptoms of paraganglioma, your doctor will order lab tests of your urine and blood to check your hormone levels.

Imaging: Your doctor will use imaging scans such as MRI, CT, and PET to look at where the tumor is and how big it is. They will also check for signs that the tumor has spread to other parts of the body.

How is paraganglioma treated?

Treatment of paraganglioma may involve many different doctors, including doctors who specialize in hormone disorders and doctors who diagnose and treat neuroendocrine tumors. Treatment options to discuss with your doctor include:

Medications: Your doctor may give you medications to control your symptoms, such as alpha blockers and may be followed by beta blockers, which are drugs to control high blood pressure.

Watch and wait: In some cases, the tumor grows very slowly. In this case it may be safest for your doctor to check your tumor regularly without treating it.

Surgery: Once paraganglioma is diagnosed, you may have surgery to remove the tumor. Sometimes surgery is not an option, in which case, your doctor will discuss other options with you.

Radiation therapy: Radiation therapy can be used to slow the tumors from growing and to help relieve symptoms.

It is important to talk with a team of specialists to decide what the right treatment is for you. You can contact MyPART to get help finding specialists.

Does paraganglioma run in families?

Yes, paraganglioma can run in families, but not always. Some of these inherited cases may be associated with a genetic condition, such as Multiple Endocrine Neoplasia Types 2a and 2b, Von Hippel-Lindau Syndrome, and Neurofibromatosis type 1.

How does paraganglioma form?

Scientists have found mutations in approximately 20 different genes that they think may lead to pheochromocytoma and paraganglioma. Mutations in the genes *RET*, *VHL*, *NF1*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *MDH2*, *IDH1*, *PHD1*/*PHD2*, *HIF2A*/*EPAS1*/*2*, *TMEM127*, *MAX*, *HRAS*, *MAML3* and *CSDE1* may play a role in forming pheochromocytoma and paraganglioma.

Scientists have found that some genetic conditions may be associated with having paraganglioma.
These genetic conditions include:

- Multiple endocrine neoplasia 2 syndrome, types A and B (MEN2A and MEN2B)
- von Hippel-Lindau (VHL) syndrome
- Neurofibromatosis type 1 (NF1)
- Hereditary paraganglioma syndrome
- Carney-Stratakis dyad (paraganglioma and gastrointestinal stromal tumor [GIST])
- Carney triad (paraganglioma, GIST, and pulmonary chondroma)

What is the prognosis for someone with paraganglioma?

The estimate of how a disease will affect you long-term is called prognosis. Every person is different and prognosis will depend on many factors, such as:

- Where the tumor is in your body
- If the cancer has spread to other parts of your body
- How much of the tumor was taken out during surgery

Patients with a small paraganglioma that has not spread to other parts of the body have a five-year survival rate of about 95%. Patients with paraganglioma that has grown back (recurred) or spread to other parts of the body have a five-year survival rate between 34% and 60%.

4. Pheochromocytoma

What is pheochromocytoma?

Pheochromocytoma is a type of neuroendocrine tumor that grows from cells called chromaffin cells. These cells produce hormones needed for the body and are found in the adrenal glands. The adrenal glands are small organs located in the upper region of the abdomen on top of the kidneys.

About 80-85% of pheochromocytomas grow in the inner layer of the adrenal gland, called the adrenal medulla. About 15-20% of pheochromocytomas grow outside of this area and are called extra-adrenal pheochromocytomas or paragangliomas.

Most pheochromocytomas are benign, which means they are not cancer and do not spread to other parts of the body. Only about 10% of pheochromocytomas spread to other parts of the body.

How common is pheochromocytoma?

It is unknown how many people have pheochromocytoma because many people are never diagnosed. Most cases of pheochromocytoma occur in people aged 30 to 50 years old. One estimate suggests about only 8 people per 1 million people have pheochromocytoma, but this estimate may be low.

How is pheochromocytoma diagnosed?

Some people with pheochromocytoma have symptoms, but others don't. Symptoms may occur as often as several times a day to a couple of times per month. Some people may feel intense symptoms that last for a short period of time, called "paroxysmal attacks". These symptoms can include:

- High blood pressure
- Headaches
- Irregular heartbeat
- Sweating

Lab Tests: If you have symptoms of pheochromocytoma, your doctor will order lab tests of your urine and blood to check your hormone levels.

Imaging: Your doctor will use imaging scans such as CT, MRI, and PET to look at where the tumor is and how big it is. They will also check for signs that the tumor has spread to other parts of the body.

How is pheochromocytoma treated?

Treatment of pheochromocytoma may involve many different doctors, including doctors who specialize in hormone disorders and doctors who diagnose and treat cancer. Treatment options to discuss with your doctor include:

Medications: Your doctor may give you medications to control your symptoms, such as alpha blockers and beta blockers, which are drugs to control high blood pressure.

Surgery: Surgery is used to remove as much of the tumor as possible. In some cases, the entire adrenal gland may be removed.

Radiation therapy and chemotherapy: Radiation and chemotherapy treatments are used when pheochromocytoma has spread to other parts of the body.

Does pheochromocytoma run in families?

In some cases, pheochromocytoma can run in families. About 25-35% of cases of pheochromocytoma may be inherited. Some of these inherited cases may be associated with a genetic condition, such as Multiple Endocrine Neoplasia Types 2a and 2b, Von Hippel-Lindau Syndrome, and Neurofibromatosis.

How does pheochromocytoma form?

Scientists have found mutations in approximately 20 different genes that they think may lead to pheochromocytoma and paraganglioma. Mutations in the genes *RET*, *VHL*, *NF1*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *MDH2*, *IDH1*, *PHD1*/*PHD2*, *HIF2A*/*EPAS1*/*2*, *TMEM127*, *MAX*, *HRAS*, *MAML3* and *CSDE1* may play a role in forming pheochromocytoma and paragangliomas. In many cases, it is not known what causes pheochromocytoma to form

If you have pheochromocytoma, you may have other genetic conditions that increased your chance of getting pheochromocytoma. These genetic conditions include:

- Multiple endocrine neoplasia 2 syndrome, types A and B (MEN2A and MEN2B)
- Von Hippel-Lindau (VHL) syndrome
- Neurofibromatosis type 1 (NF1)
- Hereditary paraganglioma syndrome
- Carney-Stratakis dyad (paraganglioma and gastrointestinal stromal tumor [GIST])
- Carney triad (paraganglioma, GIST, and pulmonary chondroma)

What is the prognosis for someone with pheochromocytoma?

The estimate of how a disease will affect you long-term is called prognosis. Every person is different and prognosis will depend on many factors, such as:

- Where the tumor is in your body
- If the cancer has spread to other parts of your body
- How much of the tumor was taken out during surgery

Doctors estimate pheochromocytoma survival rates by how groups of people with pheochromocytoma have done in the past. Patients with a small pheochromocytoma that has not spread to other parts of the body have a five-year survival rate of about 95%. Patients with pheochromocytoma that has grown back (recurred) or spread to other parts of the body have a five-year survival rate between 34% and 60%.

5. Adrenocortical Carcinoma (ACC)

What is adrenocortical carcinoma?

Adrenocortical carcinoma, or ACC, is a cancer of the adrenal glands, which are two small triangular-shaped glands that sit on top of each kidney. The outside of these glands is called the adrenal cortex. The adrenal cortex makes important hormones that help your body control water

balance, blood pressure, stress response, and cause the body to have male or female traits. ACCs form in the adrenal cortex.

An ACC may be functioning, which means it makes more hormone than normal, or non-functioning, which means it has no effect on hormone production. A functioning ACC tumor often makes too much of the hormones cortisol, aldosterone, testosterone, or estrogen.

How common is adrenocortical carcinoma?

ACC is very rare, affecting around one case diagnosed in one million people in the US. It is more common in females than males.

How is adrenocortical carcinoma diagnosed?

ACC can cause pain in the abdomen, high blood pressure, acne, overgrowth of hair, and voice deepening. Other symptoms of ACC are different for females and males, since it can change hormone levels. Females may have an overgrowth in female genitalia and facial hair. Males may have abnormal penis growth or early puberty changes like increased muscle growth and body hair.

Lab Tests: If you have symptoms of ACC, your doctor will order lab tests of your urine and blood to check your hormone levels.

Imaging: Your doctor will use imaging tests such as ultrasound, CT, X-ray, PET, and MRI scans to look at the size of the tumor and whether it has spread to other parts of your body.

Biopsy: To check if the tumor is ACC, your doctor may perform a biopsy, taking a small sample from the tumor with a needle. A pathologist will study cells from the sample under the microscope to see what kind of tumor it is.

ACC is rare but, another type of tumor in the adrenal glands, adrenocortical adenoma, is quite common. Adrenocortical adenoma is not as dangerous as ACC. It can be difficult to tell the difference between them because they are both found in the adrenal glands and the cells can look similar. Getting the correct diagnosis is very important to determine the best treatment.

How is adrenocortical carcinoma treated?

Surgery: Surgery is used to remove as much of the ACC as possible. Small ACCs are often cured with surgery.

Chemotherapy: When the ACC tumors are large, or the cancer cells have spread to other parts of the body, chemotherapy is used with surgery.

Does adrenocortical carcinoma run in families?

ACC runs in families 50% of the time. Genetic testing is recommended for all close relatives of people with ACC.

When you have ACC, you may have other conditions that increase your chance of getting cancer. Genetic testing helps determine if you or your family members are at risk of developing ACC and other diseases. Genetic counseling is often recommended to help you understand the risks to you and your family members.

How does adrenocortical carcinoma form?

Scientists are always working to understand how cancer forms but it can be hard to prove. Because ACC can run in families, we know that changes in genes linked to Li-Fraumeni Syndrome, Beckwith-Wiedemann Syndrome, and Carney complex are important in causing ACC.

What is the prognosis for someone with adrenocortical carcinoma?

The estimate of how a disease will affect you long-term is called prognosis. Every person is different and prognosis will depend on many factors, such as:

- Where the tumor is in your body
- If the cancer has spread to other parts of your body
- How much of the tumor was taken out during surgery

If you want information on your prognosis, it is important to talk to your doctor. NCI also has resources to help you understand cancer prognosis.

Doctors estimate ACC survival rates by how groups of people with ACC have done in the past. Because there are so few people with ACC, these rates may not be very accurate. They also don't consider newer treatments being developed.

If the ACC is small when it is found, prognosis is good and cure is likely. If the ACC is already large or has spread to other parts of the body, treatment is more difficult and the five-year survival rate is 36% to 46%.

6. Medullary Thyroid Cancer (MTC)

What is medullary thyroid cancer?

Medullary thyroid cancer, or MTC, is a cancer that forms in the thyroid. The thyroid is a gland located in the front of your neck, just below the Adam's apple. It is responsible for sending out hormones to the rest of your body. The inside of the thyroid is called the medulla. The medulla contains special cells called parafollicular C cells that produce and release hormones. MTC happens when the C cells become cancerous and grow out of control. MTC may also be called medullary thyroid carcinoma.

How common is medullary thyroid cancer?

Thyroid cancer is fairly common. There are four different types of thyroid cancers and MTC is the rarest type making up 3% to 4% of all thyroid cancers. About 1,000 people are diagnosed with MTC each year in the U.S.

How is medullary thyroid cancer diagnosed?

MTC can start as a lump in the throat. The tumor growing in the thyroid can make your voice hoarse by blocking your vocal chords or it can make it hard to breathe by blocking your windpipe. Sometimes people can have MTC for a long time without symptoms because the tumor remains small. MTC can spread to other organs, such as lung, liver, bones, and brain.

Imaging: MTC is diagnosed by your doctor first feeling your throat to check for a lump, followed by imaging scans of the thyroid. Imaging scans might include ultrasound, CT, or MRI.

Biopsy: The doctor will also want to take out a small amount of tissue, called a biopsy, from the thyroid using a very thin needle. A pathologist will look at the tissue under the microscope to see if there are cancer cells and, if so, what type of thyroid cancer it is.

How is medullary thyroid cancer treated?

MTC is usually treated by removing the thyroid. This surgery is called a thyroidectomy. In certain people with a high risk for MTC, such as people carrying certain gene changes, a thyroidectomy may be performed to prevent cancer. Besides surgery, sometimes other treatments are also required, including radiation therapy or chemotherapy. Also, targeted therapies are available that act on changes in DNA found in some cases of MTC. After treatment, your doctor will monitor levels of a tumor marker called CEA and the hormones produced by C cells to keep track of how well the treatment is working or if cancer has come back. CEA is a type of tumor marker found in the blood of those with MTC.

Does medullary thyroid cancer run in families?

Twenty-five percent of MTC cases run in families. MTC may be passed down when families carry a change in the *RET* gene that causes a condition called multiple endocrine neoplasia type 2, or MEN2. There are two types of MEN2: MEN2A and MEN2B.

MEN2A: If you have MEN2A, you have a high chance (90%) of getting MTC. You are also at risk (30% to 50%) for getting pheochromocytoma, a cancer of the adrenal glands. MEN2A is rare, affecting 1 in 40,000 people. MEN2A may also be called Sipple syndrome or PTC syndrome.

MEN2B: MEN2B can sometimes be passed from parent to child but most of the time, it isn't. If you have MEN2B, you have a 100% chance of getting MTC at a very young age. You also have a 50% chance of getting pheochromocytoma at some point in your life. MEN2B is also called Wagenmann–Froboese syndrome or MEN3.

How does medullary thyroid cancer form?

Scientists are always working to understand how cancer forms but it can be hard to prove. We know that some MTC cases have changes in the *RET* gene. MTC is also more common in females than males. This information gives scientists clues about how MTC forms and can lead to new treatments.

What is the prognosis for someone with medullary thyroid cancer?

The estimate of how a disease will affect you long term is called prognosis. Every person is different and prognosis will depend on many factors, such as:

- If the cancer has spread to other parts of your body
- If the cancer responds to chemotherapy
- How much of the tumor was taken out during surgery

Doctors estimate MTC survival rates by how groups of people with MTC patients have done in the past. Given that there are so few MTC patients, survival rates may not be very accurate. They also don't consider newer treatments being developed. We know that people can live with MTC for many years, even though there is no cure.

Probable Questions:

1. Discuss different types of endocrine cancer.
2. Discuss causes of endocrine cancer.
3. Discuss risk of endocrine cancers.
4. How diagnosis can be done of endocrine cancers?
5. Discuss treatment of endocrine cancers.
6. Discuss symptoms, risk factors and treatment of adrenal cancer.
7. Discuss symptoms, risk factors and treatment of parathyroid cancer.
8. Discuss symptoms, risk factors and treatment of neuroendocrine cancer.
9. Discuss symptoms, risk factors and treatment of pituitary cancer.
10. Discuss rare endocrine cancers.

Suggested Readings:

1. Molecular Cell Biology by Lodish, Fourth Edition.
2. The Cell – A Molecular Approach by Cooper and Hausman, Fourth Edition
3. Principles of Genetics by Snustad and Simmons, Sixth Edition.
4. Molecular Biology of the Cell – by Bruce Alberts
5. Cell and Molecular Biology by Gerald Karp, 7th Edition.
6. Gene cloning and DNA Analysis by T. A. Brown, Sixth Edition.
7. Genetics . Verma and Agarwal.

UNIT-XX

Hormone signaling pathways (G-protein coupled receptors, Receptor Tyrosine Kinases, and steroid hormone signalling)

Objective : In this unit we Will discuss different kinds of Hormone signalling pathways including G-protein coupled receptors, Receptor Tyrosine Kinases, and steroid hormone signaling.

Introduction:

Eukaryotic cells and bacteria release a large number of signals and establish communication. The method of action is binding the signals with the protein receptors present on surface of large cell and triggering a series of intracellular reactions called intracellular signaling or signal transduction.

Besides, many signals (steroids and bacterial autoinducer) enter the cell, interact with signaling system and establish signal transduction. For establishing intracellular signaling, one must fully understand the operation of any cell from their origin to death.

The first signaling molecule (cyclic adenosine monophosphate or cAMP) was known during early 1960s. However, importance of intracellular signaling could be realized after the discovery of changes made by mutagenesis in signaling pathway which results in cellular transformation which is now called as cancer. One could understand their function by mutagenizing their cellularfunction.

It is now known that bacteria can change the eukaryotic cell signaling and invade the cells. The bacterial toxins can hijack the control of host cells. Similarly complexity of bacterial signaling is also known. Now an enormous amount of information is available on cell signaling and signal transduction pathway both in prokaryotes and eukaryotes.

The Signaling System:

Signaling system is very complex which may be compared to electronic circuits. You know that electronic system is such that can integrate, modulate and amplify inputs and generate output signals when switched on or switched off after getting suitable signals. The signaling systems include few basic type of modules. There are four main processes, but the signaling system uses the one or more processes.

The types of signaling modules used in intracellular signaling are:

(a) Receptor kinases (e.g. tyrosine kinase, serine kinase, histidinekinase),

(b) Receptor non-kinases (e.g. serpentine, cytokine, His-Aspphosphorelay),

(c) Protein kinase (intracellular enzymes e.g. cyclin families, Aspkinase),

(d) Lipid modifying intracellular enzymes (e.g. p13K, p15K,PLC),

(e) Cyclic nucleotides (e.g. cAMP, cGMP),and

(f) Metal ions (e.g.Ca^{++})

The four main processes are as follows:

(a) Protein phosphorylation bykinases,

(b) Small molecule and protein interaction, often involvingphosphates,

(c) Protein-protein interaction, often mediated by common motifs (specific protein sequences) which frequently results in membrane recruitment when one component is tethered to a membrane,and

(d) Protein and DNA interaction that promotes gene expression or geneinhibition.

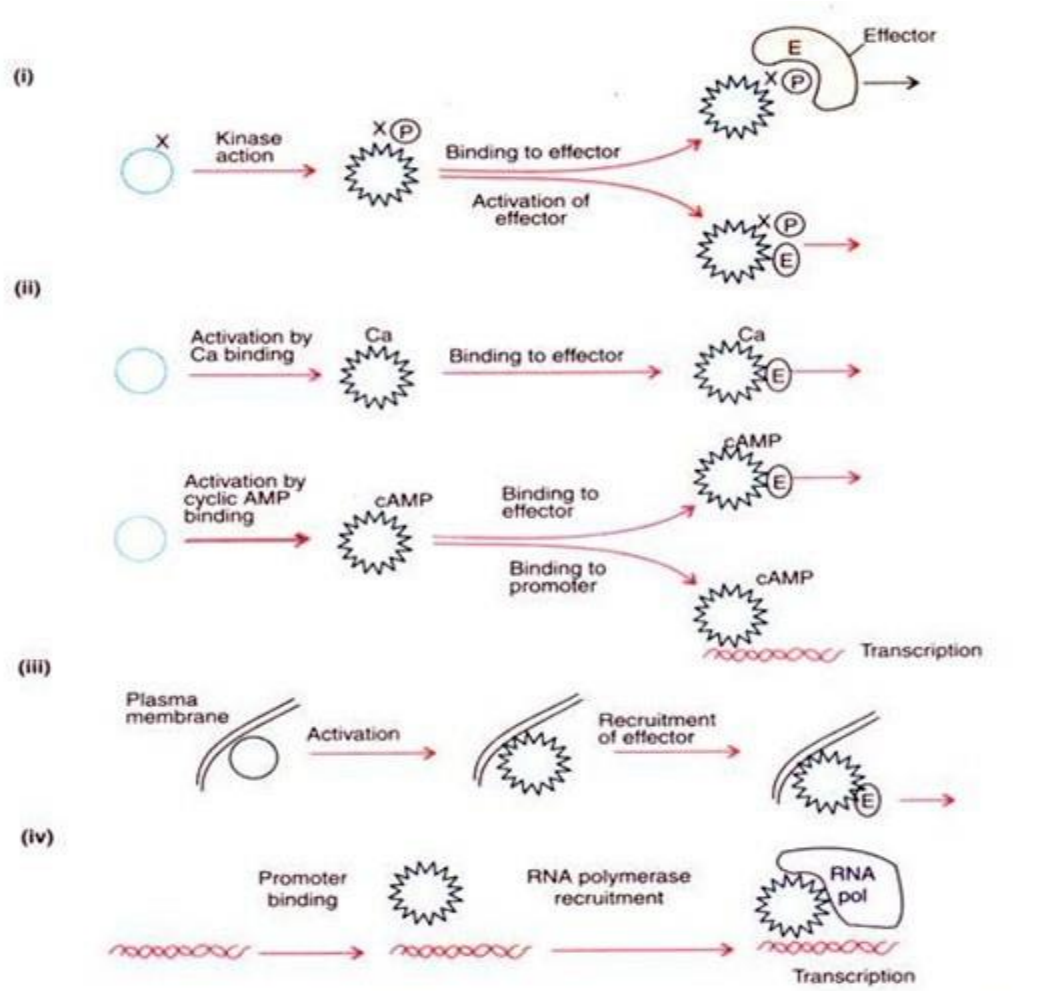


Fig. 27.8: Signalling molecules that use different types of interactions. *(i)* protein phosphorylation, where X=Tyr, Ser, Thr, His or Asp. *(ii)* interaction between small molecules and proteins, e.g. Ca^{++}, or cAMP. *(iii)* interaction between protein and protein, and *(iv)* interaction between protein and DNA which regulates transcription.

The Basic Building Blocks used in Signalling:

(a) Protein Phosphorylation: Protein phosphorylation is closely linked to cellular signaling. It exits in all signaling modules. The terminal y-phosphate is directly transferred from ARP (in some cases) to an acceptor protein by a protein kinase. The activity of the acceptor is modified example mitogen-activated protein (MAP) kinases in eukaryotes and histidyl-aspartyl phosphorelay in bacteria.

In some cases, indirect phosphorylation of protein also occurs (e.g. in G protein when binding of GTP activates their function, while GDP binding inactivates). There are secondary messengers which are used in intracellular signaling such as phosphorylated inositol's or cyclic nucleotides (cAMP, cGMP).

Kinases are regulated by any of a number of mechanisms: threonine and/or tyrosine phosphorylation, legend occupancy resulting in autophosphorylation or interaction with small molecules (e.g. cAMP or Ca^{++})

i. Histidine Kinases:

These are found in bacteria, lower eukaryotes and plants as trans membrane protein. They are stimulated to undergo self-phosphorylation by ligand occupancy.

ii. Protein Phosphatases:

Proteins which remove phosphate groups from proteins are called protein phosphatases. Protein kinases add phosphate group to proteins and play a key role in activation of signals.Specific phosphatases e.g.dephosphorylatephosphotyrosineand phosphoserine/phosphothreonme play a key role in control of proliferation, differentiation and cell cycle Phosphoproteins take part in signaling. They moderate the phosphorylation status by regulating the balance of phosphatases and kinases.

(b) Nucleotide-BindingProteins:

The three nucleotides (GTP, cGMP and cAMP) play a major role in the intracellular signaling.

iii. GTP-BindingProteins:

There is a set of eukaryotic proteins (G proteins) that show GTPase activity. They bind to GTP and remove the terminal phosphate of GTP and produce GDP bound to G protein. This cycle operates similar to ATP and ADP cycles.
When GDP dissociates from the G protein and GTP binds again, the cycle is repeated (Fig. 27. 9). G proteins are of two type: the heterotrimeric G proteins (the dominating proteins), and the small G proteins or membrane of Ras super family (the intermediate member of the signaling pathways).

The heterotrimeric G proteins consist of three different subunits- α, β and y subunits The a- subunit has GTP-binding domain; hence Gβ has a role in signal transduction.The βy subunits transmit signals by non-covalent interaction with effector molecules. Activation of G proteins and Association of α-subunit from βy subunits are given in Fig. 27.9. GTPase activity results in after binding the Gα subunit with GDP and subsequent association with Gβy and down regulation

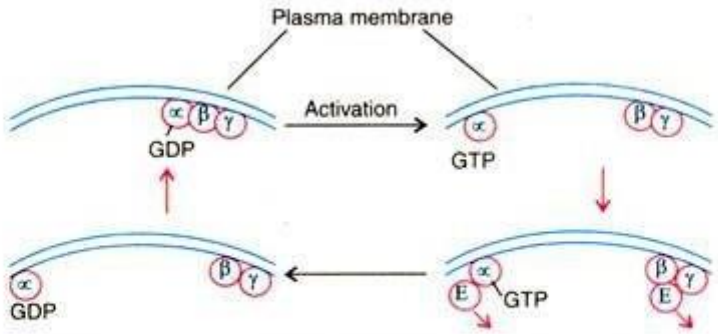


Fig. 27.9 : The function of membrane-bound heterotrimeric G proteins having α, β and γ subunits.

The small G proteins (Ras super family or p21 family) play a key role in many cellular functions such as proliferation and differentiation (Ras family), cytoskeletal organization (Rho) and nuclear membrane transport (Ran). The activity of small G proteins is modulated after interaction with several classes of proteins (Fig.27.10).

GDP-dissociation inhibitors (GDI) inhibit the loss of bound GDP and keep the G proteins in an inactive form to attenuate signaling from the activated G proteins. GTPase activity IS stimulated by GTPase-activating proteins (GAP). The removal of the bound GDP IS helped by guaninenucleotide exchange factors (GEF) which enable the GTP to bind and activate G proteins. Some of these factors have shown to be proto-oncogenes.

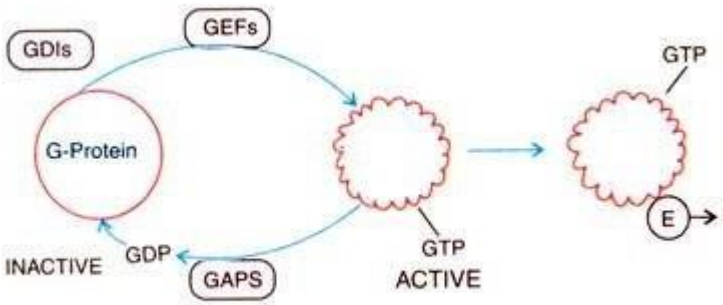


Fig. 27.10 : Functioning of small G proteins.

iv. Cyclin Nucleotide-BindingProteins:

In 1950s, cAMP was identified as the first intracellular signaling molecules. It mediates hormone action and acts as molecules transmitting the primary signal that has been received at the cell membrane). The cAMP mediates the response to chemo-attractants. The adenylate cyclase and guanylate cyclase regulate the concentration of cAMP and cGMP,respectively.

The soluble bacterial adenylate cyclases produce cAMP which binds to c AMP receptor protein (CRP) and activate them. CRP is a transcription factor. The cAMP influences the expression of many of genes. Consequently bacteria become able to express metabolic enzymes which are required during growth. The cAMP also regulates the expression of the other genes which can cause pathogenesis.In eukaryotes heterotrimeric G proteins regulate the membrane-bound adenylate cyclases which produce cAMP. G proteins are coupled to transmembrane receptors. The cAMP-dependent protein kinases (protein kinase A) are the main effects of the cAMP signals.

While in the inactive form, protein kinase A consists of a dimer of regulatory (A) subunits and two catalytic (C) sub- units. The molecules of cAMP binds to reach R subunits and induceconformational changes. Consequently activated C subunits are released. This activated protein kinase A phosphorylates many substrates on serine or threonine (Fig. 27.11).

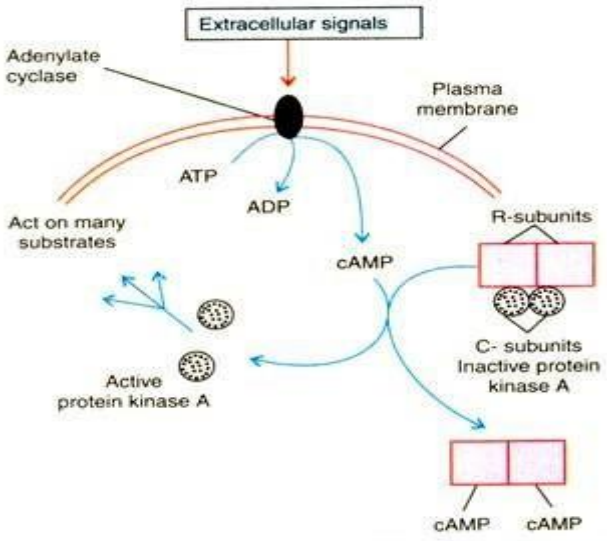


Fig. 27.11 : Production of cAMP and activation of protein kinase A.

Both the cycles work in eukaryotes by direct binding to proteins which form cation channels. Binding events result in opening of the channel.The G-protein-linked cell surface receptor generates small intracellular mediators thought cAMP pathways (Fig. 27.12).

(c) Role of Intracellular Concentration of Ca^{++} in CellSignaling:

Calcium is found in Cytoplasm and maintained in a very low concentration(10-100 nM). But its concentration varies with cell cycle, exogenous source or release from the stores. It gets complexes in membrane bound vesicles acting as stores. A highly specific protein calmodulin (CaM) binds to Ca^{++} and transmit the signal. Ca-binding to CaM brings about changes in conformation of CaM.

Consequently CaM interacts with many effectors including CaM-modulated kinase. The most extensively studied CaM is the phosphatase calcineurin which is associated with several cellular activities such as NO synthesis, apoptosis, and induction of T lymphocytes. In eukaryotic cells Ca^{++} acts as a second messenger. Fig. 27.12 shows the two major pathways by which G-protein- linked cell surface receptors generate small intracellular mediators.

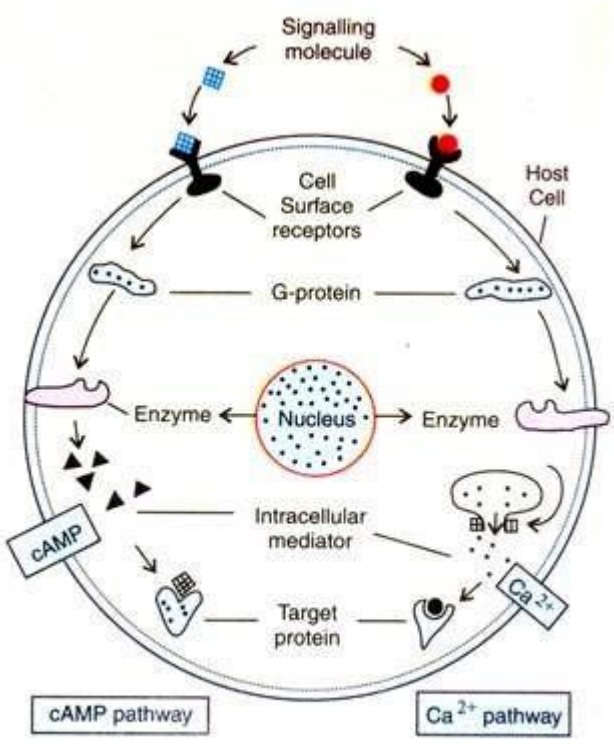


Fig. 27.12 : Generation of small intracellular mediators by G-protein-linked cell.

Role of Phosphorylated Lipids in Cell Signaling:

In eukaryotes lipids are involved in signaling process. Cellular phospholipases attack the lipid moieties of the membrane to produce different types of signaling molecules. For example, phosphatidylinositol lipids play a role in cellular stimulation. They have inositol as head, the six- membered carbon ring with a -OH group on each carbon.

On the basis of phosphorylation status of inositol head group, several phosphatidylinositols are found in the cells. The activity of three enzymes triggers their signaling role. These are:

phosphoinositide 5′-kinase (P15p, phosphoinositide 3′-kinase (P13K), and phospholipase C (PLC). Extracellular signals regulate all these enzymes.

(d) Regulation ofTranscription:

Both types of cells are able to respond to any signal by changing their gene expression. In a signaling pathway the end point acts as signal. Regulators causing changes in expression of many genes in bacteria are called 'global regulators'. In prokaryotes post-transcriptional events regulate expression of many of the transcriptional factors for example cAMP-mediated CRP- DNA interactions.

In prokaryotes, phosphorylation or protein-protein interactions regulate the control of transcriptional factors and also select the other factors to the promotors. Besides, some other factors also get translocated from cytoplasm to the nucleus and regulate transcription.

(e) Role of Cell Membrane inSignaling:

Cell membrane acts as boundary of the cell through which extracellular signal has to enter. In bacteria histidine kinases act as receptor and directs signals across the membrane. Besides, there are many signal molecules which are associated with cell membrane because the end effect is membrane-associated.

The components can be well organized in three-dimensional way in cell membrane. The signaling components recruit the other molecules to the membrane where they interact with other factors. For example, GTP-bound Ras activates Raf kinase to recruit Raf to the membrane where the membrane-bound kinase activates it throughphosphorylation.

3. Prokaryotic SignallingMechanisms:

Intracellular signaling is very complex like electronic circuit. Genome size of different bacteria varies and those organisms work according to genes present in them. In bacteria the generic mechanism of regulation is called signaling systems which includes:

a. The histidyl-aspartae phosphorylation systems (the main module of bacteria used to receive and process incoming signals such as chemotaxis, response to osmolarity, oxygen and phosphate, and virulencesystem),

c. The cAMP and CRP (involved in regulation of hundreds of genes. The cAMP is controlled at transcriptional and post-transcriptional levels. Binding of CRP- cAMP complex induces gene expression).

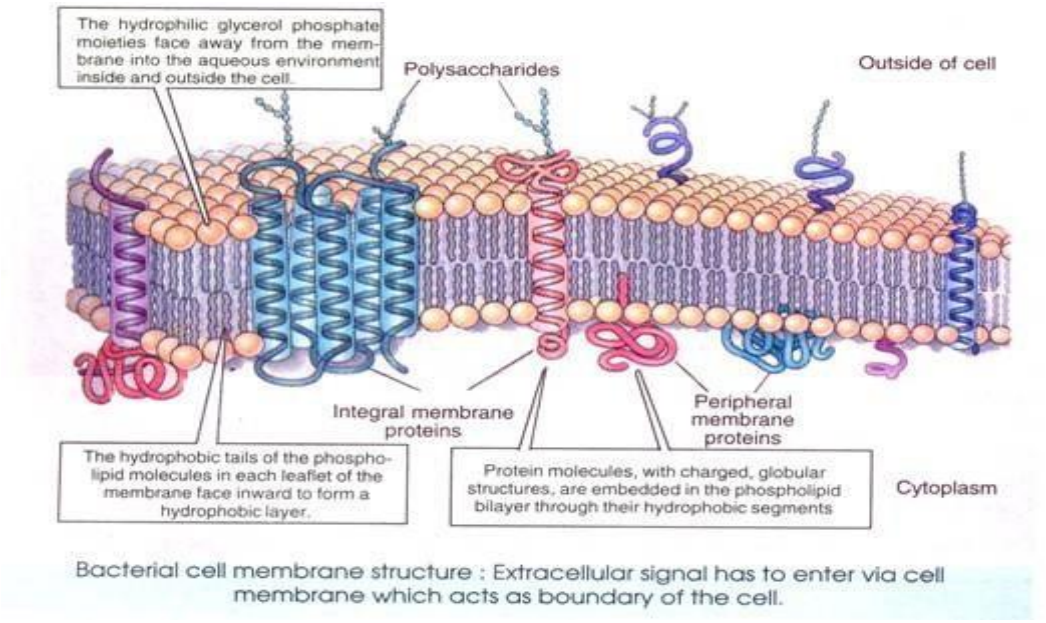
4. Eukaryotic SignallingPathway:

Earlier it was thought that signaling process in eukaryotes was very complex to understand in molecular terms. Fragmented understanding about individual components could be known.The knowledge of signaling expanded with the development of new techniques such as genome sequencing, increasing number of reagents (isolated components, specific probes e.g. antibodies for individual components and selective inhibitors).

In spite of all these, no pathway has been fully elucidated. The best characterized pathway is the Ras activation and MAP kinases of which several details are unclarified. They are interconnected and cannot work without reference to others.

The Phospholipase C/Inositol Triphosphate Pathway:

The phospholipase C, beta or gamma is activated by membrane signaling events and cleaves PIP2 to produce diacylyglycerol (DAG) and inositol triphosphate (IP_3). These activates the release of Ca^{++} ions and results in activation of protein kinase C (PKC), which phosphorylates many additional protein substrates.



Bacterial cell membrane structure : Extracellular signal has to enter via cell membrane which acts as boundary of the cell.

The Adenylate Cyclase, cAMP and Protein Kinase A Pathway:

Adenylate cyclase is activated at the membrane by interaction with the activated heterotrimeric G protein G_5. The cAMP is generated and binds to and activates protein kinase A (PKA), which phosphorylates manysubstrates.

Integrin's, the Rho Family and Organization of Cytoskeletal:

The integrins are the signalling molecules that interact with theextracellular matrix on the outside of the cell and various proteins-linked to actin on the cells interior. The proteins involved include α-actin, lalin, tensin, vinculin andpavilin.

A local adhesion is formed upon activation that includes focal adhesion kinase (FAK). The Src kinase is recruited and several proteins in the complex are activated by phosphorylation by Src and FAC. These signals lead to the Ras/Raf, Rho signaling pathway and to cytoskeletal rearrangement.In eukaryotes, the central role of signaling pathway of a cell is to define its phenotype and function. The increasing novel knowledge about the components of signaling pathways and the types of genes which they interact are already being applied in new strategy to combat the cancer. For example, genetically engineered viruses are attempted to grow in such cells that lack functional p53 and kill thesecells.

There are about 2000-5000 signal transduction proteins in mammalian cells. Bacteria have capacity to utilize eukaryotic signaling pathway during the

process of infection. These findings make a line between the signaling pathways involved in infection and the other responsible for the pathology in diseases such as cancer and inflammation.

Eicosanoids (Gr. ecosn = 20):

These are derived from arachidonic acid, a C-20 fatty acid with 4 double bonds, e.g., prostaglandins, thrombaxanes and leukotriene's. These are called local hormones because they are short lived and have autocrine and paracrine effect.

Cytokine receptors:

Cytokine receptors are receptors that bind cytokines. In recent years, the cytokine receptors have come to demand the attention of more investigators than cytokines themselves, partly because of their remarkable characteristics, and partly because a deficiency of cytokine receptors has now been directly linked to certain debilitating immunodeficiency states. In this regard, and also because the redundancy and pleiotropy of cytokines are a consequence of their homologous receptors, many authorities are now of the opinion that a classification of cytokine receptors would be more clinically and experimentally useful.

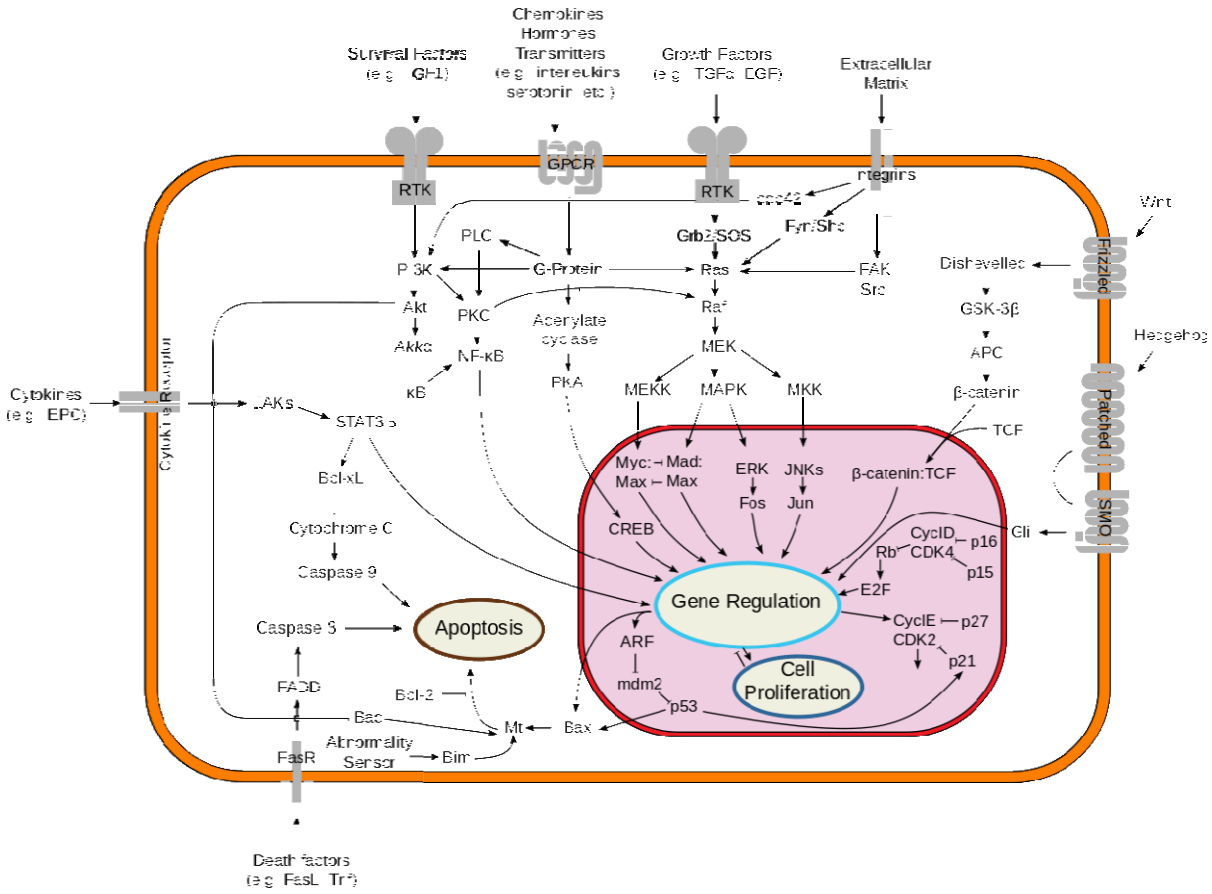


Fig : Signal transduction. (Cytokine receptor at center left.)

Classification of Cytokine Receptors

A classification of cytokine receptors based on their three- dimensional structure has been attempted.

(Such a classification, though seemingly cumbersome, provides several unique perspectives for

attractive pharmacotherapeutic targets.)

Type I cytokine receptors whose members have certain conserved motifs in their extracellular amino-acid domain. The IL-2 receptor belongs to this chain, whose γ chain (commonto several other cytokines) deficiency is directly responsible for the X-linked form of Severe Combined Immunodeficiency (X-SCID).

Type II cytokine receptors, whose members are receptors mainly for interferons.

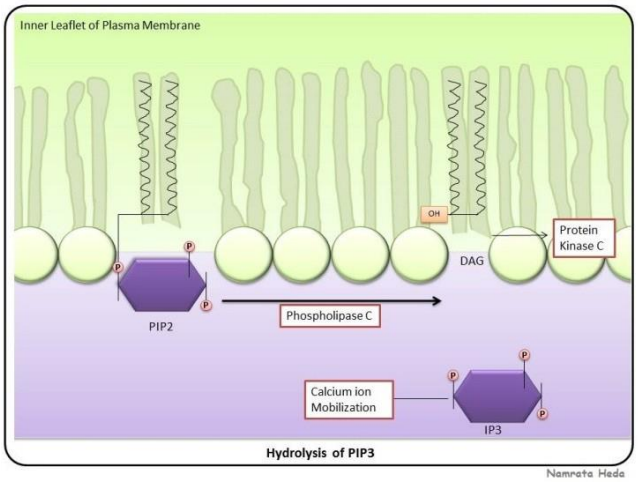
Immunoglobulin (Ig) superfamily, which are ubiquitously present throughout several cells and tissues of the vertebrate body

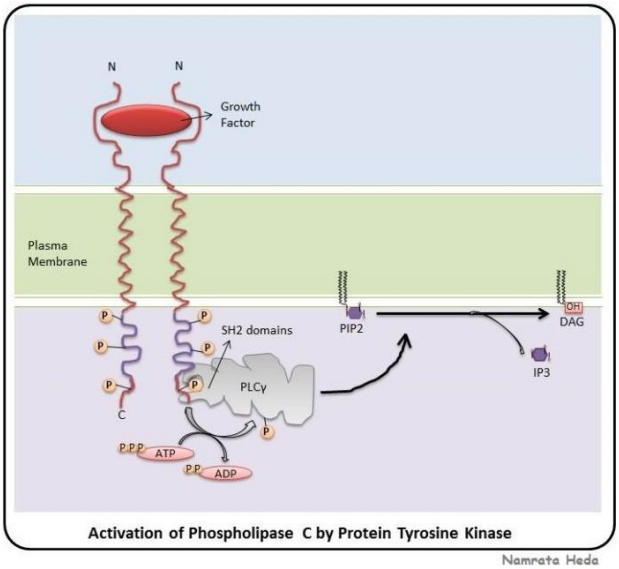
Tumor necrosis factor receptor family, whose members share a cysteine-rich common extracellular binding domain, and includes several other non-cytokine ligands like receptors, CD40, CD27 and CD30, besides the ligands on which the family is named (TNF).

Chemokine receptors, two of which acting as binding proteins for HIV (CXCR4 and CCR5). They are G protein coupled receptors.

Phospholipids and Ca ion mediated signaling :

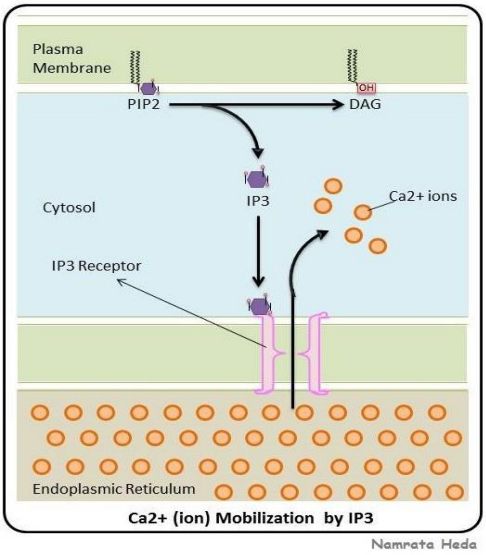
Phosphatidylinositol 4,5-bisphosphate abbreviated as PIP2 is a phospholipid present in the inner leaflet of the bilayer of the plasma membrane. The second messengers are derived from this small component (phospholipid) and the pathway is based on thesemessengers.





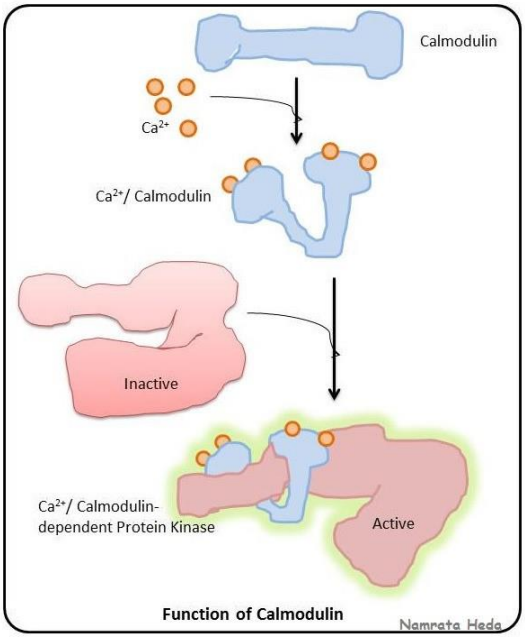
The hydrolysis of PIP2 takes place by the enzyme phospholipase C as can be seen in the adjacent figure. It is interesting to note that the enzyme phospholipase C is ultimately activated by G- protein coupled receptors (GPCRs) or protein tyrosine kinases. This is so because one form of phospholipase C (PLC-β) is stimulated by G proteins while another form of phospholipase C (PLC-γ) contains SH2 domains (as can be seen in the figure shown below) and hence it associates with activated receptor protein tyrosine kinases. This interaction helps PLC-γtolocalize to plasma membrane and also leads to its phosphorylation. This tyrosine phosphorylation increases PLC-γ activity, which in turn stimulates hydrolysis ofPIP2.

The hydrolysis of PIP2 produces two distinct second messengers as diacylglycerol and inositol 1,4,5-triphosphate which is abbreviated as IP3. Both these messengers stimulate different downstream signaling pathways thereby triggering two distinct cascades of intracellular signaling. Diacylglycerol stimulates protein kinase C mobilization while IP3 stimulates Ca2+(ions) mobilization. The diacylglycerol as second messenger activates serine/threonine kinases which belongs to the protein kinase C family which play an important role in cell growth anddifferentiation.IP3, another second messenger is released into the cytosol and it acts to release the Ca2+(ions) from intracellular stores. The level of the Ca2+(ions) inside the cell is very low and is maintained by pumping through Ca2+(ion) pumps across the plasma membrane.



The Ca2+(ions) are pumped into the ER and hence ER is considered to be the store of intracellular Ca2+(ions). Here, IP3 binds to the receptors in the ER membrane as can be seen in the adjacent diagram. These receptors are ligand-gated ion channels and hence, there is efflux of Ca2+(ions) into thecytosol.This increase of Ca2+(ions) in the cytosol has an effect on variety of proteins like protein kinases. For example, there are some members of protein kinase C (PKC) family that requires Ca2+(ions) as well as diacylglycerol for their functioning. Hence, these PKC family members are regulated by both IP3 anddiacylglycerol.

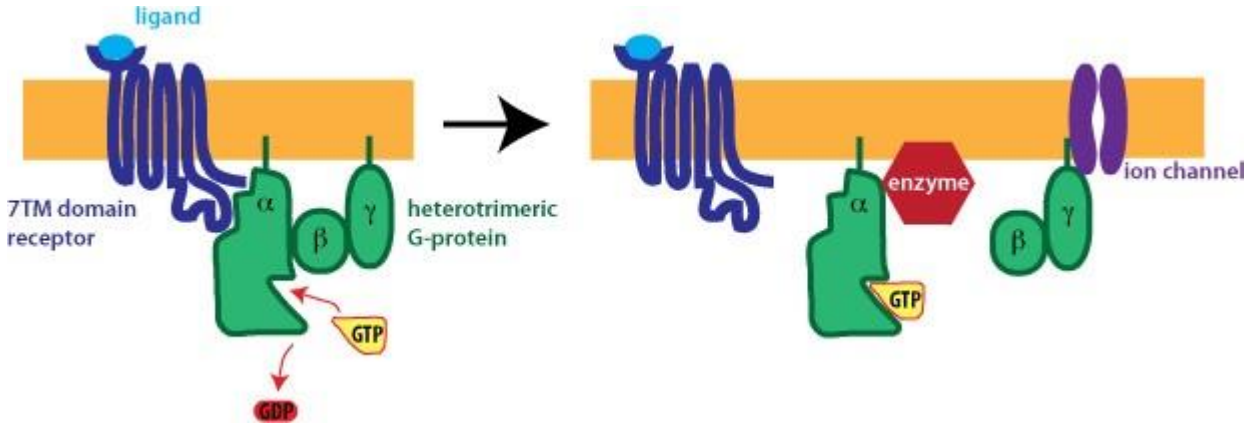
Calmodulin is another very important protein to mention while we are studying about Ca2+(ions). The word 'calmodulin' means - cal(cium) + modul(ate) + in(g). Thus, calmodulin is 'calcium modulating' protein that mediates most of the activities of Ca2+(ions). Calmodulin is dumbbell shaped protein which has four Ca2+(ions) binding sites (figure is shown below). When the Ca2+(ions) concentration in the cell increases, calmodulin is activated. This active Ca2+/calmodulin complex then binds to a variety of target proteins, like Ca2+ion/calmodulin - dependent protein kinases thereby rendering them active. The examples of Ca2+ion/calmodulin dependent-protein kinases are: myosin light-chain kinase and members.



When there is a change in plasma membrane's potential i.e.; when there is membrane depolarization, the voltage-gated Ca2+ ion channels are opened in the plasma membrane. Because of the opening, there is influx of Ca2+(ions) from the extracellular fluid into the cytosol of the cell. This increase in the levels of Ca2+(ions) further triggers the opening of the another receptor called the ryanodine receptor in the plasma membrane which further releases the Ca2+(ions) from the intracellular stores. This increase in the Ca2+(ions) results in triggering the release of neurotransmitter. Hence, we can say that Ca2+ ion plays an important role in converting electric signals to chemical signals. In muscle cells, the ryanodine receptors on the sarcoplasmic reticulum. These receptors maybe opened directly when there is membrane depolarization.

G-protein coupled receptors:

The largest family of cell surface receptors are the G-protein coupled receptors (GPCRs). There are hundreds of different GPCR proteins, and nearly a third of all drugs target this type of receptor. A diverse set of ligands bind to this type of receptor, including peptide hormones, neurotransmitters, and odor molecules. These receptors all have a similar structure with seven transmembrane domains. On the basis of their seven transmembrane domain structure, many GPCRs have been identified in the human genome. Proteins that were identified by sequence homology, but whose ligands are not known, are termed orphan receptors.



GPCRs associate with heterotrimeric G-proteins (green), that is, G-proteins composed of three different subunits: alpha, beta, and gamma. The subunits are tethered at the membrane surface by covalently attached lipidmolecules.

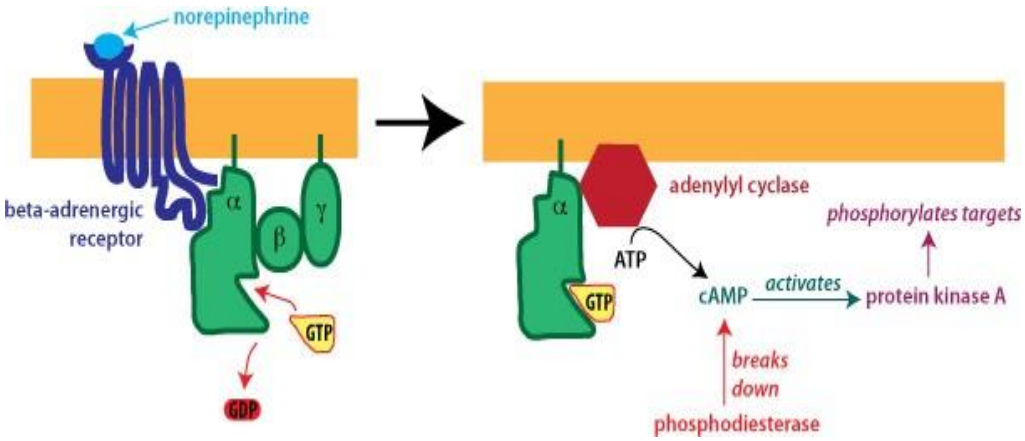
When a ligand binds, the receptor activates the attached G-protein by causing the exchange of GTP (yellow) for GDP (red). The activated G-protein then dissociates into an alpha (G-alpha) and a beta-gamma complex. G-alpha bound to GTP is active, and can diffuse along the membrane surface to activate (and sometimes inhibit) target proteins, often enzymes that generate second messengers. Likewise, the beta-gamma complex is also able to diffuse along the inner membrane surface and affect protein activity. Inactivation occurs because G-alpha has intrinsic GTPase activity. After GTP hydrolysis, G-alpha bound to GDP will reassociate with a beta-gamma complex to form an inactive G-protein that can again associate with areceptor.

The GTPase activity of the G-alpha can be made faster by other proteins--sometimes the target protein, sometimes a separate regulatory protein. Cholera toxin causes a chemical modification that *prevents* GTP hydrolysis and leads to unregulated signaling.

Different G-alpha proteins activate different second messenger pathways. There are several different classes of heterotrimeric G-proteins that are defined by their different G-alpha subunits. One type of G-alpha activates the enzyme adenylyl cyclase, which catalyzes the formation of the second messenger cyclic AMP (cAMP). Because an activated adenylyl cyclase can generate many molecules of cAMP, this is a means to amplify the signal. cAMP can have several effects, but a major effect is to bind to and activate protein kinase A (PKA; also known as cAMP- dependent kinase). PKA then phosphorylates target proteins in the cell. cAMP is rapidly broken down by phosphodiesterases, limiting the length of the signal.

A specific example of a receptor that couples to this type of G-protein is the beta-1 adrenergic receptor found in the heart. Beta 1 receptors are the

principal type of adrenergic receptor found in the heart. The ligand for this receptor is norepinephrine, the neurotransmitter that is released by sympathetic postganglionic neurons. (As well, the hormone epinephrine, released from the adrenal medulla, is also a ligand for these receptors.) Stimulation of beta-1 receptors causes increased cAMP and PKA activation. PKA phosphorylates various target proteins in cardiac cells to cause an increase in both the heart rate and the strength of cardiac muscle contraction. Beta-1 receptors are the targets of drugs (beta blockers) that are used to treat heart failure andhypertension.



Another example involving GPCR signaling that stimulates adenylyl cyclase is the regulation of secretion in the small intestine. This regulation is disrupted by cholera toxin. The effect ofcholera toxin is to lead to persistent activation of adenylyl cyclase because it destroys the GTPase activity of G-alpha. There is over-production of cAMP, continuous activation of PKA, and continuous phosphorylation of CFTR, causing excessive fluidsecretion.

A different type of G-alpha activates the enzyme phospholipase C. This type of G-alpha couples to various GPCRs found on smooth muscle, such as the oxytocin receptor shown in the example below

Phospholipase C is an enzyme that cuts PIP2, a membrane phospholipid, to generate two second messengers, IP_3 and diacylglycerol (DAG). IP_3 is water soluble, diffusing through the cytosol to bind to and open a ligand-gated Ca^{++} channel in the endoplasmic reticulum (or sarcoplasmic reticulum in muscle cells). Thus, stimulation of a receptor linked to this G-alpha is a way to increase Ca^{++} inside the cytosol. Ca^{++} in the cytosol exerts its effects by binding to Ca^{++}-binding proteins such as calmodulin. In the uterus, the increase in intracellular Ca^{++} that results from oxytocin signaling causes the smooth muscle to contract.DAG is lipid soluble and stays in the membrane. It

activates protein kinase C (PKC), which, like PKA phosphorylates particular target proteins.

Desensitization:

In the continuing p:resence of ligand, many GPCRs show desensitization. The mechanism is shown in the figure. A protein known as a G-protein Receptor Kinase (GRK) phosphorylates the receptor on particular residues. This increases its affinity for a protein called beta-arrestin (red), that binds to the receptor. This reduces signaling by *preventing* the association with the G-protein.

There are several potential outcomes once beta-arrestin binds to the receptor. One possibility is that beta-arrestin targets the receptor for endocytosis, leading to receptor downregulation (a decreased number of receptors on the cell surface). Another possibility is the activation of beta-arrestin-dependent signaling pathways that are independent of G-protein signaling. Beta-arrestincan act as a scaffold that binds and brings together other intracellular signaling proteins. The physiological significance of beta-arrestin-dependent signaling is still being worked out.

Steroid Hormone superfamily mediated signal transduction:

As already noted, all signaling molecules act by binding to receptors expressed by their target cells. In many cases, these receptors are expressed on the target cell surface, but some receptors are intracellular proteins located in the cytosol or the nucleus. These intracellular receptors respond to small hydrophobicsignalling molecules that are able to diffuse across the plasma membrane. The steroid hormones are the classic examples of this group of signaling molecules, which also includes thyroid hormone, vitamin D_3, and retinoic acid.

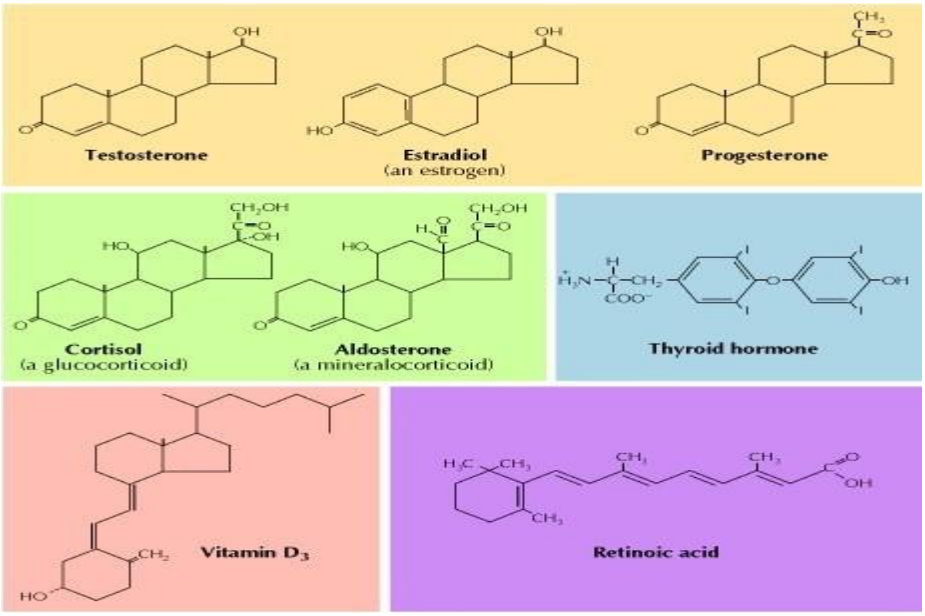


Figure : Structure of steroid hormones, thyroid hormone, vitamin D $_3$, and retinoic acid

The steroid hormones (including testosterone, estrogen, progesterone, the corticosteroids, and ecdysone) are all synthesized from cholesterol. Testosterone, estrogen, and progesterone are the sex steroids, which are produced by the gonads. The corticosteroids are produced by the adrenal gland. They include the glucocorticoids, which act on a variety of cells to stimulate production of glucose, and the mineralocorticoids, which act on the kidney to regulate salt and water balance. Ecdysone is an insect hormone that plays a key role in development by triggering the metamorphosis of larvae toadults.

Although thyroid hormone, vitamin D_3, and retinoic acid are both structurally and functionally distinct from the steroids, they share a common mechanism of action in their target cells. Thyroid hormone is synthesized from tyrosine in the thyroid gland; it plays important roles in development and regulation of metabolism. Vitamin D_3regulates Ca^{2+} metabolism and bone growth. Retinoic acidand related compounds (retinoids) synthesized from vitamin A play important roles in vertebratedevelopment.

Because of their hydrophobic character, the steroid hormones, thyroid hormone, vitamin D_3, and retinoic acid are able to enter cells by diffusing across the plasma membrane . Once inside the cell, they bind to intracellular receptors that are expressed by the hormonally responsive target cells. These receptors, which are members of a family of proteins known as the steroid

receptor superfamily, are transcription factors that contain related domains for ligand binding, DNA binding, and transcriptional activation. Ligand binding regulates their function as activators or repressors of their target genes, so the steroid hormones and related molecules directly regulate gene expression.

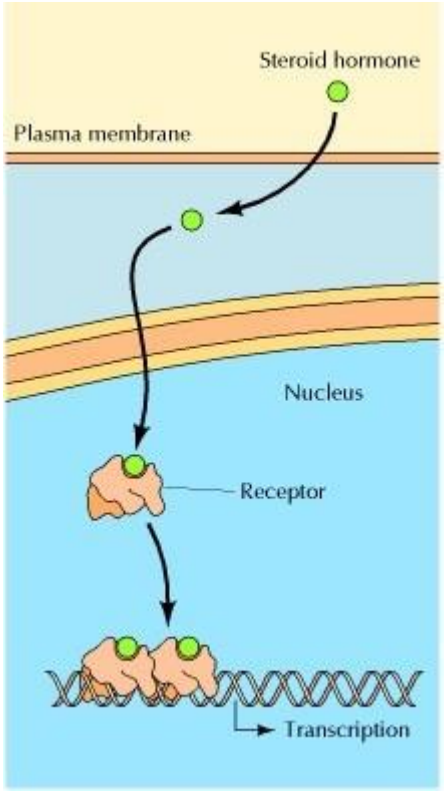


Figure: Action of steroid hormones. The steroid hormones diffuse across the plasma membrane and bind to nuclear receptors, which directly stimulate transcription of their target genes. The steroid hormone receptors bind DNA as dimers.

Ligand binding has distinct effects on different receptors. Some members of the steroid receptor superfamily, such as the estrogen and glucocorticoid receptors, are unable to bind to DNA in the absence of hormone. The binding of hormone induces a conformational change in the receptor, allowing it to bind to regulatory DNA sequences and activate transcription of target genes. In other cases, the receptor binds DNA in either the presence or absence of hormone, but hormone binding alters the activity of the receptor as a transcriptional regulatory molecule. For example, thyroid hormone receptor acts as a repressor in the absence of hormone, but hormone binding converts it to an activator that stimulates transcription of thyroid hormone-inducible genes

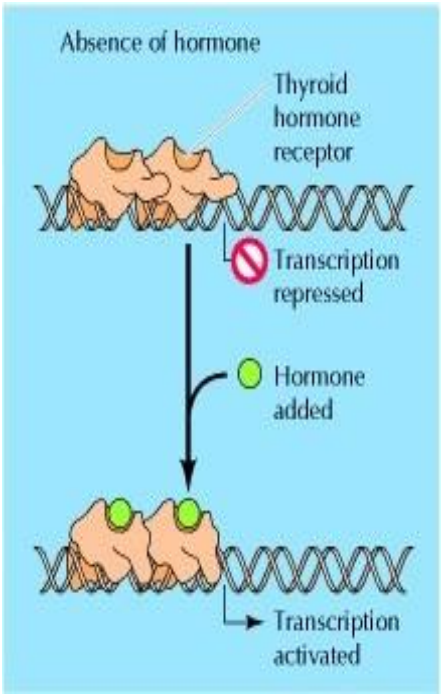


Figure: Gene regulation by the thyroid hormone receptor. Thyroid hormone receptor binds DNA in either the presence or absence of hormone. However, hormone binding changes the function of the receptor from a repressor to an activator of target genetranscription

Neurotransmitter Receptors in Cell Signaling Transduction

Chemical transmission is the major means by which nerves communicate with one another in the nervous system. Many different types of neurotransmitters play an important role in the process of chemical transdmittion. The neurotransmitters achieve cell signaling transduction through neurotransmitter receptors on the postsynaptic membrane. The neurotransmitter receptor perform large specificity and potency. Many receptors have been isolated and purified biochemically, and many have also been cloned and sequenced. Neurotransmitter receptors can also be grouped according to the type of primary effector to which they couple. This classification leads to four major categories of receptors.

Four groups of Neurotransmitter Receptor in cell signaling transduction

Group I: Ligand-gated ion channels as neurotransmitter receptors

These ligand-gated ion channels include nAChR, GluN1, GluN2A-D, GluN3A,B, GluA1-4, GluK1-3, 4-5, GABAA, GlyR, IP3-R1, IP3-R2, IP3-R3, 5HT3, P2X1-7 and Nicotiniccholinergic (muscle [αβγδε] and neuronal [α or αβ] subtypes).

Receptors in this category include those that are activated by synaptically released neurotransmitter and occur on the cell surface (mostly, the intracellular ligand-gated receptor for IP3 is present in the smooth endoplasmic reticulum). Upon the binding of an agonist to these ligand-gated ion channels, the receptors undergo a conformational change that facilitates opening of the intrinsic ion channel (some ligand gated ion channel receptors (e.g., NMDA and GABAA) are also found at extrasynaptic locations).

The permeability to specific ions is a characteristic of the receptor; for example, both the neuronal nicotinic cholinergic receptors (nAChR) and N-methyl D-aspartate (NMDA) receptors are selectively permeable to Na+ and Ca2+ ions, whereas GABAA andglycine receptors are primarily permeable to Cl- ions. As a result of the changes in ion conductance, the membrane potential may become either depolarized, as occurs for nAChRs or NMDA receptors, or hyperpolarized, as observed for GABAA or glycine receptors.

Group II: Receptors with intrinsic guanylyl cyclase activity as neurotransmitter receptors

The representative receptor with intrinsic guanylyl cyclase activity is GC-B. Receptors in the this group possess intrinsic guanylyl cyclase activity and generate cyclic GMP (cGMP) upon activation of a receptor. These receptors consist of an extracellular binding domain, a single transmembrane-spanning domain (TMD), a protein kinase–like domain and a guanylyl cyclase catalytic domain. Ligand binding results in a conformational change in the receptor and activation of the guanylyl cyclase catalytic region. Receptors with intrinsic guanylyl cyclase activity are often very highly phosphorylated in the absence of agonist and rapidly undergo dephosphorylation uponactivation.

Group III: Receptors with intrinsic or associated tyrosine kinase activity as neurotransmitter receptors:

Receptors with intrinsic or associated tyrosine kinase activity include TrkB, EGFR, FGFR1- FGFR4, IGFR-1, Trk A, ErbB2, ErbB3, ErbB4, Trk C, PDGFR α and β, gp130 + CNTFRα and LIFRβ, 2 x gp130 + IL6Rα and gp130 + LIFRβ. Receptors in the third group possess intrinsic receptor tyrosine kinase (RTK) activity themselves or are closely associated with cytoplasmic tyrosine kinases (RATK). Structurally, RTKs possess an extracellular ligandbinding domain, a single TMD and an intracellular catalytic kinase domain.

Three distinct events underlie signal transduction at RTKs: (1) Initially, upon ligand binding to an RTK, the receptor undergoes a dimerization that results in the juxtaposition of the two cytoplasmic domains. (2) Contact between these domains is thought to result in a stimulation of catalytic activity, (3) which in turn results in an intermolecular autophosphorylation of tyrosine residues both within and outside of the kinase domain. Once autophosphorylated, RTKs can recruit a number of cytoplasmic proteins and initiate a series of reactions involving protein– proteininteractions. RATKs, such as those for the neurotrophic cytokines (leukemia inhibitory factor, interleukin-6 or ciliary neurotrophic factor) do not possess intrinsic tyrosine kinase activity themselves, but upon activation, they undergo dimerization and are then able to recruit cytoplasmic tyrosine kinases (such as Janus kinase). The latter then phosphorylate the RATK on tyrosine residues (in addition to being tyrosine phosphorylated themselves) and facilitate protein–protein interactions, as observed forRTKs.

Group IV: G-protein-coupled receptors as neurotransmitter receptors

G-protein-coupled receptors within neurotransmitter receptors include Acetylcholine receptors, Adenosine receptors, ATP receptors, Dopamine receptors…… This group of receptors involves G proteins. Numerically, more diverse types of receptors have been demonstrated to operate via an intervening G protein than by any other mechanism. These G protein–coupledreceptors(GPCRs) have a characteristic seven TMD structure. G-protein-coupled neurotransmitter receptors can be further divided into four functional categories: (1) Some GPCRs, such as GABAB, α2-adrenergic, D2-dopaminergic or M2 muscarinic (mAChR), regulate the changes in K+ conductance independently of second-messenger production. (2) A second group of GPCRs is linked to the modulation of adenylyl cyclase activity. This regulation may be either positive, as in the case of activation of the β2-adrenergic receptor, or negative, as occurs following activation of the α2-adrenergic receptor. Changes in the concentrations of cAMP regulate the activity of protein kinase A (PKA). (3) A third group of GPCRs is linked to the activation of phosphoinositide-specific phospholipase C (PLC) with the attendant breakdown of PIP2 and formation of IP3 and DAG. These receptors are linked to changes in Ca2+ homeostasis and protein phosphorylation via the action of protein kinase C (PKC). Other effector enzymes that may be regulated by IP3-linked GPCRs include phospholipases A2 and D. (3) A fourth, and unique, mechanism for the activation of a GPCR is that utilized by the visual pigment rhodopsin, which structurally is a prototypical GPCR. However, in this case it is light, rather than a chemical stimulus, that triggers the activation of rhodopsin. Photoactivated rhodopsin activates transducin, a

G-protein, which is coupled to cGMP phosphodiesterase with a concomitant increased rate in the hydrolysis of cGMP toGMP.

Ras / Rafand MAP Kinase Pathway :

The gene family ras encodes small GTPases that are involved in cellular signal transduction. Ras the super-family of proteins regulates diverse cell behaviors such as cell growth, differentiation and survival. Since Ras communicates signals from outside the cell to the nucleus, mutations in ras genes can permanently activate it and cause inappropriate transmission inside the cell even in the absence of extracellular signals. Because these signals result in cell growth and division, disregulated Ras signaling can ultimately lead to oncogenesis and cancer.

Ras proteins function as binary molecular switches that control intracellular signaling networks. Ras-regulated signal pathways control processes such as actin- cytoskeletal integrity, proliferation, differentiation, cell adhesion, apoptosis, and cell migration. Ras and Ras-related proteins are often deregulated in cancers, leading to increased invasion and metastasis, and decreased apoptosis. Activated Ras activates the protein kinase activity of RAF kinase. RAFkinase phosphorylates and activates MEK. MEK phosphorylates and activates a mitogen- activated protein kinase (MAPK).

Mitogen-activated protein (MAP) kinases are serine/threonine-specific protein kinases that respond to extracellular stimuli (mitogens, osmotic stress, heat shock and pro-inflammatory cytokines) and regulate various cellular activities, such as gene expression, mitosis, differentiation, proliferation, and cell survival/apoptosis. MAPK pathways are activated within the protein kinase cascades called “MAPK cascade”. Each one consists of three enzymes, MAP kinase, MAP kinase kinase (MKK, MEKK, or MAP2K) and MAP kinase kinasekinase (MKKK or MAP3K) that are activated in series. A MAP3K that is activated by extracellular stimuli, which phosphorylates a MAP2K on its serine and threonine residues and this MAP2K activates a MAP kinase through phosphorylation on its serine and tyrosine residues.

The phosphorylation of tyrosine precedes to the phosphorylation of threonine, although phosphorylation of either residue can occur in the absence of the other. Because both tyrosine and threonine phosphorylations are required to activate the MAP kinases, phosphatases that remove phosphate from either sites will inactivate them. This MAP kinase signaling cascade has been evolutionary well-conserved from yeast to mammals. Cascades convey information to effectors, coordinates incoming information

from other signaling pathways, amplify signals, and allow for a variety of responsepatterns.

Down-regulation of MAP kinase pathways may occur through dephosphorylation by serine/threonine phosphatases, tyrosine phosphatases, or dual-specificity phosphatases and through feedback inhibitory mechanisms that involve the phosphorylation of upstream kinases. Drugs that selectively down-regulate MAP kinase cascades could prove to be valuable as therapeutic agents in the control of malignant disease.

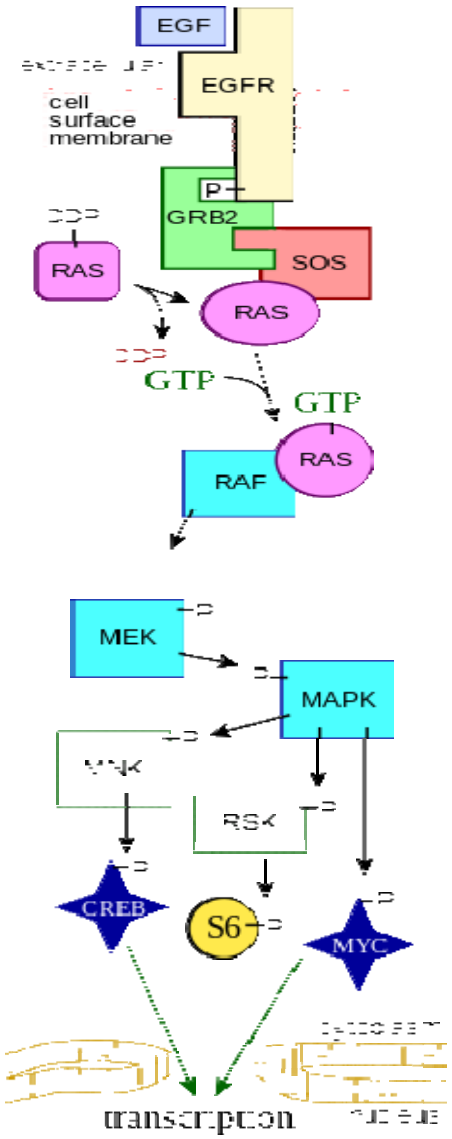


Figure: Key components of the MAPK/ERK pathway. "P" represents phosphate,whichcommunicates the signal. Top, epidermal growth factor (EGF) binds to the EGF receptor(EGFR) in the cell membrane, starting the cascade of signals. Further downstream, phosphate signal activates MAPK (also known as ERK). Bottom, signal enters the cell nucleus and causes

transcription of DNA, which is then expressed as protein.

JAK-STAT signaling pathway:

The JAK-STAT signaling pathway is a chain of interactions between proteins in a cell, and is involved in processes such as immunity, cell division, cell death and tumour formation. The pathway communicates information from chemical signals outside of a cell to the cell nucleus, resulting in the activation of genes through a process called transcription. There are three key parts of JAK-STAT signaling: Janus kinases (JAKs), Signal Transducer and Activator of Transcription proteins (STATs), and receptors (which bind the chemical signals).[1] Disrupted JAK-STAT signaling may lead to a variety of diseases, such as skin conditions, cancers, and disorders affecting the immune system.

Here are 4 JAK proteins: JAK1, JAK2, JAK3 and TYK2. JAKs contains a FERM domain(approximately 400 residues), an SH2-related domain (approximately 100 residues), a kinase domain (approximately 250 residues) and a pseudokinase domain (approximately 300 residues). The kinase domain is vital for JAK activity, since it allows JAKs to phosphorylate (add phosphate molecules to) proteins. There are 7STAT proteins: STAT1, STAT2, STAT3, STAT4, STAT5A ,STAT5B and STAT6. STAT proteins contain many different domains, each with a different function, of which the most conserved region is the SH2 domain. The SH2 domain is formed of 2 α-helices and a β-sheetand is formed approximately from residues 575-680.STATs also have transcriptional activation domains (TAD), which are less conserved and are located at the C-terminus. In addition, STATs also contain: tyrosine activation, amino-terminal, linker, coiled-coil and DNA-binding domains.

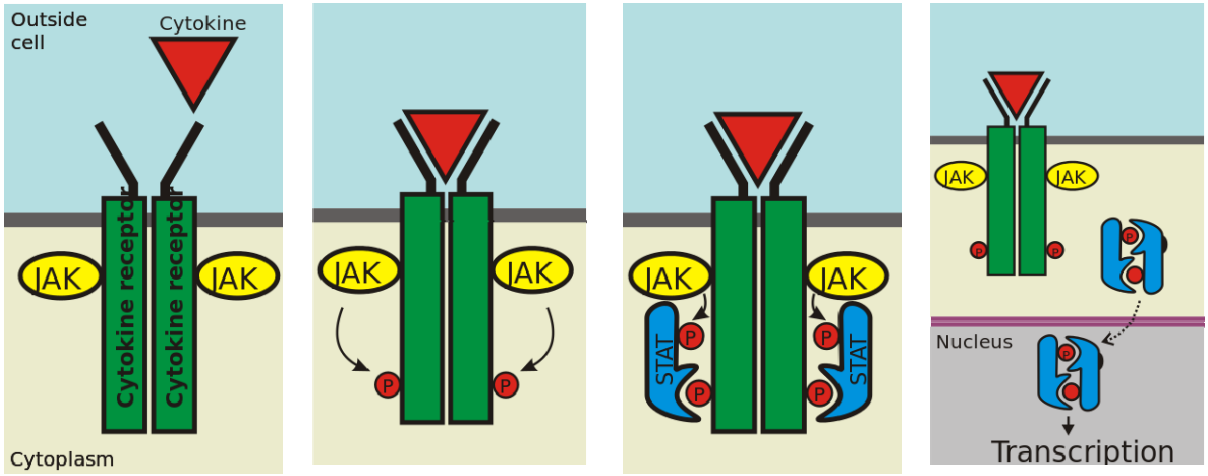


Fig: Key steps of the JAK-STAT pathway. JAK-STAT signalling is made of three major proteins: cell-surface receptors, Janus kinases (JAKs), and signal transducer and activator of transcription proteins (STATs). Once a ligand (red triangle) binds to the receptor, JAKs add

phosphates (red circles) to the receptor. Two STAT proteins then bind to the phosphates, and then the STATs are phosphorylated by JAKs to form a dimer. The dimer enters the nucleus, binds to DNA, and causes transcription of targetgenes.

Probable Questions:

1. Describe the role of protein phosphorylation in signal transduction.
2. Describe role of Intracellular Concentration of Ca^{++} in CellSignaling.
3. Describe the role of Phosphorylated Lipids in Cell Signaling.
4. Classify cytokine receptors in details.
5. Write down the role of calmodulin in signal transduction.
6. Describe the basic components of G-protein coupled receptor.
7. What is desensitization?
8. How signal is transmitted by steroid hormones.
9. Describe the role of neurotransmitter receptors in signal transduction.
10. How signal is transmitted thorough Ligand-gated ion channels ?
11. Describe Ras / Raf and MAP Kinase Pathway with suitable diagrams.
12. Describe JAK-STAT signaling pathway with suitable diagram.

Suggested readings:

1. Molecular Cell Biology by Lodish, Fourth Edition.
2. The Cell – A Molecular Approach by Cooper and Hausman, Fourth Edition
3. Principles of Genetics by Snustad and Simmons, Sixth Edition.
4. Molecular Biology of the Cell – by Bruce Alberts
5. Cell and Molecular Biology by Gerald Karp, 7^{th} Edition.
6. Gene cloning and DNA Analysis by T. A. Brown, Sixth Edition.
7. Genetics . Verma and Agarwal.

Disclaimer:

The study materials of this book have been collected from various books, e-books, journals and other e-sources.