POST GRADUATE DEGREE PROGRAMME (CBCS)
IN
BOTANY

SEMESTER - I

Course: BOTAECC

(Environmental Biology)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
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SYLLABUS
COURSE – BOTAECC
Environmental Biology
(Full Marks – 25)

Course	Group	Details Contents Structure		Study hour
BOTAECC	**Environmental Biology**	**Unit 1. Natural Resources:**	Brief overview; degradation and conservation.	**1**
		Unit 2. Environmental pollution	Air, water, soil – types of pollutants, sources, effects and remedial measures.	**1**
		Unit 3. Electronic waste	Source, types, components of e-waste, recycling of e waste, impact of e -waste on environment and their management.	**1**
		Unit 4. Ecotoxicology and biomonitoring	Principles, mechanisms, types and effects. Different aspect of biomonitoring.	
		Unit 5. Global Environmental Change and Environmental Impact Assessment	Principles and mechanisms and their impact assessment in different view point	**1**
		Unit 6. Environmental Law and Policies and Sustainable Development	Environmental toxicology- Principles and mechanisms. Environmental laws and policies. Concept; National sustainable development strategies.	**1**

Content

Environmental Biology

Soft Core Theory Paper **Credits: 2**

Content Structure:

1. Introduction

2. Objectives

3. An introduction to environmental biology.

4. Environmental pollution- Pollution and pollutant- Concept, definition and characteristics

a. Air pollution- Source and types of air pollutant and their chemistry, photochemical reactions, green house and global warming, O3 depletion, acid rain, air pollutant in India

b. Water pollution- Source and type of water pollution, effect of water pollution on ecosystem, heavy metals and their effect on biota, nuclear pollution and thermal pollution

c. Electronic waste (e waste), sources and types, constituents of e waste, recycling of e- waste, impact of e waste on environment and its management

d. Soil pollution- Sources and classes of soil pollutants and their environmental effects, solid waste-pollution and disposal problems, waste- effect disposal and management

5. Toxicology and Biomonitoring - Principles and mechanisms types and effects.

6. Global Environmental Change and Environmental laws.

7. Suggested reading

8. Assignment

1. Introduction

Environmental Biology is increasingly gaining attention, especially in these environmentally-conscience times. Malaysia enjoys the luxury of a rich biodiversity in a relatively small area. Its effective preservation and conservation requires a strong understanding and appreciation of the environment. Students are trained in fundamental and practical aspects of ecology and the environment. The interdisciplinary field of environmental biology focuses on the relationships among plants, animals and their surroundings, including their responses to environmental stimuli. Environmental biology is closely linked to and often coupled with evolutionary biology, since both involve an exploration of how organisms adapt to changing conditions.

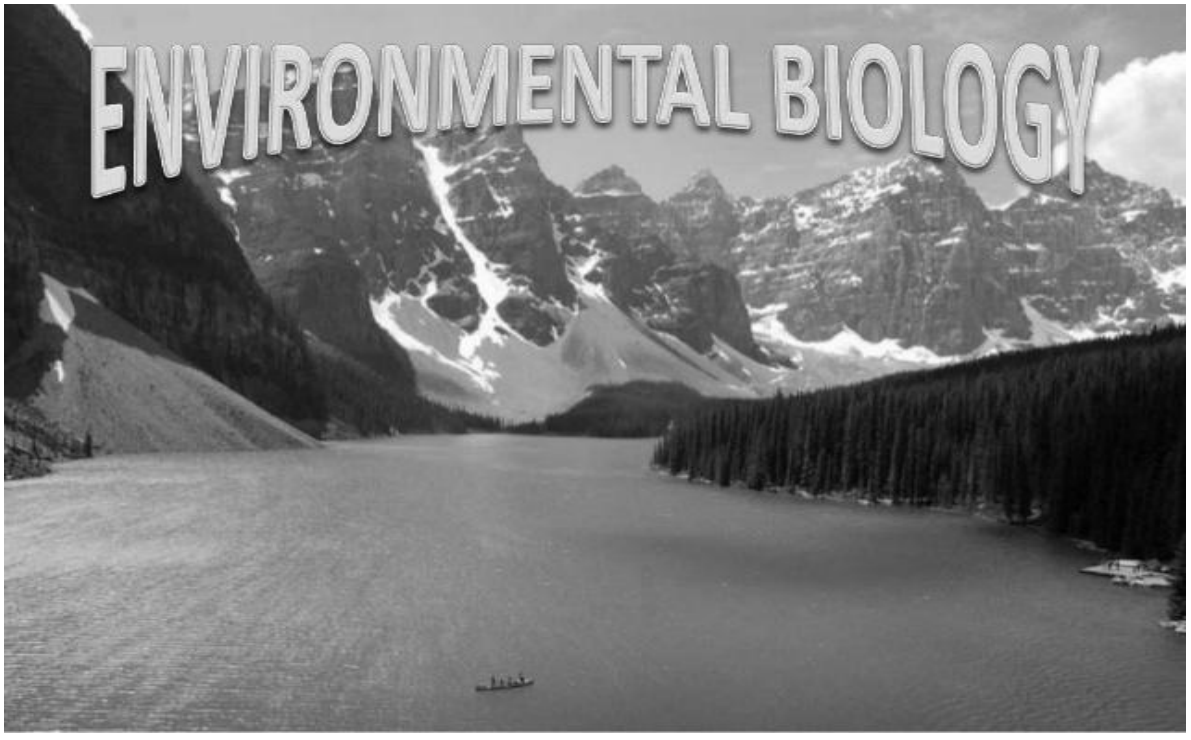
Environmental biologists may specialize in a single ecosystem, such as wetlands or forests, or in the human-wildlife interface created through development, agriculture and other man-made systems. Environmental biologists often work to preserve natural landscapes and biodiversity, protect wildlife populations and reverse ecosystem degradation.

2. Course Objectives

A student who has completed the course should have solid knowledge of:

1. Explain the ideas of environmental biology
2. Differentiate between terrestrial and aquatic biome
3. To understand about environmental pollution
4. Describe principles and mechanisms of environmental toxicology
5. To know about environmental laws and policies

3. An Introduction to Environmental Biology



What is Ecology:

This book is about ecology and conservation. Ecology is the study of organisms in their natural surroundings. The word ecology comes from two Greek words – oikos meaning home and logos meaning understanding. So ecology is all about understanding the homes of animals, plants and other organisms. The surroundings of an organism are known as its environment.

Environments consist of many components including both physical features, such as climate and soil type, and biological features, such as predators and prey. The term environmental biology has wider connotations than ecology because it includes the study of humans in the environment, so you will find such subjects as agriculture, pollution and the unnatural surroundings we create in this book too. Understanding the ecology of an area is like trying to put together a gigantic, multidimensional jigsaw. Some pieces are the individual species in the area. In an oak wood, for example (figure 1.1), the species might include bluebells, oak trees, earthworms, snails, hedgehogs, wood ants and tawny owls. Other pieces in the jigsaw are the important aspects of the physical environment, for example the pH of the rainwater, the total amount that falls in a year, how it is distributed throughout the seasons, and significant information about the temperature, sunlight and soil type. The jigsaw pieces interlock with

one another in numerous, subtle ways. In many ways ecology is a relatively new science. Indeed, the word was only coined by the German biologist Ernst Haeckel in 1869, fully ten years after Charles Darwin published his theory of natural selection. Yet, in little over a century ecology has grown to become one of the most important disciplines within biology.

Like all branches of science, it has its own language. This includes the terms habitat, population, community and ecosystem.

A habitat is the place where an organism lives. The word is Latin and literally means 'it dwells'. Actually, organisms from a single species can live in a number of habitats. For example, the common rat (Rattus norvegicus) is typically found associated with farms, refuse tips, sewers and warehouses. However, it also occurs in hedgerows close to cereal crops or sugar beet, and in salt marshes. On islands (e.g. the Isle of Man, Rhum and Lundy) rats also occupy grassland and the sea shore. With small organisms, especially those living in a restricted area such as in the soil or on a single plant or animal, it is worth being more precise about exactly where they live.

The term microhabitat – 'a small habitat' – is used to describe this. A single habitat may have many microhabitats. For example, if you are an insect living on an oak tree, life is very different depending on whether you live on the upper surface of the leaves, the lower surface of the leaves or inside them. It is even more different if you live under the bark, next to the roots or inside an acorn. Each of these different places is a microhabitat. A niche is a complete description of how the organism relates to its physical and biological environment. Just as in a jigsaw puzzle each piece has its own unique shape and pattern, and only fits in one place, so each species has a unique niche – the way it fits into its environment.

Consider a particular species, the grey heron (*Ardea cinerea*). Its habitats are water meadows, rivers, lakes and the sea shore. A complete account of its niche would include a description both of its physical environment (such as the type of water it needs, the temperature range in which it can survive and reproduce) and of its biological environment (such as the prey it eats, its competitors and the vegetation it needs for its nest). It is difficult to provide a quantitative description of an organism's niche.

Polioptila caerulea, a North American bird. This is an insectivore and the horizontal axis shows the length of the insects on which it feeds. The vertical axis shows the height above ground at which it forages. The contour lines with numbers indicate the frequency with which the birds feed at a particular height and on a particular length of prey. You can see that the birds concentrate on prey 4 mm in length, which they catch about 3–6 m off the ground. However, there are many other aspects to an organism's niche in addition to its feeding niche.

In theory, other axes could be added at right angles to those. Temperature could be shown on athird axis, risk of predation at different times of the year on a fourth, height above ground of the bird's nest on a fifth, and so on. In practice, though, no more than two or three axes can be shown on a graph.

Computers, however, can store and compute data for many more. The ecological principle that each species has its own unique niche and that no two species can coexist if they occupy the same niche is known as Gause's competitive exclusion principle. The biologist G. F. Gause gets the credit because of his research on single-celled ciliates in the genus Paramecium. A population is a group of individuals within a species that have the opportunity to breed with one another because they live in the same area at the same time. It follows from this definition that individuals from two different species cannot belong to the same population. This is because, with occasional exceptions, species are reproductively isolated from one another. Tawny owls do not breed with short-eared owls, for example. Most species are divided into many populations that are geographically separated. Bluebells in one wood, for example, will belong to a different population from the bluebells in another wood several kilometres away. Indeed, in a large wood there may be several populations of bluebells, though the boundaries between populations may be somewhat arbitrary.

A community is an association of species that live together in some common environment or habitat. Most communities are composed of a mixture of prokaryotes, protoctists, fungi, plants and animals. The organisms in a community interact with one another in all sorts of ways. For a start, there will be feeding relationships. In most communities, autotrophs (also known as producers and comprising green plants, photosynthetic algae, photosynthetic bacteria and chemosynthetic bacteria) provide food for herbivores (also known as primary consumers). In turn, herbivores are eaten by first-level carnivores (also known as secondary consumers), and these may be eaten by second-level carnivores (or tertiary consumers). Eventually organisms die and their remains are broken down by decomposers. These feeding relationships can be represented by food chains or by food webs that show the interrelationships between the various food chains in a community. The species in a community also interact with one another in other ways. They may rely on one another for reproduction, as is the case in insectpollinated plants. Or one species may act as a home for another, as a humpback whale carries barnacles. Or the interaction may be more subtle – all the species in a woodland, for example, rely on the activities of the various soil organisms which recycle nutrients.

The term 'community' is a valuable one in ecology. However, in 1935 Sir Arthur Tansley

invented the term ecosystem because he realized that the organisms that make up a community cannot realistically be considered independently of their physical environment. The term ecosystem, therefore, applies to a community of organisms and its associated physical environment. There is one other feature of ecosystems and their associated communities worth stressing. This is that ecosystems are dynamic. Indeed, some ecosystems change as new species invade and others die out. A grassland invaded by shrubs and trees will change gradually as scrubland and then woodland develops. In a mature ecosystem, such as oak woodland, the population sizes and activities of the different species will alter from season to season and year to year. The bluebells flower so beautifully in spring, but by late summer they have set seed, the leaves have died back and the bluebell bulbs are ready to lie dormant until the next spring.

Humans in the environment

We have given ourselves a Latin binomial, Homo sapiens, just like all the other species we have classified. However, it is obvious that the impact humans have on the environment is unlike that of any other species. Ancient humans evolved in Africa and migrated out into Asia and Europe a million or more years ago. A second wave of migration of modern humans spread out of Africa about 130 000 years ago resulting in the colonisation of every continent. Before humans evolved, of course, all the communities in the world were natural. In Britain, natural vegetation during the Ice Ages was treeless Arctic grassland called tundra; during warmer interglacials, after the ice sheets melted, trees invaded. In the south, forests of oaks, ash, lime and hornbeam grew; in the Scottish highlands, the main vegetation was Scots pine conifer forest. These mature forests are called the climax vegetation, but such vegetation is now rare due to human activities.

Humans learned to make and use fire early in their history, about half a million years ago – very useful during Ice Ages! Before this, only lightning started wildfires that had the potential to damage vegetation. About 10 000 years ago humans also began to change the natural vegetation by cultivating crops. Animals were domesticated at about the same time. Captive animals graze areas of vegetation in much greater densities than natural animal populations do. The practice of burning and grazing led to the vegetation in many areas of the world

developing into grasslands.

As human populations grew, their dwellings – in villages, towns and then cities – also restructured or even destroyed the vegetation. As humans are animals, human population biology might be expected to follow the same rules as those of other animal populations

(figure 1.3a and Biology 2, chapter 3). In other animal species, the population initially grows at a rate of increase related to the reproductive rate of the species. Plotting the log of the numbers of individuals in the population against a linear plot of time gives a straight line (figure 1.3b). Eventually there will be competition for resources that are in limited supply. This competition is intraspecific because it occurs between individuals belonging to the one species. The result of this increasing competition is that the population growth slows down. Eventually the population should reach the maximum size that the environment can sustain, a figure known as the environment's carrying capacity. The population may overshoot the numbers the environment can support, but will then fall to stabilise at the carrying capacity.

However, human population biology is more complex, and seems to have gone through different phases of growth. As you can see from figure 1.4, anthropologists and archaeologists think that the world's human population was stable, or only rising very slowly, up to about 10 000 years ago and that it was rather small – somewhere between 5 and 10 million. Archaeological evidence indicates that about 10 000 years ago the population started to rise more rapidly; there was a change in the rate of increase of population and in the carrying capacity. This reflects the change from mobile gatherer-hunter societies towards a more stationary agricultural lifestyle and the gradual development of the first towns and cities. There was another change in about 1750 with the onset of the industrial revolution. Since then world population has continued to rise sharply. On 12th October 1999, the world population officially reached 6 billion. That's six thousand million of us. Every day, the number increases by about 250 000. In other words, each day a quarter of a million more people are born than die. We in the West are used to thinking that this is a problem of developing countries. It is true that most industrialised countries, such as the UK, the USA and France, have population growth rates that are low compared to those in other countries. Bangladesh's population, for example, is growing 12 times faster than that of the UK. Yet the average person in the industrialised world uses about 60 times more resources than someone in the developing world. What is the carrying capacity of the UK for people? The current population of the UK is 59 million, but we have to import a large proportion of our food.

Under intensive cultivation, agricultural self-sufficiency could support around 41 million people. In other words, given a population of 41 million we should be able to provide all our nutritional needs, provided we carried on farming intensively using fertilisers and pesticides. A less intensive use of our land, which might prevent the net loss of soil through soil erosion, would probably mean a population of, at most, 35 million. So the carrying capacity of the UK

estimated from food supply may be somewhere between 35 and 41 million people.

However, if we had to rely on renewable energy sources (wind, solar, tidal, wave and geothermal) rather than on fossil fuels (coal, gas, oil and peat) or nuclear power we would probably have to reduce our population to 15–20 million. Such a reduction may seem far-fetched, though it is interesting to note that only immigration is preventing the populations of many industrialized Western European countries from falling. It has been argued that the quality of life would be much better in the UK if there were only half or a third the number of people there are today. Imagine if this were the case. There would be less pollution, more room for wildlife and no more getting stuck in traffic jams.

The effects of human activity Agriculture

Britain during the last Ice Age was treeless. Scotland and northern England were covered in a great ice sheet and the south of England was a cold, windswept landscape. After the climate warmed and the ice melted, trees colonized the area from southern and eastern Europe and dense woodland developed. At lower latitudes, in the tropics, the climate became warmer and wetter and tropical forests flourished. The ‘magic’ date of 10 000 years ago, when plants and animals started to be domesticated and the human population began to rise significantly marks the end of the last Ice Age and the start of the warm period we live in today. Past warm periods, the interglacial, lasted about 12 000–15 000 years.

We do not yet know if we are living in an interglacial, as we do not know if there is another glacial coming, when global temperatures will fall and the ice caps expand over Britain. At the moment humans are more concerned with global warming than global cooling. In the Near East 10 000 years ago a quiet revolution was about to take place. Archaeological excavations have revealed villages with evidence of early cereal crops and herded sheep and goats: farming had begun. As farming spread, human lifestyles changed and population densities increased (see pages 3–5). Gradually the natural vegetation of many areas was modified and replaced due to the action of farmers grazing their animals and planting crops. Agriculture seems to have first made an impact in Britain between 6000 and 5000 years ago. The gradual replacement of natural vegetation as a result of cultivation occurred throughout the next 3000 years with the introduction of ploughs, then better ploughs, and the increasing use of animals for ploughing and transport, milk and wool. By the time William I had the census taken which is recorded in the Domesday Book of 1086, only about 15% of England retained its original woodland. Intensive farming is now the normal method of food production in most of Europe and North America. Some of the biosocial consequences of modern agricultural practices are

discussed in chapter 2. Not every culture farms intensively. There are some groups, including the many small tribes of the Amazon basin and the Inuit of the Arctic, who still live in ways similar to those of our ancestral gatherer-hunters. Some, like the Dinka and Maasai in Africa, and nomads in the Middle East and Mongolia, herd animals, be they cattle, goats, camels or horses. Many groups in South America, India, Africa and elsewhere grow mixtures of crops local to their area such as maize, cassava, sorghum, rice, vegetables and fruits, to meet their immediate needs.

Whatever way of life a group has, whether gatherer-hunter or of industrial complexity, it is important that their way of life is sustainable. Hunters must never overhunt their prey, gatherers must leave enough seed for the next harvest, herds must not damage their grazing land beyond recovery, villagers must not take all the trees for firewood, intensive farming must not lead to soil erosion and dustbowl creation, and industry must not pollute the land, rivers or seas beyond repair. You can judge for yourself just how sustainable many human activities are as you read the rest of this book.

Pollution

Almost any substance can become a pollutant if it occurs in the wrong place, in the wrong concentration or at the wrong time. Hence fertilizers are excellent substances for increasing crop yields in intensive agricultural systems, but the same fertilizers running off the land into a river can pollute the water and cause the death of organisms in the natural ecosystem. Farming pollutants include fertilizers, pesticides and animal waste.

Our domestic lifestyle produces distinctive pollutants too – domestic refuse, car exhaust fumes and chlorofluorocarbons (CFCs) from refrigerators and aerosol sprays. Pollution from industrial processes has been around longer than you might think. Metal extraction was known as early as 6000 years ago and metal pollution has been found deep in the ice of

Greenland, which is about 4000 years old! The Romans were determined polluters. They burned coal in their underfloor heating systems and smelted all sorts of heavy metal ores to extract lead, copper, silver and zinc. Smelting ores in open fires and crude furnaces was an inefficient process that produced considerable atmospheric pollution. Although huge quantities of metals are now smelted compared with Roman times, luckily for us the methods of extraction have improved. Metal extraction is more efficient, so more metal is extracted even from poorer grade ores and less contaminating metal escapes during the process. Pollution is, however, still a big problem in the world.

Threats to biodiversity

Biodiversity is a much-heard word these days, although it was probably first used as recently as 1985. It is really shorthand for biological diversity. Biological diversity can be measured at all sorts of levels: the diversity of ecosystems in a region, the number of species in each ecosystem, and the genetic diversity within the populations of each species. Biodiversity includes all these levels of complexity and can be assessed on a local, national or global scale. Ecologists and conservationists are very concerned about the threatened and actual loss of global biodiversity.

The activities of humans over the last 100 000 years have severely compromised biodiversity. Hunting large animals for food probably led to the extinction of species such as mammoths and giant, flightless birds. Clearing natural vegetation for farmland and dwelling space and the polluting of soil, sea and atmosphere have all had the effect of reducing biodiversity.

We still have no certain idea how many species there are in the world or what many of those species are. Estimates vary a lot, but there may be as many as 15 million species, of which eight million are insects. By far the most diverse places on land (we are not so sure about the sea as the very deep oceans are mostly unexplored) are the tropical rainforests and the largest, in the Amazon basin of South America, is the richest of all. We have still not studied the rainforests enough to know exactly what is there, or how their ecosystems function. Yet it is these forests that are disappearing at an alarming rate, cleared for subsistence farming, for cattle ranching, for timber extraction, for mining, for access, and by accidental burning.

Monitoring the environment Ecology is in many ways the most complicated of all the biological sciences. Ecologists have to know something about the structure, physiology and behaviour of organisms before they can begin to understand how such organisms interact with one another and with the physical environment. For these reasons, ecology is increasingly an

experimental science. Ecologists constantly need to test their predictions either in natural environments, in seminatural experiments out in the field, or in artificial, simplified laboratory experiments. Theories can be of great value in ecology, but they must always be tested against reality, and this is where practical ecology is so important. We can only gain an understanding about the ecosystems around us by getting information about them through practical ecology. However, whole ecosystems are often far too complex to understand all in one go. It is easier to begin by choosing one or two species, or a small area of habitat, to study in detail. Practical ecology involves making observations, taking measurements and sometimes testing ideas by experimentation. Because there is so much to study in the environment and because the environment may change considerably in the next few decades (due to global warming and pollution) anyone can do valuable research. Carefully designed and long-term observations can be of great value. Just recording when the first bluebells start to flower and the first tree leaves appear, how often late frosts damage leaves or when frogs spawn each year could be

important records of the effects of changing climate. As Oliver Rackham, a leading expert on ancient woodland, put it: 'I often lament the observations which I would have begun if I had known in the 1960s what were to be the ecological problems of the 1990s ... I would urge conservation trusts to be more active in long-term research, experimentation and maintaining archives. Photographing from fixed points, recording permanently marked plots or transects, or following the fate of marked individual plants.

Natural Resources:

are resources that exist without any actions of humankind. This includes the sources of valued characteristics such as commercial and industrial use, aesthetic value, scientific interest and cultural value. On Earth, it includes sunlight, atmosphere, water, land, all minerals along with all vegetation, and animal life.

Natural resources can be part of our natural heritage or protected in nature reserves. Particular areas (such as the rainforest in Fatu-Hiva) often feature biodiversity and geodiversity in their ecosystems. Natural resources may be classified in different ways. Natural resources are materials and components (something that can be used) that can be found within the environment. Every man-made product is composed of natural resources (at its fundamental level).

A natural resource may exist as a separate entity such as fresh water, air, as well as any living organism such as a fish, or it may be transformed by extractivist industries into a economically-useful form that must be processed to obtain the resource such as metal ores, rare-earth elements, petroleum, timber and most forms of energy. Some resources are renewable resource, which means that they can be used at a certain rate and natural processes will restore them, whereas many extractive industries rely heavily on non-renewable resources that can only be extracted once. Natural-resource allocations can be at the center of many economic and political confrontations both within and between countries. This is particularly true during periods of increasing scarcity and shortages (depletion and overconsumption of resources). Resource extraction is also a major source of human rights violations and environmental damage. The sustainable development goals and other international development agendas frequently focus on creating more sustainable resource extraction, with some scholars and researchers focused on creating economic models, such as circular economy, that rely less on resource extraction, and more on reuse, recycling and renewable resources that can be sustainably managed.

There are various methods of categorizing natural resources. These include the source of

origin, stage of development, and by their renewability.

On the basis of origin, natural resources may be divided into two types:

Biotic — Biotic resources are obtained from the biosphere (living and organic material), such as forests and animals, and the materials that can be obtained from them. Fossil fuels such as coal and petroleum are also included in this category because they are formed from decayed organic matter.

Abiotic – Abiotic resources are those that come from non-living, non-organic material. Examples of abiotic resources include land, fresh water, air, rare-earth elements, and heavy metals including ores, such as gold, iron, copper, silver, etc.

Considering their stage of development, natural resources may be referred to in the following ways:

Potential resources — Potential resources are those that may be used in the future—for example, petroleum in sedimentary rocks that, until drilled out and put to use remains a potential resource

Actual resources — Those resources that have been surveyed, quantified and qualified, and are currently used in development, such as wood processing, and are typically dependent on technology.

Reserve resources — The part of an actual resource that can be developed profitably in the future

Stock resources — Those that have been surveyed, but cannot be used due to lack of technology—for example, hydrogen

On the basis of recovery rate, natural resources can be categorized as follows:

Renewable resources — Renewable resources can be replenished naturally. Some of these resources, like sunlight, air, wind, water, etc. are continuously available and their quantities are not noticeably affected by human consumption. Though many renewable resources do not have such a rapid recovery rate, these resources are susceptible to depletion by over-use. Resources from a human use perspective are classified as renewable so long as the rate of replenishment/recovery exceeds that of the rate of consumption. They replenish easily compared to non-renewable resources.

Non-renewable resources – Non-renewable resources either form slowly or do not naturally form in the environment. Minerals are the most common resource included in this category. From the human perspective, resources are non-renewable when their rate of consumption exceeds the rate of replenishment/recovery; a good example of this are fossil fuels, which are in this category because their rate of formation is extremely slow (potentially millions of

years), meaning they are considered non-renewable. Some resources naturally deplete in amount without human interference, the most notable of these being radio-active elements such as uranium, which naturally decay into heavy metals. Of these, the metallic minerals can be re-used by recycling them, but coal and petroleum cannot be recycled. Once they are completely used they take millions of years to replenish.

Management - Natural resource management is a discipline in the management of natural resources such as land, water, soil, plants, and animals—with a particular focus on how management affects quality of life for present and future generations. Hence, sustainable development is followed according to judicial use of resources to supply both the present generation and future generations. The disciplines of fisheries, forestry, and wildlife are examples of large subdisciplines of natural resource management.

Management of natural resources involves identifying who has the right to use the resources, and who does not, for defining the boundaries of the resource. The resources may be managed by the users according to the rules governing when and how the resource is used depending on local condition or the resources may be managed by a governmental organization or other central authority.

A "...successful management of natural resources depends on freedom of speech, a dynamic and wide-ranging public debate through multiple independent media channels and an active civil society engaged in natural resource issues...", because of the nature of the shared resources the individuals who are affected by the rules can participate in setting or changing them. The users have rights to devise their own management institutions and plans under the recognition by the government. The right to resources includes land, water, fisheries and pastoral rights. The users or parties accountable to the users have to actively monitor and ensure the utilization of the resource compliance with the rules and to impose penalty on those peoples who violate the rules. These conflicts are resolved in a quick and low cost manner by the local institution according to the seriousness and context of the offence. The global science-based platform to discuss natural resources management is the World Resources Forum, based in Switzerland.

Nature conservation is the moral philosophy and conservation movement focused on protecting species from extinction, maintaining and restoring habitats, enhancing ecosystem services, and protecting biological diversity. A range of values underlie conservation, which can be guided by biocentrism, anthropocentrism, ecocentrism, and sentientism, environmental ideologies that inform ecocultural practices and identities.[2] There has recently been a movement towards evidence-based conservation which calls for greater use of scientific evidence to improve the effectiveness of conservation efforts. As of 2018 15% of land and 7.3% of the oceans were

protected. Many environmentalists set a target of protecting 30% of land and marine territory by 2030.

Evidence-based conservation

Main article: Evidence-based conservation

Evidence-based conservation is the application of evidence in conservation management actions and policy making. It is defined as systematically assessing scientific information from published, peer-reviewed publications and texts, practitioners' experiences, independent expert assessment, and local and indigenous knowledge on a specific conservation topic. This includes assessing the current effectiveness of different management interventions, threats and emerging problems, and economic factors.

Evidence-based conservation was organized based on the observations that decision making in conservation was based on intuition and/or practitioner experience often disregarding other forms of evidence of successes and failures (e.g. scientific information). This has led to costly and poor outcomes. Evidence-based conservation provides access to information that will support decision making through an evidence-based framework of "what works" in conservation.

The evidence-based approach to conservation is based on evidence-based practice which started in medicine and later spread to nursing, education, psychology, and other fields. It is part of the larger movement towards evidence-based practices.

Environmental degradation is the deterioration of the environment through depletion of resources such as quality of air, water and soil; the destruction of ecosystems; habitat destruction; the extinction of wildlife; and pollution. It is defined as any change or disturbance to the environment perceived to be deleterious or undesirable.

Environmental degradation is one of the ten threats officially cautioned by the high-level PaneI on Threats, Challenges and Change of the United Nations. The United Nations International Strategy for Disaster Reduction defines environmental degradation as "the reduction of the capacity of the environment to meet social and ecological objectives, and needs". Environmental degradation comes in many types. When natural habitats are destroyed or natural resources are depleted, the environment is degraded. Efforts to counteract this problem include environmental protection and environmental resources management.

Scientists assert that human activity has pushed the earth into a sixth mass extinction event.The loss of biodiversity has been attributed in particular to human overpopulation, continued human population growth and overconsumption of natural resources by the world's wealthy. A 2020 report by the World Wildlife Fund found that human activity, specifically overconsumption, population growth and intensive farming, has destroyed 68% of vertebrate wildlife since 1970.

The Global Assessment Report on Biodiversity and Ecosystem Services, published by the United Nation's IPBES in 2019, posits that roughly one million species of plants and animals face extinction from anthropogenic causes, such as expanding human land use for industrial agriculture and livestock rearing, along with overfishing. Since the establishment of agriculture over 11,000 years ago, humans have altered roughly 70% of the earth's land surface, with the global biomass of vegetation being reduced by half, and terrestrial animal communities seeing a decline in biodiversity greater than 20% on average. A 2021 study says that just 3% of the planet's terrestrial surface is ecologically and faunally intact, meaning areas with healthy populations of native animal species and little to no human footprint. Many of these intact ecosystems were in areas inhabited by indigenous peoples.

The implications of these losses for human livelihoods and wellbeing have raised serious concerns. With regard to the agriculture sector for example, The State of the World's Biodiversity for Food and Agriculture, published by the Food and Agriculture Organization of the United Nations in 2019, states that "countries report that many species that contribute to vital ecosystem services, including pollinators, the natural enemies of pests, soil organisms and wild food species, are in decline as a consequence of the destruction and degradation of habitats, overexploitation, pollution and other threats" and that "key ecosystems that deliver numerous services essential to food and agriculture, including supply of freshwater, protection against hazards and provision of habitat for species such as fish and pollinators, are declining."

Water degradation:

One major component of environmental degradation is the depletion of the resource of fresh water on Earth. Approximately only 2.5% of all of the water on Earth is fresh water, with the rest being salt water. 69% of fresh water is frozen in ice caps located on Antarctica and Greenland, so only 30% of the 2.5% of fresh water is available for consumption. Fresh water is an exceptionally important resource, since life on Earth is ultimately dependent on it. Water transports nutrients, minerals and chemicals within the biosphere to all forms of life, sustains both plants and animals, and moulds the surface of the Earth with transportation and deposition of materials.

The current top three uses of fresh water account for 95% of its consumption; approximately 85% is used for irrigation of farmland, golf courses, and parks, 6% is used for domestic purposes such as indoor bathing uses and outdoor garden and lawn use, and 4% is used for industrial purposes such as processing, washing, and cooling in manufacturing centres. It is estimated that one in three people over the entire globe are already facing water shortages, almost one-fifth of the world population live in areas of physical water scarcity, and almost one quarter of the world's population live in a developing country that lacks the necessary infrastructure to use water from

available rivers and aquifers. Water scarcity is an increasing problem due to many foreseen issues in the future including population growth, increased urbanization, higher standards of living, and climate change.

Climate change and temperature

Climate change affects the Earth's water supply in a large number of ways. It is predicted that the mean global temperature will rise in the coming years due to a number of forces affecting the climate. The amount of atmospheric carbon dioxide (CO_2) will rise, and both of these will influence water resources; evaporation depends strongly on temperature and moisture availability which can ultimately affect the amount of water available to replenish groundwater supplies.

Transpiration from plants can be affected by a rise in atmospheric CO_2, which can decrease their use of water, but can also raise their use of water from possible increases of leaf area. Temperature rise can reduce the snow season in the winter and increase the intensity of the melting snow leading to peak runoff of this, affecting soil moisture, flood and drought risks, and storage capacities depending on the area.

Warmer winter temperatures cause a decrease in snowpack, which can result in diminished water resources during summer. This is especially important at mid-latitudes and in mountain regions that depend on glacial runoff to replenish their river systems and groundwater supplies, making these areas increasingly vulnerable to water shortages over time; an increase in temperature will initially result in a rapid rise in water melting from glaciers in the summer, followed by a retreat in glaciers and a decrease in the melt and consequently the water supply every year as the size of these glaciers get smaller and smaller.

Thermal expansion of water and increased melting of oceanic glaciers from an increase in temperature gives way to a rise in sea level. This can affect the fresh water supply to coastal areas as well. As river mouths and deltas with higher salinity get pushed further inland, an intrusion of saltwater results in an increase of salinity in reservoirs and aquifers. Sea-level rise may also consequently be caused by a depletion of groundwater, as climate change can affect the hydrologic cycle in a number of ways. Uneven distributions of increased temperatures and increased precipitation around the globe results in water surpluses and deficits, but a global decrease in groundwater suggests a rise in sea level, even after meltwater and thermal expansion were accounted for, which can provide a positive feedback to the problems sea-level rise causes to fresh-water supply.

A rise in air temperature results in a rise in water temperature, which is also very significant in water degradation as the water would become more susceptible to bacterial growth. An increase in water temperature can also affect ecosystems greatly because of a species' sensitivity to

temperature, and also by inducing changes in a body of water's self-purification system from decreased amounts of dissolved oxygen in the water due to rises in temperature.

Climate change and precipitation

A rise in global temperatures is also predicted to correlate with an increase in global precipitation but because of increased runoff, floods, increased rates of soil erosion, and mass movement of land, a decline in water quality is probable, because while water will carry more nutrients it will also carry more contaminants. While most of the attention about climate change is directed towards global warming and greenhouse effect, some of the most severe effects of climate change are likely to be from changes in precipitation, evapotranspiration, runoff, and soil moisture. It is generally expected that, on average, global precipitation will increase, with some areas receiving increases and some decreases.

Climate models show that while some regions should expect an increase in precipitation, such as in the tropics and higher latitudes, other areas are expected to see a decrease, such as in the subtropics. This will ultimately cause a latitudinal variation in water distribution. The areas receiving more precipitation are also expected to receive this increase during their winter and actually become drier during their summer, creating even more of a variation of precipitation distribution. Naturally, the distribution of precipitation across the planet is very uneven, causing constant variations in water availability in respective locations.

Changes in precipitation affect the timing and magnitude of floods and droughts, shift runoff processes, and alter groundwater recharge rates. Vegetation patterns and growth rates will be directly affected by shifts in precipitation amount and distribution, which will in turn affect agriculture as well as natural ecosystems. Decreased precipitation will deprive areas of water causing water tables to fall and reservoirs of wetlands, rivers, and lakes to empty. In addition, a possible increase in evaporation and evapotranspiration will result, depending on the accompanied rise in temperature. Groundwater reserves will be depleted, and the remaining water has a greater chance of being of poor quality from saline or contaminants on the land surface.[19]

Population growth

Graph of human population from 10000 BCE to 2000 CE. It shows exponential rise in world population that has taken place since the end of the seventeenth century.

Main articles: Human overpopulation and Population growth. The human population on Earth is expanding rapidly, which together with even more rapid economic growth is the main cause of the degradation of the environment. Humanity's appetite for resources is disrupting the environment's natural equilibrium. Production industries are venting smoke into the atmosphere and discharging chemicals that are polluting water resources. The smoke includes detrimental

gases such as carbon monoxide and sulphur dioxide. The high levels of pollution in the atmosphere form layers that are eventually absorbed into the atmosphere. Organic compounds such as chlorofluorocarbons (CFCs) have generated an opening in the ozone layer, which admits higher levels of ultraviolet radiation, putting the globe at risk.

The available fresh water being affected by the climate is also being stretched across an ever-increasing global population. It is estimated that almost a quarter of the global population is living in an area that is using more than 20% of their renewable water supply; water use will rise with population while the water supply is also being aggravated by decreases in streamflow and groundwater caused by climate change. Even though some areas may see an increase in freshwater supply from an uneven distribution of precipitation increase, an increased use of water supply is expected.

An increased population means increased withdrawals from the water supply for domestic, agricultural, and industrial uses, the largest of these being agriculture, believed to be the major non-climate driver of environmental change and water deterioration. The next 50 years will likely be the last period of rapid agricultural expansion, but the larger and wealthier population over this time will demand more agriculture. Population increase over the last two decades, at least in the United States, has also been accompanied by a shift to an increase in urban areas from rural areas, which concentrates the demand for water into certain areas, and puts stress on the fresh water supply from industrial and human contaminants. Urbanization causes overcrowding and increasingly unsanitary living conditions, especially in developing countries, which in turn exposes an increasingly number of people to disease. About 79% of the world's population is in developing countries, which lack access to sanitary water and sewer systems, giving rises to disease and deaths from contaminated water and increased numbers of disease-carrying insects.

Agriculture

Water pollution due to dairy farming in the Wairarapa in New Zealand

Agriculture is dependent on available soil moisture, which is directly affected by climate dynamics, with precipitation being the input in this system and various processes being the output, such as evapotranspiration, surface runoff, drainage, and percolation into groundwater. Changes in climate, especially the changes in precipitation and evapotranspiration predicted by climate models, will directly affect soil moisture, surface runoff, and groundwater recharge. In areas with decreasing precipitation as predicted by the climate models, soil moisture may be substantially reduced. With this in mind, agriculture in most areas already needs irrigation, which depletes fresh water supplies both by the physical use of the water and the degradation agriculture causes to the water. Irrigation increases salt and nutrient content in areas that would not normally

be affected, and damages streams and rivers from damming and removal of water. Fertilizer enters both human and livestock waste streams that eventually enter groundwater, while nitrogen, phosphorus, and other chemicals from fertilizer can acidify both soils and water. Certain agricultural demands may increase more than others with an increasingly wealthier global population, and meat is one commodity expected to double global food demand by 2050, which directly affects the global supply of fresh water. Cows need water to drink, more if the temperature is high and humidity is low, and more if the production system the cow is in is extensive, since finding food takes more effort. Water is needed in processing of the meat, and also in the production of feed for the livestock. Manure can contaminate bodies of freshwater, and slaughterhouses, depending on how well they are managed, contribute waste such as blood, fat, hair, and other bodily contents to supplies of fresh water.

The transfer of water from agricultural to urban and suburban use raises concerns about agricultural sustainability, rural socioeconomic decline, food security, an increased carbon footprint from imported food, and decreased foreign trade balance. The depletion of fresh water, as applied to more specific and populated areas, increases fresh water scarcity among the population and also makes populations susceptible to economic, social, and political conflict in a number of ways; rising sea levels forces migration from coastal areas to other areas farther inland, pushing populations closer together breaching borders and other geographical patterns, and agricultural surpluses and deficits from the availability of water induce trade problems and economies of certain areas. Climate change is an important cause of involuntary migration and forced displacement. According to the Food and Agriculture Organization of the United Nations, global greenhouse gas emissions from animal agriculture exceeds that of transportation.

Water management

A stream in the town of Amlwch, Anglesey which is contaminated by acid mine drainage from the former copper mine at nearby Parys Mountain

The issue of the depletion of fresh water has stimulated increased efforts in water management. While water management systems are often flexible, adaptation to new hydrologic conditions may be very costly. Preventative approaches are necessary to avoid high costs of inefficiency and the need for rehabilitation of water supplies, and innovations to decrease overall demand may be important in planning water sustainability.

Water supply systems, as they exist now, were based on the assumptions of the current climate, and built to accommodate existing river flows and flood frequencies. Reservoirs are operated based on past hydrologic records, and irrigation systems on historical temperature, water

availability, and crop water requirements; these may not be a reliable guide to the future. Re-examining engineering designs, operations, optimizations, and planning, as well as re-evaluating legal, technical, and economic approaches to manage water resources are very important for the future of water management in response to water degradation. Another approach is water privatization; despite its economic and cultural effects, service quality and overall quality of the water can be more easily controlled and distributed. Rationality and sustainability is appropriate, and requires limits to overexploitation and pollution and efforts in conservation.

4. The Biosphere- The Terrestrial Biomes and the Aquatic Biomes

Biosphere, relatively thin life-supporting stratum of Earth's surface, extending from a few kilometres into the atmosphere to the deep-sea vents of the ocean. The biosphere is a global ecosystem composed of living organisms (biota) and the abiotic (nonliving) factors from which they derive energy and nutrients.

Before the coming of life, Earth was a bleak place, a rocky globe with shallow seas and a thin band of gases—largely carbon dioxide, carbon monoxide, molecular nitrogen, hydrogen sulfide, and water vapour. It was a hostile and barren planet. This strictly inorganic state of the Earth is called the geosphere; it consists of the lithosphere (the rock and soil), the hydrosphere (the water), and the atmosphere (the air). Energy from the Sun relentlessly bombarded the surface of the primitive Earth, and in time-millions of years-chemical and physical actions produced the first evidence of life: formless, jellylike blobs that could collect energy from the environment and produce more of their own kind. This generation of life in the thin outer layer of the geosphere established what is called the biosphere, the "zone of life," an energy-diverting skin that uses the matter of the Earth to make living substance.

The biosphere is a system characterized by the continuous cycling of matter and an accompanying flow of solar energy in which certain large molecules and cells are self-reproducing. Water is a major predisposing factor, for all life depends on it. The elements carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur, when combined as proteins, lipids, carbohydrates, and nucleic acids, provide the building blocks, the fuel, and the direction for the creation of life. Energy flow is required to maintain the structure of organisms by the formation and splitting of phosphate bonds. Organisms are cellular in nature and always contain some sort of enclosing membrane structure, and all have nucleic acids that store and transmit genetic information.

All life on Earth depends ultimately upon green plants, as well as upon water. Plants utilize sunlight in a process called photosynthesis to produce the food upon which animals feed and to provide, as a by-product, oxygen, which most animals require for respiration. At first, the oceans and the lands were teeming with large numbers of a few kinds of simple single-celled organisms, but slowly plants and animals of increasing complexity evolved. Interrelationships developed so that certain plants grew in association with certain other plants, and animals associated with the plants and with one another to form communities of organisms, including those of forests, grasslands, deserts, dunes, bogs, rivers, and lakes. Living communities and their nonliving environment are inseparably interrelated and constantly interact upon each other. For convenience, any segment of the landscape that includes the biotic and abiotic components is called an ecosystem. A lake is an ecosystem when it is considered in totality as not just water but also nutrients, climate, and all of the life contained within it. A given forest, meadow, or river is likewise an ecosystem. One ecosystem grades into another along zones termed ecotones, where a mixture of plant and animal species from the two ecosystems occurs. A forest considered as an ecosystem is not simply a stand of trees but is a complex of soil, air, and water, of climate and minerals, of bacteria, viruses, fungi, grasses, herbs, and trees, of insects, reptiles, amphibians, birds, and mammals.

Stated another way, the abiotic, or nonliving, portion of each ecosystem in the biosphere includes the flow of energy, nutrients, water, and gases and the concentrations of organic and inorganic substances in the environment. The biotic, or living, portion includes three general categories of organisms based on their methods of acquiring energy: the primary producers, largely green plants; the consumers, which include all the animals; and the decomposers, which include the microorganisms that break down the remains of plants and animals into simpler components for recycling in the biosphere. Aquatic ecosystems are those involving marine environments and freshwater environments on the land. Terrestrial ecosystems are those based on major vegetational types, such as forest, grassland, desert, and tundra. Particular kinds of animals are associated with each such plant province.

Ecosystems may be further subdivided into smaller biotic units called communities. Examples of communities include the organisms in a stand of pine trees, on a coral reef, and in a cave, a valley, a lake, or a stream. The major consideration in the community is the living component, the organisms; the abiotic factors of the environment are excluded.

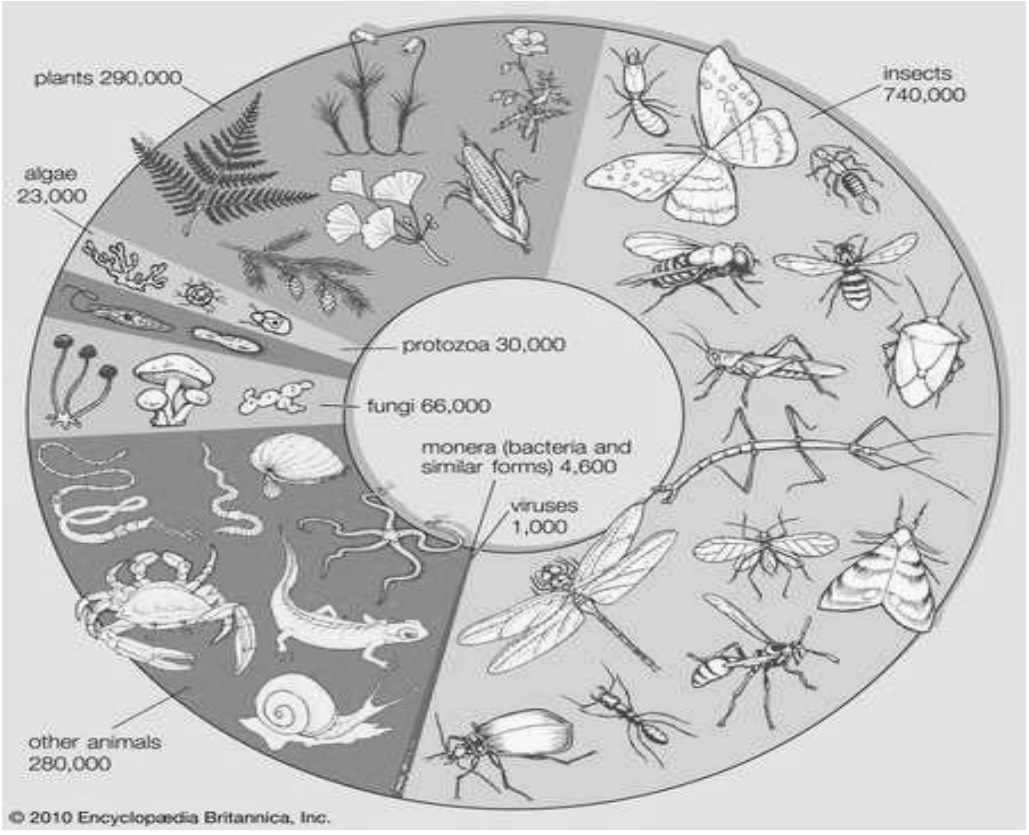
A community is a collection of species populations. In a stand of pines, there may be many species of insects, of birds, of mammals, each a separate breeding unit but each dependent on the others for its continued existence. A species, furthermore, is composed of individuals,

single functioning units identifiable as organisms. Beyond this level, the units of the biosphere are those of the organism: organ systems composed of organs, organs of tissues, tissues of cells, cells of molecules, and molecules of atomic elements and energy. The progression, therefore, proceeding upward from atoms and energy, is toward fewer units, larger and more complex in pattern, at each successive level.

This article focuses on the makeup of the biosphere and examines the relationships between its principal components, including man. The characteristics and dynamics of biological populations and communities are dealt with, as are the interactions that constitute the primary stabilizing links among the constituent organisms. Due attention is also given to the distribution patterns of these biotic units and to the processes that produced such patterns. The major aquatic and terrestrial ecosystems of the Earth are treated in some detail. Other points include energy transformations and transfers within the biosphere and the cyclic flow of materials needed for life. For the development, methodology, and applications of the study of interrelations of organisms with their environment and each other, *see* ecology. Further treatment of the various aquatic and terrestrial environments is provided in ocean, lake, river, continental landform, Arctic, and Antarctica. For a discussion of the origin of life on Earth and the varieties of and commonalities among organisms, *see* life and Earth, pregeologic history of. The characteristics and classifications of living organisms are covered in detail in algae, amphibian, angiosperm, animal, annelid, arachnid, arthropod, aschelminth, bacteria, bird, bryophyte, chordate, cnidarian, crustacean, dinosaur, echinoderm, fern, fish, flatworm, fungus, gymnosperm, insect, lampshell, mammal, mollusk, moss,animal, plant, protist, protozoa, reptile, sponge, and virus.

The Diversity of Life

The biosphere supports between 3 and 30 million species of plants, animals, fungi, single-celled prokaryotes such as bacteria, and single-celled eukaryotes such as protozoans. Of this total, only about 1.4 million species have been named so far, and fewer than 1 percent have been studied for their ecological relationships and their role in ecosystems. A little more than half the named species are insects, which dominate terrestrial and freshwater communities worldwide; the laboratories of systematists are filled with insect species yet to be named and described. Hence, the relationships of organisms to their environments and the roles that species play in the biosphere are only beginning to be understood.



Estimated number of known living species. The majority of species are still unknown *i.e.*, yet to be described by taxonomists.

The importance of the biosphere:

The continued functioning of the biosphere is dependent not only on the maintenance of the intimate interactions among the myriad species within local communities but also on the looser yet crucial interactions of all species and communities around the globe. The Earth is blanketed with so many species and so many different kinds of biological communities because populations have been able to adapt to almost any kind of environment on Earth through natural selection. Life-forms have evolved that are able to survive in the ocean depths, the frigid conditions of Antarctica, and the near-boiling temperatures of geysers. The great richness of adaptations found among different populations and species of living organisms is the Earth's greatest resource. It is a richness that has evolved over millions of years and is irreplaceable.

It is therefore startling to realize that our inventory of the Earth's diversity is still so incomplete that the total number of living species cannot be estimated more closely than between 3 and 30 million species. Decades of continuous research must be carried out by systematists, ecologists, and geneticists before the inventory of biodiversity provides a more accurate count. The research has been slow. Only recently, as the extinction rate of species has been increasing rapidly, have societies begun to realize the interdependence of species. To sustain life on Earth, more than the few animal and plant species used by humans must be preserved. The flow of energy and the cycling of nutrients through ecosystems, the regulation of populations, and the stability of biological communities, all of which support the continued maintenance of life, rely on the diversity of species, their adaptations to local physical conditions, and their coevolved relationships.

Despite the limited scientific knowledge of most species, ecological studies during the 20th century made great headway in unraveling the mechanisms by which organisms coevolve with one another and adapt to their physical environment, thereby shaping the biosphere. Each new decade has produced a steady stream of studies showing that the biological and physical elements of the Earth are more interconnected than had been previously thought. Those studies also have shown that often the most seemingly insignificant species are crucial to the stability of communities and ecosystems. Many seemingly obscure species are at risk worldwide of being dismissed as unimportant. The effect that the loss of species will have on ecosystems is appreciated only by understanding the relationships between organisms and their environments and by studying the ecological and evolutionary processes operating within ecosystems.

The need to understand how the biosphere functions has never been greater. When human population levels were low and technological abilities crude, societies' impact on the biosphere was relatively small. The increase in human population levels and the harvesting of more of the Earth's natural resources has altered this situation, especially in recent decades. Human activities are causing major alterations to the patterns of energy flow and nutrient cycling through ecosystems, and these activities are eliminating populations and species that have not even been described but which might have been of central importance to the maintenance of ecosystems.

The biologist Edward O. Wilson, who coined the term *biodiversity*, estimated conservatively that in the late 20th century at least 27,000 species were becoming extinct each year. The majority of these were small tropical organisms. The impact that this freshet of extinctions

would have on the biosphere is akin to receiving a box of engine parts and discarding a portion of them before reading the directions, assuming that their absence will have no negative repercussions on the running of the engine. The following sections describe how many of the biological and physical parts fit together to make the engine of the biosphere run and why many seemingly obscure species are important to the long-term functioning of the biosphere.

AQUATIC BIOME:

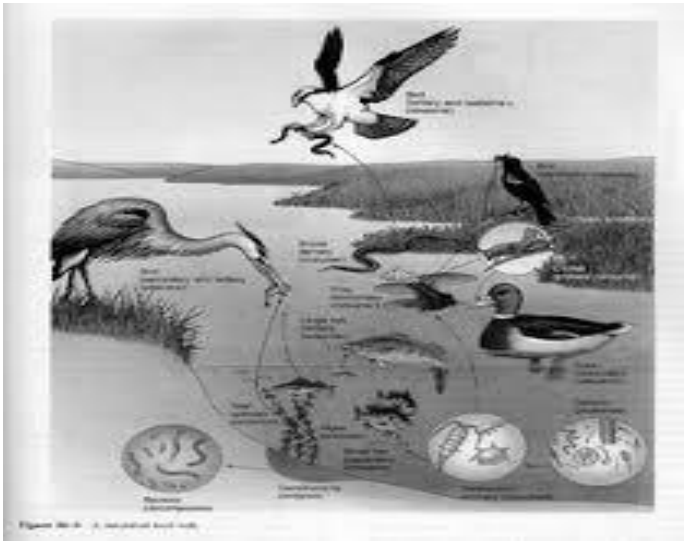
Water is the common link among the five biomes and it makes up the largest part of the biosphere, covering nearly 75% of the Earth's surface. Aquatic regions house numerous species of plants and animals, both large and small. In fact, this is where life began billions of years ago when amino acids first started to come together. Without water, most life forms would be unable to sustain themselves and the Earth would be a barren, desert-like place. Although water temperatures can vary widely, aquatic areas tend to be more humid and the air temperature on the cooler side.

The aquatic biome can be broken down into two basic regions, freshwater (i.e, ponds and rivers) and marine (i.e, oceans and estuaries).

Freshwater Regions

Freshwater is defined as having a low salt concentration—usually less than 1%. Plants and animals in freshwater regions are adjusted to the low salt content and would not be able to survive in areas of high salt concentration (i.e, ocean). There are different types of freshwater regions: ponds and lakes, streams and rivers, and wetlands. The following sections describe the characteristics of these three freshwater zones.

Ponds and Lakes-



These regions range in size from just a few square meters to thousands of square kilometers. Scattered throughout the earth, several are remnants from the Pleistocene glaciation. Many ponds are seasonal, lasting just a couple of months (such as sessile pools) while lakes may exist for hundreds of years or more. Ponds and lakes may have limited species diversity since they are often isolated from one another and from other water sources like rivers and oceans.

Lakes and ponds are divided into three different "zones" which are usually determined by depth and distance from the shoreline.

The topmost zone near the shore of a lake or pond is the *littoral zone*. This zone is the warmest since it is shallow and can absorb more of the Sun's heat. It sustains a fairly diverse community, which can include several species of algae (like diatoms), rooted and floating aquatic plants, grazing snails, clams, insects, crustaceans, fishes, and amphibians. In the case of the insects, such as dragonflies and midges, only the egg and larvae stages are found in this zone. The vegetation and animals living in the littoral zone are food for other creatures such as turtles, snakes, and ducks.

The near-surface open water surrounded by the littoral zone is the *limnetic zone*. The limnetic zone is well-lighted (like the littoral zone) and is dominated by plankton, both phytoplankton and zooplankton. Plankton are small organisms that play a crucial role in the food chain. Without aquatic plankton, there would be few living organisms in the world, and certainly no humans. A variety of freshwater fish also occupy this zone.

Plankton have short life spans—when they die, they fall into the deep-water part of the lake/pond, the *profundal zone*. This zone is much colder and denser than the other two. Little light penetrates all the way through the limnetic zone into the profundal zone. The fauna are heterotrophs, meaning that they eat dead organisms and use oxygen for cellular respiration.

Temperature varies in ponds and lakes seasonally. During the summer, the temperature can range from 4° C near the bottom to 22° C at the top. During the winter, the temperature at the

bottom can be 4° C while the top is 0° C (ice). In between the two layers, there is a narrow zone called the thermocline where the temperature of the water changes rapidly. During the spring and fall seasons, there is a mixing of the top and bottom layers, usually due to winds, which results in a uniform water temperature of around 4° C. This mixing also circulates oxygen throughout the lake. Of course there are many lakes and ponds that do not freeze during the winter, thus the top layer would be a little warmer.

Streams and river-

These are bodies of flowing water moving in one direction. Streams and rivers can be found everywhere-they get their starts at headwaters, which may be springs, snowmelt or even lakes, and then travel all the way to their mouths, usually another water channel or the ocean. The characteristics of a river or stream change during the journey from the source to the mouth. The temperature is cooler at the source than it is at the mouth. The water is also clearer, has higher oxygen levels, and freshwater fish such as trout and heterotrophs can be found there. Towards the middle part of the stream/river, the width increases, as does species diversity-numerous aquatic green plants and algae can be found. Toward the mouth of the river/stream, the water becomes murky from all the sediments that it has picked up upstream, decreasing the amount of light that can penetrate through the water. Since there is less light, there is less diversity of flora, and because of the lower oxygen levels, fish that require less oxygen, such as catfish and carp, can be found.

Wetlands:

Wetlands are areas of standing water that support aquatic plants. Marshes, swamps, and bogs are all considered wetlands. Plant species adapted to the very moist and humid conditions are called hydrophytes. These include pond lilies, cattails, sedges, tamarack, and black spruce. Marsh flora also include such species as cypress and gum. Wetlands have the highest species diversity of all ecosystems. Many species of amphibians, reptiles, birds (such as ducks and waders), and furbearers can be found in the wetlands. Wetlands are not considered freshwater ecosystems as there are some, such as salt marshes, that have high salt concentrations—these support different species of animals, such as shrimp, shellfish, and various grasses.

Marine Regions:

Marine regions cover about three-fourths of the Earth's surface and include oceans, coral reefs, and estuaries. Marine algae supply much of the world's oxygen supply and take in a huge amount of atmospheric carbon dioxide. The evaporation of the seawater provides rainwater for the land.

Oceans

The largest of all the ecosystems, oceans are very large bodies of water that dominate the Earth's surface. Like ponds and lakes, the ocean regions are separated into separate zones: intertidal, pelagic, abyssal, and benthic. All four zones have a great diversity of species. Some say that the ocean contains the richest diversity of species even though it contains fewer species than there are on land.

The *intertidal zone* is where the ocean meets the land—sometimes it is submerged and at other times exposed, as waves and tides come in and out. Because of this, the communities are constantly changing. On rocky coasts, the zone is stratified vertically. Where only the highest tides reach, there are only a few species of algae and mollusks. In those areas usually submerged during high tide, there is a more diverse array of algae and small animals, such as herbivorous snails, crabs, sea stars, and small fishes. At the bottom of the intertidal zone, which is only exposed during the lowest tides, many invertebrates, fishes, and seaweed can be found. The intertidal zone on sandier shores is not as stratified as in the rocky areas. Waves keep mud and sand constantly moving, thus very few algae and plants can establish themselves—the fauna include worms, clams, predatory crustaceans, crabs, and shorebirds.

The *pelagic zone* includes those waters further from the land, basically the open ocean. The pelagic zone is generally cold though it is hard to give a general temperature range since, just like ponds and lakes, there is thermal stratification with a constant mixing of warm and cold ocean currents. The flora in the pelagic zone include surface seaweeds. The fauna include many species of fish and some mammals, such as whales and dolphins. Many feed on the abundant plankton.

The *benthic zone* is the area below the pelagic zone, but does not include the very deepest parts of the ocean (see *abyssal zone* below). The bottom of the zone consists of sand, slit, and/or dead organisms. Here temperature decreases as depth increases toward the abyssal zone, since light cannot penetrate through the deeper water. Flora are represented primarily by seaweed while the fauna, since it is very nutrient-rich, include all sorts of bacteria, fungi, sponges, sea anemones, worms, sea stars, and fishes.

The deep ocean is the *abyssal zone*. The water in this region is very cold (3°C), highly

pressured, high in oxygen content, but low in nutritional content. The abyssal zone supports many species of invertebrates and fishes. Mid-ocean ridges (spreading zones between tectonic plates), often with hydrothermal vents, are found in the abyssal zones along the ocean floors. Chemosynthetic bacteria thrive near these vents because of the large amounts of hydrogen sulfide and other minerals they emit. These bacteria are thus the start of the food web as they are eaten by invertebrates and fishes.

Coral Reefs

Coral reefs are widely distributed in warm shallow waters. They can be found as barriers along continents (e.g., the Great Barrier Reef off Australia), fringing islands, and atolls. Naturally, the dominant organisms in coral reefs are corals. Corals are interesting since they consist of both algae (zooanthellae) and tissues of animal polyp. Since reef waters tend to be nutritionally poor, corals obtain nutrients through the algae via photosynthesis and also by extending tentacles to obtain plankton from the water. Besides corals, the fauna include several species of microorganisms, invertebrates, fishes, sea urchins, octopuses, and sea stars.

Estuaries

Estuaries are areas where freshwater streams or rivers merge with the ocean. This mixing of waters with such different salt concentrations creates a very interesting and unique ecosystem. Microflora like algae, and macroflora, such as seaweeds, marsh grasses, and mangrove trees (only in the tropics), can be found here. Estuaries support a diverse fauna, including a variety of worms, oysters, crabs, and waterfowl.

TERRESTRIAL BIOME

Differences in temperature or precipitation determine the types of plants that grow in a given area. Generally speaking, height, density, and species diversity decreases from warm, wet climates to cool, dry climates. Raunkiaer (1934) classified plant life forms based on traits that varied with climate. One such system was based on the location of the perennating organ

(Table 1). These are tissues that give rise to new growth the following season, and are therefore sensitive to climatic conditions. The relative proportions of different life forms vary with climate. In fact, life form spectra are more alike in similar climates on different continents than they are in different climates on the same continent (Figure 3). Regions of similar climate and dominant plant types are called biomes. This chapter describes some of the major terrestrial biomes in the world; tropical forests, savannas, deserts, temperate grasslands, temperate deciduous forests, Mediterranean scrub, coniferous forests, and tundra (Figure 4).

Table 1: Raunkiaer life form classification system based on location of the perennating bud

Raunkiaer life form classification system based on location of the perennating bud.		
Life form	Location of perennating tissue	Plant types
Phanerophyte	*>0.5 m*	Trees and tall shrubs
Chamaephyte	0 - 0.5 m	Small shrubs and herbs
Hemicryptophyte	Soil surface	Prostrate shrubs or herbaceous plants that dieback each year
Cryptophyte	In the soil	Rhizomatous grasses or bulb forming herbs
Therophyte	Seed	Annuals

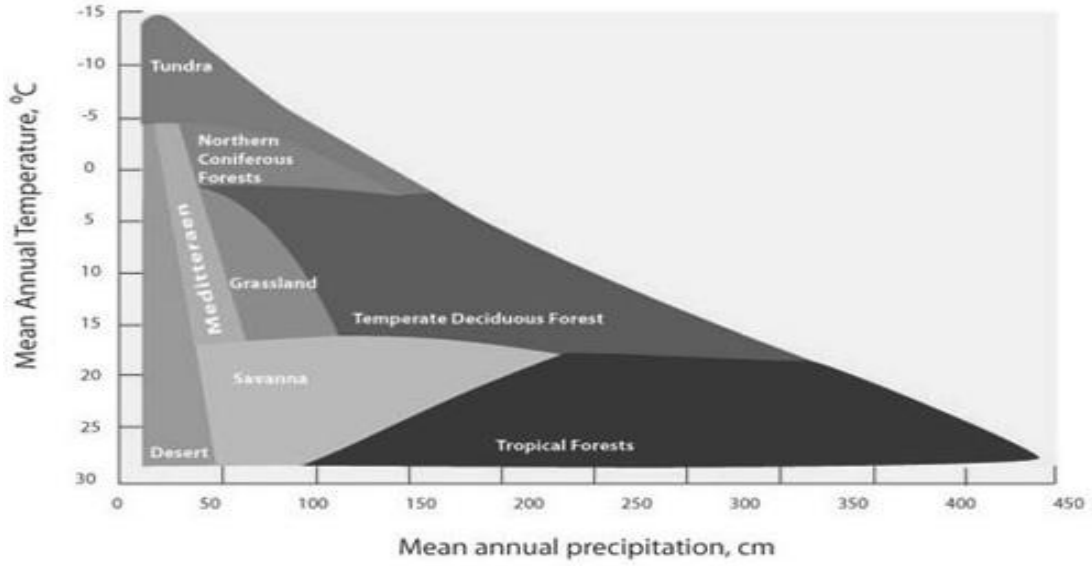
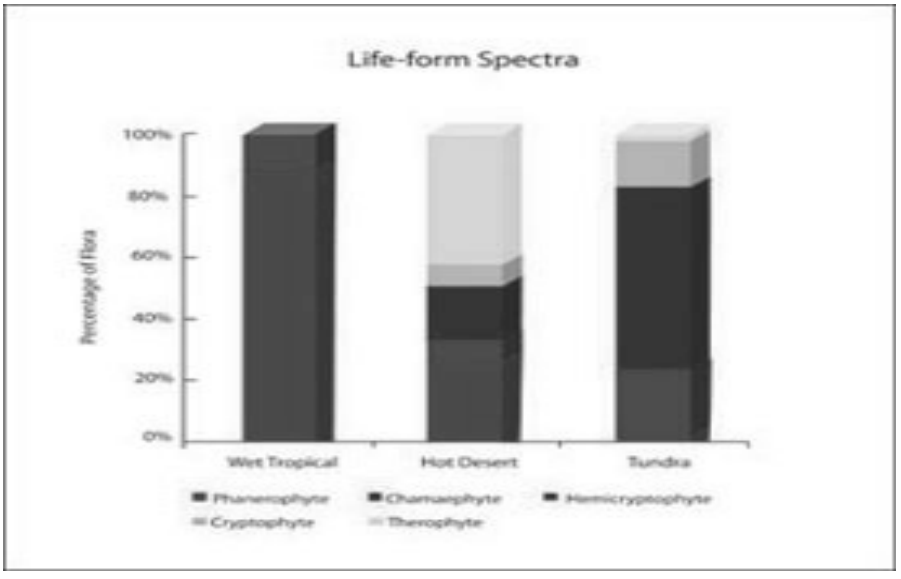


Figure 1: The distribution of vegetation types as a function of mean annual temperature and precipitation.

Tropical Forest Biomes:

Figure 2: Life-form spectra in different climates

Raunkiar classified plant life forms on traits that varied with climate, such as the perennating organ, or tissues that give rise to new growth the following season.



Tropical forests are found in areas centered on the equator (Figure 4). Central and South America possess half of the world's tropical forests. Climate in these biomes shows little seasonal variation (Figure 5), with high yearly rainfall and relatively constant, warm temperatures. The dominant plants are phanerophytes - trees, lianas, and epiphytes. Tropical rainforests have an emergent layer of tall trees over 40 m tall, an over story of trees up to 30

m tall, a sub-canopy layer of trees and tall shrubs, and a ground layer of herbaceous vegetation.

Tropical forests have the highest biodiversity and primary productivity of any of the terrestrial biomes. Net primary productivity ranges from 2–3 kg m^{-2} y^{-1} or higher. This high productivity is sustained despite heavily leached, nutrient poor soils, because of the high decomposition rates possible in moist, warm conditions. Litter decomposes rapidly, and rapid nutrient uptake is facilitated by mycorrhizae, which are fungal mutualists associated with plant roots. The tropical forest biome is estimated to contain over half of the terrestrial species on Earth. Approximately 170,000 of the 250,000 described species of vascular plants occur in tropical biomes. As many as 1,209 butterfly species have been documented in 55 square kilometers of the Tambopata Reserve in southeastern Peru, compared to 380 butterfly species in Europe and North Africa combined. The tropical forest biome is composed of several different sub-biomes, including evergreen rainforest, seasonal deciduous forest, tropical cloud forest, and mangrove forest. These sub-biomes develop due to changes in seasonal patterns of rainfall, elevation and/or substrate.

Savanna Biomes:

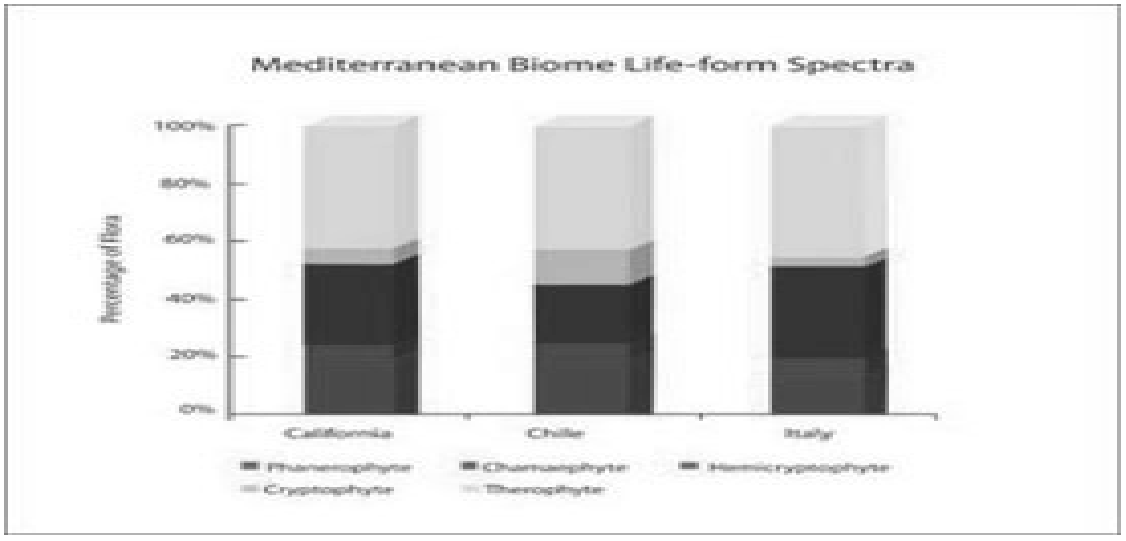


Figure 3: Life-form spectra in similar Mediterranean type climates on different continents

Located north and south of tropical forest biomes are savannas (Figure 4), with lower yearly rainfall and longer dry seasons (Figure 6). These biomes are dominated by a mix of grasses and small trees. Savannas cover 60% of Africa and represent a transition from tropical forests to deserts. Trees in savannas are usually drought deciduous. Several savanna types associated with differing rainfall patterns, height of the water table and soil depth can be distinguished

by their relative abundance of trees and grass.

Repetitive dry season fires have occurred in the African savanna over the last 50,000 years. Fire plays a major role in the balance between trees and grasses in savannas. With long periods between fires, tree and shrub populations increase. Fires release nutrients tied up in dead plant litter. Soil provides a good thermal insulator, so seeds and below ground rhizomes of grasses are usually protected fromdamage. Net primary productivity ranges from 400–600 g m^{-2} yr^{-1}, but varies depending upon local conditions such as soil depth. Decomposition is rapid and year-round, and the annual turnover rate of leaf material is high; up to 60–80%. This turnover is aided by the rich diversity of large herbivores found in savannas, where up to 60% of the biomass can be consumed in a given year. Dung beetles are important components of the nutrient cycle due to their role in breaking down animal droppings. The high herbivore diversity and production is mirrored by the great variety of predators and scavengers found in savannas.

Desert Biomes

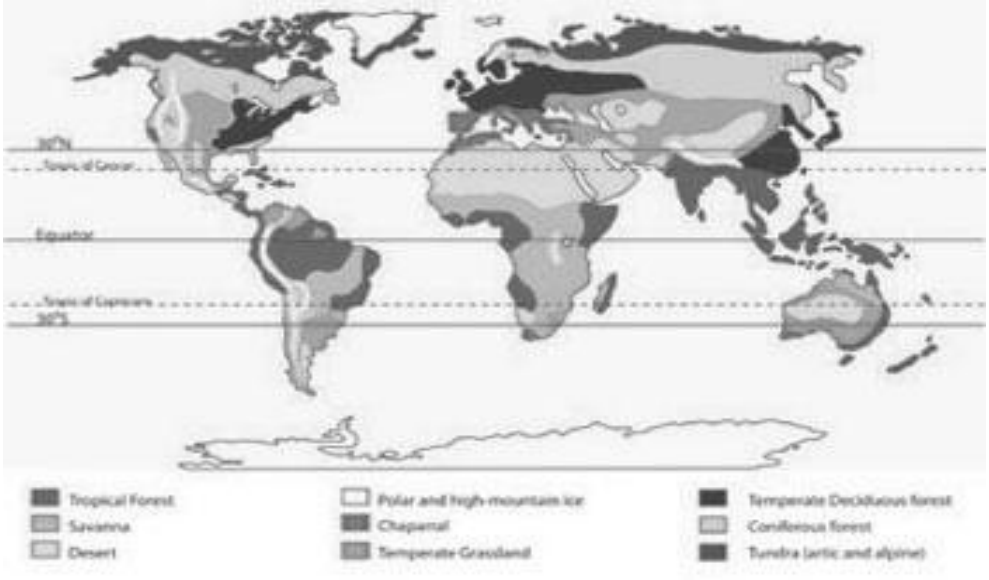


Figure 4: Biomes of the world
Biomes are regions of similar climate and dominant plant types.

Deserts generally occur in a band around the world between 15–30° N and S latitude (Figure 4). They cover between 26–35% of the land surface of the Earth. The climate of deserts is dominated by low precipitation, generally below 250 mm yr^{-1} (Figure 7). However, there is a lot of variability in desert types, with hot deserts, cold deserts, high elevation deserts, and rain shadow deserts. Consequently, there is a great deal of variation in the biodiversity, productivity and organisms found in different types of desert.

The dominant plant biomass in most deserts is composed of perennial shrubs with extensive roots and small, gray or white leaves. However, in warm deserts, therophytes (annual plants) can make up most of the species diversity (Figure 2). Desert annuals can survive unpredictable dry periods as seeds. Seeds may remain viable in the soil for several years, until the appropriate rainfall and temperature conditions occur, after which they will germinate. These annuals grow rapidly, completing their life cycle in a few weeks, then flowering and setting seed before soil water reserves are depleted. Winter desert annuals in North American deserts can generate over 1 kg m^{-2} of biomass in a wet year. With the exception of large blooms of annuals, net primary productivity in most deserts is low and extremely variable. There is a positive relationship between productivity and precipitation, and values can range from near 0 to 120 g m^{-2} yr^{-1}. Just as with savannas, productivity will vary with soil depth and local drainage patterns (e.g., washes).

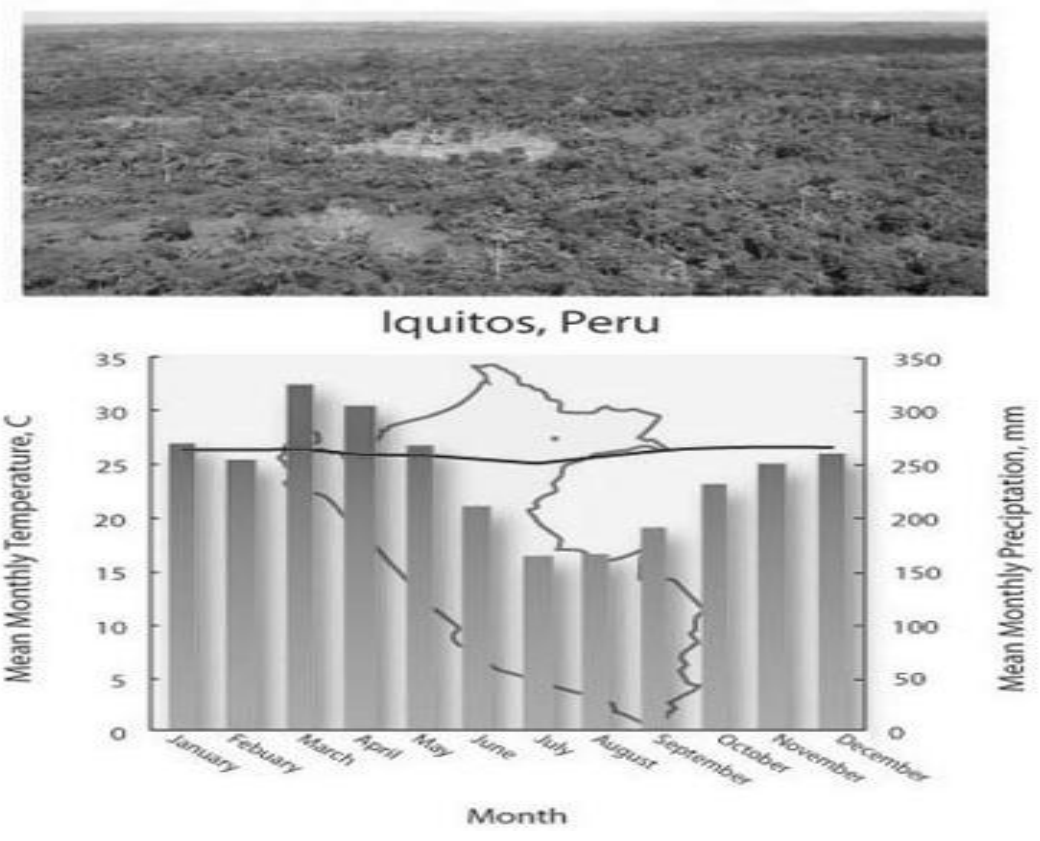


Figure 5: Tropical forest biome climate diagram
Climate in these areas show little seasonal variation with high yearly rainfall and relatively constant, warm temperatures.

Grassland Biomes

Grassland biomes occur primarily in the interiors of continents (Figure 4) and are characterized by large seasonal temperature variations, with hot summers and cold winters (Figure 8). Precipitation varies, with a strong summer peak. The type of grassland community that develops, and the productivity of grasslands, depends strongly upon precipitation. Higher precipitation leads to tall grass prairie with a high biodiversity of grasses and forbs. Lower precipitation leads to short grass prairies and arid grasslands.

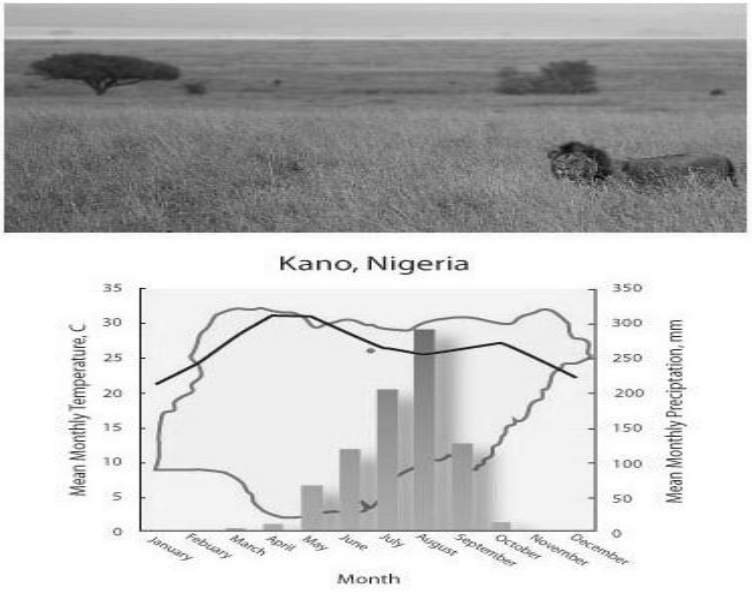


Figure 6: Savanna biome climate diagram
Savannas are located north and south of tropical forest biomes and are characterized by lower yearly rainfall and longer dry seasons.

Net primary productivity in dry grasslands may be 400 g m^{-2} yr^{-1}, while higher precipitation may support up to 1 kg m^{-2} yr^{-1}. Grasslands grade into deciduous forest biomes on their wetter margins, and deserts on their drier margins. The borders between grasslands and other biomes are dynamic and shift according to precipitation, disturbance, fire and drought. Fire and drought will favor grassland over forest communities.

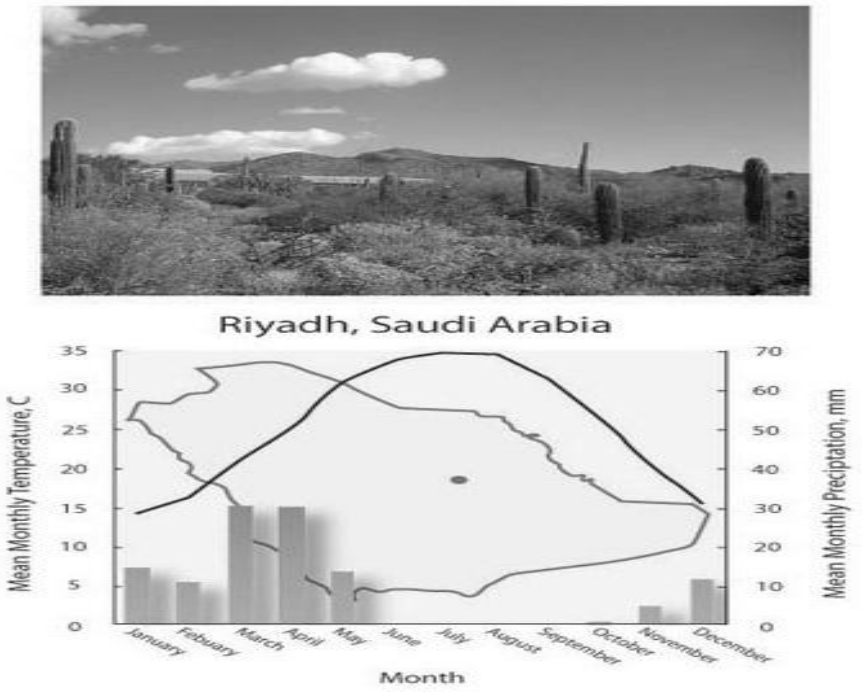


Figure 7: Desert biome climate diagram
There is a greater variability in desert types, with hot deserts, cold deserts, high elevation deserts, and rain shadow deserts.

Three major selective forces dominate the evolution of plant traits in grasslands, recurring fire, periodic drought, and grazing. These factors have led to the dominance of hemicryptophytes in grasslands with perennating organs located at or below the soil surface. Many grasses have below ground rhizomes connecting above ground shoots or tillers. Grass blades grow from the bottom up, with actively dividing meristems at the base of the leaf. Thus when grazers eat the grass blade, the meristem continues to divide and the blade can continue to grow. Grasses are often decay-resistant, and recurring cool, fast moving surface fires started by lightning at the end of summer aid in nutrient recycling. Fires stimulate productivity and the germination of fire resistant seeds.

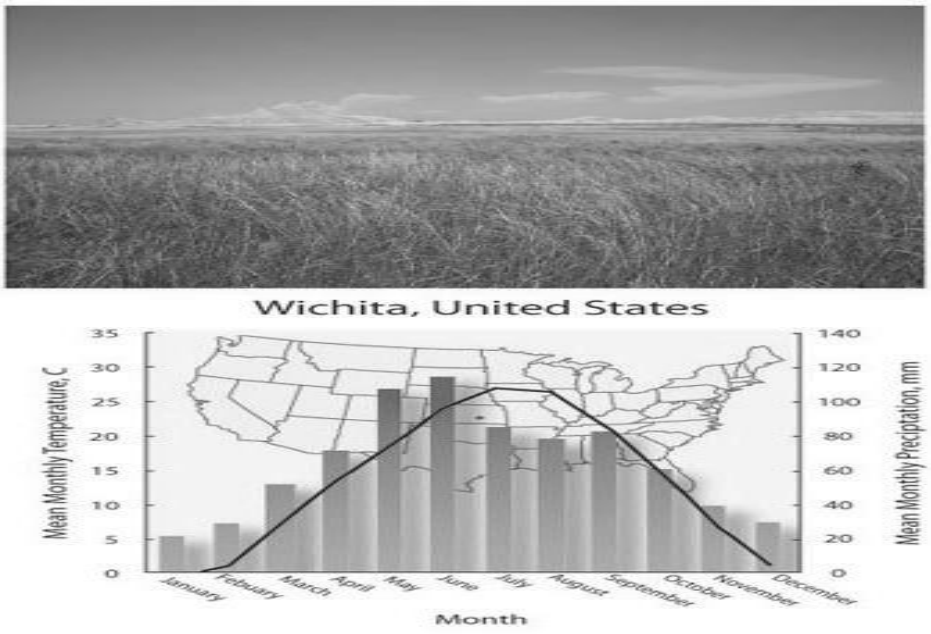


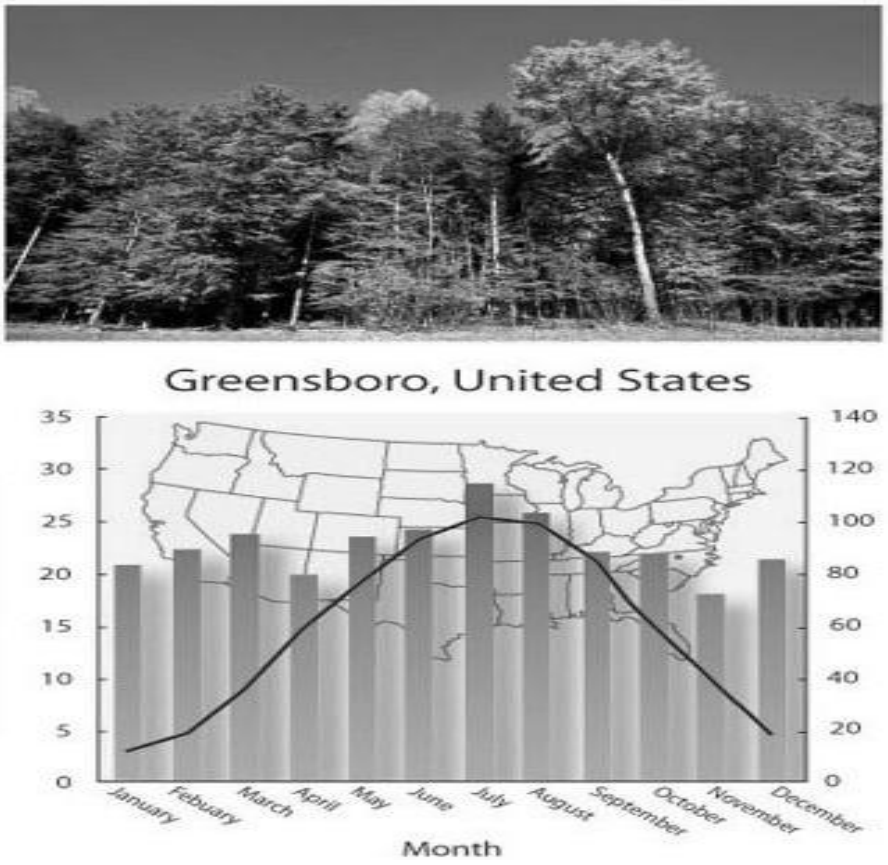
Figure 8: Grassland biome climate diagram
Grassland biomes occur primarily in the interiors of continents and are characterized by large seasonal temperature variations, with hot summers and cold winters.

Many of the world's largest terrestrial animals are found in grasslands. Animals such as gray kangaroos (*Macropus giganteus*) in Australia, Bison (*Bison bonasus*) and horses (*Equus* spp.) in Eurasia and North America were part of species rich assemblages of grazing animals, their predators, and scavengers. Remnant herds in North America suggest that disturbances due to grazers increased local biodiversity by creating openings that rare species could colonize. Large grazers also accelerated plant decomposition through their droppings, creating nutrient hotspots that altered species composition.

Temperate Deciduous Forest Biome

Temperature deciduous forests occur in mid-latitudes (Figure 4) where cool winters, warm summers, and high year round precipitation occurs (Figure 9). Net primary productivity ranges from 600–1500 g m^{-2} yr^{-1} with high litter production. Litter serves as a major pathway for nutrient recycling. This biome is named for the dominant trees that drop their leaves during the winter months. These forests may have an overstory of 20–30 m tall trees, an understory of 5–10 m trees and shrubs, a shrub layer around 1–2 m in height, and a ground

layer of herbaceous plants. Biodiversity is relatively high in this biome due to the niche partitioning allowed by the multiple forest layers. More complex forests are associated with a



greater number of animal species; for example, bird species diversity shows a positive correlation with forest height and number of layers.

Figure 9: Temperate deciduous forest climate diagram
Temperature deciduous forests occur in mid-latitudes and are characterized by cool winters, warm summers, and high year round precipitation occurs.

Mediterranean Climate Biomes2

This small biome (about 1.8 million square km) is separated into five separate regions between 30–40 degrees N and S latitude (Figure 4) with hot, dry summers, and cool, moist winters (Figure 10). Unrelated evergreen, sclerophyllous shrubs and trees have evolved independently in each of these areas, representing a striking example of convergent evolution. Net primary productivity varies from 300–600 g m^{-2} yr^{-1}, dependent upon water availability, soil depth, and age of the stand. Stand productivity decreases after 10–20 years as litter and woody biomass accumulates. Recurring fires aid in nutrient cycling and many plants show fire-induced or fire-promoted flowering. Some species are able to resprout from buds protected by the soil, while others germinate from decay-resistant seeds that lie dormant in the

soil until a fire promotes their germination. Therophytes make up a large component of the flora, and their appearance is associated with openings created by fires.

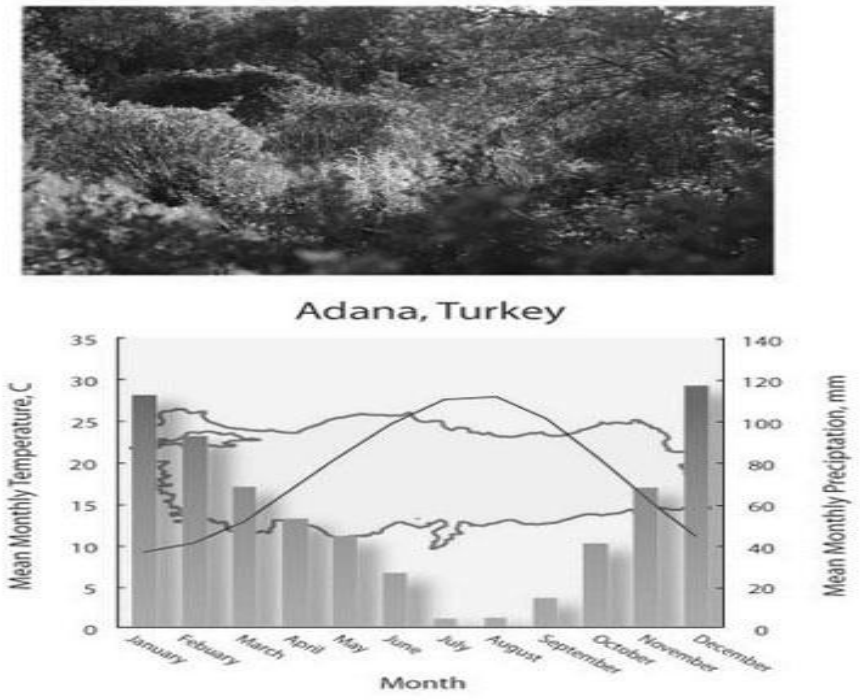


Figure 10: Mediterranean biome climate diagram
There are five separate regions between 30-40 degrees N and S latitude with hot, dry summers, and cool, moist winters.

Northern Coniferous Forest Biome

Located at higher latitudes is a biome dominated by needle-leaved, drought tolerant, evergreen trees (Figure 4), and a climate consisting of long, cold winters and short, cool summers (Figure 11). Biodiversity is low in this two-layered forest made up of an overstory of trees and a ground layer of herbs or mosses. The overstory in much of the boreal forest is made up of only one or two species. The low biodiversity is mirrored by low net primary productivity of 200–600 g m^{-2} yr^{-1}. Productivity varies with precipitation, the length of the frost-free period, and local soil drainage. In flooded areas, sphagnum bogs may develop. The acidic tissue of sphagnum, and the anoxic, flooded conditions, slows decomposition, resulting in the production of peat bogs.

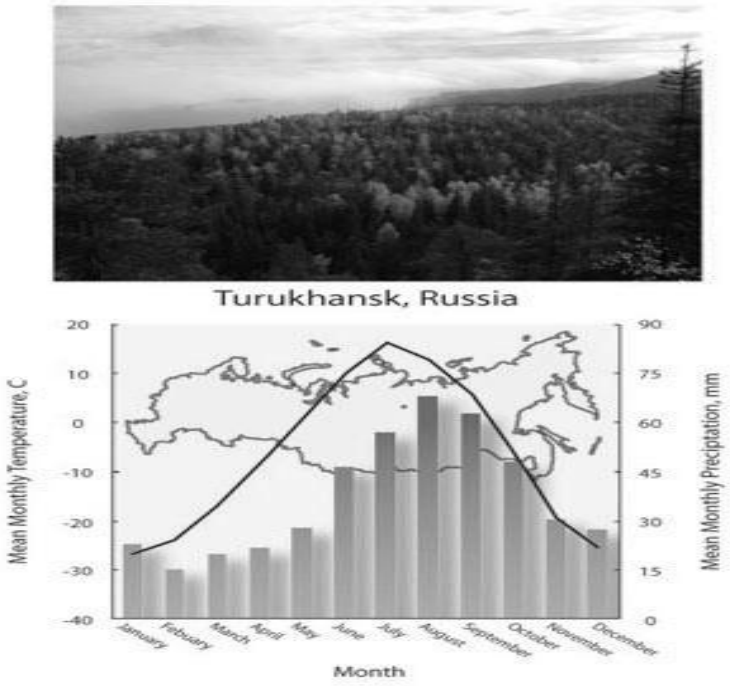


Figure 11: Boreal forest biome climate diagram
Boreal forests are characterized by needle-leaved, drought tolerant, evergreen trees, and a climate consisting of long, cold winters and short, cool summers.

Biomass in tree trunks and long-lived evergreen leaves results in nutrients being stored in the plants. Low temperatures lead to slow decomposition and high litter accumulation. Up to 60% of the biomass may be tied up in litter and humus. Soils are heavily leached, and permafrost underlies much of the soil. Consequently, trees have shallow root systems and rely on extensive mycorrhizal associations for nutrient uptake.

Tundra Biome

At latitudes beyond the boreal forest tree line lies a marshy area (Figure 4) where growing seasons are very short and temperatures are below zero degrees Celsius for much of the year (Figure 12). Because of these low temperatures and short growing seasons, net primary productivity is very low in the tundra, between 100–200 g m^{-2} yr^{-1}. Productivity varies with snowfall depth and local drainage. Rocky fields and dry meadows will have lower productivity than moist, low-lying areas and wet meadows.

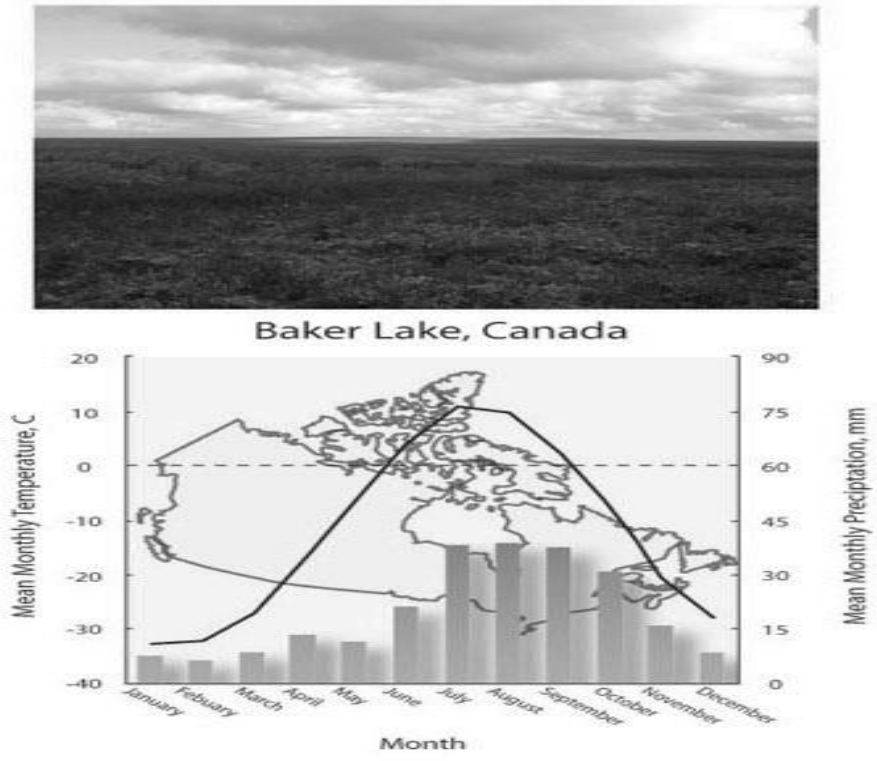


Figure 12: Tundra biome climate diagram
Very short growing seasons and temperatures that are below zero degrees Celsius for much of the year characterize tundras.

Biodiversity in the tundra is low and dominated by mosses, lichens, and low-growing perennial shrubs. The tundra biome contains only about 3% of the world's flora. Up to 60% of the flora can be made up of long-lived hemicryptophytes. Windy conditions and low temperatures select for low growing shrubs, often with tightly-packed, rounded canopies with closely spaced leaves and branches. Wind and ice damage help form this shape by pruning branches. The canopy morphology reduces wind speeds and absorbs solar radiation, resulting in canopy temperatures on sunny days more than 10° C above air temperature.

Soils are low in nutrients due to slow decomposition rates and plants retain nutrients in long-lived evergreen tissues. Nitrogen fixation by lichens with cyanobacterial components is a major source of soil nitrogen. Animals have extended hibernation periods or migrate seasonally.

4. Environmental pollution- Pollution and pollutant- Concept, definition and characteristics.

a. Air pollution- Source and types of air pollutant and their chemistry, photochemical reactions, green house and global warming, O_3depletion, acid rain, air pollutant in India

b. Water pollution- Source and type of water pollution, effect of water pollution on ecosystem, heavy metals and their effect on biota, nuclear pollution and thermal pollution

c. Soil pollution- Sources and classes of soil pollutants and their environmental effects, solid waste-pollution and disposal problems, waste- effect disposal andmanagement

a) Air pollution

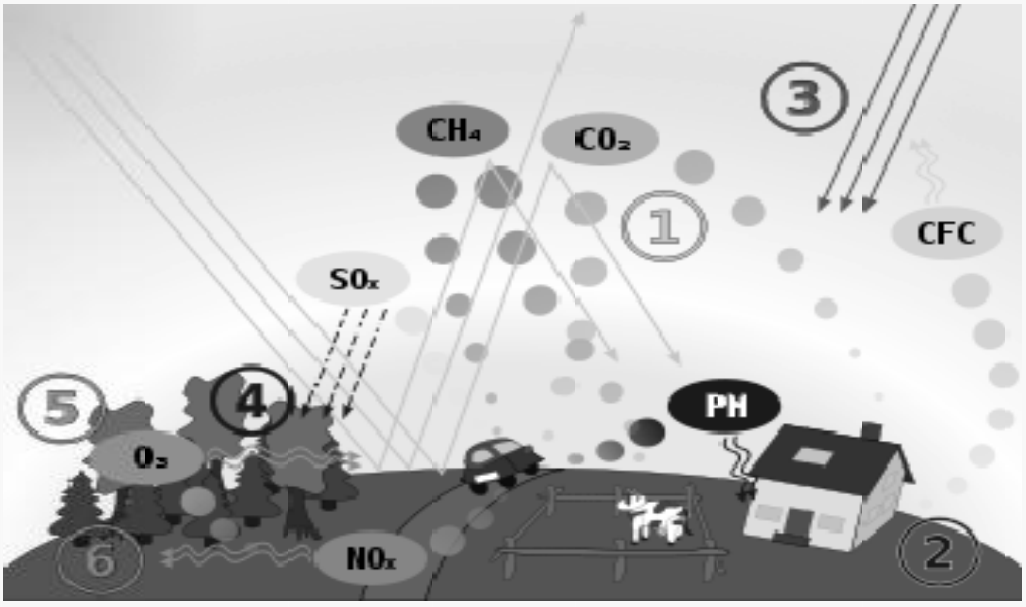
Air pollution occurs when harmful or excessive quantities of substances including gases, particles, and biological molecules are introduced into Earth's atmosphere. It may cause diseases, allergies and even death to humans; it may also cause harm to other living organisms such as animals and food crops, and may damage the natural or built environment. Both human activity and natural processes can generate air pollution.

Indoor air pollution and poor urban air quality are listed as two of the world's worst toxic pollution problems in the 2008 Blacksmith Institute World's Worst Polluted Places report. Outdoor air pollution alone causes 2.1 to 4.21 million premature deaths annually. According to the 2014 World Health Organization report, air pollution in 2012 caused the deaths of around 7 million people worldwide, an estimate roughly echoed by the International Energy Agency.

Pollutants:

An air pollutant is a material in the air that can have adverse effects on humans and the ecosystem. The substance can be solid particles, liquid droplets, or gases. A pollutant can be of natural origin or man-made. Pollutants are classified as primary or secondary. Primary pollutants are usually produced by processes such as ash from a volcanic eruption. Other examples include carbon monoxide gas from motor vehicle exhausts or Sulphur dioxide released from factories. Secondary pollutants are not emitted directly. Rather, they form in the air when primary pollutants react or interact. Ground level ozone is a prominent example of secondary pollutants. Some pollutants may be both primary and secondary: they are both emitted directly and formed from other primary pollutants.

Before flue-gas desulphurization was installed, the emissions from this power plant in New Mexico contained excessive amounts of ulphur dioxide.



Schematic drawin , causes a d effects of air pollution: (1) gr enhouse effect, (2) particulate contamination, (3) increased UV radiation, (4) acid rain, (5) increased ground-level ozone concentration, (6) increased levels of nitrogen oxides.

Thermal oxidizers are air pollution abatement options for hazardous air pollutants (HAPs), volatile organic compounds (VOCs), and odorous emissions

Pollutants emitted into the atmosphere by human activity include:

- Carbon dioxide– Because of its role as a greenhouse gas it has been described as "the leading pollutant"and "the worst climate pollution". Carbon dioxide is a natural component of the atmosphere, essential for plant life and given off by the human respiratory system. This question of terminology has practical effects, for example as determining whether the U.S. Clean Air Act is deemed to regulate CO_2 emissions.CO_2 currently forms about 410 parts per million (ppm) of earth's atmosphere, compared to about 280 ppm in pre-industrial times, and billions of metric tons of CO_2 are emitted annually by burning of fossil fuels. CO2 increase in earth's atmosphere has been accelerating.
- Sulfur oxides (SO_x) – particularly Sulphur dioxide, a chemical compound with the formula SO_2. SO2 is produced by volcanoes and in various industrial processes. Coal and petroleum often contain Sulphur compounds, and their combustion generates Sulphur dioxide. Further oxidation of SO_2, usually in the presence of a catalyst such as NO_2, forms H_2SO_4, and thus acid rain. This is one of the causes for concern over the environmental impact of the use of these fuels as power sources.
- Nitrogen oxides (NO_x) – Nitrogen oxides, particularly nitrogen dioxide, are expelled from high temperature combustion, and are also produced during thunderstorms by electric

discharge. Theycan be seen as a brown haze dome above or a plume downwind of cities. Nitrogen dioxide is a chemical compound with the formula NO_2. It is one of several nitrogen oxides. One of the most prominent air pollutants, this reddish-brown toxic gas has a characteristic sharp, biting odor.

- Carbon monoxide (CO) – CO is a colorless, odorless, toxic yet non-irritating gas. It is a product of combustion of fuel such as natural gas, coal or wood. Vehicular exhaust contributes to the majority of carbon monoxide let into our atmosphere. It creates a smog type formation in the air that has been linked to many lung diseases and disruptions to the natural environment and animals. In 2013, more than half of the carbon monoxide emitted into our atmosphere was from vehicle traffic and burning one gallon of gas will often emit over 20 pounds of carbon monoxide into the air.
- Volatile organic compounds (VOC) – VOCs are a well-known outdoor air pollutant. They are categorized as either methane (CH_4) or non-methane (NMVOCs). Methane is an extremely efficient greenhouse gas which contributes to enhance global warming. Other hydrocarbon VOCs are also significant greenhouse gases because of their role in creating ozone and prolonging the life of methane in the atmosphere. This effect varies depending on local air quality. The aromatic NMVOCs benzene, toluene and xylene are suspected carcinogens and may lead to leukemia with prolonged exposure. 1, 3-butadiene is another dangerous compound often associated with industrial use.
- Particulate matter / particles, alternatively referred to as particulate matter (PM), atmospheric particulate matter, or fine particles, are tiny particles of solid or liquid suspended in a gas. In contrast, aerosol refers to combined particles and gas. Some particulates occur naturally, originating from volcanoes, dust storms, forest and grassland fires, living vegetation, and sea spray. Human activities, such as the burning of fossil fuels in vehicles, power plants and various industrial processes also generate significant amounts of aerosols. Averaged worldwide, anthropogenic aerosols—those made by human activities—currently account for approximately 10 percent of our atmosphere. Increased levels of fine particles in the air are linked to health hazards such as heart disease, altered lung function and lung cancer. Particulates are related to respiratory infections and can be particularly harmful to those already suffering from conditions like asthma.
- Persistent free radicals connected to airborne fine particles are linked to cardiopulmonary disease.
- Toxic metals, such as lead and mercury, especially their compounds.

- Chlorofluorocarbons (CFCs) – harmful to the ozone layer; emitted from products are currently banned from use. These are gases which are released from air conditioners, refrigerators, aerosol sprays, etc. On release into the air, CFCs rise to the stratosphere. Here they come in contact with other gases and damage the ozone layer. This allows harmful ultraviolet rays to reach the earth's surface. This can lead to skin cancer, eye disease and can even cause damage to plants.
- Ammonia – emitted mainly by agricultural waste. Ammonia is a compound with the formula NH_3. It is normally encountered as a gas with a characteristic pungent odor. Ammonia contributes significantly to the nutritional needs of terrestrial organisms by serving as a precursor to foodstuffs and fertilizers. Ammonia, either directly or indirectly, is also a building block for the synthesis of many pharmaceuticals. Although in wide use, ammonia is both caustic and hazardous. In the atmosphere, ammonia reacts with oxides of nitrogen and Sulphur to form secondary particles.
- Odors — such as from garbage, sewage, and industrial processes
- Radioactive pollutants – produced by nuclear explosions, nuclear events, war explosives, and natural processes such as the radioactive decay of radon.

Secondary pollutants include:

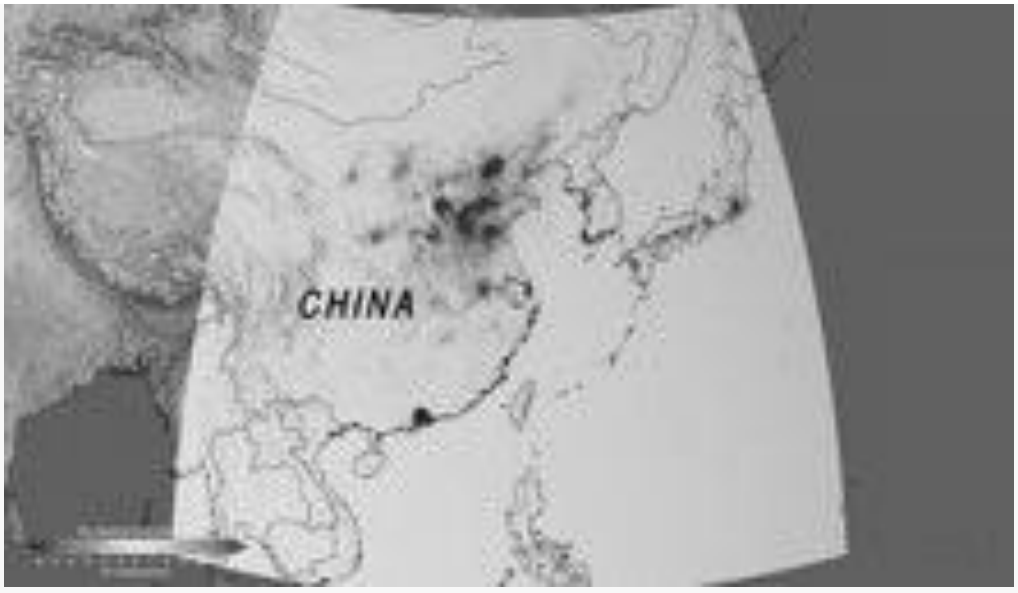
- P**articulate**s created from gaseous primary pollutants and compounds in photochemical smog. Smog is a kind of air pollution. Classic smog results from large amounts of coal burning in an area caused by a mixture of smoke and Sulphur dioxide. Modern smog does not usually come from coal but from vehicular and industrial emissions that are acted on in the atmosphere by ultraviolet light from the sun to form secondary pollutants that also combine with the primary emissions to form photochemical smog.
- **Ground level ozone** (O_3) formed from NO_x and VOCs. Ozone (O_3) is a key constituent of the troposphere. It is also an important constituent of certain regions of the stratosphere commonly known as the Ozone layer. Photochemical and chemical reactions involving it drive many of the chemical processes that occur in the atmosphere by day and by night. At abnormally high concentrations brought about by human activities (largely the combustion of fossil fuel), it is a pollutant and a constituent of smog.
- **Peroxyacetyl nitrate** ($C_2H_3NO_5$) – similarly formed from NO_x and VOCs.

Minor air pollutants include:

- A large number of minor hazardous air pollutants. Some of these are regulated in USA under the Clean Air Act and in Europe under the Air Framework Directive

A variety of persistent organic pollutants, which can attach to particulates
This video provides an overview of a NASA study on the human fingerprint on global air quality.

Persistent organic pollutants (POPs) are organic compounds that are resistant to environmental degradation through chemical, biological, and photolytic processes. Because of this, they have been observed to persist in the environment, to be capable of long-range transport, bio-accumulate in human and animal tissue, bio-magnify in food chains, and to have potentially significant impacts on human health and the environment.



Source:

Mean acidifying emissions (air pollution) of different foods per 100g of protein	
Food Types	**Acidifying Emissions (g SO_2eq per 100g protein)**
Beef	343.6
Cheese	165.5
Pork	142.7
Lamb and Mutton	139.0
Farmed Crustaceans	133.1
Poultry	102.4
Farmed Fish	65.9
Eggs	53.7
Groundnuts	22.6
Peas	8.5
Tofu	6.7

There are various locations, activities or factors which are responsible for releasing pollutants into the atmosphere. These sources can be classified into two major categories.

Anthropogenic (man-made) sources:

Controlled burning of a field outside of Statesboro, Georgia in preparation for spring planting.

Smoking of fish over an open fire in Ghana, 2018

These are mostly related to the burning of multiple types of fuel.

- Stationary sources include smoke stacks of fossil fuel power stations (see for example environmental impact of the coal industry), manufacturing facilities (factories) and waste incinerators, as well as furnaces and other types of fuel-burning heating devices. In developing and poor countries, traditional biomass burning is the major source of air pollutants; traditional biomass includes wood, crop waste and dung.
- Mobile sources include motor vehicles, marine vessels, and aircraft.
- Controlled burn practices in agriculture and forest management. Controlled or prescribed burning is a technique sometimes used in forest management, farming, prairie restoration or greenhouse gas abatement. Fire is a natural part of both forest and grassland ecology and controlled fire can be a tool for foresters. Controlled burning stimulates the germination of some desirable forest trees, thus renewing the forest.
- Fumes from paint, hair spray, varnish, aerosol sprays and other solvents. These can be substantial; emissions from these sources was estimated to account for almost half of pollution from volatile organic compounds in the Los Angeles basin in the 2010s.
- Waste deposition in landfills, which generate methane. Methane is highly flammable and may form explosive mixtures with air. Methane is also an asphyxiant and may displace oxygen in an enclosed space. Asphyxia or suffocation may result if the oxygen concentration is reduced to below 19.5% by displacement.

- Military resources, such as nuclear weapons, toxic gases, germ warfare and rocketry.
- Fertilized farmland may be a major source of nitrogen oxides.

Natural sources

Dust storm approaching Stratford, Texas.

- Dust from natural sources, usually large areas of land with little or no vegetation
- Methane, emitted by the digestion of food by animals, for example cattle
- Radon gas from radioactive decay within the Earth's crust. Radon is a colorless, odorless, naturally occurring, radioactive noble gas that is formed from the decay of radium. It is considered to be a health hazard. Radon gas from natural sources can accumulate in buildings, especially in confined areas such as the basement and it is the second most frequent cause of lung cancer, after cigarette smoking.
- Smoke and carbon monoxide from wildfires. During periods of actives wildfires, smoke from uncontrolled biomass combustion can make up almost 75% of all air pollution by concentration.[27]
- Vegetation, in some regions, emits environmentally significant amounts of Volatile organic compounds (VOCs) on warmer days. These VOCs react with primary anthropogenic pollutants—specifically, NO_x, SO_2, and anthropogenic organic carbon compounds — to produce a seasonal haze of secondary pollutants. Black gum, poplar, oak and willow are some examples of vegetation that can produce abundant VOCs. The VOC production from these species result in ozone levels up to eight times higher than the low-impact tree species.
- Volcanic activity, which produces sulphur, chlorine, and ash particulates

Emission factors:

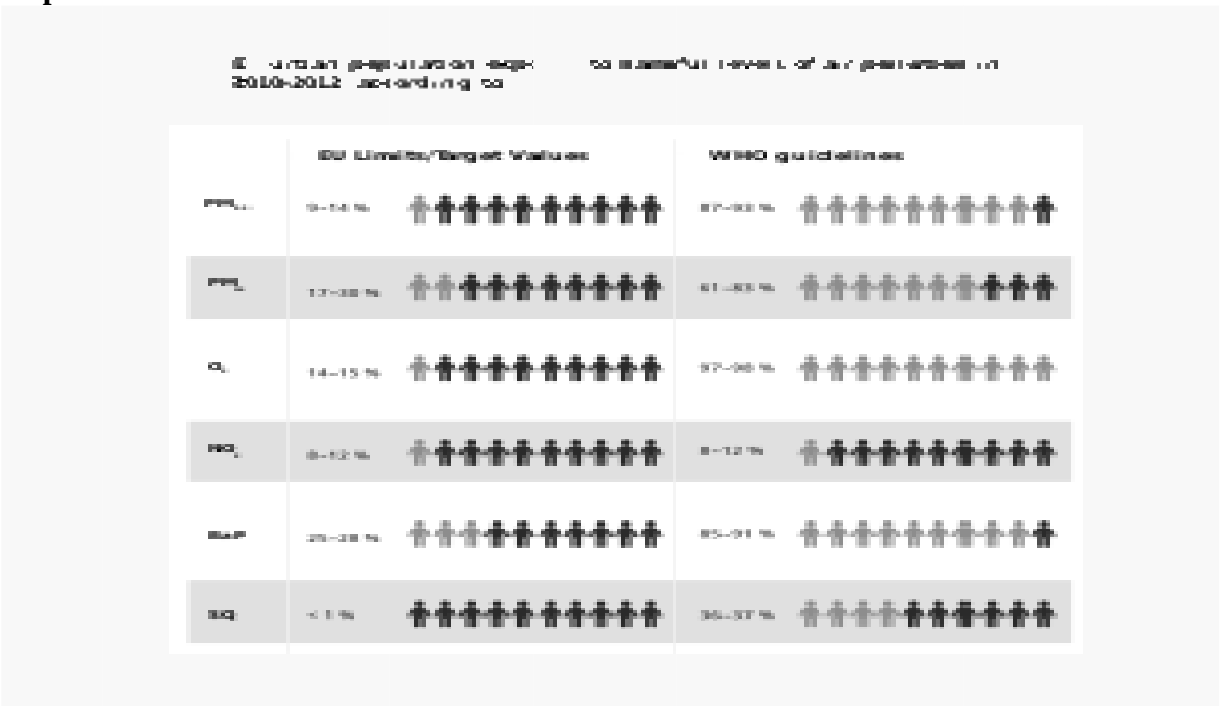
Beijing air on a 2005-day after rain (left) and a smoggy day (right)

Air pollutant emission factors are reported representative values that attempt to relate the quantity of a pollutant released to the ambient air with an activity associated with the release of that pollutant. These factors are usually expressed as the weight of pollutant divided by a unit weight, volume, distance, or duration of the activity emitting the pollutant (e.g., kilograms of particulate emitted per tonne of coal burned). Such factors facilitate estimation of emissions from various sources of air pollution. In most cases, these factors are simply averages of all available data of acceptable quality, and are generally assumed to be representative of long-term averages.

There are 12 compounds in the list of persistent organic pollutants. Dioxins and furans are two of them and intentionally created by combustion of organics, like open burning of plastics. These compounds are also endocrine disruptors and can mutate the human genes.

The United States Environmental Protection Agency has published a compilation of air pollutant emission factors for a wide range of industrial sources. The United Kingdom, Australia, Canada and many other countries have published similar compilations, as well as the European Environment Agency.

Exposure



Up to 30 % of Europeans living in cities are exposed to air pollutant levels exceeding EU air quality standards. And aroun 98 % of uropeans living in cities are exposed to levels of air polluta ts deemed damaging to health by the World Health Organization's more stringent guideli es.

Air pollution risk is a function of the hazard of the pollutant and the exposure to that pollutar t. Air pollution exposure can be expressed for an individual, for certain groups (e.g. neighbc rhoods or children li ing in a country), or for entire populations. For example, one may w ant to calculate the exp osure to a hazardous air pollutant for a geographic area, which includes the various microenvironments and age groups. This can be calculate as an i rhalation exposure. This w uld account for daily exposure in various settings (e.g. different indoor micro-environments and outdoor locations). The exposure needs to inclu e different age and other demographic groups, e pecially infants, children, pregn ant women and other sensitive subpopulations. Th e exposur to an air pollutant must integrate the concentrations of the air pollutar t with respect to the time spent in each setting and the respective inhalation rates for each su bgroup for each spe ific time that the subgroup is in the setting and en ged in particular activities (playing, cooking, reading, working, spending time in traffic, etc.). For example, a small child's inhalation rate will be less than that of an adult. A child engaged in vigorous exercise will have a higher respiration rate than the same child in a sedentary activity. The daily exposure, then, needs to reflect th e time spe t in each icro-envir nmental setting and the type of activities in these settings. The air pollutant concentration in each micro ctivity/micro-environmental setting is summed to indicate the exposure. For some pollutar ts such as black carbon, traffic related expo ures may dominate total exposure despite short exposure ti es since high concentrations coincide with proximity to major roads or participation to (m otorized) traffic. A large portion of total daily exposure occurs as short peaks of high con entrations, but it rem ins unclear how to define peaks and determine their frequency and health impact.

Indoor air quality:

Air quality monitoring, New Delhi, India.

A lack of ventilation indoors concentrates air pollution where people often spend the majority of their time. Radon (Rn) gas, a carcinogen, is exuded from the Earth in certain locations and trapped inside houses. Building materials including carpeting and plywood emit formaldehyde (H_2CO) gas. Paint and solvents give off volatile organic compounds (VOCs) as they dry. Lead paint can degenerate into dust and be inhaled. Intentional air pollution is introduced with the use of air fresheners, incense, and other scented items. Controlled wood fires in stoves and fireplaces can add significant amounts of smoke particulates into the air, inside and out. Indoor pollution fatalities may be caused by using pesticides and other chemical sprays indoors without proper ventilation.

Carbon monoxide poisoning and fatalities are often caused by faulty vents and chimneys, or by the burning of charcoal indoors or in a confined space, such as a tent. Chronic carbon monoxide poisoning can result even from poorly-adjusted pilot lights. Traps are built into all domestic plumbing to keep sewer gas and hydrogen sulfide, out of interiors. Clothing emits tetrachloroethylene, or other dry cleaning fluids, for days after dry cleaning.

Though its use has now been banned in many countries, the extensive use of asbestos in industrial and domestic environments in the past has left a potentially very dangerous material in many localities. Asbestosis is a chronic inflammatory medical condition affecting the tissue of the lungs. It occurs after long-term, heavy exposure to asbestos from asbestos-containing materials in structures. Sufferers have severe dyspnea (shortness of breath) and are at an increased risk regarding several different types of lung cancer. As clear explanations are not always stressed in non-technical literature, care should be taken to distinguish between several

forms of relevant diseases. According to the World Health Organization (WHO), these may defined as; asbestosis, lung cancer, and Peritoneal Mesothelioma (generally a very rare form of cancer, when more widespread it is almost always associated with prolonged exposure to asbestos).

Biological sources of air pollution are also found indoors, as gases and airborne particulates. Pets produce dander, people produce dust from minute skin flakes and decomposed hair, dust mites in bedding, carpeting and furniture produce enzymes and micrometer-sized fecal droppings, inhabitants emit methane, mold forms on walls and generates mycotoxins and spores, air conditioning systems can incubate Legionnaires' disease and mold, and houseplants, soil and surrounding gardens can produce pollen, dust, and mold. Indoors, the lack of air circulation allows these airborne pollutants to accumulate more than they would otherwise occur in nature.

Health effects:

In 2012, air pollution caused premature deaths on average of 1 year in Europe, and was a significant risk factor for a number of pollution-related diseases, including respiratory infections, heart disease, COPD, stroke and cancer. The health effects caused by air pollution may include difficulty in breathing, wheezing, coughing, asthma and worsening of existing respiratory and cardiac conditions. These effects can result in increased medication use, increased doctor or emergency department visits, more hospital admissions and premature death. The human health effects of poor air quality are far reaching, but principally affect the body's respiratory system and the cardiovascular system. Individual reactions to air pollutants depend on the type of pollutant a person is exposed to, the degree of exposure, and the individual's health status and genetics the most common sources of air pollution include particulates, ozone, nitrogen dioxide, and Sulphur dioxide. Children aged less than five years that live in developing countries are the most vulnerable population in terms of total deaths attributable to indoor and outdoor air pollution.

Mortality

The World Health Organization estimated in 2014 that every year air pollution causes the premature death of some 7 million people worldwide. Studies published in March 2019 indicated that the number may be around 8.8 million.

India has the highest death rate due to air pollution India also has more deaths from asthma than any other nation according to the World Health Organization. In December 2013 air

pollution was estimated to kill 500,000 people in China each year. There is a positive correlation between pneumonia-related deaths and air pollution from motor vehicle emissions. Annual premature European deaths caused by air pollution are estimated at 430,000-800,000an important cause of these deaths is nitrogen dioxide and other nitrogen oxides (NOx) emitted by road vehicles. In a 2015 consultation document the UK government disclosed that nitrogen dioxide is responsible for 23,500 premature UK deaths per annum. Across the European Union, air pollution is estimated to reduce life expectancy by almost nine months. Causes of deaths include strokes, heart disease, COPD, lung cancer, and lung infections.

Urban outdoor air pollution is estimated to cause 1.3 million deaths worldwide per year. Children are particularly at risk due to the immaturity of their respiratory organ systems.

The US EPA estimated in 2004 that a proposed set of changes in diesel engine technology (Tier 2) could result in 12,000 fewer premature mortalities, 15,000 fewer heart attacks, 6,000 fewer emergency department visits by children with asthma, and 8,900 fewer respiratory-related hospital admissions each year in the United States.

The US EPA has estimated that limiting ground-level ozone concentration to 65 parts per billion, would avert 1,700 to 5,100 premature deaths nationwide in 2020 compared with the 75-ppb standard. The agency projected the more protective standard would also prevent an additional 26,000 cases of aggravated asthma, and more than a million cases of missed work or school. Following this assessment, the EPA acted to protect public health by lowering the National Ambient Air Quality Standards (NAAQS) for ground-level ozone to 70 parts per billion (ppb).

A new economic study of the health impacts and associated costs of air pollution in the Los Angeles Basin and San Joaquin Valley of Southern California shows that more than 3,800 people die prematurely (approximately 14 years earlier than normal) each year because air pollution levels violate federal standards. The number of annual premature deaths is considerably higher than the fatalities related to auto collisions in the same area, which average fewer than 2,000 per year.

Diesel exhaust (DE) is a major contributor to combustion-derived particulate matter air pollution. In several human experimental studies, using a well-validated exposure chamber setup, DE has been linked to acute vascular dysfunction and increased thrombus formation.

The mechanisms linking air pollution to increased cardiovascular mortality are uncertain, but probably include pulmonary and systemic inflammation.

Cardiovascular disease

A 2007 review of evidence found ambient air pollution exposure is a risk factor correlating with increased total mortality from cardiovascular events (range: 12% to 14% per 10 microg/m^3 increase).

Air pollution is also emerging as a risk factor for stroke, particularly in developing countries where pollutant levels are highest. A 2007 study found that in women, air pollution is not associated with hemorrhagic but with ischemic stroke. Air pollution was also found to be associated with increased incidence and mortality from coronary stroke in a cohort study in 2011. Associations are believed to be causal and effects may be mediated by vasoconstriction, low-grade inflammation and atherosclerosis other mechanisms such as autonomic nervous system imbalance have also been suggested.

Lung disease

Research has demonstrated increased risk of developing asthma and COPD from increased exposure to traffic-related air pollution. Additionally, air pollution has been associated with increased hospitalization and mortality from asthma and COPD. Chronic obstructive pulmonary disease (COPD) includes diseases such as chronic bronchitis and emphysema.

A study conducted in 1960–1961 in the wake of the Great Smog of 1952 compared 293 London residents with 477 residents of Gloucester, Peterborough, and Norwich, three towns with low reported death rates from chronic bronchitis. All subjects were male postal truck drivers aged 40 to 59. Compared to the

subjects from the outlying towns, the London subjects exhibited more severe respiratory symptoms (including cough, phlegm, and dyspnea), reduced lung function (FEV_1 and peak flow rate), and increased sputum production and purulence. The differences were more pronounced for subjects aged 50 to 59. The study controlled for age and smoking habits, so concluded that air pollution was the most likely cause of the observed differences. More recent studies have shown that air pollution exposure from traffic reduces lung function development in children and lung function may be compromised by air pollution even at low concentrations. Air pollution exposure also cause lung cancer in nonsmokers.

It is believed that much like cystic fibrosis, by living in a more urban environment serious health hazards become more apparent. Studies have shown that in urban areas patients suffer mucus hyper secretion, lower levels of lung function, and more self-diagnosis of chronic bronchitis and emphysema.

Cancer (lung cancer)

Cancer is mainly the result of environmental factors.

A review of evidence regarding whether ambient air pollution exposure is a risk factor for cancer in 2007 found solid data to conclude that long-term exposure to PM2.5 (fine particulates) increases the overall risk of non-accidental mortality by 6% per a 10 microg/m^3 increase. Exposure to PM2.5 was also associated with an increased risk of mortality from lung cancer (range: 15% to 21% per 10 microg/m^3 increase) and total cardiovascular mortality (range: 12% to 14% per a 10 microg/m^3 increase). The review further noted that living close to busy traffic appears to be associated with elevated risks of these three outcomes – increase in lung cancer deaths, cardiovascular deaths, and overall non-accidental deaths. The reviewers also found suggestive evidence that exposure to PM2.5 is positively associated with mortality from coronary heart diseases and exposure to SO_2 increases mortality from lung cancer, but the data was insufficient to provide solid conclusions. Another investigation showed that higher activity level increases deposition fraction of aerosol particles in human lung and recommended avoiding heavy activities like running in outdoor space at polluted areas.

In 2011, a large Danish epidemiological study found an increased risk of lung cancer for patients who lived in areas with high nitrogen oxide concentrations. In this study, the association was higher for non-smokers than smokers. An additional Danish study, also in 2011, likewise noted evidence of possible associations between air pollution and other forms of cancer, including cervical cancer and brain cancer.

In December 2015, medical scientists reported that cancer is overwhelmingly a result of environmental factors, and not largely down to bad luck. Maintaining a healthy weight, eating a healthy diet, minimizing alcohol and eliminating smoking reduces the risk of developing the disease, according to the researchers.

Children

In the United States, despite the passage of the Clean Air Act in 1970, in 2002 at least 146 million Americans were living in non-attainment areas—regions in which the concentration of certain air pollutants exceeded federal standards. These dangerous pollutants are known as the criteria pollutants, and include ozone, particulate matter, Sulphur dioxide, nitrogen dioxide, carbon monoxide, and lead. Protective measures to ensure children's health are being taken in cities such as New Delhi, India where buses now use compressed natural gas to help eliminate the "pea-soup" smog. A recent study in Europe has found that exposure to ultrafine particles can increase blood pressure in children. According to a WHO report-2018, polluted air is a main cause poisoning millions of children under the age of 15 years and ruining their lives which resulting to death of some six hundred thousand children annually.

Infants:

Ambient levels of air pollution have been associated with preterm birth and low birth weight. A 2014 WHO worldwide survey on maternal and perinatal health found a statistically significant association between low birth weights (LBW) and increased levels of exposure to PM2.5. Women in regions with greater than average PM2.5 levels had statistically significant higher odds of pregnancy resulting in a low-birth weight infant even when adjusted for country-related variables.The effect is thought to be from stimulating inflammation and increasing oxidative stress.

A study by the University of York found that in 2010 exposure to PM2.5 was strongly associated with 18% of preterm births globally, which was approximately 2.7 million premature births. The countries with the highest air pollution associated preterm births were in South and East Asia, the Middle East, North Africa, and West sub-Saharan Africa.

A study performed by Wang, et al. between the years of 1988 and 1991 has found a correlation between sulphur Dioxide (SO2) and total suspended particulates (TSP) and preterm births and low birth weights in Beijing. A group of 74,671 pregnant women, in four separate regions of Beijing, were monitored from early pregnancy to delivery along with daily air pollution levels of sulphur Dioxide and TSP (along with other particulates). The estimated reduction in birth weight was 7.3 g for every 100 $\mu g/m^3$ increase in SO2 and 6.9g for each

100 μg/m^3 increase in TSP. These associations were statistically significant in both summer and winter, although, summer was greater. The proportion of low birth weight attributable to air pollution, was 13%. This is the largest attributable risk ever reported for the known risk factors of low birth weight. Coal stoves, which are in 97% of homes, are a major source of air pollution in this area.

Brauer et al. studied the relationship between air pollution and proximity to a highway with pregnancy outcomes in a Vancouver cohort of pregnant woman using addresses to estimate exposure during pregnancy. Exposure to NO, NO2, CO PM10 and PM2.5 were associated with infants born small for gestational age (SGA). Women living <50meters away from an expressway or highway were 26% more likely to give birth to a SGA infant.

"Clean" areas

Even in the areas with relatively low levels of air pollution, public health effects can be significant and costly, since a large number of people breathe in such pollutants. A study published in 2017 found that even in areas of the U.S. where ozone and PM2.5 meet federal standards, Medicare recipients who are exposed to more air pollution have higher mortality rates. A 2005 scientific study for the British Columbia Lung Association showed that a small improvement in air quality (1% reduction of ambient PM2.5 and ozone concentrations) would produce $29 million in annual savings in the Metro Vancouver region in 2010. This finding is based on health valuation of lethal (death) and sub-lethal (illness) affects.

Central nervous system

Data is accumulating that air pollution exposure also affects the central nervous system.In a June 2014 study conducted by researchers at the University of Rochester Medical Center, published in the journal Environmental Health Perspectives, it was discovered that early exposure to air pollution causes the same damaging changes in the brain as autism and schizophrenia. The study also shows that air pollution also affected short-term memory, learning ability, and impulsivity. Lead researcher Professor Deborah Cory-Slechta said that "When we looked closely at the ventricles, we could see that the white matter that normally surrounds them hadn't fully developed. It appears that inflammation had damaged those brain cells and prevented that region of the brain from developing, and the ventricles simplyexpanded to fill the space. Our findings add to the growing body of evidence that air pollutionmay play a role in autism, as well as in other neurodevelopmental disorders." Air pollutionhas a more significant negative effect on males than on females.

In 2015, experimental studies reported the detection of significant episodic (situational)

cognitive impairment from impurities in indoor air breathed by test subjects who were not informed about changes in the air quality. Researchers at the Harvard University and SUNY Upstate Medical University and Syracuse University measured the cognitive performance of 24 participants in three different controlled laboratory atmospheres that simulated those found in "conventional" and "green" buildings, as well as green buildings with enhanced ventilation. Performance was evaluated objectively using the widely used Strategic Management Simulation software simulation tool, which is a well-validated assessment test for executive decision-making in an unconstrained situation allowing initiative and improvisation. Significant deficits were observed in the performance scores achieved in increasing concentrations of either volatile organic compounds (VOCs) or carbon dioxide, while keeping other factors constant. The highest impurity levels reached are not uncommon in some classroom or office environments. Air pollution increases the risk of dementia in people over 50 years old.

Agricultural effects:

In India in 2014, it was reported that air pollution by black carbon and ground level ozone had reduced crop yields in the most affected areas by almost half in 2011 when compared to 1980 levels.

Economic effects:

Air pollution costs the world economy $5 trillion per year as a result of productivity losses and degraded quality of life, according to a joint study by the World Bank and the Institute for Health Metrics and Evaluation (IHME) at the University of Washington. These productivity losses are caused by deaths due to diseases caused by air pollution. One out of ten deaths in 2013 was caused by diseases associated with air pollution and the problem is getting worse. The problem is even more acute in the developing world. "Children under age 5 in lower-income countries are more than 60 times as likely to die from exposure to air pollution as children in high-income countries." The report states that additional economic losses caused by air pollution, including health costs and the adverse effect on agricultural and other productivity were not calculated in the report, and thus the actual costs to the world economy are far higher than $5 trillion.

Historical disasters:

The world's worst short-term civilian pollution crisis was the 1984 Bhopal Disaster in India. Leaked industrial vapors from the Union Carbide factory, belonging to Union Carbide, Inc., U.S.A. (later bought by Dow Chemical Company), killed at least 3787 people and injured from 150,000 to 600,000. The United Kingdom suffered its worst air pollution event when the December 4 Great Smog of 1952 formed over London. In six days more than 4,000 died and more recent estimates put the figure at nearer 12,000. An accidental leak of anthrax spores from a biological warfare laboratory in the former USSR in 1979 near Sverdlovsk is believed to have caused at least 64 deaths. The worst single incident of air pollution to occur in the US occurred in Donora, Pennsylvania in late October, 1948, when 20 people died and over 7,000 were injured.

Alternatives to pollution

There are now practical alternatives to the principal causes of air pollution:

- Areas downwind (over 20 miles) of major airports more than double total particulate emissions in air, even when factoring in areas with frequent ship calls, and heavy freeway and city traffic like Los Angeles. Aviation biofuel mixed in with jet fuel at a 50/50 ratio can reduce jet derived cruise altitude particulate emissions by 50-70%, according to a NASA led 2017 study (however, this should imply ground level benefits to urban air pollution as well).
- Ship propulsion and idling can be switched too much cleaner fuels like natural gas. (Ideally a renewable source but not practical yet)
- Combustion of fossil fuels for space heating can be replaced by using ground source heat pumps and seasonal thermal energy storage.
- Electric power generation from burning fossil fuels can be replaced by power generation from nuclear and renewables. For poor nations, heating and home stoves that contribute much to regional air pollution can be replaced by a much cleaner fossil fuel like natural gas, or ideally, renewables.
- Motor vehicles driven by fossil fuels, a key factor in urban air pollution, can be replaced by electric vehicles. Though lithium supply and cost is a limitation, there are alternatives. Herding more people into clean public transit such as electric trains can also help. Nevertheless, even in emission-free electric vehicles, rubber tires produce significant amounts of air pollution themselves, ranking as 13th worst pollutant in Los Angeles.

- Reducing travel in vehicles can curb pollution. After Stockholm reduced vehicle traffic in the central city with a congestion tax, nitrogen dioxide and PM10 pollution declined, as did acute pediatric asthma attacks.
- Bio digesters can be utilized in poor nations where slash and burn is prevalent, turning a useless commodity into a source of income. The plants can be gathered and sold to a central authority that will break it down in a large modern bio digester, producing much needed energy to use.
- Induced humidity and ventilation both can greatly dampen air pollution in enclosed spaces, which was found to be relatively high inside subway lines due to braking and friction and relatively less ironically inside transit buses than lower sitting passenger automobiles or subways.

Reduction efforts:

Various air pollution control technologies and strategies are available to reduce air pollution. At its most basic level, land-use planning is likely to involve zoning and transport infrastructure planning. In most developed countries, land-use planning is an important part of social policy, ensuring that land is used efficiently for the benefit of the wider economy and population, as well as to protect the environment.

Because a large share of air pollution is caused by combustion of fossil fuels such as coal and oil, the reduction of these fuels can reduce air pollution drastically. Most effective is the switch to clean power sources such as wind power, solar power, hydro power which don't cause air pollution. Efforts to reduce pollution from mobile sources includes primary regulation (many developing countries have permissive regulations), expanding regulation to new sources (such as cruise and transport ships, farm equipment, and small gas-powered equipment such as string trimmers, chainsaws, and snowmobiles), increased fuel efficiency (such as through the use of hybrid vehicles), conversion to cleaner fuels or conversion to vehicles. Titanium has been researched for its ability to reduce air pollution. Ultraviolet light will release free electrons from material, thereby creating free radicals, which break up VOCs and NOx gases. One form is super hydrophilic

In 2014, Prof. Tony Ryan and Prof. Simon Armitage of University of Sheffield prepared a 10 meter by 20 meter-sized poster coated with microscopic, pollution-eating nanoparticles of titanium dioxide. Placed on a building, this giant poster can absorb the toxic emission from around 20 cars each day.

A very effective means to reduce air pollution is the transition to renewable energy. According to a study published in Energy and Environmental Science in 2015 the switch to

100% renewable energy in the United States would eliminate about 62,000 premature mortalities per year and about 42,000 in 2050, if no biomass were used. This would save about $600 billion in health costs a year due to reduced air pollution in 2050, or about 3.6% of the 2014 U.S. gross domestic product.

Control devices

The following items are commonly used as pollution control devices in industry and transportation. They can either destroy contaminants or remove them from an exhaust stream before it is emitted into the atmosphere.

- **Particulate control**
  - Mechanical collectors (dust cyclones, multicyclones)
  - Electrostatic precipitators an electrostatic precipitator (ESP), or electrostatic air cleaner is a particulate collection device that removes particles from a flowing gas (such as air), using the force of an induced electrostatic charge. Electrostatic precipitators are highly efficient filtration devices that minimally impede the flow of gases through the device, and can easily remove fine particulates such as dust and smoke from the air stream.
  - Bag houses Designed to handle heavy dust loads, a dust collector consists of a blower, dust filter, a filter-cleaning system, and a dust receptacle or dust removal system (distinguished from air cleaners which utilize disposable filters to remove the dust).
  - Particulate scrubbers Wet scrubber is a form of pollution control technology. The term describes a variety of devices that use pollutants from a furnace flue gas or from other gas streams. In a wet scrubber, the polluted gas stream is brought into contact with the srubbing liuid, by spraying it with the liquid, by forcing it through a pool of liquid, or by some other contact method, as to remove the pollutants.

Hotspots:

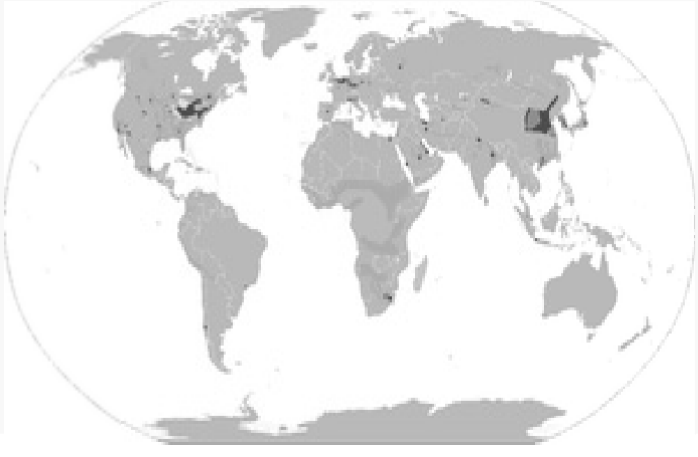
Air pollution hotspots are areas where air pollution emissions expose individuals to increased negative health effects. They are particularly common in highly populated, urban areas, where there may be a combination of stationary sources (e.g. industrial facilities) and mobile sources (e.g. cars and trucks) of pollution. Emissions from these sources can cause respiratory disease, childhood asthma, cancer, and other health problems. Fine particulate matter such as diesel soot, which contributes to more than 3.2 million premature deaths around the world each year, is a significant problem. It is very small and can lodge itself within the lungs and enter the

bloodstream. Diesel soot is c ncentrated in densely populated areas, and one in six eople in the U.S. live near a diesel pollution hot spot.

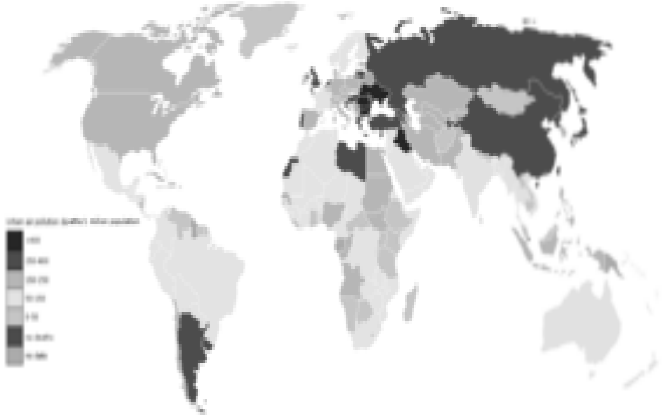
While air pollution hotspots affect a variety of populations, some groups are more likely to be located in hotspots. Previous studies have shown disparities in exposure to pollution by race and/or income. Hazardous land uses (toxic storage and disposal facilities, manufacturing facilities, major roadways) tend to be located where property values and income levels are low. Low socioeconomic status can be a proxy for other kinds of social vulnerability, including race, a lack of ability to influence regulation and a lack of ability to move to neighborhoods with less environmental pollution. These communities bear a disproportionate burden of environmental pollution and are more likely to face health risks such as cancer or asthma.

Studies show that patterns in race and income disparities not only indicate a higher exposure to pollution but also higher risk of adverse health outcomes. Communities characterized by low socioeconomic status and racial minorities can be more vulnerable to cumulative adverse health impacts resulting from elevated exposure to pollutants than more privileged communities. Blacks and Latinos generally face more pollution than whites and Asians, and low-income communities bear a higher burden of risk than affluent ones. Racial discrepancies are particularly distinct in suburban areas of the Southern United States and metropolitan areas of the Midwestern and Western United States. Residents in public housing, who are generally low-income and cannot move to healthier neighborhoods, are highly affected by nearby refineries and chemical plants.

Cities



Nitrogen dioxide oncentrati ns as measured from atellite 20 2–2004



Deaths from air pollution in 200

Air pollution is usually concentrated in densely p pulated m tropolitan areas, especially in developing countries where environmental regulations are relatively lax or nonexistent. However, even populated areas in developed countries attain unhealthy levels of pollution, with L s Angeles and Rome being two examples. Between 2002 and 2011 the incidence of lung cancer in Beijing near d ubled. While smokin remains t e leading cause of lung cancer in China, the number of smokers is falling while lung cancer rates are rising.

Most olluted cities by PM	
Particulate matt r, µg/m^3 (004)	**City**
168	Cairo, Egypt
150	Delhi, India
128	Kolkata, India (Cal utta)
125	Tianjin, China
123	Chongqing, China
109	Kanpur, India
109	Luckno , India
104	Jakarta, Indonesia
101	Shenyang, China

Governing urban air pollution:

European Court of Justice ruled that under this directive citizens have the right to require national authorities to implement a short term action plan that aims to maintain or achieve compliance to air quality limit values.

This important case law appears to confirm the role of the EC as centralised regulator to European nation-states as regards air pollution control. It places a supranational legal obligation on the UK to protect its citizens from dangerous levels of air pollution, furthermore superseding national interests with those of the citizen.

In 2010, the European Commission (EC) threatened the UK with legal action against the successive breaching of PM10 limit values. The UK government has identified that if fines are imposed, they could cost the nation upwards of £300 million per year.

In March 2011, the Greater London Built-up Area remains the only UK region in breach of the EC's limit values, and has been given 3 months to implement an emergency action plan aimed at meeting the EU Air Quality Directive. The City of London has dangerous levels of PM10 concentrations, estimated to cause 3000 deaths per year within the city. As well as the threat of EU fines, in 2010 it was threatened with legal action for scrapping the western congestion charge zone, which is claimed to have led to an increase in air pollution levels.

In response to these charges, Boris Johnson, Mayor of London, has criticised the current need for European cities to communicate with Europe through their nation state's central government, arguing that in future "A great city like London" should be permitted to bypass its government and deal directly with the European Commission regarding its air quality action plan.

This can be interpreted as recognition that cities can transcend the traditional national government organizational hierarchy and develop solutions to air pollution using global governance networks, for example through transnational relations. Transnational relations include but are not exclusive to national governments and intergovernmental organizations, allowing sub-national actors including cities and regions to partake in air pollution control as independent actors.

Particularly promising at present are global city partnerships. These can be built into networks, for example the C40 Cities Climate Leadership Group, of which London is a member. The C40 is a public 'non-state' network of the world's leading cities that aims to curb their greenhouse emissions. The C40 has been identified as 'governance from the middle' and is an alternative to intergovernmental policy. It has the potential to improve urban air quality as participating cities "exchange information, learn from best practices and consequently

mitigate carbon dioxide emissions independently from national government decisions".

Green house and global warming

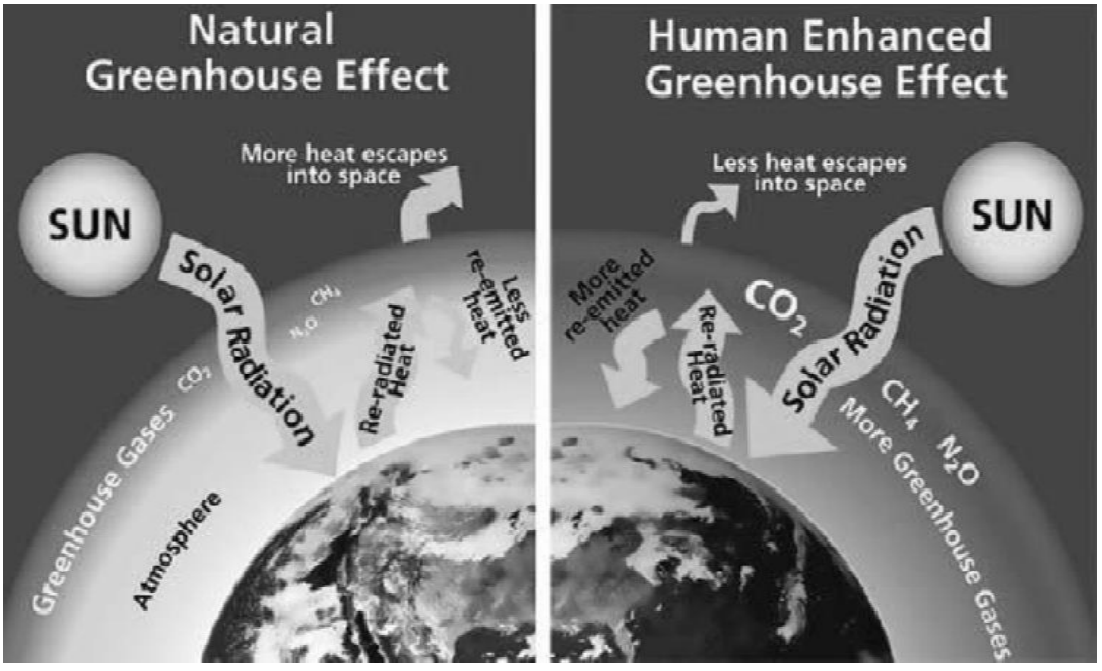
The continuous rise in temperature of the planet is really upsetting. The root cause for this is global warming. Global warming begins when sunlight reaches the Earth. The clouds, atmospheric particles, reflective ground surfaces and surface of oceans then sends back about 30 % of sunlight back into the space, whilst the remaining is absorbed by oceans, air and land. This consequently heats up the surface of the planet and atmosphere, making life feasible. As the Earth warms up, this solar energy is radiated by thermal radiation and infrared rays, propagating directly out to space thereby cooling the Earth. However, some of the outgoing radiation is re-absorbed by carbon dioxide, water vapors, ozone, methane and other gases in the atmosphere and is radiated back to the surface of Earth. These gases are commonly known as greenhouse gases due to their heat-trapping capacity. It must be noted that this re-absorption process is actually good as the Earth's average surface temperature would be very cold if there was no existence of greenhouse gases. The dilemma began when the concentration of greenhouse gases in the atmosphere was artificially increased by humankind at an alarming rate since the past two centuries. As of 2004, over 8 billion tons of carbon dioxide was pumped thermal radiation is further hindered by increased levels of greenhouse gases resulting in a phenomenon known as human enhanced global warming effect. Recent observations regarding global warming have substantiated the theory that it is indeed a human enhanced greenhouse effect that is causing the planet to heat up. The planet has experienced the largest increase in surface temperature over the last 100 years. etween 1906 and 2006, the Earth's average surface temperature augmented between 0.6 to 0.9 degrees Celsius, however out per year. Millions of pounds of methane gas are generated in landfills and agricultural decomposition of biomass and animal manure. Nitrous oxide is released into the atmosphere by various nitrogen-based fertilizers including urea and di-ammonium phosphate and other soil management utilizations. Once released, these greenhouse gases stay in the atmosphere for decades or even longer. According to Intergovernmental Panel on Climate Change (IPCC), carbon dioxide and methane levels have increased by 35 % and 148 % since the industrial revolution of 1750.

Greenhouse Effect

While other planets in the solar system of the Earth are either roasting hot or bitterly cold, Earth's surface has relatively mild, steady temperatures. Earth enjoys these temperatures

because of its atmosphere, which is the thin layer of gases that cover and protect the planet. However,97 % of climate scientists and researchers agreethat humans have changed the Earth's atmosphere in dramatic ways over the past two centuries, resulting in global warming. To understand global warming, it is first necessary to become familiar with the greenhouse effect. As Fig.1 depicts, the natural greenhouse effect normally traps some portion of heat in such a way that our planet is safe from reaching freezing temperatures while human enhanced greenhouse effect leads to global warming. This is due to burning of fossil fuels which increase the amount of greenhouse gases (carbon dioxide, methane and oxides of nitrogen) present in the atmosphere.

Fig.1 Types of greenhouse effects



The trade of incoming and outgoing radiation that heats up the Earth is often referred to as the greenhouse effect because a greenhouse works in a similar way (Fig.2). Incoming ultraviolet radiation easily passes through the glass walls of a greenhouse and is absorbed by the plants and hard surfaces inside. Weaker infrared radiation, however, has difficulty passing through the glass walls and is trapped inside, therefore, warming the greenhouse. This effect lets tropical plants prosper inside a greenhouse, even during a cold season

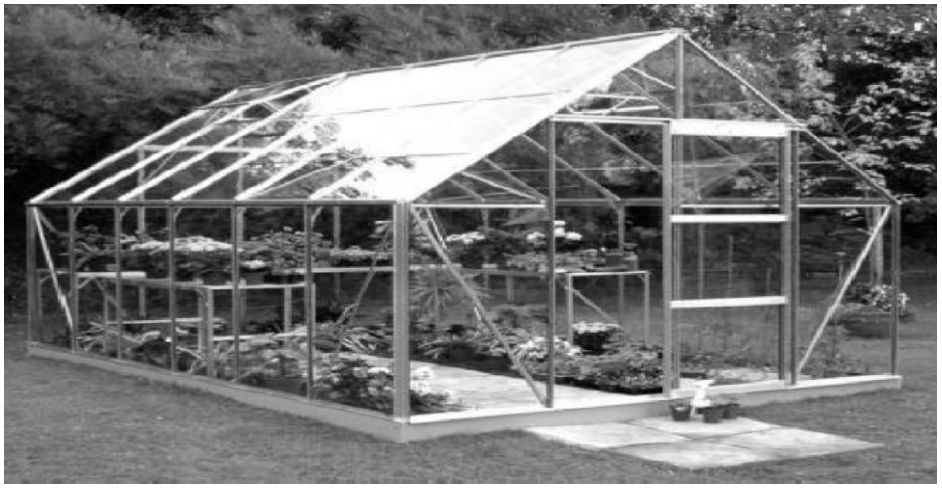


Fig. 2 Plants embodied in a greenhouse

A similar phenomenon takes place in a car which is parkedoutsideon a cold sunny day. Incoming solar radiation warms the interior of the car but outgoing thermal radiation is trapped inside the closed windows of the cars. This entrapment basically warms up the car. This trapping occurs in such a way that the hot air does not rise and does not lose energy though convention. This phenomenon is depicted in Fig. 3.

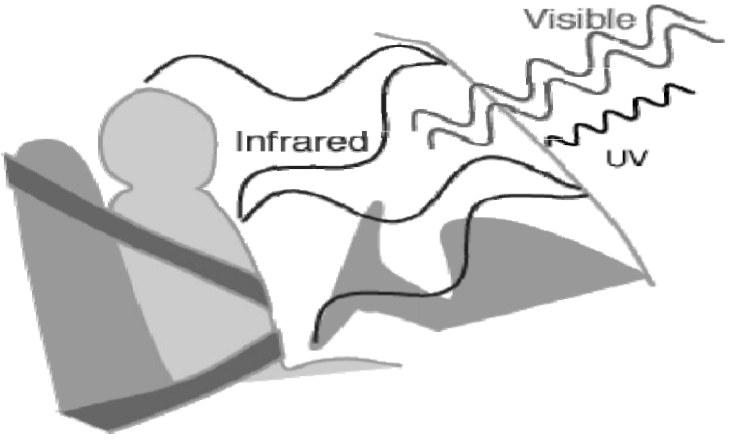


Fig. 3 Greenhouse effect example

In the words of Michael Daley, an Associate Professor of Environmental Science at Lasell College: "Gas molecules that absorb thermal infrared radiation, and are in significant enough quantity, can force the climate system. These types of gas molecules are called greenhouse gases". Carbon dioxide and other greenhouse gases act like a mantle,

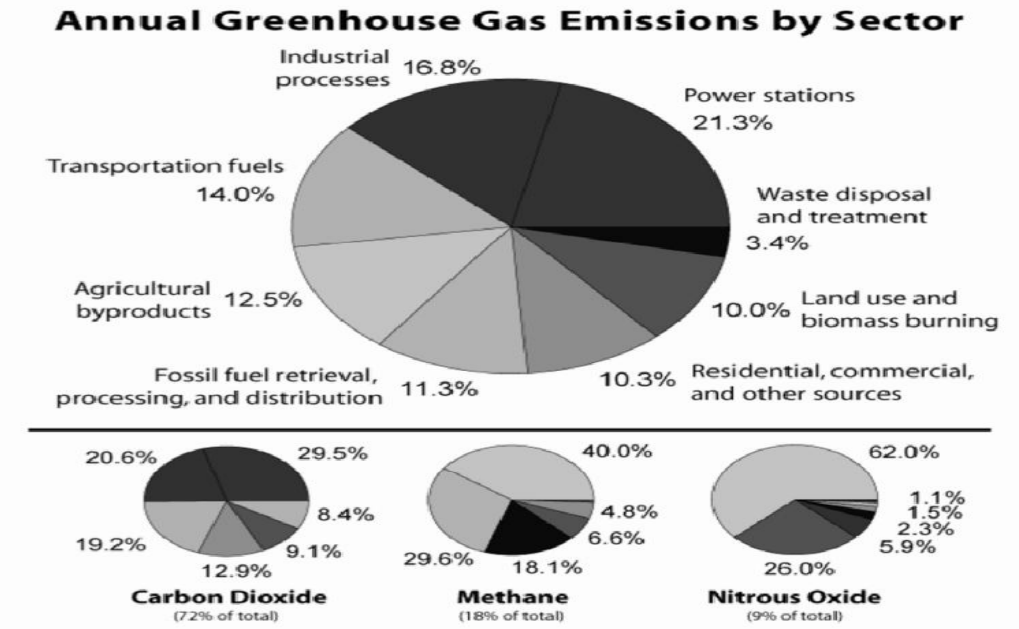
absorbing infrared radiation and preventing it from escaping into the outer space. The net effect is the regular heating of the Earth's atmosphere and surface.

The greenhouse effect, combined with increasing levels of greenhouse gases and the resulting global warming, is expected to have philosophical implications. If global warming continues unrestrained and nothing effective is done to limit this evil, it will cause significant climate change, a rise in sea levels, extreme weather events and other ruthless natural, environmental and social impacts.

Greenhouse Gases: A Hazard

There are many greenhouse gases which are mainly emitted by human activity. The first and foremost in the list is carbon dioxide. Excessive burning of fossil fuels like coal and oil is the major factor for producing this gas. Moreover, deforestation i.e. removal of trees for acquiring lands also causes large amount of carbon dioxide in the atmosphere. Cement manufacture also contributes carbon dioxide to atmosphere when calcium carbonate is heated generating lime and carbon dioxide. The second culprit gas is methane, commonly known as natural gas. It is produced as a result of agricultural activities such as livestock digestion, paddy rice farming and use of manure. Methane is also produced due to improper management of waste. Nitrous oxides are generated mainly by fertilizers. Moreover, fluorinated gases such as

chlorofluorocarbons (CFCs) are chiefly a result of various industrial processes and refrigeration. Fig.4 shows pictorially the distribution of greenhouse gases. These gases are playing their negative part in increasing the havoc of global warming. They are continuously



causing an increase in the earth's temperature.

Causes of Global warming:

The major cause of global warming is the greenhouse gases. They include carbon dioxide, methane, nitrous oxides and in some cases chlorine and bromine containing compounds. The build-up of these gases in the atmosphere changes the radiative equilibrium in the atmosphere. Their overall effect is to warm the Earth's surface and the lower atmosphere because greenhouse gases absorb some of the outgoing radiation of Earth and re-radiate it back towards the surface. The net warming from 1850 to the end of the 20th century was equivalent to nearly 2.5 W/m^2 with carbon dioxide contribution about 60 % to this figure, methane about 25 per cent, with nitrous oxides and halocarbons providing the remainder. In 1985, Joe Farman, of the British Antarctic Survey, published an article showing the decrease in ozone levels over Antarctica during the early 1980s. The response was striking: large scale international scientific programmes were mounted to prove that CFCs (used as aerosol propellants in industrial cleaning fluids and in refrigeration tools) were the cause of the problem. Even more important was abrupt international action to curb the emissions of CFCs. The second major cause of global warming is the depletion of ozone layer. This happens mainly due to the presence of chlorine containing source gases. When ultraviolet light is present, these gases dissociate releasing chlorine atoms which then catalyses ozone destruction. Aerosols present in the atmosphere are also causing global

warming by changing the climate in two different ways. Firstly, they scatter and absorb solar and infrared radiation and secondly, they may alter the microphysical and chemical properties of clouds and perhaps affect their lifetime and extent. The scattering of solar radiation acts to cool the planet, while absorption of solar radiation by aerosols warms the air directly instead of permitting sunlight to be absorbed by the surface of the Earth. The human contribution to the amount of aerosols in the atmosphere is of various forms. For instance, dust is a by-product of agriculture. Biomass burning generates a mixture of organic droplets and soot particles. Many industrial processes produce a wide diversity of aerosols depending on what is being burned or generated in the manufacturing process. Moreover, exhaust emissions from various sorts of transport produce a rich mixture of pollutants that are either aerosols from the outset or are transformed by chemical reactions in the atmosphere to form aerosols.

Global Warming: The Effects

Predicting the consequences of global warming is one of the most difficult tasks faced by the climate researchers. This is due to the fact that natural processes that cause rain, snowfall, hailstorms, rise in sea levels is reliant on many diverse factors. Moreover, it is very hard to predict the size of emissions of greenhouse gases in the future years as this is determined majorly through technological advancements and political decisions. Global warming produces many negative effects some of which are described here. Firstly, extra water vapour which is present in the atmosphere falls again as rain which leads to floods in various regions of the world. When the weather turns warmer, evaporation process from both land and sea rises. This leads to drought in the regions where increased evaporation process is not compensated by increased precipitation. In some areas of the world, this will result in crop failure and famine particularly in areas where the temperatures are already high. The extra water vapour content in the atmosphere will fall again as extra rain hence causing flood. Towns and villages which are dependent on the melting water from snowy mountains may suffer drought and scarcity of water supply. It is because the glaciers all over the world are shrinking at a very rapid rate and melting of ice appears to be faster than previously projected. According to Intergovernmental Panel on Climate Change (IPCC), about one-sixth of the total population of the world lives in the regions which shall be affected by a decrease in melting water. The warmer climate will likely cause more heat waves, more violent rainfall and also amplification in the severity of hailstorms and thunderstorms. Rising of sea levels is the most deadly effect of global warming, the rise in

temperature is causing the ice and glaciers to melt rapidly. This will lead to rise of water levels in oceans, rivers and lakes that can pilot devastation in the form of floods.

As evident from Fig. 5, temperature anomalies are projected to increase in coming years. Before, the 20th century, the situation was well under control but the beginning of the current century, the situation started to worsen .This was all due to increase in global warming majorly due to the fact that new industries and power houses started operation and emitted harmful gases which cause the planet to heat up. This data is based on the research carried out by different climate and environmental research agencies.

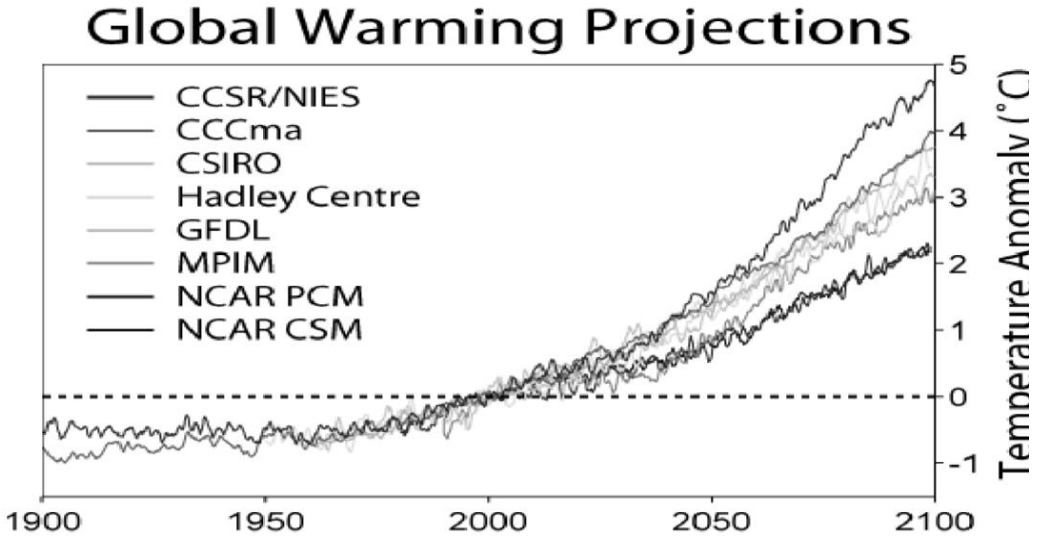


Fig. 5 Global warming projections by various

Science and Engineering research agencies

Similarly, Fig.6 elaborates the risks and impacts of global warming in years to come. As can be inferred from figure, we are currently experiencing severity of extreme climate events in the form of thunderstorms, floods and earthquakes. This destruction will take a sharp hike if nothing is done to stop this menace. Fig. 7 depicts global mean temperature in the recent years according to National Aeronautics and Space Administration (NASA). The trend clearly puts up a serious question for us. How will we survive on earth given the rise in temperature to prevail?

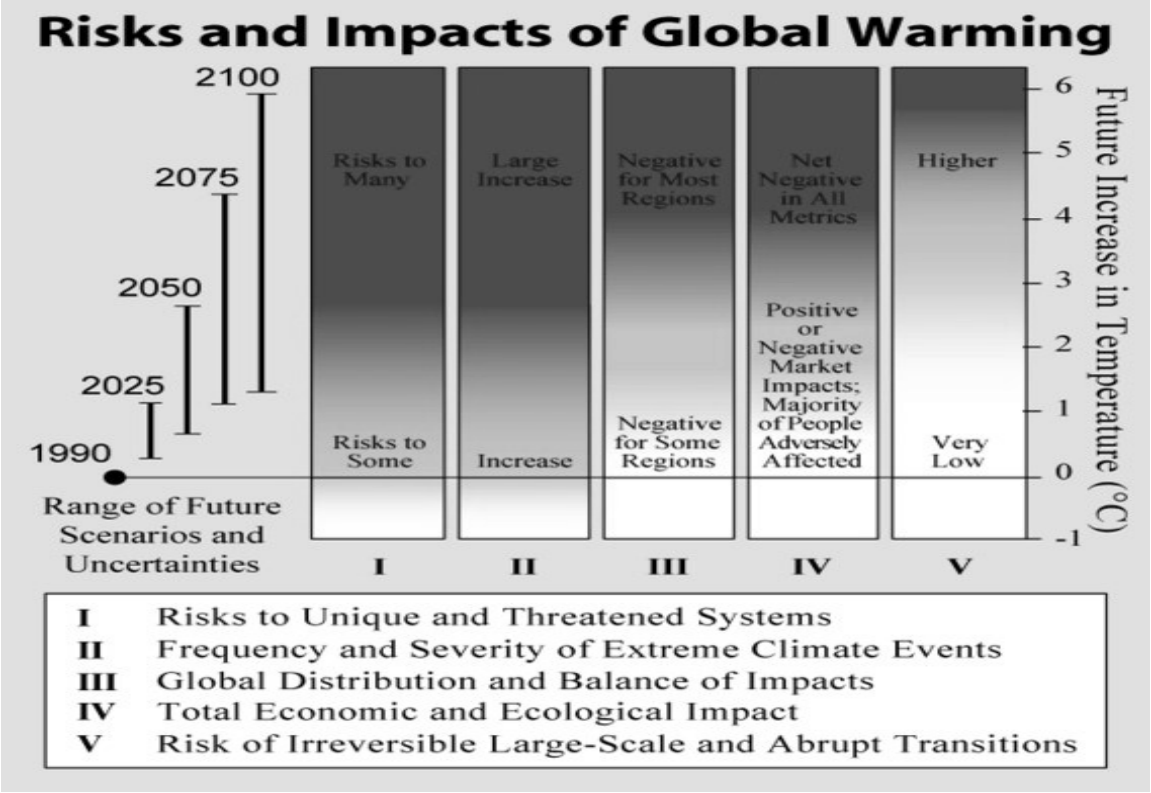


Fig. 6 An assessment of the relative impact and risks connected with global warming. The bars are color-coded to show level of impact/concern for each factor as a function of temperature increase

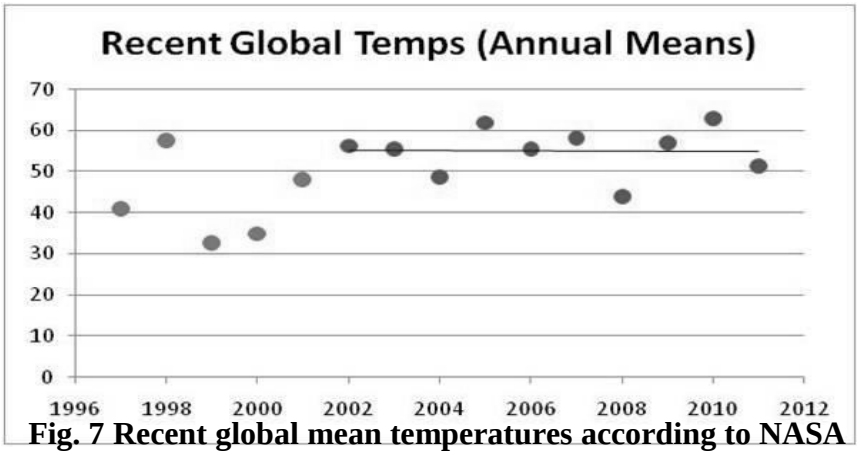


Fig. 7 Recent global mean temperatures according to NASA

Effects on Living Beings

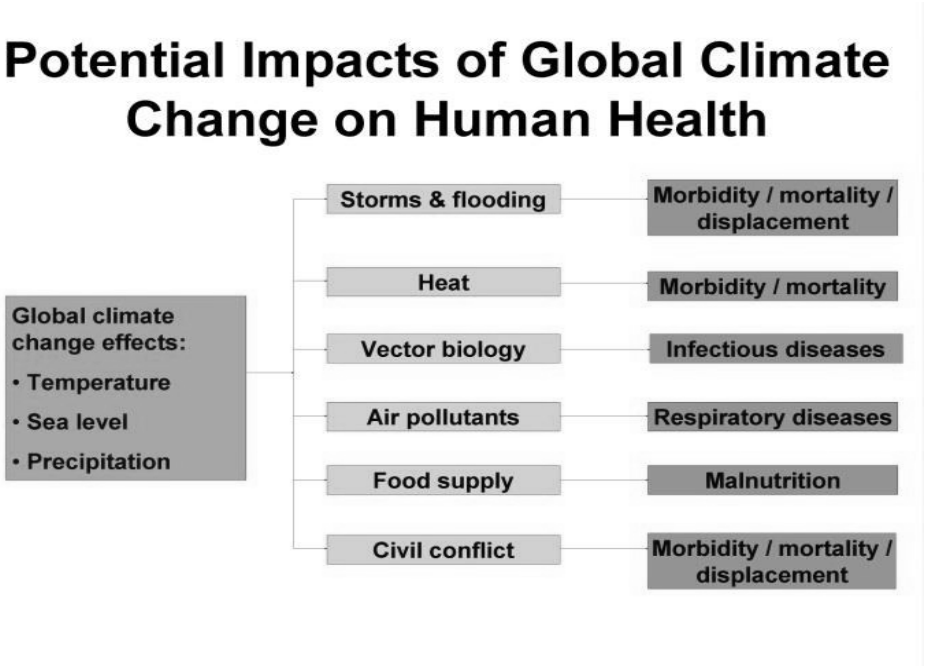
Global warming can severely affect the health of living beings. Excess heat can cause stress which may lead to blood pressure and heart diseases. Crop failures and famines, which are a direct consequence of heating up of earth, can cause a decline in human body resistance to viruses and infections. Global warming may also transfer various diseases to other regions as people will shift from regions of higher temperatures to regions of

comparatively lower temperatures. Warmer oceans and other surface waters may lead to severe cholera outbreaks and harmful infections in some types of sea food.

Moreover, it is an established fact that warmer temperatures lead to dehydration which is a major cause of kidney stones. A medical team from

The Children's Hospital of Philadelphia examined the health proceedings of more than 60,000 Americans alongside weather records. They discovered that individuals were most likely to be hospitalized with kidney stones three days after a temperature rise. Since 1994, kidney stone incidence has risen from about one in 20 people to one in 11. This trend is likely to increase as the globe gets hotter. According to Luis Ostrosky, M.D. of the Division of Infectious Diseases at The University of Texas Health Science Centre at Houston Medical School and medical director for epidemiology at Memorial Hermann-Texas Medical Centre: “One infection that is definitely making a weird pattern is valley fever”. In his words, “This is a fungal infection we used to see only in California, Arizona, New Mexico and a little in Texas, but last year we found it for the first time in Washington State. “This potentially deadly condition caused apprehension in California when the number of cases increased drastically during 2010 and 2011.Valley fever infections have been on the rise, probably because of warming climates and drought causing dust storms. Dry soil and wind can carry spores that spread the virus. Hotter and drier climates are projected to increase the amount of dusting carrying this disease. Researchers have already noticed a rise in mosquito-borne disease like dengue fever and malaria due to warmer and longer summers. Perhaps the most prominent mosquito-borne disease, West Nile Virus, has already experienced a sharp increase in annual cases. According to the U.S. Centres for Disease Control and Prevention, the summer of 2012 was the nastiest West Nile season on record, the likely reason was that summer's scorching heat and drought. Lyme disease is another dangerous disease which is transmitted mainly through bites from certain tick species.

Fig. 8 describes in the form of a block diagram that how alterations in global climate can affect human health. The bitterest fact is that it can cause various diseases and deprive



human beings of the food.

Global warming is also affecting animals. They need to move to cooler places in order to survive. This process has been observed in various places, for instance, in the Alps, in mountainous Queensland in Australia, and in the misty forests of Costa Rica. Fish in the North Sea have been reported to move northwards too .The impacts on species are becoming noteworthy to such an extent that their movements can be used as a sign of a warming world. They are the silent witnesses of the swift changes being inflicted on the Earth. Scientists and researchers predict that global warming is gradually damaging the ecosystems of various species and is playing a very unconstructive role in making them extinct. For instance Asia's only ape – the orangutan – is in bottomless trouble. Its last remaining strongholds in the rainforests of Indonesia are being endangered by a range of pressures, including climate change, putting the animal at the menace of extinction within a few decades. With global warming continually increasing the duration and frequency of droughts, bushfires are occurring more often in these heavily logged forests, further fragmenting the orang-utan's living domain. Similarly, in Africa, elephants face a series of threats including shrinking living space, which brings them more regularly into divergence with people. With this reduced living space, elephants will be unable to escape any changes to their natural habitat caused by global warming, including more common and longer dry periods, placing further pressure on their survival.

Alternative Energy Sources

The hazards caused by global warming are tremendous. Excessive use of fossil fuels such as coal, natural gas and oil play a part in it too. The usage of fossil fuels should be discontinued immediately. The most significant solution to put an end to this disaster is the use of alternative energy sources. They include wind, solar, bio mass, geothermal and hydro. The most noteworthy point in using these sources is their clean nature. They do not produce any sort of pollution or toxic gases that can lead to global warming. They are environmentally friendly and pose no threat to ecological balance. However, their high installation and setup costs may drive energy companies away from them at first but in the long run they are surely beneficial for everyone. Most importantly, fossil fuels will deplete one day and sooner or later, we have to turn to renewable energy sources for energy production. Thus, the eventual solution to end global warming is to use alternative energy sources. Fig. 9 depicts in a pictorial way that earth can be saved from the hazards of global warming if we utilize renewable energy sources.

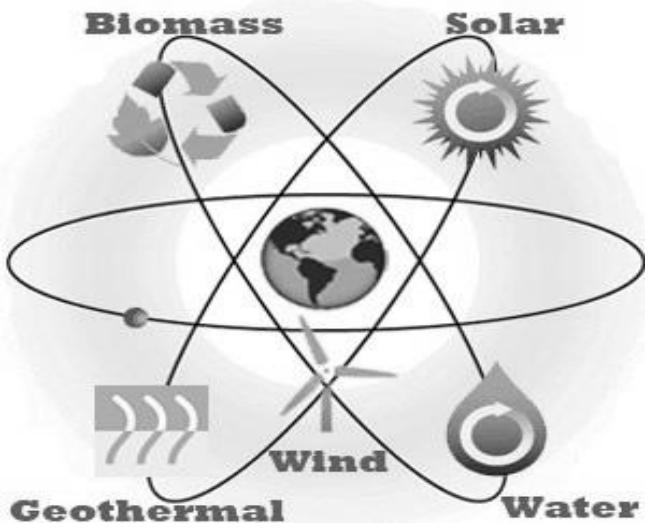


Fig. 9 save earth from global warming by using renewable energy sources

To counteract the medical hazards of global warming, it is essential to turn to renewable energy sources. Public, in general, should be responsible about their decisions on energy conservation methods. This will ensure a healthy atmosphere and stable climate for our future generations. Governments should devise and pass policies which encourage the energy companies and people, in general, to use renewable energy instead of conven ional energy, Nongovernmental organizations (NGOs) should distribute pamphlets to people motivating them to use alternative sources of energy and discourage them from using fossil fuels. They should also explain to them the hazards which the usage of fossil fuels will cause. Many developed countries are already generating huge amounts of power using renewables. These countries should extend their helping hand to developing countries to combat the evil of global warming collectively. Using renewable energy is the most effective way to curtain the emission of gases which play a major role in global warming.
Fig. 10 and Fig. 11 show that the use of renewables is gradually increasing. The figure should be much more than present so that we can tackle the problem of global warming timely and effectively

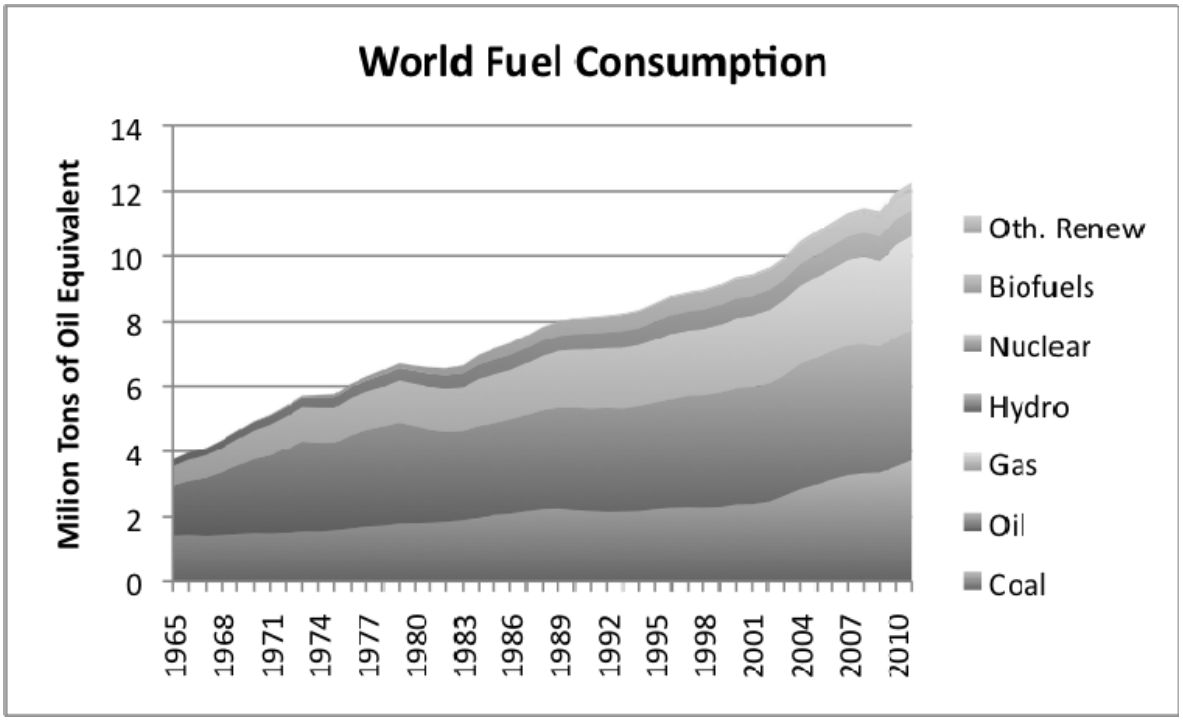


Fig. 10 World fuel consumption in recent years

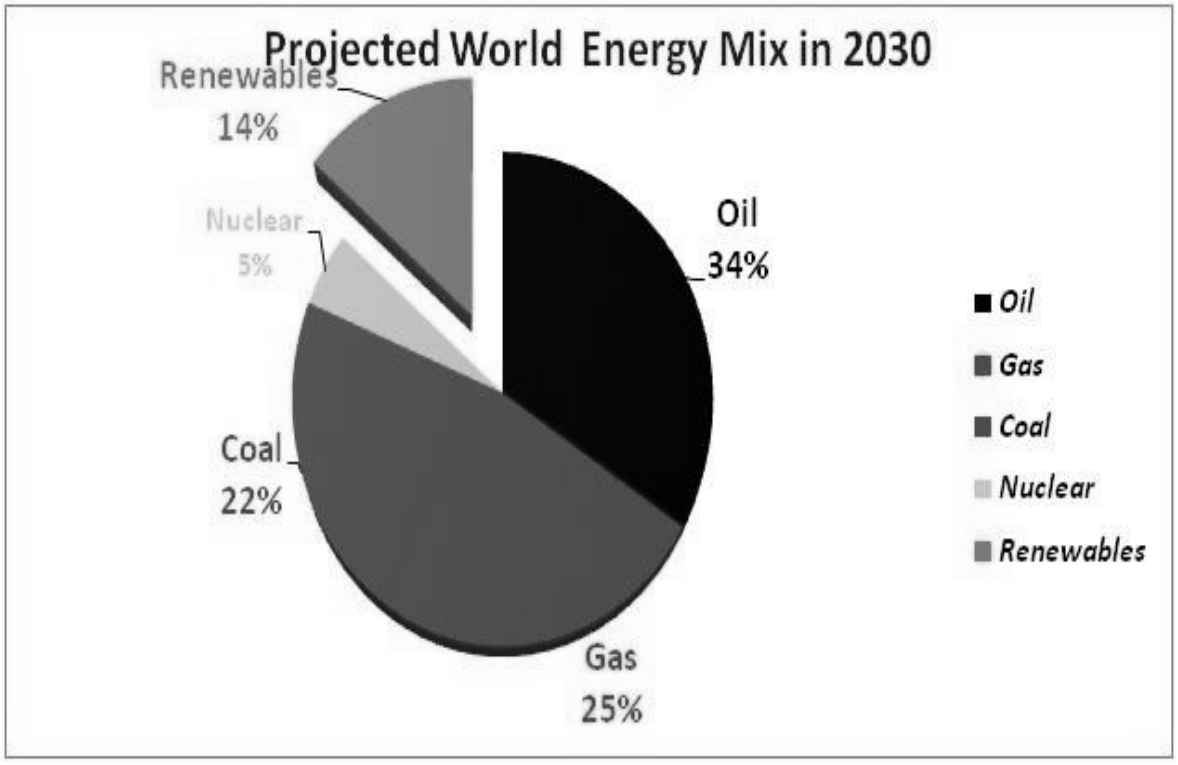


Fig .11 Projected world energy mix in 2030

Other Solutions

As elaborated earlier, toxic emissions are a major cause of global warming, a likely solution to reduce harmful emissions is to cut the usage of vehicles which produce them. This has not been met with much success as many people refuse to cut down their practice of using cars. No doubt, some people have started to use bicycles and public transport, whereas some other prefer to walk but these numbers are relatively small. It should be noted that fuel economy and emission rates are chief factors to consider regarding the car choice. Hybrid cars have higher efficiency and lower emission rates. Keeping the tires inflated will help improve mileage and air filters should be frequently replaced to cut down harmful emissions. People should share the ride with friends or co-workers to reduce the total number of vehicles on the road. Print and social media can play an effective role in curbing the problem. It should use the philosophy of automobile advertisements to encourage drivers to conserve energy and reduce pollution. Awareness campaigns can be started using placards, posters and logos similar to shown in Figures 12-14. They are a very useful way to demonstrate that global warming is not good for the planet. Recycling is also a good way to reduce global warming. People should use rechargeable batteries instead of disposable ones. Quality products should be bought that have a long life. Shopping should be done from local markets which reduce transportation. Even small

individual efforts like lowering the thermostats in winter and using compact fluorescent lamps instead of incandescent lamps can aid to address the issue of global warming. Reforestation schemes must be started to grow a large number of trees. Forest degradation and deforestation must be discouraged at government level. Nuclear power is also a possible solution as this power results in fewer emissions but this method should be used with care as it can lead to severe accidents therefore, the major hurdle is to overcome the security, propagation, waste disposal and high costs of nuclear power if this method has to be made practical.

Fig. 12 shows symbolically how global warming is causing the earth to melt

Fig. 13 showing a symbolic representation to stop global warming

Fig. 14 depicting that how human beings are destroying the earth for their own benefits

The scientific and environmental community is on the same page regarding the bitter reality of global warming and the involvement of human factor in it. The paper discussed here has only dented the surface of what is a very intricate line of scientific and engineering exploration. Global warming is a big hazard and appropriate measures must be taken to tackle this serious problem. This problem is not only causing trouble to the human beings but also to animals and plants. Melting of polar ice caps will lead to floods which can cause mayhem everywhere. Rise of sea levels will devastate agricultural and fishing activities. To embark upon these problems, some remedial steps must be timely taken which include but are not limited to the use of renewable sources of energy and stopping deforestation. Innovative solutions must be brought forward to end this hazard once and forever.

Ozone Layer Depletion and Its Effects

The ozone layer is a layer in Earth's atmosphere which contains relatively high concentrations of ozone (O_3). This layer absorbs 93-99% of the sun's high frequency ultraviolet light, which is potentially damaging to life on earth [1]. Over 91% of the ozone in Earth's atmosphere is present here. It is mainly located in the lower portion of the stratosphere from approximately 10 km to 50 km above Earth, though the thickness varies seasonally and geographically. The ozone layer was discovered in 1913 by the French physicists Charles Fabry and Henri Buisson. Its properties were explored in detail by the British meteorologist G. M. B. Dobson, who developed a simple spectrophotometer (the Dobson meter) that could be used to measure

stratospheric ozone from the ground. Between 1928 and 1958 Dobson established a worldwide network of ozone monitoring stations which continues to operate today. The "Dobson unit", a convenient measure of the total amount of ozone in a column overhead, is named in his honor.

Ozone

Without ozone, life on Earth would not have evolved in the way it has. The first stage of single cell organism development requires an oxygen-free environment. This type of environment existed on earth over 3000 million years ago. As the primitive forms of plant life multiplied and evolved, they began to release minute amounts of oxygen through the photosynthesis reaction (which converts carbon dioxide into oxygen)

The buildup of oxygen in the atmosphere led to the formation of the ozone layer in the upper atmosphere or stratosphere. This layer filters out incoming radiation in the "cell-damaging" ultraviolet (UV) part of the spectrum. Thus with the development of the ozone layer came the formation of more advanced life forms. Ozone is a form of oxygen. The oxygen we breathe is in the form of oxygen molecules (O_2) - two atoms of oxygen bound together. Normal oxygen which we breathe is colourless and odourless. Ozone, on the other hand, consists of three atoms of oxygen bound together (O_3). Most of the atmosphere's ozone occurs in the region called the stratosphere. Ozone is colourless and has a very harsh odour. Ozone is much less common than normal oxygen. Out of 10 million air molecules, about 2 million are normal oxygen, but only 3 are ozone. Most ozone is produced naturally in the upper atmosphere or stratosphere. While ozone can be found through the entire atmosphere, the greatest concentration occurs at altitudes between 19 and 30 km above the Earth's surface. This band of ozone-rich air is known as the "ozone layer". Ozone also occurs in very small amounts in the lowest few kilometres of the atmosphere, a region known as the troposphere. It is produced at ground level through a reaction between sunlight and volatile organic compounds (VOCs) and nitrogen oxides (NO_x), some of which are produced by human activities such as driving cars. Ground-level ozone is a component of urban smog and can be harmful to human health. Even though both types of ozone contain the same molecules, their presence in different parts of the atmosphere has very different consequences. Stratospheric ozone blocks harmful solar radiation - all life on Earth has adapted to this filtered solar radiation. Ground-level ozone, in contrast, is simply a pollutant. It will absorb some incoming solar radiation, but it cannot make up for ozone losses in the stratosphere.

Ozone Hole

In some of the popular news media, as well as in many books, the term "ozone hole" has and

often still is used far too loosely. Frequently, the term is employed to describe any episode of ozone depletion, no matter how minor. Unfortunately, this sloppy language trivializes the problem and blurs the important scientific distinction between the massive ozone losses in Polar Regions and the much smaller, but nonetheless significant, ozone losses in other parts of the world. Technically, the term "ozone hole" should be applied to regions where stratospheric ozone depletion is so severe that levels fall below 200 Dobson Units (D.U.), the traditional measure of stratospheric ozone. Normal ozone concentration is about 300 to 350 D.U. Such ozone loss now occurs every springtime above Antarctica, and to a lesser extent the Arctic where special meteorological conditions and very low air temperatures accelerate and enhance the destruction of ozone loss by man-made ozone depleting chemicals (ODCs).

Ozone Layer

The ozone layer is not really a layer at all, but has become known as such because most ozone particles are scattered between 19 and 30 kilometers (12 to 30 miles) up in the Earth's atmosphere, in a region called the stratosphere. The concentration of ozone in the ozone layer is usually under 10 parts ozone per million. Without the ozone layer, a lot of ultraviolet (UV) radiation from the Sun would not be stopped reaching the Earth's surface, causing untold damage to most living species. In the 1970s, scientists discovered that chlorofluorocarbons (CFCs) could destroy ozone in the stratosphere. Ozone is created in the stratosphere when UV radiation from the Sun strikes molecules of oxygen (O2) and causes the two oxygen atoms to split apart. If a freed atom bumps into another O2, it joins up, forming ozone (O3). This process is known as photolysis. Ozone is also naturally broken down in the stratosphere by sunlight and by a chemical reaction with various compounds containing nitrogen, hydrogen and chlorine. These chemicals all occur naturally in the atmosphere in very small amounts. In an unpolluted atmosphere there is a balance between the amount of ozone being produced and the amount of ozone being destroyed. As a result, the total concentration of ozone in the stratosphere remains relatively constant. At different temperatures and pressures (i.e. varying altitudes within the stratosphere), there are different formation and destruction rates. Thus, the amount of ozone within the stratosphere varies according to altitude. Ozone concentrations are highest between 19 and 23 km. Most of the ozone in the stratosphere is formed over the equator where the level of

sunshine striking the Earth is greatest. It is transported by winds towards higher latitudes. Consequently, the amount of stratospheric ozone above a location onthe Earth varies naturally with latitude, season, and from day-to-day. Under normal circumstances highest ozone values are found over the Canadian Arctic and Siberia, whilst thelowest values are found around the equator. The ozone layer over Canada is normally thicker in winter and early spring, varying naturally by about 25% between January and July. Weather conditions can also cause considerable daily variations.

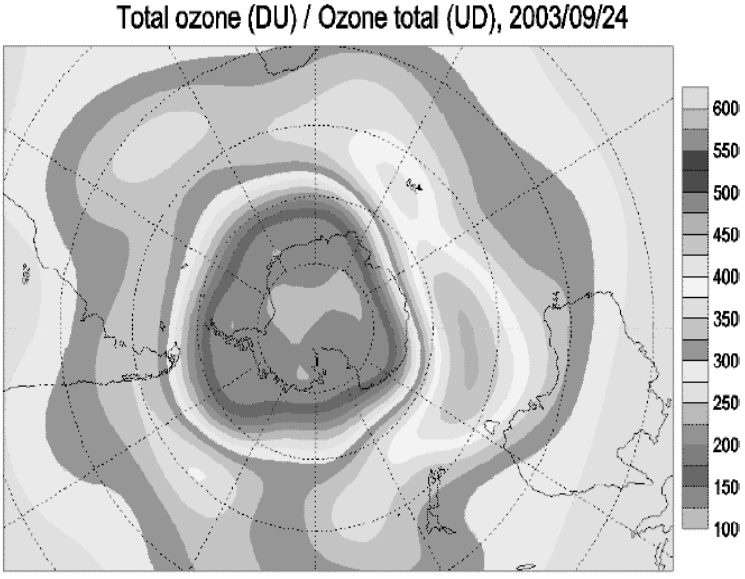


Fig.1 ozone layer depletion over Antarctica Ozone depletion over India

With so much worry about the rapid ozone depletion taking place in various parts of the earth, Indian scientists are closely monitoring the ozone layer over India for possible depletion trends. Opinions are many and varied. According to S K

Srivastava, head of the National Ozone Centre in New Delhi, there is no trend to show total ozone depletion over India. V.Thaphyal and S M Kulshresta of the Indian Meteorological

Department also point out that for the period 1956 to 1986 "ozone measurements exhibit year to year variability, but do not show any increasing or decreasing trend over India."

However, former director of the National Ozone Centre, K Chatterji, now with Development Alternatives, warns that there is no case for complacency. He asserts that his calculations exhibit an ozone depletion trend in the upper, layers of the stratosphere over New Delhi and Pune from 1980 to 1983 in the month of October when the Antarctic ozone hole is at its maximum. Since India already receives high doses of ultraviolet (UV-B) radiation, and is at the threshold go to speak, effects of ozone layer depletion could he far more disastrous in India. A P Mitra, former director general of the Council of Scientific and Industrial Research, clarifies that while there is no trend in the total ozone value, there is some evidence of ozone depletion at higher altitudes - at about 30 to 40 km - even over the tropics. He argues, however, that there is insufficient data and that the depletion may be due to solar cycles and other natural phenomena. However, the effects of CFCs and belong cannot be ruled out. Total column ozone data has been recorded over India for a long time. A network of stations using Dobson spectrophotometers to mea- sure total ozone, some six times a day, covers Srinagar, New Delhi, Varanasi, Ahmedabad, Pone and Kodaikanal. Ozone profiles are also regularly recorded using balloons. Ozone levels are the lowest during November and December and the highest in summer. Across the country, variations do exist. In Kodaikanal, the total ozone is 240 to 280 Dobson units (DU), in New Delhi 270 to 320 DU and in Srinagar 290 to 360 DU. One Dobson unit is the equivalent of 0.01 mm of compressed gas at a pressure of 760 rare mercury and 0°C.B N Srivastava of the National Physical Laboratory, who been working on incident UV radiation levels, says that during summer, at noon, the UV-B radiation with a wavelength of 290 nanometer (nm) is equivalent to levels attained in the Antarctica during the ozone hole period. He warns that even a slight depletion of the ozone layer over India may lead to large percentage changes in UV-B radiation over the country.

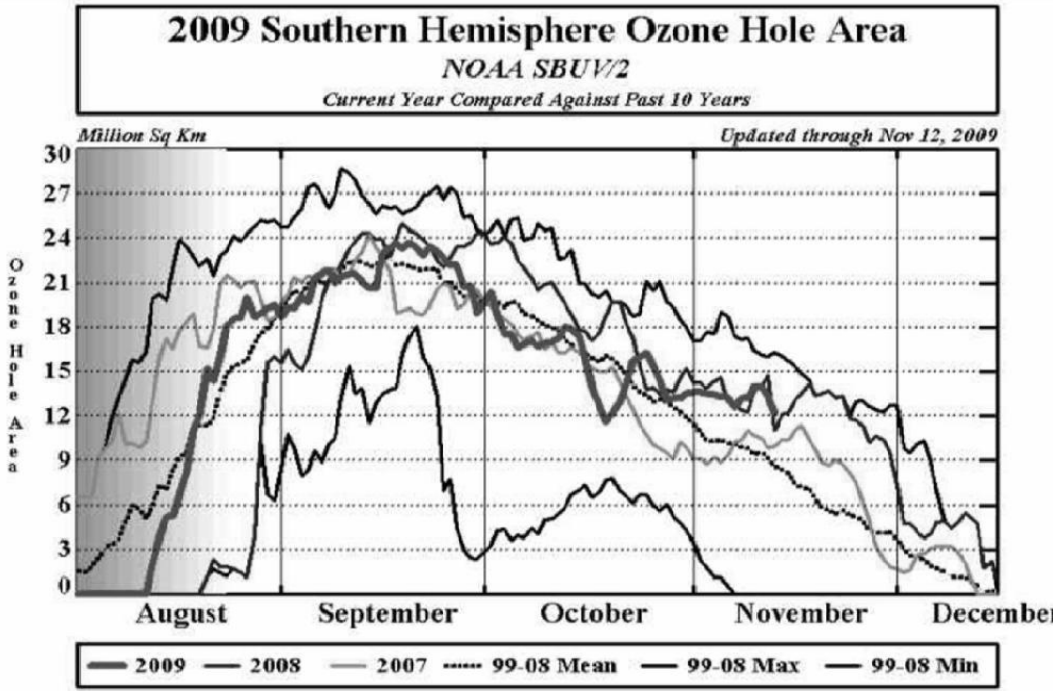
Measuring Ozone Depletion

The most common stratospheric ozone measurement unit is the Dobson Unit (DU). The Dobson Unit is named after the atmospheric ozone pioneer G.M.B. Dobson who carried out the earliest studies on ozone in the atmosphere from the 1920s to the 1970s. A Dobson Unit measures the total amount of ozone in an overhead column of the atmosphere. Dobson Units are measured by how thick the layer of ozone would be if it were compressed into one layer at 0 degrees Celsius and with a pressure of one atmosphere above it. Every 0.01 millimeter

thickness of the layer is equal to one Dobson Unit. The average amount of ozone in the stratosphere across the globe is about 300 DU (or a thickness of only 3mm at 0°C and 1 atmospheric pressure!). Highest levels of ozone are usually found in the mid to high latitudes, in Canada and Siberia (360DU). When stratospheric ozone falls below 200 DU this is considered low enough to represent the beginnings of an ozone hole. Ozone holes of course commonly form during springtime above Antarctica, and to a lesser extent the Arctic.

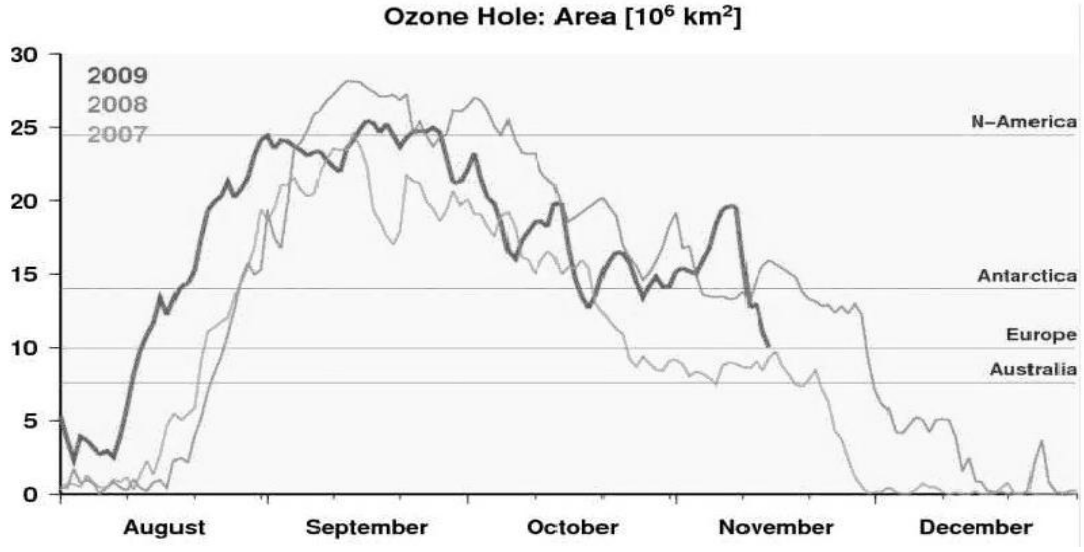
The Ozone Hole 2009- Situation at 2009

November the 2009 ozone hole is now waning, with much of the continent experiencing a stratospheric spring warming. The residual vortex is over the Weddell Sea and Antarctic Peninsula and here minimum values are around 160 DU and depletion exceeds 50%. Ozone values outside the polar vortex have dropped to near 400 DU, and inside the vortex ozone values are increasing as the atmosphere warms. The temperature of the ozone layer over Antarctica is now rising, though a small area is still cold enough for polar stratospheric clouds (PSCs) to exist. During the early winter, the polar vortex was often rather more elliptical than it was in 2008, and this lead to some early depletion in circumpolar regions as stratospheric clouds became exposed to sunlight. It reverted to a more circular circulation as winter progressed and this led to another relatively slow start to the growth of the ozone hole (as measured by NASA/SBUV2), with the "hole" not beginning until mid-August. The vortex became more elliptical again in late August, with South Georgia being affected by the fringes of the ozone hole between September 2 and 6. The hole grew to reach an area of around 24 million square kilometers by mid-September, but had declined to 12 million square kilometres by mid-November. It is now a little larger than the average for the past decade. The tip of South America and South Georgia were affected by the fringes of the ozone hole from



September 24 to September 30 and again from October 3 to October 7.

Fig.2 ozone hole area variation



Ozone Layer Recovery

The ozone depletion caused by human-produced chlorine and bromine compounds is expected to gradually disappear by about the middle of the 21st century as these compounds are slowly removed from the stratosphere by natural processes. This environmental achievement is due to the landmark international agreement to control the production and use of ozone-depleting substances. Full compliance would be required to achieve this expected recovery. Without the Montreal Protocol and its Amendments, continuing use of chlorofluorocarbons (CFCs) and other ozone-depleting substances would have increased the stratospheric abundances of chlorine and bromine tenfold by the mid-2050s compared with the 1980 amounts. Such high chlorine and bromine abundances would have caused very large ozone losses, which would have been far larger than the depletion observed at present. In contrast, under the current international agreements that are now reducing the human-caused emissions of ozone-depleting gases, the net troposphere concentrations of chlorine- and bromine-containing compounds started to decrease in 1995. Because 3 to 6 years are required for the mixing from the troposphere to the stratosphere, the stratospheric abundances of chlorine are starting to reach a constant level and will slowly decline thereafter. With full compliance, the international agreements will eventually eliminate most of the emissions of the major ozone-depleting gases. All other things being constant, the ozone layer would be expected to return to a normal state during the middle of the next century. This slow recovery, as compared with the relatively rapid onset of the ozone depletion due to CFC and bromine-containing halons emissions, is related primarily to the time required for natural processes to eliminate the CFCs and halons from the atmosphere. Most of the CFCs and halons have atmospheric residence times of about 50 to several hundred years.

Ozone depletion occurs when the natural balance between the production and destruction of stratospheric ozone is tipped in favour of destruction. Although natural phenomena can cause temporary ozone loss, chlorine and bromine released from man-made compounds such as CFCs are now accepted as the main cause of this depletion. It was first suggested by Drs. M. Molina and S. Rowland in 1974 that a man-made group of compounds known as the chlorofluorocarbons (CFCs) were likely to be the main source of ozone depletion. However, this idea was not taken seriously until the discovery of the ozone hole over Antarctica in 1985 by the Survey. Chlorofluorocarbons are not "washed" back to Earth by rain or destroyed in reactions with other chemicals. They simply do not break down in the lower atmosphere and they can remain in the atmosphere from 20 to 120 years or more. As a consequence of their

relative stability, CFCs are instead transported into the stratosphere where they are eventually broken down by ultraviolet (UV) rays from the Sun, releasing free chlorine. The chlorine becomes actively involved in the process of destruction of ozone. The net result is that two molecules of ozone are replaced by three of molecular oxygen, leaving the chlorine free to repeat the process:

$$Cl + O_3 = ClO + O_2$$

$$ClO + O = Cl + O_2$$

Ozone is converted to oxygen, leaving the chlorine atom free to repeat the process up to 100,000 times, resulting in a reduced level of ozone. Bromine compounds, or halons, can also destroy stratospheric ozone. Compounds containing chlorine and bromine from man-made compounds are known as industrial halocarbons. Emissions of CFCs have accounted for roughly 80% of total stratospheric ozone depletion. Thankfully, the developed world has phased out the use of CFCs in response to international agreements to protect the ozone layer.

However, because CFCs remain in the atmosphere so long, the ozone layer will not fully repair itself until at least the middle of the 21st century. Naturally occurring chlorine has the same effect on the ozone layer, but has a shorter life span in the atmosphere.

Chlorofluorocarbons

Chlorofluorocarbons or CFCs (also known as Freon) are non-toxic, non-flammable and non-carcinogenic. They contain fluorine atoms, carbon atoms and chlorine atoms. The 5 main CFCs include CFC-11 (trichlorofluoromethane - $CFCl_3$), CFC-12 (dichloro-difluoromethane - CF_2Cl_2), CFC-113 (trichloro-trifluoroethane - $C_2F_3Cl_3$), CFC-114 (dichloro-tetrfluoroethane - $C_2F_4Cl_2$), and CFC-115 (chloropentafluoroethane - C_2F_5Cl).CFCs are widely used as coolants in refrigeration and air conditioners, as solvents in cleaners, particularly for electronic circuit boards, as a blowing agents in the production of foam (for example fire extinguishers), and as propellants in aerosols. Indeed, much of the modern lifestyle of the second half of the 20th century had been made possible by the use of CFCs.Man-made CFCs however, are the main cause of stratospheric ozone depletion. CFCs have a lifetime in the atmosphere of about 20 to 100 years, and consequently one free chlorine atom from a CFC molecule can do a lot of damage, destroying ozone molecules for a long time. Although emissions of CFCs around the developed world have largely ceased due to international control agreements, the damage to the stratospheric ozone layer will continue well into the 21st century.

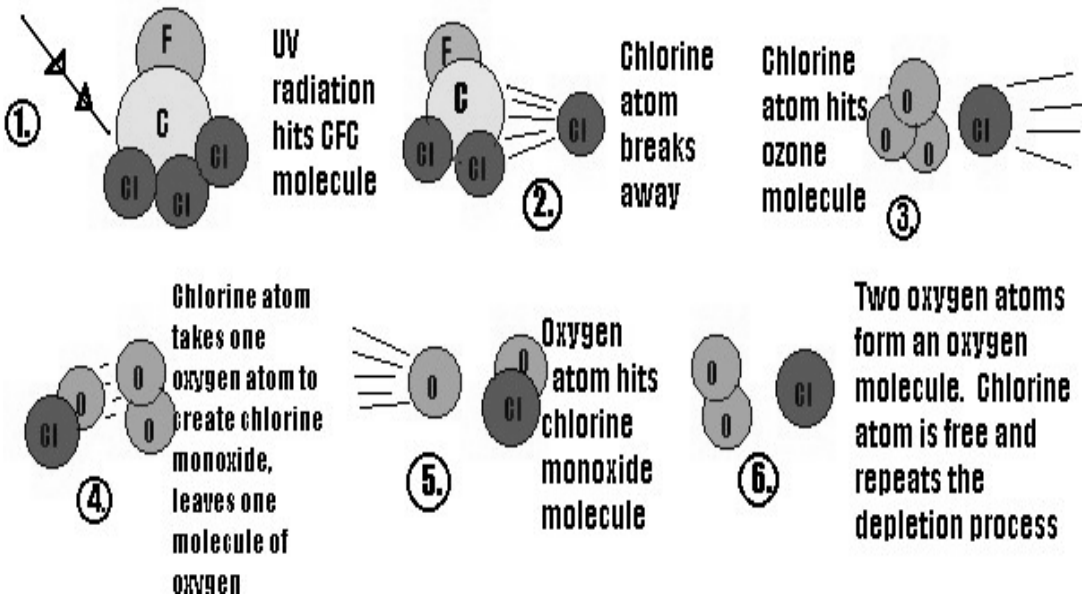


Fig.4 ozone depletion reaction

Rocket Launches:

The global market for rocket launches may require more stringent regulation in order to prevent significant damage to Earth's stratospheric ozone layer in the decades to come, according to a new study by researchers in California and Colorado. Future ozone losses from unregulated rocket launches will eventually exceed ozone losses due to chlorofluorocarbons, or CFCs, which stimulated the 1987 Montreal Protocol banning ozone-depleting chemicals, said Martin Ross, chief study author from The Aerospace Corporation in Los Angeles. The study, which includes the University of Colorado at Boulder and Embry-Riddle Aeronautical University, provides a market analysis for estimating future ozone layer depletion based on the expected growth of the space industry and known impacts of rocket launches." As the rocket launch market grows, so will ozone-destroying rocket emissions," said Professor Darin Toohey of CU-Boulder's atmospheric and oceanic sciences department. "If left unregulated, rocket launches by the year 2050 could result in more ozone destruction than was ever realized by CFCs."Since some proposed space efforts would require frequent launches of large rockets over extended periods, the new study was designed to bring attention to the issue in hopes of sparking additional research, said Ross. "In the policy world uncertainty often leads to unnecessary regulation," he said. "We are suggesting this could be avoided with a more robust understanding of how rockets affect the ozone layer." Current global rocket launches deplete the ozone layer by no more than a few hundredths of 1 percent annually, said Toohey. But as the space industry grows and other ozone-depleting chemicals decline in the Earth's stratosphere, the issue of ozone depletion from rocket launches is

expected to move to the forefront. Highly reactive trace-gas molecules known as radicals dominate stratospheric ozone destruction, and a single radical in the stratosphere can destroy up to 10,000 ozone molecules before being deactivated and removed from the stratosphere.

Ozone depletion

In 1974, after millions of tons of CFCs had been manufactured and sold; chemists F. Sherwood Rowland and Mario Molina of the University of California began to wonder where all these CFCs ended up. Rowland and Molina theorized that ultraviolet (UV) rays from the Sun would break up CFCs in the stratosphere, and that the free chlorine atoms would then enter into a chain reaction, destroying ozone. Many people, however, remained unconvinced of the danger until the mid-1980s, when a severe springtime depletion of ozone was first monitored by the British Antarctic Survey above Antarctica. The depletion above the South Pole was so severe that the British geophysicist, Joe Farman, who first measured it, assumed his spectrophotometer must be broken and sent the device back to England to be repaired. Once the depletion was verified, it came to be known throughout the world through a series of NASA satellite photos as the Antarctic Ozone Hole.Laboratory studies backed by satellite and ground-based measurements, show that free chlorine reacts very rapidly with ozone. They also show that the chlorine oxide formed in that reaction undergoes further processes that regenerate the original chlorine, allowing the sequence to be repeated up to 100,000 times. This process is known as a "chain reaction". Similar reactions also take place between bromine and ozone. Observations of the Antarctic ozone hole have given a convincing and unmistakable demonstration of these processes. Scientists have repeatedly observed a large number of chemical species over Antarctica since 1986. Among the chemicals measured were ozone and chlorine monoxide, which is the reactive chemical identified in the laboratory as one of the participants in the ozone-destroying chain reactions. The satellite maps shown in the figure below relate the accumulation of chlorine monoxide observed over Antarctica and the subsequent ozone depletion that occurs rapidly in a few days over very similar areas.

EFFECT OF OZONE LAYER DEPLETION

i) Effect on human and animal health-

Increased penetration of solar UV-B radiation is likely to have profound impact on human health with potential risks of eye diseases, skin cancer and infectious diseases. UV radiation is known to damage the cornea and lens of the eye. Chronic exposure to UV-B could lead to cataract of the cortical and posterior subcapsular forms. UV-B radiation can adversely affect the immune system causing a number of infectious diseases. In light skinned human populations, it is likely to develop nonmelanoma skin cancer (NMSC). Experiments on animals show that UV exposure decreases the immune response to skin cancers, infectious agents and other antigens.

ii) Effect on terrestrial plant-

Antarctic springtime ozone hole will shrink by five to 10 per cent between 2000 and 2020. In sharp contrast, the cosmic It is a known fact that the physiological and developmental processes of plants are affected by UV-B radiation. Scientists believe that an increase in UV-B levels would necessitate using more UV-B tolerant cultivar and breeding new tolerant ones in agriculture. In forests and grasslands increased UV-B radiation is likely to result in changes in species composition (mutation) thus altering the bio-diversity in different ecosystems. UV-B could also affect the plant community indirectly resulting in changes in plant form, secondary metabolism, etc. These changes can have important implications for plant competitive balance, plant pathogens and bio-geochemical cycles.

iii) Effects on Aquatic Ecosystems-

While more than 30 percent of the world's animal protein for human consumption comes from the sea alone, it is feared that increased levels of UV exposure can have adverse impacts on the productivity of aquatic systems. High levels of exposure in tropics and subtropics may affect the distribution of phytoplanktons which form the foundation of aquatic food webs. Reportedly a recent study has indicated 6-12 percent reduction in phytoplankton production in the marginal ice zone due to increases in UV-B. UV-B can also cause damage to early development stages of fish, shrimp, crab, amphibians and other animals, the most severe effects being decreased reproductive capacity and impaired larval development.

iv) Effects on Bio-geo-chemical Cycles-

Increased solar UV radiation could affect terrestrial and aquatic bio-geo-chemical cycles

thus altering both sources and sinks of greenhouse and important trace gases, e.g. carbon dioxide (CO2), carbon monoxide (CO), carbonyl sulphide (COS), etc. These changes would contribute to biosphere-atmosphere feedbacks responsible for the atmosphere build-up of these gases.

v) Effects on air quality-

Reduction of stratospheric ozone and increased penetration of UV-B radiation result in higher photo dissociation rates of key trace gases that control the chemical reactivity of the troposphere. This can increase both production and destruction of ozone and related oxidants such as hydrogen peroxide which are known to have adverse effects on human health, terrestrial plants and outdoor materials. Changes in the atmospheric concentrations of the hydroxyl radical (OH) may change the atmospheric lifetimes of important gases such as methane and substitutes of chlorofluoro carbons (CFCs).

vi) Effects on Materials-

An increased level of solar UV radiation is known to have adverse effects on synthetic polymers, naturally occurring biopolymers and some other materials of commercial interest. UV-B radiation accelerates the photo degradation rates of these materials thus limiting their lifetimes. Typical damages range from discoloration to loss of mechanical integrity. Such a situation would eventually demand substitution of the affected materials by more photo stable plastics and other materials in future. In 1974, two United States (US) scientists Mario Molina and F. Sherwood Rowland at the University of California were struck by the observation of Lovelock that the CFCs were present in the atmosphere all over the world more or less evenly distributed by appreciable concentrations. They suggested that these stable CFC molecules could drift slowly up to the stratosphere where they may breakdown into chlorine atoms by energetic UV-B and UB-C rays of the sun. The chlorine radicals thus produced can undergo complex chemical reaction producing chlorine monoxide which can attack an ozone molecule converting it into oxygen and in the process regenerating the chlorine atom again. Thus the ozone destroying effect is catalytic and a small amount of CFC would be destroying large number of ozone molecules. Their basic theory was then put to test by the National Aeronautic Space Authority (NASA) scientists and found to be valid, ringing alarm bells in many countries and laying the foundation for international action.

vii) Effects on Climate Change-

Ozone depletion and climate change are linked in a number of ways, but ozone depletion is not a major cause of climate change. Atmospheric ozone has two effects on the

temperature balance of the Earth. It absorbs solar ultraviolet radiation, which heats the stratosphere. It also absorbs infrared radiation emitted by the Earth's surface, effectively trapping heat in the troposphere. Therefore, the climate impact of changes in ozone concentrations varies with the altitude at which these ozone changes occur. The major ozone losses that have been observed in the lower stratosphere due to the human-produced chlorine- and bromine-containing gases have a cooling effect on the Earth's surface. On the other hand, the ozone increases that are estimated to have occurred in the troposphere because of surface-pollution gases have a warming effect on the Earth's surface, thereby contributing to the "greenhouse" effect. In comparison to the effects of changes in other atmospheric gases, the effects of both of these ozone changes are difficult to calculate accurately. In the figure below, the upper ranges of possible effects for the ozone changes are indicated by the open bars, and the lower ranges are indicated by the solid bars.

viii) Effects on Ultraviolet Radiation-

The depletion of the ozone layer leads, on the average, to an increase in ground-level ultraviolet radiation, because ozone is an effective absorber of ultra-violet radiation. The Sun emits radiation over a wide range of energies, with about 2% in the form of high-energy, ultraviolet (UV) radiation. Some of this UV radiation (UV-B) is especially effective in causing damage to living beings, the largest decreases in ozone during the past 15 years have been observed over Antarctica, especially during each September and October when the ozone hole forms. During the last several years, simultaneous measurements of UV radiation and total ozone have been made at several Antarctic stations. In the late spring, the biologically damaging ultraviolet radiation in parts of the Antarctic continent can exceed that in San Diego, California, where the Sun is much higher above the horizon. In areas where smaller ozone depletion has been observed, UV-B increases are more difficult to detect. In particular, detection of trends in UV-B radiation associated with ozone decreases can be further complicated by changes in cloudiness, by local pollution, and by difficulties in keeping the detection instrument in precisely the same condition over many years. Prior to the late 1980s, instruments with the necessary accuracy and stability for measurement of small long-term trends in ground-level UV-B were not available. Therefore, the data from urban locations with older, less-specialized instruments provide much less reliable information, especially since simultaneous measurements of changes in cloudiness or local pollution are not available. When high-quality measurements have been made in other areas far from

major cities and their associated air pollution, decreases in ozone have regularly been accompanied by increases in UV-B. This is shown in the figure below, where clear-sky measurements performed at six different stations demonstrate that ozone decreases lead to increased UV-B radiation at the surface in amounts that are in good agreement with that expected from calculations (the "model" curve).

INTERNATIONAL ACTIONS:

The first international action to focus attention on the dangers of ozone depletion in the stratosphere and its dangerous consequences in the long run on life on earth was focused in 1977 when in a meeting of 32 countries in Washington D.C. a World plan on action on Ozone layer with UNEP as the coordinator was adopted. As experts began their investigation, data piled up and in 1985 in an article published in the prestigious science journal, “Nature” by Dr. Farman pointed out that although there is overall depletion of the ozone layer all over the world, the most severe depletion had taken place over the Antarctica. This is what is famously called as "the Antarctica Ozone hole". His findings were confirmed by Satellite observations and offered the first proof of severe ozone depletion and stirred the scientific community to take urgent remedial actions in an international convention held in Vienna on March 22, 1985. This resulted in an international agreement in 1987 on specific measures to be taken in the form of an international treaty known as the Montreal Protocol on Substances That Deplete the Ozone Layer. Under this Protocol the first concrete step to save the Ozone layer was taken by immediately agreeing to completely phase out chlorofluorocarbons (CFC), Halons, Carbon tetrachloride (CTC) and Methyl chloroform (MCF) as per a given schedule

A. Montreal Protocol:

In 1985 the Vienna Convention established mechanisms for international co-operation in research into the ozone layer and the effects of ozone depleting chemicals (ODCs). 1985 also marked the first discovery of the Antarctic ozone hole. On the basis of the Vienna Convention, the Montreal Protocol on Substances that Deplete the Ozone Layer was negotiated and signed by 24 countries and by the European Economic Community in September 1987. The Protocol called for the Parties to phase down the use of CFCs, halons and other man-made ODCs. The Montreal Protocol represented a landmark in the international environmentalist movement. For the first time whole countries were legally bound to reducing and eventually phasing out altogether the use of CFCs and other ODCs.

Failure to comply was accompanied by stiff penalties. The original Protocol aimed to decrease the use of chemical compounds destructive to ozone in the stratosphere by 50% by the year 1999. The Protocol was supplemented by agreements made in London in 1990 and in Copenhagen in 1992, where the same countries promised to stop using CFCs and most of the other chemical compounds destructive to ozone by the end of 1995. Fortunately, it has been fairly easy to develop and introduce compounds and methods to replace CFC compounds. In order to deal with the special difficulties experienced by developing countries it was agreed that they would be given an extended period of grace, so long as their use of CFCs did not grow significantly. China and India, for example, are strongly increasing the use of air conditioning and cooling devices

B. Australian Chlorofluorocarbon Management Strategy

It provides a framework for the responsible management and use of CFCs in Australia. The strategy recognizes some continuing need for these chemicals in pharmaceutical and laboratory uses, but commits to their gradual phasing out.

C. Environmental Protection (Ozone Protection) Policy2000

This WA policy aims to minimize the discharge of ozone-depleting substances into the environment, and has been extended to cover use of alternative refrigerants (where relevant). This has been done to prevent current stocks of ozone-depleting substances from being released to the atmosphere by trade's people that are not accredited, or with inadequate training and/or equipment working on systems that contain these substances.

D. United Nations Environment Programme:

Has published several assessments of the environmental effects of ozone depletion (United Nations Environment Programme, 1998; World Meteorological Organization, 2002).

E. Ozone Protection and Synthetic Greenhouse Gas Management Act 1989 **(and associated regulations and amendments)**

Was implemented by the Commonwealth Government to meet its commitments under the Montreal Protocol.

F. Ultraviolet index forecast

The Bureau of Meteorology has developed a model to predict the amount of ultraviolet exposure and the times of day at which it will occur for 45 WA locations. It is designed to help people minimize their exposure to dangerous levels of ultraviolet radiation.

Under the auspices of United Nations Environment Programme (UNEP), Governments of the world, including the United States have cooperatively taken action to stop ozone depletion with the "The Montreal Protocol on Substances that Deplete the Ozone Layer",

signed in 1987. Scientists are concerned that continued global warming will accelerate ozone destruction and increase stratospheric ozone depletion. Ozone depletion gets worse when the stratosphere (where the ozone layer is), becomes colder. Because global warming traps heat in the troposphere, less heat reaches the stratosphere which will make it colder. Greenhouse gases act like a blanket for the troposphere and make the stratosphere colder. In other words, global warming can make ozone depletion much worse right when it is supposed to begin its recovery during the next century. Maintain programs to ensure that ozone-depleting substances are not released and ongoing vigilance is required to this effect. In fact, global warming, acid rain, ozone layer depletion, and ground-level ozone pollution all pose a serious threat to the quality of life on Earth. They are separate problems, but, as has been seen, there are links between each. The use of CFCs not only destroys the ozone layer but also leads to global warming.

Acid rain: Causes, Effects and Solutions:

Acid rain, or acid deposition, is a broad term that includes any form of precipitation that contains acidic components, such as sulfuric acid or nitric acid, according to the Environmental Protection Agency (EPA).

The precipitation is not necessarily wet or liquid; the definition includes dust, gasses, rain, snow, fog and hail. The type of acid rain that contains water is called wet deposition. Acid rain formed with dust or gasses is called dry deposition

Dead trees line a riverbank near Norilsk, Russia, formerly a major industrial center in Siberia, and one of the most polluted cities in the world, according to National Geographic

Causes-

The term acid rain was coined in 1852 by Scottish chemist Robert Angus Smith, according to the Royal Society of Chemistry, which calls him the "father of acid rain." Smith decided on the term while examining rainwater chemistry near industrial cities in England and Scotland. He wrote about his findings in 1872 in the book "Air and Rain: The Beginnings of a Chemical Climatology."

In the 1950s, scientists in the United States started studying the phenomenon, and in the 1960s and early 1970s, acid rain became recognized as a regional environmental issue that affected Western Europe and eastern North America.

Though manmade pollutants are currently affecting most acidic precipitation, natural disasters can be a factor as well. For example, volcanoes can cause acid rain by blasting pollutants into the air. These pollutants can be carried around the world in jet streams and turned into acid rain far from the volcano.

After an asteroid supposedly wiped out the dinosaurs 65.5 million years ago, sulfur trioxide was blasted into the air. When it hit the air, it turned into sulfuric acid, generating a downpour of acid rain, according to a paper published in 2014 in the journal Nature Geoscience.

Even before that, over 4 billion years ago, it is suspected that the air may have had 10,000 times as much carbon dioxide as today. Geologists from the University of Wisconsin-Madison backed up this theory be studying rocks and publishing the results in a 2008 issue of the journal Earth and Planetary Science Letters. "At [those levels of carbon dioxide], you would have had vicious acid rain and intense greenhouse [effects]. That is a condition that will dissolve rocks," said study team member John Valley. [Early Earth Marred by Acid Rain]

Sulfur dioxide (SO_2) and nitrogen oxides (NOx) released into the air by fossil-fuel power plants, vehicles and oil refineries are the biggest cause of acid rain today, according to the EPA. Two thirds of sulfur dioxide and one fourth of nitrogen oxide found in the atmosphere come from electric power generators.

A chemical reaction happens when sulfur dioxide and nitrogen oxides mix with water, oxygen and other chemicals in the air. They then become sulfuric and nitric acids that mix with precipitation and fall to the ground. Precipitation is considered acidic when its pH level is about 5.2 or below, according to Encyclopedia Britannica. The normal pH of rain is around 5.6.

Effects-

Acid rain affects nearly everything. Plants, soil, trees, buildings and even statues can be transformed by the precipitation.

Acid rain has been found to be very hard on trees. It weakens them by washing away the protective film on leaves, and it stunts growth. A paper released in the online version of the journal of Environmental Science and Technology in 2005 showed evidence of acid rain stunting tree growth.

"By providing the only preserved soil in the world collected before the acid rain era, the Russians helped our international team track tree growth for the first time with changes in soil from acid rain," said Greg Lawrence, a U.S. Geological Survey scientist who headed the effort. "We've known that acid rain acidifies surface waters, but this is the first time we've been able to compare and track tree growth in forests that include soil changes due to acid rain."

Acid rain can also change the composition of soil and bodies of water, making them uninhabitable for local animals and plants. For example, healthy lakes have a pH of 6.5 or higher. As acid rain raises the level of acidity, fish tend to die off. Most fish species can't survive a water pH of below 5. When the pH becomes a 4, the lake is considered dead, according to National Atmospheric Deposition Program.

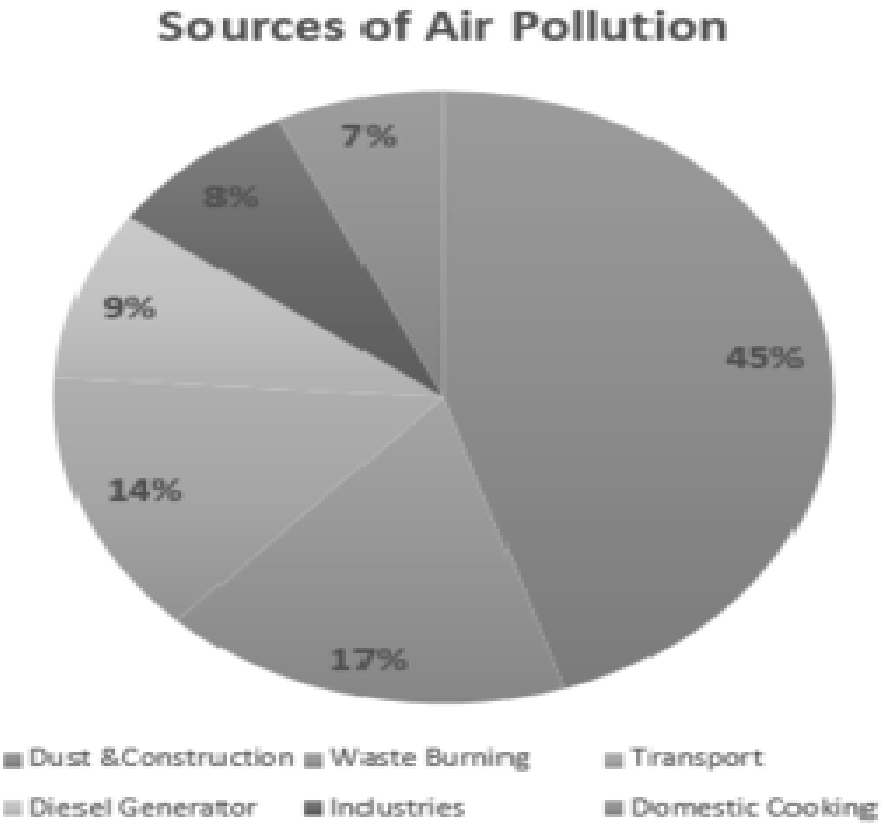
It can additionally deteriorate limestone and marble buildings and monuments, like gravestones.

Solutions-

There are several solutions to stopping manmade acid rain. Regulating the emissions coming from vehicles and buildings is an important step, according to the EPA. This can be done by restricting the use of fossil fuels and focusing on more sustainable energy sources such as solar and wind power.

Also, each person can do their part by reducing their vehicle use. Using public transportation, walking, riding a bike or carpooling is a good start, according to the EPA. People can also reduce their use of electricity, which is widely created with fossil fuels, or switch to a solar plan. Many electricity companies offer solar packages to their customers that require no installation and low costs.

Air pollution in India:



Dust & Construction contribute about 45% to the air pollution in India, which is followed by Waste Burning. Dust & Construction activities are mostly in the urban areas while Waste Burning is in the rural areas (agriculture).Air pollution occurs when harmful or excessive quantities of substances including gases, particles, and biological molecules are introduced into the Earth's atmosphere. Air pollution in India is a serious issue, ranking higher than smoking, high blood pressure, child and maternal malnutrition, and risk factors for diabetes. At least 140 million people breathe air 10 times or more over the WHO safe limit and 13 of the world's 20 cities with the highest annual levels of air pollution are in India. Air pollution contributes to the premature deaths of 2 million Indians every year. In urban areas, most

emissions come from vehicles and industry, whereas in rural areas, much of the pollution stems from biomass burning for cooking and keeping warm. In autumn and winter months, large scale crop residue burning in agriculture fields – a low cost alternative to mechanical tilling – is a major source of smoke, smog and particulate pollution India has a low per capita emissions of greenhouse gases but the country as a whole is the third largest after China and the United States. A 2013 study on non-smokers has found that Indians have 30% lower lung function compared to Europeans.

The Air (Prevention and Control of Pollution) Act was passed in 1981 to regulate air pollution and there have been some measurable improvements. However, the 2016 Environmental Performance Index ranked India 141 out of 180 countries.

In 2015, Government of India, together with IIT Kanpur launched the National Air Quality Index. In 2019, India launched 'The National Clean Air Programme's with tentative national target of 20%-30% reduction in PM2.5 and PM10 concentrations by 2024, considering 2017 as the base year for comparison. It will be rolled out in 102 cities that are considered to have air quality worse than the National Ambient Air Quality Standards.

Causes:

Cooking fuel in rural India is prepared from a wet mix of dried grass, fuel wood pieces, hay, leaves and mostly cow/livestock dung. This mix is patted down into disc-shaped cakes, dried, and then used as fuel in stoves. When it burns, it produces smoke and numerous indoor air pollutants at concentrations 5 times higher than coal.

Fuel and biomass burning

A rural aburo stove using biomass cakes, fuel wood and trash as cooking fuel. Surveys suggest over 100 million households in India use such stoves (chullahs) every day, 2–3 times a day. Clean burning fuels and electricity are unavailable in rural parts and small towns of India because of poor rural highways and limited energy generation infrastructure.Fuel wood and biomass burning is the primary reason for near-permanent haze and smoke observed above rural and urban India, and in satellite pictures of the country. Fuelwood and biomass cakes are used for cooking and general heating needs. These are burnt in cook stoves known as chullah or chulha piece in some parts of India. These cook stoves are present in over 100 million Indian households, and are used two to three times a day, daily. Some reports, including one by the World Health Organization, claim 300,000 to 400,000 people die of indoor air pollution and carbon monoxide poisoning in India because of biomass burning and use of chullahs the air pollution is also the main cause of the Asian brown cloud which is delaying the start of the monsoon. Burning of biomass and firewood will not stop unless electricity or clean burning fuel and combustion technologies become reliably available and widely adopted in rural and urban India.

India is the world's largest consumer of fuelwood, agricultural waste and biomass for energy purposes. From the most recent available nationwide study, India used 148.7 million tonnes coal replacement worth of fuel-wood and biomass annually for domestic energy use. India's national average annual per capita consumption of fuel wood, agricultural waste and biomass cakes was 206 kilogram coal equivalent. The overall contribution of fuelwood, including sawdust and wood waste, was about 46% of the total, the rest being agri waste and biomass dung cakes. Traditional fuel (fuelwood, crop residue and dung cake) dominates domestic energy use in rural India and accounts for about 90% of the total. In urban areas, this traditional fuel constitutes about 24% of the total.India burns tenfold more fuelwood every

year than the United States; the fuelwood quality in India is different from the dry firewood of the United States; and, the Indian stoves in use are less efficient, thereby producing more smoke and air pollutants per kilogram equivalent.

Fuel adulteration

Some Indian taxis and auto-rickshaws run on adulterated fuel blends. Adulteration of gasoline and diesel with lower-priced fuels is common in South Asia, including India.Some adulterants increase emissions of harmful pollutants from vehicles, worsening urban air pollution. Financial incentives arising from differential taxes are generally the primary cause of fuel adulteration. In India and other developing countries, gasoline carries a much higher tax than diesel, which in turn is taxed more than kerosene meant as a cooking fuel, while some solvents and lubricants carry little or no tax.

As fuel prices rise, the public transport driver cuts costs by blending the cheaper hydrocarbon into highly taxed hydrocarbon. The blending may be as much as 20–30 percent. For a low wage driver, the adulteration can yield short term savings that are significant over the month. The consequences to long term air pollution, quality of life and effect on health are simply ignored. Also ignored are the reduced life of vehicle engine and higher maintenance costs, particularly if the taxi, auto-rickshaw or truck is being rented for a daily fee.

Adulterated fuel increases tailpipe emissions of hydrocarbons (HC), carbon monoxide (CO), oxides of nitrogen (NO_x) and particulate matter (PM). Air toxin emissions - which fall into the category of unregulated emissions - of primary concern are benzene and polyaromatic hydrocarbons (PAHs), both well known carcinogens. Kerosene is more difficult to burn than

gasoline, its addition results in higher levels of HC, CO and PM emissions even from catalyst-equipped cars. The higher sulfur level of kerosene is another issue. Fuel adulteration is essentially an unintended consequence of tax policies and the attempt to control fuel prices, in the name of fairness. Air pollution is the ultimate result. This problem is not unique to India, but prevalent in many developing countries including those outside of south Asia. This problem is largely absent in economies that do not regulate the ability of fuel producers to innovate or price based on market demand.

Traffic congestion

Traffic congestion is severe in India's cities and towns. Traffic congestion is caused for several reasons, some of which are: increase in number of vehicles per kilometer of available road, a lack of intra-city divided-lane highways and intra-city expressways networks, lack of inter-city expressways, traffic accidents and chaos due to poor enforcement of traffic laws.

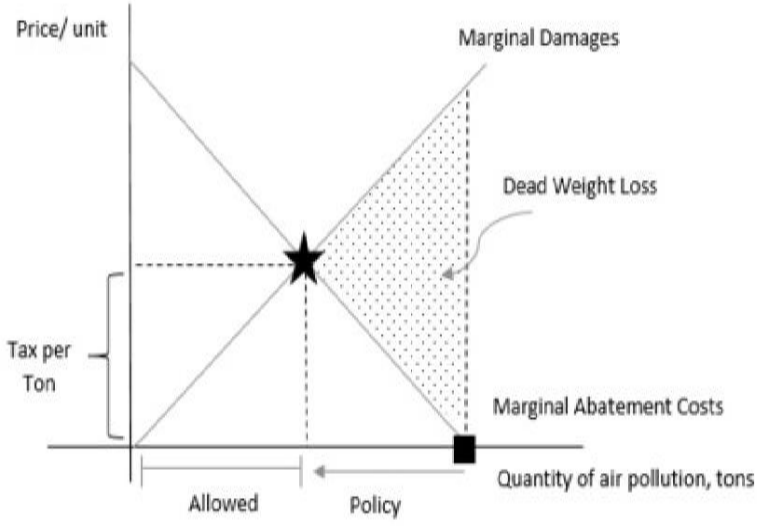
Traffic congestion reduces average traffic speed. At low speeds, scientific studies reveal, vehicles burn fuel inefficiently and pollute more per trip. For example, a study in the United States found that for the same trip, cars consumed more fuel and polluted more if the traffic was congested, than when traffic flowed freely. At average trip speeds between 20 and 40 kilometers per hour, the cars pollutant emission was twice as much as when the average speed was 55 to 75 kilometers per hour. At average trip speeds between 5 and 20 kilometers per hour, the cars pollutant emissions were 4 to 8 times as much as when the average speed was 55 to 70 kilometers per hour. Fuel efficiencies similarly were much worse with traffic

congestion.

Traffic gridlock in Delhi and other Indian cities is extreme. The average trip speed on many Indian city roads is less than 20 kilometers per hour; a 10 kilometer trip can take 30 minutes, or more. At such speeds, vehicles in India emit air pollutants 4 to 8 times more than they would with less traffic congestion; Indian vehicles also consume a lot more carbon footprint fuel per trip, than they would if the traffic congestion was less. Emissions of particles and heavy metals increase over time because the growth of the fleet and mileage outpaces the efforts to curb emissions.

In cities like Bangalore, around 50% of children suffer from asthma

Greenhouse gas emissions

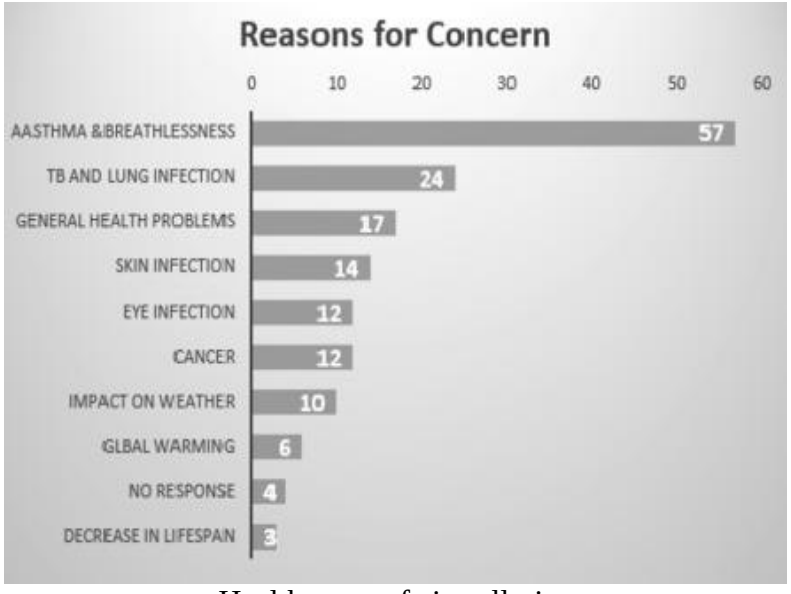


Market for Air Pollution: The box represents the current status quo and the star represents the ideal, socially optimal point to be at. The shaded area represents the dead weight loss. The MAC curve denotes the additional cost of achieving one more unit decrease in level of emissions. MD denotes the additional damage caused by an additional unit of emission.

India was the third largest emitter of carbon dioxide in 2017 at 6.82% share of CO2 emissions, after China (27.21%) and the United States (14.58%). According to a report by the Global Carbon Project, "“after low growth during 2014 to 2016, fossil CO2 emissions have now risen two years in a row, with a 1.6 per cent rise in 2017 and a projected 2.7 per cent (range 1.8 per cent to 3.7 per cent) rise expected in 2018, reaching a record high of 37.1 (plus or minus 2) billion tonnes of CO2. The peak in global CO2 emissions is not yet in sight.”About 65 percent of India's carbon dioxide emissions in 2009 was from heating, domestic uses and power sector. About 9 percent of India's emissions were from

transportation (cars, trains, two wheelers, aeroplanes, others). India's coal-fired, oil-fired and natural gas-fired thermal power plants are inefficient and offer significant potential for CO_2 emission reduction through better technology. Compared to the average emissions from coal-fired, oil-fired and natural gas-fired thermal power plants in European Union (EU-27) countries, India's thermal power plants emit 50 to 120 percent more CO_2 per kWh produced.This is in significant part to inefficient thermal power plants installed in India prior to its economic liberalisation in the 1990s

Effects



Health costs of air pollution

Asthma is the leading health problem faced by Indians. Not surprisingly, it accounts for more than 50% of the health problems caused by air pollution.One of the most important reasons for concern for the growing air pollution in the country is its effects on the health of individuals. Exposure to particulate matter for a long time can lead to respiratory and cardiovascular diseases such as asthma, bronchitis, lung cancer and heart attacks. The Global Burden of Disease Study for 2010, published in 2013, had found that outdoor air pollution was the fifth-largest killer in India and around 620,000 early deaths occurred from air

pollution-related diseases in 2010.According to a WHO study, 13 of the 20 most-polluted cities in the world are in India; however, the accuracy and methodology of the WHO study was questioned by the Government of India.

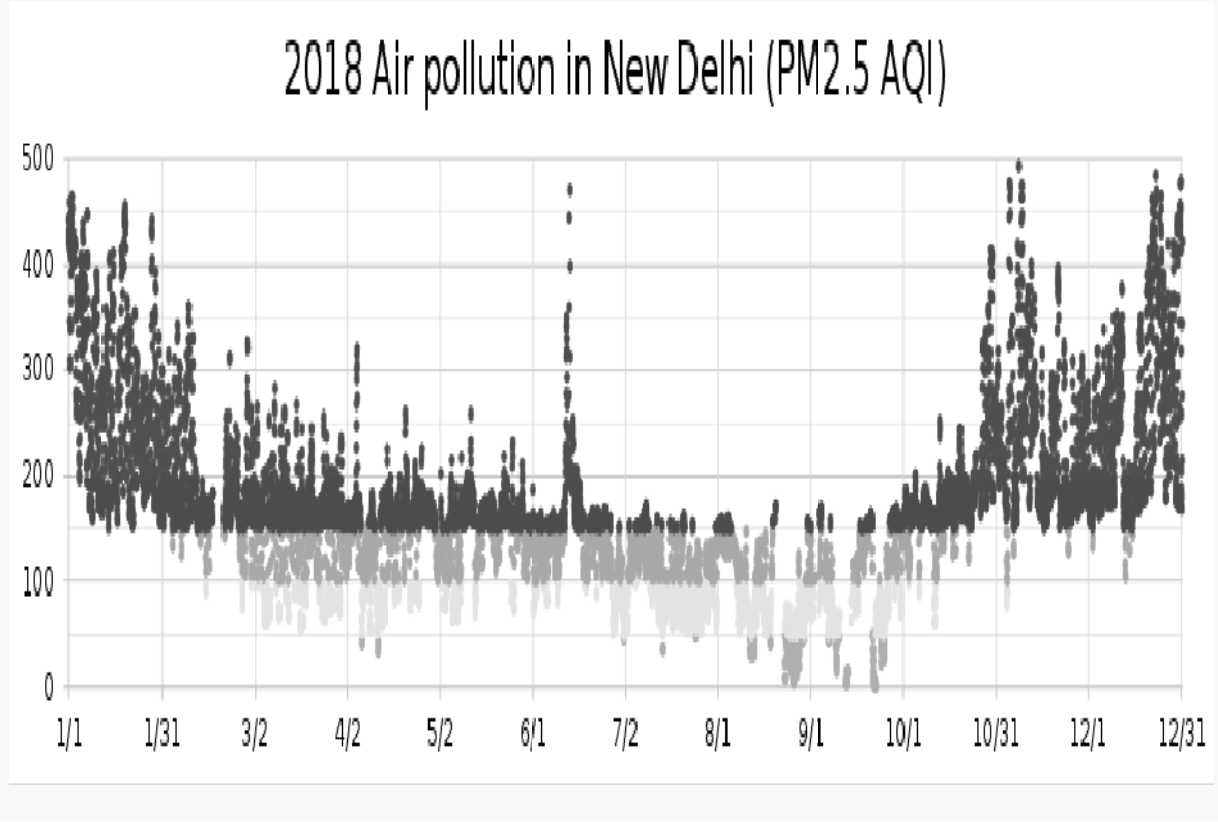
Over a million Indians die prematurely every year due to air pollution, according to the non-profit Health Effects Institute. Over two million children -- half the children in Delhi -- have abnormalities in their lung function, according to the Delhi Heart and Lung Institute. Over the past decade air pollution has increased in India significant. Asthma is the most common health problem faced by Indians and it accounts for more than half of the health issues caused by air pollution.

State-Wise Trends:

According to the WHO, India has14 out of the 15 most polluted cities in the world in terms of PM 2.5 concentrations. Other Indian cities that registered very high levels of PM2.5 pollutants are Delhi, Patna, Agra, Muzzaffarpur, Srinagar, Gurgaon, Jaipur, Patiala and Jodhpur, followed by Ali Subah Al-Salem in Kuwait and a few cities in China and Mongolia.

Air Quality Index (AQI) is a number used to communicate the level of pollution in the air and it essentially tells you the level of pollution in the air in a given city on a given day. The AQI of Delhi was placed under the "severe-plus category" when it touched 574, by the System of Air Quality and Weather Forecasting And Research. In May 2014 the World Health

Organisation announced New Delhi as the most polluted city in the world.In November 2016, the Great smog of Delhi was an environmental event which saw New Delhi and adjoining areas in a dense blanket of smog, which was the worst in 17 years.



2018 Air Pollution in NewDelhi (PM2.5 AQI). A surge on June 14 was caused by dust storms brought on by a combination of extreme heat and powerful downdraft winds.
Hazardous Very Unhealthy Unhealthy Unhealthy for Sensitive Groups Moderate Good

Top 20 Cities in India with the highest level of PM 2.5	
Cities	PM2.5 Levels
Delhi	153
Patna	149
Gwalior	144
Raipur	134
Ahmedabad	100
Lukhnow	96
Firozabad	96
Kanpur	93
Amritsar	92
Ludhiana	91
Allahbad	88
Agra	88
Khanna	88

India's Central Pollution Control Board now routinely monitors four air pollutants namely sulphur dioxide (SO2), oxides of nitrogen (NOx), suspended particulate matter (SPM) and respirable particulate matter (PM10). These are target air pollutants for regular monitoring at 308 operating stations in 115 cities/towns in 25 states and 4 Union Territories of India. The monitoring of meteorological parameters such as wind speed and direction, relative humidity and temperature has also been integrated with the monitoring of air quality. The monitoring of these pollutants is carried out for 24 hours (4-hourly sampling for gaseous pollutants and 8-hourly sampling for particulate matter) with a frequency of twice a week, to yield 104 observations in a year.

The key findings of India's central pollution control board are:

- Most Indian cities continue to violate India's and world air quality PM10 targets. Respirable particulate matter pollution remains a key challenge for India. Despite the general non-attainment, some cities showed far more improvement than others. A decreasing trend has been observed in PM10 levels in cities like Solapur and Ahmedabad over the last few years. This improvement may be due to local measures taken to reduce sulphur in diesel and stringent enforcement by the government.

- A decreasing trend has been observed in sulphur dioxide levels in residential areas of many cities such as Delhi, Mumbai, Lucknow, Bhopal during last few years. The decreasing trend in sulphur dioxide levels may be due to recently introduced clean fuel standards, and the increasing use of LPG as domestic fuel instead of coal or fuelwood, and the use of CNG instead of diesel in certain vehicles.
- A decreasing trend has been observed in nitrogen dioxide levels in residential areas of some cities such as Bhopal and Solapur during last few years. The decreasing trend in sulphur dioxide levels may be due to recently introduced vehicle emission standards, and the increasing use of LPG as domestic fuel instead of coal or fuelwood.
- Most Indian cities greatly exceed acceptable levels of suspended particulate matter. This may be because of refuse and biomass burning, vehicles, power plant emissions, industrial sources.
- The Indian air quality monitoring stations reported lower levels of PM10 and suspended particulate matter during monsoon months possibly due to wet deposition and air scrubbing by rainfall. Higher levels of particulates were observed during winter months possibly due to lower mixing heights and more calm conditions. In other words, India's air quality worsens in winter months, and improves with the onset of monsoon season.
- The average annual SOx and NOx emissions level and periodic violations in industrial areas of India were significantly and surprisingly lower than the emission and violations in residential areas of India
- Of the four major Indian cities, air pollution was consistently worse in Delhi, every year over 5-year period (2004–2018). Kolkata was a close second, followed by Mumbai. Chennai air pollution was least of the four.

Steps Taken/ Policy Recommendations

- The government in Delhi launched an Odd-Even Rule in November, 2017 which is based on the Odd-Even rationing method: This meant that cars running with number plates ending in Odd digits could only be driven on certain days of the week, while the even digit cars could be driven on the remaining days of the week.
- Local governments of various states also implemented measures such as tighter vehicle emissions' norms, higher penalties for burning rubbish and better control of road dust
- The Indian government has committed to a 50% reduction in households using solid

fuel for cooking

- Some goals set for future are:
  - Clean up the transportation sector by introducing 1,000 electric public transport buses to its 5,50-string feet
  - Meet a goal of 25% of private vehicles to be electricity powered by 2023
  - Provide farmers with a machine called a Happy Seeder which converts agricultural residue to fertilizer
  - Analyze health data and study the efficiency of different room filtration systems in areas where indoor air pollution is highest
  - Identify effective ways to inform the public about air pollution data
  - Launch new citizen science programs to better document exposures
  - Reduce Carbon Emissions: "According to Inter-governmental Panel on Climate Change, to limit warming well below 2 degree Celsius, CO2 emissions should decline by about 20 per cent by 2030 and reach net zero around 2075; to limit warming below 1.5 degree Celsius, CO2 emissions should decline by 50 per cent by 2030 and reach net zero by around 2050.

b) Water Pollution

British poet W. H. Auden once noted, "Thousands have lived without love, not one without water." Yet while we all know water is crucial for life, we trash it anyway. Some 80 percent of the world's wastewater is dumped—largely untreated—back into the environment, polluting rivers, lakes, and oceans.

This widespread problem of water pollution is jeopardizing our health. Unsafe water kills more people each year than war and all other forms of violence combined. Meanwhile, our drinkable water sources are finite: Less than 1 percent of the earth's freshwater is actually accessible to us. Without action, the challenges will only increase by 2050, when global demand for freshwater is expected to be one-third greater than it is now.

But while most Americans have access to safe drinking water, potentially harmful contaminants—from arsenic to copper to lead—have been found in the tap water of every single state in the nation.

Still, we're not hopeless against the threat to clean water. To better understand the problem and what we can do about it, here's an overview of what water pollution is, what causes it, and how we can protect ourselves.

What Is Water Pollution?

Water pollution occurs when harmful substances—often chemicals or microorganisms—

contaminate a stream, river, lake, ocean, aquifer, or other body of water, degrading water quality and rendering it toxic to humans or the environment.

Water pollution is the contamination of water bodies, usually as a result of human activities. Water bodies include for example lakes, rivers, oceans, aquifers and groundwater. Water pollution results when contaminants are introduced into the natural environment.

What Are the Causes of Water Pollution?

Water is uniquely vulnerable to pollution. Known as a "universal solvent," water is able to dissolve more substances than any other liquid on earth. It's the reason we have Kool-Aid and brilliant blue waterfalls. It's also why water is so easily polluted. Toxic substances from farms, towns, and factories readily dissolve into and mix with it, causing water pollution.

Categories of Water Pollution

Groundwater-

When rain falls and seeps deep into the earth, filling the cracks, crevices, and porous spaces of an aquifer (basically an underground storehouse of water), it becomes groundwater—one of our least visible but most important natural resources. Nearly 40 percent of Americans rely on groundwater, pumped to the earth's surface, for drinking water. For some folks in rural areas, it's their only freshwater source. Groundwater gets polluted when contaminants—from pesticides and fertilizers to waste leached from landfills and septic systems—make their way into an aquifer, rendering it unsafe for human use. Ridding groundwater of contaminants can be difficult to impossible, as well as costly. Once polluted, an aquifer may be unusable for decades, or even thousands of years. Groundwater can also spread contamination far from the original polluting source as it seeps into streams, lakes, oceans.

Surface water-

Covering about 70 percent of the earth, surface water is what fills our oceans, lakes, rivers, and all those other blue bits on the world map. Surface water from freshwater sources (that is, from sources other than the ocean) accounts for more than 60 percent of the water delivered to American homes. But a significant pool of that water is in peril. According to the most recent surveys on national water quality from the U.S. Environmental Protection Agency, nearly half of our rivers and streams and more thanone-third of our lakes are polluted and unfit for swimming, fishing, and drinking. Nutrient pollution, which includes nitrates and phosphates, is the leading type of contamination in these freshwater sources. While plants and animals need these nutrients to grow, they have become a major pollutant due to farm waste and

fertilizer runoff. Municipal and industrial waste discharges contribute their fair share of toxins as well. There's also all the random junk that industry and individuals dump directly into waterways

Ocean water

Eighty percent of ocean pollution (also called marine pollution) originates on land—whether along the coast or far inland. Contaminants such as chemicals, nutrients, and heavy metals are carried from farms, factories, and cities by streams and rivers into our bays and estuaries; from there they travel out to sea. Meanwhile, marine debris—particularly plastic—is blown in by the wind or washed in via storm drains and sewers. Our seas are also sometimes spoiled by oil spills and leaks—big and small—and are consistently soaking up carbon pollution from the air. The ocean absorbs as much as a quarter of man-made carbon emissions.

Point source-

When contamination originates from a single source, it's called point source pollution. Examples include wastewater (also called effluent) discharged legally or illegally by a manufacturer, oil refinery, or wastewater treatment facility, as well as contamination from leaking septic systems, chemical and oil spills, and illegal dumping. The EPA regulates point source pollution by establishing limits on what can be discharged by a facility directly into a body of water. While point source pollution originates from a specific place, it can affect miles of waterways and ocean.

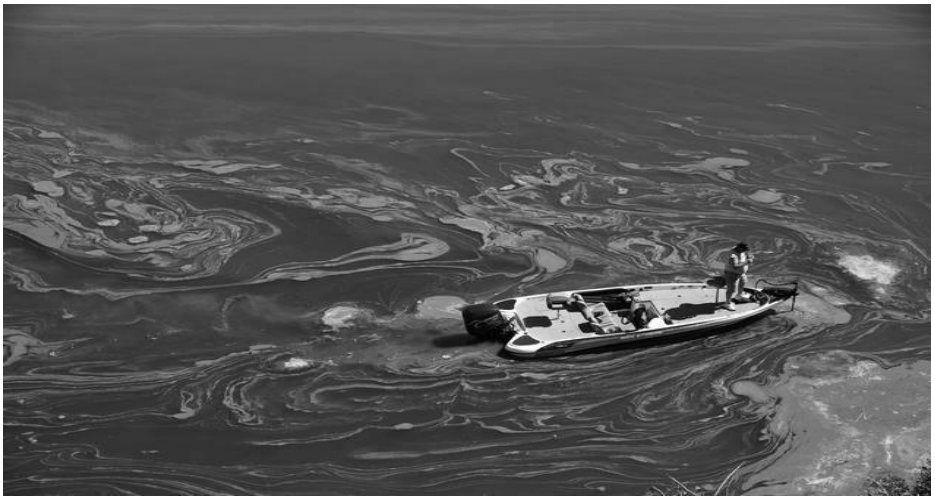
Nonpoint source-

Nonpoint source pollution is contamination derived from diffuse sources. These may include agricultural or stormwater runoff or debris blown into waterways from land. Nonpoint source pollution is the leading cause of water pollution in U.S. waters, but it's difficult to regulate, since there's no single, identifiable culprit.

Transboundary-

It goes without saying that water pollution can't be contained by a line on a map. Transboundary pollution is the result of contaminated water from one country spilling into the waters of another. Contamination can result from a disaster—like an oil spill—or the slow, downriver creep of industrial, agricultural, or municipal discharge.

The Most Common Types of Water Contamination
Agricultural



Toxic green algae in Copco Reservoir, northern California

Not only is the agricultural sector the biggest consumer of global freshwater resources, with farming and livestock production using about 70 percent of the earth's surface water supplies, but it's also a serious water polluter. Around the world, agriculture is the leading cause of water degradation. In the United States, agricultural pollution is the top source of contamination in rivers and streams, the second-biggest source in wetlands, and the third main source in lakes. It's also a major contributor of contamination to estuaries and groundwater. Every time it rains, fertilizers, pesticides, and animal waste from farms and livestock operations wash nutrients and pathogens—such bacteria and viruses—into our waterways. Nutrient pollution, caused by excess nitrogen and phosphorus in water or air, is the number-one threat to water quality worldwide and can cause algal blooms, a toxic soup of blue-green algae that can be harmful to people and wildlife.

Sewage and wastewater

Used water is wastewater. It comes from our sinks, showers, and toilets (think sewage) and from commercial, industrial, and agricultural activities (think metals, solvents, and toxic sludge). The term also includes storm water runoff, which occurs when rainfall carries road salts, oil, grease, chemicals, and debris from impermeable surfaces into our waterways.

More than 80 percent of the world's wastewater flows back into the environment without being treated or reused, according to the United Nations; in some least-developed countries, the figure tops 95 percent. In the United States, wastewater treatment facilities process about 34 billion gallons of wastewater per day. These facilities reduce the amount of pollutants such

as pathogens, phosphorus, and nitrogen in sewage, as well as heavy metals and toxic chemicals in industrial waste, before discharging the treated waters back into waterways. That's when all goes well. But according to EPA estimates, our nation's aging and easily overwhelmed sewage treatment systems also release more than 850 billion oil pollution

Big spills may dominate headlines, but consumers account for the vast majority of oil pollution in our seas, including oil and gasoline that drips from millions of cars and trucks every day. Moreover, nearly half of the estimated 1 million tons of oil that makes its way into

marine environments each year comes not from tanker spills but from land-based sources such as factories, farms, and cities. At sea, tanker spills account for about 10 percent of the oil in waters around the world, while regular operations of the shipping industry—through both legal and illegal discharges—contribute about one-third. Oil is also naturally released from under the ocean floor through fractures known as seeps.

Radioactive substances

Radioactive waste is any pollution that emits radiation beyond what is naturally released by the environment. It's generated by uranium mining, nuclear power plants, and the production and testing of military weapons, as well as by universities and hospitals that use radioactive materials for research and medicine. Radioactive waste can persist in the environment for thousands of years, making disposal a major challenge. Consider the decommissioned Hanford nuclear weapons production site in Washington, where the cleanup of 56 million gallons of radioactive waste is expected to cost more than $100 billion and last through 2060. Accidentally released or improperly disposed of contaminants threaten groundwater, surface water, and marine resources.

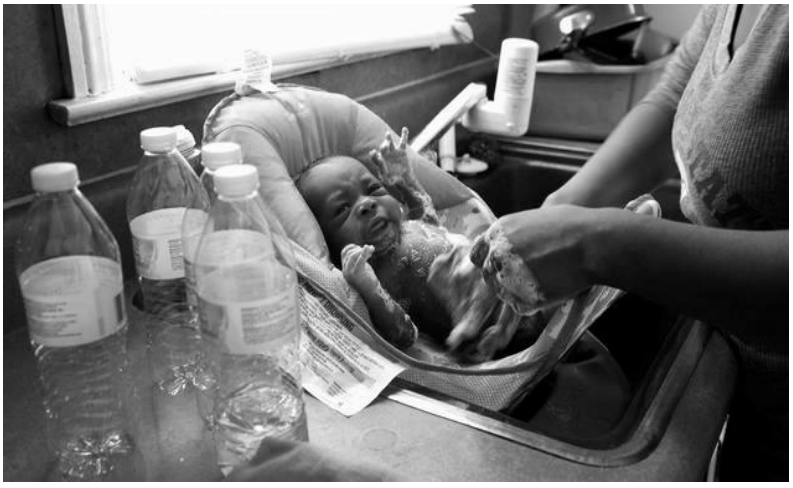
What Are the Effects of Water Pollution?

On human health

To put it bluntly: Water pollution kills. In fact, it caused 1.8 million deaths in 2015, according to a study published in The Lancet. Contaminated water can also make you ill. Every year, unsafe water sickens about 1 billion people. And low-income communities are disproportionately at risk because their homes are often closest to the most polluting industries.

Waterborne pathogens, in the form of disease-causing bacteria and viruses from human and animal waste, are a major cause of illness from contaminated drinking water. Diseases spread by unsafe water include cholera, giardia, and typhoid. Even in wealthy nations, accidental or illegal releases from sewage treatment facilities, as well as runoff from farms and urban areas, contribute harmful pathogens to waterways.

Thousands of people across the United States are sickened every year by Legionnaires' disease (a severe form of pneumonia contracted from water sources like cooling towers and piped water), with cases cropping up from California's Disneyland to Manhattan's Upper East Side.



A woman using bottled water to wash her three-week-old son at their home in Flint, MichiganTodd McInturf/The Detroit News/AP Meanwhile, the plight of residents in Flint, Michigan—where cost-cutting measures and aging water infrastructure created the recent lead contamination crisis—offers a stark look at how dangerous chemical and other industrial pollutants in our water can be. The problem goes far beyond Flint and involves much more than lead, as a wide range of chemical pollutants— from heavy metals such as arsenic and mercury to pesticides and nitrate fertilizers—are getting into our water supplies. Once they're ingested, these toxins can cause a host of health issues, from cancer to hormone disruption to altered brain function. Children and pregnant women are particularly at risk.

Even swimming can pose a risk. Every year, 3.5 million Americans contract health issues such as skin rashes, pinkeye, respiratory infections, and hepatitis from sewage-laden coastal waters, according to EPA estimates.

On the environment

In order to thrive, healthy ecosystems rely on a complex web of animals, plants, bacteria, and fungi—all of which interact, directly or indirectly, with each other. Harm to any of these organisms can create a chain effect, imperiling entire aquatic environments.

When water pollution causes an algal bloom in a lake or marine environment, the proliferation of newly introduced nutrients stimulates plant and algae growth, which in turn reduces oxygen levels in the water. This dearth of oxygen, known as eutrophication, suffocates plants and animals and can create "dead zones," where waters are essentially devoid of life. In

certain cases, these harmful algal blooms can also produce neurotoxins that affect wildlife, from whales to sea turtles.

Chemicals and heavy metals from industrial and municipal wastewater contaminate waterways as well. These contaminants are toxic to aquatic life—most often reducing an organism's life span and ability to reproduce—and make their way up the food chain as predator eats prey. That's how tuna and other big fish accumulate highquantities of toxins, such as mercury. Marine ecosystems are also threatened by marine debris, which can strangle, suffocate, and starve animals. Much of this solid debris, such as plastic bags and soda cans, gets swept into sewers and storm drains and eventually out to sea, turning our oceans into trash soup and sometimes consolidating to form floating garbage patches. Discarded fishing gear and other types of debris are responsible for harming more than 200 different species of marine life.

Meanwhile, ocean acidification is making it tougher for shellfish and coral to survive. Though they absorb about a quarter of the carbon pollution created each year by burning fossil fuels, oceans are becoming more acidic. This process makes it harder for shellfish and other species to build shells and may impact the nervous systems of sharks, clownfish, and other marine life

What Can You Do to Prevent Water Pollution?

With your actions

It's easy to tsk-tsk the oil company with a leaking tanker, but we're all accountable to some degree for today's water pollution problem. Fortunately, there are some simple ways you can prevent water contamination or at least limit your contribution to it:Reduce your plastic consumption and reuse or recycle plastic when you can.

- Properly dispose of chemical cleaners, oils, and non-biodegradable items to keep them from ending up down the drain.
- Maintain your car so it doesn't leak oil, antifreeze, or coolant.
- If you have a yard, consider landscaping that reduces runoff and avoid applying pesticides and herbicides.
- If you have a pup, be sure to pick up its poop.

c) Soil Pollution

Soil contamination Soil contamination or soil pollution as part of land degradation is caused by the presence of xenobiotics (human-made) chemicals or other alteration in the natural soil environment. It is typically caused by industrial activity, agricultural chemicals or improper disposal of waste. The most common chemicals involved are petroleum hydrocarbons, polynuclear aromatic hydrocarbons (such as naphthalene and benzo(a)pyrene), solvents, pesticides, lead, and other heavy metals. Contamination is correlated with the degree of industrialization and intensity of chemical substance. The concern over soil contamination stems primarily from health risks, from direct contact with the contaminated soil, vapours from the contaminants, and from secondary contamination of water supplies within and underlying the soil. Mapping of contaminated soil sites and the resulting cleanups are time consuming and expensive tasks, requiring extensive amounts of geology, hydrology, chemistry, computer modeling skills, and GIS in Environmental Contamination, as well as an appreciation of the history of industrial chemistry. In North America and Western Europe the extent of contaminated land is best known, with many of countries in these areas having a legal framework to identify and deal with this environmental problem. Developing countries tend to be less tightly regulated despite some of them having undergone significant industrialization. Causes Pesticides and herbicides Agents of war Health effects Ecosystem effects Cleanup options By country People's Republic of China European Union United Kingdom Canada India See also References External links Soil pollution can be caused by the following (non-exhaustive list) Excavation showing soil contamination at a disused gasworks in England. Contents Causes Micro plastics Oil spills Mining and activities by other heavy industries Accidental spills may happen during activities, etc. Corrosion of underground storage tanks (including piping used to transmit the contents) Acid rain Intensive farming Agrochemicals, such as pesticides, herbicides and fertilizers Petrochemicals Industrial accidents Road debris Drainage of contaminated surface water into the soil Ammunitions,

chemical agents, and other agents of war Waste disposal Oil and fuel dumping Nuclear wastes Direct discharge of industrial wastes to the soil Discharge of sewage Landfill and illegal dumping Coal ash Electronic waste The most common chemicals involved are petroleum hydrocarbons, solvents, pesticides, lead, and other heavy metals. Any activity that leads to other forms of soil degradation (erosion, compaction, etc.) may indirectly worsen the contamination effects in that soil remediation becomes more tedious. Historical deposition of coal ash used for residential, commercial, and industrial heating, as well as for industrial processes such as ore smelting, were a common source of contamination in areas that were industrialized before about 1960. Coal naturally concentrates lead and zinc during its formation, as well as other heavy metals to a lesser degree. When the coal is burned, most of these metals become concentrated in the ash (the principal exception being mercury). Coal ash and slag may contain sufficient lead to qualify as a "characteristic hazardous waste", defined in the USA as containing more than 5 mg/l of extractable lead using the TCLP procedure. In addition to lead, coal ash typically contains variable but significant concentrations of polynuclear aromatic hydrocarbons (PAHs; e.g., benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(cd)pyrene, phenanthrene, anthracene, and others). These PAHs are known human carcinogens and the acceptable concentrations of them in soil are typically around 1 mg/kg. Coal ash and slag can be recognised by the presence of off-white grains in soil, gray heterogeneous soil, or (coal slag) bubbly, vesicular pebble-sized grains. Treated sewage sludge, known in the industry as biosolids, has become controversial as a "fertilizer". As it is the byproduct of sewage treatment, it generally contains more contaminants such as organisms, pesticides, and heavy metals than other soil. In the European Union, the Urban Waste Water Treatment Directive allows sewage sludge to be sprayed onto land. The volume is expected to double to 185,000 tons of dry solids in 2005. This has good agricultural properties due to the high nitrogen and phosphate content. In 1990/1991, 13% wet weight was sprayed onto 0.13% of the land; however, this is expected to rise 15 fold by 2005. Advocates say there is a need to control this so that pathogenic microorganisms do not get into water courses and to ensure that there is no accumulation of heavy metals in the top soil. Pesticides and herbicides A pesticide is a substance used to kill a pest. A pesticide may be a chemical substance, biological agent (such as a virus or bacteria), antimicrobial, disinfectant or device used against any pest. Pests include insects, plant pathogens, weeds, mollusks, birds, mammals, fish, nematodes (roundworms) and microbes that compete with humans for food, destroy property, spread or are a vector for disease or cause a nuisance. Although there are benefits to the use of

pesticides, there are also drawbacks, such as potential toxicity to humans and other organisms. Herbicides are used to kill weeds, especially on pavements and railways. They are similar to auxins and most are biodegradable by soil bacteria. However, one group derived from trinitrotoluene (2:4 D and 2:4:5 T) have the impurity dioxin, which is very toxic and causes fatality even in low concentrations. Another herbicide is Paraquat. It is highly toxic but it rapidly degrades in soil due to the action of bacteria and does not kill soil fauna. Insecticides are used to rid farms of pests which damage crops. The insects damage not only standing crops but also stored ones and in the tropics it is reckoned that one third of the total production is lost during food storage. As with fungicides, the first insecticides used in the nineteenth century were inorganic e.g. Paris Green and other compounds of arsenic. Nicotine has also been used since the late eighteenth century. There are now two main groups of synthetic insecticides –

1. Organochlorines include DDT, Aldrin, Dieldrin and BHC. They are cheap to produce, potent and persistent. DDT was used on a massive scale from the 1930s, with a peak of 72,000 tonnes used 1970. Then usage fell as the harmful environmental effects were realized. It was found worldwide in fish and birds and was even discovered in the snow in the Antarctic. It is only slightly soluble in water but is very soluble in the bloodstream. It affects the nervous and endocrine systems and causes the eggshells of birds to lack calcium causing them to be easily breakable. It is thought to be responsible for the decline of the numbers of birds of prey like ospreys and peregrine falcons in the 1950s – they are now recovering. As well as increased concentration via the food chain, it is known to enter via permeable membranes, so fish get it through their gills. As it has low water solubility, it tends to stay at the water surface, so organisms that live there are most affected. DDT found in fish that formed part of the human food chain caused concern, but the levels found in the liver, kidney and brain tissues was less than 1 ppm and in fat was 10 ppm, which was below the level likely to cause harm. However, DDT was banned in the UK and the United States to stop the further buildup of it in the food chain. U.S. manufacturers continued to sell DDT to developing countries, who could not afford the expensive replacement chemicals and who did not have such stringent regulations governing the use of pesticides.

2.Organophosphates e.g. parathion, methyl parathion and about 40 other insecticides are available nationally. Parathion is highly toxic, methyl-parathion is less so and Malathion is generally considered safe as it has low toxicity and is rapidly broken down in the mammalian liver. This group works by preventing normal nerve transmission as cholinesterase is prevented from breaking down the transmitter substance acetylcholine, resulting in

uncontrolled muscle movements. The disposal of munitions, and a lack of care in manufacture of munitions caused by the urgency of production, can contaminate soil for extended periods. There is little published evidence on this type of contamination largely because of restrictions placed by governments of many countries on the publication of material related to war effort. However, mustard gas stored during World War II has contaminated some sites for up to 50 years and the testing of Anthrax as a potential biological weapon contaminated the whole island of Gruinard. Contaminated or polluted soil directly affects human health through direct contact with soil or via inhalation of soil contaminants which have vaporized; potentially greater threats are posed by the infiltration of soil contamination into groundwater aquifers used for human consumption, sometimes in areas apparently far removed from any apparent source of above ground Agents of war Health effects contamination. This tends to result in the development of pollution-related diseases. Health consequences from exposure to soil contamination vary greatly depending on pollutant type, pathway of attack and vulnerability of the exposed population. Chronic exposure to chromium, lead and other metals, petroleum, solvents, and many pesticide and herbicide formulations can be carcinogenic, can causecongenital disorders, or can cause other chronic health conditions. Industrial or man-made concentrations of naturally occurring substances, such as nitrate and ammonia associated with livestock manure from agricultural operations, have also been identified as health hazards in soil and groundwater. Chronic exposure to benzene at sufficient concentrations is known to be associated with higher incidence of leukemia. Mercury and cyclodienes are known to induce higher incidences of kidney damage and some irreversible diseases. PCBs and cyclodienes are linked to liver toxicity. Organophosphates and carbonates can induce a chain of responses leading to neuromuscular blockage. Many chlorinated solvents induce liver changes, kidney changes and depression of the central nervous system. There is an entire spectrum of further health effects such as headache, nausea, fatigue, eye irritation and skin rash for the above cited and other chemicals. At sufficient dosages a large number of soil contaminants cancause death by exposure via direct contact, inhalation or ingestion of contaminants in groundwater contaminated through soil. The Scottish Government has commissioned the Institute of Occupational Medicine to undertake a review of methods to assess risk to human health from contaminated land. The overall aim of the project is to work up guidance that should be useful to Scottish Local Authorities in assessing whether sites represent a significant possibility of significant harm (SPOSH) to human health. It is envisaged that the output of the project will be a short document providing high level guidance on health risk assessment with reference to existing published guidance and methodologies that have been identified as being particularly relevant and helpful. The project will examine how policy guidelines have been developed for determining the acceptability of risks to human health and propose an approach for assessing what constitutes unacceptable risk in line with the criteria for SPOSH as defined in the legislation and the Scottish Statutory Guidance. Not unexpectedly, soil contaminants can have significant deleterious consequences for ecosystems. There are radical soil chemistry changes which can arise from the

presence of many hazardous chemicals even at low concentration of the contaminant species. These changes can manifest in the alteration of metabolism of endemic microorganisms and arthropods resident in a given soil environment. The result can be virtual eradication of some of the primary food chain, which in turn could have major consequences for predator or consumer species. Even if the chemical effect on lower life forms is small, the lower pyramid levels of the food chain may ingest alien chemicals, which normally become more concentrated for each consuming rung of the food chain. Many of these effects are now well known, such as the concentration of persistent DDT materials for avian consumers, leading to weakening of egg shells, increased chick mortality and potential extinction of species. Effects occur to agricultural lands which have certain types of soil contamination. Contaminants typically alter plant metabolism, often causing a reduction in crop yields. This has a

secondary effect upon soil conservation, since the languishing crops cannot shield the Earth's soil from erosion. Some of these chemical contaminants have long half-lives and in other cases derivative chemicals are formed from decay of primary soil contaminants. Cleanup or environmental remediation is analyzed by environmental scientists who utilize field measurement of soil chemicals and also apply computer models (GIS in Environmental Contamination) for analyzing transport and fate of soil chemicals. Various technologies have been developed for remediation of oil-contaminated soil and sediments. There are several principal strategies for remediation: Excavate soil and take it to a disposal site away from ready pathways for human or sensitive ecosystem contact. This technique also applies to dredging of bay muds containing toxins. Ecosystem effects Cleanup options Aeration of soils at the contaminated site (with attendant risk of creating air pollution) Thermal remediation by introduction of heat to raise subsurface temperatures sufficiently high to volatize chemical contaminants out of the soil for vapor extraction. Technologies include ISTD, electrical resistance heating (ERH), and ET-DSP. Bioremediation, involving microbial digestion of certain organic chemicals. Techniques used in bioremediation include landfarming, biostimulation and bioaugmentating soil biota with commercially available microflora. Extraction of groundwater or soil vapor with an active electromechanical system, with subsequent stripping of the contaminants from the extract.Containment of the soil contaminants (such as by capping or paving over in place). Phytoremediation, or using plants (such as willow) to extract heavy metals. Myco-remediation, or using fungus to metabolize contaminants and accumulate heavy metals. Remediation of oil contaminated sediments with self-collapsing air microbubbles. Surfactant leaching Various national standards for concentrations of particular contaminants include the United States EPA Region 9 Preliminary Remediation Goals (U.S. PRGs), the U.S. EPA Region 3 Risk Based Concentrations (U.S. EPA RBCs) and National Environment Protection Council of Australia Guideline on Investigation Levels in Soil and Groundwater. The immense and sustained growth of the People's Republic of China since the 1970s has exacted a price from the land in increased soil pollution. The State Environmental Protection Administration believes it to be a threat to the environment, to food safety and to sustainable agriculture. According to a scientific sampling, 150 million mu (100,000 square kilometres) of China’s cultivated land have been polluted, with contaminated water being used to irrigate a further 32.5 million mu (21,670 square kilometres) and another 2 million mu (1,300 square kilometres) covered or destroyed by solid waste. In total, the area accounts for one-tenth of China’s cultivatable land,

and is mostly in economically developed areas. An estimated 12 million tonnes of grain are contaminated by heavy metals every year, causing direct losses of 20 billion yuan ($2.57 billion USD). According to the received data from Member states, in the European Union the number of estimated potential contaminated sites is more than 2.5 million and the identified contaminated sites around 342 thousand. Municipal and industrial wastes contribute most to soil contamination (38%), followed by the industrial/commercial sector (34%). Mineral oil and heavy metals are the main contaminants contributing around 60% to soil contamination. In terms of budget, the management of contaminated sites is estimated to cost around 6 billion Euros (€) annually. Generic guidance commonly used in the United Kingdom are the Soil Guideline Values published by the Department for Environment, Food and Rural Affairs (DEFRA) and the Environment Agency. These are screening values that demonstrate the minimal acceptable level of a substance. Above this there can be no assurances in terms of significant risk of harm to human health. These have been derived using the Contaminated Land Exposure Assessment Model (CLEA UK). Certain input parameters such as Health Criteria Values, age and land use are fed into CLEA UK to obtain a probabilistic output. Guidance by the Inter Departmental Committee for the Redevelopment of Contaminated Land (ICRCL) has been formally withdrawn by DEFRA, for use as a prescriptive document to determine the potential need for remediation or further assessment. By country People's Republic of China European Union United Kingdom the CLEA model published by DEFRA and the Environment Agency (EA) in March 2002 sets a framework for the appropriate assessment of risks to human health from contaminated land, as required by Part IIA of the Environmental Protection Act 1990. As part of this framework, generic Soil Guideline Values (SGVs) have currently been derived for ten contaminants to be used as "intervention values". These values should not be considered as remedial targets but values above which further detailed assessment should be considered; see Dutch standards. Three sets of CLEA SGVs have been produced for three different land uses, namely residential (with and without plant uptake) allotments commercial/industrial It is intended that the SGVs replace the former ICRCL values. The CLEA SGVs relate to assessing chronic (long term) risks to human health and do not apply to the protection of ground workers during construction, or other potential receptors such as groundwater, buildings, plants or other ecosystems. The CLEA SGVs are not directly applicable to a site completely covered in hard standing, as there is no direct exposure route to contaminated soils. To date, the first ten of fifty-five contaminant SGVs have been published, for the following: arsenic, cadmium, chromium, lead, inorganic mercury, nickel, selenium ethyl benzene, phenol and toluene. Draft SGVs for benzene,

naphthalene and xylene have been produced but their publication is on hold. Toxicological data (Tox) has been published for each of these contaminants as well as for benzo[a]pyrene, benzene, dioxins, furans and dioxin-like PCBs, naphthalene, vinyl chloride, 1,1,2,2 tetrachloroethane and 1,1,1,2 tetrachloroethane, 1,1,1 trichloroethane, tetrachloroethene, carbon tetrachloride, 1,2-dichloroethane, trichloroethene and xylene. The SGVs for ethyl benzene, phenol and toluene are dependent on the soil organic matter (SOM) content (which can be calculated from the total organic carbon (TOC) content). As an initial screen the SGVs for 1% SOM are considered to be appropriate. In March 2009, the issue of Uranium poisoning in Punjab attracted press coverage. It was alleged to be caused by fly ash ponds of thermal power stations, which reportedly lead to severe birth defects in children in the Faridkot and Bhatinda districts of Punjab. The news reports claimed the uranium levels were more than 60 times the maximum safe limit. In 2012, the Government of India confirmed that the ground water in Malwa belt of Punjab has uranium metal that is 50% above the trace limits set by the United Nations' World Health Organization (WHO). Scientific studies, based on over 1000 samples from various sampling points, could not trace the source to fly ash and any sources from thermal power plants or industry as originally alleged. The study also revealed that the uranium concentration in ground water of Malwa district is not 60 times the WHO limits, but only 50% above the WHO limit in 3 locations. This highest concentration found in samples was less than those found naturally in ground waters currently used for human purposes elsewhere, such as Finland. Research is underway to identify natural or other sources for the uranium.

Soil Pollution has gradually become a major challenge that we need to overcome for establishing a healthy environment. Weathering of earth’s crusts by different processes leads to the formation of soil that accumulates over the centuries. The soil is the home for a large part of bacterial biodiversity and other microscopic and macroscopic living organisms.

Example of Soil Pollution

However, let us consider our very own country India. Indian economy is largely dependent on agriculture. Thus, we Indians give very high priority to the development of agriculture, fisheries, and livestock. Therefore, for surplus production, it is very important to protect crops from any type of damage that occurs due to insects, weeds, rodents and other crop diseases.

So, how do we protect crops? The very obvious answer is pesticides and herbicides. However, do you know these pesticides and herbicides is a leading cause of soil pollution? Therefore, it is very important to judiciously use pesticides because it contains lots of different harmful chemicals. Therefore, to improve soil and prevent soil pollution it is important to limit the use of pesticides and herbicides.

Causes of Soil Erosion

Definition of Soil Pollution

Soil pollution refers to anything that causes contamination of soil and degrades the soil quality. It occurs when the pollutants causing the pollution reduce the quality of the soil and convert the soil inhabitable for microorganisms and macro organisms living in the soil.

Soil contamination or soil pollution can occur either because of human activities or because of natural processes. However, mostly it is due to human activities. The soil contamination can occur due to the presence of chemicals such as pesticides, herbicides, ammonia, petroleum hydrocarbons, lead, nitrate, mercury, naphthalene, etc in an excess amount.

The primary cause of soil pollution is a lack of awareness in general people. Thus, due to many different human activities such as overuse of pesticides the soil will lose its fertility. Moreover, the presence of excess chemicals will increase the alkalinity or acidity of soil thus degrading the soil quality. This will in turn cause soil erosion. This soil erosion refers to soil pollution.

Causes of Soil Pollution

Soil pollution can be natural or due to human activity. However, it mostly boils down to the activities of the human that causes the majority of soil pollution such as heavy industries, or pesticides in agriculture.

5. Electronic waste - Source, types, components of e-waste, recycling of e waste, impact of e -waste on environment and their management.

Electronic waste

E-waste or electronic waste is created when an electronic product is discarded after the end of its useful life. The rapid expansion of technology means that a very large amount of e-waste is created everyminute.

Electronic waste or e-waste may be defined as discarded computers, office electronic equipment, entertainment device electronics,mobile phones,television sets, and refrigerators. This includes used electronics which are destined for reuse, resale, salvage, recycling, or disposal as well as re-usables (working and repairable electronics) and secondary scraps (copper, steel, plastic, etc.). The term "waste" is reserved for residue or material which is dumped by the buyer rather than recycled, including residue from reuse and recycling operations, because loads of surplus electronics are frequently commingled (good, recyclable, and non-recyclable). Several public policy advocates apply the term "e-waste" and "e-scrap" broadly to all surplus electronics. Cathode ray tubes (CRTs) are considered one of the hardest types to recycle.

CRTs have relatively high concentration of lead and phosphors (not to be confused with phosphorus), both of which are necessary for the display. The United States EnvironmentalProtection Agency (EPA) includes discarded CRT monitors in its category of "hazardous household waste"but considers CRTs that have been set aside for testing to be commodities if they are not discarded, speculatively accumulated, or left unprotected from weather and other damage. These CRT devices are often confused between the DLP Rear Projection TV, both of which have a different recycling process due to the materials they are composedof.

Environmental impact

The processes of dismantling and disposing of electronic waste in developing countries led to a number of environmental impacts as illustrated in the graphic. Liquid and atmospheric releases end up in bodies of water, groundwater, soil, and air and therefore in land and sea animals – both domesticated and wild, in crops eaten by both animals and human, and in drinking water.

One study of environmental effects in Guiyu, China found the following:

- Airborne dioxins – one type found at 100 times levels previouslymeasured
- Levels of carcinogens in duck ponds and rice paddies exceeded international standards for agricultural areas and cadmium, copper, nickel, and lead levels in rice paddies were above internationalstandards
- Heavy metals found in road dust – lead over 300 times that of a control village's road dust and copper over 100 times

The environmental impact of the processing of different electronic waste components

E-Waste Component	Process Used	Potential Environmental Hazard
Cathode ray tubes (used in TVs, computer monitors, ATM, video cameras, and more)	Breaking and removal of yoke, then dumping	Lead, barium and other heavy metals leaching into the ground water and release of toxic phosphorus
Printed circuit board (image behind table – a thin plate on whichchips and other electronic components	De-soldering and removal of computer chips; open burning and acid baths to remove metals after chips are	Air emissions and discharge into rivers of glass dust, tin, lead, brominated dioxin, beryllium cadmium, and mercury
Chips and other gold plated components	Chemical stripping using nitric and hydrochloric acid and burning ofchips	PAHs, heavy metals, brominated flameretardants discharged directly into rivers acidifying fish and flora. Tin and lead contamination of surface and groundwater. Air emissions of brominated dioxins, heavy metals, and PAHs
Plastics from printers, keyboards, monitors, etc.	Shredding and low temp melting to be reused	Emissions of brominated dioxins, heavy metals, and hydrocarbons
Computer wires	Open burning and stripping to remove copper	PAHs released into air, water, and soil.

Information security

E-waste presents a potential security threat to individuals and exporting countries. Harddrives that are not properly erased before the computer is disposed of can be reopened, exposing sensitive information. Credit card numbers, private financial data, account information, and records of online transactions can be accessed by most willing individuals. Organized criminals in Ghana commonly search the drives for information to use in local scams. Electronic files about government contracts have been discovered on hard drives found in Agbogbloshie. Multimillion-dollar agreements fromUnitedStates security institutions such as the Defense Intelligence Agency (DIA), the Transportation Security Administration, and Homeland Security have all resurfaced in Agbogbloshie.

E-waste management Recycling

Computer monitors are typically packed into low stacks on wooden pallets for recycling and then shrink-wrapped.

E-waste can be managed by properly disposing and managing your electronic waste .We should give our waste to the e waste dealers and should follow the precautions as they contain many heavy metals.

Recycling

One of the major challenges is recycling the printed circuit boards from the electronic wastes. The circuit boards contain such precious metals as gold, silver, platinum, etc. and such base metals as copper, iron, aluminum, etc. One way e-waste is processed is by melting circuit boards, burning cable sheathing to recover copper wire and open- pit acid leaching for separating metals of value.

Consumer awareness efforts

A campaign to promote e-waste recycling in Ghana.

The U.S. Environmental Protection Agency encourages electronic recyclers to become certified by demonstrating to an accredited, independent third party auditor that they meet specific standards to safely recycle and manage electronics. This should work so as to ensure the highest environmental standards are being maintained. Two certifications for electronic recyclers currently exist and are endorsed by the EPA. Customers are encouraged to choose certified electronics recyclers. Responsible electronics recycling reduces environmental and human health impacts, increases the use of reusable and refurbished equipment and reduces

Energy use while conserving limited resources. The two EPA-endorsed certification programs are Responsible Recyclers Practices (R2) and E-Stewards. Certified companies ensure they are meeting strict environmental standards which maximize reuse and recycling, minimize exposure to human health or the environment, ensure safe management of materials and require destruction of all data used on electronics.[60] Certified electronics recyclers have demonstrated through audits and other means that they continually meet specific high environmental standards and safely manage used electronics. Once certified, the recycler is held to the particular standard by continual oversight by the independent accredited certifying body. A certification board accredits and oversees certifying bodies to ensure that they meet specific responsibilities and are

competent to audit and provide certification.

Benefits of recycling

Recycling raw materials from end-of-life electronics is the most effective solution to the growing e-waste problem. Most electronic devices contain a variety of materials, including metals that can be recovered for future uses. By dismantling and providing reuse possibilities, intact natural resources are conserved and air and water pollution caused by hazardous disposal is avoided. Additionally, recycling reduces the amount of greenhouse gas emissions caused by the manufacturing of new products.Another benefit of recycling e-waste is that many of the materials can be recycled and re-used again. Materials that can be recycled include "ferrous (iron-based) and non-ferrous metals, glass, and various types of plastic." “Non-ferrous metals, mainly aluminum and copper can all be re-smelted and re- manufactured. Ferrous metals such as steel and iron can be also be re-used." Due to the recent surge in popularity in 3D printing, certain 3D printers have been designed (FDM variety) to produce waste that can be easily recycled which decreases the amount of harmful pollutants in the atmosphere. The excess plastic from these printers that comes out as a byproduct can also be reused to create new 3D printed creations.

6. Ecotoxicology and Biomonitoring - Principles and Mechanisms

Environmental toxicology

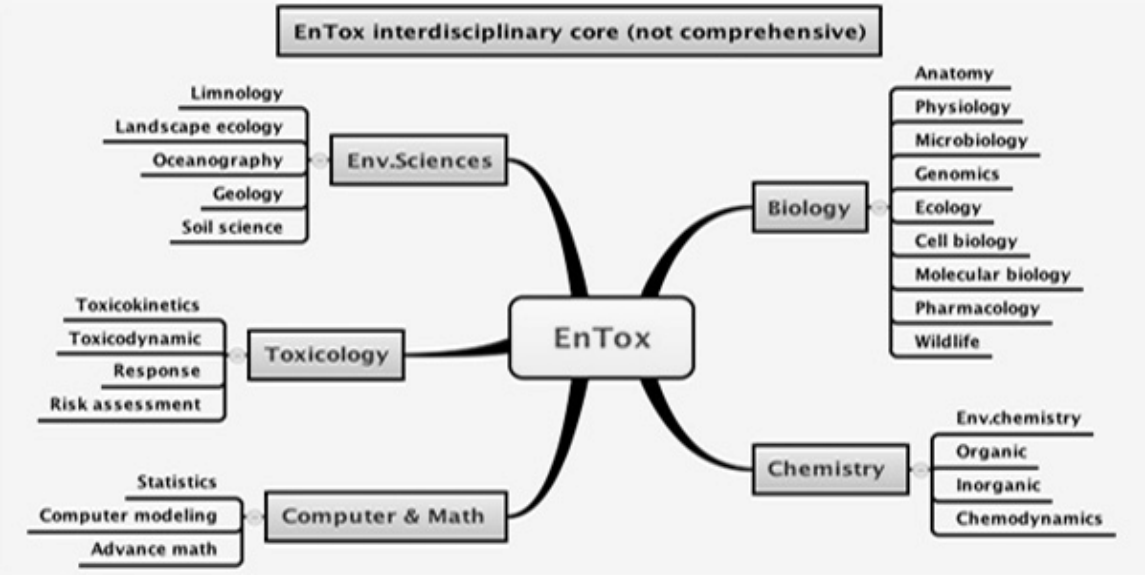
Environmental toxicology is a multidisciplinary field of science concerned with the study of the harmful effects of various chemical, biological and physical agents on living organisms. Ecotoxicology is a subdiscipline of environmental toxicology concerned with studying the harmful effects of toxicants at the population and ecosystemlevels.

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.

Harmful effects of such chemical and biological agents as toxicants from pollutants, insecticides, pesticides, and fertilizers can affect an organism anditscommunity by reducing its species diversity and abundance. Such changes in population dynamics affect the ecosystem by reducing its productivity and stability.

Although legislation implemented since the early 1970s had intended to minimize harmful effects of environmental toxicants upon all species, McCarty (2013) has warned that "longstanding limitations in the implementation of the simple conceptual model that is the basis of current aquatic toxicity testing protocols" may lead to an impending environmental toxicology "dark age"



Overview of the interdisciplinarity of environmental toxicology

Biomonitoring:

In analytical chemistry, biomonitoring is the measurement of the body burden of toxic chemical compounds, elements, or their metabolites, in biological substances. Often, these measurements are done in blood and urine. Biomonitoring is performed in both environmental health, and in occupational safety and health as a means of exposure assessment and workplace health surveillance.

The two best established environmental biomonitoring programs in representative samples of the general population are those of the United States and Germany, although population-based programs exist in a few other countries. In 2001, the U.S. Centers for Disease Control and Prevention (CDC) began to publish its biennial National Report on Human Exposure to Environmental Chemicals, which reports a statistically representative sample of the U.S. population.

Biomonitoring involves the use of organisms to assess environmental contamination, such as of surrounding air or water. It can be done qualitatively by observing and noting changes in organisms, or quantitatively by measuring accumulation of chemicals in organism tissues. By observing or measuring the effects the environment has on its resident organisms, pollution may be suspected or inferred.

Historically, public health regulations [where?] have been based on theoretical risk calculations according to known levels of chemical substances in air, water, soil, food, other consumer products and other sources of potential exposure.[citation needed] Human biomonitoring offers the opportunity to analyze the actual internal levels of bodily substances from all potential routes

of exposure at one time, which may contribute to improving risk assessments. [better source needed]

Scientific advancements have made it possible to detect a greater number of chemical substances in smaller concentrations in the body, with some chemicals detectable at levels as low as parts per trillion.[better source needed] A single biomonitoring measurement is only one snapshot in time and may not accurately reflect the level of exposure over longer periods. The presence of an environmental chemical in the body does not necessarily indicate harm. The analytical chemistry of detecting chemicals has advanced more rapidly than the ability to interpret the potential health consequences. Health risks are usually established from toxicity studies in laboratory animals and epidemiological evidence in humans. Lead is a well studied chemical with a CDC action level of concern, currently at 10 µg/dL, or 100 parts per billion, in blood; however, neurobehavioral impairment has been noted below this level.[12] Because this approach requires establishment of cause and effect in epidemiological studies and a thorough understanding of human dose response, data to support these types of action levels exist for only a few environmental chemicals. The concept of Biomonitoring Equivalents (BEs) has been developed as an alternative approach to aid in interpreting and communicating biomonitoring results in the context of potential risks to health.

There are different types of biomarkers that indicate exposure, effect, or susceptibility.

Methodology

Chemicals and their metabolites can be detected in a variety of biological substances such as blood, urine, exhaled air, hair, nails, feces, semen, breast milk, or saliva. Blood and urine are the most commonly used in occupational safety and health. Breast milk is a favored matrix (substance) to measure lipophilic (fat-loving) persistent, bioaccumulative, and toxic (PBT) compounds during lactation; this exposure route is dominant for breastfeeding children. A lipophilic compound might also be detected in blood, while a hydrophilic (water-loving) compound might be detected in urine.

Analytical methods used by the CDC include isotope dilution mass spectrometry, inductively coupled plasma mass spectrometry, or graphite furnace atomic absorption spectrometry. Others include gas chromatography or high-performance liquid chromatography coupled with various detectors such as ultraviolet, electron capture, flame ionization, atomic emission, or mass spectrometric detectors. Ligand-binding assays and immunoassays are also used. As biomonitoring necessarily involves working with human subjects and specimens, biosafety procedures are necessary to prevent the transmission of pathogens.

Biomonitoring equivalents

Scientists performing biomonitoring testing are able to detect and measure concentrations of natural and manmade chemicals in human blood and urine samples at parts-per-billion to parts-per-quadrillion levels. A 2006 U.S. National Research Council report found that while scientists were capable of detecting the chemicals at these levels, methods for interpreting and communicating what their presence meant regarding potential health risks to an individual or population were still lacking. The report recommended that scientific research be done to improve the interpretation and communication of biomonitoring results through the use of existing risk assessments of specific chemicals.

To address this situation, several groups recognized that exposure guidance values, such as reference dose and tolerable daily intake, could, with sufficient data, be translated into corresponding estimates of biomarker concentrations for use in the interpretation of biomonitoring data. In 2007, the initial methodology for the systematic translation of exposure guidance values into corresponding screening values for biomonitoring data, dubbed Biomonitoring Equivalents, was published by scientists from Summit Toxicology. Subsequently, an expert panel from government, industry and academia, convened to develop detailed guidelines for deriving and communicating these Biomonitoring Equivalents.

Biomonitoring Equivalents can be used for evaluation of biomonitoring data in a risk assessment context. Comparing biomonitoring data for a chemical with its Biomonitoring Equivalent provides a means for assessing whether population exposures to chemicals are within or above the levels considered safe by regulatory agencies. Biomonitoring Equivalents can thus assist scientists and risk managers in the prioritization of chemicals for follow-up or risk management activities.

Since 2007, scientists have derived and published Biomonitoring Equivalents for more than 110 chemicals, including cadmium, benzene, chloroform, arsenic, toluene, methylene chloride, triclosan, dioxins, volatile organic compounds, and others. Several have been developed through collaborations of scientists from the U.S. Environmental Protection Agency, CDC and Health Canada. Researchers from the German Human Biomonitoring Commission have also proposed a concept for deriving screening values similar to Biomonitoring Equivalents.

Communication

The National Research Council's 2006 report emphasized that accurate communication of results is essential for the proper use of biomonitoring surveys, but at the same time noted "there is no accepted standard for good biomonitoring communications." In 2007, the Boston University School of Public Health organized a panel on this topic. An expert panel on Biomonitoring

Equivalents has published guidelines for communicating information to the general public and health care providers.

Charles McKay of the Connecticut Poison Control Center is interviewed in a video titled "A Medical Doctor's Perspective on Biomonitoring", which is focused on helping the general public better understand biomonitoring.

Biomonitoring in environmental health

In 2006 the U.S. National Research Council published a report, Human Biomonitoring for Environmental Chemicals. The report recognized the value of biomonitoring for better understanding exposure to environmental chemicals, and included several findings and recommendations to improve the utility of biomonitoring data for health risk assessment. In summary, the report called for more rigorous health-based criteria for selecting chemicals to include in biomonitoring studies; the development of tools and techniques to improve risk-based interpretation and communication of biomonitoring data; integration of biomonitoring into exposure assessment and epidemiological research; and exploration of bioethical issues around biomonitoring, including informed consent, confidentiality of results, and others.

The issue of exposure to environmental chemicals has received attention as a result of televised reports by Bill Moyers for PBS and Anderson Cooper for CNN's "Planet in Peril" series. The book Our Stolen Future, with a foreword by former Vice President Al Gore, also raised awareness by focusing on endocrine disruption.

Surveys of human exposure to chemicals do not usually integrate the number of chemical compounds detected per person and the concentration of each compound. This leaves untested relevant exposure situations; e.g., whether individuals with low concentrations of some compounds have high concentrations of the other compounds. Analyses of the concentrations of a given compound usually show that most citizens have much lower concentrations than a certain minority. A study based on a representative sample of the population of Catalonia (Spain), which integrated the number of compounds detected per person and the concentration of each compound, found that more than half of the population had concentrations in the top quartile of 1 or more of the 19 persistent toxic substances (PTS) (pesticides, PCBs) analyzed. Significant subgroups of the population accumulated PTS mixtures at high concentrations. For instance, 48% of women 60–74 years had concentrations of 6 or more PTS in the top quartile; half of the entire population had levels of 1 to 5 PTS above 500 ng/g, and less than 4% of citizens had all PTS in the lowest quartile. Thus, PTS concentrations appear low in most of the population only when each individual compound is looked at separately. It is not accurate to state that most of the population has low concentrations of PTS. The assessment of mixture effects must address the

fact that most individuals are contaminated by PTS mixtures made of compounds at both low and high concentrations.

Surveys by country

United States

In the United States, the CDC first tested samples from the general population for lead and a few pesticides in 1976. In the late 1990s, the National Health and Nutrition Examination Survey (NHANES) program had a major expansion.

National Report on Human Exposure to Environmental Chemicals

The CDC's Division of Laboratory Sciences within the National Center for Environmental Health has developed a National Biomonitoring Program, and has published the biennial National Report on Human Exposure to Environmental Chemicals since 2001. As the selection of chemicals is controversial, the CDC has identified influential criteria:Evidence of exposure in a U.S. population, presence and significance of health effects after a given level of exposure, desire to track public health initiatives to reduce exposure to a given agent, existing method for accurately measuring biologically relevant concentrations of the chemical, sufficient tissue specimens, in particular, blood and/or urine samples and cost-effectiveness.

CDC established three criteria for removing chemicals from future surveys: a new replacement chemical (i.e., a metabolite or other chemical) is more representative of exposure than the chemical currently measured, or if after three survey periods, detection rates for all chemicals within a method-related group are less than 5 percent for all population subgroups (i.e., two sexes, three race/ethnicity groups, and the age groups used in the National Report), or if after three survey periods, levels of chemicals within a method-related group are unchanged or declining in all demographic subgroups documented in the National Report.

The National Children's Study plans to follow 100,000 children across the United States from birth until age 21. The study was authorized as part of the Children's Health Act of 2000 as the largest effort undertaken to address the effects of social, economic and environmental factors on a child's health. the CDC's Environmental Health Laboratory announced in 2009 it would play a key role in the biomonitoring of the ongoing National Children's Study. In collaboration with the National Institute of Child Health and Development, National Institute of Environmental Health Sciences and U.S. Environmental Protection Agency.

Some U.S. states have received federal support and established biomonitoring programs. In 2001, the CDC awarded planning grants to 33 states to assist in capacity building for expanding biomonitoring.

The California Environmental Contaminant Biomonitoring Program (CECBP) was established by

law in 2006 and is administered by the California Department of Public Health.

Minnesota's Biomonitoring Pilot Program was established by law in 2007 and is run by the Minnesota Department of Health.

Germany

The German Environmental Survey (GerES) has been performed since 1985, and in 1992 the Human Biomonitoring Commission of the German Federal Environment Agency was established.

Canada

Statistics Canada administers the Canadian Health Measures Survey, which includes biomonitoring for environmental chemicals. Health Canada administers a program called Mother-Infant Research on Environmental Chemicals, which focuses on 2,000 pregnant women and their infants.

Occupational biomonitoring

In occupational safety and health, biomonitoring may be done for reasons of regulatory compliance, workplace health surveillance and research, confirming effectiveness of hazard controls, or as a component of occupational risk assessment. It can also be used to reconstruct exposures following acute or accidental events, and to assess the effectiveness of personal protective equipment. It is useful for dermal exposures, for which sampling methods are often not readily available, and for finding unexpected exposures or routes. There are also biomarkers not just for chemical hazards, but also other types such as noise and stress. Occupational health differs from environmental health in that the former has smaller number of exposed individuals, but with a wider range of exposure levels.

Biomonitoring is complementary to exposure monitoring in that it measures the internal dose of a toxicant within the body rather than its concentration outside the body, with the advantage that it confirms whether not only exposure but uptake has actually occurred. It also takes into account differences in metabolism, physical exertion, and mixtures of toxicants between individuals that affect the internal dose. It can be done in an individual or collective manner.

A major use of occupational toxicology data is for determining what biomarkers (including both the a toxicant and its metabolites) may be used for biomonitoring, and establishing biological exposure indices. These are used during exposure assessment and workplace health surveillance activities to identify overexposure, and to test the validity of occupational exposure limits. These biomarkers are intended to aid in prevention by identifying early adverse affects, unlike diagnostics for clinical medicine that are designed to reveal advanced pathologic states.

In the US, the Occupational Safety and Health Administration as of 2017 has three regulations

that require biomonitoring: after exposure to benzene in an unplanned release, and for employees exposed to cadmium or lead at or above a specified level over a specified amount of time. In the European Union, biological limit values are health-based, while biological guidance values are statistically derived and indicate background exposures in the general population. As of 2020 lead is the only substance that has a binding biological limit value in the EU.Voluntary lists of biological exposure limits or action levels are maintained by the American Conference of Governmental Industrial Hygienists, German Research Foundation, UK Health and Safety Executive, France's ANSES, and the Swiss Accident Insurance Fund. Biomonitoring for research purposes is performed by the U.S. National Institute for Occupational Safety and Health as part of its Adult Epidemiology and Surveillance program, as well as other occupational health studies.

7. Environmental Laws and Policies

Environmental laws around the world International law

Global and regional environmental issues are increasingly the subject of international law. Debates over environmental concerns implicate core principles of international law and have been the subject of numerous international agreements and declarations.

Customary international law is an important source of international environmental law. These are the norms and rules that countries follow as a matter of custom and they are so prevalent that they bind all states in the world. When a principle becomes customary law is not clear cut and many arguments are put forward by states not wishing to be bound. Examples of customary international law relevant to the environment include the duty to warn other states promptly about icons of an environmental nature and environmental damages to which another state or states may be exposed, and Principle 21 of the Stockholm Declaration.

Numerous legally binding international agreements encompass a wide variety of issue-areas, from terrestrial, marine and atmospheric pollution through to wildlife and biodiversity protection. International environmental agreements are generally multilateral (or sometimes bilateral) treaties (a.k.a. convention, agreement, protocol, etc.). Protocols are subsidiary agreements built from a primary treaty. They exist in many areas of international law but are especially useful in the environmental field, where they may be used to regularly incorporate recent scientific knowledge. They also permit countries to reach agreement on a framework that would be contentious if every detail were to be agreed upon in advance. The most widely known protocol in international environmental law is the Kyoto Protocol, which followed from the United Nations Framework Convention on Climate Change.

While the bodies that proposed, argued, agreed upon and ultimately adopted existing international agreements vary according to each agreement, certain conferences, including 1972's United Nations Conference on the Human Environment, 1983's World Commission on Environment and Development, 1992's United Nations Conference on Environment and Development and 2002's World Summit on Sustainable Development have been particularly important. Multilateral environmental agreements sometimes create an International Organization, Institution or Body responsible for implementing the agreement. Major examples are the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the International Union for Conservation of Nature (IUCN).

International environmental law also includes the opinions of international courts and tribunals. While there are few and they have limited authority, the decisions carry much weight with legal commentators and are quite influential on the development of international environmental law. One of the biggest challenges in international decisions is to determine an adequate compensation for environmental damages. The courts include the International Court of Justice (ICJ), the international Tribunal for the Law of the Sea (ITLOS), the European Court of Justice, European Court of Human Rights and other regional treaty tribunals.

Asia

The Asian Environmental Compliance and Enforcement Network (AECEN) is an agreement between 16 Asian countries dedicated to improving cooperation with environmental laws in Asia. These countries include Cambodia, China, Indonesia, India, Maldives, Japan, Korea, Malaysia, Nepal, Philippines, Pakistan, Singapore, Sri Lanka, Thailand, Vietnam, and Lao PDR.

European Union

The European Union issues secondary legislation on environmental issues that are valid throughout the EU (so called regulations) and many directives that must be implemented into national legislation from the 28 member states (national states). Examples are the Regulation (EC) No. 338/97 on the implementation of CITES; or the Natura 2000 network the centerpiece for nature & biodiversity policy, encompassing the bird Directive (79/409/EEC/ changed to 2009/147/EC)and the habitats directive (92/43/EEC). Which are made up of multiple SACs (Special Areas of Conservation, linked to the habitats directive) & SPAs (Special Protected Areas, linked to the bird directive), throughout Europe.

EU legislation is ruled in Article 249 Treaty for the Functioning of the European Union

(TFEU). Topics for common EU legislation are:

- Climatechange
- Air pollution
- Water protection andmanagement
- Wastemanagement
- Soilprotection
- Protection of nature, species andbiodiversity
- Noisepollution
- Cooperation for the environment with third countries (other than EU memberstates)
- Civilprotection

Middle East

The U.S. Environmental Protection Agency is working with countries in the Middle East to improve "environmental governance, water pollution and water security, clean fuels and vehicles, public participation, and pollution prevention."

Australia

The Environment Protection and Biodiversity Conservation Act 1999 is the center piece of environmental legislation in the Australian Government. It sets up the "legal framework to protect and manage nationally and internationally important flora, fauna, ecological communities and heritage places". It also focuses on protecting world heritage properties, national heritage properties, wetlands of international importance, nationally threatened species and ecological communities, migratory species, Commonwealth marine areas, GreatBarrier Reef Marine Park, and the environment surrounding nuclear activities.Commonwealth v Tasmania (1983), also known as the "Tasmanian Dam Case", is the most influential case for Australian environmental law.

Brazil

The Brazilian government created the Ministry of Environment in 1992 in order to develop better strategies of protecting the environment, use natural resources sustainably, and enforce public environmental policies. The Ministry of Environment has authority over policies involving environment, water resources, preservation, and environmental programs involving the Amazon.

China

Ministry of Environmental Protection of the People's Republic of China

According to the U.S. Environmental Protection Agency, "China has been working with great determination in recent years to develop, implement, and enforce a solid environmental law framework. Chinese officials face critical challenges in effectively implementing the laws, clarifying the roles of their national and provincial governments, and strengthening the operation of their legal system." Explosive economic and industrial growth in China has led to significant environmental degradation, and China is currently in the process of developing more stringent legal controls. The harmonization of Chinese society and the natural environment is billed as a rising policypriority.

India:

Indian environmental law

In India, Environmental law is governed by the Environment Protection Act, 1986. This act is enforced by the Central Pollution Control Board and the numerous State Pollution Control Boards. Apart from this, there are also individual legislations specifically enacted for the protection of Water, Air, Wildlife, etc. Such legislations include :-

- The Water (Prevention and Control of Pollution) Act,1974
- The Water (Prevention and Control of Pollution) Cess Act,1977
- The Forest (Conservation) Act,1980
- The Air (Prevention and Control of Pollution) Act,1981
- Air (Prevention and Control of Pollution) (Union Territories) Rules,1983
- The Biological Diversity Act, 2002 and the Wild Life Protection Act,1972
- Batteries (Management and Handling) Rules,2001
- Recycled Plastics, Plastics Manufacture and Usage Rules,1999
- The National Green Tribunal established under the National Green Tribunal Act of

2010has jurisdiction over all environmental cases dealing with a substantial environmental question and acts covered under the Water (Prevention and Control of Pollution) Act,1974.

- Water (Prevention and Control of Pollution) Cess Rules,1978
- Ganga Action Plan,1986
- The Forest (Conservation) Act,1980
- The Public Liability Insurance Act, 1991 and the Biological Diversity Act, 2002. The acts covered under Indian Wild Life Protection Act 1972 do not fall within the jurisdiction of the National Green Tribunal. Appeals can be filed in the Hon'ble Supreme Court ofIndia.
- Basel Convention on Control of TransboundaryMovements on Hazardous Wastes and Their Disposal, 1989 and ItsProtocols
- Hazardous Wastes (Management and Handling) Amendment Rules,2003

Japan

The Basic Environmental Law is the basic structure of Japan's environmental policies replacing the Basic Law for Environmental Pollution Control and the Nature ConservationLaw. The updated law aims to address "global environmental problems, urban pollution by everyday life, loss of accessible natural environment in urban areas and degrading environmental protection capacity in forests and farmlands."

Russia

The Ministry of Natural Resources and Environment of the Russian Federation makes regulation regarding "conservation of natural resources, including the subsoil, water bodies, forests located in designated conservation areas, fauna and their habitat, in the fieldof hunting, hydrometeorology and related areas, environmental monitoring and pollution control, including radiation monitoring and control, and functions of public environmental policy making and implementation and statutory regulation."

Environmental law has developed in response to emerging awareness of and concern over issues impacting the entire world. While laws have developed piecemeal and for a variety of reasons, some effort has gone into identifying key concepts and guiding principles common to environmental law as a whole.[15] The principles discussed below are not an exhaustive list and are not universally recognized or accepted. Nonetheless, they represent important principles for the understanding of environmental law around the world.

Principles

Sustainable development

Main article: Sustainable development

Defined by the United Nations Environment Programme as "development that meets the needs of the present without compromising the ability of future generations to meet their own needs," sustainable development may be considered together with the concepts of "integration" (development cannot be considered in isolation from sustainability) and "interdependence" (social and economic development, and environmental protection, are interdependent). Laws mandating environmental impact assessment and requiring or encouraging development to minimize environmental impacts may be assessed against this principle.

The modern concept of sustainable development was a topic of discussion at the 1972 United Nations Conference on the Human Environment (Stockholm Conference), and the driving force behind the 1983 World Commission on Environment and Development (WCED, or

Bruntland Commission). In 1992, the first UN Earth Summit resulted in the Rio Declaration, Principle 3 of which reads: "The right to development must be fulfilled so as to equitably meet developmental and environmental needs of present and future generations." Sustainable development has been a core concept of international environmental discussion ever since, including at the World Summit on Sustainable Development (Earth Summit 2002), and the United Nations Conference on Sustainable Development (Earth Summit 2012, or Rio+20).

Equity

Further information: Intergenerational equity

Defined by UNEP to include intergenerational equity - "the right of future generations to enjoy a fair level of the common patrimony" - and intragenerational equity - "the right of all people within the current generation to fair access to the current generation's entitlement to the Earth's natural resources" - environmental equity considers the present generation under an obligation to account for long-term impacts of activities, and to act to sustain the global environment and resource base for future generations. Pollution control and resource management laws may be assessed against this principle.

Trans boundary responsibility

Defined in the international law context as an obligation to protect one's own environment, and to prevent damage to neighboring environments, UNEP considers transboundary responsibility at the international level as a potential limitation on the rights of the sovereign state. Laws that act to limit externalities imposed upon human health and the environment may be assessed against this principle.

Public participation and transparency

Identified as essential conditions for "accountable governments,... industrial concerns," and organizations generally, public participation and transparency are presented by UNEP as requiring "effective protection of the human right to hold and express opinions and to seek, receive and impart ideas,... a right of access to appropriate, comprehensible and timely information held by governments and industrial concerns on economic and social policies regarding the sustainable use of natural resources and the protection of the environment, without imposing undue financial burdens upon the applicants and with adequate protection of privacy and business confidentiality," and "effective judicial and administrative proceedings." These principles are present in environmental impact assessment, laws requiring publication and access to relevant environmental data, and administrative procedure.

Precautionary principle

Main article: Precautionary principle

One of the most commonly encountered and controversial principles of environmental law, the Rio Declaration formulated the precautionary principle as follows:

In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.

The principle may play a role in any debate over the need for environmental regulation.

Prevention

The concept of prevention . . . can perhaps better be considered an overarching aim that gives rise to a multitude of legal mechanisms, including prior assessment of environmental harm, licensing or authorization that set out the conditions for operation and the consequences for violation of the conditions, as well as the adoption of strategies and policies. Emission limits and other product or process standards, the use of best available techniques and similar techniques can all be seen as applications of the concept of prevention.

Polluter pays principle

Main article: Polluter pays principle

The polluter pays principle stands for the idea that "the environmental costs of economic activities, including the cost of preventing potential harm, should be internalized rather than imposed upon society at large." All issues related to responsibility for cost for environmental remediation and compliance with pollution control regulations involve this principle.

International environmental law:

Global and regional environmental issues are increasingly the subject of international law. Debates over environmental concerns implicate core principles of international law and have been the subject of numerous international agreements and declarations.

Customary international law is an important source of international environmental law. These are the norms and rules that countries follow as a matter of custom and they are so prevalent that they bind all states in the world. When a principle becomes customary law is not clear cut and many arguments are put forward by states not wishing to be bound. Examples of customary international law relevant to the environment include the duty to warn other states promptly about icons of an environmental nature and environmental damages to which another state or states may be exposed, and Principle 21 of the Stockholm Declaration ('good neighbourliness' or sic utere).

Given that customary international law is not static but ever evolving and the continued increase of air pollution (Carbon Dioxide) causing climate changes, has led to discussions on whether basic customary principles of international law, such as the jus cogens (peremptory norms) and erga omnes principles could be applicable for enforcing international environmental law.

Numerous legally binding international agreements encompass a wide variety of issue-areas, from terrestrial, marine and atmospheric pollution through to wildlife and biodiversity protection. International environmental agreements are generally multilateral (or sometimes bilateral) treaties (a.k.a. convention, agreement, protocol, etc.). Protocols are subsidiary agreements built from a primary treaty. They exist in many areas of international law but are especially useful in the environmental field, where they may be used to regularly incorporate recent scientific knowledge. They also permit countries to reach agreement on a framework that would be contentious if every detail were to be agreed upon in advance. The most widely known protocol in international environmental law is the Kyoto Protocol, which followed from the United Nations Framework Convention on Climate Change.

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The Biological Diversity Act, 2002 and the Wild Life Protection Act, 1972

Batteries (Management and Handling) Rules, 2001

Recycled Plastics, Plastics Manufacture and Usage Rules, 1999

The National Green Tribunal established under the National Green Tribunal Act of 2010[60] has jurisdiction over all environmental cases dealing with a substantial environmental question and acts covered under the Water (Prevention and Control of Pollution) Act, 1974.

Water (Prevention and Control of Pollution) Cess Rules, 1978

Ganga Action Plan, 1986

The Forest (Conservation) Act, 1980

Wildlife protection Act, 1972

The Public Liability Insurance Act, 1991 and the Biological Diversity Act, 2002. The acts covered under Indian Wild Life Protection Act 1972 do not fall within the jurisdiction of the National Green Tribunal.[61] Appeals can be filed in the Hon'ble Supreme Court of India.[62]

Basel Convention on Control of TransboundaryMovements on Hazardous Wastes and Their Disposal, 1989 and Its Protocols

Hazardous Wastes (Management and Handling) Amendment Rules, 2003.

8. SuggestedReadings

1. Jones Allan M. Environmental Biology (1997) by Routledge New Fetter Lane, London EC4P 4EE2.
2. Shukla, R.S. & Chandel, P.S. Plant Ecology, Latest Ed., S. Chandel andCo.
3. Dhaliwal, G. S., Sngha, G. S. and Ralhan, P. K. 1998. Fundamentals of Environmental Science. KalyaniPublishers.
4. Kaushik, A. and Kaushik, C. P. 2014. Perspective in environmental studies. New Age International Ltd. Publishers, NewDelhi.
5. https://www.wikipedia.org/
6. http://www.biologydiscussion.com/
7. Ecology Global insights and Investigation by Peter Stiling
8. Douglas, J. Futuyma (1998). Evolutionary Biology, (3rd Edition). Sinauer Associates.
9. Eldon, D., Enger, Bradley, Smith, F. (1995). Environmental Science. W C Brown Publications.
10. Grant, W. E. and Swannack, T. M. (2008). Ecological Modelling. Blackwell.

9. Assignment

1. Define ecosystem and ecology.
2. What are food chain and food web?
3. Describe carbon cycle with diagram.
4. Describe nitrogen cycle with diagram.
5. Discuss different laws for the protection of environment.
6. Define water pollution. State its cause and effects.
7. Define air pollution. State its cause and effects
8. Discuss the laws and policies in environmental pollution
9. Montreal protocol
10. Xenobiotics

All the materials are self written and collected from ebook, journals and websites.

***** ***** *****

POST GRADUATE DEGREE PROGRAMME (CBCS)

IN

BOTANY

SEMESTER - I

Course: BOTCOR T101

(Microbiology & Immunology)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

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Kalyani, Nadia

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SYLLABUS

COURSE– BOTCOR T101

Microbiology & Immunology

(Full Marks–75)

Course	Group	Details Contents Structure		Study hour
BOTCOR T101	Microbiology	Unit 1. History and Development of Microbiology:	Contributions of Leeuwenhoek, Koch, Pasteur, Jenner and Fleming.	1
		Unit 2. Bacterial Systematics:	Three kingdom concept of Haeckel, five kingdom concept of Whittaker and three domain classification of Woese; Characters used in bacteriology; Classification- phenetic and phylogenetic; Major groups of microorganisms.	1
		Unit 3. Thermodynamic Principles in Microbiology:	Concept of free energy, Entropy, Enthalpy, Energy rich bonds, Chemical potential, Membrane potential, Diffusion potential.	1
		Unit 4. Bacterial Morphology:	Structure, chemistry and function of capsule, pili, flagella, cell wall, cell membrane,	1
		Unit 5. Bacterial Morphology:	Ribosome, chromosome and plasmid, reserve materials and cytoplasmic inclusions; endospore (structure, formation, germination).	1
		Unit 6. History of Development of Virology:	nature, classification and nomenclature of viruses; structural organization and chemistry of viruses; assay of viruses, chemical and physical determination, assays of infectivity; Virus diseases in plants,	1

			symptoms of diseases, general transmission of viruses; Bacteriophages- isolation and demonstration, structure of adenoviruses, tobacco mosaic viruses and coliphage T4;	
		Unit 7. Virology:	Multiplication of a virulent phage (lytic cycle); Lysogeny- nature of lysogeny, vegetative cycle, lysogenic state, prophage cycle, induction of a lysogenic cell; Relation of viruses and plasmids in tumour formation- formation of tumours, formation of animal tumours by DNA viruses and RNA viruses;	**1**
		Unit 8. Virology:	Brief idea about SARS virus, MARS v Zica virus, Nipah virus, Ebola virus Hanta virus; General account of vi virusoids, and prions.	**1**
		Unit 9. Microbial Growth and Nutrition:	Nutritional types and requirements; Typ media (natural, synthetic, semisynthetic, complex, selective); Growth- phases of growth, kinetics of growth, factors influencing growth; Batch culture, continuous culture, synchronous culture, Diauxic.	**1**
		Unit 10. Control of Microorganisms	Physical, chemical and chemotherap agents; antibiotic resistance; control of using chemicals and interferon.	**1**
		Unit 11. Genetic Recombination:	Transformation, transduction conjugation, detection of recombi overview of bacterial genetic map.	**1**
		Unit 12.	Nitrification, Denitrification, Ammonification; Mechanism of biologi	**1**

		Microbes in Nitrogen and Sulphur Cycle:	N2 fixation and structure and regulation nif gene; Microbialoxidation and reducti of sulphur.	
		Unit 13. Medical Microbiology:	Air borne diseases, water borne dise food borne diseases.	**1**
		Unit 14. Industrial Microbiology:	Industrial microorganisms, improvement, production of eth penicillin.	**1**
		Unit 15. Cosmetic Microbiology:	Production of ethanol, penicillin and vit B12. Concept and current trends about cosmetic biology.	**1**
	Immunology	Unit 1. Fundamentals of Immunology:	Innate and Acquired immunity, T-cell, B-cell, MHC, Cytokines, Antigen - types and characteristics:	**1**
		Unit 2. Fundamentals of Immunology:	Structure and functions of immunoglobins, Cell mediated and Humoral Immunity;	**1**
		Unit 3. Fundamentals of Immunology:	Ag-Ab reactions and Immunological techniques.	**1**

Content

Course Content:

1. History and Development of Microbiology: contributions of Leeuwenhoek, Koch, Pasteur, Jenner and Fleming.

2. Bacterial Systematics: Three kingdom concept of Haeckel, five kingdom concept of Whittaker and three domain classification of Woese; Characters used in bacteriology; Classification- phenetic and phylogenetic; Major groups of microorganisms.

3. Thermodynamic Principles in Microbiology: Concept of free energy, Entropy, Enthalpy, Energy rich bonds, Chemical potential, Membrane potential, Diffusion potential.

4. Bacterial Morphology: Structure, chemistry and function of capsule, pili, flagella, cell wall, cell membrane, ribosome, chromosome and plasmid, reserve materials and cytoplasmic inclusions; endospore (structure, formation, germination).

5. History of Development of Virology: nature, classification and nomenclature of viruses; structural organization and chemistry of viruses; assay of viruses, chemical and physical determination, assays of infectivity; Virus diseases in plants, symptoms of diseases, general transmission of viruses; Bacteriophages- isolation and demonstration, structure of adenoviruses, tobacco mosaic viruses and coliphage T4; Multiplication of a virulent phage (lytic cycle); Lysogeny- nature of lysogeny, vegetative cycle, lysogenic state, prophage cycle, induction of a lysogenic cell; Relation of viruses and plasmids in tumour formation-formation of tumours, formation of animal tumours by DNA viruses and RNA viruses; Brief idea about SARS virus, MARS virus, Zica virus, Nipah virus, Ebola virus and Hanta virus; General account of viroids, virusoids, and prions.

6. Microbial Growth and Nutrition: Nutritional types and requirements; Types of media (natural, synthetic, semisynthetic, complex, selective); Growth- phases of growth, kinetics of growth, factors influencing growth; Batch culture, continuous culture, synchronous culture, Diauxic.

7. Control of Microorganisms: physical, chemical and chemotherapeutic agents; antibiotic resistance; control of virus using chemicals and interferon.

8. Genetic Recombination: transformation, transduction and conjugation, detection of recombinants, overview of bacterial genetic map.

9. Microbes in Nitrogen and Sulphur Cycle: Nitrification, Denitrification, Ammonification; Mechanism of biological N2 fixation and structure and regulation of nif gene; Microbialoxidation and reduction of sulphur.

10. Medical Microbiology: Air borne diseases, water borne diseases, food borne diseases.

11. Industrial Microbiology: Industrial microorganisms, strain improvement, production of ethanol, penicillin and vitamin B12.

12. Cosmetic Microbiology: concept and current trends.

13. Fundamentals of Immunology: Innate and Acquired immunity, T-cell, B-cell, MHC, Cytokines, Antigen - types and characteristics: Structure and functions of immunoglobins, Cell mediated and Humoral Immunity; Ag-Ab reactions and Immunological techniques.

1. History and Development of Microbiology:

1. Contributions Of Leeuwenhoek, Koch, Pasteur, Jenner, And Flemming

The existence of small creatures was impossible to see with naked eyes. However, the discovery of the microscope in the nineteenth century helped to identify the so small creatures that present our surrounding environment or everywhere.

1.1 Contributions of Robert hook: An English scientist Robert Hook was the first to discover cells in the 17th century. He uses a lance to observe the smallest unit of tissue which is referred to as a cell.

1.2 Contributions of Antonie Van Leeuwenhoek: The Dutch Dorper and amateur biologist Antoni Van Leeuwenhoek was the first person to see the bacteria in his homemade microscope. He made a very simple microscope containing only one lance to examine the microorganism. Leeuwenhoek discovered bacteria in 1676 and reported his observation to the Royal Society of London. He draws the microorganism by observing his microscope and he referred to them as 'animalcules'. Then the next 150 years the progression of technology is helping to understand and nature of the bacteria. Leeuwenhoek first describes the correct description of protozoa and bacteria by using his microscope. Because of his extraordinary contribution to microbiology, he is known as the "Father of Microbiology". He is also considered the 'Father of bacteriology' and protozoology. Robert Koch and Louis Pasture from their research institute began the golden age of microbiology.

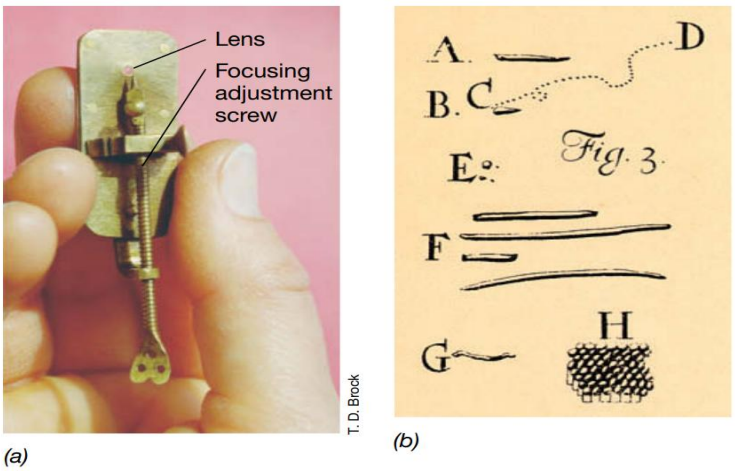


Figure1: Leeuwenhoek microscope(a), Drawimg bacteria by Leeuwenhoek (b).

1.3 Contributions of Louis Pasture: Louis Pasture was first given the concept of spontaneous generation theory from his experiment of the swan-neck flask. In this experiment, he boiled the broth in a small neck flask and exposed it to air then the culture broth bear organism to grow. When this experiment he did with the swan-necked flask thus no organism is found in the air-exposed broth because the swan neck significantly slows down the motion of the airflow through the tube and the air particles such as microorganisms are become trapped on the moisture on its inner surface, thus the microbes are not reached to the broth and the broth is sterile, but such that microorganism laden dust contact to the broth thus broth become putrefies. From this experiment, he proved that there was an organism present on-air dust that growing in the broth. In 1858 pasture proved that microorganisms arise from another microorganism.

When Pasture was the head of the science department in the university of Lille in 1854, he observed the problem of 'souring' of wine. It was noticed that when the wine-making in the aerobic condition turned wain sour but he showed that fermentation occurs in anaerobic conditions (in the absence of oxygen or air) that give the sour free wine. It gives the concept of life is present in absence of air and the souring of the wine is caused by the invasion of the wrong organism. Pasture concludes that air inhibits the fermentation process is referred to as the **'Pasture effect'**. The observation of fermentation processes by pasture laid a solid foundation for the **'Germ theory'**. Pasture also determines that the microorganism is responsible for the spoilage of wine during fermentation thus he suggests that mild heat at 62.8^0C for 30 min is destroyed the microbes and does not affect the taste of the product rather than boiling this process is referred to as pasteurization. The pasteurization process is first introduced in the US in 1892. He is known as the 'Father of Morden Microbiology' or 'Father of Bacteriology.

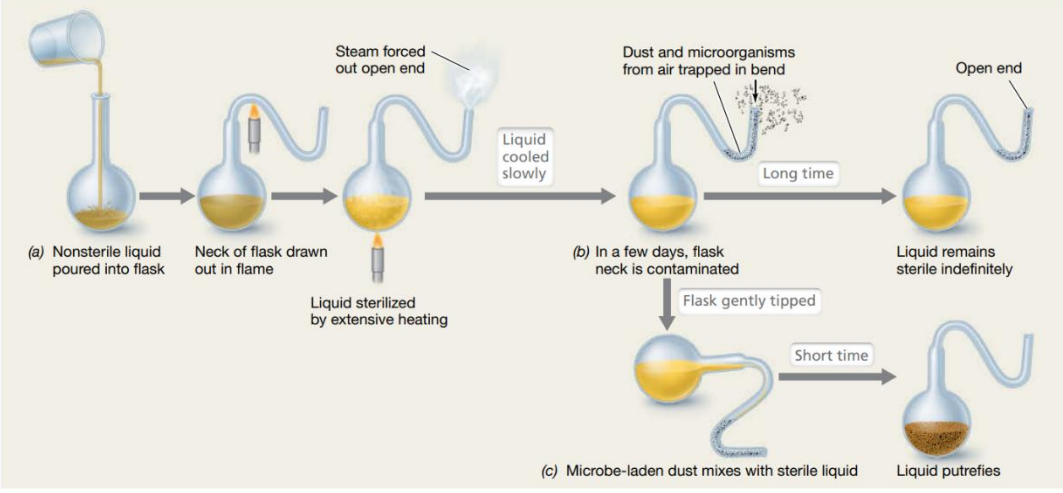


Figure2: Swan-neck flask experiment by Pasture.

1.4 Contributions of Robert Koch: Robert Koch was working around the same time when Pasture was doing his experiment. Koch gives direct documentation of the role of bacteria in causing disease. He was the first isolated causal agent of anthrax disease caused by bacteria *Bacillus anthesis* in 1876. He discovers the technique of pure culture for isolating the bacteria and also use the solid culture by using the solidify agent in 1881. He discovers *Mycobacterium tuberculosis* in 1882 that cause tuberculosis disease, He develop the criteria for linking cause and effect in infectious disease is known as Koch postulates in 1884.

According to Koch's postulate:

a. The disease-causing organism found only in infected or seek people, it is absent in healthy people.
b. The causal organism isolates and grows in pure culture.
c. The organism causes disease to the healthy animal when it is introduced.
d. The organism of an infected person is recovering shows the same as the organism was infected.
e. The germ theory of disease is introduced by Pasture and Koch according to this theory the invasive microorganism can cause disease.

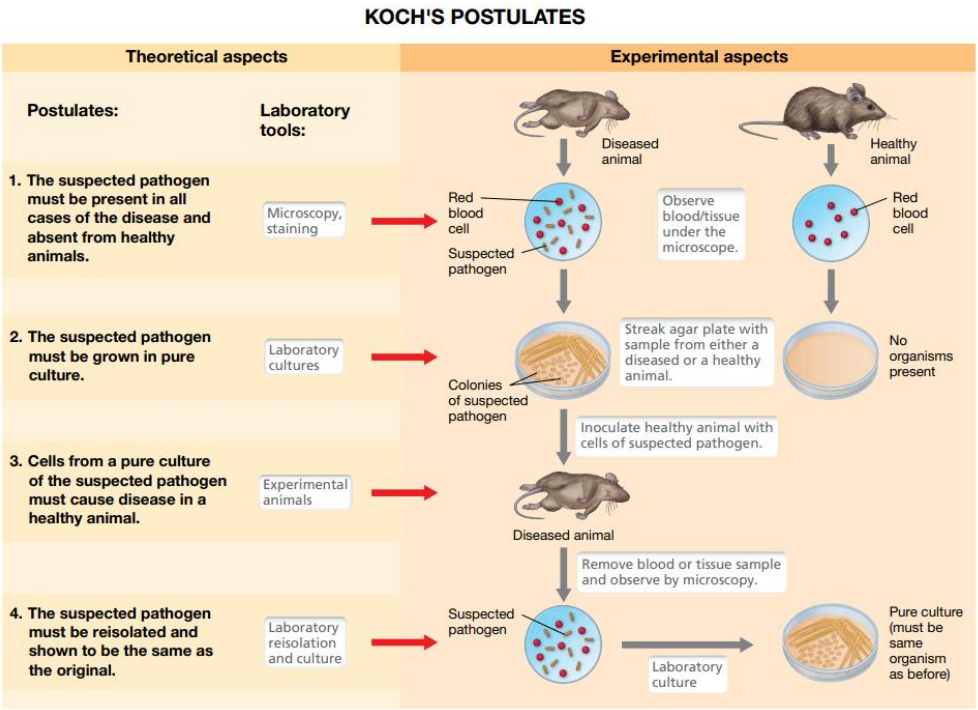


Figure3: Proving cause and effect of infectious disease by Koch's postulate.

1.5 Contributions of Edward Jenner: Edward Jenner was an English physician. He has first prevented smallpox. In 1976 he observed that a person who was attacked by cowpox later the person is not attacked by smallpox. The milk-man when comes in contact with cow-pox during his

work the immunity is develop in the person because the cowpox is more related to the smallpox virus. Thus, when the immunity is built, he is not attacked by the smallpox virus. Jenner has utilized this knowledge to develop the process is referred to as vaccination based on the Latin word 'Vacca' means cow. Thus, the use of the cowpox virus develops immunity against the actual smallpox material.

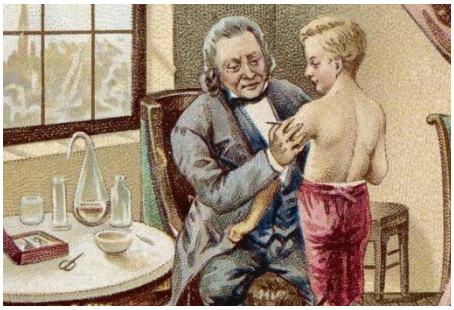


Figure4: Jenner's experiment.

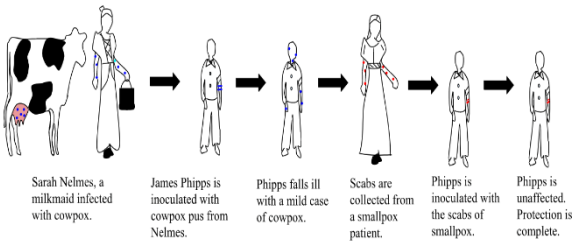


Figure5: Edward Jenner vaccination.

In 1879, Pasture observed the chicken-cholera causing bacteria is lost its pathogenicity in the old culture. He found that the non-virulent bacteria were introduced in healthy animals the immunity is developed against the nonvirulent derivative. The process by which the virulent bacteria lost their pathogenicity is known as attenuation.

Jenner's experiment principle is help to pasture to prevent anthrax. He makes vaccines against hydrophobia or rabis (bites by a dog, cat, etc.). The vaccination helps to modern immunization programs against different diseases like tetanus, Diphtheria, pressures, polio, etc.

1.6 Contributions of Alexander Fleming: Fleming was a Scottish physician and bacteriologist in England. In 1929 he discovered the 'wonder drug' penicillin. During the first world war (1914-1918) he was interested in searching for something that kill the pathogen. The discovery of antibiotics was completely by accident in 1920. A solid culture of Petri dish of bacteria *staphylococcus* left for longer than usual thus the fuzzy fungus growing in the patch become mouldy. The bacterial colonies around the fungus colony are reduced in size rather the bacterial colony rest from the fungal colony on the plate. The poor bacterial colonies produce because the compound is found to be responsible for the anti-bacterial action. This anti-bacterial compound produced by the fungus is known as penicillin. The antibiotics were letter used to treat the people who suffer from different types of bacterial infection and use many other applications.

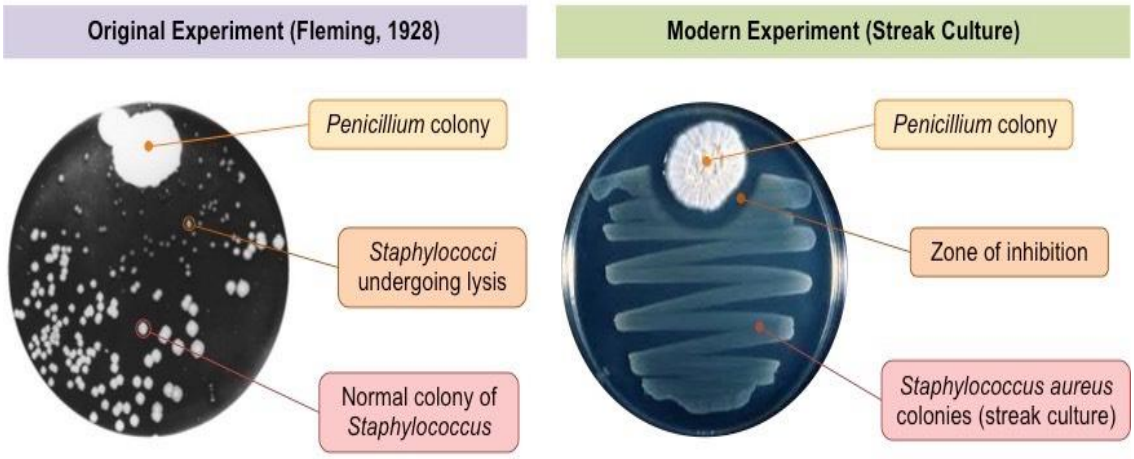


Figure 6: Discovery of antibiotic penicillin by A. Fleming in 1928

2. Bacterial systematics

The term systematics is derived from the Latinised Greek word and ‘systema’ means ‘together’. The systematics partly overlaps with taxonomy and originally used to des-cribe the system of classification prescribed by early biologists. Linnaeus applied the word “Systematics” in the system of classi-fication in his famous book ‘Systema Natu-rae’ published in 1735.

Blackwelder and Boyden (1952) gave a definition that “sys-tematics is the entire field dealing with the kinds of animals, their distinction, classifica-tion and evolution”. C. G. Simpson (1961) considers that “Systematics is the scientific study of the kinds and diversity of organ-isms and of any and all relationships among them”.

The simpler definition by Ernst Mayr (1969), and Mayr and Ashlock (1991) is “Sys-tematics is the science of the diversity of organisms”. Christoffersen (1995) has de-fined systematics as “the theory, principles and practice of identifying (discovering) systems, i.e., of ordering the diversity of organisms (parts) into more general systems of taxa according to the most general causal processes”.

The systematics includes both taxonomy and evolution. Taxonomy includes classifi-cation and nomenclature but inclines heavily on systematics for its concepts. So study of systematics includes a much broader aspect that includes not only morphology and ana-tomy but also genetics, molecular biology, behavioral aspects and evolutionary biology.

The recent approach to the science of biology has added a new dimension to the science of classification and the new systematics has emerged as a synthesis of progress in all the major disciplines of Biology.

Branches of Systematics:

The new systematics may be divided into following branches:

1. Numerical systematics:

This type of systematics is based on bio-statistical method in identification and classifi-cation of animals. This branch is called biometry.

2. Biochemical systematics:

This branch of systematics deals with classification of animals on the basis of biochemical analysis of protoplasm.

3. Experimental systematics:

This branch of systematics deals with identification of various evolutionary units within a species and their role in the process of evolution. Here mutation is considered as evolutionary unit.

2.1 Three Kingdom System (Hackel's Concept)

As the knowledge of the properties of various groups of microbial life exploded around the middle of the 19th century, it became apparent that at this level of biological knowledge a division of the living world into two kingdoms cannot really be maintained on a logical and consistent ground.

Although the two kingdom system suited at the level of well-developed advance organisms, it failed to satisfy biologists at the level of microbial forms of life.

Many of the microbes possessed both "plant-like" and "animal-like" characteristics simultaneously, and many others enjoyed such characteristics which were unique to them and not found in either plants or animals.

Slime moulds, which were considered to be protozoan and grouped under the kingdom Animalia, were found phagotrophic and amoeboid (animal-like) in their vegetative state but they resembled true fungi in their reproductive state (plant-like).

Motility by means of flagella was found the only animal-like character in flagellate protozoa: many of them possessed cell wall and were phototrophs (plant-like). Bacteria were found having very little in common with either plants or animals.

Many algae and fungi, which were earlier thought to be immotile thus classified as plants, were found either motile or producing motile structures (zoospores, gametes etc.) during their life.

In view of the foregoing, attempts were made to find a solution and the same was proposed in 1866 by E. Haeckel, a German zoologist and Darwin's disciple.

Haeckel suggested that the inconsistencies of the two kingdom system could be avoided by the recognition of a third kingdom, and he proposed Protista as a new kingdom to accommodate organisms exhibiting characters either common to both plants and animals, or unique to their own.

Thus, a three kingdom system consisting of kingdoms Protista, Plantae, and Animalia came into being. This arrangement by Haeckel was done on the basis of morphological complexities and tissue system, division of labour, and mode of nutrition.

Organisms lacking morphological complexities, tissue system, division of labour, and enjoying diversified type of modes of nutrition were segregated and put under the kingdom Protista (algae, fungi, protozoa and bacteria).

Organisms having diverse tissue-system with well- defined division of labour and maximum morphological complexities in their body remained segregated from protists and were bifurcated into two categories: those enjoying autotrophic mode of nutrition were considered to be plants and

put under kingdom Plantae, and those that have entirely holophagic (phagotrophic) mode of nutrition were considered to be animals and put under kingdom Animalia. According to this system, all known microorganisms came to be recognized as protists; neither plants nor animals.

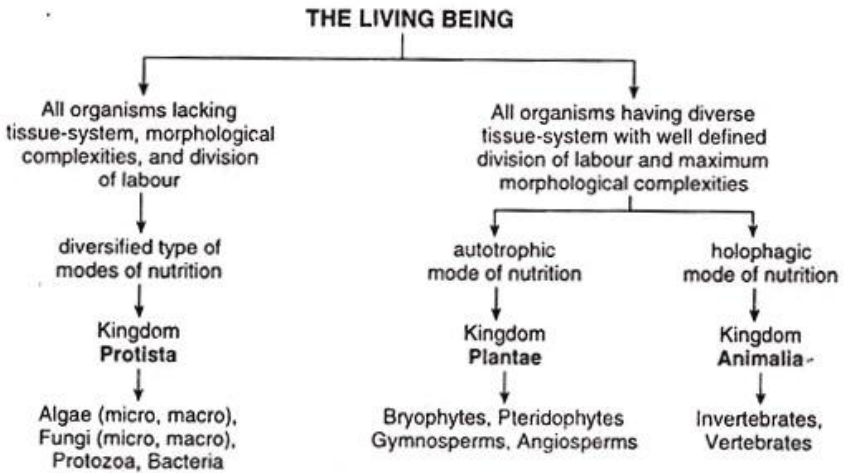


Figure: Three kingdom system of classification

2.2 Five Kingdom Systems (Whittakar's Concept)

Although most of the inconsistencies of protists came to an end with the proposal of two separate kingdoms (Monera and Protoctista) in four kingdom system, the heterogeneity among protoctists were still discernible. All fungi were non-photosynthetic and enjoyed very distinct mode of nutrition (absorptive; osmotrophic).

Similarly, some of the algae have lost their photosynthetic ability (e.g., some euglenophycophytes), lacked cell wall, moved freely and thus were considered closely related with protozoa; other algal forms exhibiting more extensive organizational development and totally photosynthetic nature were considered more closely related with plants.

Considering such inconsistencies of protoctistans. R.H. Whittakar (1969) proposed a comprehensive five kingdom system comprising of kingdom — Monera, Protista, Fungi (Mycophyta), Plantae, and Animalia

He retained bacteria and cyanobacteria (blue green algae) under kingdom Monera, retained microalgae (the pliytoplanktonic algal forms considered closely related with protozoa), protozoa, and slime moulds under Kingdom Protista (he adopted term Protista rather than Protoctista of Copeland); separated, fungi completely form protoctists and accommodated them under a new kingdom Fungi (Mycophyta); segregated extensively developed completely photosynthetic algal

forms (the macroalgae considered closely related with plants) from protoctists and accommodated them along with plants under kingdom Plantae; and, retained invertebrate and vertebrate animals as such under kingdom Animalia. In this way, the microorganisms spread into three kingdoms (Monera, Protista, Fungi) and came to be recognised as monerans, protistans and fungi (mycophytans); neither plants nor animals.

Whittaker's system of organisms classification is based on evolutionary relationship of phenotypic (observable) characteristics in which three levels of cellular organization are thought to have evolved along three different lines of nutritional strategies: photosynthesis, absorption, and ingestion.

The phenotypic characteristics taken into account to raise five kingdoms by Whittaker are:

(1) Cell type-prokaryotic or eukaryotic,

(2) Level of organization—solitary and colonial unicellular organization or multicellular, and

(3) Nutritional type.

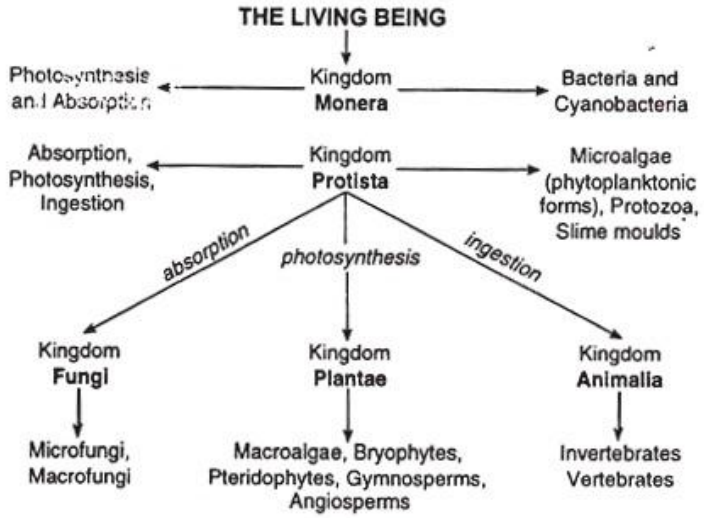


Figure: Five kingdom system of classification

1. Kingdom Monera (Archaebacteria, Bacteria, and Cyanobacteria):

(i) The monerans consist of all the prokaryotes, and majority of them are represented by the smallest organisms on earth.

(ii) The nuclei of the monerans are not organized with nuclear membrane, nucleoplasm, chromatin fibres and nucleoli, and are referred to as 'incipient nuclei'.

(iii) The moneran cells typically lack in cell organelles such as chloroplasts, mitochondria and other membrane-bound organelles like endoplasmic reticulum, Golgi bodies, etc.

(iv) The photosynthetic pigments are present in the form of chromatophores, which can be compared with a single lamella of a granum in the plastids (chloroplasts) of algae and higher plants.

(v) The respiratory enzymes, which are mainly the concern of mitochondria in other organisms, however, are present along the infoldings of plasma membrane called 'mesosomes'.

(vi) Nutrition absorptive, chemosynthetic, photohetetrophic, or photoautotrophic. Since many of the monerans are photosynthetic, they possess photosynthetic pigments but the same are different from those of other photosynthetic organisms.

(vii) The monerans possess cell walls (except mycoplasmas and some archaebacteria) beyond any doubt but the cell wall composition is unique. Unlike other organisms, the chief constituent of the monerans cell wall is 'peptidoglycan' except archaebacteria in which the main constituent is thought to be usually proteinaceous).

(viii) Flagella, if present, are 8 stranded lacking 9 + 2 arrangement; each strand is made up of a protein named flagellin.

(ix) The monerans are also unique in having specific type of ribosomes distributed in cytoplasm having 70 sedimentation co efficient (70S) as against 80 sedimentation coefficient (80S) ribosomes arrayed on membranes in other organisms.

(x) Monerans reproduce asexually, they lack true sexual reproduction.

(xi) The mode of recombination of hereditary characters in monerans can be attributed to alternative pathways of sexuality (parasexuality), namely, transformation, conjugation, transduction and mutation.

(xii) The nuclear genetic material in monerans is represented by a single molecule of DNA per cell and the cell division does not involve any precision in the distribution of genetic material among the daughter cells. This is accomplished by a process known as amitosis.

2. Kingdom Protista (Phytoplanktonic Algae, Protozoa, and Slime Moulds):

(i) Eukaryotic with solitary or colonial unicellular organization without any differentiation into tissues and organs.

(ii) Mostly aquatic forms called planktons; the planktons may be photosynthetic and cell-walled (phytoplanktons) or may be non-photosynthetic and wall-less (zooplanktons).

(iii) Nutrition absorptive, photosynthetic, or ingestive. Photosynthetic pigments are chlorophylls present in plastids (chloroplasts).

(iv) The protistan cells possess cell organelles such as mitochondria, Iysosomes, centrioles and other membrane-bound organelles like endoplasmic reticulum, Golgi bodies, etc.

(v) The cellular organization is of two envelop type, i.e., besides plasma membrane, internal membranes occur around certain organelles.

(vi) Genetic material is organized in the form of a true nucleus. DNA is associated with histone proteins.

(vii) Flagella, if present, are 11 stranded with 9 + 2 arrangement and are made up of a protein named tubulin.

(viii) All forms reproduce asexually; many have true sexual reproduction with plasmogamy, karyogamy, and meiosis. However, an embryo stage is absent.

3. Kingdom Fungi (The Fungi):

(i) Fungi are ubiquitous found in any conceivable habitat.

(ii) They are versatile by virtue of their high degree of adaptability. Anything that can be decomposed to yield energy invites fungi to colonize it.

(iii) They are eukaryotes but their eukaryotism is specific in some aspects. The fungal nuclei are minute to the extent that they cannot be easily observed in compound microscope. They are so plastic that they pass easily through minute septal pores. The nuclear division of fungi is intra-nuclear, i.e., the nuclear membrane is persistent and stages of division complete within it by the process called karyochorisis.

(iv) One-to-all known fungi are heterotrophic and absorb food from the environment employing extracellular digestion.

(v) The vegetative body of fungi is usually filamentous; the filament is called a hypha, which is thread-like, extensively branched, and surrounded by cell wall.

(vi) The vegetative body, even when it forms tissues, is never differentiated into root and shoot and, most important of all, has no specialized vessels for internal transport of nutrients.

(vii) The cell wall is characteristically mainly constituted of chitin (commonly called fungal cellulose); recently, cellulose has been found as chief constituent of cell wall (members of Oomycetes).

(viii) Fungi reproduce usually asexually and sexually by means of asexual and sexual spores, respectively. The main asexual spores formed are either sporangiospores formed inside sporangia or they are conidiospores (conidia) formed exogenously on hyphae or specialized hyphal branches called conidiophores. The sexual spores are oospores, zygospores, ascospores and basidiospores.

(ix) The reserve food material is glycogen (animal starch).

4. Kingdom Plantae (Macroalgae and Plants):

(i) Primarily autotrophic; some heterotrophic, a few saprophytic.

(ii) Organisms multicellular (except some algae) with walled and frequently vacuolate eukaryotic cells.
(iii) Simple multicellular to advanced tissue organization.
(iv) Autotrophism by means of photosynthesis; photosynthetic pigments are chlorophylls present in plastids (chloroplasts).
(v) Reproduction primarily sexual, with haploid and diploid stages alternating with each other (alternation of generations); haploid stage reduced in higher members of the kingdom.
(vi) Development of individuals, as a result of sexual reproduction, proceeds from solid embryos except in the algal groups.
(vii) Food reserve is usually starch and fat.
(viii) Growth is usually indefinite; growing points well-defined.

5. Kingdom Animalia (Invertebrate and Vertebrate Animals):
(i) Individuals multicellular with wall-less eukaryotic cells.
(ii) Multi-cellularity accompanied with cellular tissue and organ-system levels of organization with complex cell junctions.
(iii) Nutrition primarily ingestive with digestion in an internal cavity, but some forms are absorptive and some lack digestive cavity.
(iv) Reproduction mainly sexual with meiosis forming gametes; haploid stages other than gametes almost lacking above lowest phyla.
(v) Zygote develops into an embryo.
(vi) Animals are motile or mobile (except sponges).

(vii) Muscle cells present for mobility and nerve cells for conduction of impulses.
(viii) Centrioles occur in cells.

2.3 Three domain System (Carl Woese's Classification)

The three-domain system was first introduced by Carl Woese in 1990 that is why its called Carl Woese's Classification. This classification system also is known as the Six Kingdoms and Three Domains Classification because it divides the life forms into three domains and six kingdoms.
The three-domains of Carl Woese's Classification system include archaea, bacteria, eukaryote, and six kingdoms are Archaebacteria (ancient bacteria), Eubacteria (true bacteria), Protista, Fungi, Plantae, Animalia.

This classification system divides the life based on the differences in the 16S ribosomal RNA (rRNA) structure and as well as the cell's membrane lipid structure and its sensitivity to antibiotics. The main difference from earlier classification systems is the splitting of archaea from bacteria.

The evaluating rRNA structure is very helpful. As a result of rRNA molecules, throughout nature perform the identical function, their structure modifications very little over time. Subsequently, similarities and dissimilarities in rRNA nucleotide sequences are a very good indication of how associated or unrelated completely different cells and organisms are.

In this classification, Carl Woese uses 16S ribosomal RNA (rRNA) as a 'Chronometer', because of;

- It is universally distributed means its presence in all species.
- It is functionally similar in all organisms.
- It can change its sequence slowly.
- Its sequences can be aligned, or matched up, between 2 organisms.

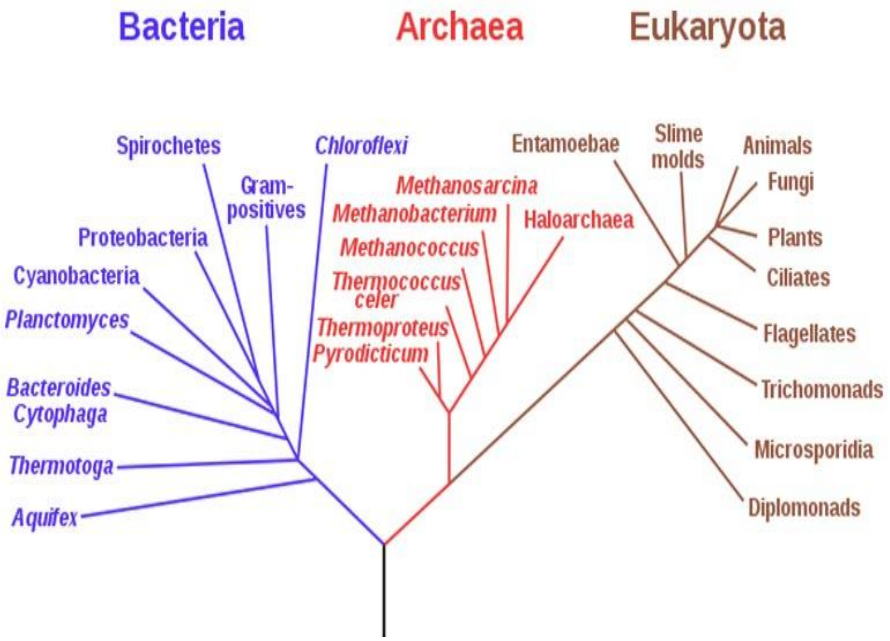


Figure: Three domain system of Woese

Carl Woese's Classification is made of three domains such asDomain Archaea, Domain Bacteria, Domain Eukarya.

a. Domain Archaea

The Archaea domain includes all prokaryotic cells, they lack nuclear membrane; have distinct biochemistry; contain RNA markers from bacterial cells. The Archaea are considered as the oldest

species of organisms on Earth. They can survive in extreme, harsh environments that differentiated them from other domains. The cell wall of archaea lacks peptidoglycan. Archae also contains ether leakage in their membranes.

Archae has three phyla such as;

Crenarchaeota: They can survive at extremely high temperatures and extremely low temperatures.

Euryarchaeota: Some of them are known as extremely halophiles, which can prevent inhibit highly saline environments.

Konarchaeota: It includes all those species were found in a single hot spring, Obsidian Pool, present in Yellowstone National Park (USA).

b. Domain Bacteria

These are also prokaryotic cells with bacterial rRNA and contain diacyl glycerol diester lipids in their membrane. They are also called eubacteria or "true bacteria". Their cell membrane contains ester linkage between unbranched fatty acid chains and glycerol. Their cell wall made up of peptidoglycan.

There are present 5 phyla of the bacterial domain such as;

Proteobacteria: The example of proteobacteria is E. coli, Salmonella typhus, Legionella, Heliobacter pylori (cause of many ulcers), Neisseria gonorrhea (cause of gonorrhea).

Cyanobacteria: The example of Cyanobacteria is Photosynthetic 'blue-green' bacteria which produces O2 gas.

Eubacteria: The example of Eubacteria is Clostridium (tetanus, botulism), Bacillus, Mycoplasma (walking pneumonia).

Chlamydias: The example of Chlamydias is Giardia, Chlamydia (STD), etc.

Spirochaetes: The example of Spirochaetes is Spiral bacteria that cause syphilis, Lyme disease.

c. Domain Eukarya

These are eukaryotic cells with a membrane-bound nucleus. Their membranes contain their membranes between unbranched fatty acid chains and glycerol. They lack peptidoglycans on their cell wall. Eukarya inhibit the antibacterial antibiotics but sensitive to antibiotics that affect eukaryotic cells.

There are presently four Kingdoms of Eukarya such as;

Protista: These are slime molds, euglenoids, algae, and protozoans.

Fungi: These are sac fungi, club fungi, yeasts, and molds.

Plantae: These are mosses, ferns, conifers, and flowering plants.

Animalia: These are sponges, worms, insects, and vertebrates.

2.4 Phenetic Classification:

For a very long time, microbial taxonomists had to rely exclusively on a phenetic system, which classifies organisms according to their phenotypic similarity. This system succeeded in bringing order to biological diversity and clarified the function of morphological structures. For example, because motility and flagella are always associated in particular microorganisms, it is reasonable to suppose that flagella are involved in at least some types of motility. Although phenetic studies can reveal possible evolutionary relationships, this is not always the case. For example, not all flagellated bacteria belong to the same phylum. This is why the best phenetic classification is one constructed by comparing as many attributes as possible.

An approach to biological classification which uses overall similarity to assess relationships is called Phenetics or numerical taxonomy. Phenetic classification makes no attempt to reflect evolution; taxa are related based on similarity and difference of character states regardless of the evolutionary content of the characters and states reflected. The character of choice in phenetics is the Unit character or Single character.

In phenetics each unit character is given the same or no weight while in phyletic and cladistic classification the weighting of character is believed to be evolutionarily important. Phenetic classification are based on overall similarities of characters.

Dendrogram:

It is branching diagram in the form of tree and depicts degree of relationship.

Phenogram:

It is representation of phenetic relationship.

Cladogram:

It is the depiction of cladistic relationship.

Modern Phenetic Methods (Taxometrics):

Phylogenetic classifications of plants faced many difficulties and uncertainties. It led to new method of classification with Phenetic approach instead of phylogenetic approach. It was given by Sneath and Sokal (1957).

It was a coincidence that numerical taxonomy has come to be almost synonymous with phenetic classification. Numerical taxonomy neither produces new data nor is a new system of classification but it is a new method of organizing data and obtaining from them a classification.

R.H.A. Sneath or R.R. Sokal published a textbook “Principle of Numerical Taxonomy” in 1963 and revised it in 1973 as Numerical Taxonomy. They have suggested seven main advantages of numerical taxonomy over conventional taxonomy.

These seven principles are as follows:

(1) The greater the content of information in the taxa of a classification and the more characters on which it is based, the better a given classification will be.

(2) A Priori every character is of equal weight in creating natural taxa.

(3) Overall similarity between any two entities is a function of their individual similarities in each of the many characters in which they are being compared.

(4) Distinct taxa can be recognized because correlation of characters differs in the group of organisms under study.

(5) Phylogenetic inferences can be drawn from the taxonomic structure of a group and from character correlations, given certain assumptions about evolutionary pathways and mechanism.

(6) Taxonomy is practiced as an empirical sciences as opposed to interpretative or intrutive science.

(7) Classification are based on phenetic similarity.

Most of these principles bear resemblance to the aims and methods of Adanson and are therefore, known as Neo-Adansonian principles.

Numerical Taxonomy is based on Phenetic evidences, i.e., on similarities by observed and recorded characters of taxa, and not on phylogenetic probabilities. Since, numerical taxonomy is operational in the sense, it is divided into a series of repeated steps, allowing its results to be checked back step by step.

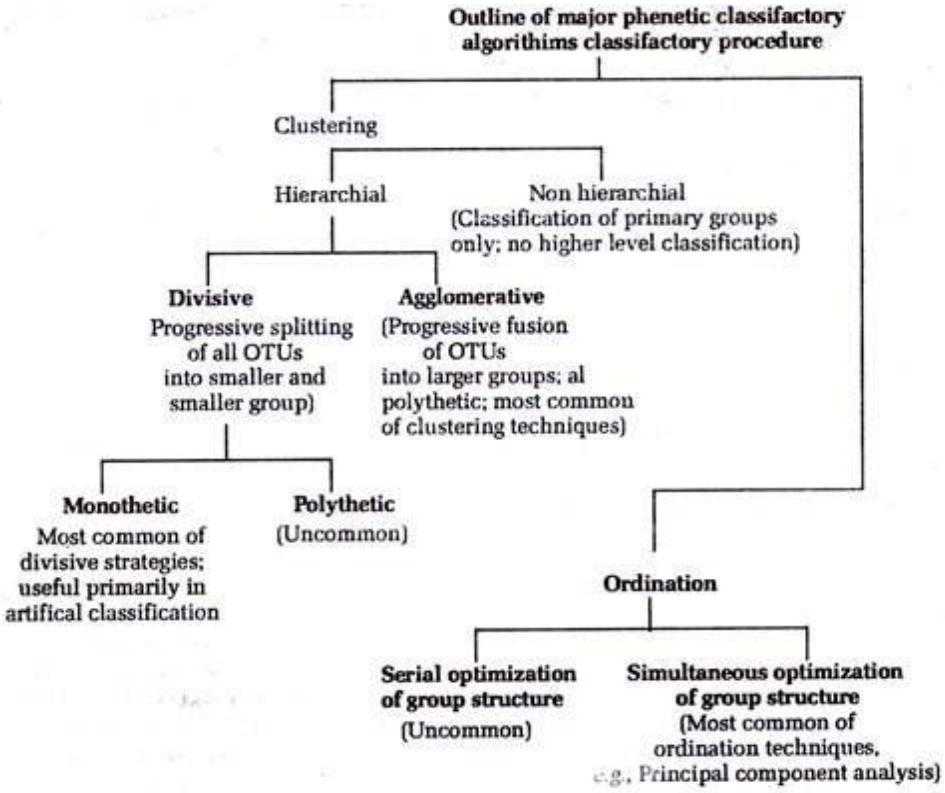


Figure: Outline of phonetic classification

2.5 Phylogenetic Classification:

With the publication in 1859 of Charles Darwin's On the Origin of Species, biologists began developing phylogenetic or phyletic classification systems that sought to compare organisms on the basis of evolutionary relationships. The term phylogeny (Greek phylon, tribe or race; genesis, generation or origin) refers to the evolutionary development of a species. Scientists realized that when they observed differences and similarities between organisms as a result of evolutionary processes, they also gained insight into the history of life on Earth. However, for much of the twentieth century, microbiologists could not effectively employ phylogenetic classification, primarily because of the lack of a good fossil record. When Carl Woese and George Fox proposed using small subunit (SSU) rRNA nucleotide sequences to assess evolutionary relationships among microorganisms, the door opened to the resolution of long-standing inquiries regarding the origin and evolution of the majority of life forms on Earth—microbes. Whole genome sequencing is the most recent analytical tool to elucidate phylogenetic relationships among microbes.

Phyletic or Evolutionary or Phylogenetic characters are used primarily in phylogenetic classification. The term Phylogenetic is used in many ways.

Haeckel (1886) used the term in evolutionary history of a group. Recently a particular approach to classification (cladism) have used phylogenetic to refer to reconstructing only the branching sequence of phylogeny. This approach is called cladistics with phylogenetics retained in its original and broader usage.

The character distinction is in between homologous versus analogous. Homologue means the same organ in different animals under every variety of form and function. Analogue means a part or organ in one animal which has the same function as another part or organ in a different animal.

After Darwin's theory of evolution the homologous organs were viewed as structural modification of the same organ, inherited from a common ancestor. Analogues are those features developed by different organs to the same selection pressure.

The detection of homologous character is a difficult task for a phylogenetic reconstruction. Because of complexities, botanists have tended to deal with the problem obliquely.

Phylogenetic and Ontogenetic characters are simply features which are presumed to reflect information about the phylogeny of the group; and developmental features. A regressive character is one in which loss of appendages occur. In context of evolution these are also known as adaptive or non-adaptive characters.

Phylogenetic classification attempt to reflect the geneology or evolutionary history of a particular group of plants.

Cladistic Characters:

The characters have developed from the cladistic approach to classification attempting to determine branching sequence of evolution and base a classification upon them. Only derived character states are regarded as significant cladistically.

Characters are Primitive vs. derived character states; or as synonyms, general vs. unique; generalized vs. Specialized, Primitive vs. advanced, Plesiomorphic vs. apomorphic and Pfesiotypic vs. apotypic etc. Plesiotypic and Apotypic terms were used by cladists like Wiley (1981), Wagner (1983) etc. Shared derived character states between and among the taxa are called synapomorphies (or synapotypies) and shared primitive states are symplesiomorphies (or symplesiotypies).

Automorphy:

Derived character state occurring only in one evolutionary line and has no direct use in constructing branching sequences.

Compatible character:

Useful cladistical characters are called compatible characters where evolutionary directionality of the states within each character is the same.

Problems with Phylogenetic Classifications:

i. Convergent Evolution:

Species with similar selection pressures look alike i.e., appear alike, and hence, can 'trick' a taxonomist.

ii. Lack of Fossils:

Fossilization is a sporadic process. Some events happened so quickly that fossils may not adequately document the changes i.e., the angiosperms appeared very rapidly in fossil records.

iii. Strict Evolutionary Classification:

It assumes a monophyletic origin of groups i.e., the ancestors can only came from one group; they cannot be polyphyletic e.g., the members of your immediate family i.e., grandparents, parents, siblings make up a group with a single origin (monophyletic). Neighbors are not included in this group since they have a different origin.

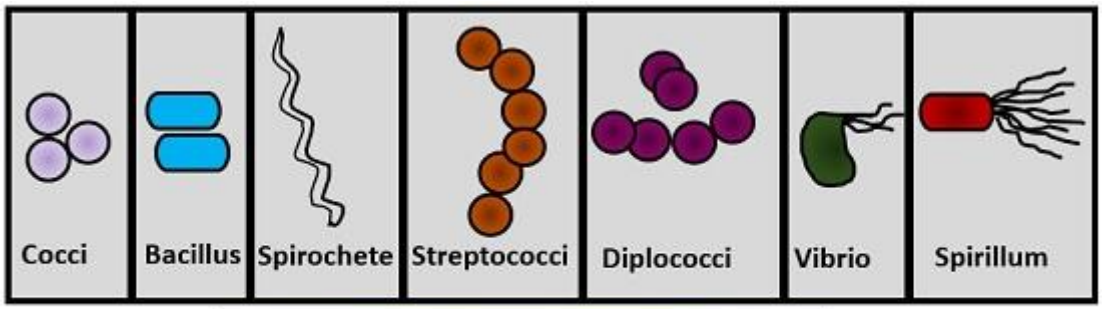
2.6 Major Groups of Microorganisms:

Microorganisms are microscopic, which includes bacteria, fungi, archaea or protists. Microorganisms differ from each other in size and structure, habitat, metabolism, and many other characteristics. Most bacteria are harmless or helpful, but some are pathogens, causing disease in humans and other animals.

Bacteria:

They are single-celled disease-causing micro-organisms. They can be spiral or rod-shaped.

- **Cell properties**: Bacteria is a prokaryotic cell (having a primitive nucleus) and unicellular.
- **Size:** It ranges from 0.2-100 µm.
- **Shape:** Bacteria are pleomorphic in nature. It possesses variable shapes



Different forms of bacteria

- **Distribution:** Worldwide.
- **Habitat**: Soil, water, earth crust, dead organic matter, hot springs etc.
- **Movement:** For its movement, it has a whip-like structure called "Flagella".
- **Nucleus:** True nucleus absent.
- **Genetic material:** The genetic material of bacteria can be either DNA or RNA.
- **Types:** Bacteria can be either Gram-positive or Gram-negative based on cell wall characteristics. Depending upon the cell shape, bacteria typically exist in five forms, viz. Bacillus, Coccus, Vibrio and Spirillum.
- **Resistance:** Some strains of the bacteria are resistant to adverse conditions like high Ph, temperature, high salt concentration and many antibiotics etc.
- **Nutrition type:** Heterotrophic or autotrophic
- **Chlorophyll:** Present in photosynthetic bacteria like purple and green bacteria.
- **Reproduction:** Bacteria reproduce by both asexual and sexual method. Asexual methods include budding, fragmentation and most commonly binary fission. Sexual methods include transformation, transduction and conjugation.

- **Absorption:** Bacteria absorb nutrients with the help of flagella that is used to trap the food or other organisms.
- **Nature:** Some bacteria are symbiotic and parasitic in nature.
- **Morphology:** Simple
- **Carotenoids:** Wide variety of carotenoids are present in a class of bacteria.
- **Oxygen requirement:** On the basis of oxygen requirement bacteria, can be aerobic, anaerobic and facultative anaerobes.

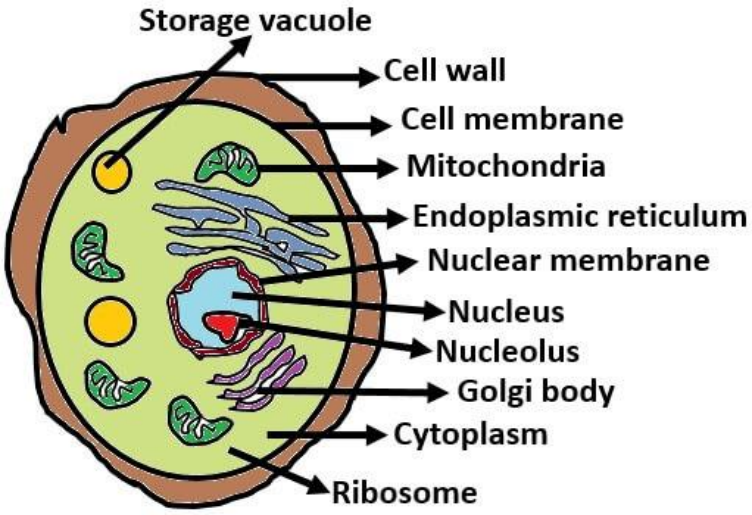
Fungi:

They are mostly multicellular disease-causing microbes. Bread moulds are common examples of fungi.

- **Cell properties:** Fungi are eukaryotic and it can be multicellular or unicellular.
- **Size:** The size of moulds ranges from 2.0-10.0 µm and the size of yeast ranges from 5.0-10.0 µm.
- **Shape:** A fungus has two distinct morphological shapes. Its vegetative stage is characterized by the formation of a hyphal network, while the reproductive stage is characterized by the formation of fruiting bodies through the hyphae.
- **Distribution:** Worldwide.
- **Habitat:** Deserts, deep-sea sediments, soil, dead organic matter etc.
- **Movement:** Fungi have no locomotory apparatus, there is only the movement of spores through air or wind.
- **Nucleus:** True nucleus present.
- **Genetic material:** In fungi, either DNA or RNA is present.
- **Types:** On the basis of cell type, fungi are broadly classified into yeast and moulds. different forms of fungi
- **Resistance:** Fungal spores are also resistant to many antibiotics, chemicals, ph, temperature etc.
- **Nutrition type:** Most of them are heterotrophic.
- **Chlorophyll:** Present.
- **Reproduction**: Fungi reproduce by vegetative, asexual and sexual methods. Vegetative methods include binary fission and budding. Asexual reproduction includes hyphae fragmentation,

chlamydospore formation, transverse cell division etc. Sexual reproduction includes gamete fusion, gametangial contact, gametangial copulation, spermatization and somatogamy.

- **Absorption:** Fungi absorb food and nutrients by their hyphae.
- **Nature:** Fungi are also symbiotic and parasitic in nature.
- **Morphology:** Complex.
- **Carotenoids:** Present.
- **Oxygen requirement:** Most of the fungi grow in the presence of oxygen i.e. aerobic.



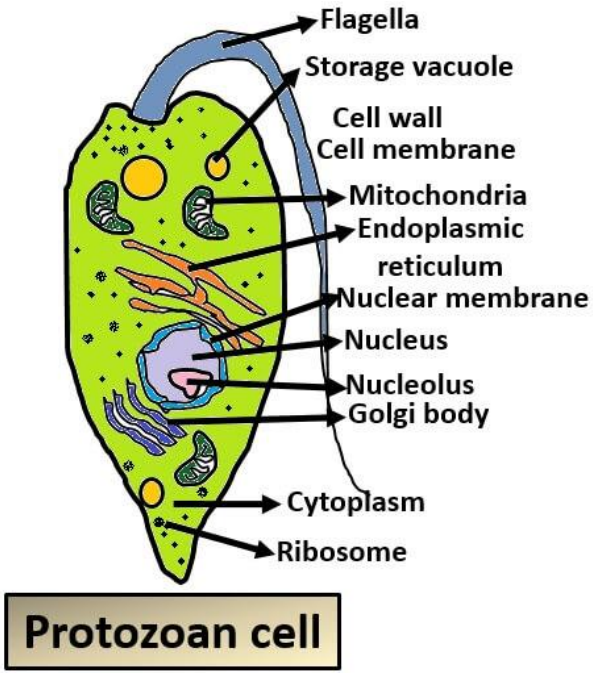
Fungal cell

Protozoa:

They mainly include organisms such as Amoeba, Plasmodium, etc. They can be unicellular or multicellular.

- **Cell properties:** These are eukaryotic and multicellular.
- **Size:** It ranges from 2.0-200 µm.
- **Shape:** Protozoa are variable in shape.
- **Distribution:** Cosmopolitan.
- **Habitat:** Soil, plant, marine water, and freshwater etc.
- **Movement:** Protozoa move through the help of cilia or flagella.
- **Nucleus:** It contains a vesicular nucleus.
- **Genetic material:** DNA.
- **Types:** Flagellates, ciliates, amoeboid and sporozoans are the four types of protozoa.

- **Resistance:** Some parasitic protozoans are drug-resistant.
- **Nutrition type:** Most of the protozoans are heterotrophic and a few are autotrophic.
- **Chlorophyll:** Few protozoans contain green chlorophyll pigment.
- **Reproduction:** Protozoans reproduce by budding, binary fission, schizogony, multiple fission etc.
- **Absorption:** Protozoa uptake food by cytosome present on the cell wall with the help of flagella or pseudopodia.
- **Nature:** Protozoans are Paraphyletic in nature.
- **Morphology:** Complex.
- **Carotenoids:** Present.
- **Oxygen requirement**: Most of the protozoans are aerobic.

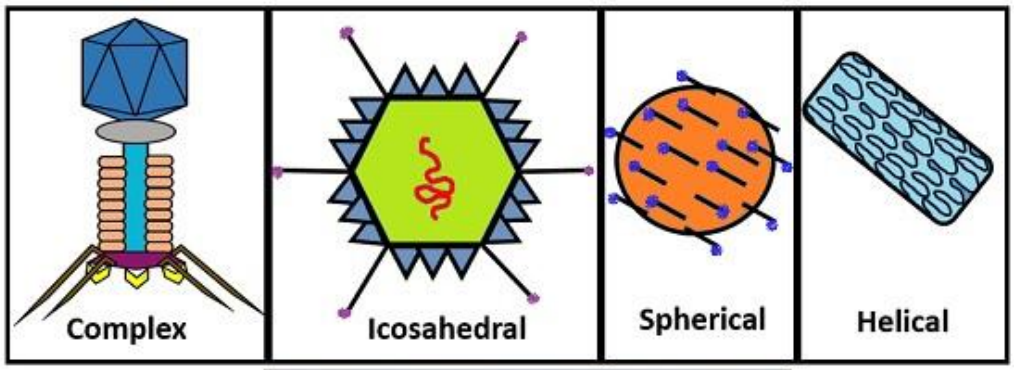


Virus:

Viruses are disease-causing microbes that reproduce only inside the host organism.

- **Cell properties:** Virus are prokaryotic and acellular microorganisms.
- **Size:** It ranges from 0.015-0.2 µm
- Shape: Its shape is generally icosahedral, while a few are spherical, helical and complex. different forms of virus
- **Distribution:** Ubiquitous.
- **Habitat:** These are mainly living inside the host.

- **Movement:** They move into the host cell by recognition of receptor site through tail pin and tail fibres by various methods like endocytosis and exocytosis.
- **Nucleus:** Absent.
- **Genetic material:** In viruses either DNA or RNA is present.
- **Types:** Viruses are broadly classified into a plant, animal and human virus on the basis of their effect.
- **Resistance:** These are resistant to many drugs or antibiotics.
- **Nutrition Type:** Parasitic.
- **Chlorophyll:** Absent.
- **Reproduction:** Viruses replicate inside the host via lytic and lysogenic replication cycle.
- **Absorption:** Its absorption is through tail fibres.
- **Nature:** Viruses only share a parasitic relationship with other organisms.
- **Morphology:** Simple.
- **Carotenoids:** Absent.

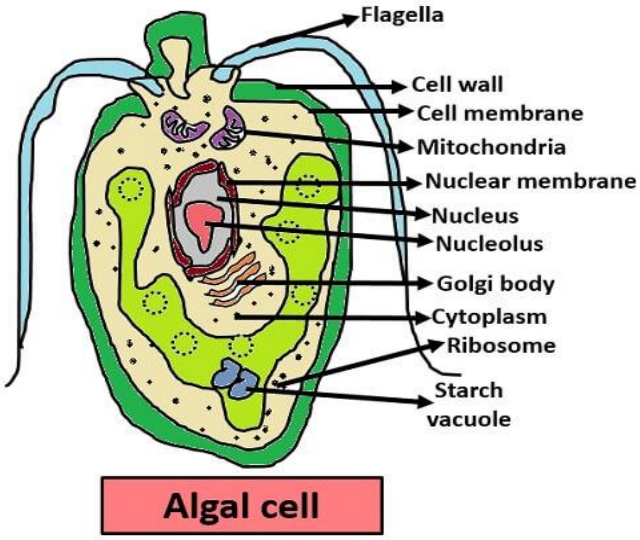


Different forms of viruses

Algae: They include multicellular, photosynthetic organisms such as Spirogyra, Chlamydomonas, etc.

- **Cell properties:** Algae are eukaryotic and it can be unicellular, multicellular or colonial.
- **Size:** It ranges from 1.0 µm to several feet.
- **Shape:** Algae exist in variable shapes or irregular structure.
- **Distribution:** Worldwide.
- **Habitat:** Freshwater, marine water, brackish water, and moist soil etc.
- **Movement:** They move through their flagella.
- **Nucleus:** True nucleus present.

- **Genetic material:** DNA.
- **Types:** Euglenoids, golden-brown algae, fire algae, green algae, red algae, brown algae and yellow-green algae are common types.
- **Resistance**: Some are resistant to radiation.
- **Nutrition type**: Autotrophic.
- **Chlorophyll:** Present.
- **Reproduction:** Algae reproduce by vegetative, asexual and sexual reproduction. A vegetative method includes budding, binary fission, through hormogonia etc. An asexual method includes reproduction through spores like zoospores, aplanospores, tetraspores, akinetes, exospores, endospores. A sexual method includes gamete fusion such as autogamy, hologamy, isogamy, anisogamy and oogamy.
- Absorption: Algae prepare food by their own in the presence of sunlight and chlorophyll i.e. through photosynthesis.
- **Nature:** Heterotrophic and parasitic in nature.
- **Morphology:** Complex.
- **Carotenoids:** Present.
- **Oxygen requirement:** Algae can be aerobic or anaerobic.



3. Thermodynamic Principles in Microbiology

3.1 Concept of free energy:

Two fundamental laws of thermodynamics:

- **The first law** is the principle of the conservation of energy: for any physical or chemical change, the total amount of energy in the universe remains constant; energy may change form or it may be transported from one region to another, but it cannot be created or destroyed.
- The **second law** of thermodynamics, which can be stated in several forms, says that the universe always tends toward increasing disorder: in all natural processes, the entropy of the universe increases.

Gibbs free energy, G

Gibbs free energy expresses the amount of an energy capable of doing work during a reaction at constant temperature and pressure. In thermodynamics, the **Gibbs free energy** (or **Gibbs energy**) is a thermodynamic potential that can be used to calculate the maximum reversible work that may be performed by a thermodynamic system at a constant temperature and pressure .When a reaction proceeds with the release of free energy (that is, when the system changes so as to possess less free energy), the free-energy change has a negative value and the reaction is said to be exergonic. In endergonic reactions, the system gains free energy and gibbs free energy (ΔG) is positive.

3.2 Entropy, S

Entropy is a quantitative expression for the randomness or disorder in a system. When the products of a reaction are less complex and more disordered than the reactants, the reaction is said to proceed with a gain in entropy.

3.3 Enthalpy, H

It is the heat content of the reacting system. It reflects the number and kinds of chemical bonds in the reactants and products. When a chemical reaction releases heat, it is said to be exothermic; the heat content of the products is less than that of the reactants and ΔH has, by convention, a negative value. Reacting systems that take up heat from their surroundings are endothermic and have positive values of ΔH.

Units

The units of ΔG and ΔH are joules/mole or calories/mole (1 cal = 4.184 J); units of entropy are joules/ mole·Kelvin (J/mol·K).

- Under the conditions existing in biological systems (including constant temperature and pressure), changes in free energy, enthalpy, and entropy are related to each other quantitatively by the equation

  Δ G =ΔH - T ΔS[Δ G =change in Gibbs free energy of the reacting system

  Δ H = change in enthalpy of the system,

  T = absolute temperature

  ΔS = change in entropy of the system]

- ΔS has a positive sign when entropy increases
- ΔH has a negative sign when heat is released by the system to its surroundings.
- . Either of these conditions, which are typical of energetically favourable processes, tend to make ΔG negative. In fact, **ΔG of a spontaneously reacting system is always negative.**

3.4 Energy Rich Bonds:

Energy rich compounds in cells comprise five kinds of high energy bonds-

1. Phosphoanhydride
2. Acyl Phosphate
3. Enolphosphate
4. Guanidine phosphate
5. Thioester bonds

Phosphoanhydride Bond

Phosphoanhydride bond is formed between two molecules of phosphoric acids.These types of bonds we can typically found in nucleotides. Typical representative of a high energy compound with phosphoanhydride bond is adenosine triphosphate or ATP. In this compound, are two high energy diphosphate bonds (phosphoanhydride bonds).The third phosphate bond between phosphate and ribose is not energy rich bond, basically it is phosphate ester bond.

Similar diphosphate bonds are present in all di- and triphosphates of purine and pyrimidine nucleosides.Energy of diphosphate bonds has a significant impact in metabolism of a cell.ATP molecule serves as the principal donor of free energy in biological systems (Such as in most endergonic reactions of the cell, in the active transport of molecules across the membrane, muscle contraction, transmission of nerve impulse, and certain other processes that requires energy).ATP is also the principal donor of energy or the source of energy for some metabolic pathways.

Apart from ATP, the energy of diphosphate bonds of another nucleotides such as GTP (donor of energy in gluconeogenesis),UTP (important in the metabolism of saccharides) and CTP (in the metabolism of lipids) are also used in various biological processes.

Acyl phosphate bond

Acyl phosphate bond is formed by the reaction of carboxylic acid with phosphate group. This type of bond present in 1,3 bisphosphoglycerate and is formed in the glycolysis and can also transferred from this compound to ADP to form ATP. This type of high energy bond is formed also in the activation of fatty acids when these react with ATP.

Enolphosphate bond

This type of bond is formed when phosphate group is attached to the hydroxyl group which is bounded to carbon with double bond. This bond is energetically richest bond as in hydrolysis of this bond approximately 61 KJ /mol bond energy is liberated.

Such bond is present in phosphoenol pyruvate which is formed during glycolytic pathway in the breakdown of glucose. This energy rich bond can be transferred by means of kinase to ADP to form ATP. This process can be called as the phosphorylation of substrate.

Guanidine phosphate bond

This type of bond is formed if phosphate group is attached to guanidine group. Phosphocreatine is one of the important compounds in muscle cells.

In some animals, as a storage form of energy is arginine phosphate where phosphate group remains bound to guanidine group of arginine.

Thioester Bond

It can not be classified as typical high energy bond because here is not energy rich phosphate, but here is acyl rest of carboxylic acid attached to sulphur from –SH group. Thioesters are the product of esterification between a carboxylic acid and a thiol. In cells, this type of bond is formed when the rest of the carboxylic acid is attached to coenzyme A. This acid is by this way activated and can enter the different chemical reactions without the supplement of energy.

Table: Energy bonds

TYPES OF ENERGY BOND	COMPOUND	BOND ENERGY IN kJ/mol
Phosphoanhydride	ATP	30.5
Enol phosphate	Phosphoenol pyruvate	61
Acyl phosphate	1,3 bisphosphoglycerate	49
Guanidine Phosphate	Phosphocreatine	43
Thioester	Acetyl CoA	41

3.5 Chemical Potential

In thermodynamics, the **chemical potential** of a species is the energy that can be absorbed or released due to a change of the particle number of the given species, e.g. in a chemical reaction or phase transition. The chemical potential of a species in a mixture is defined as the rate of change of free energy of a thermodynamic system with respect to the change in the number of atoms or molecules of the species that are added to the system. Thus, it is the partial derivative of the free energy with respect to the amount of the species, all other species' concentrations in the mixture remaining constant. The molar chemical potential is also known as **partial molar free energy.**

Under the most common thermodynamic condition of constant temperature and pressure, chemical potential determines the stability of substances, such as chemical species, compounds, and solutions, and their tendency to chemically react to form new substances, to transform to new physical states, or to migrate from one spatial location to another.

Chemical potential is **a measure of the capability of a substance to cause either a chemical or electrochemical reaction in its environment**, due to its internal chemical energy or external energy. In a chemical system, it is a measure of disequilibrium of reaction products and reactants.

3.6 Membrane Potential

Membrane potential (also called transmembrane potential or membrane voltage) is the difference in electric potential between the interior and the exterior of a biological cell.

For the exterior of the cell, typical values of membrane potential, normally given in units of millivolts and denoted as mV, range from-70 or –80 mV to –40 mV.

All animal cells are surrounded by a membrane composed of a lipid bilayer with proteins embedded in it.The membrane serves as both an insulator and a diffusion barrier to the movement of ions. Transmembrane proteins (also known as ion transporter or ion pump proteins) actively push ions across the membrane and establish concentration gradients across the membrane. Simultaneously, ion channels allow ions to move across the membrane down those concentration gradients.Ion pumps and ion channels are therefore responsible for creating a voltage between the two sides of the membrane.

Almost all plasma membranes have an electrical potential across them, with the inside usually negative with respect to the outside.

In non-excitable cells, and in excitable cells in their baseline states, the membrane potential is held at a relatively stable value. It is called as the resting potential(A relatively static membrane potential which is usually referred to as the ground value for trans-membrane voltage).

The opening and closing of ion channels can induce a departure from the resting potential. Two states can be defined in this regard, which are-

- Depolarization-If the interior voltage becomes less negative (as for example if the resting potential changes from –70 mV to –60 mV or -30 mV).
- Hyperpolarization- If the interior voltage becomes more negative (say from –70 mV to –80 mV), then it is called hyperpolarized.

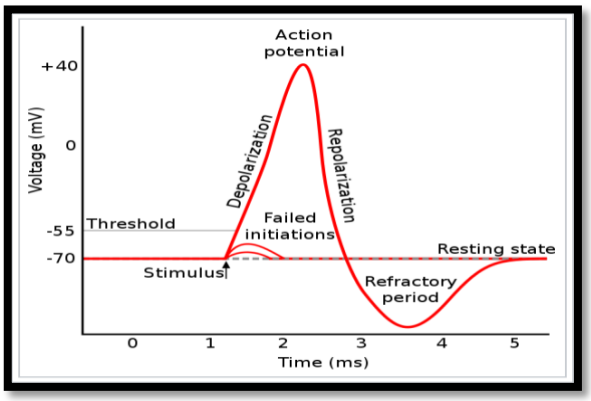
  In excitable cells, a sufficiently large depolarization can evoke an action potential, in which the membrane potential changes rapidly and significantly for a short time (Action potential occurs in excitable cells

  such as neurones, muscle cells etc.). Action potentials are generated by special types of voltage gated ion channels which remain embedded in the plasma membrane of cell.

  Simply it can be said that differences in the concentrations of ions on opposite sides of a cellular membrane lead to a voltage called the **membrane potential [**Many ions have a concentration gradient across the membrane, including potassium (K^+), which is at a high concentration inside and a low concentration outside the membrane. Sodium (Na^+) and chloride (Cl^-) ions are at high concentrations in the extracellular region, and low concentrations in the intracellular regions. These concentration gradients provide the potential energy to drive the formation of the membrane potential. This voltage is established when the membrane has permeability to one or more ions].So, he resting membrane potential is determined mainly by two factors-

❖ The differences in ion concentration of the intracellular and extracellular fluids.

❖ The relative permeabilities of the plasma membrane to different ions.



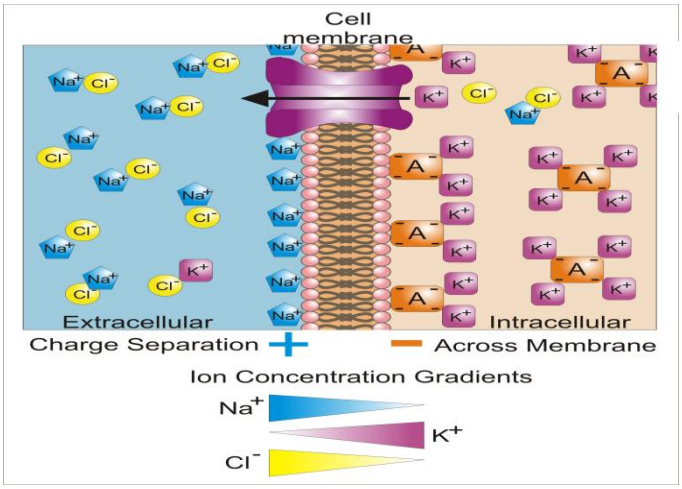


Fig: Membrane potential

3.7 Diffusion Potential

In general, diffusion can be defined as the net movement of anything (as for example, atoms, ions, molecules, energy etc.) generally from a region of higher concentration to a region of lower concentration.

A diffusion potential is the potential difference generated across a membrane when a charged solute (an ion) diffuses down its concentration gradient. Therefore, a diffusion potential is caused by diffusion of ions.

A diffusion potential can be generated only if the membrane is permeable to that ion. Furthermore, if the membrane is not permeable to the ion, no diffusion potential will be generated no matter how large a concentration gradient is present.

The **magnitude** of a diffusion potential is measured in millivolts (mV).The magnitude of diffusion potential depends on the size of the concentration gradient, where the concentration gradient is the driving force.The sign of the diffusion potential depends on the charge of the diffusing ion.

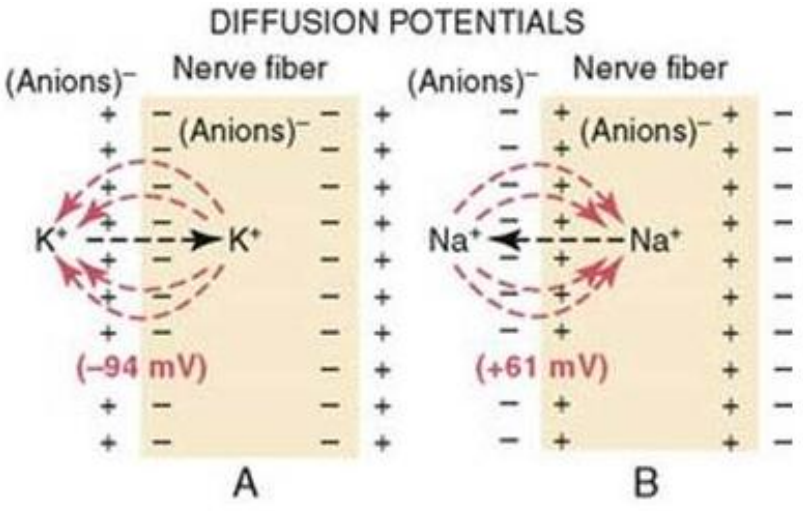


Figure: Diffusion Potentials.

4. Bacterial morphology

4. Bacterial morphology:

Bacterial cells are varied according to their size, shape, arrangement of flagella, cell structure, etc.

4.1 Size: The bacterial cell varied greatly according to their size. The average range of diameter is from 0.5 µm- 2.0 µm. The smallest bacterial cell is Mycoplasma pneumoniae size 0.2 µm and the largest bacteria size is 750 µm of *Thiomargaritanamibiensis.* There are different bacteria varies in their size, these are as follows:

Table: Different sizes of bacteria

Bacteria		Size
Thiomarguritanamibiensis	:	750 µm
Escherichia coli	:	1 -2 µm
Clostridium botulinum	:	3.8 µm
C. tetani	:	2-5 µm
Mycobacterium tuberculosis	:	0.5-4 µm
Salmonella typhi	:	0.5-4 µm
*Staphylococcus*sp	:	0.8 µm
Streptococcus pneumoniae	:	1.25 µm
Corynebacterium diphtheriae	:	1-8 µm
Mycoplasma pneumoniae	:	0.2 µm

4.2 Shape: The bacterial cell varies greatly in shape. There are different kinds of shapes of bacteria like spherical, rod-shaped or bacilli, spiral, etc. these are as follows:

a) **Spherical or coccus bacteria:** The spherical or elliptical bacteria are called cocci. The diameter is of those cells is 0.5-1.25 µm, without any flagella, they are usually nonmotile. They occur in a single or group in a different orientation.

- **Micrococci:** The single coccus cell is known as micrococci. E.g., *Micrococcus nigra, M. lutens* etc.
- **Diplococcus:** When the cocci occur in pairs is referred to as diplococci. E.g.: *Diplococcus pneumoniae.*
- **Tetracocci:** When the cocci form a fore cell group is referred as tertacocci. e.g.: *Pedicoccus cerevisiae.*
- **Staphylococci:** When the spherical bacteria form an irregular group is referred to as staphylococci. E.g.: *Staphylococcus aureus.*
- **Streptococci:** When the cocci cells occur in the long-chain are referred to as streptococci. E.g.: *Streptococcus lactis.*

- **Sercinae:** When the spherical bacteria are as a cube-like structure is called sarcinae form. E.g.: *Sarcina ventriculi, S. lutea.*

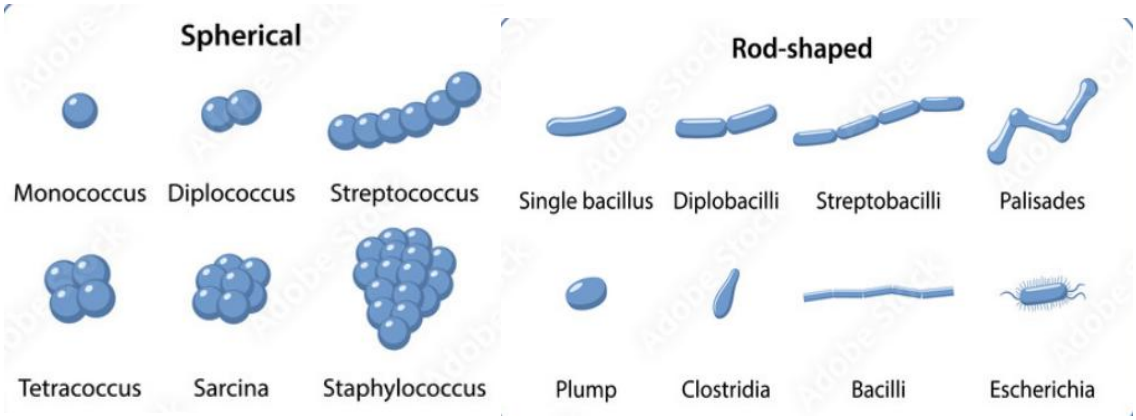


Figure: Different spherical and rod-shaped shaped cells of bacteria.

b) **Rod-shaped or bacilli bacteria:** when the bacterial cell is cylindrical, rod-shaped in appearance is referred to as bacilli. They are motile or nonmotile, rod narrow or long, and have blunt or round ends. The cells that occur are single or group. These are as follows:

- **Bacillus:** When the rod-shaped bacilli occur in singly is known as bacillus. E.g.: *Lactobacillus* sp.
- **Diplobacillus:** When the rod-shaped bacteria occur in pairs is known as diplobacillus. E.g.: *Corynebacterium diphtheriae.*
- **Streptobacillus:** when the bacilli occur in the chain is referred to as streptobacillus. E.g.: *B. tuberculosis.*

C) **Spiral bacteria:** Large spiral coil cell is referred to as spiral which has more than one turn of a helix one or more flagella at each pole. E.g.: *Spirillum minus.*

Figure: Different spiral-shaped bacterial cells.

Vibrio: Cylindrical, curved like a coma-shaped cell with flagella. E.g.: *Vibrio cholerae.*

Filamentous: Some bacteria are filamentous. E.g.: Thiothrix sp.

Pleomorphic: Some bacteria can change their size and shape according to their surrounding environment is called pleomorphic. E.g.: Mycoplasma sp

4.3 Flagella: Some bacterial cells bear a hair-like structure on the cell surface for locomotion movement which is referred to as flagella.

These are surface appendages 20-30 nm in diameter and about 15 µm in length. Based on the number and arrangement of flagella the bacteria may be atrichias i.e., no flagella on the surface, others are as follows:

Atrichias: The bacteria without any flagella. E.g.: *Lactobacillus* sp

Monotrichous: Bacteria with one flagellum at one pole. E.g.: *Vibrio cholerae.*

Lophotrichous: Bacteria with many flagella at one pole. E.g.: *Spirillum*

Amphitrichous: Bacteria with many flagella on two poles. E.g.: *Pseudomonas.*

Peritrichous: Bacteria with many flagella throughout the body surface. E.g.: *Proteus* sp.

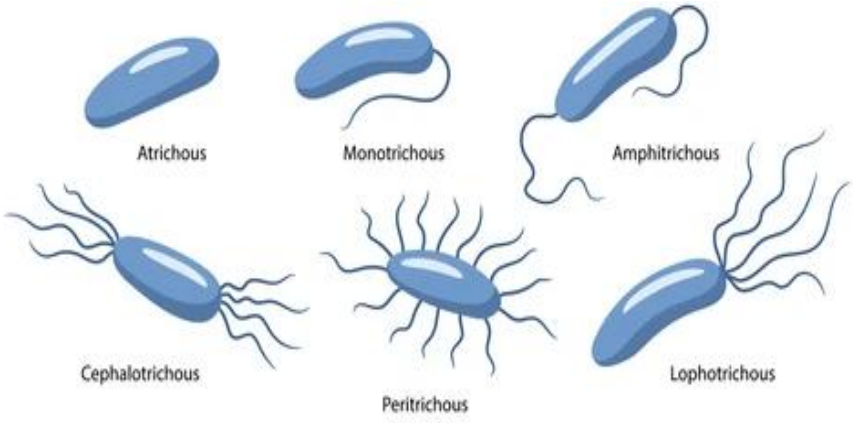


Figure: Arrangement of bacterial flagella.

Flagella structure: A flagellum consist of a basal body, hook, and filament.

Basal body: The basal body is the basal part of flagella by which it is attached to the cell wall and cell membrane. It is composed of the central rod which is inserted through two pairs of rings- A proximal pair and distal pair.

In gram-negative bacteria, the proximal pair of rings are M-ring (membrane), S ring (surface). The distal pair are P-ring (peptidoglycan) and L-ring (Lipopolysaccharide).

M-ring is anchored in the membrane, S-ring is located on the surface, P-ring is anchored in peptidoglycan layer and L-ring is attached to lipopolysaccharide layer. L-ring is absent in gram-positive bacteria. Later it was found that the M ring and S ring are comprised of the same protein and work together as same domain and another ring is present in the cell is C-ring (Cytoplasmic ring), which consists of three proteins FilG, FilM, and FilN. The rotor and stator are produced in the motor complex. In gram-negative bacteria, the rotor consists of MS ring and C ring, and the stator consists of MotA and MotB which form a channel in the plasma membrane for proton movement.

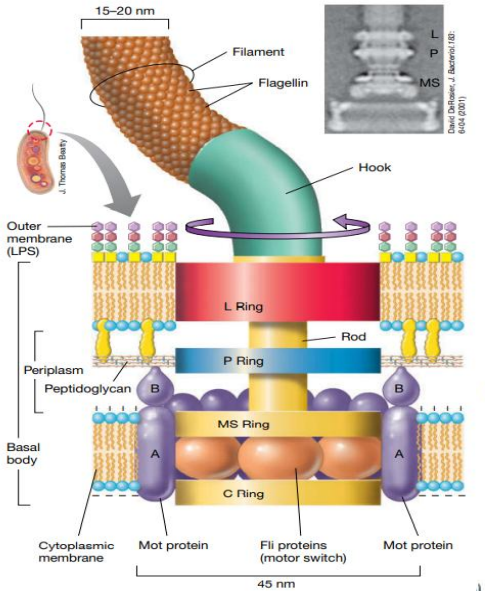


Figure: Structure of Flagella.

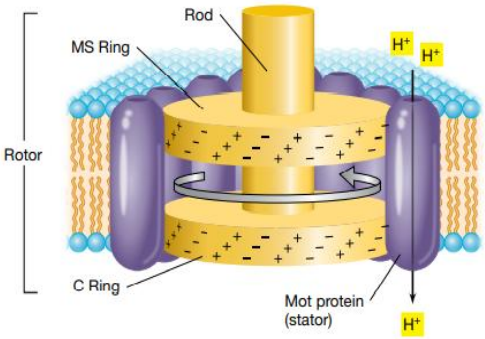


Figure: Function of rotation by Mot proteins.

Hook: The hook connects the filament with the basal body. It is comprised of protein. It is longer in gram-positive but short in gram-negative.

Filament: The main elongated part of flagella is filament; it is made up of a globular protein called flagellin. The flagellin is aggregated into the chain. The flagellin chain is now helically coiled thus forming a hollow tube-like structure with a central hollow core.

At pH 3-4 flagella break into flagellin but at pH 5-6, flagellin unite to form flagella

Endo flagella: In some bacteria have Endo flagella, which are located in outer periplasmic space, i.e., in the space between the cytoplasmic membrane and outer membrane. E.g., *Spirochaete* sp.

The function of flagella: Flagella serve in locomotion in trophic movement in bacteria. The locomotion movement takes place by rotation of flagella, the rotation is about 40 rpm.

4.4 Pili: Bacterial cell surface provides with some short fibrous structure which is referred to as pili. Pili is mainly served in conjugation. It is a thin appendage-like structure found on the surface of the bacterial cell. The pili are made up of a small protein called pilin.

Type of Pili: Ottow in 1975 classified pili or fimbriae into six group

Group I: Peritrichous 300/cell

Group II: Sex pili 2-10 per cell. Serve in conjugation.

Group III: Thick hollow tube up to 10 per cell.

Group IV: Flexible, Polar 2 per cell

Group V: Contractile, Serve in Conjugation

Group VI: Short with the antigenic property.

Structure: F-pili is a tube-like structure with a terminal knob made up of globular proteins which are called pilin. Pilin proteins are aggregated and form a chain this chain is helically coiled and form a hollow structure with a central core.

Function: Pili help in conjugation to the formation of conjugation tube; Pili is the receptor for many viruses etc.

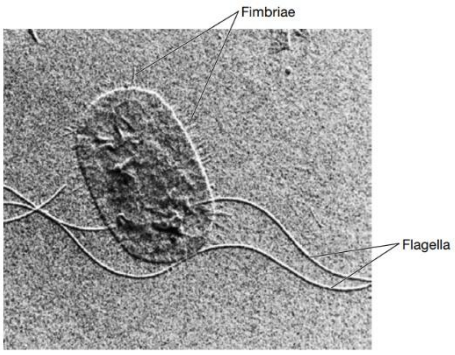


Figure: Flagella and pili.

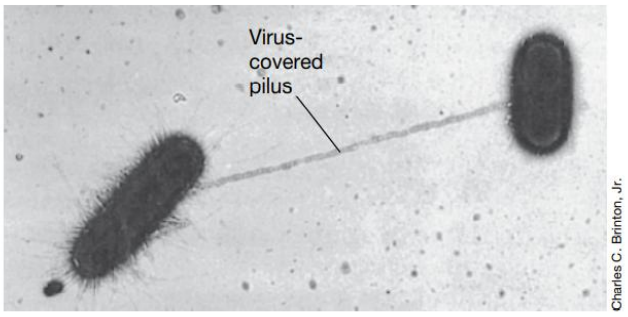


Figure: Pilus undergo conjugation.

4.5 Fimbriae: The filamentous appendages are present on the bacteria surface are known as fimbriae. It is found in both gram-positive and gram-negative bacteria. The fimbriae are produced by small helically coiled proteins. It is present up to 1000 per cell.

Function: It helps to attach the bacterial cell to the surface.

4.6 Capsule: Some bacterial cells are surrounded by an extracellular gelatinous material around the cell wall which is referred to as a capsule. It is well organized and can not be easily washed out. E.g.: Streptococcus pneumoniae. Sometimes it is called the slime layer which is unorganized loosely associated and can be washed out.

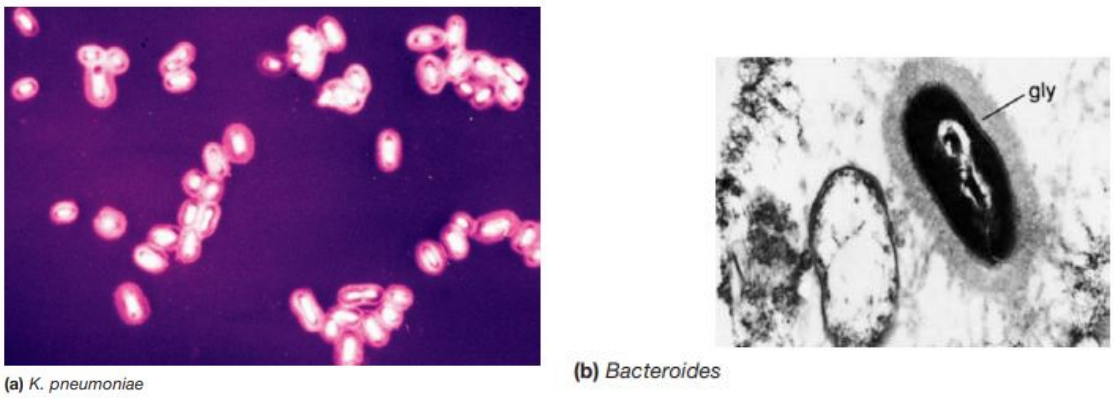


Figure: Bacterial capsule.

Organization: Capsule or slime layer is made up of either polysaccharide (Klebsiella Pneumoniae) or polypeptide (Bacillus arthrosis) or both the polysaccharide may be homopolysaccharide (Acetobacter xylinum) or heteropolysaccharide (Pseudomonas aeruginosa) The capsule is species-specific and differs in different species.

Function: Capsule serve in different function these are as follows:

1) It protects the bacterial from phagocytosis by macrophage, thus it is pathogenic and pathogenicity depends on the capsule.
2) It protects the bacterial cell from desiccation because it is hygroscopic and maintains viscosity which also can absorb nutrients for the bacterial cell.
3) Capsule is sticky thus bacteria can grow on a different smooth surface.

4.7 Cell wall or peptidoglycan layer: Bacterial cell is surrounded by a well which is made up of three main chemicals i.e., a. Peptidoglycan, b. Lipopolysaccharide c. Teichoic Acid, in addition to this protein, lipoprotein and phospholipid are also found.

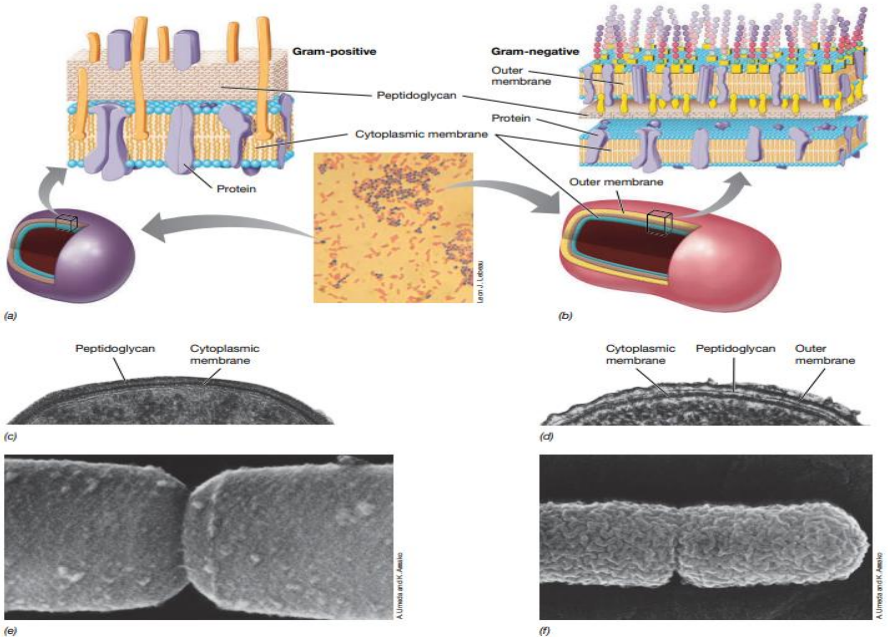


Figure: Structure of bacterial cell wall

a) **Peptidoglycan:** It is the main cell wall component which is 90-95% of the dry weight of cell wall in gram-positive bacteria but in gram-negative bacteria it is very thin and about 5-10%.

Peptidoglycan consists of three parts:

i) **Heteropolymer chain of amino glucose:** This chain has consisted of N acetyl glucose amin (NAG) alternated with N acetyl muramic acid (NAM).

ii) **Tetrapeptide side chain**: Each muramic acid is linked with a tetrapeptide side chain consisting of L-alanine, D-glutamine, L-lysine, D-alanine.

iii) **Pentapeptide cross-bridge:** Two tetrapeptides of two parallel chain is connected by a cross bridge. In gram-positive bacteria, the tetrapeptide chain is joined by the glycine side chain of D-alanine with L-lysine. In gram-negative bacteria, the tetrapeptide side chain is joined by D-alanine with diamino-pimelic acid (DAP).

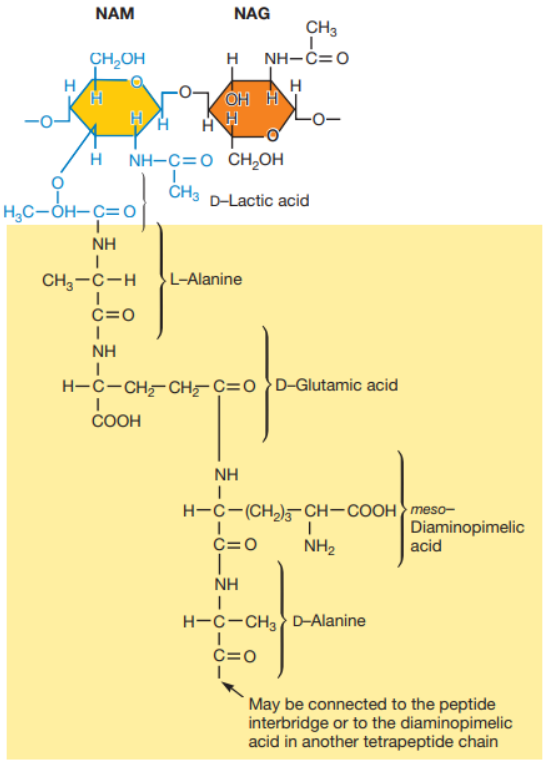


Figure: Composition of peptidoglycan.

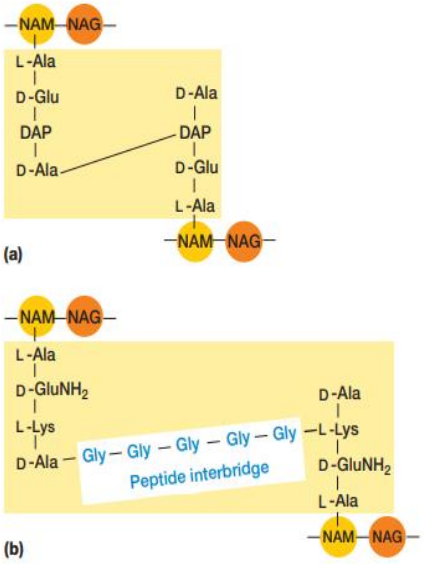


Figure: Cross-link Peptidoglycan.

The glycoside bond in between N-acetylglucosamine and N-acetylmuramic acid is hydrolyzed by lysozyme but in Streptomyces aureus is resistant to lysine because they have an o-acetyl group on carbon 6 of some muramic acid.

b) **Lipopolysaccharide:** Lipopolysaccharide from the outer wall or outer membrane of gram-negative bacteria. It consists of a polysaccharide core, polysaccharide side chain, and lipid-A. Polysaccharide core contains glucose, galactose, etc. Polysaccharide side chains contain many glucans which have antigenic properties. Lipid-A is a hydrocarbon.

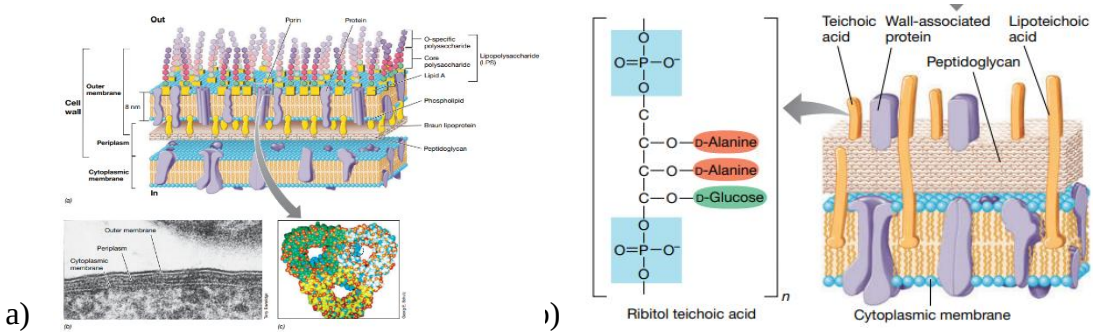


Figure: a) Structure of gram-negative and b) gram-positive bacterial cell wall.

The outer membrane is anchored with peptidoglycan by Braun's lipoprotein which has toxic property is known as endotoxin. The lipid-A is responsible for toxicity.

c) **Teichoic acid**: It is the polymer of polyhydric alcohol phosphate e.g., glycerol phosphate in *Bacillus*; ribityl phosphate in streptomyces aureus.

d) **Protein:** Outer membrane or lipopolysaccharide (LPS) of gram-negative bacteria have porin protein which is a non-selective pore.

e) **Mycolic acid**: Mycolic acid is found in acid-fast bacteria, e.g., *Mycoplasma* sp.

4.8 Plasma membrane:

Definition: Internal to call wall there is a membrane which is the boundary of a cell separated cytoplasm and E.C.M (Extracellular metric) which are referred as the plasma membrane or cell membrane or cytoplasm membrane. [E.C.M= Extracellular metric is the material of the cell outside plasma membrane is gram $^{+ve}$ bacteria, in gram $^{-ve}$ bacteria it is cell wall outer membrane and periplasmic space, in the animal cell it is glycocalyx and adhesive protein.

Usually, plasma membranes are located just below the cell wall at a distance of 9 nm, but at some places, the membrane invaginates inward and forms a mesosoma. Sometimes they produce vesicle or lamellar vesicles. Lamellar vesicle is found in *Nitrobacter*, a vesicle is found in *Chromatium* sp.

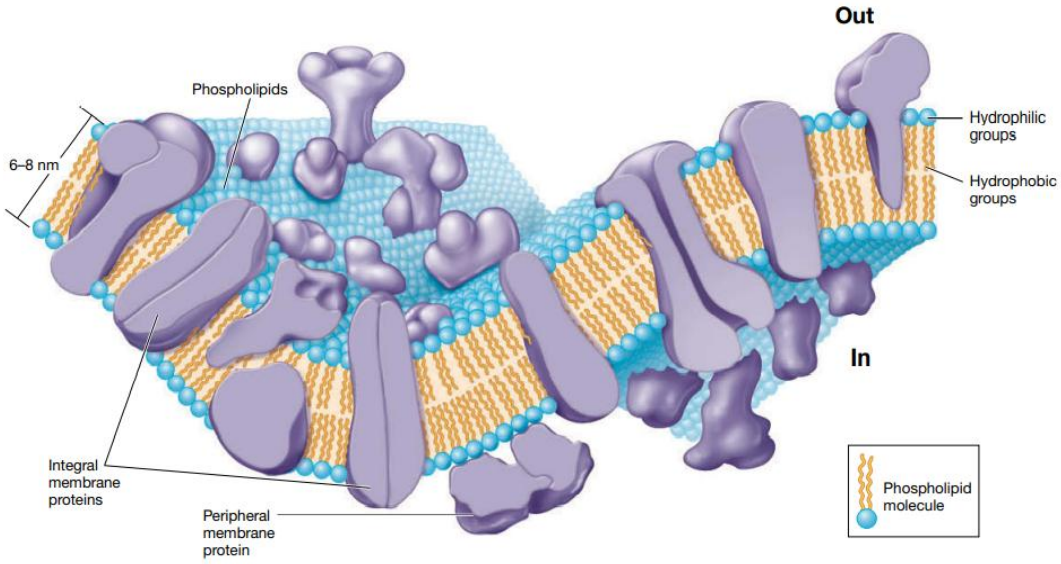


Figure: Structure of plasma membrane or cytoplasmic membrane.

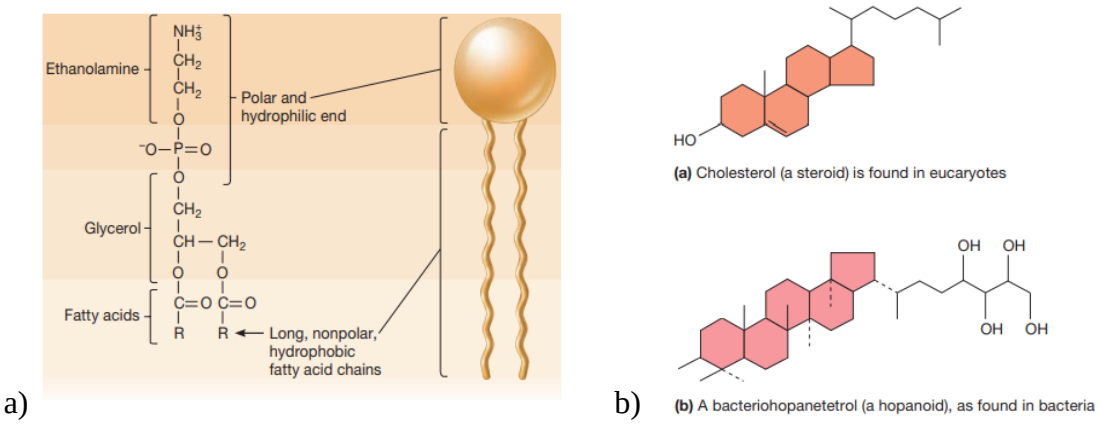
Structure: - The unit membrane structure is a lipid bilayer attached with some protein. Jonathan Singer and Garth Nicolson 1972 proposed a fluid mosaic model of membrane structure. It is comprised of a lipid fluid and solid protein mosaic. Lipid in fluid consistency state to permed another membrane component to make laterally or flipping. The protein part occurs as a mosaic of discontinuous particles. The protein part may be intrinsic or extrinsic. The extrinsic protein part is

loosely attached by an ionic bond or calcium bridge. Intrinsic proteins are embedded in the lipid bilayer.

Chemistry or chemical structure: -The lipid mainly consists of phospholipid and glycolipid; some members have sterol and hopanoid.

Phospholipid: -It is the main lipid of lipid bilayer where glycerol is esterified with two fatty acids and phosphatidic acid. Phosphatidic acid is again linked with amino acid, alcohol, etc.

Glycolipid: -Lipid attached with glycan is referred to as glycolipid.



a) b)

Figure: a) Structure of Phosoholipid, Phosphatidylethanolamine, b) Structure of steroid or hopanoid.

Sterol: - Sterol is a diterpenoid it is found in mycoplasma (it has no cell wall) or PPLO (Pleuro pneumonia-like organism).

Hopanoid: - Some bacteria have hopanoid which has some function as a sterol.

Protein: - Based on chemistry the membrane protein is grouped into three categories integral protein (Transmembrane protein), surface protein, and lipid anchored protein.

Integral Protein: - The protein which penetrates lipid bi-layer is referred to as an integral protein. It may consist of three domains (functional part). There is a transmembrane domain, E.C.M domain, cytoplasmic domain.

Surface protein: -The protein which occurs outside the lipid bi-layer and is attached with the noncovalent bond is referred to as a surface protein.

Lipid anchored protein: -The protein is located outside the lipid bilayer but associated with the covalent bond.

Function: -Membrane serve in many purposes these are as follows-

1) It serves as a boundary between cytoplasm and E.C.M thus protecting cytoplasm from the external environment.
2) Bacterial membrane provides with all respiratory requirements i.e.; E.T.C (Electron transport chain) and A.T. pose.
3) All other syntheses are localized in the membrane.
4) Membrane has all types of the transporter (protein) thus they can transport different types of ions, food sources, amino acid, growth factors.
5) A.T.P. production takes place on membranes because they provide with A.T. phase.
6) Mesozome is attached with DNA. where DNA replicates.
7) Mesozome divide two daughter cells by formation of the new wall.
8) Lamellar vesical provide with photosynthetic pigment thus serve in photosynthesis (Nitrobacter)
9) Vesical is linked with intracellular transport.

4.9 Ribosome: -

Prokaryote cells contain many small granular structures scattered in the cytoplasm which represents ribosomes. A cell has about 10,000 ribosomes.

A bacterial ribosome during function is of 70s type but in the cytoplasm, they existed us 2 subunit – 30s and 50s. During protein synthesis, they are united and form 70s ribosomes.

Structure: - According to A.lake 1981, the smaller subunit consists of a head, a base, and a platform. In between head and platform, there is a cleft.

50s subunit consists of a stalk, a central protuberance, and a ridge. In between ridge and central protuberance, there is a valley.

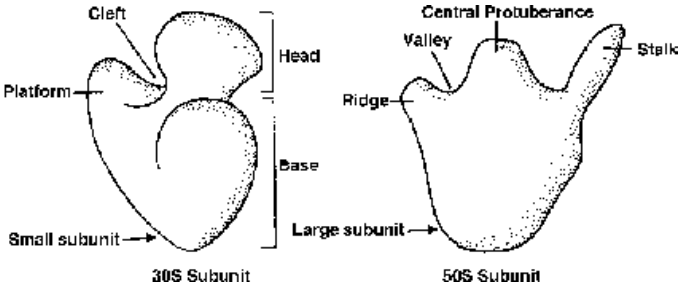


Figure: Structure of ribosome.

Chemistry (chemical structure): -

30s → 16s rRNA 1600 nucleotide

21 proteins (s1, s2, ...s21)

50s→ 5s rRNA –120 nucleotide.

23s rRNA – 3200 nucleotides. [S= Small and L = Large]

34 proteins (L1, L2, ...L34).

ribosome	subunit	rRNAs	r-proteins
70S	50S	23S (2904 nt)	31
		5S (120 nt)	
	30S	16S (1542 nt)	21

30s subunit consists of a single rRNA which is 16s rRNA made up of 1600 nucleotides and consists of 21 types of protein which are referred to as s1, s2, ...s21.

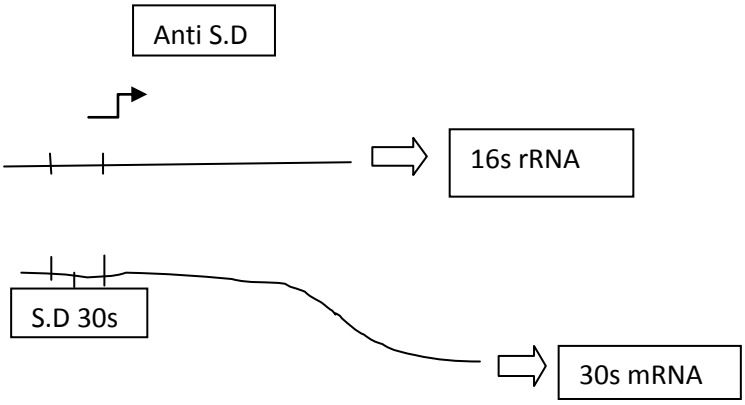
50s subunit is comprised of 2 types of rRNA, 5s rRNA of 120 nucleotides 235 rRNA of 3200 nucleotide and 34 types of protein which designated by L1, L2, ...L34.

S20 and L26 are similar thus there is a total of 54 types of protein (34+21-1=54).

Function: -

1) Ribosome is translational machinery on which protein is synthesized with quality control. It serves as a scaffold (platform) for the ordered instruction of all molecules involved in protein synthesis.

2) 16s rRNA of 30s ribosome have anti-S.D (Shine Dalgarno) sequence which is complementary to S.D mRNA thus It recognized mRNA.



3) Ribosome have three sites-

A—site (Aminoacyl site), a site recognized and whorled, aminoacyl tRNA

P—Site (Peptide site), site hold peptide, here peptide bond formation takes place.

E—Site (Exit site), site release free t-RNA after transpeptidation.

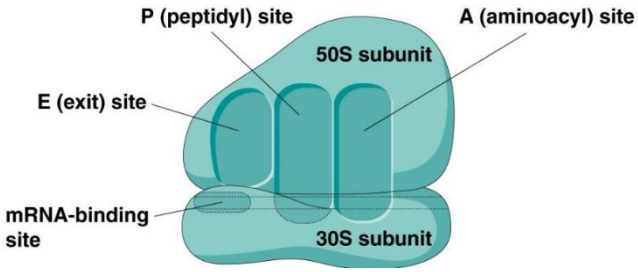


Figure: Sites of the ribosome.

4) 5s rRNA has a sequence that is complementary to the tΨCG loop of tRNA thus it attracts the particular loop.

5) A part of 23S serves as an enzyme (peptide) transfer as which is referred to as P.T.C. (Peptide transfer center), Rodnine 2007.

6) Ribosome acts attraction of translation independent amino acid (e.g., - Alanine, etc) and the quality control is done by the ribosome rqc2 (Ribosome quality control protein).

6) Codon, anticodon mismatch amino acid is releasing another quality control process which is referred to as proofreading.

4.10 Cytoplasmic inclusions and reserve materials

PHB: - Poly B-hydroxy butyric acid

CH3.CH2.CH2—COOH

ϒ ß α

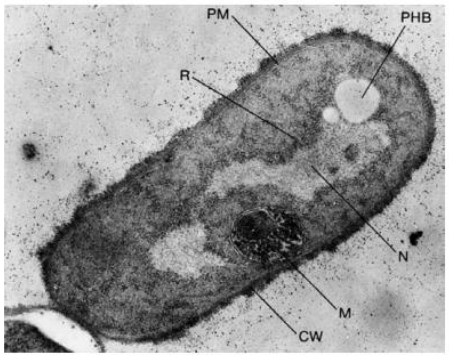
CH3--CH--CH2--COOH
|
OH—CH—CH2—COOH
|
CH3

Some lipid storage granules with a diameter of about 0.2 – 0.7 mm are found in the cytoplasm of some bacteria. e.g., Azotobactors, Mycobacterium, etc. Which are made up of polymer of B—Hydroxy butyric acid which is referred to as PHB. β-Hydroxy butyric are united by an ester bond.

Characteristic: -The granules are chloroform soluble they can be absorbed by Sudan dye or Nile blue. Within the cytoplasm, it occurs as granules. The electron microscope reveals it as a clear blue area. In *Bacillus*sp., it is about 60% of total dry weight when grown or acetate or butyrate.

Synthesis: - PHB is synthesized by condensation of acetyl s coA.

Function: - It is cell carbon reserve that is used as a source of energy during starvation or the absence of nutrients.

a) 

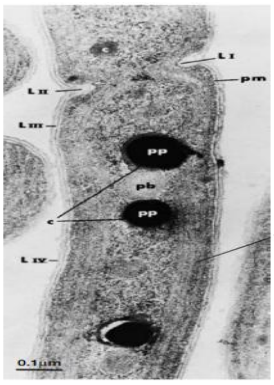
b) 

Figure: a) PHB granule, b) Volutin or polyphosphate (pp) granaule.

Volutin: -Volutin is polyphosphate granules found in the cytoplasm of some bacterial cells. It is a linear polymer of orthophosphate joint by ester bonds. It is found in bacteria algae, fungi, etc.

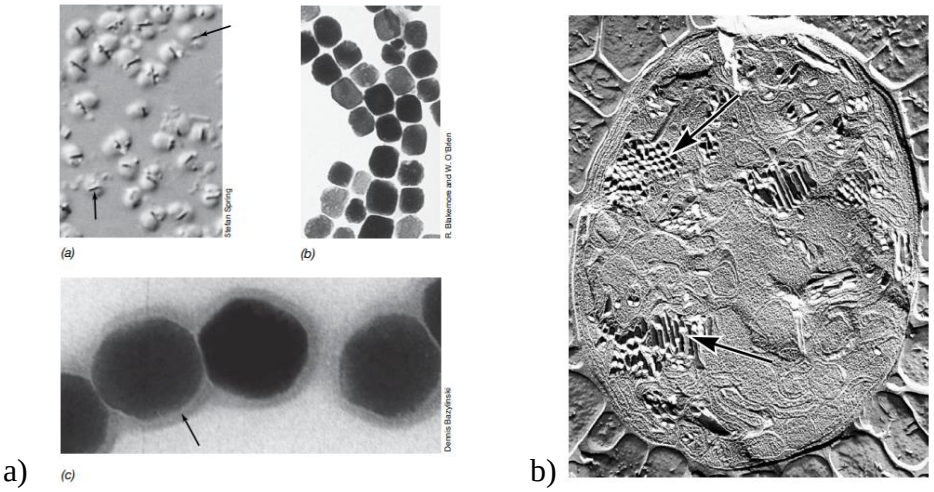
It is also referred to as metachromatic granules because when it is stained with methane blue it becomes red.

Function: - Bacteria produce ATP from volutin during the synthesis of nucleic acid and in absence of carbon nutrients.

Synthesis: The non-functional bacteria produce volutin from ATP.

Magnetosome: It is a membrane-bounded structure consisting of crystals of magnetic minerals surrounded by a lipid bilayer. It is present in magnetotactic bacteria they are mostly motile and aquatic. The magnetosome usually consists of the crystal of magnetite (Fe_3O_4) or greigite (Fe_3S_4). E.g., *Magnetospirillummagnetotacticum.*

Gas vesicle: It is a hollow spindle-like structure made of protein. Impermeable for water. Air can enter easily into the gas vesicle; it mainly stores air. It has a rigid structure of very low compressibility. It decreases the cell density and provides bouncy to the bacterial cell.



a) b)

Figure: a) Magnetosome. b) Gas vesicle and vacuole.

4.11 Plasmid: Extrachromosomal dispensable cytoplasmic circular DNA which is a self-replicated hereditary determinant carries many genes like resistance, toxin production, enzyme production, etc., and which can move from one cell to another cell and where they can recombine, these are referred to as plasmid.

Occurrence: They are usually found in bacteria and some eukaryotic cell organelles like chloroplast, mitochondria, etc. They may occur as one to many in a cell.

Character: The characters are as follow

a. Made up of circular DNA.
b. Smaller than chromosomal DNA.
c. Capable of autonomous replication.
d. Plasmids may be inserted in bacterial chromosomes when they are referred to as episome.
e. transferable from one bacterium to another bacteria cell or from one organelle to another organelle which is referred to as promiscuity.
f. They bear many genes like resistance, toxin production, etc.

Type: Plasmids are of various types according to the inherited character. E.g.: F-plasmid, R-plasmid, col-plasmid (colicine), Ti-plasmid (tumour-inducing plasmid), nif- plasmid, bt-plasmid (*Bacillus thuringiensis*).

Replication: Replication is semiconservative. Initiation is controlled by the plasmid gene, elongation and termination are controlled by the bacteria gene.

Importance: They are used in biotechnology as a vector for the production of a transgenic plant, animal, etc.

4.12 Bacterial chromosome: Bacterial chromosome is also referred genophore because they do not have chromatin.

It is a thousand times longer than a cell. A typical genophore consists of circular DNA, a small amount of RNA and protein are also found. However, a genophore consists of about 60% DNA which provides a cote from which 12-80 loops are radiated out (average 10 loops). The loops are sensitive to RNA which is a plcetonamic loop.

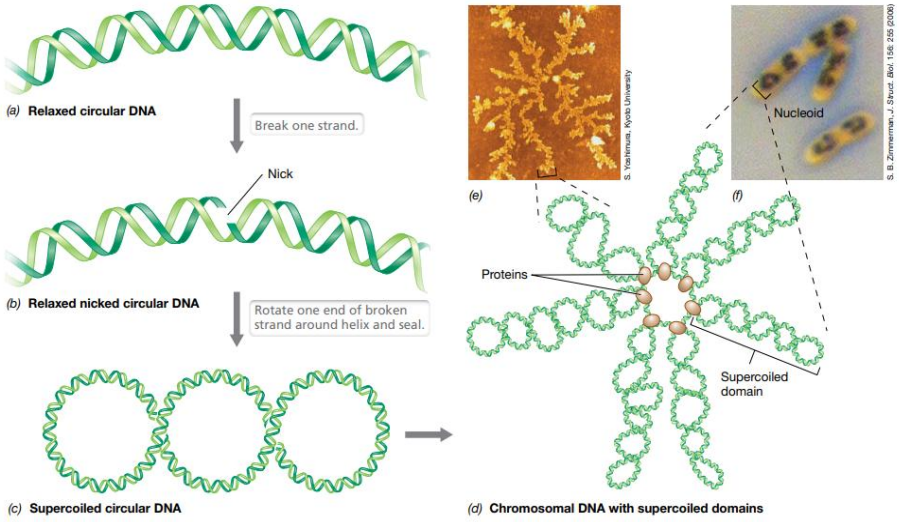


Figure: Bactial supercoil DNA

The loops are plectonamic organized into the superhelical domain. The DNA of the loops are highly compacted by a balance of force like supercoiling, protein, DNA-RNA interaction. An average loop contains 40 Mb DNA.

The number of genophores: A typical bacteria contains a single genophore but multiple genophores may be found in some bacteria. E.g.: *Rhodobacter spheroids.* Two chromosomes are found in *Bacillus militants.*

Replication: Circular genophore allow replication without telomere at ori point.

4.13 Endospore: Non-reproductive solitary resistance thick-walled single spore from in cell of some bacteria to tide over unfavourable condition is referred as endospore.

Shape and location: The endospore may be elliptical, spherical, or ovoid and it may be central, terminal, sub-terminal. E.g.: Central elliptical endospore is found in *Bacillus*sp., terminal spherical found in Clostridium tetani, Sub-terminal oval endospore found in *Clostridium subterminae.*

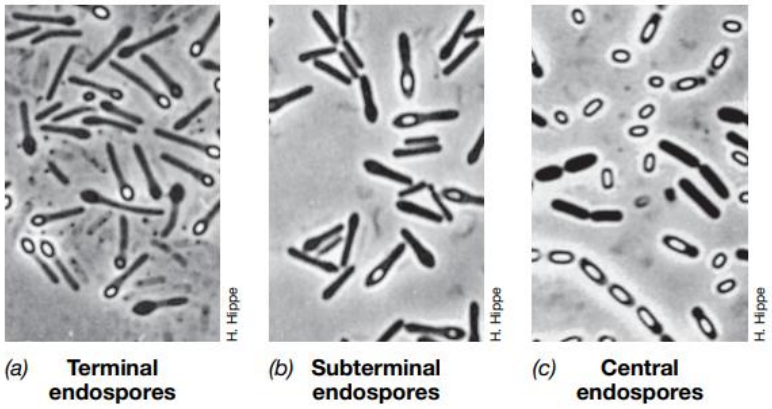


Figure: Types of endospore.

Endospore former: The bacteria which produce endospore is referred to as endospore former. It is found in Phylum: Firmicute which includes mainly gram^{+ve} bacteria and for gram^{-ve} bacteria.

Structure: The endospore structure is as follows:

- **Exosporium:** Exosporium is the outermost layer of the endospore. It is a thick multi-layered wall that is apparently loose.
- **Spore cote:** Below the exosporium, there is the middle layer which is referred to as spore cote.
- **Cortex:** Below the spore cote there is a thick layer of the cortex. The cortex is surrounded central core.
- **Core:** Below the cortex, there is a central core. The core is surrounded by a thin membrane called core membrane or core wall or intine. The central core is composed of DNA, ribosome, tRNA, dipicolinic acid, ca^{2+}, and enzyme, etc.

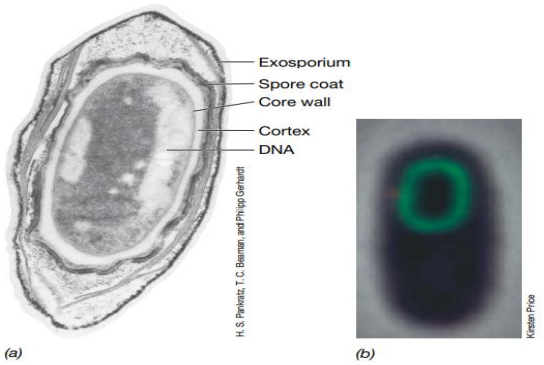


Figure: bacterial endospore structure.

Function: Endospore tide over the unfavourable condition and they germinate into the vegetative cell when favourable conditions return. They remain viable for up to 100 years.

Resistance property: Endospore is resistant to desiccation, high temperature, toxin, radiation staining, etc.

- **Desiccation resistance:** Endospore is desiccation resistant because of the low amount of water and presence of thick impermeable spore cote.
- **High-temperature resistance:** It is temperature resistance because the wall is dehydrated and it provides with ca^{2+} and dipicolinic acid complex. Which is 5-15% 0f dry weight.

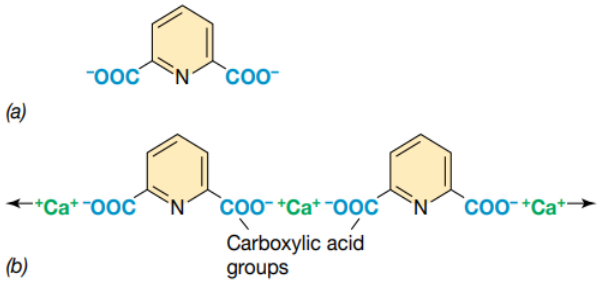


Figure: a) Structure of dipicolicic acid. b) Binding of dipicolinic acid with Ca^{2+}

- **Toxin:** Due to impermeable spore cote the toxin cannot enter it and it cannot stain but 'Schaeffer Fulton 'stain can enter.
- **Radiation resistance:** It is radiation resistance because it is highly refractile in nature because it provides SASP i.e., small acid-soluble protein. Moreover, DNA damage can be repaired because they have a DNA repairing enzyme system.

It is associated with food poisoning and infection however, it can be controlled by pasteurization.

Endospore formation: Endospore development was studied in Bacillus and Clostridium. Active growing phase the spore formation is stopped. The sporulation occurs due to a lack of nutrients an unfavourable condition. The formation of endospore is as follows:

- **Nucleoid division:** The first change during spore formation is the division of the nucleoid. The nucleoid produces an axial chromatin filament and divides into two nuclei one is large and another is small. After division, they migrate to the opposite pole.
- **Spore septa formation:** The membrane grows inward and formed spore septa. The spore septa enclose the chromosome and a part of the cytoplasm.

- **Fore spore formation:** The small nuclei are surrounded by spore septa is referred to as fore spore which ultimately develops into endospore. The large portion has surrounded the forespore and gradually increased its size which becomes highly refractive is called an endospore.

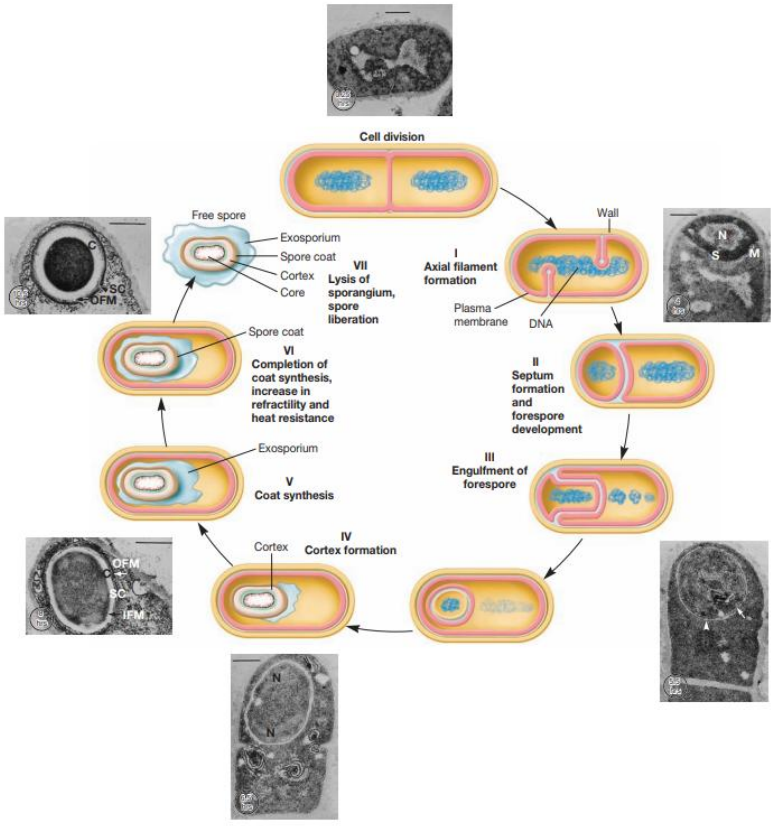


Figure: Formation of endospore of *Bacillus megaterium.*

Germination: When the favourable condition returns the endospore germinates into a vegetative cell. The germination of endospore is as follows:

- **Activation:** One or more factors like acidic pH, heat (60^0C for 1 hour), compound containing free SH group is helping to activation of the endospore.
- **Initiation:** Binding of a substance like L-alanine, adenosine on the medium activates the autolysin that destroys the peptidoglycan layer and the complex of ca^{2+} dipicolinic acid. Therefore, water ins enter, and ca^{2+} dipicolinc acid is released which are responsible for heat resistance.
- **Outgrowth:** The spore wall is swelling and the thick cortex layer is disintegration is helped to emerge the germ cell by breaking of spore cote. Thus, it develops in the vegetative cell.

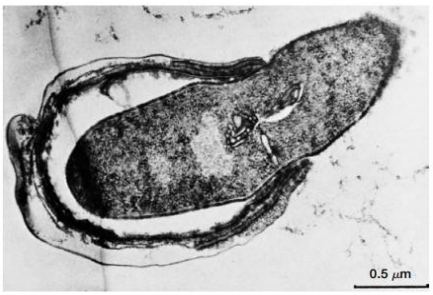


Figure: Germination of endospore and formation of a vegetative cell.

5. History of Development of Virology

5.1 Nature of Viruses

A virus is a genetic element that can multiply only inside a living cell, called the host cell. Not considered living entities, viruses are not found on the tree of life. Viruses rely on the host cell for energy, metabolic intermediates, and protein synthesis, and so they are obligate intracellular parasites. However, viruses possess their own nucleic acid genomes and in this sense are independent of the host's genome. Viruses infect cells in all three domains of life and are responsible for many infectious diseases of humans, plants, and other organisms. Although viruses are not cells, their genomes encode those functions needed to multiply and they have a structurally intricate extracellular form, called the virion; the virion allows the virus to travel from one host cell to another. Viruses cannot reproduce unless the virion itself, or in some cases its genome only, has gained entry into a suitable growing host cell, a process called infection.

Properties common to all viruses

1. Viruses have a nucleic acid genome of either DNA or RNA.
2. Compared with a cell genome, viral genomes are small, but genomes of different viruses range in size by over 100-fold (ca 3000 ntd to 1,200,000 bp)
3. Small genomes make small particles – again with a 100-fold size range.
4. Viral genomes are associated with protein that at its simplest forms the virus particle, but in some viruses this nucleoprotein is surrounded by further protein or a lipid bilayer.
5. The outermost proteins of the virus particle allow the virus to recognize the correct host cell and gain entry.
6. Viruses can only reproduce in living cells: they are obligate parasites.

Table: Comparison between Viruses and Bacteria

	Bacteria		
	Typical Bacteria	Rickettsias/ Chlamydias	Viruses
Intracellular Parasite	No	Yes	Yes
Plasma Membrane	Yes	Yes	No
Binary Fission	Yes	Yes	No
Pass through Bacteriological Filters	No	No/Yes	Yes
Possess Both DNA and RNA	Yes	Yes	No
ATP-Generating Metabolism	Yes	Yes/No	No
Ribosomes	Yes	Yes	No
Sensitive to Antibiotics	Yes	Yes	No
Sensitive to Interferon	No	No	Yes

5.2 Classification of Viruses

1. Classification on the basis of nucleic acid

Viral genome is DNA

i) Double stranded DNA virus: eg. Adenovirus, Herpesvirus

ii) Single stranded DNA virus: eg. Parvovirus, φ174 virus

Genome is RNA

i) Double stranded RNA virus: eg.Reo virus

ii) Single stranded RNA virus: these are further classified into two groups

- Positive sense RNA (+RNA): Polio virus, Hepatitis A
- Negative sense RNA (-RNA): Rabies virus, Influenza virus

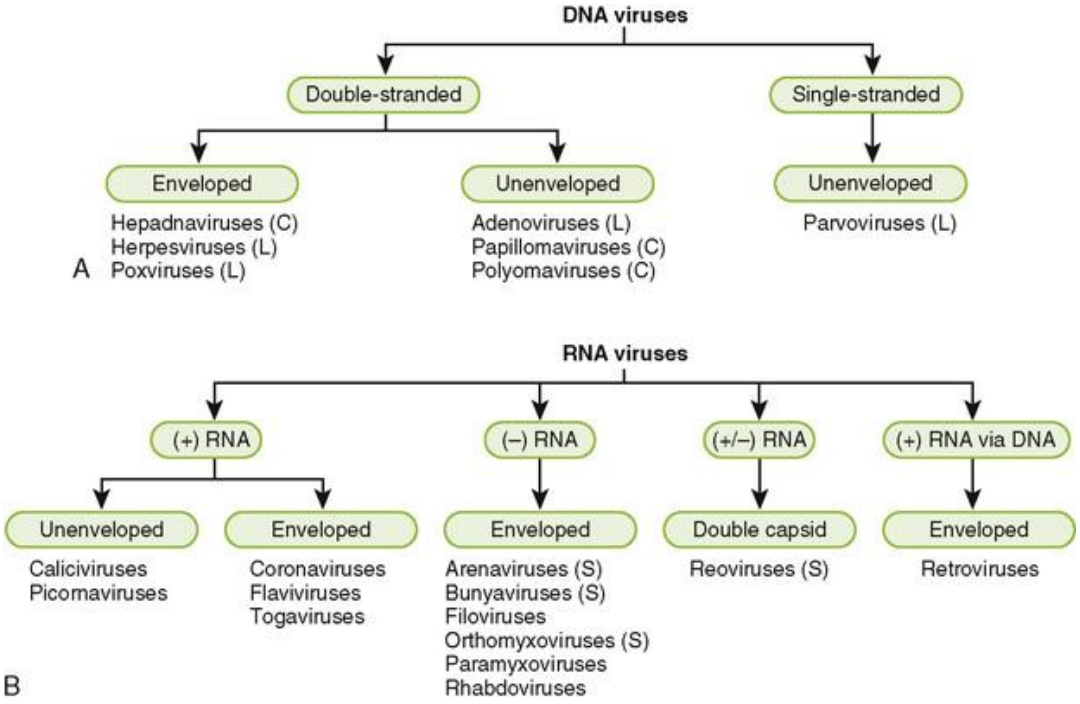


Figure: DNA and RNA viruses

2. Classification on the basis of structure or symmetry

- **Cubical virus:** They are also known as icosahedral symmetry virus Eg.Reo virus, Picorna virus
- **Spiral virus:** They are also known as helical symmetry virus Eg. Paramyxovirus, orthomyxovirus
- **Radial symmetry virus:** Bcateriophage
- **Complex virus:** Pox virus

3. Classification on the basis of replication properties and site of replication

- **Replication and assembly in cytoplasm of host:** All RNA virus replicate and assemble in cytoplasm of host cell except Influenza virus
- **Replication in nucleus and assembly in cytoplasm of host:** Influenza virus, Pox virus
- **Replication and assembly in nucleus of host**: All DNA viruses replicate and assemble in nucleus of host cell except Pox virus.
- **Virus replication through ds DNA intermediate:** All DNA virus, Retro virus and some tumor causing RNA virus replicates through ds DNA as intermediates.
- **Virus replication through ss RNA intermediate:** All RNA virus except Reo virus and tumor causing RNA viruses.

4. Classification on the basis of host range

- **Bacteriophage:**Phage are virus infecting bacteria. Eg, λ phage, T2, T4, φ174, MV-11

- **Plant virus:** Those virus that infects plants. Eg. TMV, cauliflower mosaic virus
- **Animal virus**: Those virus that infects animals. Eg. Polio virus, Retro virus, Herpes virus, Adeno virus
- **Insect virus:** Virus that infects insects. Eg. Baculovirus, Sacbrood virus, Entomopox virus, Granulosis virus

5. Classification on the basis of mode of transmission

- **Virus transmitted through respiratory route:** Swine flu, Rhino virus
- **Virus transmitted through faeco-oral route**: Hepatitis A virus, Polio virus, Rota virus
- **Virus transmitted through sexual contacts:** Retro virus
- **Virus transmitted through blood transfusion**: Hepatitis B virus, HIV
- **Zoonotic virus:** Hepatitis B virus, HIV

Classifying viruses- The Baltimore Scheme

Viruses exhibit great diversity in terms of morphology, genome structure, mode of infection, host range, tissue tropism, disease (pathology), etc. Classification on thes grounds does not give a good basis for unifying discussions of virus replication processes. To circumvent these problems, Nobel Laureate David Baltimore proposed a classification scheme which encompasses all viruses based on the nature of their genomes, and their modes of replication and gene expression.

The original Baltimore classification scheme was based on the fundamental importance of messenger RNA (mRNA) in the replication cycle of viruses. Viruses do not contain the molecules necessary to translate mRNA and rely on the host cell to provide these. They must therefore synthesize mRNAs which are recognized by the host cell ribosomes. In the Baltimore scheme, viruses are grouped according to the mechanism of mRNA synthesis. By convention, all mRNA is designated as positive (or 'plus') sense RNA. Strands of viral DNA and RNA which are complementary to the mRNA are designated as negative (or 'minus') sense and those that have the same sequence are termed 'positive' sense. Using this terminology, coupled with some additional information about the replication process, a modified classification scheme based on the original proposed by Baltimore defines seven groups of viruses, with each commonly being referred to by the nature of the virus genomes it includes:

Class 1 contains all viruses that have doublestranded (ds) DNA genomes. In this class, the designation of positive and negative sense is not meaningful since mRNAs may come from either strand.

Class 2 contains viruses that have singlestranded (ss) DNA genomes. The DNA can be of positive or negative sense, depending on the virus being studied.

Class 3 contains viruses that have dsRNA genomes. All known viruses of this type have segmented genomes and mRNA is only synthesized from one template strand of each segment.

Class 4 contains viruses with ssRNA genomes of the same (positive) sense as mRNA and which can be translated

Class 5 contains viruses that have ssRNA genomes which are complementary in base sequence to the mRNA (negative-strand RNA viruses)

Class 6 contains viruses that have ssRNA genomes and which generate a dsDNA intermediate as a prelude to replication, using an enzyme carried in the virion

Class 7 More recently, it has been suggested that some viruses, termed reversiviruses, should be transferred from class 1 into a new class 7. This is based on their replication from dsDNA via a positive sense ssRNA intermediate back to dsDNA

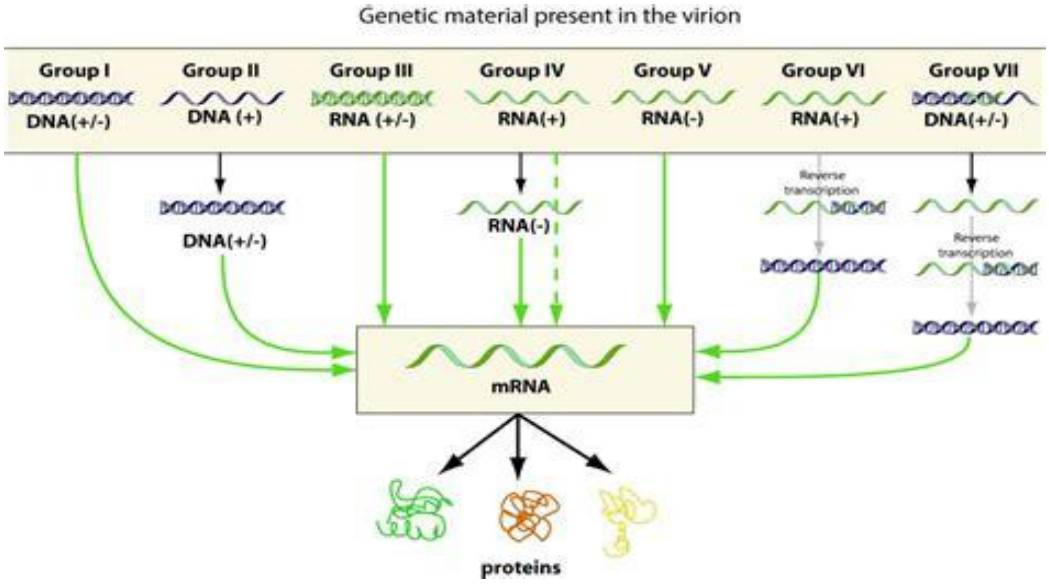


Figure: The Baltimore classification

Table: Mode of genome replication

Group	Description
Double-stranded (ds) DNA viruses	Genome replication: dsDNA → dsDNA mRNA synthesis: dsDNA → mRNA
Single-stranded (ss) DNA viruses	Genome replication: ssDNA → dsDNA → ssDNA mRNA synthesis: ssDNA →dsDNA →mRNA
Double-stranded RNA viruses	Genome replication: dsRNA → ssRNA → dsRNA mRNA synthesis: dsRNA → mRNA
Plus-strand RNA (+RNA) viruses	Genome replication: +RNA → −RNA → +RNA mRNA synthesis: +RNA = mRNA → −RNA → mRNA
Negative-strand RNA (−RNA) viruses	Genome replication: −RNA → +RNA → −RNA mRNA synthesis: −RNA → mRNA
Retroviruses	Genome replication: ssRNA → dsDNA → ssRNA mRNA synthesis: ssRNA → dsDNA → mRNA
Reverse transcribing DNA viruses	Genome replication: dsDNA → ssRNA → dsDNA mRNA synthesis: dsDNA → mRNA

5.3 Nomenclature of viruses

It was realized by the virologists that binomial system of nomenclature proposed by Carolus Linnaeus and successfully used for both animals and plants cannot be used for viruses. Johnson (1927) was the first to attempt to name the viruses. According to him, the name of a virus consists of at least three parts: Common name of the host on which it was first discovered and described, the term 'virus' and an Arabic number indicating chronologically the order in which it was described on that host. As per this system Tobacco Mosaic virus may be named a Tobacco virus 1. A decade later, Smith (1937) modified the Johnson's system by replacing the common name of the host with the Latin generic name. Thus, Tobacco virus 1 became Nicotiana virus 1. Holmes (1948) proposed new generic names to virus on the basis of symptoms induced while virus species was derived from the names of host plants. Accordingly, Tobacco mosaic virus was named as Marmortabacci. Hansen (1968) developed a complicated system of nomenclature in which name of the genus is constructed by putting together different syllable, each syllable being based on different property of the virus. Thus, Tobacco Mosaic virus became Minochorda, wherein M means that virus is mechanically transmitted-in-signifies that specific vector is unknown, and —Chorda signifies that the virus particles are rod -shaped and stiff.

According to Thornberry (1968), Viruses of higher plants were named as Phytovirus, those of pteridophytes as Pteridovirus, viruses of Bryophytes as Bryovirus and those of Thallophytes as Thalloviruses. Specific name suffixed to the generic names, contained the name of host plant and symptoms produced. Thus, Tobacco Mosaic Virus is named as Phytovirusnicomosaicum.

Gibbs (1969) opined that introduction of binomials to viruses would create more problems than solving and result in confusion. Earlier in 1953, the Sixth International Congress of Microbiology, at Rome took the decision that application of binomials to the viruses is undesirable and should be discouraged.

Therefore, the International Committee of Virus nomenclature at its meeting held in Rio de Janerio also concluded that the viruses be designated by non-lineal binomials and proposed a new system of nomenclature. According to this system of nomenclature, the name of the virus consists of two parts: the first part represents the common name of virus while the second part contains coded information about the virus.

Aside from physical data, genome structure and mode of replication are criteria applied in the classification and nomenclature of viruses, including the chemical composition and configuration of the nucleic acid, whether the genome is monopartite or multipartite. The genomic RNA strand of single-stranded RNA viruses is called sense (positive sense, plus sense) in orientation if it can serve as mRNA, and antisense (negative sense, minus sense) if a complementary strand synthesized by a viral RNA transcriptase serves as mRNA. Also considered in viral classification is the site of capsid assembly and, in enveloped viruses, the site of envelopment.

5.4 Structural organization and Chemistry of Virus particles

Virions are composed of a protein shell, called the capsid, and the virus genome that the capsid contains. Most bacterial and plant viruses are naked, with no further layers, whereas many other types of viruses, especially animal viruses, have an outer layer. If the layer is composed of a phospholipid bilayer taken from the host cell membrane, it is called the envelope. In enveloped viruses, the inner structure of nucleic acid plus capsid protein is called the nucleocapsid. The virion protects the viral genome when the virus is outside the host cell, and proteins on the virion surface are important in attaching it to its host cell. The virion may also contain one or more virus-specific enzymes that play a role during infection and replication. Once inside the host cell, a viral genome can orchestrate one of two quite different events. The virus may replicate and destroy the host in a virulent infection via a lytic pathway. In a lytic infection, the virus redirects the host cell's metabolism from growth to support virus multiplication and the assembly of new virions. Eventually, new virions are released, and the process can repeat itself within new host cells. Alternatively, some viruses can cause a lysogenic infection; in this case, the host cell is not destroyed and the viral genome becomes part of the host genome.

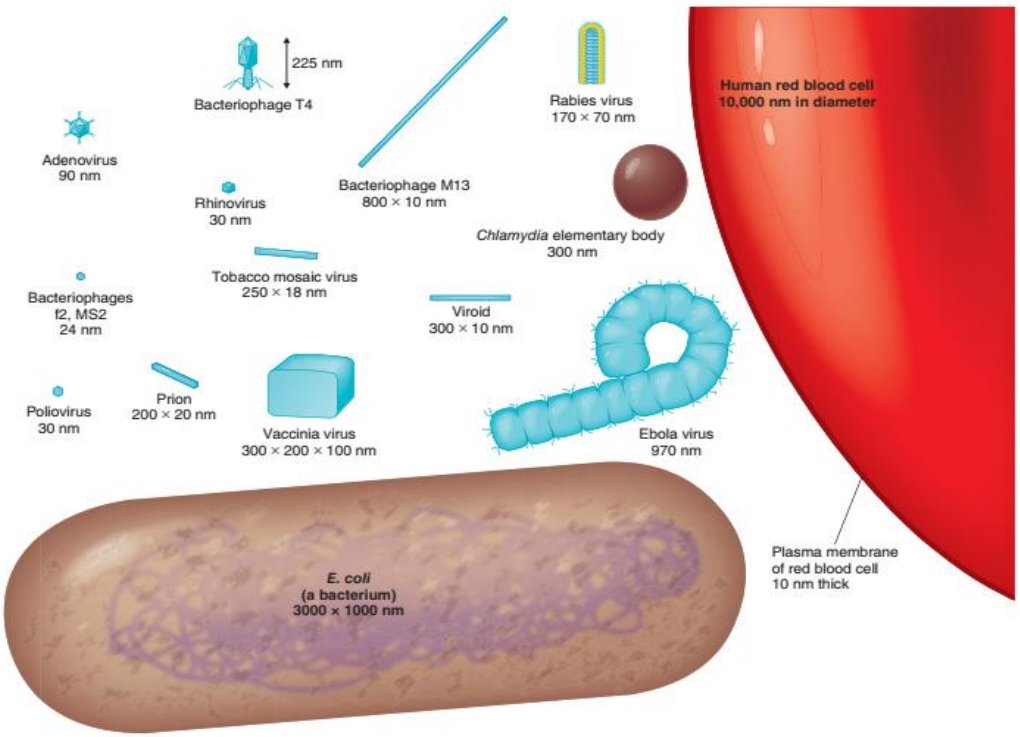


Figure: The sizes of several viruses (teal blue) and bacteria (brown) are compared with a human red blood cell, shown to the right of the microbes

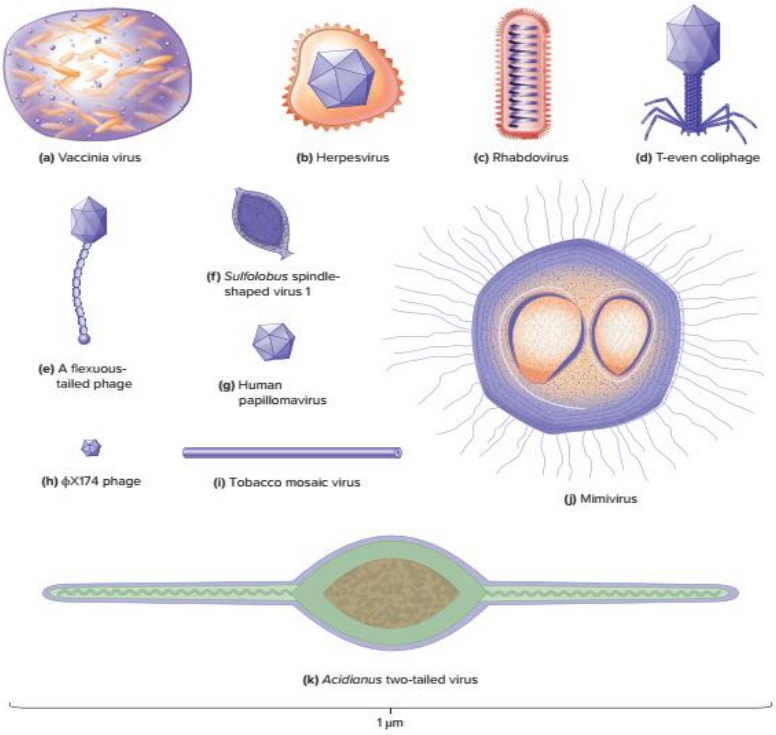


Figure: The Size and Virion Morphology of Selected Viruses.

Nonenveloped viruses construct a capsid from many copies of one protein and a few minor proteins. Each subunit is termed a protomer, and thousands of protomers self-assemble to form the capsid. In contrast, enveloped viruses require both nucleocapsid proteins and additional proteins to anchor the membrane. Some viruses use non-capsid proteins as scaffolding upon which the capsids are assembled. Probably the most important advantage of this design strategy is that the viral genome is used with maximum efficiency. For example, the tobacco mosaic virus (TMV) capsid is constructed using a single type of protomer. Recall that the building blocks of proteins are amino acids and that each amino acid is encoded by three nucleotides. The TMV protomer is 158 amino acids in length. Therefore only about 474 nucleotides are required to code for the coat protein. The entire TMV genome consists of only 6,400 nucleotides. Thus only a small fraction of the genome is used to code for the capsid. Suppose, however, that the TMV capsid were composed of six different protomers, all about 150 amino acids in length. If this were the case, about 2,700 nucleotides in the TMV genome would be required just for capsid construction, and much less genetic material would be available for other purposes.

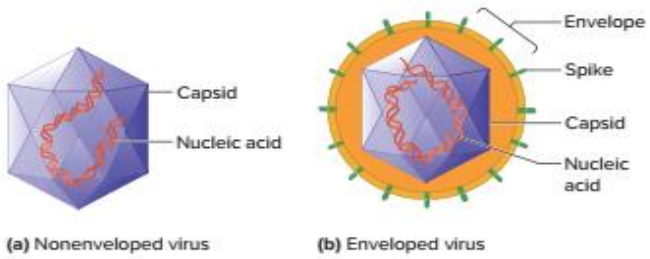


Figure: Generalized Structure of Virions. (a) The simplest virion is that of a nonenveloped virus, consisting of a capsid assembled around its nucleic acid (nucleocapsid). (b) Virions of enveloped viruses are composed of a nucleocapsid surrounded by a membrane called an envelope. The envelope usually has viral proteins called spikes inserted into it (Source: *Prescott's Microbiology*)

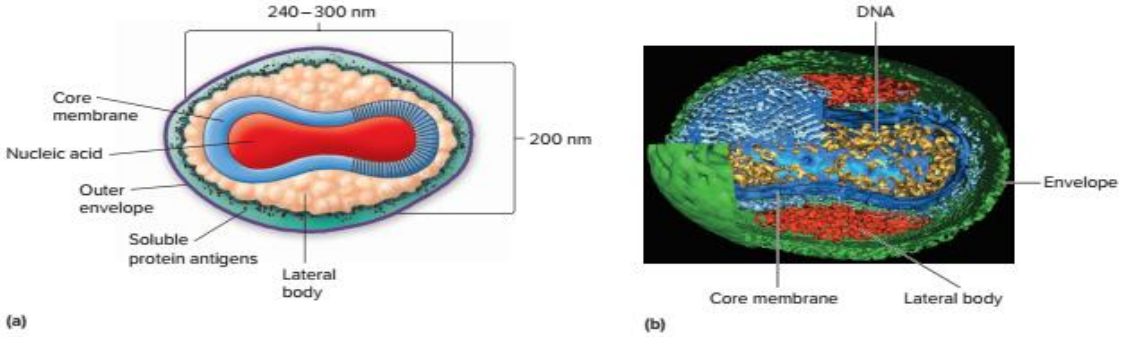


Figure: Morphology of Vaccinia Virus Virions. (a) Diagram of virion structure. (b) Electroncryotomogram of the virion

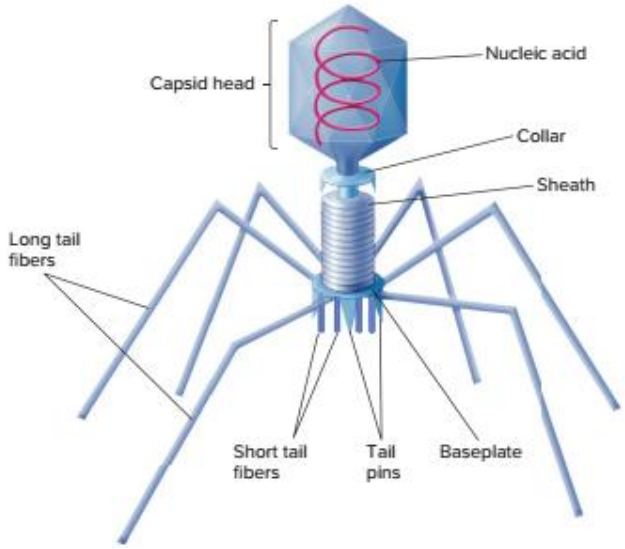


Figure: T4 Phages of E. coli. The structure of T4 bacteriophage virion.(Source: *Prescott's Microbiology*)

5.5 Assay of Viruses

Viruses can either be detected directly – by identification of their nucleic acid genomes or proteins in samples – or else indirectly – by their pathological effects in cell culture or whole organisms, by their interaction with antibody or by identifying the presence of antibody that is specific for a known virus. Once isolated, viruses can be studied using a wide range of techniques. Targeted manipulation of viral genomes by reverse genetics has proved a powerful means of identifying gene function.

Perhaps the most important techniques in virology are those that allow the enumeration of viruses. Almost all methods to measure the amount of virus will actually determine its concentration in the sample. The virus concentration is often referred to as the virus titre, expressed in virus units per ml of sample. There are two different measures of virus titre that are useful: a physical particle count, i.e. the concentration of particles present in a sample; and an infectivity count, i.e. the concentration of particles present are that are capable of completing successfully a full infectious cycle in a susceptible host system – cell or animal.

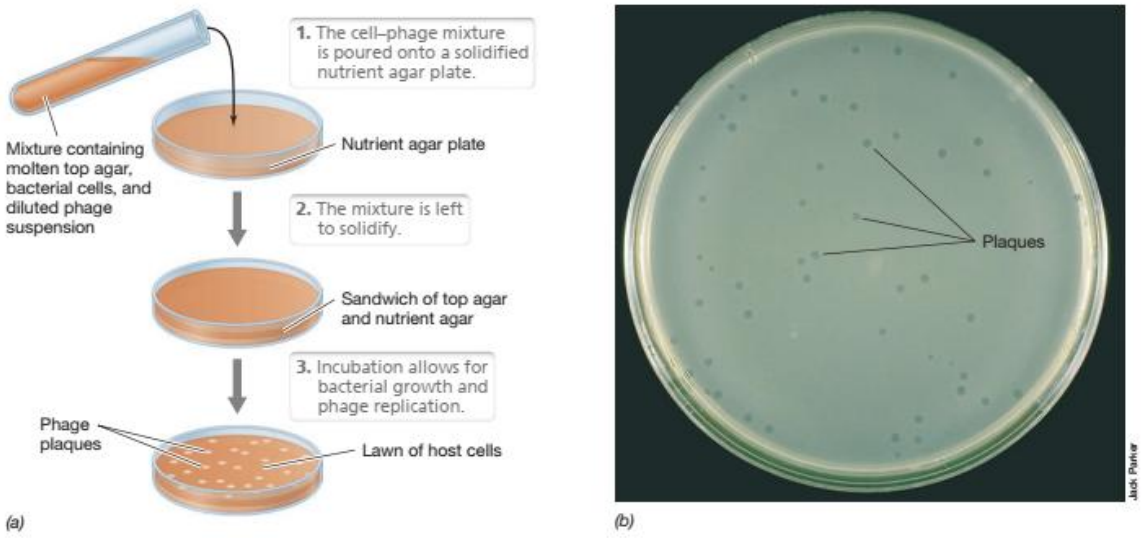


Figure: Quantification of bacterial virus by plaque assay. (a) "Top agar" (a dilute agar solution) containing a dilution of virions mixed with permissive host bacteria is poured over a plate of "bottom agar" (a more concentrated and thus stiffer agar). Infected cells begin to grow but then are lysed, forming plaques in the lawn. (b) Plaques (about 1–2 mm in diameter) formed by bacteriophage

1. **Total virus particles count:**

Electron microscopy and hemagglutination are the two meth-ods used for estimation of total virus particles.

Electron microscopy: EM is useful to count virus particlesdirectly in a negatively stained viral suspension. In this method, the virus suspension is mixed with a known concentration of latex particles, and the number of virus particles in the suspension is estimated by a ratio between the virus and latex particles demonstrated by EM.

Hemagglutination assay: Quantitation of hemagglutinating virusesis carried out by determination of hemagglutination titers. Although it is not a sensitive method, it is used as a convenient method of virus assay. For example, approximately, 107 influenza virions are essential to produce microscopic agglutination in cultured cells. Many animal virus particles bind to the surface of red blood cells. If the ratio of virions to cells is large enough, virions will join the red blood cells together; that is, they agglutinate, forming a network that keeps the red blood cells in suspension. In practice, red blood cells are mixed with diluted samples of the virus particles, and each mixture is examined. The hemagglutination titer is the highest dilution of the virus preparation (or the reciprocal of the dilution) that still causes hemagglutination

2. Assay of infectivity of viruses

Quantitative and quantal assays are the two types of assays, which are carried out to determine assay of infectivity of viruses.

Quantitative assay of infectivity: Quantitative assay is usedto estimate the presence of actual number of viable infectious viral particles in the inoculum. Two methods are available for the purpose, which include plaque assay in monolayer cell culture and pock assay on chick embryo CAM.

Plaque assay: It was introduced by Dulbecco in 1952 as a modification of bacteriophage plaque assay. This is based on the principle that each infectious viral particle gives rise to a localized focus of infected cells that can be visualized by the naked eye. Such foci are called plaques, and each plaque indicates an infectious virus. Therefore a count of the plaques produced at a particular dilution can be used to estimate the number of virions in the original sample. The resulting value is expressed as plaque-forming units (PFU) rather than as virions, for several reasons. First, not all virions may be infective. Furthermore, even though there are far fewer viruses than host cells, it is still possible for more than one virion to infect the same cell. Finally, it is possible for two infected cells to be plated to the same area, which would then give rise to a single plaque. The test is performed by adding a viral inoculum to a monolayer of culture cells in a bottle or Petri dish. After sometime, this allows adsorption of viruses. The liquid medium is removed and replaced with a solid agar gel to ensure that the spread of progeny virus is confined to the immediate vicinity of infected cells.

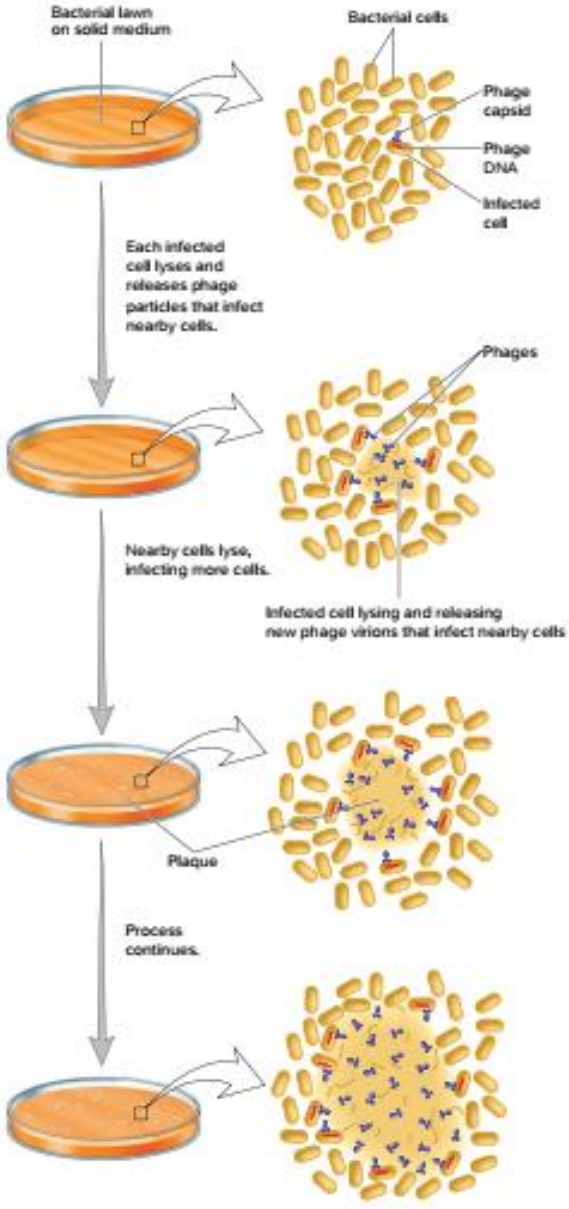


Figure: Formation of Phages Plaques. When phages and host bacterial cells are mixed at an appropriate ratio, only a portion of the cells are initially infected. When this mixture is plated, the infected cells will be separated from each other. The infected cells eventually lyse, releasing progeny phages. They infect nearby cells, which eventually lyse, releasing more phages. This continues and ultimately gives rise to a clear area within a lawn of bacteria. The clear area is a plaque.

Pock assay: Viruses that form pocks on CAM can beassayed by counting the number of pocks formed on the inoculated CAM. Each pock on CAM arises from a single virus particle. This is known as pock assay. Vaccinia and variola viruses can be assayed by pock assay.

Quantal assays of infectivity: Quantal assays of infectivity can be carried out to quantitate a virus by quantitating the virus particles in animals, in embryonated eggs, or in tissue culture. This method of assay of infectivity only indicates the presence or absence of infectious viruses, but it does not indi-cate actual number of viruses. The endpoints used for infectivity titrations are estimated by the (a) development of CPE in cell cultures, (b) production of hemagglutination in allantoic fluid of embryonated egg, or (c) death of experimentally infected animals. The titer of virus is usually expressed as the "50% infectious dose (ID50)" per mL, which indicates the highest dilution of the inoculum that initiates detectable symptoms, antibodies, or other responses in 50% of inoculated test animals, eggs, or cell cultures.

Sometimes rather than counting virions, the nucleic acid of a virus can be quantified by quantitative-polymerase chain reaction (qPCR).This is possible because PCR amplifies specific nucleic acids in a mixture of nucleic acids

5.6 Structure of Adenoviruses

Adenoviruses are a group of small and naked icosahedral viruses that contain linear double-stranded DNA genomes. Adenoviruses are of minor health importance, causing mild respiratory infections in humans, but they have unique stature in virology because of the mechanism by which they replicate their genomes. Attached to the 5′ end of adenoviral genomic DNA is a protein called the adenoviral terminal protein, and it is essential for replication of the adenoviral genome. The complementary DNA strands also have inverted terminal repeats that play a role in the replication process. Following infection, the adenoviral nucleocapsid is released into the host cell nucleus, and transcription of the early genes proceeds by activity of the host RNA polymerase. Most early transcripts encode important replication proteins such as the terminal protein and a viral DNA polymerase. Replication of the adenoviral genome begins at either end of the DNA genome and the terminal protein facilitates this process because it contains a covalently bound cytosine that functions as a primer for DNA polymerase. The products of this initial replication are a completed double-stranded viral genome and a single-stranded minus-sense DNA molecule. At this point, a unique replication event occurs. The single DNA strand cyclizes by means of its inverted terminal repeats, and a complementary (plus-sense) DNA strand is synthesized beginning from its 5′ end. This mechanism is unique because double-stranded DNA is replicated without the formation of a lagging strand, as occurs in conventional semiconservative DNA replication. Once sufficient copies of the adenoviral genome have formed and virion structural components accumulate in the host cell, mature adenoviral virions are assembled and released from the cell following lysis.

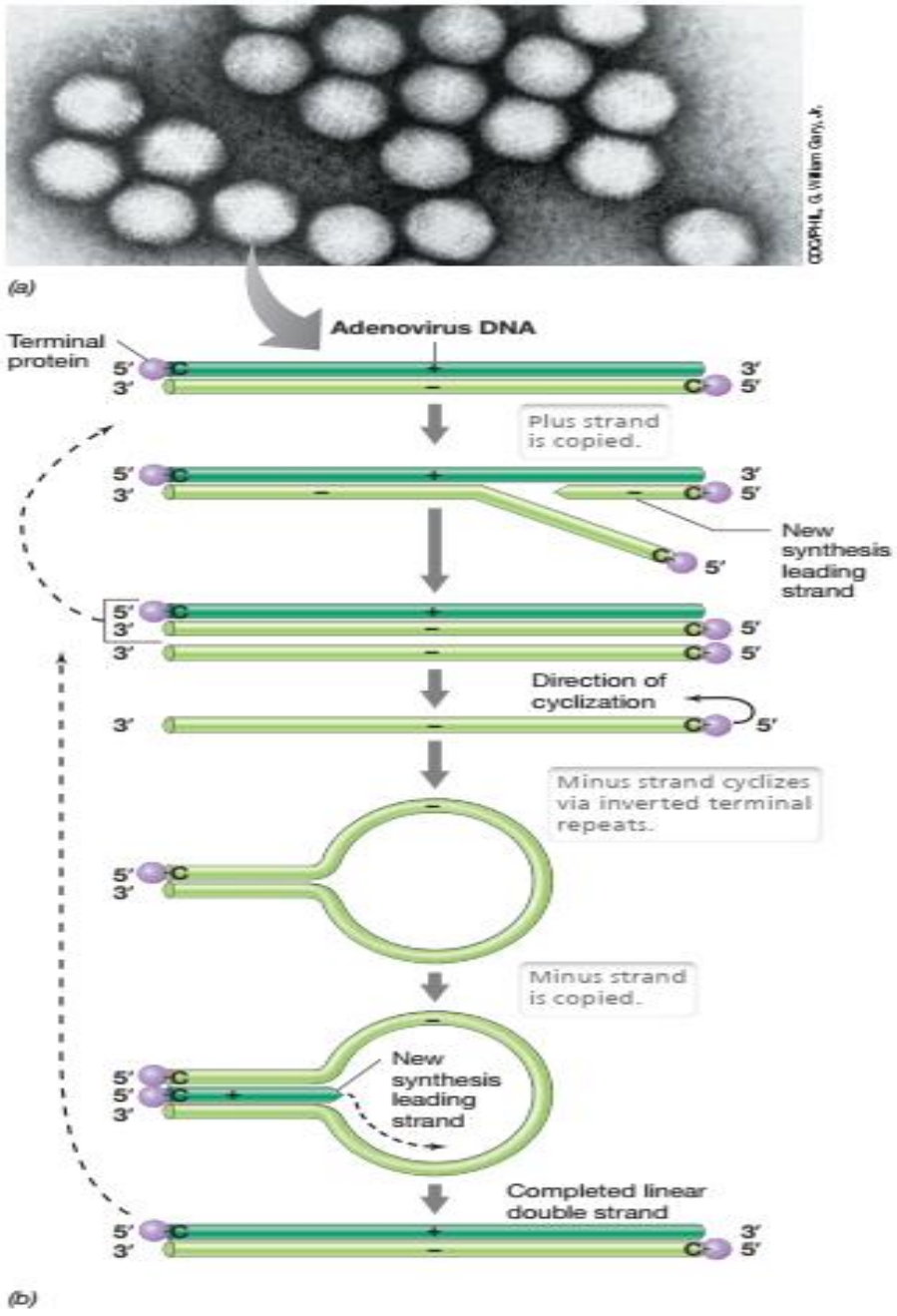


Figure: Adenovirus genome replication

All adenovirus particles are similar; particles are medium-sized, non-enveloped having 90-100 nm diameters (Fig. 17.5). The particles have icosahedral symmetry which can easily be visible in the electron microscope by negative staining. Viral particles are composed of 252 capsomers: 240 hexons forming the faces and 12 pentons at vertices of icosahedron (2-3-5 symmetry). Each penton bears a slender fiber. The penton fibres consist of a slender shaft with a globular head. They are involved in the process of attachment of the virus particle to the host cell via the coxsackie-adenovirus receptor on the surface host cell. The thin fibres protruding from each vertex of the

icosahedral particle are just visible and the triangular faces of the icosahedral particle can be made out. But during preparation for electron microscopy, the fibres easily become detached. The hexons consist of a trimer of protein II with a central pore, there is no protein I. The proteins VI, VIII and IX are the minor polypeptides which are also associated with the hexon. They are thought to be involved in stabilization and/or assembly of the particle. The pentons are more complex; the base consists of a pentamer of protein III, 5 molecules of IlIa are also associated with the penton base. The pentons have a toxin-like activity. A trimeric fibre protein extends from each of the 12 vertices (attached to the penton base proteins) and is responsible for recognition and binding to the cellular receptor. A globular domain at the end of the adenovirus fiber is responsible for recognition of the cellular receptor. There are at least 10 proteins in the adenovirus capsid. The double-stranded linear DNA is associated with two major core proteins: terminal protein (TP) and VII. Terminal protein is covalently attached to the 5′ ends of the genome strands. Protein VII (1070 copies/particle) is arginine-rich basic protein (similar to histones) which is covalently associated with the genome forming a 'chromatin-like' substance.

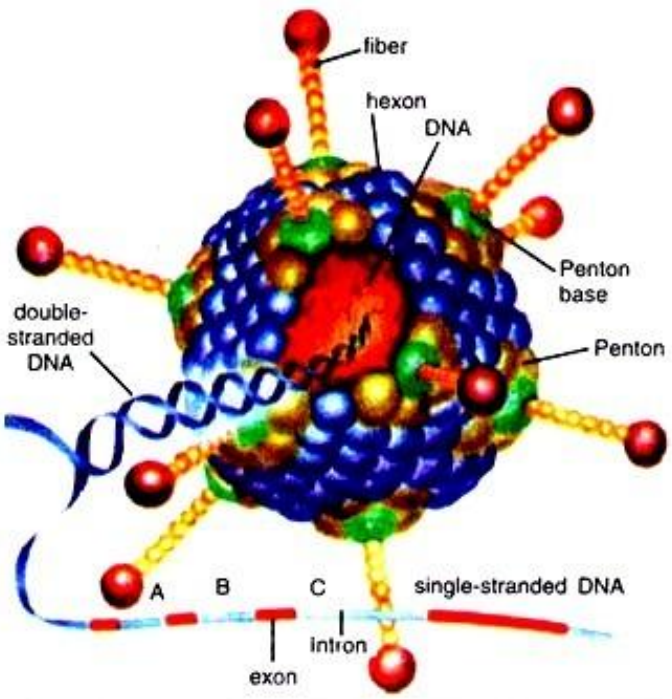


Figure: Structure of an Adenovirus

5.7 Structure of TMV

The TMV particle consists of a single molecule of positive sense ssRNA, embedded in a framework of small, identical protein molecules (A protein), and arranged in a right-handed helix, with each protein binding to 3 nucleotides of RNA. The protein coat is technically called 'capsid'. R. Franklin

estimated 2,130 sub-units, namely, capsomeres in a complete helical rod and 49 capsomeres on every three turns of the helix; thus there would be about 130 turns per rod of TMV. The diameter of RNA helix is about 80 Å and the RNA molecule lies about 50 Å inward from the outer-most surface of the rod. The central core of the rod is about 40 Å in diameter. Each capsomere is a grape like structure containing about 158 amino acids and having a molecular weight of 17,000 dalton as determined by Knight. The ssRNA is little more in length (about 3300 Å) slightly protruding from one end of the rod. The RNA molecule consists of about 7300 nucleotides; the molecular weight of the RNA molecule being about 25,000 dalton.

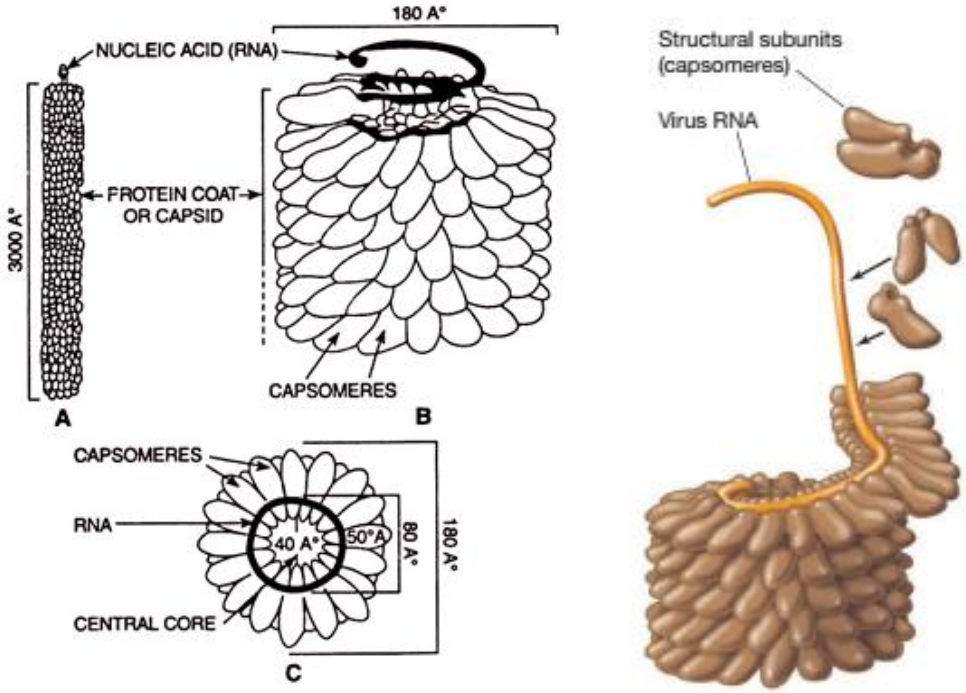


Figure: The arrangement of RNA and protein coat in tobacco mosaic virus, a simple naked virus (Left side). The RNA forms a helix surrounded by the protein subunits (capsomeres). The center of the virus particle is hollow (right side).

Life Cycle of TMV

Plant viruses like TMV penetrate and enter the host cells in toto and their replication completes within such infected host cells (Fig. 13.21). Inside the host cell, the protein coat dissociates and viral nucleic acid becomes free in the cell cytoplasm. Although the sites for different steps of the viral multiplication and formation of new viruses have not yet been determined with absolute certainty, the studies suggest ha alter becoming free in the cell cytoplasm the viral-RNA moves into the nucleus (possibly into the nucleolus). The viral-RNA first induces the formation of specific enzymes called ‘RNA polymerases’ the single-stranded viral-RNA synthesizes an additional RNA

strand called replicative RNA. This RNA strand is complementary to the viral genome and serves as 'template' for producing a new RNA single strand which is the copies of the parental viral-RNA. The new viral-RNAs are released from the nucleus into die cytoplasm and serve as messenger-RNAs (mRNAs). Each mRNA, in cooperation with ribosomes and t-RNA of the host cell directs the synthesis of protein subunits. After the desired protein sub-units (capsomeres) have been produced, the new viral nucleic acid is considered to organize the protein subunit around it resulting in the formation of complete virus particle, the virion. No 'lysis' of the host cell, as seen in case of virulent bacteriophages, takes place. The host ells remain alive and viruses move from one cell to the other causing systemic infection. When transmitted by some means the viruses infect other healthy plants.

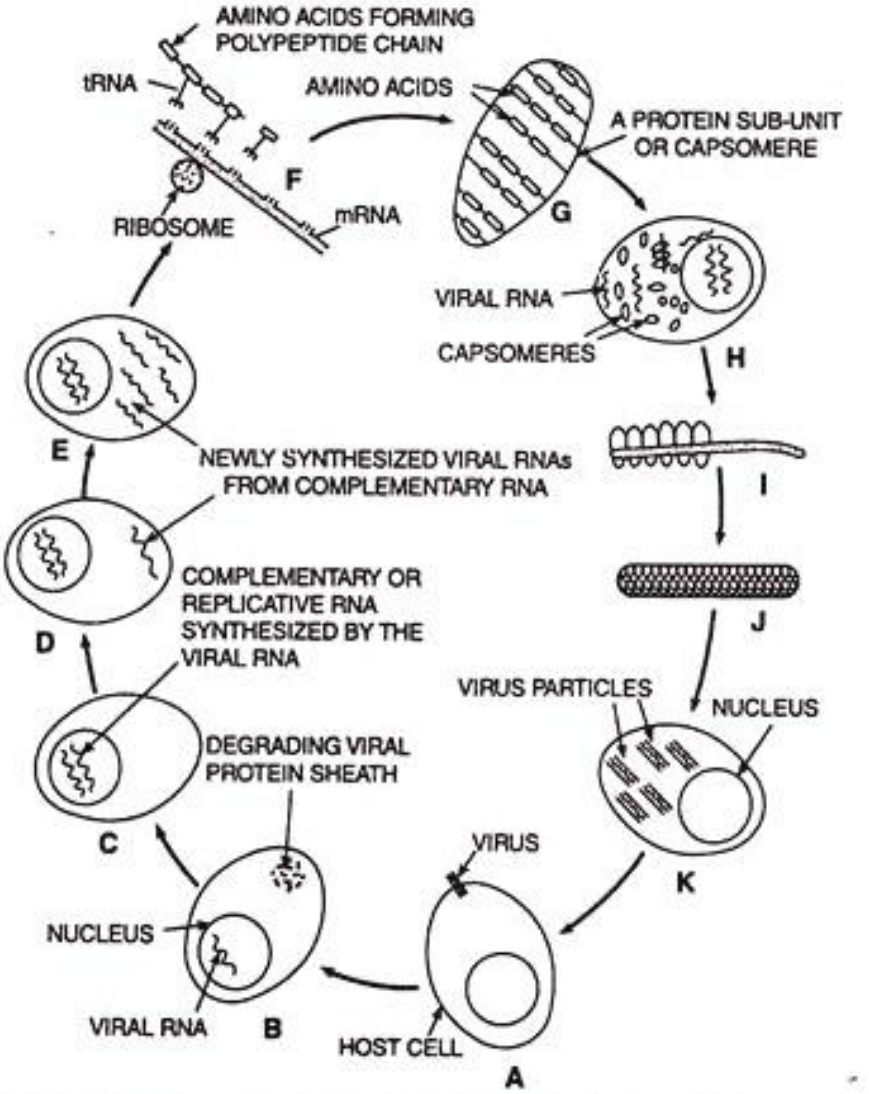


Figure: Replication of TMV (diagrammatic)

(Source: https://www.biologydiscussion.com/viruses/tobacco-mosaic-virus-tmv-structure-and-replication/54903)

5.8 Structure of Coliphage T4

Bacteriophage T4 is the most well-studied member of Myoviridae, the most complex family of tailed phages. T4 assembly is divided into three independent pathways: the head, the tail and the long tail fibers. The prolate head encapsidates a 172 kbp concatemeric dsDNA genome. The 925 Å-long tail is surrounded by the contractile sheath and ends with a hexagonal baseplate. Six long tail fibers are attached to the baseplate's periphery and are the host cell's recognition sensors. The sheath and the baseplate undergo large conformational changes during infection. X-ray crystallography and cryo-electron microscopy have provided structural information on protein–protein and protein–nucleic acid interactions that regulate conformational changes during assembly and infection of Escherichia coli cells.

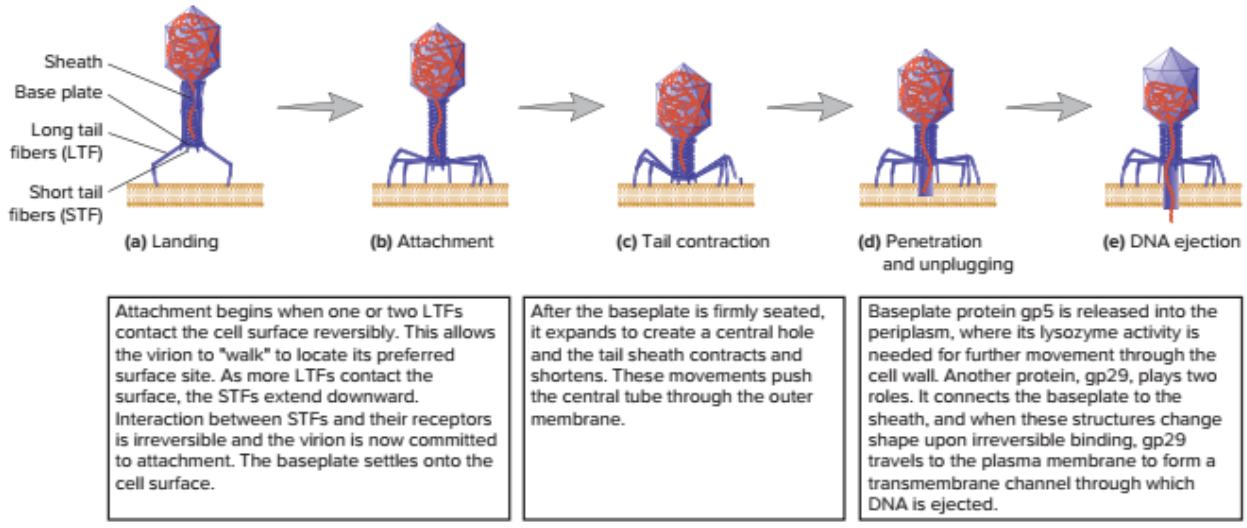


Figure: T4 Phage Adsorption and DNA Entry. (a–e) Adsorption and viral DNA entry into the E. coli host are mediated by the phage's tail fibers and base plate. (f) An electron micrograph of an E. coli cell being infected by T-even phages. These phages have released their dsDNA into the cell and have empty capsids.

The life cycle of T4 bacteriophage (family Myoviridae, species Enterobacteria phage T4) serves as our example of a virulent (lytic) dsDNA phage. Cell lysis is the outcome of an infection with a lytic bacteriophage. As with most viruses (except plant viruses), the first step of viral infection is attachment (adsorption) to the host cell surface. T4 attachment begins when a long tail fiber contacts either the lipopolysaccharide or certain proteins in the outer membrane of its Escherichia

coli host. As more long tail fibers make contact, the baseplate settles down on the surface). Both baseplate and sheath change shape, and the tail sheath shortens. As the sheath becomes shorter and wider, the central tube located within the sheath is pushed through the bacterial cell wall. Finally, the linear DNA is extruded from the head into the host cell Within 2 minutes after entry of T4 DNA into an E. coli cell, the E. coli RNA polymerase starts synthesizing T4 mRNA. This mRNA is called early mRNA because it is made before viral DNA is made. One of the earliest T4 genes to be expressed encodes a protein that binds to the host enzyme RNaseE and directs it to degrade host mRNA. The consequence of host mRNA degradation is that ribonucleotides and ribosomes are now free for transcription and translation of T4 genes. Within 5 minutes, viral DNA synthesis commences, catalyzed by a virus-encoded DNA-dependent DNA polymerase. DNA replication is initiated from several origins of replication and proceeds bidirectionally from each. Viral DNA replication is followed by the synthesis of late mRNAs, which are important in later stages of the infection.

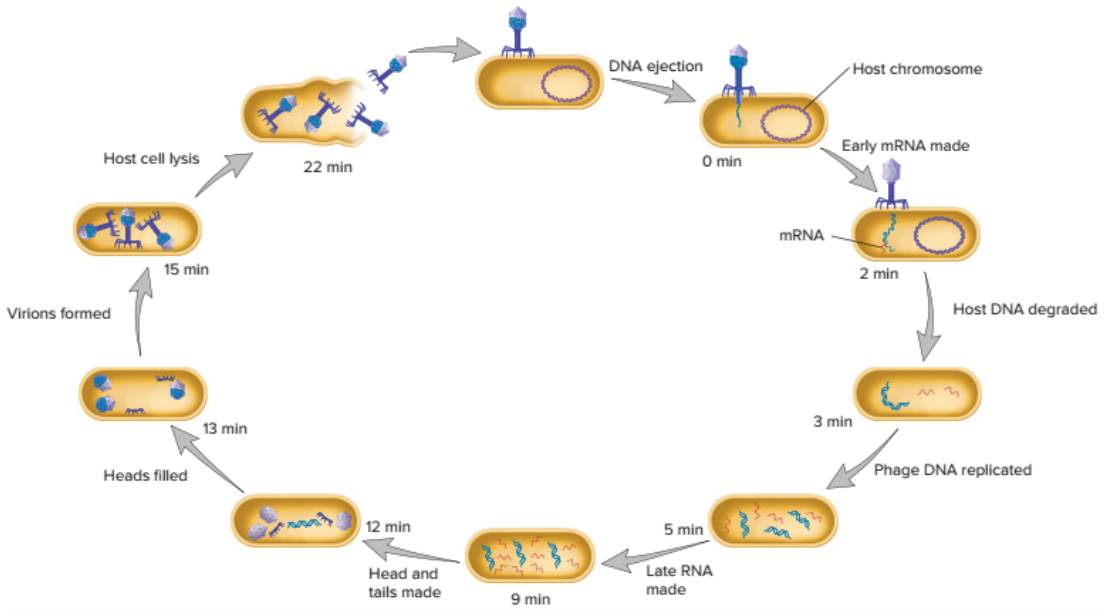


Figure: The Life Cycle of Bacteriophage T4. A diagram depicting the life cycle with the minutes after DNA ejection given for each stage

5.9 Lytic Cycle:

The lytic cycle is one of two cycles that a virus can use to reproduce inside a host cell. The lytic cycle is a series of stages in which a virus hijacks a host cell, uses its components to manufacture more of the virus, destroys and exits the cell, and then goes on to infect other cells. This cycle stands in contrast with the lysogenic cycle, which involves the insertion of the virus's genome into

the genome of the host cell. The lytic cycle is typically considered the main method of virus reproduction inside any permissive host (a host cell that can't prevent the reproduction of viruses).

Lytic Cycle Steps

Whether we are discussing coronavirus, an influenza virus, or a simple bacteriophage, the majority of viruses reproduce the same way: using the lytic cycle. The six stages of the lytic replication cycle are commonly described as follows:

- **Attachment -** A virus attaches itself to the exterior host cell.
- **Injection -** The virus's genetic material (either RNA or DNA) is injected into the host cell through a newly-created hole in the cell membrane.
- **Integration -** The virus's genetic material gives the cell a new set of instructions: build more of the virus.
- **Replication -** The ribosomes inside the host cell build the components of the virus.
- **Assembly** - The host cell assembles the components into new copies of the virus.
- **Lysis -** The cell bursts open, resulting in its death and also in the release of more of the virus which can now go on to infect other cells. (The words lysis and lytic come from the Greek word lusis, which means "loosening.")

Life cycle of phage λ is 45 minutes long, as compared to 22-25 minutes long life cycle of T4 phage. In general the life cycle of most phages at 37°C varies between 22 and 60 minutes. After injection, the linear phage DNA is circularized.

The cos site results in a circular form of DNA which starts the two events:

(i) Transcription and translation of DNA, and

(ii) (ii) Replication of DNA.

As a result of replication numerous DNA molecules are formed in the form of concatemers. Structural proteins for head and tail are synthesized through translation of mRNA. During maturation assembly of phage particles occurs. The lytic cycle ends with lysis of host cell envelope and releases lambda particle.

i) Circularization of Phage DNA:

After injection the linear phage DNA is delivered into bacterial cell. The cohesive ends form hydrogen bonds. The E.coli DNA seals the breaks and the DNA is converted into closed circle within three minutes after infection.

(ii) Transcription:

After circularization of phage DNA, transcription begins by the modified RNA polymerase of host. The modification enables the polymerase to ignore certain termination sites. Fig. 18.15 shows a detailed version of genetic map of phage.

There are three regulatory genes era, N and Q, three promoters, left promoter (PL), right promoter (PR1 and PR2). The DNA replication genes O and P, and five termination sites (tL1, tR1, tR2, tR3 and tR4). The L and R series are transcribed leftward and rightward, respectively from the complementary DNA strands.

On the basis of transcription the genes are grouped into three classes, immediate early genes (N and cro), delayed early genes (located left to N e.g. cIII, gam, red, xis and int, and right to cro e.g. cll, O, P and Q) and late genes.

Consequently three classes of mRNAs are transcribed e.g. immediate early, delayed early and late mRNAs. Early mRNAs are transcribed from both left and right strands, whereas late mRNAs are transcribed from left to right, and the left transcribes from right to left.

Genetic Map of Phage λ showing Genes arranged in different Groups and their Association with Leftward and Rightward Transcription. First of all O and P genes are transcribed whose products are necessary for DNA synthesis. This is followed by transcription of gene coding for structural proteins, packaging system and finally lytic proteins.

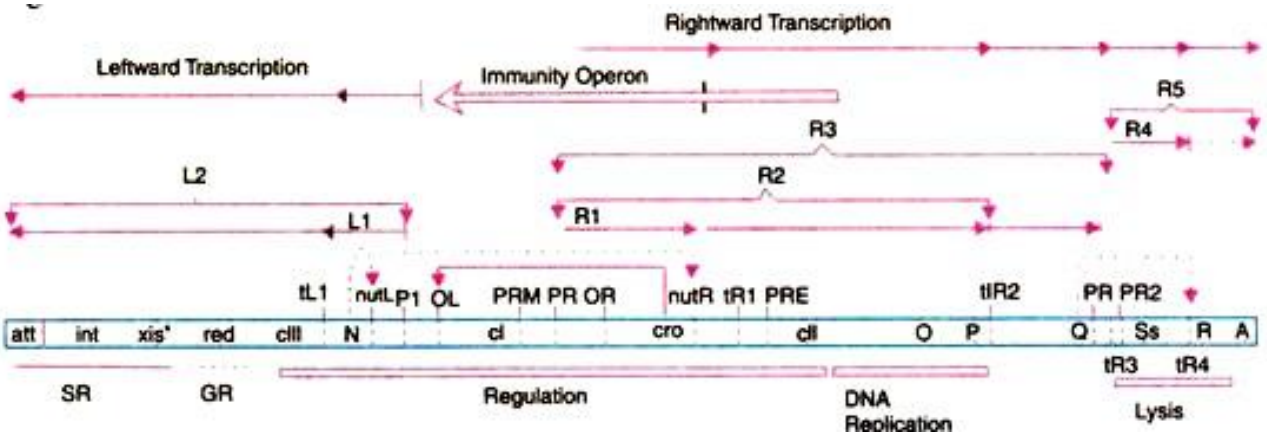


Figure: genetic map of phage lambda showing genes in different groups and their association with leftward and rightward transcription

Before transcription of O and P genes, two immediate early mRNA transcripts are formed that codes for regulatory proteins responsible for turning ‘on’ and ‘off to the leftward transcription and rightward transcription whenever required.

The phage has two promoters, PL and PR which initiates the synthesis of RNA molecules L1 and L2. Initially transcription terminates at the sites tL1 and tR1. L1 encodes gene product N (gpN) which is the delayed early gene product and a major regulatory factor that controls certain regions of DNA when it is transcribed.

After synthesis, gpN binds to nutL and nutR sites present at left and right side of the promoters. When RNA polymerase moves along with the DNA, it picks up the gpN. The gpN enables the polymerase to ignore the termination sites (tL1 and tR1) resulting in longer transcripts L1 and L2. Therefore, the gpN acts as anti-terminator and neutralizes the effect of tL1 and tR2. Hence, the gpN controls the expression of most vital function.

After inhibition of termination, transcription occurs in leftward direction and extends upto b2 region, and the rightward transcription extends upto Q gene. The rightward transcription permits the synthesis of O and P gene products. The leftward transcript consists of a red region that codes for two proteins required for genetic recombination.

The O, P and red proteins have catalytic property; therefore, they are not made continuously. When sufficient amount of gpcro (gene product of cro) encoded in R1 is available, it binds to leftward operator (OL), and the repressor activity of cro turns off the synthesis of all leftward mRNA.

The tR3 is the termination site of mRNA even after modification by gpN. Therefore, the rightward transcription terminates at tR3. However, during the time of early transcription sufficient amount of mRNA is produced by rightward transcription. Thus the O and P proteins become sufficient for DNA replication.

After binding of gpcro to OL the concentration of gpcro increases, and also binds to OR and turns off the rightward mRNA synthesis. Therefore, wasteful synthesis of O and P proteins does not occur, and aberrant and deleterious DNA synthesis are also checked.

The gene product of cll (gpcll) is encoded in the same transcript containing O and P during the early transcription. The sufficient amount of gpcll acts as late promoter and delays late mRNA synthesis. After turning of rightward transcription by gpcro, the gpcll is not synthesized.

This relieves the inhibition of late mRNA synthesis. At this time the gpQ (a positive regulator) is required that begins the late mRNA synthesis and neutralizes the third right terminator (tR3) and allow to proceed transcription through vegetative genes to J gene and into the b2 region of DNA molecule.

The gpQ turns on late mRNA synthesis which translates structural and assembly proteins, maturation system and the lysis enzymes. The gpQ is also called anti-terminator which binds the qut sequence and taken up by RNA polymerase. Therefore, the RNA polymerase ignores tR4. Taking advantage of it R4 is extended and forms R5 transcript of late mRNA which synthesizes the head, tail and lysis proteins.

(iii) Replication:

The phage λ replicates autonomously during lytic as well as lysogenic cycles by using only exogenous precursors. The host chromosome is not degraded by the phage, unlike T4 phage.

The replication is accomplished in two stages, early replication and late replication stages:

(a) Early replication (theta mode of replication):

During early replication the circular DNA molecule is associated with host's cell membrane, and replicates to produce circular copies of DNA molecule. On the genome an origin for replication (ori site) is situated within O gene.

The gpO and gpP nick the circular DNA at ori site. Replication is bidirectional and proceeds in opposite directions from the ori site (Fig. 18.16A). In another temperate phage P2, replication is unidirec-tional. The replication fork moves around the circle and forms the Greek letter theta (0); therefore, it is called theta mode of replication.

The two branch points are called replication forks at which the non-replicated original duplex joins the two daughter chromosomes. At the end of replication two identical copies of circular DNA molecule are formed. Thus the theta mode increases the number of templates for transcription and further replication. For the first time Cairns (1963) reported the theta mode of replication in E. coli.

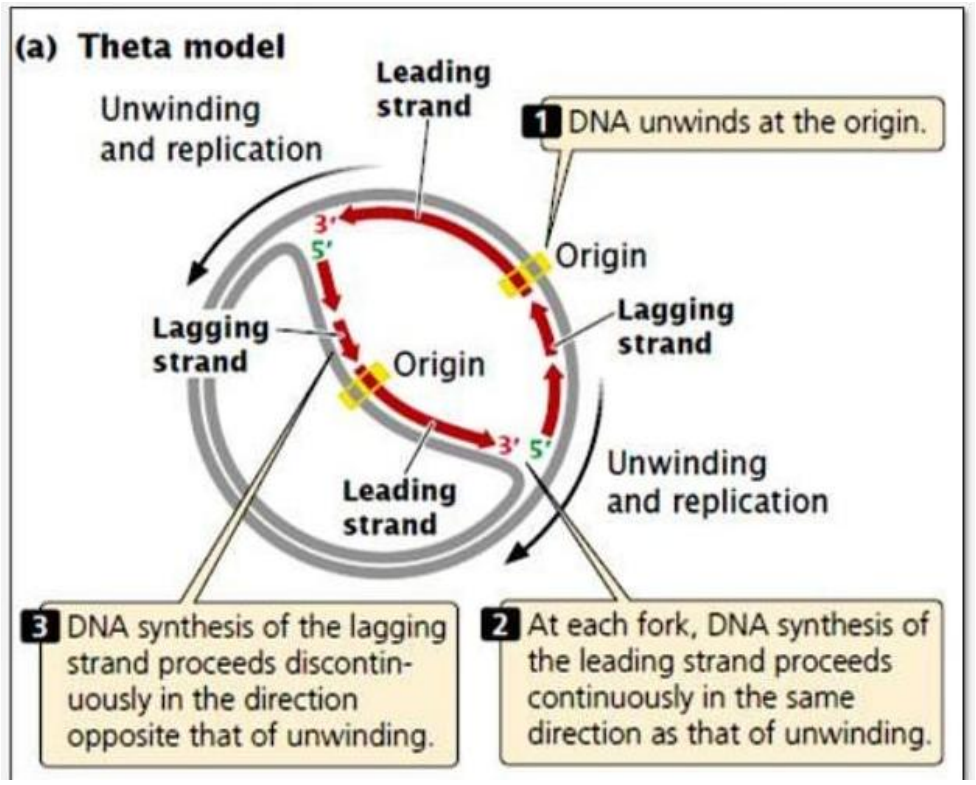


Figure: Theta mode of replication

(b) Late replication (rolling circle mode of replication):

After the synthesis of circular copies of DNA the progenies dissociate from the cell membrane and switch over from theta to rolling circle model of replication. By the time heads and tails have been synthesized and the sequence- specific cutting system called the terminase (Ter) system (Ter proteins are the components of an empty head) becomes active resulting in predominance of rolling circles.

A nick is made at a point on outer strand of duplex (Fig. 18.16 B). Circle rolls and a new strand is synthesized at 3′ end. The 5′ end single stranded DNA is displaced. Finally, the displaced strand contains a long ssDNA molecule of one parental strand and other newly synthesized strand. The rolling circle has the two types of cos sites, one in the circle and the second in the linear branch. In the linear branch there are several cos sites. Such branch is called concatemeric branch.

During replication the cos site of rolling circle does not open because in the open strand replication cannot occur. If two cos sites are present in circular DNA, one is cut by Ter system resulting in free end in concatemer and removal of phage DNA. Thus, Ter-cutting requires two cos sites or one cos site and a free cohesive end on a single DNA molecule. The Ter system was first identified-by genetic analysis of tandem di-lysogens i.e. cells having two adjacent prophage.

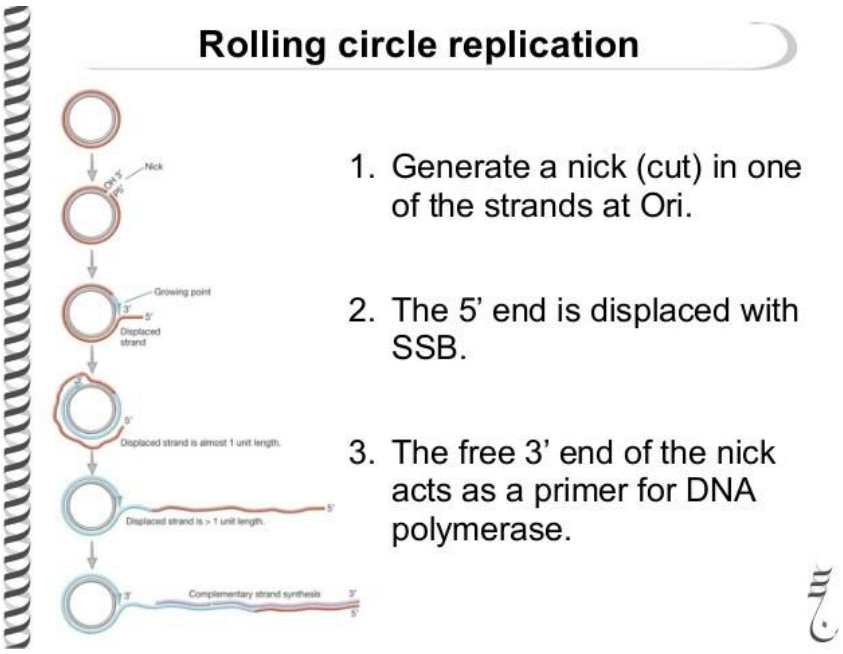


Figure: Rolling circle mode of replication

Replication of Bacteriophage λ DNA

The cut dsDNA of phage thus liberated from the concatemeric branch contains 12 nucleotide long ssDNA that acts as cohesive ends. The unit length genome synthesized by this mechanism contains exactly the phage genome. The gpgam is required to inhibit recBC endonuclease V of the host. Otherwise the concatemers would be broken down.

The two process cutting at cos sites and packaging of phage genome are coupled.

(iv) Assembly:

Hohn and Katsura (1977) have described about the structure and assembly of phage. During the process of maturation, the phage particles (head and tail) are independently assembled. The genes encoding for DNA maturation and phage head proteins are: nul, A, W, B, C, nu3, D, E and F. The genes that code for phage tail are: Z, U, V, G, H, M, K, L, I and J. There are bacterial genes (groES and groEL) that also help in assembly of phage particles.

Different steps in DNA packaging in phage λ head assembly have been described by Kaiser et al. (1975). The process of assembly begins with aggregation of several copies of four head proteins which built up a scaffolded pro-head (Fig.18.17). The pro-head is only a sphere supported with an internal supporting system. Therefore, it looks like a wheel. Many phages form this type of scaffolded pro-head.

In the second stage, gene product of groES and groEL genes i.e. GroEs and GroEL proteins interact and form GroES-GroEL complex. This complex binds to scaffold prohead. The scaffolding is removed by bacterial protease. In some phages, scaffolding falls away and is reused. Gene products of nul and A (gpnul and gpA) that contain Ter system interact a short base-sequence near one cos site with a point on the head.

Later on, it becomes the region for head-tail attachment. The phage DNA folds into the head. A change in conformation in E protein occurs after a small amount of DNA enters into the head. The gpE and the changed E protein causes the formation of icosahedral head. The gpF1 plays a role in expansion of spherical particles to icosahedron. A small amount of gpD enters into head which is filled with λ DNA by the unknown mechanism.

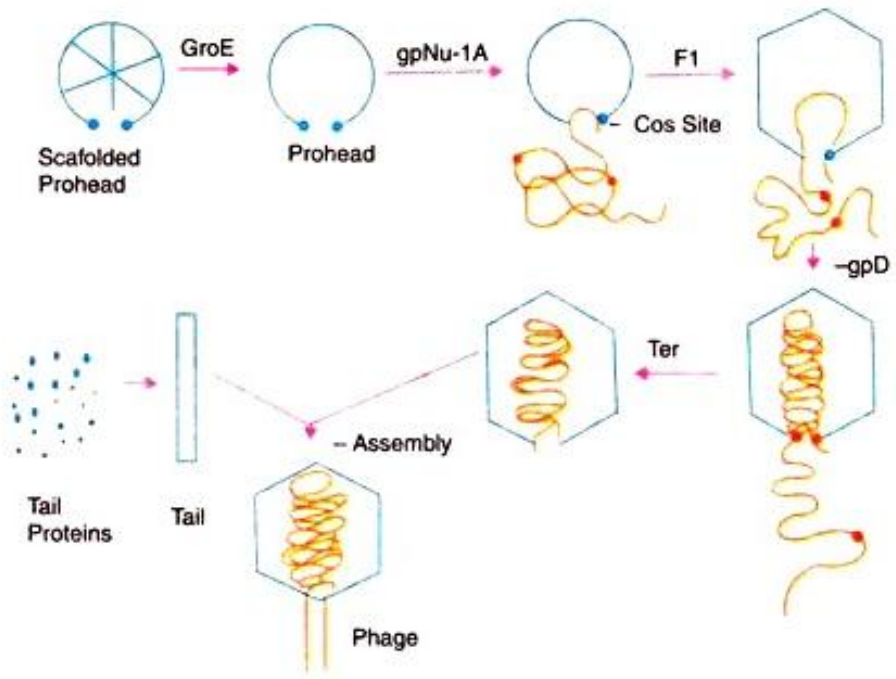


Figure: Diagrammatic representation of assembly of phage Lambda

When the next cos site reaches the head during the process of filling, it is cut by Ter system generating the sticky ends. The unpacked DNA is released from the filled head. Insertion of phage λ DNA occurs till the cos site comes. The fully packed particle is called black particle.

During this process tail is assembled by several tail proteins, and terminated by a head-tail connector protein. The complete tail is bound to the head through the short piece of ssDNA and head protein present in the neck.

The free ssDNA of released DNA binds to the neck of the second pro-head, and so on. In this way the complete phage particles are formed. Murialdo (1991) has reviewed the bacteriophage λ DNA maturation and packaging.

(v) Lysis:

Inside the bacterial cell about 100 particles are assembled within an hour. The two genes S and R of λ take part in bacterial lysis. The gpS stops metabolism of bacterial cell, and gpR lyses the cell wall. Finally progenies are released from the destroyed cell.

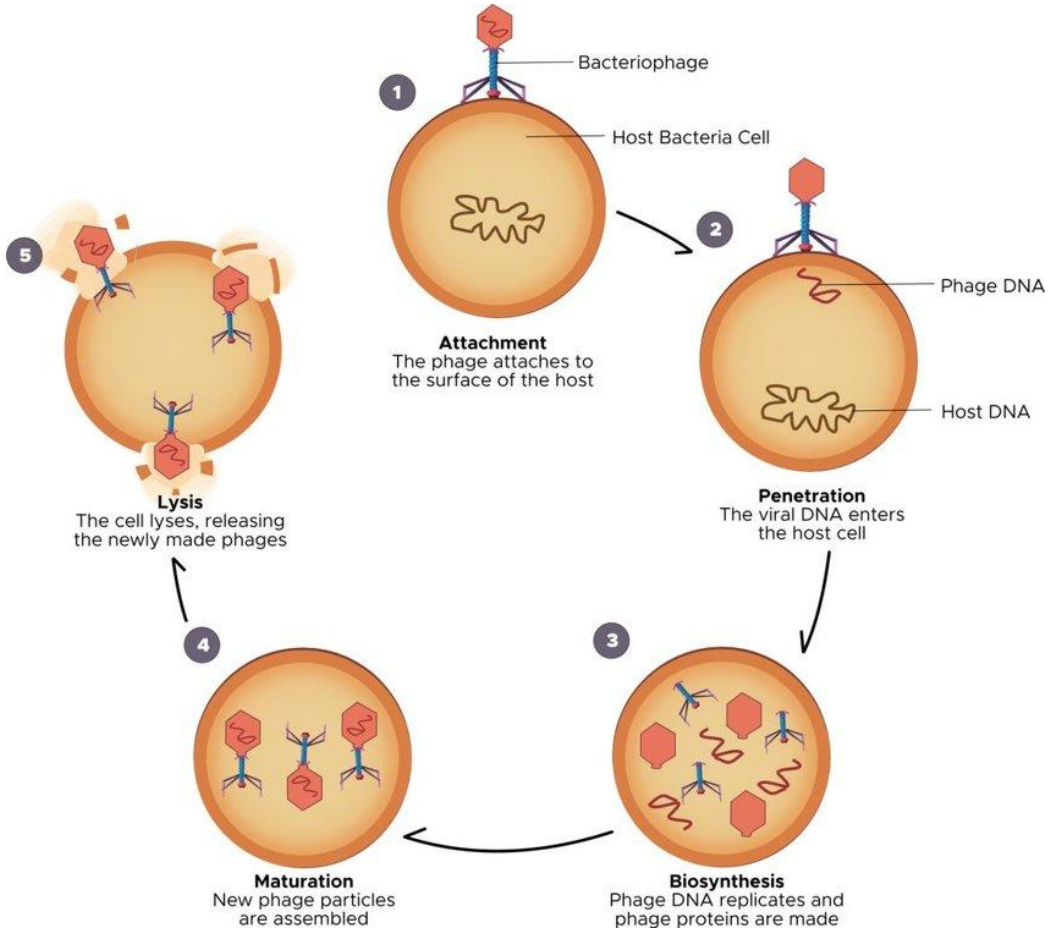


Figure: Lytic Cycle of Bacteriophages

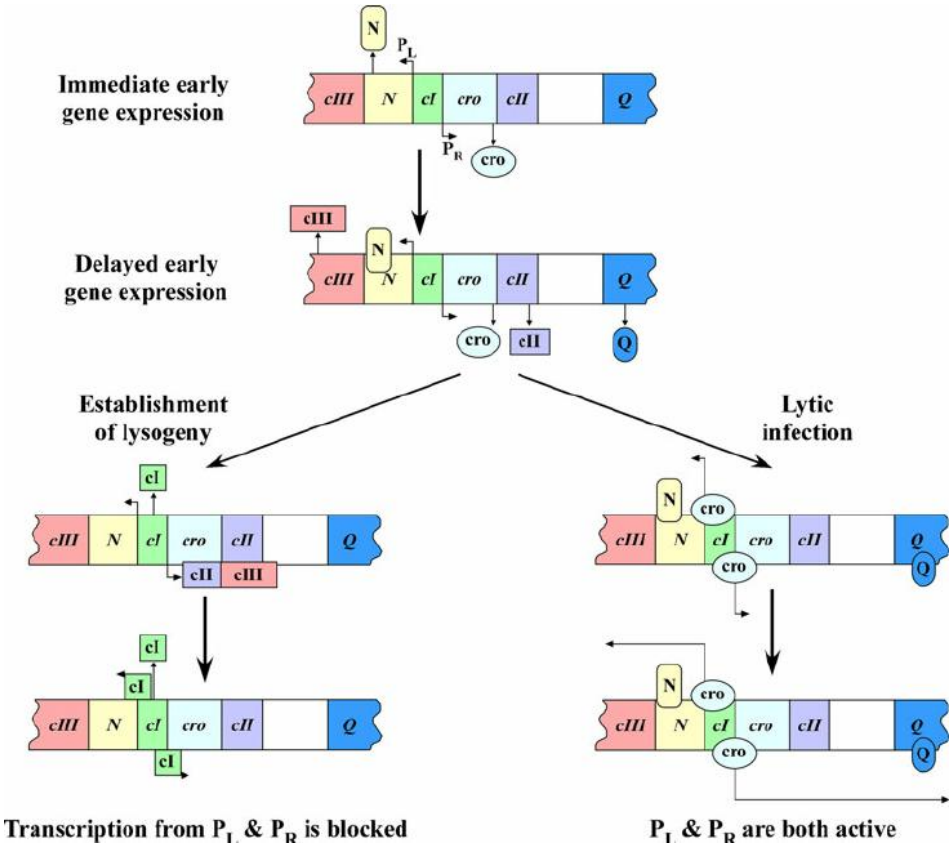


Figure: Establishment of Lytic or lysogenic pathway

5. 10 Lysogenic Cycle:

The alternative cycle of phage X where progenies are not produced is called lysogenic pathway. The phage genome is integrated into bacterial chromosomes. The host cell survives for indefinite time. The host cells that contain integrated phage DNA, i.e. prophage, is called the lysogen.

The prophage multiplies for several generations. The prophage is excised from the bacterial chromo-somes due to stimulation by UV irradiation or mitomycin C. After excision of DNA, the phage leads lytic cycle and the host cell is killed.

The lysogenic bacteria bear the two key features, immunity to super-infection by other phage λ and induction under certain environmental conditions to enter into lytic cycle. The immunity to super-infection and establishment of lysogeny in lysogens is conferred in the presence of λ repressor coded by cl gene. In a lysogen, repressor is always synthesized to bind operators OL and OP resulting in blocking of RNA polymerase activity.

Thus the repressor prevents the transcription of all prophage genes except its own. As a result of blocking of PL, transcription of N gene does not occur. Similarly blocking of PR prevents the early transcriptional genes i.e. O, P and O genes.

The gpcll activates the specific promotor site (PRE) for transcription of cl gene and synthesis of the repressor (gpcl). Therefore, the maintenance of lysogenic state requires that the synthesis of repressor must be continued.

Establishment of lysogenic state occurs as described below:

(i) Integration (Insertion):

The process of firmly joining of phage DNA with bacterial chromosomes is called integration or insertion. The significant features of insertion mechanism have been given by Allen Campbell in 1962 who obtained a λ prophage map by three factor crosses with two I genes and a gal (galactose utilizing) gene marker of bacterial chromosomes.

The two important features of the Campbell model are:

(i) The formation of a circular DNA from the linear DNA, and

(ii) At specific loci in a phage and bacterial DNA, a single reciprocal recombination results in the insertion of phage DNA into the bacterial chromosome.

Campbell Model of Integration (A) of Phage λ DNA into the Bacterial Chromosome and its Excision (B)

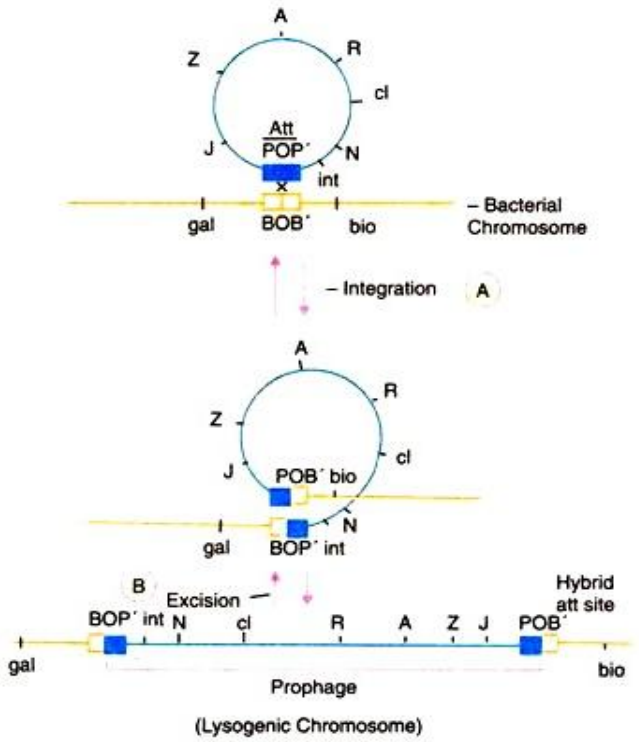


Figure: Campbell model of integration A. of phage lambda DNA into bacterial chromosome and its excision B.

(a) The Attachment sites:

The specific loci are called the attachment sites (Fig.18.18). The attachment site of phage is designed as attP which consists of two halves, P.P. Similarly, the attachment site of bacterial chromosomes designated as attB, and its two halves as B.B.

The dots (.) between the two att sites are the points where crossing over occurs. This point has a common base pairs and designated as O. Thus the complete att sites are designated as POP' and BOB'. The phage att site is located between the int and J genes, and bacterial att site is situated between gal (galactose) and bio (biotin) genes.

(b) Mechanism of Integration:

The essence of mechanism which is called Campbell model is the circularization of DNA followed by physical breakage and rejoining of phage and bacterial DNA between the POP' and BOB'. The Ter endonuclease makes staggered nicks in A, DNA. The gpcll stimulates transcription of the int gene at the same time as that of cl gene.

The int gene codes for synthesis of an integrase enzyme which becomes in plentiful before I repressor turns off transcription. The attPOP' and attBOB' match each other. The integrase with the help of a special bacterial protein catalyzes the physical exchange of viral and bacterial DNA strand.

The circular DNA is integrated into the E.coli chromosomes as a linear DNA between gal and bio genes, and is called prophage (Fig 18.18). The phage DNA joins with bacterial chromosomes by covalent bonds. The process by which the X DNA is inserted into the bacterial chromosome is called site-specific recombination. The point recognizes the att sites and brings about the reciprocal crossing over between the POP' and BOB' sites.

(ii) Replication:

The bacterium containing a complete set of phage genes is called lysogen and the life cycle as lysogenic cycle. The process of formation of a lysogen by a temperate phage is called lysogenization. Now the prophage replicates normally under the control of the bacterium by normal bacterial replication mechanism. The replication prophage contributes to viral growth and produces phage particles.

However, integration is not an absolute requirement for lysogeny. In E.coli phage, P1 is similar to X which circularizes after infection and starts synthesizing repressor. Therefore, it remains as an

independent circular DNA molecule in the lysogen and replicates as the chromosome. After the bacterial cell divides, the daughter cell contains one or two copies of phage genome.

(iii) Excision:

When the host cell is unable to survive, the λ prophage leaves the E.coli genome and begins the production of new phages. This process is known as induction which is triggered by a drop in X repressor level. Whenever, the repressor will decline, the lytic cycle will commence. In addition, induction occurs in response to environmental factors e.g. UV light or chemical mutagens that damage host DNA.

This damage causes the synthesis of recA protein, which acts as protease and cleaves repressor chain between the two domains. RecA protein binds to X repressor and stimulates it to proteolytically cleave itself. An early gene (xis gene) codes for synthesis of excision are that binds to the integrase (int gene product) and enables it to excise the prophage. Thus, the excision is the reversal process of integration.

After excision phage is converted to its circular form and enters the lytic cycle. During the course of excision, as a result of mistake, the bacterial gal or bio gene remains included in phage genome. This mistake occurs at a frequency of one in a million.

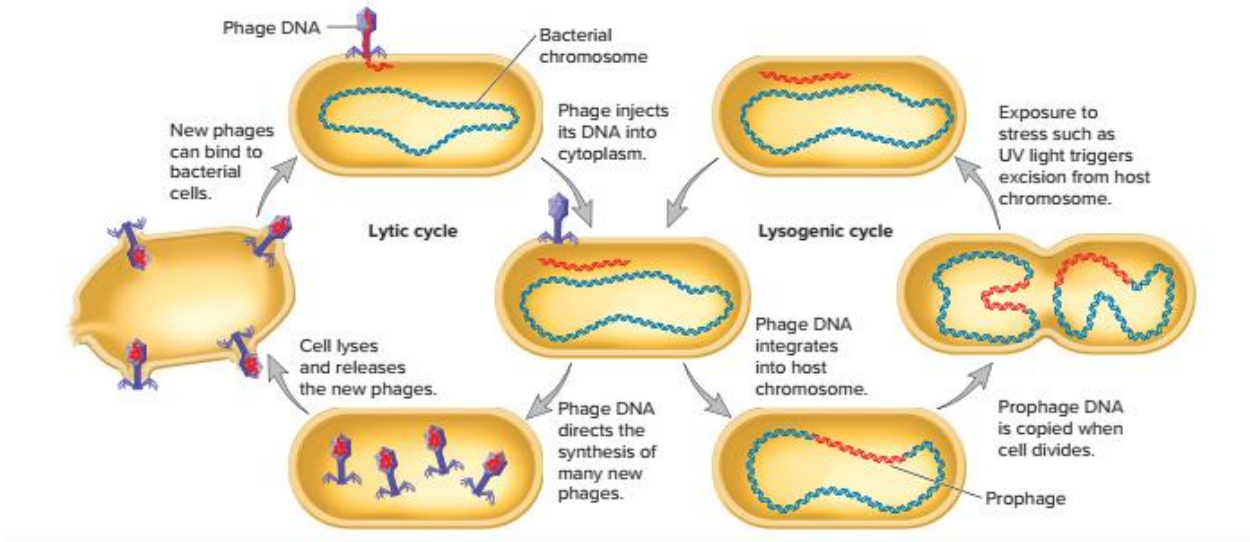


Figure: Lytic and Lysogenic Cycles of Temperate Phages. Temperate phages have two phases to their life cycles. The lysogenic cycle allows the genome of the virus to be replicated passively as the host cell's genome is replicated. Certain environmental factors such as UV light can cause a switch

from the lysogenic cycle to the lytic cycle. In the lytic cycle, new virus particles are made and released when the host cell lyses. Virulent phages are limited to just the lytic cycle.

5.11 SARS Virus:

Coronaviruses are a large family of viruses that can cause diseases ranging from the common cold to Severe Acute Respiratory Syndrome (SARS). SARS is a viral respiratory illness caused by a coronavirus called SARS-associated coronavirus (SARS-CoV). SARS was first reported in Asia in February 2003. The illness spread to more than two dozen countries in North America, South America, Europe, and Asia before the SARS global outbreak of 2003 was contained. In general, SARS begins with a high fever (temperature greater than 100.4°F [>38.0°C]). Other symptoms may include headache, an overall feeling of discomfort, and body aches. Some people also have mild respiratory symptoms at the outset. About 10 percent to 20 percent of patients have diarrhea. After 2 to 7 days, SARS patients may develop a dry cough. Most patients develop pneumonia. The main way that SARS seems to spread is by close person-to-person contact. The virus that causes SARS is thought to be transmitted most readily by respiratory droplets (droplet spread) produced when an infected person coughs or sneezes. Droplet spread can happen when droplets from the cough or sneeze of an infected person are propelled a short distance (generally up to 3 feet) through the air and deposited on the mucous membranes of the mouth, nose, or eyes of persons who are nearby. The virus also can spread when a person touches a surface or object contaminated with infectious droplets and then touches his or her mouth, nose, or eye(s).

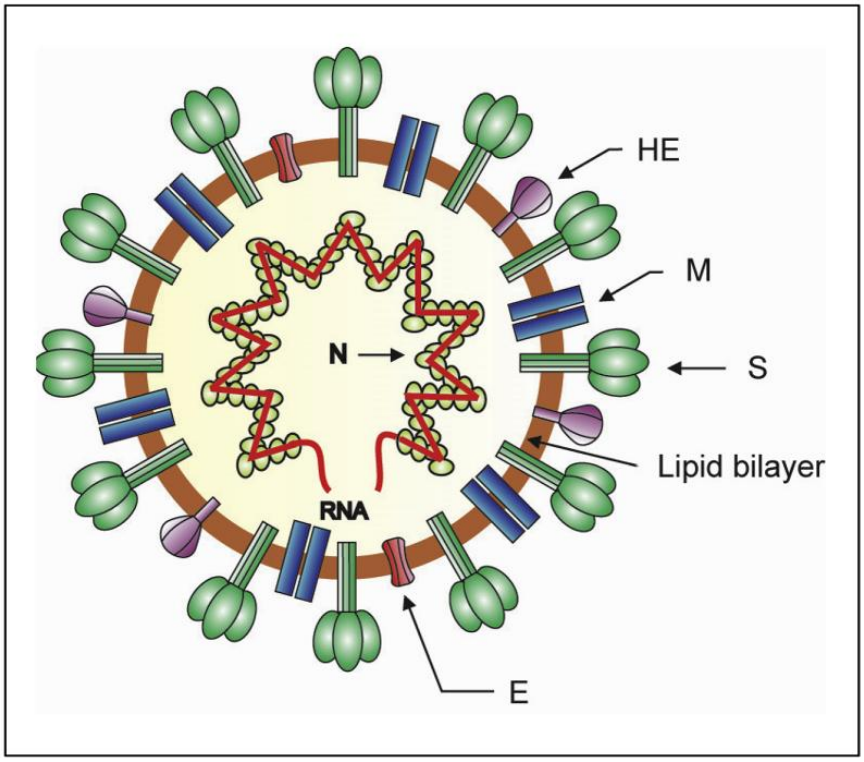


Figure: Schematic diagram of the coronavirus virion. Together with membrane (M) and envelope (E) transmembrane proteins, the spike (S) glycoprotein projects from a host cell-derived lipid bilayer, giving the virion a distinctive appearance.

5.12 MERS Virus

Middle East respiratory syndrome (MERS) is a viral respiratory disease caused by a novel coronavirus (Middle East respiratory syndrome coronavirus, or MERS- CoV) that was first identified in Saudi Arabia in 2012. Both SARS and MERS coronavirus infections in humans involve severe respiratory disease. Typical MERS symptoms include fever, cough and shortness of breath. Pneumonia is common, but not always present. Gastrointestinal symptoms, including diarrhea, have also been reported. Some laboratory-confirmed cases of MERS-CoV infection are reported as asymptomatic, meaning that they do not have any clinical symptoms, yet they are positive for MERS-CoV infection following a laboratory test. Most of these asymptomatic cases have been detected following aggressive contact tracing of a laboratory-confirmed case.

MERS-CoV is a zoonotic virus, which means it is a virus that is transmitted between animals and people. Studies have shown that humans are infected through direct or indirect contact with infected dromedary camels. MERS-CoV has been identified in dromedaries in several countries in the Middle East, Africa and South Asia.

No vaccine or specific treatment is currently available, however several MERS-CoV specific vaccines and treatments are in development. As a general precaution, anyone visiting farms, markets, barns, or other places where dromedary camels and other animals are present should practice general hygiene measures, including regular hand washing before and after touching animals, and should avoid contact with sick animals. The consumption of raw or undercooked animal products, including milk and meat, carries a high risk of infection from a variety of organisms that might cause disease in humans. Animal products that are processed appropriately through cooking or pasteurization are safe for consumption, but should also be handled with care to avoid cross contamination with uncooked foods.

5.13 Zika Virus

Zika virus is a mosquito-borne flavivirus that was first identified in Uganda in 1947 in monkeys. It was later identified in humans in 1952 in Uganda and the United Republic of Tanzania. The first recorded outbreak of Zika virus disease was reported from the Island of Yap (Federated States of Micronesia) in 2007. This was followed by a large outbreak of Zika virus infection in French Polynesia in 2013 and other countries and territories in the Pacific. In March 2015, Brazil reported a

large outbreak of rash illness, soon identified as Zika virus infection, and in July 2015, found to be associated with Guillain-Barré syndrome. To date, a total of 86 countries and territories have reported evidence of mosquito-transmitted Zika infection.

The incubation period (the time from exposure to symptoms) of Zika virus disease is estimated to be 3–14 days. The majority of people infected with Zika virus do not develop symptoms. Symptoms are generally mild including fever, rash, conjunctivitis, muscle and joint pain, malaise, and headache, and usually last for 2–7 days.

Zika virus infection during pregnancy is a cause of microcephaly and other congenital abnormalities in the developing fetus and newborn. Zika infection in pregnancy also results in pregnancy complications such as fetal loss, stillbirth, and preterm birth. Zika virus infection is also a trigger of Guillain-Barré syndrome, neuropathy and myelitis, particularly in adults and older children. Research is ongoing to investigate the effects of Zika virus infection on pregnancy outcomes, strategies for prevention and control, and effects of infection on other neurological disorders in children and adults.

Zika virus is primarily transmitted by the bite of an infected mosquito from the Aedes genus, mainly Aedes aegypti, in tropical and subtropical regions. Aedes mosquitoes usually bite during the day, peaking during early morning and late afternoon/evening. This is the same mosquito that transmits dengue, chikungunya and yellow fever. Zika virus is also transmitted from mother to fetus during pregnancy, through sexual contact, transfusion of blood and blood products, and organ transplantation.

There is no treatment available for Zika virus infection or its associated diseases. Symptoms of Zika virus infection are usually mild. People with symptoms such as fever, rash, or arthralgia should get plenty of rest, drink fluids, and treat pain and fever with common medicines. If symptoms worsen, they should seek medical care and advice.

5.14 Nipah Virus

Nipah virus (NiV) is a zoonotic virus (it is transmitted from animals to humans) and can also be transmitted through contaminated food or directly between people. In infected people, it causes a range of illnesses from asymptomatic (subclinical) infection to acute respiratory illness and fatal encephalitis. The virus can also cause severe disease in animals such as pigs, resulting in significant economic losses for farmers. Nipah virus was first recognized in 1999 during an outbreak among pig farmers in, Malaysia. No new outbreaks have been reported in Malaysia since 1999. It was also

recognized in Bangladesh in 2001, and nearly annual outbreaks have occurred in that country since. The disease has also been identified periodically in eastern India.

During the first recognized outbreak in Malaysia, which also affected Singapore, most human infections resulted from direct contact with sick pigs or their contaminated tissues. Transmission is thought to have occurred via unprotected exposure to secretions from the pigs, or unprotected contact with the tissue of a sick animal. During the later outbreaks in Bangladesh and India, Nipah virus spread directly from human-to-human through close contact with people's secretions and excretions.

Human infections range from asymptomatic infection to acute respiratory infection (mild, severe), and fatal encephalitis. Infected people initially develop symptoms including fever, headaches, myalgia (muscle pain), vomiting and sore throat. This can be followed by dizziness, drowsiness, altered consciousness, and neurological signs that indicate acute encephalitis. Some people can also experience atypical pneumonia and severe respiratory problems, including acute respiratory distress. Encephalitis and seizures occur in severe cases, progressing to coma within 24 to 48 hours. The incubation period (interval from infection to the onset of symptoms) is believed to range from 4 to 14 days. However, an incubation period as long as 45 days has been reported.

Nipah virus infection can be diagnosed with clinical history during the acute and convalescent phase of the disease. The main tests used are real time polymerase chain reaction (RT-PCR) from bodily fluids and antibody detection via enzyme-linked immunosorbent assay (ELISA). Other tests used include polymerase chain reaction (PCR) assay, and virus isolation by cell culture.

Fruit bats of the family Pteropodidae – particularly species belonging to the Pteropus genus – are the natural hosts for Nipah virus. There is no apparent disease in fruit bats. The virus is highly contagious in pigs. Pigs are infectious during the incubation period, which lasts from 4 to 14 days.

5.15 Ebola Virus:

The Ebola virus causes an acute, serious illness which is often fatal if untreated. EVD first appeared in 1976 in 2 simultaneous outbreaks, one in what is now Nzara, South Sudan, and the other in Yambuku, DRC. The latter occurred in a village near the Ebola River, from which the disease takes its name. Ebola then spreads through human-to-human transmission via direct contact (through broken skin or mucous membranes) with: 1. Blood or body fluids of a person who is sick with or has died from Ebola 2. Objects that have been contaminated with body fluids (like blood, feces, vomit) from a person sick with Ebola or the body of a person who died from Ebola.

The incubation period, that is, the time interval from infection with the virus to onset of symptoms is from 2 to 21 days. A person infected with Ebola cannot spread the disease until they develop symptoms. Symptoms of EVD can be sudden and include Fever, Fatigue, Muscle pain, Headache, Sore throat. This is followed by: Vomiting, Diarrhea, Rash, Symptoms of impaired kidney and liver function. In some cases, both internal and external bleeding (for example, oozing from the gums, or blood in the stools). Laboratory findings include low white blood cell and platelet counts and elevated liver enzymes.

Confirmation that symptoms are caused by Ebola virus infection are made using the following diagnostic methods: antibody-capture enzyme-linked immunosorbent assay (ELISA), antigen-capture detection tests, serum neutralization test, reverse transcriptase polymerase chain reaction (RT-PCR) assay, electron microscopy, virus isolation by cell culture.

Supportive care - rehydration with oral or intravenous fluids - and treatment of specific symptoms improves survival. A range of potential treatments including blood products, immune therapies and drug therapies are currently being evaluated. Health-care workers should always take standard precautions when caring for patients, regardless of their presumed diagnosis. These include basic hand hygiene, respiratory hygiene, use of personal protective equipment (to block splashes or other contact with infected materials), safe injection practices and safe burial practices.

5.16 Hantavirus

Hantaviruses are a family of viruses spread mainly by rodents and can cause varied disease syndromes in people worldwide. Infection with any hantavirus can produce hantavirus disease in people. Hantaviruses in the Americas are known as “New World” hantaviruses and may cause hantavirus pulmonary syndrome (HPS). Other hantaviruses, known as “Old World” hantaviruses, are found mostly in Europe and Asia and may cause hemorrhagic fever with renal syndrome (HFRS).

Each hantavirus serotype has a specific rodent host species and is spread to people via aerosolized virus that is shed in urine, feces, and saliva, and less frequently by a bite from an infected host. The most important hantavirus in the United States that can cause HPS is the Sin Nombre virus, spread by the deer mouse.

Hantavirus Pulmonary Syndrome (HPS) is a severe, sometimes fatal, respiratory disease in humans caused by infection with hantaviruses. Hemorrhagic fever with renal syndrome (HFRS) is a group

of clinically similar illnesses caused by hantaviruses from the family Bunyaviridae. HFRS includes diseases such as Korean hemorrhagic fever, epidemic hemorrhagic fever, and nephropathiaepidemica. The viruses that cause HFRS include Hantaan, Dobrava, Saaremaa, Seoul, and Puumala.

Symptoms of HFRS usually develop within 1 to 2 weeks after exposure to infectious material, but in rare cases, they may take up to 8 weeks to develop. Initial symptoms begin suddenly and include intense headaches, back and abdominal pain, fever, chills, nausea, and blurred vision. Individuals may have flushing of the face, inflammation or redness of the eyes, or a rash. Later symptoms can include low blood pressure, acute shock, vascular leakage, and acute kidney failure, which can cause severe fluid overload.

As of the end of 2019*, 816 cases of hantavirus disease were reported in the United States since surveillance began in 1993. These were all laboratory-confirmed cases and included HPS and non-pulmonary hantavirus infection.

Rodent control is the primary strategy for preventing hantavirus infections. Rodent populations near human communities should be controlled, and rodents should be excluded from homes. Individuals should avoid contact with rodent urine, droppings, saliva, and nesting materials, and the safety measures described below should be followed when cleaning rodent-infested areas.

5.17 Viriods

Viroids are infectious RNA molecules that lack a protein component. Viroids are small, circular, single-stranded RNA molecules that are the smallest known pathogens. They range in size from 246 to 399 nucleotides and show a considerable degree of sequence homology to each other, suggesting that they have common evolutionary roots. Viroids cause a number of important plant diseases and can have a severe agricultural impact. A few well-studied viroids include coconut cadang-cadang viroid (246 nucleotides) and potato spindle tuber viroid (359 nucleotides). No viroids are known that infect animals or microorganisms. They cause over 20 different plant diseases, including potato spindle-tuber disease, exocortis disease of citrus trees, and chrysanthemum stunt disease.

The extracellular form of a viroid is naked RNA; there is no protein capsid. Although the viroid RNA is a single-stranded, covalently closed circle, its extensive secondary structure forms a hairpinshaped double-stranded molecule with closed ends. This makes the viroid sufficiently stable to exist outside the host cell and protects the viroid while inside the cell from cellular ribonucleases. Because it lacks a capsid, a viroid does not use a receptor to enter the host cell. Instead, the viroid

enters a plant cell through a wound, as from insect or other mechanical damage. Once inside, viroids move from cell to cell via plasmodesmata, which are the thin strands of cytoplasm that link plant cells. Viroid RNAs do not encode proteins and thus the viroid is totally dependent on its host for replication. Plants have several RNA polymerases, one of which has RNA replicase activity, and this is the enzyme that replicates the viroid. The replication mechanism itself resembles the rolling circle mechanism used for genome synthesis by some small viruses. The result is a large RNA molecule containing many viroid units joined end to end. The viroid has ribozyme activity and uses it to self-cleave the large RNA molecule, releasing individual viroids.

Viroids are currently divided into two families. Viroids in family Pospiviroidae have circular RNA that exists as a rodlike shape due to intrastrand base pairing, which forms double-stranded regions with single-stranded loops. The circular RNA of viroids in the family Avsunviroidae is shaped like a rod with a highly branched structure at one end, rather like a tree trunk with its roots. Each branch is formed by intramolecular base pairing of the RNA that creates a stemloop structure. The two types of viroids replicate in different locations within the infected plant cell.

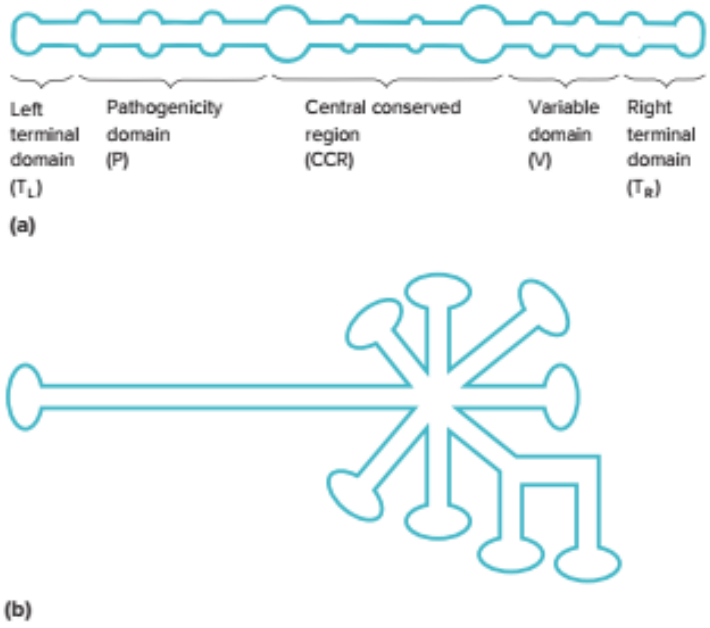


Figure: a) This schematic diagram shows the general organization of a viroid belonging to the family Pospiviroidae. The closed single-stranded RNA circle has extensive intrastrand base pairing and interspersed unpaired loops. Also shown are the five domains identified in the molecule. Most changes in viroid pathogenicity arise from variations in the P and T L domains. (b) Schematic

diagram of a viroid belonging to Avsunviroidae. These viroids lack the central conserved region observed in pospiviroid

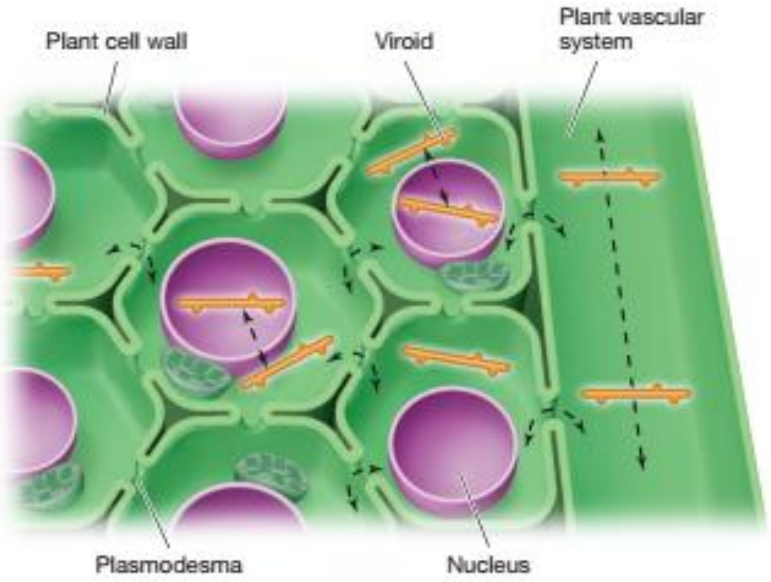


Figure: Viroid movement inside plants. After entry into a plant cell, viroids (orange) replicate either in the nucleus or the chloroplast. Viroids can move between plant cells via the plasmodesmata (thin threads of cytoplasm that penetrate the cell walls and connect plant cells). On a larger scale, viroids can also move around the plant via the plant vascular system.

5.18 Prions:

Prions (for proteinaceous infectious particles) are dramatically simpler than viruses, comprising only a single protein. They cause a variety of neurodegenerative diseases in humans and other animals, including scrapie in sheep, bovine spongiform encephalopathy (BSE or "mad cow disease"), and the human diseases kuru, fatal familial insomnia, Creutzfeldt-Jakob disease (CJD), and Gerstmann-Strässler-Scheinker syndrome (GSS). All result in progressive degeneration of the brain and eventual death. At present, no effective treatment exists.

The best-studied prion is the scrapie prion. Scrapie is caused by an abnormal form of a cellular protein. The normal cellular protein is called PrPC. Although its exact function is unknown, it has been shown to reside on the cell surface of neurons and other cell types, and play a role in brain development. The abnormal form is called PrPSc (for scrapie-associated prion protein). Evidence supports a model in which entry of PrPSc into the brain of an animal causes the PrPC protein to

change from its normal conformation to an abnormal form that aggregates. The newly produced PrPSc molecules then convert more PrPC molecules into the abnormal PrPSc form. How the PrPSc causes this conformational change is unclear. However, the best-supported model is that the PrPSc directly interacts with PrPC, causing the change. It is noteworthy that mice lacking the PrP gene cannot be infected with PrPSc. An important characteristic of PrPSc is its ability to oligomerize—that is, to form short chains of PrPSc proteins. This is important because oligomers may play a central role in continued conversion of PrPC to PrPSc, as well as in the spread of PrPSc from cell to cell and from one individual to another. Furthermore, evidence suggests that the toxic form of PrPSc is a trimer. However, how the PrPSc trimer causes disease is not understood.

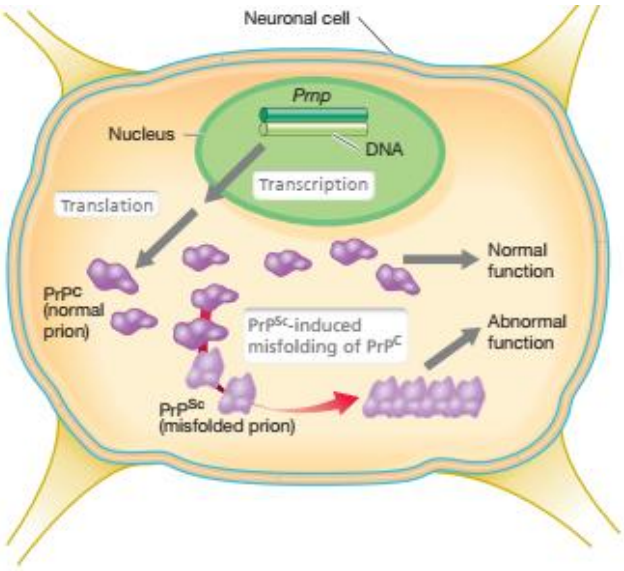


Figure: Mechanism of prion misfolding. Neuronal cells produce the native form of the prion protein. The pathogenic form catalyzes the refolding of native prions into the pathogenic form. The pathogenic form is protease resistant, insoluble, and forms aggregates in neural cells. This eventually leads to destruction of neural tissues (see part a) and neurological symptoms. (Source: *Brock's Biology*)

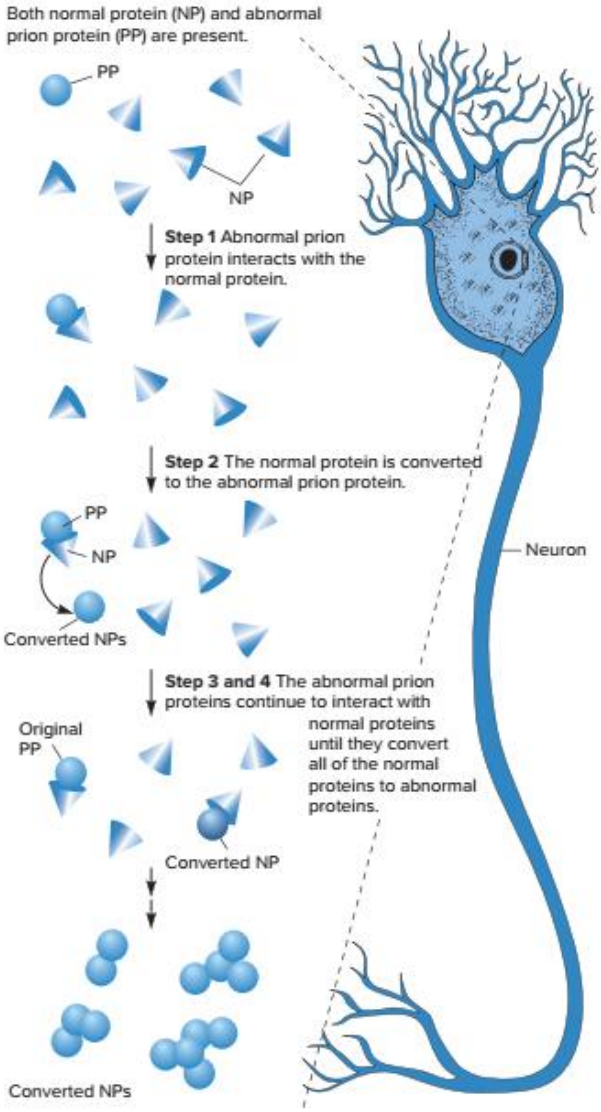


Figure: One Proposed Model for Prion Replication. The normal and prion proteins differ in their tertiary structures. Recent studies suggest that cofactors may function in prion replication. One proposed cofactor is RNA, which is thought to act as a catalyst for the conversion of PrPC to PrPSc. (Source: *Prescott's Microbiology*)

PrPSc is identical in amino acid sequence to PrPC from the same animal species, but it has a different conformation. For example, native prion proteins are largely a-helical, whereas the pathogenic forms contain less a-helix and more b-sheet secondary structure. As the pathogenic prions accumulate and aggregate, they form insoluble crystalline fibers referred to as amyloids in neural cells; this leads to disease symptoms including the destruction of brain and other nervous tissue. PrPC functions in the cell as a cytoplasmic membrane glycoprotein, and it has been shown that membrane attachment of pathogenic prions is necessary before disease symptoms commence.

Mutant versions of PrPSc that can no longer attach to nerve cell cytoplasmic membranes still aggregate but no longer cause disease.

Besides the transmissible spongiform encephalopathies, amyloids are also associated with debilitating human diseases such as Alzheimer's, Huntington's, Parkinson's, and type 2 diabetes.

6. Microbial growth and nutrition

In biology, growth can be defined as an irreversible increase in cellular mass due to active synthesis of all constituents. Growth results in increase of cell number (except in coenocytic organisms).In case of multicellular organisms, the increase of cell number leads to the increase of size as the daughter cells are also remained together.In contrast, in case of unicellular organisms, cell multiplication leads to the increase in number of individuals in a population i.e; population size is increased.

As we are discussing about the **microbial growth**, growth may be defined as the increase in number of cells in the population in case of bacteria(unicellular organism).

6.1 Nutritional types & requirements

Sources of Carbon, Energy and electrons:

Carbon Source-

- Carbon di-oxide is the sole or principal biosynthetic carbon source in case of **autotrophs**.
- Organisms that use reduced, preformed organic molecules as their carbon source for growth is called **heterotrophs.**

Energy Source-

- **Phototrophs** use light as their energy source.
- **Chemotrophs** obtain energy from the oxidation of chemical compounds(either organic or inorganic).

Electron Source-

- **Lithotrophs** use reduced inorganic substances as their electron source
- **Organotrophs** extract electrons from reduced organic compounds.

Table: Major nutritional types of microorganisms

Nutritional type	Carbon source	Energy source	Electron source	Example
Photolithoautotroph	CO_2	Light	Inorganic electron donor	Purple and Green sulphur bacteria,

				cyanobacteria, Diatom
Photoorganoheterotroph	Organic carbon	Light	Organic electron donor	Purple non sulphur bacteria, green non sulphur bacteria
Chemolithoautotroph	Co2	Inorganic Chemicals	Inorganic electron donor.	Sulphur oxidizing bacteria, hydrogen oxidizing bacteria, methanogens, nitrifying bacteria, iron oxidizing bacteria.
Chemolithoheterotroph	Organic carbon	Inorganic chemicals	Inorganic electron donor	Some sulphur oxidizing bacteria
Chemoorganoheterotroph	Organic carbon	Organic chemicals, often same as C source .	Organic electron donor, often same as C source.	Most non photosynthetic microbes, including most pathogens, fungi and many protists and archaea.

Environmental factors that influences on microbial growth:

- Solutes and Water activity
- pH (Acidic/alkaline)
- Temperature
- Oxygen concentration
- Pressure
- Radiation etc.

Microbial responses to various envioronmental factors-

1. **Solutes and Water activity:**

Osmotolerant- Able to grow over wide ranges of water activity or osmotic concentration**.**

Halophile-To grow, they requires high levels of sodium chloride(high salt conc.),usually above 0.2 M.

2. pH

Acidophile- They can grow optimally between pH
range 0-5.5.

Neutrophile- Optimum growth can be observed between pH
5.5-8.0.

Alkaliphile- Grow optimally between pH 8.0-11.5.

3.Temperature

Psychrophile- Grows at 0 °and shows optimum growth temperature of 15° C or lower.

Psychotroph-They can grow at 0-7° C and has an optimum growth between 20-30° C and shows maximum growth at around 35° C.

Mesophile- Shows optimal growth between 20-45° C

Thermophile-They can grow at 55° C or higher temperature; exhibit optimum growth between 55-65° C.

Hyperthermophile- Shows optimum growth between 85° C & 113° C.

4.Oxygen concentration

Obligate aerobe-They completely depend on atmospheric oxygen for growth.

Facultative anaerobe- They does not require oxygen for growth; but they shows better growth in the presence of oxygen.

Aerotolerant anaerobe- They can grow equally well either in presence of oxygen or in absence of oxygen.

Obligate anaerobe- These type of organisms can not tolerate oxygen and eventually dies in the presence of oxygen.

Microaerophile-They requires oxygen level 2-10% for growth and damaged by atmospheric oxygen level(20%).

5. Pressure

Peizophile (barophile)- These types of microorganisms grow rapid at high hydrostatic pressures.

6.2 Types of media

Culture media:

Majority of bacteria can be cultivated under artificial conditions on suitable culture media. A culture medium must contain all the raw materials that are needed by the particular microorganism to build up its cellular constituents such as carbohydrate, protein, lipids, nucleic acids etc. All the essential constituents that are needed in their natural habitat should be provided in culture media. Since, water is the most important constituent of all living systems, microorganisms can best thrive in an aqueous medium. So, the other ingredients of culture medium are present in dissolved condition.

Specialized media are essential for isolation and identification of specific microorganisms. Although all microorganisms need sources of energy and macro & micro nutrients, the precise composition of a satisfactory medium depends on that particular species to be grown and it's specific nutritional requirements. Sometimes, the natural habitat of the particular microorganisms give the idea about the nutritional requirements which lead to the appropriate selection of culture media.

Culture media can be classified based on several parameters: the chemical constituents from which they are made, their physical nature, and their function.

Basis of classification-

- Chemical composition- Such as Synthetic media , complex media etc.
- Physical Nature- Such as Liquid, semisolid , solid.
- Function– Supportive (general purpose), enriched media, selective media, differential media etc.

- According to chemical composition ; media can be classified into-

Synthetic media- A medium in which all chemical components are known is called a defined or synthetic medium.

It can be in liquid form (broth) or solidified by solidifying agent Agar. Defined media or synthetic media are often used to culture photoautotrophs such as cyanobacteria and photosynthetic protists. Many chemoorganoheterotrophs can also be grown in this type of media.

Example- A simple synthetic medium which supports growth of common bacteria such as *Escherichia coli* can be prepared by following ingredients (nutritional requirements)- Glucose , Na2HPO4, KH2PO4, (NH4)2 SO4 , MgSO4.7H2O , CaCl2 , FeSO4 .7H2O & pH 6.8-7.0

Complex media

Media that contains some ingredients of unknown chemical composition are known as Complex media. Complex media are very useful because a single complex medium may be able to meet all the nutritional requirements of many different microorganisms. Complex media are often needed because sometimes the nutritional requirement of a particular microorganism are unknown and thus a defined or synthetic medium cannot be constructed.

Most complex media contains undefined components such as Peptones, meat extract & yeast extract. Peptones are protein hydrolysates prepared by partial proteolytic digestion of meat, casein ,soya meal, gelatine ,and other protein sources & they serve as sources of carbon, energy and nitrogen. Beef extract contains amino acids , peptides ,nucleotides, organic acids , vitamins and minerals. Yeast extract serves as excellent source of Vitamin B as well as nitrogen and carbon compounds. Three commonly used complex media are **nutrient broth** , **tryptic soy broth** and **MacConkey agar**.

A complex bacteriological medium commonly used to obtain rapid growth of microorganisms is nutrient broth which contains beef-extract, peptone and sodium chloride.

For the growth of saprophytic fungi, a common complex medium is used called **malt-extract agar**.

Potato-dextrose Agar containing extracts of boiled potato, glucose and some salts is another media for growing fungi.

Straw-infusion broth is a good complex medium for growth of soil Actinomycetes.

Blood Agar , Serum Albumin agar are often used for the growth of pathogenic bacteria.

- According to function, several functional types of media can be classified-
- **Supportive Media**- Some media are called general purpose or supportive media because they sustain the growth of many microorganisms. Media such as tryptic soy broth and tryptic soy agar are called general purpose media or supportive media.
- **Enriched Media**- Additional nutritional requirements are sometimes added to supportive media in order to enrich the media & design the media to encourage the growth of specific groups of

microorganisms , specifically fastidious microbes(Having complex or particular nutritional requirements). Example- Blood Agar medium.

- **Selective Media**- Selective media allow the growth of particular microorganisms, while inhibiting the growth of other microorganisms. As for example, Gram- negative bacteria will grow on media containing bile salts or dyes such as basic fuchsin and crystal violet; however, the growth of gram positive bacteria is inhibited.

  Other examples of selective media are **Eosin methylene blue agar & MacConkey agar** which are widely used for the detection of *E . coli* and related bacteria. These selective media suppress the growth of Gram-positive bacteria.

  If one researcher is intended to study the types of **nitrogen-fixing bacteria** present in a certain soil, the designed selective medium would contain other ingredients except any nitrogenous compound because in such a medium, organisms are capable of utilizing atmospheric nitrogen alone for it's growth.

  If we want to find out **antibiotic resistant strains** in certain population, the selective medium would contain the particular antibiotic at a concentration which is inhibitory for the sensitive strains.

- **Differential Media-** Differential media are capable to distinguish among different group of microbes and permit tentative identification of microorganisms based on their biological characteristics.

  Blood Agar is an example of **both differential medium & an enriched medium.** It can distinguish between haemolytic and nonhemolytic bacteria. Some haemolytic bacteria(Some Streptococci & Staphylococci) produce clear zones around their colonies because of red blood cell destruction. Blood agar is a growth medium in which blood (usually sheep blood) provides protein, carbohydrate, lipid ,iron , vitamins and some other necessary growth factors for the cultivation of fastidious microorganisms.

6.3 Phases of growth-

Bacterial growth phases can be divided into –

- ❖ Lag Phase.
- ❖ Exponential Phase/ Log Phase.
- ❖ Stationary Phase.

❖ Senescence & Death Phase.

Lag Phase-

When microorganisms are introduced into fresh culture medium, cell number is not increased immediately. It is not the time of inactivity , rather cells synthesize new components during this phase. The cells may be old and depleted of ATP , essential cofactors, and ribosomes ; these must be synthesized before the growth can begin. So , the first phase of bacterial growth can be defined as Lag phase when cells synthesize essential components at the advent of growth.

Exponential Phase (Log Phase)-

During the exponential(log phase), microorganisms grow and divide at the maximal rate possible depending upon their genetic potential ,nature of the respective medium and the environmental conditions. The rate of growth is constant during the exponential (log) phase as they completes the cell cycle and doubling in number at regular intervals. The growth rate during log phase depends upon several factors such as nutrient availability. The rate of microbial growth also increases with nutrient concentration but in a hyperbolic manner much like that seen in case of many enzymes. The shape of the curve reflects the rate of nutrient uptake by microbial transport proteins. At sufficiently high nutrient levels, the transport systems become saturated and the growth rate does not rise further with increasing nutrient concentration.

Stationary Phase-

In a closed system such as a batch culture, population growth eventually ceases and growth curve becomes horizontal. Final population size depends on nutrient availability and other factors as well as the type of microorganism cultured in the growth media. In stationary phase , the total number of viable microorganisms remain constant. The reason behind the constant number of viable microorganisms in stationary phase may be due to the balance between the cell division and cell death or the population may simply cease to divide but remain metabolically active.

One obvious reason microorganisms enter the stationary phase is the nutrient limitation. If an essential nutrient become severely depleted, population growth will slow and eventually stop.

Senescence and Death-

During this phase of bacterial growth, the number of viable cells often declines exponentially with cells dying at a constant rate. It is thought that nutrient deprivation and build up of toxic wastes

cause irreparable harm to the cells. For this reason when bacterial cells are transferred to the fresh medium, no cellular growth can be observed. Because loss of viability not always means the loss of total cell number. Perhaps cells died but did not undergo lysis.

❖ Sometimes the later part of Lag phase is called **acceleration phase and** the early part of stationary phase is called **deceleration phase.**

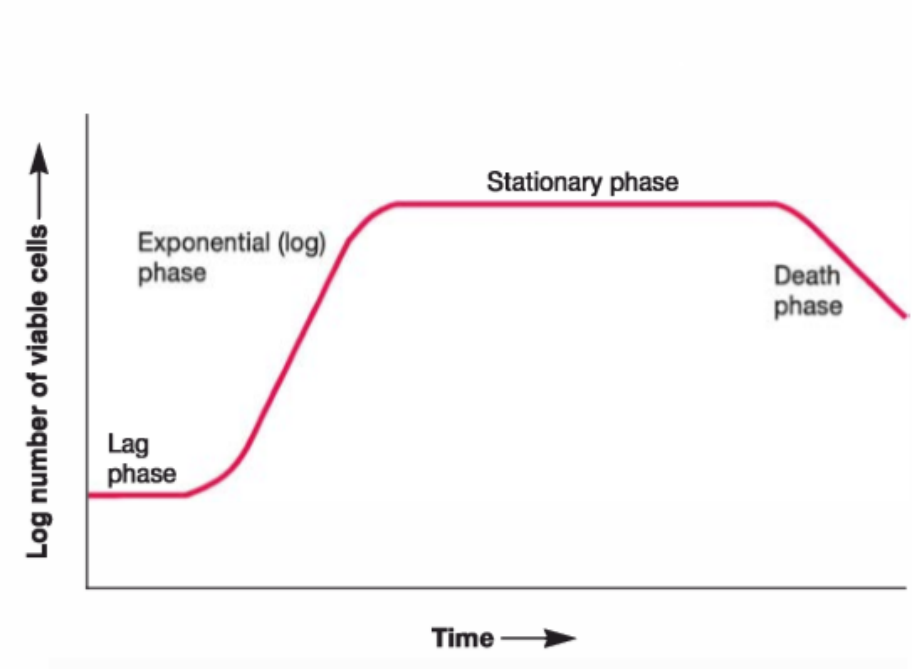


Figure: Growth curve

6.4 Batch culture, continuous culture, synchronous culture, Diauxic

Batch Culture:

Bacterial growth in a flask or any kind of big containers such as industrial fermenters holding a microbial growth supporting medium is known as **batch culture**.

Actually, a batch culture represents a 'closed system' because the initially present nutrient levels are gradually consumed during growth producing metabolic end products which gradually accumulates in the culture vessel. These accumulated metabolic end products in the culture vessel changes the pH value of the medium. In the batch culture, there is no provision for addition of fresh nutrients or

for the removal of end products or adjustment of pH value. Such type of culture procedure in a closed system is called the Batch culture. The environment of batch culture is subjected to constant changes such as depletion of nutrients, shifting of pH value, accumulation of toxic metabolites etc. Due to such changes, the duration of active growth phase is limited.

Continuous culture-

A continuous culture represents an 'Open' system in contrast to batch culture. For many experiments, it is very necessary to maintain a culture in an active state of growth over a prolonged time. To achieve this, a continuous culture can be developed which provides a constant environment for the growth of an organism.

One of the devices for growing microorganisms in a continuous culture is the Chemostat.

The simplest form of **chemostat** consists of a culture vessel provided with an inlet (for regulated entry of fresh culture medium) , an arrangement for pumping in sterilized air (for adequate aeration) and an outlet system for maintaining a constant volume of liquid in the culture vessel .The design of chemostat ensures a constant, regulated flow of fresh medium in a culture vessel and removal of equal quantity of toxic metabolites from the culture vessel through an outlet system. The continuous inflow of fresh medium prevents the depletion of essential nutrients and continuous outflow of accumulated toxic metabolites leads to the prolonged growth in a chemostat.

Figure: Chemostat system

The second type of continuous culture system called **turbidostat**. Turbidostat system has a photocell measures the turbidity of the culture in the growth vessel. The flow rate of media through the vessel is automatically regulated to maintain a predetermined turbidity as turbidity is related to cell density. Turbidostat maintains a desired cell density.

Synchronous Culture-

A synchronous culture is that type of culture in which all the cells of a particular population are in the same stage of development and they divide simultaneously.

In the batch culture, the cell population divides asynchronously that is the population contain cells in all possible stages of development (Some cells just have been produced by fission, others are in intermediate stages , and others may be ready for cell division).

In a synchronous culture, cell number increased in a step wise fashion because there would be no increase in cell number between two successive divisions.

But in synchronous culture , the cell mass still shows linear increase with time because the newly born cells increase in their mass between two successive divisions , though their number does not increase.

6.6Diauxic Growth/ Diauxic:

Diauxic growth or diauxic or Diphasic growth is any cell growth characterized by cellular growth in two phases. The diauxic growth can be represented by diauxic growth curve.

Diauxic growth, meaning double growth is caused by the presence of two sugars on a culture growth media, one of which is easier for the target bacterium to metabolize.

The preferred sugar is consumed first, which leads to rapid growth, followed by a lag phase. During the lag phase the cellular machinery used to metabolize the second sugar is activated and subsequently the second sugar is metabolized.

This can also occur when the bacterium in a closed batch culture consumes most of its nutrients and is entering the stationary phase when new nutrients are suddenly added to the growth media. The bacterium then enters a lag phase where it tries to ingest the food. Once the food starts being utilized , it enters to a new log phase showing a second peak on the growth curve.

Actually biphasic or diauxic growth is often observed when microbes are grown in a chemically defined medium containing two sugars (for example glucose and lactose). Typically, the two growth stages are separated by an often lengthy phase of arrested growth , the so called lag phase. Diauxic growth can be interpreted as an adaptation to maximise population growth in multi-nutrient environments.

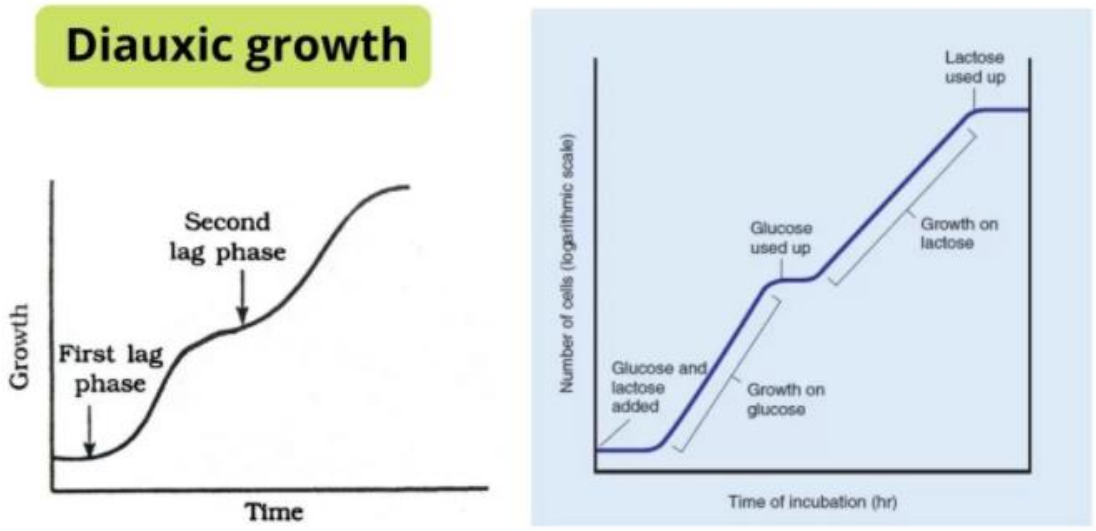


Figure: Diauxic growth

6.4 Kinetics of Growth

Calculation of the growth rate constant

Let N_0 = the initial population number

N_t = the population at time t

n = the number of generations in time t

For populations reproducing by binary fission

$$N_t = N_0 \times 2^n$$

Solving for n, the number of generations, where all logarithms are to the base 10,

$$\log N_t = \log N_0 + n \cdot \log 2, \text{ and}$$

$$n = \frac{\log N_t - \log N_0}{\log 2} = \frac{\log N_t - \log N_0}{0.301}$$

The growth rate constant (k) is the number of generations per unit time $\left(\frac{n}{t}\right)$. Thus

$$k = \frac{n}{t} = \frac{\log N_t - \log N_0}{0.301t}$$

Calculation of generation (doubling) time

If a population doubles, then

$$N_t = 2N_0$$

Substitute $2N_0$ into the growth rate constant equation and solve for

$$k = \frac{\log (2N_0) - \log N_0}{0.301g} = \frac{\log 2 + \log N_0 - \log N_0}{0.301g}$$

$$k = \frac{1}{g}$$

The generation time is the reciprocal of the growth rate constant.

$$g = \frac{1}{k}$$

Mathematics of Growth

During the exponential phase, each microorganism divides at a constant interval. Thus , the population doubles in number during specific length of time or in a specific time interval is called **Generation time**.

Suppose, a culture tube is inoculated with one cell that divides in every 20 minutes. Thus if there is 2 cells after 20 minutes, 4 cells will be there after 40 minutes and so on. Because the population is doubling in every generation, so the increase in population is always 2^n where 'n' is considered as the number of generations.

Table: Exponential growth

Time[1]	Division Number	2^n	Population[2] ($N_0 \times 2^n$)	$\log_{10} N_t$
0	0	$2^0 = 1$	1	0.000
20	1	$2^1 = 2$	2	0.301
40	2	$2^2 = 4$	4	0.602
60	3	$2^3 = 8$	8	0.903
80	4	$2^4 = 16$	16	1.204

1 The hypothetical culture begins with one cell having a 20-minute generation time.
2 Number of cells in the culture.

- Mathematics of growth during the exponential phase shows the calculation of two important values- **Growth rate constant & generation time.**

7. Control of Microorganisms

7.1 Control of Microorganisms: physical, chemical, and chemotherapeutic agents:

The control and destruction of microorganisms is a topic of immense practical importance. Although most of the microorganisms are beneficial but some microorganism cause undesirable consequences, such as food spoilage and disease. So, it is very essential to be able to kill a wide variety of microorganisms or inhibit their growth to minimize their destructive effects. Some disease causing pathogenic microorganisms exist in the nature and some are responsible for the contamination of water, food, and other substances also. So, the main goal of microbial control is to destroy the pathogenic microorganisms and their transmission and also eliminate microorganisms responsible for the contamination of water, food, and other substances.

Control of microorganisms by physical, chemical, and biological agents is very crucial. Any chemical, physical, or biological product that controls microorganisms is referred to as an antimicrobial agent.

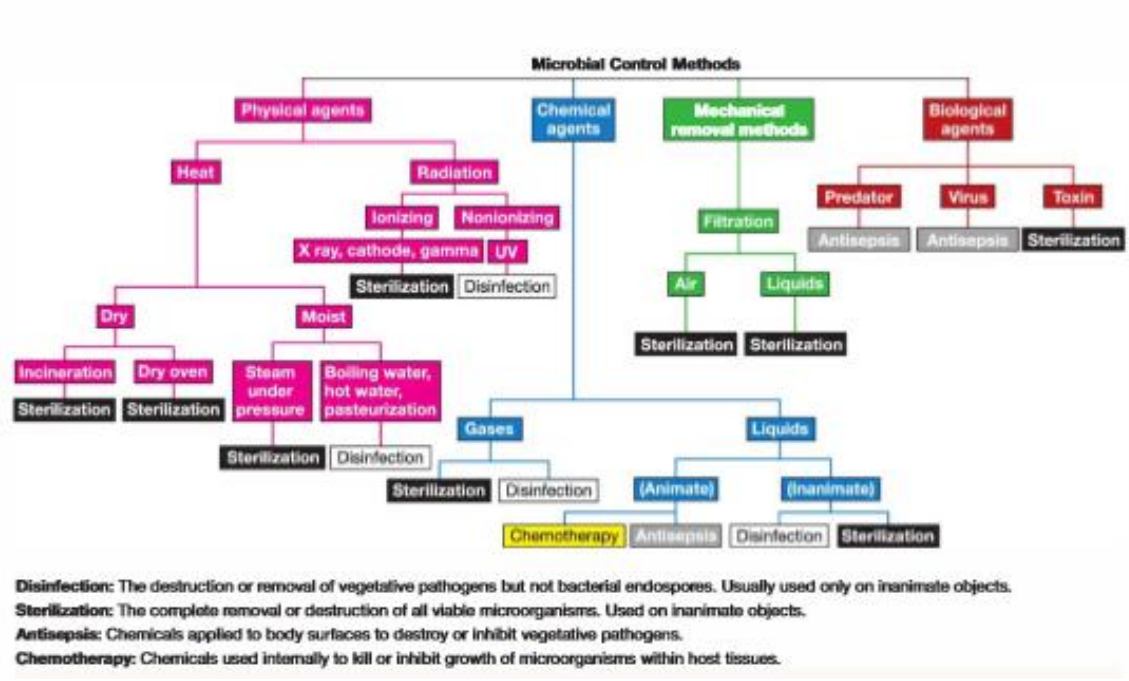


Figure: Microbial control methods

Physical Control Method-

Heat and other physical agents are normally used to control microbial growth and sterilize objects.The most frequently employed physical agents are heat and radiation

1) Heat

- We all know that moist heat readily destroys viruses, bacteria, and fungi. Moist heat kills by degrading nucleic acids and denaturing enzymes and other essential proteins. It also disrupts cell membranes.
- Exposure to boiling water for 10 minutes is sufficient to destroy vegetative cells and eukaryotic spores.
- But sometimes problem arises as sometimes the temperature of boiling water (100°C or 212°F at sea level) is not sufficient to destroy bacterial spores, which may survive hours of boiling.

  To destroy bacterial endospores, moist heat sterilization must be carried out at temperatures above 100°C which requires the use of saturated steam under pressure. Steam sterilization is carried out with an **autoclave** (usually 121^{o}C and 15 pounds of pressure). At this temperature, saturated steam destroys all vegetative cells and spores.
- Many heat-sensitive substances, such as milk, are treated with controlled heating at temperatures well below boiling, a process known as **pasteurization.**

2) Radiation

Ultraviolet (UV) radiation around 260 nm is quite lethal and it causes thymine-thymine dimerization of DNA, preventing replication and transcription. Ionizing radiation is an excellent sterilizing agent and penetrates deep into objects.

Ionizing radiation leads to destruction of bacterial spores and all microbial cells. However, ionizing radiation is not always effective against viruses.

Gamma radiation from a cobalt 60 source and accelerated electrons from high-voltage electricity are used in the cold sterilization of antibiotics, hormones, sutures, and plastic disposable supplies (such as syringes).

Gamma radiation and electron beams are also used to sterilize and "pasteurize" meat and other foods.

Chemical control agents

Chemicals are more often employed in disinfection and antisepsis.

Some of disinfectants and antiseptics are enlisted below-

- Phenolics
- Alcohols

- Halogens

  The halogens iodine and chlorine are important antimicrobial agents. Chlorine is used as disinfectant for municipal water supplies and swimming pools and is employed in the dairy and food industries also.

  Iodine is used as a skin antiseptic and kills by oxidizing cell constituents and iodinating cell proteins. At higher concentrations, it may even kill some spores.

  Iodine often is applied as tincture of iodine (effective antiseptic).

  Iodine can be complexed with an organic carrier to form an iodophor (minimize skin burns and irritation, used in hospitals for cleansing preoperative skin and in hospitals and laboratories for disinfecting).
- Heavy Metals
- Quaternary Ammonium Compounds
- Aldehydes
- Sterilizing Gases

Chemotherapeutic Agents

The term Chemotherapy was introduced by Paul Ehrlich in 1904. Ehrlich's efforts resulted in the development of several arsenic-containing compounds including *Salvarsan* which was effective against syphilis causing spirochaete. The first successful chemotherapeutic drug was discovered by Domagk. In 1935, he reported a dye called *prontosil* could cure bacterial diseases in mice. Domagk found that the sulfanilamide part was responsible for the antibacterial action of prontosil. However, sulfanilamide was too toxic for internal application to human. So, less toxic derivatives of sulfanilamide were synthesized chemically for the appropriate use in human. These synthetic compounds were called as **sulfa-drugs** which served as most powerful agents for combating bacterial diseases until the discovery of penicillin and other antibiotics.

General Characteristics of Antimicrobial Drugs-

Selective Toxicity-A successful chemotherapeutic agent has selective toxicity. It means that chemotherapeutic drug kills or inhibits the microbial pathogen without damaging the host tissue or showing minimum damage to the host (as little as possible).

The degree of selective toxicity may be expressed in terms of- **Therapeutic dose**-the drug level required for clinical treatment of a particular infection.

Toxic dose-the drug level at which the agent becomes too toxic for the host.

The superiority of chemotherapeutic agent can be defined in terms of **therapeutic index**. The therapeutic index is the ratio of the toxic dose to the therapeutic dose. The larger the therapeutic index, the better the chemotherapeutic agent in general.

A drug having greater selective toxicity and a higher therapeutic index means it is highly effective against target microorganism but have minimal or no toxicity to the host.

A drug having low therapeutic index can damage the host, show undesirable effects or side effects on the host. If the therapeutic index is small, the drug must be dosed carefully and the person receiving the drug should be monitored closely for any signs of drug toxicity.

Drugs vary considerably in their range of effectiveness-

Narrow-spectrum drugs- Effective only against a limited variety of pathogens.

Broad-spectrum drugs- Capable to attack many different kinds of bacteria.

Cidal /Static Property-

- Static agents reversibly inhibit growth. If the agent is removed, the microorganisms will recover and grow again.
- A cidal agent kills the target pathogen.
- The effect of an chemotherapeutic agent also varies with the target species. It is also possible that one chemotherapeutic agent or antimicrobial drug may be cidal for one species and static for another species. Elimination of infection depends on host's own immunity mechanisms.

Minimal Inhibitory Concentration (MIC) & Minimum Lethal Concentration (MLC)-

The MIC is the **lowest concentration** of a drug that **prevents growth of a particular pathogen.**

On the other hand, MLC is the **lowest drug concentration** that **kills the pathogen.**

- **Antibiotics** are also chemotherapeutic agents. They were defined by S. A. Waksman in 1945 as substances produced by microorganisms which can kill or inhibit growth of other microorganisms at a very low concentration.

  The term antibiotic was derived from 'antibiosis', the phenomenon of antagonism of one organism by another.

  Like other chemotherapeutants, an ideal antibiotic is one which attacks exclusively to pathogens without causing damage to host. Antibiotic may be bacteriostatic (inhibition of growth of pathogen) , bactericidal(Killing of pathogen) or bacteriolytic (Killing of bacteria or pathogen by lysis).

  However, all antibiotics are not effective on all bacteria. The range of different types of bacteria that can be inhibited , killed or lysed by an antibiotic determines the spectrum of antibiotic.

When an antibiotic substance shows antibacterial activity against both Gram-positive and Gram-Negative groups, it can be called as broad-spectrum antibiotic.

Antibiotic should be quickly absorbed to attain an effective concentration in blood and excreted without leaving undesirable side effects. It is desirable that an oral antibiotic should not be inactivated in the acidic gastric juice. Another desirable property of antibiotic is it's ability to withstand inactivating enzymes secreted by other bacteria.

For example, many bacteria can produce an enzyme, penicillinase which causes hydrolysis of the β-lactam ring of penicillins which leads to the formation of an inactive product called penicilloic acid. Bacteria also produce similar other enzymes for the inactivation of several other antibiotics. These are the strategies taken by bacteria to become resistant to several antibiotics.

Sometimes , the natural antibiotics (produced by different organisms such as bacteria, fungi , actinomycetes) do not possess all the desired properties. So, they are modified chemically to give these properties, such modified antibiotics are called semi-synthetic antibiotic. Large number of semi-synthetic derivatives of penicillins and cephalosporins are available which are resistant to β-lactamase and are capable to resist acidic pH of gastric juice.

Antibacterial antibiotics can be classified based on their **mode of action**; according to the mode of action, antibiotics can be classified into five major groups-

I. Inhibition of cell wall synthesis.
II. Damage of the cell membrane.
III. Inhibition of protein synthesis.
IV. Interference of nucleic acids.
V. Inhibition of specific enzymes.

Table: Properties of some common antibacterial drugs

Antibiotic Group	Primary Effect	Mechanism of Action	Members	Spectrum	Common Side Effects
Cell Wall Synthesis Inhibition					
Penicillins	Cidal	Inhibit transpeptidation enzymes involved in cross-linking the polysaccharide chains of peptidoglycan Activate cell wall lytic enzymes	Penicillin G, penicillin V, methicillin Ampicillin, carbenicillin	Narrow (Gram-positive) Broad (Gram-positive, some Gram-negative)	Allergic reactions (diarrhea, anemia, hives, nausea, renal toxicity)
Cephalosporins	Cidal	Same as above	Cephalothin, cefoxitin, cefaperazone, ceftriaxone	Broad (Gram-positive, some Gram-negative)	Allergic reactions, thrombophlebitis, renal injury
Vancomycin	Cidal	Prevents transpeptidation of peptidoglycan subunits by binding to D-Ala-D-Ala amino acids at the end of peptide side chains. Thus it has a different binding site than that of the penicillins.	Vancomycin	Narrow (Gram-positive)	Ototoxic (tinnitus and deafness), nephrotoxic, allergic reactions
Cell Membrane Disruption					
Polymyxin B	Cidal	Binds to plasma membrane and disrupts its structure and permeability properties	Polymyxin B, polymyxin topical ointment	Narrow—mycobacterial infections, principally leprosy	Can cause severe kidney damage, drowsiness, dizziness
Protein Synthesis Inhibition					
Aminoglycosides	Cidal	Bind to small ribosomal subunit (30S) and interfere with protein synthesis by directly inhibiting synthesis and causing misreading of mRNA	Neomycin, kanamycin, gentamicin Streptomycin	Broad (Gram-negative, mycobacteria) Narrow (aerobic Gram-negative)	Ototoxic, renal damage, loss of balance, nausea, allergic reactions
Tetracyclines	Static	Same as aminoglycosides	Oxytetracycline, chlortetracycline	Broad (including rickettsia and chlamydia)	Gastrointestinal upset, teeth discoloration, renal and hepatic injury
Macrolides	Static	Bind to 23S rRNA of large ribosomal subunit (50S) to inhibit peptide chain elongation during protein synthesis	Erythromycin, clindamycin	Broad (aerobic and anaerobic Gram-positive, some Gram-negative)	Gastrointestinal upset, hepatic injury, anemia, allergic reactions
Chloramphenicol	Static	Same as macrolides	Chloramphenicol	Broad (Gram-positive and -negative, rickettsia and chlamydia)	Depressed bone marrow function, allergic reactions

Nucleic Acid Synthesis Inhibition					
Quinolones and Fluoroquinolones	Cidal	Inhibit DNA gyrase and topoisomerase II, thereby blocking DNA replication	Norfloxacin, ciprofloxacin, Levofloxacin	Narrow (Gram-negatives better than Gram-positives) Broad spectrum	Tendonitis, headache, light-headedness, convulsions, allergic reactions
Rifampin	Cidal	Inhibits bacterial DNA-dependent RNA polymerase	R-Cin, rifacilin, rifamycin, rimactane, rimpin, siticox	*Mycobacterium* infections and some Gram-negatives (e.g., *Neisseria meningitidis* and *Haemophilus influenzae* b)	Nausea, vomiting, diarrhea, fatigue, anemia, drowsiness, headache, mouth ulceration, liver damage

Antimetabolites					
Sulfonamides	Static	Inhibit folic acid synthesis by competing with *p*-aminobenzoic acid (PABA)	Silver sulfadiazine, sulfamethoxazole, sulfanilamide, sulfasalazine	Broad spectrum	Nausea, vomiting, and diarrhea; hypersensitivity reactions such as rashes, photosensitivity
Trimethoprim	Static	Blocks folic acid synthesis by inhibiting the enzyme tetrahydrofolate reductase	Trimethoprim (in combination with a sulfamethoxazole)	Broad spectrum	Same as sulfonamides but less frequent
Dapsone	Static	Thought to interfere with folic acid synthesis	Dapsone	Narrow—mycobacterial infections, principally leprosy	Back, leg, or stomach pains; discolored fingernails, lips, or skin; breathing difficulties, fever, loss of appetite, skin rash, fatigue
Isoniazid	Cidal if bacteria are actively growing, static if bacteria are dormant	Exact mechanism is unclear but thought to inhibit lipid synthesis (especially mycolic acid); putative enoyl-reductase inhibitor	Isoniazid	Narrow—mycobacterial infections, principally tuberculosis	Nausea, vomiting, liver damage, seizures, "pins and needles" in extremities (peripheral neuropathy)

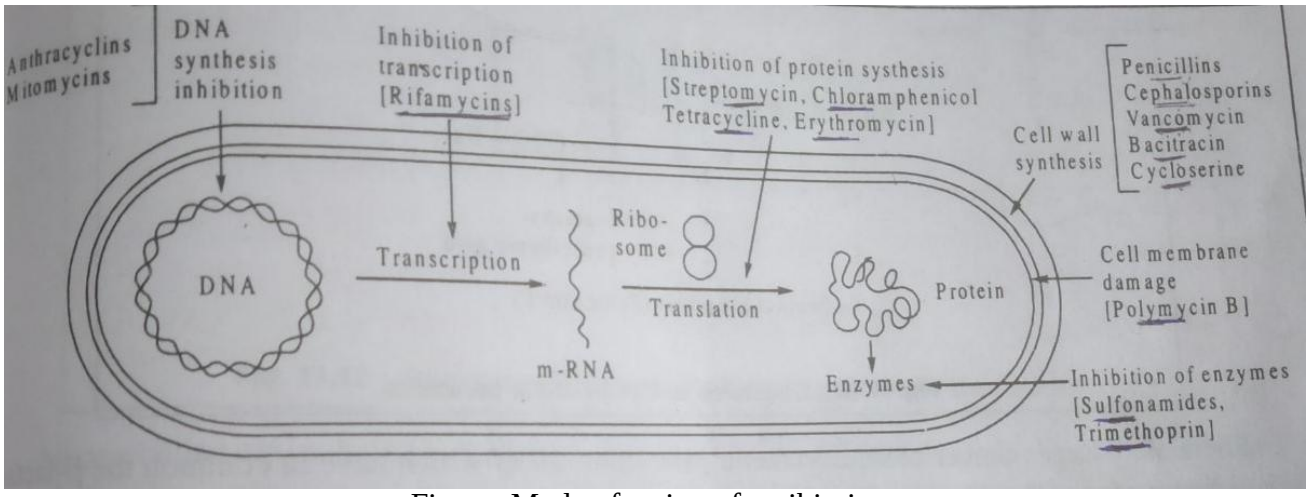


Figure: Mode of action of antibiotics

7.2 antibiotic resistance:

There are several mechanisms to become antibiotic resistant-

1. **Limiting Access of the Antibiotic**

I. Outer membrane porin
II. Reduce uptake of antibiotic across the cell membrane
III. Active efflux of antibiotic (Efflux pumps such as Tetracycline efflux pumps Tet A, Tet B & ABC transporters)

2. **Enzymatic Inactivation of Antibiotic**

I. β-lactamase
II. Aminoglycoside modifying enzyme
III. Chloramphenicol Acetyl Transferase
IV. Oxidation of Tetracycline

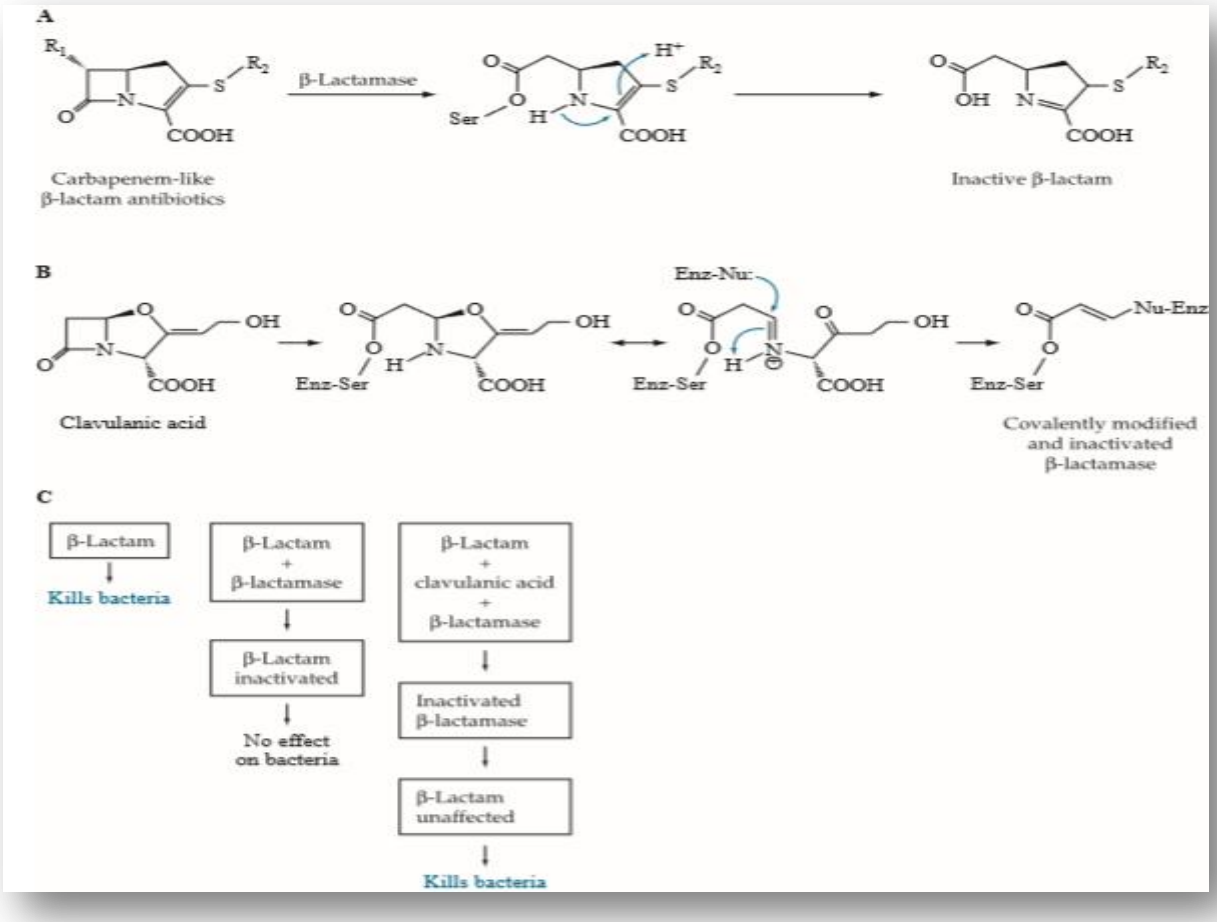


Figure: Modes of action and inhibitors of β-lactamase. (A, B) Modes of action of β-lactamase on β-

lactam antibiotics (A) and clavulanic acid (B), a suicide substrate inhibitor of β-lactamase. (C) Clavulanic acid inactivates the β-lactamase so that β-lactam antibiotics can kill the bacteria.

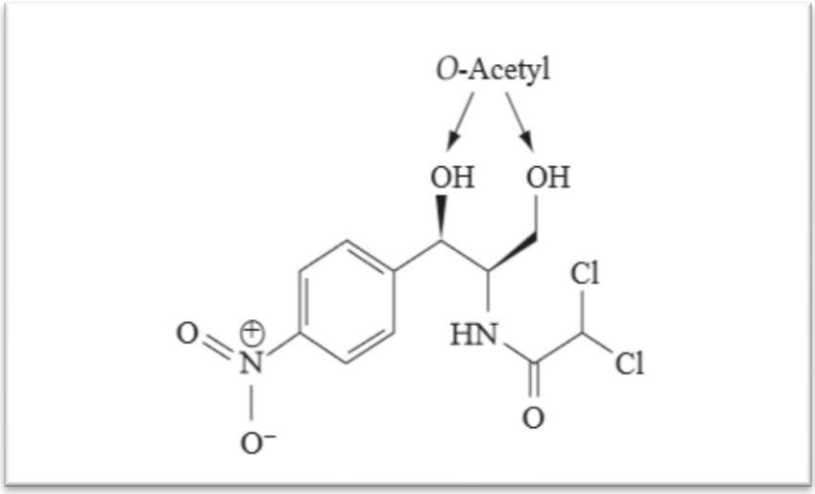


Figure: Action of chloramphenicol acetyltranferase enzyme

3. Modification or protection of the Antibiotic Target

I. Accumulation of the spontaneous mutation in target site (Mutational change in residue A2058 in bacterial 23S rRNA to G2058 results less tight binding of macrolide to ribosomal 23S rRNA. Thus, the bacteria become resistant to macrolide such as erythromycin.)

II. Chemical addition or change in target site (Such as addition of methyl groups which impede antibiotic binding to target site).

a) Resistance to β-lactam (Here the binding specificity of Penicillin-binding protein/PBP is altered so that they no longer bind to β-lactam ring).

b) Resistance to Glycopeptide antibiotics

Vancomycin is an antibiotic which prevents the cross linking of peptidoglycan by binding to the D-ala-D-ala at the end of muropeptide. Resistance to vancomycin can be achieved by three essential enzymes-

- A ligase (encoded by either VanA or VanB) that makes D-ala-D-lactate.
- Lactate dehydrogenase (encoded by VanH) to produce D-lactate from pyruvate.
- VanX, a dipeptidase that cleaves D-ala-D-ala but not D-ala-D-lactate resulting resistance to vancomycin.

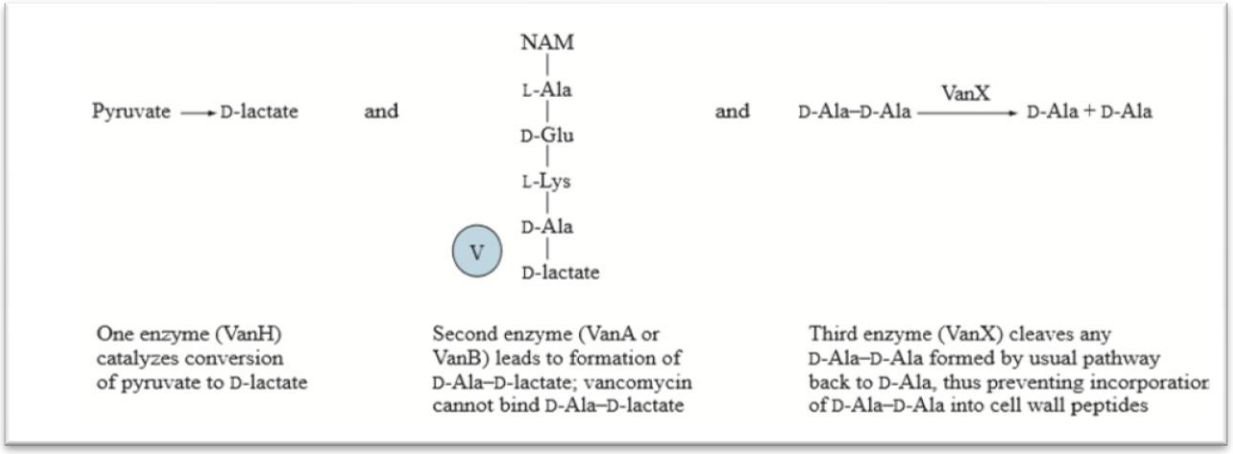


Figure: Mechanism of vancomycin resistance

c)Resistance to Tetracycline

Ribosome protection is conferred by a cytoplasmic protein calledTetM, TetO and TetQ in different bacteria that protects ribosome from tetracycline inhibition.

d) Resistance to Macrolide ,Streptogramin and Lincosamide

Addition of 1 or 2 methyl groups to the A2058 adenine in 23S rRNA leads to the resistance to Macrolide , Streptogramin and Lincosamide.

e) Resistance to Quinolones, Rifampin and Streptomycin

Resistance to quinolone commonly involves amino acid changes that alter the way these antibiotics interact with the A or B subunit of DNA gyrase. Mutation of this essential enzyme leads to resistance. Rifampicin resistance is caused by mutation that result in amino acid changes in the β subunit of RNA polymerase.Streptomycin resistance results from specific amino acid changes in the S-12 protein in the 50S ribosomal subunit.

f) Resistance to Trimethoprim and Sulfonamide

Resistance to trimethoprim and sulfonamides arises from mutations in the folate pathway biosynthetic enzymes inhibited by these antibiotics. The mutant forms of the enzymes no longer bind the antibiotic with a higher affinity than their natural substrate. Mutations conferring resistance to sulfonamides or to trimethoprim occur rather frequently, but simultaneous double mutations that

confer resistance to both types of antibiotic occur only rarely. For this reason, a combination of trimethoprim and one of the sulfonamides is currently used for antibacterial therapy.

4. **Failure to activate an antibiotic**

Metronidazole, which is often used to treat dental plaque caused by Porphyromonasgingivalis and gastric ulcers caused by Helicobacter pylori, must be activated before it can attack bacterial DNA. Acquisition of resistance to metronidazole by H. pylori, the cause of ulcers, is an ominous development for ulcer sufferers. Resistance to metronidazole is poorly understood, but in at least some cases, mutations that decrease the expression of the activation enzyme flavodoxin, which is required to convert metronidazole into its active form, can occur.

Isoniazid is one of the mainstays of antituberculosis therapy. It must be activated by a catalase (KatG) of mycobacteria. One known mechanism of resistance to isoniazid is a mutation that inactivates KatG.

7.3 Control of virus using chemicals –Antiviral Drugs

viruses enter host cells and make use of host cell enzymes and constituents, it was long thought that a drug that blocked virus multiplication would be toxic for the host.

However, the discovery of inhibitors of virus-specific enzymes and replication cycle processes has led to the development of antiviral drugs.

Antiviral Drugs

- Probably most publicized antiviral agent is Tamiflu (generically, oseltamivir phosphate). Tamiflu inhibits the viral molecule neuraminidase, which is essential for release of newly synthesized influenza A virus particles from host cells.
- Amantadine and rimantadine can also be used to prevent influenza A illness. When given within the first 48 hours of infection, these drugs reduce the incidence of influenza by 50% to 70% in an exposed population. Amantadine blocks the penetration and uncoating of influenza virus particles.
- Several drugs are commonly used to treat illnesses caused by viruses with DNA genomes. Adenine arabinoside (vidarabine) disrupts the activity of viral DNA polymerase and several other enzymes involved in DNA and RNA synthesis and function. It is given intravenously or applied as an ointment to treat herpes infections.
- Acyclovir, is also used in the treatment of herpes infections. Upon phosphorylation, acyclovir also inhibits viral DNA polymerase.

Unfortunately, acyclovir-resistant strains of herpes have developed. Effective acyclovir derivatives are now available. Valacyclovir is an orally administered prodrug form of acyclovir.

Another kind of drug named foscarnet also inhibits the virus's DNA polymerase. It is very effective at treating illnesses caused by herpes simplex viruses and cytomegalovirus.

- Drug HPMPC, also known as cidofovir is effective against papovaviruses (warts), adenoviruses (respiratory diseases), herpesviruses (oral and genital sores), and poxviruses (chickenpox). The drug acts on the viral DNA polymerase as a competitive inhibitor. It is structurally similar to deoxycytosine triphosphate (dCTP), a substrate of DNA polymerase. Thus, it blocks viral DNA synthesis.
- Nowadays, much effort has been focused on developing new drugs for HIV treatment. Now four categories of drugs used in combination to manage HIV infection-

1. **Nucleoside reverse transcriptase inhibitors (NRTIs)**-These are nucleoside analogues that produce faulty viral DNA (e.g., azidothymidine or AZT).
2. **Non-nucleoside reverse transcriptase inhibitors (NNRTIs)-** They canprevent HIV DNA synthesis by selectively binding to and inhibiting the reverse transcriptase enzyme.
3. **Protease inhibitors (PIs)-** Block the activity of the HIV protease that is needed for the production of all viral proteins. Most used PIs are saquinavir, indinavir, and ritonavir.
4. **Fusion inhibitors (FIs)-** New category of drugs that prevent HIV entry into cells.

Inhibition of reverse transcription, (which catalyzes the conversion of the virus's RNA genome into double-stranded DNA) blocks viral DNA synthesis and halts HIV replication. Protease inhibitors are effective because HIV, like many RNA viruses, synthesizes polyproteins that must be cleaved into the individual proteins required for virus replication. Protease inhibitors mimic the peptide bond that is normally attacked by the protease. Fusion inhibitors are particularly interesting as an effective blockade to viral entry into host cells, essentially preventing disease.

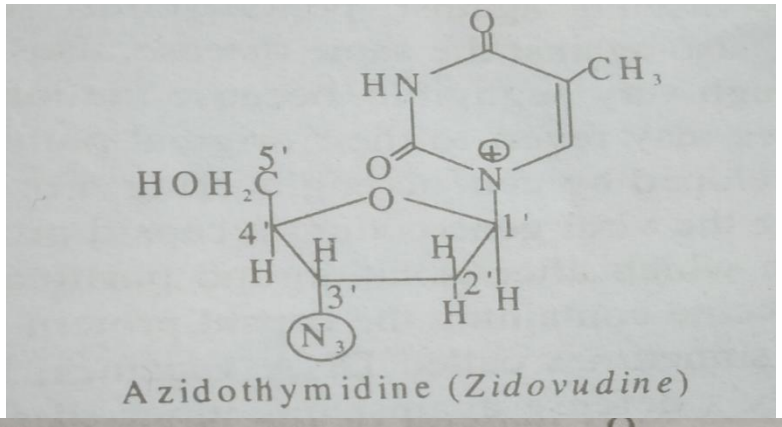
- The combination of the two reverse transcriptase inhibitors AZT and 3TC, and the protease inhibitor ritonavir reduces HIV concentrations in plasma almost to zero. However, the treatment does not eliminate proviral HIV DNA that still resides in certain cells of the immune system (e.g., memory T cells) and possibly other cells.
- Recent studies have led to CDC a pre-HIV exposure prophylaxis (prevention) strategy to men and women.

❖ There are three types of antiviral agents-

Virucidal Agents- Which directly inactivate virus.

Antiviral Agents- Inhibit Viral replication.

Immunomodulators- Which boost the host immune response.



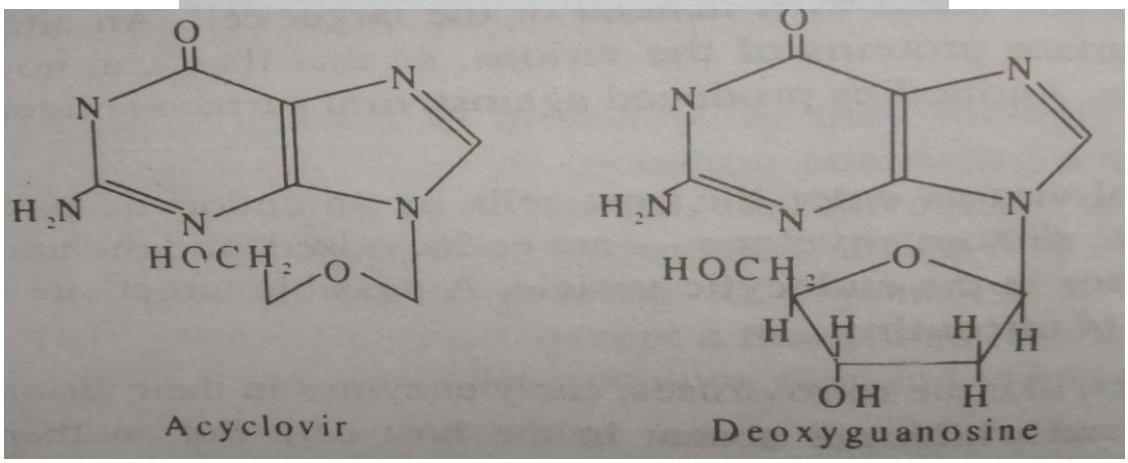


Figure: Some common antiviral drugs

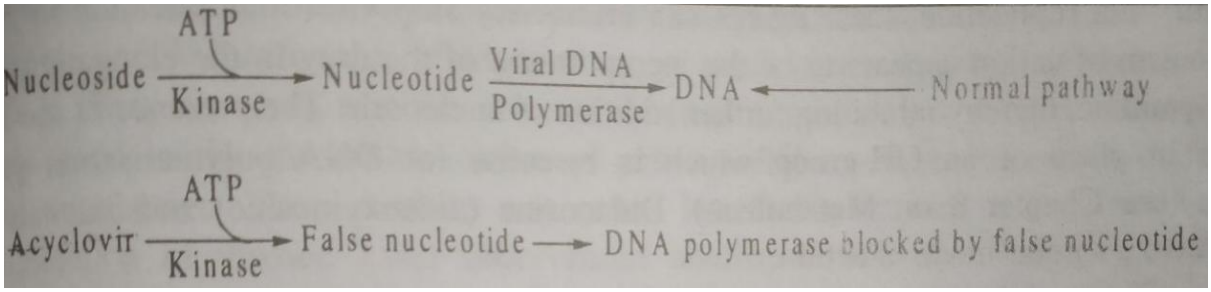


Figure: Inhibition of viral DNA synthesis by nucleoside analogues like acyclovir

Control of virus by interferons

Interferons are natural glycoproteins produced by virus-infected eukaryotic cells which protect host cell from virus infection. The substance interferon was so named because it interfered with intracellular multiplication of viruses.

Interferons do not interact directly with viruses. Interferons induce virus infected cells to synthesize antiviral proteins which inhibit viral multiplication. Interferon production starts after initiation of viral maturation and continues for 20 to 50 hr after that. Then the production stops due to the

formation of a repressor which is formed or activated only when the interferon concentration in the producing cell exceeds a certain threshold concentration.

Mechanism of Action

- Type I interferons include α-IFN and β-IFN. These interferons do not interact with viruses directly causing their inhibition, but they induce the formation of antiviral proteins which are activated to inhibit viral multiplications. These interferon-regulated proteins block the synthesis of macromolecular components necessary for viral multiplication. Several interferon-regulated host proteins have been identified. Among the better known of these proteins are a protein kinase and an enzyme catalyzing the formation of short polymer of adenylic acid, the 2′, 5′-oligoadenylate synthetase (2′-5′ A synthetase).

  The protein kinase induced by Type I interferon catalyses the phosphorylation of initiation factor (eIF-2) thereby causing inhibition of protein synthesis. The 2′, 5′-oligoadenylate synthetase is an enzyme also induced by Type I interferons which require activation by ds-RNA like the protein kinase. The activated synthetase acts as an activator of an endonuclease, RNase L. The activated RNase degrades viral ss-RNA.

- Type II interferon includes gamma interferon. Type II interferon induces the major histocompatibility antigens of human cells. IFN induced expression of these major histocompatibility antigens represent an important contribution of the antiviral activity of gamma-IFN through enhancement of the activity of cytotoxic T lymphocyte.

8. Genetic recombination

The movement of genetic information from one organism to another is referred to as gene transfer. Gene transfer is either horizontal or vertical. If genetic information is transferred from mother to offspring is known as vertical gene transfer, mostly found in eukaryotes and gene transfer between two organisms is referred to as lateral or horizontal gene transfer.

There are three types of horizontal gene transfer mechanisms in bacteria i.e.

I) Transformation

II) Transduction

III) Conjugation

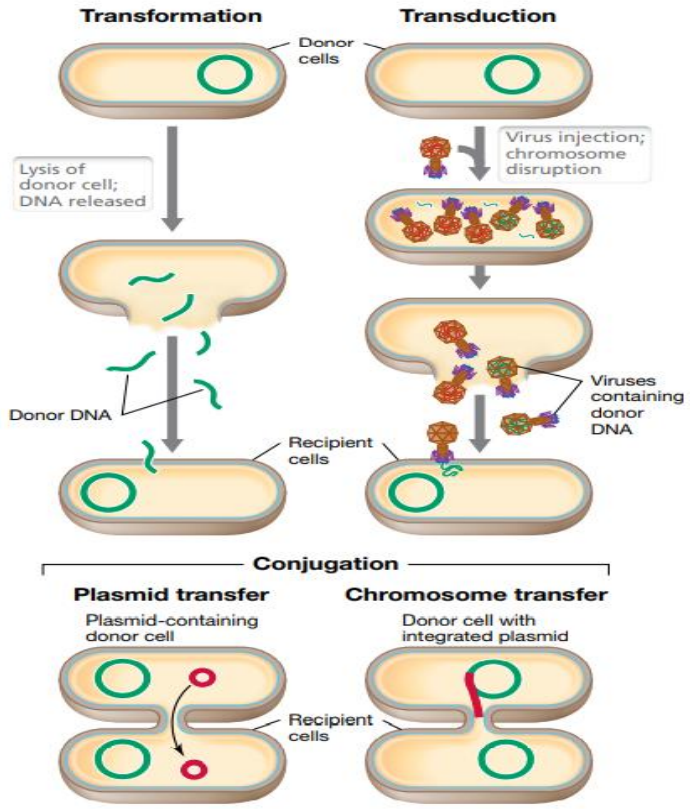


Figure: Gene transfer prosses of donor bacterial cell to the recipient cell.

8.1: Transformation:

I) **Transformation:** Genetic recombination of bacteria which takes place direct uptake of naked DNA or exogenote from the surrounding media is referred to as transformation.

a) History of discovery: Bacterial transformation was first reported by Fred Griffith in 1928 on *Streptococcus pneumonia* is associated with the transformation of rough strain to smooth strain. Subsequently, the transformation has been reported in many other bacteria. In gram-positive bacteria (*Streptococcus pneumonia, Bacillus subtitles*) as well as Gram-negative bacteria (*Haemophilus influenza*).

b) Competence: All bacteria are not able for transformation in all conditions but it takes place at the competent stage. The ability of a bacteria to take naked DNA from surrounding media and insert the naked DNA into its chromosomal DNA and transform is referred to as competence.

Frei Felder 1987 defines competence as a physiological state which permits a cell to take up transforming DNA. It is not a permanent feature of bacteria but occurs only for a short duration during the life cycle. This stage occurs at the end of the log phase but just before the stationary phase and the presence of suitable media, temperature, etc.

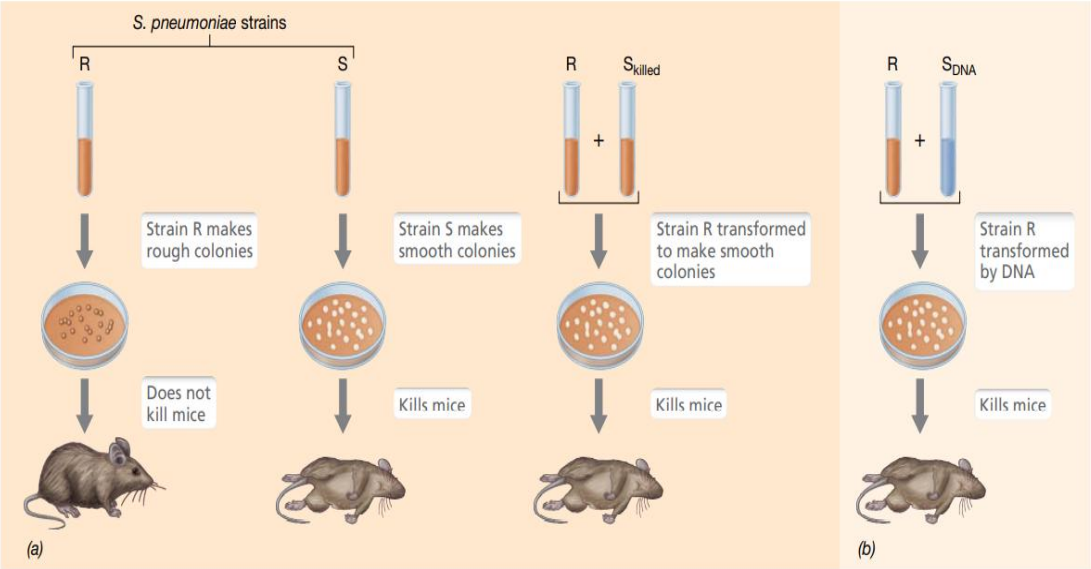


Figure: Griffith 1928, experiment. The rough or R strain is non-pathogenic thus, it does not kill the mouse but smooth or S strain is pathogenic than kill the mouse. He kills the S strain by heating and mixing with the R strain then injects it in mice thus, mice die. This experiment indicates that the R strain is transformed by DNA to S strain and become pathogenic and kill the mice.

c) Mechanism of transformation: Notani and Setlow in 1974 describe the mechanism of transformation. It includes the following features:

- Competence induction: In *S. pneumoniae* the competence state is induced by competence activator protein of molecular weight 1000 da. This molecule is synthesized within cells bind with plasma membrane receptors and triggers the synthesis of 10 new proteins which accelerate the transport of foreign DNA from outside to inside.

  In some bacteria e.g., *Haemophilus influenza* there is no competence factor but the competence state is associated with the change of cell envelope (membrane) which produces some vesicle which is referred to as transformosome bud which binds with DNA with conserved sequence.

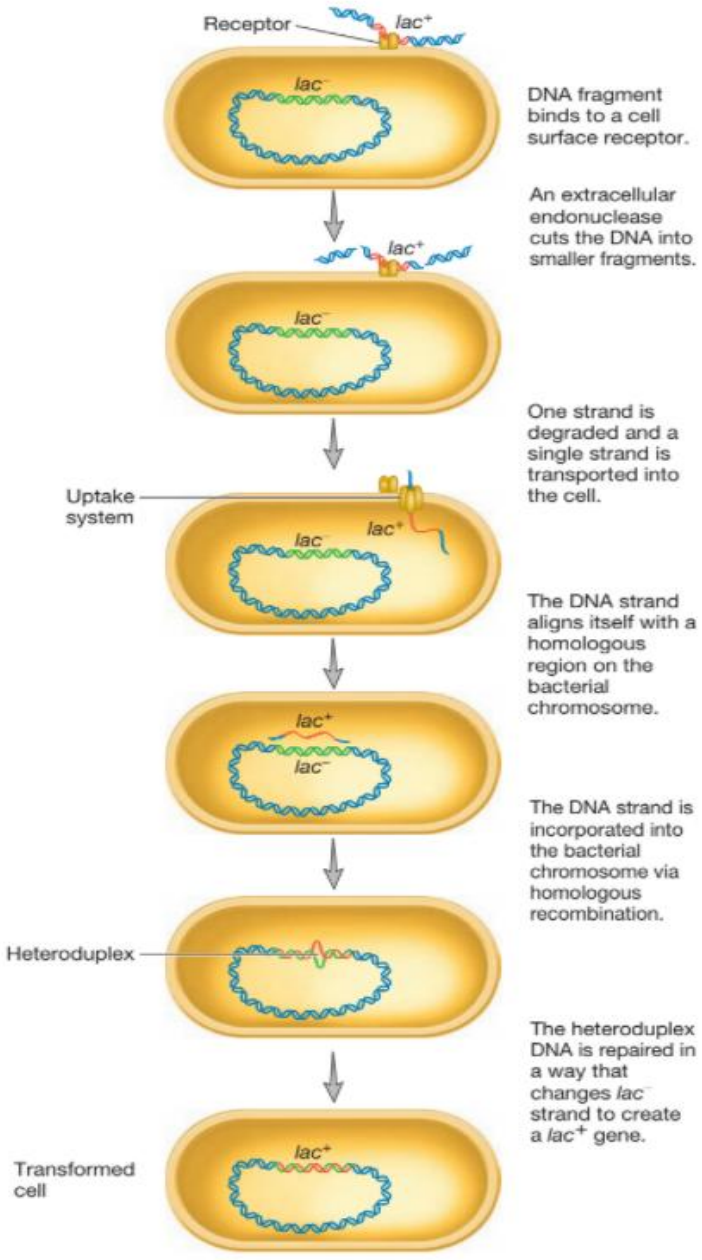


Figure: Bacterial transformation as seen in *S. pneumoniae.*

- DNA binding: As a result of collision when DNA comes in contact with the cell surface of competent bacteria it is attached. The attachment is at first temporary for 2 min and within 5 min the attachment is permanent. For about 10 min no transformation takes place which is referred to as the transformation eclipse phase.

  In B. subtilis membrane vesicles bind with DNA. In *H. influenzae*transformosome bud bind with DNA with a conserved sequence **5′ AAGTGCGG TCA 3′**. The uptake site contains two proteins of 28 kDa and 52 kDa.

- DNA penetration: Endonuclease I is located at the surface of competent bacteria. This endonuclease now attacks donor DNA and degrade one strand of dsDNA and produce ssDNA. This ssDNA now enters in cell thus endonuclease I serve as DNA translocase. However, in *Bacillus subtilis* dsDNA enter the cell. Successful transformation occurs with donor DNA of molecular weight 3x106 – 8x106 Da.

- Pairing or synopsis: The ssDNA coated with SSB (single-strand binding protein) for maintaining of single-strand nature. Now DNA is inserted into the bacterial DNA or recipient DNA. In *Escherichia coli*RecA protein (recombination protein) facilitate DNA pairing during recombination. RecA cause local unwinding of dsDNA now synapsis occurs between homologous ssDNA and recipient DNA. Unwinding continues and base-pairing continues, thus invading DNA increase which is referred to as branch migration.

- Integration: Now endonuclease cut unpaired donor DNA and recipient DNA, these prosses are referred to as trimming. Now the nick is sealed by ligase. Thus, a heteroduplex segment of DNA is formed which has some mismatch pairing. These mismatches may repair. The repairing enzyme repairs the mismatch by scanning the DNA. The repairing enzymes are replacing the base of the new strand. If this region is replicated before the repairing thus recombination takes place in one daughter strand and the other strand remains identical to the mother strand.

8.2: Transduction:

II) **Transduction:** The genetic recombination in bacteria that takes place with the help of temperate phage through the lysogenic cycle is referred to as transduction.

This phenomenon was first time reported by Zinder and Lederberg in 1952. As phage is host-specific hence transduction occurs in related species. E.g.: *E. coli, Salmonella, Shigella*. Several traits are usually transduced. E.g., fermentation potential, antigen property, resistance property, etc.

Type of transduction: There are two types of transductions i.e., generalized transduction and specialized transduction or restricted transduction.

a) **Generalized transduction:** The process of recombination in which any gene is transduced through a defective phage that contains a portion of donor bacterial chromosome is referred to as generalized transduction.

It is associated with the lytic cycle. Generalized transduction was first discovered in *Salmonella enterica* with phage p22 and also extensively studied in *E. coli* with phage P1.

Mechanism of generalized transduction: This process takes place under the following points:

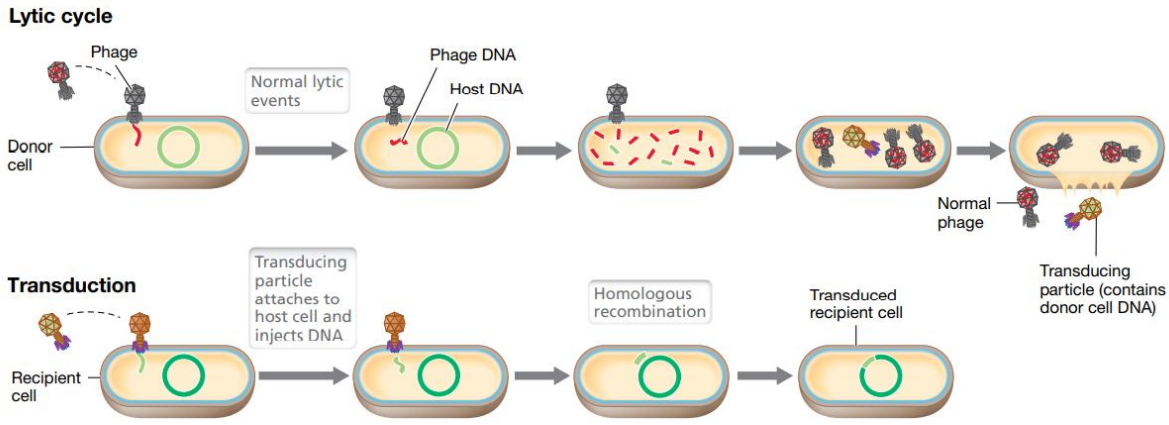


Figure: Mechanism of generalized transduction.

- Penetration by Phage: When the P1 phage genome reaches bacterial cytoplasm it produces a viral enzyme that hydrolyzed the bacterial chromosome and produces many small pieces of DNA fragments.
- Virion assembly: The viral genome now multiplies and produces a protein coat that assembly then produces a virion. Erotically the virion may pick up bacterial gene rather than phage --genome, thus the virus will be defective for any bacterial gene.
- Recombination: When this defective virion that contains any portion of the bacterial chromosome is attacked by a fresh bacterium then some marker gene may recombine through transduction.

Since a capsid can whole up to 44 kb DNA thus some or all virus DNA is left behind. The quantity of DNA transduction depends on the size of the capsid. In this way, many marker genes i.e., Thr^{+}, Leu^{+}, $Azi^{+,}$ etc. are obtained in *E. coli*.

b) **Specialized transduction:** The process of transduction in which a gene in a particular locus is recombined with the recipient cell is referred to as specialized or restricted transduction.

It is done by λ phage of E. coli k12.

Mechanism of restricted transduction: When the λ phage penetrates the *E. coli* then enters its genetic material into the bacterium cell. The phage genome is integrated into the bacterial chromosome through the lysogen process. The recombination at a locus in between galand bio gene. When the phage genome is excised, it may contain a part of some bacterial gene like gal^{+}, $bio^{+,}$ etc. thus the virus will be defective this process is taken place by reverse integration.

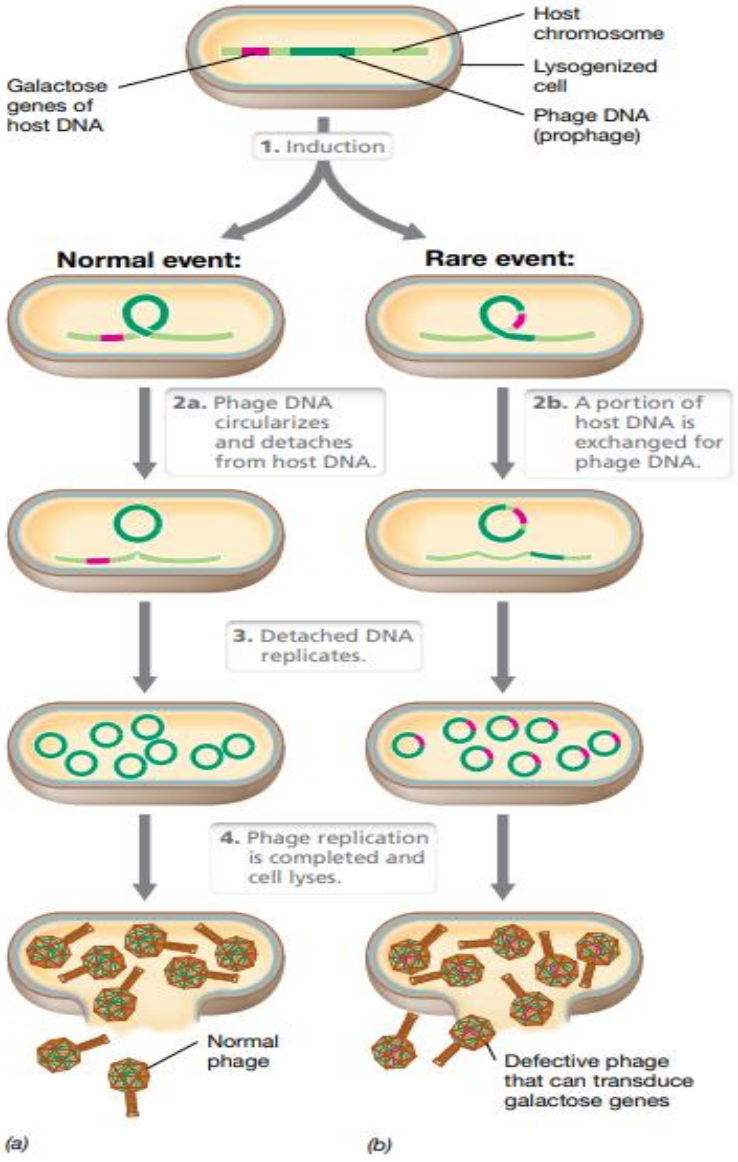


Figure: Mechanism of specialized transduction or restricted transduction.

When this defective phage infects fresh bacteria with marker gene gal$^-$ the homologs DNA part of gal$^-$ and gal$^+$ recombine thus the bacteria is transduced for gal locus. However, the defective phage cannot reproduce themselves because of missing some essential genes for replication.

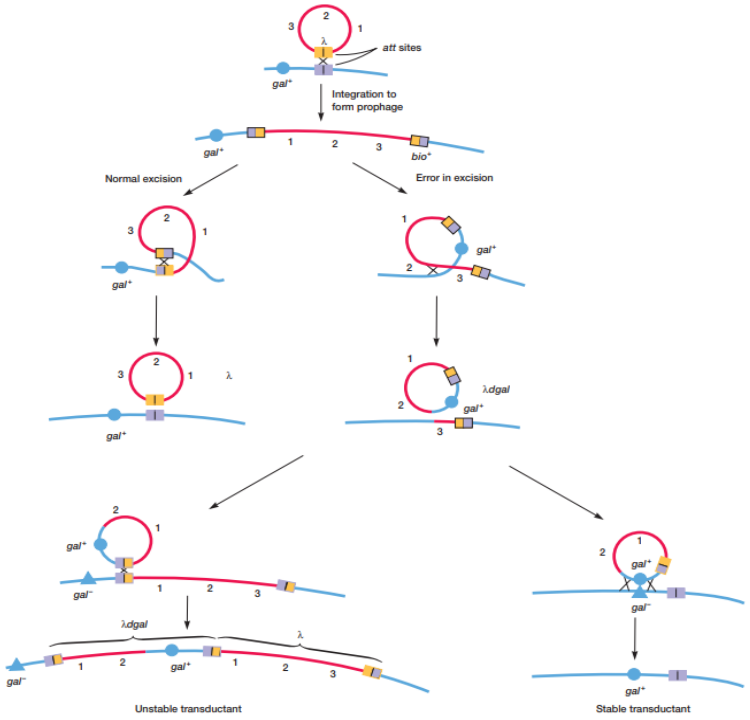


Figure: Mechanism of gene integration and produce stable of unstable transductant.

8.3: Conjugation:

III) **Conjugation:** It is a process of transfer of genetic material from one bacterial cell to another bacterial cell through contact by the formation of a conjugation tube is referred to as bacterial conjugation.

The conjugation was first time observed by J. Lederberg and E.L. Tatum (1946). The conjugation takes place by the F-plasmid (fertility factor) which transfer from donor cell to recipient cell. The bacterial cell which contains the F plasmid or F-factor is called donor or male cell and the bacterial cell which have no F plasmid

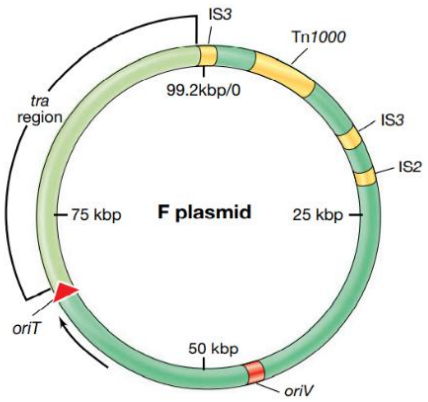


Figure: Structure of F plasmid.

is known as the recipient cell or female cell. The conjugation may be associated with genetic recombination or not it can discus under the following points:

a) F^+ X F^- conjugation
b) Hfr X F^- conjugation
c) $F^/$ X F^- conjugation

a) F^+ X F^- conjugation: F^+ strain contain F plasmid with F factor and F^- strain do not have F plasmid or the F factor. The F^+ also referred to as donor and F^- is referred to as recipient. When two such strains come close to each other F-pili is produce a conjugation tube of F^+ strain. Now F plasmid is replicated and transferred from the donor cell to the F- cell or recipient cell. Thus, the F^- cell becomes F^+ so here no recombination takes place.

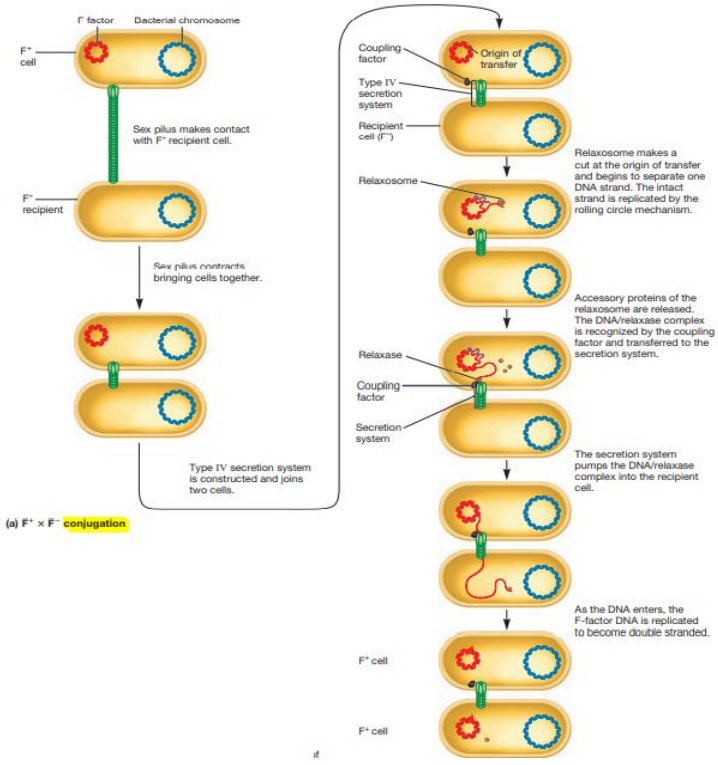


Figure: Mechanism of F^+ x F^- conjugation.

b) Hfr X F^- conjugation: The F plasmid is integrated into the bacterial chromosome thus the F^+ cell becomes Hfr (High-frequency recombination). The Hfr strain is produced by the integration of the F plasmid into the bacterial chromosome due to the presence of IS (insertion sequence) element and RecA protein that helps to integrate the F factor into the bacterial chromosome.

Then the Hfr strain is conjugated with an F^- or recipient cell by the formation of a conjugation tube then transfer the genetic material. Then the bacterial chromosome of Hfr replicates at the oriT locus (origin of replication) Where episome is located now one copy is transferred to F^- cell which can carry some bacterial gene from a donor.

Wollman et al in 1956 observed that chromosome of Hfr donor maybe transfer to F^- where the donor is the wild type with Leu^+, Lac^+, gal^+, and F^- is

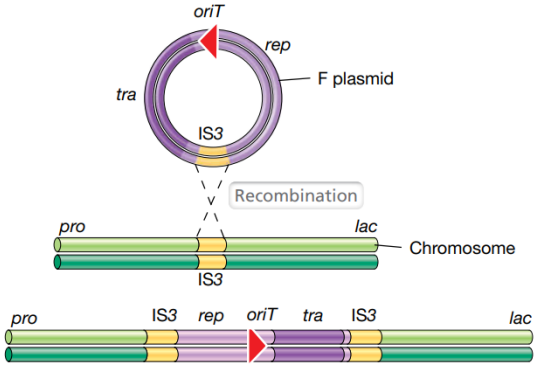


Figure: Integration of F plasmid into the bacterial chromosome and produce Hfr strain.

mutant with Leu$^-$, Lac$^-$, gal$^-$ marker. F factor along with some chromosomal genes is transfer. Now F$^-$ may become wild concerning marker gene. The number of changes of the marker gene is a proposal to the time or duration of conjugation. The number increase with the increase of the duration of the marker gene.

Whatever maybe when the gene enters in F$^-$ the homologs portion is paired, recombine in presence of RecA through a process of branch migration, mismatch repairing, etc.

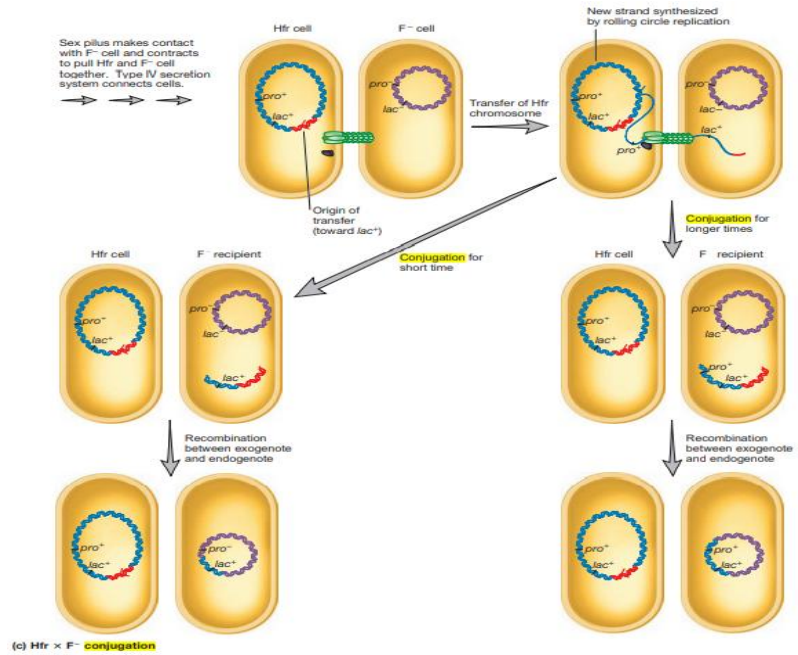


Figure: Mechanism of Hfr x F$^-$ conjugation, here the donor cell is transferred some chromosomal part to the recipient thus the gene is undergoing homologous recombination and the recombination takes place.

c) $F^{/}$ X F^{-} conjugation: The integrated F factor of the Hfr strain can be released by the reversal process from the bacterial chromosome along with some bacterial genes. Now the F plasmid is referred to as $F^{/}$ plasmid and the bacteria is referred to as $F^{/}$ strain.

When the $F^{/}$ strain comes in contact with the F^{-} strain the $F^{/}$ plasmid replicates and migrates to F^{-}, now the F^{-} is referred to as a merozygote and the process is referred to as sex duction.

The homologs part of the $F^{/}$ plasmid is now recombined with the $F^{/}$ marker gene.

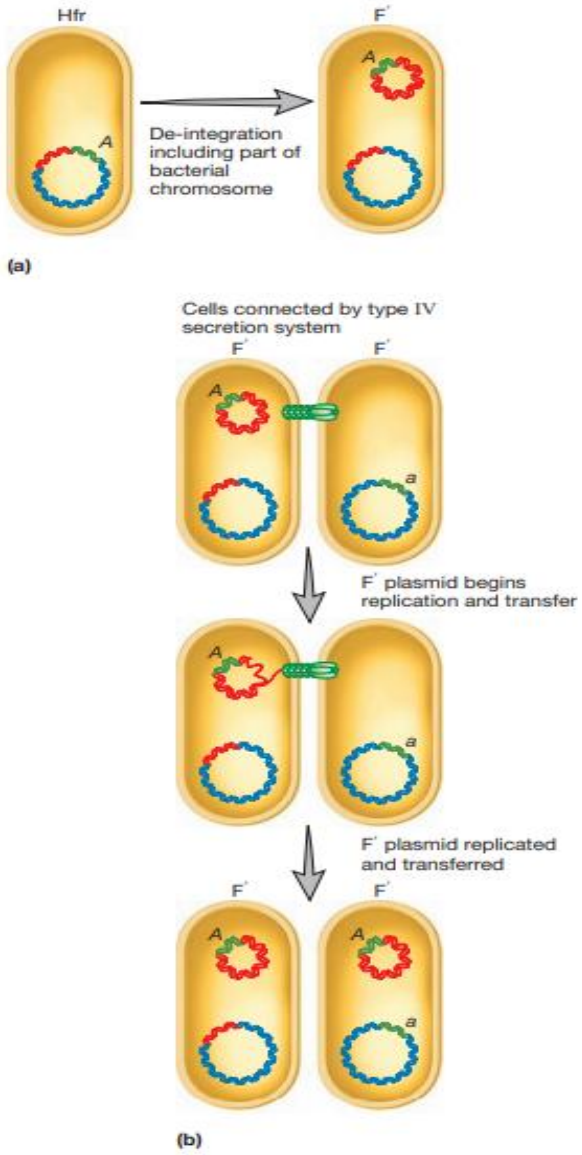


Figure: Mechanism of F x F conjugation, here the F plasmid is transferred to the recipient with some chromosomal gene of the donor that forms merozygote.

8.4 Detection of recombinants: Detection of a recombinant bacterial cell by growing them in a minimal medium after the gene transfer process. The prototrophic bacteria which have all genes that

grow any kind of media but for bacterial strain were selective. For e.g., in the bacterial strain that lacks leu gene thus, this bacterial strain in not grow on a minimal medium that lacks leucine. If the nutrient is provided to the minimal media the strain can grow is referred to as auxotroph.

After gene transfer from donor to the recipient like leu gene thus, the bacteria are prototroph to leucine and grow easily in the minimal media by synthesizing all the compounds.

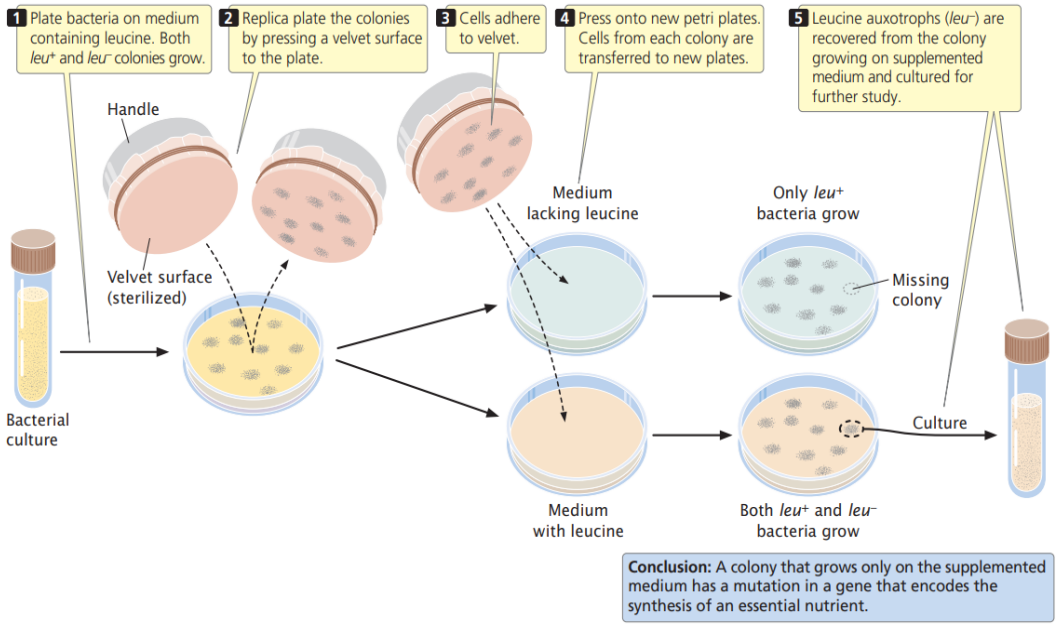


Figure: Detection of recombinant by using leu- auxotroph grow in the minimal media after gene transfer.

8.5 Bacterial gene mapping: The transfer of chromosomal DNA from the Hfr strain to the F- strain during conjugation is helps to map the bacterial gene. The transfer of the Hfr chromosome to the recipient cell-like *E. coli* takes time 100 min. Only the part of the recipient chromosome is transferred from the Hfr to the F- recipient that is recombined with the homologous portion of the recipient cell.

The chromosome transfer is always starting from the portion where the F factor is integrated. After begin the gene transfer is process is continuous to the recipient and the gene is transferred according to their position on the chromosome. The individual gene requires time for transfer that indicates the position of this gene located on the chromosome. The gene map of bacteria is made by interrupting conjugation. Minute is the basic unit of distance.

Francois Jacob and Elie Wollman were first developing the method of gene mapping. The use of two cells one is Hfr or donor and the other is recipient cell.

The donor or Hfr cell has str^s leu^+thr^+ ari^rton^r lac^+ gal^+.

str^s: Susceptible to the streptomycin antibiotic.

leu^+: Prototrophic for leucine.

thr^+: Prototrophic for threonine.

ari^r: Resistance to sodium azide.

ton^r: Resistance to infection by bacteriophage T1.

lac^+: Ability to break down lactose.

gal^+: Ability to break down galactose.

The recipient cell F^- have str^r leu^-thr^- ari^s ton^s lac^- gal^-.

str^r: Resistance to the streptomycin antibiotic.

leu^-: Auxotrophic for leusine

thr^-: Auxotrophic for threonine

ari^s: Susceptible to sodium azide.

ton^s: Susceptible to infection by bacteriophage T1.

lac^-: No ability to break down lactose

gal^-: No ability to break down galactose.

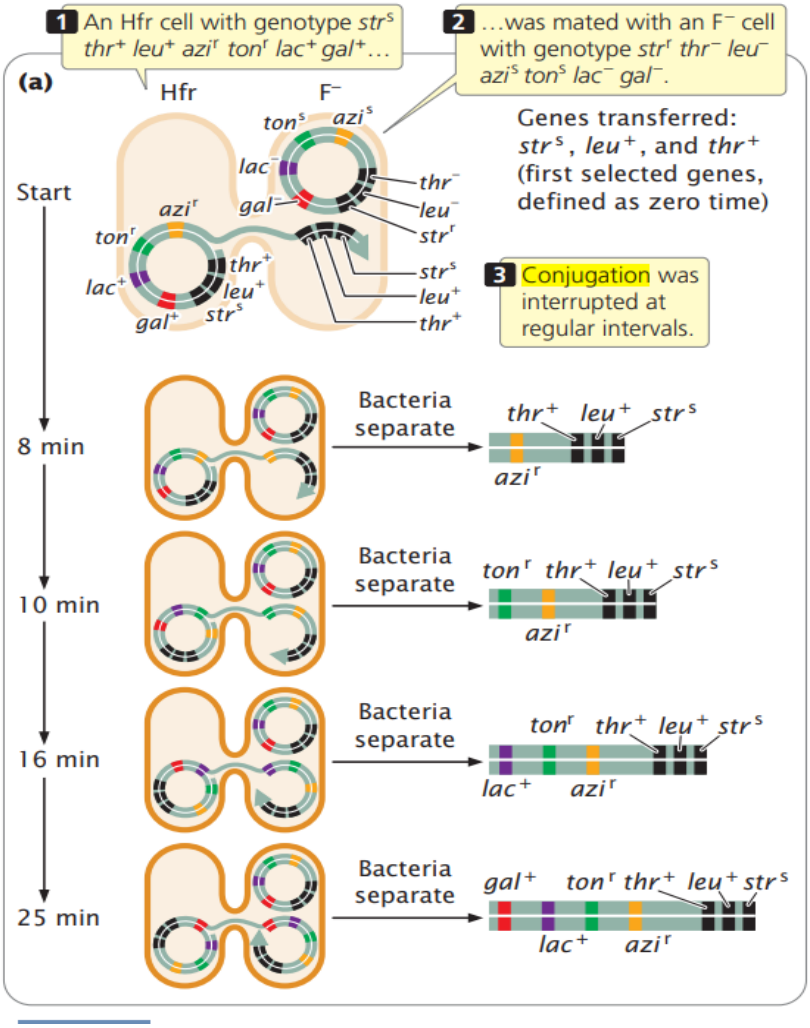


Figure: Bacterial gene map by conjugation process.

In a nutrient media, the two strains were mixed and allowed for conjugation. After a few minutes, the medium is diluted, the sample was removed at regular intervals. The cell from each sample is placed on minimal media which contains antibiotic streptomycin and lacks lue and thr. The donor cell is str^r so it is not gram and the recipient is auxotrophic to lue^- and thr^- gene thus it would not grow. The transformed cell which contains str^r, lue^+, thr^+ after transformation can grow on the minimal medium. So that gene were transformed. The cell was tested gene were transformed. The cell was tested for the presence of another gene that transfers from Hfr to F^- recipient. The azi^r is the first donor gene is appearing in all recipient cells. The gene ton^r is appear next to ari^r after 10 min. The lac^+ and gal^+ appear in the recipient cell at 16 min and 25 min respectively.

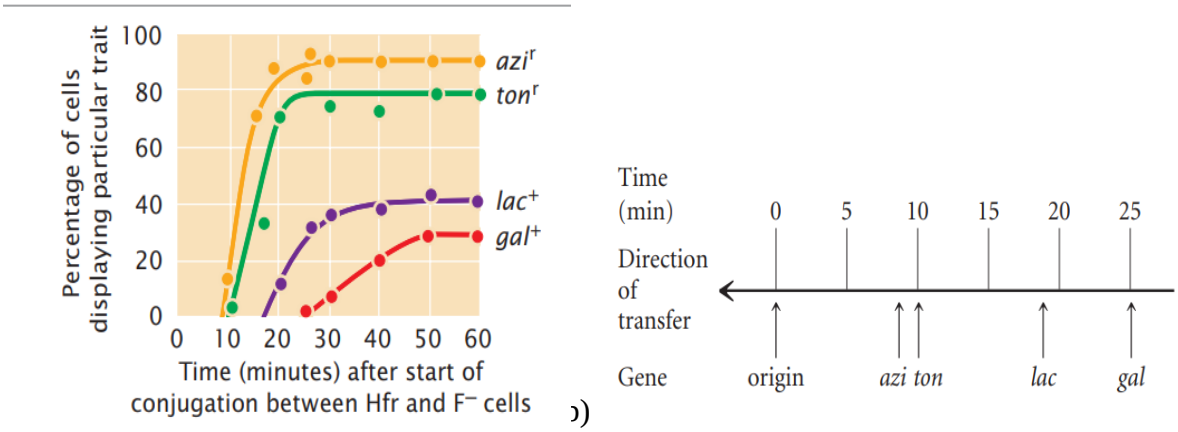


Figure: a) Order and relative distance between genes indicate by time. b) Time indicates the position of the gene on the chromosome.

The frequency of gene transfer from donor to the recipient cell is decreased for that gene which is located fur from the F factor. In this case, the gal^+ gene has a very low frequency thus this gene is present in a few recipient cells and the gene which is located near the F-factor has a high frequency for transfer to the recipient rather than the genes transfer later.

The direction of gene: The chromosome transfer is always starting from where the F factor is located. In Hfr1 the F factor is located between the leu and azi genes thus, the leu gene has high frequency for transfer than azi.

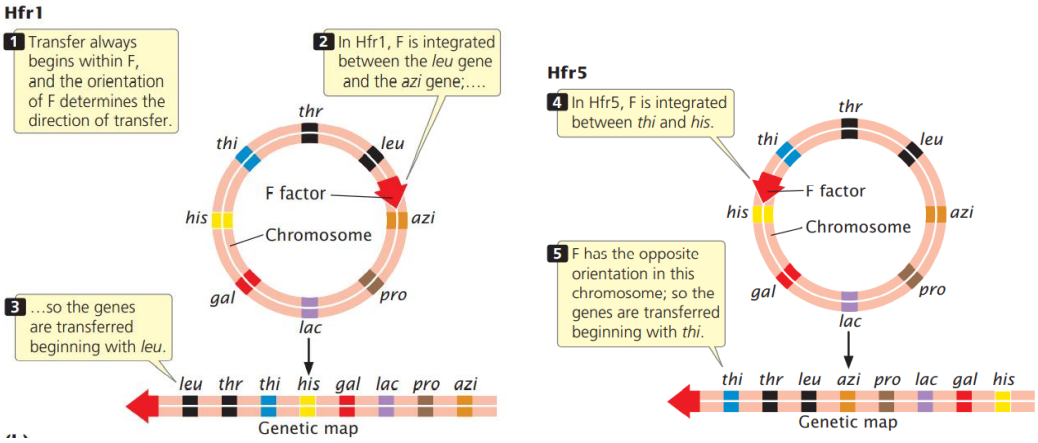


Figure: Direction of gene transfer in Hfr1 and Hfr5 strain.

In the case of Hfr5 where the F factor is located between his and the genes thus, the gene has a high frequency for transfer and is has a low frequency. The data give evidence that the bacterial chromosome is circular.

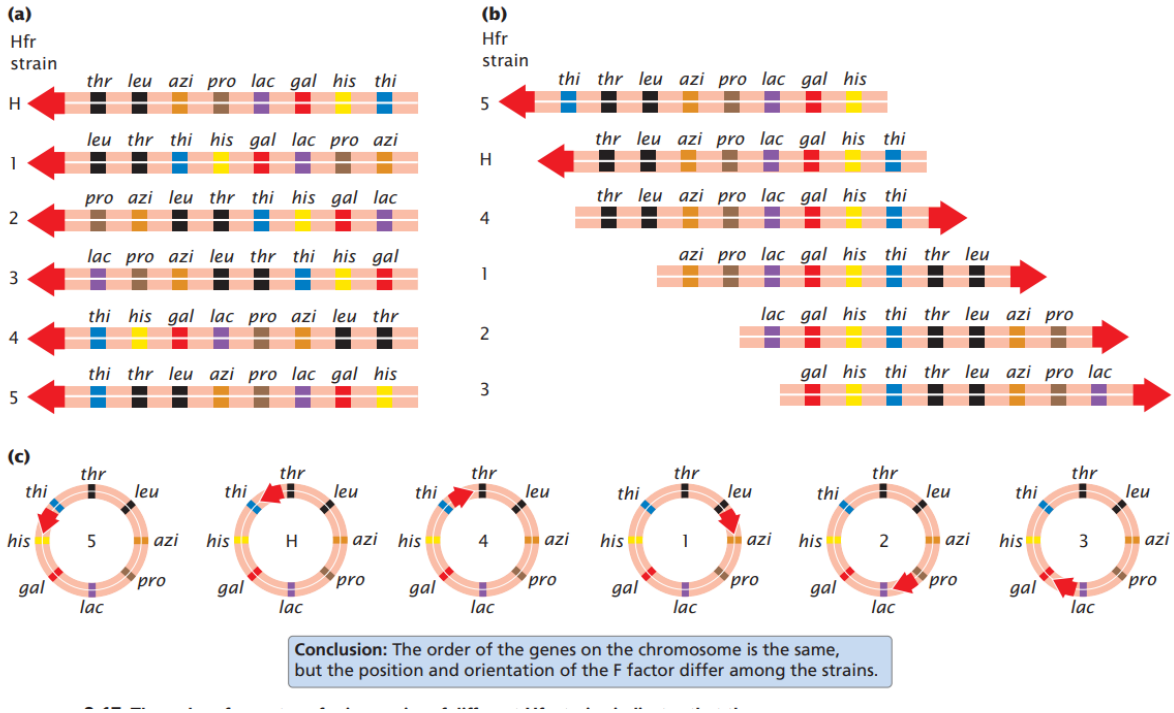


Figure: Different Hfr strains show the transfer of gene of a circular chromosome of *E. coli* cell.

9. Microbes in Nitrogen and Sulphur Cycle

9.1 Nitrification:

Nitrification, the oxidation of NH3 to NO3 -, is a major process in well-drained oxic soils at neutral pH and is carried out by the nitrifying Bacteria and Archaea. Nitrification was long considered an obligatory two-step process in which some species oxidize NH3 to NO2 - and then other species oxidize NO2 - to NO3 -. In the first step, ammonium is oxidized to nitrite ($N0_2^-$), and in the second step, N 0_2^- is oxidized to nitrate ($N0_3^-$). Many species of both Bacteria and Archaea can oxidize NH3, whereas only species of Bacteria are known that oxidize NO2 -.

However, some Bacteria, such as certain *Nitrospira* species, can oxidize ammonia completely to nitrate; these have been called comammox (for complete ammonia oxidizer) bacteria and seem to be especially important nitrifiers in freshwater lakes and rivers, drinking water systems, coastal sediments, and paddy soils.

Table: Nitrifying organisms

Processes	Example organisms
Nitrification ($NH_4^+ \rightarrow NO_3^-$)	
$NH_4^+ \rightarrow NO_3^-$	Comammox (*Nitrospira* species)
$NH_4^+ \rightarrow NO_2^-$	*Nitrosomonas, Nitrosopumilus* (*Archaea*)
$NO_2^- \rightarrow NO_3^-$	*Nitrobacter*

9.2 Denitrification:

Nitrogen gas (N2) is the most stable form of N and is a major reservoir for N on Earth. Only a relatively small number of Bacteria and Archaea are able to use N2 as a cellular N source by the process of nitrogen fixation . The N recycled on Earth is mostly already “fixed N”; that is, N in combination with other elements, such as in ammonia (NH_3) or nitrate (NO_3 -).

In most of the cases ,the end product of NO_3 - reduction is N_2, nitric oxide (NO), or nitrous oxide (N_2O). The reduction of NO_3 - to these gaseous N compounds, called denitrification , is the main means by which N_2 and N_2O are formed biologically.

Denitrification can be either beneficial or harmful. On the one hand, denitrification is a detrimental process. For example, if agricultural fields fertilized with NO_3- fertilizer become waterlogged following heavy rains, anoxic conditions can develop and denitrification can be extensive; this removes fixed N from the soil.

On the other hand, denitrification can aid in wastewater treatment. By converting NO_3 - in wastewater to volatile forms of N, denitrification minimizes the load of fixed N in discharge waters that triggers algal growth and loss of water quality.

The production of N_2O and NO by denitrification can have other environmental consequences. Nitrous oxide can be photochemically oxidized to NO in the atmosphere. Nitric oxide reacts with ozone (O3) in the upper atmosphere to form nitrogen dioxide (NO_2), which is further oxidized to form nitric acid (HNO_3) that returns to Earth as acid rain. In addition, N_2O is a very potent greenhouse gas.

Denitrification ($NO_3^- \rightarrow N_2$)	*Bacillus, Paracoccus, Pseudomonas*

9.3 Ammonification:

Ammonia is released during the decomposition of organic N compounds such as amino acids and nucleotides, a process called ammonification. In simple terms, ammonification is the process of converting natural nitrogen compounds into ammonia.

Another process contributing to the generation of NH_3 is the respiratory reduction of NO_3 - to NH_3, called dissimilative nitrate reduction to ammonia.

Ammonification is a subpart of nitrogen cycle. In this process, microorganisms like various decomposing bacteria act in combination on dead organic matter to convert their nitrogen into simple ammonia form. Actually when an organism excretes wastes or dies , the nitrogen in its tissues is in the form of organic nitrogen. Various fungi and prokaryotes then decompose the tissue and release inorganic nitrogen back into the ecosystem as ammonia in the process known as ammonification.

Ammonification (organic-N $\rightarrow NH_4^+$)

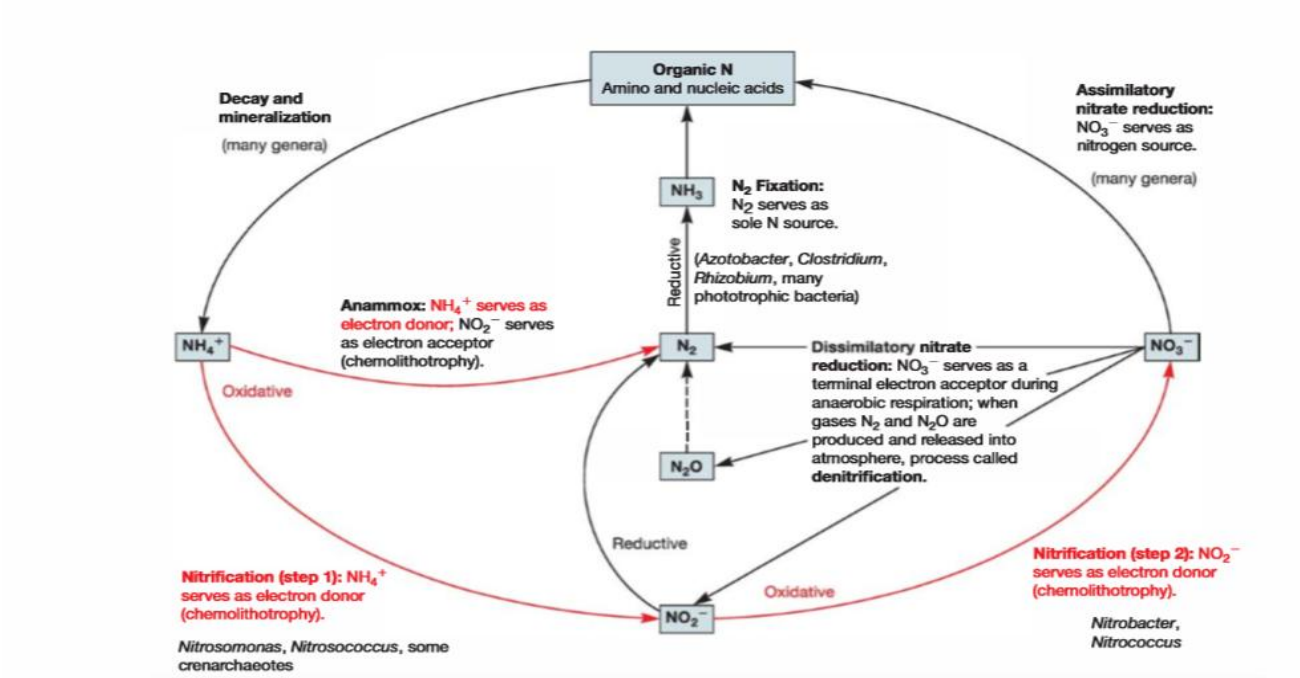


Figure: Nitrogen cycle

Anammox Reaction

The anammox reaction (anoxic ammonium oxidation) is an anaerobic reaction performed by chemolithotrophs in the phylum Planctomycetes. Here ,ammonium ion (NH_4+) serves as the electron donor and nitrite ($N0_2$-) as the terminal electron acceptor; it is reduced to nitrogen gas (N_2). In effect, the anammox reaction is a shortcut to N_2, proceeding directly from ammonium and nitrite, without having to cycle first through nitrate.

In the anammox reaction, NH_3 is oxidized anaerobically with NO_2- as the electron acceptor, forming N_2 as the final product, which is released to the atmosphere. Anammox bacteria affiliate with five genera (*Brocadia, Kuenenia, Scalindua, Anammoxoglobus,* and*Jettenia*) in a single phylogenetically cohesive family (Brocadiaceae) within the Planctomycetes. Since anammox bacteria have yet to be isolated in pure culture, the genus and species names are prefaced by the term *Candidatus* to indicate their tentative taxonomic status.

In wastewater processing plants, anammox is highly beneficial because like denitrification, it removes fixed nitrogen from sewage and reduces the ability of wastewater effluents to trigger algal and other microbial blooms.

9.4 Mechanism of biological N2 fixation

Biological Nitrogen fixation accounts for most of the conversion of atmospheric N_2 into ammonium, and thus serves as a key entry point of molecular nitrogen into biogeochemical cycle of nitrogen.

Biological Nitrogen fixation, like industrial nitrogen fixation, produces ammonia from molecular nitrogen. The overall reaction is-

$$N_2 + 8\,e^- + 8\,H^+ + 16\,ATP \rightarrow 2\,NH_3 + H_2 + 16\,ADP + 16\,P_i$$

The reduction of N_2 to $2NH_3$, a six electron transfer, is coupled to the reduction of two protons to evolve H_2. The nitrogenise enzyme complex catalyzes this reaction. The nitrogenise enzyme complex can be separated into two components-the Fe protein and the MoFe protein.

- The Fe protein is the smaller of the two components and has two identical subunits that vary in mass from 30 to 72 kD each, depending on the bacterial species. Each subunit contains an iron-sulphur cluster which participates in the redox reactions that convert N_2 to NH_3. The Fe protein is irreversibly inactivated by O2 with typical half decay times of 30 to 45 seconds.
- The MoFe protein has four subunits, with a total molecular mass of 180 to 235 kDa, depending on the bacterial species. Each subunit has two Mo-Fe-S clusters. The MoFe protein is also inactivated by O_2, with a half decay time in air of 10 minutes.
- The enzyme complex catalyzing the reaction is called Nitrogenase and it is basically composed of two proteins. Both proteins are highly sensitive to oxygen and they lose catalytic property in presence of free oxygen. One of these two proteins is MoFe protein which contains molybdenum and iron. It is also called Dinitrogenase. The second component of nitrogenise complex is known as Fe-S protein because it contains non-heme iron and acid-labile sulphur, but no molybdenum. This protein is called dinitrogenase reductase.
- The mechanism of reduction of N_2 to NH_3 involves a transfer of an electron from the reduced electron donor-ferredoxin or flavodoxin-to the Fe atom of an oxidized form of the Fe-S protein which is thereby reduced.The electrons are transferred from reduced ferredoxin or flavodoxin or other effective reducing agents to Fe-protein component which gets reduced. From reduced Fe-protein, the electrons are given to MoFe-protein component which in turn gets reduced and is accompanied by hydrolysis of ATP into ADP and inorganic phosphate (Pi). Two Mg^{++} and 2 ATP molecules are required per electron transferred during this process.Binding of 2 ATPs to reduced

Fe-protein and subsequent hydrolysis of 2 ATPs to 2 ADP + 2 Pi is believed to cause a conformational change of Fe-protein which facilitates redox (reduction-oxidation) reactions. From reduced MoFe-protein, the electrons are finally transferred to molecular nitrogen (N_2) and 8 protons, so that two ammonia and one hydrogen molecule are produced.

- At first glance, it might be expected that six electrons and six protons would be required for reduction of one N_2 molecule to two molecules of ammonia. But, the reduction of N_2 is obligatorily linked to the reduction of two protons to form one H_2 molecule also.

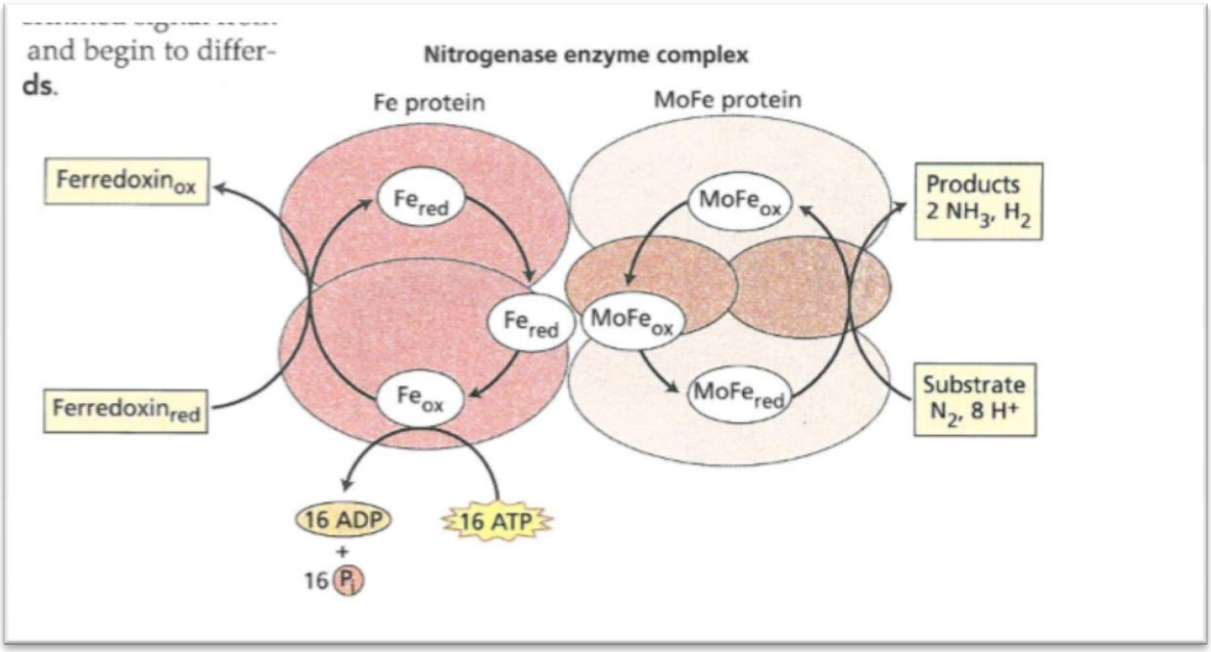


Figure: Nitrogenase enzyme complex

Structure and regulation of nif gene

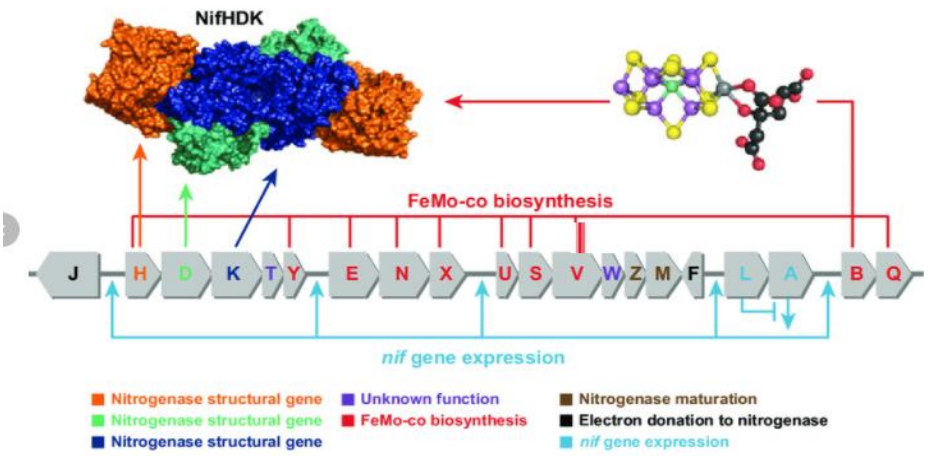
The *nif* genes are genes encoding enzymes involved in the fixation of atmospheric nitrogen into a form of nitrogen available to living organisms.The primary enzyme encoded by the *nif* genes is the nitrogenase complex which is in charge of converting atmospheric nitrogen (N_2) to other nitrogen forms such as ammonia which the organism can use for various purposes. Besides the nitrogenase enzyme, the *nif* genes also encode a number of regulatory proteins involved in nitrogen fixation.The expression of the *nif* genes is induced as a response to low concentrations of fixed nitrogen and oxygen concentrations.

Regulation:

- In most bacteria, regulation of *nif* genes transcription is done by the nitrogen sensitive NifA protein.

- When there isn't enough fixed nitrogen available for the organism's use, NtrC triggers NifA expression, and NifA activates the rest of the *nif* genes.
- If there is a sufficient amount of reduced nitrogen or oxygen is present, another protein is activated: NifL. NifL inhibits NifA activity resulting in the inhibition of nitrogenase formation.
- NifL is regulated by the products of *glnD* and *glnK*. The *nif* genes can be found on bacterial chromosomes, but in symbiotic bacteria they are often found on plasmids or symbiosis islands with other genes related to nitrogen fixation (such as the nod genes).

The expression and regulation of *nif* genes, while sharing common features in all or most of the nitrogen-fixing organisms in nature, have distinct characters and qualities that differ from one diazotroph to another. As for example- Klebsiella pneumoniae is a a free-living anaerobic nitrogen-fixing bacterium.It contains a total of 20 *nif* genes located on the chromosome in a 24-Kb region. *nifH*, *nifD*, and *nifK* encode the nitrogenase subunits, while *nifE*, *nifN*, *nifU*, *nifS*, *nifV*, *nifW*, *nifX*, *nifB*, and *nifQ* encode proteins involved the assembly and incorporation of iron and molybdenum atoms into the nitrogenase subunits. *nifF* and *nifJ* encode proteins related to electron transfer taking place in the reduction process and *nifA* and *nifL* are regulatory proteins in charge of regulating the expression of the other *nif* genes.



Fig; Nif gene clusture of *Klebsiella pneumoniae* (Source-Internet)

9.5 Microbial oxidation of sulphur:

Under oxic conditions, sulphide rapidly oxidizes spontaneously at neutral pH.Sulphur-oxidizing chemolithotrophic bacteria, most of which are aerobes, can catalyze the oxidation of sulphide. However, because of the rather rapid spontaneous reaction, microbial sulfide oxidation is significant only in areas where H_2S emerging from anoxic environments meets the atmosphere. Where light is

available, there can be anoxic oxidation of sulphide, catalyzed by the phototrophic purple and green sulphur bacteria . S^0 is chemically stable but is readily oxidized by sulphur-oxidizing chemolithotrophic bacteria such as *Thiobacillus* and *Acidithiobacillus* .

Sulphur-oxidizing microbes are the third major group of chemolithotrophs. The metabolism of *Thiobacillus* and *Acidithiobacillus* spp. has been best studied. These bacteria oxidize sulphur (S^0), hydrogen sulphide (H_2S), thiosulfate ($S_20_3^{2-}$), and other reduced sulphur compounds to sulfuric acid; therefore they have a significant ecological impact.

Due to formation of sulfuric acid (H_2SO_4), S^0 oxidation characteristically lowers the pH in the environment, sometimes drastically. For this reason, small amounts of S^0 can be added to alkaline agricultural soils as an inexpensive and natural way to lower the pH, relying on the ubiquitous sulfur chemolithotrophs to carry out the acidification process.

Sulfide/sulfur oxidation ($H_2S \rightarrow S^0 \rightarrow SO_4^{2-}$)	
Aerobic	Sulfur chemolithotrophs (*Thiobacillus, Beggiatoa,* many others)
Anaerobic	Purple and green phototrophic bacteria, some chemolithotrophs

Reduction of sulphur:

Hydrogen sulphide (H2S) is a major volatile sulphur compound. Hydrogen sulphide is produced from bacterial sulphate reduction.

$$(SO_4^{2-} + 4\,H_2 \rightarrow H_2S + 2\,H_2O + 2\,OH^-)$$

Depending on the oxidation state of the sulphur species, it can serve as an electron acceptor, an electron donor, or both. Sulphate, the fully oxidized species, is reduced by plants and microbes for use in amino acid and protein biosynthesis; this is described as **assimilatory sulphate reduction**. By contrast, when sulphate diffuses into anoxic habitats, it provides an opportunity for microbial **dissimilatory sulphate reduction..** Here sulphate serves as a terminal electron acceptor during anaerobic respiration by a variety of microbes, including delta proteobacteria such as *Desulfovibrio* and *Desulfonema* spp. and archaea belonging to the genus *Archaeoglobus*.

Sulphide can then serve as an electron source for anoxygenic photosynthetic microorganisms and chemolithoautotrophs, including members of the phylum Chlorobi and the genus *Thiobacillus*, respectively. These microbes convert sulphide to elemental sulphur and sulphate.

Sulfate reduction (anaerobic) ($SO_4^{2-} \rightarrow H_2S$)
Desulfovibrio, Desulfobacter
Archaeoglobus (Archaea)

Sulfur reduction (anaerobic) ($S^0 \rightarrow H_2S$)
Desulfuromonas, many
hyperthermophilic *Archaea*

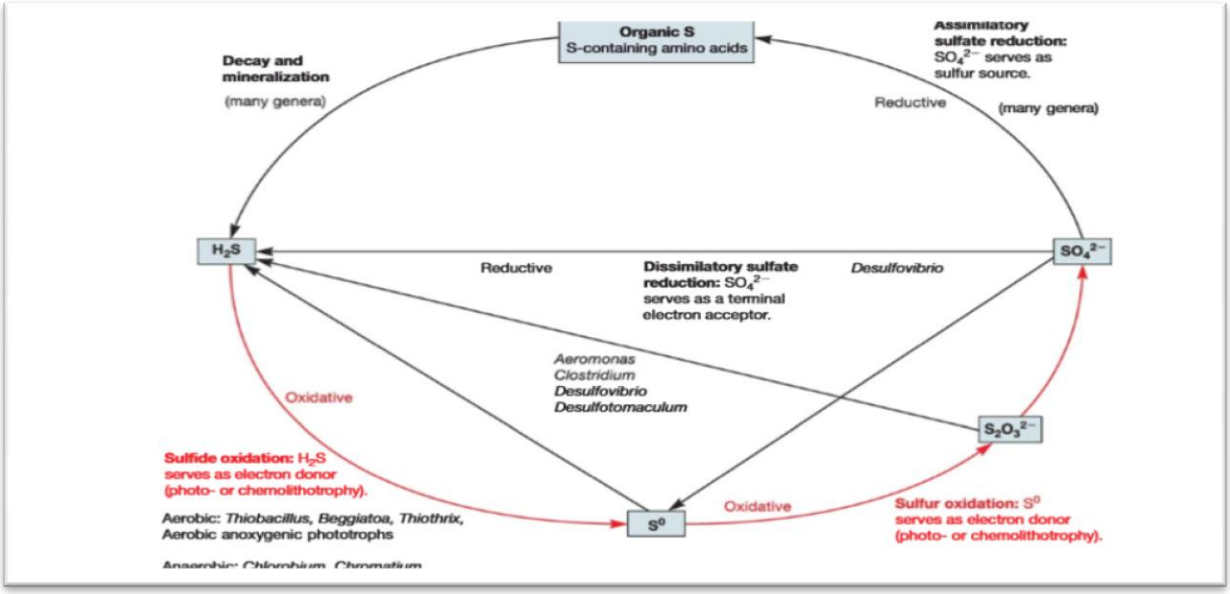


Figure: Sulphur cycle

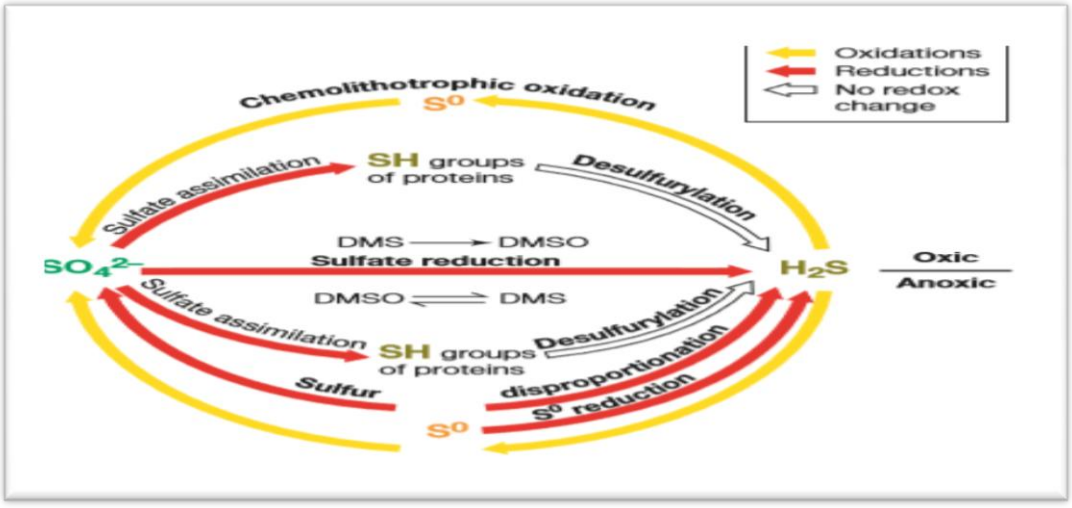


Figure: Sulphur assimilation

10. Medical Microbiology

10.1 Airborne diseases:

Chickenpox:

Chickenpox (Varicella) and Shingles (Herpes Zoster) Chickenpox (varicella) is a highly contagious skin disease primarily of children 2 to 7 years of age. Humans are the reservoir and the source for this virus, which is acquired by droplet inhalation into the respiratory system. The virus is highly infectious with secondary infection rates in susceptible household contacts of 65% to 86%. In the pre-vaccine era, about 4 million cases of chickenpox occurred annually in the United States, resulting in approximately 11,000 hospitalizations and 100 deaths. The causative agent is the enveloped, DNA varicella-zoster virus (VZV), a member of the family Herpesviridae. The virus produces at least six glycoproteins that play a role in viral attachment to specific receptors on respiratory epithelial cells. Their recognition by the human immune system results in humoral and cellular immunity. This virus has been shown to inhibit the expression of MHC molecules by infected cells; however, this inhibition only temporarily interferes with immune recognition of the virus, perhaps as a way of increasing its transmission. Following an incubation period of 10 to 23 days, small vesicles erupt on the face or upper trunk, fill with pus, rupture, and become covered by scabs. Healing of the vesicles occurs in about 10 days. During this time intense itching often occurs. Laboratory confirmation of varicella virus is by detection of varicella-zoster immunoglobulin M (IgM) antibody; detection of VZV, demonstration of VZV antigen by the direct fluorescent antibody and by a polymerase chain reaction in clinical specimens or a significant rise in serum IgG antibody level to VZV. However, laboratory testing for VZV is not normally required, as the diagnosis of chickenpox is typically made by clinical assessment. Laboratory confirmation is recommended, though, to confirm the diagnosis of severe or unusual cases of chickenpox. As the incidence of chickenpox continues to decline due to vaccination, fewer cases are seen clinically, resulting in the likelihood of misdiagnosis. Furthermore, in persons who have previously received varicella vaccination, the disease is usually mild or atypical and can pose particular challenges for clinical diagnosis. Therefore, laboratory confirmation of varicella cases is becoming more important. Chickenpox can be prevented or the infection shortened with an attenuated varicella vaccine or the drug acyclovir (Zovirax or Valtrex). It should be noted that Valtrex (valacyclovir) is an orally administered prodrug of Zovirax or acyclovir. Valtrex is the valyl ester of acyclovir and is rapidly hydrolyzed to acyclovir in the body. Individuals who recover from chickenpox are subsequently immune to this disease; however, they are not free of the virus, as viral DNA resides

in a dormant (latent) state within the nuclei of cranial nerves and sensory neurons in the dorsal root ganglia. This viral DNA is maintained in infected cells but virions cannot be detected. When the infected person becomes immunocompromised by such factors as age, neoplastic diseases, organ transplants, AIDS, or psychological or physiological stress, the viruses may become activated (figure 37.2b). They migrate down sensory nerves, initiate viral replication, and produce painful vesicles because of sensory nerve damage. This syndrome is called postherpetic neuralgia. To manage the intense pain, corticosteroids or the drug gabapentin (Neurontin) can be prescribed. The reactivated form of chickenpox is called shingles (herpes zoster). Most cases occur in people over 50 years of age. Except for the pain of postherpetic neuralgia, shingles do not require specific therapy; however, in immunocompromised individuals, acyclovir, valacyclovir, vidarabine (Vira-A), or famciclovir (Famvir) are recommended. More than 500,000 cases of herpes zoster occur annually in the United States.

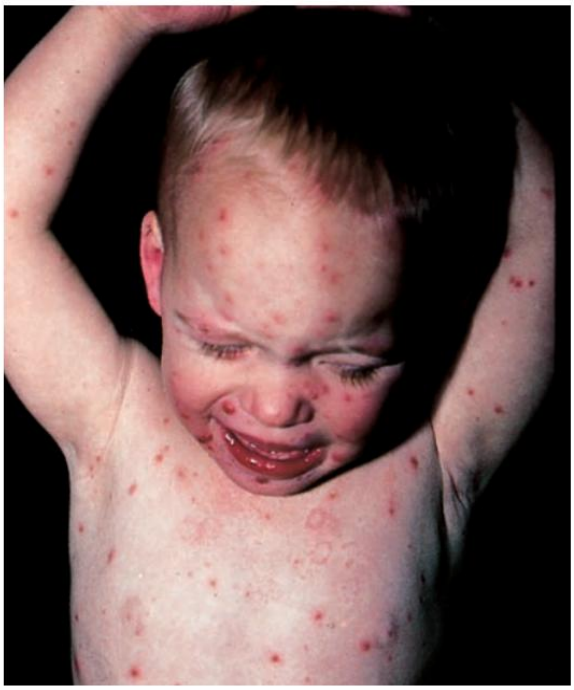


Figure showing infection of Chickenpox

Influenza:

Influenza (Flu) Influenza [Italian, to be influenced by the stars—un influenza di freddo], or the flu, is a respiratory system disease caused by negative-strand RNA viruses that belong to the family Orthomyxoverride. There are four groups: influenza A, influenza B, influenza C. Influenza is characterized by chills, fever (usually 102°F, 39°C), headache, malaise, cough, sore throat, and

general muscular aches and pains. These symptoms arise from the death of respiratory epithelial cells, probably due to attacks by activated T cells. These symptoms are more debilitating than are symptoms of the common cold. Recovery usually occurs in 3 to 7 days, during which cold-like symptoms appear as the fever subsides. Influenza alone usually is not fatal. However, death may result from pneumonia caused by secondary bacterial invaders such as Staphylococcus aureus, Streptococcus pneumoniae, and Haemophilus influenza. A commercially available identification technique is Directigen FLU-A (an enzyme immunoassay [EIA] rapid test). This test can detect the influenza A virus in clinical specimens in less than 15 minutes. As with many other viral diseases, only the symptoms of influenza usually are treated. Amantadine (Symmetrel) rimantadine (Flumadine), zanamivir (Relenza), and oseltamivir (Tamiflu) have been shown to reduce the duration and symptoms of type A influenza if administered during the first two days of illness. Unfortunately, 91% of the virus samples (representing the predominant influenza strain) tested by the CDC in December 2005 were resistant to rimantidine and amantidine, compared to 11% in the previous year. Amantadine and rimantadine are chemically related, antiviral drugs known as adamantanes. These usually have activity against influenza A viruses but not influenza B viruses. Amantadine and rimantadine are thought to interfere with influenza A virus M2 protein, a membrane ion channel protein. They also inhibit virus uncoating, which inhibits virus replication, resulting in decreased viral shedding. Zanamivir and oseltamivir are chemically related antiviral drugs known as neuraminidase inhibitors that have activity against both influenza A and B viruses. Neuraminidase inhibitors attack the virus directly by plugging the catalytic site of the enzyme neuraminidase. With the enzyme inactivated, viral particles can't travel from cell to cell. Importantly, aspirin (salicylic acid) should be avoided in children younger than 14 years to reduce the risk of Reye's syndrome. The mainstay for prevention of influenza since the late 1940s has been inactivated virus vaccines, especially for the chronically ill, individuals over age 65, residents of nursing homes, and health-care workers in close contact with people at risk. Clinical disease in these patients is most likely to be severe. Because of influenza's high genetic variability, efforts are made each year to incorporate new virus subtypes into the vaccine. Even when no new subtypes are identified in a given year, annual immunization is still recommended because immunity using the inactivated virus vaccine typically lasts only 1 to 2 years.

Yellow Fever:

Yellow fever is less lethal than other viral diseases caused by a flavivirus, and no longer occurs in the U.S. It is featured here because it remains a public health problem in Africa and South America, causing over 200,000 infections and 30,000 deaths each year. The disease received its first name, yellow jack because jaundice is a prominent sign in severe cases. The jaundice is due to the deposition of bile pigments in the skin and mucous membranes because of liver damage. The disease is spread through a population in two epidemiological patterns. In the urban cycle, human-to-human transmission is by Aedes aegypti mosquitoes. In the sylvatic cycle, the mosquitoes transmit the virus between monkeys and from monkeys to humans (sylvatic means in the woods or affecting wild animals). Once inside a person, the virus spreads to local lymph nodes and multiplies; from this site, it moves to the liver, spleen, kidneys, and heart, where it can persist for days. Yellow fever has an abrupt onset after an incubation period of 3 to 6 days, and usually includes fever, prostration, headache, sensitivity to light, low-back pain, extremity pain, epigastric pain, anorexia, and vomiting. The illness can progress to the liver and renal failure, and haemorrhagic symptoms and signs caused by thrombocytopenia (low platelet count) and abnormal clotting and coagulation can occur. The fatality rate of severe yellow fever is approximately 20%. Diagnosis of yellow fever is made by culture of virus from blood or tissue specimens or by identification of viral antigen or nucleic acid in tissues using immunohistochemistry (IHC), ELISA antigen capture, or PCR. There is no specific treatment for yellow fever. An active immunity to yellow fever results from initial infection or vaccines containing the attenuated yellow fever 17D strain or the Dakar strain virus. Prevention and control of this disease involve vaccination and control of the insect vector.

Common cold:

Common Cold The common cold [coryza: Greek koryza, discharge from the nostrils] is one of the most frequent infections experienced by humans of all ages. The incidence of infection is greater during the winter months, likely due to increased population density (indoors), the effect of dry winter air on mucous membranes, and the decreased immune function that results from the direct effect of cold temperatures. About 50% of the cases are caused by rhinoviruses [Greek rhinos, nose], which are nonenveloped, single-stranded RNA viruses in the family Picornaviridae. There are over 115 distinct serotypes, and each of these antigenic types has a varying capacity to infect the nasal mucosa and cause a cold. In addition, immunity to many of them is transitory. Several other respiratory viruses are also associated with colds (e.g., coronaviruses and parainfluenza viruses).

Thus colds are common because of the diversity of rhinoviruses, the involvement of other respiratory viruses, and the lack of a durable immunity. Rhinoviruses provide an excellent example of the medical relevance of research on virus morphology. The complete rhinovirus capsid structure has been elucidated with the use of X-ray diffraction techniques. The results help explain rhinovirus resistance to human immune defences. The capsid protein that recognizes and binds to cell surface molecules during infection lies at the bottom of a surface cleft (sometimes called a "canyon") about 12 Å deep and 15 Å wide. Thus the binding site is well protected from the immune system while it carries out its functions. Moreover, with greater than 100 serotypes of human rhinoviruses, immunity to one strain may not protect against the other strain, making vaccine development problematic. Possibly drugs that could fit in the cleft and interfere with virus attachment can be designed. Viral invasion of the upper respiratory tract is the basic mechanism in the pathogenesis of a cold. The virus enters the body's cells by binding to the adhesion molecule ICAM-1. The clinical manifestations include familiar nasal stuffiness, sneezing, scratchy throat, and a watery discharge from the nose. The discharge becomes thicker and assumes a yellowish appearance over several days. General malaise is commonly present. Fever is usually absent in uncomplicated colds, although a low grade (100–102°F) fever may occur in infants and children. The disease usually runs its course in about a week. Diagnosis of the common cold is made from observations of clinical symptoms. There are no procedures for direct examination of clinical specimens or for serological diagnosis. Sources of cold viruses include infected individuals excreting viruses in nasal secretions, airborne transmission over short distances by way of moisture droplets, and transmission on contaminated hands or fomites. Epidemiological studies of rhinovirus colds have shown that the familiar explosive, non-contained sneeze (see figure 36.9) may not play an important role in virus spread. Rather, hand-to-hand contact between a rhinovirus "donor" and a susceptible "recipient" is more likely. The common cold occurs worldwide with two main seasonal peaks, spring and early autumn. Infection is most common early in life and generally decreases with an increase in age. Nothing is available for treating the common cold except additional rest, extra fluids, and the use of anti-inflammatory agents for alleviating local and systemic disc.

Chlamydial Pneumonia:

Chlamydial pneumonia is caused by Chlamydophila (Chlamydia) pneumoniae. Clinically, infections are generally mild; pharyngitis, bronchitis, and sinusitis commonly accompany some lower respiratory tract involvement. Symptoms include fever, a productive cough (respiratory secretion brought up by coughing), sore throat, hoarseness, and pain on swallowing. Infections with *C. pneumoniae* are common but sporadic; about 50% of adults have antibody to the chlamydiae.

Evidence suggests that C. pneumoniae is primarily a human pathogen directly transmitted from human to human by droplet (respiratory) secretions. Diagnosis of chlamydial pneumonia is based on symptoms and a micro immunofluorescence test. Tetracycline and erythromycin are routinely used for treatment. In seroepidemiological studies, *C. pneumoniae* infections have been linked with coronary artery disease as well as vascular disease at other sites. Following a demonstration of C. pneumoniae-like particles in atherosclerotic plaque tissue by electron microscopy, *C. pneumoniae* genes and antigens have been detected in artery plaque. Rarely, however, has the microorganism been recovered in cultures of atheromatous tissue (i.e., artery plaque). As a result of these findings, the possible etiologic role of *C. pneumoniae* in coronary artery disease and systemic atherosclerosis is under intense scrutiny.

10.2 Water borne diseases:

Cholera:

Cholera Throughout recorded history, cholera [Greek chole, bile] has caused seven pandemics in various areas of the world, especially in Asia, the Middle East, and Africa. The disease has been rare in the United States since the 1800s, but an endemic focus is believed to exist on the Gulf Coast of Louisiana and Texas. Cholera is caused by the comma-shaped, gram-negative Vibrio cholerae bacterium of the family Vibrionaceae. V. cholerae is actively motile by way of its single, polar flagellum. Although there are many serogroups, only O1 and O139 have exhibited the ability to cause epidemics. V. cholerae O1 is divided into two serotypes, Inaba and Ogawa, and two biotypes, classic and El Tor. Class Gammaproteobacteria: Order Vibrionales Individuals acquire cholera by ingesting food or water contaminated by faecal material from patients or carriers. Shellfish are natural reservoirs. In 1961 the El Tor biotype emerged as an important cause of cholera pandemics, and in 1992 the newly identified strain V. cholerae O139 emerged in Asia. This novel toxigenic strain does not agglutinate with O1 antiserum but possesses epidemic and pandemic potential. In Calcutta, India, serogroup O139 of V. cholerae has displaced El Tor V. cholerae serogroup O1, an event that has never before happened in the recorded history of cholera. Once the bacteria enter the body, the incubation period is 12 to 72 hours. The bacteria adhere to the intestinal mucosa of the small intestine, where they are not invasive but secrete choleragen, a cholera toxin. Choleragen is an AB toxin composed of two functional subunits—an enzymatic A subunit (the toxic component) and an intestinal receptor-binding B subunit. The A subunit enters the intestinal epithelial cells and activates the enzyme adenylate cyclase by the addition of an ADP-ribosyl group in a way similar to that employed by diphtheria toxin. As a result, choleragen stimulates hypersecretion of water and

chloride ions while inhibiting the absorption of sodium ions. The patient loses massive quantities of fluid and electrolytes, causing abdominal muscle cramps, vomiting, fever, and watery diarrhoea. The voided fluid is often referred to as “rice water-stool” because of the flecks of mucus floating in it. Diarrhoea can be so profuse that a person can lose 10 to 15 litters of fluid during the infection. Death may result from the elevated concentrations of blood proteins, caused by reduced fluid levels, which leads to circulatory shock and collapse. The cholera toxin gene is carried by the CTX filamentous bacteriophage. The phage binds to the pilus used to colonize the host’s gut, enters the bacterium, and incorporates its genes into the bacterial chromosome. Evidence indicates that passage through the human host enhances infectivity, although the exact mechanism is unclear. Before V. cholerae exits the body in watery stools, some unknown aspect of the intestinal environment stimulates the activity of certain bacterial genes. These genes, in turn, seem to prepare the bacteria for ever more effective colonization of their next victims, possibly fuelling epidemics. V. cholerae can also be free-living in warm, alkaline, and saline environments. Laboratory diagnosis is by the culture of the bacterium from faeces and subsequent identification by agglutination reactions with specific antisera. Treatment is by oral rehydration therapy with NaCl plus glucose to stimulate water uptake by the intestine; the antibiotics of choice are tetracycline, trimethoprim-sulfamethoxazole, or ciprofloxacin. The most reliable control methods are based on proper sanitation, especially water supplies. The mortality rate without treatment is often over 50%; with treatment and supportive care, it is less than 1%. Fewer than 20 cases of cholera are reported each year in the United States.

10.3 Food borne diseases:

Botulism:

Botulism Foodborne botulism [Latin botulus, sausage] is a form of food poisoning caused by an exotoxin produced by Clostridium botulinum, an obligately anaerobic, endospore-forming, gram-positive rod found in soil and aquatic sediments. The most common source of infection is home-canned food that has not been heated sufficiently to kill contaminating C. botulinum spores. The spores then germinate, and a toxin is produced during vegetative growth. If the food is later eaten without adequate cooking, the active toxin results in disease. Class Clostridia (section 23.4); The bacterial endospore. The botulinum toxin is a neurotoxin that binds to the synapses of motor neurons. It selectively cleaves the synaptic vesicle membrane protein synaptobrevin, thus preventing exocytosis and the release of the neurotransmitter acetylcholine. As a consequence, muscles do not contract in response to motor neuron activity and flaccid paralysis results.

Symptoms of botulism occur within 12 to 72 hours of toxin ingestion and include blurred vision, difficulty in swallowing and speaking, muscle weakness, nausea, and vomiting. Without adequate treatment, one-third of the patients may die of either respiratory or cardiac failure within a few days. Laboratory diagnosis is restricted to Laboratory Response Network facilities and is by demonstration of the toxin in the patient's serum, stools, or vomitus. In addition, recovery of C. botulinum in stool cultures is diagnostic. Treatment relies on supportive care and polyvalent antitoxin. Fewer than 100 cases of botulism occur in the United States annually. Infant botulism is the most common form of botulism in the United States and is confined to infants under a year of age. Approximately 100 cases are reported each year. It appears that ingested spores, which may be naturally present in honey or house dust, germinate in the infant's intestine. C. botulinum then multiplies and produces the toxin. The infant becomes constipated, listless, generally weak, and eats poorly. Death may result from respiratory failure. Prevention and control of botulism involve (1) strict adherence to safe food-processing practices by the food industry, (2) educating the public on safe home-preserving (canning) methods for foods, and (3) not feeding honey to infants younger than 1 year of age.

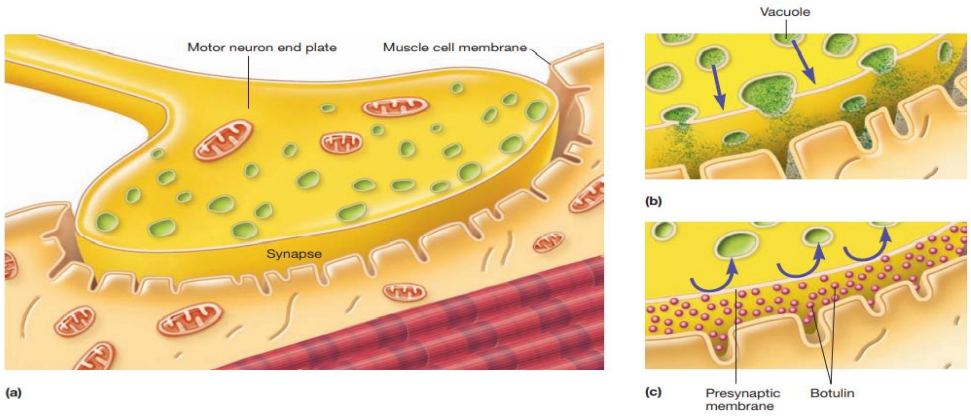


Figure 38.23 The Physiological Effects of Botulism Toxin. **(a)** The relationship between the motor neuron and the muscle at the neuromuscular junction. **(b)** In the normal state, acetylcholine released at the synapse crosses to the muscle and creates an impulse that stimulates muscle contraction. **(c)** In botulism, the toxin enters the motor end plate and attaches to the presynaptic membrane, where it blocks release of the chemical. This prevents impulse transmission, and keeps the muscle from contracting.

Staphylococcal Food Poisoning:

Staphylococcal food poisoning is the major type of food intoxication in the United States. It is caused by ingestion of improperly stored or cooked food (particularly foods such as ham, processed meats, chicken salad, pastries, ice cream, and hollandaise sauce) in which Staphylococcus aureus has grown. Class Bacilli: Order Bacillales (section 23.5) S. aureus (a gram-positive coccus) is very resistant to heat, drying, and radiation; it is found in the nasal passages and on the skin of humans

and other mammals worldwide. From these sources, it can readily enter a food. If the bacteria are allowed to incubate in certain foods, they produce heat-stable enterotoxins that render the food dangerous even though it appears normal. Once the bacteria have produced the toxin, the food can be extensively and properly cooked, killing the bacteria without destroying the toxin. Intoxication can therefore result from food that has been thoroughly cooked. Thirteen different enterotoxins have been identified; enterotoxins A, B, C1, C2, D, and E are the most common. (Recall that enterotoxins A and B are superantigens.) These toxins appear to act as neurotoxins that stimulate vomiting through the vagus nerve. Toxigenicity (section 33.4) Typical symptoms include severe abdominal pain, cramps, diarrhea, vomiting, and nausea. The onset of symptoms is rapid (usually 1 to 8 hours) and of short duration (usually less than 24 hours). The mortality rate of staphylococcal food poisoning is negligible among healthy individuals. Diagnosis is based on symptoms or laboratory identification of the bacteria from foods. Enterotoxins may be detected in foods by animal toxicity tests or antibody-based methods. Treatment is with fluid and electrolyte replacement. Prevention and control involve avoidance of food contamination and control of personnel responsible for food preparation and distribution.

11. Industrial Microbiology

11.1 Industrial microorganisms

Microbes Are the Source of Many Products of Industrial Importance. Microorganisms which are used for industrial production must meet certain requirements.

- The organism must be able to grow in a simple medium and should preferably not require growth factors (i.e. pre-formed vitamins, nucleotides, and acids) outside those which may be present in the industrial medium in which it is grown.
- The organism should be able to grow vigorously and rapidly in the medium in use. A slow growing organism no matter how efficient it is, in terms of the production of the target material, could be a liability.
- Not only should the organism grow rapidly, but it should also produce the desired materials, whether they are cells or metabolic products, in as short a time as possible.
- Its end products should not include toxic and other undesirable materials, especially if these end products are for internal consumption.
- The organism should have a reasonable genetic, and hence physiological stability. An organism which mutates easily is an expensive risk. It could produce undesired products if a mutation occurred unobserved. The result could be reduced yield of the expected material, production of an entirely different product or indeed a toxic material.
- The organism should lend itself to a suitable method of product harvest at the end of the fermentation. If for example a yeast and a bacterium were equally suitable for manufacturing a certain product, it would be better to use the yeast if the most appropriate recovery method was centrifugation. This is because while the bacterial diameter is approximate
- Wherever possible, organisms which have physiological requirements which protect them against competition from contaminants should be used.
- The organism should be reasonably resistant to predators such as *Bdellovibrio*spp or bacteriophages. It should therefore be part of the fundamental research of an industrial establishment using a phage-susceptible organism to attempt to produce phage-resistant but high yielding strains of the organism.
- Where practicable the organism should not be too highly demanding of oxygen as aeration (through greater power demand for agitation of the fermenter impellers, forced air injection etc.) contributes about 20% of the cost of the finished product.
- Lastly, the organism should be fairly easily amenable to genetic manipulation to enable the establishment of strains with more acceptable properties.

11.2 Strain improvement:

Microbes produce a variety of products in very low concentrations which have been used as antibiotics, drugs, vitamins, enzymes, bulk organic compounds, polymers, amino acids, biofuels, etc. Prerequisite for efficient biotechnological processes at industrial scale requires the use of microbial strains which produce high titre of the desired product. However, this is not an inherent property of the selected microorganism(s); hence, modifications in their genetic material could possibly help in overcoming this limitation. Thus, industrially relevant microbes are subjected to a variety of treatments using physical, chemical or genetic tools to overproduce the desired metabolite and make the process cost efficient. This process of enhancing the biosynthetic capabilities of microbes to produce desired product in higher quantities is defined as microbial strain improvement.

11.3 Ethanol production:

Microbial energy conversion includes the microbial transformation of organic materials into biofuels, such as ethanol and hydrogen that can fuel cars or other machines. Currently ethanol is used as an additive to gasoline because it is easy to store and can be burned in cars designed to use pure gasoline. Corn is the substrate most commonly used for generating ethanol in the United States, while sugar and palm oil are used in other countries. Ethanol production involves the microbial degradation of plant starch using amylases and amyloglucosidases. The resulting sugars are then fermented to ethanol. However, the use of corn and other foods to make bioethanol has unintended consequences including increased pesticide and fertilizer use, and inflating worldwide food prices. Cellulose (or cellulosic) biofuel production using crop residues is beginning to replace corn-based biofuel production. Crop residues are plant materials left in the field after harvest as well as plants like switchgrass that grow on land not fertile enough for crops. This material consists of cellulose and hemicellulose—polymers of five different hexoses and pentoses: glucose, xylose, mannose, galactose, and arabinose. Degradation of cellulose and hemicellulose to release these monomers is commonly done by heating the plant material and treating it with acid, which is energy intensive and corrosive. This is an expensive and potentially hazardous process.

Even with cheaper, more sustainable plant materials for ethanol production, ethanol as a biofuel has several disadvantages. One is that it absorbs water and cannot be shipped through existing pipelines because they almost always contain some water. So ethanol must be distilled twice to remove the water: once when it is generated and then again after shipping. Furthermore, ethanol contains far

less energy than other fuels, including gasoline and higher molecular weight alcohols such as butanol, which are easier to generate.

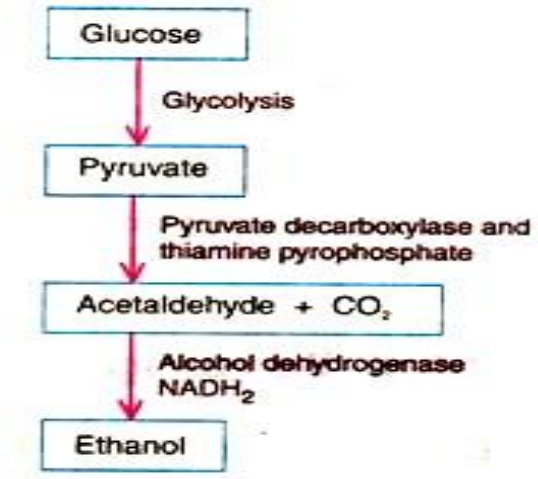


Figure: Ethanol production by microbes

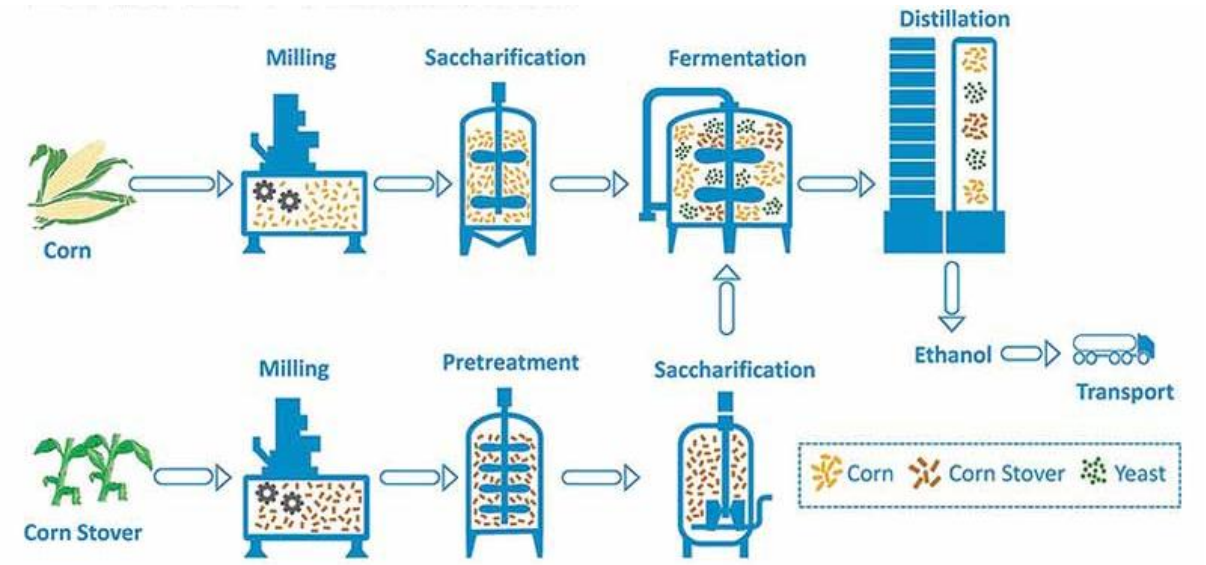


Figure: An outline of ethanol production

Table: Major microbial products and processes of industrial importance.

Substances	Microorganisms
Industrial Products	
Ethanol (from glucose)	*Saccharomyces cerevisiae*
Ethanol (from lactose)	*Kluyveromyces fragilis*
Acetone and butanol	*Clostridium acetobutylicum*
2,3-butanediol	*Enterobacter, Serratia*
Enzymes	*Aspergillus, Bacillus, Mucor, Trichoderma*
Agricultural Products	
Gibberellins	*Gibberella fujikuroi*
Food Additives	
Amino acids (e.g., lysine)	*Corynebacterium glutamicum*
Organic acids (citric acid)	*Aspergillus niger*
Nucleotides	*Corynebacterium glutamicum*
Vitamins	*Ashbya, Eremothecium, Blakeslea*
Polysaccharides	*Xanthomonas*
Medical Products	
Antibiotics	*Penicillium, Streptomyces, Bacillus*
Alkaloids	*Claviceps purpurea*
Steroid transformations	*Rhizopus, Arthrobacter*
Insulin, human growth hormone, somatostatin, interferons	*Escherichia coli, Saccharomyces cerevisiae*, and others (recombinant DNA technology)
Biofuels	
Hydrogen	Photosynthetic microorganisms
Methane	*Methanothermobacter*
Ethanol	*Zymomonas, Thermoanaerobacter*

11.4 Penicillin Production

More than 65% of all antibiotics are produced by microorganisms. Currently most antibiotics are produced by members of the genus Streptomyces and by filamentous fungi. Antibiotics are secreted by these microbes and either used in the native state or chemically modified to produce semisynthetic derivatives. Semisynthetic derivatives were developed to treat a broader spectrum of bacteria or bacteria that had become resistant to the parent drug. The synthesis of penicillin and its derivatives illustrates how an antibiotic is commercially produced. As with any industrially important natural product, the manufacturer will determine how to maximize product yield. In this case, the growth of the fungus *Penicillium chrysogenum*requires careful adjustment of the medium, so that the slowly hydrolyzed disaccharide lactose is used in combination with a limited supply of nitrogen to stimulate a greater accumulation of penicillin after growth has stopped. If particular penicillin is needed, a specific precursor is added to the medium. For example, phenylacetic acid is added to maximize production of penicillin G, which has a benzyl side chain. After about a week, the broth is separated from the fungal hyphae and processed by absorption, precipitation, and crystallization to yield the final product. This basic product can then be modified by chemical procedures to yield a variety of semisynthetic penicillin. Manufacture of semisynthetic β-lactam

antibiotics, such as ampicillin and amoxicillin, begins with natural penicillin. The β-lactam ring is preserved, but the side chain is modified, in this case to provide a broader spectrum of activity.

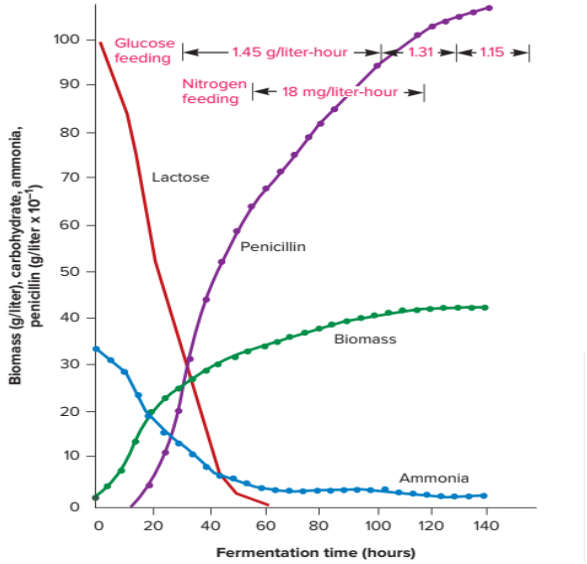


Figure: Penicillin Fermentation Involves Precise Control of Nutrients. The synthesis of penicillin begins when nitrogen from ammonia becomes limiting. After most of the lactose has been degraded, glucose is added along with a low level of nitrogen. This stimulates maximum transformation of the carbon sources to penicillin.

Vitamin B_{12} Production

Vitamin B12 (cyanocobalamin) is a water soluble vitamin with complex structure. The empirical formula of cyanocobalamin is C63H90N14O14PCO. The structure of vitamin B12 consists of a corrin ring with a central cobalt atom. The corrin ring is almost similar to the tetrapyrrole ring structure found in other porphyrin compounds e.g. heme (with Fe) and chlorophyll (with Mg). The corrin ring has four pyrrole units. Cobalt present at the center of the corrin ring is bonded to the four pyrrole nitrogen's. Cobalt also binds to dimethylbenzimidazole and amino isopropanol. Thus, cobalt atom present in vitamin B12 is in a coordination state of six.

Vitamin B12 is exclusively synthesized in nature by microorganisms. The biosynthesis of B12 is comparable with that of chlorophyll and hemoglobin. Many of the reactions in the synthesis of vitamin B12 are not yet fully understood.

Vitamin B12 is commercially produced by fermentation. It was first obtained as a byproduct of Streptomyces fermentation in the production of certain antibiotics (streptomycin, chloramphenicol, or neomycin). But the yield was very low. Later, high-yielding strains were developed. And at

present, vitamin B12 is entirely produced by fermentation. It is estimated that the world's annual production of vitamin B12 is around 15,000 kg.

High concentrations of vitamin B12 are detected in sewage-sludge solids. This is produced by microorganisms. Recovery of vitamin B12 from sewage-sludge was carried out in some parts of United States. Unlike most other vitamins, the chemical synthesis of vitamin B12 is not practicable, since about 20 complicated reaction steps need to be carried out. Fermentation of vitamin B12 is the only choice. Several microorganisms can be employed for the production of vitamin B12, with varying yields. Glucose is the most commonly used carbon source. The most commonly used microorganisms are — *Propionibacterium freudenreichii*, *Pseudomonas denitrificans*, *Bacillus megaterium* and *Streptomyces olivaceus.*

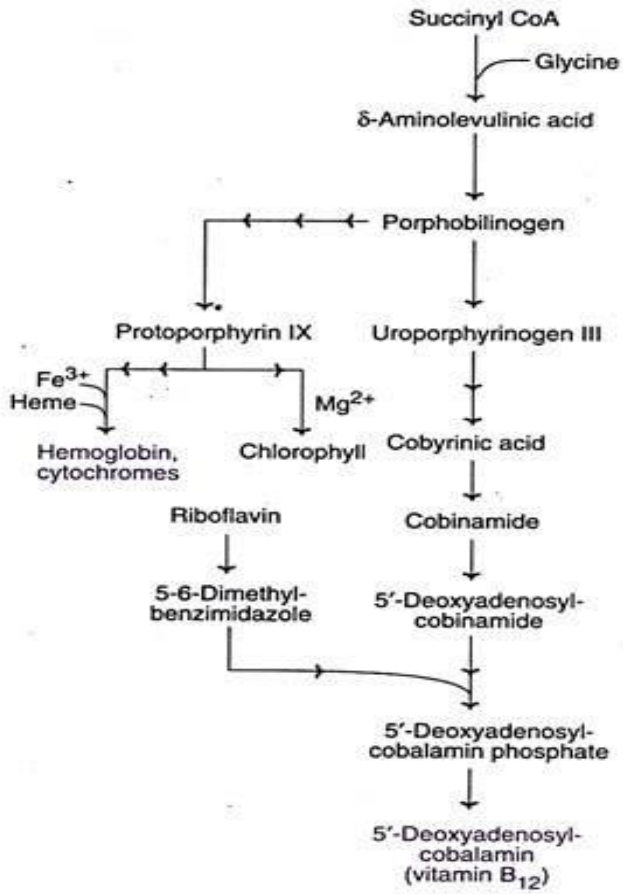


Figure: An outline of the biosynthesis of vitamin B12(Source: https://www.biologydiscussion.com/vitamins/microbial-production-of-vitamins-an-overview/10372)

By employing modern techniques of genetic engineering, vitamin B12 production can be enhanced. A protoplast fusion technique between *Protaminobacter* rubber and *Rhodopseudomonasspheroides*

resulted in a hybrid strain called *Rhodopseudomonasprotamicus*. This new strain can produce as high as 135 mg/l of vitamin B12 utilizing carbon source.

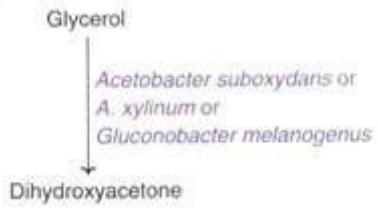
12. Cosmetic Microbiology:

Cosmetics in microbiology

All the steroids possess the basic structure namely cyclopentanoperhydrophenanthrene. Steroids as hormones (glucocorticoids, mineralocorticoids, androgens, estrogens) perform a wide range of functions. They are very useful therapeutically. For instance, cortisone, due to its anti-inflammatory action is used in the treatment of rheumatoid arthritis and skin diseases; derivatives of progesterone and estrogens are employed as contraceptives. Certain derivatives of cortisone (e.g., prednisolone) are more effective in their therapeutic action

12.1. Concept:

Dihydroxyacetone is used in cosmetics and suntan lotions. Certain acetic acid bacteria can convert glycerol to dihydroxyacetone through the process of biotransformation.



Good oxygen supply, temperature 26-28°C, and pH 6.0 are ideal for the optimal biotransformation.

12.2 Current trends:

Indigo can be synthesized by microbial transformation. This has been made possible by cloning a single Pseudomonas gene that encodes naphthalene di-oxygenase in the creation of E. coli. The relevant reactions of biotransformation for the production of indigo are depicted in

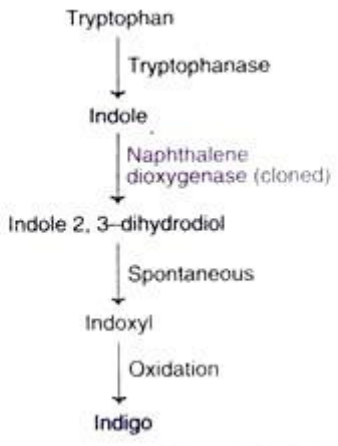


Figure: Microbial production of indigo

3. Not all cyanobacteria live in moist environments - many live in the desert! The extracellular polymeric substances that I mentioned earlier help keep these colonies from drying out. If you take away these polymers, cells become very sensitive to desiccation.

4. The urea and propylene glycol used in conventional moisturizers absorb moisture, but they don't retain it. The cyanobacteria polymers do both, and they do it well. For example, the moisture retention value for urea is 15.9% - it's 28% for cyanobacterial polymers. Once again, these compounds may be coming to a moisturizer near you.

5. Mycosporine-like amino acids (MAAs) are small, colourless molecules that hang out in the cytoplasm and outer sheath of cyanobacteria. (They're also made by dinoflagellates.) Mycosporines (a broad class of photo protectant compounds) from *Porphyraumbilicalis* (a red alga) are already used in two commercially available sunscreens - Helioguard365 and Helionori.

The MAAs used in Helionori are photo- and heat-stable, and they help reduce sunburn. Because they block UV radiation, they also protect DNA from UV-mediated damage - they block the formation of thymine dimers, for example. MAAs are also effective antioxidants - mycosporine-glycine quenches hydroxyl radicals. Because free radicals make our skin age, don't be surprised when MAAs show up in your cosmetics and anti-aging creams.

6. Scytonemin is a lipid-soluble pigment that coats cyanobacterial colonies or larger mats to protect the cells from short-wave UV radiation. Not only can scytonemin block up to 90% of the sun's UV rays, it's also an antioxidant capable of scavenging free radicals. Chances are, scytonemin will also make its way into cosmetics in the near future.

13. Fundamentals of immunology

13.1 Innate and Acquired immunity:

Immunity is the ability of an organism to resist infection. The human immune system employs a two-pronged defense against invading pathogens. Innate immunity, the first of these interconnected defensive mechanisms, is the built-in capacity of the immune system of multicellular organisms to target pathogens that are seeking to colonize the host. Depending on the virulence of the pathogen, the infectious dose, and the immune competence of the individual, innate mechanisms alone may be insufficient to eliminate the pathogen. In such cases, and assuming that the pathogen has not quickly killed the host, adaptive immunity is triggered within the individual against those specific pathogens that have overcome the innate defenses

Innate immunity:

Innate immunity is a noninducible, preexisting ability of the body to recognize and destroy a broad range of pathogens or their products. Innate immune responses develop quickly, typically within several hours of exposure to a pathogen or its product, and they do not require previous exposure to a pathogen or its products for activation. Eukaryotes have functionally similar pathogen recognition mechanisms that lead to rapid and effective host defense. For example, pathogen recognition in the insect Drosophila (fruit fly) is carried out in much the same way as it is in humans, using immune cells that show structural and evolutionary homology. In addition to a variety of physical and chemical barriers to infection, innate immunity is largely dependent on the activity of phagocytes cells that ingest, kill, and digest microbial pathogens. Phagocytes include neutrophils, macrophages, dendritic cells, and eosinophils, and we define their distinctive features in Section 26.4. In addition to phagocytes, cells of the innate immune system consist of mast cells and basophils, both of which trigger inflammation when activated and natural killer (NK) cells, which identify and destroy host cells that have become compromised and are therefore dangerous to the host, such as cells that are infected by a pathogen or are cancerous.

Phagocytes: Most white blood cells are mobile phagocytes (or eating cells), called neutrophils or polymorphonuclear cells (PMNs), that patrol the blood in search of invading microbes. Other primary phagocytic cells are part of the mononuclear phagocyte system, and include monocytes and macrophages. Monocytes are present in the blood and settle in the tissues as macrophages (MØ). These phagocytes are attracted to sites of infection (chemotaxis), bind to the microbe (adhere),

ingest (phagocytose) and kill the microbe. Molecules coating a microbe, such as complement or antibody, enhance contact and ingestion (opsonization) of the microbe.

Natural killer (NK) cells: NK cells are found throughout the tissues of the body but mainly in the circulation, and are important for protection against viruses and some tumors. Changes in the surface molecules of cells as the result of virus infection allow NK cells to bind to and kill infected cells by releasing perforins and inducing apoptosis. In addition, on binding to virus-infected cells, NK cells secrete interferon gamma (IFNγ) which protects adjacent cells from infection by viruses and helps to activate T-cell-mediated immunity.

Mast cells and basophils: Mast cells (in connective tissues) and basophils (in the circulation) are produced in the bone marrow and have similar morphology and functions. When activated, these cells degranulate releasing pharmacological mediators which cause vasodilation, increased vascular permeability and leukocyte migration.

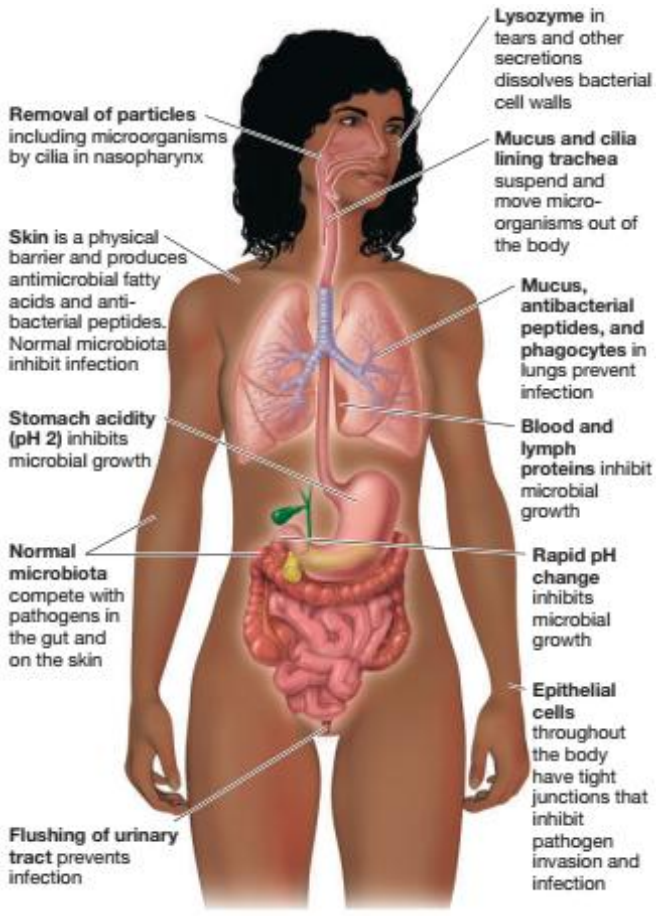


Figure: Barrier to infection in the human body. These barriers provide natural resistance to colonization and infection by pathogens. (Source: Brock's Biology)

Dendritic cells: There are three main kinds of dendritic cells (Langerhans cells, interdigitating cells, follicular dendritic cells). They represent a critical interface between innate and adaptive immunity. Their role is to recognize microbial antigens through innate receptors and process and present their peptides to T cells of the adaptive immune system. Follicular dendritic cells in specialized areas of lymphoid tissues hold unmodified antigens for recognition by B cells.

Other cells of innate immunity: A variety of other cells, including eosinophils, platelets and erythrocytes play a role in immune defense. Eosinophils are granular leukocytes that attack and kill parasites by releasing the toxin, major basic protein. Platelets, on activation, release mediators that activate complement leading to attraction of leukocytes. Erythrocytes bind and remove small immune complexes.

Adaptive Immunity

When innate immune responses fail to eliminate an invading pathogen, adaptive immunity is activated. Adaptive immunity is the acquired ability to recognize and destroy a specific pathogen or its products. Adaptive responses show specificity because they are directed at unique pathogen components or products called antigens, which often define a particular strain of pathogen or type of foreign material. Macrophages and dendritic cells ingest and process pathogens to present peptide antigens to T lymphocytes (T cells), which play a key role in initiating the adaptive response. Another adaptive immune cell, the B lymphocyte (B cell), also presents antigen to T cells. The presented antigen peptides, called epitopes, bind specific receptors on the lymphocyte surface, triggering the expression of genes that cause the lymphocytes to proliferate and differentiate. Differentiated B cells called plasma cells specialize in the production of antigen-specific proteins called antibodies (immunoglobulins), which bind the pathogen and mark it for destruction.

Unlike the comparatively rapid innate response, a protective adaptive response usually takes several days to develop, and the strength of the adaptive response increases as the numbers of antigen-reactive lymphocytes increase. Although adaptive immune responses are typically slower than innate response mechanisms, they are highly specific and result in immune memory, the ability of lymphocytes to quickly respond after subsequent exposure to a previously encountered antigen.

Four characteristics distinguish adaptive immunity:

1. Discrimination between self and non-self. While both innate and adaptive immune cells must discriminate between self and non-self, the adaptive immune system responds much more selectively to non-self.

2. Specificity. Recognition of non-self-antigen is directed against one particular pathogen or foreign substance (among trillions); the immunity to one pathogen or substance usually does not confer immunity to others.

3. Diversity. To mount these very specific responses, the system is able to generate an enormous diversity of cellular receptors and antibodies that recognize trillions of unique foreign substances.

4. Memory. When reexposed to the same pathogen or substance, the host reacts so quickly there is usually no noticeable illness. By contrast, the reaction time for innate defenses is just as long for later exposures to a given antigen as it was for the initial one.

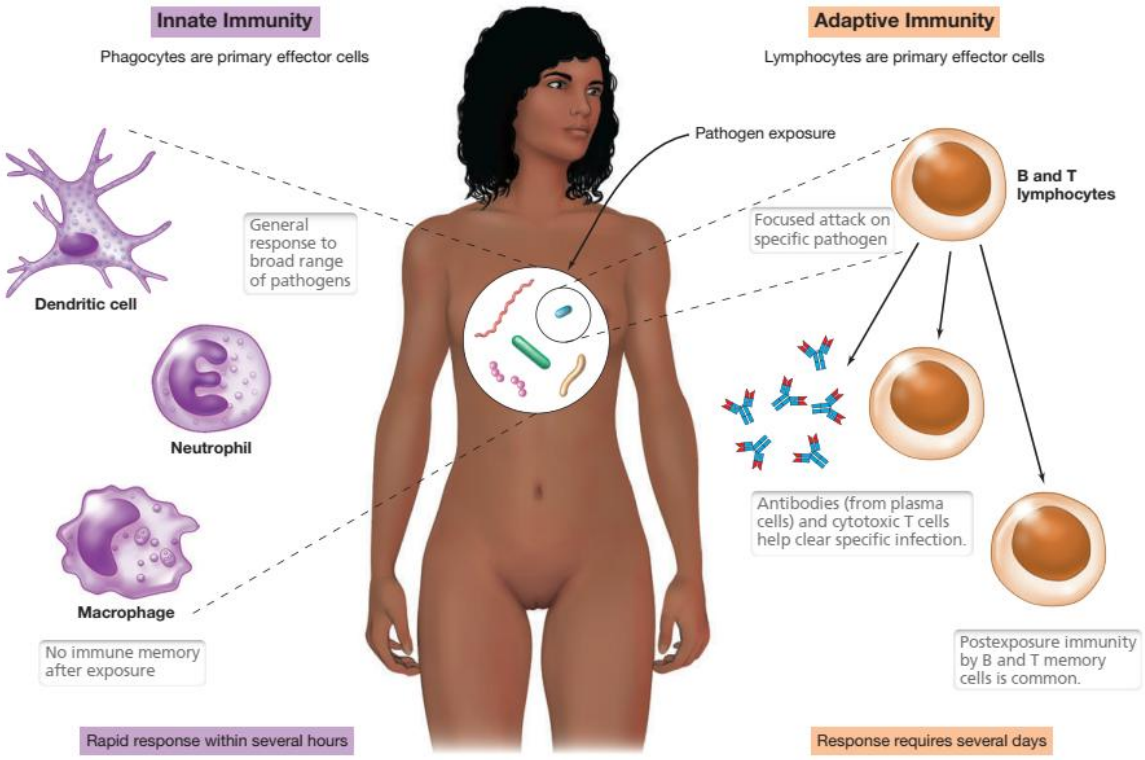


Figure: Overview of the two prolonged immune system

Lymphocytes

Lymphocytes are responsible for the specificity and memory in adaptive immune responses. They are produced in the primary lymphoid organ and function in the secondary lymphoid organs/tissues where they recognize and respond to foreign antigens. There are three types of lymphocytes – NK cells, T cells and B cells, although only T and B cells have true antigen specificity and memory. T

cells and B cells mature in the thymus and bone marrow, respectively. In the resting state both T and B lymphocytes have a similar morphology with a small amount of cytoplasm. They have specific but different antigen receptors and a variety of other surface molecules necessary for interaction with other cells.

Table: Characteristics of human B and T cells

	T cells	B cells
Site of maturation	Thymus	Bone marrow
Antigen receptor	TCR	Antibody
Requirement of MHC for recognition	Yes	No
Characteristic 'markers'	All have TCR, CD3 Th – CD4 Tc – CD8	Surface Ig, CD19, CD20, CD21 CD79
Main location in lymph nodes	Paracortical area	Follicles
Memory cells	Yes	Yes
Function	Protect against intracellular microbes Provide help for Ab responses	Protect against extracellular microbes
Products	Th1 – IFNγ, TNFα Th2 – IL-4, IL-5, IL-6 Tc – Perforins	Antibodies (B cells mature into plasma cells)

There are two classes of T lymphocytes, T helper (Th) cells and T cytotoxic (Tc) cells. All T lymphocytes have antigen receptors (TCR) (Topic F2) that determine their specificity and CD3, which is essential for their activation (Topic F4). These molecules also serve as 'markers' to identify T cells. B lymphocytes make and use antibodies as their specific antigen receptor. They have molecules similar to CD3, i.e. CD79, which are important in their activation. B lymphocytes can mature into plasma cells that produce and secrete large amounts of antibody.

13.2 T Cell

T cell precursors derived from hemopoietic stem cells (HSC) in the bone marrow. In the thymus, these precursors differentiate into functional T lymphocytes under the influence of thymic stromal cells and cytokines. Molecules important to T cell function such as CD4, CD8 and the T cell

receptor develop at different stages during the differentiation process. Millions of T cells, each with receptors specific for different antigens, are generated by gene rearrangement from multiple (inherited) germline genes. Each of the T cells produced in the thymus has only one specificity coded for by its antigen receptor.

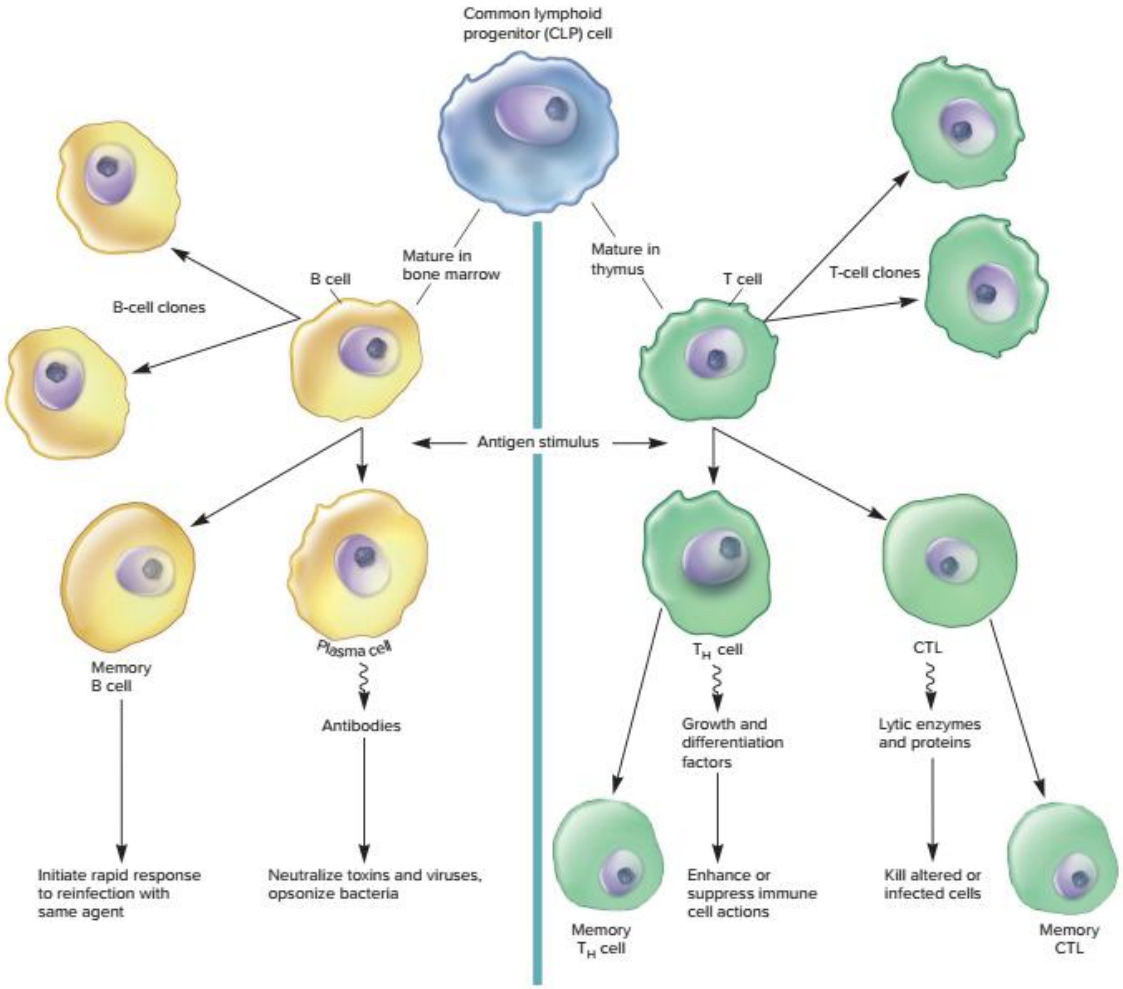


Figure: Development and function of B and T lumphocytes

Common lymphoid progenitor (CLP) cells in the bone marrow develop into B cells, which then migrate to secondary lymphoid tissue and the spleen where they can be activated to multiply (B-cell clones) and differentiate into antibody-producing plasma cells or long-lasting memory cells. CLP cells in the thymus differentiate into T cells that become two main types of cells: T-helper cells (T_H cells) and cytotoxic T lymphocytes (CTLs). Mature, naïve T cells leave the thymus and migrate to secondary lymphoid tissues where, upon antigenic stimulation, they replicate and become activated T_H cells and CTLs. TH cells mediate the responses of other immune cells while CTLs kill host cells infected with intracellular pathogens and malignant host cells. Like B cells, T_H cells and CTLs can also differentiate into memory

Once produced in the thymus, T cells undergo selection using their newly produced receptors. T cells with receptors that bind weakly to MHC molecules are selected whilst those with receptors which bind strongly to MHC and self-antigens die through apoptosis and are removed by phagocytic macrophages. T cells which survive the selection process mature into functionally distinct subsets. These cells migrate to the peripheral lymphoid tissues where they complete their functional maturation and provide protection against invading microbes. T cells can be identified using monoclonal antibodies specific for characteristic molecules such as the T cell receptor (TCR) or CD3. These cells function to control intracellular microbes and to provide help for B cell (antibody) responses. Two different kinds of T cells are involved in these functions, T helper (Th) cells and T cytotoxic (Tc) cells. Th cells provide help for B cells through direct cell surface signaling and by producing cytokines that are critical to B cell growth and differentiation. In addition to TCR and CD3, Th cells also express cell surface CD4 molecules that bind to MHC class II molecules, an interaction required for their activation by antigen.

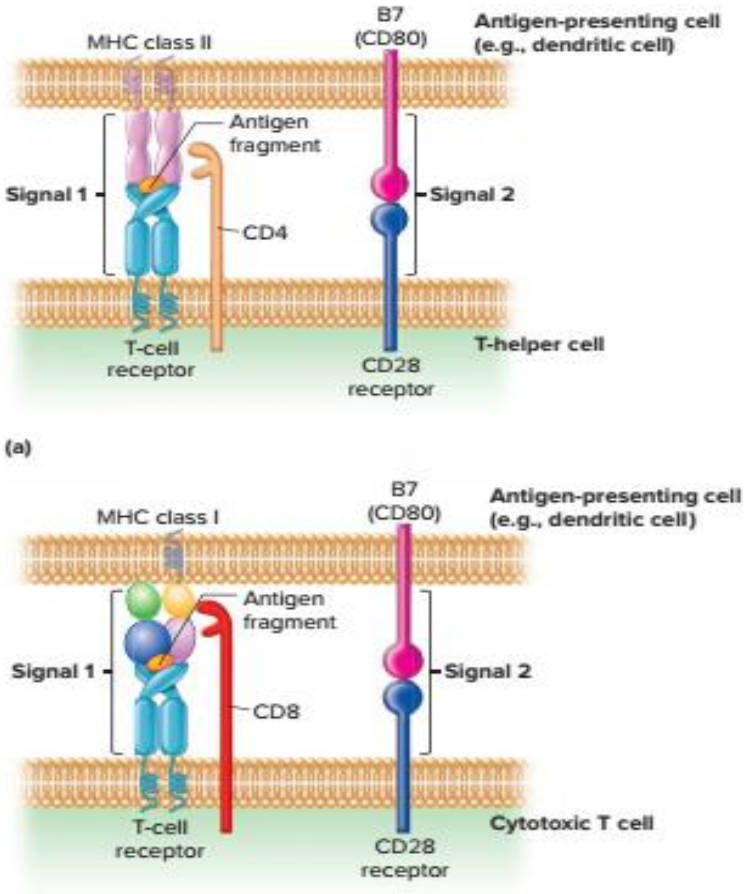


Figure: Three Signals Are Essential for T-Cell Activation. The first signal is the presentation of the antigen fragment by a dendritic cell. (a) The CD4 coreceptor on a T-helper cell mediates

recognition of a MHC class II molecule on an APC. (b) The MHC class I molecule is recognized by the CD8 coreceptor on cells that will be activated to become CTLs. The second signal occurs when the dendritic cell presents the B7 (CD80) protein to the T helper or CTL with its CD28 protein receptor. The third signal is the release of cytokines by the APC and the T cells themselves, which drives the T cells to differentiate into memory and effector cells, which proliferate and become fully activated.

13.3 B Cell

B cells develop from hemopoietic stem cells primarily (perhaps exclusively) in the microenvironment of the fetal liver and, after birth, the bone marrow. He two main functions of the bone marrow as a primary lymphoid organ are to: (a) produce large numbers of B cells, each with unique antigen receptors (antibodies) such that, overall, there is sufficient B cell diversity to recognize all of the antigens in our environment (generate diversity); (b) eliminate B cells with antigen receptor for self-molecules. The early stages of B cell development (like that of T cells) is independent of exogenous antigen. Two kinds of B cells (B1 and B2) have been identified. The B2 cells are produced in the bone marrow (conventional B cells) as described and with the help of Th cells produce IgG, IgA and IgE antibodies. However, B1 cells arise early in ontogeny, express mainly IgM antibodies encoded by germline antibody genes, mature independently of the bone marrow and generally recognize multimeric sugar/lipid antigens of microbes and are thymus independent.

When activated by antigen and, in most cases, with T cell help, B cells proliferate and mature into memory cells or plasma cells. Memory cells only produce antibody for expression on their cell surface and remain able to respond to antigen if it is reintroduced. In contrast, plasma cells do not have cell surface antibody receptors. Rather, these cells function as factories producing and secreting large amounts of antibody of the same specificity as the antigen receptor on the stimulated parent B cell. The morphology of a plasma cell is consistent with its primary function – high-rate glycoprotein (antibody) synthesis. This includes extensive endoplasmic reticulum, mitochondria and Golgi apparatus. It should be noted that a plasma cell only produces antibodies of one specificity, one class and one subclass.

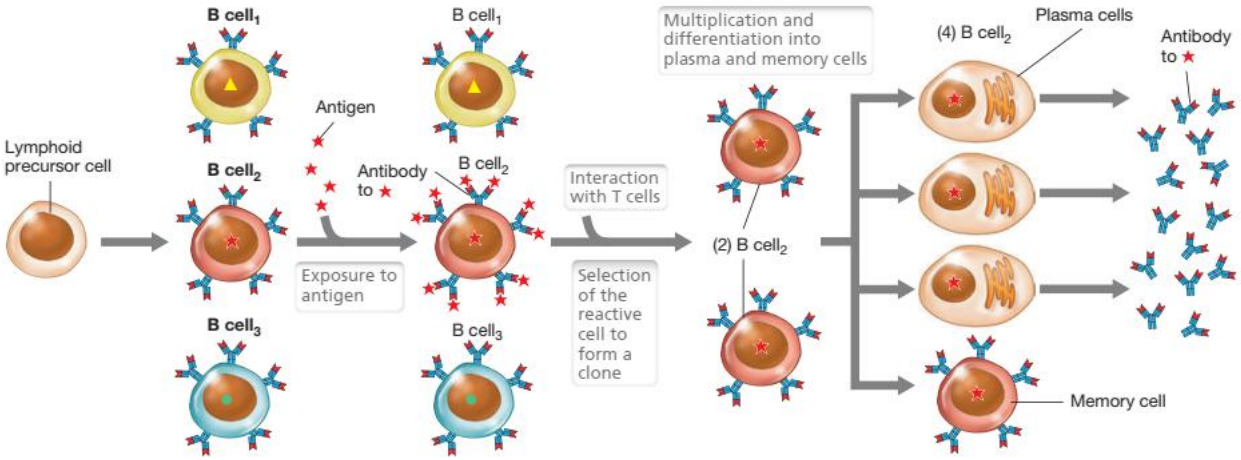


Figure: B cell clonal selection and expansion. Among the repertoire of individual B cells (represented by B cell1, B cell2, and B cell3), only B cell2 specifically interacts with the introduced antigen (shown as a star). The antigen drives selection and, following interaction with a Th cell, proliferation of B cell2 to form a clone of antigen-specific B cells. Clonal copies of the original antigen-reactive B cell have the same antigen-specific surface antibody. Continued exposure to antigen results in continued expansion of the clone. Clones differentiate further into many antibody-producing plasma cells and comparatively few long-lived memory B cells (Source: *Brock's Biology*)

13.4 The major histocompatibility complex

The major histocompatibility complex (MHC) is a collection of genes encoding proteins that enable the host to distinguish between self and non-self. The term histocompatibility is derived from the Greek word for tissue (histo) and the ability to get along (compatibility). Human MHC is located on chromosome 6 and is called the human leukocyte antigen (HLA) complex. HLA proteins can be divided into three classes: Class I are found on all types of nucleated body cells; class II appear only on cells that can process antigens and present them to T lymphocytes.

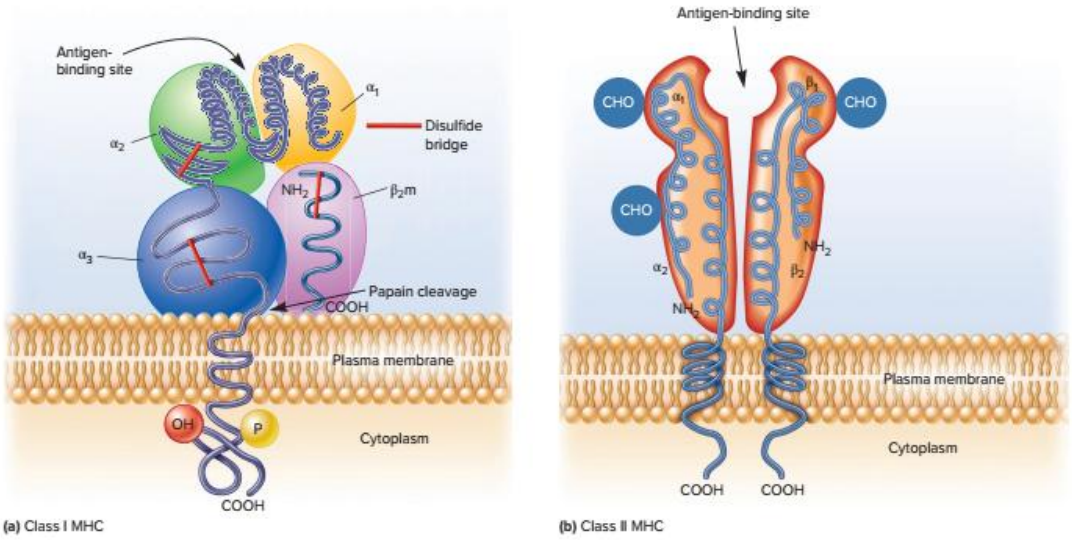


Figure: The Membrane-Bound Class I and Class II Major Histocompatibility Complex Molecules (a) The class I molecule is a heterodimer composed of the alpha protein, which is divided into three domains: α1, α2, and α3, and the protein β2 microglobulin (β2m). (b) The class II molecule is a heterodimer composed of two distinct proteins called alpha and beta. Each is divided into two domains α1, α2 and β1, β2, respectively. (Source: Prescott's Biology)

Class I MHC

Class I MHC molecules include HLA types A, B, and C, and identify all nucleated cells of the body as "self." They consist of a complex of two protein chains, a larger alpha chain (45,000 Da), and a smaller chain called β2-microglobulin (12,000Da). Only the alpha chain spans the plasma membrane. Both chains interact to form a pocket that projects from the cell surface. This pocket can bind either self-antigen, thereby marking the cell as a host cell, or it can bind a peptide extracted from an intracellular pathogen. HLA proteins differ among individuals; the closer two people are related, the more similar are their HLA molecules. This is because many forms of each HLA gene exist (i.e., HLA genes are polymorphic). Throughout evolution, multiple alleles of each gene have arisen by gene mutation, recombination, and other mechanisms. Variation among individuals is further amplified because HLA genes are codominant; thus each person expresses alleles from both mom and dad at each A, B, and C locus. Thus a person produces six different class I MHC proteins. Because MHC class I proteins are found on all nucleated cells (i.e., only red blood cells lack them), they stimulate an immune response when cells from one host are introduced into another host with different class I molecules.

Class II MHC

Class II MHC molecules are produced only by certain immune cells, such as DCs, activated macrophages, mature B cells, and some innate lymphocyte cells (ILCs). As we will learn, class II molecules on DCs and B cells are key to initiating an adaptive immune response. Like class I MHC proteins, class II MHC molecules are transmembrane proteins consisting of two distinct chains. However, MHC class II molecules fold to form a three-dimensional antigen-binding pocket into which only a non-self-peptide fragment can be inserted for presentation to other cells of the immune system. MHC class II molecules also differ from MHC class I because they have a much deeper groove into which a longer peptide derived from a foreign substance can bind. The important differences between the two classes are therefore that MHC I molecules have smaller binding pockets capable of tethering self and non-self-peptides, while MHC II molecules bind only larger, non-self-peptides. Non-self-peptide antigen fragments in the MHC groove must be present to activate T cells, which in turn activate other immune cells

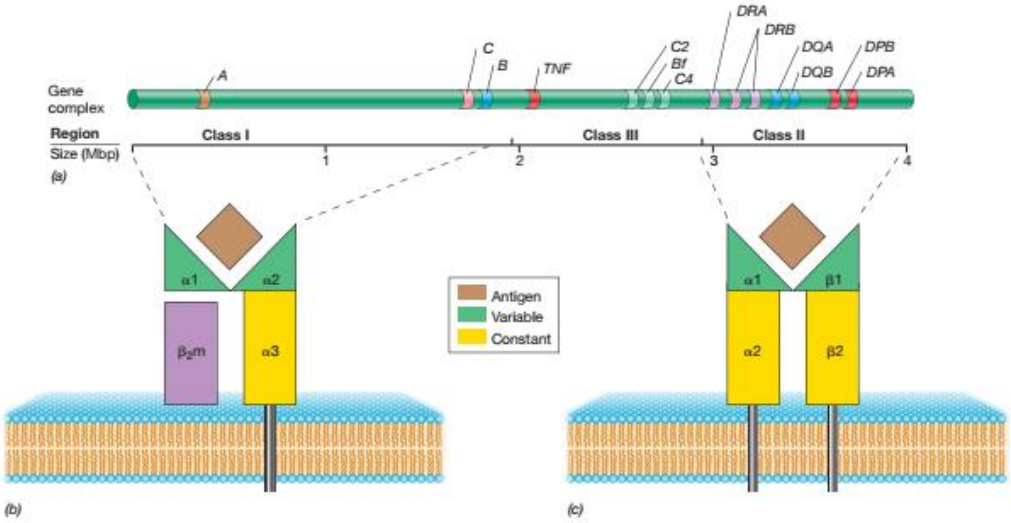


Figure: (a)The HLA complex, located on human chromosome 6, contains more than 4 megabase pairs (Mbp). Class II genes DPA and DPB encode class II proteins DPa and DPb; DQA and DQB encode DQa and DQb; DRA and two DRB loci encode DRa and DRb proteins. The class I MHC proteins HLA-A, HLA-C, and HLA-B are encoded by genes A, C, and B. The class II and class I loci are highly polymorphic and encode peptide-binding proteins. (b) Schematic of MHC class I protein. The α1 and α2 domains interact to form the peptide antigen–binding site. (c) Schematic of MHC class II protein. The α1 and β1 domains combine to form the peptide antigen–binding site. (Source: Brock's Biology)

13.5 Cytokines:

Cytokines are small molecules, secreted by cells in response to a stimulus. They may have an effect on the cell that produces them and are critical to signaling between cells, with each cytokine often inducing several different biological effects. Many different cells release cytokines, but each cell type releases only certain of these molecules. Cytokines may induce growth, differentiation, chemotaxis, activation, and/or enhanced cytotoxicity. Moreover, it is not uncommon for different cytokines to have similar activities and for many cytokines, some with opposing activities, to be released by a particular stimulus. Thus, the resulting biological effect is a factor of the sum of all of these activities.

To some extent cytokines can be grouped by the cell populations that secrete them. Monokines are cytokines secreted by cells of the myeloid series (monocytes, macrophages) and lymphokines are cytokines secreted primarily by lymphocytes, although some cytokines are produced by both lymphocytes and myeloid cells. The term interleukin (IL) is often used to describe cytokines produced by leukocytes, although some interleukins are also produced by other cell populations. A group of small heparin-binding cytokines, chemokines, direct cell migration, and may also activate cells in response to infectious agents or tissue damage. Interferons are produced by a variety of cells in response to viral infection.

Properties of Cytokines

- Cytokines are a group of low-molecular- weight regulatory proteins secreted by WBC and other cells in the body.
- Cytokine secretion is very specific and self- limited event as because they are not usually stored as performed molecules. Cytokine synthesis is initiated by new gene transcrip-tion as a consequence of cellular activation. Once synthesized, cytokines are rapidly secreted, resulting in a burst of release when needed.
- After secretion, cytokines (almost 60 different types of cytokines) regulate immune and inflammatory reactions
- Cytokines bind to specific receptors on the membrane of target cells, triggering signal- transduction pathways that ultimately alter gene expression in the target cells. The nature of the target cell for a particular cytokine is determined by the presence of specific membrane receptors. Cytokines are so specific due to their high affinity for which Pico molar concentrations of cytokines can mediate a biological effect.
- Cytokine actions may be local or systemic

i) Most of the cytokines act close to where they are produced, A particular cytokine when binds to receptors on the membrane of the same cell from where it has been secreted is called autocrine action.

(ii) When secreted cytokines bind to receptors on a target cell in close proximity to the producer cells, it is called paracrine action

(iii) In most of the cases, cytokines act on cells that are in contact with the cytokine producers but when cytokines are pro-duced in large amounts, it may enter the circulation and act at a distance from the site of production this is called endocrine action.

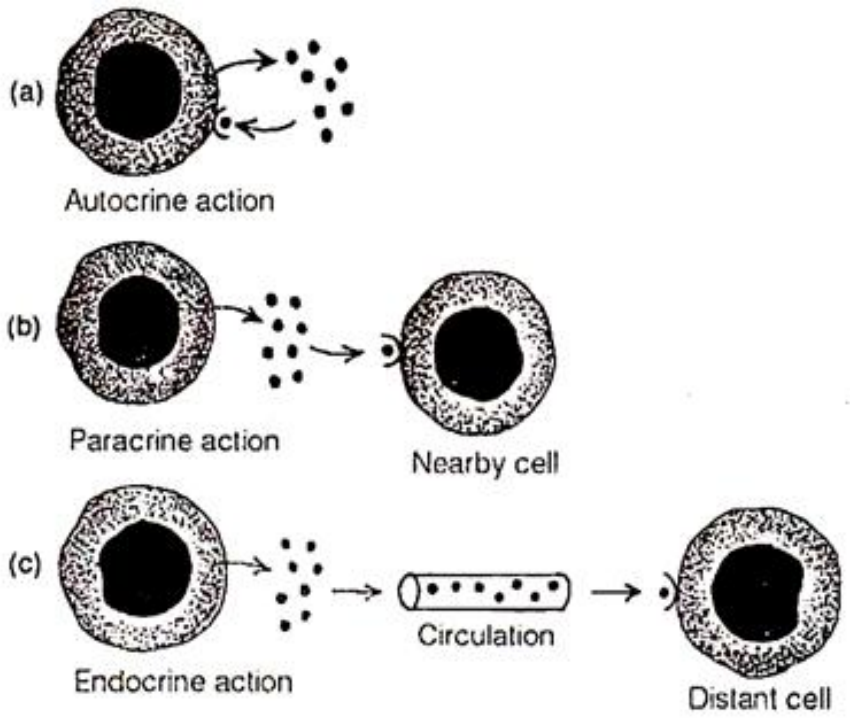


Figure: Most cyanobacteria exhibit autocrine and/or paracrine action; fewer exhibit endocrine actions

- Cytokines often influence the synthesis and actions of other cytokines and different immune cells. The ability of one cytokine to stimulate production of others, leads to cas-cade in which a second or third cytokine may mediate the biological effects of the first. Cytokines regulate the intensity and duration of the immune response by stimulating or inhibiting the activation, proliferation and differentiation of various cells.

- The cytokines secreted by a single lympho-cyte following antigen-specific activation can influence the activity of various cells involved in the immune response. For example, cytokines produced by activated TH cells (T-helper cells) can influence the activity of B-cells, Tc cells, natural killer cells (NK cells), macrophages, granulocytes and hematopoietic stem cells. This means that it activates an entire network of interacting cells.

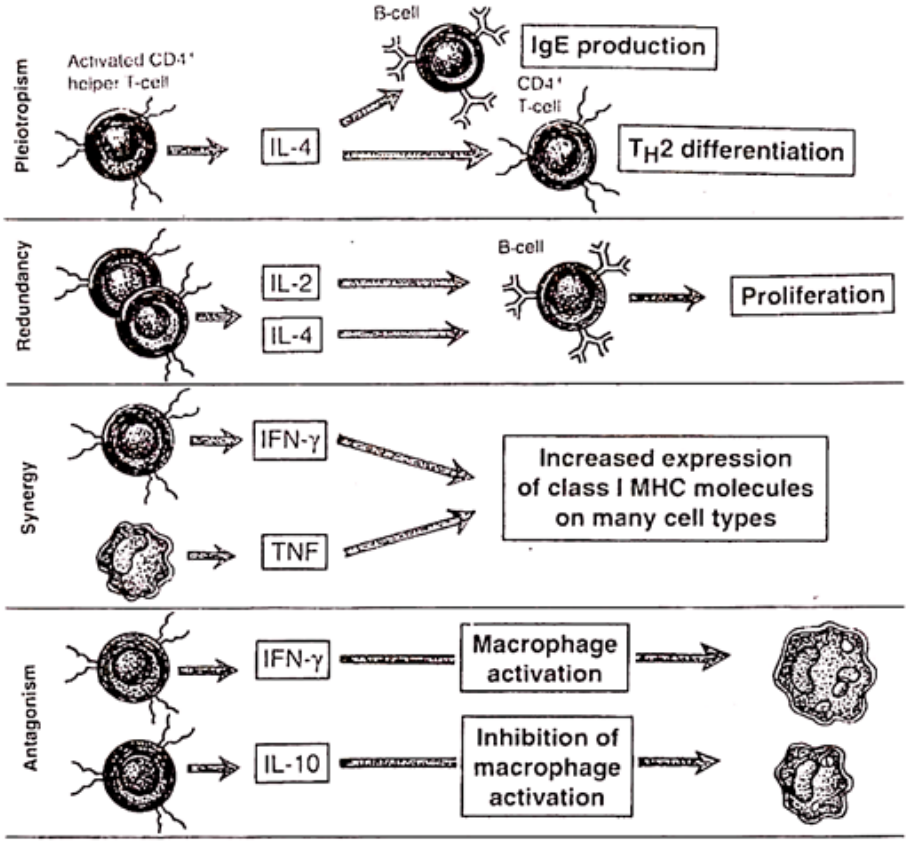


Figure: Properties of Cytokines

- Cytokines exhibit the attributes of pleiotropy, redundancy, synergy and antagonism.

  Pleiotropism refers to the ability of one cytokine to act on different cell types which action; fewer exhibit endocrine action allows a cytokine to mediate diverse biologi-cal effects.

  Redundancy refers to the property of multiple cytokines i.e. two or more cytokines having the same functional effects. This property of cytokine makes it difficult to explain a parti-cular activity to a single cytokine.

  Synergism exhibits to the phenomenon when the combined effect of two cytokines on cellu-lar activity is greater than the additive effects of the individual cytokines.

  Antagonism indicates the property that is just opposite to the synergism as in this case, the effects of one cytokine inhibit or offset the effects of another cytokine.

- Cytokine activity is also being regulated by external signals, the expression of cytokine receptors vary and also the responsiveness of cells to cytokines. As for e.g., stimulation of T or B-lymphocytes by antigens leads to increased expression of cytokine receptors.
- Many of the changes in gene expression induced by cytokines result in differentiation of T and B-lymphocytes and activation of effector cells such as macrophages.
- Besides activation, cellular responses to cyto-kines can also include feedback inhibitory signals to the cytokine activity. These mechanisms include cytokine induction of gene encoding inhibitors of the cytokine receptors. These inhibitors may inhibit the function of cytokine receptors expressed on the cell sur-face, molecules that block interactions of signaling kinases, phosphatases.

Interferons

Interferons (INF) are produced by human and animal cells in minute quantities mainly in response to viral infections, and help eliminate such infections. Interferon appears to be body's first line of defense against viral infection and is considered as nonspecific resistance factor because it does not exhibit specificity towards a particular virus.

That is, an interferon produced in response to one virus is also effective in eliminating the infection caused by another virus. Although interferon is nonspecific toward the viral pathogen it, is specific for the host, i.e., the interferon produced by human cells proves effective only in human cells and not in others.

All the interferons are proteins. The human alpha interferons (IFN-α) are proteins having 165 or 166 amino acids as they are produced by the cells. Some smaller forms of these interferons have been found in culture medium. B and large, all the alpha interferons are proteins that are not-glycosylated although some IFN-α species are glycoproteins. In contrast, IFN-β, IFN-ϒ and IFN-ω appear to be glycoproteins as produced from natural sources.

The interferons represent proteins with antiviral activity that are secreted from cells in response to a variety of stimuli. There are two types of interferons, Type I and Type II, and interferon-like cytokines. Type I interferons consist of seven classes—IFN-α, IFN-β, IFN-ε, IFN-κ, IFN-ω, IFN-δ, and IFN-τ. Type II interferon consists of IFN-ϒ only.

Table: Comparison between Type I and Type II interferon

	Type I (IFN-α/β)	Type II (IFNγ)
Chromosomal location	9	12
Origin	All nucleated cells, especially fibroblasts, macrophages and dendritic cells	NK cells and Th1, γδ and CD8 T cells
Induced by	Viruses, other cytokines, some intracellular bacteria and protozoans	Antigen-stimulated T cells
Functions	Antiviral, increases MHC class I expression, inhibits cell proliferation	Antiviral, increases MHC I and II expression, activates macrophages

Mechanism of action:

The mechanism of action of interferons is interesting in the sense that the interferons produced by host cells in response to viral attack have no direct effect on the viruses, rather they induce an antiviral state in neighbors healthy host cells that prevents viral replication in such cells. Double-stranded RNA viruses are the most potent inducers of interferon synthesis. When a virus attacks the host cell and releases its genome into the host cytoplasm, the viral genome interacts with the host genome and regulates the synthesis of interferon at the level of transcription. The interferons so synthesized in infected host cells are released and migrate to neighboring unaffected host cells. When interferons move to neighboring uninfected cells, they bind to specific receptor sites on the surface of these cells and stimulate them to produce certain antiviral proteins within them. It is considered that these antiviral proteins are translational inhibitory proteins and block the translation of mRNA molecules of the host cells and, therefore, the viral replication is ultimately blocked in such host cells. Interferons work in a complex manner and involve a series of molecular events to create antiviral state in the host cells. Interferons bound to specific receptor sites on the surface of uninfected host cells stimulate them to produce at least two enzymes: 2′, 5′-oligoadenylate synthetase and protein kinase.

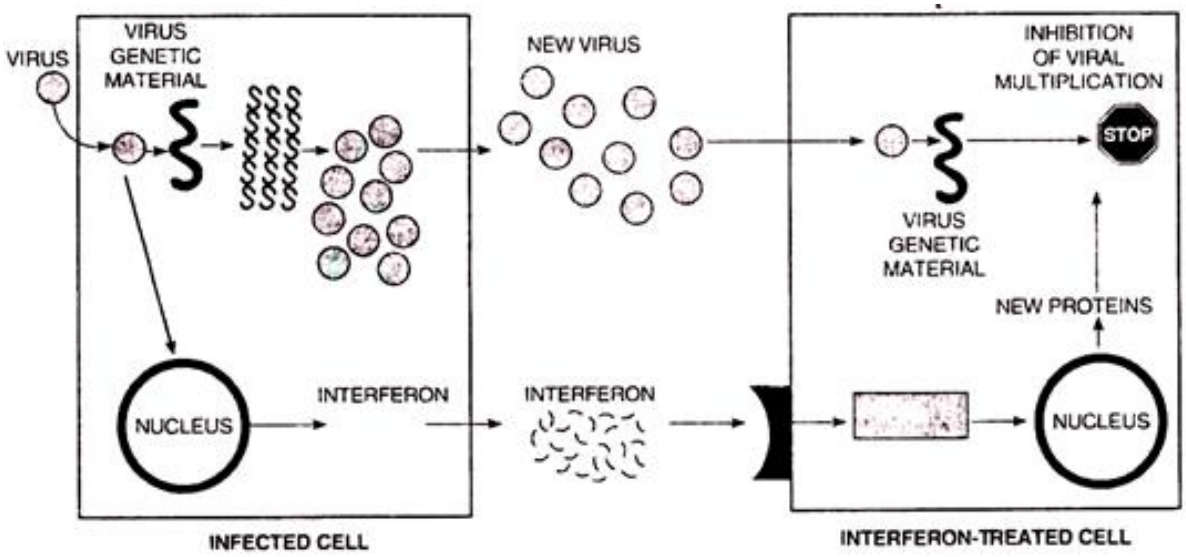


Figure: Diagrammatic representation of the Mechanism of action of interferons

The 2′ 5′- oligoadenylate synthetase catalyzes the synthesis of 2′, 5′-oligoadenyIate, an unusual polymer which activates mRNA endonuclease. The activated mRNA endonuclease cleaves and thereby inactivates the viral RNA- genome. The protein kinase, however, is activated only if the viral genome is double-stranded RNA. The activated protein kinase catalyzes the phosphorylation of a factor elF-2a.

The factor elF-2a is required tor the initiation of protein synthesis but it becomes inactive when phosphorylated. As a result of the phosphorylation of elF-2a factor, the protein synthesis is stopped and. therefore, the synthesis of viral proteins too ceases.

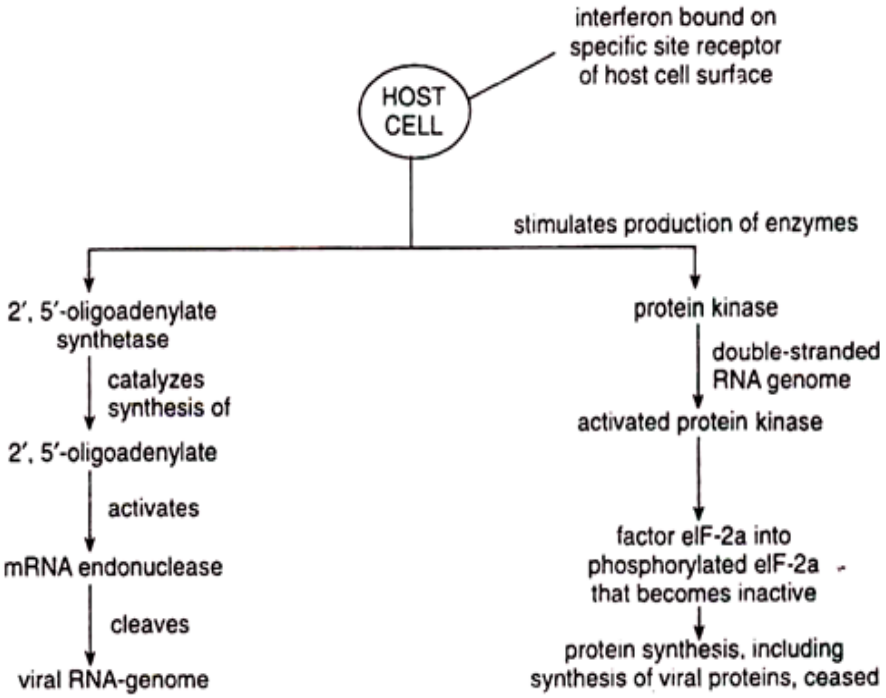


FIG. 11.16. Schematic representation of the details of the complexity of interferon action.

Figure: Schematic representation of the details of the complexity of interferon action

13.6 Antigen types and characteristics

Antigens are large molecules of proteins, present on the surface of the pathogen- such as bacteria, fungi viruses, and other foreign particles. When these harmful agents enter the body, it induces an immune response in the body for the production of antibodies.

The properties of antigens are as follows:

- The antigen should be a foreign substance to induce an immune response.
- The antigens have a molecular mass of 14,000 to 6,00,000 Da.
- They are mainly proteins and polysaccharides.
- The more chemically complex they are, the more immunogenic they will be.
- Antigens are species-specific.
- The age influences the immunogenicity. Very young and very old people exhibit very low immunogenicity.

There are some essential factors which influ-ence the power of antigen. Those are i. Molecular size, ii. Structural stability, iii. Degradability, iv. Foreign-ness, v. Chemical composition and heterogeneity, vi. Antigen processing and presentation, vii. Confor-mation and viii. Accessibility.

Types of Antigen

- **On the basis of origin**

There are different types of antigens on the basis of origin:

Exogenous Antigens

Exogenous antigens are those antigens which enter within the host body from their surroun-dings or external environments. These are basically of pollutants, microorganisms, pollens, drugs etc. Different infectious diseases, are caused by these type of introduced or foreign external agents are normally called communicable diseases, e.g., influenza virus, malarial protozoa etc.

Endogenous Antigens

Endogenous antigens are again classified under three sub-categories named as:

Xeno-genic or Heterogenic antigens

Xeno-genic antigens are those groups of foreign items which are related with tissue transplanta-tion and serology. Normally, these are called heterogenic antigens as they are related with phylogenetically unrelated species.

When a piece of tissue or graft is transplanted to an individual, it may be treated as foreign, then those molecules are considered as xeno-antigens. Similar foreign recognition may be resulted in serology. Cross-reactions are very common in between antisera to certain erythrocytic surface antigens or some bacterial antigens.

Antisera is formed against surface antigens. Produced anti-sera cross-react with cells or body fluids of animals belonging to different species due to presence of mucopolysaccharide and lipid based chemical determinants.

Allogenic antigens

Allogenic antigens are those antigens which are genetically determined, polymorphic in nature and help to differentiate one individual of one species from another individual belonging to the same species. When an individual (recipient) receives a blood transfusion or undergoes trans-plantation operation (like plastic surgery, kidney etc.)

These phenomena lead to incompatibility, agglutination and graft rejection. In case of human beings these types of anti-genic determinants are located on erythrocytes, leukocytes, platelets, cell surface markers, serum proteins and histocompatibility antigens.

Autologous antigens

This group of antigens is very rare and unnatural. In normal condition, self-components are non-immunogenic in nature, but in an abnor-mal condition self-body components are started to be considered as non-self or antigenic component.

- **On the Basis of Immune Response**

On the basis of the immune response, antigens can be classified as:

Immunogen

These may be proteins or polysaccharides and can generate an immune response on their own.

Hapten

These are non-protein, foreign substances that require a carrier molecule to induce an immune response.

- **Antigens can also be classified into two broad categories:**

1. Comparison of T-independent and
2. T-dependent antigens.

- **Comparison of T-independent and T-dependent antigens:**

	Properties	T-independent antigens	T-dependent antigens
1.	Chemical nature	Polymers (Polysaccharides), Glycolipids, Nucleic acid	Proteins
2.	Antibody response in:		
	• Athymic mice	+ve	–ve
	• T-cell depleted cultures	Chance of reduction	–ve
3.	Affinity maturation	No	Yes
4.	Isotype switching	No (usually)	Yes
5.	Secondary immune response (memory B-cells)	No	Yes
6.	Ability to induce delayed type hypersensitivity	No	Yes
7.	Type	2 types: Type I and Type II	No such types

13.7 Structure of Immunoglobulin:

Immunoglobulins are glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies. The immunoglobulins derive their name from the finding that they migrate with globular proteins when antibody-containing serum is placed in an electrical field.

Although different immunoglobulins can differ structurally, they all are built from the same basic units.

A. Heavy and Light Chains

All immunoglobulins have a four chain structure as their basic unit. They are composed of two identical light chains (23kD) and two identical heavy chains (50-70kD)

B. Disulfide bonds

1. **Inter-chain disulfide bonds** - The heavy and light chains and the two heavy chains are held together by inter-chain disulfide bonds and by non-covalent interactions The number of inter-chain disulfide bonds varies among different immunoglobulin molecules.

2. **Intra-chain disulfide binds** - Within each of the polypeptide chains there are also intra-chain disulfide bonds.

C. Variable (V) and Constant (C) Regions

When the amino acid sequences of many different heavy chains and light chains were compared, it became clear that both the heavy and light chain could be divided into two regions based on variability in the amino acid sequences. These are the:

1. Light Chain - VL (110 amino acids) and CL (110 amino acids)
2. Heavy Chain - VH (110 amino acids) and CH (330-440 amino acids)

D. Hinge Region

This is the region at which the arms of the antibody molecule forms a Y. It is called the hinge region because there is some flexibility in the molecule at this point.

E. Domains

Three dimensional images of the immunoglobulin molecule show that it is not straight. Rather, it is folded into globular regions each of which contains an intra-chain disulfide bond. These regions are called domains.

1. Light Chain Domains - VL and CL
2. Heavy Chain Domains - VH, CH1 - CH3 (or CH4)

F. Oligosaccharides

Carbohydrates are attached to the CH2 domain in most immunoglobulins. However, in some cases carbohydrates may also be attached at other locations.

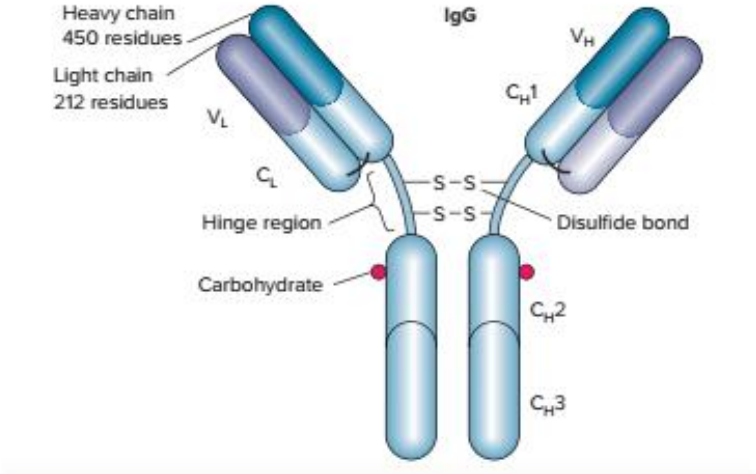


Figure: The basic structure of Human IgG

Structure of the variable region

A. Hypervariable (HVR) or complementarity determining regions (CDR)

Comparisons of the amino acid sequences of the variable regions of immunoglobulins show that most of the variability resides in three regions called the hypervariable regions or the complementarity determining regions. Antibodies with different specificities (i.e. different combining sites) have different complementarity determining regions while antibodies of the exact same specificity have identical complementarity determining regions (i.e. CDR is the antibody combining site). Complementarity determining regions are found in both the H and the L chains.

B. Framework regions

The regions between the complementarity determining regions in the variable region are called the framework regions. Based on similarities and differences in the framework regions the immunoglobulin heavy and light chain variable regions can be divided into groups and subgroups. These represent the products of different variable region genes.

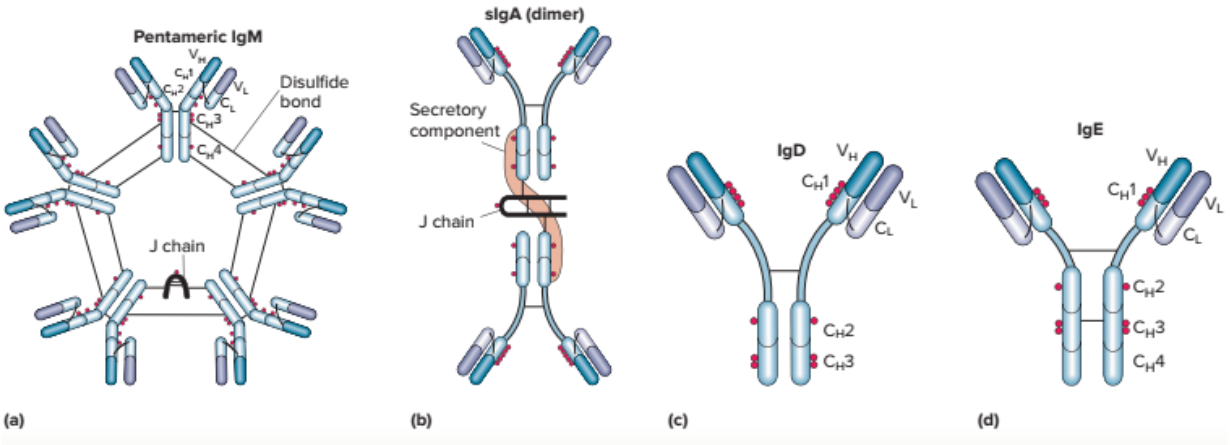


Figure: Immunoglobulins M, A, D, and E. (a) The pentameric structure of human IgM. The disulfide bonds linking peptide chains are shown in black. Note that 10 antigen-binding sites are present. (b) The dimeric structure of human secretory IgA. Notice the secretory component (tan) wound around the IgA dimer and attached to the constant domain of each IgA monomer. (c) The structure of human IgD showing the disulfide bonds that link protein chains (shown in black). (d) The structure of human IgE. Carbohydrate side chains are in red.

Human immunoglobulin classes, subclasses, types and subtypes

A. Immunoglobulin classes

The immunoglobulins can be divided into five different classes, based on differences in the amino acid sequences in the constant region of the heavy chains. All immunoglobulins within a given class will have very similar heavy chain constant regions. These differences can be detected by sequence studies or more commonly by serological means (i.e. by the use of antibodies directed to these differences).

1. IgG - Gamma heavy chains

2. IgM - Mu heavy chains

3. IgA - Alpha heavy chains

4. IgD - Delta heavy chains

5. IgE - Epsilon heavy chains

B. Immunoglobulin Subclasses

The classes of immunoglobulins can de divided into subclasses based on small differences in the amino acid sequences in the constant region of the heavy chains. All immunoglobulins within a subclass will have very similar heavy chain constant region amino acid sequences. Again these differences are most commonly detected by serological means.

1. IgG Subclasses

a) IgG1 - Gamma 1 heavy chains

b) IgG2 - Gamma 2 heavy chains

c) IgG3 - Gamma 3 heavy chains

d) IgG4 - Gamma 4 heavy chains

2. IgA Subclasses

a) IgA1 - Alpha 1 heavy chains

b) IgA2 - Alpha 2 heavy chains

C. Immunoglobulin Types

Immunoglobulins can also be classified by the type of light chain that they have. Light chain types are based on differences in the amino acid sequence in the constant region of the light chain. These differences are detected by serological means.

1. Kappa light chains

2. Lambda light chains

D. Immunoglobulin Subtypes

The light chains can also be divided into subtypes based on differences in the amino acid sequences in the constant region of the light chain.

1. Lambda subtypes

a) Lambda 1

b) Lambda 2

c) Lambda 3

d) Lambda 4

Function of Immunoglobulin:

A. Antigen binding

Immunoglobulins bind specifically to one or a few closely related antigens. Each immunoglobulin actually binds to a specific antigenic determinant. Antigen binding by antibodies is the primary function of antibodies and can result in protection of the host. The valency of antibody refers to the number of antigenic determinants that an individual antibody molecule can bind. The valency of all antibodies is at least two and in some instances more.

B. Effector Functions

Frequently the binding of an antibody to an antigen has no direct biological effect. Rather, the significant biological effects are a consequence of secondary "effector functions" of antibodies. The immunoglobulins mediate a variety of these effector functions. Usually the ability to carry out a particular effector function requires that the antibody bind to its antigen. Not every immunoglobulin will mediate all effector functions. Such effector functions include:

1. Fixation of complement - This result in lysis of cells and release of biologically active molecules

2. Binding to various cell types - Phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind immunoglobulins. This binding can activate the cells to perform some function. Some immunoglobulins also bind to receptors on placental trophoblasts, which results in transfer of the immunoglobulin across the placenta. As a result, the transferred maternal antibodies provide immunity to the fetus and newborn

Table: Physicochemical properties of human immunoglobulin classes

	IMMUNOGLOBULIN CLASSES				
Property	IgG[1]	IgM	IgA[2]	IgD	IgE
Heavy chain	γ_1	μ	α_1	δ	ε
Mean serum concentration (mg/ml)	9	1.5	3.0	0.03	0.00005
Percent of total serum antibody	80–85	5–10	5–15	<1	<1
Valency	2	5(10)	2(4)	2	2
Mass of entire molecule (kDa)[3]	146	970	160[3]	184	188
Placental transfer	+	–	–	–	–
Half-life in serum (days)[4]	23	5	6	3	2
Complement activation Classical pathway Alternative pathway	 ++ –	 +++ –	 – +	 – –	 – –
Induction of mast cell degranulation	–	–	–	–	+
% carbohydrate	3	7–10	7	12	11
Major characteristics	Most abundant Ig in body fluids; neutralizes toxins; opsonizes bacteria	First to appear after antigen stimulation; activates classical complement pathway; agglutinator; monomer functions as part of BCR complex	Secretory antibody; protects mucous membranes	Serves as part of BCR complex	Anaphylactic-mediating antibody; resistance to helminths

13.8 Cell mediated and Humoral immunity:

Cell mediated immunity

While humoral immunity protects the body from extracellular pathogenic agents and soluble antigens like toxins, cell-mediated immunity is directed against intracellular pathogens. The infected body cells are recognized by the immune cells and are destroyed.

Also, abnormal body cells, like cancer cells, are recognized as non-self due to the presence of tumour-specific antigens on their surface and are destroyed by the cell-mediated immune system. Similarly, transplanted cells and tissues become target of attack of this type of immunity.

Cell-mediated immunity is due to the activity of T-lymphocytes, just as humoral immunity is due to the activity of B-lymphocytes, though other cells of immune system are also involved.

(i) T-Lymphocytes (T-Cells):

T-cells, as also B-cells, originate from lymphopoietic stem cells. The precursors of T-cells proliferate, differentiate and are matured in thymus gland. Within the thymus gland, they are generally called thymocytes. In the thymus gland, the thymocytes proliferate in large numbers and are differentiated, but majority of them are eliminated by apoptosis.

Such selection is based on the ability of T-cells to bind to self-antigens. The surviving T-cells are those which bind weakly to the MHC proteins. These cells then migrate via blood stream to the secondary lymphoid organs, like lymph nodes and spleen. But before they leave the thymus, each T-cell acquires a specific acceptor for an antigenic determinant.

This is called the T-cell acceptor (TCR). Also, during differentiation in the thymus, T-cells acquire the CD4 or CD8 surface proteins which confer their functional behaviour (see Fig. 10.13). The T-cells come across the antigens for the first time in the secondary lymphoid tissues and clonal selection occurs, as it happens also in case of B-cells.

There are two main functional types of T-cells which are T-helper (Th) cells and cytotoxic T-cells (CTL). These two types are distinguished by possession of different surface proteins, viz. CD4 protein on TH-cells and CD8 protein on CTL. In addition to these two main types, there are two other types. These are delayed hypersensitivity T-cells (TD) which also possess CD8 protein, and suppressor T-cells (Ts) which possess CD4 protein.

The major function of cell-mediated immunity is to kill an infected body cell. This function is carried out by the CTL. On the other hand, the TH-cells help in activation of other cells. The function of Ts and TD cells are less well-known.

The CTLs bind with the help of CD8 protein to the infected body cells which express the antigenic determinants embedded in MHC Class I protein. A CTL recognizes the antigenic determinant with the help of its cognate TCR, while the CD8 binds to the MHC Class I protein. On the other hand, a TH-cell binds with its TCR to an antigen-presenting cell, like a macrophage or a dendritic cell or a B-cell which expresses the antigenic determinant embedded in MHC Class II protein. In this case, the CD4 protein of TH-cell binds to the MHC protein.

In both CTL and, TH-cell, recognition of the specific antigenic determinant is made by their cognate TCRs and the binding is stabilized by CD8 and CD4 proteins, respectively. In addition to CD8 and CD4, other surface proteins are involved in effective binding with their target cells.

(ii) T-Cell Receptor (TCR):

Just as each B-cell possesses a specific antigen-receptor, so also every T-cell — irrespective of its type — has a receptor, called a T-cell receptor (TCR) which can recognize and bind to a specific antigenic determinant. A TCR can recognize an antigenic determinant only when it is complexed with an MHC protein. In contrast; a B-cell, in which the antigen-receptor is an Ig molecule of one class or other, can recognize both cell-bound as well as free antigens. A TCR is a heterodimer

consisting of two polypeptide chains, α and β, having molecular weights of 50 and 39 kilo Daltons, respectively. The two chains are joined to each other by disulphide bonds and both are anchored into the membrane of the T-cell. Each chain has a variable domain and a constant domain. The variable domains of the α and β chain constitute the antigen-binding site TCR. Thus, it is seen that TCRs resemble the Ig molecules in many respects and actually they belong to the immunoglobulin super-family. At the same time, TCRs differ in several respects from Igs.

For example, TCRs are always anchored to T-cell membrane, and they are never found free in the plasma or body fluids as Igs are. Again, TCRs can interact only with antigens complexed with MHC proteins of target cells, whereas Ig molecules can react with both cell-bound or free antigens.

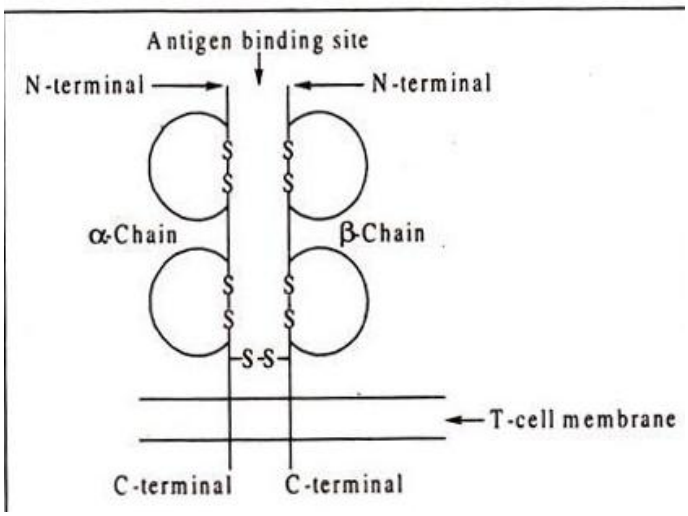


Figure: Structure of T-Cell Receptor

(iii) T-Cell Diversity:

As one particular T-cell can recognize only one specific antigenic determinant, the total T-cell population necessarily represents an enormously large number of diverse clones of these cells. T-cell diversity is due to the T-cell receptor. The basic mechanism of creating this diversity is similar to that which causes antibody diversity. Just like the H- and L-chains of Ig molecules, the α- and β-chains of TCR contain different domains.

Each of these domains is coded by multiple DNA segments in the germ-lines. About one million different β-chains and 25 different α-chains are present, yielding about 25 x 106 different TCRs. The α-chains are made of V, J and C domains, and P-chains possess V, D, J and C domains. The genes coding these domains of polypeptides are located in human chromosome 7 (for α-chains) and chromosome 14 (for β-chains).

As in case of immunoglobulin's, gene rearrangement by random selection of DNA segments of one V-segment, one J-segment and the C-segment leads to formation of the gene for α-chain. In case of β-chain, there is one extra domain D coded by two DNA segments, D1 and D2. It can be seen that the genes controlling the β-chain are scattered (unlinked).

Rearrangements of TCR genes starts soon after the precursors (lymphopoietic stem cells) enter into the thymus gland. Diversity arises primarily through random somatic recombination of the V, J, D and C segments of DNA in case of the β-chains, and V, J and C segments in case of α-chains. But further diversity is also added by imprecise recombination (junctional diversity), as it has been explained in case of antibody diversity.

After TCR polypeptides have been synthesized, they are expressed as TCR on the surface of the thymocytes. Those T-cells which bind strongly to the MHC proteins (self-antigens) are eliminated by programmed cell-death (apoptosis). The surviving T-cells (less than 5% of the total thymocytes produced from stem-cells) which bind weakly to self-antigens then come out from thymus as immuno-competent cells.

(iv) T-Cell Activation:

Cell-mediated immunity, also known as cellular immunity, provides protection against intracellular pathogens, mostly viruses, but also some bacteria and fungi which are able to grow within infected body cells. The immuno-potent T-cells destroy such infected cells, thereby making the pathogens open to attack by the antibodies.

The key-role in killing the infected body cells is played by the cytotoxic T-cells (CTLs) but for doing it, CTLs need to be activated. In this process of activation, TH cells are actively involved. On the other hand, TH-cells themselves have to be activated for performing its role in activation of other cells, including CTLs.

The role of TH-cells in activation of B-cells, as also it has been seen how TH-cells are themselves activated before they interact with B-cells (see Fig. 10.28 and Fig. 10.29). Let us now examine how activation of CTLs occurs with the help of TH-cells.

A pathogen entering into the body is first challenged by the phagocytic cells of innate defence system. These cells, like macrophages and dendritic cells ingest the pathogen and digest them with their hydrolytic enzymes. The resulting peptides derived from the proteins of the infecting microbe act as antigenic determinants and these are embedded in the matrix of the MHC Class I proteins and expressed on the surface of the phagocytes.

The phagocytes are now antigen-presenting cells (APC) and they migrate to the lymphoid tissues in search of appropriate CTLs with TCRs which match with the antigenic determinants present on their (APCs) surface. The CTLs are still in an inactive state. A CTL now recognizes the specific antigenic determinant present on an APC with the help of its TCR and binds to it. Such recognition is also helped by the CD8 protein which binds to MHC Class I protein of the APC. The binding of a CTL with an APC is the first step in activation of CTL.

The APCs with antigenic determinants on MHC Class II proteins can recognize and interact with TH-cells carrying cognate TCRs. The interaction between an APC and a TH cell leads to their binding between the TCR and the antigenic determinant on one hand, and the CD4 protein of TH-cell and MHC protein of the APC on the other. This leads to stimulation of both the TH-cell and the APC and they produce a series of cytokines. The cytokine, interleukin-1, produced by the APC activates the TH– cell and the activated TH-cell produces interleukin-2 which induces the CTLs to proliferate and become killer cells.

The activated CTLs are now capable of attacking infected body cells. Such infected cells are recognized by the CTLs with the help of TCR which bind to the antigenic determinants expressed on the surface of body cells embedded in MHC Class I proteins. The CD8 protein of CTL binds to the MHC Class I protein of the infected body cells. The activation of TH-cells and CTLs is diagrammatically shown Fig. 10.43.

(v) Role of Cytotoxic T-Cells:

The cytotoxic T-cells play the key role in cell-mediated immunity. After activation, the CTLs become killer cells capable of destroying infected body cells, as well as malignant cells. These killer CTLs come out from the lymphoid tissues and enter into blood and lymph. While circulating they encounter the target cells and destroy them by the so-called ‘lethal hit’.

The activated CTLs have large granules in their cytoplasm. These contain the proteolytic enzymes, called granzymes, as also a toxic protein called perforin. The target cells which are mostly body cells infected by intracellular pathogens like viruses, some bacteria and occasionally some fungi, display the pathogen’s antigenic determinants on their surface complexed with MHC Class I proteins.

When a circulating killer CTL encounters an infected body cell, it recognizes the antigenic determinant and binds to it with its TCR and CD8 protein in a manner similar to its interaction with an APC. After a contact with an infected cell, the granules of the CTL migrate towards the point of

contact and they release perforin molecules which polymerize to form a hole in the membrane of the target cell.

Through these pores, the granzymes enter into the cytoplasm of the target cell causing destruction of their contents. The target cell eventually undergoes lysis. This is called a 'lethal hit'. The CTL can again synthesise the cytoplasmic granules and can attack another target cell.

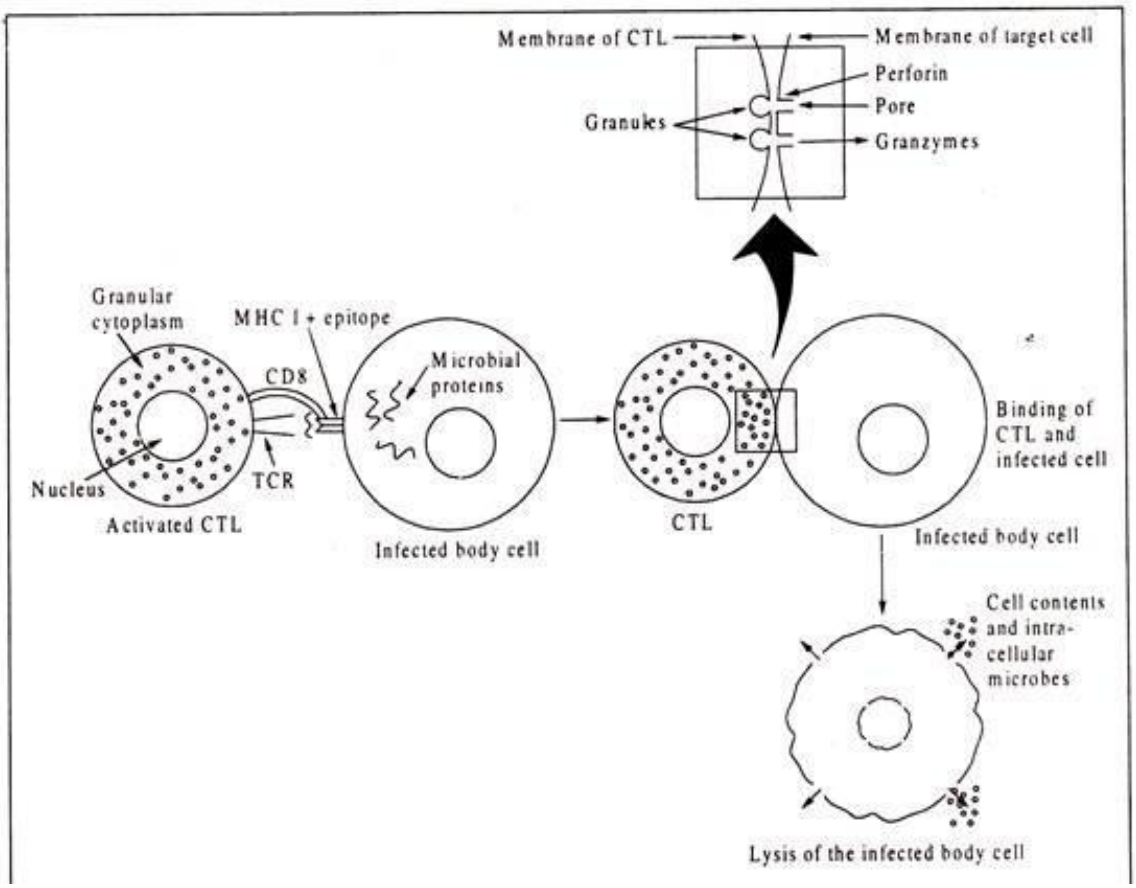


Figure: Mechanism of lethal hit by a killer CTL on an infected body cell

In addition to infected body cells, activated CTLs can also inflict a lethal hit to cancer cells. These cells, like infected body cells, express specific tumour antigens complexed with MHC Class I on their surface and thus become target of CTL activity.

The circulating CTLs keep a strict watch on the appearance of such tumour cells and eliminate them promptly causing lysis. This is known as immune surveillance and it constitutes a very important phenomenon in keeping the body free of cancer cells which arise by transformation of normal body cells.

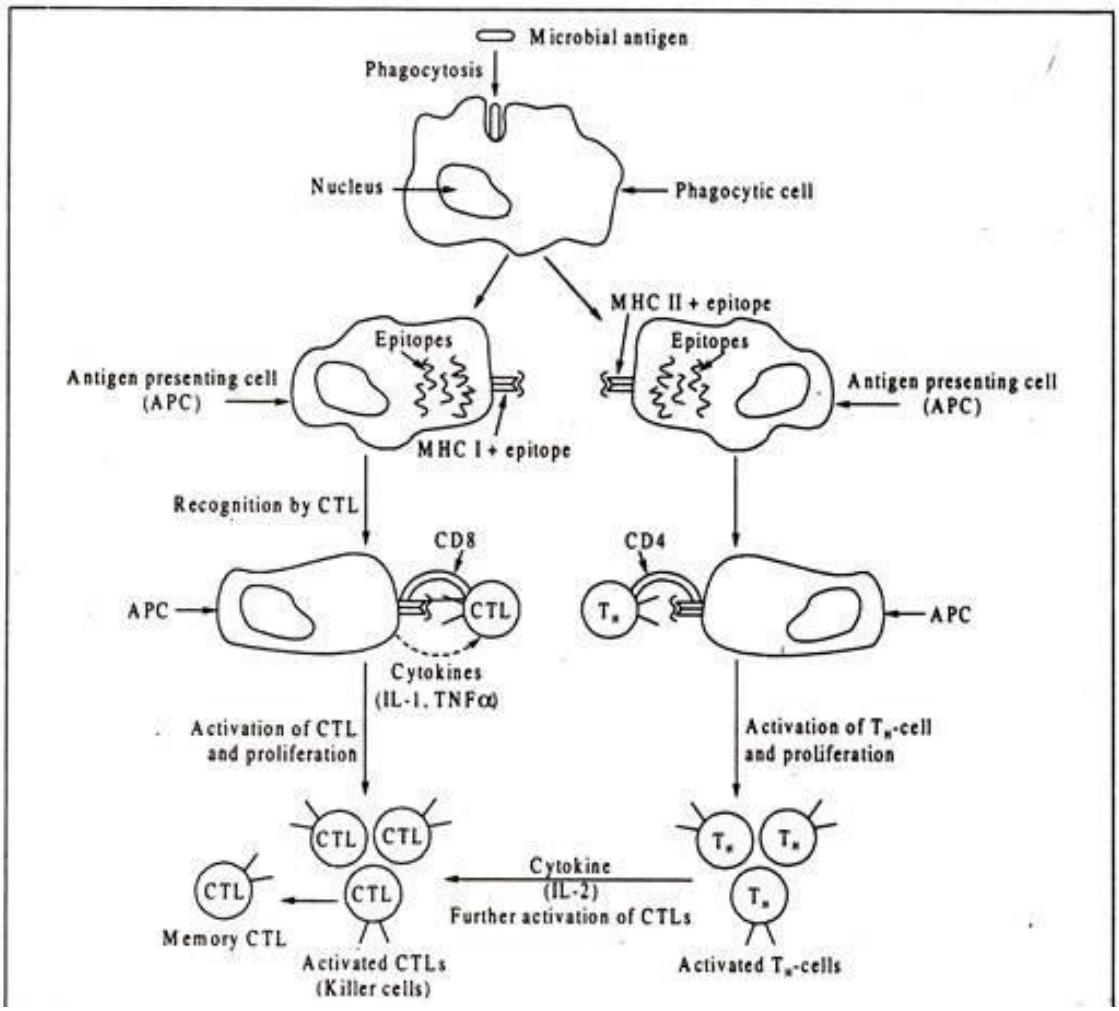


Figure: Activation of T- cells

Another important aspect of T-cell response is the production of a clone of memory T-cells. Like memory B-cells, these cells remain dispersed throughout the body and search for the antigen which induced the initial T-cell response. The memory cells immediately pounce upon the antigen and destroy it. During this second encounter, the memory T-cells proliferates rapidly to induce a stronger secondary T-cell response.

In addition to direct killing of infected body cells and tumour cells, cytotoxic T-cells also produce different cytokines which are involved in defence against foreign invaders. Among these is a protein, called macrophage activating factor, which attracts macrophages to the site of infection and activates them. Another protein produced by the T-cells is the migration inhibitor which prevents macrophages to leave the infection site, so that the macrophages remain restricted to the site of infection.

The main function of the CTLs is on intracellular pathogens which are not attacked by the antibodies produced in humoral immunity. Through lethal hits resulting in lysis, such intracellular pathogen are exposed to the action of antibodies and they are eliminated through phagocytosis and neutralization. Thus, the cell-mediated immunity and humoral immunity work together to protect the body.

Antibody mediated or Humoral immunity

The antibody-mediated or humoral immunity is that where the B-lymphocytes synthesize antibodies response to the detection of antigens and these antibodies counteract with those antigens. Antibody-mediated immunity is often referred to as humoral immunity because the antibody molecules flow extracellularly through the blood and other vital body fluids which are called humours in Greek.

The precursors of B-lymphocytes, which originate from the stem cells of bone marrow, processed within thymus-independent tissues of the lymphatic system (the spleen, tonsils, intestine, appendix, and lymph nodes) and become immunologically competent. In response to antigenic stimulation, the immunologically competent B-lymphocytes convert into two different cell populations primary B-lymphocytes and secondary B- lymphocytes.

The primary B-lymphocytes show response to the first antigenic stimulation and immediately enter into the process of their conversion into plasma cells. In contrast, the secondary B-lymphocytes do not show any response to the first antigenic stimulation and constitute memory cells which transform into plasma cells in response to subsequent exposure to antigens, perhaps years later. However, these are the plasma cells which secrete antibodies.

Once thought to be totally independent system, the conversion of immunologically competent B-lymphocytes into antibody producing plasma cells is cooperated by helper T-lymphocytes (helper T-cells). An antigen with at-least two different antigenic determinants first attaches on the surface of a macrophage cell which then migrates to lymphatic tissues and interact with helper T-lymphocyte.

Once the helper T-lymphocyte binds with the antigen present on the surface of microphage cell, it releases interleukin-2 that stimulates multiplication of helper T-lymphocytes and also the release of B-lymphocyte growth factor, which in turn enhances the division of immunologically competent B-lymphocytes and their conversion into plasma cells.

However, the antibodies secreted by plasma cells clump together (agglutinate) with antigens present in body's circulatory system forming antibody-antigen-complexes which are up-taken by scavenger white blood cells.

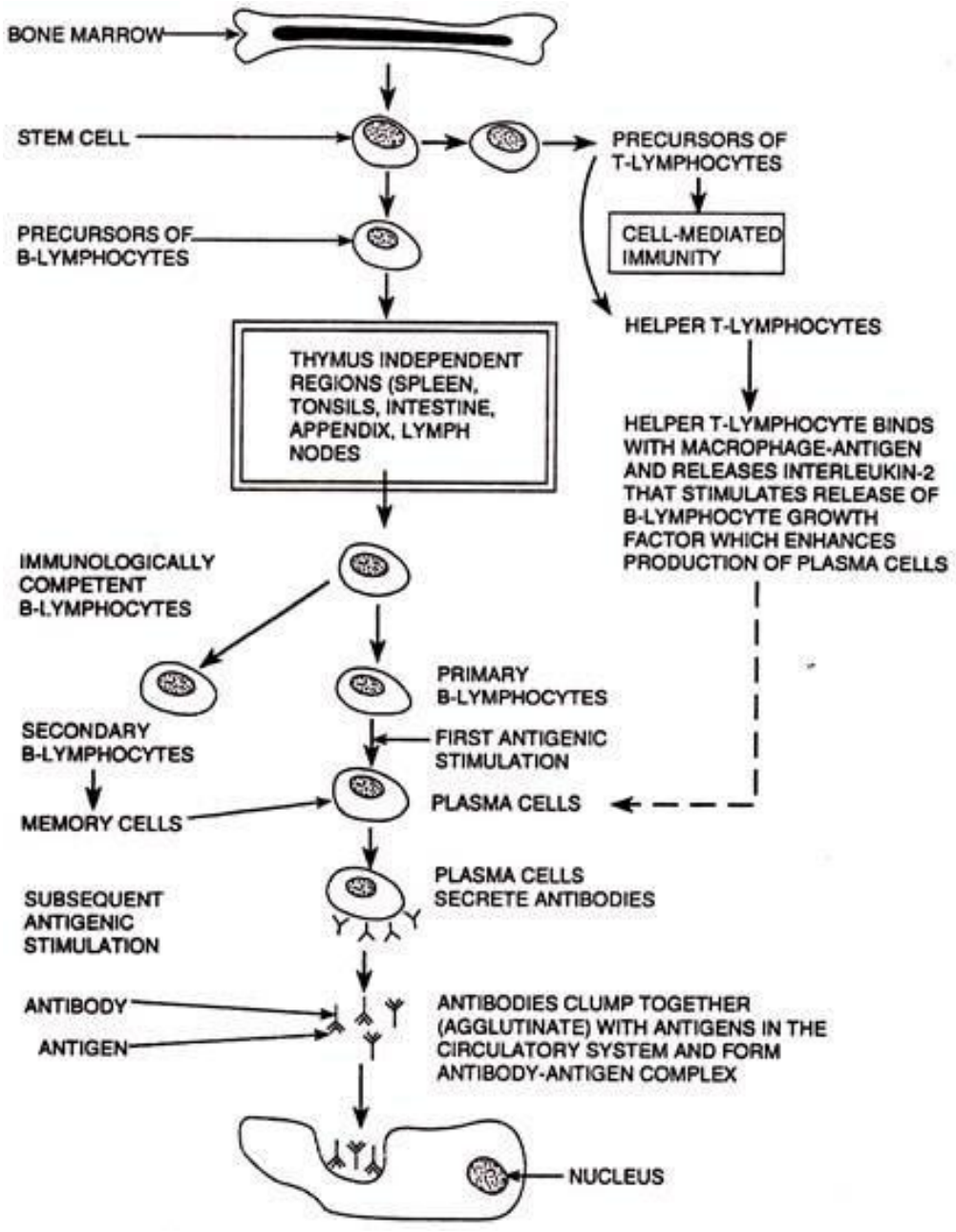


Figure: Diagrammatic representation of antibody mediated immunity

13.9 Antigen-Antibody reactions

Antigen (Ag) antibody (Ab) reactions occur when an antigen combines with a corresponding antibody to produce an immune complex. Therefore, an antigen-antibody reaction is thus a bimolecular association which is similar to an enzyme-substrate interaction but the only difference is that antigen-antibody reaction does not lead to an irreversible chemical interaction.

The basis for antigen-antibody reactions are the non-covalent interactions like hydrogen bonds, ionic bonds, van der Waal interactions, hydrophobic interactions, etc. These interactions are individually weak, therefore, a large number of such interactions work together in an antigen-antibody reaction. The in vitro study of antigen antibody reactions is known as serology.

The principle for all diagnostic immunological tests is serological reactions. The binding of an antibody with an antigen of the type that stimulated the formation of the antibody, results in agglutination, precipitation, complement fixation, greater susceptibility to ingestion and destruction by phagocytes, or neutralization of an exotoxin.

The main use of antigen-antibody reactions is in the determination of blood groups for transfusion, serological ascertainment of exposure to infectious agents, and development of immunoassays for the quantification of various substances.

Schematically an Antigen-Antibody Reaction can be represented as:

Ag + Ab [Ag-Ab] → Aggregation → Precipitation/Agglutination/Neutralization

For diagnostic immunological tests, the serological tests must possess high specificity and sensitivity. Specificity is the ability of an antibody to recognize a single specific antigen. There is a high degree of specificity in antigen-antibody reactions.

Antibodies can distinguish differences in:

i. Primary structure of an antigen,

ii. Isomeric forms of an antigen, and

iii. Secondary and tertiary structure of an antigen.

Therefore specificity implies that:

a. Antibody is specific for a single and specific antigen.

b. Antibody wills not cross-react with other antigens.

c. It will not give false positive results

Sensitivity means the lowest amount of antigen that can be detected. If in a diagnostic test an antibody is capable of detecting a single antigen molecule, then such a test possesses highest sensitivity. The amount of antigen detected in a test is directly proportional to the amount of antibody used. Enzyme Linked Immuno Sorbent Assays (ELISA) is the most sensitive serological tests.

Agglutination	Antigenic particle + specific Ab results in aggregation of particles
Precipitation	Soluble Ag + specific Ab results in lattice formation and precipitation
C activation	Ag in solution or on particle + specific Ab results in activation of C
Cytolysis	Cell + anti-cell Ab + C may result in lysis of the cell
Opsonization	Antigenic particle + Ab + C enhances phagocytosis by Mo, MØ, PMNs
Neutralization	Toxins, viruses, enzymes, etc. + specific Abs may result in their inactivation

13.11 Immunological techniques

A variety of other assays have been developed which provide specific qualitative and quantitative measurement of Ag or Ab for both research and diagnostic purposes. The presence of Ab to a particular Ag in the serum of a patient can be determined using very sensitive radioimmunoassay (RIA) or enzyme-linked immunosorbent assays (ELISA). Such assays are of particular value in demonstrating Ab to Ags of infectious agents, e.g. virus, bacteria, etc. The presence of an Ab of a particular isotype can also be determined using a modification of these assays. The radioallergosorbent test (RAST) uses as detecting ligand a radiolabeled Ab to human IgE and permits the measurement of specific IgE Ab to an allergen. ELISA and RIA also provide very specific and sensitive measurement of toxins, drugs, hormones, pesticides, etc., not only in serum, but also in water, foods and other consumer products. Based on these procedures, assays for nearly any Ag or Ab can be readily developed.

RIA

RIA is a highly sensitive method of estimation of antigens or haptens. The principle of RIA is a competition between a radioactive antigen or a hapten (a ligand) and a non-radioactive counter-part of the same antigen or hapten for binding to the sites of the cognate antibody molecules. The antigen is generally labelled with a gamma- emitting isotope as for e.g.125I.

Technique of RIA is of two types:

(i) Direct RIA

When antigens or primary antibodies are directly labelled with a radionuclide, it forms the basis of Direct RIA. This technique utilizes radio-labelled antibody or its ligand (antigen). Antibody is incubated with ligand and unbound reactants are removed from the system (phase separation).It may utilize precipitation of bound reactants (quantitative precipitin reaction), particulate antigens (such as bacteria), the immobilization of the nonradioactive reactant onto a solid matrix (such as plastic), and so on.

(ii) Indirect RIA

Anti-immunoglobulin antibody (secondary antibody) is radio-labelled and used in the indi-rect RIA. This technique uses radiolabeled secondary antibody (anti-immunoglobulin) to detect the binding of a primary antibody**.**

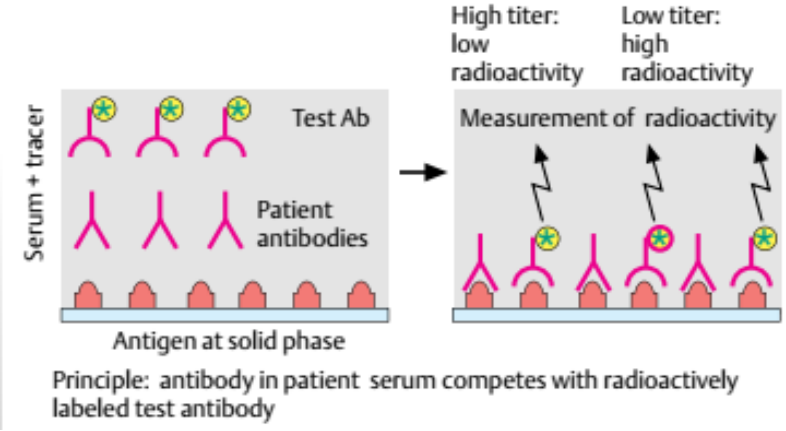


Figure: Indirect RIA

Enzyme-Linked Immunosorbent Assay (ELISA):

The most important of the immuno-enzyme assays are the Enzyme-linked immunosorbent assays, commonly called ELISA. It is a type of solid-phase enzyme immunobinding assay. In ELISA antigen is linked to a solid phase anchored antibody in such a way that retains both immunological and enzymatic activity. The solid phase may be of polystyrene/polyvinylchloride.

So ELISA can be called as a qualitative or quanti-tative assay for antibodies i.e., an assay for quantitating either antibody or antigen by use of an enzyme-linked antibody and a substrate that forms a coloured reaction product.

A number of enzymes are usually used for ELISA, such as, alkaline phosphatase, horseradish peroxidase and β-galactosidase. Besides enzymes, some auto-antibodies are also detected by enzyme-linked immunosorbent assay (ELISA).

The basic principle of ELISA depends on the presence of enzyme. An enzyme conjugated to an antibody reacts with a colorless substrate to gene-rate a coloured reaction product. ELISA is similar to RIA in principle, depends on an enzyme but avoiding the involvement of radioactive label.

A number of variations of ELISA have been developed. Each type of ELISA can be used quali-tatively to detect the presence of antigen/antibody.

I. Indirect ELISA

Antibody can be detected or quantitated with an indirect ELISA.

1. Serum or some other sample contains pri-mary antibody (Ab1) is added to an antigen- coated microtiter well and allowed to react with the bound antigen.

2. After reactions, free Ab1 is washed away and antigen bound antibodies are present within the microtiter well.

3. Addition of enzyme conjugated secondary antibody (Ab2) to the microtiter well which will bind with the primary antibody.

4. After sometime, a wash is done to remove excess Ab2 from the set up.

5. Now, specific substrate for specific antibody is added and as a result, a coloured reaction product is formed.

6. The coloured reaction product is measured by specialized spectro-photometric plate reader

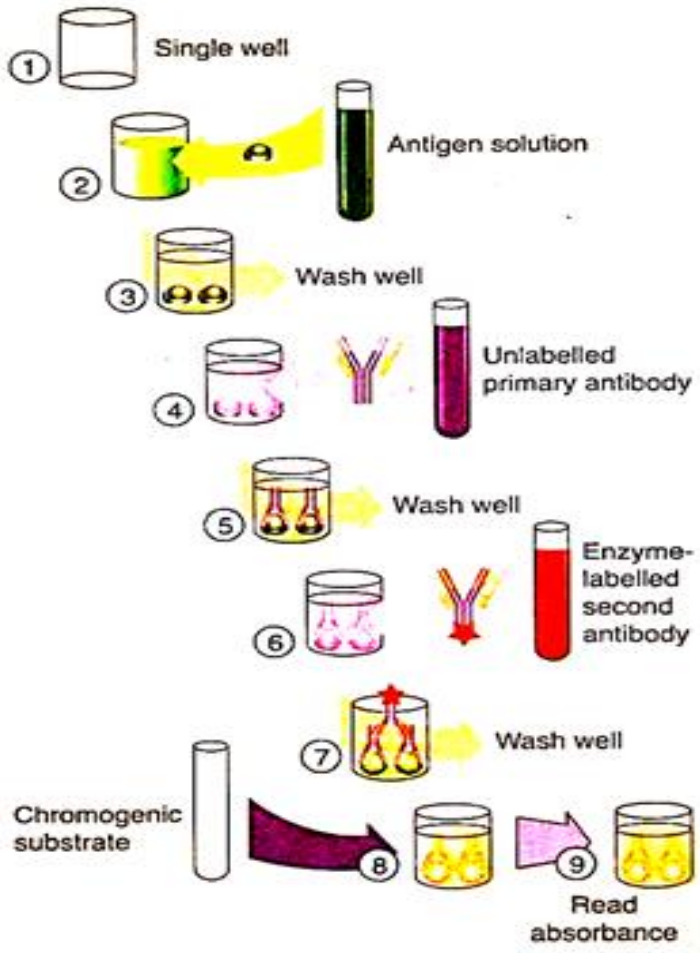


Figure: Steps involved in indirect ELISA method

II. Sandwich ELISA

Antibody can be detected or quantitated by a sandwich ELISA.

1. The antibody (Ab1) is placed (immobilized) on a microtiter well.

2. A sample contains antigen is added and allowed to react with the bound antibody (Ab1).

3. A wash is taken to remove excess free anti-body from the well.

4. After that, a second antibody (Ab2) specifi-cally bound with enzyme is added which binds with different epitope present on the bound antigen.

5. A second wash is needed to remove free second antibody.

6. Again a specific substrate is added and the coloured reaction product is measured

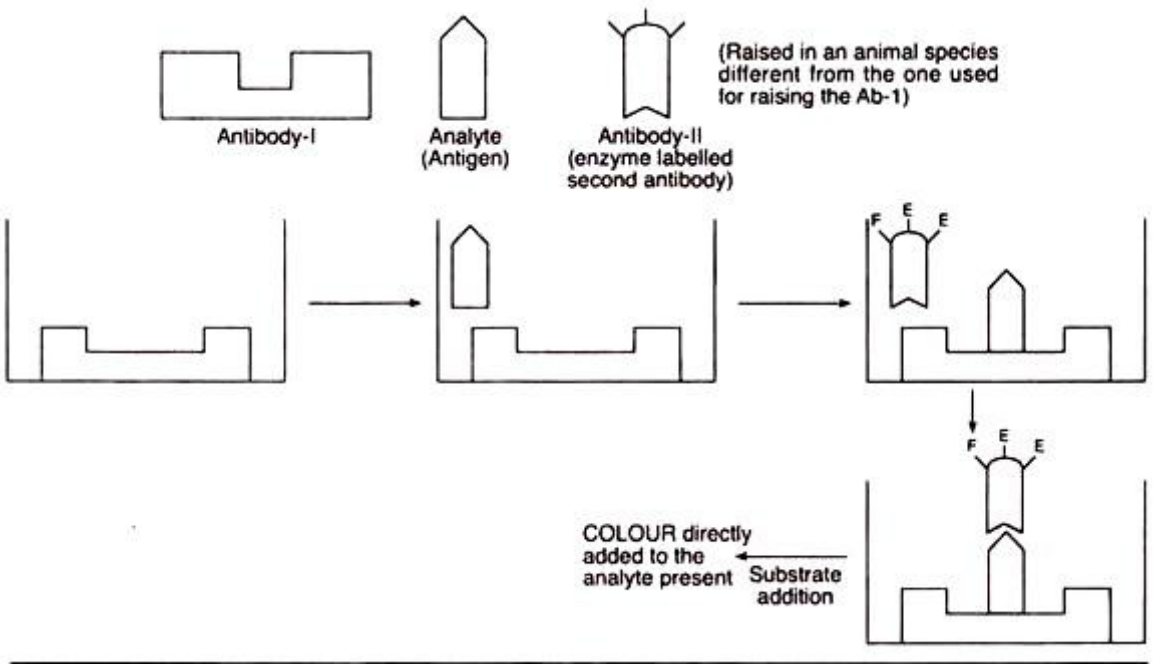


Figure: ELISA sandwich method for antigen outline

III. Competitive ELISA

Another variation for measuring amounts of antigen is Competitive ELISA.

1. In this technique antibody is first incubated in solution with a sample containing antigen.

2. The antigen-antibody mixture which is thus formed, is then added to an antigen-coated microtiter well.

3. The more antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well.

4. An enzyme conjugated secondary antibody (Ab2) specific for the iso-type of the primary antibody is added.

5. Now, primary antibody is used to quantitate the amount of primary antibody bound to the well.

6. Colour changes are being monitored. More the colour is observed less will be the analyte in the test specimen, i.e., in the competitive assay, the higher will be the concentration of antigen in the original sample, indicates the lower absorbance

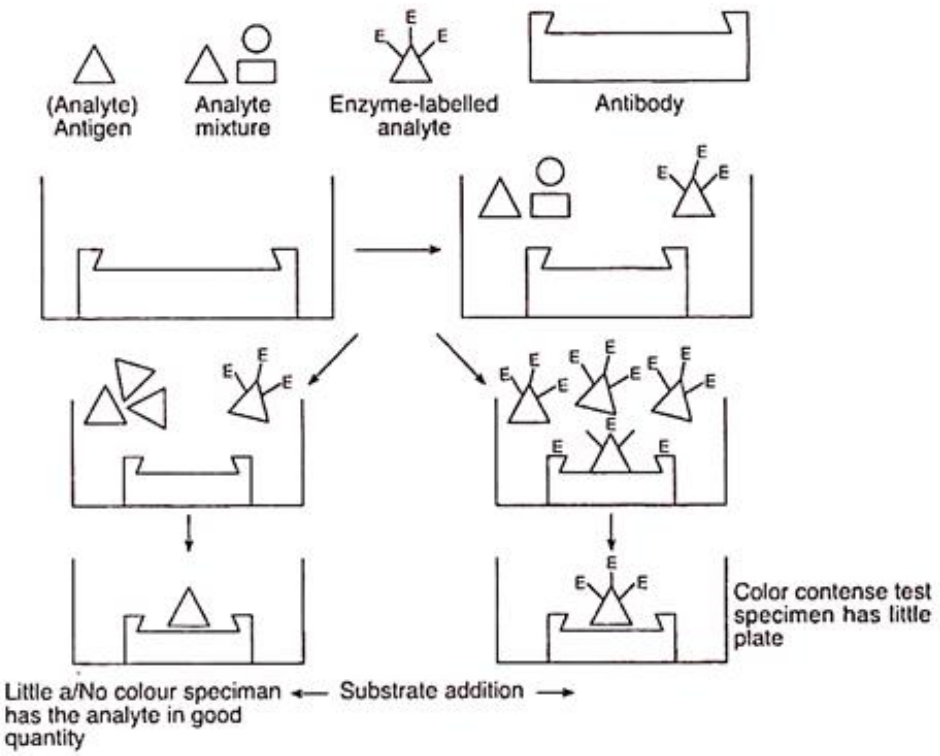


Figure: ELISA competitive method for antigen outline

Immunoblotting:

Another immunologic technique used in the microbiology laboratory is immunoblotting, also known as Western blotting. Western blotting involves polyacrylamide gel electrophoresis to separate the proteins in a patient specimen. The proteins are then transferred to sheets of filter paper (nitrocellulose or polyvinyl difluoride). This immobilizes the proteins on a sturdy substrate. Protein bands are then visualized by treating the filter papers with solutions of enzyme-tagged antibodies. This procedure was considered more specific than ELISA and lateral flow assays for many applications. However, the use of monoclonal antibodies in these other assays for antigen capture or antibody detection, along with other technological advances, has reduced the need for Western blotting, a more complex and time-consuming process. Western blotting is still used to confirm certain infections, such as Lyme disease.

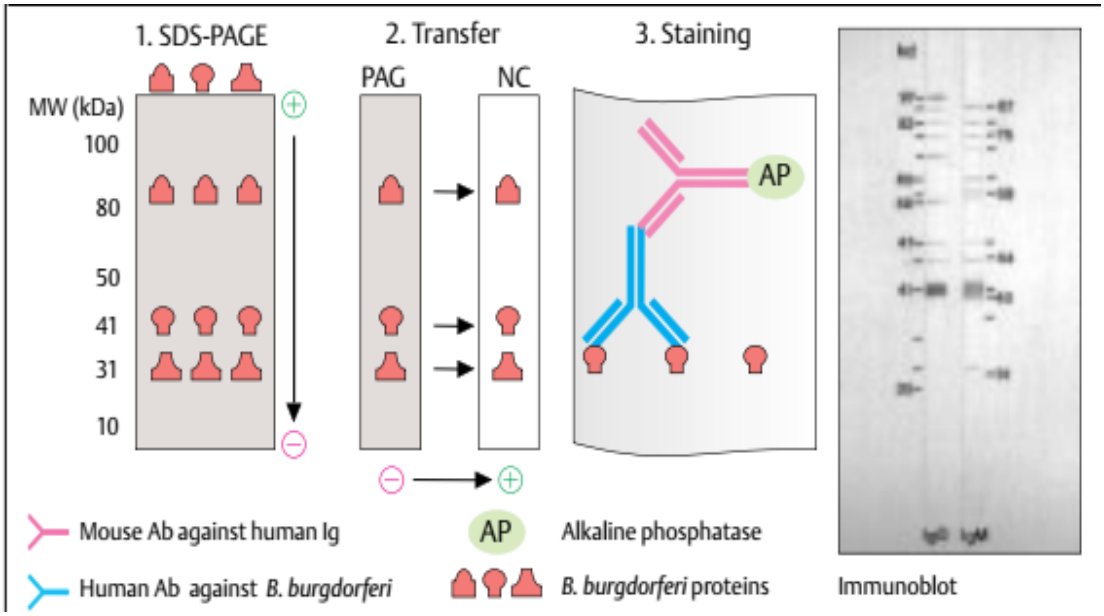


Figure: Steps involved in immunobloting

Immunoprecipitation:

The immunoprecipitation technique detects antibodies that when bound to antigens are called precipitins. The precipitin reaction occurs when antibodies and antigens are mixed in the proper proportions. The antibodies link the antigen to form a large antibody-antigen network or lattice that settles out of solution when it becomes sufficiently large. Immunoprecipitation reactions occur at what is known as the zone of equivalence. This describes conditions within the assay tube or plate when there is an optimal ratio of antigen to antibody so that almost all antigen is bound by antibody and an insoluble lattice forms. If the precipitin reaction takes place in a test tube a precipitation ring forms in the area in which the optimal ratio or equivalence zone develops.

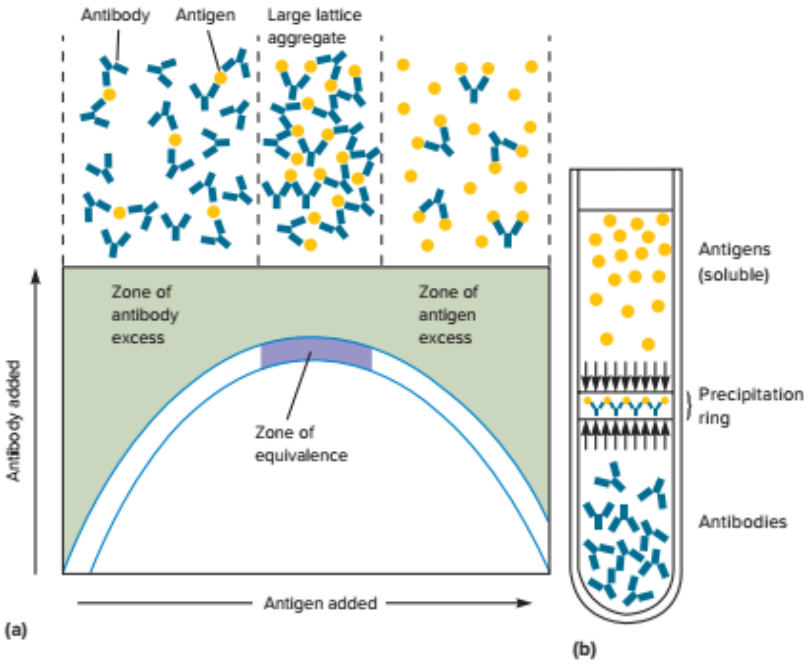


Figure: Immunoprecipitation. (a) Graph showing that a precipitation curve is based on the ratio of antigen to antibody. The zone of equivalence represents the optimal ratio for precipitation. (b) A precipitation ring test. Antibodies and antigens diffuse toward each other in a test tube. A precipitation ring is formed at the zone of equivalence.

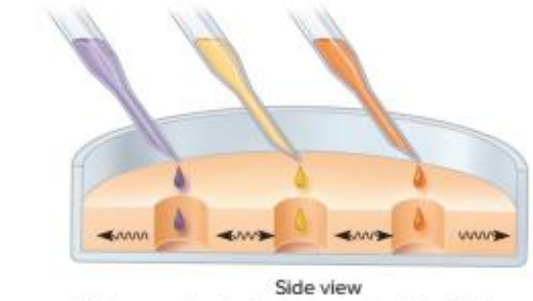
Immunodiffusion:

Immunodiffusion refers to a precipitation reaction that occurs between an antibody and antigen in an agar gel. Two techniques are routinely used: single radial immunodiffusion and double diffusion in agar.

The single radial immunodiffusion (RID) assay or Mancini technique quantitates antigens. Antibody is added to agar and the mixture is poured onto slides and allowed to set. Wells are cut in the agar and known amounts of standard antigen added. The unknown test antigen is added to a separate well. The slide is incubated for 24 hours or until equilibrium has been reached, during which time the antigen diffuses out of the wells to form insoluble complexes with antibodies. The size of the resulting precipitation ring surrounding various dilutions of antigen is proportional to the amount of antigen in the well (the wider the ring, the greater the antigen concentration). This is because antigen concentration drops as it diffuses farther out into the agar. The antigen forms a precipitin ring in the agar when its level has decreased sufficiently to reach equivalence and combine with the antibody to produce a large, insoluble network. This method can be used to quantitate serum immunoglobulins, complement proteins, and other substances.

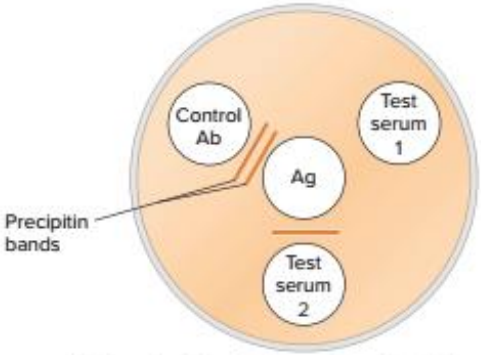
The double diffusion agar assay (Öuchterlony technique) is based on the principle that diffusion of both antibody and antigen (hence double diffusion) through agar form stable and easily

observable immune complexes. Test solutions of antigen and antibody are added to separate wells punched in agar. The solutions diffuse outward, and when antigen and the appropriate antibody meet, they combine and precipitate at the equivalence zone, producing an indicator line (or lines). The visible line of precipitation permits a comparison of antigens for identity (same antigenic determinants), partial identity, or nonidentity against a given selected antibody. Immunodiffusion assays are most commonly used to confirm certain fungal infections.



Side view

(a) In one method of setting up a double-diffusion test, wells are punctured in soft agar, and antibodies and antigens are added to separate wells in a pattern. As the contents of the wells diffuse toward each other, a number of reactions can result, depending on whether antibodies meet and precipitate antigens.



(b) Example of test pattern and results. Antigen (Ag) is placed in the center well and antibody (Ab) samples are placed in outer wells. The control contains known Abs to the test Ag. Note bands that form where Ab and Ag meet. The other wells (1, 2) contain unknown test sera. One is positive and the other is negative. Double bands indicate more than one antigen and antibody that can react.

Figure: Immunodiffusion. Double diffusion agar assay showing characteristics of identity (test serum 2) and a reaction of nonidentity (test serum 1)

Flow Cytometry:

Flow cytometry allows detection of specific lymphocyte subsets (CD4+ T cells, for example, in HIV-infected blood) in an easy, reliable, fast way. It has become a routine technique for counting CD4+ and CD8+ lymphocytes, as well as lymphocytes in varying stages of development as needed

in the diagnosis of leukemia and lymphoma. This technique has also enabled the development of quantitative methods to assess antimicrobial susceptibility and drug cytotoxicity in a rapid, accurate, and highly reproducible way.

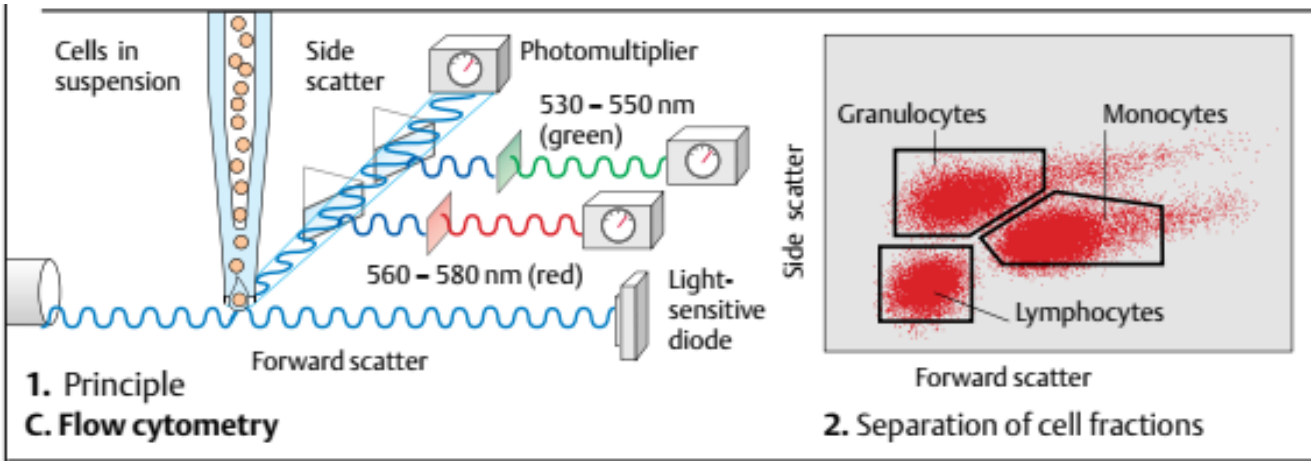


Figure: Flow Cytometry technique

Immunofluorescence:

In direct immunofluorescence, the antibodies are already conjugated with a fluorescent dye. In indirect fluorescence, on the other hand, a fluorochrome-labeled secondary anti body is added in second step after the anti gen-specific primary antibody has been bound. Direct immunofluorescence can be used to study two or more antigens at a time. Indirect immunofluorescence improves the visualization of weakly expressed antigens because several molecules of the labeled antibody can bind to the primary antibody. The sample can be fixed to make the cell membrane permeable enough for identification of intracytoplasmic antigens. This is also an effective way to stain cells in suspension, tissue sections, or cytospins.

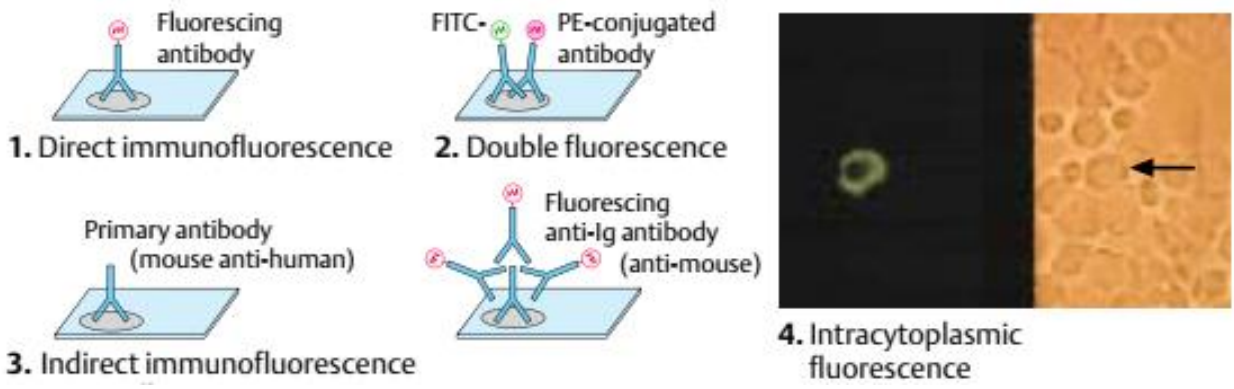


Figure: Steps involved in immunofluorescence

References:

1. Brock Biology of Microorganisms, 16th edition, Michael T. Madigan, Kelly S. Bender, Daniel Buckley, W Matthew Sattley, David A. Stahl.
2. Prescott's Microbiology,11th Edition, Joanne Willey and Kathleen Sandman and Dorothy Wood.
3. Fundamentals of Microbiology and Immunology, Banerjee & Banerjee.
4. Microbiology: Principles and Explorations, 10th Edition, Jacquelyn G. Black, Laura J. Black.
5. Molecular Biology: Principles of Genome Function, Third Edition, Nancy Craig, Rachel Green, Carol Greider, Gisela Storz, Cynss.
6. Immunology, Janis Kuby, Barbara A. Osborne