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G.C.E. (Advanced Level)

Biology

Grade 12
Resource Book

Muthunu Sandumal Akurugodagama

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Department of Science
Faculty of Science and Technology
National Institute of Education
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Message from the Director General

The National Institute of Education takes opportune steps from time to time for the development of quality in education. Preparation of supplementary books for respective subjects is one such initiative.

The additional reading book has been c omposed by the National Institute of Education to implement grades 12 and 13 syllabi and Teachers' Guides successfully in the classroom.

It is our behalf that provision of essential staff relating to respective syllabi by this additional book will facilitate learning the relevant subject stream.

I wish to express my gratitude to the NIE staff and external experts who made their academic contribution to make this material available to you.

Dr. (Mrs.) T. A. R. J. Gunasekara
Director General
National Institute of Education
Maharagama.

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Message from the Director

From 2017, a rationalized curriculum is in effect for the G.C.E (A/L) in the education system in Sri Lanka. It means updating the curriculum that was in implementation.

In this effort, revisions were made in the content, form and curricular materials of the G.C.E (A/L) subjects Physics, Chemistry and Biology and in concurrence to that, certain changes in the learning teaching methodology, evaluation and assessment were expected. The volume of the subject matter in the curriculum was largely reduced and several alterations in the learning teaching sequence were also made. A new teachers' hand book was introduced in place of the old curricular material, the teachers' instruction manual.

The teachers' instruction manual contained a line-up of subject matter expected to be learnt but the newly introduced teacher's hand book doesn't accommodate any subject matter. Yet, it provides a guideline for teachers to mould the learning events and for evaluation. Though the teacher's hand book implicitly enunciates the limits of the subject by way of the learning outcomes, the teachers' hand book only is not sufficient to identify holistically the confines of the facts. Thus emerged the need of a resource book which simply describes the subject content. This book comes to you as a result of an attempt to fulfill that requirement.

When implementing the previous curricula, the use of internationally recognized standard textbooks published in English has been an imperative for Advanced Level Science subjects. But, the contradictions of facts related to subject matter and inclusion of material beyond the limits of the local curriculum in them, usage of those books was not an easy task for teachers and students.

This book, offers students an opportunity to study the relevant subject content in the mother tongue within the limits of the local curriculum. It also provides both students and teachers a source of reliable information expected by the curriculum dispensing with seedling information from various print media and extra classes.

This book authored by specialist subject teachers and university lectures is presented to you after the approval of the Academic Affairs Board and the Council of the NIE. Thus it can be recommended as a material of high standard.

Mr. R. S. J. P. Uduporuwa
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01 Introduction to Biology

Nature scope and importance of biology with reference to challenges faced by the mankind

Biology is a Science which is focused on studying of living organisms. (Bios- Life, logos ”study of”)

The conceptof “LIFE” is not easy to define. Still scientists are unable to provide an acceptable definition for life.

“Life” is something special and unique which cannot be explained using laws of chemistry and physics.

Biology is a subject which is very complex and vast. Hence for the convenience of studying, it has been divided into three primary braches: Zoology (the study of animals), Botany (study of plants) and Microbiology (study of microorganisms). Some areas of study in these branches:

- Cell Biology (studying cells)
- Histology (studying tissues)
- Anatomy (studying about gross structure of the body)
- Physiology (studying function)
- Biochemistry (studying biological molecules)
- Genetics (studying inheritance)
- Ecology (studying environment)

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Issues pertaining to Biology

Understanding biological Diversity

At present our planet is rich in diversity. Life on earth formed around 3.8 billion years ago. The first formed organisms are believed to be heterotrophic, anaerobic prokaryotes. Since then the evolutionary process resulted in the extensive biodiversity which exists in the biosphere now.

Scientists assume, based on their studies that there are about 10 to over 100 million species in the world. There is a dynamic relationship between living world and the inanimate world and each and every organism has a specific role in the environment for existence of the Biosphere.

The variety of life on earth, the number of species of plants, animals and microorganisms, the diversity of genes in these species, the different ecosystems on the earth such as deserts, rainforests and coral reefs are all part of a biologically diverse earth.

Understanding the human Body and its functions

When studying biology,especially by studying histology, anatomy of the human body,one can gain the knowledge about the structure of the organs. This results in understanding and appreciation of the organization of the human body and understanding the functions of different organ systems and the relationship between structure and functions.

Sustainable use and Management of natural resources and Environment

Natural resources are sources of materials and energy found naturally which are used in everyday life and for economic development.

These natural resources are limited on earth. Due to the increase of growth rate of human population, overuse of natural resources is taking place. It causes threat of depletion of natural resources.

Due to over exploitation of natural resources, various environmental problems arise such as;

- Environmental pollution
- Loss of Biodiversity
- Desertification

Hence to overcome the above problems management of natural resources and Environment should be practiced. Knowledge of Biology is useful to bring about remedies for the above problems.

Sustainable Food production

Sustainable food production is the production of sufficient amounts of food for the

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human population using environmentally safe methods.

The current human population is about 7 billion and expected to be double in less than 40 years. Therefore, for the survival of human beings sustainable food production is necessary.

To maintain sustainable food production following methods can be applied, which are based on knowledge in biology.

- Production of high yielding varieties of plants and animals.
- Production of disease resistant plants and animal varieties.
- Improve the post harvest technological methods.

Understanding plant life

Plants are the primary producers in the world. All the animals depend directly or indirectly on plants. Therefore understanding plant life is important. As the time human population is increasing we need to increase the productivity. Therefore understanding plant function and biology is important to produce high yielding plants, disease resistant plants, etc.

Understanding diseases and causes

To maintain healthy human body one should have the knowledge of causes of the diseases and their effects.

Some dangerous diseases which exist in current world are non communicable diseases such as cancers, heart diseases, diabetes, chronic renal diseases and communicable diseases such as dengue, AIDS, etc.

Cancers- causes for this is not fully understood yet. Cancers are one of the leading causes of death .

AIDS- is a viral disease which is a serious and growing health problem worldwide.

Heart diseases- This is also a serious and growing health problem worldwide. Causes are not fully understood yet.

Chronic renal diseases- In Sri Lanka, recentlyCKDu has become a serious health problem.

Currently scientists are working on prevention, remedial measures and cures for such diseases.

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Solving some legal and ethical issues

Knowledge and application of biological concepts is important in solving some legal issues, such as parentage testing, in criminal investigations and to solve immigration disputes.

DNA fingerprinting is used in above circumstances.

The nature and the organizational patterns of the living world

In accordance with different criteria we can see a diversity among living organisms. Organisms are diverse based on size, shape, form and habitats.

- Living organisms show a wide range of variation in size, shape, form and habitat.
- Size – Bacteria – 0.25 μm – 2 μm to Giant Sequoia (Giant Red Wood)– 100m
- Shape – Organisms are diverse in shape, Ex: Cylindrical (earth worm), streamline shape(birds, fish)
- Form –Unicellular (Amoeba), multicellular (any plant or animal)
- Habitat – Terrestrial (Rat), aquatic (Fish), arboreal (Loris), aerial(Birds)

Characteristics of organisms

In order to survive, each organism whether simple or complex must be able to perform certain functions. Following features are the characteristics of organisms.

(i) Order and organization

From molecular level to biosphere there is an order and organization in organisms to perform their biological activities efficiently.

Lower level components are organized in a methodical pattern in upper level to make it most efficient.e.g: plant leaf and human eyes.

(ii) Metabolism

The sum of all chemical activities taking place in an organism is its metabolism. It includes catabolic reactions and anabolic reactions.

(iii) Growth and development

All organisms begin their life as a single cell. During growth an irreversible increase in dry mass occurs, which is characterized only by the living. Irreversible changes that occur during the life span of an organism is development.Growth and development are two consequent processes that happen in the life span of organisms.

(iv) Irritability and coordination

Irritability is the ability to respond to stimuli from both internal and external environment. Movement of organisms occurs as a result of irritability and coordination. In animals

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this happens as a result of coordinated efforts of nervous, hormonal, muscular and skeletal systems

(v) Adaptation

Adaptation is a peculiarity of structure, physiology or behavior that promotes the likelihood of an organism's survival and reproduction in a particular environment. E.g: Sunken stomata in Xerophytes, Viviparity in some mangroves, Splayed out foot in camels.

(vi) Reproduction

Ability to produce offspring for continuous existence of species

(vii) Heredity & Evolution

Organisms have genes that pass from one generation to the next and control specific physiological, morphological and behavioral characters of organisms.

Ability of organisms to change over time as a result of genetic modification is evolution

Many non living entities may have one or more of these characteristics but not all of them e.g., crystals grow, waves move but only living organisms display all these characteristics simultaneously or at some point during their life cycle.

By considering this it can be said that these are occurring in single celled organisms as well as highly complex organisms such as humans and Anthophytes (flowering plants).

Hierarchiel levels of organization of living things

The cell is the basic structural and functional unit of life. Some organisms are unicellular while others are multicellular.Cell is composed of several organells which are formed by different organic molecules.Then hierarchieal levels of organization of living things can be constructed by using relevant examples at each level.

Molecules,Organells, Cells, Tissues, Organs, Organ systems, Organisms, Populations, Communities, Ecosystems, Biosphere

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02

Chemical and cellular basis of life

Elemental composition of living matter

There are about ninety two elements naturally occur in earth's crust. Of which, about 20-25% elements are essential to continue healthy life and reproduction. (about 25- elements are essential for humans and about 17 for plants).

Oxygen (O), Carbon (C), Hydrogen (H), and Nitrogen (N) make up 96% of living matter.

Calcium (Ca), Phosphorous (P), potassium (K) and sulphur (S)- make up most of the remaining 4% of the mass of the organism.

In humans, C, H, O, N- accounts for 96.3% of the body mass and Ca, P, K, S, Na, Cl, Mg and trace elements accounts for the remaining 3.7%. (e.g. B (Boron) , Co (Cobalt), Cu (Copper), Cr (Chromium), F (Fluorine), I (Iodine), Fe (Iron),

Mo (Molybdenum), Mn (Manganese), Se (Selenium), Si (Silicon), Sn (Tin), V (Vanadium), Zn (Zinc)

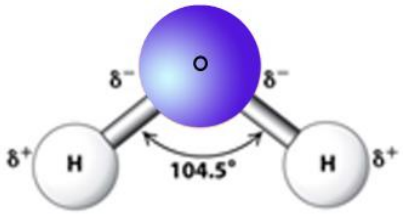
Physical and chemical properties of water important for life

Water is a vital inorganic molecule; life could not exist on this planet without water. It is important due to following reasons,

1. Vital chemical constituent of living cell
2. Provides a biological medium for all organisms

Most of above properties are based on the chemical structure of water molecule. Physical and chemical properties of water molecule provide the ability to render the vitality. Water molecule is a small, polar and angular molecule.

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δ+ partial positive

δ-partial negative

Fig 2.1: Chemical structure of the water molecule

Polarity is an uneven charge distribution within a molecule. In water molecule, oxygen atom is slightly negative and hydrogen atom is slightly positive. Weak attractions between the slightly polar hydrogen atom of one water molecule and the slightly polar oxygen atom of adjacent water molecuole are known as hydrogen bonds.These hydrogen bonds play a major role in maintaining all the properties of water.

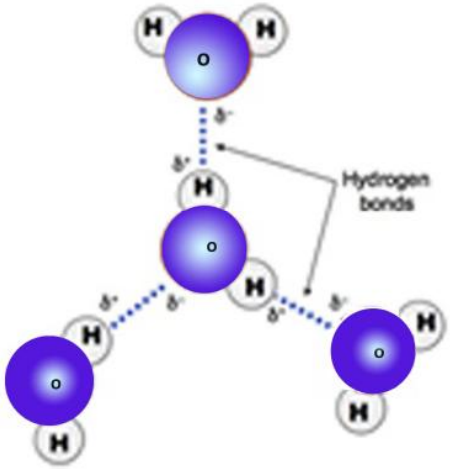


Fig 2.2: Hydrogen bonding in water

The properties of water arise due to attractions of different water molecules. When the water is in liquid form its H bonds are very fragile. H bonds form, break and reform with great frequency.

Four major properties of water to maintain life on earth

- Cohesive behavior
- Ability to moderate temperature
- Expansion upon freezing
- Versatility as a solvent

Properties of water related to functions

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1. Cohesive behavior

Attraction between water molecules due to hydrogen bonding is known as cohesion. Attraction between water molecules and other substances are known as adhesion. Both of the above properties of water allow it to act as a transport medium.

Due to cohesion between water molecules, water and dissolved substances such as minerals and nutrients transport through vascular tissues, xylem and phloem against gravity.

Adhesion between water molecules and cell walls also helps in conduction of water and dissolved substances.

Water has a high surface tension. This ability is given to water molecules, due to cohesion between the water molecules. Therefore, in an aquatic system, upper surface water molecules are attracted by lower surface molecules and it forms a water film. Small insects e.g. water skaters can walk on the surface of a pond.

2. Ability to moderate temperature

Water can absorb or release a relatively high amount of heat energy by a slight change in its own temperature.

Due to the high specific heat, water will function as thermal buffer in living system and aquatic bodies during the temperature fluctuations on earth.

Due to the high heat of vaporization, with the minimum loss of water an organism can release much heat energy. Therefore, body surface of an organism maintained as cool surface.

e.g. Prevent from overheating.

Evaporation of sweat from human skin helps to maintain the body temperature at constant level.

Transpiration in plants keeps the plant body surface as a cool surface and prevent from becoming too warm in the sunlight.

3. Expansion upon freezing

Generally, in an increase in temperature of any substances, reduces their density and on the other hand, in a decrease in temperature increases their density. When the temperature of water falls below 4 ˚C, it begins to freeze and forms a crystalline lattice called ice cubes. Therefore water has the maximum density at 4˚C.Hence, ice floats on the surface of water bodies. It is an important property of water in polar regions, where, organisms in aquatic bodies can survive during the winter.

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4. Versatility as a solvent

This ability is given to water due to their polarity. Polar molecules (e.g. Glucose), non polar ionic (e.g. NaCl), both polar and ionic (e.g. lysozymes) can dissolve in water, because water molecules surround each of the solute molecules and form hydrogen bonds with them. Solubility depends on polarity and not in their ionic nature.

Chemical Nature and Functions of Main Organic Compounds of Organisms

Carbohydrates

Most abundant group of organic compound on earth is carbohydrates. Major elemental composition is C, H, and O. Hydrates of carbon contain the same proportion of H: O which equals to 2:1 as in water.General formula is $C_x(H_2O)_y$. Three major groups of carbohydrates are monosaccharides, disaccharides and polysaccharides.

Generally carbohydrates include sugars (monosaccharides and disaccharides) and polysaccharides.

Monosaccharides

The simplest form of carbohydrates having general molecular formula as $(CH_2O)_n$ are monosaccharide. Where C varies from 3-7 . All monosaccharide are reducing sugars, water soluble and occur in crystalline form.

According to the number of carbon atoms, they are named as;

- 3C- Triose e.g. Glyceraldehydes (Phosphoglyceraldehyde is a derivative of Triose)
- 4C- Tetroses.g. Erythrose (rare in nature)
- 5C- Pentoses.g. Ribose, Deoxyribose, Ribulose (RUBP is a derivative of ribulose)
- 6C- Hexoses e.g. Glucose, Fructose, Galactose

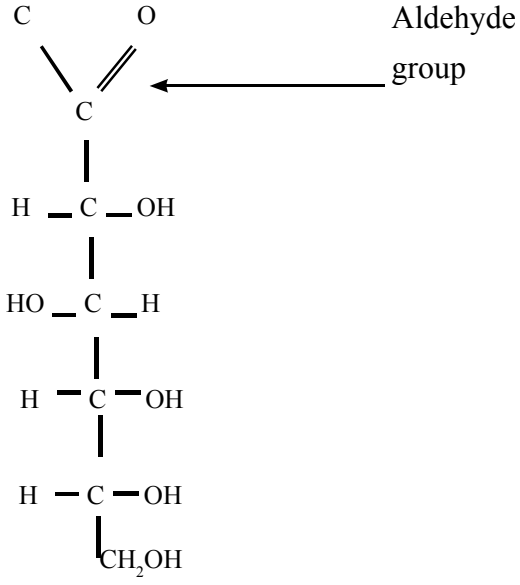
According to the type of carbonyl (Keto, aldo)group, they are classified as;

a. Aldoses-glucose, galactose

b. Ketoses-fructose

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Aldose



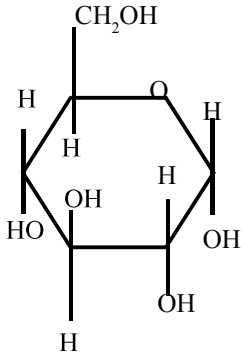
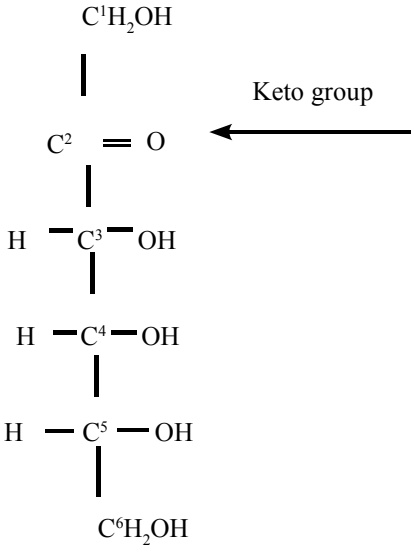


Fig 2.3: Solid form of glucose

Fig 2.4: Aqueous form of Glucose molecule

Ketose



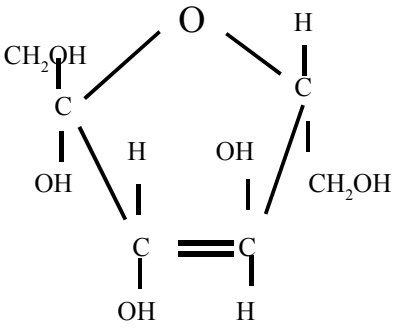


Fig 2.5: Solid form of fructose

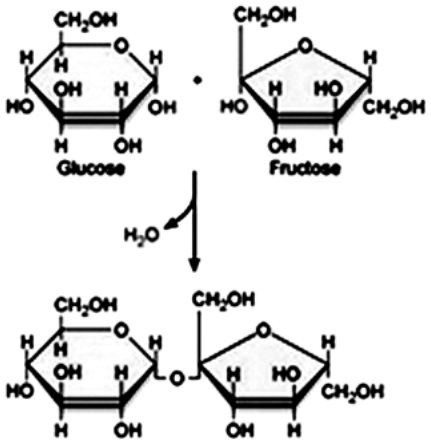
Fig 2.6: Aqueous form of fructose

In aqueous media some monosaccharides are in ring form (No need to memorize the chemical structures)

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Disaccharides

They are sugars formed by joining two monosaccharides by a glycosidic bond.



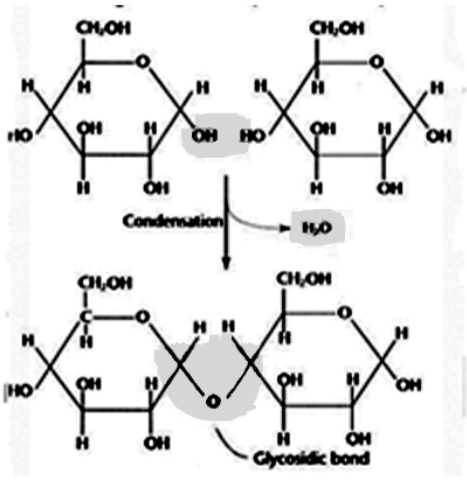


Fig 2.7: Formation of sucrose ***Fig 2.8: Formation of maltose***

(no need to memorize the chemical structures)

Glycosidic bond is formed by removal of a water molecule from two adjacent monosaccharides by a condensation reaction. Water molecule is formed from OH group of one monosaccharide molecule and H from adjoining monosaccharide molecule.

Glucose + Glucose $\xrightarrow{\text{Condensation}}$ Maltose + H_20

Glucose + fructose $\xrightarrow{\text{Condensation}}$ Sucrose + H_20

Glucose + Galactose $\xrightarrow{\text{Condensation}}$ Lactose + H_20

Maltose and lactose are reducing sugars and sucrose is a non reducing sugar.

Polysaccharides

They are macromolecules and biopolymers. Polysaccharides are made up of few hundred to a few thousand monosaccharide subunits

They are non crystalline, water insoluble, and not considered as sugars.

Some polysaccharides act as storage components where others contribute to the structure of living organisms. Based on their function they are categorized as storage polysaccharides and structural polysaccharides.

- Storage- Starch, Glycogen
- Structural- Cellulose, Hemicellulose, Pectin

Based on their architecture they are categorized as

- Linear forms- Cellulose, Amylose
- Branched forms- Glycogen, Amylopectin, Hemicellulose

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Table 2.1: Major polysaccharides, their monomers and functions

Polysaccharide	**Monomer**	**Functions**
Starch	Glucose	Stored in plants
Glycogen	Glucose	Stored in animals and fungi
Cellulose	Glucose	Component of Cell wall
Inuline	Fructose	Stored in tubers of Dhalia
Pectin	Galacturonic acid	Component of Middle lamella of plant cell wall
Hemicellulose	Pentose	Component of Plant cell walls
Chitin (nitrogen containing polysaccharide)	Glucosamine	Component of Fungal cell walls and exoskeleton of Arthropods

Functions of carbohydrates

Monosaccharides

- Energy source
- Building blocks of disaccharides and polysaccharides (disaccharides such as maltose, sucrose and polysaccharides such as starch, glycogen)
- Components of nucleotides (DNA, RNA)

Disaccharides

- Storage sugar in milk- Lactose
- Translocation in phloem –Sucrose
- Storage sugar in sugarcane- Sucrose

Polysaccharides

a.) Storage polysaccharides-

- starch stores glucose as energy source in plants and chlorophytes
- glycogen stores glucose as energy source in animals and fungi
- inulin stores fructose as energy source in Dahlia tubers

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b.) structural polysaccharides-

- Cellulose in the cell walls of plants and chlorophytes
- Pectin in the middle lamella of plant tissues.
- Hemicellulose in cell walls of plants.
- Peptidoglycan in the cell walls of prokaryotes.
- Chitin in the cell walls of fungi and in exoskeleton in Arthropods.

Lipids

- Diverse group of hydrophobic molecules
- Large biological molecules but not considered as polymers or macromolecules.
- Consist of C, H, O and H:O ratio is not 2:1. Comparatively more H are present.
- Biologically important types of lipids: Fats, Phospholipids and Steroids.

Fats

Fats are made up of glycerol and fatty acids; Glycerol belongs to alcohol group having 3 carbons where each of them bear single hydroxyl group. Fatty acids are hydrocarbon chains with long (16-18) carbon skeleton with a carboxyl group at its one terminal.

Fatty acid molecules bind to each hydroxyl group of glycerol by ester bond. Resulting fat molecules are called as triacylglycerol.

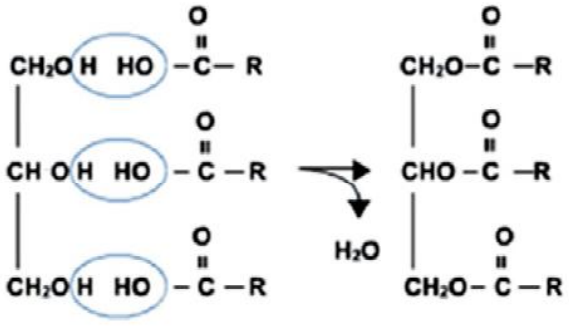


Fig 2.9: Formation of Triacylglycerol

Hydrocarbon chains of fatty acids contribute to the hydrophobic nature of the fats. Based on the nature of hydrocarbon chains of fatty acids, they are categorized as

a) Saturated fats- fats are made up of saturated fatty acids: fatty acids with hydrocarbons having no any double bonds. Usually animal fats come under this category. They are mostly solid at room temperature. e.g: butter

b) Unsaturated fats- fats are made up of unsaturated fatty acids- fatty acids with hydrocarbons having one or more double bonds. Usually plant fats come under

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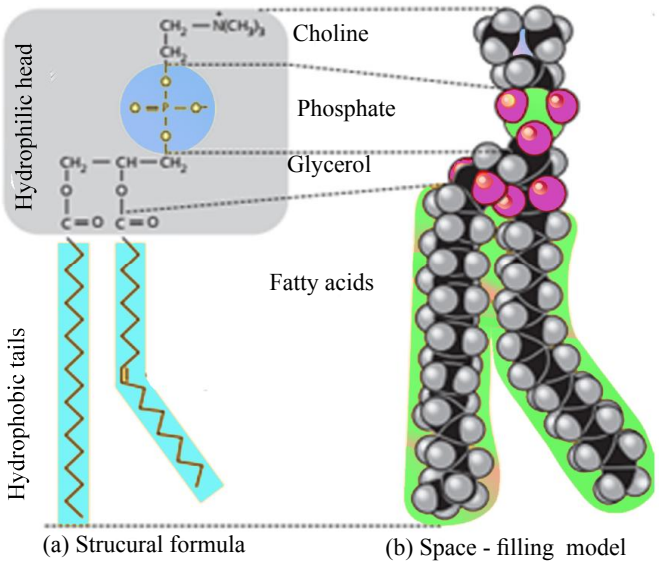
this category. They are mostly liquid in room temperature. e.g: vegetable oils. Unsaturated fats may classify based on the nature of their double bonds. a) *Cis* Unsaturated fat b) *Trans* Unsaturated fat

Consumption of excess saturated fats and trans unsaturated fats contribute arthrosclerosis.

Phospholipids

Phospholipids are major components of the cell membranes. They are composed of two fatty acids and one phosphate group attached to one glycerol molecule. The phosphate group gives the negative electrical charge to the phospholipid molecule. Typically an additional polar molecule or small charged molecule is also linked to the phosphate group e.g. choline.

The two ends of the phospholipids show different behavior. The hydrocarbon tails are hydrophobic while phosphate group and its attachment (head) are hydrophilic.



(no need to memorize the structure)

Fig 2.10: structure of the phospholipid molecule

Functions of Lipids

- food reserve as energy source (triglycerides such as fats and oils)
- maintain the fluidity of plasma membrane (phospholipids, cholestrol)
- act as signaling molecules (eg. Hormones) that travel through the body
- found as components of animal cell membrane (cholesterol)

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Protein

Proteins are made up of amino acids. Twenty different amino acids are involved in the formation of proteins. Elemental composition is C, H,O,N and S. At the centre of the amino acid is an asymmetric carbon atom except in glycine. Each amino acid is composed of an amino group, a carboxyl group, a hydrogen atom and a variable group symbolized by R, which is an alkyl group. In the case of glycine R is replaced by H atom. The R group also called the 'side chain' differs with each amino acid where as the other groups are in the ' back bone' (including the H atom).

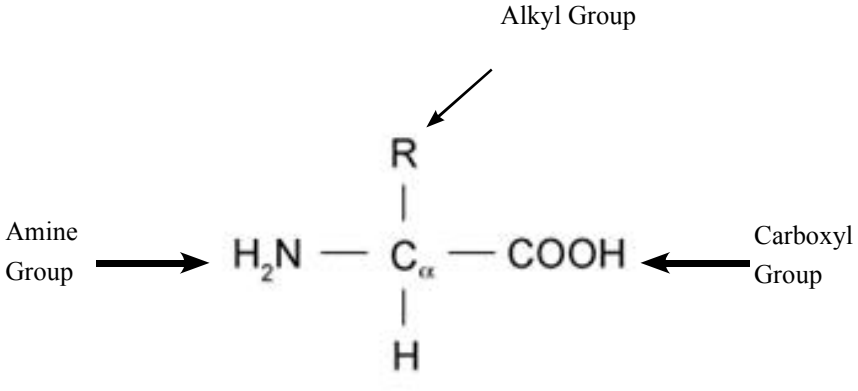


Fig 2.11: Structure of an Amino acid molecule

Amino acids may have one or more carboxyl groups and amino groups. Amino group has alkaline nature and carboxyl group has acidic nature. When both characteristics are found in one molecule they are known as amphoteric molecules. Therefore, amino acids are amphoteric.

Two Amino acids undergo condensation reaction by removing a water molecule from both and result a bond known as peptide bond;

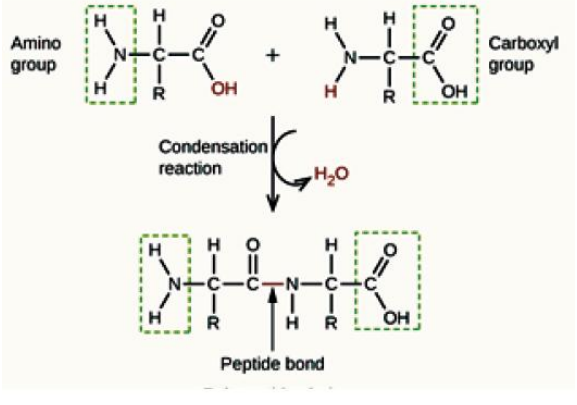


Fig 2.12: Formation of peptide bond

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Protein is composed of one or more polypeptide chains which are composed of amino acids.

Levels of protein structures

There are four levels of structure which play important roles in their functions;

a) Primary
b) Secondary
c) Tertiary
d) Quaternary

a) Primary structure

The unique sequence of linearly arranged amino acids linked by peptide bonds is the primary structure of proteins.

b). Secondary structure

The primary structure of a single polypeptide chain coils and folds, as a result of intra molecular hydrogen bonds between the oxygen atoms and the hydrogen atoms attached to the nitrogen atoms, of the same poly peptide chain backbone, to form the secondary structure, which is either β pleated or alpha helical.

- Alpha helix- e.g.Keratin.
- β pleated sheet e.g.spider's silk fiber

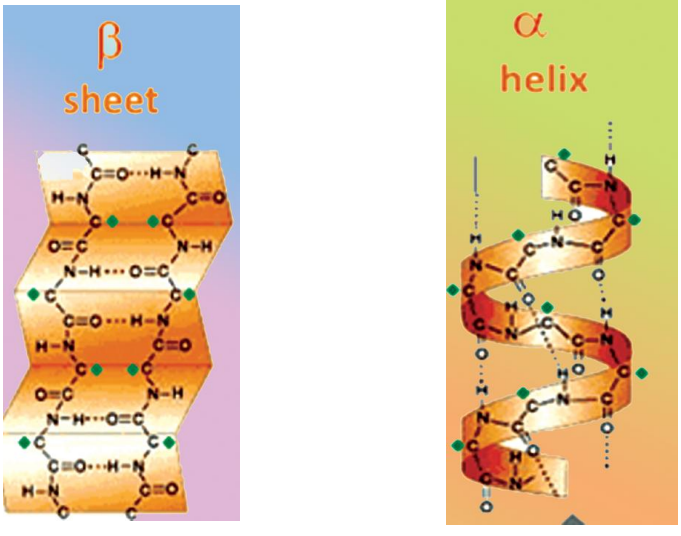


Fig 2.13: beta pleated sheet and alpha helix of secondary structures of proteins

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b) Tertiary structure

Usually the secondary polypeptide chain bends and folds extensively forming a precise compact unique, functional and three-dimensional shape resulting from following interactions between the side chain/ R-group of amino acids;

- H bonds
- Disulphide bonds
- Ionic bonds
- Van der Waals interactions/ Hydrophobic interactions

e.g.most of the enzymes, myoglobin, albumin

c) Quaternary structure

Aggregation of two or more polypeptide chains involve in the formation of one functional protein. Separate chains are called protein subunits which were held together by inter and intra-molecular interactions.

e.g.Haemoglobin, Collagen

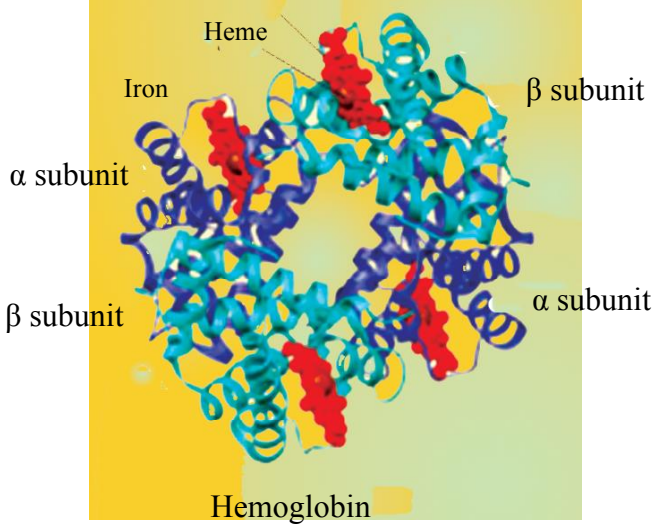


Fig 2.14: structure of the hemoglobin molecule

Denaturation of proteins

Denaturation of protein is the loss of specific chemical three dimensional shape due to the alteration of weak chemical bonds and interactions.

Agents affecting the denaturation

1. High temperature and high energy radiation
2. Strong acids, alkaline and high concentrations of salts
3. Heavy metals
4. Organic solvents and detergents

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Functions of the proteins

Table 2.2 Functions of Proteins

Type of protein	Example	Functions
Catalytic protein	Pepsin, Amylase	Catalyze biochemical reaction
Structural protein	Keratin,	Prevent desiccation
	Collagen	Provide strength and support
Storag e	Ovalbumin	Storage protein in egg
	Casein	Storage protein in milk
Transport	Haemoglobin	Transport O_2 and CO_2
	Serum albumin	Transport fatty acids
Hormones	Insulin	Regulate blood glucose level
	Glucagon	
Contractile/ Motor	Actin/Myosin	Contraction of muscle fibres
Defensive	Immunoglobins	Eliminate foreign bodies

Nucleic acids

Nucleic acids are Polymers exist as polynucleotides made up of monomers called nucleotides. They contain C, H, O, N and P. Nucleic acids are macromolecules, biopolymers. There are two types of Nucleic acids: DNA (Deoxyribo nucleic acids) and RNA (Ribonucleic acids).

Structure of nucleotides

Nucleotides have 3 components; namely pentose sugar, nitrogenous base and a phosphate group

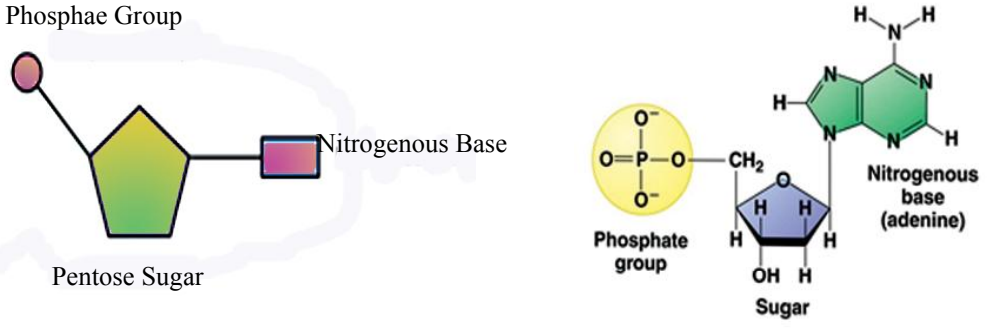


Fig 2.15: Structure of nucleotides (no need to memorize chemical structures)

A nucleotide without a phosphate group is called a nucleoside.e.g. Adenosine, Guanosine

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Pentose sugar

Pentose sugars are two types; namely Deoxy ribose and ribose (in deoxyribose one oxygen atom is less than in ribose)

Nitrogenous bases

There are two major groups of nitrogenous bases:

1. Purines- larger in size with two rings
2. Pyrimidines- smaller in size with a single ring

In purines there are two types; namely Adenine, Guanine. In pyrimidens there are three types, Thyamine, Uracil and Cytocine. Bases are commonly represented by letters A, G, T, U and C respectively.

Phosphate group

It gives the nucleic acids the acidic nature.

Formation of nucleic acids

Millions of nucleotides join by phospho-di-ester bond to form polynucleotide chains by condensation between the –OH group of the phosphate of one nucleotide with the –OH attached to 3rd carbon of pentose sugar of the other. These bonds results in a backbone with a repeating pattern of sugar-phosphate units. Nucleic acids are linear polymers of nucleotides. There are two kinds of nucleic acids depending on the type of the sugar molecules involved. If the sugar molecule in the nucleotide is deoxyribose,the nucleic acid is (DNA). If the pentose sugar is ribose, then the nucleic acid is RNA. DNA contains Adenine, Thymine, Guanine and Cytosine and RNA contains Adenine, Guanine, Cytosine and Uracil.

Structure of DNA molecule (Watson and Crick model)

DNA molecules have two anti-parallel polynucleotide chains that spiral around an imaginary axis, forming a double helix. The two sugar-phosphate backbones run in opposite directions from each other, and the arrangement is referred to as anti-parallel. The sugar phosphate backbones are on the outside of the helix, and the nitrogenous bases are paired in the interior of the helix. The two strands are held together by hydrogen bonds between the paired nitrogen bases.

Base pair rule

Always a purine base, pairs with a specific,pyrimidine base,

A=T (2 hydrogen bonds)

G≡C (3 hydrogen bonds)

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Hence two chains (strands) are said to be complementary to each other. These pairs are known as complementary base pairs.In this original double helical structure, one complete turn consists of ten base pairs as shown in the diagram.

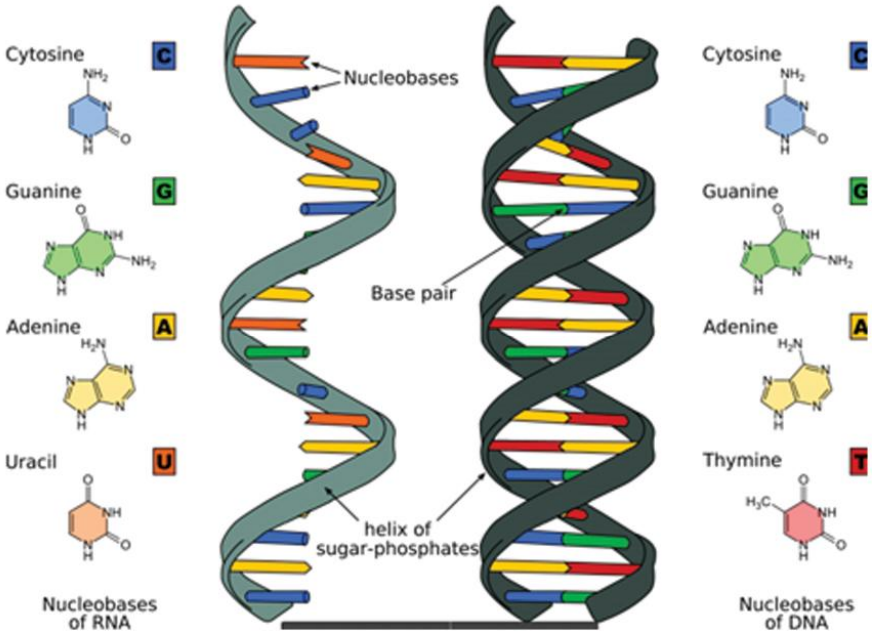


Fig2.16: The structure of the DNA and RNA molecules (no need to memorize chemical structures)

Functions of DNA

- Store and transmit genetic information from one generation to the next generation
- Store the genetic information for protein synthesis

Structure of RNA

This is normally a single stranded nucleic acid composed of ribo-nucleotides containing bases, Uracil (U), Cytosine (C), Guanine (G), Adenine (A). Complementary base pairing between two RNA molecules or within the same molecule may occur in some. Complementary base pairing facilitates three dimensional shapes essential for their functioning. Adenine binds with Uracil with two hydrogen bonds and Guanine binds with Cytosine with three hydrogen bonds. There are three types of RNA present in cells,

1. Messenger RNA (mRNA)
2. Transfer RNA (tRNA)
3. Ribosomal RNA (rRNA)

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1. Messenger RNA

Messenger RNA is a linear molecule and is the least abundant type of RNA in a cells comparatively. There are two functions;

- Copies the genetic information stored in DNA molecule as a sequence of nitrogenous bases
- Transports genetic information from nucleoplasam to the site of protein synthesis (ribosome) through nucleopores

2. Transfer RNA (tRNA)

Smallest RNA molecule. Linear, but forms three- looped structure as shown in the diagram.

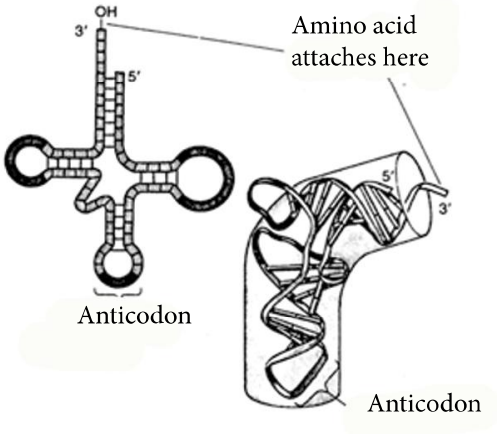


Fig 2.17: Structure of the tRNA molecule

Function - transportation of amino acids to the site of protein synthesis.

3. Ribosomal RNA

It is the most abundant type of RNA. rRNA has a complex irregular structure. It provides the site where polypeptide chains are assembled.

Differences between DNA and RNA

1. DNA is double stranded molecule while RNA is a single stranded molecule.
2. DNA consists of A, T, G and C and absence of U, while RNA consists of A,U, G and C and absence of T
3. Sugar molecule in RNA is ribose, while in DNA it is deoxyribose.

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Nucleotides other than those found in nucleic acids

ATP, NAD+, NADP+, FAD and their functions

Functions of ATP

- Universal energy carrier

Functions of NAD+

- Act as a coenzyme
- Act as an electron carrier
- Function as an oxidizing agent during respiration

Functions of NADP+

- Act as coenzymes
- Act as an electron carrier
- NADP+ act as a reducing agent in photosynthesis

Functions of FAD

- Act as a coenzyme
- Act as an electron carrier

Contribution of microscope to the expansion of knowledge on cells and cellular organization

Advancement of the cytology is mostly based on the microscopy. The discovery and early study of cells progressed with the invention of microscope.

Light microscope

Visible light is passed through the specimen and then through glass lenses. The lenses refract the light in such a way that the image of the specimen is magnified as it is projected into the eye.The simplest microscope is a single lens.

The compound light microscope

Compound light microscopes are commonly used in school laboratories and it is used in medical laboratories as a diagnostic tool.

Resolution power and magnification are important parameters which can be seen in a microscope.

Magnification is ratio of an object's image size to its actual size. Usually the maximum magnification of light microscope is 1000 times the actual size of the specimen)

Resolution power is minimum distance between two points that can be distinguished as separate points (resolution power of light microscope is 0.2μm). It is a measure of

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the clarity of the image.

Magnification is limited due to the resolution.

Light from an object (specimen on the slide) passes first through objective lens. Then produce a magnified image.

Above image then acts as an object for the second lens (the eye piece lens) which further magnifies it.

The total magnification is hence the product of the magnification of each lens.

$$\text{Total magnification} = \text{Magnification of objective lens} \times \text{Magnification of objective lens}$$

e.g- .If magnification of Objective lens = ×40, eyepiece =×15

Total is =15 × 40= ×600 time magnified

The Electron Microscope

The limitation imposed upon the resolution power of the light microscope by the wavelength of light. The resolution power is inversely proportional to the wavelength. Due to this, scientists considered the use of other forms of radiations with comparatively shorter wavelengths.

As a result, electron microscopes were developed. In electron microscopy, a beam of electrons is focused through the specimen or on to its surface.

This means, that in theory, the electron microscope should be able to magnify objects up to 1×10^8 times. In practice, it magnifies just over 5×10^5 times.

Electron microscopes have revealed many organelles and other sub cellular structures those were impossible to resolve with the light microscopes.

There are two types of electron microscopes.

1. Transmission electron microscopes (TEM)
2. Scanning electron microscopes(SEM)

Transmission electron microscopes

It is used to study the internal structures of cells.In this microscope, a beam of electrons is passed through a thin, especially prepared slice of material. A very thin specimen is used. Specimens stained with heavy metals which attach more to certain cellular structures than other areas. Image reflects the pattern of electrons passed through the specimen, displays on a screen. While electrons pass through the specimen, more electrons may get displayed in regions where structures were densely stained.

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Scanning electron microscopes

In this instrument, a fine beam of electrons is reflected from the surface of specimen. Specimen is mostly coated with gold prior to observation. Here the specimen scatters many electrons whereas others are absorbed. This instrument is ideal to observe the surface view in three dimensional appearances.

Table 2.3: Differences between light and electron microscope

Light Microscope	Electron microscope
Glass lenses are used to focus the light rays	Powerful magnets are used to focus beam of electrons
Image is directly detected by naked eye	Not directrly detected by naked eye, micrographs are used
Living and non living objects can be observed	Only non-living objects are observed
Actual color of the object can be observed	Actual color cannot be observed. Images are developed
Dyes used to stain the object	Heavy metals are used to stain the object

Historical background of the cell and analyses the structure and functions of the sub cellular units

Cell theory

All organisms are composed of cells.

Recall the hierarchy of life, the levels of organization mentioned earlier. The basic unit which can be called “living” is the cell, which may form a single celled organism (e.g.*Chlamydomonas*, Yeast) or a multi-cellular plant or animal. The cell is the basic structural and functional unit of life.

The level of organization of matter represented by a cell shows all the characteristics of life. Any stage below level of a cell cannot be considered living, whether it is a single celled organism or multi-cellular plant or an animal.

Robert Hooke (1665) examined a cork using simple microscope and gave the term “CELL” to describe the basic units.

Anton Van Leeuwenhook (1650), a contemporary of Robert Hooke, was the first to describe and record living single celled organisms, *Euglena* & bacteria

Matthias Schleiden (1831), a botanist, studying plant tissues concluded that all plants are made up of cells.

Theodore Schwann a zoologist (1839) concluded that animal tissues are also made up of cells.

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Rudolf Virchow (1855) showed that all cells arise from pre-existing cells by cell division,

Schleiden, Schwann and Virchow presented the 'Cell Theory' which included the following.

1.All organisms are composed of one or more cells.

2.The basic structural and functional unit of organisms is the cell.

3.All cells arise from pre-existing cells.

Organization of cells

There are two kinds of cellular organization - Prokaryotic and Eukaryotic

All cells share certain basic features. They are;

- All cells are bounded by a plasma membrane which is a selective barrier
- Within the cell have, a semifluid, jelly like substance which is called cytosol. Subcellular components are suspended within the cytosol.
- They carry DNA as genetic materials.
- Ribosomes are found in all cells

Table 2.4: The differences between Prokaryotic cells and Eukaryotic cells

Feature	Prokaryote	Eukaryote
organism	Bacteria, Archaebacteria	Protists,Fungi, plants, animals
Cell size	Average diameter 1-5µm	10µm-100µm diameter
Form	Mainly unicellular	Mainly multicellular (except most of protista and some fungi are unicellular)
Evolutionary origin	3.5 billion years ago	1.8 billion years ago ,evolved from prokaryotes
Cell division	Binary fission, no mitosis and meiosis	Mitosis, meiosis, or both;
Genetic material	DNA is circular and lies free in the cytoplasm. This region is called nucleoid, DNA is naked and not associated with proteins	DNA is linear and contained in a nucleus. DNA is associated with proteins
Type of ribosomes	70s ribosome (smaller)	Both 70s (Mitochondria and Chloroplast) and 80s ribosomes (larger) present (may attach to endoplasmic reticulum)

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Organelles	Few organelles, none are surrounded by membrane Internal membranes scarces; if present usually associated with respiration, photosynthesis and N_2 fixation.	Many organelles, membrane bounded organelles present. Great diversity of organelles. e.g. nucleus, mitochondria, chloroplasts bounded by two membranes. e.g. Lysosomes, Vacuole, bounded by single membrane.
Cell walls	Peptidoglycan present in Bacteria and cyanobacteria, polysaccharide and protein present in Archae bacteria	Cell walls of green plants and fungi are rigid and contain polysaccharides; cellulose in plant cell walls and chitin in fungal walls (none in animal cells)
Flagella	Simple, lacking microtubules; extracellular (not enclosed by cell surface membrane) 20nm diameter	Complex, with '9+2' arrangement of microtubules; intracellular (surrounded by cell surface membrane) 200nm diameter
Respiration	Mostly by mesosomes	Mitochondria for aerobic respiration
Photosynthesis	No chloroplasts; takes place on membranes which show no stacking	Chloroplasts containing membranes which are usually stacked into lamellae or grana
Nitrogen fixation	Some have the ability	None have the ability

Bacteria, Cyanobacteria and Achaea are prokaryotic cells. All the other organisms have eukaryotic cells.

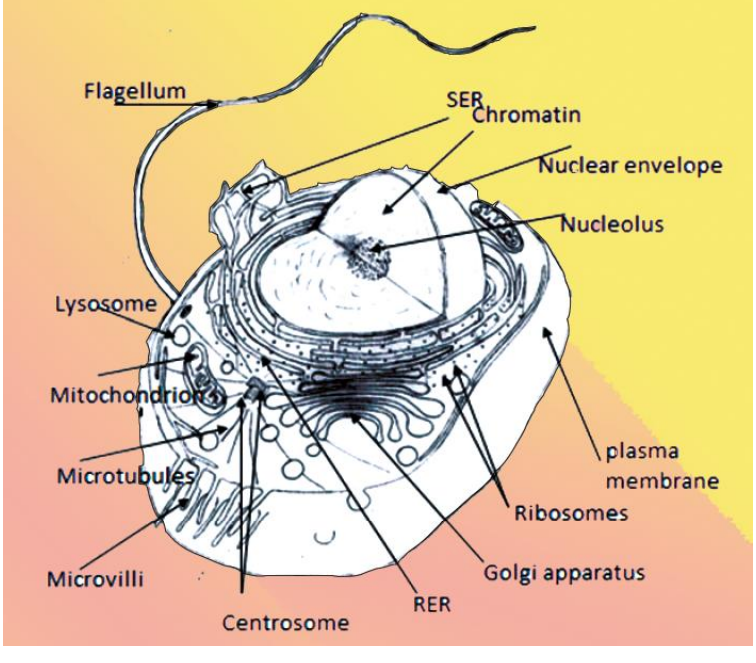


Fig 2.18: Structure of an animal cell

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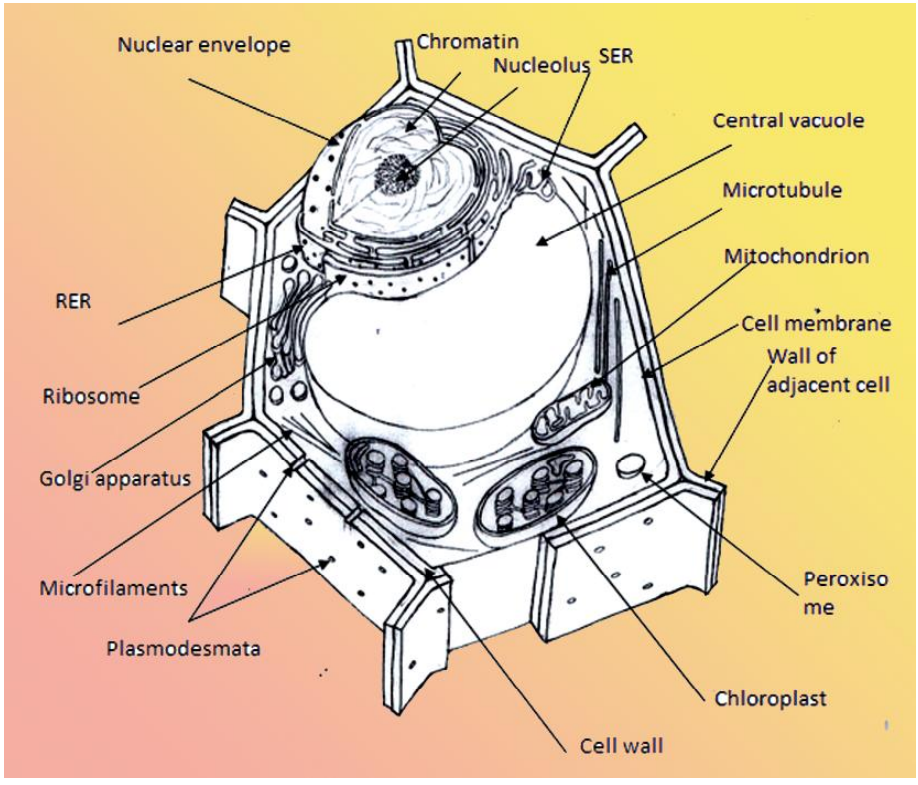


Fig 2.19: Structure of plant cell

Structures and functions of organelles and other subcellular components

Plasma membrane: Plasmamembrane is the outer limit of cytoplasm. All cellular membranes resemble the ultra structure of plasma membrane. In 1972, Singer and Nicolson put forward the fluid mosaic model of cell membrane. It is mainly composed of;

- Phospholipids (most abundant type of lipid in plasma membrane)
- Protein

The Plasma membrane has the following features. It is about 7nm thick. It is mainly made up of a phospholipid bilayer. Phospholipids are amphipathic molecules. The hydrophilic heads of the phospholipids face outwards into the aqueous environment of both inside and outside of the cell.

The hydrophobic hydrocarbon tails face inwards and create a hydrophobic interior.

Plasmamembrane is compared to the fluid mosaic model. Since phospholipid molecules are moveable, they provide the fluid nature to the membrane.

Protein molecules embedded randomly contribute to its mosaic nature.

Some of the protein molecules penetrate all the way through the membrane, called transmembrane proteins and some others penetrate only part of the way into the membrane. These are called **integral proteins.**

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Most of the integral proteins are transmembrane proteins which have hydrophilic channels. These act as pores through which ions and certain polar molecules can pass.

Some proteins are not embedded in the lipid bilayer at all, and are loosely bound to the inner surface of the membrane, called **peripheral proteins.**

Some proteins and lipids have short branching carbohydrate chains like antennae, forming **glycoprotein** and **glycolipids,** respectively.

Animal's cell membrane may contain few **cholesterol molecules** randomly integrated into the lipid bilayer.

These cholesterol molecules provide flexibility and stability to the membrane by reducing membrane fluidity at moderate temperatures and prevent membrane solidification at low temperatures.

The two sides of the membrane may differ in composition and function.

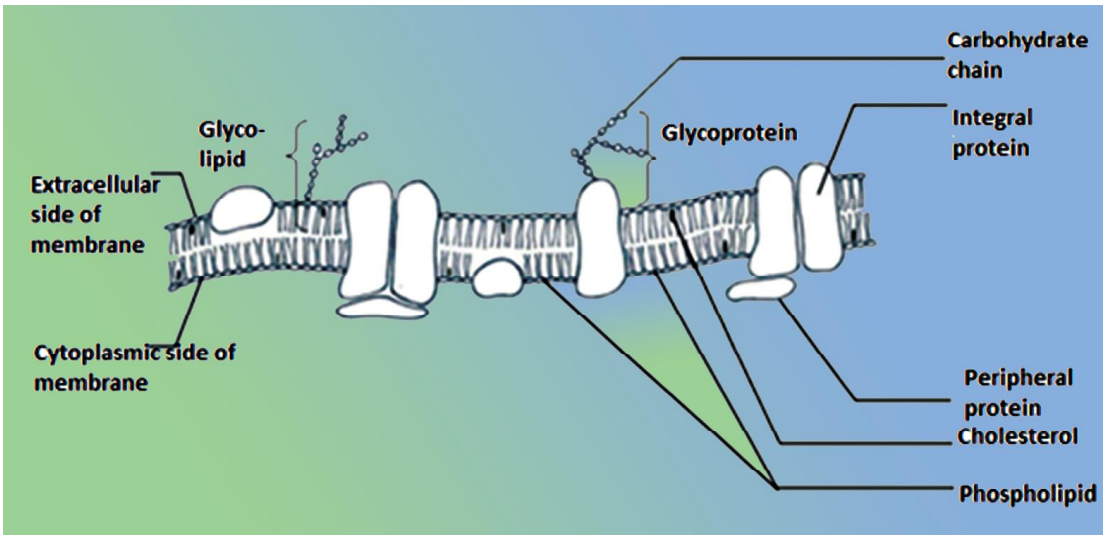


Fig 2.20: Structure of the plasma membrane

Functions

- The plasma membrane surrounds the cytoplasm of living cell physically separating the intracellular components from the extracellular environment.
- Plasma membrane is selectively permeable and able to regulate the exchange of material needed for survival.
- Proteins embedded in the plasma membrane identify the cell, enabling nearby cells to communicate with each other (involved in cell recognition).
- Some protein molecules act as receptor molecules for interacting with specific biochemical, such as hormones, neurotransmitters and immune proteins.
- Some proteins in the cell membrane attach to some cytoskeletal fibers and help

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to maintain the shape of the cell.

- Some proteins in the membrane act as enzymes. (e.g.Microvillus on epithelial cell lining of some parts of the gut contains digestive enzymes in their cell surface membrane.)

Subcellular components

There are many sub-cellular components in the cell. Some of them are organelles, which are bound by membranes and suspended in the cytosol of eukaryotic cell to perform specialized functions.

Nucleus

Most prominent organelle, consist most of the genes, having an average diameter of $5\mu m$ and enclosed by a double membrane cover called nuclear envelope.

- Nuclear envelope- composed of two membranes, inner and outer membranes, separated by a space of 20-40 nm. Nuclear envelope is perforated by nuclear pores which has pore complex to regulate the entry and exit of substances. It has nuclear lamina, made up of protein filaments which line the interior side of the nuclear envelope.
- Nuclear matrix is made up of protein filaments and extended throughout the interior of the nucleus. Chromatin and nucleolus are embedded in the nuclear matrix.
- Nucleolus- appears as darkly stained granules with fibers adjoining part of the chromatin.
- Chromatin –appears as a diffused mass in electron micrographs of non dividing cells. It is a complex of DNA and proteins. During nuclear divisions, chromatin condenses, tightly coils and form threads, called chromosomes. Each species has a constant number of chromosomes. (e.g. typical human cell has 46 chromosomes).

Functions

- Control all cellular activities.
- Synthesize DNA to produce new nuclei for cell divisions.
- Synthesize rRNAs and ribosomal subunits required for protein synthesis, through nucleolus.
- Synthesize mRNA and tRNA according to the information present on the DNA.
- Store and transport genetic information.

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Ribosomes

These are subcellular components which carryout protein synthesis. They consist of two subunits; larger subunit and smaller subunit. They are composed of rRNA and protein. Ribosomes are found in two types; 70S and 80S. 70S ribosomes are found freely on the cytoplasam of prokaryotes, matrix of mitochondria and stroma of chloroplasts. 80S ribosomes are found only in eukaryotes. Based on the nature of presence, 80S ribosomes are categorized as two types; free ribosomes and bound ribosomes.

Free ribosomes: freely available as group in cytoplasam. Bound ribosomes are attached to the membrane surface of rough endoplasmic reticulum.

Functions

Protein synthesis

Endoplasmic reticulum

It is a network of internal membranes forming flattened or tubular sacs separating cytosol from ER lumen. It is continuous with the outer membrane of nuclear envelope. There are two types of ER; Rough ER and Smooth ER

Rough ER

Rough ER consists of flattened sacs, and ribosomes bound to surface. Proteins synthesized by ribosomes move into lumen of ER.

Functions

- Transport protein synthesized by ribosomes
- Synthesizing glycoproteins
- Produce transport vesicles
- Facilitate the growth of own membrane by adding phospholipids proteins and carbohydrates. Therefore called as membrane factory

Smooth ER

Smooth ER is a network of tubular sacs without ribosomes. Membrane bound enzymes are present.

Functions

- It synthesizes lipids including oils, steroids and phospholipids.
- Metabolism of carbohydrates.
- Produce transport vesicles to transport within cell.
- Involves in detoxification.
- Stores Ca^{2+} ions.

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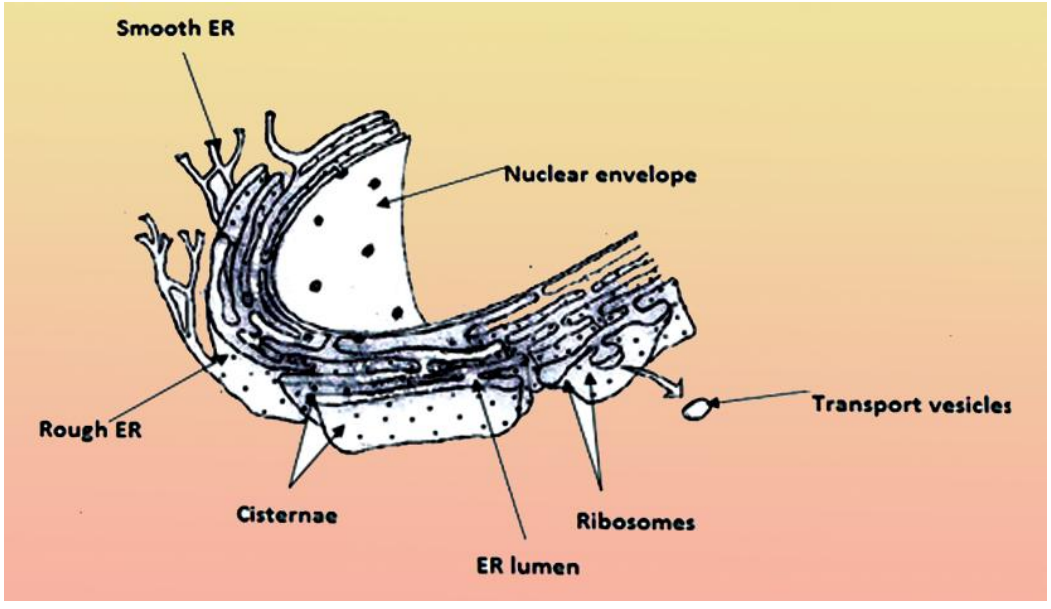


Fig 2.21: structure of the endoplasmic reticulum

Golgi apparatus

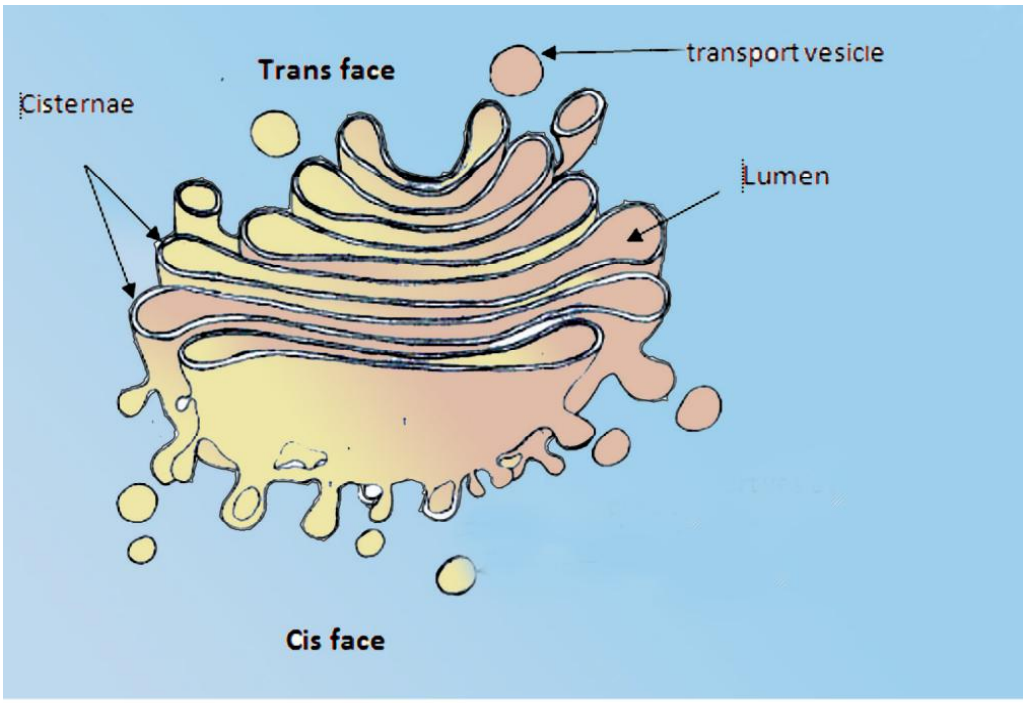


Fig 2.22: Structure of the golgi apparatus

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Golgi apparatus is a stacks of flattened sacs or Cisternae. Inner and outer surfaces can be identified as cis face and transface respectively. Cis face is located near the E.R to receive vessicles from E.R. Trans face give rise to secretory vessicles which budded off and travel other side. Golgi complex is abundant in secretory cells.

Functions

- Collecting, packaging and distribution of materials
- Manufacturing cellulose and non cellulose cell wall components such as pectin
- Produce lysosomes

Lysosomes

They are single membrane bounded vesicles contributing to digestive activity. They contain hydrolytic enzymes which catalyze breakdown of carbohydrates, proteins, lipids and nucleic acids.

Functions

- Digest food particles received by phagocytosis
- Transport residue material out of cell by exocytosis.
- Digest worn out organelles
- Autolysis causing cell death.

Peroxisome

They are single membrane bounded vesicles with oxidizing enzymes.They are present in both plants and animals. Enzymes in peroxysome catalyze the breakdown of H_2O_2.

Functions

- Detoxification of peroxides
- Photorespiration in plants

Specialized peroxysomes called glyoxysomes are found in fat storing tissues in plants.

Glyoxysomes converts fatty acids into sugar.

Mitochondria

It is one of the most common organelles in eukaryotic cells. It is an elongated organelle with two enclosing membranes. Outer membrane is smooth but the inner membrane is convoluted to form cristae. Cristae increase the surface area and they contain stalk particles. The gap/space in between inner and outer membranes of the mitochondrion is called intermembrane space. The inner most part of the organelle is known as mitochondrial matrix, which consists of 70 s ribosomes circular DNA

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molecule (mitochondrial DNA), phosphate granules and enzymes. The matrix carries enzymes for the reactions in Krebs cycle (in cellular respiration). Further, cristae composed of proteins and enzymes essential for electron transport chain and oxidative phosphorylation.

Functions

- Synthesize ATP in aerobic respiration
- Involve in Photorespiration

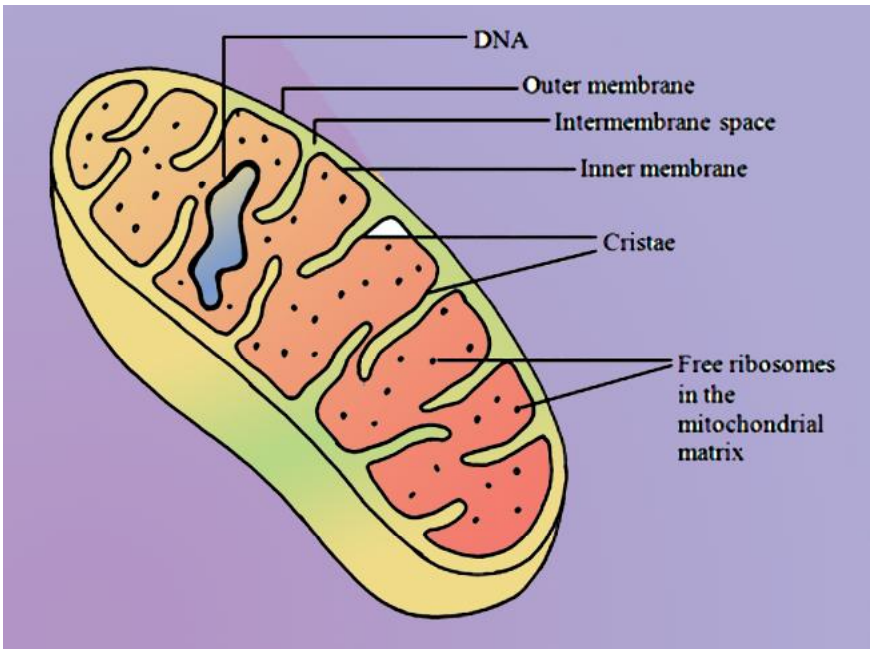


Fig 2.23: Structure of the mitochondria

Chloroplast

It is a biconvex lens shaped organelle with two membranes which is found in plants and some protists. The outer and inner membranes are smooth and are separated by a very narrow intermembrane space. Inside the chloroplast there is another membrane system. This membrane produces flattened and interconnected sacks called thylakoids. Thylakoids contain complexes called photosystems which are made up of photosynthetic pigments. Thylakoids stacked to form a granum. The grana are interconnected by inter granal lamellae. The fluid outside the thylakoid is stroma which contain circular DNA (chloroplast DNA), 70s ribosomes, many enzymes, starch granules and lipid droplets.

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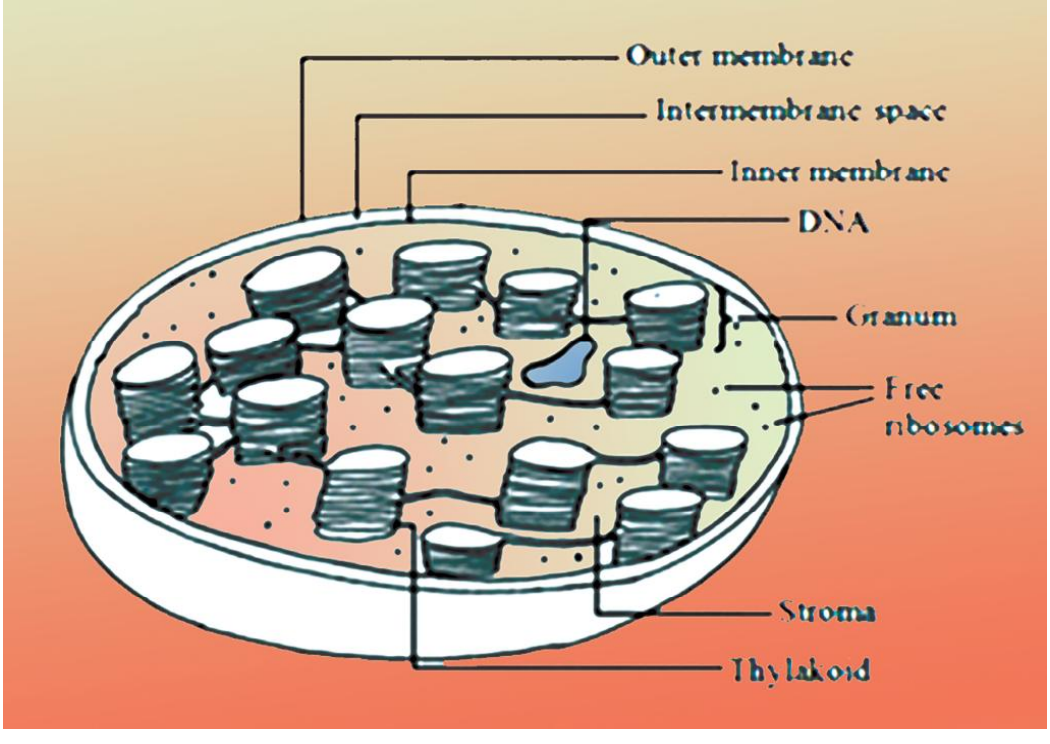


Fig 2.24: Structure of the chloroplast

Functions

- Photosynthesis

Cytoskeleton

Cytoskeleton is the supporting structure of the cell and maintains its shape. It is more important for animal cells which lack cell walls. Cytoskeleton is made out of microtubules and protein filaments. Additionally, it is Dynamic hence, has the ability to break and reform as needed.

There are three types of components in the Cytoskeleton as follows;

- Microtubules
- Actin filaments or Microfilaments,
- Intermediate filaments

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Table 2.5: Differences between Microtubules, Microfilaments and intermediate filaments

Property	**Microtubules (Tubulin polymers)**	**Microfilaments (Actin filaments)**	**Intermediate filaments**
Structure	Hollow tubes; wall consists of 13 columns of tubulin molecules	Two intertwined strands of actin, each strand is a polymer of actin subunitrs	Fibrous proteins supercoiled into thicker cables
Protein subunits	Tubulin	Actin	One of several different proteins (e.g. Keratin), depending on the cell type.
Main functions	Maintenance of cell shape Cell motility (as in cilia or flagella) Chromosome movements in cell division Organelle movements	Maintenance of cell shape (tension-bearing elements) Changes in cell shape Muscle contraction Cytoplasmic streamingin plant cells Cell motility (as in pseudopodia) Cell division in animal cells (cleavage furrow formation)	Maintaining of cell shape (tension-bearing elements) Anchorage of nucleus and certain other organelles.Formation of nuclear lamina

Functions

- Provide strength to the cytoplasm
- Anchorage organelles and cytosolic enzymes of the cell
- Movement of cytoplasm, cytoplasmic streaming, positioned organelles and move chromosomes when necessary.
- Maintain the shape of the cell (mainly in animal cells)

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Cilia and Flagella

Cilia and flagella share a common structure. Flagella are long elongated structures and Cilia are short cellular projections that are often organized in rows. Cilia are more numerous than flagella on the cell surface. They are made of microtubules, with a 9+2 structure (Nine doublets of microtubules are arranged in a ring, with two single microtubules in its center). They are covered by plasma membrane and bound to a basal body which anchors the cilium or flagellum to the cell. The Basal body has 9 + 0 arrangement (no microtubules in its center)

Functions

- Act as locomotor appendages
- Can move fluid over the surface of the tissue
- Cilia lining in oviducts help move an egg toward the uterus

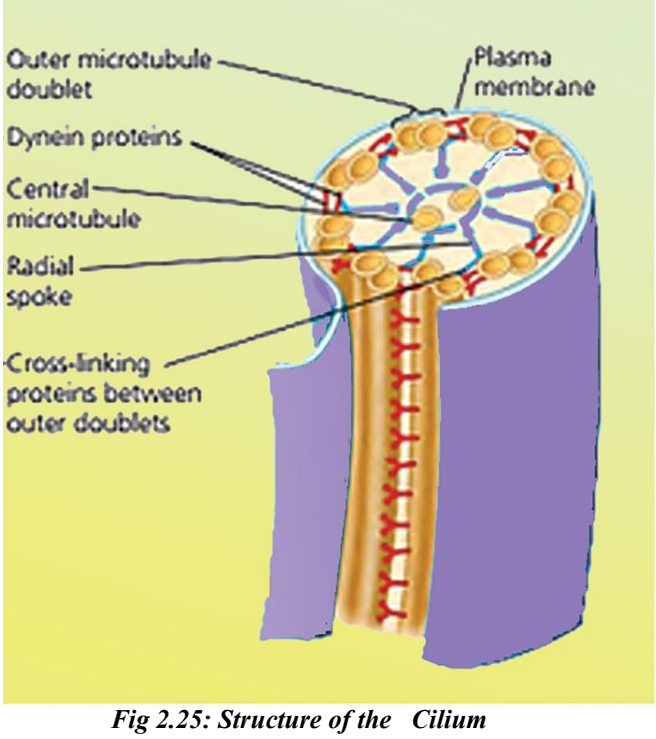


Fig 2.25: Structure of the Cilium

Centrioles

Centriole is made up of cylindrically arranged microtubules which are non membrane bounded subcellular component present only in animal cells. Each centriole composed of nine sets of triplet microtubules arranged in a ring (9+0). A pair of centrioles which arranged perpendicular to each other are located in a region called centrosome near the nucleus.

Functions

- Produce aster and spindle in cell division

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Central Vacuole

Central vacuole is a large structure, bound by tonoplast, filled with liquid called cell sap found in plant cells. The composition of sap differs from cytosol and it contains water, ions such as Potassium and Chloride and sometimes water soluble colored pigments such as anthocyanin.

Functions

- Stores water and other materials such as sugars, ions and pigments.
- Maintains water balance of the cell
- Gives turgidity and support to cell.
- Produce colours in some plants with sap pigments
- Stores soluble substances needed for cellular activities.

Extracellular components

1. Cell wall

Cell wall is an extracellular structure of plant cells. Animal cells do not have cell walls. However, prokaryotes, fungi and some protists also have a thin and flexible cell wall .The chemical composition of the wall greatly varies from species to species and even from one cell type to another even in the same plant. Nevertheless in Plants, cell wall is generally made up of cellulose, pectin, hemicellulose, lignin and suberin (in some plant cells only).

Plants generate two types of cell walls: primary and secondary walls. Young cells first secrete primary cell wall: it is the wall laid down during plant cell division.

Just outside the primary wall there is a thin layer (middle lamella) which is rich in sticky polysaccharides called pectins (magnesium and calcium pectate).Middle lamella glues adjacent cells together. Due to the deposition of hardening substances on the primary wall a secondary cell wall is generated secondarily.

Primary cell wall is permeable, relatively thin, flexible, composed mainly of cellulose fibers which are laid unevenly running through the extracellular matrix (middle lamella)Water can move freely through the free spaces of cell wall

Secondary cell wall lies between plasma membrane and primary cell wall. It contains several layers of hard materials, forming a rigid structure. In addition to cellulose, impermeable substances such as lignin and suberine are also incorporated in to the secondary wall. Lignin cement anchors cellulose fibers together providing hard and rigid matrix, giving the cell wall an extra support.

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Cell wall has pits through which cytoplasm of adjoining cells join through plasmodesmata.

Functions

- Protection and support
- Allows development of turgidity when water enters the cell
- Prevents bursting during turgidity
- Limits and control cell growth
- Component of appoplast pathway
- Maintaining cell shape
- hold the plant up against the force of gravity

2. Cell junctions

Cell junctions are structures at which neighbouring plasma membranes are joined. They are also interact and communicate via sites of direct physical contacts.

Functions

- Connects the internal chemical environment of adjacent cells.
- Cell junctions are structures at which cytoplasm of adjoining cells are joined. There are three types of cell junctions in animal cells
- Tight junctions – connect the plasma membranes of adjacent cells tightly bound by specific proteins forming continous seals around the cells. Prevent leakages of extracellular fluids through intercellular space. e.g. skin epithelium
- Desmosomes/Anchor junctions – mechanically attach the cytoskeletons of adjoining cells by intermediate filaments for strong binding.e.g. muscle tissue
- Gap junctions /Communicating junctions – provide cytoplasmic channels from one cell to an adjescent cell. Gap junctions consists of special membrane proteins that surround the pore through which ions, sugars amino acids may pass. They allow signal and material exchange between adjacent cells through direct connections. e.g.heart muscles, animal embryo.

Plasmodesmata

- Microscopic channels which runs through plant cell walls. They are cytoplasmic living connections between cytoplasm of adjoining cells. These are membrane lined channels filled with cytoplasm.

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Extracellular matrix of animal cells

Although animal cells lack cell walls they do have elaborate extracellular matrix (ECM). Main components of the ECM are glycoproteins and other carbohydrates containing molecules secreted by the cells. Most abundant glycoprotein in the ECM of most animal cell is collagen which forms strong fibres outside the cell. The collagen fibres are embedded in a network woven out of proteoglycan secreted by cells.

Functions

- Forms a protective layer over the cell surface
- Linking extra cellular matrix and cytoskeleton.
- Influences the cell behavior by Involving in the mechanical and chemical signaling.

The cell cycle and the process of cell division

The sequence of events that takes place in the cell from the end of one cell division to the end of the next cell division is referred to as cell cycle. At the end of the cell division, two genetically identical daughter cells resembling the parent cell are produced in mitosis.

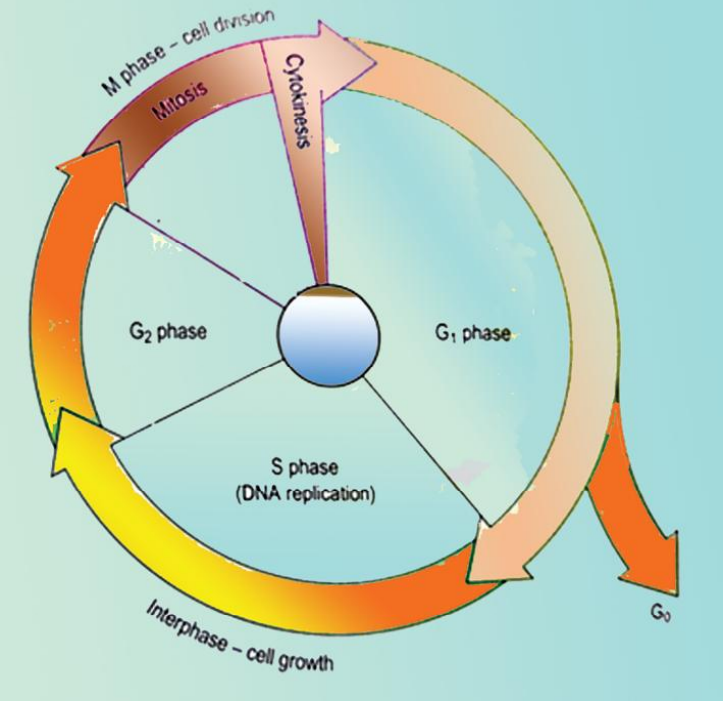


Fig 2.26: The cell cycle

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Eukaryotic cell cycle

Mitosis

Eukaryotic cell cycle may divided into two major phases.

- Interphase
- Mitotic phase/ M-phase

Interphase is the longer phase of cell division. It covers about 90% of the cell cycle. Interphase could be divided into three phases;

- G_1 phase (first gap phase)
- S phase (synthetic phase)
- G_2 phase (second gap phase)

G1 phase

In this phase synthesis of proteins and production of cellular organelles leading to cell growth occur. Proteins essential for S phase are produced during this phase.

S phase

DNA replication occurs and synthesis of histone proteins takes place. DNA wind around histone beads and form chromatin.

G_2 phase

Cells continue to grow through protein synthesis as well as cellular organelles. Proteins essential for mitotic phase will be synthesized. Duplication of centrosomes takes place.

There are cell cycle-controlling checkpoints available at G_1, G_2 and M phases to ensure that the cell is ready for moving into upcoming phases of cell division. Some cells receive a go-head signal at the G_1 check point, it will usually complete the G_1, S, G_2 and M phases and divide. If it does not receive a go head signal at that point it may exit the cycle, entering into a non dividing stage called the Go phase. The most cells of the human body are actually in the Go phase. e.g. nerve cells and muscle cells.

Mitotic phase/ M phase

M phase covers only about 10% of cell cycle. This includes mitosis and cytokinesis.

Mitosis

Mitosis is referred to the nuclear division which gives rise to two genetically identical daughter nuclei from a mother nucleus. This may get divided into five stages; prophase, prometaphase, metaphase, anaphase and telophase in order to ease the learning of activities of cell cycle.

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1. Prophase

Chromatin fibers get condensed by shortening and thickening and transformed into chromosomes. As a result chromosomes will be visible through light microscope. Nucleoli get disappeared and chromosomes appear with two sister chromatids attached at the centromere. Chromosomal arms of sister chromatids attached by special proteins called cohesion. The formation of mitotic spindles begins. Spindle includes the centrosomes, the spindle microtubules and the aster.

Centrosomes move toward opposite poles of the cell due to the lengthening of microtubules between them.

2. Prometaphase

The nuclear envelope fragments. Chromosomes get even more condensed. A special protein called kinetochore attaches the sister chromatids of each chromosome at their centromere. Some of the microtubules that attach to the kinetochore of the chromosomes move the chromosomes back and forth. Microtubules which are not attached to the kinetochore interact with those from the opposite poles.

3. Metaphase

Centrosomes reach the opposite poles. The chromosomes have arrived to a place called metaphase plate which is located in equal distance from each pole. The centromeres of all chromosomes are located in the metaphase plate. At the end of this phase, each chromosome of the cell get attached to the kinetochore microtubule at their centromere and aligned at the metaphase plate.

4. Anaphase

Sister chromatids are separated at the centromere. Microtubules attached to kinetochore get shorten and pull sister chromatids towards the opposite poles. Cell elongates as the non kinetochore microtubules are lengthen. By the end of anaphase equal and complete set of chromosomes found at each pole of the cell.

5. Telophase

Nuclear envelope reforms around each set of chromosomes at opposite poles. Nucleoli reappears. Spindle microtubules get deplolymerized. Chromosomes unwind and become less condense to form chromatin. Two genetically identical daughter nuclei are formed.

Cytokinesis

The division of the cytoplasam starts at the end of the telophase. Therefore at the end of the mitosis two genetically identical daughter cells are produced.

In animal cells- a cleavage furrow forms. This produces two genetically identical daughter cells.

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In plant cells- cell plate forms as a result of vesicle produced by golgi apparatus. This divides the cytoplasm in to two and generates two genetically identical daughter cells to the parent cell.

Significances of mitosis

1. Maintains the genetic stability
2. Growth and development
3. Cell repair, replacement and regeneration
4. Asexual reproduction

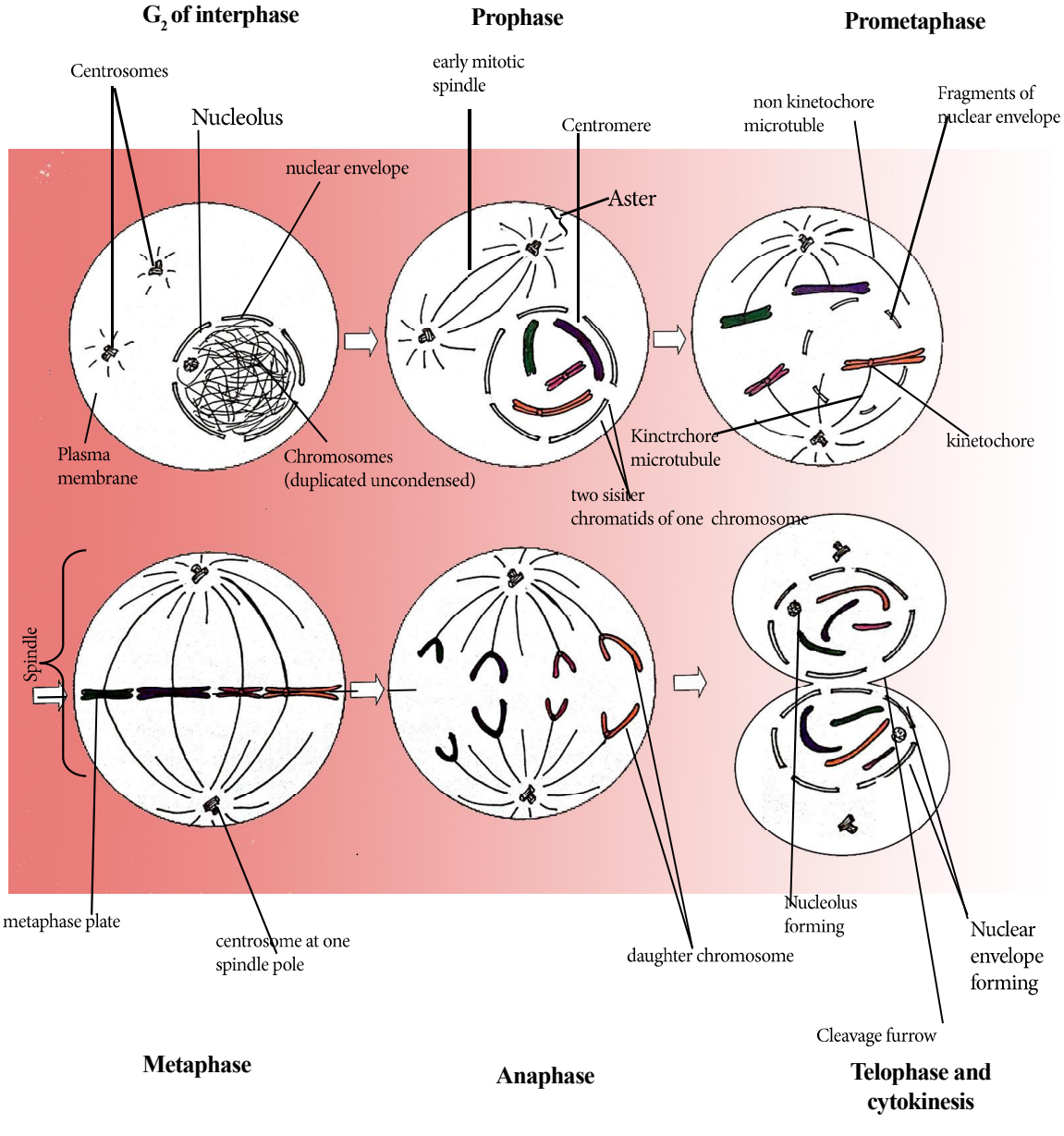


Fig 2.27 : The phases of mitotic cell cycle

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Meiosis

Sexually reproducing organisms undergo different type of cell division called meiosis.

Meiosis

Meiosis is a type of nuclear division which gives rise to four haploid, genetically non identical daughter nuclei, from a diploid mother nucleus.

Meiosis involves two consecutive nuclear divisions, Meiosis I and Meiosis II.

Meiosis I is a reduction division and Meiosis II is similar to mitosis, each stage consists of four sub-phases: prophase, metaphase, anaphase, and telophase.

Before meiosis one cell is in interphase, during S phase of the interphase DNA replication occur.

Meiosis I

1. Prophase I

Cell enters to the prophase from interphase.Chromosomes begin to condense. Nucleolus begins to disappear. Next the formation of zipper like structure called the synaptonemal complex by a specific proteins holds two homolg tightly together. The pairing and physical connection of homologous chromosomes is called synapsis.

During synapsis part of the DNA molecule of non-sister chromatids paired homologous chromosomes break, exchange and rejoin at corresponding point. This process is called crossing over. These points of crossing over become visible as chiasmata after the synaptonemal complex dissembles and the homologous chromosomes slightly apart from each other

Nuclear envelop breaks. Centrosomes move towards opposite poles forming spindle in animal cells.

The kinetochore of each homologue attach to microtubule from one pole or the other. The homologous pair then moves toward the metaphase plate.

2. Metaphase I

The pair of homologous chromosomes get arranged on the metaphase plate with one chromosome of each pair faces each pole. Both chromatids of a homologue are attached to kinetochore microtubules from one pole and those of the other homolog are attached to kinetochore microtubules from the opposite pole. Homologous chromosome arrange randomly at metaphase plate.

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3. Anaphase I

Kinetochore microtubules of the spindle get shorten. Homologous pair separates and one chromosome of each pair moves towards the opposite pole. Sister chromatids of each chromosome remain attached at the centromere and move as a single unit towards the same pole.

4. Telophase I

One complete haploid set of chromosomes accumulate at each pole. Nuclear envelope reforms around each set of chromosomes.Nucleoli reappear. Spindle disintegrates. Chromosomes decondensed into chromatin.Genetically non identical, haploid, two daughter nuclei are formed within one cell.

Cytokinesis

Usually occurs simultaneously with telophase I. Genetically non identical, haploid, two daughter cells are formed. In animal cells, cleavage furrow is formed. In plant cells a cell plate is formed.

No DNA replication occurs between meiosis I and meiosis II

Meiosis II

1. Prophase II

Centrosomes start producing spindle apparatus (spindle fibers, aster centrosome). Chromatin fibers condense and produce chromosomes with two sister chromatids. Nuclear envelope breaks down into fragments. Nucleolus disappears. During the late prophase II centromere of the chromosomes are moved to the metaphase II plate.

2. Metaphase II

All Chromosomes get attached to the microtubules at their centromere and aligned on the metaphase plate. Kinetochores of sister chromatids are attached to microtubules extending from both poles.

Due to the crossing over in meiosis I, the two sister chromatids of each chromosome are not genetically identical.

Meiosis II usually takes place in the perpendicular direction of Meiosis I. Therefore, metaphase plate of meiosis II is perpendicular to the metaphase plate of meiosis I.

3. Anaphase II

Due to the breakdown of proteins attaching sister chromatids, they are separated at centromere. As a result of shortening of microtubules , sister chromatids of each chromosome move towards opposite poles.

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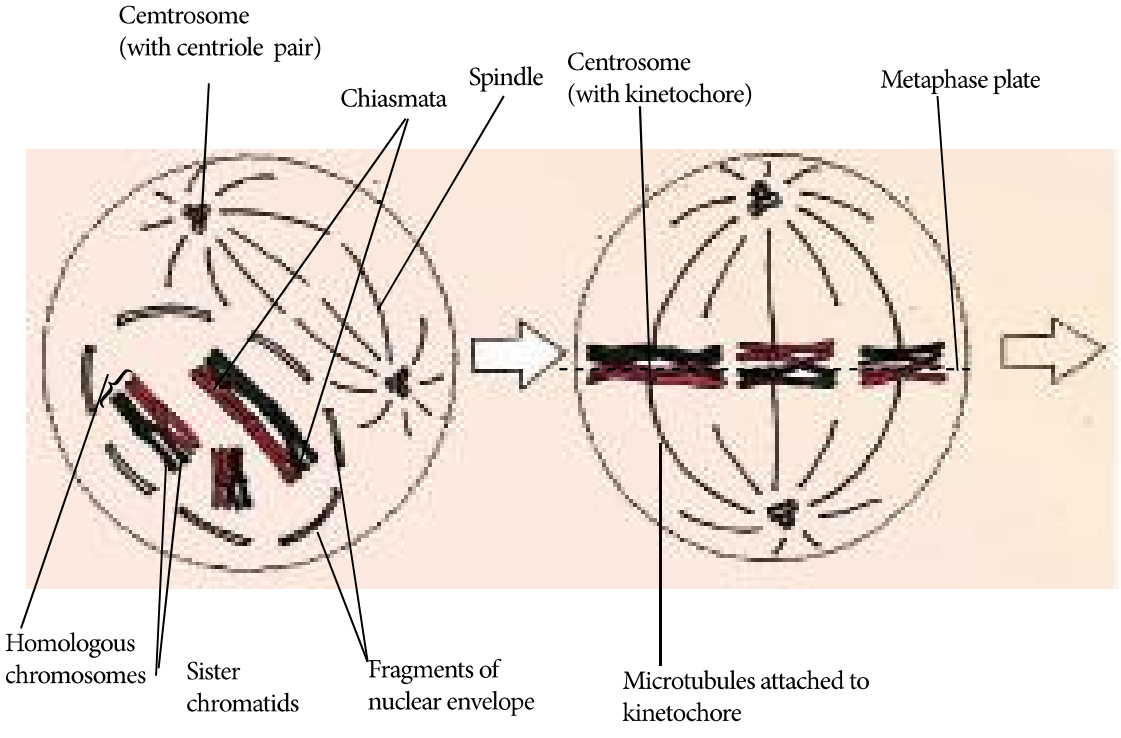
4. Telophase II

Nuclear envelope and nucleolus reform. Chromosomes decondense into chromatin. Spindle disassembles. Genetically non identical,haploid, four daughter nuclei are formed from one parent cell.

Cytokinesis

Cytokinesis occurs as in mitosis. Genetically non identical, haploid, four daughter cells are formed. These four daughter cells are not even identical to their parent cell.

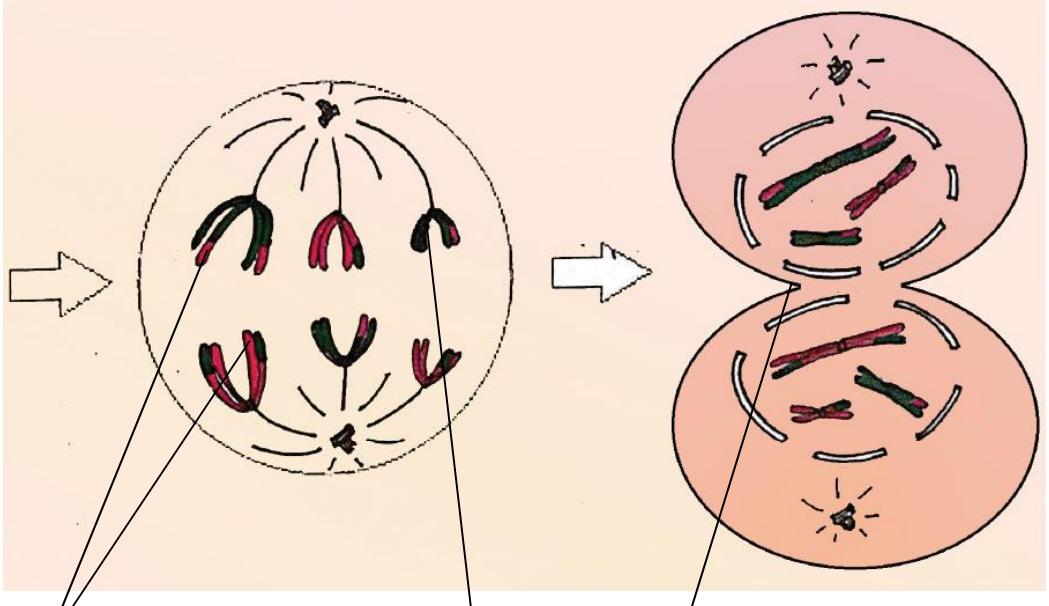
Centrosomes or centrioles are not available in plant cells. However, spindle is formed during cell division from accumulated microtubule complex.



Meiosis I
Prophase I

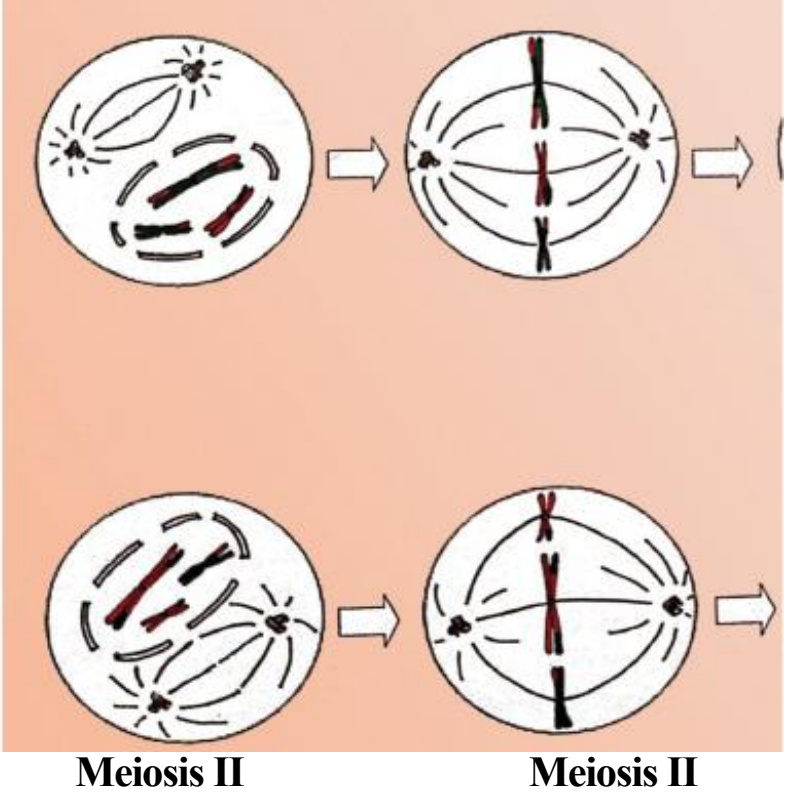
Meiosis I
Metaphase I

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Meiosis I
Anaphase I

Meiosis I
Telophase I and cytokinesis



Meiosis II
Prophase II

Meiosis II
Metaphase II

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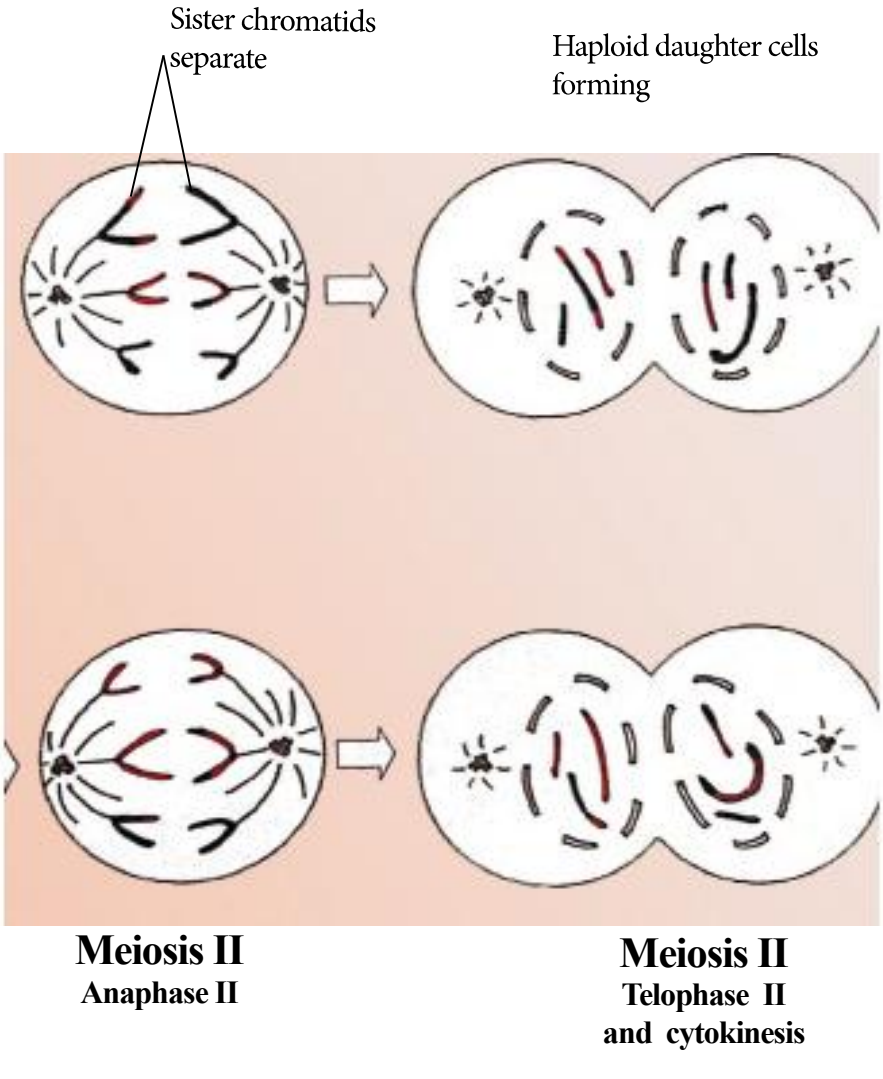


Fig 2.28: The phases of meiosis

Significance of meiosis

- Maintains the constant number of chromosomes through generations in sexually reproducing species.
- Producse new genetic variations leading to evolution.
- Genetic variation occurs due to crossing over ,recombination and independent assortment.

Tumor, cancer and galls

- Cell division is driven by external and internal factors. They may be chemical or physical factors
- Cancer cells do not respond to normally to the body's control mechanism
- They divide excessively and invade other tissues. If unchecked they can kill the organism.

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- Cancer cells do not consider the normal signals that regulate the cell cycle.
- They do not need growth factors. They may make required growth factors themselves or giving signals to continue cell cycle without growth factors. Another possibly is an abnormal cell cycle control system.
- The problem begins when a single cell in a tissue undergoes transformation, the process converts a normal cell to abnormal cell.
- If the body immune system can not recognize and destroy it, it may leads to proliferation of cells and formation of a tumor.
- If the abnormal cells remain at the original site, the lump is called benign tumor. Most benign tumors do not cause serious problems and can be completely removed by a surgery.
- A malignant tumor becomes invasive and attack one or more organs. An individual with a malignant tumor is said to have a cancer.
- A few tumor cells may separate from the original tumor, enter blood vessels or lymph vessels and travel to other parts of the body. They may proliferate and form a new tumor.
- This spread of cancer cells to locations distant from their original site is called metastasis.

Galls in plants

- This occurs due to uncontrolled mitotic division of plant cell.
- The plant cell division is controlled by maintaining a proper balance between plant growth regulators such as auxins and cytokinins. When this balance is lost plant cells produce undifferentiated mass of cells.
- Galls are the bumbs and growths that develop on different parts of plants after being invaded by some very unique organisms.
- Galls have range of causes, including viruses, fungi, bacteria, insects and mites.
- Usually the gall causers in some way attack or penetrate the plants growing tissues and causes the host to reorganize its cells and to develop an abnormal growth.

The energy relationships in metabolic processes

Sum of all biochemical reactions of living being is known as the metabolism and it consists of all catabolic and anabolic reactions.

Catabolism is breaking down of complex molecules into simple molecules by releasing free energy. Therefore it is an exergonic reaction. Anabolism is making complex molecules from the simple molecules by absorbing free energy. Hence it is an endergonic reaction.

Biochemical reactions involved in usage of energy released by catabolic reactions in

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living system are called as anabolic reactions. ATP acts as the energy carrier in all living organism including the simplest bacteria. Therefore the ATP is known as the universal currency of energy transactions.

Energy can be defined as the capacity to do work. All living organisms require energy for their living process in many ways. Such processes are;

- Synthesis of substances
- Active transport across plasma membrane
- Transmission of nerve impulses
- Muscle contraction
- Beating of cilia and flagella
- Bioluminescence
- Electrical discharges.

Overall idea of the energy relations of living system on biosphere is composed of following steps.

- Energy flows into biological systems from the environment through solar radiation. (Primary energy source is the Sun)
- Light energy is captured in the cells having photosynthetic pigments (chlorophyll) by the process of photosynthesis and stored as chemical energy in the organic compounds such as carbohydrates
- Captured energy in organic food is transformed into chemical energy in ATP by a process called cellular respiration.
- The energy stored in ATP is utilized in various energy requiring processes.

ATP (Adenosine Tri Phosphate)

ATP is a nucleotide, consisting of,

- Ribose- sugar
- Adenine - nitreogenous base
- A chain of three phosphate groups.

During the hydrolysis of ATP, ADP and Pi are produced. As a result, a very high energy is released. This is because the reactants (ATP and water) contain more energy in comparison to products (ADP and Pi). Therefore it yields energy and is an exergonic reaction.

When ATP is hydrolyzed, the free energy yield of each of the two end phosphate groups is -30.5kJ/mol.

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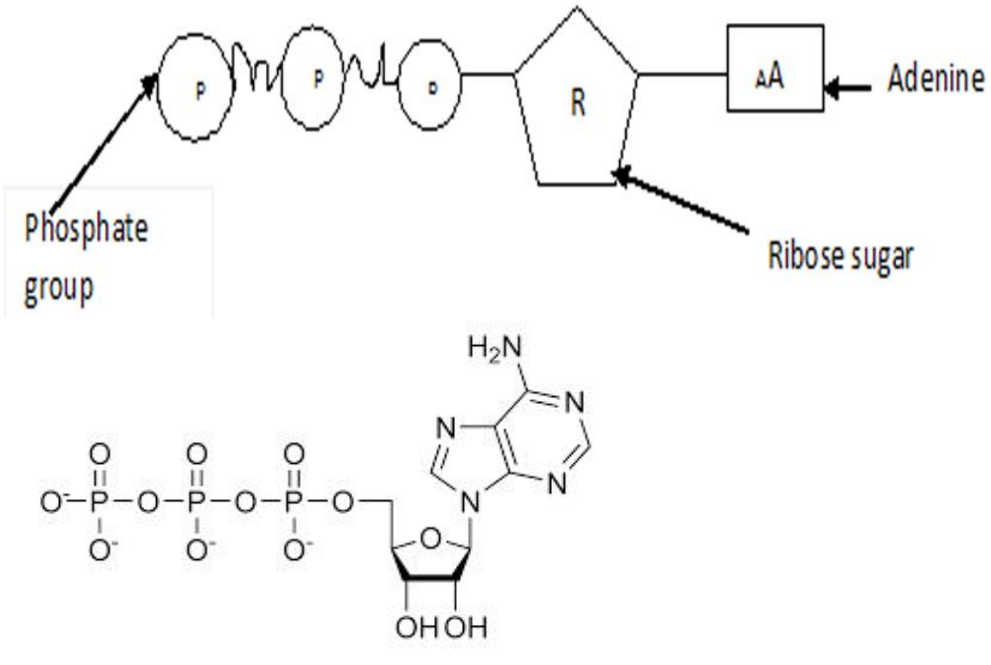


Fig 2.29: Chemical structure of ATP molecule (need not be memorized)

Most biological reactions use the energy released during breaking of the terminal phosphate bond. ATP is mobile. Therefore it can carry energy to anywhere in the cell, for any energy consuming reaction.

ATP can be produced within living cells within a short period of time, using ADP, inorganic phosphate (Pi) and energy. Production of ATP within cells is called phosphorylation. According to the energy source phosphorylation is divided as;

i. Photophosphorylation – synthesis of ATP using solar energy in photosynthesis

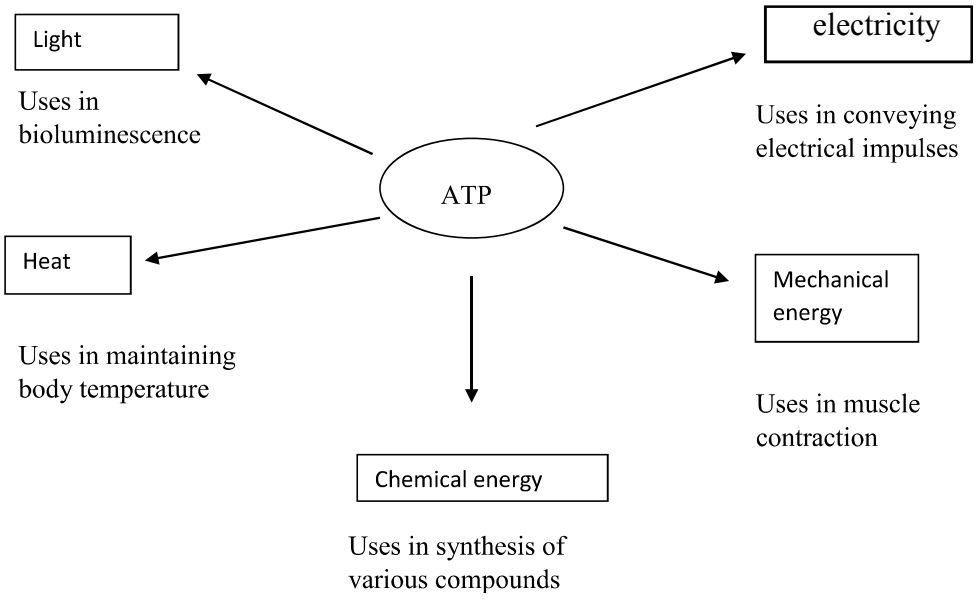
ii. Substrate phosphorylation – synthesis of ATP using energy released by the breaking down of complex molecules into simple ones.

iii. Oxidative phosphorylation – synthesis of ATP using energy released as a result of oxidation of molecules.

} In cellular respiration

In living cells energy in ATP is transformed in to various energy forms which are used for different functions.

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The role of Enzymes in regulating metabolic reactions

An enzyme is a macromolecule, which acts as a biological catalyst. Enzymes are produced in living cells/

General characteristics of an enzyme:

1. Most of the enzymes are globular proteins.
2. Enzymes are biological catalysts. They lower the activation energy of the reaction they catalyze (increases the rate of reaction).
3. Most enzymes are heat liable/ sensitive
4. Their presence does not alter the nature or properties of the end products ` of any reaction.
5. Enzymes are highly specific to the substrate (substrate specific)
6. Most enzyme catalyzed reactions are reversible.
7. The rate of enzyme activity is affected by pH, temperature and substrate concentrations.
8. They are not being used up during the reaction.
9. Enzymes possess active sites where the reaction takes place.
10. Some enzymes need non-proteinous components to catalyse the reaction which are known as cofactors.

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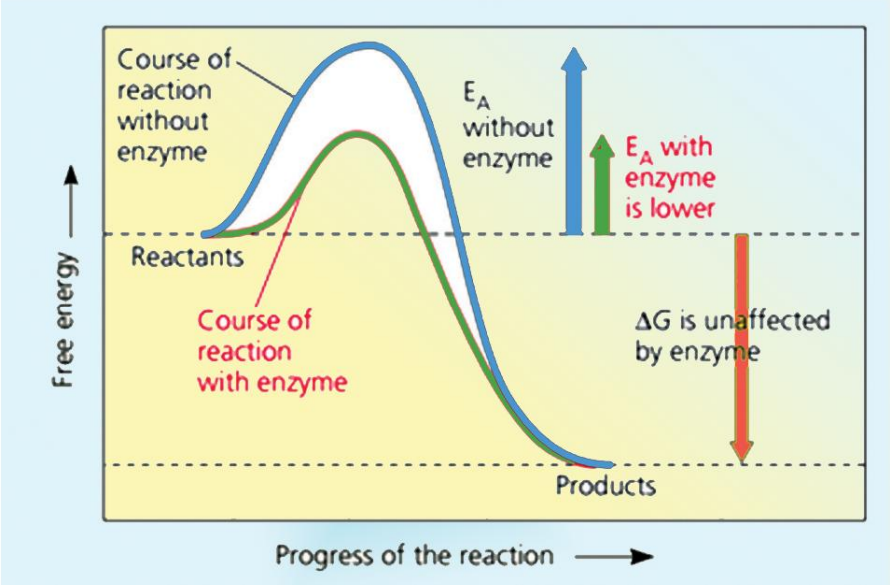


Fig 2.30 - The relationship between activation energy and the enzyme

The mechanisms of enzyme action

The reactant and enzyme acts on is referred to as the substrate. The enzyme binds to its substrate forming enzyme-substrate complex. While enzyme and substrate form their complex, catalytic action of the enzyme converts the substrate to the product.

Enzyme + substrate ⇔ Enzyme-substrate complex ⇔ Enzyme + Product

The reaction catalyzed by each enzyme is very specific. The specificity of an enzyme results from its shape. The substrate binds to a specific region of the enzyme. This region is called the active site. The active site is formed by only a few amino acids. Other amino acids are needed to maintain the shape of the enzyme molecule. The shape of the active site is complementary to the shape of the specific substrate of the enzyme, and hence important in the substrate specificity of the enzyme. The shape of the active site of an enzyme is not always fully complementary to its substrate. As enzymes are not rigid structures, the interactions between substrate and active site may slightly change the shape of the active site, so that the substrate and the active site become complementary to each other. This is called induced fit mechanism. The tight fit not only brings the substrate molecules and the active site close to each other, but also ensures the correct orientation of the molecules to help the reaction to proceed and catalyzes the conversion of substrate to product. Thereafter, the product departs from the active site of the enzyme. The enzyme is then free to take another substrate molecule into its active site.

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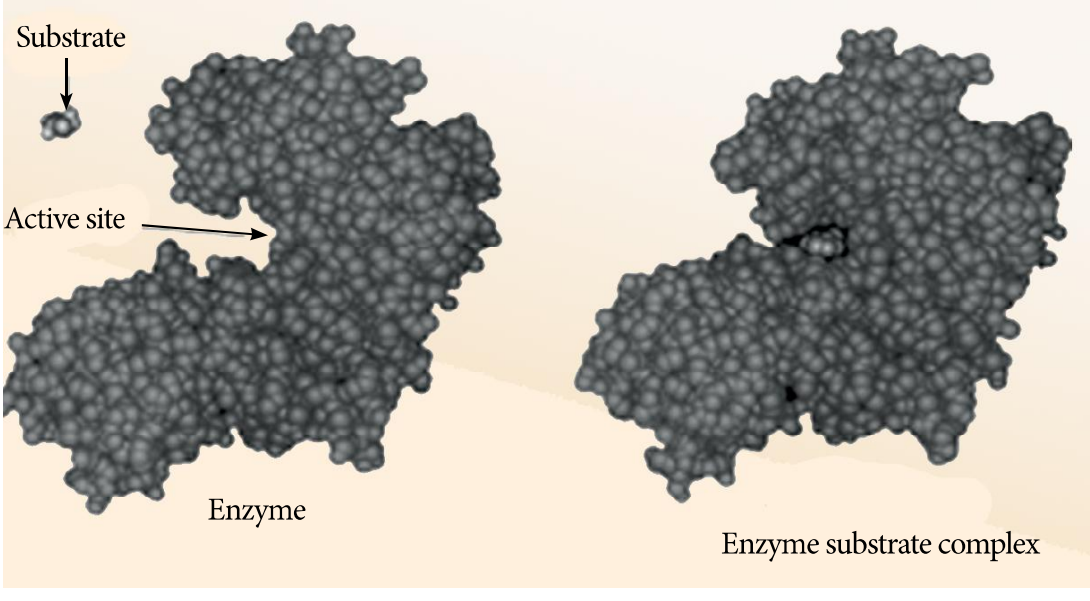


Fig 2.31: Induced fit between an enzyme and its substrate

Cofactors

Non-proteinuos components which are essential for the catalytic activities of certain enzymes are called cofactors.

These cofactors bind to the enzymes in two ways. Some tightly bind and remain permanently and others loosely bind temporarily. Loosely bound cofactors are reversible under certain circumstances.

Organic cofactors are called co-enzymes. e.g. derivatives of vitamins e.g. NAD, FAD and biotin

Inorganic co-factors – e.g. Zn^{2+}, Fe^{2+}, Cu^{2+}

Factors affecting the rate of enzymatic reactions

1. Temperature
2. pH
3. Substrate concentration
4. Enzyme concentration
5. Inhibitors

Temperature

Increase in temperature increases molecular motion. Therefore the speed of the moving molecules of both enzymes as well as the substrate will be accelerated. This will enhance the colliding probability for both enzyme active sites and substrate molecules. More collision between the enzyme active sites and substrate molecules generate greater chances for the reaction to occur. This can continue up to a certain

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point, after which there is a rapid decline in enzyme activity. This point is referred to as optimum temperature. This may vary from organism to organism.

e.g. most of the human enzymes have optimum temperature around the body temperature (35˚C-40˚C). Optimum temperature of bacteria in hot springs is about 70˚C.

When the temperature increases beyond the optimum temperature, the hydrogen bonds, ionic and other weak chemical bonds of enzyme active sites may be disrupted. This will result a change in the shape of the active site of enzyme which will alter the complementary nature of the active site of enzyme molecules. Therefore, the complementary binding of enzyme active sites and substrate molecules will be prevented. The above event is called as denaturation of enzyme molecules.

Therefore the rate of enzyme catalyzed reaction will start to decline when the temperature increases beyond the optimum temperature and stops completely at certain temperature, although rate of collision will keep on increasing.

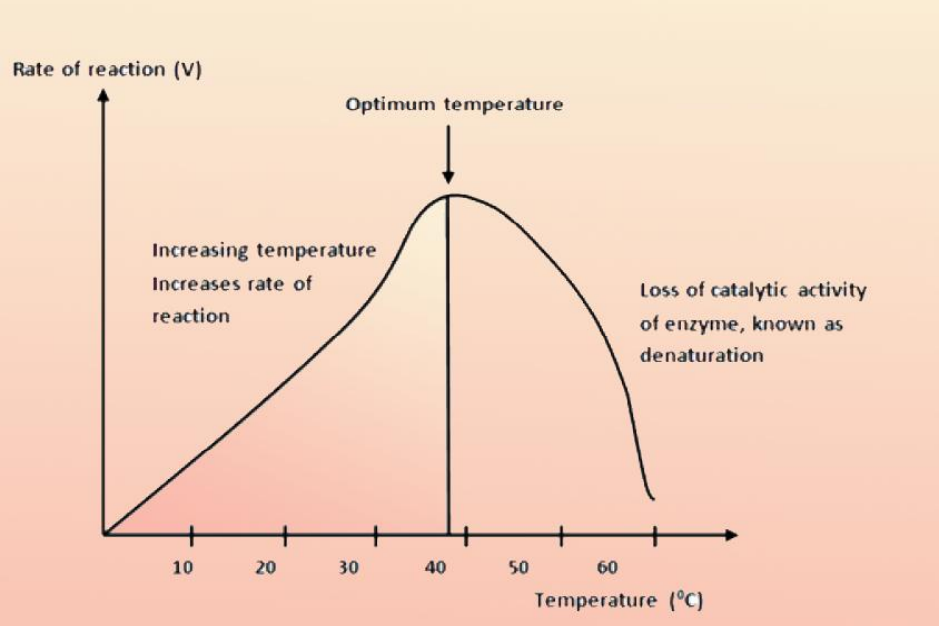


Fig -2.32 The graph of Rate of reaction (V) vs Temperature(T)

pH

Enzymes function most efficiently within a certain pH range despite maintaining temperature of the environment constant.

The narrow range of pH in which a particular enzyme catalyzed reaction takes place is named as the pH range. The pH at which the highest rate of reaction occurs is the optimum pH of the enzyme.The alteration in pH above or below the optimum pH may lead to decline in enzyme activity. This is due to the alteration of chemical bonds involving in formation of enzyme substrate complex. In most enzymes optimum pH range is 6-8, but there are exceptions. Pepsin works best at pH 2 and optimum pH for Trypsin is 8.

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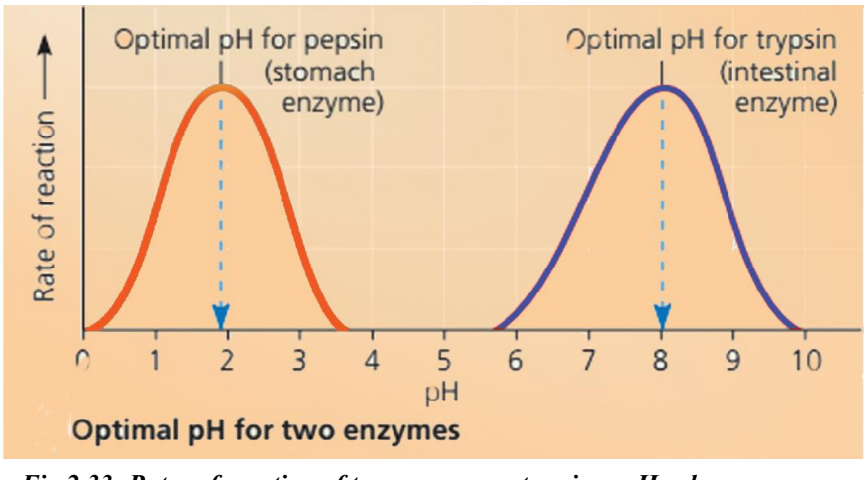


Fig 2.33- Rates of reaction of two enzymes at various pH values

Substrate concentration

Increasing substrate concentration increases the probability of collision between the enzyme and substrate molecules with correct orientation. However the enzyme molecules will be saturated after a particular concentration and therefore there will not be any further increase in the rate of reaction.

Enzyme inhibitors

Certain molecules or ions selectively bind permanently or temporarily to the enzyme molecules and prevent them from forming enzyme-substrate complex. These substances are called inhibitors.

They are either binding reversibly with weak interactions or binding irreversibly through covalent bonds.

e.g. Irreversible inhibitors: toxins, poisons

Reversible inhibitors- drugs used against microbes

Competitive inhibitors

Most of these are reversible inhibitors. These chemicals resemble the shape and nature of the substrate. Therefore they compete with the substrate selectively for the active site of certain enzymes. As a result of the above, the number of active sites available for the enzymes may decline and therefore reduces the rate of enzyme catalyzed reactions.

The above situation may be reversed by increasing the substrate concentration.

e.g. Protease inhibitor of drugs against HIV.-change

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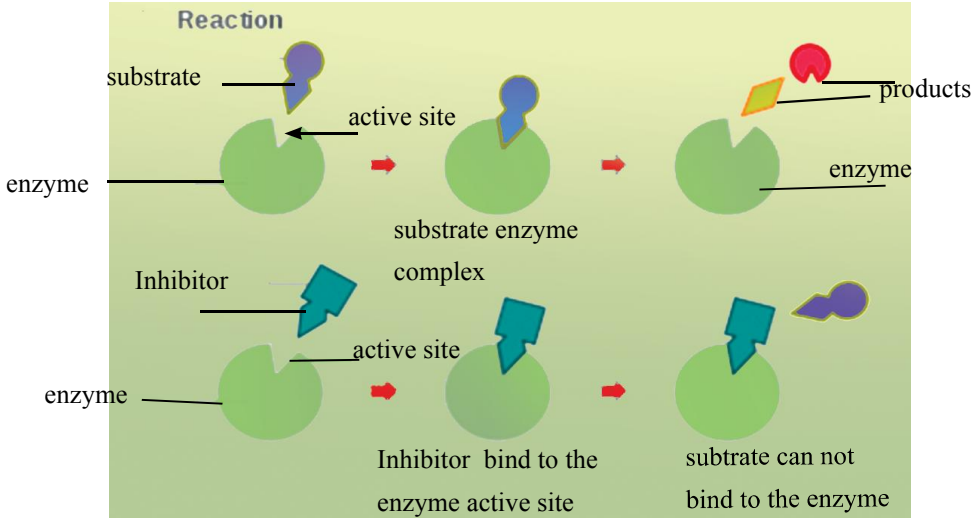


Fig 2.34: Competitive inhibitors

Non-competitive inhibitors

These chemicals do not compete with substrate molecules. They interrupt enzymatic reaction by binding to a part of the enzyme other than the active site. This causes the enzyme molecule to change its shape in such a way that the active site becomes less effective for the formation of enzyme substrate complex.

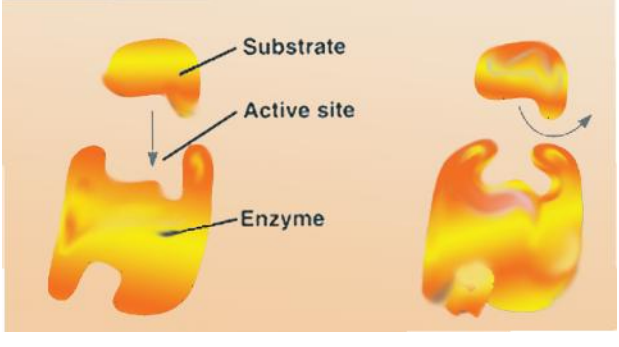


Fig 2.35: noncompetitive inhibitors

Regulation mechanism of enzymatic activity in cells

Allosteric regulation of enzymes

In many cases, the molecules that naturally regulate enzyme activity in a cell behave like reversible non-competitive inhibitors. Regulatory molecules (either activators or inhibitors) bind to specific regulatory sites elsewhere (other than the active site) of the molecule via non-covalent interactions and affect the shape and function of the enzyme. It may result in either inhibition or stimulation of an enzyme activity.

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a.) Allosteric activation and inhibition

Most enzymes regulated by allosteric regulation are made from two or more subunits. Each sub unit composed of a polypeptide chain with its own active site. The entire complex oscillates between two different shapes one catalyzing active and other inactive. In this two forms regulatory molecules bind to a regulatory site called allosteric site, often located where subunits join.

When an activator binds with this regulatory site, stabilizes the shape with functional active sites. Whereas the inhibitor binds with the regulatory site, it stabilizes the inactive form of enzyme. Subunits of enzyme arranged in a way through which they transmit the signals quickly other subunits. Through the interaction of subunits even a single activator or inhibitor molecule that bind to one regulatory site will affect the active site of all sub units. e.g. ADP function as allosteric activator bind to the enzyme and stimulates the production of ATP by catabolism. If the supply of ATP exceed demand catabolism shows down as ATP bind to the same enzyme as inhibitor.

b.) cooperativity

This is another type of allosteric activation. Binding of one substrate molecule can stimulate binding or activity at other active site. Thereby increase the catalytic activity.

e.g. hemoglobin (not an enzyme) is made up of four subunits each with an O_2 binding site. The binding of a one molecule of O_2 to one binding site increases the affinity for O_2 of the remaining binding site.

c.) Feedback inhibition

In feedback inhibition, a metabolic pathway is stopped by the inhibitory binding of its end product of a process to an enzyme. Thereby limit the production of more end products than required and thus wasting chemical resources.

Feedback inhibition

Feedback inhibition is an essential process regulates the end products produced in metabolism.

e.g. ADP function as allosteric activator and stimulates the production of ATP during the catabolism.

In case ATP supply exceeds demand, catabolism slows down as ATP molecules function as allosteric inhibitor.

Energy needed for all living processes is obtained directly from ATP. ATP is mainly produced by a process call ed cellular respiration, in living cells.

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Photosynthesis as an energy fixing mechanism

Photosynthesis

Photosynthesis is a metabolic process by which light energy is trapped and converted to chemical energy. Chemical energy is stored in chemical bonds of carbohydrates, fats, oils, and proteins. All life on Earth depends on photosynthesis either directly or indirectly. Photosynthesis also occurs in algae and certain prokaryotes.

Global importance of photosynthesis;

- All life on earth depends on photosynthesis, directly or indirectly
- Fulfill both carbon and energy requirements of organisms
- Provide O_2 for respiration of aerobic organisms
- Maintain O_2 and CO_2 balance in the atmosphere
- Production of fossil fuel
- Maintenance of global temperature

During photosynthesis $CO_2$is reduced by the H of H_2O and simple sugars are made using light energy. In eukaryotic photosynthetic cells, chloroplasts are the sites of photosynthesis.

Process of photosynthesis consists of two main stages and they are integrated.

- Light-dependent reaction
- Calvin cycle

There are two types of photosynthetic mechanisms (path ways) based on the number of C atoms of the first stable product of the CO_2 fixation.

- C_3 Mechanism – No of C atom of the first stable compound is three
- C_4 Mechanism – No of C atom of the first stable compound is four

Light-dependent reactions of photosynthesis take place in the membrane system of thylakoids. They are flattened fluid-filled sacs, which form stacks called grana at intervals. Chlorophylls, carotenoids and electron acceptors are located on this membrane system of thylakoids.

Stroma is a gel like structure containing soluble enzymes and other chemicals, which is the site of the Calvin cycle.

Photosynthetic pigments are substances which absorb visible light. In a leaf we see green colour because chlorophylls absorb violet, blue and red light and therefore, they transmit and reflect green colour. Different pigments absorb different wavelengths of light. In chloroplast, there are two types of chloroplast pigments such as chlorophylls and carotenoids. Chlorophyll a is the key light capturing pigment and they participate directly in the light reaction of photosynthesis.

According to the action spectrum, chlorophyll a is more effective for blue and red light. Chlorophyll b and caraotenoids (carotenes and xanthophylls) are effective in absorption of specific range wavelengths of corresponding to different colours.

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Other important function of some carotenoids is photoprotection. Photoprotection is absorption and dissipation of excessive light energy, if not that excessive light may cause damage to the chlorophylls or interact with oxygen and form reactive oxidative molecules which are dangerous to the cell.

Absorption spectrum

An absorption spectrum is a graph of the relative amounts of light absorbed at different wavelengths by a pigment.

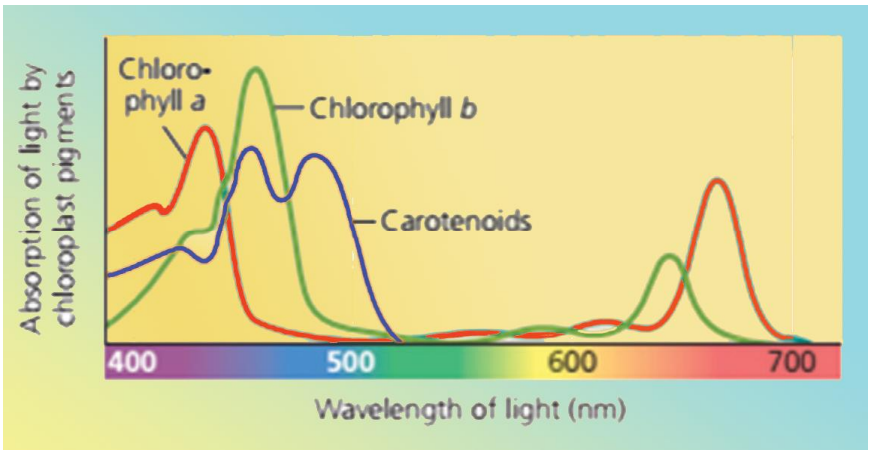


Fig. 2.36: Absorption spectrum

Action spectrum

An action spectrum is a graph showing the effectiveness of different wave lengths of light in stimulating the photosynthesis.

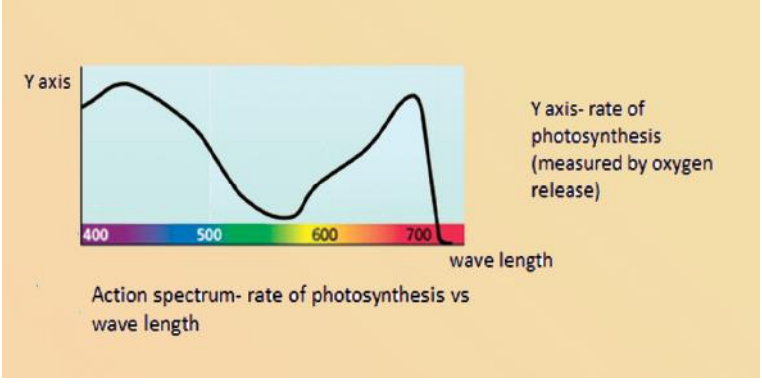


Fig 2.37: Action spectrum

Excitation of chlorophyll by light

When a molecule of chlorophyll or other photosynthetic pigment absorbs light it becomes excited. The energy from the light is used to boost electrons to a higher level and become positively charged. The excited state is unstable and returns to their original lower energy state. The excited electrons may pass through several electron carriers until they reach the final electron acceptor.

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Chlorophyll —(Light energy)→ Chlorophyll+ + e- (electron)

Therefore chlorophyll is oxidized and electron acceptor is reduced.

Photosystems

Chlorophyll molecules, other organic molecules and proteins are organized into complexes in the thylakoid membrane of chloroplasts. They are called photosystems.

A photosystem contains a reaction centre complex and light harvesting complexes. The reaction centre complex also contains a primary electron acceptor.

There are two types of photosystems found in the thylakoid membrane. They are Photosystem I (PS I) and photosystem II (PS II).In the PS I the chlorophyll a molecule is known as P700 since they absorb light at 700nm wave length effectively. In the PS II the reaction centre contains a chlorophyll a molecule which is known as P680 which absorbs light having a wavelength of 680 nm.

Light-dependent reaction /Light reaction photosynthesis

Linear electron flow

Light is absorbed by the photosynthetic pigments and synthesize ATP and NADPH due to the excitation of Photosystem I and Photosystem II which are embedded in the thylakoid membrane of chloroplast. The key to this energy transformation is a flow of the electron in one direction through the photosystems and other molecular components built in the thylakoid. This process is called linear electron flow.

The striking of photons of light on the pigments results in the excitation of electrons from the photosystem II to the higher energy state.

These electrons will be accepted by the primary electron acceptor of photosystem II.

Splitting of water takes place as a result of an enzyme catalyzed reaction and yields O_2 (g), H^+ ions and electrons.

Electrons released as a result of hydrolysis may neutralize excited photosystem II (P680).

Striking of photons of light on the pigments results in the excitation of electrons from photosystem I (P700) to the higher energy state. Excited electrons will be accepted by a primary electron acceptor of PSI.

Excited electrons of PS II at primary electron acceptor of PS II will pass through an electron transport chain to PS I and neutralize the excited PS I. The energy released due to the passage of electrons from higher energy state to lower energy result in the synthesis of ATP. This is known as photophosphorylation. Excited electrons of PS I at primary electron acceptor of PSI will pass through an electron transport chain and

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reduce NADP and yield NADPH. The reduction of NADP is catalyzed by an enzyme called NADP reductase.

Cyclic electron flow

This occurs in photosystem I but not in Photosystem II. Here some photoexcited electrons uses alternative cyclic pathway. This produces ATP but not NADPH and Oxygen are released.

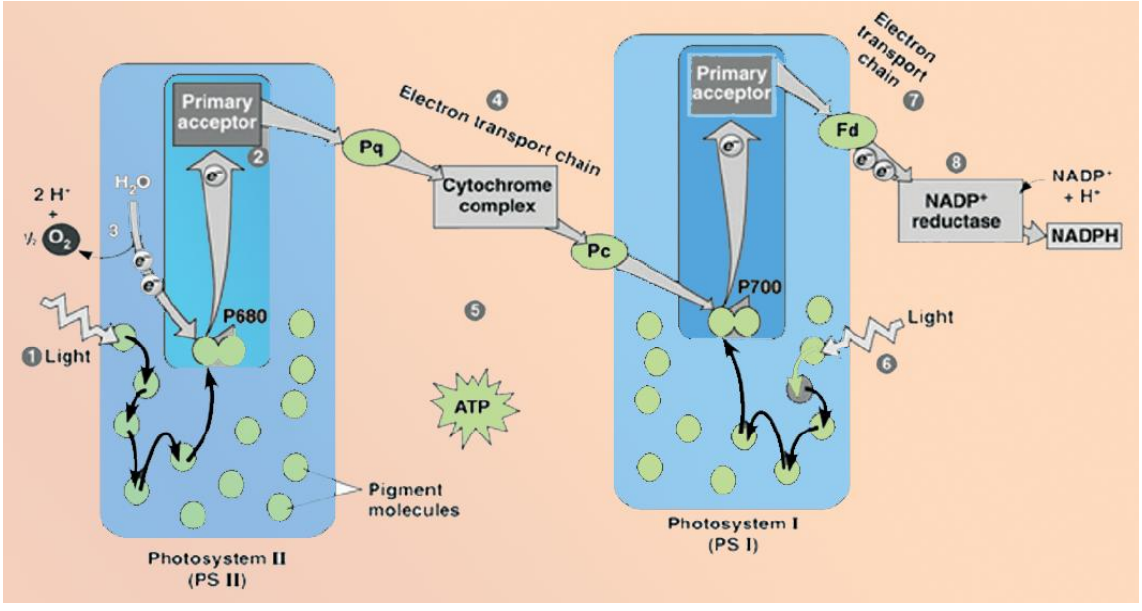


Fig 2.39 : Linear electron flow in the light reaction of photosynthesis

The Calvin cycle

The Calvin cycle takes place in the stroma of the chloroplast. Energy from ATP and NADPH produced by the light reaction are used to reduce CO_2. The reactions are catalyzed by enzymes and their sequence was discovered by scientist Calvin. This is an anabolic reaction. The first stable product of the Calvin cycle is glyceraldehyde 3-phosphate (G3P). For the net synthesis of one molecule of G3P, the cycle must take place three times.

The Calvin cycle of photosynthesis can be described in three steps;

Carboxylation (Carbon fixation)

Reduction

Regeneration of carbondioxide acceptor

Carbon fixation

The CO_2 acceptor is a 5 C sugar, Ribulose bisphosphate (RuBP). The addition of CO_2 to a RuBP is called carboxylation. The enzyme involves in this reaction is RuBP carboxylase oxygenase or Rubisco.

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The first product of RuBP carboxylation is a 6C molecule which is unstable and breaks down immediately in to two molecules of 3-phosphoglycerate (3-PGA). This is the first stable product of photosynthesis. The enzyme RuBP carboxylase oxygenase (Rubisco) is present in large amounts in the chloroplast stroma.

Reduction phase

1,3-Bisphosphoglycerate will be reduced to Glyceraldehyde 3- phosphate (G3P) through step by step. Enzyme catalyzed reactions utilizing NADPH and ATP from light reaction. G3P will act as a precursor for carbohydrate synthesis (glucose).

Regeneration of RuBP

RuBP is regenerated by undergoing a series of complex reactions. This process uses energy from ATP generated in light reaction.

Photorespiration

As its name suggests, Rubisco is capable of catalyzing two distinct reactions, acting as both a carboxylase and as an oxygenase.

In the oxygenase reaction of Rubisco uses the same substrate, RUBP, but reacts it with O_2. The reaction is catalyzed on the same active site as the carboxylation reaction. Thus CO_2 and O_2 are competitive substrates. Therefore CO_2 inhibits the oxygenase and O_2 Inhibits the carboxylase reaction.

The oxygenase reaction forms just one molecule of 3-PGA plus a two carbon product, 2-phosphoglycolate which is of no immediate use in the Calvin cycle and in higher concentrations it is toxic for the plant. It therefore has to be processed in a metabolic pathway called photorespiration. The photorespiratory pathway involves enzymes in the chloroplasts, peroxisome and mitochondria. (detail of this pathway is not expected).

Photorespiration is not only energy demanding, but furthermore leads to a net loss of CO_2 Each time Rubisco reacts with O_2 instead of CO_2 the plants makes 50% less 3-PGA than it would have done if CO_2 had been used. This potentially eliminates the net gain in photosynthetic carbon and loose the productivity.

These two factors result in an increase in photorespiration relative to photosynthesis so that an increasing proportion of carbon is lost as the temperature rises.

The CO_2. required for photosynthesis enters a leaf via stomata. However, stomata are also the main avenues of transpiration. On a hot, dry day, most plants close their stomata in order to conserves water. At the same time O_2. released from the light reactions begins to increase and this leads to further reduction of (CO_2.) to (O_2) ratio in the cytosol. These conditions within the leaf favor a wasteful process photorespiration under high temperature, dryness and high light intensities.

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Therefore plants developed different way to cope with this situation during the evolution that resulted a most successful solution to concentrate CO_2 around Rubisco provided by C4 photosynthetic pathway.

The establishment of C4 photosynthetic pathway includes several biochemical and anatomical modifications that allow plants with this pathway to concentrate CO_2 at the site of Rubisco. Thereby its oxygenase reaction and the following photorespiration are largely repressed in C_4 plants.

In most C4 plants the CO_2 concentration mechanism is achieved by a division of labor between two distinct specialized leaf cell types, the mesophyll and the bundle sheath cells. Compared to C3 plants the bundle sheath cells of C4 plants have expanded physiological functions. This is reflected by the enlargement and higher organelle content of these cells in C4 species. For the efficient function of the C4 pathway a close contact between mesophyll and bundle sheath cells are tightly interconnected to each other by high numbers of plasmodesmata. The bundle sheath cells enclose the vascular bundles and are themselves surrounded by the mesophyll cells and this type of leaf anatomy was termed Kranz anatomy.

Since Rubisco can operate under high CO_2 concentrations in the bundle sheath cells, it works more efficiently than in C3 plants. Because of the CO_2 concentration mechanism they can acquire enough CO_2 even when keeping their stomata more closed and minimize the water loss by transpiration.

C4 pathway of photosynthesis

In the mesophyll cells of C4 plants CO_2 is converted to bicarbonate by carbonic anhydrase and initially fixed by phosphoenolpyruvate carboxylase using PEP as CO_2 acceptor. The resulting oxaloacetate (OAA) is composed of four carbon atoms, which is the basis for the name of this metabolic pathway. Oxaloacetate is rapidly converted to the more stable C4 acids malate or aspertate that diffuse to the bundle sheath cells. Here, CO_2 is released by decarboxylating enzymes and the released CO_2 is refixed by Rubisco, which exclusively operates in the bundle sheath cells in C4 plants.

Chloroplasts found in mesophyll cells are different in anatomy in comparison to chloroplasts of bundle sheath cells.

Since chloroplasts of mesophyll cells carryout only light reaction, they are rich in grana. The grana of mesophyll chloroplasts are large and highly differentiated for light reaction. Bundle sheath chloroplasts possess a very few, less differentiated grana or grana are absent. Moreover, that PSII in the bundle sheath cells are depleted in order to lower oxygen production in these cells.

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$CO_2 + H_2O \xrightarrow{\text{carbonic anhydrase}} HCO_3^- + H^+$

$PEP + HCO_3^- \xrightarrow{\text{PEP carboxylase}} \text{Oxaloacetate (4C)}$

This PEP carboxylase enzyme is much more efficient than the enzyme of RUBP carboxylase for two reasons.

1. It reacts with bicarbonate (HCO^-_3) rather than with CO_2, The advantage of this is that there is a 50-fold higher concentration of HCO_3- than CO_2 in solution in the cytosol.
2. It has no affinity for O_2

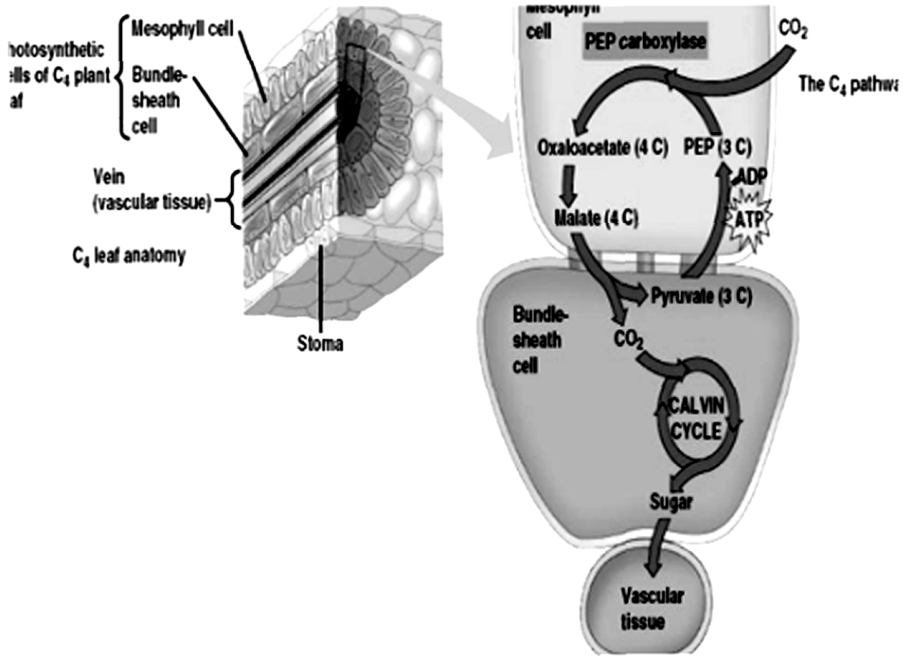


Fig 2.39 : The C4 Pathway

Significance of the C4 pathway

- Helps plants to improve the efficiency of CO_2 fixation at lower CO_2 concentrations by preventing the gateways for photorespiration by spatially separating Rubisco.
- In hot-dry climate, it is essential for the stomata to close to prevent water loss through transpiration. This reduces CO_2 intake of particular plants. Therefore, plants in tropical zones or hot climate may suffer from CO2 deficiency. At lower CO_2 concentrations, C4 mechanism increases the efficiency of photosynthesis by concentrating CO_2 in the bundle sheath cells.

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C4 plants exhibit better water-use efficiency than C3 plants because of the CO_2 concentration mechanism they can acquire enough CO_2 even when keeping their stomata more closed. Thus water loss by transpiration is reduced.

Since Rubisco can operate under high CO_2 concentrations in the bundle sheath cells, it woks more efficiently than in C3 plants, consequently C4 plants need less of this enzyme, this leads to a better nitrogen-use efficiency of C4 compared to C3 plants.

Table 2.6: Comparisons of C3 and C4 plants

Characteristics	C3 plants	C4 plants
Representative species	Wheat, rice barley	Maize, sugarcane, grasses
Temperature optimum for photosynthesis (^{0}C)	15-25	50% greater at 35 ˚C
CO_2 fixation	Occurs once	Occurs twice, first in mesophyll cells, second in bundle sheath cells
CO_2 acceptor	5C, RuBP	3C, PEP mesophyll cells 5C RuBP in bundle sheath cells
CO_2 fixing enzyme	Rubisco	PEP carboxylase in mesophyll cells which is very efficient Rubisco in bundle sheath cells, working efficiently under high CO_2 concentration
First product of CO_2 fixation	C3 acid, 3- phosphoglycerate (3-PGA)	4C acid, oxaloacetate (OAA)
Leaf anatomy	Bundle sheath cells, if present, are not green (non photosynthetic), photosynthesis occurs in Mesophyll cells	Kranz anatomy with photosynthesis occurring in both mesophyll cells and bundle sheath cells
Productivity	Yield is usually lower	Yield is usually high

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Factors affecting photosynthesis

The rate of photosynthesis is an important factor in crop production. Rate is affected by various factors.

e.g. light intensity, CO_2 concentration, temperature, water, pollutants and inhibitors

The photosynthesis involves a series of reactions. Therefore various factors are involved in it.

Blackman who is the scientist first proposed the idea of principal of limiting factors.

When a chemical process is affected by more than one factor, its rate is limited by the factor which is nearest its minimum value.

e.g. Light intensity

Light Intensity

The rate of photosynthesis increases linearly with increasing light intensity. Gradually the rate of increase falls off as the other factors become limiting. Very high light intensities chlorophyll may bleach and slow down photosynthesis. However, plants exposed to such conditions are usually protected by devices such as thick cuticles, hairy leaves.

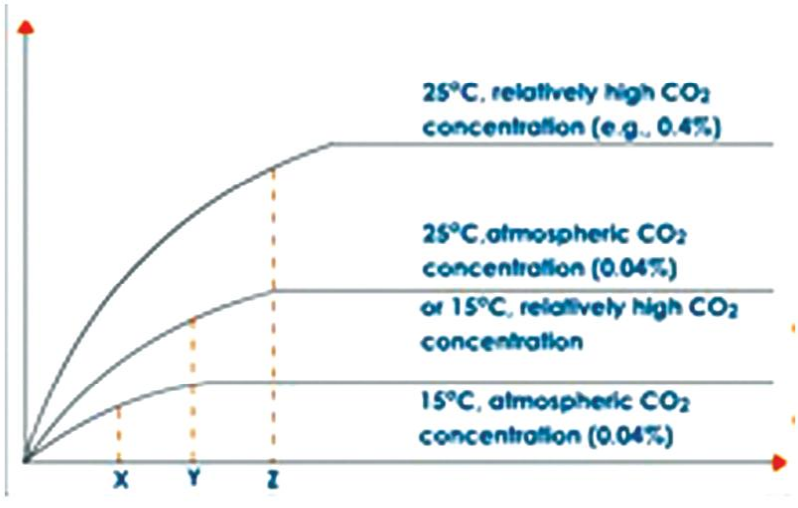


Fig 2.40: Rate of photosynthesis with light intensity at different temperatures

Under normal conditions, CO_2 is the major limiting factor in photosynthesis. Increase in photosynthetic rate is achieved by increasing CO_2 concentration. For example some greenhouse crops such as tomatoes are grown in CO_2 enriched atmosphere.

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Cellular respiration as a process of obtaining energy

Cellular respiration is the process by which chemical energy in organic molecules such as carbohydrates is released by stepwise oxidative process, catalyzed by enzymes and made available in living cells in the form of ATP. Cellular respiration is divided as

a) aerobic respiration

b) anaerobic respiration

Aerobic respiration:-

The process of synthesize ATP from the respiratory substrates such as glucose in the presence of molecular oxygen (O_2) known as aerobic respiration. Glucose is found to be the major respiratory substrate in living cells.

The aerobic respiration of glucose molecules can be represented by the following balanced chemical equation.

$$C_6H_{12}O_6 + 6O_2(g) \longrightarrow 6CO_2(g) + 6H_2O + \text{Energy (ATP+ heat)}$$

This process consist three main steps. They are;

a) Glycolysis

b) Pyruvate oxidation and citric acid cycle (Kreb's cycle)

c) oxidative phosphorylation (Electron transport chain)

Glycolysis

It takes place in the cytosol of the cell, because all enzymes that catalyze reactions of the glycolysis are found in the cytosol of the cell. This process does not depend on O_2.

During the above process a six carbon (6C) glucose molecule is broken down step by step into two three-carbon (3C) pyruvate molecules.

Two ATP molecules are used up to initiate the process.

Four hydrogen molecules and electrons released from glucose breakdown reduce two NAD^+ and produce two NADH. At the end of glycolysis there will be four ATP molecules produced. Since two ATP molecules were used up for the initiative step, the net yield will be two ATP molecules.

Only when O_2 is present, the pyruvate molecules will enter the mitochondria and further steps will take place.

Oxidation of Pyruvate/ Link reaction

These two pyruvate molecules enter mitochondrion by active transport through the membrane. In the matrix of mitochondria, Pyruvate is converted to acetyl group by releasing two CO_2 molecules. Then this acetyl group combines with co-enzyme A to

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produce Acetyl co-A. In this reaction two NAD^+ is converted to two NADH molecules. Therefore this step can be represented as follows.

$2C_3H_4O_3 + 2CoA + 2NAD^+ \longrightarrow 2$ Acetyl Co-A $+ 2CO_2 + 2$ NADH

Oxidation of pyruvate is a linking reaction of glycolysis and citric acid cycle.

Acetyl Co-A will feed its acetyl group for citric acid cycle.

Citric acid cycle

This takes place in the matrix of mitochondria using specific enzymes. As the first product of this cyclic pathway is citric acid, it is known as citric acid cycle. The pathway was discovered by a German-British scientist Hans Krebs. Hence,it is named as Kreb's cycle. Citric acid contains three carboxylic acid groups. This cycle is also known as Tricarboxylic Acid cycle or TCA cycle.In the citric acid cycle 4 C compound oxalo acetate combines with 2 C compound acetyl Co -A to form 6 C compound, citric acid. Then citric acid undergoes a series of reactions to regenerate oxaloacetate by releasing two CO_2 molecules by decarboxylation reaction. One ATP molecule is produced by substrate level phosphorylation. One $FADH_2$ and three NADH will be generated as a result of oxidation reactions. These are the products of a single acetyl group led into citric acid cycle and hence these numbers should double when the yield for a glucose molecule is considered.

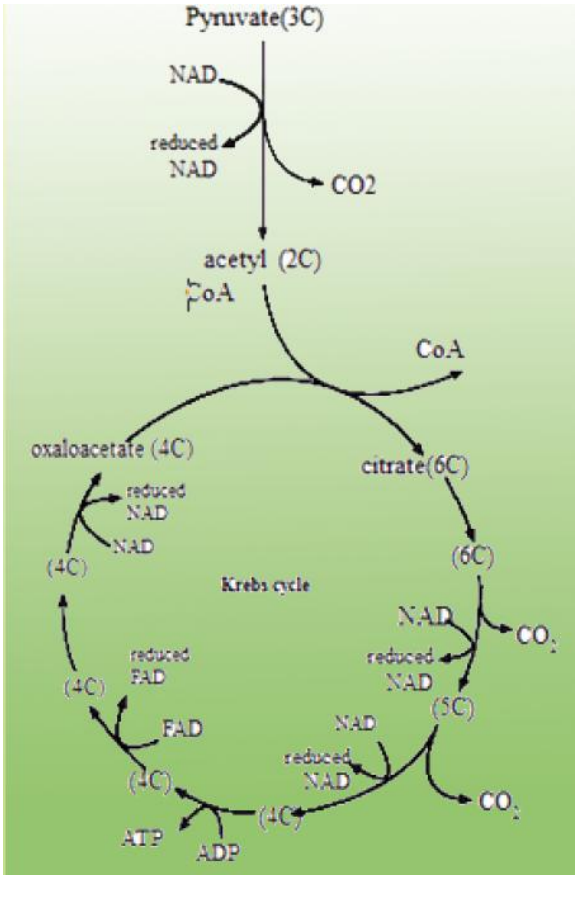


Fig 2.41: Krebs cycle (mechanism is not necessary for the examination)

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Electron transport chain

This step is taken place across the inner membrane (cristae) of mitochondria. The folding of cristae increases surface area for oxidative phosphorylation. NADH and $FADH_2$ products in the early stages of aerobic respiration are oxidized by transferring electrons, through the electron transport chain and finally to molecular oxygen (O_2). The electron transport chain is located in the inner membrane of mitochondrion and composed of series of protein and non-protein molecules involving in the movement of electrons and protons across cristae. Therefore, the Molecular oxygen(O_2)is the final electron acceptor in aerobic respiration. In the electron transport chain, ATP is synthesized by oxidative phosphorylation.

In this electron transport chain, energy is released progressively from NADH and $FADH_2$ and that energy is used to synthesize ATP. When one molecule of NADH is oxidized in the electron transport chain, 2.5 molecules of ATP in average are generated due to oxidative phosphorylation. When one molecule of $FADH_2$ is oxidized 1.5 molecules of ATP in average are produced due to oxidative phosphorylation. Total number of ATP that is produced in this step is 28.

This is true in the active cells such as liver cells and cardiac muscle cells but not in other cells where two ATP produced in glycolysis is used to transport 2NADH from cytosol to mitochondrial matrix. In those cells total number of ATP produced by one molecule of glucose is (32-2) = 30 ATP.

Total number of ATP molecules produced from one molecule of glucose, during aerobic respiration.

In glycolysis;

As ATP $\longrightarrow$ 2ATP

From 2NADH $\longrightarrow$ 5ATP (oxidation phosphorylation)

In pyruvate oxidation;

From 2NADH $\longrightarrow$ 5ATP (oxidation)

In Citric acid cycle;

As ATP $\longrightarrow$ ATP (substrate level phosphorylation)

From 6NADH $\longrightarrow$ 15ATP (oxidative phosphyrlation)

From 2 $FADH_2$ $\longrightarrow$ 3ATP

$\therefore$ Total Number of ATP = 32 ATP

Anaerobic respiration

Anaerobic respiration is breaking down of glucose in the absence of molecular oxygen which is regulated by enzymes of the cells occurring in cytosol. In the absence of molecular oxygen pyruvate molecules cannot be broken down further. ATP generated is utilized to fulfill energy requirements. However, NADH produced during glycolysis cannot be utilized. Therefore, since $NAD^+$is limited it is essential for the cell to recycle NADH to enhance the availability of NAD^+ other than anaerobic respiration

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fermentation is a method of production of ATP in the absence of O_2 . There are many types of fermentation, differing from end products formed by pyruvate. The two common types are;

1. Ethyl alcohol fermentation
2. Lactic acid fermentation

Ethyl alcohol fermentation

- Like in aerobic respiration, the first step of this is also Glycolysis.
- Therefore one molecule of glucose is converted to 2 molecules of pyruvate giving 2 molecules of ATP and two molecules of NADH
- Then this pyruvate involve in two steps. In the 1st step pyruvate is converted in to Acetyldehyde, releasing a molecule of CO_2.
- In the second step acetyldehyde is reduced to ethanol using NADH that is produced in Glycolysis.
- Therefore final hydrogen acceptor in ethyl alcohol fermentation is acetylaldehyde (organic compound)
- Many bacteria carry out ethyl alcohol fermentation. The most common organism which carries out ethyl alcohol fermentation is yeast.

Lactic acid fermentation

- As in ethyl alcohol fermentation, Glycolysis takes place as the first step of lactic acid fermentation.
- Therefore one molecule of glucose produces two molecules of pyruvate, two molecules of ATP and two molecules of NADH.
- Then pyruvate is reduced directly by NADH for lactic acid as an end product with no release of CO_2. Therefore final H acceptor is also organic compounds.
- Certain fungi and bacteria carryout lactic acid fermentation but the most common organisms are lactic acid bacteria involved in formation of yoghurt and curd.

Respiratory quotient

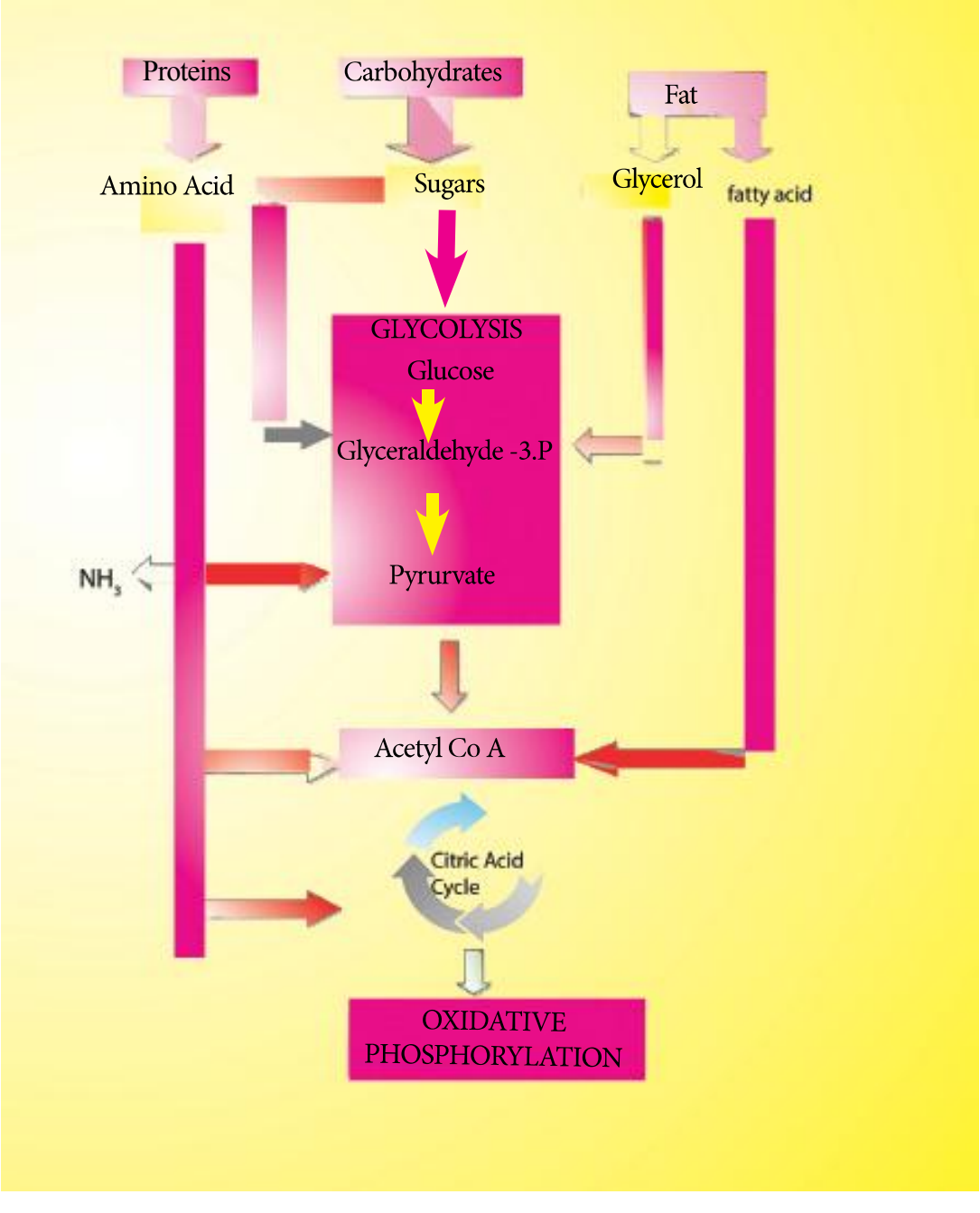
It is the ratio of CO_2 evolved and the volume of O_2 consumed in a given time for the respiratory substrate.

$RQ = V\,CO_2 / VO_2$

RQ of respiration of carbohydrates, fats and proteins are 1.0, 0.7 and 0.8 respectively.

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Use of proteins, Carbohydrates, fats in respiration



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03

Evolution and Diversity of Organisms

The theories of origin of life and natural selection to analyze the process of evolution of life

Origin of life on earth

Condition of earth before life

Earth and the other planets of the solar system were formed about 4.6 billion years ago. At the beginning of the solar system, planet Earth was being bombarded by chunks of rocks and ice.

The first atmosphere was probably thick with water vapour, along with various compounds released by volcanic eruptions, including nitrogen and its oxides, carbon dioxide, methane, ammonia, hydrogen and hydrogen sulphide. The neutral atmosphere then turned to be a reducing one. The first atmosphere had little oxygen. Later earth was cooled down and the water vapour condensed into the ocean. Some of the hydrogen quickly escaped into the space. Volcanic eruptions, lightening, extreme UV radiation, hydro thermal vents and alkaline vents along with the Earth's reducing atmosphere favored the synthesis of organic molecules essential for the origin of life. These simple organic molecules then polymerized to form macromolecules such as proteins and nucleic acids. Further, the formation of self-replicating organic molecules made life possible on earth.

Evolution of Biological Diversity

1. Biochemical evolution

Direct evidence for life on early earth comes from fossils of micro-organisms that are about 3.5 billion years old. Observations and experiments in chemistry, geology and physics have provided evidence, for the appearance of the first living cells. The theory of the biochemical evolution arouse from the hypothesis based on chemical and physical processes on early earth. The emerging force of natural selection could have produced the first cells through a sequence of four main stages.

1. Atmospheric conditions of early earth facilitated the abiotic synthesis of small organic molecules such as amino acids and nitrogenous bases from inorganic molecules

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2. Polymerization of the above small organic molecules leads to the formation of organic macromolecules.

a. Amino acids $\xrightarrow{\text{polymerization}}$ proteins

b. N-base + sugar+ phosphate $\longrightarrow$ Nucleic acids

3. Organic macromolecules were packed into membranes, to produce protocells

4. Nucleic acids gained self replicating capability, which made inheritance possible for the cells.

2. Origin of protocell

Haldane suggested that the early oceans were a solution of organic molecules "primitive soup" in which life arose. Recent studies related to the volcanic-atmosphere and alkaline vents show the abiotic synthesis of organic molecules. Another source of organic molecules may have been meteorites. RNA accumulated into lipid bound vesicles and formed "protocells" which exhibited enzyme catalyzed activities and were able to grow, replicate and evolve. The early genes and enzymes would have been RNA which enabled replication of RNA. Other molecules that were in the primitive soup were also collected in the protocell. Growth occurred by addition of lipids to the membrane by collision of micelles. When the protocell becomes too large, it divided to form two protocells with RNA included.

3. Origin of photosynthetic organisms

Fossils of the first photosynthetic organism, today's cyanobacteria, originated before 2.7 billion years ago. As a result of photosynthesis iron (Fe^{2+}) ion were oxidized. Once all of the dissolved iron has precipitated, additional O_2 dissolved in the water until the water bodies became saturated with O_2. The increase of photosynthetic bacteria contributed to the increment of the amount of atmospheric oxygen which had accelerated the origin of chloroplast.

4. The origin of first eukaryote

The fossils of the first eukaryotic organisms were estimated as from about 1.8 billion years ago. These eukaryotic single cellular organisms later evolved in to multicellular organisms. The appearance of structurally complex eukaryotic cells sparked the evolution of greater morphological diversity than was possible for the simple prokaryotic cells. After the first eukaryotes appeared, a great range of unicellular forms evolved. It gave rise to diversity of some single-celled eukaryotes which evolved in to multicellular forms, such as the varieties of algae, plants, fungi and animals. Fossils of the oldest known protists similar to small red algae were dated as 1.2 billion years ago.

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5. Diversification of Eukaryotes

Many present day animal phyla appeared in the early Cambrian period. Several animal groups which include, porifera, sponges, cnidarians (Sea anemones and their relatives) and molluscs appeared in the late Proterozoic. According to the DNA analysis, sponges evolved 700 million years ago. Ancestors of arthropods, chordates and other animal phyla originated 670 million years ago. The first food chains on earth appeared when animals started to depend on algae or plants as consumers and with the arrival of many groups of animals, functioning food webs began to appear. Colonization of land by fungi, plants and animals began after about 500 million years ago. Plants that colonized land possess vascular systems to transport water and minerals and water proof coating of wax to prevent the water loss. With the emergence of large trees, differentiation as roots, stems and leaves began and diversified since 40 million years ago. Plants and fungi colonized the land together by interacting with each other. Arthropods (insects and spiders) were the first group of animals to colonize the land. The earliest tetrapods formed about 365 million years ago which were evolved from lobed-finned fish. The divergence of human lineage from other primates was initiated 6-7 million years ago. The origin of the human species took place 195,000 years ago.

Geological eons and eras of evolution

- Eons: Hadean, Archaean, Proterozoic, Phanerozoic
- Eras: Eon Phanerozoic covers the 3 eras, Palaeozoic, Mesozoic, Cenozoic

1. Hadean eon

- Origin of Earth

2. Archaean eon

- Oldest known rocks on Earth's surface
- Oldest fossils of cells (prokaryotes) appeared
- Concentration of atmospheric oxygen begins to increase

3. Proterozoic eon

- Diverse algae and soft-bodied invertebrate animals appeared
- Oldest fossils of eukaryotic cells appeared

Eon Phanerozoic covers the 3 eras; Palaeozoic, Mesozoic, Cenozoic

1. Palaeozoic era

- Sudden increase in diversity of many animal phyla
- Marine algae becomes abundant; colonization of land by diverse fungi, plants, and animals
- Diversification of vascular plants
- Diversification of bony fishes, first tetrapods and insects appeared
- Amphibians dominated
- Extensive forests of vascular plants

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- First seed plants appeared
- Origin and radiation of reptiles
- Origin of most present-day groups of insects
- Extinction of many marine and terrestrial organisms
- Diversification of early vascular plants

2. Mesozoic era

- Cone-bearing plants (gymnosperms) dominated
- Dinosaurs evolved, radiated
- Origin of mammals
- Gymnosperms continued as dominant plants, dinosaurs dominated, abundant and diverse
- Flowering plants (angiosperms) appeared and diversified, many organisms including dinosaurs become extinct

3. Cenozoic era

- Major radiation of mammals, birds, and pollinating insects
- Dominance of angiosperm increased and their radiation continued, radiation of most present day mammalian orders
- Origins of many primate groups, continued radiation of mammals and angiosperms, earliest direct human ancestors
- Appearance of bipedal human ancestors
- Origin of genus Homo

Theories of evolution

Evolution can be defined as a change in the genetic composition of a population from generation to generation (descent with modification) over a long period of time. This may take millions of years.

Theories of evolution are

- Theory of Lamarck.
- Darwin - Wallace theory (Theory of Natural selection)
- Neo Darwinism

Theory of Lamarck

Lamarck published his hypothesis in 1809. He explained his hypothesis using two principles.

1. Use and disuse
2. Inheritance of acquired characteristics

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1. Use and disuse - The parts of the body that are used extensively become larger and stronger. If not used, they deteriorate.

   e.g.- Giraffe stretching its neck to reach leaves on higher branches.

2. Inheritance of acquired characteristics – Organism acquired adaptation during their life time according to the needs of enviro off spring is better adapted to live in that environment e.g. long muscular neck of the giraffe had evolved over many generations as giraffes stretch their necks even higher

Darwin - Wallace theory (Theory of natural selection)

Darwin observed two phenomena from the environment. His observations were;

The populations of a species vary in characteristics among their inheritance traits.

Each species produces more offspring than their environment could accommodate.

The above observations were interpreted by Charles Darwin as,

Certain traits of a population which are capable of exhibiting qualities for better surviveal and their reproduction can produce more offspring.

Variation in abilities for survival and production among a population may enhance the abundance of favorable characteristics in that population.

Some favorable characteristics for survival and reproduction are;

- Escaping from predators - defense
- Tolerating physical conditions – stress conditions
- Obtaining food
- Resistance against disease
- Fertilizing probability
- Number of offspring produced

Process of natural selection

- Over production
- Variation
- Competition and survival of the fittest
- Natural selection of favourable traits

Neo-Darwinism

Neo-Darwinism generally denotes the integration of Charles Darwin's theory of natural selection, Mendelian genetics as the basis for biological inheritance and knowledge of population genetics.

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Hierarchy of taxa on scientific basis

Methods of artificial and natural classification

Arrangement of organisms into groups on the basis of the common characteristics is called classification. Taxonomy is the scientific study on classification, identification, nomenclature and description. This includes placing groups of organisms in a hierarchical sequence.

Two methods of classification

(1) Artificial classification - grouping is based on a few pre selected unifying characters.

- The characters are selected first according to convenience and organisms are grouped based on the selected criteria
- Evolutionary relationships are ignored
- Only system used before 18th century
- Easy to use, easy to expand by adding more groups

e.g. Plants can be classified as cereals, ornamental plants, medicinal plants, poisonous plants etc. Animals can be classified as two legged, four legged, six legged, eight legged etc.

(2) Natural classification - grouping based on true relationships.

- Represent s evolutionary relationships based on phylogeny- evolutionary history of a species or groups of species
- Systems developed after then study of evolution.
- Based on many characteristics.

Characteristics used can be morphological, anatomical, cytological or molecular biological such as DNA and RNA base sequences

e.g. Plants can be classified into phyla; Bryophyta, Lycophyta, Pterophyta, Cycadophyta, Coniferophyta and Anthophyta etc. Animals can be classified into Cnidaria, Platyhelminthes etc.

History of classification

The early classification systems were all artificial systems and were mostly based on human uses.

Aristotle was the first to classify organisms scientifically. He divided organisms into plants and animals. Animals were further classified according to criteria such as mode of locomotion, reproduction and presence or absence of red blood cells. Aristotle's pupil Theophrastus classified plants according to habit. e.g. trees, shrubs and herbs, and according to lifespan e.g. annuals, biennials and perennials.

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Up to the time of Linnaeus scientists have used many different methods for naming of organisms. Carolus Linnaeus(1753), Swedish botanist, introduced binomial nomenclature and also classified about 6,000 plants into a hierarchical order of taxa, classification level such as; Species, genus, order, and class. His classification of flowering plants was based on the number of stamens and styles of flower. He identified two kingdoms of organisms; plants and animals.

With the discovery of the microorganisms the scientists understood that there were organisms which could not be assigned into either plants or animals. To get over this difficulty Ernest Haeckel (1866) introduced a third kingdom: Protista. He also introduced the taxon Phylum and classified many organisms.

With the discovery of the electron microscope biologists identified prokaryotic and eukaryotic cellular organization. Robert H Whittaker (1969) introduced the five kingdom system of biological classification; Monera, Protista, Fungi, Plantae and Animalia. His classification was based on the nature of cellular organization, unicellular or multicellular and mode of nutrition.

With the acceptance of Darwin's theory of the evolution and unitary origin of life, taxonomists began to use natural systems with interpreting evolutionary relationships. With the recent advancement of molecular biology and the use of molecular methods in studying evolutionary relationships it became apparent that in the very early evolution some prokaryotes differ as much from each other as do from eukaryotes. Such difficulties have lead biologists to adopt three Domain system of classification. The three domains are Bacteria, Archea and Eukarya, which are taxonomic ranks higher than the Kingdom. Carl Woese (1977) introduced this three domain system.,

In this tree of life the first major split in the history of life occurred when bacteria diverged from others. Eukarya and Archea are mostly related to each other than bacteria.

Present system of classification and its basis

The present system of classification is mainly based on the rapid advance of molecular biology and the new information on the evolutionary relationships of organisms.

- the sequence of bases of DNA of important genes
- the sequences of bases of DNA of mitochondria and chloroplasts
- the base sequence of ribosomal RNA
- the sequence of amino acids in common proteins
- the molecular structure of cellular components

are used as important taxonomic criteria in modern systematics.

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However, the kingdom Protista is not a natural group. It is an artificial group including organisms which have different evolutionary origins.

Viruses do not have cellular organization, and therefore do not belong to any of the kingdoms. They are also an artificial group considered separately.

Hierarchy of Taxa from Domains to Species

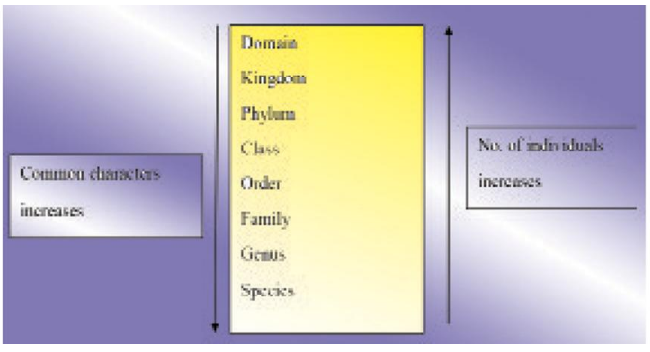
The taxonomic unit at any level/ rank of the hierarchy is called a taxon (plural-taxa). Each taxon has a rank and a name.

e.g. *Panthera* is a taxon at the Genus level/ rank

Mammalia is a taxon at the Class level/ rank

Under the hierarchical system there are levels/ ranks of taxa. Each Domain is divided into kingdoms. A Kingdom is divided into phyla (singular phylum), phylum into classes .etc. Many of these categories may also be subdivided.

e.g. Super class, Sub-family, Subspecies, etc.



From domain to species, the number of shared characteristics among the members in the taxa increases. From species to domain, the number of individuals in the taxon increases.

Biological definition of a species

Species is a group of organisms who shares similar characteristics and has the ability to interbreed and produce viable and fertile offspring.

Other definitions of species

- Morphological species concept- use of morphological criteria to distinguish species such as body shape and other structural features

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- Ecological species concept–defines species in terms of its ecological niche and the sum of how members of the species interact with the non living and the living components of their environment
- Phylogenetic species concept – defines the species as the smallest group of individuals that share a common ancestor.

Binomial nomenclature

In classification, use of common names for organisms, causes confusion. More over some common names do not actually reflect the kind of organism they signify.

e.g. Jelly fish (a Cnidarian)
Cray fish (a Crustacian)
Silver fish (an insect)
Star fish (an Echinoderm)

Further, a given organism has different names in different languages. Carolous Linnaeus (1707-1778) proposed a binomial system of nomenclature of species, which was accepted worldwide to avoid ambiguity.

According to the binomial nomenclature the name of an organism has two parts:

First is the generic name, to which the species belongs and the second is a specific epithet, the unique for each species within the genus. Generic name is usually a noun and the specific epithet an adjective describing a particular feature.

e.g. *Homo sapiens- Homo* means man, *sapiens* means intelligent

Related species have the same generic name with different specific epithets.

e.g. *Dipterocarpus zeylanicus* and *Dipterocarpus grandiflorus*

Dipterocarpus zeylanicus means fruit with two wings, and endemic to Sri Lanka.

Dipterocarpus grandiflorus means fruit with two wings and having large flowers.

International codes of Binominal nomenclature

Biologists have adopted sets of rules or Codes of nomenclature. These codes are slightly different for plants, animals, bacteria and viruses. Some of the important rules for naming plants, fungi, bacteria and animals are as follows.

- Two species of organisms cannot have the same name.
- Each species has a generic name and a specific epithet, both together forming the species name or scientific name.
- The Name should be made up of Latinized words written in the Roman script.
- It should be underlined when hand written and italicized when printed.
- The first letter of the generic name must be capitalized the and specific epithet must be in simple letters.

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In scientific writing, the name of the author who gave the name is indicated by a capital letter, an abbreviation or full word at the end of the name, which is not Latinized.

e.g. *Cocos nucifera L.,* (L for Linnaeus).

A third word can be used to represent a subspecies or a variety, example *Panthera parduskotiya* (Sri Lankan leopard).

- **Use of keys**
  - Used to group organisms and identify them
  - Keys do not show the evolutionary relationships
  - The Commonly used key is the dichotomous key
  - Some examples are given below

Example 1: **Silverfish, Butterfly, House fly, Beetle**

1. Possesses wings ……. (2)
   Do not possess wings …….. Silverfish
2. Possess two pairs of wings …… (3)
   Do not possesses two pairs of wings……Housefly
3. Possesses a proboscis ……...……....Butterfly
   Do not possess a proboscis …….....…….....,.Beetle

Example 2: **Snake, Earthworm, Frog, Sea anemone, Butterfly**

1. Radially symmetrical body …….Sea anemone
   Not having a radially symmetrical body ……...…… … ……..(2)
2. Possess legs……...……(3)
   Do not possesses legs ……...(4)
3. Wings present……..... Butterfly
   Wings absent……..……..... Frog

4 Body covered by scales ...…… Snake
Body is not covered by scales …… Earthworm

Domains

There are three domains. They are;

a) Domain – Bacteria- consists of one kingdom. Kingdom - Bacteria
b) Domain –Archaea-consists of one kingdom. Kingdom - Archaebacteria
c) Domain –Eukarya-consists of four kingdoms.

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Kingdom - Protista

Kingdom - Fungi

Kingdom - Plantae

Kingdom - Animalia

The diversity of organisms within the Domain Bacteria

Key characteristics of Domain Bacteria

- They are prokaryotic
- They are unicellular, colonial, filamentous
- Most of them are found in size between 0.5 to 5μm
- Well adapted to most of the 'normal' habitats (both land and water)
- Most of them contain peptidoglycan in their cell walls
- According to the amount of peptidoglycan present in the cell wall they are classified as Gram positive and Gram negative bacteria
- Most of their cell walls are surrounded by a sticky layer of polysaccharides or proteins called capsule
- Most of them have flagella for motility. Bacterial flagellum differs from eukaryotic flagellum as they are not covered by a plasma membrane and absence of 9+2 structure of microtubules.
- Possess diverse nutritional modes-Autotrophs, heterotrophs
- Posses diverse metabolic modes- obligate aerobes, obligate anaerobes, facultative anaerobes, etc.
- Some are capable of performing nitrogen fixation- e.g. *Rhizobium sp.*, some cyanobacteria
- Rapid reproduction by binary fission. Some perform conjugation as a sexual method.
- Certain bacteria use bacterial chlorophyll as a photosynthetic pigment.

Key Characteristics of Cyanobacteria

- Prokaryotic organisms
- Photosynthetic
- Most are unicellular and oxygen generating and solitary. But some are linked to form filaments or colonies sheathed in mucous
- Some have the ability of fixing atmospheric nitrogen

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Key characteristics of Domain Archaea

- They are prokaryotic and unicellular.
- They lack peptidoglycan in their cell walls which are made up of proteins and polysaccharides
- The size of most of them is between 0.5-5 μm
- They include extreme halophiles and extreme thermophiles
- Some Archaeabacteria live in more moderate environments-Methanogens
- Other species inhabits the anaerobic guts of cattle, termites and other herbivores

Key characteristics of Domain Eukarya

- They are Eukaryotic
- Vary in size
- Most of them are multicellular
- Habitats are diverse
- Diverse in nutrition
- Mostly aerobes
- Most of them exhibit sexual reproduction (some protists are only known to reproduce asexually)

Table 3.1: A comparison of the three domains of life

	Characteristic	Bacteria	Archea	Eukarya
1	Cellular organization	Prokaryotic	Prokaryotic	Eukaryotic
2	Cell wall composition	Peptidoglycan	Proteins and polysaccharides (lack peptidoglycan)	Cellulose, Hemicellulose, Pectin and Chitin
3	Membrane lipids	Unbranched hydrocarbons	Some branched hydrocarbons	Unbranched hydrocarbons
4	Genetic Composition			
	Histones associated with DNA	Absent	Present in some species	Present
	Circular chromosomes	Present	Present	Absent
	Introns in genes	Very rare	Present in some genes	Present in many genes

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5	Protein synthesis			
	RNA polymerase	One kind	Several kinds	Several kinds
	Initiator amino acids for protein synthesis	Formyl-methionine	Methionine	Methionine
6	Response to antibiotics Streptomycin and Chloramphenicol	Growth inhibited	Growth not inhibited	Growth not inhibited
7	Growth at temperatures > 100˚C	No	Some species	No
8	Habitats	Diverse habitats	extreme environmental conditions-volcanic pits/ hot springs/ salt marshes etc.	Diverse habitats
9	Examples	Bacteria, cyanobacteria; *Nostoc, Anabaena ,, Escherichia coli, Salmonella typhi*	Archaebacteria; *Methanococcus Thermococcus., Helobacteria* .	Protists fungis plants and animals

The diversity of organisms within the kingdom Protista

Key characteristics of Kingdom Protista

- Most of them are unicellular, although there are some colonial and multi cellular species
- It is a polyphyletic group (originated from more than one ancestor) and an artificial group in classification.
- Found in freshwater, marine and damp soil, some are symbionts.
- Unicellular, colonial or multicellular.
- Some are photoautotrophs, some are heterotrophs and some are mixotrophs (combination of photoautotrophic and heterotrophic nutrition).

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Euglena



- Unicellular, lack cell wall and pellicle present.
- Chloroplasts are present.
- They have one or two flagella
- They have eye spot
- Contractile vacuole is present
- They have a pocket at one end of the cell from which one or two flagella emerged.

Paramecium



- Habitat is freshwater
- Lack cell wall but pellicle is present, unicellular
- Cilia may completely cover the cell surface
- They have two types of nuclei- mega nucleus and micronucleus
- Contractile and food vacuoles are present
- Oral groove is present

Amoeba



- Aquatic (marine and freshwater) forms are free living others are parasitic.
- Lack cell walls, unicellular organisms
- They form pseudopodia which are used to locomote and feed
- They do not have definite shape.
- Food vacuoles are present

Ulva



- Macroscopic marine forms.
- Cell wall present
- Multicellular thallus differentiated into leaf like blades and root like holdfast.
- Green in colour (green algae)

Gelidium



- Marine.
- Cell walls present
- Multicellular thallus with hold fast.
- It is greenish red in colour (red algae)

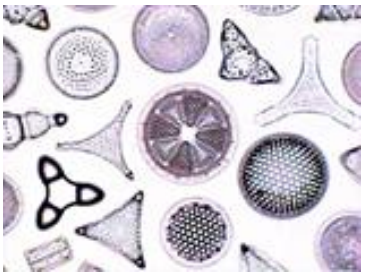
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Sargassum



- Marine
- Comparatively larger and complex
- Thallus is plant- like; it consists of a root like holdfast ,stem like stipe and leaf like blade.
- Multi cellular, thallus is supported by gas filled bulb shape floats.
- Appear in olive green or brown colour (brown algae)

Diatoms



- It is aquatic (fresh water and marine)
- Unicellular, having glass like, wall consists of two parts that overlap (presence of silica)
- Highly diverse group regarding the shape and markings in the surface
- Golden brown in colour (golden brown algae)

The diversity of organisms within the kingdom Plantae

Kingdom Plantae

Evolutionary relationships among major groups of plants

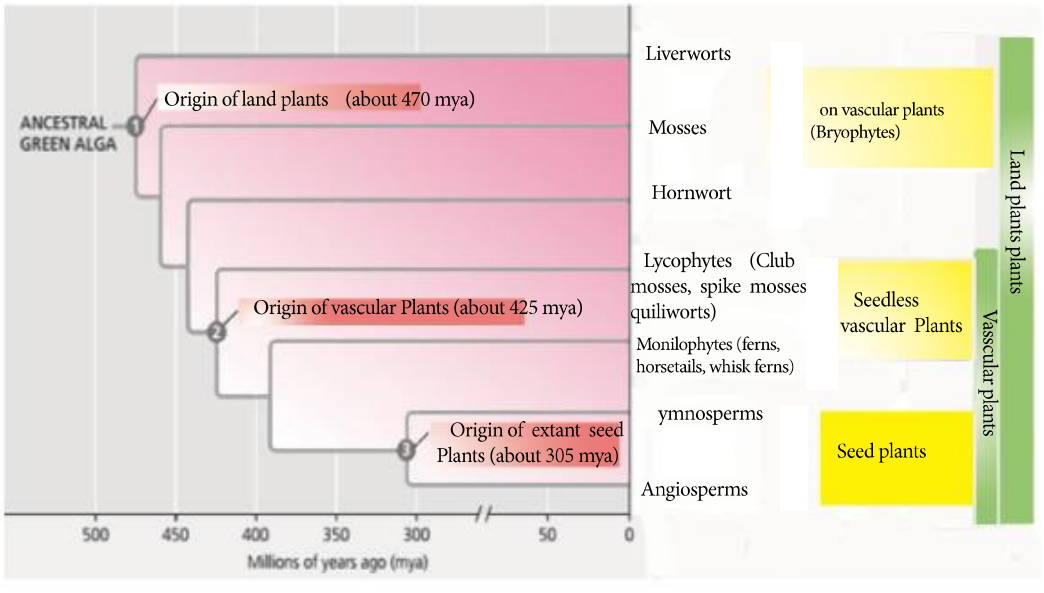
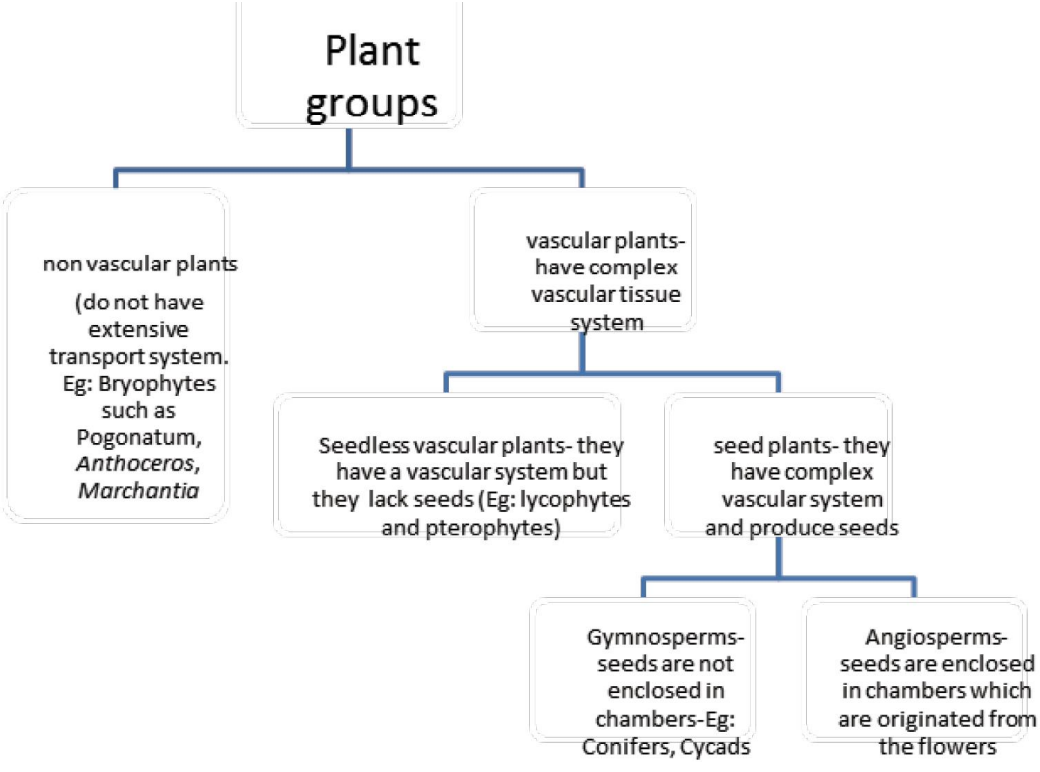


Fig 3.1 Evolutionary relationship of major groups of plants

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It is believed that members of the kingdom Plantae were evolved from a group of chlorophytes/ green algae. Most of them are terrestrial organisms. Chlorophyte algae lack key traits of land plants, walled spores produced in sporangia, multicellular gametangia, dependent embryo and apical meristem. They evolved in the terrestrial environment.

Plant groups can be distinguished based on the presence or absence of an extensive system of vascular tissue. Based on that there are two major groups of plants the can be seen; they are Vascular plants and Non-vascular plants.



Diversification of Kingdom Plantae

- Non-vascular plants
  - Phylum Bryophyta - Mosses- *Pogonatum*
  - Phylum Hepatophyta- *Marchantia*
  - Phylum- Anthocerophyta- *Anthoceros*
- Vascular seedless plants
  - Phylum – Lycophyta- *Selaginella*
  - Phylum – Pterophyta- *Nephrolepis*
- Vascular seed plants

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- Gymnosperms- Phylum – Cycadophyta (*Cycas sp.*),
  Phylum- Coniferophyta (*Pinus*), Phylum – Gnetophyta (*Gnetum*)
- Angiosperms- Phylum – Anthophyta (all flowering plants)

Non-vascular plants

One way to distinguish a group of plants is to see whether or not they have an extensive system of vascular tissue that transport water and nutrients throughout the plant body. Most present day plants do have a complex vascular tissue system and therefore, are called vascular plants. Plants that do not have an extensive transport system are described as non vascular plants. Non vascular plants are informally named as Bryophytes.

e.g. *Marchantia, Pogonatum, Antheroceros*

Bryophytes share some derived traits with vascular plants, but lackin many innovations of vascular plants such as presence of true stems, roots and leaves.

Diversity of Bryophytes

Phylum- Hepatophyta e.g. *Marchantia* (liverworts).

Phylum Anthocerophyta e.g. *Anthoceros* (hornworts)

Phylum Bryophyta e.g. *Pogonatum* (mosses)

Characteristic features of phylum Bryophyta

e.g. *Pogonatum*

- Especially common in moist terrestrial places.
- Haploid gametophyte is dominant stage of the life cycle, photosynthetic and independent.
- Gametophytes are differentiated into ‘leaves’, ‘stems’ and rhizoids. They have no vascular tissues. Archegonia and antheridia are typically carries on separate female and male gametophyte. Therefore gametophyte is dioecious.
- Male plant produces flagellated sperm which can swim through a film of water for fertilization.
- Sporophytes are usually green and photosynthetic when young. However, they are not independent. They attach to their parental gametophytes and absorb nutrients and water from the female gametophyte.
- Sporophytes have specialized pores called stomata which are also found in all vascular plants.
- They are homosporous.

Vascular plants cover about 93% of the existing plant species. They can be further divided into two groups.

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1. Seedless vascular plants
2. Seed plants



Seedless vascular plants

Seedless vascular plants lack seeds and disperse by means of spores. They are categorized into two groups.

1. Lycophytes
2. Pterophytes

Even though, both pterophytes and lycophytes are seedless plants, pterophytes share a more recent common ancestor with seed plants.

Fossils and living seedless vascular plants provide evidence for plant evolution during Devonian and Carboniferous periods. The ancestors of vascular plants already have had some derived traits of modern vascular plants; however, they lack roots and some other adaptations.

Fossils suggest that, the ancestors of vascular plants had gametophyte and sporophytes that were about equal in size. However among the living vascular plants sporophyte generation is large and more complex. For example in ferns, leafy plants are the sporophytes

Significant features of seedless vascular plants;

1. Transportation through Xylem and Phloem

Vascular plants have two types of vascular tissues; Xylem and Phloem

Xylem consists of tracheids, fibers and parenchyma cells- conducts water and minerals.

Cell walls of tracheids and fibers are strengthened by the polymer lignin. These tissues permit plants to grow tall. This may facilitate them to obtain a high amount of light for photosynthesis and ease the spore dispersal.

Phloem- this tissue has cells arranged in tubes. They distribute sugars, amino acids and other organic products among different parts of the plant.

2. Evolution of roots

Roots are organs that absorb water and nutrients from the soil. They anchor the plants and allow the shoot system to grow taller. They are to replace the rhizoids seen in bryophytes. Root tissues of living plants resemble stem tissues of the early vascular plants preserved in fossils.

3. Evolution of leaves- There are two types of leaves. They are microphylls and megaphylls. Microphylls are single veined and smaller in size while megaphylls are large, flattened with branched veins.

Leaves with branched vascular tissues increase the surface area for efficient photosynthesis (megaphylls).

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Sporophylls and spore variations

Modified leaves that bear sporangia are known as sporophylls. Most seedless vascular plant species produce one type of sporangium and one type of spores. Therefore, they are known as homosporous.

Some plant species produce two types of sporangia and produce two kinds of spores called mega spores and microspores. This condition is known as heterosporous. Mega spores develop into female gametophyte while microspores develop into male gametophyte.

Phylum Lycophyta

- Lycophytes are terrestrial and some are epiphytes.
- The dominant plant is sporophyte.
- They produce upright stems and ground hugging stems.
- In upright stems small leaves can be found.
- Ground hugging stems produce dichotomously branching roots.
- They have strobili. In many club mosses and spike mosses sporophylls are clustered into club shaped cones/ strobili.
- They are homosporous or heterosporous.

Spike mosses are usually relatively smaller and often grow horizontally.

- All Club mosses are homosporous;
  e.g. *Lycopodium* sp.
- All Spike mosses are heterosporous.
  e.g. *Selaginella*





In some species the tiny gametophyte live above the ground and are photosynthetic. Others live below the ground and are nourished by symbiotic fungi.

Phylum Pterophyta

- Most sporophytes have rhizome (an underground stem)
- At the tip of the rhizome they produce leaves, called fronds
- Many fronds are highly dissected and feathery.
- All species are homosporous which develop into a bisexual gametophyte.
- Sporophytes are dominant
  e.g. *Nephrolepis.*

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Seed plants



Vascular plants consist of seeds and are a called "seed plants". They represent the vast majority of living plant species.

Seed plants can be divided into two groups, based on the absence or presence of enclosed chambers in which seeds mature.

1. Gymnosperms
2. Angiosperms

Gymnosperms are "naked seed" plants as their seeds are not enclosed in chambers.

Angiosperms are "seed plant" group accommodating all flowering plants. Angiosperm seeds develop inside chambers called ovaries; which originate within flowers and mature into fruits.

Significant features of seed plants

1. Production of seed

A seed consists of an embryo and endosperm. Endosperms supply food to the embryo. This endosperm is surrounded by a protective coat which is known as seed coat. When seeds are mature they can be dispersed through various dispersal methods.

Seeds are key adaptations that help seed plants to become the dominant producers on land and to exhibit the vast plant diversity of today.

2. Reduced gametophyte

The evolutionary trend of gametophyte reduction continued further in the vascular plants and led to seed plants. The gametophyte of seed vascular plants is not visible to the naked eye. They are mostly microscopic.

Tiny gametophytes develop from spores and are retained within the sporangia of sporophyte. This arrangement protects the gametophyte from environmental stresses. The moist reproductive tissues of the sporophyte shield the gametophyte from UV radiation and protect them from drying out. This relationship also enables the dependant gametophyte to obtain nutrients from the sporophyte.

3. Heterospory

Seed plants are heterosporous (produce both megaspores and microspores). Each megasporangium has a single functional megaspore and each microsporangium contains a large number of microspores.

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4. Production of ovules and eggs

Seed plants are unique in retaining the megasporangium within the parent sporophyte. A layer of sporophyte tissue called integument envelops which protects the megasporangium.

The entire structure, containing megasporangium, megaspore and integuments is called an ovule. Inside each ovule female gametophyte develops from a megaspore and produces one or more eggs.

5. Production of pollen and sperms

A microspore develops into pollen grain that consists of a male gametophyte enclosed within the pollen wall. The wall of the pollen is tough as it is made up of the polymer sporopollenin, which protects the pollen grain during pollination. The transfer of pollen grain towards the ovule is called pollination. When a pollen germinates, it gives rise to a pollen tube that discharges sperm (male gametes) into the female gametophyte located within the ovule.

Inside a pollen grain, a sperm producing male gametophyte is present. The sperms of seed plants do not require motility as they are carried directly in to the eggs by pollen tubes. Some gymnosperms retain the ancient flagellated condition, however flagella have been lost in the sperm of most gymnosperms and all angiosperms.

Phylum Gnetophyta

e.g. *Gnetum*

- Only gymnosperms have vessels in xylem
- Leaves of gymnosperms look like those of the flowering plants. Their seeds, look like fruits of angiosperms.



Phylum Cycadophyta

- They have palm like leaves and large cones
- They have flagellated sperms similar to those of seedless vascular plants

e.g. *Cycas*



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Phylum Coniferophyta

e.g: *Pinus*



- Large trees are included such as Cyperes and Red woods.
- In conifers two types of spores are produced by separate cones.

Phylum Anthophyta - Angiosperms

Presence of flowers -

- Stamens produce microspores and these microspores develop into pollen grains
- These pollen grains contain male gametophyte/ gametes
- Carpels produce megaspores and these megaspores produce female gametophytes/ embryo sac
- Seeds are enclosed within the carpels
- Production of fruits- seeds are protected by fruits which help in their dispersal. This is one of the unique features of phylum Anthophyta. A fruit typically consists of a fertilized ovary and sometimes include other persistent floral parts. After fertilization, the ovary wall thickens and develops into fruit. Ovules develop as seed of the fruit. Thr fruit protects dormant seeds and aid in their dispersal.

Diversity of Angiosperms

The flowering plants (angiosperms) are divided into two groups based mainly on the number of cotyledons in their embryo.

These two groups are;

1. Monocotyledons- species with one cotyledons
2. Dicotyledons- species with two cotyledons

Table 3 .2- Comparison of monocots and Dicots

Class – Monocotyledoneae	Class – Dicotyledoneae
The embryos have only one cotyledon	Embryos have two cotyledons
Fibrous root system	Tap root system
Parallel veins in leaves	Reticulate veins in leaves
Flower parts are trimerous	Flowers are pentamerous or tetramerous
Perianth present in flowers (No distinct calyx and corolla)	Distinct calyx and corolla present in flowers

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Pollen grains are with one opening/ aperture	Pollen grains are with three openings/ apertures
Vascular bundles in the stem do not have cambia and are scattered e.g. grasses, coconut, rice	Vascular bundles in the stem have cambia and arranged in a ring e.g. Rose, shoe flower, cucurbits

The diversity of organisms within the kingdom Fungi

Kingdom Fungi

Characteristic features of Kingdom Fungi

- Eukaryotic
- Cell walls are made up of chitin a strong but flexible polysaccharide.
- They are absorptive and heterotrophs - many of them secrete extra cellular enzymes which aid in the breaking down of complex molecules into small molecules.
- Different species live as decomposers, parasites or mutualistics.
- Few are unicellular, others forming multicellular filaments called hyphae .
- Septa can be found in hyphae. (division of hyphae into cells by septa – cross walls).
    - Septum has a hole which enables the movement of mitochondria, ribosomes, nuclei etc.
    - Fungi lack septa are known as coenocytic fungi (with many nuclei)
- Fungal hyphae produce mycelium
- Some fungi produce haustoria (to penetrate and absorb or exchange nutrients between plants and the fungi)
- Multicellular fungi produce mycelia. (a network of branched hyphae adapted for absorption of nutrition)
- They show sexual and asexual reproduction.
- They produce spores.

Characteristic features of Phylum Chytridiomycota

e.g.: *Chytridium*

- Aquatic or terrestrial .
- Some are decomposers while others are parasitic.

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- Multicellular or unicellular when multicellular it is coenocytic.
- They produce zoospores which are flagellated.
- Cell walls are made up of chitin.
- Some of them form colonies with hyphae while others exit as single spherical cell.

Characteristic features of Phylum Zygomycota

e.g. *Mucor , Rhizopus*

- Most of them are saprotrophs and some of them are parasites or commensals.
- Mycelium is coenocytic and aseptate. Septa found only where reproductive cells are formed.
- Asexual reproduction: Produce sporangia in which genetically identical haploid spores are produced. Also by endospores produced in sporangia.
- Sexual reproduction: A Zygosporangium is produced which is a sturdy structure produced by plasmogamy and karyogamy. Zygosporangium is resistant to unfavorable environmental conditions.
  - Zygosporangium is a multinucleated structure which is resistant to drying and freezing.
  - They are metabolically inactive in adverse environmental conditions.
- Zygosporangium produces genetically diverse haploid spores when environmental conditions are favourable.

Characteristic features of Phylum Ascomycota

e.g. *Aspergillus, Saccharomyces, Penicillium*

- Marine or freshwater or terrestrial
- Parasitic or symbiotic.
- Most of them are decomposers.
- Unicellular or filamentous, multicellular.
- In asexual reproduction conidia are produced at the tip of the conidiphores which are specialized hyphae. (Exospores in clusters or chains)
- In sexual reproduction fusion of sexually differentiated gametangia takes place and produce sac like structure called asci.
- Ascospores are produced within asci. Generally there are eight ascospores are produced in each ascus.
- Most of these fungi produce ascocarps enclosing asci.

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Characteristic features of Phylum Basidiomycota

e.g. *Agaricus,* Puffballs, Shell fungi

- They are Terrestrial.
- They are major decomposers and some are symbionts.
- Filamentous with septae and dikaryotic.
- Mycelium is the dominant stage of the life cycle.
- They produce fruiting bodies called basidiocarps during sexual reproduction. Produce basidia on the gills of the basidiocarp.
- Produce basidiospores on basidium and exogenous.

The diversity of organisms within the kingdom Animalia

Kingdom Animalia

Characteristic features of Kingdom Animalia

- Multicellular
- Heterotrophic eukaryotes- they ingest food and digest them in the body using enzymes
- Cells of the animals are organized into tissues.
- Most of them reproduce by sexually.
- Some show radial symmetry and some others show bilateral symmetry.

Phylum Cnidaria

Characteristic features of each example are not necessary

e.g. *Hydra,* Sea anemone, *Obelia,* Corals and Jelly fish

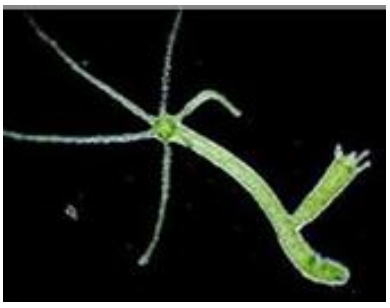


Obelia,



Jelly fish

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Hydra



Coral polyp

- Majority of them are marine, except a few fresh water species. Some are macroscopic.
- Simple organization: diploblastic or just 2 body cell layers- an outer layers of ectoderm and inner layer of endoderm sandwitched between these two layers is a acellular layer of mesoglea
- They have a simple gastrovascular cavity which is a sac with a central digestive compartment. This cavity is lined by endodermis with a single opening (mouth) only.
- They show radial symmetry with two body forms polyp and medusa. Polyps are cylindrical forms attached to the substrate by the aboral end of the body. Tentacles are found around the mouth.
- Medusa resembles a flattened mouth down version of the polyps and they are free living.
- Some cnidarians exist only as polyps or only as medusa. Others have both polyp and medusa forms in their lifecycles.
- Tentacles are armed with cnidocytes which functioning defense and capturing prey.
- They have nematocysts which contain stinging thread.

Phylum Platyhelminthes

Characteristic features of each examples are not necessary

e.g. *Planaria, Taenia , Fasciola*



Fasciola



Planaria

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- Commonly known as flatworms.
- Free living (*Planaria*) or parasitic (flukes and tapeworms).
- They are found in marine, fresh water and in damp terrestrial habitats.
- Body is dorsoventrally flattened. Some have elongated tape like body forms without true segmentation.
- Triploblastic with all three germ layers (ectoderm, mesoderm and endoderm). Signs of cephalization present but are not distinct.



Taenia

- No body cavities, circulatory, respiratory and skeletal systems.The gaseous exchange is by simple diffusion through body wall.
- Sensory organs are found only in free living examples. Eye spots are found in the head.
- First appearance of little complex nervous and sensory system. A pair of anterior ganglion and two longitudinal nerve cords on central nervous system.
- Appearance of separate organs for excretion: Nitrogenous excretory system consists of protonephridia. These are a network of tubules with ciliated structures called flame bulb. These are used to maintain the osmotic balance.
- They have incomplete digestive system only with mouth without anus. Branched gastrovascular cavity is present for digestion. Some are having eversible pharynx.
- Free living examples have cilia for the locomotion.
- Some show asexual reproduction by regeneration. All are bisexual. Except tape worms (*Taenia*). Others have cross fertilization which is internal. In parasitic forms there are several larval stages, direct development in free living examples without larval stages.

Phylum Nematoda (charactcristic features of eachexamples are not necessary)

e.g. round worms, hook worms, pin worms



round worm



hook worm



pin worm

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- Most of them are free living in marine, few are fresh water and damp soil environments and parasitic in plants and animals.
- They are bilateral symmetrical. Triploblastic with pseudocoelomic .Their body forms are cylindrical with tapered ends. Body size varies from microscopic to macroscopic. They do not show distinct cephalization and segmentation. The sensory papillae are found on the anterior end of the body. Body is covered by tough cuticle and undergoes ecdysis.
- No circulatory and respiratory systems. Gaseous exchange is by simple diffusion through body wall. They have an Alimentary canal.
- Body wall is composed only of longitudinal muscles. They do not have special locomotary structures. Longitudinal muscles in the body wall are involved in locomotion.
- The Sexual reproduction is by internal fertilization. Sexes are separated and females are larger than males.

Phylum Annelida

Characteristic features of each examples are not necessary

e.g. Earthworms, Leeches and regworms.



Earth worm



Leech

- They can be marine, freshwater or in damp soil.
- They are segmented worms with cylindrical bodies
- They are Triploblastic.
- Coelom (true body cavity) is present for the first time.
- The first animals to show cephalization.
- Nervous system well developed; dorsal cerebral ganglion, ventral nerve chord, circumenteric connectives.
- Clitellum, Parapodia, setae and suckers are found in some examples. Clitellum is for external fertilization. Parapodia is used for locomotion and respiration. Seate are present for locomotion and suckers for locomotion and ingestion in ecto parasitic forms.

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Phylum Mollusca

Characteristic features of each examples are not necessary

e.g. Oysters, Clams, Slugs, Snails, Octupus, Squids, Chitons and tusks shells



Squid



Octopus



Snail



clam



chiton



tusk shell



Oyster

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- Majority are marine. Some inhabit freshwater and land. Some are bilateral symmetrical and few are asymmetrical.
- They are soft bodied and non-segmented. Calcareous shell is secreted as a protective exoskeleton. Coelomic.
- Body is divided into three parts:
  - muscular foot is used for locomotion
  - visceral mass contains most of the internal organs
  - mantale is to secrete the shell
- Shell could be internal or external.
- Many molluscs possess radula (a minutely toothed, chitinous ribbon) in the mouth for feeding.
- Most molluscs have separated sexes and their gonads are located in visceral mass.

Phylum Arthropoda

Characteristic features of each examples are not necessary

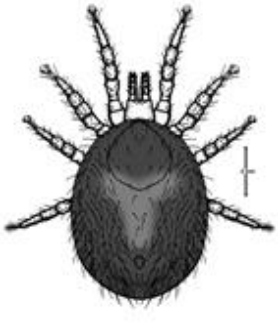
e.g. Insects, Spiders, Prawns, Crabs, Scorpions, Ticks, Mites, Millipedes and Centipedes.



Spider



Scorpion



Mite



Tick



Centepede



Millepede

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- One of the most successful animal groups on earth with the highest number of species. They live everywhere – air, water, soil
- They have segmented bodies with "jointed legs"
- They have a chitinous exoskeleton (skeleton on the outside) Because of exoskeleton these animals can't grow continuously and needs periodic molting
- The nervous system is well developed with primitive dorsal brain.
- The nerve cord is solid, segmented and ventrally located.
- They have many and varied sense organs.
- They have an open blood circulatory system; Blood is pumped by a heart into the body cavities (haemocoel), where tissues are surrounded by the blood. No capillaries.
- Respiration
  - In aquatic animals- Gills
  - In terrestrial animals- Tracheal system of chitinous tubes
  - In arachnids - Book lungs
- Excretion is by Malphigian tubules They excrete uric acids
- Reproduction: Sexes separate [Dioecious]

Phylum Echinodermata

Characteristic features of each examples are not necessary

e.g.sea stars, brittle stars, sea lily, feather star, sea cucumber, sea urchins and sand dollars



Sea star



Sea lily



Brittle star



Sand dollar

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Sea cucumber

- They are exclusively marine. Triploblastic and coelomic, slow moving or sessile.
- Adults are penta radial symmetrical without head and segmentation.
- Deuterostomes.
- Thin epidermis covers the endoskeleton of hard calcareous plates.
- Water vascular system is a network by hydraulic canals branching into tube feet which function in locomotion and feeding.
- Digestive system is usually complete, but the mouth is on the underside and the anus on the top surface of the animal.
- Circulatory system is reduced and closed without a heart. Sexes are separated with external fertilization. Larval forms are bilaterally symmetrical.
- Well developed nervous system. Intelligent animals.

The characteristic features to study organisms belonging to phylum Chordata

Phylum Chordata

Characteristic features of Phylum Chordata

- Longitudinal, flexible rod called notochord located between digestive tube and nerve cord. It is extending from anterior to posterior providing support in at least embryonic stage.
- Dorsal, hollow, single nerve cord located dorsal to the notochord.
- In all chordate embryos there are slits or clefts in pairs either side of pharynx (pharyngeal sits) that opens to the outside of body. In terrestrial, adult chordates it disappears and remains in the aquatic adults and larval forms of terrestrial chordates as respiratory structures.
- Muscular tail that extends posterior to the anus present in the embryonic stages. In some terrestrial adult it is reduced.

(Characteristic features of each examples of following classes are not necessary)

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Characteristic features of class Chondrichthyes

e.g. Skates, Sharks

- All are aquatic
- Skeleton composed predominantly of cartilage
- Fins for locomotion
- Caudal fin is heterocercal.
- Gills without operculum.
- Body is covered with placcoid scales.
- Eggs are fertilized internally. Some are ovoviviparous and others are oviparous or viviparous.
- Reproductive tract, excretory duct and digestive tract empty into the cloaca, a common chamber that has a single opening to the outside.

Characteristic features of Class Osteichthyes

- All are aquatic
- Having a skeleton composed of bones.
- Gills are covered by a bony flap called operculum.
- Swim bladder for control the buoyancy.
- Caudal fin is homocercal.
- Body is covered by flatten bony scales called ctenoid and cycloid scales.
- Most are fertilized externally some have internal fertilization.
- Most species are oviparous.

e.g. Carp, Tuna,

Characteristic features of Amphibia

- First animals to invade land but need water to complete life cycle, live in both water and on land.
- They are found only on land or fresh waters. No marine species.
- First species to poses limbs, body is somewhat elevated by these limbs to help locomotion in terrestrial environment.
- Some are limbless but some are tetrapods.
- Ectothermic- changes body temperature according to environmental temperature. This restricts metabolism.
- Body is covered with thin, moist skin. No scales. Sensitive to environmental changes.
- Nictitating membrane covers the eye and tympanic membrane is found behind the eye.
- Most amphibians show external fertilization. Eggs without shells.

e.g. Toad, Frog, *Ichthyophis*

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Characteristic features of Reptilia

- They are the first animal to live a complete terrestrial life.
- Possess limbs for locomotion and digits.
- Body is covered with keratinized scales to prevent from desiccation and abrasion.
- Poses lungs for aerial respiration.
- They are ectothermic (cold blooded)
- Live in terrestrial and aquatic habitats.
- Internal fertilization. They lay shelled eggs (calcareous) on land.

e.g. Lizards, Snakes, Turtles, Crocodiles and Alligators

Characteristic features of Aves

- Body is covered by keratinized feathers.
- Hind limbs are converted to flight.
- Many adaptations to help flying: light body, wings, bones with air cavities, high metabolism, restrictions in body size
- They are having a beak without teeth.
- They are endothermic.
- Birds have colour vision and excellent eye sight.
- Internal fertilization, lay shelled eggs

e.g. Crow, Parrot, Humming birds, Eagles etc.

Characteristic features of Mammalia

- Nourish young by producing milk with mammary glands.
- Body covered with hair for insulation.
- They are endothermic group of animals and most of them have high metabolic rate.
- They have differentiated teeth.
- They have an efficient respiratory system with lungs.
- A complete circulatory systems and a four chambered heart.
- Muscular diaphragm is found to help respiration.
- They have a larger brain with compared to the other group of vertebrates. Very intelligent animals. Learning skills and a good memory.
- Different methods of communication.
- Show relatively long periods of parental care.

e.g. Bat, whales, monkeys, cows

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04

Plant form and function

Structure, Growth and Development of Plants

The main focus of this unit is on structure, growth and development of vascular plants.

Plants consist of a root system and a shoot system and roots and shoots grow at their tips, which are with meristematic properties and called as apices, buds or meristems.

Types of plant tissues, structure- function relationship

A Tissue is a group of one or more cell types which carries out specialized function(s).

Meristems, locations and role in plant growth

Plants have undifferentiated tissues called meristems, consisting of cells which constantly divide under suitable conditions and produce new cells. Some of these cells then elongate and differentiate to produce new tissues of the plant body and others remain as meristems. Meristems may have dormant periods. Due to the action of meristem new cells are added. Subsequently these cells get differentiated and therefore plant growth occurs by making new plant tissues.

Characteristics of meristematic cells

All cells in the meristems have common characteristics. They;

- are living cells
- are isodiametric (roughly spherical)
- are structurally and functionally undifferentiated
- have a central nucleus
- have a dense cytoplasm
- have ability to multiply

In meristem there are three overlapping zones of cells consisting of cells at successive stages of

- cell division
- cell elongation and
- differentiation

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There are three types of meristems. They are;

1. apical meristems
2. lateral meristems
3. intercalary meristems

Apical meristems

These meristems are located at root tips and shoot tips. They add new cells that enable increase in length. This process is known as primary growth.

Lateral meristems

Vascular cambium and cork cambium are lateral meristems. They are found in woody plants and involve in the secondary growth in increasing circumference of roots and stems. The vascular cambium produces secondary xylem and secondary phloem. The cork cambium produces thick and tough periderm, replacing epidermis.

Intercalary meristems

Some monocots such as grasses show meristematic activity at the bases of stems and leaves (nodes). These are known as intercalary meristems. They allow rapid regrowth in damaged leaves.

Primary growth of roots

Elongation of root due to the activity of primary meristems located on root apex is called primary growth of the root.

During the growth three processes take place.

i. Cell division - due to mitotic division.
ii. Cell elongation
iii. Cell maturation - due to differentiation.

These stages are found in three overlapping regions starting from meristems.

The zone of cell division includes the root apical meristem and its derivatives. In this region, new cells are produced to both sides. Cells produced outward to the apical meristem are differentiated to form root cap which prevents damaging the root apical meristem from friction when grows through soil.

Cells produced inward to the meristem undergo elongation, in the zone of cell elongation. Root cells elongate, sometimes to more than ten times their original length. Hence the root is pushed forward through soil.

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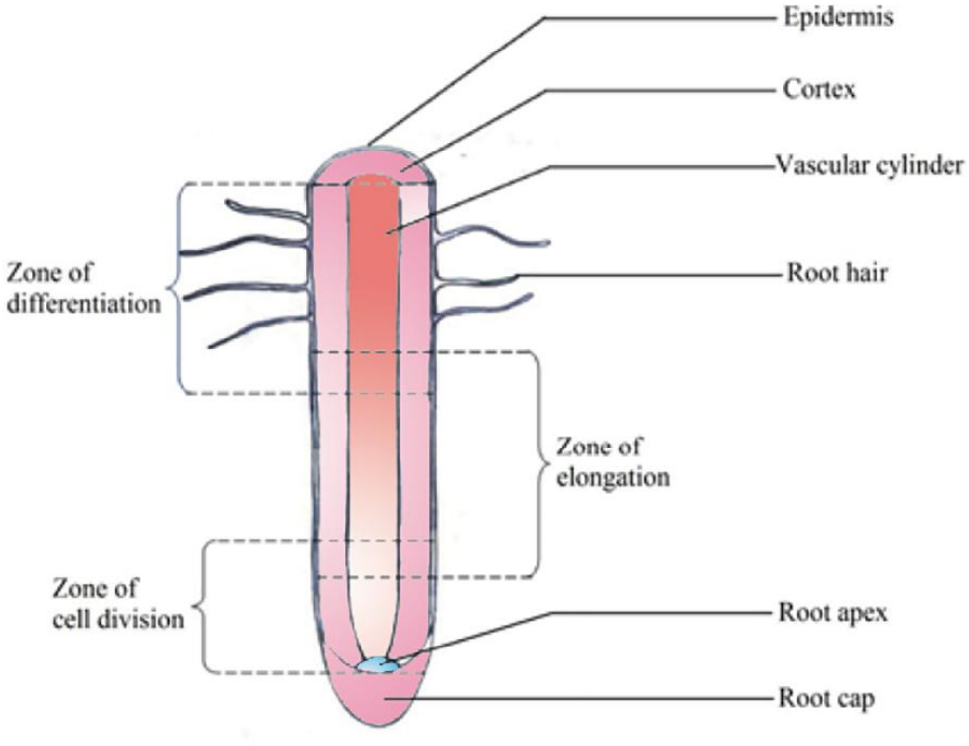


Fig. 4.1 The different zones of the root (zone of differentation , Zone of elonjation and zone of cell divisions

In the zone of maturation, the cells begin specializing in structure and function where cells complete their differentiation and become functionally mature. Primary structure of the root is formed as a result of primary growth.

Primary growth of the shoot:

Elongation of shoot is due to the activity of primary meristem located in shoot apex, and is called primary growth of the shoot.

A shoot apical meristem is a dome-shaped mass of dividing cells located at the shoot tip.

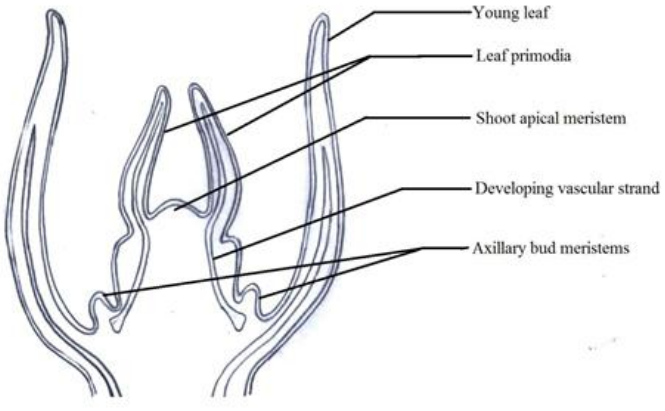


fig 4.2 .The Ls of the Shoot

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Leaves develop from leaf primordia, finger-like projections along the sides of the apical meristems. Normally these primordia cover the shoot apical meristem.

Shoot apical meristem produces new cells only towards the stem, due to mitosis. After cell elongation, cell differentiation takes place.

Then the primary tissues of the stem are formed due to cell differentiation. Therefore, the height of the stem is increased due to primary growth.

Table 4.1 Differences between shoot apex and root apex

Shoot apex	Root apex
Found at tips of shoot	Found at the tip of the root
Protected by leaf primordial	Protected by root cap
Produces new cells only inwards	Produce new cells both sides outwards and inwards

Plant tissue systems

The new cells originating from the meristems are differentiated to perform specialized functions and form a plant tissue system. During differentiation process, they undergo changes in cytoplasm, organelles and cell wall. Therefore, several types of plant cells can be recognized according to their structure and function.

A tissue consists of group of one or more cell types which carries out specialized function(s).

Vascular plants have three main tissue systems. They are;

1. dermal tissue systems
2. ground tissue systems
3. vascular tissue systems

Dermal tissue system

This is the outer protective covering of plants.

e.g. Epidermis

- Protective layer in the stems and roots of the primary plant body and leaves
- Tightly packed single cell layer
- Normally covered by a cuticle which is a waxy epidermal coating in aerial parts
- Specialized cells such as guard cells, trichomes and root hairs are also found in epidermis

Functions of epidermis:

- Defense against physical damage and pathogens
- Cuticle helps to prevent water loss

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- Root hairs involve in absorption of water and mineral ions
- Guard cells help gaseous exchange
- Trichomes (epidermal outgrowths such as hairs and glands) ;
  - o hair like trichomes reduce water loss, shiny hairs reflect excess light
  - o Some trichomes secrete chemicals involved in defense against insects/ pathogens/ herbivores,

Epidermis in older regions of stems and roots is replaced by a protective layer called periderm after the secondary growth

Ground tissue system

Ground tissue fills the gap between dermal tissue and vascular tissue, mainly consists of cortex (outer to vascular tissue) and pith (inner to vascular tissue). The ground tissue includes cells specialized for functions such as storage, photosynthesis, support and short distance transport.

Three main types of cells are present in ground tissue. They are;

1. parenchyma cells
2. collenchyma cells
3. sclerenchyma cells

Parenchyma cells

- Living even at functional maturity
- Mature cells have primary cell walls which are relatively thin, flexible and most of the cells lack secondary walls
- They have a large central vacuole

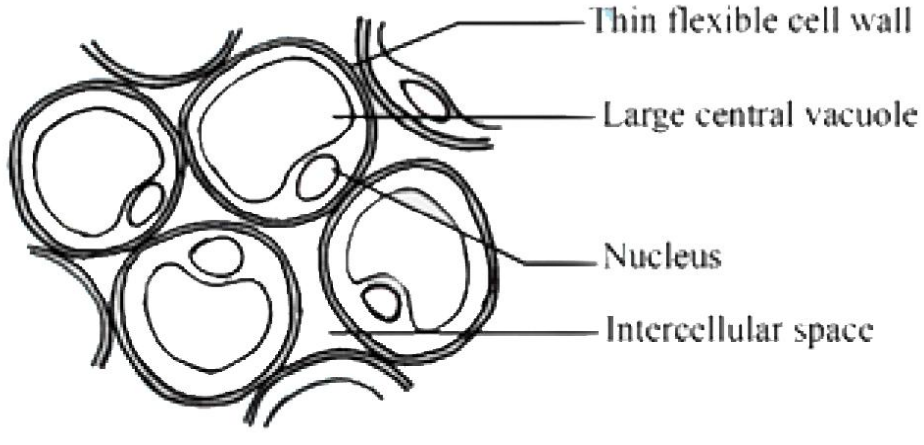


Fig 4.3 Diagram of General Parenchyma cells

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Functions

- Perform most of the metabolic functions of the plants.

  e.g. synthesis of various organic products
- Storage-

  e.g. some cells in root and stems contain plastids (leucoplasts) which store starch.
- Most of the parenchyma cells retain the ability to divide and differentiate under suitable conditions. This ability is important in wound repair. These abilities also make it possible to multiply and differentiate cells even from a single parenchyma cell in tissue culture practices.

Collenchyma cells

- They are generally elongated
- They have thicker primary walls than parenchyma cells
- Their walls are unevenly thickened
- Young stems and petioles often have strands of collenchyma cells just below the epidermis
- Even at functional maturity they are living, flexible and elongating with stems and leaves they support

Functions

- Give mechanical support to leaves and stems without restraining growth

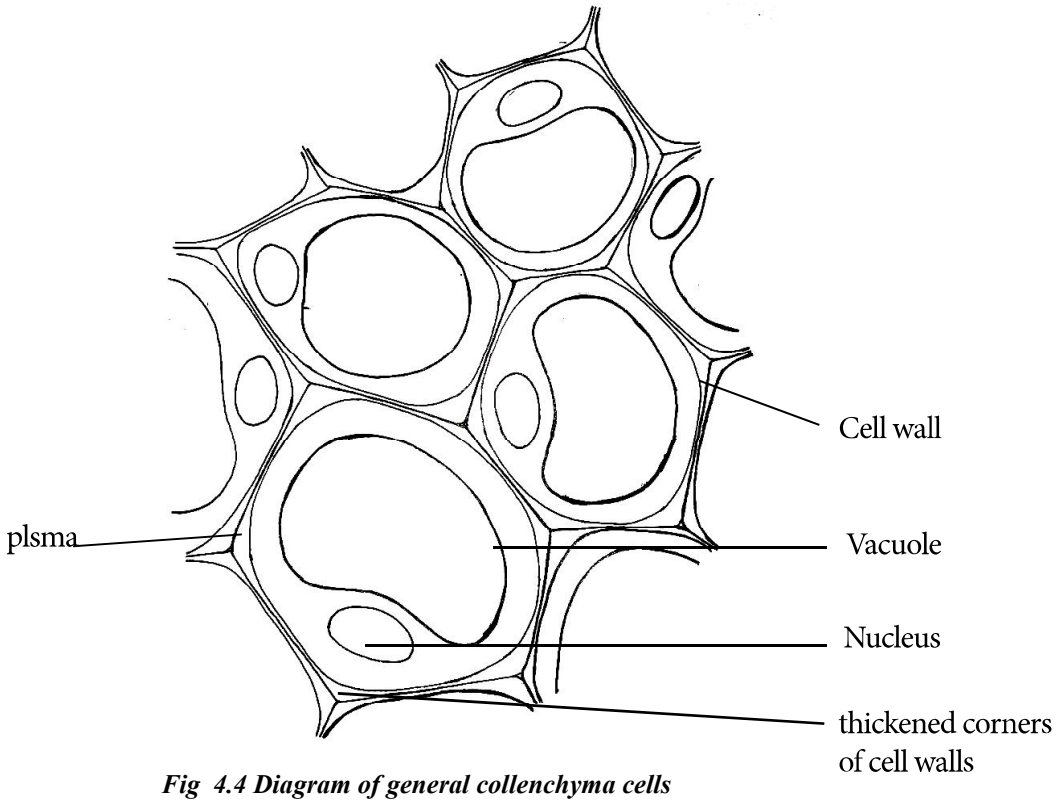


Fig 4.4 Diagram of general collenchyma cells

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Sclerenchyma cells

- Secondary cell walls are produced after cell elongation
- They have secondary cell walls thickened by large amount of lignin
- They are dead cells at maturity

There are two types of sclerenchyma cells;

1. sclereids
2. fibers

Sclereids are shorter and wider than fibers and irregular in shape. They have very thick lignified secondary cell walls. They are found in places where growth has stopped e.g. nut shells, seed coats and flesh of coarse fruit.

Fibers are usually grouped in strands. They are long, slender and tapered. Used commercially to obtain fibers. e.g: coconut husk fiber, hemp fibers

Functions

Sclereids and fibers are specialized to provide support and strength.

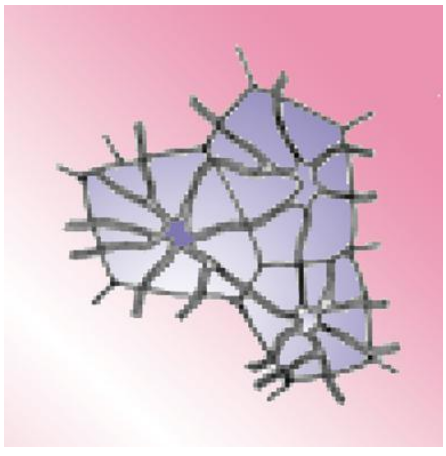


Fig 4.5 : Diagram of T.s of sclereids

Vascular tissues- Xylem and phloem

Xylem tissue

- It consists of vessel elements, tracheids, fibers and parenchyma cells in angiosperms and some of the gymnosperms.
- Vessel elements and tracheids mainly conduct water.
- They are dead at functional maturity.
- Fibers give mechanical strength.
- Parenchyma functions in storage and in radial transportation.

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Vessel elements

- In all angiosperms and some gymnosperms, contain vessel elements
- They are cylindrical and long.
- They are wider, shorter and have thinner walls than tracheids
- Secondary walls are thickened by lignin
- They provide support to prevent collapse under tension of water transport
- Perforation plates are present at end walls of vessel elements. Other walls are interrupted by pits
- They form xylem vessel by aligning end to end with perforation plates
- Water flows freely through perforation plates

Tracheids

- Found in all vascular plants
- Long, thin cells with tapering ends
- Secondary walls are thickened with lignin and often interrupted by pits
- Water moves from end to end through pits
- Thickening by lignin provides support to prevent collapse under water transport

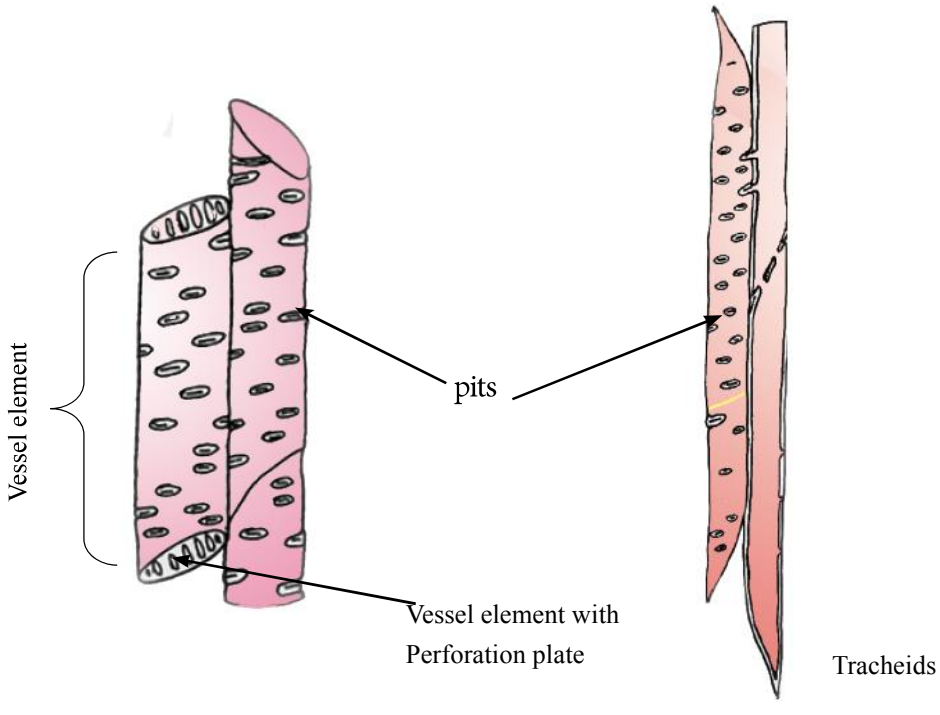


Fig 4.6 Diagram of L.S of vessel elemetmt amd tracheids

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Phloem tissue

- It consist of sieve tube elements, companion cells, parenchyma cells and fibers in angiosperms
- Except fibers other phloem cells are living cells
- In seedless vascular plants and gymnosperms sieve tube elements and companion cells are absent. Instead of sieve tube elements, long narrow cells called sieve cells are present in these plants.

Sieve tube elements

- Sieve tube elements lack nucleus, ribosomes, a distinct vacuole, and cytoskeletal elements-
- cytoplasm reduced into a thin peripheral layer.
- Absence of these allow passing of nutrients more freely
- Chains of sieve tube elements are aligned to form sieve tubes
- The end walls between sieve tube elements contain porous plate called sieve plate.
- Sieve plate allows movement of fluid from one sieve element to the next.

Companion cells

- They are non-conducting cells.
- Found alongside in each sieve tube element and connects with sieve tube element by numerous plasmodesmata
- Nucleus and ribosomes of these cells also serve to adjacent sieve tube element
- Some companion cells in leaves help in phloem loading and in other organs unloading

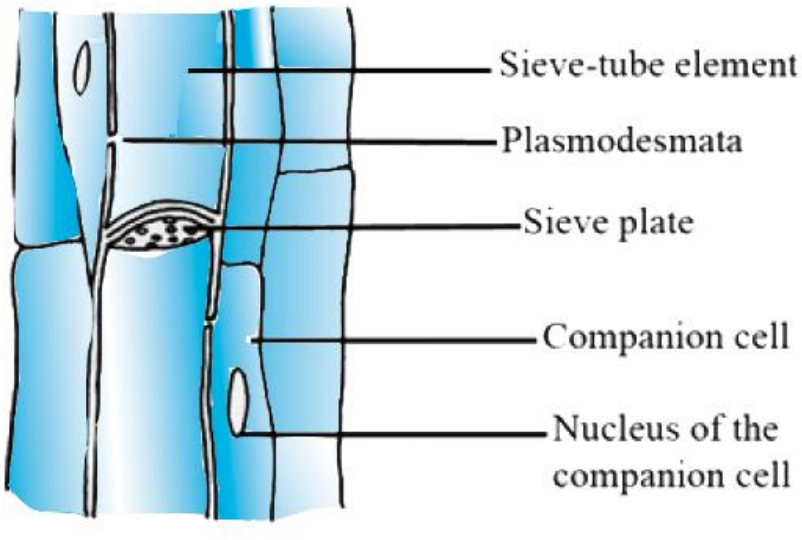


Fig -4.7 Longitiudinal view of sieve tube elements and companion cells

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Growth and development process of a plant

Plant growth

Growth involves irreversible increase of dry mass associated with the development of an organism. Often it is associated with increase of cell number as a result of producing more cells from the meristem accompanied by cell elongation.

Plants continue growth throughout the life known as indeterminate growth.

Primary structure root

Apart of the distribution pattern of xylem and phloem tissue structures of both monocot and dicot roots are more or less similar.

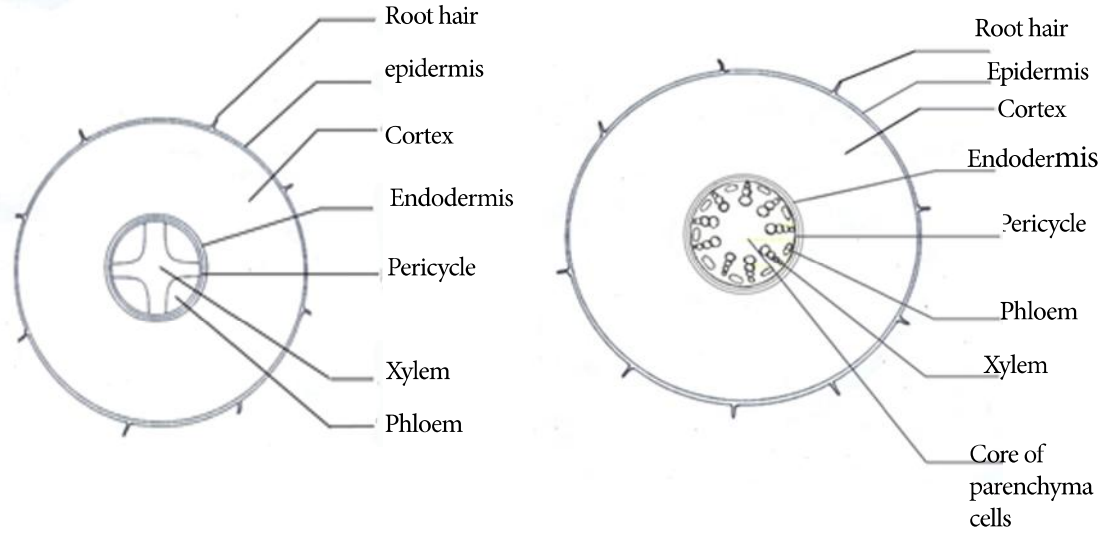


Fig- 4.8 Primary structure of typical Dicot and Primary structure of typical monocot root

- Outermost cell layer is epidermis. Cells have unicellular outgrowths called root hairs. Epidermis protects inner parts while root hairs involve in the absorption of water and minerals.
- Between epidermis and vascular cylinder there is a ground tissue known as cortex which is made up of mostly parenchyma cells with intercellular spaces.
- Cortex mainly stores carbohydrates, and also ransports water and minerals towards the endodermis.
- Innermost single cell layer of the cortex is the endodermis.
- Endodermis contains a suberin belt called casparian strip and no inter-cellular spaces. Therefore, it blocks cortical apoplast from the vascular apoplast.
- Interior to endodermis there is a pericycle containing two or three parenchyma cell layers. These cells in dicot roots have meristematic function and involve in the formation of lateral roots and secondary growth of the root.

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- Inner to pericycle there is vascular tissues as a solid core. Xylem can be found in the middle and it is star shaped in a cross section of a dicot root. Phloem is located in the groove between the arms of xylem.
- In monocot roots, vascular tissue consists of a central core of parenchyma cells surrounded by a ring of alternating xylem and phloem. Pericycle in monocot roots is not meristematic

Primary structure of dicotyledonous plant stem :

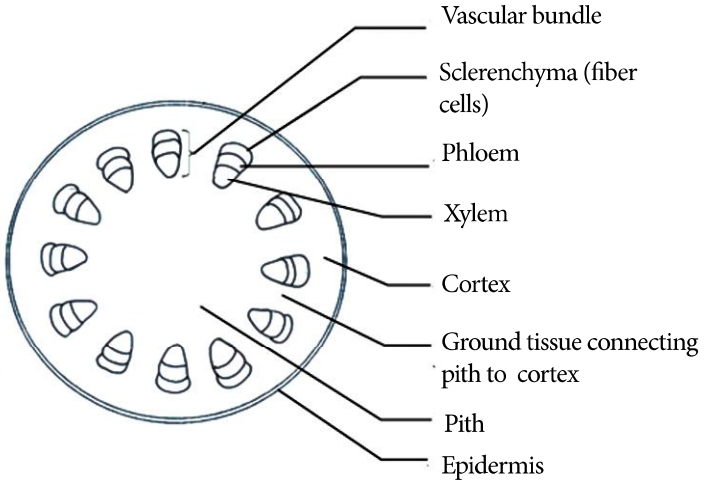


Fig 4.9 Primary structure of T.S of dictolyledonous plant stem

- The outermost epidermal cell layer protects inner parts from desiccation and infections. The epidermis is interrupted by pores called stomata.
- Interior to epidermis is cortex mostly containing parenchyma cells.
- Collenchyma cells may also be present just beneath the epidermis to provide strength.
- Sclerenchyma such as fibers are also present in the cortex to provide additional support.
- Vascular bundles arranged as a ring. Vascular bundle contains primary phloem towards cortex primary xylem towards pith and in-between a cambium tissue.
- Outside vascular bundle, there is a cluster of sclerenchyma cell.
- Inner to vascular bundles large pith which is also made up of parenchyma cells can be found.
- Lateral shoots develop from axillary buds

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Primary structure of the monocotyledonous stem:

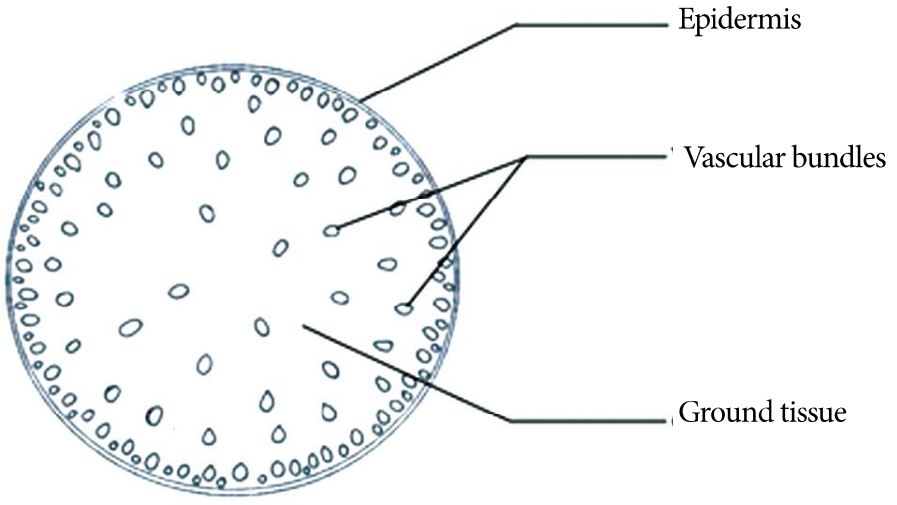


Fig :4.10 Primary structue of cross section of typical monocot stem

- Ground tissue of monocot shoot is not differentiated into cortex and pith
- The vascular bundles are scattered throughout the ground tissue in most monocot stems.
- Each vascular bundle is surrounded by sclerenchyma. It consists of a xylem tissue and a phloem tissue but no cambium inbetween xylem and phloem.

Secondary growth :

- Increase in the diameter of stems and roots in plants due to the new cells produced by lateral meristems is called secondary growth.
- This occurs in stems and roots of woody perennial plants including, all gymnosperms species and many dicot species.
- Lateral meristems, namely vascular cambium and cork cambium produce cells and tissues in the secondary growth.
- The vascular cambium adds secondary xylem (wood) towards primary xylem and secondary phloem towards primary phloem, increasing vascular flow and support for the shoots.
- The cork cambium produces tough thick covering consisting mainly of wax impregnated cells that protect the stem from water loss and from invasion of insects, bacteria and fungi.
- In woody plants, primary growth and secondary growth occur simultaneously. As the primary growth adds new cells and lengthens stems and roots in the younger regions of a plant, secondary growth increases the diameter of stems and roots inolder regions where. primary growth has ceased.

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- Secondary vascular tissue is produced by the action of vascular cambium.
- In a typical woody stem, the vascular cambium consists of a continuous cylinder of undifferentiated cells of often only a single cell layer in thickness, located outside the pith and primary xylem and to the inside of the cortex and primary phloem.
- In a typical woody root, the vascular cambium forms laterally exterior to the primary xylem and interior to the primary phloem and pericycle.
- As these meristematic cells divides they increase circumference of the vascular cambium and also add secondary xylem to the inside of the cambium and secondary phloem to the outside.
- Viewed in a cross section, the vascular cambium appears as a ring of initials.
- Some initials are elongated and are oriented with their long axis parallel to the axis of stem or root.
- They produce cells such as tracheids, vessel elements, parenchyma and fibers of the xylem, as well as sieve-tube elements companion cells, phloem fibers and phloem parenehyma.
- The other initials are shorter and oriented perpendicular to the axis of the stem or root.
- They produce vascular rays-mostly parenchyma cells that connect secondary xylem and phloem, store carbohydrates and aid in wound repairing.
- As the secondary growth continues over many years, layers of secondary xylem (wood) accumulate.
- The walls of the secondary xylem cells are heavily lignified and account for the hardness and strength of wood.
- During early stages of secondary growth, the epidermis pushed outwards, causing it to split, dry and falls off the stem or root.
- It is replaced by two tissues produced by cork cambium, a cylinder of dividing cells that arises in the outer layer of cortex in stems and in the outer layer of pericycle in the roots.
- Cork cambium produces cork cells to exterior.
- Cork cambium and tissues it produces are collectively called periderm
- As the cork cells mature, they deposit a waxy, hydrophobic material called suberin in their walls and they become dead cells.
- The cork tissues function as a barrier that helps protect the stem or root from water loss, physical damages and pathogens.
- Each cork cambium and the tissues it produces comprise a layer of periderm which is impermeable to water and gasses.

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- For gaseous exchange small pores are present in the periderm known as lenticels which are formed by loosely arranged cork cells. They appear as horizontal slits.
- Further growth of stem or root breaks the layer of cork cambium and it lacks its meristematic activity and its cells become cork cells
- A new cork cambium is initiated inside which with produce a new layer of periderm.
- As new cells are added, the outer regions of cork will crack and peel off in many tree trunks.
- Due to the tissue layers produced by vascular cambium and cork cambium, girth of the stem or root increases in secondary growth.
- Bark is all tissues out of the vascular cambium (cork is commonly and incorrectly referred to as bark). Its main components are secondary phloem and periderm.

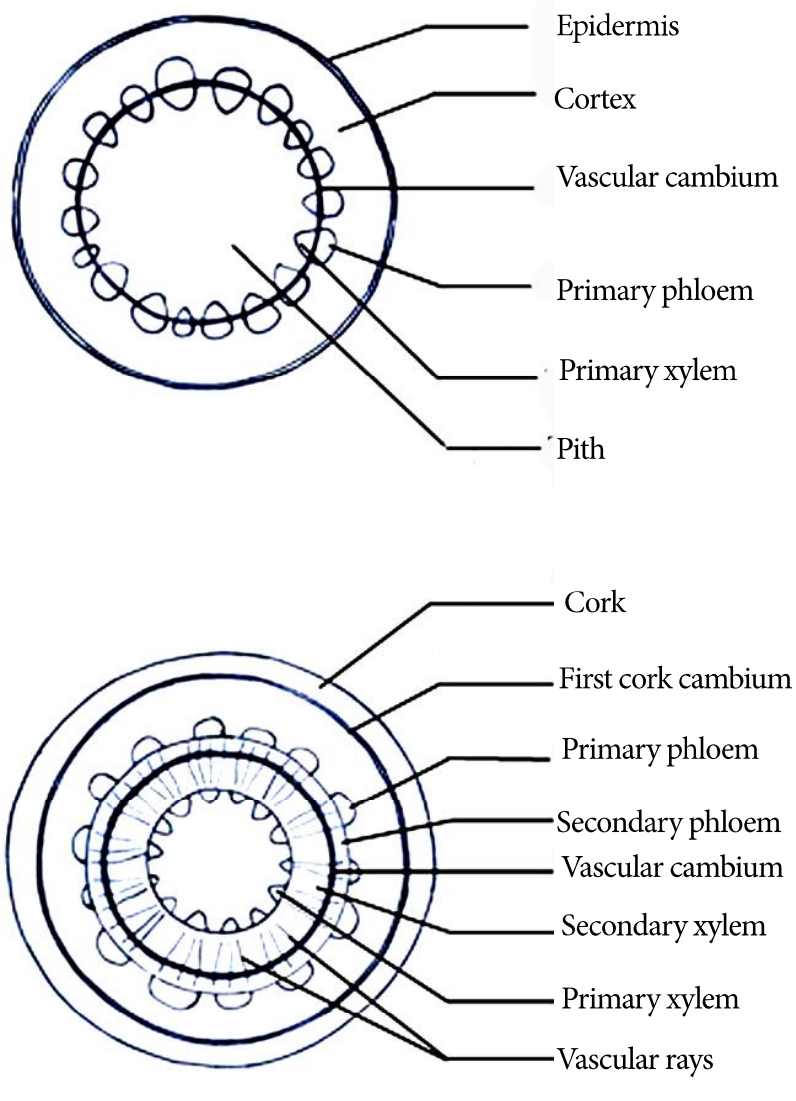


Fig. 4.11- The process of secondary growth of dicot woody plant

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Heart Wood and Sap wood:

- As a woody plant ages, the older layers of secondary xylem no longer transport water and minerals.
- These layers are called heartwood because they are close to the centre of the stem or root.
- The newest outer layers of secondary xylem, still transport xylem sap are known as sapwood.
- The heartwood is generally darker than sapwood because of resins and other compounds that permeate the cell cavities and help protect the core of the tree from fungi and wood-boring insects.
- Only the young secondary phloem functions in phloem translocation and old secondary phloem is sloughed off.

Hard wood and soft wood

- Hard wood is the secondary xylem of dicot angiosperms while wood of gymnosperms are named soft wood
- Xylem vessels are absent in soft wood

Growth rings:

- The thickness of secondary xylem and the lumen of vessels are larger in periods of warm and wet seasons compared to other growth season of the year. These differences are visible in a cross section as lighter and darker rings. These are referred as growth rings.
- In temperate regions, wood that develops early in spring is known as spring wood. This xylem tissue consists of xylem vessels with large lumens and thin walls. This structure maximizes delivery of water to new leaves.
- The wood produced during rest of the growing season is called summer wood. These xylem tissues consist of xylem vessels with thick walls and small lumen, do not transport much water but provide more support.
- These two woods collectively known as an annual ring. A year's growth appears as distinct ring in the cross section of most tree trunks and roots. Therefore age of the tree can be estimated by counting annual rings in trees growing in temperate regions.

Shoot, architecture and light capture

- Length of the stem and branching pattern are designed to capture maximum light.
- Plants grow tall to avoid shading from neighboring plants.

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Stem

- Most tall plants have thick stem with strong mechanical support.
- Woody plants undergo secondary growth thereby make their tall stem stronger.
- Vines rely on other objects to reach higher levels to capture more light.

Branching pattern

- There is a variety in branching pattern.
- Some plants are unbranched and still others are well branched.
- This variation in branching pattern enables the plant to absorb maximum light in the ecological niche it occupies.

Leaves

Leaf size

- Size of the leaf vary, based on the place where the plant grows.
- Largest leaves are found in plants growing in rain forests.
- Smallest leaves are found in plant species inhabiting dry or very cold environments.

Phyllotaxy

- This is the arrangement of leaves on the stem.
- The arrangement may be one leaf, two leaves or several leaves per node.
- Phyllotaxy helps the plant to capture maximum sunlight.

Leaf orientation

- Leaves may be horizontally oriented.
- They capture light efficiently in low light conditions.
- Some plants have vertically arranged leaves. e.g. Grasses
- This is to avoid the possible damage caused by exposure of leaf to the over intense light. When leaves are nearly vertical, light rays are parallel to the leaf surfaces, so no leaves receive too much of light.

Process of Gaseous Exchange in Plants

Anatomy of typical dicot and monocot leaves

In most vascular plants, leaves are the main photosynthetic organs. The exchange of gases occurs through stomata in the upper and lower epidermis. Epidermis is usually a single cell layer. In between the upper and lower epidermis, there is a ground tissue called the mesophyll. This tissue consists of parenchyma cells, specialized for photosynthesis.

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In dicot leaves, stomata are, mainly found in the lower epidermis. The mesophyll consists of two distinct layers called palisade and spongy. Palisade mesophyll consists of elongated cells that are arranged in one or more layers. This can be found in the upper part of the leaf, just beneath the upper epidermis.

The spongy mesophyll can be found between the palisade layer and lower epidermis. They are loosely arranged with many air spaces. Spongy mesophyll cells have less chloroplasts than in palisade mesophyll cells.

The vascular tissue of the leaf is continuous with vascular tissue of the stem. Veins in the leaf is highly branched (net like venation) in the mesophyll layer. Each vein is protected by a bundle sheath layer.

In monocot leaves, stomata are present in both lower and upper epidermis. Mesophyll is not differentiated into palisade and spongy layers. Chloroplasts are abundant in all mesophyll cells. Veins are parallelly arranged (parallel venation).

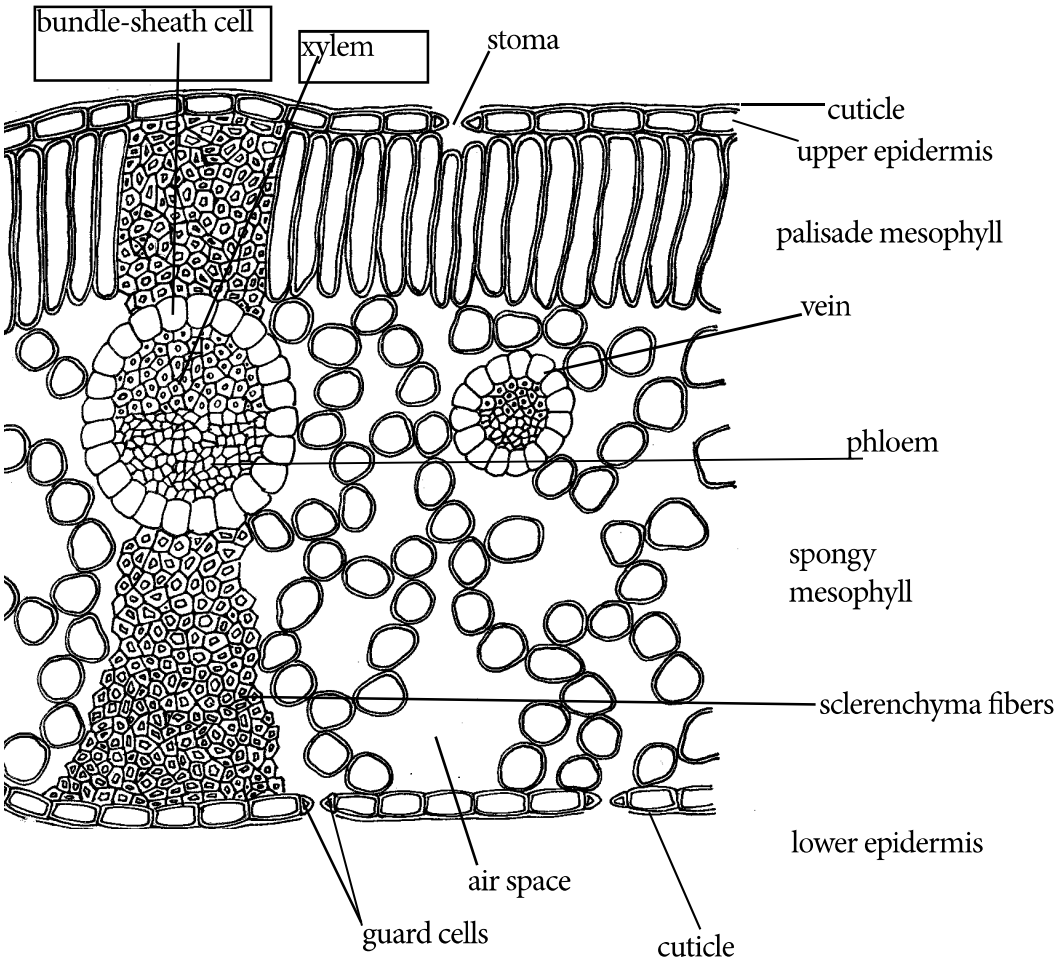


Fig. 4.12 T.S of typical Dicot Leaf

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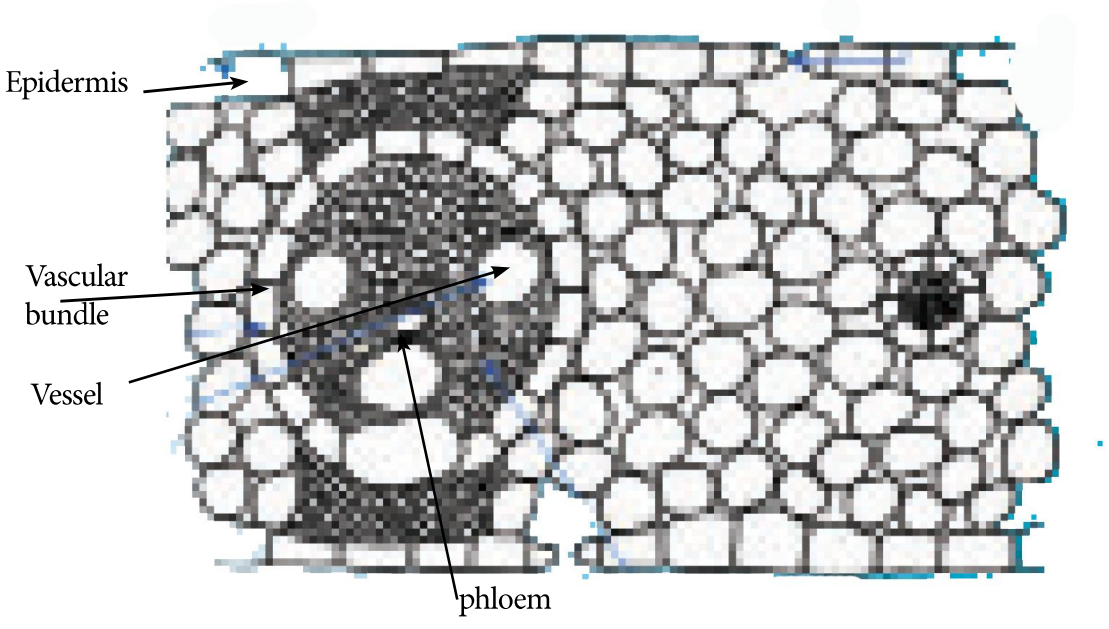


Fig 4.13 T.S of typical Monocot Leaf

Structure of Stomata

Stomata are pores surrounded by guard cells in the epidermis of the leaves and stems of plants which can open and close. Guard cells are modified epidermal cells which have a distinct shape and are the only epidermal cells that contain chloroplasts. Guard cells are typically bean shaped in angiosperms. The guard cell walls are unevenly thickened. The inner cellulose wall is thicker and less elastic than the outer wall. Some of the cellulose microfibrils are radially arranged to form inelastic hoops around guard cells.

Guard cells regulate the diameter of the stomata by changing shape, widening or narrowing the gap between the pair of guard cells.

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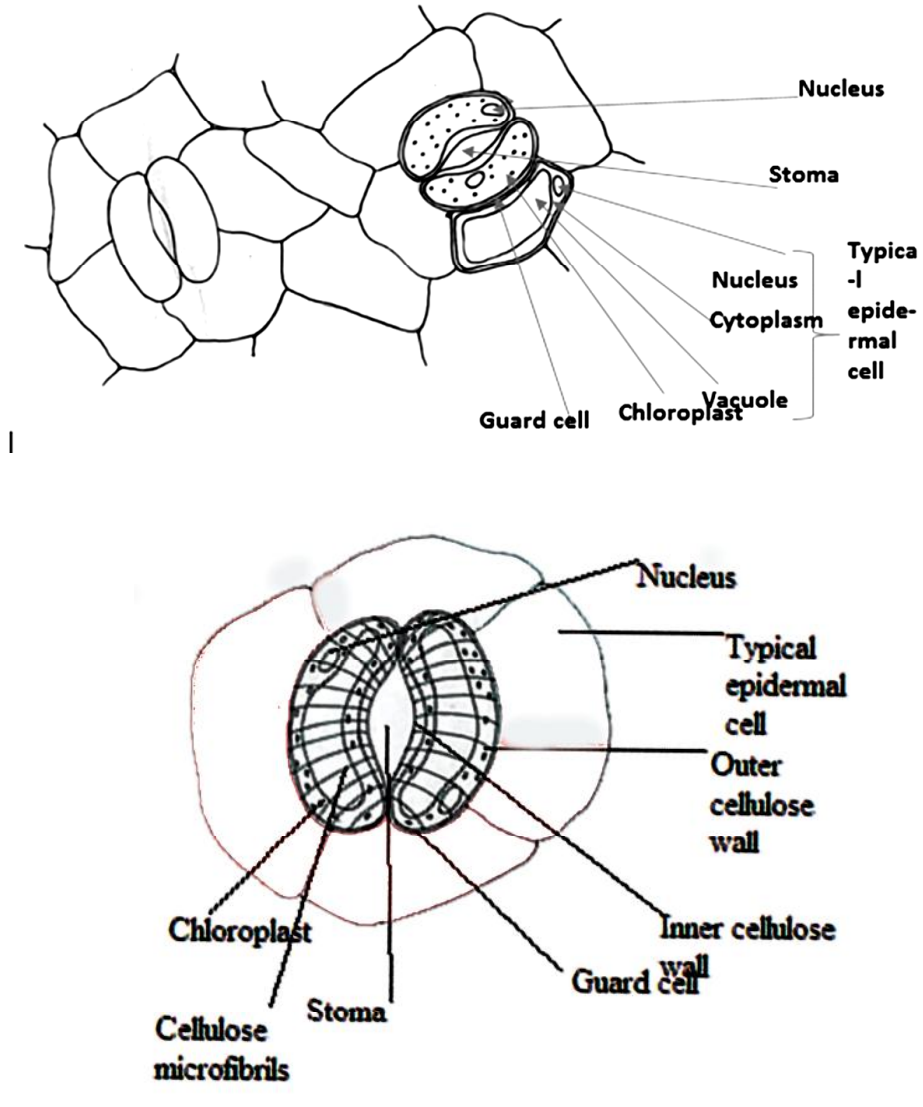


Fig - 4.14 Structure of the stomata

Gaseous exchange

Gaseous exchange is the exchange of gases between the cells of the organism and the environment. In plants gaseous exchange is possible via stomata and lenticels. In addition to these a small amount of gases can be exchanged via cuticle. There is no special system within plants for the transport of O_2 and CO_2.These gases move entirely by diffusion.

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Mechanism of opening and closing of stomata

Stomatal opening and closing depends on changes in turgor of the guard cells. If water flows into the cells by osmosis their turgor increases, and they expand, but they do not expand uniformly in all directions. The relatively inelastic inner walls make them bend and draw away from each other. The result is that the pore opens. If the guard cells lose water, the reverse happens- their turgor decreases, and their inner walls become straighter thus closing the pore.

The K+ influx hypothesis explains the mechanism.

K+ influx hypothesis

During the day time, the guard cells actively accumulate K+ from neighboring epidermal cells, thus lowering their water potential that leads to the inflow of water by osmosis from the surrounding epidermal cells. As a result the turgor pressure in guard cells increase, opening stomata.

The accumulation of K+ in the guard cells requires the energy which is provided by the transfer of electrons during photosynthesis of the chloroplast in guard cells.

Stomatal closing occurs by loss of K+ from guard cells to neighbouring epidermal cells. It leads to exosmosis of water from guard cells. As a result the turgor pressure in guard cells decrease, closing stomata.

Abscisic acid (ABA) also plays a role in K+ influx mechanism

Role of ABA in stomatal closure in drought

- ABA is produced in roots and leaves in response to water deficiency.
- Production of ABA leads to close the stomata by removal of K+in guard cells.
- This prevents the wilting of the plant.

Factors affecting stomatal action

- Stomata open during day and mostly closed at night. Light stimulates accumulation of K+ in guard cells.
- Decrease in CO_2 concentration in substomatal cavity lead to open stomata
- Internal clock in the guard cells controlling their daily rhythm of opening and closing of stomata.
- Environmental stresses such as drought, high temperature and wind can cause stomata to close during the day time.

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Acquisition of water and minerals

Need for transport

As land plants evolved and increased in number, competition for light, water and nutrients also increased. As a result, the size and complexity of plant body increased. Therefore the simple ways of transportation of water and material became inadequate leading to the evolution of vascular tissues, consisting of xylem and phloem to carryout long distance transport in plants.

e.g. the xylem transports water and minerals from roots to shoots.

the phloem transports products of photosynthesis from where they are made or stored to where they are needed.

Methods of water and solutes movement

Both active and passive transport mechanisms occur in plants

- Active transport
- Passive transport
  - Diffusion
  - Osmosis
  - Imbibitions
  - Facilitated diffusion
  - Bulk flow- long distance

Short distance

Passive transport occurs spontaneously, and it does not require metabolic energy (ATP).

Movement of some materials across membranes takes place using ATP and that process is called an active transport.

Diffusion

Molecules have an energy called thermal energy, due to their constant motion. One result of this motion is diffusion.

In the absence of other forces, the movement of molecules of a substance from a place where it is more concentrated to place where it is less concentrated, due to random motion of molecules is called diffusion.

The motion of a molecule is random, but movement of a population of molecules by diffusion is directional.

Therefore, diffusion takes place according to a concentration gradient, spontaneously and not using metabolic energy (ATP).

Diffusion takes place across the membrane also, if the membrane is permeable to those molecules.

e.g. Water and soluble materials can diffuse through the cellulose cell wall

O_2 and CO_2 can diffuse through the plasma membrane

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Osmosis

Osmosis is a special type of diffusion. The diffusion of free water molecules across a selectively permeable membrane is called osmosis.

Free water is water molecules that are not bound to solutes or surfaces.

Imbibition

The physical adsorption of water molecules by hydrophilic materials is called imbibition.

e.g. adsorption of water molecules by the cellulose cell walls.

Facilitated diffusion

Movement of water and hydrophilic solutes across the membranes passively with the help of transport protein that span the membrane is called facilitated diffusion.

Transport proteins are very specific. They transport some substances but not the others. This movement also takes place along concentration gradient and it is a passive movement.

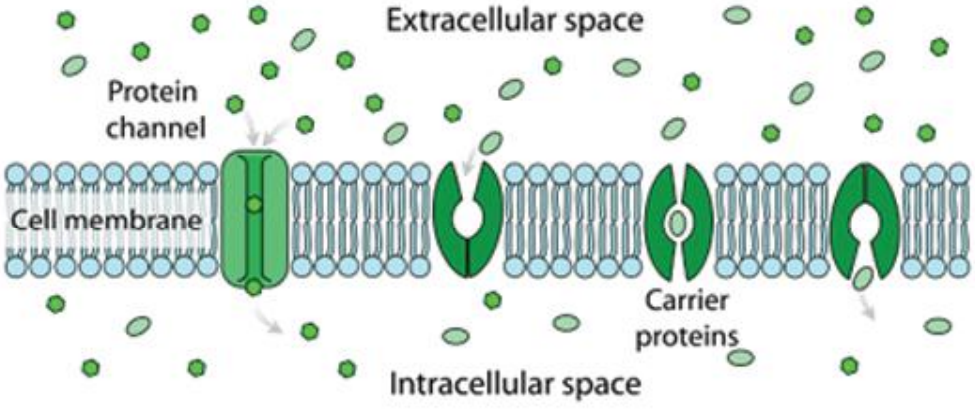


Fig 4.15 -The process of as facilitated morcment

Bulk flow

Bulk flow is the movement of liquid and the materials (entire solution) in response to pressure gradient. Always the bulk flow transports materials from higher pressure to lower pressure region.

It is a long-distance transport method. This flow does not occur through the membranes and occurs at much greater speed than diffusion. This method of transport is independent of solute concentration gradient.

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Concept of water potential

The physical property that predicts the direction in which water will flow governed by solute concentration and applied pressure is called water potential. Water potential is related to potential energy of water molecules.

Any system that contains water has a water potential. Free water moves from regions of higher water potential to regions of lower water potential, if there is no barrier to its flow.

Water potential is denoted by Ψ. Ψ is measured in a unit of megapascal (MPa). Arbitrary the Ψ of pure water in a container open to the atmosphere under standard conditions (at sea level and room temperature) is 0 MPa.

Both solute concentration and physical pressure can affect water potential; as expressed in the water potential equation.

$\Psi = \Psi s + \Psi p$

Ψ= water potential

Ψs= solute potential

Ψp= pressure potential

Solute potential

Solute potential (Ψs) is directly proportional to the molarity of a solution. (Ψs is also called osmotic potential. Solutes affect the direction of osmosis.)
The solutes in plants are typically mineral ions and sugars.

Ψ of pure water is 0 MPa.

When solutes are added, they bind water molecules and reduce free water molecules, reducing capacity of the water to move and do work.

In this way an increase in solutes has a negative effect on water potential. Therefore, Ψs of a solution is always expressed as a negative number. As the solute concentration increases, Ψs will become more negative.

e.g: Ψs of the 0.1M sugar solution is -0.23MPa

Pressure potential

Pressure potential (Ψp) is the physical pressure on a solution. Ψp can be positive or negative relative to atmospheric pressure.

e.g: Ψp of a xylem vessel is usually less than -2 MPa as xylem vessels are under tension (negative pressure)

Ψp of a living cell is a positive value because living cell is usually under positive pressure due to osmotic uptake of water.

The cell contents press the plasma membrane against the cell wall. Then press against the protoplast, producing a pressure called turgor pressure. When turgor pressure increases, the Ψ of cell also increases.

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Water potential of a cell

Cell is a system containing water. Therefore, it has a water potential. The protoplast is an aqueous system and it contains solutes. Therefore, it has a solute potential (Ψs) which is negative. Because of Ψs, Ψ of the cell is decreased.

Due to the turgor pressure internal pressure of protoplast increases and increases pressure potential (Ψp) of the cell. Because of Ψp, Ψ of cell increased.

Therefore, water potential (Ψ) of a cell is given by the following equation.

$\Psi = \Psi s + \Psi p$

Entry of water into vacuolated cell across the cell membrane

If a cell is placed in a solution, direction of water movement depends on the water potential of external solution and protoplast.

Take a fully flaccid cell; (as a result of water losing)

The Ψp of that cell is 0;

$\Psi p = 0$; $\Psi s = \Psi$

Ψs of pure water is 0 and addition of solutes will increase the negative value of Ψs or become more negative. Suppose this flaccid cell is placed in a solution of higher solute concentration (more negative solute potential) than the cell itself, since the external solution has a lower (more negative) water potential, water diffuse out of the cell. The protoplast of the cell shrinks and pulls away from the cell wall. This process is known as plasmolysis.

Suppose this flaccid cell is placed in pure water (Ψ=0 MPa). The cell has a lower water potential than the pure water as it contains solutes. Therefore, water enters the cell by osmosis. Then the protoplast of the cell begins to swell and press the plasma membrane against the cell wall. The partially elastic cell, exerting a turgor pressure, contains the pressurized protoplast. Therefore, Ψp is increased gradually. The maximum value of Ψp= Ψs of the cell. Therefore, Ψ becomes 0. This matches the water potential of extracellular environment, 0 Mpa. Then a dynamic equilibrium is formed and there is no further net water movement. If the cell has the maximum value for Ψp, (it equal to the Ψs of the cell) the cell is said to be in fully turgid state. (fully turgid or fully flaccid cells are not found in nature).

Therefore, if non-woody tissue is placed in a solution with higher water potential, it is stiffened and is very rigid. Therefore, turgor pressure helps support of non woody plants. Turgor pressure is also important in cell elongation. Loss of turgor results in wilting, a condition where leaves and stem droop.

Movement of water and minerals from soil solution to plant root

The cells near the root tips of the roots are important because most of the absorption of water and minerals occurs there. In this region, the epidermal cells are permeable

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to water and many are differentiated to root hairs. Root hairs account for much of the absorption of water by roots, due to increase in surface area.

The root hairs absorb the soil solution, which consists of water molecules and dissolved mineral ions that are not bound tightly to soil particles. This absorption takes place across the plasma membrane. Water can enter root hair by osmosis, a passive movement along the concentration gradient.

But in the root hairs concentration of mineral ions is greater than that of soil solution. K+ concentration in the root hair is hundreds of time greater than in the soil solution. Therefore, mineral ions transport occurs against concentration gradient, by an active transport.

The soil solution is also absorbed into hydrophilic walls of the epidermal cells and passes freely along the cell walls and the extracellular spaces into the root cortex.

Radial transport

Transport of water and minerals entered from soil to root cortex into the xylem of the root is known as radial transport.

The endodermis, the innermost layer of cells in the cortex, functions as the last check point for selective passage of the minerals from the cortex into the vascular cylinder. All materials which enters root through cell walls and extracellular spaces should cross the membranes of endodermis. Therefore, unwanted materials can be selectively excluded.

Three routes are used in the radial transport. They are:

1. apoplastic route
2. symplastic route
3. transmembrane route

Apoplastic route

The apoplastic route consists of everything external to the plasma membrane of living cells and includes cell walls, extracellular spaces and the interior of dead cells such as vessel elements and tracheids.

Water and solutes move along continuum of the cell walls and extracellular spaces and it is known as apoplastic route.

Uptake of soil solution by the hydrophilic walls of root hairs provides access to the apoplast. Water and minerals then can diffuse into cortex along this matrix of walls and extracellular spaces.

Endodermis blocks apoplastic route by a barrier located in the transverse and radial walls of endodermal cells, called the casparian strips. It is a belt made of suberin which is impervious to water and mineral salts. Thus water and minerals cannot cross the endodermis and enter the vascular cylinder via apoplast. Therefore, water and minerals

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cross the selectively permeable plasma membrane before entering the vascular tissue and keep unneeded and toxic materials out.

The endodermis also prevents solutes that have accumulated in the xylem from leaking back into the soil solution.

Symplastic route

The symplast consists of the entire mass of cytosol of all living cells in a plant, as well as plasmodesmata, the cytoplasmic channels that interconnect them.

In the symplastic route, water and solutes move along the continuum of cytosol. This route requires substance to cross a plasma membrane once, when they first enter the plant. After entering one cell, substances can move from cell to cell via plasmodesmata.

Transmembrane route

The transmembrane route requires repeated crossing of plasma membranes as water and solutes exit one cell and enter the next.

As the soil solution moves along the apoplast, some water and minerals are transported into the protoplast of the cells of the epidermis and cortex and then move via the symplast.

Some substances can use more than one route. The least resistence for the transport is found in apoplastic route. Therefore, more water use apoplastic route.

Finally, water and minerals enter into the tracheids and vessel elements of xylem. These waters conducting cells lack protoplasts when mature and therefore they are parts of the apoplast. Endodermal cells and living cells of the vascular tissues discharge minerals from their protoplast to their own cell walls. Both diffusion and active transport involve in transport of solutes from symplast to apoplast. Then water and minerals can enter the tracheids and vessel elements to the transport to shoot system by bulk flow only through the apoplast.

Upward movement of water and minerals in a plant

Water and minerals which enter to vascular cylinder are transported to upper parts of the plant and this transport is known as ascent of xylem sap.

Xylem sap, the water and dissolved minerals in the xylem, gets transported by bulk flow, which is much faster than diffusion.

To explain the process involved in the ascent of xylem sap, cohesion-tension hypothesis is put forward. According to this hypothesis, transpiration provides pull for the ascent of xylem sap and cohesion of water molecules transmits this pull along the entire length of xylem from shoots to roots. Hence xylem sap is normally under tension (negative pressure).

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The negative pressure potential helps water to move up through xylem and water moves according to the water potential gradient.

Adhesion and cohesion facilitate transport water by bulk flow. Due to high adhesion water molecules are attracted to cellulose molecules in the xylem walls. Cohesion of water molecules is unusually high due to hydrogen bonds among water molecules. Therefore, a continuous water column is formed within xylem vessels and tracheids. Transpiration pull can extend down to the root only through an unbroken chain of water.

As water evaporates from the mesophyll cells, water potential of mesophyll reduces, and water moves from cells of petioles to the mesophyll cells. It reduces the water potential of cells of petioles. then water pulls upward due to this transpiration pull.

The xylem sap is driven by difference in pressure potential. Therefore, the water potential gradient within xylem is essentially a pressure gradient.

The tensile force on xylem sap is transmitted all the way from the leaves to the root tips and even into the soil. Therefore, water potential gradient between the soil solution and atmosphere through the plant body also help ascent of xylem sap, against the gravity.

The plants do not need energy to lift the xylem sap.

Mechanism of mineral absorption into root

Mineral ions are absorbed by the plant roots mainly from the soil solution. Epidermal cells are permeable to water and many epidermal cells are modified to form root hairs. Root hair cells are unicellular structures which absorb dissolved mineral ions from the soil solution. Soil solution has a lower concentration of ions than that of the cell sap of root hair cells. Therefore, absorption takes place against a concentration gradient.

The process involved in transport of material in phloem

Basic characteristics of phloem transport

The transport of the product of photosynthesis is carried out by the phloem tissue, known as phloem translocation.

In angiosperms, the sieve-tube elements of the phloem are specialized cells for translocation.

Phloem sap, the aqueous solution that flows through sieve tubes differs from xylem sap mainly because it contains sucrose (as 30% by weight) and it may also contain amino acids, hormones and minerals.

Phloem sap moves from sites of sugar production to site of sugar use or storage. Therefore, it takes place from sugar source to a sugar sink.

Sugar source is an organ that is a net producer of sugar, by photosynthesis or by breakdown of starch.

Plant leaves are sources whereas growing roots, stems, buds and fruits are sinks.

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Storage organs such as tubers and bulbs, may be a source or a sink, depending on its function.

Mechanism of phloem translocation

Sinks usually receive sugar from the nearest sugar sources. For each sieve tube, the direction of transport depends on the locations of the sugar source and sugar sink that are connected by that tube. Therefore, neighbouring sieve tubes may carry sap in opposite directions if they originate and end in different locations.

The first step in translocation of sugar is to transport or load into sieve tube elements. In some species, it moves from mesophyll cells to sieve tube elements via symplast, passing through plasmodesmata.

In many plants, sugar movements into phloem requires active transport because sucrose is more concentrated in sieve tube element and companion cells than mesophyll cells.

Sucrose is unloaded at the sink end of the sieve tube. The process varies by species and organ. However, the concentration of free sugar in sink is always lower than in the sieve tube because the unloaded sugar is consumed during growth and metabolism of cells of sinks or converted to insoluble polymers such as starch. As a result of concentration gradient, sugar molecules diffuse from phloem into the sink and water follows by osmosis.

Phloem sap moves from source to sink at a rate about 1m/hr and it moves by bulk flow driven by positive pressure, known as pressure flow.

Phloem translocation of angiosperms is explained by pressure flow hypothesis. In this translocation, following processes take place.

1. Loading of sugar into the sieve tube reduces water potential inside the sieve tube elements at the source
2. This causes the sieve tube to take up water from the xylem by osmosis.
3. This uptake of water generates a positive pressure that forces the sap to flow along the tube
4. The pressure is reduced by unloading of sugar and consequent loss of water from phloem to the xylem at the sink

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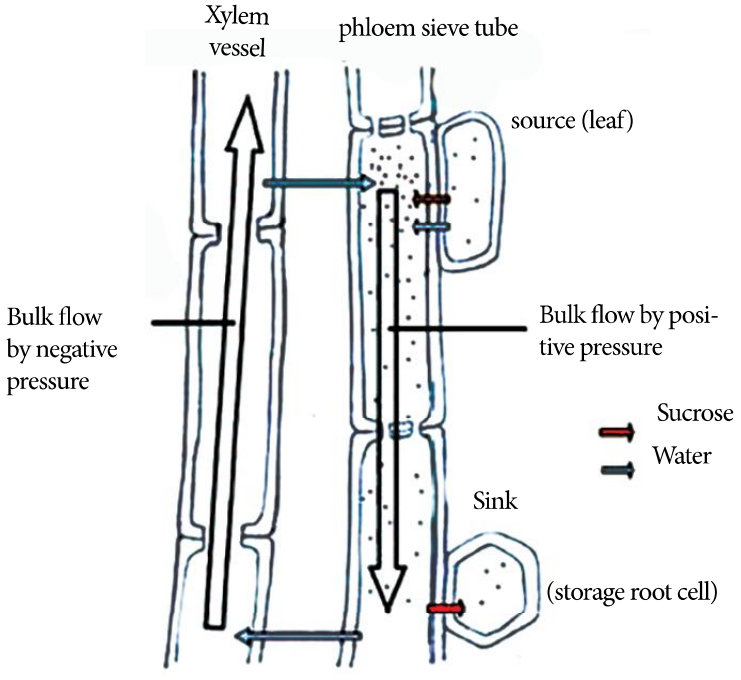


Fig 4.16 : The Process phloem transportation

Process of water loss in plants

Transpiration

Removal of water from leaves and other aerial parts of the plant body as water vapour by diffusion is known as transpiration.

This water loss takes place in plants mainly;

through stomata.-stomatal transpiration

upto some extent through cuticle-cuticular transpiration

and through lenticels- lenticular transpiration.

About 95% of water in plants is lost through stomatal transpiration.

In the day time, air in the intercellular air spaces is saturated with water vapour because they are in contact with the moist cell walls. Normally air outside the plant is drier than inside. Hence water potential of outside air is lower than that of inside. Therefore water vapour in the air spaces of the plant diffuses down its water potential gradient and exits the plant.

Stomatal Transpiration

Water is brought to the leaf in the xylem of vascular bundle and subsequently spread from a fine branching network throughout the leaf. These branches end in one or few xylem vessels or tracheids possessing little lignification. Therefore water can release easily through their cellulose walls to mesophyll cells. Water moves through

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the mesophyll cells by apoplast, symplast and transmembrane pathways according to water potential gradient.

Then water evaporate from the wet walls of the mesophyll cells into the intercellular air spaces particularly into the large substomatal air spaces. From here water vapour diffuses through the stomata to the atmosphere.

Immediately next to the leaf is a thin layer of stationary air through which water vapour diffuses out and swept away by moving air.

There is a diffusion gradient from the stationary layer back to the mesophyll cells. Each stomata has a diffusion gradient or diffusion shell around it. The diffusion shell of neighbouring stomata overlap in still air to form one overall diffusion shell (Layer). Thickness of the diffusion shell depends on the surface features of the leaf and wind speed.

Factors affecting the rate of transpiration

1. Light intensity
2. Temperature
3. Humidity
4. Wind speed
5. Concentration of CO_2
6. Available water in soil

Light intensity

Stomata usually open in the light and close in darkness. With the increase of light intensity the rate of transpiration increases.

Temperature

In the presence of light, the external factor which has the greatest effect on transpiration is temperature. The higher the temperature, the greater the rate of evaporation of water from mesophyll cells which result the greater saturation of the leaf atmosphere with water vapour. At the same time, a rise in temperature lowers the relative humidity of the air outside the leaf. Both events result in a steeper concentration gradient of water molecules from leaf to external atmosphere. The steeper this gradient is the faster the rate of diffusion.

Humidity

Low humidity outside environment of the leaf increases transpiration, because it makes the diffusion gradient of water vapour from the moist leaf atmosphere to drier

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external atmosphere. As the concentration of water vapour in the external atmosphere is high when humidity rises, the diffusion gradient becomes less steep result in lower transpiration.

Wind speed

In still air, a shell of highly saturated air builds up around the leaf thus reducing the steepness of the diffusion gradient between leaf atmosphere and external atmosphere which makes the transpiration rate low.

In windy condition, flow of air will generally sweep away the shell. Therefore, windy condition increases transpiration rate.

Availability of soil water

As soil dries out, water usually binds more tightly to soil particles reducing the amount of available water. The soil solution becomes more concentrated and its water potential decreases. Therefore, tendency for water to enter by osmosis is lower. This reduces water uptake by plants and as a result transpiration rate is also reduced. There is greater resistance to movement of water through the plant due to less steep water potential gradient from the soil through the plant to the atmosphere.

Significance of transpiration to plants

1. Distribute minerals and water throughout the plant.
2. Ascent of water in the xylem.
3. Uptake of water and minerals by roots from the soil solution.

Root pressure and guttation

At night, when the relative humidity is high approaching 100%, transpiration rate is very low or zero. Root cells continuously pump water and mineral ions into xylem tissue. The endodermis prevents ions from leaking back into the cortex and soil. Therefore more mineral ions accumulate in the vascular cylinder and reduce water potential. Therefore water moves from the cortex. It generates a root pressure and upward push of xylem sap.

Due to root pressure, more water enters to the leaves than lost by transpiration. This results in removal of water droplets from leaf tips or leaf margins of some herbaceous plants. That process is known as guttation. Guttation fluid differs from dew, which is condensed moisture of the atmosphere.

Many plants do not generate any root pressure and therefore there is no guttation. Even in plants that display guttation, root pressure cannot match the water loss by transpiration after sun rise and therefore no guttation is seen in the day time, because then xylem sap is not pushed but pulled upward by transpiration.

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Root pressure is never sufficient to push water up distance over meters. Guttation takes place through the hydathode which are formed by special groups of cells located near the ends of small veins and does not take place through the stomata.

e.g. *Alocasia,* *Colocasia*

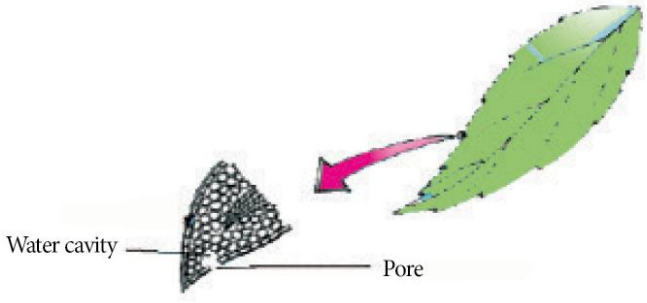


Fig 4,17: The general structure of the hydathode

Diversity of nutritional processes in plants

Nutrition is the process of acquiring raw materials and energy from the environment for the metabolic activities of organisms.

Plants require nutrients for their growth, development and reproduction.

Modes of nutrition in plants

Autotrophic nutrition (autotrophism)

The organisms who exhibit autotrophism are referred to as autotrophs. Autotrophs synthesize organic materials from CO_2 and inorganic materials.

Plants are photoautotrophs which utilizes light energy in order to synthesize organic molecules from inorganic material.

- **Symbiosis**

Symbiosis is an ecological relationship in which two species live in close contact with each other. There are three types; they are mutualism, parasitism, and commensalism.

- **Mutualism**

A symbiotic relationship in which both participants are benefited.

e.g: legume root nodules with nitrogen fixing bacteria (*Rhizobium*)

mycorrhizae- symbiotic association of roots of higher plants with fungi

corolloid root of *Cycas with Anabaena*

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- **Commensalism**

It is an interaction between two species in which benefits one of the species and neither harm nor benefits the other.

e.g: epiphytic orchids

- **Parasitism**

It is a close association between two different species which is beneficial to one (the parasite) and harmful to the other (the host).

e.g. semi parasitic–*Loranthus* and host plant

parasitic - *Cuscuta* (Dodder plant) and host plants

- **Special mode of nutrition**

Carnivorous plants

These plants are photosynthetic but obtain nitrogen and minerals by killing and digesting insects and other small animals. They live in habitats where the soil is poor in nitrogen and other minerals.

e.g : *Nepenthes, Drosera , Utricularia*

Nutritional requirements for the optimal growth of plants

Essential elements: Elements which are required for a plant to complete its life cycle and produce another generation.

Seventeen essential elements are needed by all plants

C,O,H,N,P,S,K,Ca, Mg, Cl, Fe, Mn, B, Zn, Cu, Ni, Mo,

Essential elements are two types

Macronutrients

Macronutrients: plants need these elements in large amounts.

e.g: C,O,H,N,P,S,K,Ca, Mg (9 elements)

Micronutrients

Plants require these elements in small amounts.

e.g: Cl, Fe, Mn, B, Zn, Cu, Ni, Mo

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Table 4.2- Macro elements and their functions and deficiency symptoms

Element	Form/ forms of intake	Source	Function	Deficiency symptoms
C	CO_2	Atmospheric air	One of the major components of organic molecules in plants	Poor growth
O	CO_2	Atmospheric air and soil solution	Major components of organic molecules in plants	Poor growth
H	H_2O	Soil solution	Major components of organic molecules in plants	Poor growth, wilting
N	NO_3^-, NH_4^+	Soil solution	Component of amino acids, proteins, nucleotides, nucleic acids, chlorophyll, coenzymes, enzymes	Stunted growth and strong chlorosis, particularly of older leaves
K	K^+	Soil solution	operation of stomata, cofactors of many enzymes	Yellow and brown leaf margins , weak stems, poorly developed roots
Ca	Ca^{2+}	Soil solution	Component of cell walls and middle lamella, maintenance of membrane structure and permeability, signal transduction	Crinkling of young leaves, death of terminal buds
Mg	Mg^{2+}	Soil solution	Component of chlorophyll molecule, Activates many enzymes.	Chlorosis between veins, found in older leaves
P	H_2PO^24-	Soil solution	Component of ATP and nucleic acids, phospholipids	Healthy appearance but very slow development, thin stems, purpling of veins, poor flowering and fruiting
S	SO_4^{-2-}	Soil solution	Components of some amino acids and proteins	Chlorosis in younger leaves

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Micro elements, their functions and deficiency symptoms

Element	Form/ form of intake	Source	Function	Deficiency symptoms
Cl	Cl^-	Soil solution	Osmosis and ionic balance, in photosynthesis	Wilting, stubby roots, leaf mottling (uncommon)
Fe	Fe^{2+},Fe^{3+}	Soil solution	Chlorophyll synthesis in photosynthesis, N_2 fixation	Chlorosis between veins, particularly in young leaves
Zn	Zn^{2+}	Soil solution	Activator of many enzymes and activate formation of Chlorophyll, need for DNA transcription	Crinkled leaves, reduced internode length
B	$H_2BO_3^-$	Soil solution	Cofactor in chlorophyll synthesis, role in cell wall function, pollen tube growth	Death of meristems, thick leathery, and discolored leaves
Cu	Cu2+ , Cu+	Soil solution	Activator or component of certain enzymes	Light green color throughout young leaves, with drying of leaf tips, roots stunted and excessively branched
Mo	MoO_4^{2-}	Soil solution	Nitrogen metabolism	Death of root and shoot tips, chlorosis in older leaves
Ni	Ni^{2+}	Soil solution	Nitrogen metabolism	Death of leaf tips, chlorosis in older leaves
Mn	Mn^{2+}	Soil solution	Activates some enzyme require in photosynthesis	chlorosis between veins, found in young leaves

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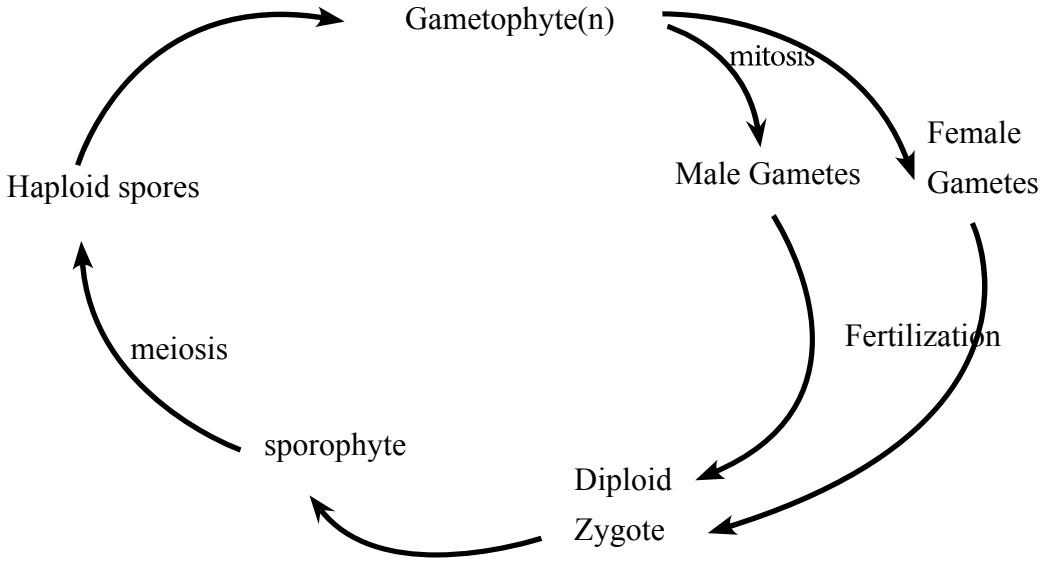
Reproductive process in Plants

Trends in life cycles to relate the adaptations of plants for a terrestrial life

Sexual reproduction of terrestrial plants

- The life cycles of all land plants exhibit alternation of generations, which means the presence of haploid generation and diploid generation alternatively, with each producing the other.
- The two multicellular body forms that alternate in the life cycles of land plants are the haploid gametophyte and diploid sporophyte which are morphologically different. Therefore called heteromorphic alternation of generations. Their reproductive organs (gametangia and sporangia) are protected by sterile cell layers to prevent desiccation of mother cells. (gamete forming cells and spore forming cells).
- Gametophytes produce gametes by mitosis.
- All land plants carryout internal fertilization to prevent desiccation of gametes.
- Female egg (ovum) is retained in the archegonium and male gametes (antherozoids) are released from the antheridium. Seedless plants depend on external water for fertilization, but seed plants do not depend on external water for their fertilization.
- After fertilization, diploid zygote is retained within the gametophyte to produce an embryo which is nourished by the gametophyte. Embryo develops into the diploid sporophyte.
- Delay of meiosis after fertilization results in creating a diploid sporophytic generation.
- Diploid sporophyte produces haploid spores by meiosis.
- Spores grow into haploid gametophytes.
- In the course of evolution of land plants, diploid sporophytic generation acquire adaptations needed for successful colonization on land and become dominant plant in the life cycle. Gametophytic generation gradually reduced and has become dependent on the sporophytic generation in seed plants.

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Life cycle of Pogonatum

- Gametophyte is the dominant plant, larger and longer-living than sporophyte.
- Gametophyte is photosynthetic.
- 'Stem', 'leaves', and rhizoids are present in the gametophyte
- Gametophytes are dioecious (unisexual). Mature male gametophytes produce antheridia in which several sperms are produced.
- Female gametophytes produce archegonia. A single egg is produced within the archegonium
- The egg is not released.
- Flagellated, motile sperm swims through external water towards egg, entering the archegonium in response to chemical attractants.
- Sperm fuses with the ovum resulting diploid zygote. This occurs in the archegonium.
- After fertilization zygote develops into the embryo.
- The embryo is also retained within the archegonium and develops into the diploid sporophyte by obtaining nutrients from the gametophyte.
- The sporophyte remains attached to the gametophyte.
- The sporophyte consists of a foot, seta and a capsule (sporangium).
- The foot absorbs nutrients and water from the gametophyte.
- The capsule produces spores by meiosis. Homosporous.
- If spores are dispersed to a favourable habitat, (such as moist soil or tree bark) they may germinate and grow into a green, branched filament called protonema.
- Protonema produces buds that grow into gametophytes.

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Life cycle of *Nephrolepis*

- Sporophyte is dominant
- Gametophyte is reduced and short lived.
- Both sporophytes and gametophytes are independent and photosynthetic.
- Sporophytes have more complex structure.
  - Plant body is differentiated into roots, stem and leaves.
  - Cuticle is found on aerial parts of the plant body
  - Stomata are developed on aerial parts for gaseous exchange.
  - Two types of vascular tissues, xylem and phloem are developed
  - They have fiddlehead young leaves
  - Stem is an underground rhizome
  - Leaves are compound pinnate leaves
  - Long underground branches called stolons arise from the rhizome \ which gives rise to new plantlets.
  - Sporangia are developed as clusters called sori on the underside of mature leaflets. Sori are covered by the indusium, protecting the young sporangia from desiccation. Spores are produced in the sporangium by meiosis and are homosporous.
- When the sorus matures, indusium dries up and shrivels, exposing mature sporangia.
- Under dry environmental conditions sporangium wall ruptures, releasing spores.
- Spores are dispersed by wind.
- When spores are dispersed to a favourable habitat they may germinate and grow into a gametophyte
- Gametophyte is a small heart shaped, macroscopic, green coloured photosynthetic thallus.
- Rhizoids develop on the ventral surface.
  - Gametophytes are monoecious (bisexual). Antheridia and archegonia are developed on the ventral side.
  - Antheridium produces flagellated sperms and releases them into the external environment.
  - Archegonium produces one egg and retains it.
  - Motile sperms swim through external water towards egg entering the archegonium in response to chemical attractants.
  - Sperm fuses with the egg resulting the diploid zygote.
  - After fertilization zygote develops into the embryo and then to the young sporophyte while retained in the gametophyte.
  - All the developmental stages are nourished by the gametophyte.

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- When the young sporophyte develops its photosynthetic tissues, it becomes an independent plant.

Life cycle of *Selaginella*

- Sporophytes are dominant and photosynthetic.
- Gametophytes are reduced in structure and short-lived, partially depend on the sporophyte.
- Sporophyte plant body is differentiated into roots, stem and leaves. Vascular tissues present. Herbaceous.
- Heterophyllous leaves are arranged as pairs.
- Stem is dorsiventrally flattened.
- Sporangia are borne on the specialized leaves called sporophylls.
- Sporophylls are compactly arranged in a terminal strobilus.
- Two types of sporophylls called megasporophyll and microsporophyll are arranged in the same strobilus.
- Megasporophyll produces a single megasporngium and microsporophyll produces a single microsporangium.
- Morphologically two different types of spores are produced. This nature is called heterospory.
- Megasporangium produces four large megaspores by meiosis.
- Microsporangium produces numerous small microspores by meiosis.
- Both types of spores have thick/tough walls.
- Microspores are retained in the microsporangium and develop into young male gametophytes.
- Young male gametophytes are enclosed by the wall of microspore which, are released by the microsporangium.
- In the external environment they become mature male gametophytes.
- Male gametophytes is microscopic, enclosed in the microspore wall, non-photosynthetic, depend on stored food.
- Male gametophytes produce flagellated sperms and release them into the external environment.
- Megaspores are released into the external environment. In the external environment they develop into female gametophytes.
- Female gametophyte is multicellular, surrounded by the thick wall of megaspore, Few rhizoids develop.
- Photosynthetic, but partially depend on stored food in the megaspore.
- Archegonia develop at the superficial regions and are fully embedded in the gametophytic tissue.

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- One egg is produced inside the archegonium.
- Sperm swims towards the egg (n) using flagella through external water, entering into the archegonium and fertilizes the egg (n) resulting in a zygote(2n).
- Zygote develops to form an embryo and then embryo develops to form a young sporophyte by obtaining nutrients from the female gametophyte.
- Sporophyte generation is the larger and more complex form in the alternation of generation

Life cycle of *Cycas*

- Sporophytes are the dominant photosynthetic plants in the life cycle, gametophytes are reduced and depend on the sporophyte throughout its life.
- Sporophytes are a perennial tree with roots, stem and leaves.
- Stem is unbranched columnar and woody.
- Leaves are arranged in crowns.
- Compound leaves show xerophytic adaptations and young leaves are fiddleheads.
- Sporophytes are heterosporous and dioecious. Sporophytes have developed a tap root system.
- Secondary growth is present.
- Sporophytes which produce megaspores are called female plants and those which produce microspores are called male plants.
- Mature female plant produces a crown of megasporophylls.
- Megasporangium is enclosed in the protective layer called integument to form the ovule. Integument has a small pore in the distal end of the ovule called micropyle.
- One of the cells in the megasporangial tissue differentiates into a single megaspore mother cell. Megaspore mother cell undergoes meiosis to form four haploid megaspores out of which only one remains functional.
- The remaining megasporangial tissue functions as nucellus which provides nourishment.
- Megaspores are not released to the external environment megaspore develop into the female gametophyte (n) while within the ovule. Mature ovule contains the female gametophyte. The female gametophyte produces several archegonia. Each archegonium produces a single egg cell within it.
- Mature male plants produce male cones with microsporophylls which consist of microsporangia on the lower surface. Large numbers of microspores (n) are produced from microspore mother cells (2n) within the microsporangium by meiosis.

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- They develop into pollen grains within the sporangium and then discharge.
- Pollen grains are dispersed by wind and deposited on the micropyle of a mature ovule is called pollination.
- Pollen grains enter into pollen chamber of the ovule through the micropyle. In the pollen chamber, pollen grains develop into male gametophytes. Male gametophyte consists of a branched pollen tube which involves in absorption of nutrients from the nucellus. Male gametophyte is short-lived.
- Produce two large sperms with a spiral band of numerous cilia.
- The basal end of the pollen tube ruptures releasing sperms into the archegonial chamber of the ovule. Sperms swim through the liquid medium and fertilize the egg resulting the 2n zygote.
- Zygote develops into the embryo
- Remaining female gametophyte becomes the endosperm which providesnutrients for the developing embryo during seed germination. Integument becomes the seed coat.
- The ovule becomes the seed.
- Seed is the dispersal unit which contains the embryo and stored food that are enclosed in the seed coat.
- Seeds are dispersed and under favorable environmental conditions seeds germinate producing the seedlings (young Sporophyte).

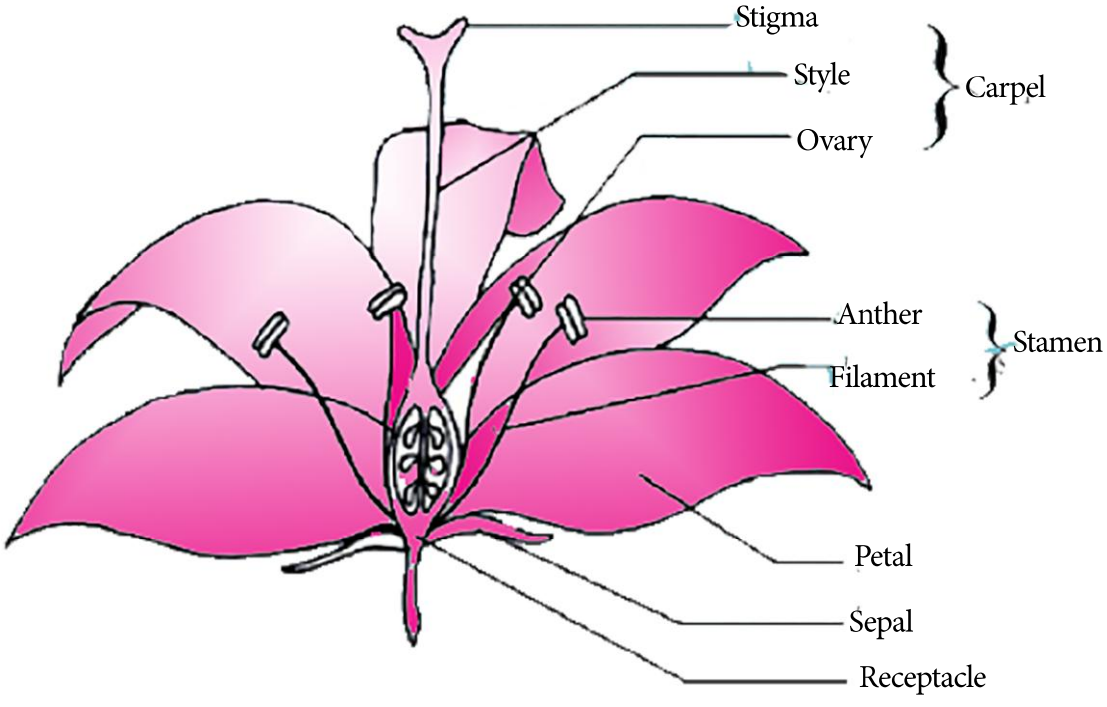
structures and functions associated with sexual reproduction in flowering plants

Life cycle of flowering plants

- Sporophyte is the dominant plant. Gametophytes are short-lived, microscopic, entirely depend on the sporophyte.
- Sporophyte produces the reproductive structures called flowers.
- A flower is a specialized shoot with four whorls of modified leaves named sepals, petals, stamens and carpels.
- Sepals are usually green, enclose and protect the flower before it opens.
- Petals are brightly coloured in most flowers and aid in attracting pollinators. (But wind pollinated flowers generally lack brightly coloured parts).
- The sepals and petals are sterile floral organs. They do not directly involve in reproduction.
- Stamens are the microsporophylls.
- The stamen consists of a stalk called filament and two terminal lobes called anther.

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4.16 Structure of a typical angiosperm flower

- Anther is made up of microsporangia (pollen sacs) containing microspore mother cells which produce microspores by meiosis. Microspores develop into pollen grains within the anther.
- A pollen grain contains two nuclei, the tube nucleus and generative nucleus.
- Carpels are the megasporophylls. At the tip of the carpel is a sticky stigma that receives pollens. The swollen base of the carpel forms the ovary. Ovary contains one or more ovules A long, slender neck called style connects ovary with stigma.
- Ovule produces four megaspores by meiosis of which only one becomes functional.
- Functional megaspore develops into the female gametophyte called the embryo sac. It is a highly reduced microscopic structure.
- The mature embryo sac consists of eight nuclei contained within seven cells- three antipodal cells, two polar nuclei in the central cell, two synergids and one egg.
- Transfer of pollen grains to a mature stigma is known as pollination.
- In some plant species, pollen grains are transferred from an anther of a flower on to the stigma of the same flower. This is self-pollination
- Pollen may be transferred to a stigma of a different flower. This is cross pollination.

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- Most angiosperm plants are adapted for cross pollination.
- Typical characteristics of flowers like such as colour and odour. favour cross pollination.
- In addition, some plants show special types of adaptations cross pollination.
  e.g. heterostyly, self infertility, unisexuality

Significance of cross pollination

Cross pollination results in cross fertilization. Cross fertilization allows shuffling of genes within a species, producing new genetic combinations resulting increased genetic variation within the species. These features are very important for survival and also might lead to evolution.

Fertilization

- The pollen grain germinates after it is placed on the stigma.
- It extends a pollen tube that grows down through the style of the carpel.
- The generative nucleus divides forming two sperm nuclei.
- When the pollen tube reaches the ovary, it moves through the micropyle (The pore in the integuments of the ovule) and discharges two sperm nuclei into the embryo sac.
- One sperm nucleus fuses with the egg cell forming a diploid zygote and the other sperm nucleus fuses with the 2 polar nuclei. This type of fertilization is called double fertilization and is unique to angiosperms.
- After double fertilization, the ovule matures into a seed. The zygote develops into the embryo. The triploid nucleus develops into the endosperm that store food.
- The significance of double fertilization is that it synchronizes with the development of the embryo.
- If fertilization does not occur that prevents plants from wasting nutrients on infertile ovules.
- The seed consists of the embryo, endosperm with store food and a seed coat.
- Seeds are enclosed in the fruit.
- Fruit is an enlarged and developed ovary, usually after being stimulated by fertilization. Fertilization triggers hormonal changes that cause the ovary to form a fruit.
- If a flower has not been pollinated, fruit does not develop, and entire flower falls away.
- During fruit development, the ovary wall gets converted to the pericarp.
  - In some plants fruits develop from the ovary without fertilization. This is called

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parthenocarpy. Parthenocarpic fruits do not develop seeds. Parthenocarpy occurs naturally in some species.

e.g. Banana

- It also can be induced with plant growth substances to get seedless fruits.

e.g. Grapes, Orange

- In some plants, seeds develop without fertilization. This is called parthenogenesis.

e.g. certain grasses

- In parthenogenesis,
  - the egg is resulted by mitosis and hence is diploid,or
  - haploid ovum fuses with a polar nucleus, or
  - the genetic content of the egg is duplicated to become diploid, enabling seed development without fertilizing by the sperm.

Significance of development of seed and fruit

Seed

- Seed is the dispersal unit of seed plants which contains the embryo and stored food, surrounded by the seed coat
- The seed habit has a strategy for life on land: The presence of,
  - seed coat- helps to survive in extreme conditions
  - food reserves-provide nourishment to the embryo during development
  - dormancy period helps to survive during unfavorable conditions
  - adaptations for dispersal give a better chance for growth , development and survival.

Fruits

- Protects the enclosed seeds
- When mature, aids in their dispersal by wind , water or animals.
- After being dispersed, if environmental conditions are favourable, a seed may germinate to form a seedling.
- Inhibition of embryo within the seed at one stage of maturation, naturally prevents germination of seeds within fruit, which is called seed dormancy.
- Many seeds have mechanisms of inhibiting germination and remain dormant.
- Presence of inhibitors, presence of thick/strong seed coats, presence of seed coats impervious to water are common causes of seed dormancy.
- After breaking seed dormancy, when water, oxygen and suitable temperature are provided, seeds start to germinate.
- Absorption of water, activation of enzymes, mobilization of food resources

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(nutrients) followed by rapid growth process of the embryo extending radical through the seed coat is called seed germination. Radical shows positive geotropism and plumule shows negative geotropism.

Plant responses to internal and external Signals

Responses of plants to different stimuli

Photomorphogenesis:

- Light triggers many key events in plant growth and development, collectively known as photomorphogenesis.
- Light reception also allows plants to measure the passage of days and seasons.
- Plants detect not only light signals, but also the direction, intensity and wave length (Color)
- A graph called an action spectrum depicts the relative effectiveness of different wave lengths of radiation in carrying out a particular process, such as photosynthesis.
- Action spectrum reveals that red and blue light are the most important colors in regulating plant's photomorphogenesis.
- The two major classes of light receptors in plants are the blue light photoreceptors and phytochromes (which absorb mostly red light)
- Blue- Light photo receptors initiates a variety of responses in plants, including phototropism, the light induced opening of stomata and the light induced slowing of hypocotyl elongation that occurs when a seedling breaks ground.
- Phytochrome photoreceptors regulate many plant responses to light, including seed germination and shade avoidance.

Effect of light on:

seed germination.

- As the nutrient reserves are limited, many types of seeds (especially small ones) germinate only when the light environment and other conditions are nearly optimal.
- Such seeds often remain dormant for years until light conditions change. (e.g.- Plowing a field or a death of a shady tree may create a favorable light environment for germination)

plant spacing

- Phytochromes provide the plant with information about the quality of light which enables the plant to get adapted to changes in outside light conditions.

e.g. "Shade Avoidance" response of a forest tree (below the canopy) that requires

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relatively high light intensity. As the forest canopy absorbs more red light allowing only far red light to pass through, the tree below the canopy will allocate more of its resources to grow taller.

- In contrast, exposure to direct sunlight increases the proportion of far red: red light and thereby stimulates branching and inhibits vertical growth.

flowering

- Photoperiod is the interval in a 24hour period in which the plant gets exposed to light.
- Photoperiod controls flowering in many types of plants.

Shoot elongation and Phototropism

- The growth of a shoot towards light (positive) or away from it (negative) is called phototropism.
- Positive phototropism strengthen photosynthesis.
- This response results from a differential growth of cells on opposite sides of the shoot; the cells in the darker side elongate faster than the cells on the brighter side.

Response to Gravity

Gravitropism

- Shoot of the plant grows upwards while root grows downwards, due to their response to gravity or gravitropism.
- Gravitropism can be either positive or negative.

  e.g. Roots display positive gravitropism while shoot display negative gravitropism.
- Gravitropism occurs as soon as a seed germinates. This ensure that the root grows into the soil and shoot grows towards sunlight.
- Plants may detect gravity by the settling of statoliths.
- Statoliths of vascular plants are specialized plastids containing dense starch grains.
- They can settle under the gravity, to the lower portions of the cell.
- In roots, they are located in certain cells of the root cap.

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The statolith hypothesis:

The aggregation of statoliths at the low points of root cap cells triggers re-distribution of Ca^{2+} which causse lateral transport of auxin within the root. As a result, Ca and auxin get accumulated at lower side of elongation zone of root. At high concentration of auxin, cell elongation is inhibited resulting slow growth on lower side and more rapid elongation on upper side. Consequently, the root grows downwards.

Response to mechanical stimuli

Trees grow in windy environment normally have shorter stockier trunks than same species growing in normal environmental conditions. Advantage of this is that the tree could stand high winds. This exhibits the sensitivity of mechanical stress of plants. the changes in plant form due to mechanical disturbances is called thigmomorphogenesis.

During evolution, some plant species have become 'touch specialists'. Climbing plants have tendrils that coil rapidly around support. Tendril usually grows straight until it touches a support. The contact stimulates differential growth on opposite sides of the tendril. The directional growth of tendril towards support is called thigmotropism.

Other touch specialists, respond to touch by rapid leaf movements. E.g. Mimosa pudica collapses its leaflets when touched. Touching results in a sudden loss of turgor of cells in a specialized motor organ called pulvini, causing the leaflets to collapse. This response is called thigmonasty.

The role of plant growth substances/ hormones/ regulators in response to different stimuli

Hormones in general are signaling molecules which are produced in small quantities, get transported from the place they are produced to other parts of the organism and trigger responses in target cells. or/and effect on plant growth and development. With this definition, its hard to explain some physiological processes in plants. In addition, some signaling molecules that are considered as plant hormones act locally. Thus the broader term plant growth regulators seem more appropriate.

Plant growth regulators are natural or synthetic organic compounds which modify or control specific physiological process in plants.

Plant biologists prefer to use the term plant growth regulators rather than plant hormones, as there are certain differences in plant hormones and animal hormones.

Therefore, plant hormones and plant growth substances are considered as equal. But plant hormones are active even at very low concentration.

Major types of plant hormones/ growth regulators are auxins, gibberlin, cytokinin, abscisic acid, ethylene and Jasmonate (jasmonic acid).

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Hormone	Functions
Auxin	• stimulates stem elongation in low concentration • promotes the formation of lateral and adventitious roots • regulates development of fruit • enhances apical dominance • functions in phototropism • functions in gavitotropism • promotes vascular differentiation • retards leaf abscission
Gibberellins	• stimulate stem elongation • stimulate pollen development • stimulate pollen tube growth • stimulate fruit growth • stimulate seed development and germination • regulate sex determination and transition from juvenile to adult phase
cytokinins	• regulate cell division in shoots and roots • modify apical dominance and promote lateral bud growth • promote movement of nutrients into sink tissues • stimulate seed germination • delay leaf senescence
Abscisic acid	• inhibits growth • promotes stomatalclousure during drought stress • promotes seed dormancy and inhibits early germination • promotes leaf senescence • promote dessication tolerance
Ethylene	• promotes ripening of many types of fruit • promote leaf abscission • promote triple response in seedlings (inhibition of stem elongation, promotion of lateral expansion, and horizontal growth) • enhance the rate of senescence • promote roots and root hair formation • promotes flowering in the pineapple family

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Response of plants to some biotic and abiotic stresses

Stress

Certain factors in the environment may have a potentially adverse effects on a plants' survival, growth and reproduction.

Two types of stresses,

1. Abiotic stress
2. Biotic stress

Response of plants to some biotic and abiotic stresses

Stress

Certain factors in the environment may have potentially adverse effects on plants' survival, growth and reproduction.

Two types of stresses;

3. Abiotic stress(due to nonliving factors)
4. Biotic stress (due to living factors)

Abiotic Stress

Among several common abiotic stresses. Following three stresses are discussed.

1. Drought stress
2. Cold stress
3. Salt stress

1. Drought stress: Plants may wilt when water loss by transpiration exceeds water absorption. Prolonged drought may even kill a plant. Plants have control systems that enable them to cope with the drought/ water deficit conditions.

Water deficit stimulates increased synthesis and release of abscisic acid (ABA), which acts on guard cell membrane, closing stomata to reduce transpiration.

In grasses the leaves roll in to a tube-like shape which reduces the surface area to reduce transpiration. Some plants shed their leaves during seasonal drought.

2.Cold stress: When cell membrane cools below a critical temperature it looses its fluidity due to the lipids become locked in to crystalline structure. This blocks the transport across the membrane and affects the function of the cell.

Plants respond to cold stress by altering the lipid composition of their membranes. They increase the proportion of unsaturated fatty acids which keeps the membranes more fluid at low temperature.

Freezing is another cold stress. Water in the cell wall and intercellular spaces freezes before freezing the solute-rich water in the cytosol. The reduction of liquid water in the cell wall lowers the extracellular water potential causing water in the cytosol to leave.

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This results high concentration of solutes in the cytoplasm which is harmful and may lead to cell death.

Before the onset of winter, the cell of frost-tolerant plants increases cytoplasmic levels of specific solutes such as sugars that help to reduce the loss of water from the cell preventing dehydration.

3. Salt stress: An excess of salts (high salinity) in soil lowers the water potential of soil resulting reduced water potential gradient from soil to root. This leads to reduction of water uptake by roots.

In general too high salinity in soil is toxic toplants.

Many plants can respond to moderate soil salinity by producing solutes that are well tolerated at high concentrations. These are organic compounds that keep the water potential of cell more negative than that of the soil solution.

A few plants that are salt-tolerant (halophytes) have developed salt glands, which secrete excess salts out of the plant across leaf surfaces. e.g. many mangrove plants

Biotic stress –

How plants defend themselves against pest and pathogens attack;

In plant defense mechanisms, some compounds and structures are already existed whilst some others are formed after infection or pest attack. Therefore, two categories of defense mechanisms called preexisting and induced mechanisms can be identified.

Preexisting structural and chemical defense mechanisms;

- Amount and quality of wax and cuticle that cover the epidermal cells
- The structure of the epidermal cell walls and thickness
- The size, location and shapes of stomata
- Toxic compounds, alkaloids (eg. Nicotine), phenolics (eg. Flavonoids, lignin & tannins), terpenoids (eg. Azadirachtin) and lectin
- Thorns, pricks, trichromes

Induced structural and chemical defense mechanisms;

- Morphological changes in the cell wall
- Formation of cork and abscission layers
- Phenolic compounds
- Toxic compounds
- Enzymes that can degrade fungal cell walls or damage insect organs

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05 Animal form and function

Relate the Structure of Animal Tissues to their function

There are four major types of animal tissues : Epithelial tissue, Connective tissue, Muscle tissue and Nervous tissue.

1. Epithelial tissue: -

Characteristics

It covers external or internal free surfaces and organs. The cells of the tissues are closely packed. Cells of epithelial tissue have both apical and basal surfaces where apical surface remains free and the basal surface attached to the basement membrane. No blood vessels in the tissue. Tissue gets nutrients and oxygen from the underneath connective tissue.

Functions-

- Protection (e.g. barrier against mechanical injury, pathogens, barrier against fluid loss
- Secretion (e.g. enzymes, hormones, mucus, sweat)
- Absorption (e.g. nutrients, respiratory gases)

There are two general types according to the number of cell layers on the basement membrane.

- Simple epithelia – Single cell layer (e.g simple squamous, simple cuboidal, simple columnar and pseudostratified)
- Compound epithelia – several cell layers (e.g. stratified squamous, transitional)

Simple squamous epithelium –

It is a single layer with plate like cells. This type of epithelium is thin and leaky. They are found in places where materials exchange by diffusion.

e.g: blood capillaries, alveoli,

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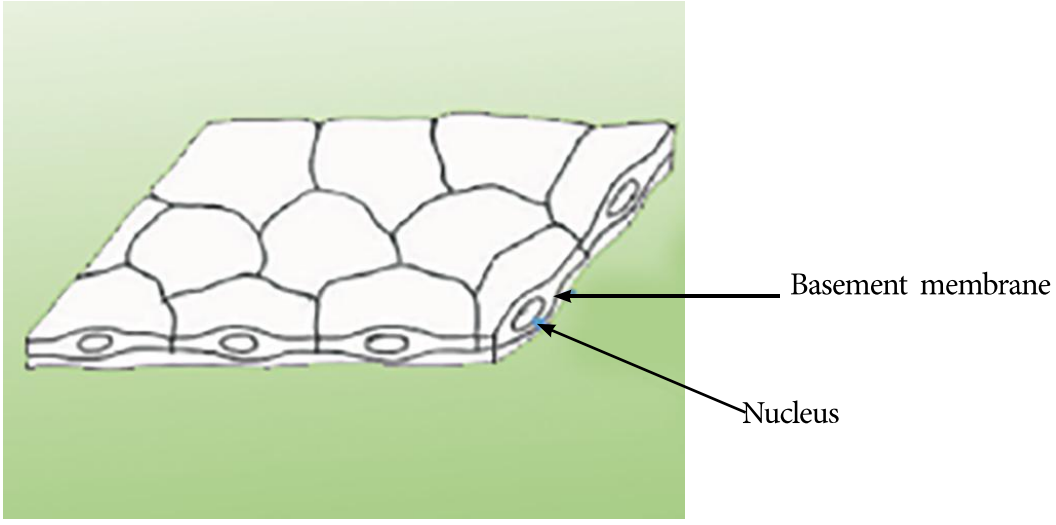


Fig 5.1: simple squamous epithelium

Simple cuboidal epithelium –

It is a single cell layer with dice shaped cells specialized for secretion. It is found in kidney tubules, many glands such as thyroid glands and salivary glands

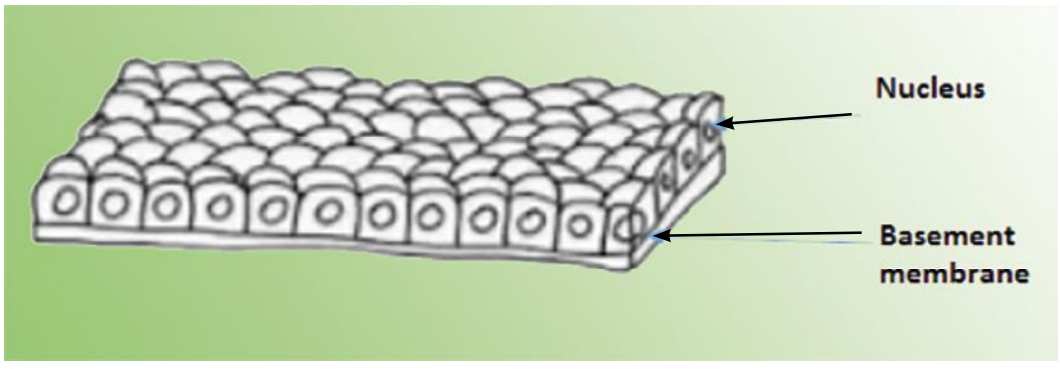


Fig 5.2: simple caboildal epithelium

Simple columnar epithelium

It is a single layer with large and brick-shaped cells. It is often found in places where secretion or active absorption is important. e.g. intestinal lining

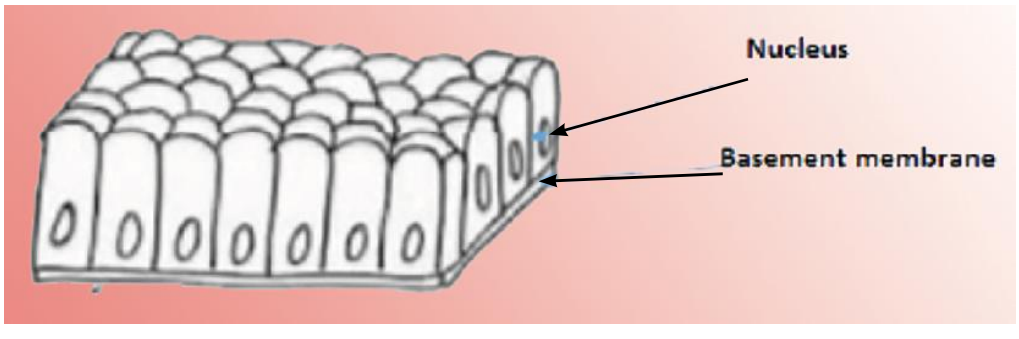


Fig 5.3 : simple coloumnar epithelium

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Pseudostratified columnar epithelium

This tissue consists of a single cell layer. Cells are not of equal height. Nuclei of cells are located at different level. Appear as several layers. In many vertebrates this epithelium has ciliated cells that form a mucous membrane and the cilia help to sweep the mucous along the surface. e.g. nasal passage, trachea.

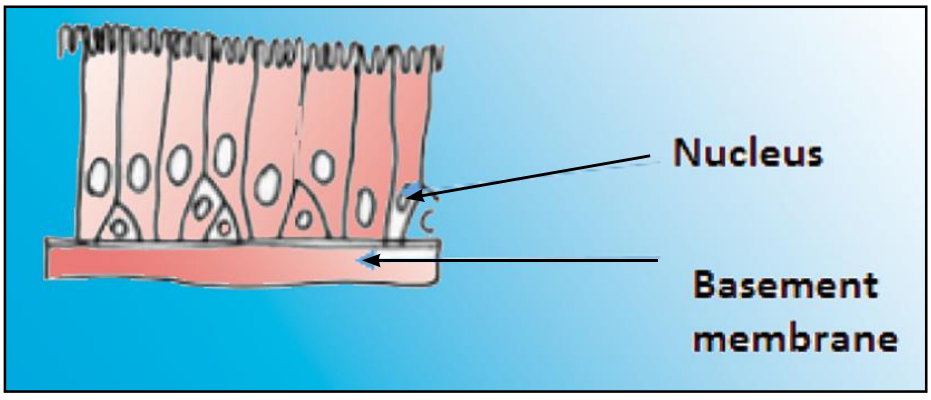


Fig 5.4 : Pseudostratified squamous epitheium

Compound epithelia –

Stratified squamous epithelium:

This tissue is composed of a number of layers of cells. This epithelium regenerates rapidly. Cell division produces new cells near the basement membrane. The old cells are sloughed off and replaced by the new cells . This epithelium is found on surfaces where they are subjected to abrasion such as outer skin, lining of mouth, anus, vagina

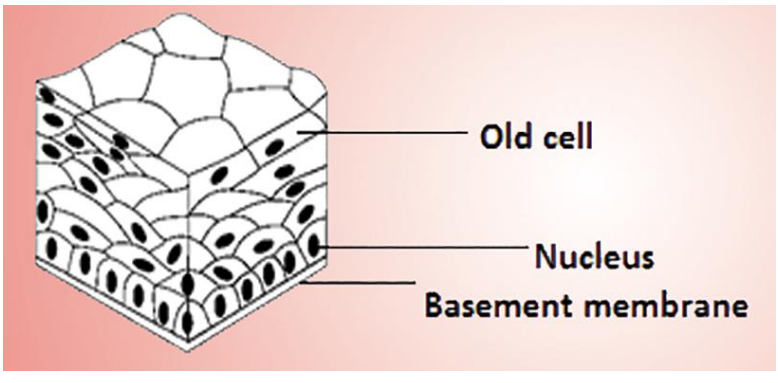


Fig 5.5: Stratified squamous epithelium

2. Connective tissue

Characteristics

Connective tissues are the most abundant tissues in the body that help to connect organs and tissues together structurally and functionally. These types of tissue consist of different types of cells scattered in a large amount of extracellular matrix containing different types of fibres. Matrix may be a semisolid (Jelly-like), liquid or

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solid (dense and rigid). Different types of cells can be found in the matrix such as fibroblasts (secrete fiber proteins), macrophages (engulf foreign particles or any cell debris by phagocytosis) and mast cells (secrete heparin and histamine) in addition fat cells (storage and insulation) and leukocytes (protection) are found in some connective tissues.

There are three different types of fibers. They are collagen fibers (provide strength and flexibility), reticular fibers (join connective tissues to adjacent tissues) and elastic fibers (make tissue elastic).

Functions

- Binding and structural support
- Protection
- Transport of materials
- Insulation

There are different types of connective tissues. They are loose connective tissue (Areolar tissue), Fibrous connective tissue (Dense connective tissue), adipose tissue, blood, cartilage and bone .

Loose connective tissue (Areolar Tissue)

This tissue is the most widely distributed connective tissue type in the vertebrate body. This can be considered as the generalized type of connective tissue. Types of cells in this tissue are fibroblasts, macrophages, mast cells, leukocytes and fat cells.

All three types of fibres are found in this tissue. Fibers are loosely arranged and wavy in nature. This tissue binds epithelia and the underlying tissue. Therefore this holds organs in place. This tissue is found under the skin and throughout the body.

Fibrous connective tissue (Dense connective tissue)

This tissue is densely packed with collagen fibers. Therefore the matrix is relatively reduced and contains fewer cells (fibrocytes). This tissue is found in tendons (attach muscle to bones) and ligaments (connect bones and joints) where tensile strength is required.

Adipose tissue

This tissue is packed with adipose cells. Each adipose cell contains a large fat droplet. This is a specialized type of loose connective tissue which pads and insulates the body and stores fuel as fat molecules. For instance, it is found under the skin where it act as a thermal insulator and energy store.

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Blood tissue

It is a specialized connective tissue where the matrix is not secreted by the cells and fibers are formed only during blood clotting process. The extracellular matrix of the blood is liquid. It is called as plasma. The blood plasma contains salts, water and dissolved proteins. Red blood cells (transport respiratory gases), white blood cells (defense) and platelets (blood clotting) are suspended in the blood plasma. Main functions of blood tissues include transport of materials, protection, and osmoregulation. (For further details refer pg 195)

Cartilage

This tissue consists of a matrix composed of chondroitin sulphate which is a rubbery protein-carbohydrate complex. Collagen fibers and chondrocytes are embedded in the matrix. Chondrocytes secrete chondroitin sulphate and collagen fibers. This tissue provides support and flexibility in places such as trachea, intervertebral discs.

Bone

It is a mineralized connective tissue. Matrix consists of collagen fibres and inorganic salts. Inorganic salts are Calcium, magnesium and phosphate ions. Cells are osteoblasts (bone forming cells) and osteocytes (mature bone cells that maintain bone tissue). Osteocytes are enclosed within lacunae. The mammalian hard bone has repeating units called osteons. Each osteons has a concentric layer of mineralized materials. At the centre of the osteon is a central canal containing blood vessels and nerves. This tissue forms the endoskeleton of most vertebrates and provides support and strength to the body.

3. Muscle tissue

Muscle tissue is responsible for movement. The cells in the muscle tissue are composed of actin and myosin proteins. This tissue is able to contract and relax. There are three basic types of muscle tissues found in the vertebrate animal body. They are smooth muscle, skeletal muscle and cardiac muscle tissues.

Smooth muscle tissue

The cells of the smooth muscle tissue are spindle shaped and uninucleated. Cells lack striations. This tissue is responsible for involuntary body functions (e.g. churning of stomach, constriction of arteries). This tissue is found in digestive tract, urinary bladder, arteries and other internal organs.

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Skeletal muscle tissue

They are composed of bundles of long cells with multi nuclei. Cells are striated. The contractile units of this muscle cells are called sarcomers. This gives the striated appearance to the muscle cells. The arrangement of the sarcomere gives the striated appearance. The muscles are generally attached to the skeletal system and helps mainly in voluntary body movements.

Cardiac muscle tissue

They are composed of uninucleated cells which are interconnected via intercalated discs. Cells are striated with sarcomeres. Cardiac muscle tissue is responsible for involuntary heart contractions. Intercalated disks help relay signals from cell to cell and synchronize heart contraction. Cardiac muscle tissue is only found in the wall of the heart.

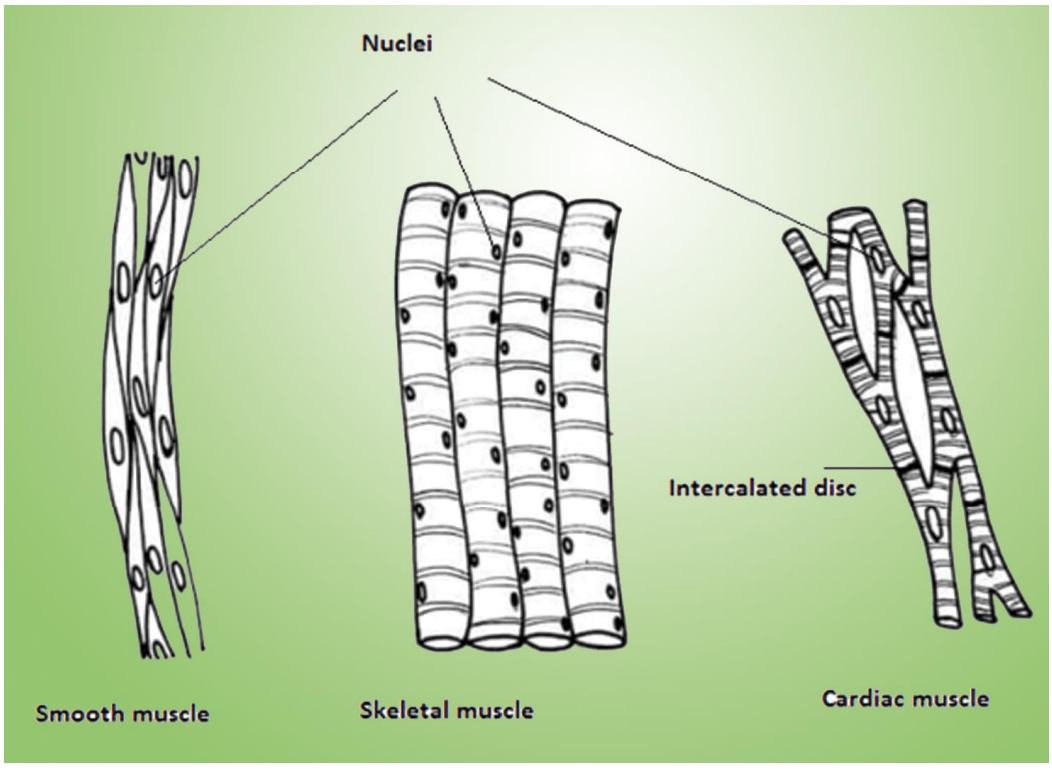


Fig 5.6: Muscle tissue

4. Nervous tissue

Nervous tissue contains neurons and glial cells. Neurons receive, process and transmit nerve impulses. Neuroglia (Glial cells) support the neurons.

Neurons: A neuron has cell body, dendrites and axon. The basic structural unit of the nervous system is the neuron. Dendrites and cell body is used to receive nerve impulses from other neurons. Axon is used to transmit impulses to other neurons, cells or muscles. Axons are bundled together into nerves.

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Neuroglia (Glial cells): Neuroglial cells are supportive cells of neurons. Their functions include nourishment of nerve cells, insulation of nerve cells, replenishing neurons and sometimes modulate neuron functions.

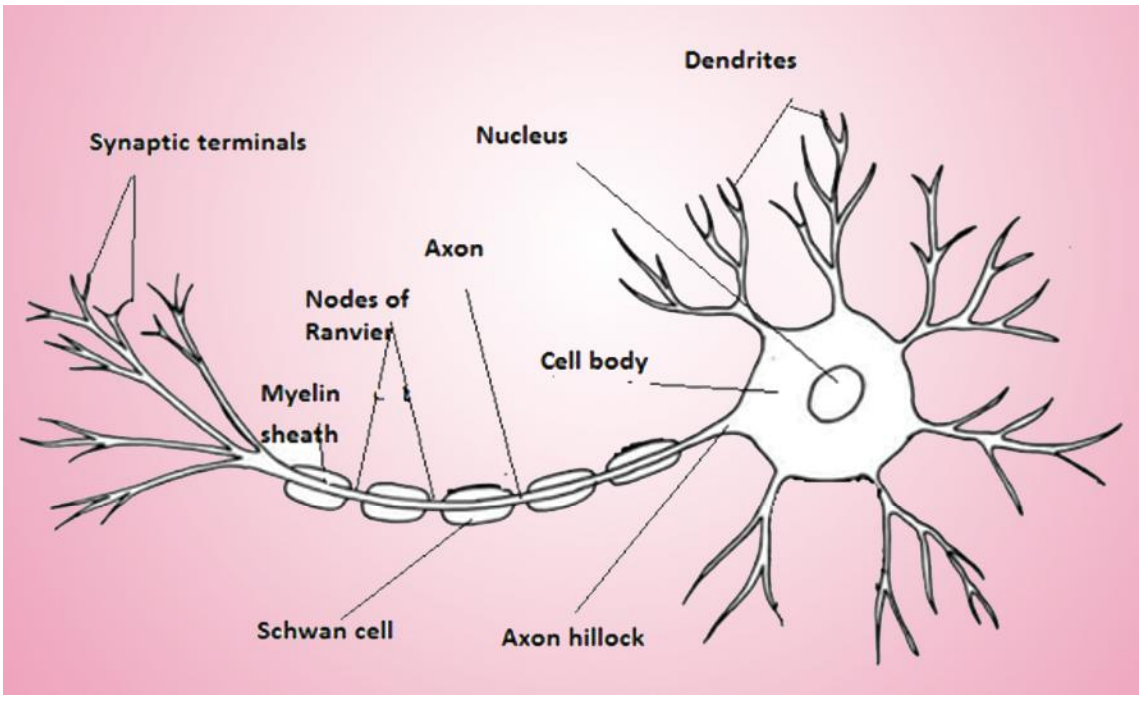


Fig 5.7: The structure of the Motor neuron

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Nutrition in animals

Animal nutrition is the process by which food is taken in for make use in different body functions. Typically food is broken into smaller molecules and absorbed prior to utilization. Animals are heterotrophs.

Heterptrophic nutrition is the process by which the organisms obtain organic food molecules by ingesting other organisms or by substances derived from other organisms.

Other than animals, fungi and majority of bacteria are heterotrophs. There are two type of heterotrophic nutrition namely, holozoic nutrition and symbiosis.

Holozoic nutrition

Most animals are holozoic where they ingest food into their alimentary canal. This mode of nutrition consists of five main stages: Ingestion, digestion, absorption, assimilation and elimination/ Egestion.

Main stages in holozoic nutrition

- Ingestion: This is the first stage where the act of eating or feeding happens. Food sources differ among animal species and they possess different modes of ingestion according to the diet or environment.
- Digestion: Food is broken down into molecules small enough to pass through the membranes and enter the cells of organisms. Digestion could occur mechanically (by teeth or muscle contractions) and chemically (by enzymes). During mechanical digestion, food is broken down in to smaller fragments thus increase the surface area for efficient chemical digestion. During chemical digestion enzymes break bonds in large molecules into small molecules.

The last two stages of food processing occur after the food is digested.

- **Absorption:** In this stage, the animal's cells take up small molecules. e.g.simple sugars, amino acids
- **Assimilation:** Assimilation is the process of utilization of absorbed nutrients for various functions of the body.
- **Elimination:** In this process undigested materials are passed out from the alimentary canal.

Feeding Mechanisms of animals

- **Filter feeders:** They strain suspended food particles from the surrounding watery medium. They use several mechanisms such as capturing, trapping, etc.

  e.g. - clams and oysters - feed on small pieces of food in the water that passes over their gills. Cilia in the gills sweep the food particles to the animal's mouth in a film of mucus.

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- **Fluid feeders:** They suck nutrient rich fluid from a living host using well adapted mouth parts.

  e.g: Mosquitoes-suck human blood, Aphids- suck phloem sap of plants, Bees and humming birds suck -honey from flowers

- **Substrate feeders:** These animals live inside their food source or on the food source eating its way through the food.

  e.g. The leaf miner caterpillar - eating through the soft tissues of a leaf

  Maggots (fly larvae) - burrow into animal carcasses

- **Bulk feeders:** Animals which eat comparatively large pieces of food. These animals have different types of adaptations to tear the food or to capture the pray (e.g. jaws, teeth, tentacles, claws, poisonous fangs).

  e.g. - Most animals including human

- **Symbiosis**

  This is an ecological relationship between organisms of two different species that live closely together. It is divided into three groups such as Mutualism, Parasitism and Commensalism

- **Mutualism:** It is a close association between two living organisms of different species which benefits both partners.

  e. g. - Cellulose digesting microorganisms in Ruminants and Termites

- **Parasitism:** It is a close association between two living organisms of different species which is beneficial to one (parasite) and harmful to the other (host). Parasites live either within or on the host and derive its nourishment

  e.g. –Tape worm and humans, Lice and humans

- **Commensalism:** It is a close association between two living organisms of different species which is beneficial to one and does not affect the other (neither harmful or beneficial)

  e.g.- Barnacles attached to whales

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Human Digestive system

Structure and function of the human digestive system

Human alimentary canal is a long tube/ tract which connects with external environment and can fulfill steps of holozoic mode of nutrition. Human digestive system consists of alimentary canal and associated glands. The alimentary canal consists of the following parts: oral cavity, pharynx, esophagus, stomach, small intestine, large intestine, rectum and anus. The associated glands include salivary glands, pancreas and liver.

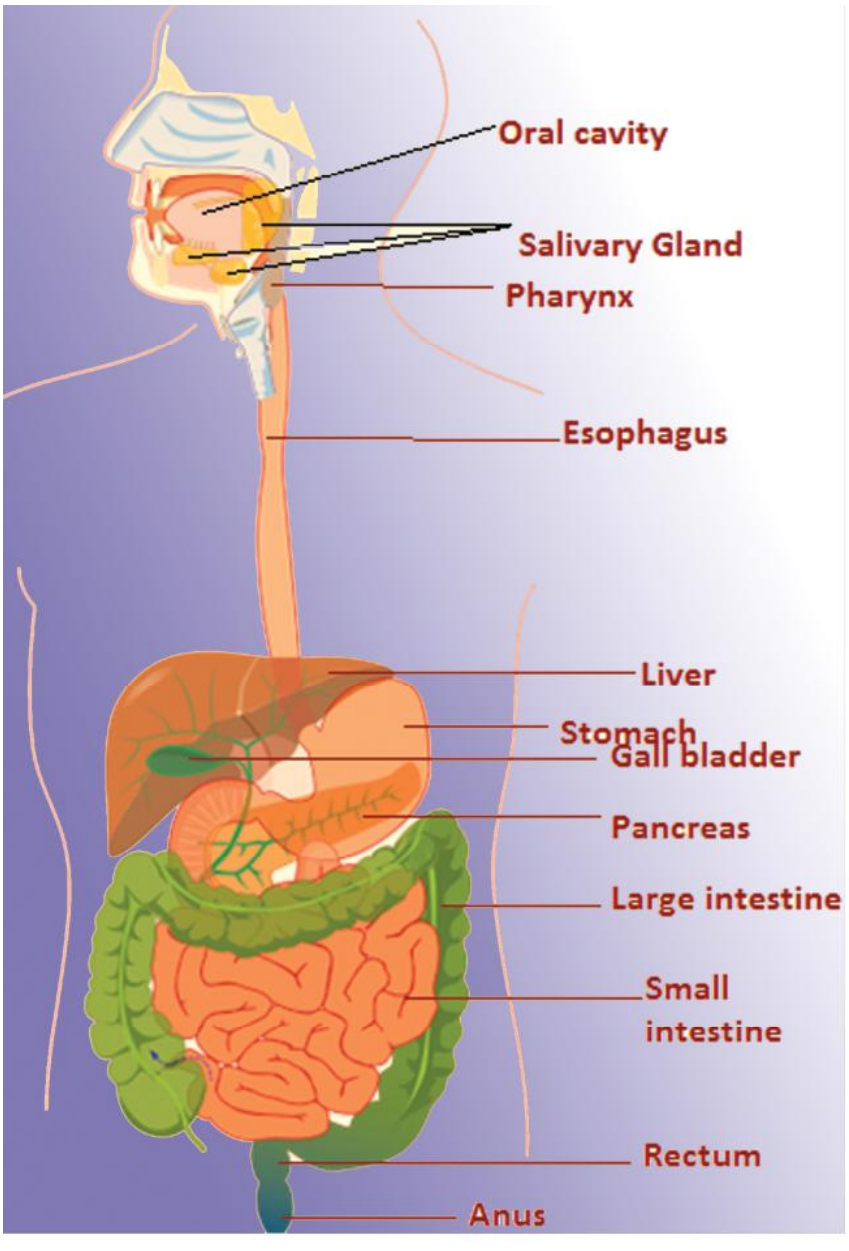


Fig 5.8 :- Structure of the Human Digestive tract

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Mouth/ Oral cavity: Oral cavity consists of the tongue, teeth and salivary glands. Ingestion and initial steps of digestion are carried out in the oral cavity. In the mouth both mechanical and chemical digestion is carried out. There are four types of teeth in the mouth: incisors, canines, premolars and molars. Food is cut, mashed and ground by different types of teeth with different shapes. This makes it easier to swallow food and increase the surface area for digestion.

Salivary glands release saliva into the oral cavity through ducts. Releasing saliva into the oral cavity occurs when food enters the oral cavity due to a nervous reflex. Saliva is also released into the mouth before food is ingested due to various other stimuli. e.g. sight, odor of food, etc.

Saliva contains water, amylase, mucus (a viscous mixture of salts, cells and slippery glycoprotein called mucins). In addition to that, saliva composed of buffers and anti microbial components.

Functions of the saliva

- Salivary amylase: Chemical digestion of polysaccharides (e.g. starch) into smaller polysaccharides and disaccharide maltose.
- Water: Liquefy food and provide watery medium for chemical digestion. Aids in taste reception.
- Mucus: Lubrication of food which makes it easier for swallowing. Clean the mouth and protects the lining of the mouth from abrasion.
- Antimicrobial substances such as immunoglobulin and lysozymes: Protect against bacteria that enter the mouth.
- Buffers prevent tooth decay by neutralizing acid

Tongue: composed of skeletal muscles. Helps to mix the food with saliva and make bolus of food that makes easier for swallowing. Then helps to push the bolus into the posterior part of the oral cavity and into the pharynx.

Pharynx: a common passage of the respiratory tract and the digestive tract. The pharynx leads to the esophagus.

Esophagus:

It is a long tube which connects the pharynx and the stomach. It is found in the thoracic cavity. The wall of the esophagus consists of both skeletal and smooth muscles. The skeletal muscles are located towards the uppermost part of the esophagus and these muscles function during swallowing. The rest of the esophagus consists of smooth muscles which are involved in the process called peristalsis. During this process the food bolus is pushed along the esophagus which is a alternative wave of rhythmic contractions and relaxations of the smooth muscles lining the esophagus.

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Stomach- The stomach is a J- shaped dilated sac in the abdominal cavity. The inner surface of the stomach is highly folded and contains large number of pits that leads to gastric glands. Gastric glands contain three types of cells: mucus cells, chief cells and parietal cells. The stomach wall is very elastic. Distal part of the stomach connects with the small intestine. Sphincters are found at the junctions between esophagus and stomach (cardiac sphincter) and stomach and the small intestine(pyloric sphincter). They are made up of circular smooth muscles. These sphincters help to regulate the passage of materials between these organs.

Chemical digestion in the stomach

The gastric glands of the stomach secrete gastric juice. The gastric juice mainly consists of mucus, pepsinogen and HCl. Mucus and pepsinogen (inactive form of pepsin) are secreted by mucous cells and chief cells respectively. The parietal cells release hydrogen ions and chloride ions separately into the stomach lumen where HCl is formed. Pepsinogen is initially converted into pepsin by HCl. These activated pepsin help to activate remaining pepsinogen molecules. This activated pepsin initiates the chemical digestion of proteins in the stomach. The churning action of the stomach facilitates the chemical digestion. This is a series of muscle contraction and relaxation. This process mixes the swallowed food with gastric juice. Proteins are hydrolyzed to small polypeptides by pepsin. In the stomach food is mixed with gastric juice forming chyme (partially digested semisolid, acidic, food mass).

The stomach lining is protected from the digestion of HCl and pepsin in several ways: Enzymes are secreted in to the lumen as an inactive enzyme; Gastric glands secrete mucus that protect against self-digestion of the stomach lining ; Every three days, cell division adds a new epithelial cell layer which replaces the destroyed/ damaged cells in the lining of the stomach.

Functions of the stomach

- Act as a temporary reservoir for food due to high convolution and very elastic wall.
- Mechanical digestion of food by churning action due to muscular contraction.
- Produce gastric juice which starts the chemical digestion of proteins to polypeptide by pepsin
- Absorption of some materials such as water, alcohol and some drugs
- Non specific defense-HCl kills microbes
- Small jets of gastric contents push out through pyloric sphincter as chyme
- Secretion of gastrin hormone which regulates digestion in the stomach.

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Small intestine-

It is the longest organ in the alimentary canal. It is divided into three regions: duodenum, jejunum and ileum. The duodenum: C shaped curve, around the head of the pancreas.

The jejunum is middle part of the small intestine. The ileum is the terminal part of the small intestine. The surface area of the small intestine is greatly increased by permanent circular folds and villi. The villi are tiny finger like projections of the intestinal wall. Most of the digestion is completed in the duodenum. The major sites for nutrient absorption are jejunum and ileum.

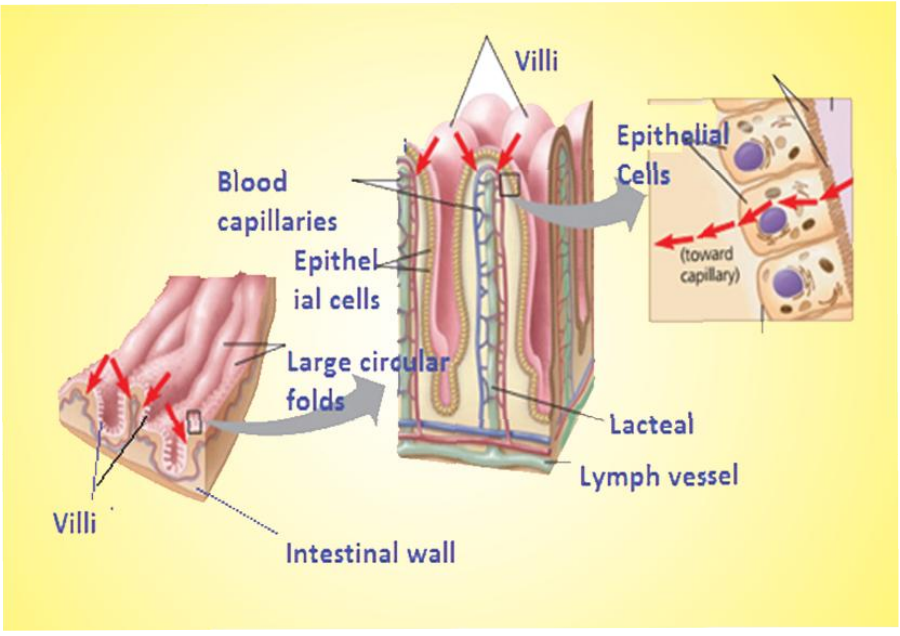


Fig 5.9 Structure of the villus

Chemical digestion in the small intestine

Small intestine receives chyme from the stomach. Peristaltic contractions aid the receiving of this chyme. Pyloric sphincter regulate the passing of chyme into the small intestine. The chyme is mixed with secretions of glands in intestinal wall and secretions of pancreas and liver. (refer pages no: 169,170). The epithelium of the duodenum secretes several digestive enzymes. Glands of the intestinal wall secrete enzymes such as Disaccharidases, Dipeptidases, Carboxypeptidases, Aminopeptidases, Nucleotidases, Nucleosidases and Phosphotases. Some of these enzymes are secreted to the lumen and others are bound to the surface of the epithelium.

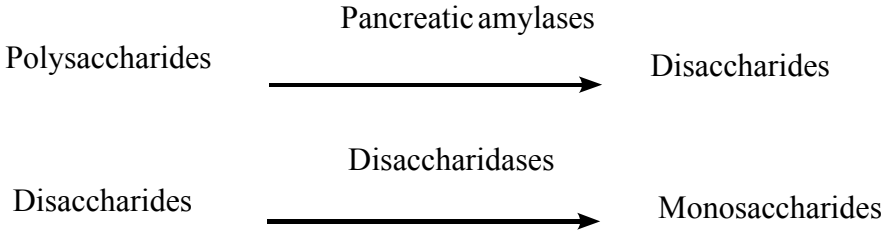
Two hormones namely cholecystokonin and secretin secreted by duodenum stimulates the release of pancreatic juice and the bile. Pancreatic juice contains enzymes such as Trypsin, Chymotrypsin , Pancreatic amylase , Pancreatic Carboxypeptidases, Pancreatic Nucleases and Pancreatic Lipases. In addition it also contains bicarbonates. The liver secretes Bile which is stored in the gall bladder until released into the duodenum.

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The Bile contains Bile salts which act as emulsifiers that help in fat digestion and absorption.

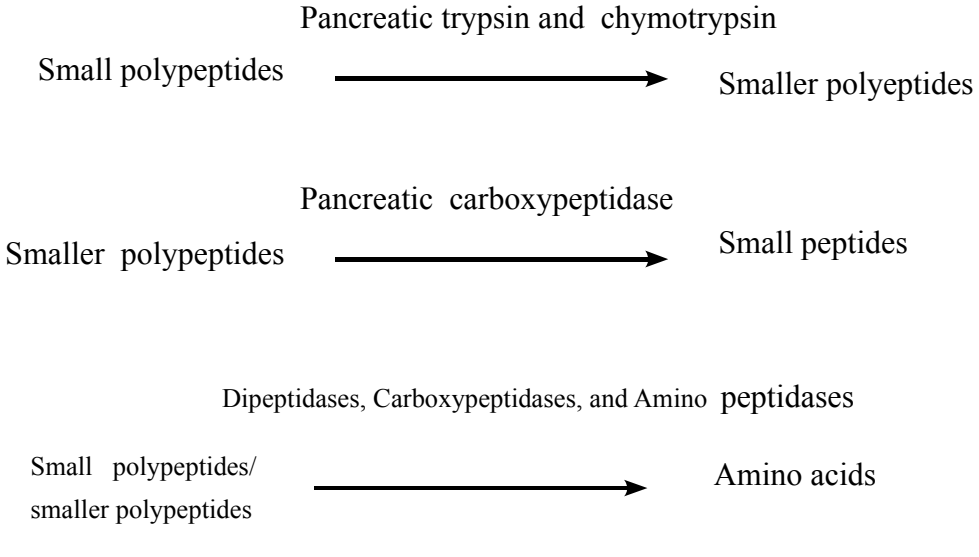
Carbohydarte digestion

Pancreatic amylase catalyze the conversion of polysaccharides (e.g. starch) into disaccharides. Intestinal disaccharidases catalyze the conversion of disaccharides into monosaccharides.



Protein digestion

Tripsin and Chymotripsin catalyse the conversion of small polypeptides into smaller polypeptides. These smaller polypeptides are converted to small peptides and amino acids by the catalytic action of Panceratic carboxypeptidases. Proteases secreted by the intestinal epithelium (Dipeptidases, Carboxypeptidases and Aminopeptidases) catalyse the conversion of small peptides into amino acids.



Fat digestion

Digestion of fats (triglycerides) starts in the small intestine. First bile salts emulsify fats. Next Pancreatic lipase catalyse the conversion of these fats into fatty acids, glycerol and monoglycerides.

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Pancreatic lipase

Fat (triglycerides) ⟶ Glycerol, fatty acids, monoglycerides

Nucleic acid digestion

Digestion of nucleic acids start in the small intestine. Pancreatic nucleases catalyse the conversion of DNA and RNA into nucleotides. These nucleotides are eventually converted into nitrogenous bases, sugars and phosphates by the catalytic action of intestinal Nucleotidases, Nucleosidases and Phosphatases.

Pancreatic nucleases

DNA, RNA ⟶ Nucleotides

Nucleotidases/Nucleosidases and phosphatatases

Nucleotides ⟶ Nitrogenous bases, sugars, phosphates

Absorption in the small intestine

- For effective absorption, the surface area of the intestinal wall has been increased with three structural modifications: heavy permanent foldings, finger like projections called villi in the intestinal wall and finger like microscopic projections called microvilli in the epithelial cells of the villi. These micro villi are exposed into the intestinal lumen, it gives the appearance of brush (brush border).
- Transport of the nutrients across the epithelium may be active or passive. For example Fructose is absorbed by facilitated diffusion. Amino acids, small peptides, vitamins, and most glucose molecules are actively transported into the epithelial cells.
- Then these nutrients from the epithelial cells are transported into the blood capillaries in the villi. Those blood capillaries are converged into the hepatic portal veins. These nutrients are carried in to the liver via the hepatic portal veins. From the liver, this nutrient filled blood is transported into the tissues.
- But absorption of some products of fat digestion takes place in a different pathway: Fatty acids and monoglycerides are absorbed into the cell through microvilli. Within the cells triglycerides are reformed and they are incooperated into water soluble globules called chylomicrons. These chylomicrons are transported into the lacteal and then into the blood vessels through lymph. Then they are transported throughout the body via the circulatory system.

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- In addition to nutrient absorption, recovery of water and ions mostly occur in the small intestine. In addition to the water intake (about 2 L) digestive juices add more water (about 7 L) into the small intestine. Most of this water is reabsorbed via osmosis.

Large intestine – The large intestine is the terminal end of the alimentary canal. It is divided into three regions: colon (proximal part), cecum and rectum. The small intestine is connected to the large intestine at a 'T' shaped junction. One arm of the 'T' junction is colon and the other arm is a small pouch called cecum. A finger like projection in the cecum is called the appendix. The colon leads to the rectum and anus. Cecum is important for fermentation of indigested materials by microbes, especially in animals that eat large amount of plant matter.

Functions of the large intestine

- The colon: completes the reabsorption of water, synthesize some Vitamin B complexes, Vitamin K and folic acid with the help of microbes and move feces (consists of undigested matter such as fibres) along the colon by peristalsis.
- The rectum stores feces until they are eliminated. Presence of two sphincters between the rectum and anus can regulate feces movement. Strong contractions in the colon trigger the defecation.

Associated glands

Pancreas

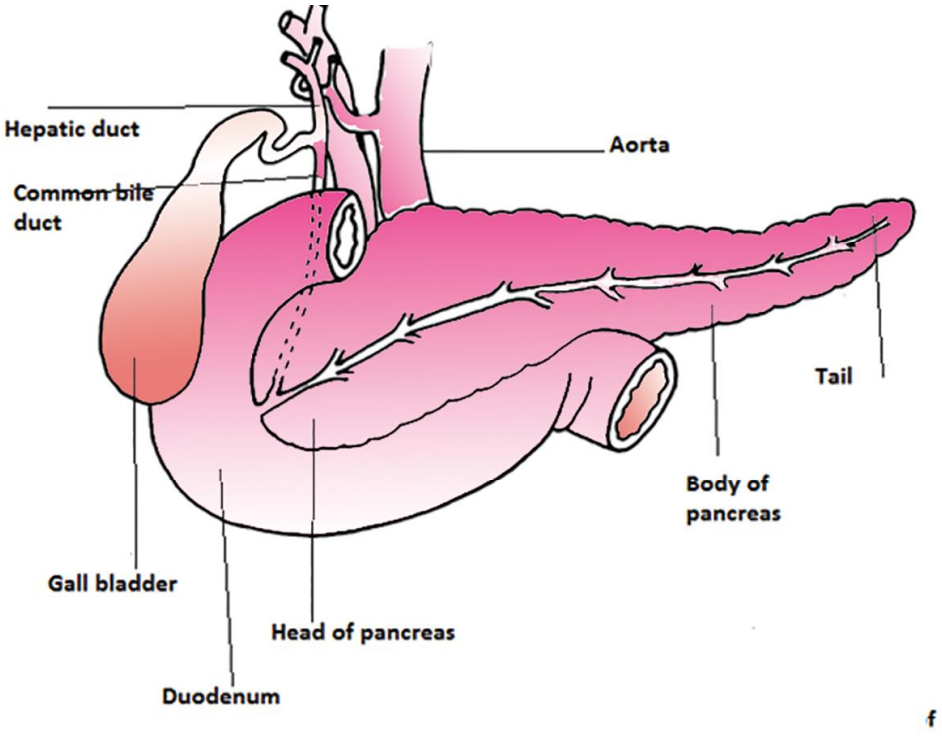


Fig 5.10: The pancreas in relation to the duodenum and biliary tract

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Histological structure of pancreas

The pancreas is a pale grey gland which consists of a broad head, a body and a narrow tail. Head is in the curve of the duodenum. Pancreas is both an exocrine and endocrine gland.

The exocrine part consists of a large number of lobules made up of small acini, the walls of which consist of secretary cells. Each lobule is drained by a tiny duct and these unite eventually to form the pancreatic duct which joins with bile duct to form hepato pancreatic duct and opens into the duodenum at its midpoint. Exocrine part of the pancreas secretes pancreatic juice. The components of the pancreatic juice are bicarbonate, carbohydrate digesting enzymes (pancreatic amylase), pancreatic lipase, nucleases and inactive form of protein digesting enzymes(trpsinogen and chymotrypsinogen). These inactive enzymes are converted to active enzymes(trypsin and chymotrypsin) upon secretion into the lumen of the duodenum.

Endocrine part of the pancreas is the islets of Langerhans, which consist of group of specialized cells. They do not have ducts. Islets of Langerhans secrete hormones, glucagan and insulin which are involved in glucose homeostasis.

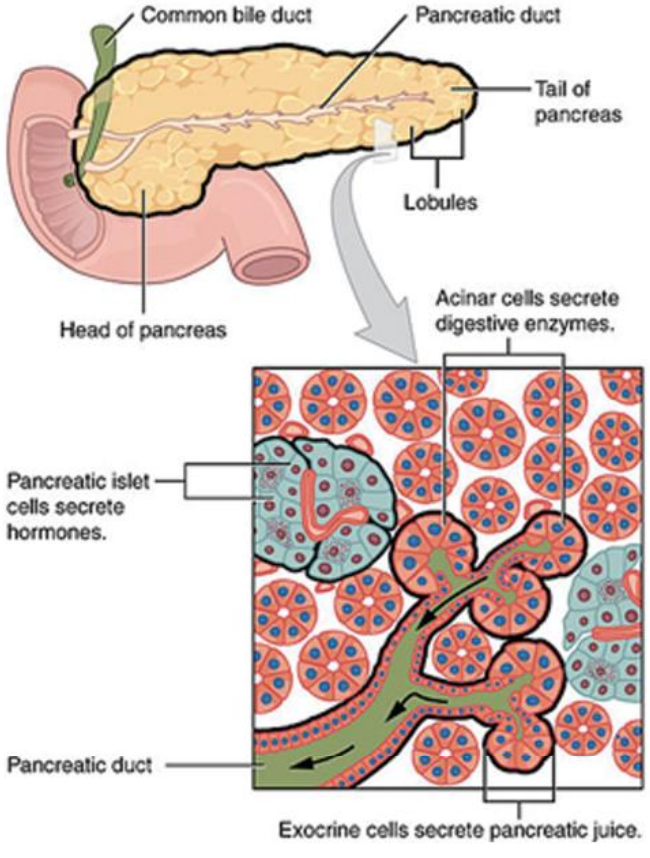


Fig 5.11: Histological structure of the pancreas

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Liver

Liver is the largest gland in the body. Its upper and anterior surfaces are smooth and convex. Its posterior surface is irregular in outline. Liver contains four lobes. Each lobe is made up of tiny hexagonal shape lobules which are the functional unit. These lobules are made up of cuboidal cells called hepatocytes which are arranged in pairs of columns radiating from a central vein. Between two pairs of column of cells there are sinusoids (blood vessels with incomplete walls) containing mixture of blood from the tiny branches of the portal vein and hepatic artery. This arrangement allows venous blood (high concentration of nutritional materials) to mix with arterial blood and come into close contact with liver cells. Hepatic macrophages (Kupffer cells) are found in the lining of the sinusoids. Blood drains from the sinusoids into central veins which joins with veins from the other lobules, forming larger veins and eventually the hepatic vein. (fig 5.12). Bile canaliculi run between columns of liver cells. Canaliculi join up to form larger bile canals. In the corner of the hexagonal structure a branch of hepatic artery, a branch of the hepatic portal vein and intra lobular bile duct can be found.

Liver is a vital organ that performs many important functions. In addition to its role in food digestion it is also perform functions such as metabolism of carbohydrates, fats and proteins, detoxification of drugs and toxic substances, defense against microbes, some hormone inactivation and heat production.

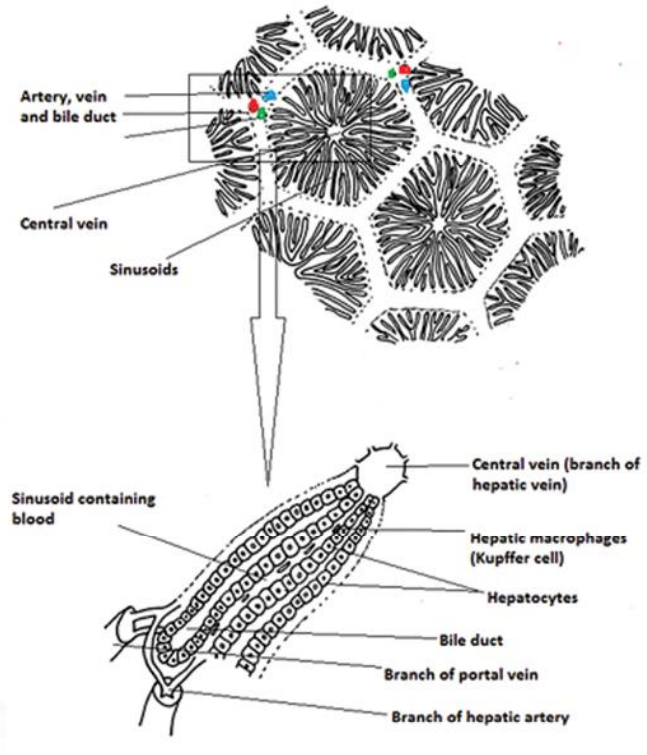


Fig 5.12: Histological structure of the liver

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Function of liver related to digestion

The liver secretes bile which is stored in the gall bladder until released into the duodenum. The bile contains bile salts which act as emulsifiers that help in fat digestion and absorption.

Most of the absorbed nutrients reach the liver and it regulates the distribution of nutrients to the rest of the body. Excess glucose is stored as glycogen in the liver cells. Glycogen deposition and break down in the liver cells are regulated by insulin and glucagon hormones. Fat soluble Vitamins (A,D,E and K) and some water soluble vitamins (B12), iron and copper are also stored in liver.

Regulation of digestion in man

Digestion in man is regulated by two ways: nervous regulation and endocrine regulation. Nervous regulation is mainly by nervous reflexes. For example nervous reflex stimulates the release of saliva when food reach the mouth. Arrival of food in the stomach trigger churning and release of gastric juices. Endocrine system plays a critical role in digestion especially in the stomach and small intestine.

when food arrives the stomach, the stomach wall is stretched. This triggers to release the hormone gastrin. Gastrin circulates via the blood stream and arrives the stomach. Then gastrin stimulates the production of gastric juice at the stomach.

Fatty acids and amino acids in the chyme trigger the release of Cholecystokinin and Secretin from the duodenum. Cholecystokinin triggers release of bile from the gall bladder and digestive enzymes from the pancreas. Secretin stimulates the release of bicarbonate from the pancreas. Bicarbonate neutralize the chyme received from the stomach

When the chyme is rich in fat, food digestion in the stomach slows down due to high levels of Cholecystokinin and Secretin secreted by duodenum. These hormones act on the stomach and inhibit peristalsis and gastric juice secretion.

Balanced Diet

The balanced diet contains the all essential nutrients required for health in the appropriate proportions. Essential components of the balanced diet contain carbohydrates, proteins, lipids, fibers, minerals, vitamins and water. Carbohydrates and lipids supply energy. Energy requirements vary with age, sex, body size and activity. Twenty amino acids are needed to synthesize proteins in the body. Most of these amino acids are synthesized within the body. They are known as non essential amino acids (e.g. Alanine, cystine, etc). Other amino acids (e.g. lysine and histidine, etc.) must be obtained from the diet as they can't be synthesized within the body. They are called essential amino acids. Animal proteins contain all the essential amino acids in proper proportions. However most plant proteins lack one or more essential amino acids. Therefore vegetarian based diet requires several sources of plant proteins to obtain all the essential amino acids.

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Components of foods and their functions

- Carbohydrates
- Proteins
- Lipids
- Vitamins
- Mineral elements
- water
- fibers

Carbohydrates

Carbohydrates are sugars and polysaccharides. They can be found in a variety of foods such as rice, bread, biscuits, cereals, hoppers, etc. during digestion most carbohydrates are broken down into monosaccharides which are absorbed into blood stream.

Functions of digestible carbohydrates

- Provide energy and heat: breakdown of carbohydrates provides ATP for body functions and generates heat.
- Act as an energy stores. e.g. excess carbohydrates are converted into glycogen and fat
- Facilitates protein sparing- proteins are not used to get energy when there is an adequate carbohydrates in the food

Proteins

Proteins are made up of amino acids during the digestion, proteins are broken down into amino acids and absorbed into the blood stream.

The amino acids are grouped into two groups i.e. essential amino acids and non essential amino acids. These essential amino acids cannot be synthesized in the body, therefore they should be obtained into the body through the diet. The non essential amino acids can be synthesized within the body. Therefore it is not necessary to obtain them through the diet.

Functions of proteins in the diet

- The amino acids which are supplied from proteins
  - are used for growth and repair of body cells and tissues
  - are used for synthesis of plasma proteins, enzymes, antibodies and some hormones
- Act as an energy source for body functions

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Lipids

Lipids in the diet are mainly composed of fats and oils. Fatty acids are composed of fats and oils. Fatty acids can be grouped as essential and non essential fatty acids. Essential fatty acids cannot be synthesized within the body while the non essential fatty acids can be synthesized within the body. Therefore essential fatty acids should be obtained through the food.

Functions of lipids in the diet

- Provide energy and heat (on weight basis fats and oils provide more energy compared to carbohydrates and proteins)
- Help in transport and storage of fat soluble vitamins such as Vitamin A, D, E and K
- Store energy as fat in the adipose tissues
- Help to synthesize steroid hormones from cholesterol.
- Provide insulation: (e.g fat found in subcutaneous layer in the skin reduces heat loss , constituents of myelin sheath of neurons)

Vitamins

Vitamins are organic compounds required in small amounts for the maintenance of normal health and metabolism. Vitamins cannot be produced in the body and therefore should be provided in the diet. If the vitamins are insufficiently taken into the body, that may lead to the deficiency diseases. Vitamins are two types they are fat soluble vitamins (Vitamin A, D, E and K) and water soluble vitamins (Vitamin B and C).

Main Functions of Vitamins

- Vitamin A- form visual pigments in the eye, epithelial tissue maintenance, promotion of growth and immunity
- Vitamin B- components of coenzymes such as FAD and NAD, promote red blood cell production
- Vitamin C- act as an antioxidant, used in collagen synthesis
- Vitamin D- aids in absorption and use of Calcium and Phosphorous
- Vitamin E- act as an antioxidant
- Vitamin K- important in blood clotting

Minerals

Minerals are inorganic substances and they are also important for normal health and many body functions. Major mineral elements needed by humans are Ca, P, S, K, Cl, Na, Mg, Fe, F and I . In addition minerals needed in trace amounts include Co, Cu, Mn, Mo, Se and Zn.

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Main functions of minerals include

- Ca- form bones and teeth, helps clotting blood and nerve and muscle function
- P- form bones and teeth, help maintain in acid base balance
- S- components of some amino acids
- K- help maintain in acid base balance and water balance, nerve function
- Cl- help maintain in acid base balance, maintain osmotic balance, nerve function
- Na- help maintain in acid base balance and water balance, nerve function
- Mg- act as enzyme cofactor
- Fe- components of hemoglobin and electron carriers, act as an enzyme cofactor
- F- maintenance of tooth structure
- I- component of thyroid hormone

Water

Water accounts for around 60% of the body mass in humans. Normally water is lost through urine, sweating and feces. Therefore amount of water in the body should be balanced within the body. This water can be taken in to the body by drinking and via foods.

Functions of water in human body

- Provides the moist internal environment for all living cells.
- Major component of blood and tissue fluid therefore helps to transport materials around the body and to exchange materials between blood and tissues and body cells
- Regulate body temperature mainly through evaporative cooling
- Dilute waste products and toxins and provide a medium for their excretion
- Moisten the food and that make easier to swallow

Fibres

Dietary fibres (non starch polysaccharides) are made up of indigestible polysaccharides in the diet. Fibres are rich in fruit, vegetable and cereals.

Functions of dietary fibres

- Provide bulk to the diet and satisfy the appetite.
- Prevent constipation by attracting water to increase faecal bulk and stimulating peristalsis leading to defecation.
- Adequate fibres in the diet protect against some gastro-intestinal disorders such as cancers in the colon and rectum.

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Essential nutrients: Essential nutrients are the substances that cannot be synthesized in the body from simple precursors and must therefore be taken through the diet. These essential nutrients include essential amino acids, essential fatty acids, vitamins and minerals. Essential nutrients have key functions in bio synthetic reaction in the body cells. If these essential nutrients are not supplied in correct proportions in the diet that will lead to malnutrition. Therefore it is essential to obtain them in correct amounts.

Essential amino acids: Essential amino acids are the amino acids that must be obtained from the food since they cannot be synthesized within the body from organic precursor molecules. Of the 20 amino acids required to make proteins in the body 8 amino acids are essential amino acids. Examples for essential amino acids are leucine and methionine. The animal protein products (e.g. eggs, meat, cheese, etc.) will provide all essential amino acids in correct proportions required for body functions. Most plant proteins are "incomplete" as they are deficient in one or more amino acids. Therefore vegetarian diet should contain a variety of plant proteins in order to obtain all the essential amino acids required.

Essential fatty acids: Essential fatty acids are the fatty acids that should be obtained from the diet since they cannot be synthesized in the body from organic precursors. Seeds, grains and vegetables provide enough amounts of essential fatty acids.

Table : Dietary sources and deficiency symptoms of vitamins and minerals

Vitamin/ Mineral	**Main dietary sources**	**Deficiency symptoms**
Fat soluble vitamins		
Vitamin A (retinol)	Dark green vegetables, orange vegetables and fruits, dairy products	Blindness, skin disorders, immunity impairment
Vitamin D	Egg yolk, dairy products	Bone deformities(rickets) in children, bone softening in adults
Vitamin E	Vegetable oils, nuts, seeds	Nervous system degeneration
Vitamin K	Green vegetables, tea, produced by colon bacteria	Defective blood clotting
Water soluble vitamins		
Thiamine (Vitamin B_1)	Legumes, peanuts, whole grains, pork	Beriberi(characterized by tingling, poor coordination, susceptibility to infection, reduced heart function)
Riboflavin (Vitamin B_2)	Dairy products, meats, vegetables, enriched grains	Skin lesions (cracks at corners of mouth)
Niacin (Vitamin B_3)	Grains, nuts, meats.	Pellagra(characterized by lesions in skin, mental confusion and diarrhea)
Pantothenic acid (Vitamin B_5)	Dairy products, fruits, vegetables, grains	Fatigue, numbness, tingling of hands and feet

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Vitamin/ Mineral	**Main dietary sources**	**Deficiency symptoms**
Pyridoxine (Vitamin B_6)	Whole grains, Meats, vegetables	Irritability, anemia
Biotin (Vitamin B_7)	Meats, legumes, vegetables	Neuro- muscular disorders, scaly skin inflammation
Folic acid (Vitamin B_9)	Green vegetables, whole grains	Anemia, birth defects
Cobalamin(Vitamin B_{12})	Dairy products, eggs, meats	Loss of balance, numbness, anemia
Ascorbic acid (Vitamin C)	Citrus fruits, broccoli, tomatoes	Scurvy (characterized by degeneration of skin and teeth) , delayed wound healing
Minerals		
Calcium (Ca)	Dairy products, dark green vegetables, legumes	Loss of bone mass, impaired growth
Iron (Fe)	Whole grains, green leafy vegetables, legumes, meats, eggs	Anemia, weakness, impaired immunity
Phosphorus (P)	Rice, bread, milk, dairy products, fish, red meat	Decaying of teeth and bones, weakness
Potassium (K)	Fruits, vegetables, meat , dairy products, grains	Muscle weakness, nausea , paralysis, heart failure
Iodine(I)	Sea foods , vegetables, iodized salt	Goiter(enlarged thyroid glands)
Sulfur (S)	Foods containing proteins	Fatigue, Impaired growth, swelling
Chlorine (Cl) and Sodium (Na)	Table salt	Reduced appetite, muscle cramps
Magnesium (Mg)	Green leafy vegetables, grains	Disturbance in nervous system
Fluorine (F)	Tea, sea food, drinking water	Tooth decay

Basal metabolic rate (BMR)

- Basal metabolic rate is defined as the minimum metabolic rate at rest, when in a post absorptive stage (at least 12 hour fasting) and is not experiencing stress.
- BMR is measured under a “comfortable” temperature range.
- BMR of humans averages for adult males 1,600-1,800 kcal per day and 1,300-1,500 kcal for adult females.

Energy budget: An energy budget is a balance sheet of energy intake against energy expenditure in a particular animal. The basic model of energy budget can be shown as follows;

C=M+U+F+P

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Where C=Energy content in the food sources taken in

M= Energy spent for metabolic activities

U= Energy associated with urinary loss

F= Energy associated with fecal loss

P= Production (Energy available for growth and reproduction)

In energy budgets, energy content in the food intake is compared with energy expenditure which includes energy spent for basal metabolism and extra activities (M), energy associated with excretory products namely urinary loss (U) and fecal loss (F). The energy differences between the energy intake and the energy expenditure for metabolism and excretion are available for production which includes growth and reproduction. Energy budget can be calculated for each animal based on energy measurements from field and laboratory. Energy budgets are useful for estimating energy available for growth and reproduction.

Food for healthy life: For a healthy life diet should contain correct proportions of carbohydrates, proteins, lipids, water, fiber, essential mineral elements and vitamins. Dietary deficiencies can have negative impact on health. When food intake exceeds daily energy requirements especially in inactive individuals can lead to ill health conditions specially diabetes mellitus and heart diseases. Some individuals develop allergic reactions to foods such as pineapple, peanuts and tomatoes. Such individuals should avoid these types of foods. Antioxidants present in the food material (e.g. vitamin C and vitamin E) are also important in avoiding disorders in the alimentary canal and maintaining a healthy life. As humans cannot synthesize all the required antioxidants some of them should be obtained from the diet.

Malnutrition: Malnutrition can arise due to failure of obtaining an adequate nutrition when the diet lacks one or more essential nutrients or consistently supplies less chemical energy than the energy required by the body. According to the WHO if, body mass index (BMI) is less than 18.5 is said to be malnutrition. BMI of a person is calculated as follows,

BMI= Mass/ height^2 (kg/ m^2)

Obesity: Obesity arises when energy expenditure of a person is much less than the energy intake. According to the WHO criteria, if the BMI is at 30.0 or over it is known as obesity. This condition is a growing issue worldwide. Obesity can lead to many diseases such as diabetes mellitus, cardiovascular diseases, some cancers etc.

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Common disorders in the alimentary canal

Gastritis: gastritis is a condition resulting inflammation of the stomach which can be due to several reasons. In gastritis, glands of the stomach wall are stimulated and secrete excess HCl causing damage to the mucosa. Due to the damages of mucosa layer of the stomach, blisters can be formed. Prolonged starvation and mental stress are one of the reasons for the secretion of excess HCl. some drugs like aspirin can also induce gastritis conditions. Longer lasting gastritis conditions is usually associated with the infection by the acid tolerance bacterium *Helicobacter pylori.* As prolonged starvation is one of the reasons for developing gastritis proper food habits should be practiced to control this condition.

Constipation: Constipation occurs due to the slow movement of feces that promotes the water reabsorption and as a result feces become more solid. Inhibition of the reflex action in defecation may also leads to constipation. This leads to pain in the anus and difficulty in defecating. Constipation can be controlled by developing behavioral adjustments to carry out defecation properly. Intake of adequate fiber in the diet can help prevent constipation.

Circulatory Systems in Animals

Need of a circulatory system

A circulatory system is required in animals for transportation of materials within the body and exchange of materials with the external environment. The simple animals (e.g. Cnidarians, Flat worms) lack specialized system for the transport and distribution of material because many or all cells are in direct contact with the external environment. In these animals exchange of materials over the body surface through direct diffusion is sufficient for their needs. In these animals those materials can be transported by diffusion through short distance within the body.

As organisms increase in size and complexity, the amount of materials moving in and out of the body also increases. The distance that materials have to be transported within the body also increases and many cells are not in direct contact with their external environment. Therefore diffusion is not sufficient to transport materials throughout the body. Therefore circulatory systems have evolved in such organisms to exchange materials between cells and their immediate surroundings.

Substances that are transported in the body

Substances that are transported in the body include respiratory gases (oxygen, carbon dioxide), nutrients (glucose, amino acids, fatty acids, vitamins, etc.), waste products of metabolism (urea, ammonia, etc), hormones and antibodies.

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Blood circulatory systems in the animal kingdom

A circulatory system has three basic components: a muscular pumping device (heart), interconnected vessels and a circulatory fluid (blood/ hemolymph). Circulatory fluid flows through the vessels mainly due to the pressure generated by the heart. The circulatory system functionally connects the fluid environment of the body cells to the organs that exchange gases, absorb nutrients and dispose of wastes by transporting fluids throughout the body. Various degrees of complexity in the arrangement of circulatory systems can be seen in the animal kingdom.

Main circulatory systems in the animal kingdom

There are two types of circulatory systems among the animals: open circulatory system and closed circulatory system.

The open circulatory system: It is a circulatory system in which a fluid called hemolymph that bathes the tissues and organs directly. There is no distinction between the circulatory fluid and the interstitial fluid surrounding cells. The heart pumps hemolymph through the circulatory vessels into the spaces (interconnected sinuses) which surround body tissues. Chemical exchange occurs directly between the hemolymph and body cells. Back flow of the hemolymph takes place via the pores (ostia) with valves found in the heart during the relaxation. The open circulatory system has evolved in some invertebrate phyla such as Arthropoda and Mollusca (some mollusc groups).

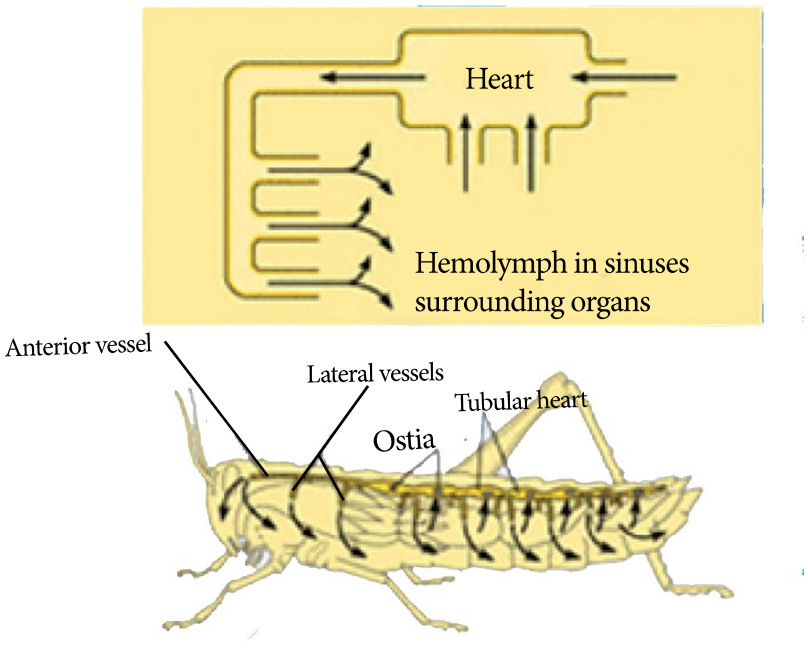


Fig 5.13:Circulatory system of a Grass hopper

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The closed circulatory system: It is a circulatory system in which blood is restricted to vessels and kept apart from the interstitial fluid. Blood is pumped by the heart/s into large vessels. These large blood vessels branches into small vessels and they penetrate into the organs. Chemical exchange occurs between the blood and the interstitial fluid and interstitial fluid and body cells. It may contain one or more hearts. This type of circulatory systems can be seen in vertebrates and invertebrates such as Annelids. In comparison to open circulatory system enable effective delivery of oxygen and nutrients to the cells of larger and more active animals due to relatively high blood pressure.

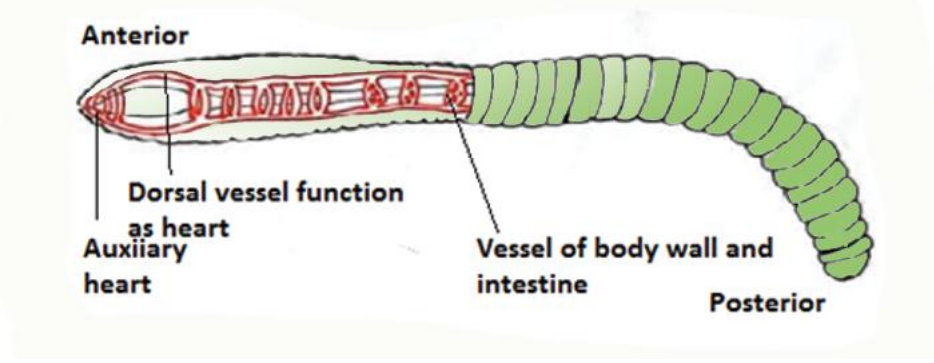


Fig 5.14: Closed blood Circulatory system of an Annelid

Organization of vertebrate circulatory systems: Single circulation and double circulation

Vertebrates have a closed circulatory system where there are three main types of blood vessels: arteries, veins and capillaries. Within each type blood flows in only one direction. The blood vessels which carry blood from the heart to organs are called arteries. When these arteries branch into smaller vessels within organs they are called arterioles. They pass blood to capillaries which are microscopic vessels with thin and porous walls. These are the places where materials exchange occurs between blood and interstitial fluid surrounding body cells through diffusion. Capillaries converge into venules and they converge into veins which carry blood back to the heart.

Single circulation

During single circulation, in a complete circulation through the entire body, blood passes through the heart only once. In animals that demonstrate single circulation, posses two chambers in the heart. They are atrium and ventricle.

During a single circulation, the oxygen poor blood returning from the body enters atrium and pass into the ventricle. Then the ventricle is contracted, blood is pumped into the capillary bed in the gills. There gas exchange occurs between the capillaries and the external environment. The O_2 is diffused into the blood while CO_2 is removed from the blood. Next the oxygen enriched blood circulates throughout the body and reaches the body cells through body capillaries. e.g.: Bony fishes, Cartilagenous fishes such as rays and sharks.

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Double circulation

During double circulation, in a complete circulation through the entire body, blood passes through the heart twice. Such circulatory system consists of separate pulmonary and systemic circuits in which blood passes through the heart after completing each cycle. e.g. Amphibians, Reptiles, Aves, Mammals. Amphibians and most reptiles have a three chambered heart: two atria and one ventricle. Birds and mammals have a four chambered heart where heart is completely divided into left and right sides. This arrangement allows the complete separation of oxygen enriched and oxygen poor blood. The oxygen poor blood from systemic circulation flows to the right atrium of the heart and then to the right ventricle. Right ventricle pumps blood into the lungs. Oxygen enriched blood from the lungs reaches the left atrium and pass to the left ventricle. The left ventricle pumps oxygen enriched blood into the systemic circulation.

Double circulation is more effective in supplying blood to all body organs and tissues especially brain and muscles due to the higher pressure exerted by the heart in the systemic circulation. This in contrast to single circulation where blood flows under reduced pressure from the gas exchange organs to other organs.

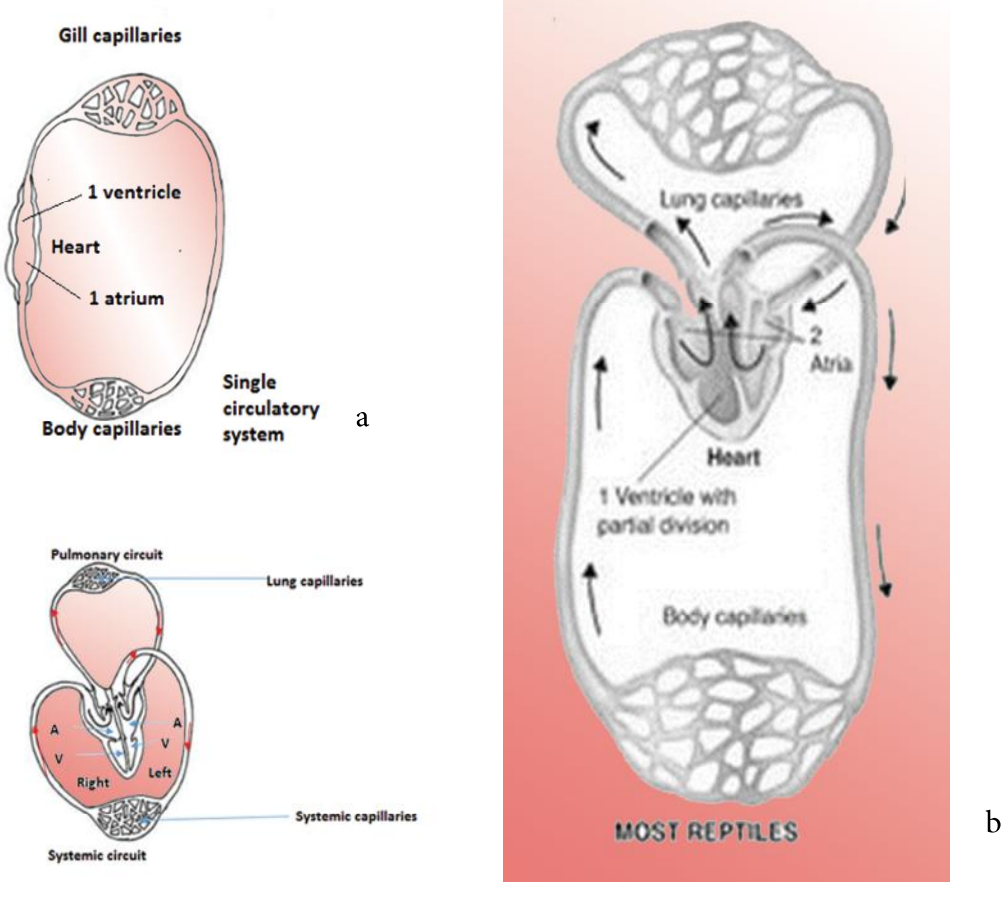


Fig 5.15: Single and double circulatory systems in animals: a. Single circulation (fish), b. double circulation (amphibian) c. double circulation (mammal)

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Basic plan of human blood circulatory system and lymphatic system

Basic plan of human blood circulatory system

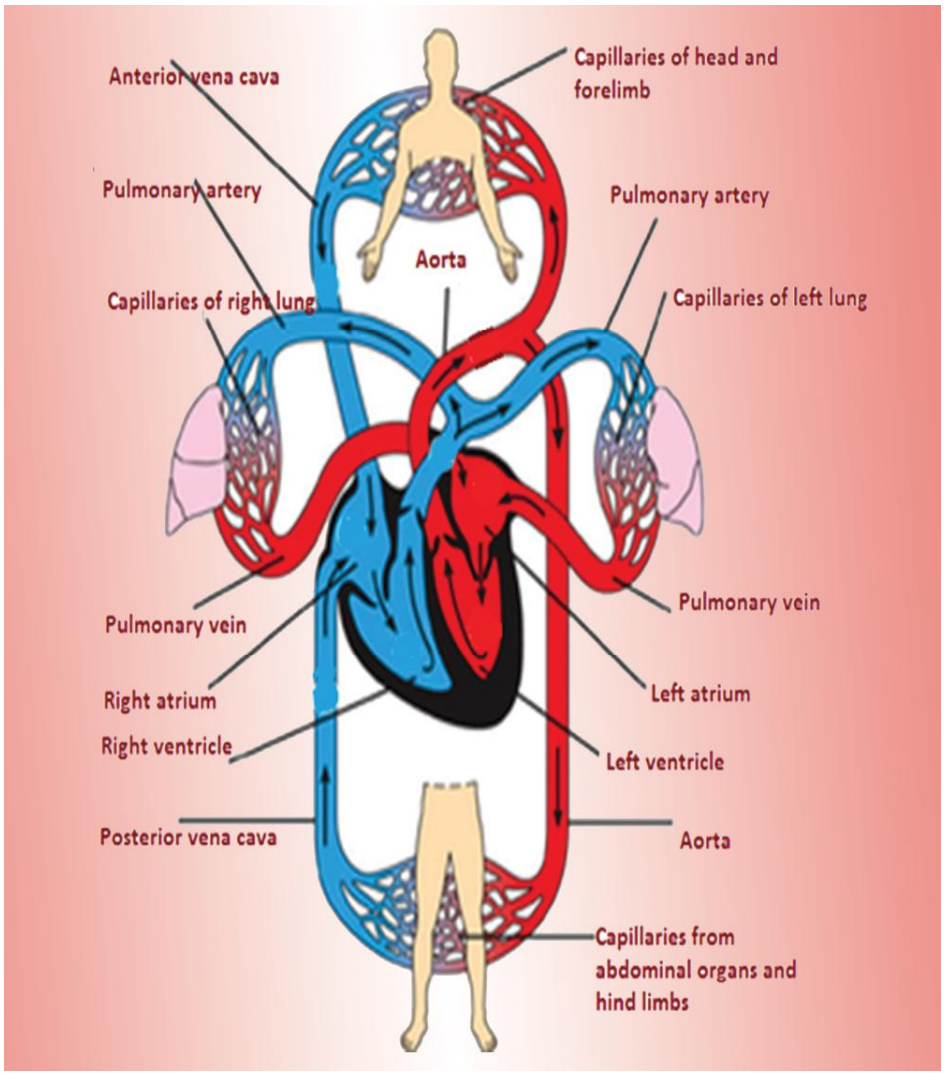


Fig 5.16: Basic plan of the human blood circulatory system

Figure (5.16) depicts the basic plan of the human blood circulatory system. The human heart consists of four chambers; they are two ventricles and two atria. There is a dual circuit which operates simultaneously. The pulmonary circuit takes oxygen poor blood to the respiratory surface, the lungs and returns the oxygen enriched blood back to the heart while the systemic circuit supplies oxygen enriched blood to all organs and tissues in the body and returns oxygen poor blood from organ and tissues back to the heart. Each circuit consists of major arteries/ arteries, arterioles, capillary beds, venules and veins/major veins.

During ventricular contraction, the right ventricle pumps the oxygen poor blood into the two lungs via the pulmonary arteries. Then in the lungs O_2 is loaded into the blood through diffusion while CO_2 is unloaded from blood into the external environment.

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This process occurs in the capillary beds in the left and right lungs. Then the oxygen rich blood is transported into the left atrium via the two pulmonary veins. During atrial contraction, this oxygen rich blood is transported into the left ventricle. During ventricular contraction oxygen rich blood is pumped into the aorta. Through arteries the aorta conveys this oxygen rich blood throughout the body. First the aorta branches into the coronary arteries which supplies blood into the heart muscles. Then the aorta branches into arteries, arterioles leading to capillary beds in the head and arms and the capillary beds in the abdominal organs and legs. The exchange of gases occurs in the capillary beds where O_2 rich blood is diffused into the tissues while the CO_2 rich blood diffused into the blood capillaries. These blood capillaries rejoined to form venules which direct oxygen poor blood into veins. Oxygen poor blood from trunk and hind limbs is drained into the inferior vena cava and oxygen poor blood from the head, neck and fore limbs are directed into the superior vena cava. The blood from both inferior and superior vena cava is pumped into the right atrium where it is passed into the right ventricle. This blood is directed to the pulmonary circuit as explained above.

Basic plan of human lymphatic system

The lymphatic system is closely connected with the blood circulatory system both structurally and functionally. It consists of lymph vessels through which lymph travels. Other structure in the lymphatic system includes lymph nodes, lymphoid tissues (tonsils) and lymphoid organs (e.g. spleen and thymus). Lymph vessels consist of tiny vessels and larger vessels. Tiny lymph vessels are in close contact with the capillaries of the blood circulatory system. Lymph nodes are composed of connective tissues and white blood cells.

The lymphatic system returns lost fluid and proteins from the blood capillaries back into the blood. The lost fluid from the blood capillaries is called lymph when they are inside the lymphatic system. The composition of the lymph is same as interstitial fluid. Lymph vessels have valves. That prevents the backflow of the lymph. The lymph is drained into veins at the base of the neck via two large ducts The rhythmic contraction of the lymph vessel walls and skeletal muscle contraction help to move the lymph.

The functions of human lymphatic system include tissue drainage to maintain the blood volume in the blood circulatory system, absorption of fat and fat soluble vitamins from the small intestine and for immune responses.

Structure and function of the human heart

The human heart is roughly a cone shaped hollow muscular organ. The heart wall is composed of three layers of tissues: Pericardium, Myocardium, Endocardium.

Pericardium: The pericardium is the outer most layers. It is made up of two sacs: The outer fibrous pericardium and inner serous pericardium.

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Myocardium: Myocardium is the middle layer of the heart wall. It is composed of specialized cardiac muscle found only in the heart. Running through the myocardium is also a network of specialized conducting fibres responsible for transmitting the heart's electrical signals.

Enodocardium: Endocardium is the inner layer of the heart wall. It lines the chambers and valves of the heart. It is a smooth membrane and consists of flattened epithelial cells. It is continuous with the endothelium lining of the blood vessels.

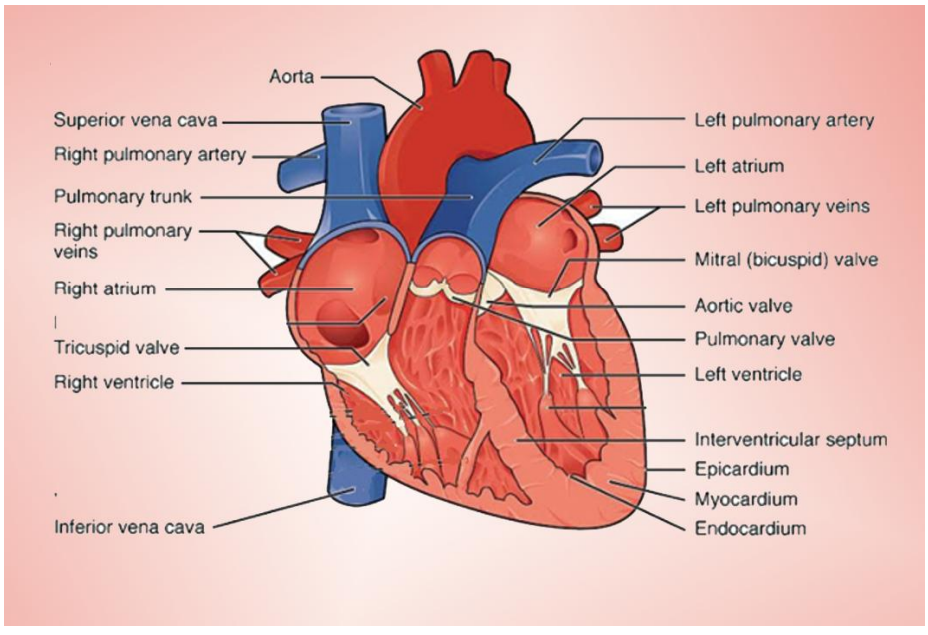


Fig 5.17: Interior of the human heart

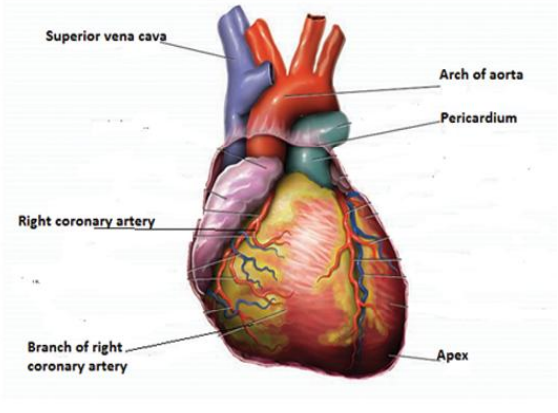


Fig 5.18: External appearance of the human heart

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There are four chambers in the heart two upper atria and two lower ventricles. Ventricles have to pump blood to the whole body where as atria pump blood to the ventricles only. Therefore walls of the ventricles are thicker than walls of the atria. Wall of the left ventricle is thicker than walls of the right ventricle because the right ventricle pumps blood to the lungs which are closer to the heart whereas the left ventricle has to pump blood throughout the body.

Therefore the blood entering the aorta from the left ventricle is at much higher blood pressure than the blood entering the pulmonary artery from the right ventricle.

The heart is completely divided into a right and left side by a septum. The atria and ventricles on each side are divided by an atrio-ventricular valve (AV). The right atrioventricular valve has three flaps hence known as tricuspid valve and the left atrioventricular valve has two flaps hence known as bicuspid valve. Conical shaped papillary muscles are extensions of the inner wall of the ventricles. Atrio-ventricular valves attached to the papillary muscles by fibrous cords which are called chordae tendineae. Atrio-ventricular valves are anchored by strong fibres. This prevents the valves from being turned inside out. Semilunar valves are found at the points where the pulmonary artery and aorta leave the right and left ventricles respectively. These valves prevent the backflow of blood into the ventricles.

Two pulmonary arteries with oxygen poor blood leave the heart from the upper part of the right ventricle. Two pulmonary veins from each lung carry oxygen rich blood back to the left atrium. The aorta with oxygen rich blood leaves from the upper part of the left ventricles. The superior vena cava and inferior vena cava open into the right atrium and empty their contents into the right atrium. The heart is supplied with arterial blood by the right and left coronary arteries which branch from the aorta immediately after the aortic valve.

The conducting system of the heart

Heart generates its own electrical impulses and beats independently of nervous or hormonal control. However it is supplied with both sympathetic and parasympathetic nerve fibers which increase and decrease respectively the intrinsic heart rate. In addition to that heart responds to a number of circulating hormones including adrenaline and thyroxine.

Small group of specialized neuromuscular cells in the myocardium initiate and conduct impulses. The conducting system of the heart consists of following specialized system.

- SA node (Sinoatrial node)
- AV node (Atrioventricular node)
- Atrioventricular bundle (bundle of His), bundle branches and Purkinje fibres

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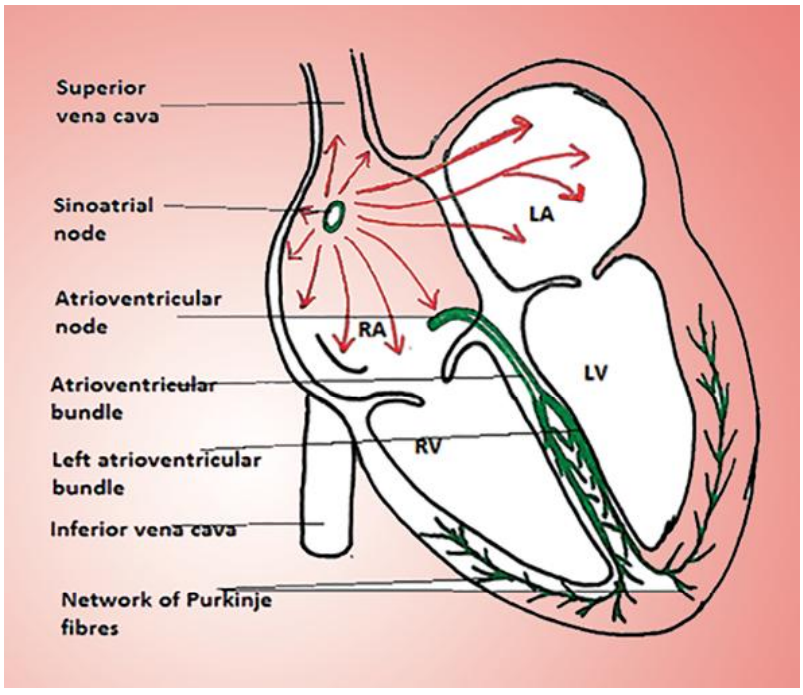


Fig5.19: The conducting system of the human heart

SA node / Sinoatrial node

SA node is a small mass of specialized cells. It lies in the myocardium of the right atrium near the opening of the superior vena cava. The stimulus for contraction of the heart originates in the 'SA node'. The SA node initiates the heart beat and sets the rhythm of the heart beat so it is called the pace maker. But the heart rate can be varied by the stimulation from the autonomic nervous system, hormones such as adrenaline, thyroxine and temperature.

AV node

AV node is also a small mass of specialized cells. It is situated between wall of the left and right atria. The AV node transmits the electrical signals from the atria into the ventricles.

Atrioventricular bundle (bundle of His), bundle branches and Purkinje fibres

AV bundle is a mass of fibers. It originates from the AV node. The AV bundle crosses the fibrous rings that separate atria and ventricle at the upper end of the ventricular septum it divides into right and left bundle branches. Within the ventricular myocardium the branches break up into fine fibres. These fine fibers are Purkinje fibers. The AV bundle branches and the Purkinje fibers transmit electrical impulse from the AV node to the apex of the myocardium. As a result of this impulse, wave of ventricular contractions begin. Then the contraction sweeps upwards and outwards pumping blood simultaneously into the pulmonary artery and the aorta.

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The cardiac cycle

The cardiac cycle refers to the sequences of events that take place in a complete heart beat. During this process one complete cycle of pumping and filling of blood into the heart occurs. Complete cardiac cycle lasts for 0.8 second. It occurs as follows:

1. Atrial systole – Contraction of the atria
2. Ventricular systole - Contraction of the ventricles
3. Complete cardiac diastole – Relaxation of the atria and ventricles

At rest the healthy adult heart is likely to beat at a rate of 60- 80 beats per minute. During a single heart beat, the heart contracts (systole) and then relaxes (diastole). During a single contraction the amount of blood pumped by a ventricle is called the stroke volume.

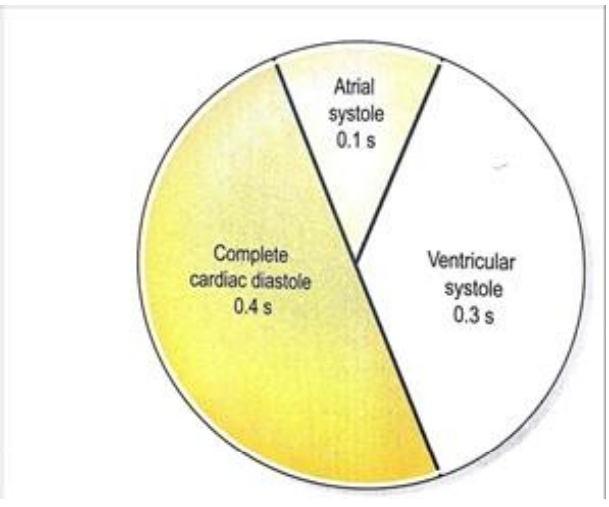


Fig 5.20:The stages of Cardiac cycle

Complete cardiac diastole

This lasts for 0.4 second. Both atria and ventricles are relaxed and blood return to the heart. The superior vena cava and the inferior vena cava transport oxygen poor blood into the right atrium. At the same time as the four pulmonary veins bring oxygen rich blood into the left atrium. The pressure of the atria is more than the pressure of the ventricles. Therefore atrioventricular valves are open and some blood flows passively through to the ventricles.

Atrial systole

When blood flows into the atrium SA node is stimulated. Then SA node triggers a wave of contractions that spreads over the myocardium of both atria. Hence the remaining blood in the atria flows into the ventricles thereby emptying the atria. This lasts for 0.1 second.

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Ventricular systole

Through the atrial muscles the electrical impulses reaches the AV node. Then AV node triggers its own electrical impulses which quickly spread to the ventricular muscles via the AV bundle, the bundle branches, and Purkinje fibers. This results in a wave of contractions which sweep upwards from the apex of the heart across the wall of the ventricles. As a result both ventricles contract.

The pressure in the right ventricle is more than the pressure in the pulmonary artery and the pressure in the left ventricle is more than pressure in the aorta. Therefore pulmonary valve and aortic valves open and blood flows into pulmonary artery and aorta respectively.

The high pressure generated during ventricular contractions force the atrioventricular values to close preventing backflow of blood into the atria. Ventricular systole lasts for 0.3 seconds. When ventricles relax, the pressure within them falls. The pulmonary and aortic valves close. Pressure within the pulmonary artery and aorta is more than pressure within the ventricles. The valves of the heart and great vessels open and close according to the pressure within the chambers of the heart. The sequence of opening and closing of valves ensure that blood flows only in one direction.

Electrocardiogram (ECG)

Electrical activity in the heart can be detected on the surface of the skin by placing electrodes on the chest or limbs as the body tissues and fluids conduct electricity well. Such a recording is called an electrocardiogram (ECG). The ECG indicates the spread of the electrical signal generated by the SA node as it travels throughout the heart. The normal ECG tracing of a healthy individual shows five waves which by convention have been named P, Q, R, S and T.

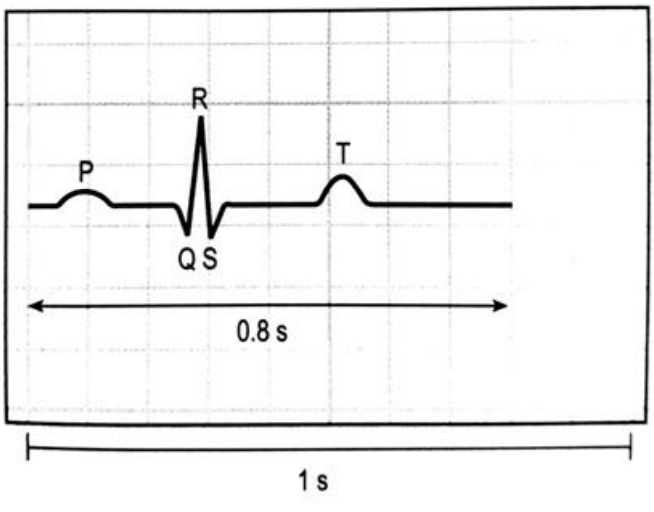


Fig 5.21: Electrocardiogram of one cardiac cycle

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P wave - It represents the impulse from the SA node as it sweeps over the atria (atrial depolarization).

QRS wave complex – Represents rapid spread of impulse from the AV node throughout the ventricles and electrical activity of the ventricular muscles.(ventricular depolarization)

T wave – Represents ventricular repolarization and relaxation of the ventricular muscles. Due to the larger QRS complex, atrial repolarization which occurs during ventricular contraction is not seen.

Information about the heart function of a person (state of the myocardium and the cardiac conduction system) can be obtained by examining the pattern of waves and the time interval between cycles and parts of cycles.

Blood Pressure

The force that the blood exerts on the walls of blood vessels as it travels is referred to as blood pressure. Blood pressure in the arteries of systemic circulation maintains the essential flow of blood into and out of the organs of the body.

It is very important to keep blood pressure within normal limits. High blood pressure could lead to damage blood vessels resulting in formation of clots or bleeding from damaged sites. If the blood pressure falls too low, there will be inadequate blood flow through tissue capillary beds. This will adversely affects the normal functioning of vital organs such as the brain, heart and kidneys.

Blood pressure varies according to the time of day, the posture, gender, age, activity, exercise and stress (Emotional states) of an individual. Blood pressure falls at rest and during sleep. Blood pressure increases during excitement, fear or anxiety.

Systolic and diastolic pressure

Systolic pressure

Systolic pressure is the pressure produced within the arterial system when the left ventricle contracts and pushes blood into the aorta. At rest , systolic pressure in a normal healthy adult systolic pressure is about 120 mmHg.

Diastolic pressure

Diastolic blood pressure is the blood pressure within the arteries following ejection of blood at complete cardiac diastole(when the heart is at rest. In a normal healthy adult diastolic pressure is about 80 mmHg.

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Arerial blood pressure is measured by a sphygmomanometer. It is expressed as

$$\frac{\text{Systolic pressure (mm Hg)}}{\text{Diastobic pressure (mm Hg)}}$$

120/80 mmHg

Hypertension and Hypotension

Hypertension

Sustained elevated blood pressure above normal limits is called hypertension.

Consequences of hypertension are kidney damage, adrenal gland disorders, heart attack (because of the increased heart rate and cardiac contraction), stroke(caused by cerebral haemorrhage), damaged blood vessles which can lead to death

Risk Factors for Hypertension

- Obesity
- Diabetes mellitus
- Family history
- Smoking
- A sedentary life style
- High intake of salts
- High intake of alcohol
- Stress
- Deposition of low density lipoprotein (LDL) on artery walls.

Hypotension

Sustained reduction of blood pressure below normal limits is called hypotension. Hypotension usually occurs as a complication of other condition such as shock, Dengue hemorrhage fever, standing up suddenly from sitting or lying position, over bleeding/ hemorrhage condition, fasting, low nutrition etc. Low blood pressure leads to inadequate blood supply to the brain. Depending on the cause unconsciousness may be brief (fainting) or prolonged possibly causing death.

Coronary circulation

The heart is supplied with arterial blood by the right and left coronary arteries which branch from the aorta immediately distal to the aortic valve.

The coronary arteries travel the heart wall eventually forming a vast network of capillaries. Most of the venous blood is collected into a number of cardiac veins that joins to form the coronary sinus which opens into the right atrium. The remainder passes directly into the heart chambers through little venous channels.

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Consequences of blockage of coronary arteries

The inner lining of the arteries can be thickened and harden leading to the condition called atherosclerosis which occurs as a result of fatty deposits especially cholesterol particles. This can affect the normal blood supply to the organs and tissues.

One or more branches of coronary arteries can be blocked due to atherosclerosis which can be complicated by thrombosis (blood clot). Depending on the place (s) of the block in the coronary arteries and the degree of blockage related parts of the heart muscle will be deprived of oxygen and nutrients. Hence, narrowing of the arteries leads to chest pain (Angina). The complete occlusion due to the blockage of one or more coronary arteries leads to heart attacks (Myocardial infarction) which refers to the damage or death of cardiac muscle tissue due to lack of adequate oxygen and nutrients. Due to this, heart beat rhythm may be abnormal and the heart may cease to be an effective pump. The other vital organs such as brain may be deprived of inadequate supply of oxygen rich blood and heart attack may be fatal if not treated on time.

Stroke – Similarly blockage due to atherosclerosis or rupture of arteries supplying blood to the brain may cause the death of nervous tissue due to lack of oxygen and nutrients. This is refered to as stroke.

Respiratory pigments

Respiratory pigments are organic compounds which can combine with oxygen where the partial pressure of oxygen is high and release oxygen where partial pressure of oxygen is low. Since oxygen is less soluble in watery medium including blood, transportation of oxygen from respiratory surface to the tissues/ organs is a problem for complex animals. To overcome this problem animals have evolved the respiratory pigments.

Different respiratory pigments can be seen in the animal kingdom:

- Haemoglobin- present in blood of human, other vertebrate and annelids
- Haemocyanin- present in hemolymph of arthropods and molluscs.
- Chlorocruorin- present in the blood of many annelids
- Haemoerythrin- present in the blood of marine invertebrates (some annelids)
- Myoglobin- present in vertebrate muscles

All these respiratory pigments except myoglobin transport oxygen from respiratory surfaces to the tissues and organs and while transporting carbon dioxide from tissue/ organs to the respiratory surface for elimination. Myoglobin present in the muscle tissue has an oxygen storage function.

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Transport of respiratory gases in human blood

Transport of oxygen

It is the hemoglobin molecule found in the erythrocytes which is responsible for the transport of oxygen around the body. Hemoglobin is composed of four subunits. Each subunit is composed of a globin protein and the haem group. Haem groups are responsible for the characteristic red colour of the blood. A ferrous (iron) atom is located within each haem group and each of these can combine reversibly with one molecule of oxygen. Therefore each hemoglobin molecule can carry up to four oxygen molecules.

$Hb + 4O_2 \rightleftharpoons HbO_8$

(Hemoglobin molecules) (Oxyhemoglobin)

Combination of oxygen with hemoglobin to from oxyhemoglobin

Transport of Carbon dioxide

Carbon dioxide is carried by the blood in different ways.

- As HCO_3^- ions in the plasma (about 70%): When CO_2 diffuses into the red blood cells the enzyme carbonic anhydrase catalyze the combination of CO_2 with water to form bicarbonate (HCO_3^-) and H^+ ions. The HCO_3^- moves out of the erythrocytes into the plasma
- As carbaminohemoglobin (about 23%): CO_2 combined with protein group of hemoglobin and form carbaminohemoglobin. Therefore CO_2 does not compete with oxygen binding sites in hemoglobin.
- Dissolved in plasma (about 7%): as free gas.

Composition and major functions of human blood

Blood which is a connective tissue is composed of cells and plasma. Cellular components of the blood are three types namely red blood cells, white blood cells and platelets. ((fig. 5.22) Red blood cells, leukocytes and platelets are developed from the bone marrow in the bones such as ribs, vertebrae, sternum and pelvis. Erythropoietin hormone (from kidneys) stimulates the generation of red blood cells.

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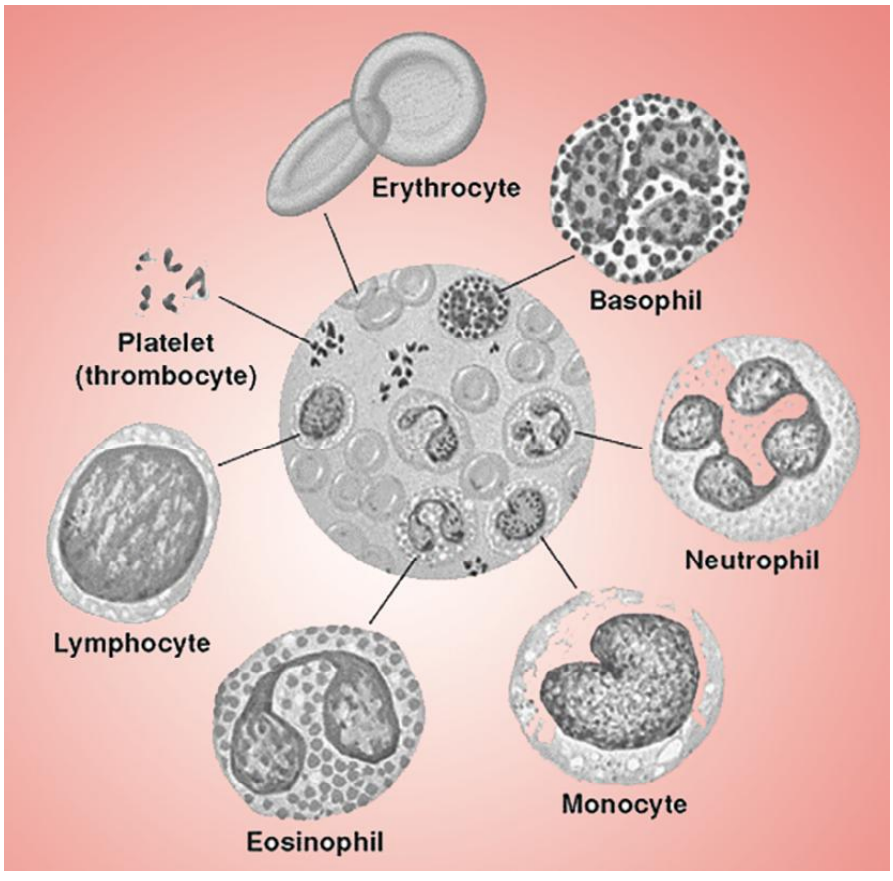


Fig 5.22: The composition of blood

Red blood cells (erythrocytes)

They are small biconcave disk-like cells. Mature erythrocytes lack nuclei. This character helps to carry more hemoglobin molecules within the cell. They also lack mitochondria thus they produce ATP via anaerobic respiration. If they produce ATP by aerobic respiration that will reduce the O_2 transport efficiency. They have about 120 days of life span.

Generally a micro liter of blood contains 4- 6 million red blood cells. This figure can be varied depending on the gender and health status.

Main function of red blood cells is the transportation of O_2 molecules. They also transport CO_2 molecules.

White blood cells (Leukocytes)

There are five types of leukocytes. They are Basophils, Lymphocytes, Eosinophils, Neutrophils and monocytes. Main functions of leukocytes are body defense, phagocytic engulfing and digesting microorganisms. Lymphocytes develop into T cells and B cells. These cells increase immune response against foreign substances.

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Platelets

Platelets are derived from bone marrow cells. They also do not have nuclei and they play a major role in blood clotting.

Blood plasma

Blood plasma consists of inorganic ions in dissolved forms, plasma proteins such as albumin, antibodies and fibrinogen, nutrients, metabolic wastes, respiratory gases and hormones. pH of human blood is around 7.4. Protein concentration in plasma is higher than in interstitial fluid.

The dissolved ions in the plasma buffer and maintain the osmotic balance in the blood.

Albumin in the plasma also buffers the blood and antibodies are involved in defense. Fibrinogen in the plasma aids in blood clotting. When the clotting factors are removed from the plasma it is called as serum.

Major Functions of blood

- Transport of oxygen to organs and removal of carbon dioxide from the organs and tissues
- Transport of soluble excretory materials to organs of excretion
- Transport of nutrients
- Transport of hormones from the glands where they are produced to target organs
- Defence against foreign invasions
- Aids in osmoregulation

Blood clotting

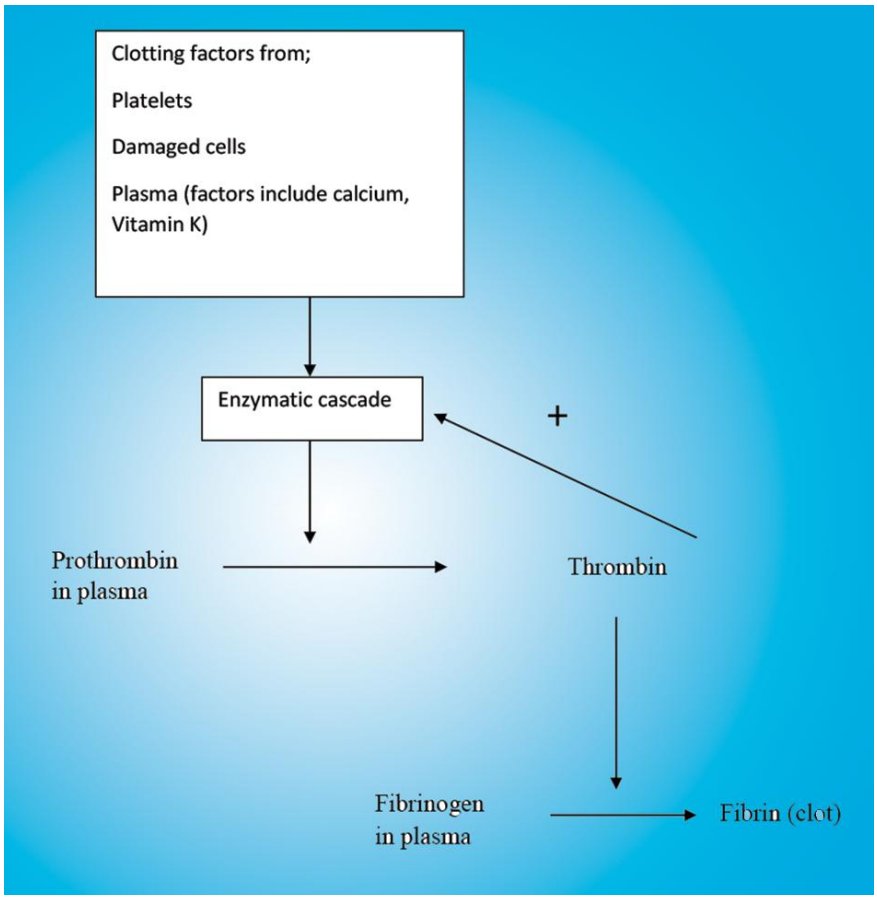
When a tissue is damaged, blood flows from it and coagulates to form a blood clot. This prevents further blood loss and entry of pathogenic micro organisms which is of clear survival value. In general the blood in undamaged vessels does not clot.

A highly complex series of reactions takes place in order for coagulation to occur as well as prevent unwanted clotting. When the blood vessel is damaged the connective tissues of the vessel wall is exposed. Therefore platelets in the blood adhere to the collagen fibers in the connective tissue and release substance that makes close by platelets sticky. This platelet plug provides instant protection against blood loss.

Then platelets release clotting factors. They trigger the formation of thrombin. Then thrombin converts fibrinogen into fibrin. Next this fibrin aggregates into threads that form a network of the clot. The activated thrombin is also involved in formation of more thrombin which completes the formation of blood clot.

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The cascade reaction during blood clotting is given below;



Clotting does not occur in undamaged blood vessels because the lining of the vessels is very smooth and does not promote platelet aggregation or cell rupture. Also some substances such as Heparin prevent clotting. Heparin prevent the conversion of prothrombin into thrombin and fibrinogen to fibrin and is widely used clinically as an anticoagulant.

Grouping of blood

The surface of the red blood cells carries antigens called aggulitinogen (antigen A and antigen B). In addition individuals have antibodies in plasma (anti–A and anti-B). According to ABO blood grouping system there are four blood groups namely A, B, AB and O. A person with a specific antigen in red cells does not possess the corresponding antibody in the plasma. e.g. Anyone with antigen A on the red blood cell membrane has no anti–A antibody in the plasma.

If red blood cells have antigen A and plasma has antibodies b (anti-B) that person's blood group is A

If red blood cells have antigen B and plasma with antibodies a (anti–A) that person's blood group is B

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If red blood cells have both antigen 'A' and 'B' and plasma has no anti-A or anti- B antibodies that person's blood group is 'AB'

If red blood cells have no antigen A or B but plasma has both antibodies (anti–A and anti-B) that person's blood group is 'O'

When a patient receives a blood transfusion it is vital that they receive blood that is compatible with their own. If it is incompatible a type of immune response occurs. This is because the donor's red cell membranes possess glycoprotein which act as antigens and react with antibodies (agglutinin) in the recipient's plasma. The result is that the donor's cells are agglutinated.

Therefore when transfusion occurs it is important to know blood group of donor and recipient. People with blood group AB make neither anti–A nor– Anti-B antibodies. Transfusion of type A, B or AB blood into these individuals is likely to be safe since there are no antibodies to react with them. Person with AB blood group is known as a universal recipient.

Individual with blood group O has neither antigen A nor antigen B on their plasma membrane of red blood cells. but they do have antibodies (anti–A and anti-B) in their plasma. So these individuals having blood group O can donate blood to the persons with any blood group. A person with the blood group O is known as a universal donor. Therefore prior to transfusion cross-matching is still required to ensure that there is no reaction between donor and recipient blood.

The Rhesus system

Some individuals have antigen called Rhesus factor on the plasma membrane of red blood cells. Individuals having this factor on the red blood cells are called Rh+ and those who do not have this factor are called Rh-.

Rh+ individuals do not have anti Rhesus antibodies in the plasma while individuals who are Rh- have anti-Rhesus antibodies in their plasma. However if Rh^+ blood enters a Rh^- negative individual the recipient responds by manufacturing Rh antibodies in the blood plasma.

When a Rh^- mother bears a Rhesus positive (Rh^+) child during delivery few Rh^+ red blood cells of the fetus may enter the mother's circulation and cause the mother to produce Rh antibodies in her plasma. If the mother is pregnant for the second time with a Rhesus positive fetus, the Rh antibodies developed in her plasma in response to the first child's red blood sells can pass across the placenta to the fetus and destroy fetal red cells. Normally Rh antibodies are not formed in large enough quantities in the mother's plasma to affect the first born child. However subsequent Rh^+ children can suffer destruction of their red blood cells.

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Gas exchange in animals

Need of respiratory structures in animals and evolution of complexity of respiratory structures in different animal groups

Respiratory gas exchange (uptake of oxygen into the body and release of carbon dioxide into the external environment) occurs by diffusion. In simple animals e.g. cnidarians and flat worms every cell in the body is close enough to the external environment so that gas exchange can occur directly between all cells and the environment. Diffusion through body surface is adequate as these animals have a simple body form and low energy requirement.

In large animals, body complexity and energy requirement is high and the bulk of the cells in the body lack immediate access to the external environment. Hence, gas exchange through the body surface is not adequate to full fill their energy requirements. As a result, specialized surfaces referred to as respiratory surfaces have evolved where gaseous exchange occurs.

With the increase of the body size and complexity the surface area to volume ratio (A/V) of animals decreases. However, a large surface area is required for efficient gas exchange and thus diverse respiratory structures with large surface area with folding and branching has evolved for efficient gas exchange. Examples for such respiratory structure are Gills, Trachea, and lungs. External projections of the body such as gills were evolved in aquatic animals for efficient extraction of dissolved oxygen from water. On the other hand, surface invaginations like trachea and lungs were evolved in terrestrial animals for efficient extraction of oxygen from the atmosphere.

Characteristics of respiratory surfaces

An effective respiratory surface must have the following properties.

- It must be permeable, and wet so that gases can pass through by dissolving.
- It must be thin because diffusion is only efficient over short distances.
- It should possess a large surface area to allow a sufficient volume of gases to be exchanged according to the organism's need.
- It should possess a good blood supply (maintain a steep diffusion gradient)

Respiratory structures in animals

- Body surface: Cnidarians, Flatworms, Earthworms
- Gills

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- External Gills: marine annelids
- Internal Gills: Fish, shrimps, prawns

- Tracheal systems: Insects
- Lungs: Mammals (Human), Reptiles, Birds.
- Skin: Amphibians
- Book lungs: Spiders, Scorpions

Human Respiratory System

Gross structure and function of the human respiratory system

Human respiratory system consists of the following major parts: nostrils, nasal cavity, pharynx, larynx, a series of branching ducts starting from the trachea, two bronchi one leading to each lung and smaller bronchioles, which finally end in air sacs called alveoli. The bronchioles and the alveoli are contained within the paired, cone-shaped lungs located in the thoracic cavity. The two lungs differ slightly in shape and size. The left lung is slightly smaller than the right because the apex of the heart is slightly to the left of the median plane and it has 2 lobes while the right lung has 3 lobes. Each lung is surrounded by two membranes. The inner membrane, called the visceral pleura adheres to the outer surface of the lungs while the outer membrane called the parietal pleura adheres to the wall of the thoracic cavity. Between these two membranes there is a thin, fluid filled space.

During respiration, air enters the respiratory system through the nostrils. In the nasal cavity air is filtered by hairs and is warmed and humidified as it travels through spaces in the nasal cavity. The nasal cavity leads to the pharynx which is a common passage for both air and food. That means air passage and food passage cross each other. During swallowing of food the larynx moves upwards which allows the epiglottis to close the opening of the larynx called the glottis. This allows food to go down the esophagus to the stomach. The rest of the time the glottis is open so that air can move from the pharynx through the larynx to the trachea. In the larynx are vocal cords which are made up of largely elastic fibers. These vocal cords help produce sound when expired air rushes across the stretched or tensed vocal cords, causing them to vibrate. Both the walls of the larynx and trachea are strengthened by cartilage that help these airways to keep open. The air passes from the trachea into the two bronchi that lead into each lung. Within the lungs the air passes through smaller and smaller branches of the bronchi called bronchioles.

The epithelium found in the major branches of this respiratory tract has cilia and a thin film of mucus. The mucus helps trap the dust and other particulate contaminants in the inhaled air. Then the beating of cilia moves this mucus upwards towards the pharynx where it is swallowed into the esophagus. This process is referred to as the “mucus escalator”. It helps clean the respiratory system.

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At the tips of tiniest bronchioles are large number of small air sacs clustered together. The air passes into these air sacs called alveoli where gas exchange occurs. The walls of the alveoli are made up of a single layer of flattened epithelial cells that lack cilia. This inner lining of the alveoli is coated by a thin film of fluid. The lungs contain millions of alveoli. This allows a large surface area for gas exchange. Each alveolus is also surrounded by a network of capillaries. Oxygen in the air that enters the alveoli dissolves in the moist film and rapidly diffuse across the thin epithelium into the capillaries. Meanwhile a net diffusion of carbon dioxide occurs from the capillaries into the alveoli. Since there are no cilia in the alveoli, there are white blood cells in the alveoli to engulf foreign particles. Alveoli are also coated with a surfactant that reduces the surface tension thereby preventing the collapse of alveoli due to high surface tension.

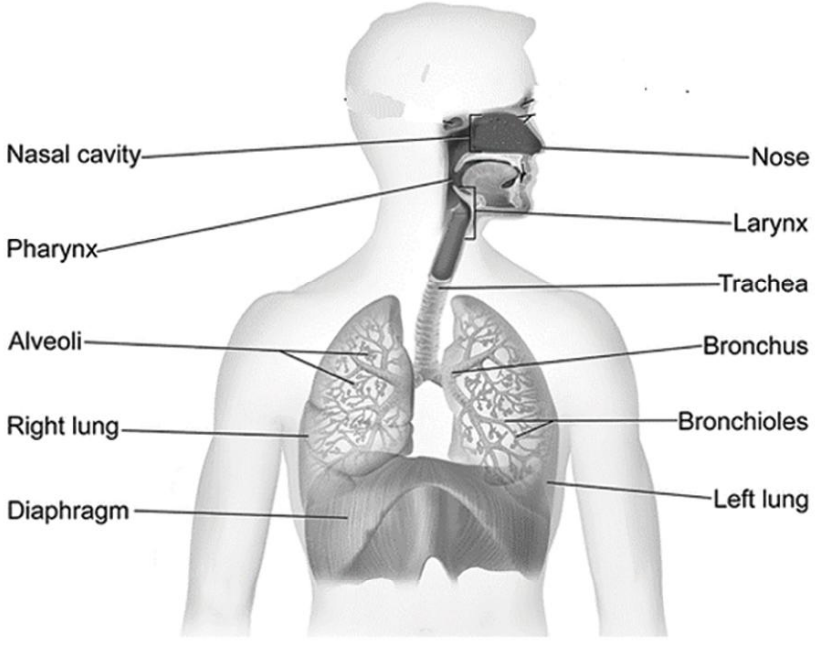


Fig 5.23: Gross structure of human respiratory system

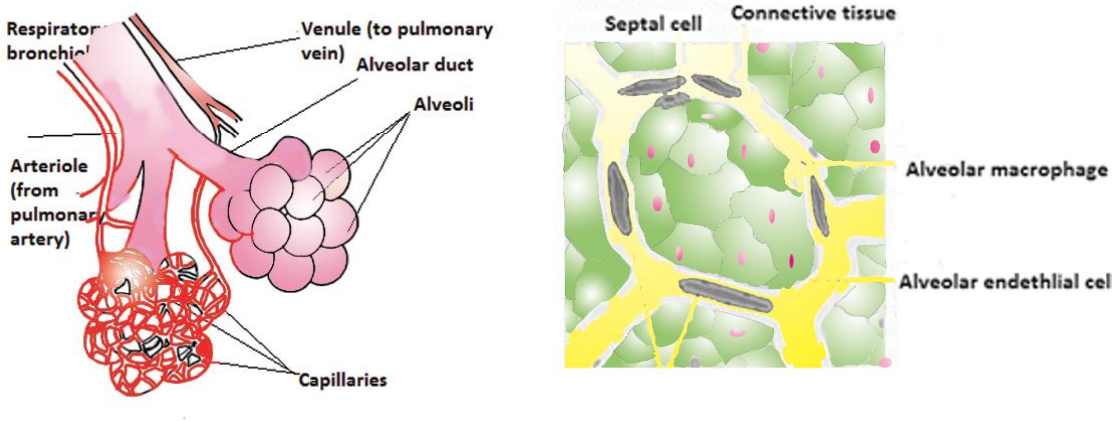


Fig 5.24: The alveoli and their capillary network

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Mechanism of ventilation of the lungs

- Ventilation of the lungs is necessary to maintain high oxygen and low carbon dioxide concentrations in the alveoli or the gas exchange surface.
- Ventilation is accomplished by breathing, which is the alternating movement of air into (inhalation) and out of (exhalation) the lungs.
- Humans employ what is referred to as negative pressure breathing where air is pulled rather than pushed into lungs.
- Inhalation is an active process. Contraction of rib muscles or intercoastal muscles and the diaphragm which is a sheet of skeletal muscle that forms the bottom of the thoracic cavity leads to the expansion of the thoracic cavity.
- The visceral and parietal pleurae surrounding the lung stick together due to the surface tension of the fluid between these two membranes. This allows the two membranes to slide smoothly past each other. Hence, as the volume of the thoracic cavity increases, the lung volume increases as well.
- As a result, the pressure within the lungs decrease relative to the outside air.
- This creates a pressure gradient between the atmosphere and the lungs.
- Thus, air flows from a high pressure gradient in the atmosphere to a lower pressure gradient in the lungs.
- During exhalations which is usually a passive process, the rib muscles and the diaphragm relax. This cause the volume of the thoracic cavity to reduce.
- As a result, the pressure inside the lungs increase in relation to the air outside. This pressure forces air to move out of the lungs through the respiratory tubes into the atmosphere.
- When a man is at rest contraction of rib muscles and contraction of diaphragm are enough for breathing.
- However, depending on the activity level, additional muscles may be used to aid breathing such as muscles of the neck, back and chest. These muscles further help to increase the volume of the thoracic cavity by raising the rib cage. e.g. during exercise

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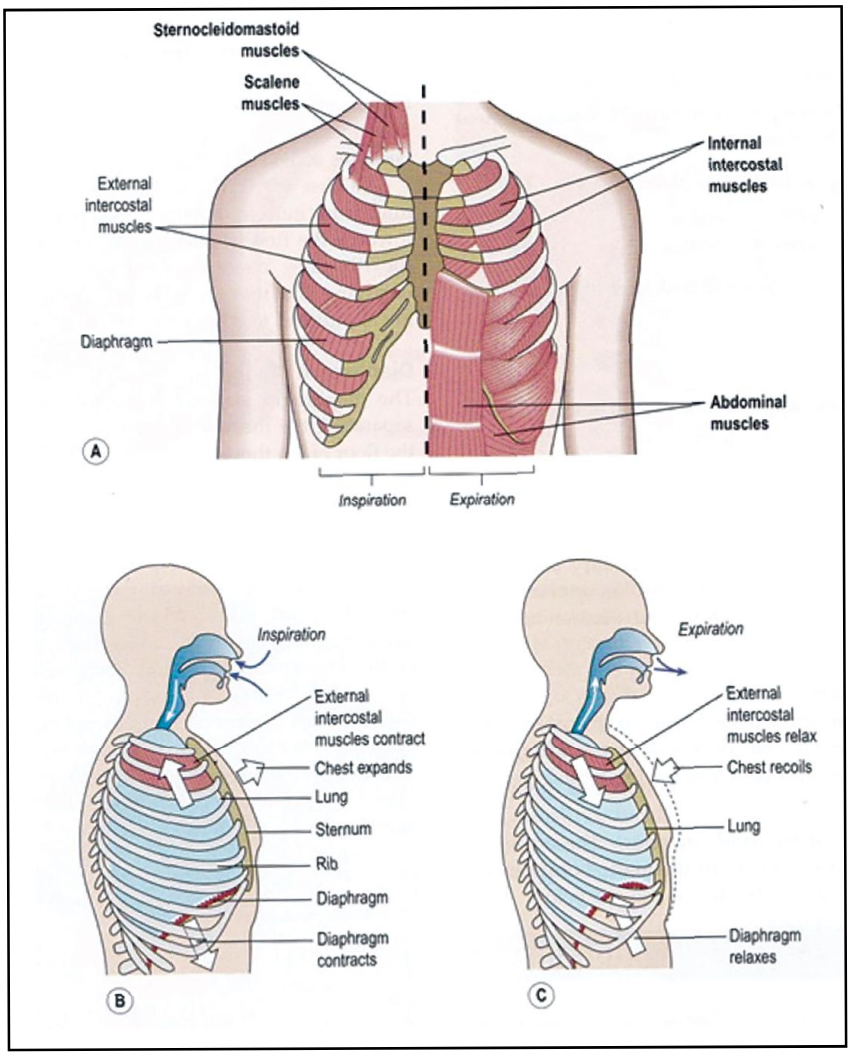


Fig 5.25: (A) Muscles involved in respiration; (B) and (C) Changes in chest volume during inspiration and expiration

- The lungs serve as an efficient respiratory surface because:
- Alveoli create a large surface area for gas exchange.
- Alveoli and capillary walls are both lined by simple squamous epithelia which reduce the distance the gasses need to travel by diffusion.
- The alveoli surface is moist to dissolve respiratory gasses for diffusion.
- Alveoli are highly vascularized which enables the maintenance of a steep diffusion gradient of respiratory gasses

Gas exchange at the alveoli and in the tissues is a continuous process. It requires transport of O_2 from the lungs to the blood and movement of CO_2 from the blood (referred to as external respiration) and movement of O_2 from blood to the tissues and CO_2 from tissues to the blood (referred to as internal respiration).

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Diffusion of O_2 and CO_2 requires partial pressure gradients between the alveolar air in the lungs and blood (during external respiration) and blood and tissues (during internal respiration).

During inhalation, fresh air mixes with the stale air in the lungs. This mixture in the lungs has a higher partial pressure of oxygen (PO_2) and a lower partial pressure of carbon dioxide (PCO_2) than the blood in the alveolar capillaries. There is thus a concentration gradient favoring the diffusion of these two gases in opposite directions. Net diffusion of O_2 takes place from the air in the alveoli to the blood and net diffusion of CO_2 takes place from the blood into the alveoli.

When O_2 molecules diffuses into blood capillaries they bind to heamoglobin in the red blood cells. Four molecules of O_2 bind reversibly with one molecule of haemoglobin and form oxyhaemoglobin.

$$Hb + 4\ O_2 \rightleftharpoons HbO_8$$

When blood leaves the alveolar capillaries the oxygen and carbon dioxide partial pressures are in equilibrium with those of alveoli air. Once this blood returns to the heart, it is pumped through the systemic circuit.

Blood reaching the tissues in the systemic capillaries have a higher PO_2 and a lower PCO_2 than in the tissues. These partial pressure gradients result in the net diffusion of O_2 from the blood stream into the tissue and CO_2 diffusion from the cells into the blood stream across the extracellular fluid/interstitial fluid. This is called unloading of O_2 and loading of CO_2. Then the blood returns to heart and pumped to lungs again.

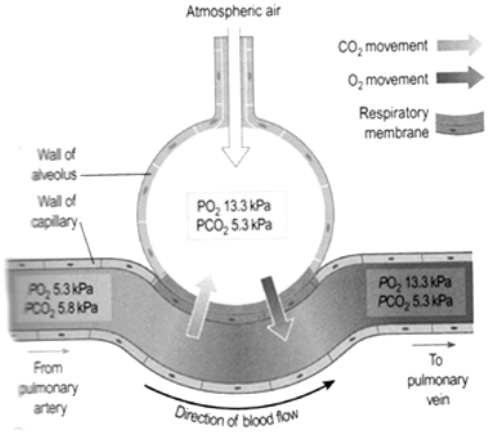


Fig 5.26: External respiration

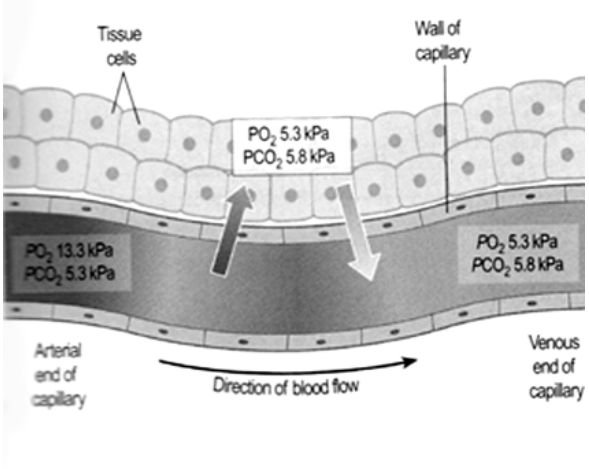


Fig 5.27:Internal respiration

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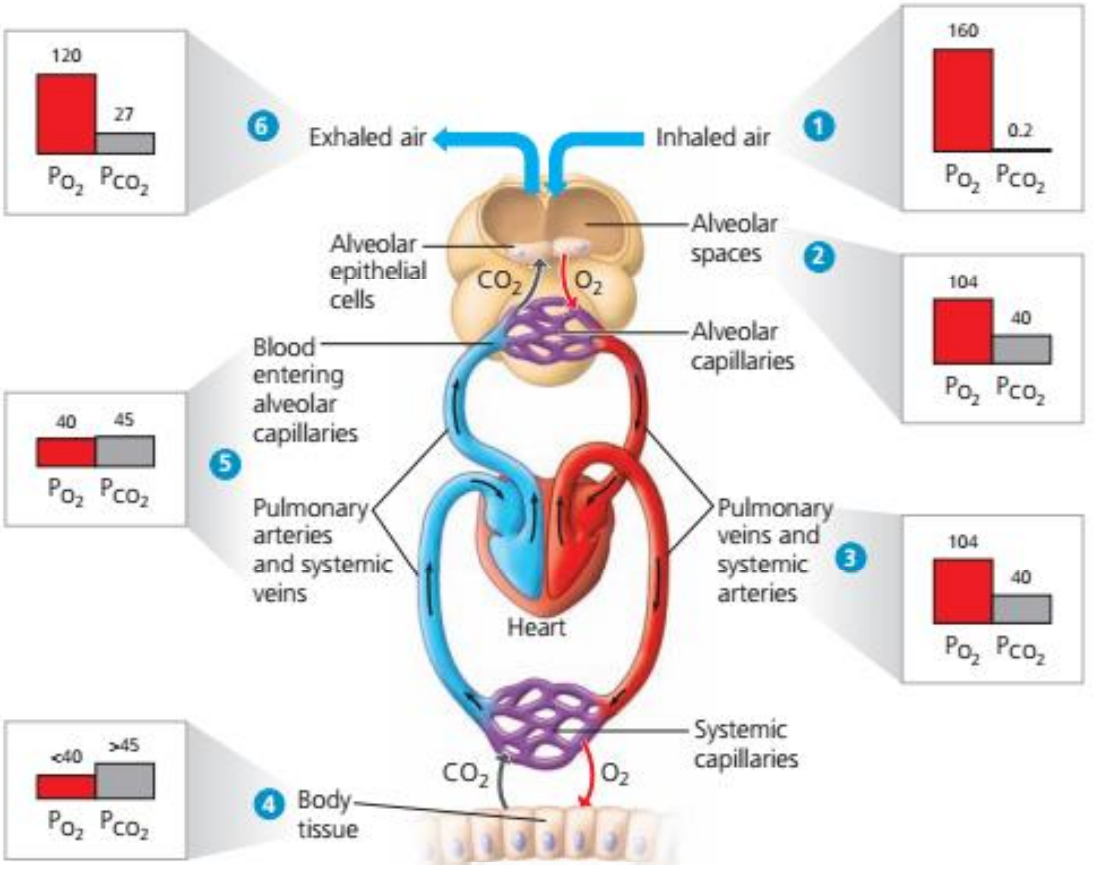


Fig 5.28: Loading and unloading of respiratory gases

Homeostatic control of Breathing

- Typically breathing is regulated by involuntary mechanisms. These involuntary mechanisms help coordinate gas exchange with blood circulation and metabolic demands
- Medulla oblongata is the main breathing regulating center found at the base brain. There are a pair of breathing control centers found in medulla and they are responsible for regulating the breathing rhythm.
- A negative-feedback mechanism is involved in regulating this process. Sensors which detect stretching of the lung tissues are found in the lungs. During inhalation, these sensors send nerve impulses to the neurons that act as control circuits in the medulla and further inhalation is inhibited and this prevents the lungs from over expanding.
- To regulate breathing, the medulla depends on pH changes in tissue fluids. The pH of tissue fluid is an indicator of blood carbon dioxide concentration. For

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example, when metabolic activities increase, the concentration of CO_2 in the blood increases. Because CO_2 diffuse into the cerebrospinal fluid, this results in an increase of CO_2 concentration in the cerebrospinal fluid as well. There CO_2 reacts with water and form carbonic acid (H_2CO_3). H_2CO_3 dissociate into HCO_3^- and H^+

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

- Hence, a high CO_2 concentration results in an increase in H+ concentration, thereby a lower pH.
- This pH change is detected by the sensors in the medulla and in major blood vessels called arteries and aorta.
- Sensors in the medulla and major blood vessels detect this decrease in pH. In response, the control circuits in medulla increase the depth and rate of breathing until the excess CO_2 is removed in exhaled air and the pH of blood comes to its normal value which is 7.4
- The O_2 level has little influences on the breathing control centers. But, when O_2 concentration becomes very low, O_2 sensors found in the aorta and the carotid arteries send impulses to the medulla to increase the breathing rate.
- The regulation of breathing is also modulated by additional neural circuits in the pons, a part of the brain stem found above the medulla.

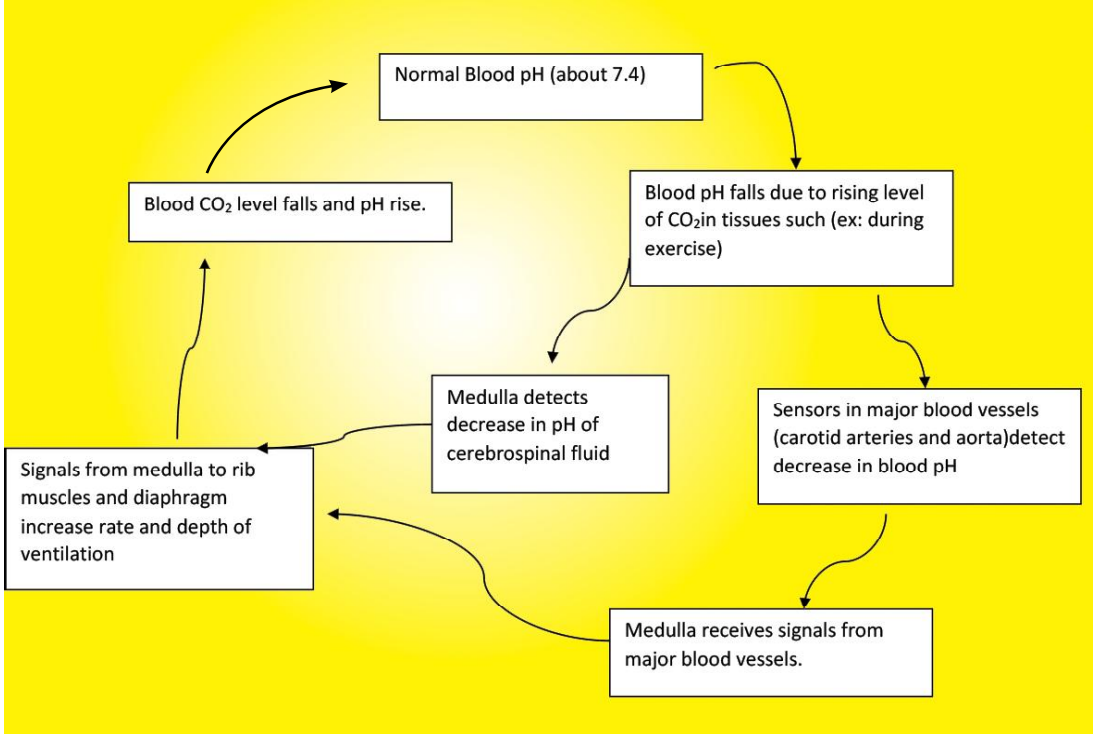


Fig5.29: Homeostatic control of breathing

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Disorders of the respiratory system

The effect of smoking on the smooth functioning of the respiratory system

- Cigarette smoke harms nearly every organ in the body including the organs of the respiratory system and increase the risk of illness, disability and death. Smokers inhale large number of chemicals which mainly come from burning tobacco. Some of these inhaled compounds are chemically active and can trigger damaging changes in the body.
- Nicotine is among the compounds inhaled in tobacco smoke which is the addictive drug in the smoke. It temporarily increases the rate of heart beat and constriction of peripheral blood vessels causing a temporary increase in blood pressure.
- Cigarette smoke stimulates the secretion of mucus by the goblet cells and inhibits the action of cilia in the respiratory tract causing accumulation of mucus in bronchioles and blocking them, leading to bronchial inflammation or bronchitis. As a result, breathing may become difficult.
- Some chemicals such as hydrogen cyanide in cigarette smoke stops the cilia from working properly. Due to loss of action of cilia, dust and other particulate matter get collected in the lung, resulting in an increase in phagocytic cells in the lung tissue. Due to release of large amounts of lytic enzymes by these cells, the alveolar tissue is destroyed thus reducing the effective area available for gas exchange.
- Carbon monoxide (CO) present in tobacco smoke is absorbed into the blood and is able to bind to hemoglobin better than oxygen and combines irreversibly with hemoglobin. Thus it decreases the amount of oxyhaemoglobin produced. Therefore, oxygen transport through blood is decreased.
- Tobacco smoke also contains a large number of cancer-causing substances (carcinogens). Nearly 90% of lung cancers are due to smoking. Long term exposure to such chemicals in cigarette smoke results in the proliferation of cells in the bronchial epithelium, forming a mass of abnormal cells. A cancer may develop among these cells. If these cells break free, the cancer may spread to other parts of the lungs and or to other organs.
- Passive or second hand smoking will also result in the above mentioned ill effects.

Silicosis

This may be caused by long-term exposure to dust containing silica compounds. High risk industries are,

- Quarrying granite, slate, sandstone

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- Mining hard coal, gold, tin, copper
- Stone masonry and sand blasting
- Glass and pottery work

When silica particles are inhaled they accumulate in the alveoli. These particles are ingested by macrophages, some of which remain in the alveoli and come out in to the connective tissue around bronchioles and blood vessels close to the pleura. Progressive fibrosis is stimulated which eventually obliterates the blood vessels and respiratory bronchioles. Gradual destruction of lung tissue eventually leads to pulmonary hypertension and heart failure.

Asbestos related diseases - Asbestosis

Those who are involved in making or using products containing asbestos are at risk. This occurs when asbestos fibers are inhaled with dust. In spite of their large size the particles penetrate the level of respiratory bronchioles and alveoli. Macrophages accumulate in the alveoli and the shorter asbestos fibers are ingested. The larger fibers are surrounded by macrophages, protein materials and iron deposits. The macrophages that have engulfed fibers move out of the alveoli and accumulate around respiratory bronchioles and blood vessels, stimulating the formation of fibrous tissue. These cause progressive destruction of lung tissue and pulmonary hypertension.

Lung cancer

Nearly 90% of lung cancer is due to smoking. When one smokes, the nasal hairs, mucus and cilia in the respiratory tract that otherwise is sufficient to protect the lung from chemical and biological irritants, are overwhelmed and eventually stop functioning. As a result, irritants, free radicals, carcinogens and pathogens accumulate in the lungs. Eventually these cause lung cancer.

Tuberculosis (TB)

Tuberculosis is an infectious disease caused by the bacterium *Mycobacterium tuberculosis.* The bacterium spreads when an infected person coughs and the bacterium enters the body of an uninfected person through inhaled air. The most common form is Pulmonary TB which affects the lungs. Other organs may also be affected. Transmission of pulmonary TB is by inhaling the bacterium into the lungs. This bacterium can survive in the air and in the house dust for long periods. Malnutrition and other infectious can reduce resistance to the disease.

Symptoms

Loss of appetite, loss of weight, excessive sweating, fever, a racking cough and spitting up blood.

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Asthma

Asthma is characterized by wheezing and chest tightness causing breathing difficulty. This is caused by the sudden contractions of smooth muscles in the walls of the bronchioles which causes the bronchioles to narrow or even close. During this time breathing causes whistling or wheezing sound. The cause of asthma is an over reaction of an immune response to stimuli like pollen, dust, mites, spores, particular food, cold air, exercise, smoking gases. Anti-inflammatory drugs help control.

Respiratory cycle and lung volumes and capacities

Inhalation and exhalation during a single breath is referred to as a respiratory cycle. The amount of air that flows in and out of the lungs depends on the conditions of inspiration and expiration. Thus, four respiratory volumes are described.

- **Tidal volume (TV)**: This is the volume of air inhaled and exhaled with each breath during normal breathing. On average it is about 500 ml in a resting adult human.
- **Inspiratory reserve volume (IRV):** This is the extra volume of air that can be forcibly inhaled beyond the tidal volume.
- **Expiratory reserve volume (ERV):** The extra volume of air which can be expelled from the lungs after a tidal expiration.
- **Residual volume (RV):** The volume of air that remains in the lungs even after forceful expiration. This is on average is about 1,200 ml.

Specific combinations of respiratory volumes are called respiratory capacities. Thus, respiratory capacities always consist of two or more lung volumes. The respiratory capacities are important to determine the respiratory status of a person.

- **Inspiratory capacity (IC):** The total volume of air that can be inspired after a tidal expiration.

  Thus, IC = TV + IRV
- **Functional residual capacity (FRC):** The volume of air remaining in the lungs at the end of a tidal expiration.

  Thus, FRC = RV + ERV
- The functional residual capacity is important for continuous exchange of gas in the alveoli and to prevent the collapse of the alveoli during expiration.
- **Vital capacity (VC):** The maximum volume of air which can be inhaled and exhaled. It is normally around 3100 mL in women and 4800 mL in men.

  VC = TV + IRV + ERV

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- **Total lung capacity (TLC):** The maximum volume of air the lungs can hold or the sum of all lung volumes. This is normally around 6000 mL.

In addition, some of the inspired air fills the system of branching conducting tubes (trachea, bronchi and bronchioles) and never contributes to the gas exchange in the alveoli. This volume is referred to as the anatomical dead space and it is typically about 150 mL.

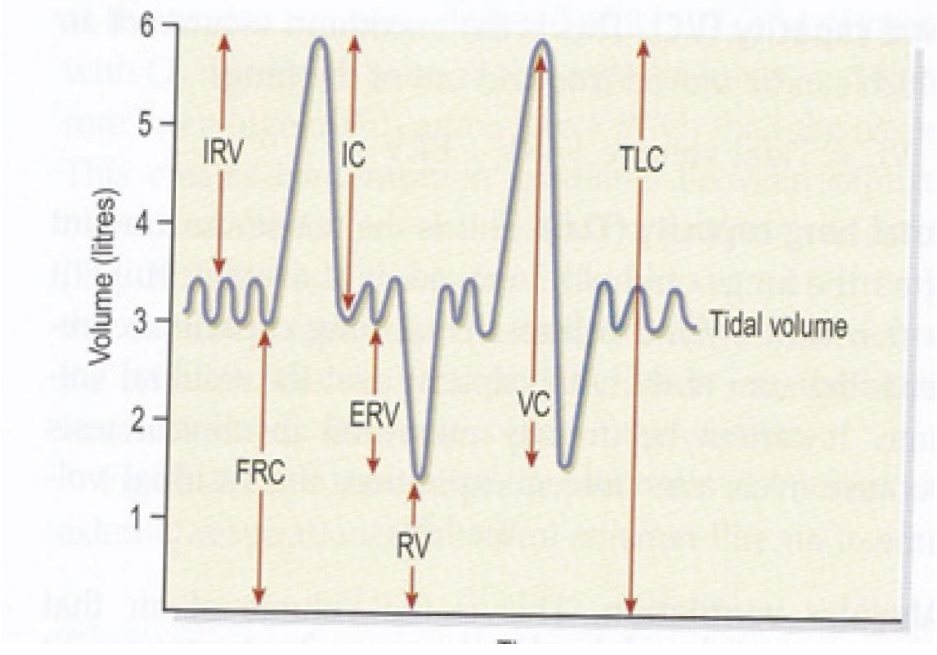


Fig 5.30: Lung volumes and capacities

IRV: Inspiratory reserve volume

IC: Inspiratory capacity

FRC: Functional residual capacity

ERV: Expiratory reserve volume

RV: residual volume

VC: Vital capacity

TLC: Total lung capacity

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Immunity

The state of being resistance to injury, invading pathogens and foreign substances through defensive mechanisms in the body is known as immunity. Pathogens which are the agents that can cause infectious diseases include some bacteria, virus, and fungi. The substances that can be recognized as foreign to the body include chemical components of pollen grains, incompatible blood cells and transplanted tissues. Defenses in the animal body against these foreign invasions make up the immune system. Immune system of animals should be able to distinguish own body cells (self) from foreign cells/particles within the body (non-self) for initiating defensive immune responses for destruction of the foreign agents such as pathogens. Special cell types in the body fluid and tissues of most animals can interact with these foreign invasions and destroy them. Immune cells produce receptor molecules that bind specifically to molecules from foreign cells and agents and activate defense responses.

Immune responses in animals can be divided into two types:

- Innate immunity
- Acquired immunity (Adaptive immunity)

Innate immunity

Innate immunity is the ability to resist damage or diseases in the body through inherent body defenses which offer rapid responses against a broad range of pathogens and foreign substances. In innate immunity, recognition and defense responses will depend on characters common to groups of pathogens. Innate immunity lacks specific responses to specific invaders and its protective mechanisms functions the same way regardless of the type of the invader. Hence innate immunity is also known as non-specific defense. Innate immune responses include defense mechanisms that provide immediate but general protection against foreign invasion. Innate immunity is found in both invertebrates and vertebrates. Innate defense mechanisms can be broadly divided into two types: External barriers (External defenses/ barrier defense) and Internal nonspecific defenses (Internal defenses).

External defenses/ barrier defense in innate immunity

External barriers discourage pathogens and foreign substances from penetrating the body. So they are considered as the first line of defense. For innate immunity in the human body, external defenses/barriers are found in the skin, mucus membranes and secretions of various organs. They act as physical and chemical barriers.

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- Human **skin** with its many layers of closely packed, keratinized cell layers in the epidermis provides a significant physical barrier to entrance of microbes. In addition periodic shedding of epidermal cells helps remove microbes from the skin surface.
- The **mucous membranes** which line the body cavities provide a physical barrier to entrance of many microbes (e.g. the linings of the respiratory tract, digestive tract, urinary tract and reproductive tract). The mucous membranes produce mucus which traps microbes and other particles. In the respiratory tract, ciliated epithelial cells sweep mucus and any entrapped material upward. Coughing and sneezing speed up the mucus movement and its entrapped pathogens out of the body preventing their entry to the lungs.
- **Secretions** by various organs (e.g. tears, saliva, mucus) help as physical and chemical barriers to protect epithelial surface of the skin and mucous membranes. Tears in the eyes provide protection against irritants and microbes. Tears in the eyes provide continuous washing action that helps to dilute microbes and prevent settling on the surface of eyes. Saliva washes microbes from the mouth surface and the flow of saliva reduces the colonization of microbes in the mouth. Mucus secretions which bathe various exposed epithelia provide a continual washing action to dilute and inhibit colonization microbes such as bacteria and fungi. Lysozyme (an enzyme) present in tears, saliva, perspiration and mucous secretions can destroy cell walls of some bacteria. Gastric juice which provides an acidic environment in the stomach can destroy many bacteria and bacterial toxins ingested with food. Secretions of the sweat and sebaceous glands of the skin give acidity of the skin which helps to prevent growth of bacteria.

Internal defenses in Innate immunity

When the pathogens penetrate the external defensive barriers in the skin and mucus membranes in the human body, they encounter a second line of innate immunity responses called internal defenses. Within the body, detection of non-self is accomplished by molecular recognition in which receptor molecules on specific cells in the immune system will bind specifically to molecules of foreign agents such as pathogens.

In innate immunity, internal defenses consist of Phagocytic cells, Natural killer cells, Antimicrobial proteins and Inflammatory responses.

- **Phagocytic cells:** These are specialized cells that can ingest microbes, foreign particles and cell debris for intra cellular digestion and destruction. Phagocytes use the receptor molecules to detect components of foreign agents and particles. Neutrophils and Macrophages are the two main types of phagocytic cells in man. While circulating in the blood, neutrophils are attracted first to the infected site

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by signals from affected tissues. Then neutrophils can ingest and destroy infected pathogens. Macrophages are larger and more potent phagocytic cells.

- **Natural killer cells:** These are a type of lymphocytes present in the blood and some tissue/organs such as spleen and lymph nodes which function in nonspecific defense. They can detect the cells with abnormal surface molecules (e.g. virus-infected body cells and some cancerous cells) and kill them. Natural killer cells do not engulf these abnormal cells but upon binding they can release chemicals to kill the virus-infected cells and cancerous cells which could inhibit further spread of the virus or cancer.
- Antimicrobial proteins: They are proteins present in the blood and interstitial fluids which function in innate defense by attacking microbes directly or impeding their reproduction. Interferons and Complement proteins are two such antimicrobial proteins that discourage microbial growth. **Interferons** are proteins secreted by virus-infected body cells that protect uninfected host cells from viral infections by interfering with the viral replication. Once released by virus-infected cells, interferons diffuse to uninfected neighboring cells where they are stimulated to produce "anti-viral proteins" which inhibit viral replication. Some interferons activate macrophages which enhance the phagocytic activity. Complement proteins are a group of normally inactive proteins in the blood plasma and plasma membranes. When they are activated by different substances present on the surfaces of microbes, a cascade of biochemical reaction occurs which lead to lysis of invaded cells. They also promote phagocytosis and inflammatory response.

- **Inflammatory response:** This is an innate immune defense response in the body to tissue damage triggered by microbial infections or injury of the tissues. This involves the release of substances that promote increased permeability and dilation of blood vessels, enhance migration of phagocytes, destruction of invading pathogens and aid in tissue repair (Figure 5.31). Inflammation attempts to destroy the microbes at the site of the injury preventing the spread to other tissues and promote tissue repair.

Inflammatory response is brought about by various signaling molecules upon infection or injury. Histamine is one of the important inflammatory signaling molecules released mainly by mast cells in the connective tissues at the site of damage. Histamine causes increased permeability and dilation of nearby blood vessels (blood capillaries). Increased permeability of the blood vessels enhance the infiltration of white blood cells, antimicrobial proteins and clotting elements to enter the injured area from the blood that aid in destruction of invading pathogens and tissue repair. Dilation of blood vessels allows more blood to flow through the damage area which helps to remove dead cells. Activated phagocytes (macrophages and neutrophils) moved from the

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blood to the damaged tissue area can also discharge signaling molecules (cytokines) which also promote blood flow to the injured or infected site. During inflammation, activated complement proteins can cause further histamine release which attracts more phagocyte cells to enter injured tissue and carry out additional phagocytosis. This process can digest the microbes and cell debris at the site of injury.

Signs and symptoms of inflammation are redness, heat, swelling and pain. Dilation of blood vessels causes redness and heat production due to high metabolism in the area. Increased permeability of blood vessels triggers localized swelling due to leaking of tissue fluid into neighboring tissues. Pain results from injury to neurons and microbial toxins. As a result of most inflammatory responses, pus may be accumulated. It is a fluid rich in dead phagocytes, dead pathogens and cell debris from the damaged tissue. Minor injury or infection causes a localized inflammatory response. If the injury or infection is severe it may lead to a systemic response (throughout the body) leading to fever. Elevated body temperature within limits may enhance the phagocytosis and accelerate tissue repair by speeding up the chemical reactions.

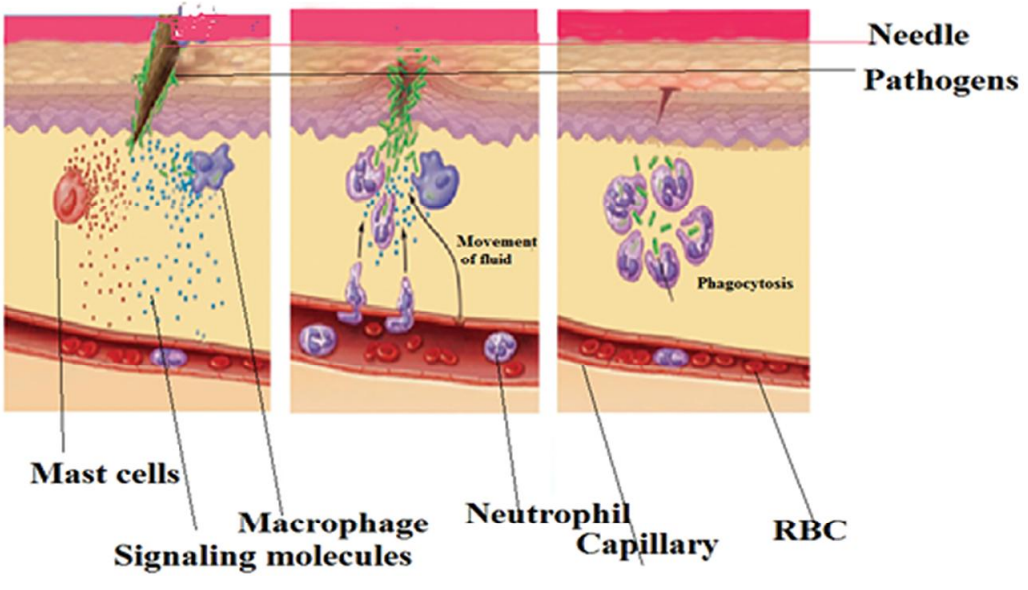


Fig 5.31: Major events in inflammatory response

Acquired Immunity (Adaptive Immunity)

Acquired immunity is the ability of the body to defend itself against invading foreign agents (e.g. pathogens) through specific defense responses mediated by diverse **T lymphocytes and B lymphocytes**. Acquired immunity shows (i) specificity for particular foreign molecules (ii) recognition of animals own molecules (self-molecules) from non-self-molecules and (iii) memory for most previously encountered pathogens such that the subsequent encounter causes a stronger and more rapid response (immunological memory). In the animal kingdom, acquired immunity is found only in the vertebrates.

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The cells that have developed the ability to carry out acquired immune responses if they are activated against foreign agents are called T **lymphocytes** and **B** lymphocytes. In man, both types of lymphocytes are originated from stem cells in the bone marrow. Some of the lymphocytes that migrate to thymus for maturation are called T lymphocytes (T cells). The lymphocytes that remain in the bone marrow for completion of development are called B **lymphocytes** (B cells). Before leaving these lymphocytes to the secondary lymphatic tissues, their plasma membranes acquire diverse specific protein receptors (antigen receptors) which have the ability to recognize specific foreign invasions (There can be over 100,000 antigen receptors on the surface of a single B lymphocyte or T lymphocyte)

An **antigen** is a substance that has the ability to stimulate an immune response through T lymphocytes and B lymphocytes and to react with the specific cells or antibodies that resulted from the stimulated immune response. Viral proteins, bacterial toxins and chemical components of bacterial structures such as flagella and cell walls can be antigenic. Structural components of incompatible blood cells, transplanted tissues can also be antigenic. Antigens are usually large foreign molecules such as proteins and polysaccharides. In general not the entire antigen, but certain parts of a large antigen molecule act as the triggers for the acquired immune responses. The small accessible portion of the antigen that binds to a specific antigen receptor of a T lymphocyte or B lymphocyte is called an epitope (for exp a group of amino acids in a large protein). Can serve as an epitofo. Usually a single antigen has several epitopes (Figure 5.32) each can bind with a specific antigenic receptor of the single T or B lymphocyte.

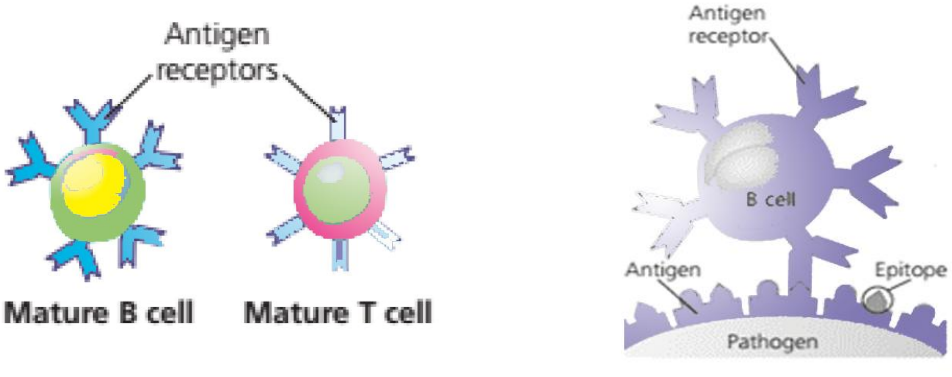


Figure 5.32: (a). Mature T lymphocyte and mature B lymphocyte with antigen receptors on the plasma membrane, (b). B cell receptor binds with an Epitope on antigen

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In acquired immunity, two types of immune responses are mediated by T lymphocytes and B lymphocytes. They are Cell mediated immune responses and Humoral immune responses. Humoral immune response is also called as Antibody mediated immune response. Both immune responses are triggered by antigens. A given pathogen may provoke both types of immune responses.

Cell mediated immune response

Cell mediated immune response is a type of acquired immunity in which specifically sensitized T lymphocytes attach to the antigen undergo proliferation and eventually differentiate into "Cytotoxic T cells" that can directly kill the cells with the invading antigen . In addition "Memory T cells" are formed that can cause stronger and more rapid response at the subsequent encounter of the same antigen to the body. This is particularly effective against infected cells (fungi, parasites and virus that are present within host cells), some cancer cells and foreign transplanted cells. Cell mediated immunity always involves cells attacking cells.

Humoral immune response

Humoral immune response is a type of acquired immunity in which specifically sensitized B lymphocytes attach to a particular antigen undergo proliferation and eventually differentiate into "Plasma cells" that secrete circulating antibodies that can neutralize and inactivate the specific toxins and pathogens in the blood and lymph. In addition "Memory B cells" are formed that can cause stronger and more rapid response at subsequent encounter of the same antigen. Humoral immune response works mainly against antigens present in body fluids and extracellular pathogens (mainly bacteria) that multiply in the body fluids.

Antibodies

Antibodies are proteins secreted by plasma cells (differentiated B lymphocytes) in response to specific antigens; the antibody binds with that antigen to neutralize, inhibit or destroy it. Antibodies can neutralize and inactivate the specific toxins and pathogens in the body fluids. The antibodies do not directly kill the pathogens but can interfere with activity of the pathogen or mark the pathogen for inactivation and destruction. Antibody-antigen complexes can activate complement system and phagocytosis to destroy the pathogen. Antibodies are also called as immunoglobulins. Immunoglobulin has the same Y shaped structure as B lymphocyte antigen receptors but are secreted than membrane bound.

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Role of T lymphocytes and B lymphocytes in acquired immunity

Recognition of the antigen, binding to the antigen and sensitization:

For an acquired immune response to occur, some T lymphocytes or B lymphocytes must first recognize that a foreign antigen is present in the body. Even though there are vast variety of antigen receptors present on different B lymphocytes and T lymphocytes, only a very small fraction are specific for a particular epitope. Hence, antigen should be presented to the B lymphocytes and T lymphocytes until a match is made. Recognition of the antigen occurs through successful match between an epitope of the antigen and an antigen receptor on small number of B lymphocytes or T lymphocytes. As specific antigen receptors produced by a single T cell or B cell can be identical they can bind to the same epitope. Hence, both T and B cells can respond to any pathogen that produces molecules containing that same epitope. But B cells and T cells encounter antigens in different ways. T lymphocytes only recognize the fragments of antigenic proteins that are presented to the cells by a special cells called "antigen presenting cells" (macrophages, dendritic cells and B cells). However, B lymphocytes can recognize and bind to the antigens present in blood plasma, lymph and interstitial fluid. The binding of an antigen to the specific antigen receptor results in sensitization (activation) of a specific T lymphocyte or B lymphocyte which initiates cell mediated and antibody mediated immune response as described below.

Proliferation and differentiation into Effector cells: Once activated the T lymphocyte or B lymphocyte undergoes multiple cell divisions (proliferations) resulting a clone, a population of cells that are identical to the original lymphocyte. Some cells of clones become Effectors cells which are short lived cells that take effect immediately against antigen to provide primary immune responses.

Elimination of invaders: The effector forms of T lymphocyte are "Cytotoxic T cells" and "Helper T cells". Cytotoxic T cells use toxic proteins to kill the cells infected with the pathogen. Signals from Helper T cells activate cytotoxic T cells to kill the infected cells. Signals from Helper T cells can also activate B lymphocytes to initiate antibody production. Effector forms of B lymphocytes are "Plasma cells". A single activated B lymphocyte can form thousands of identical Plasma cells. The plasma cells begin producing and secreting a soluble form of the B lymphocyte antigen receptor (antibodies) in large quantities which are released to the blood and lymph. Hence, circulating antibodies can neutralize and inactivate the specific toxins and pathogens in the body fluids.

Provide immunological memory: Following differentiation into Effector T cells (Cytotoxic T cells and Helper T cells), other T lymphocytes in the clones remain as "Memory T cells" which are long lived that can give rise to Effector T cells if the same

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antigen is encountered later in the life. Similarly the remaining B lymphocytes in the clones are "Memory B cells" which are long lived that can give rise to Plasma cells if the same antigen is encountered later in the life. These Memory T cells and Memory B cells can cause stronger and more rapid response at subsequent encounter of the same antigen to the body. This immunological memory is called secondary immune responses.

Active immunity

Active immunity is a long lasting immunity mediated by the action of B lymphocytes and T lymphocytes in the body and the resulting B and T memory cells specific for a pathogen. Active immunity can be developed as a result of natural infection of a pathogen or artificial immunization.

Naturally acquired active immunity

Long lasting immunity developed in the body against various infectious diseases in response to natural infections of pathogens is called naturally acquired active immunity. In response to a disease causing agent entering the body naturally for the first time (e.g. Virus of Chickenpox), some T lymphocytes and B lymphocytes in the body become activated and eventually produce specific cytotoxic T cells and antibodies to destroy the pathogen. Memory B cells and T cells produced in this process are long lived that will provide a stronger and rapid immune responses to destroy the particular antigen if the same antigen (e.g. Virus of Chickenpox) is encountered later in the life. In this way the body can resist to subsequent infections of the same antigen.

Artificially acquired active immunity

Long lasting immunity induced artificially in the body against various infectious diseases through vaccination (immunization) of attenuated (virulence-reduced) pathogens is called artificially acquired active immunity. Immunization can be carried out with preparations of antigens (vaccines) from many sources such as killed or weakened pathogens, inactivated bacterial cells or genes encoding microbial proteins. These vaccines act as the antigens and stimulate cell mediated and antibody mediated immune responses leading to production of long lived memory B and T cells to destroy the antigen. If the pathogen from which the antigen was derived, is encountered naturally later in the life, long lived memory cells can provide a stronger and rapid immune responses to destroy the particular pathogen. In general, the antigens used in the vaccines are pretreated to be immunogenic but not pathogenic. For example, BCG vaccine which is used against tuberculosis disease in man, has been prepared from a strain of the attenuated live tuberculosis bacteria. Polio vaccine consists of live attenuated poliovirus strains. Polio vaccine produces antibodies in the blood against polio virus, and in the event of infection, this protects the individual by preventing the spread of poliovirus to the nervous system.

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Passive Immunity

Passive immunity is the short term immunity developed within the body due to the transfer of antibodies produced by another individual. Passive immunity provides immediate protection, but the body does not develop memory as passive immunity does not involve recipients' T cells and B cells. Passive immunity persists only until the transferred antibodies last (few weeks to few months). Therefore the recipient is at risk of being infected by the same pathogen later unless they acquire active immunity or vaccination. Passive immunity can be developed as a result of transferring antibodies to the recipient naturally or artificially.

Naturally acquired passive immunity

Short term antibody mediated immunity for some infectious diseases can be developed within the body of the fetus or nursing infant due to the natural transfer of antibodies produced by the mother. The immunity occurs due to the transfer of antibodies to the fetus blood from mother's blood across the placenta. Antibodies also can pass from mother to the nursing infant through the colostrum and the milk during breast feeding. The baby develops the resistance against some infectious diseases for a short time. In this way the infant may be protected from these diseases until its own immunity system is fully functional. This is known as naturally acquired passive immunity.

Artificially acquired passive immunity

Artificially acquired passive immunity is a temporarily induced defensive protection achieved by the transfer of antibodies artificially to the blood of the recipient from another source. These readymade antibodies can be administered as blood plasma or serum (human or animal), or as injections of pooled human immunoglobulin from immunized donors or as monoclonal antibodies. Passive transfer of antibodies is used to prevent some infectious diseases when infectious agents are suspected to have accidently entered the body (e.g. readymade human serum antibodies for hepatitis A virus). It is also used in the treatment of several types of acute infections (e.g. readymade human anti-tetanus immunoglobin for acute conditions of tetanus). Passive immunization is also used to treat poisoning from venomous snake bite (e.g. antivenin, serum prepared from horses that have been immunized against snake venom). Immunity derived from artificially acquired passive immunization lasts for few weeks to four months.

Allergies

Some persons are overly reactive to substances that are tolerated by most other people. Antigens that induce hypersensitive reactions in some persons are called allergens. Exaggerated responses of the body to certain antigens (allergens) are called allergies. Common allergens include pollens, dust, some food (e.g. shellfish), some antibiotics

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(e.g. penicillin), venom from honey bees and wasps. Whenever an allergic reaction takes place the tissue injury occurs. The most allergens stimulate production of plasma cells which secrete antibodies specific for the antigen. When the same allergen enter the body later, it become attach to the antibodies specific to the allergen which induce the mast cells to release histamine and other inflammatory chemicals. Acting on a variety of cell types these signals bring about typical allergy symptoms such as sneezing, runny nose, teary eyes and smooth muscle contractions in the airways of the lungs that can result in breathing difficulties. An acute allergic conditions sometimes lead to death of the person due to breathing difficulties and low blood pressure with a few seconds of exposure to an allergen.

Autoimmune diseases

In some persons, the immune system becomes active against particular self-molecules of the body and begins to attack the person's own tissues leading to an autoimmune disease. Possible causes for autoimmune diseases may be genetic factors, gender and unknown environmental triggers. Many autoimmune diseases affect females than males. A variety of mechanisms produce different autoimmune diseases. Some involve the production of autoantibodies that can affect normal functioning of certain body molecules. Some involve activation of Cytotoxic T cells that destroy certain body cells. Examples for autoimmune diseases include Type 1 Diabetes mellitus, Multiple sclerosis and Rheumatoid arthritis. In Type 1 Diabetes mellitus, T cells attack the insulin producing pancreatic beta cells. In Multiple sclerosis, T cells attack myelin sheaths around neurons. In Rheumatoid arthritis, painful inflammations of the cartilage and bones occur as the immune system mistakenly sends antibodies to the lining of the joints, where they attack the tissue surrounding the joints.

Immunodeficiency diseases

Immunodeficiency disease is a disorder in which responses of the immune system to antigens are defective or absent. An immunodeficiency can lead to frequent and recurrent infections and increased susceptibility to certain cancers. An inborn immunodeficiency results from a genetic or developmental defects in the production of immune system cells or specific proteins such as antibodies or proteins of the complement system. Acquired immunodeficiency can be developed later in life due to the exposure to chemicals or biological agents. Drugs used to fight autoimmune diseases or prevent transplant rejections suppress the immune system leading to an immunodeficiency state. The human immunodeficiency virus (HIV), the pathogen that cause Acquired Immunodeficiency Syndrome (AIDS) escapes and attacks the immune system of man. The HIV causes progressive destruction of immune responses in the person leading to frequent infections and increased susceptibility to certain cancers which can cause death.

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Osmoregulation and excretion

Osmoregulation is processes by which organisms control solute concentrations and water balance within the body. Simple unicellular organisms such as *Amoeba, Paramecium* etc. use contractile vacuoles for osmoregulation. But animals have developed different structures for osmoregulation. The chemical reactions that occur in organisms result in the formation of waste products, often toxic, which must be disposed in some way. The removal of the nitrogenous metabolite and other metabolic waste products from the body is called excretion. Defecation is not considered under excretion as it involves the removal of undigested food from the gut. In many animals excretory and osomoregulatory systems are linked structurally and functionally.

Importance and need of osmoregulation and excretion

For effective body functioning and survival animals have to maintain a constant internal environment specially the relative concentrations of water and solutes within favorable limits. Therefore animals need to regulate the chemical composition of body fluids by balancing uptake and loss of water and solutes. Animal cells will swell and burst if water uptake is excessive. On the other hand, animal cells will shrink and die if water loss is high. The driving force of loss of water in animals as in plants is the concentration gradient of solutes across the cell membrane. Animals have evolved different osmoregulatory strategies depending on the environment in which they live for their survival.

Animals have to get rid of toxic products produced during metabolism in order to safeguard the composition of their internal environment. Otherwise these excretory end products become toxic to the body cells. For example protein and nucleic acids are broken down within the body cell during metabolism and the amine group is converted to ammonia which is highly toxic. Ammonia also acts as a weak base. Oxidation of glucose during metabolism will release CO_2 which is a weak acid. Accumulation of such weak acids and bases will alter the acid base balance in the internal environment. Changes in acid base balance will lead to adverse effects such as denaturation of proteins. Therefore removal of excretory products from the body is essential to maintain the internal environment within favorable limits for effective body functioning and survival.

Relationship between metabolic substrates and excretory products

Metabolic substrates in the cells are carbohydrates, fats, proteins and nucleic acids. The excretory products of these substrates will vary depending on several factors such as the chemical structure and the composition, availability of enzymes, oxygen availability and the habitat in which they live.

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When carbohydrates are metabolized within the body cells when oxygen is available final excretory end products are CO_2 and water. If they are subjected to anaerobic respiration in general lactic acid is produced.

When fats are subjected to aerobic metabolism final excretory products are CO_2 and water. Since proteins contain amine groups in their structure, during metabolism of excess amino acids ammonia is produced. Since nucleic acids contain nitrogenous bases ammonia is produced as an excretory product during their metabolism. Depending on the habitat and the availability of enzymes ammonia will be further converted to other nitrogenous waste products such as urea and uric acid.

Relationship between the nitrogenous excretory products and living environment

Nitrogenous excretory products of animals are ammonia, uric acid and urea. These different forms vary significantly in their toxicity and the energy costs of producing them.

Since ammonia is highly toxic, a large volume of water is needed to excrete ammonia. Therefore typically the organisms that live in water such as bony fishes, many aquatic invertebrates and aquatic amphibians specially tadpoles excrete ammonia since they have ready access to water. Energy cost for producing ammonia for excretion is comparatively low.

Terrestrial animals do not have access to sufficient water to excrete ammonia as the main excretory product. Instead most terrestrial animals such as mammals and adult amphibians mainly excrete urea as the main nitrogenous excretory product. Urea is less toxic. However animals must expend more energy to produce urea from ammonia. Some marine fishes such as sharks also excrete urea (which they use for osmoregulation) as the main nitrogenous waste.

Some terrestrial animals such as birds, many reptiles, land snails and insects excrete uric acid as the main excretory product. Uric acid is relatively non-toxic and generally insoluble in water Therefore it is excreted as a semisolid with trace amount of water. However uric acid production from ammonia requires more energy than urea production

.

The diversity of excretory structures of animals (Fine structures are not necessary)

Body Surface - The cells of some animals which are in direct contact with the environment and eliminate excretory products by diffusion. e.g. cnidarians

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Flame cells- These are specialized excretory cells connected to a network of tubule which opens to the outside of the animal. E.g. flatworms.

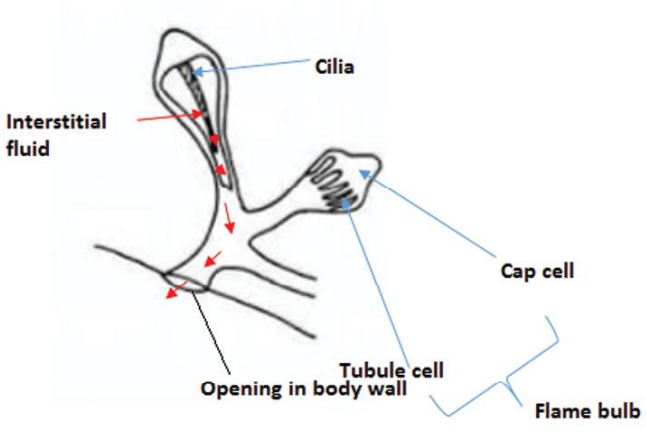


Fig 5.33: Structure of the flame cell

Nephridia – They are multi cellular, tubular structures. One end of the tubule is open to the coelom while the other end opens to the outside. e.g. Annelids

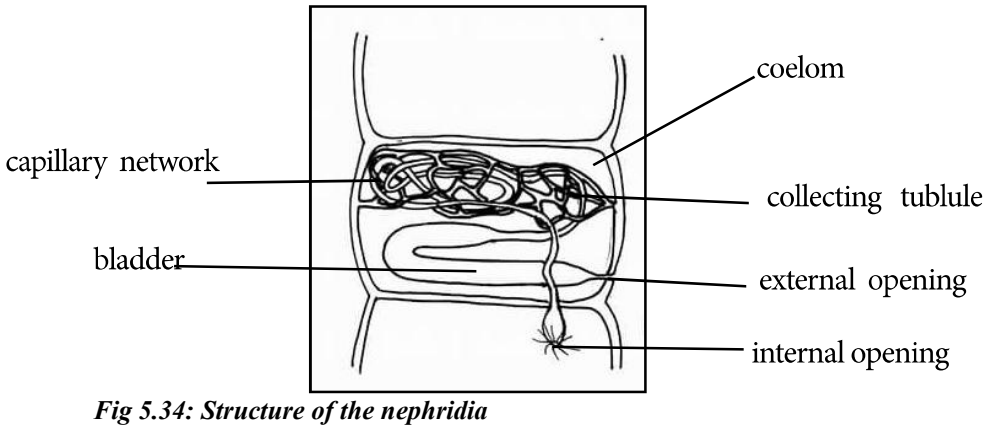


Fig 5.34: Structure of the nephridia

Malpighian tubules – These are extensive blind end tubules immersed in hemolymph and opens in to the digestive tract. e.g.: Insects and other terrestrial arthropods

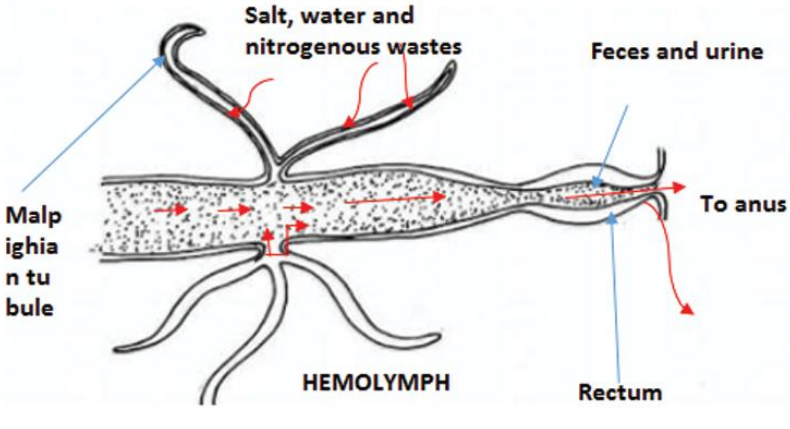


Fig 5.35: Malphigian tubules

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Green glands / Antennal glands –Two large glands found ventrally in the head and anterior to the oesophagus. e.g. Crustaceans

Sweat glands –There are coiled tubular glands situated in the dermis and connected to a sweat duct which open as a pore on the surface of the skin. E.g. human skin.

Salt glands –They are paired glands found near the eyes to excrete excess salts. e.g. Marine birds and marine reptiles.

Kidney – These are the major excretory and osmoregulatory organs of all vertebrates.

Human Urinary System

Human urinary system consists of two kidneys, two ureters, urinary bladder and urethra. Their main functions are given in the following table;

Part	Main Function
Kidney	Produce urine to excrete waste products while maintaining osmotic balance and acid base balance.
Ureter	Receives urine from kidney and send it to bladder
Urinary bladder	Temporary storage of the urine
Urethra	Provide the passage through which urine stored in the bladder leaves the body

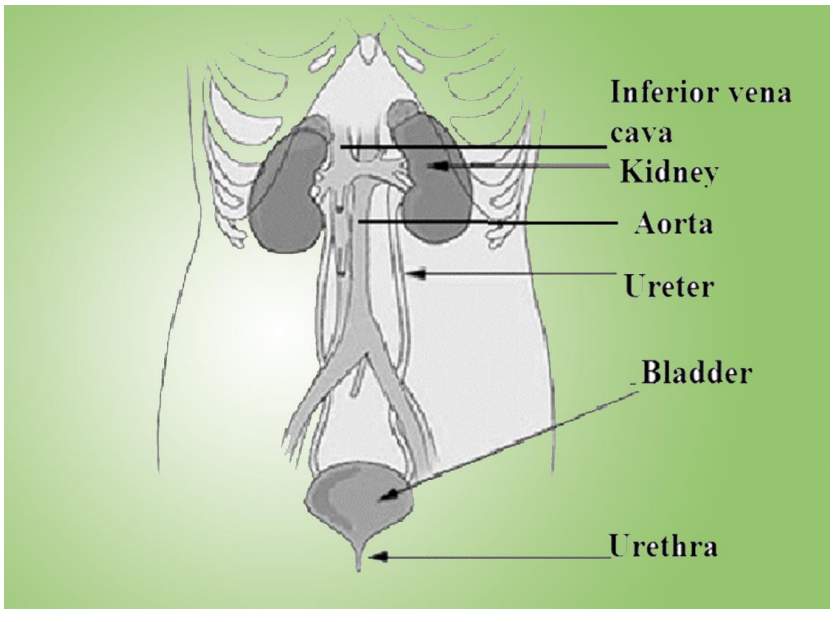


Fig 5.36: The parts of the human urinary system

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Location of kidneys

Two kidneys are located on the posterior abdominal wall one on either side of the vertebral column, behind the peritoneum and below the diaphragm. Right kidney is slightly lower than the left.

Blood supply

The kidneys receive blood from aorta via the renal arteries and renal veins return blood to the inferior vena cava.

Gross structure of the kidney

Kidney is a bean shaped organ which is held in position by a mass of fat and both are surrounded by a fibrous connective tissue. In the longitudinal section of the kidney three areas of tissues can be seen to the naked eye. They are outer fibrous capsule, renal cortex and inner renal medulla. Cortex and medulla are supplied with blood vessels and tightly packed with excretory tubules. Renal Cortex is granulated due to the presence of glomeruli. Medulla is composed of renal pyramids, which have striated appearance. Apices of pyramids project in to the renal pelvis through renal papillae. Renal pelvis leads into the ureter. Renal artery and renal vein pass through the pelvis.

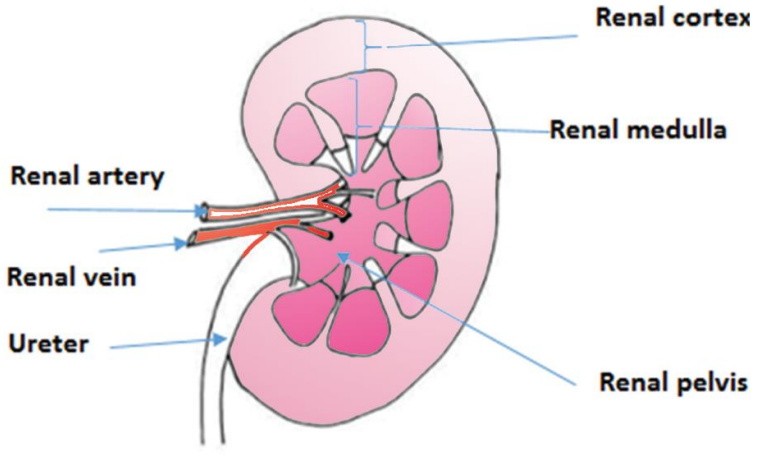


Fig 5.37: Longitudinal section of human Kidney

Structure of Nephron

Nephron is the structural and functional unit of the kidney. There are over millions of nephrons in each kidney. There are two types of nephrons; they are cortical nephrons (reach short distance to the medulla) and juxta medullary nephrons (extend deep into the medulla).

Majority of the nephrons are cortical nephrons.

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A nephron consists of a single long tubule and a ball of capillaries called the glomerulus. The tubule is closed at one end forming Bowman's capsule which surrounds the glomerulus. The other end of the tubule joins with the collecting duct.

Tubule consists of;

- Bowman's capsule
- Proximal convoluted tubule
- Loop of Henle
- Distal convoluted tubule

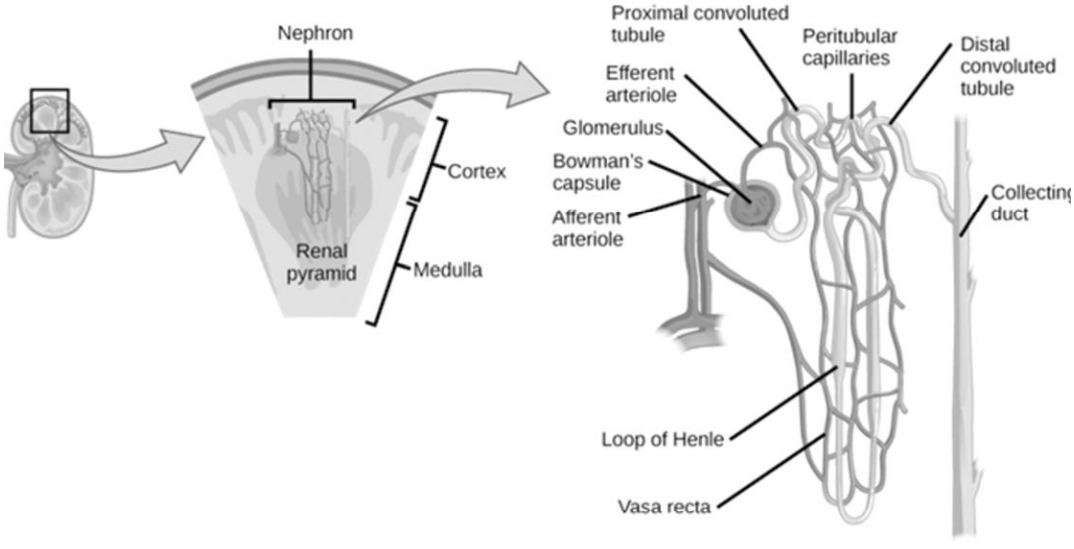


Fig 5.38: A nephron and associated blood vessels

Bowman's capsule (Glomerular capsule)

This is expanded and closed end of the tubular structure of the nephron. It is a cup shaped and double walled structure. Inner layer of Bowman's capsule consists of a single layer of flattened epithelial cells specialized for filtration. Outer layer of the Bowman's capsule composed of simple squamous epithelium. Space between the inner and outer layers is known as capsular space which is responsible for receiving of glomerular filtrate. The glomerular filtrate pass through three major tubular regions of the nephron: proximal convoluted tubule, loop of Henle and distal convoluted tubule.

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Glomerulus

The glomerulus is a ball of capillaries which is surrounded by Bowman's capsule. The nephron is supplied with blood from the afferent arteriole. The blood vessel leaving away from the glomerulus is the efferent arteriole. The efferent arteriole has a smaller diameter than the afferent arteriole. This modification is important for increasing blood pressure in the glomerulus for ultrafiltration.

The efferent arteriole form two capillary networks, one form the peritubular capillaries which surrounds the proximal and distal convoluted tubules and the other network form the vasa recta which extend towards the medulla surrounding the loop of Henle.

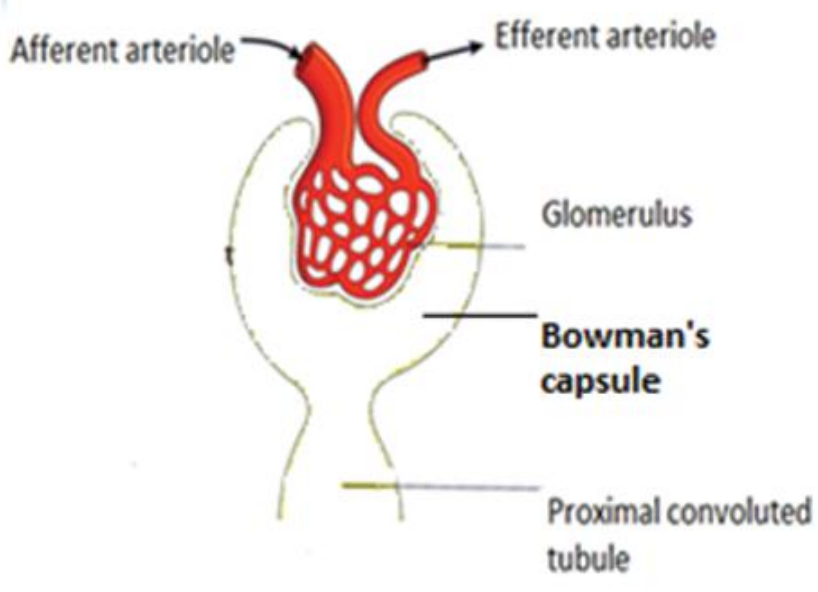


Fig 5.39 The Glomerulus and the Bowman's capsule

Proximal convoluted tubule

It is comparatively longer and wider than the distal convoluted tubule. This is lined by simple epithelium which has been specialized for selective reabsorption of substances(nutrients, ions and water) from the glomerular filtrate

Loop of Henle

It is a 'U' shaped part of the nephron with descending limb and ascending limb which are lined by simple epithelium.

Lining of the descending limb of loop of Henle is specialized for water reabsorption as it allows free movement of water. But lining of the ascending limb of loop of Henle is impermeable to water.

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Distal convoluted tubule

It is lined by simple epitheliam which has been specialized for selective reabsorption of specific ions and water. It leads into collecting duct.

Main steps in urine formation

There are 3 processes involved in urine formation. They are;

- ultrafiltration
- selective reabsorption
- secretion

Ultra filtration

- Filtration of the blood under high pressure into the cavity of the Bowmans' capsule is called ultrafiltration.
- Filtration occurs through the capillary walls of glomerulus and inner wall of Bowman's capsule.
- Blood capillaries of glomerulus are porous and cells lining the Bowmans' capsule are specialized for filtration of small size molecules and ions. These specializations allow passage of water and small solutes through the blood capillary walls into the Bowman's capsule. But due to their large size, blood cells, platelets and large molecules such as plasma proteins do not pass into the Bowmans' capsule.
- The filtrate in the Bowmans' capsule contains salts, amino acids, glucose, vitamins, nitrogenous wastes and other small molecules. The composition of the glomerular filtrate is similar in composition to plasma with exceptions of blood cells, platelets and plasma proteins.

Selective reabsorption

- The process through which useful molecules, ions and water from the glomerular filtrate are recovered and returned to the interstitial fluid and then into capillary network of the tubules is called selective reabsorption.
- Most of the reabsorption from the glomerular filtrate back into the blood takes place in the convoluted tubule. Ions, water and valuable nutrients are reabsorbed either active or passive transport from initial filtrate.

Secretion

- The process by which foreign materials and substances not required to the body including waste are cleared from the peritubular capillaries and interstitial fluid into the filtrate is called secretion

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- Secretion is required because such substances may not be entirely filtered due to the short time they remain in the glomerulus.
- Substances that are secreted in to the filtrate include H^+, NH_3, creatinine, drugs (e.g. penicillin, aspirin) and excess K^+. Tubular secretion of H^+ and NH_3 are important to maintain the normal pH in the blood by formation of NH_4^+ in the urine. NH_3 can combine with H^+ to form NH_4^+
- Secretion occurs in the proximal and distal convoluted tubules. Secretion may be either active or passive depending on the location and / or the substance.

Process of urine formation

Glomerular filtrate in the Bowmans' capsule which contains all the substances in the blood except blood cells, platelets and large molecules pass to the proximal convoluted tubule. At this region selective reabsorption of ions, water and valuable nutrients from the initial filtrate occurs. Nutrients especially glucose and amino acids are actively transported to the interstitial fluid. Cells lining the tubule actively transport Na^+ into the interstitial fluid and this transfer a positive charge out of the tubule drives the passive transport of Cl^-. Proximal tubule also reabsorbs K^+ and most of the HCO_3^- by passive transport. Reabsorption of HCO_3^- in the filtrate contributes to the pH balance in body fluids. As solutes move from the filtrate to interstitial fluid water is reabsorbed passively by osmosis. A major portion of water reabsorption from the filtrate occurs at this site. As the filtrate pass through the proximal convoluted tubule, secretion of specific substances into the filtrate takes place. Cells lining the tubule secrete H^+ (by active transport) and ammonia (by passive transport) into the lumen of the tubule. Secreted ammonia act as a buffer to trap H^+ forming NH_4^+. In addition some materials such as drugs and toxins that have been metabolized in the liver are actively secreted into the lumen of the proximal convoluted tubule. As a result of water reabsorption and secretion of different substances, the filtrate becomes more concentrated as it passes through the proximal convoluted tubule.

As the filtrate moves into the descending limb of loop of Henle passive reabsorption of water through osmosis continues and filtrate becomes more concentrated. The filtrate reaches the ascending limb of the loop of Henle via the tip of the loop. Ascending limb is impermeable to water so that no water absorption takes place but a considerable amount of NaCl reabsorption occurs at this site. Most of the Na^+ is transported into the interstitial fluid by active transport. As a result of losing NaCl but not water the filtrate become more diluted as it moves towards the distal convoluted tubule.

The distal convoluted tubule plays an important role in regulating K^+ and NaCl concentration of body fluids. The amount of K^+ secreted (by active transport) into the filtrate and the amount of NaCl (by active transport) reabsorbed from the filtrate can be varied at this site according to the needs of the body. Distal tubule also contributes

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to pH regulation by controlled secretion of H^+ and reabsorption of HCO_3-. At the distal convoluted tubule passive water reabsorption can be increased under the influence of Antidiuretic hormone (ADH) to form concentrated urine. Aldesterone secreted by the adrenal gland stimulates increase reabsorption of Na^+ and water and excretion of K^+ . This filtrate of the distal convoluted tubule finally leads to the collecting duct.

As the filtrate pass along the collecting duct filtrate become concentrated and urine is formed. At this site water reabsorption can also be increased under the influence of ADH and urine become more concentrated. Aldesterone hormone stimulates active reabsorption of Na+ and passive reabsorption of water at the collecting duct. Because of the high urea concentration in the filtrate at this region some urea diffuses into the interstitial fluid. Final processing of the filtrate at the collecting duct forms the urine.

When producing dilute urine the kidney actively reabsorb salts without allowing water to be reabsorbed by osmosis.

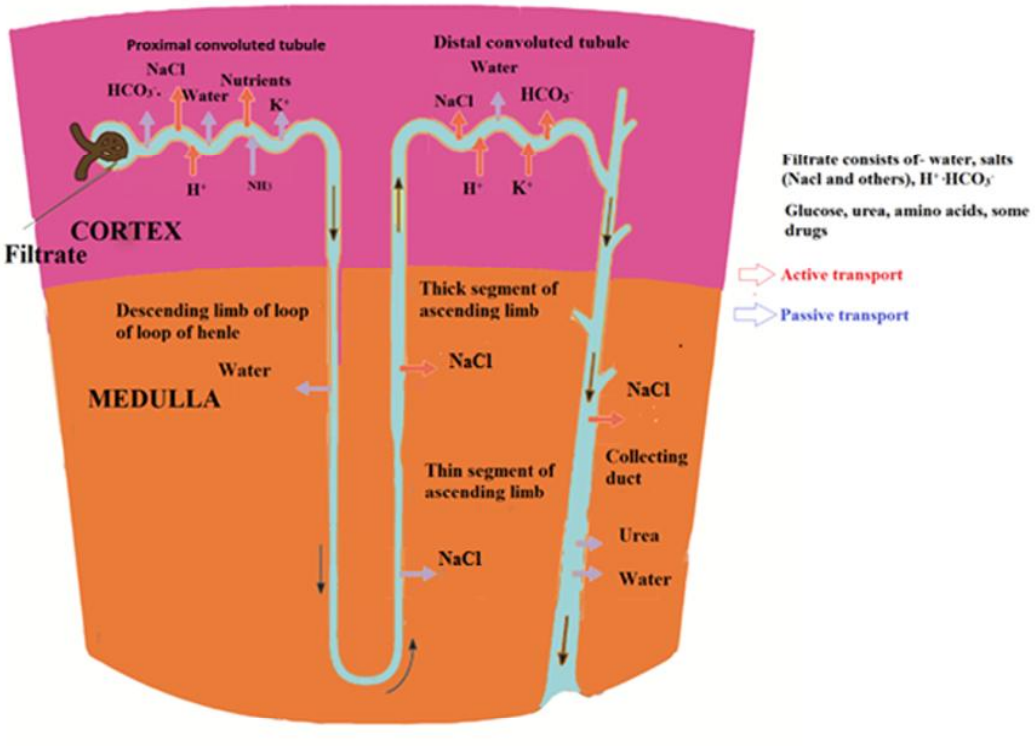


Fig 5.40 Reabsorption and secretion of different molecules and ions in the nephron and formation of urine in the collecting duct

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Role of hormones on the functions of the kidney

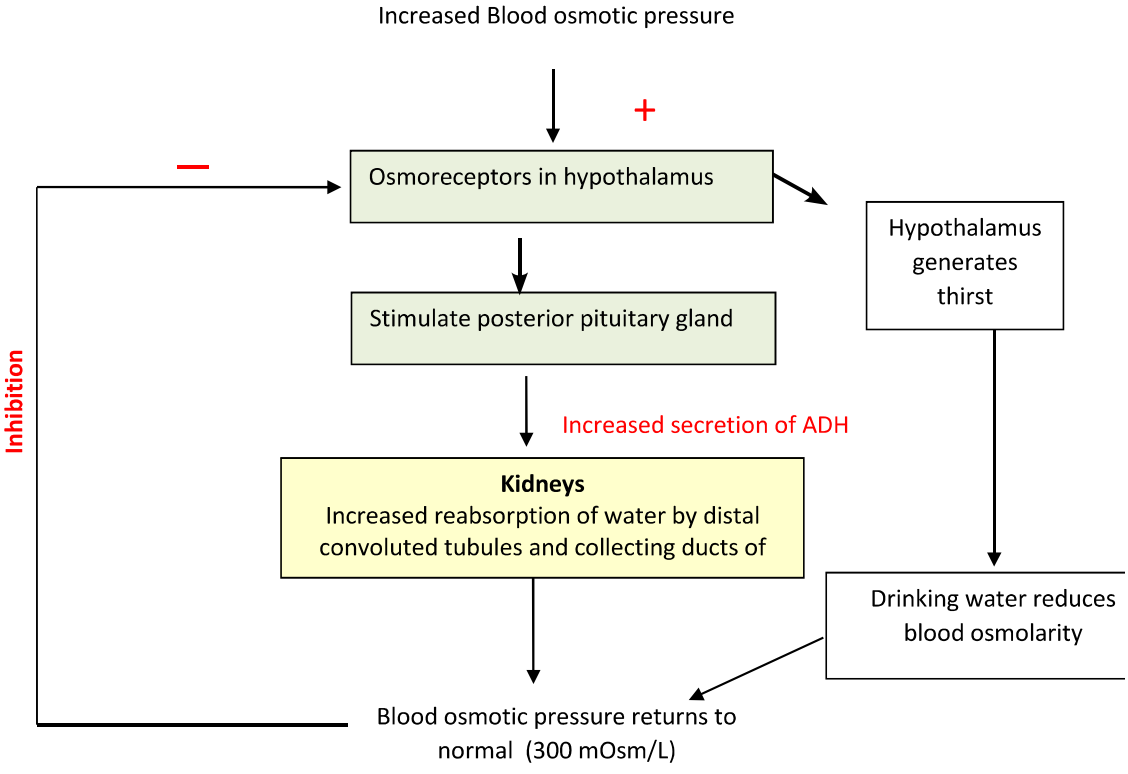


Fig5.41 Regulation of blood osmotic pressure and fluid retention in the kidney by ADH

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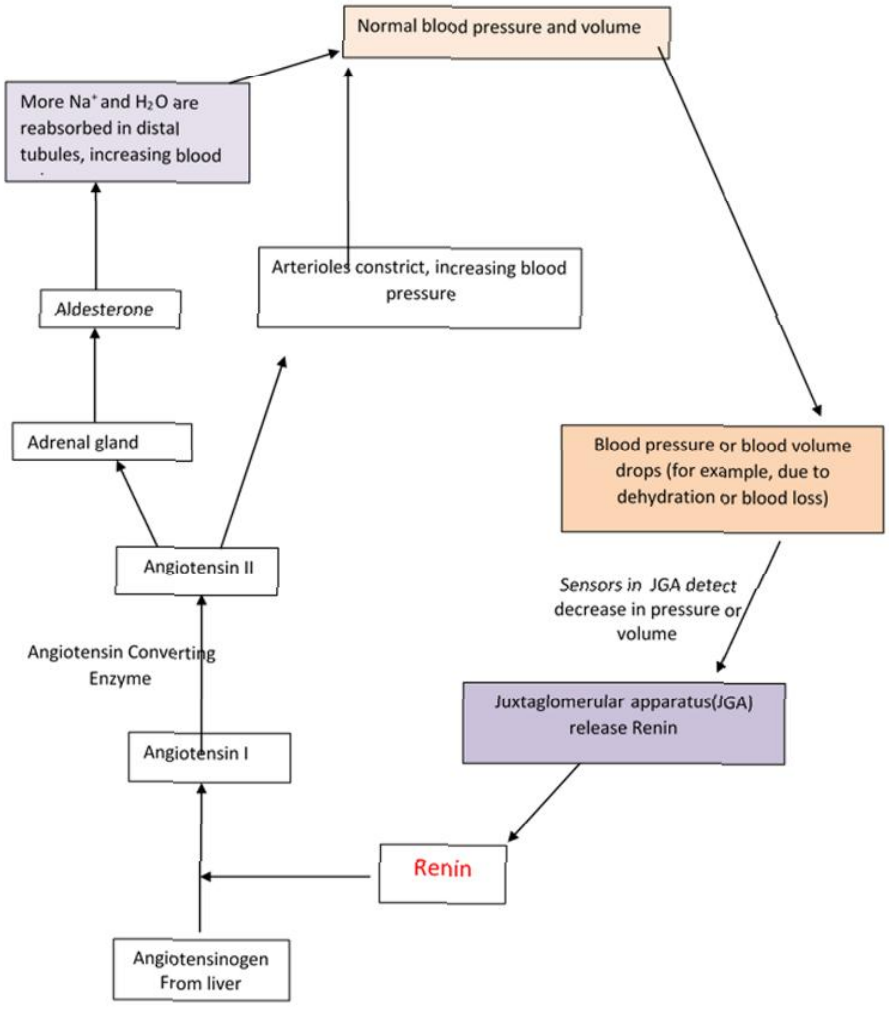


Fig5.42: Regulation of blood osmotic pressure and fluid retention in the kidney by ADH

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Role of kidney in homeostasis

- Maintaining electrolyte and water balance in the body fluids (osmoregulation).
- Excretion of toxic waste products from the body.
- Regulating blood pH through acid base balance.
- Controlling blood volume and blood pressure.
- Secretion of erythropoeitein hormone that stimulates red blood cell production.
- Production and Secretion of renin an enzyme important in control of blood pressure.

Disorders related to human urinary system

Bladder and kidney stones

These are formed due to precipitation of urinary constituents (usually oxalates and phosphates) normally in urine. They are also called renal calculi.

Causes include

- Dehydration due to not drinking sufficient amount of fluids.
- Alkaline nature of urine.
- Infections that can alter pH of urine
- Metabolic condition.
- Family history.

Measures for prevention

- Drinking plenty of water

Kidney failure

This is due to the inability of the kidneys to function properly. Therefore waste products and excess fluid will be accumulated in the blood.

Reasons for kidney failure

- Diabetes
- High blood pressure
- Having family history
- Getting older

Chronic kidney disease (CKD)

It is a condition of gradual loss of kidney function over time. There are many reasons for Kidney failure:

- Diabetes
- High blood pressure

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- Having family history
- Getting older

Prevent CKD

Follow a low salt, low fat diet

Doing proper exercise

Having regular check ups

Prevent smoking

Dialysis

Dialysis is done for the patients with kidney failure. It is a process of removing excretory products, excess solutes and toxins from the blood by an artificial method.

Chronic kidney disease of unknown/uncertain etiology (CKDu) in Sri Lanka

- It is a condition of gradual loss of kidney function over time. The root cause of CKDu has not been definitively established yet – hence it is referred to as 'Chronic kidney disease of unknown/uncertain etiology'. However it is a different form of chronic kidney disease (CKD), which is associated with conventional risk factors such as diabetes and high blood pressure, genetic disorders and urinary tract problems
-
- The onset of the disease appears to be asymptomatic, and by the time the patient seeks treatment the kidneys have reached a stage of irreversible damage -end stage renal disease (ESRD).
- In Sri Lanka, initially CKDu was prevalent among rural communities in North Central province (Medawachchiya, Kabithigollawa, Padaviya, Medirigiriya, regions), Uva (Girandurukotte), Eastern Provinces (Dehiattakandiya). CKDu is also reported in North Western, Southern and Central provinces, and parts of the Northern Province of the island.

Hypothesized reasons for CKDu

Cause of CKDu seems to be multifactorial.

- Exposure to Heavy metal/ metaloid such as Arsenic (As) and Cadmium (Cd) through food and water.
- Usage of low quality utensils for preparation of foods.
- High Flouride (F) levels in water.
- Exposure to pesticides.
- Genetic factors.
- Malnutrition and dehydration.

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Notes:

This is to acknowledge that some of the diagrams used in this book have been taken from various electronic sources using internet . This book is not published to make profit and sold only to cover cost.

The resource book is prepared according to the subject content and learning outcomes of the G.C.E. (A.L) Biology new syllabus which is implemented from 2017.

The content of this Resource book declares the limitation of the G.C.E. (A.L) Biology new syllabus which is implemented from 2017.

References

1. Biology ,A Global Approach, 10th edition (by Campbell Reece, Urry, Cain, Wasserman. Minorsky, Jackson)
2. Ross and Wilson Anatomy and physiology in Health and Illness (12th edition by Anne Waugh and Allison Grant)

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General Certificate of Education

(Advanced Level)

BIOLOGY

Grade 13

Resource Book

Animal form and function-Part II

Genetics

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G.C.E. (Advanced Level)
Biology
Grade 13
Resource Book
Animal form and function Part II
Genetics

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Message from the Director General

The National Institute of Education takes opportune steps from time to time for the development of quality in education. Preparation of supplementary resource books for respective subjects is one such initiative.

Supplementary resource books have been composed by a team of curriculum developers of the National Institute of Education, subject experts from the national universities and experience teachers from the school system. Because these resource books have been written so that they are in line with the G. C. E. (A/L) new syllabus implemented in 2017, students can broaden their understanding of the subject matter by referring these books while teachers can refer them in order to plan more effective learning teaching activities.

I wish to express my sincere gratitude to the staff members of the National Institute of Education and external subject experts who made their academic contribution to make this material available to you.

Dr. (Mrs.) T. A. R. J. Gunasekara
Director General
National Institute of Education
Maharagama.

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Message from the Director

Since 2017, a rationalized curriculum, which is an updated version of the previous curriculum is in effect for the G.C.E. (A/L) in the general education system of Sri Lanka. In this new curriculum cycle, revisions were made in the subject content, mode of delivery and curricular materials of the G.C.E. (A/L) Physics, Chemistry and Biology. Several alterations in the learning teaching sequence were also made. A new Teachers' Guide was introduced in place of the previous Teacher's Instruction Manual. In concurrence to that, certain changes in the learning teaching methodology, evaluation and assessment are expected.

The previous Teachers' Instruction Manual contains a line-up of subject matter expected to be learnt, but the newly introduced Teachers' Guide does not accommodate any subject matter. Yet, it provides learning outcomes, a guideline for teachers to mould the learning events, assessment and evaluation. Thus, the need of a resource book, which simply describes the subject content emerges. This book comes to you as a result of an attempt to fulfil that requirement. When implementing the previous curricula, the use of internationally recognized standard textbooks published in English was imperative for the Advanced Level science subjects. Due to the contradictions of facts related to the subject matter between different textbooks and inclusion of the content beyond the limits of the local curriculum, the usage of those books was not convenient for both teachers and students.

As this book is available in Sinhala, Tamil, and English, the book offers students an opportunity to refer the relevant subject content in their mother tongue as well as in English within the limits of the local curriculum. It also provides both students and teachers a source of reliable information expected by the curriculum instead of various information gathered from the other sources.

This book authored by experienced subject teachers and subject experts from the universities is presented to you followed by the approval of the Academic Affairs Board and the Council of the National Institute of Education. Thus, it can be recommended as a material of high standard.

Dr. A. D. Asoka De Silva
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05

Animal Form and Function

Processes and systems involved in coordination

Coordination between stimuli and responses is needed to maintain constant internal environment inside the body of an organism for existence.

Systems contributing to coordination

Animals unlike plants have two different but related systems for coordination of body function. They are the nervous system and the endocrine system.

Table 5.1: Similarities and differences (in relation to coordination) of the nervous system and endocrine system

Feature	Nervous coordination	Hormonal coordination
Transmission	through neurons	through blood
Nature of transmitter	chemical and electrical	chemical
Response	localized	diffused
Time taken to start the response	fast acting	slower action
Duration of response	short	long

Organization of nervous systems in different animal Phyla

Animals have specialized systems of neurons to sense their surroundings and respond rapidly. In the animal kingdom, cnidarians are the simplest animals having a nervous system. They have a diffuse nerve net which is composed of interconnected individual neurons.

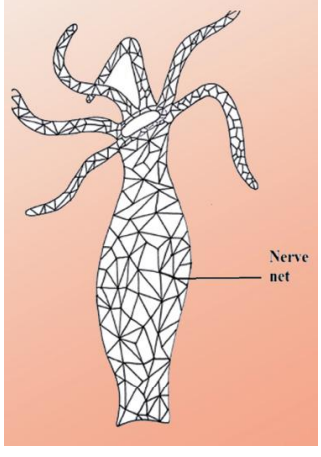
In more complex animals, the nervous systems contain groups of neurons organized into nerves, and often ganglia and a brain. In some platyhelminthes such as Planaria, the nervous system contains a pair of ganglia in the anterior region (brain) and a pair of ventral nerve cords that runs longitudinally. In planarians, the eye spots which are located near the ganglia act as photoreceptors. Annelids and arthropods have a somewhat complicated brains and ventral nerve cords. The ventral nerve cord contains ganglia. They are segmentally arranged. Nervous system

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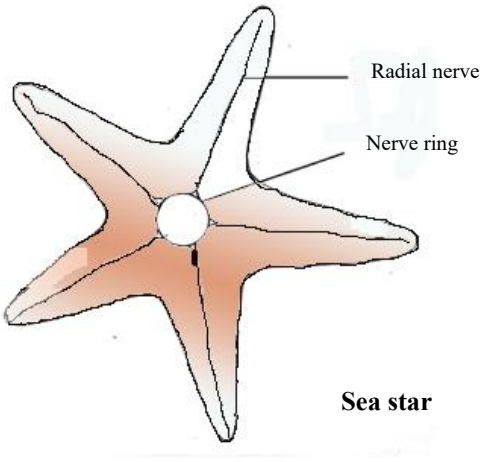
of echinodermates is composed of radial nerves and a nerve ring. Nervous system of the chordates consists of a central nervous system (CNS) and a peripheral nervous system (PNS). The CNS is composed of the brain and the spinal cord. The PNS is composed of nerves and ganglia.

Table 5.2: Different organism phyla and their nervous organization

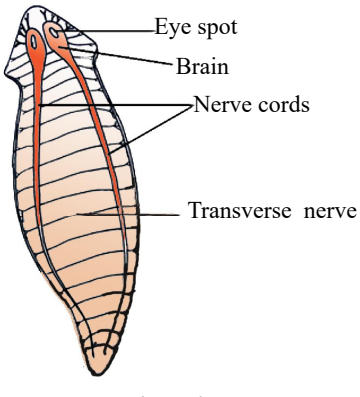
Phylum	Organization	Example
Cnidaria	Nerve net	Hydra
Platyhelminthes	Brain, longitudinal nerve cords	Planaria
Annelida	Brain, ventral nerve cord, segmental ganglia	Leech
Arthropoda	Brain, Ventral nerve cord, Segmental ganglia	Cockroach
Echinodermata	Nerve ring and radial nerves	Sea star
Chordata	Brain, spinal cord (dorsal nerve cord), nerves and ganglia	Gecko



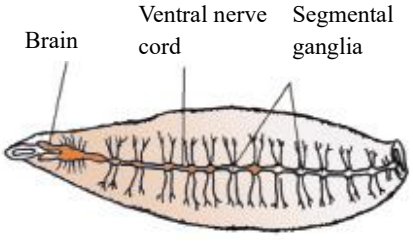
Hydra



Sea star

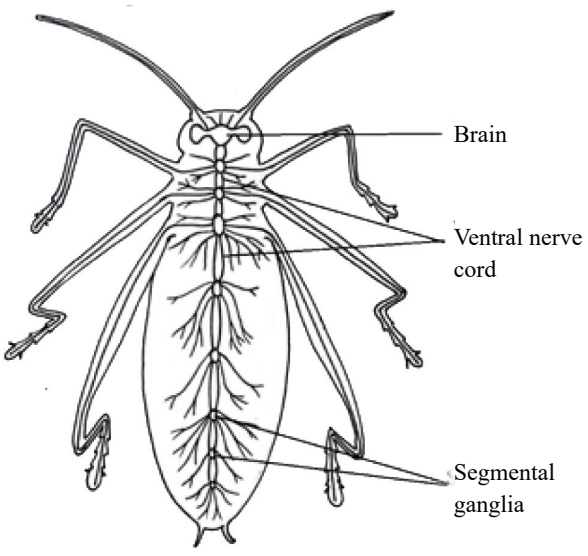


Planaria

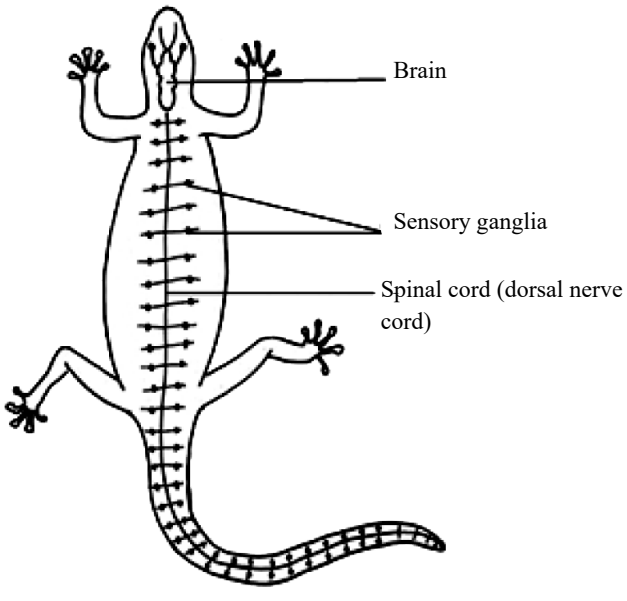


Leech

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Insect



Gecko

Figure 5.1: Organization of nervous systems in different animal phyla

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The gross structure and the functions of the human nervous system

Organization and main parts of the human nervous system

Human nervous system consists of central and peripheral nervous systems. In vertebrates, the brain and the spinal cord form the central nervous system. Nerves and ganglia forms the main components of the peripheral nervous system.

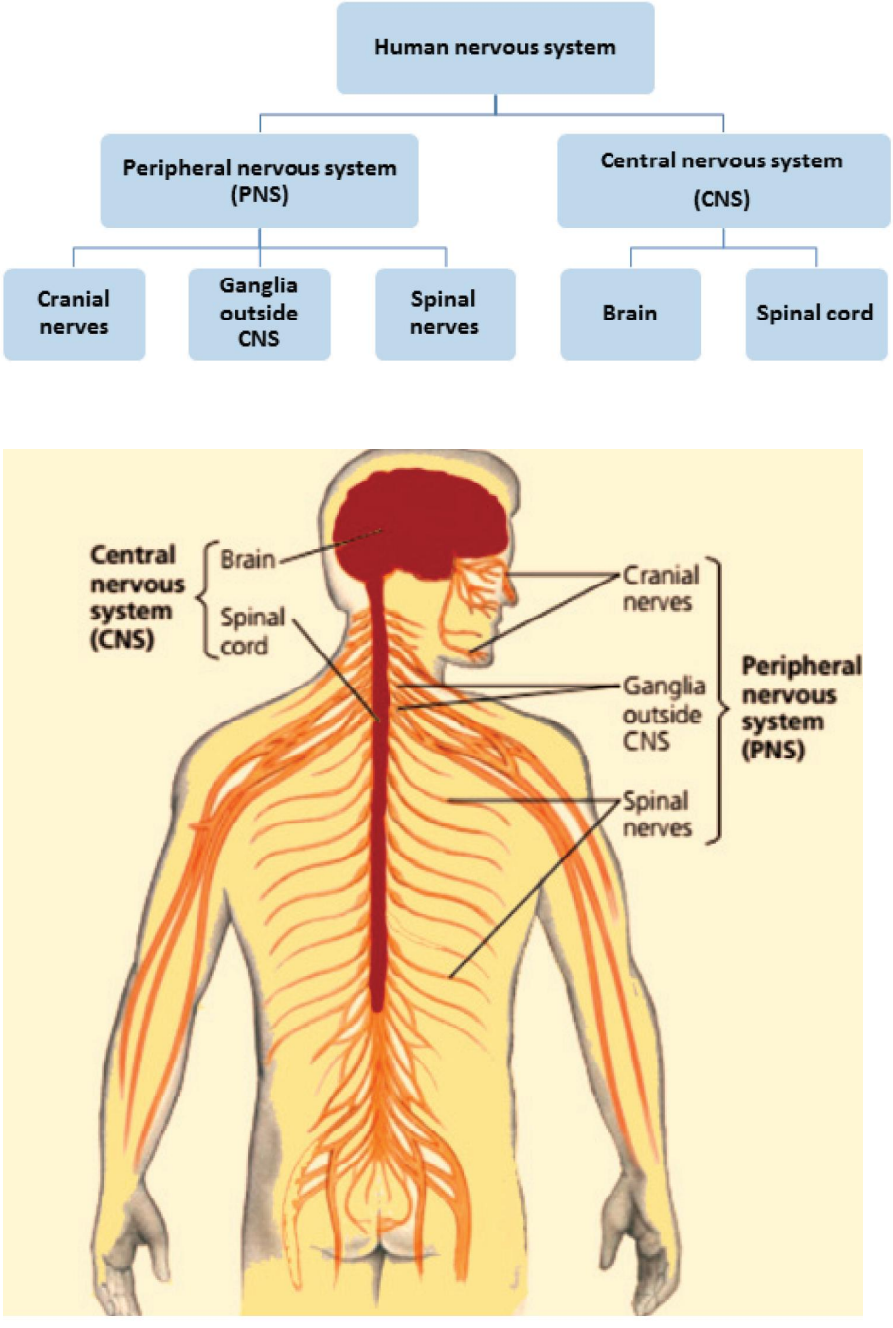


Figure 5.2: The organization of human nervous system

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Central nervous system (CNS)

Central nervous system consists of the brain and the spinal cord. In vertebrates, the CNS develops from the hollow dorsal nerve cord during embryonic development. Anterior part of the central nervous system enlarges and forms the brain which has three major regions: forebrain, midbrain and hindbrain. The central canal in the brain forms four irregular shaped cavities called ventricles. The brain contains four ventricles: three ventricles are present in the fore brain and one ventricle is in the hind brain. This central canal continues in the spinal cord. The ventricles and central canal contains cerebrospinal fluid. This fluid helps to maintain uniform pressure within the CNS and act as a shock absorber between the brain and skull. It also helps to circulates nutrients and hormones as well as to remove waste products.

The brain and the spinal cord have several adaptations to be protected from physical injuries. The brain is enclosed by a skull. The spinal cord is surrounded by vertebrae which forms the vertebral column. Further protection to the CNS is given by three layers of tissues called the meninges. The outermost layer is called the dura mater, the innermost layer as pia mater and in between these two layers is the arachnoid mater.

Main parts of the human Brain

The forebrain, midbrain and hindbrain of the human embryo develops into the adult brain. The forebrain gives rise to the cerebrum, thalamus, hypothalamus and pineal body. The mid brain gives rise to part of the brain stem. The hind brain gives rise to cerebellum, pons varoli and medulla oblongata. The brain stem consists of the midbrain, pons Varolii and medulla oblongata.

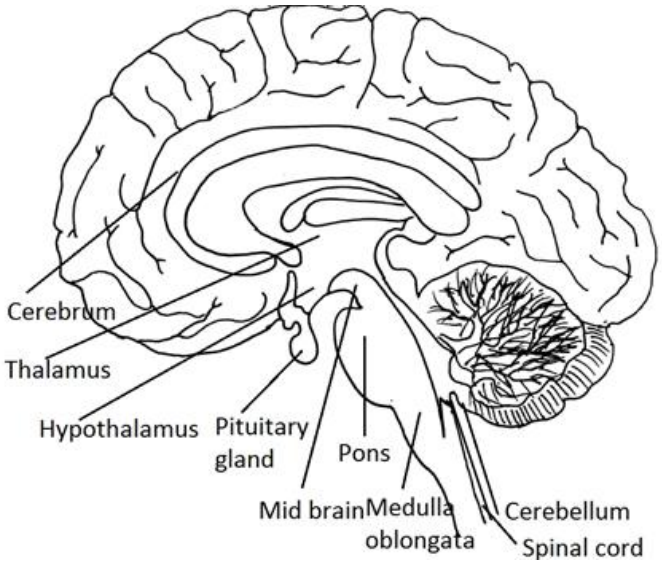


Figure 5.3 : The longitudinal view of the human brain

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Cerebrum

Cerebrum is the largest part of the human brain. It is divided by a deep cleft into right and left cerebral hemispheres. The superficial part of the cerebrum is composed of nerve cell bodies (or grey matter) forming the cerebral cortex and deeper layers consist of nerve fibers (or white matter). The two cerebral hemispheres are connected by corpus callosum which is a mass of white matter. The cerebral cortex shows many infoldings to increase the surface area of the cerebrum. The cortex of each cerebral hemisphere is divided into four lobes: frontal lobe, temporal lobe, parietal lobe and occipital lobe.

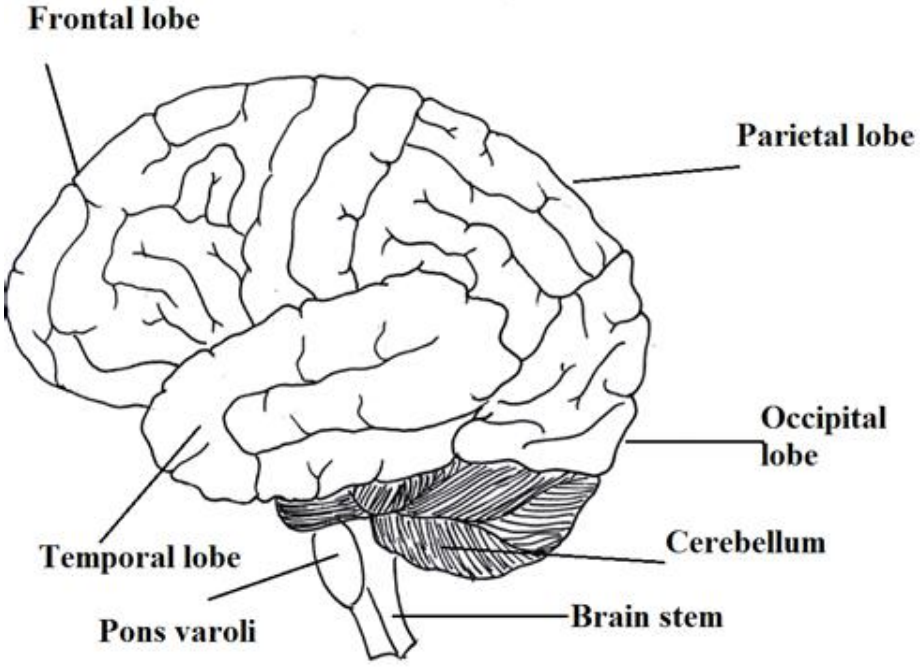


Figure 5.4 : The human cerebral cortex

Three main functional areas of the cerebral cortex have been identified. They are;

- Sensory areas which receive and process sensory information including the perception of pain, temperature, touch, sight, hearing, taste and smell
- Association areas which are responsible for recognition and interpretation of sensory information and integration and processing of complex mental functions such as memory, intelligence, reasoning, judgment and emotions
- Motor areas which are responsible for directing skeletal (voluntary) muscle movement through the initiation and control of voluntary muscle contraction

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Thalamus

Thalamus is situated within the cerebral hemispheres just below the corpus callosum. It consists of two masses comprising grey and white matter.

Functions:

Thalamus acts as the main input centre of sensory information from special sense organs and sensory receptors in the skin and integral organ. This sensory information is sorted and directed to specific location of the cerebral cortex for further processing and perception. The thalamus relays and redistributes nerve impulses from most parts of the brain to cerebral cortex.

Hypothalamus

Hypothalamus is situated below and in front of the thalamus, immediately above the pituitary gland. It is linked to the posterior lobe of the pituitary gland by nerve fibers and to the anterior lobe by a complex system of blood vessels.

Functions:

- Regulates body temperature
- Regulates thirst and water balance
- Regulates appetite
- Regulate sleep and wake cycles
- Control of autonomic nervous system
- Initiates fight-or-flight response
- Source for posterior pituitary hormones and releasing hormones that act on anterior pituitary.
- Plays a role in sexual behaviours

Mid brain

Mid brain is the upper part of the brain stem. It is situated between the cerebrum above and the pons below surrounding the cerebrospinal fluid filled connection of the third and fourth ventricles. Mid brain contains aggregates of nerve cell bodies and nerve tracts which connect the cerebrum with lower brain and spinal cord.

Functions:

- Acts as relay stations for ascending and descending nerve fibers
- Receives and integrates sensory information (auditory and visual) and sends it to particular regions of the forebrain, coordinates auditory and visual reflexes

Pons Varolii

Pons Varolii, (a part of the brain stem) is located in front of the cerebellum, below the mid brain and above the medulla oblongata. It contains nerve fibers that form a bridge between the two hemispheres of the cerebellum. It also contain nerve fibers passing between higher levels of brain

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and spinal cord. Groups of nerve cell bodies in the pons form centers that regulate respiration. Some nerve cell bodies in the pons act as relay stations.

Functions:

- Transfers information between PNS and the midbrain and forebrain
- Coordinates large scale body movements such as climbing and running
- Together with the medulla oblongata helps regulate respiration.

Medulla oblongata

Medulla oblongata is the lowest part of the brain stem which extends from the pons above and is continuous with the spinal cord below. It consists of cardiovascular centre, respiratory center and reflex centers.

Functions:

- Transfers information between PNS and the mid brain and the fore brain
- Coordinates various body movements such as running, climbing
- Controls several autonomic, homeostatic functions including breathing, heart and blood vessel activities (contains respiratory centre, cardiovascular centre)
- Controls involuntary reflexes such as vomiting, swallowing, coughing, sneezing through reflex centres

Cerebellum

Cerebellum is located behind the pons Varolii and below the posterior portion of the cerebrum. It is also made up of two hemispheres.

Functions

- Coordinates voluntary muscular movements
- Maintains posture and balance
- Helps in learning and remembering motor skills

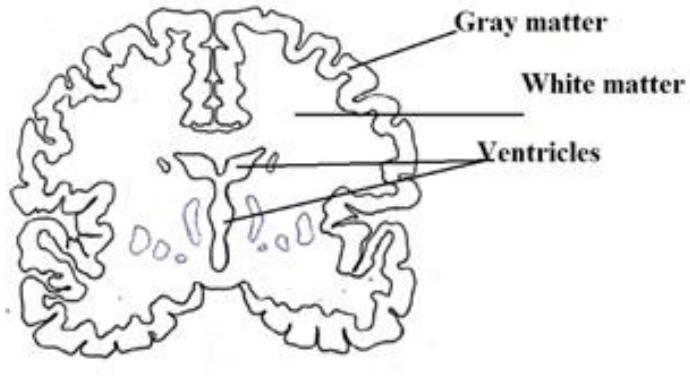


Figure 5.5 : T.S of the human brain

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Spinal cord

The spinal cord is an elongated cylindrical structure suspended in the vertebral canal. It is continuous with the medulla oblongata. Centre of the spinal cord contains the central canal which is surrounded by grey matter. Outer region of the spinal cord is made up of white matter.

Functions:

- Links the central nervous systems to sensory and motor neurons and facilitates nerve impulse propagation towards the brain and away the brain
- Coordinates and produces reflexes

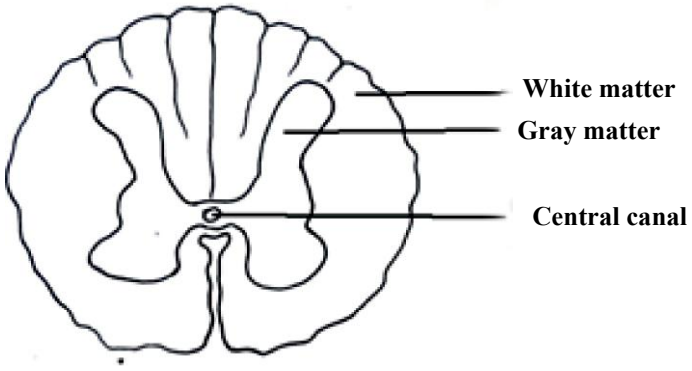


Figure 5.6: T.S of the spinal cord

Peripheral nervous system (PNS)

Peripheral nervous system is made up of cranial nerves, spinal nerves and autonomic nervous system (with ganglia). It transmits impulses to and from CNS regulating both an animal's movement and its internal environment.

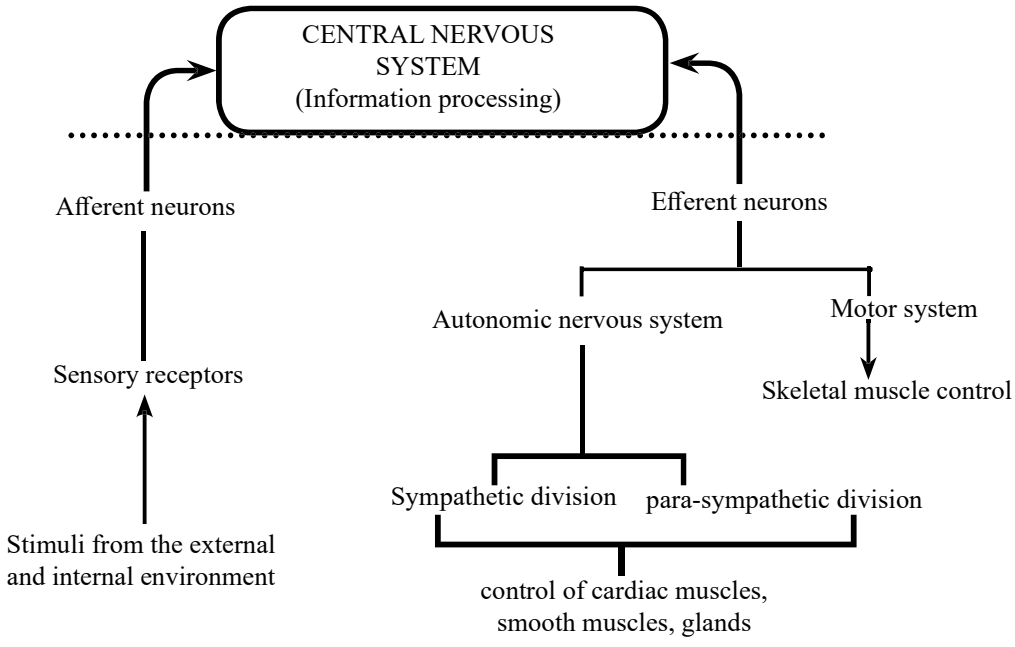


Figure 5.7 : Peripheral nervous system of a vertebrate (Functional hierarchy)

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Sensory information from sensory receptors reaches the CNS along PNS neurons referred to as afferent neurons (sensory neurons). Within the CNS this information is processed and instructions are transmitted to effector tissues/organs (muscles, glands and endocrine cells) along PNS neurons referred to as efferent neurons (motor neurons).

PNS consists of two efferent components;

- The motor system- It consists of neurons that carry nerve impulses to skeletal muscles. So it controls voluntary activities.
- Autonomic nervous system- It generally controls the involuntary activities of the body. Autonomic nervous system consists of neurons which carry impulses to control activities of smooth muscles, cardiac muscles and glands.

Autonomic nervous system consists mainly of two divisions:

- Sympathetic division
- Parasympathetic division

Sympathetic and parasympathetic nervous system

The majority of the body organs are supplied by both sympathetic and parasympathetic nerves which have antagonistic (opposite) functions. Sympathetic stimulations prepare the body to deal with exciting/ stressful situations and energy generating situations (fight- or -flight). Parasympathetic division causes opposite responses that promote calming or a return to self-maintenance functions (rest and digest).

The two divisions differ in overall functions, organization and the signal released. Parasympathetic nerves exit the CNS at the base of the brain or the spinal cord as cranial nerves or spinal nerves respectively. On the other hand sympathetic nerves exit only from the spinal cord. Different neurotransmitters enable the two systems to bring about two opposite effects in different organs such as lung, heart, intestine and bladder. For example, when the neurotransmitter secreted by the parasympathetic division is acetylcholine, the sympathetic division secretes norepinephrine.

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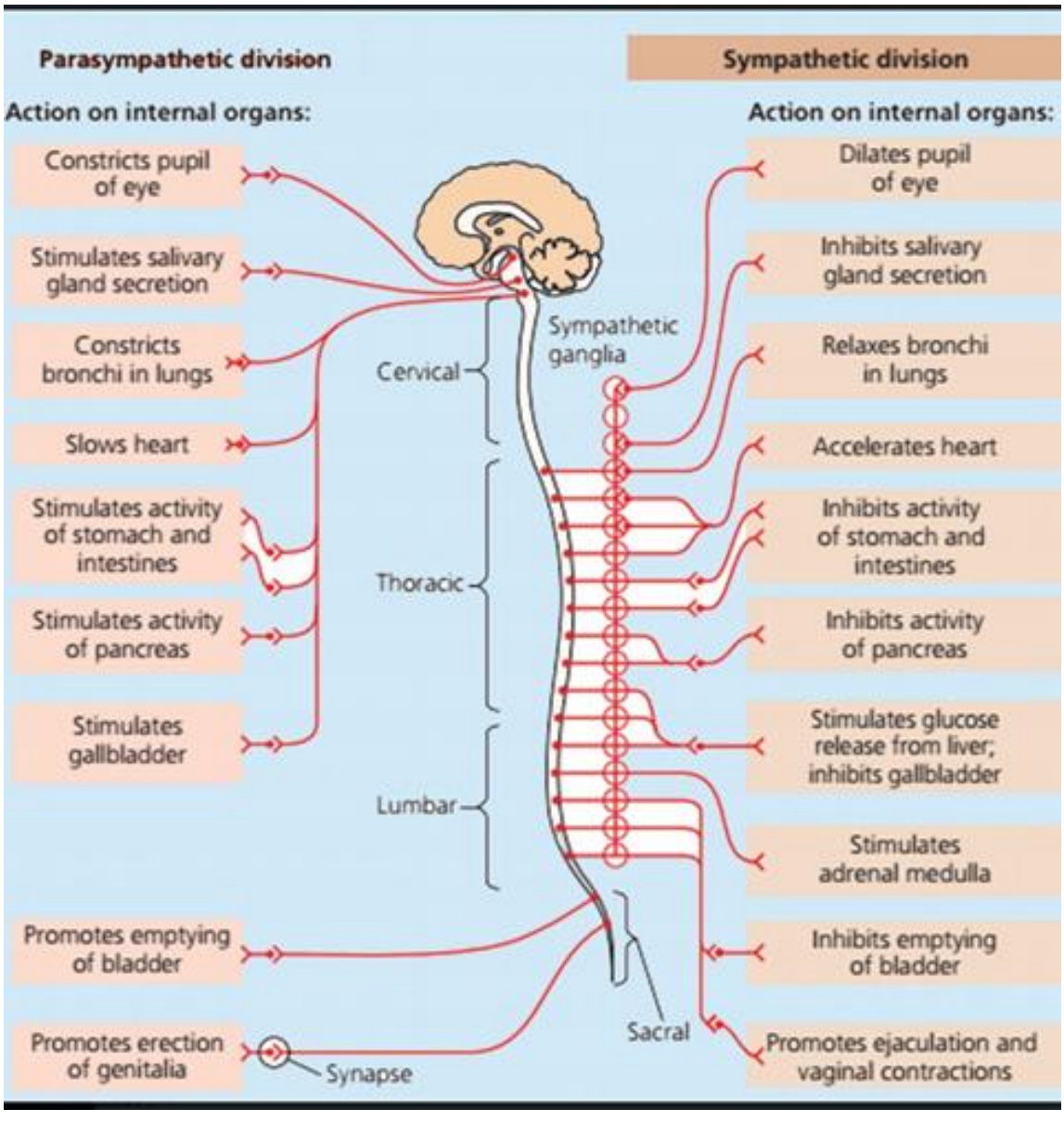


Figure 5.8: The autonomic nervous system (Parasympathetic and sympathetic division)

How nerve impulses are generated and transmitted

In all cells including neurons, ions are distributed unequally between the cell interior and exterior (extra cellular fluid). Generally the inside of the cell is negatively charged whereas the exterior is positively charged. These opposite charges are attracted across the plasma membrane and as a result it creates a voltage difference across the membrane that is referred to as membrane potential.

Resting potential

When a neuron is at rest (when not sending a signal/non conducting), the membrane potential is called the resting potential. In a non-conducting neuron the resting potential is typically between -60 mV and -80 mV.

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The resting membrane potential is maintained by;

- Distribution of ion concentrations inside and outside of the neuron- In a non conducting neuron the concentration of K+ is higher inside the cell while concentration of Na+ is higher outside. In addition there are Cl- and other large anions (proteins) inside the cell. As a result a neuron has a negative charge inside the cell and positive charge outside the cell.
- Selective permeability of the plasma membrane to K+ and Na+ ions- There are potassium channels and sodium channels, which are membrane bound proteins that are able to open or close in response to stimuli. Potassium channels allows only K+ ions to pass whereas sodium channels allow only Na+ to pass. These channels allow K+ and Na+ to diffuse according to their concentration gradient. However there are more potassium channels open than sodium channels. As a result there is a net negative charge inside the cell.
- Sodium-potassium pump- This pump helps to maintain Na+ and K+ gradient across the membrane by transporting three Na+ out of the cell for every two K+ that it transports in. This pump uses ATP to actively transport these ions.

Action potential

An action potential occurs due to a change in membrane potential above a threshold value due to a stimulus. The action potential has the following phases: depolarization, repolarization and hyperpolarization.

Depolarization: A change in the cell's membrane potential such that the inside of the membrane is made less negative relative to the outside. Depolarization results due to Na + inflow in response to a stimulus.

Repolarization: Sodium channels close blocking Na+ inflow. However most potassium channels open permitting K+ outflow. This makes the inside of the cell negative.

Hyperpolarization: Sodium channels are closed but potassium channels are opened. As a result the inside of the membrane is more negative.

Refractory period

Refractory period is the short time immediately after an action potential in which the neuron cannot respond to another stimulus, owing to the inactivation of sodium channels. This prevents the reverse conduction of an impulse in an axon.

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Generation of action potential

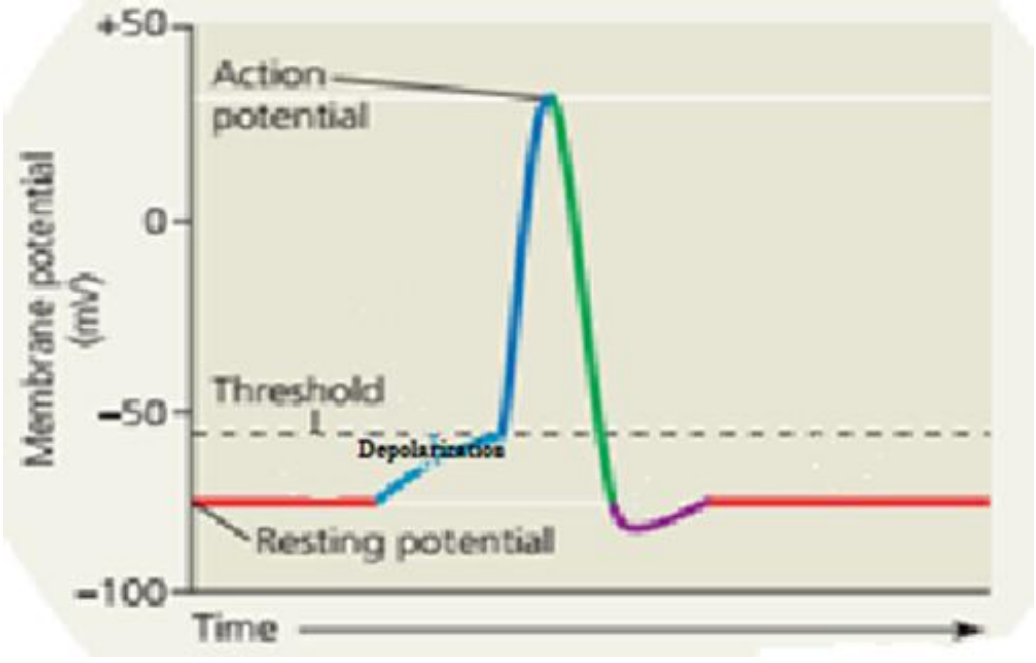


Figure 5.9: Graph of generating action potential

Conduction of action potential (nerve impulse)

- A series of action potentials that move along an axon is defined as a nerve impulse.
- An action potential is generated due to Na+ inflow (depolarization) at one location in the axon.
- This axon potential spreads to the neighboring location while the initial location repolarizes.
- This depolarization-repolarization process is repeated through the axon.

The speed of conduction depends on:

Diameter of the axon- The conduction speed increases with the increase in axon diameter.

Presence of myelinated axon (in myelinated neuron, axon potential jumps from one node of Ranvier to the next)

Synapses

A synapse is the junction where a neuron (presynaptic cell) communicates with another cell (postsynaptic cell) across a narrow gap (synaptic cleft). Postsynaptic cell may be another neuron, muscle cell or secretory cell. This junction where one neuron communicates with the next cell using a chemical (neurotransmitter) is called a chemical synapse. Some neurons can also communicate through direct electrical connections (electrical synapse).

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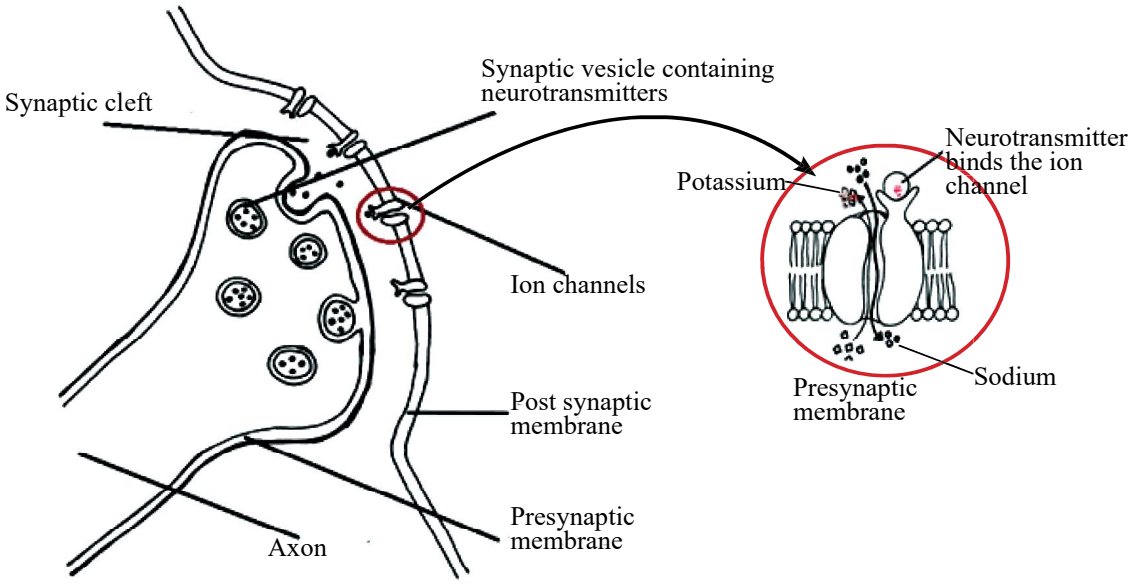


Figure 5.10: A synapse which communicates through a neurotransmitter

Mechanism of transmission of nerve impulses through chemical synapses

- An action potential at an axon terminal depolarizes the plasma membrane of presynaptic cell.
- Depolarization at the presynaptic terminal causes Ca^{2+} to diffuse into the terminals.
- The rise in Ca^{2+} causes binding of synaptic vesicles containing neurotransmitters to the presynaptic membrane.
- This results in the release of the neurotransmitters into the synaptic cleft.
- Neurotransmitters diffuse across the synaptic cleft.
- Neurotransmitters bind and activates specific receptors in the postsynaptic cell membrane.
- If acetylcholine is taken for example, the binding of neurotransmitters to the post synaptic membrane allows Na^{+} and K^{+} to diffuse across the post synaptic membrane.
- Depolarization takes place in the post synaptic membrane and it reaches the action potential
- After passing the nerve impulse to the postsynaptic cell, the signal is terminated either by:
  - Enzymatic hydrolysis of neurotransmitters
  - Recapture of neurotransmitter into the presynaptic terminals.

Neurotransmitters

Neurotransmitters are the molecules that are released from the synaptic terminals of presynaptic neuron and diffuse across the synaptic cleft, bind to the receptors at the postsynaptic membrane, triggering a response.

Common neurotransmitters are;

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- Acetylcholine
- Some amino acids
- Biogenic amines
- Neuropeptides
- Some gases

Reflex arc

Reflex arcs are the functional unit of the vertebrate nervous system. Typically a reflex arc consists of three neurons. They are

1. Afferent/ Sensory neuron
2. Interneuron
3. Efferent/ Motor neuron

A sensory neuron transmits impulses from a sensory receptor to the central nervous system where it synapses with an associated neuron called interneuron. This impulse is transmitted to a motor neuron. The motor neuron conveys the signal to effector tissues/organs.

Common disorders of the nervous system

Common disorders of the nervous system are Schizophrenia, Depression, Alzheimer's disease and Parkinson's disease.

- **Schizophrenia:** This is a severe mental disturbance characterized by psychotic episodes in which patients have a distorted perception of reality. They experience voices that only they can hear. They think that others are plotting to harm them. Evidence suggests that this disorder affects neural pathways that use dopamine as a neurotransmitter.

- **Depression:** Depression is likely to be due to a complex combination of factors that include: Changes in neurotransmitter levels in the brain, genetics, psychological, social, environmental factors .People who are suffering from this disorder show depressed mood, abnormalities in sleep, appetite and energy level. In some conditions once enjoyable activities are no longer pleasurable or interesting. Some conditions involve extreme mood swings. Effective therapies are available to increase activity of some neurotransmitters in the brain

- **Alzheimer's disease:** This is a severe mental deterioration (dementia) characterized by confusion and memory loss. Patients are gradually becoming less able to dress, bathe and feed themselves. They lose their ability to recognize people including their immediate family members. Cause of the disease is due to progressive and irreversible degeneration of neurons in the brain especially in cerebral cortex with deteriorating mental functioning. The disease affects elderly people. Genetic factors may be involved. So far, there is no cure for this disease.

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- **Parkinson disease:** This is a progressive motor disorder that leads to lack of control and coordination of muscle movements. The patients show slowness of movements, difficulty in intiating movements, poor balance; fixed muscle tone causing lack of facial expression; speech problems and muscle tremor of extremities: e.g. shaking a hand, fingers in one hand, shaking head. This disease is associated with gradual degeneration of dopamine neurotransmitter releasing neurons in the brain (mid brain, basal ganglia). The disease is common in the elderly people. Genetic factors may be involved. Disease can be treated but not cured.

Human sensory structures and functions

A sensory receptor is a specialized structure which can detect a specific stimulus and convert the stimulus energy to a changing membrane potential to be transmitted to the central nervous system as action potentials for sensory perception and interpretation. Sensory receptor can be a specialized cell or an organ or a subcellular structure that could detect the stimuli. Some sensory cells are specialized neurons. Sensory receptors can inform the central nervous system about the conditions inside and outside the body in order to maintain homeostasis. Specific sensory receptors detect the stimuli that arise in the external environment whereas internal receptors sense the stimuli that arise inside the body.

Basic characteristic of sensory receptors.

- A specialized structure (cell / organ / subcellular structure) designed to receive a specific stimuli.
- Detect the stimulus if the stimulus is at or above threshold level.
- Convert the energy of the stimulus (e.g. light energy, sound energy) into a changing membrane potential to be later transmitted as an action potential.
- Always connected with the nervous system.
- During the conversion of stimulus energy into the action potential, sensory signal can be strengthened which is called amplification.
- If the stimulation is continuous, many receptors show decrease in responsiveness which is called sensory adaptation (For example upon continuous exposure to a strong smell, perception of that smell gradually decreases and stops within few minutes).

Types of sensory receptors

Sensory receptors can be categorized based on the nature of the stimulus they detect. Several types of sensory receptors are found in the human body. They are chemoreceptors, thermoreceptors, photoreceptors, mechanoreceptors and pain receptors.

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- **Chemoreceptors**

  These are sensory receptors that respond to chemical stimuli. Chemical substances should always be dissolved in water to stimulate sensory cells. Chemoreceptors include taste receptors and olfactory receptors. These receptors mediate the senses of taste and smell. Some chemoreceptors can detect specific chemicals such as CO_2 in the circulating blood.

  - **Taste receptors:** Five basic sensations of taste have been described: sweet, sour, bitter, salt and umami (savoury taste). Receptor cells for taste are modified epithelial cells organized into taste buds. Taste buds are found in papillae which are small projections of the tongue. A taste bud consists of taste cells, supporting cells and sensory nerve endings. Substances to be tasted should be dissolved in the fluid surrounding the sensory cells and diffuse to receptor cells.

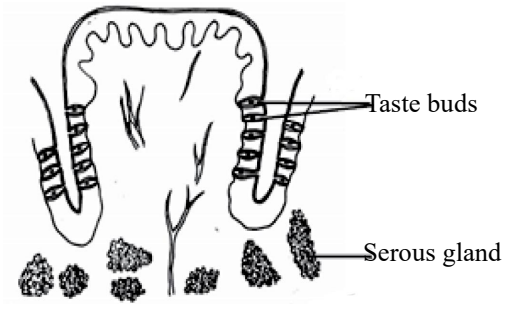


Figure 5.11: A section of a papilla

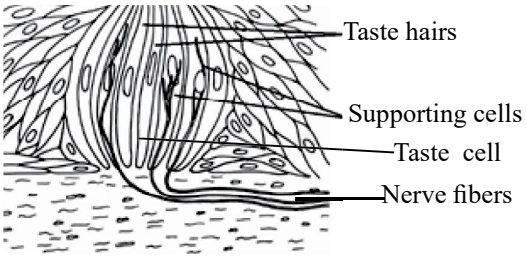


Figure: 5.12-A magnified taste bud

  - **Olfactory receptors:** In olfaction, receptor cells are neurons. Olfactory receptor cells are located within the epithelium of the upper portion of the nasal cavity. Receptive ends of the cells extend into the mucus layer of the nasal cavity. When odorants diffuse into this region, receptor cells are stimulated and the nerve impulse is sent along their axons to the olfactory bulb in the brain.

- **Thermoreceptors**

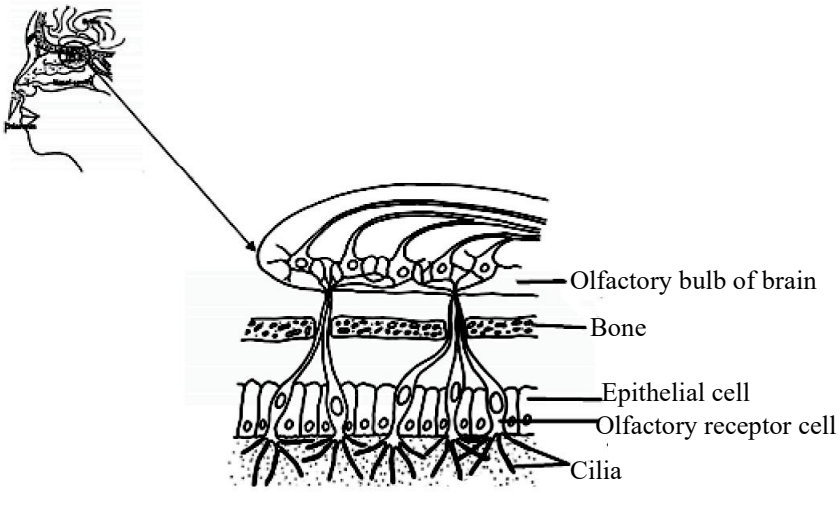


Figure 5.13: Location of olfactory receptors in humans

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Thermoreceptors are specialized temperature sensitive receptors which detect heat and cold on the body surface and in the internal environment of the body. Thermoreceptors located in the skin detect the body surface temperature whereas thermoreceptors found in hypothalamus detects the temperature of the blood circulating through the internal organs (core temperature). Thermoreceptors found in the skin are: Krause end bulbs (detect cold), Ruffini corpuscles (detect warmth) and free nerve endings (detect both cold and warmth). Thermoreceptors found in the hypothalamus are specialized neurons.

- **Photoreceptors**

Photoreceptors are sensitive for light. Humans have two main types of photoreceptor cells called rods and cones.

- **Rods:** They are more sensitive to light but do not distinguish colours, they enable us to see at night but only in black and white.
- **Cones:** They provide colour vision. But they contribute very little to night vision as they are not much sensitive. There are three types of cones. Each has a different sensitivity across the visible spectrum providing an optimal response to red, green, or blue light.

- **Mechanoreceptors**

Mechanoreceptors respond to stimuli arising from mechanical energy deformation such as pressure, touch, stretch, motion and sound. Mechanoreceptors in the human body include the following.

- Touch receptors: They are mostly present close to the surface of the skin. Examples for touch receptors are Meissner corpuscles (sensitive to light pressure), Merkel discs (sensitive to light touch) and free nerve endings.
- Pressure receptors: Example for pressure receptors are Pacinian corpuscles which are present in the deep skin. They are sensitive to deep pressure.
- Vibration receptors: Most of the touch receptors can also detect vibrations (e.g. Meissner corpuscles, Pacinian corpuscles). Specific hair cells in the organ of Corti in the inner ear detect sound vibrations. Hair cells of the vestibule of the inner ear detect the gravity whereas hair cells of the semicircular canals detect the motion.

- **Pain receptors**

Pain receptors detect stimuli that reflect harmful conditions that could arise from extreme pressure or temperature and certain chemicals that could damage the tissues. Special nerve endings in different parts of the body can detect the tissue damage. Ultimately the pain is perceived by the brain.

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Basic structure and functions of the human eye

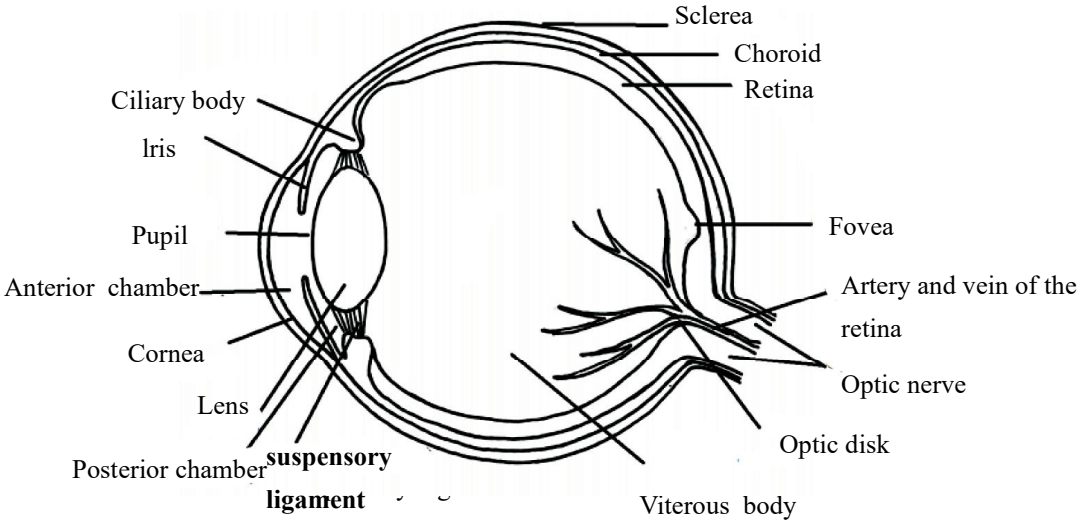


Figure 5.14: The basic structure of the human eye

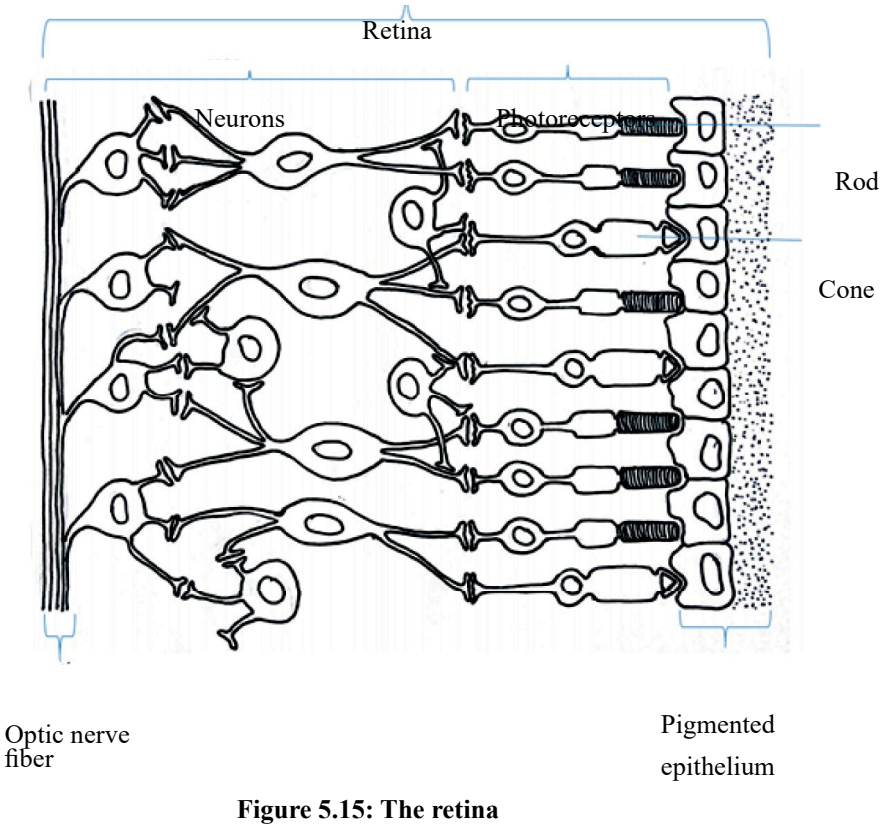


Figure 5.15: The retina

The eye is the organ responsible for sight. There is a fine transparent membrane that lines the iris and front of the eye ball; this is called conjunctiva. The walls of the eye are made up of three layers of tissue: The outer fibrous layer (sclera and cornea), the middle vascular layer (choroid,

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cilliary body and iris) and the inner nervous layer (Retina). Inside the eyeball contains the lens, aqueous fluid and vitreous body.

Sclera and cornea

- Sclera is white and opaque. It is the outermost layer of the posterior and lateral aspects of the eye ball. It connects anteriorly with the clear transparent epithelial membrane called cornea. Sclera maintains the shape of the eye and gives attachment of the extrinsic muscles of the eye.
- Cornea is the passage through which light rays reach the retina. It is devoid of blood vessels. The cornea is convex anteriorly and is involved in refracting light rays to focus on the retina.

Choroid, ciliary body and iris

- Choroid is located just beneath the sclera. It is a thin pigmented layer and rich with blood vessels.
- Cilliary body is the anterior continuation of the choroid layer consisting of smooth muscle fibers (ciliary muscle) and sensory epithelial cells. Most of these smooth muscle fibers are circular. Therefore ciliary muscles act as a sphincter. The ciliary body holds the lens in place by suspensory ligaments. The size and thickness of the lens can be controlled by contraction and relaxation of the ciliary muscle fibers attached to these suspensory ligaments. Epithelial cells secrete aqueous humor.
- Iris is a circular coloured body composed of pigment cells. It is located at the front of the eye. It extends anteriorly from the ciliary body and present behind the cornea and in front of the lens. It contains two layers of smooth muscle fibers which are arranged as circular and radial bundles. In the center of iris is a hole called pupil. Iris controls amount of light entering the pupil by changing size which is mediated by the autonomic nervous system. Pigments prevent penetration of excessive light.

Lens

The lens is lying immediately behind the pupil. It is an elastic, biconvex transparent disc made up of protein enclosed within a transparent capsule. It refracts light rays reflected by objects in front of the eye and focuses them on the retina to form the image. By changing the thickness, the lens can vary its refractive power in order to focus rays on the retina.

Aqueous fluid (aqueous humour) and vitreous body (vitreous humour)

In front of the lens, a clear watery substance is present which is called aqueous fluid (Blockage of ducts that drain this fluid can produce glaucoma causing vision loss). Aqueous fluid supplies nutrients and removes wastes from the cornea, lens and lens capsule which have no blood supply. Behind the lens a colourless and transparent jelly like vitreous humour is present. It maintains enough intra ocular pressure to support the retina against choroid and prevents the eye ball from collapsing.

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Retina

- Retina is the innermost lining of the eye. It consists of three layers: Outer pigmented epithelium, middle photoreceptive layer and inner layer with neurons. Photoreceptor layer consists of sensory cells (rods and cones) which contain photosensory pigments that can convert light rays into nerve impulses. Retina is thickest at the back. At the centre of the posterior part of the retina, macula lutea (yellow spot) is present. In the center of the yellow spot there is a little depression called the fovea centralis which contains only cones. Towards the anterior part of the retina there are fewer cones than rods. About 0.5 cm to the nasal side of the macula lutea all the nerve fibers of the retina converge to form the optic nerve. The small area of retina where the optic nerve leaves eye is the blind spot (optic disk). It lacks photoreceptors.

- **Photoreceptor cells:** There are two types: rods and cones. Within the outer segment of these cells is a stack of membranous disks in which visual pigments are embedded. In the retina, more rods are present than cones. In the rods visual pigment is rhodopsin. They are sensitive to light but do not distinguish colours. They enable us to see at night but only in black and white. In the cones, visual pigment is photopsin. They provide colour vision. They contribute very little to night vision as they are less sensitive. There are three types of cones each of which has a different sensitivity across the visible spectrum providing an optimal response to red, green or blue light.
- Neurons in the retina: several types of neurons are present including bipolar cells and ganglion cells.

Functioning of the human eyes

Light is reflected into the eye by the objects in the field of vision. In order to achieve clear vision, light reflected from the object within the visual field should be refracted mainly by the lens and focused on the retina of each eye. The processes which are involved in producing a clear image on the retina are refraction of light rays, changing the size of the pupil and accommodation. Then photoreceptor cells in the retina convert light energy to voltage changes leading to action potentials which are sent through the optic nerve to the brain for perception of visual objects. In the retina, stimulation of rods leads to black and white vision. Cones are sensitive to light and colour therefore bright light is needed to activate them and give sharp clear colour, vision. The different wavelengths of visible light activate light sensitive pigments in the cones which result in perception of different colours.

- **Refraction of light rays**

  Light rays coming from the visual field pass through the conjunctiva first, then successively through cornea, aqueous fluid, lens and vitreous body before reaching the retina. During this

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process light rays are refracted (bent) to focus them on the retina as they all are denser than the air. Lens has changing refractory power while all the other parts (conjunctiva, cornea, aqueous fluid and vitreous body) have constant refractory powers. Light rays are mostly refracted by the biconvex lens.

- **Changing the size of the pupil and accommodation**

For clear vision, the amount of light entering the eye is controlled by changing the size of the pupil which is mediated by the autonomic nervous system. Light rays coming from the distant objects need least refraction but as the object comes closer, the amount of refraction needs to be increased to focus light rays on the retina. Hence for near vision the eye must make some adjustments.

- Constriction of the pupil: In bright light, pupils are constricted to avoid entering too much light into the eye and damage the sensitive retina. In dim light, the pupils are dilated to allow entering sufficient light to activate photoreceptors which would eventually enable the vision.
- Movement of the eye ball (convergence): As light rays from near objects enter the two eyes at different angles, for clear vision they must stimulate corresponding areas of two retina. Muscles attached to the eye ball rotate the eyes to achieve the convergence. This is under autonomic controls.
- Changing the refractory power of the lens: Parasympathetic nervous supply to the ciliary body controls the contraction of ciliary muscles and accommodation of eye. Accommodation is important in near vision for focusing on near objects. In near vision the ciliary muscles contract thereby moving the ciliary body inwards towards lens. As a result convexity of the lens is increased due to the reduction of the pull of the suspensory ligaments on the lens. Thus light waves from the near objects are focused on the retina. When seeing a distant object, ciliary muscles relax, then ciliary body moves away from the lens that increases the pull of the suspensory ligaments on the lens so convexity of the lens is reduced. Thus light rays from distant objects are focused on the retina.

Focusing the image on the retina and converting the light energy to action potential to be transmitted to the brain

- The light waves coming from the object are bent (refracted) and focused on the retina. This process produces an image on the retina which is upside down. Once light rays reach the retina, chemical changes happen in the photoreceptive cells (rods and cones).
- Bipolar cells receive information from photoreceptor cells and each ganglion cell gathers inputs from several bipolar cells. In addition, specific neurons in the retina can integrate information across the retina. Ganglion cells form the optic nerve fibers that transmit sensation from the eyes as action potential to the brain. This change will generate a nerve impulse.
- The optic nerve transmits this nerve impulse into occipital lobes (visual area) of the cerebrum. There the visual objects are perceived in the correct way (the right way up) by the brain.

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- Choroid functions in absorption of light after the entered light stimulate sensory receptors in the retina.

Monocular vision and binocular vision in human

In humans, both eyes are located in front of the face which facilitates coordinated vision from the two eyes. However it is possible to see visual fields with one eye. Seeing the visual field using only one eye is called monocular vision. However when one eye is used, three dimensional vision is impaired especially in relation to the judgment of speed and distance.

Seeing the visual field using two eyes with greater overlapping fields of view is called binocular vision. The left eye views more on the left of the visual fields. The right eye views more on the right of the visual fields. Even though each eye views a scene from a slightly different angle, in the middle the visual fields are overlapped. However only one image is perceived due to the fusion of left, middle and right of the visual field images from the two eyes in the occipital lobe of the cerebrum.

Unlike monocular vision, binocular vision enables three dimensional views. So binocular vision is very important in judging the speed and distance of an approaching object such as a vehicle. It gives more accurate assessment of one object relative to another in relation to distance, depth, height and width. In some individuals, binocular vision may be impaired. Such individuals face difficulties to judge the speed and distance of an approaching object.

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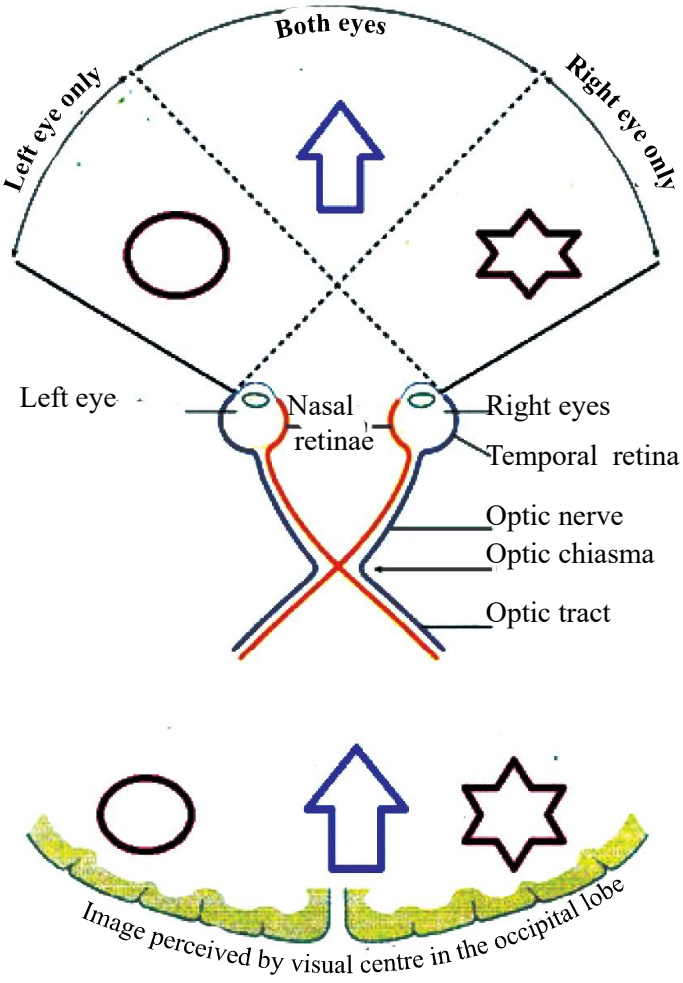


Figure 5.16: Visual fields

Structure of the human ear

Human ear is divided into three parts; outer ear, middle ear and inner ear.

Outer ear consists of pinna and auditory canal. Auditory canal is a slightly "S " shaped tube and lined by hairy skin with numerous modified sweat glands which secrete ear wax. Auditory canal extends to the tympanic membrane which is located in between the middle and the outer ear.

Middle ear (tympanic cavity) is an air filled cavity within the temporal bone. It is lined by simple epithelium. In the medial wall of the middle ear, there are two openings called oval window and round window. Oval window is covered by a small bone called stapes. Round window is covered by a fine fibrous tissue. Three very small bones (ear ossicles) called malleus, incus and stapes extend across the middle ear from tympanic membrane to the oval window. They form movable joints with each other and the medial wall of the cavity at the oval window. Malleus is in contact with the tympanic membrane and form a movable joint with the incus. Incus articulates with the stapes which fits with the oval window. A long tube called Eustachian tube connects the middle ear to the pharynx.

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Inner ear is formed from a network of channels and cavities in temporal bone which are called bony labyrinth. Within the bony labyrinth, a network of fluid filled membranes called membranous labyrinth is present which lines and fills the bony labyrinth. Inner ear is composed of three main regions: vestibule, three semicircular canals and cochlea. Vestibule is the expanded part near the middle ear. Oval and round windows are present in its lateral walls. Vestibule contains two membranous sacs called utricle and saccule. Semicircular canals are three tubes arranged at right angles to one another so that one is situated in each of the three planes of space. They are continuous with the vestibule. Cochlea is a coiled structure with the broad base which is continuous with the vestibule. Cochlea has three compartments: an upper vestibular canal, a lower tympanic canal and middle cochlear duct which is a small canal that separates the upper and lower canals. Vestibular canal originates at the oval window and tympanic canal ends at the round window. The two canals are continuous with each other and filled with perilymph. The cochlear duct is a part of the membranous labyrinth and filled with endolymph. The floor of the cochlear duct is called the basilar membrane which bears the organ of Corti (spiral organ). It contains supporting cells and specialized cochlear hair cells containing mechanoreceptors (auditory receptors) of the ear. Hairs of the cochlear hair cells project into the cochlear duct. Many hairs are attached to the tectorial membrane that hangs over the organ of Corti. Auditory receptors are dendrites of sensory nerves that combine to form the auditory nerve to the brain.

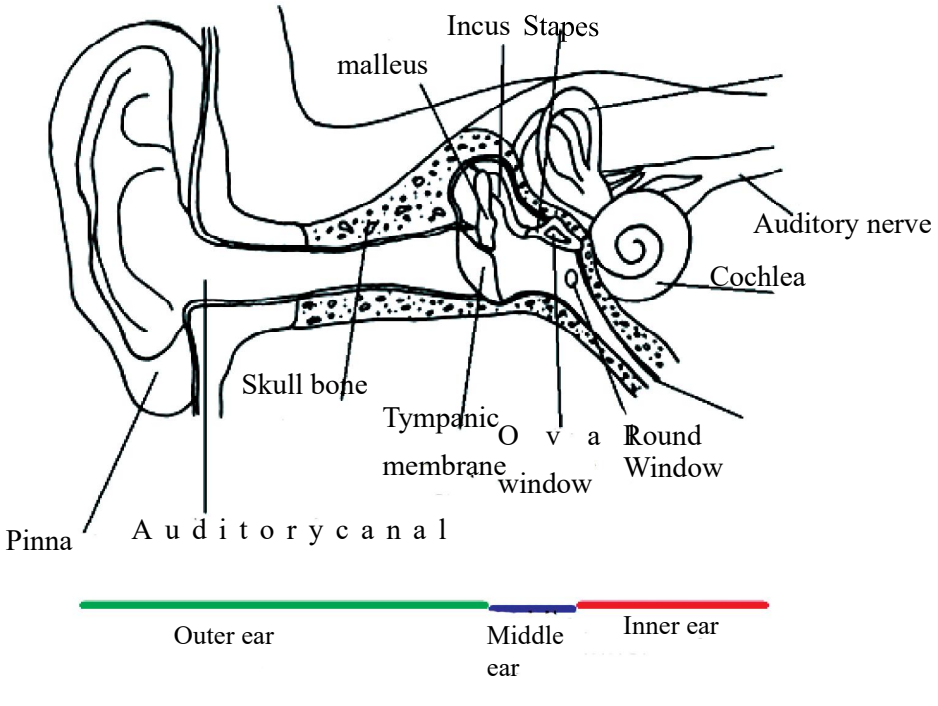
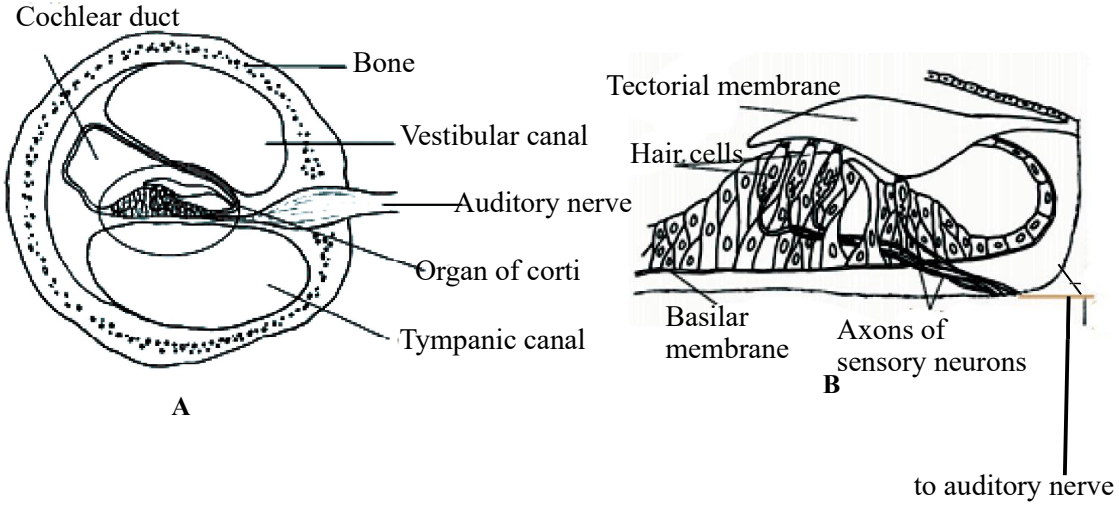
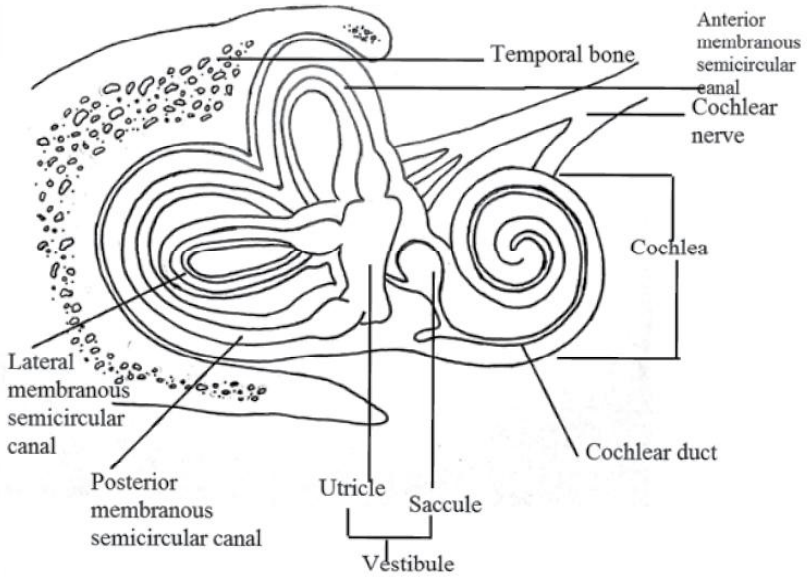


Figure:5.17-The typical structure of the human ear

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Figure 5.18: (a) . The Cochlea (b) . The organ of Corti (c) The semicircular canals

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Functions of the human ear

Hearing

Vibrating objects produce pressure waves in the surrounding air. In hearing, the ear transduces these pressure waves (mechanical stimulus) into nerve impulses that are transmitted to the brain which perceives as sound.

The outer ear collects and concentrates the sound waves and directs them along the auditory canal towards the tympanic membrane. This causes the tympanic membrane to vibrate. Tympanic membrane vibrations are transmitted and amplified through the middle ear by the movement of three jointed ear ossicles.

The ear ossicles transmit the vibrations to the oval window which is located on the membrane of the cochlear surface. When the stapes vibrates against the oval window, pressure waves are created in the perilymph inside the cochlea. When the fluid pressure waves enter the vestibular canal they push down on the cochlea duct and the basilar membrane. As a result, the basilar membrane and attached hair cells vibrate up and down. This causes bending of hair projecting from the hair cell against the fixed tectorial membrane which lies above the hair cells. This results in the stimulation of auditory receptors in the auditory hair cells and generation of nerve impulses. These nerve impulses are passed to the auditory area of the brain (temporal lobe of the cerebrum) for sound perception.

After the sound perception, the fluid wave is finally dissipated into the middle ear by vibration of the membrane of the round window. Eustachian tube maintains the air pressure on both sides of tympanic membrane at the atmospheric pressure level.

Equilibrium

Semicircular canals and vestibule located in the inner ear provide information about the position of the head in space and contribute to maintain the posture and balance.

Utricle and saccule of the vestibule perceive position with respect to gravity or linear movements. Each of these perilymph filled chambers contain hair cells that project into a gelatinous material in which small calcium carbonate particles (otolith) are embedded. When the head is tilted otoliths press on the hairs projecting into the gels. Hair cell receptors transform this deflection into an electrical signal and pass into cerebellum.

The semicircular canals, arranged in three spatial planes detect angular movements of the head. Within each canal, hair cells form a cluster with the hairs projecting into a gelatinous cap. Changes in the position of the head causes movements in the perilymph and endolymph. As a result hair cells are stimulated and resulting nerve impulses are transmitted to the brain.

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Basic structures and functions of the human skin

In the human body skin is the largest organ. It consists of two main layers which are the epidermis and the dermis. The layer underneath the skin is called subcutaneous layer which is composed of adipose tissue and areolar tissue.

Epidermis

Epidermis is the outermost layer of the skin which consists of stratified keratinized squamous epithelium. Epidermis is not supplied with blood vessels. But its deeper layers are provided with nutrients and oxygen by the interstitial fluid of the dermis finally drained away as lymph. There are several layers of cells in the epidermis. The deepest layer is the germinative layer from which epidermal cells are originated constantly. These cells undergo gradual changes as they progress towards the surface of the skin. The cells on the surface are flat, thin, non-nucleated and dead, in which the cytoplasm has been replaced by keratin which is a fibrous protein. The surface cells are constantly rubbed off and replaced by the cells underneath. In areas where the skin is subjected to wear and tear, the epidermis is thicker (e.g. palms and fingers of the hand, sole of the foot)

Melanocytes in the deep germinative layer secretes a dark pigment called melanin contribute to the skin colour. In addition extent of oxygen saturation in the circulating blood in the dermis, excessive levels of bile pigments and carotenes in the fat layer can affect the skin colour.

Dermis

Dermis is composed of areolar connective tissue. The matrix contains collagen fibers interlaced with elastic fibers. Collagen fibers bind water and give the skin its tensile strength. Fibroblasts, macrophages and mast cells are the main cells found in the dermis.

The structures present in dermis are

- blood and lymph vessels
- sensory nerve endings
- sweat glands
- sebaceous glands
- hair, arrector pili muscles
- sensory receptors (Meissner's corpuscle, Pacinian corpuscle, free nerve endings, bulb of Krause, organ of Ruffini, Merkle discs)

Functions of the human skin

- **Protection:** skin act as a defensive barrier against invasion by microorganisms, entrance of chemicals and physical agents and dehydration. The skin contains keratinized epithelium which is relatively water proof . This layer can protect deeper and more delicate structures. The skin contains specific immune cells which can phagocytose foreign invasions. The melanin pigments protect against the harmful effects of UV radiations.

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- **Regulation of the body temperature:** The skin contributes to regulation body temperature as it provides passage through which heat can be lost or gained depending on the body requirements. When body temperature is increased above the normal range, sweat glands secrete sweats onto the skin surface. Evaporation of sweat cools the body surface. When heat stressed, heat loss can be promoted by increasing the blood flow through the skin capillaries by dilating arterioles. When the body temperature falls beyond the normal range heat loss through the skin capillaries can be minimized by constricting arterioles in the dermis. When cold stressed, contraction of erector pili muscles attached to the hair can generate body heat and contribute to the heat production.
- **Cutaneous sensation:** Skin contains sensory receptors which are sensitive to touch, pressure, temperature and pain. Upon stimulation, nerve impulses are generated which are transmitted to the brain for sensory perception.
- **Synthesis of vitamin D:** Exposure to the sunlight can convert a lipid based substances in the skin to vitamin D.
- **Excretion:** Skin serve as a minor excretory organ. Sodium chloride, urea, and aromatic substances such as garlic can be excreted in sweat.

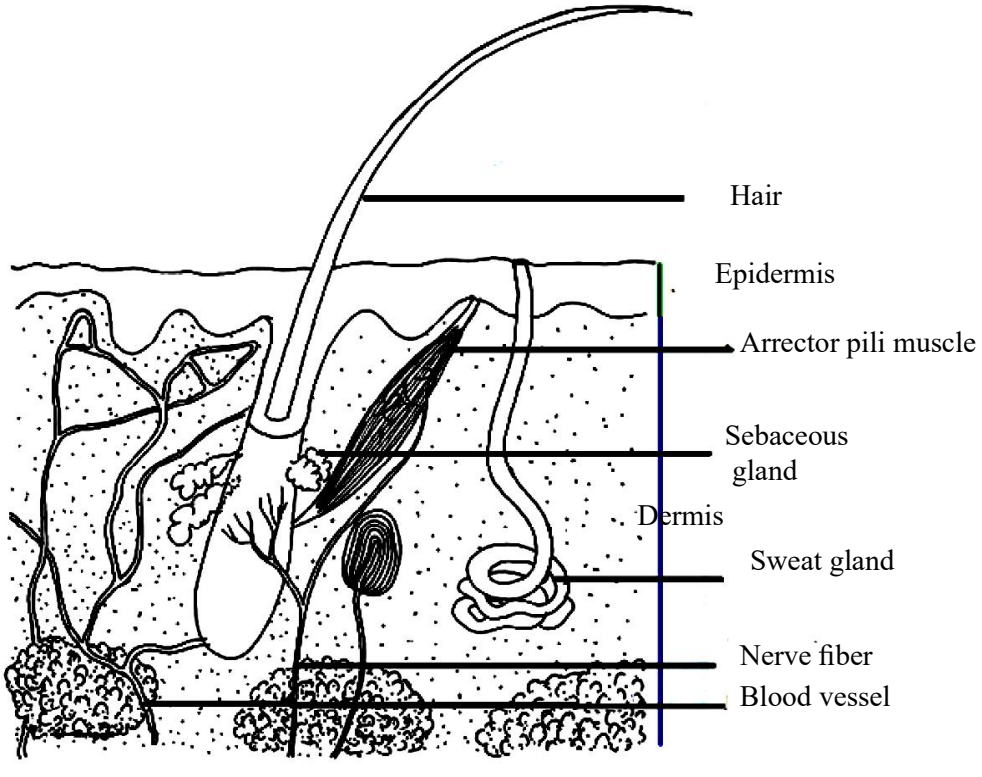


Figure 5.19: Typical structure of the skin

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The role of human endocrine system

Endocrine system is one of the two basic systems for coordination and regulation of activities in the human body. Compared to the nervous system, endocrine control is mainly involved in slower but more precise adjustments in maintaining homeostasis in the body. The endocrine system functions through "chemical signaling" by hormones which are secreted by specific endocrine glands or endocrine cells.

Endocrine glands are ductless glands consisting of groups of specialized cells which secrete hormones (chemical messengers) that diffuse directly into the bloodstream and reach the specific target organs/tissues that may be located quite distantly. Diffusion of hormones from these endocrine glands to the bloodstream is facilitated by the extensive capillary networks surrounding the glands.

Hormone is a specific type of signaling molecules secreted by an endocrine gland/endocrine cells and travels in the blood and acts on specific target cells elsewhere in the body, changing the target cell functioning. Although a specific hormone can reach all body cells, only the cells (target cells) which have matching receptors for that hormone are responsive to the chemical signal. When the hormone binds to the specific receptor of the target cell, it act as a switch influencing chemical/ metabolic reactions within the cell. Through chemical signals, hormones can communicate regulatory messages throughout the body.

Human endocrine system mainly consists of specific endocrine glands that are widely separated from each other. Location of endocrine glands of the human endocrine system is shown in the **Figure (Location of human endocrine glands).** Endocrine glands of the human endocrine system include hypothalamus, pituitary gland, thyroid gland, parathyroid glands, adrenal glands, islets of Langerhans (in the pancreas), gonads, thymus gland and pineal gland. In addition to these endocrine glands, isolated endocrine cells are found in some organs and tissues (e.g. stomach, small intestine, kidneys etc.) which secrete specific hormones (e.g. isolated endocrine cells in the stomach secrete the hormone, gastrin).

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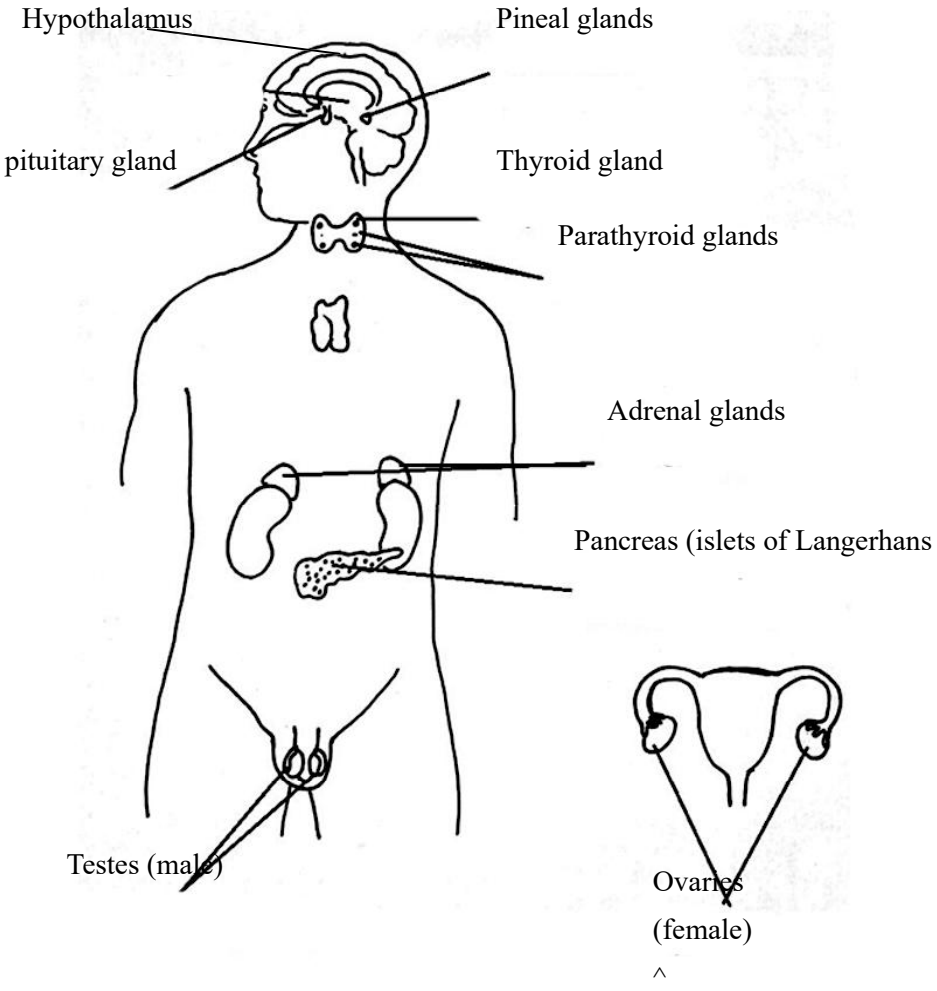


Figure 5.20: Location of human endocrine glands

Hypothalamus

Hypothalamus is located at the base of the fore brain just below the thalamus and connected to the pituitary gland. Seven hormones that are produced and released by the hypothalamus (five releasing hormones and two release inhibiting hormones), act on the anterior pituitary (target site). These hypothalamic hormones regulate the secretion of anterior pituitary hormones (Table Hypothalamic hormones that act on the anterior pituitary gland). Two other hormones produced

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by the hypothalamus (oxytocin and antidiuretic hormone) are stored in the posterior pituitary until they are released into the bloodstream and act on specific target sites.

Table 5.3: Hypothalamic hormones that act on the anterior pituitary gland

Hypothalamic hormone	Function
Growth hormone releasing hormone (GHRH)	Stimulates the secretion of growth hormone (GH) from anterior pituitary
Thyrotropin releasing hormone (TRH)	Stimulates the secretion of thyroid stimulating hormone (TSH) from anterior pituitary
Corticotropin releasing hormone (CRH)	Stimulates the secretion of adrenocorticotropic hormone (ACTH) from anterior pituitary
Gonadotropin releasing hormone (GnRH)	Stimulates the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from anterior pituitary
Prolactin releasing hormone (PRH)	Stimulates the secretion of prolactin hormone from anterior pituitary
Prolactin inhibiting hormone(PIH)	Inhibits the secretion of prolactin hormone from anterior pituitary
Growth hormone release inhibiting hormone (GHRIH)	Inhibits the secretion of GH and TSH from the anterior pituitary

Pituitary gland

Pituitary gland is situated in the fore brain just below the hypothalamus to which it is attached by a stalk. Pituitary gland consists of two main parts (anterior pituitary and posterior pituitary) which are actually two fused glands that perform different functions.

The anterior pituitary synthesizes specific hormones (**Table 5.4: Anterior pituitary hormones, their target sites and functions)**. The anterior pituitary connects with the hypothalamus through portal blood vessels. In response to specific releasing hormones secreted from the hypothalamus (Table: Hypothalamic hormones that act on the anterior pituitary gland), anterior pituitary secretes its specific hormones to the blood stream. Some hormones secreted by the anterior pituitary redirect the chemical signals from hypothalamus to other endocrine glands. These anterior pituitary hormones are called tropic hormones (TSH, ACTH, FSH and LH) as their specific target site is another endocrine gland or endocrine cell. The hormone prolactin which is secreted by the anterior pituitary is not a tropic hormone as its target sites are non-endocrine tissues. Prolactin promotes only non-tropic effects. Growth hormone (GH) secreted by the anterior pituitary has a "tropic as well as non-tropic effects" as its target sites can be endocrine cells as well as non-endocrine cells. GH is the most abundant hormone synthesized by the anterior pituitary.

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Table 5.4: Anterior pituitary hormones, their target sites and functions

Hormone	Target site	Function
Growth hormone (GH)	All body cells	Promotes tissue growth (especially bones and muscles) by stimulating protein synthesis; Regulates metabolism
Thyroid stimulating hormone (TSH)	Thyroid	Stimulates secretion of thyroid hormones (triiodothyronine and thyroxin); Stimulates growth of thyroid gland,
Prolactin	Mammary gland	Stimulates milk production; Together with other hormones promotes milk secretion by the mammary glands.
Adrenocorticotropic hormone (ACTH)	Adrenal cortex	Stimulates secretion of adrenal cortex hormones (Glucocorticoid hormones)
Follicle stimulating hormone (FSH)	Ovary	Stimulates growth and development of ovarian follicle
	Testis	Stimulate spermatogenesis
Luteinizing hormone (LH)	Ovary	Ovulation; promote formation of corpus luteum in the ovary (structure formed after ovulation) and stimulates progesterone hormone secretion by the corpus luteum.
	Testis	Stimulates secretion of testosterone hormone

The posterior pituitary which is an extension of the hypothalamus connecting via axons, does not synthesize hormones but secretes two hypothalamic hormones (oxytocin and antidiuretic hormone) to the bloodstream. Oxytocin and antidiuretic hormone (ADH) synthesized in the hypothalamic neurons travel through the long hypothalamic axons that reach into the posterior pituitary. These hormones are stored in the axon ends located in the posterior pituitary until they are released into the blood stream in response to nerve impulses transmitted from the hypothalamus. The target sites and functions of the hormones secreted by the posterior pituitary are given in the Table 5.5 **(Posterior pituitary hormones, their target sites and functions).**

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Table 5.5: Posterior pituitary hormones, their target sites and functions

Hormone	Target site	Function
Antidiuretic hormone (ADH)	Distal convoluted tubules of the nephrons and collecting ducts in the kidney	Stimulates resorption of water by increasing permeability to water
Oxytocin	Mammary gland	Stimulates milk ejection by stimulating contraction of smooth muscles
	Uterine muscles	Promotes parturition by contraction of smooth muscles

Thyroid gland

Thyroid gland is located in the neck just below the larynx and in front of the trachea. It has two lobes. Thyroid gland secretes triiodothyronine (T3) and thyroxin (T4) which are collectively called thyroid hormones. Thyroid hormones increase the basal metabolic rate and heat production; regulate the metabolism of carbohydrates, proteins and fats. Thyroid hormones are needed for normal growth and development of especially the skeletal and nervous systems. Thyroid hormones also help maintain normal blood pressure, heart rate and muscle tone and regulate digestive and reproductive functions. Calcitonin is another hormone secreted by the thyroid gland. Calcitonin helps to lower blood calcium ion level if it is raised above the normal limit. This hormone acts on bone cells and promotes storage of calcium within bone tissues. The hormone also acts on kidney tubules and inhibit calcium reabsorption enhancing calcium excretion.

Parathyroid glands

Parathyroid glands (a set four small glands) are embedded in the posterior surface of the thyroid gland located in the neck. Two glands are embedded in each lobe of the thyroid gland. Parathyroid glands secrete parathyroid hormone (PTH). Main function of the PTH is to promote high calcium levels in the blood by stimulating calcium reabsorption from the kidney tubules and calcium absorption through the small intestine. If these sources supply inadequate calcium, PTH acts on bone destroying cells and promotes release of calcium from the bones into the blood. PTH has the opposite effect of calcitonin hormone (released by the thyroid gland) on the blood calcium level.

Thymus gland

Thymus gland is located in the upper part of the chest, directly behind the sternum and between the lungs. Thymus gland secretes the hormone thymosin. Thymosin acts on the lymphocytes (originated from the stem cells in the bone marrow) and regulates development and maturation of T lymphocytes which are important components of specific immunity.

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Pineal gland

Pineal gland is located in the brain. Melatonin secreted by the pineal gland is involved in the regulation of biological rhythms related to reproduction and daily activity levels. Melatonin seems to be associated with coordination of circadian and diurnal rhythms of many tissues and inhibition of growth and development of sex organs before puberty.

Adrenal glands

Adrenal glands are paired structures, one of which lies superior to each kidney. Each gland is consists of two parts: adrenal cortex (outer) and adrenal medulla (inner). The structure and functions of these two parts are different. Hormones secreted by the adrenal cortex and the adrenal medulla can mediate the stress responses in the body.

The hormones mainly produced by the adrenal cortex are glucocorticoids and mineralocorticoids. These hormones mediate "long term stress responses" and participate in homeostatic regulation of metabolism. Glucocorticoids have a main effect on glucose metabolism and promote glucose synthesis from non-carbohydrate sources such as protein and fat so that more glucose is available in the blood circulation for cellular energy production. These hormones can promote breakdown of skeletal muscle proteins for synthesis of glucose when the body requires more glucose. Cortisol is the main glucocorticoid produced by the adrenal gland. Main mineralocorticoid produced by the adrenal gland is aldosterone which is involved in maintaining water and electrolyte balance. Aldosterone stimulates the reabsorption of sodium ions by the kidney tubules and excretion of potassium ions in the urine. As sodium reabsorption is accompanied by the water retention, blood volume and blood pressure can be increased. Hence aldosterone hormone is also involved in the regulation of blood volume and blood pressure.

The hormones produced in the adrenal medulla are adrenaline (epinephrine) and noradrenaline (norepinephrine) which could mediate 'short term stress responses'. Upon extensive sympathetic nervous stimulation, adrenal medulla secretes these hormones which can potentiate the "fight or flight response" by increasing the heart rate and blood pressure, diverting blood to essential organs (i.e heart, brain, skeletal muscles) and increasing metabolic rate etc. The hormones secreted by adrenal medulla are mainly involved in increasing the availability of chemical energy for immediate use. These hormones promote glucose release into the circulating blood by increasing the rate of glycogen breakdown (in liver and skeletal muscles) and fatty acids release (from fat cells) for energy production within the body cells.

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Islets of Langerhans in the pancreas

Pancreases can be considered an endocrine gland as well as an exocrine gland. It is located behind the stomach in the curve of the duodenum. The endocrine part of the pancreas is the islets of Langerhans which are clusters of cells scattered throughout the pancreas. These pancreatic islets mainly secrete two hormones, glucagon and insulin which control the blood glucose level by opposing actions. Alpha cells of the pancreatic islets secrete glucagon which mainly promote the blood glucose level increase. Beta cells of the pancreatic islets secrete insulin which promotes lowering of blood glucose level. Liver and skeletal muscles are the main target sites of these hormones (Refer the section on homeostatic control of blood glucose).

Gonads

Paired female gonads (ovaries) are located in the pelvic cavity. Paired male gonads (testes) lie in the scrotum. In addition to the reproduction, ovaries and testes have endocrine functions. (Refer the section on human male and female reproductive systems for more details).

Ovarian follicle produces the hormone estrogen. Corpus luteum (the structure formed from the ovarian follicle after ovulation) produces progesterone. These female sex hormones along with FSH and LH from the anterior pituitary regulate menstrual cycle, maintain pregnancy and prepare mammary glands for lactation. They also help establish and maintain feminine sexual characteristics. The ovaries also produce the hormone inhibin that inhibits secretion of FSH from anterior pituitary.

The main hormone produced and secreted by the testes (interstitial cells) is the male sex hormone, testosterone. Testosterone regulates production of sperm and stimulates the development and maintenance of masculine secondary sex characteristics. In addition, the testes (Sertoli cells) produce inhibin that inhibits secretion of FSH.

Feedback mechanisms related to the endocrine system

Variety of physiological processes in the human body including the actions of hormones on target cells are regulated by feedback mechanisms. Feedback refers to the regulation of a process by its output or end product.

Most hormonal controls in the human body use negative feedback mechanisms where accumulation of an end product of a process (the response to the stimulus) slows that process (reduces the effect of the initial stimulus). The endocrine gland will release its hormone into the blood only when the gland is stimulated and the response at the target site will in turn reverse or reduce the stimulus through the negative feedback. In the absence of stimulation, the blood level of hormone will

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decrease. Some hormone levels in the blood can be directly controlled by the blood levels of the stimulus (e.g. insulin or glucagon by blood glucose levels). For example high blood glucose levels stimulate the release of insulin hormone (from the pancreas) to the circulating blood which acts on specific target tissues to lower the blood glucose level. When glucose level in the blood reaches normal range, blood glucose level can in turn directly control the secretion of insulin levels from the pancreas and prevent further lowering of the glucose level in the blood. (Refer the section on homeostatic control of blood glucose level).

A few hormonal regulatory systems operates using "positive feedback mechanism" which is a form of regulation in which an output (or end product) of a process speeds up that process thereby reinforcing or amplifying the change. Positive feedback mechanisms involving oxytocin hormone operate in childbirth and breast milk ejection. During labour, contractions of uterus are stimulated by oxytocin hormone released by the posterior pituitary. These contractions force the baby's head into the uterine cervix stimulating its stretch receptors. In response to stimulation of stretch receptors, sensory neurons are stimulated again triggering more oxytocin release from the posterior pituitary enhancing contractions of the uterus. This process repeats until the baby is born. Afterwards oxytocin secretion stops as the stimulus (stretching of the cervix) is no longer present. Another positive feedback mechanism involving oxytocin hormone operates when releasing milk from the mammary glands **(Figure5.21: Positive feedback mechanism related to oxytocin hormone action)**. During suckling, sensory neurons send the nerve impulses to the posterior pituitary triggering release of oxytocin hormone to the circulating blood. Then oxytocin acts on the mammary glands and induces contractions of smooth muscles in the mammary glands to release milk. Milk release increases the sensory stimulus forming a positive feedback that amplifies the stimulus. In response to the positive feedback, more oxytocin is released enhancing milk ejection

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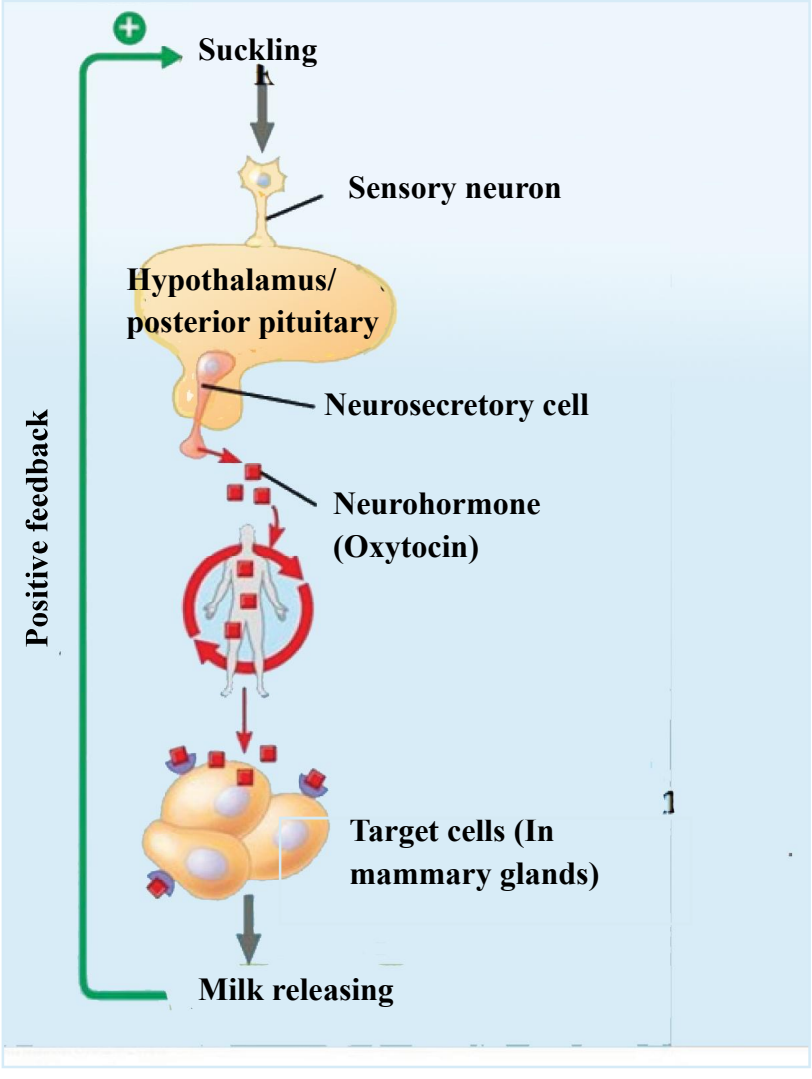


Figure 5.21: Positive feedback mechanism related to oxytocin hormone action

Some endocrine disorders in human

- **Diabetes mellitus:** Diabetes mellitus is a common disorder associated with insulin hormone produced by the pancreatic islets of Langerhans. Primary sign of this disorder is the increase in blood glucose levels above the normal limits. High blood glucose levels lead to excretion of glucose with urine, excessive production of urine and the thirst. Diabetes mellitus is mainly classified into two types: Type 1 diabetes and Type 2 diabetes.

  Type 1 diabetes was known as insulin dependent diabetes mellitus. This disorder usually appears in children and young adults. This is an autoimmune disorder caused by the destruction of beta cells of the islets of Langerhans by the immune system in the body. As

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a result, insulin secretion is severely deficient or absent in the affected individuals. Genetic factors and environment factors seem to be associated with this disorder. Type 1 diabetes may be controlled by taking meals with less carbohydrates and fats, regular monitoring of blood glucose levels and periodic insulin injections.

Type 2 diabetes was known as non-insulin dependent diabetes mellitus. This condition is not dependent on insulin production. Even though insulin is produced and secreted into the blood, target cells fail to take up glucose from the blood. Hence blood glucose levels remain elevated but glucose may be deficient inside the body cells. The cause for this type of diabetes is multifactorial. Predisposing factors include obesity, lack of exercise (sedentary lifestyle), increasing age and genetic factors. Type 2 diabetes may be controlled by the diet with less carbohydrates and fats, balancing sugar intake with exercise and taking suitable medicine.

- **Hyperthyroidism and hypothyroidism:** These conditions are associated with abnormal secretion of thyroid hormones (T3 and T4) which may occur due to abnormal functioning of the thyroid gland and disorders of pituitary or hypothalamus. Persistence of he these conditions may lead to enlargement of the thyroid gland (goiter).

  Hyperthyroidism: This condition occurs due to exposure of body tissues to excessive levels of T_3 and T_4. Common effects include increased basal metabolic rate, weight loss, warm, sweaty skin and diarrhea. Some conditions lead to bulging of eyes (exophthalmos) and goiter. Treatment may include surgical removal of part or all of the thyroid gland and using medicine to block thyroid hormone synthesis.

  Hypothyroidism: Insufficient secretion of thyroid hormones (T3 and T4) from the thyroid gland causes hypothyroidism. This can be due to lack of TSH production by anterior pituitary or iodine deficiency in diet. Common effects include low basal metabolic rate, weight gain, lethargy, dry, cold skin and constipation. The condition may be controlled by increasing dietary iodine intake or/and oral thyroid hormone treatment.

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Maintenance of constant internal environment within limits in the human body

Homeostasis

Homeostasis is a steady state condition where body's internal environment remains relatively constant within narrow physiological limits despite significant changes in the external environment. Surroundings outside of the body are referred to as the external environment. Internal environment is the immediate surroundings of the body cells which provides the cells with the medium in which they have to live. Examples for the internal environment of the body are interstitial fluid and the blood. Many animals and humans exhibit homeostasis for a range of physical and chemical properties. For example, humans maintain a fairly constant body temperature, blood pH, blood glucose and blood osmolality within narrow physiological limits. Homeostasis is important for maintaining the internal environment in a steady and balance state and to establish optimum conditions for the human body.

Homeostatic control systems in the human body mainly depend on negative feedback mechanisms to maintain a constant level thereby preventing serious changes in the internal environment. Homeostasis is achieved by maintaining a variable (e.g. body temperature, blood glucose) at or near a particular value (set point). A fluctuation in the variable above or below the set point serves as the stimulus detected by a sensor (detector). When a signal is received from the sensor, a control center generates output that triggers a response, a physiological activity that helps to return the variable towards the set point level. The set point level is achieved by the negative feedback control of the stimulus by the response.

Homeostatic regulation of body temperature in humans

As the temperature affects the rate of chemical reactions, homeostatic control keeps the human body at optimum operating temperature. The normal body temperature of man is typically 37^0C (36.5^0C – 37.5^0C). Human body temperature is controlled by negative feedback mechanisms. If the body temperature is outside the normal range, a group of nerve cells in the hypothalamus of the brain ("body's temperature control center") functions as a thermostat and responds to the temperature increase or decrease by activating heat loss mechanisms or promoting heat gain mechanisms respectively until the body temperature reaches the preset level.

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High peripheral temperature (e.g. when the person is in hot surroundings) is detected by warm receptors in the skin. High deep body temperature (e.g. due to high body heat generation after exercise) is detected by hypothalamic temperature sensitive nerve endings when warm blood pass through the hypothalamus. These nerve impulses are sent to the "body's temperature control center" (thermostat) in the hypothalamus. In response to the increase in body temperature above the preset level, the 'thermostat" in the hypothalamus sends impulses to activate heat loss mechanisms and to inhibit heat gain mechanisms that lower the body temperature until the set point.

The following heat loss mechanisms promote the decrease in body temperature.

- dilation of blood vessels in the skin which causes filling of blood capillaries with warm blood and radiating heat from the skin surface
- increase sweat secretion from the sweat glands which promotes heat dissipation through evaporative cooling

When body temperature is within the normal range again, the warm temperature sensitive receptors are no longer stimulated and their signals to the 'hypothalamic thermostat" stops due to the negative feedback mechanism. Then, additional heat loss mechanisms stop and blood flow to the peripheries returns to normal.

Low peripheral temperature (when in cold surroundings) is detected by cold receptors in the skin. Low deep body temperature (due to more heat loss and low heat generation in the body) is detected by temperature sensitive nerve endings in the hypothalamus. These nerve impulses are sent to the body's temperature control center (thermostat) in the hypothalamus. If the body temperature decreases below the preset level, the thermostat in hypothalamus sends impulses to activate heat gain mechanisms and inhibit the heat loss mechanisms thereby increasing the body temperature until the preset point.

The following heat conservation and heat gain mechanisms promote the increase in body temperature.

- constriction of blood vessels in the skin which divert the blood from the skin to deeper tissues thereby reducing heat loss through the skin surface
- shivering: rapid repetitive contractions of skeletal muscles to generate heat
- contracting hair erector muscles to generate heat to some extent
- stimulating secretions of more thyroid hormones (e.g. thyroxin) and adrenalin into the blood which increase the metabolic rate and cellular metabolism (especially oxidation of fat in the liver) to produce more heat

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When body temperature returns to the normal range, the cold temperature sensitive receptors are no longer stimulated and their signals to the hypothalamic thermostat stop due to negative feedback mechanism. Then, additional heat generating mechanisms in the body stop and blood flow to the peripheries returns normal.

Homeostatic regulation of blood glucose level

In humans, normal blood glucose level is 70 – 110 mg/100 mL (while fasting) which is sufficient for immediate needs of the body cells. The blood glucose levels can fluctuate throughout the day within physiological limits in non-diabetic persons. In the human body, blood sugar levels are homeostatically controlled by opposing actions of two hormones secreted by the pancreas: insulin and glucagon.

High blood glucose levels exceeding the normal limits, stimulate the secretion of insulin hormone from beta cells of the islets of Langerhans into the circulating blood. Insulin acts on specific target tissues to promote lowering of the blood glucose level. Insulin in the circulating blood stimulates transport of glucose into the body cells and use of glucose by body cells for ATP production (glucose may be broken down into carbon dioxide and water), conversion of glucose to glycogen in liver and skeletal muscle cells for storage, and conversion of glucose to fatty acids and storage of fat in adipose tissues. When glucose level in the blood reaches normal range, blood glucose level can in turn directly control the secretion of insulin levels from the pancreas through negative feedback. This mechanism prevents further lowering of the glucose level in the blood beyond the normal limits.

Low blood glucose levels below the normal limit, stimulate the secretion of glucagon from alpha cells of the islets of Langerhans into the circulating blood. Glucagon acts on specific target tissues to promote increase of the blood glucose level. Glucagon promotes the breakdown of glycogen in the liver and skeletal muscles and release of glucose into blood. When glucose level in the blood reaches normal range, blood glucose level can in turn directly control the secretion of glucagon levels from the pancreas through negative feedback which prevents further increasing of the glucose level in the blood beyond the normal limits.

Osmoregulation

Osmoregulation is the process of maintaining water and salt balance (osmotic balance) across membranes within the body's fluids relative to the surrounding. When there is osmotic balance, amount of water and concentration of salts is same inside and outside the cells. Osmoregulation is important for organisms to keep a constant, optimal osmotic pressure within the body. In the humans, osmoregulation ensures that the total blood volume and the concentration of dissolved substances in the plasma and tissue fluids remain constant within a favourable range.

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In the human body, osmotic balance is achieved by two ways: controlling the amount of water and controlling the amount of salt gained and lost by the body. Blood water homeostasis is controlled by the hypothalamus. Hypothalamus contains osmoreceptors which can detect the osmolarity of the blood passing through the brain. In response to the osmolarity (or osmotic pressure) of the blood, the hypothalamus controls the sensation of thirst, and secretion of the hormone ADH from the posterior pituitary.

When blood osmolarity is increased beyond the physiological limits, it is sensed by the osmoreceptors in the hypothalamus which stimulates the posterior pituitary to release ADH to the blood circulation. ADH acts on the kidney tubules and stimulates the reabsorption of water through distal convoluted tubules of the nephrons and collecting ducts producing concentrated urine. When blood osmolarity is decreased, ADH is not secreted, so water reabsorption through distal convoluted tubules of the nephrons and collecting ducts stop thereby producing diluted urine. In addition, low blood volume and low blood sodium ions stimulate the kidneys to produce angiotensin II which stimulates the adrenal cortex to secrete aldosterone hormone. Aldosterone stimulates the reabsorption of sodium ions by the kidney tubules which is accompanied by the water retention, thereby increasing blood volume and blood pressure. Hence, kidneys play a major role in osmoregulation in the human body.

Role of the liver in homeostasis.

Liver is an active organ and plays an important role in maintaining homeostasis of the human body. The functions of the liver include the following.

- **Carbohydrate metabolism:** The liver plays an important role in maintaining blood glucose levels within normal ranges. When blood glucose is increased (e.g. after a meal), glucose is stored as glycogen under the stimulation of insulin. If blood glucose level is reduced (e.g. starvation), glycogen is converted back to glucose under the influence of glucagon hormone.
- **Fat metabolism:** When the body needs excess energy, fats that are stored in the liver cells are metabolized to produce ATP
- **Protein metabolism:** In the liver cells, nitrogen part of some amino acids that are not needed for new protein synthesis are removed (deamination) and excreted in urine or transferred to carbohydrates to synthesize new nonessential amino acids (transamination). Liver also synthesizes plasma proteins (e.g. albumin, globulins) from amino acids.
- **Breakdown of erythrocytes and defense against microbial infections:** In humans, liver is a site for red blood cell breakdown. Macrophages located in the liver are involved in microbial defense.
- **Detoxification of drugs and toxicants:** The liver plays an important role in detoxification.
- **Production of heat:** Liver is the major heat producing organ of the body as it has a high metabolic rate.

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- **Storage of nutrients:** Glycogen, fat soluble vitamins (A, D, E,K), some water soluble vitamins- B12 and essential metals such as iron, copper are stored in liver.
- **Inactivation of hormones:** Some hormones are inactivated by the liver after their biological action.
- **Secretion of bile:** Liver cells synthesize the components present in the bile which is important in fat digestion and excretion of bilirubin (a breakdown product of red blood cells).

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Reproduction

Modes of reproduction seen among animals

Reproduction is a biological process through which a new generation of individuals is produced form the existing organisms. Two modes of reproduction are found among the animals: Asexual reproduction and Sexual reproduction. For the most animals, reproduction is mainly or entirely sexual. Several forms of asexual reproduction are also seen especially among invertebrates.

Asexual reproduction

Asexual reproduction is a mechanism through which new individuals are generated from a single parent without the fusion of egg and sperm. Asexual reproduction relies entirely on mitotic cell division. Asexual reproduction allows the rapid multiplication of individuals from single parents. Hence no time or energy is spent in searching the mates for the reproduction. The produced offspring are genetically identical to each other and to the single parent. Several methods of asexual reproduction are found among invertebrates. They include budding, fragmentation and regeneration, and parthenogenesis.

- **Budding:** Budding is a form of asexual reproduction in which new individuals arise form outgrowths of the animal. e.g. Hydra – A localized mass of mitotically dividing cells, develops into a small Hydra, which can eventually detach from the parent.

- **Fragmentation an**d regeneration: This is a form of asexual reproduction which involves breaking of the body or part of the body into several pieces, followed by the growth of a separate individual from each piece. Each fragment develops into a complete animal by regrowth of lost body parts (regeneration). e.g. Certain annelid worms, numerous sponges, cnidarians.

- **Parthenogenesis:** This is an unusual form of asexual reproduction in which an egg develops into a complete individual without being fertilized. Among invertebrates, parthenogenesis occurs in some animals such as bees, ants, aphids and wasps. The progeny can be haploid or diploid. In honey bees – males (drones) are fertile haploid adults that develop through parthenogenesis, whereas females including both sterile workers and the fertile queen, are diploid adults that developed from fertilized eggs. Parthenogenesis has also been observed very rarely among vertebrates (e.g. some lizards and fish).

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Sexual reproduction

Sexual reproduction is a mechanism through which a new individual is developed from a diploid zygote as a result of the fusion of two haploid gametes (the sperm and the egg) which are produced by two individuals (the male and the female parents respectively). The female gamete, the egg, is large and non-motile, while the male gamete, the sperm, is generally much smaller and motile. The fusion of the female and male gametes forms a diploid cell called the zygote. The animal that develops mitotically from a zygote can in turn give rise to gametes by meiosis. For the most animals, reproduction is mainly or entirely sexual.

- **Gamete formation:** In animals, reproductive cells called gametes (sperms and eggs) are the vehicles that transmit genes from one generation to the next. Each gamete is a haploid, unicellular reproductive cell. Gametes in animals are formed by meiosis in special organs called gonads.

- **Bisexual organism and Unisexual organism:** In a bisexual organism (Hermaphrodite), one individual has both female and male reproductive structures and therefore is able to produce both male and female gametes by the same individual. e.g. Earthworms. In unisexual organisms (dioecious), one individual bears either male or female reproductive structures. Therefore production of male or female gametes occurs in separate organisms. e.g. Humans

- **Fertilization:** Union of egg and sperm (gametes) culminating in fusion of their nuclei is called fertilization. Fertilization can be either external or internal.

  External fertilization occurs in aquatic environments. In species with external fertilization, the female releases eggs while the male releases sperm into the external environment and fertilization occurs in the water. A moist habitat is almost always required for external fertilization both to prevent the gametes from drying out and to allow the sperm to swim toward the eggs. e.g. Many invertebrates, amphibians, most bony fish.

  In internal fertilization, sperms are deposited in or near the female reproductive tract and fertilization occurs within the female reproductive tract. e.g. insects, reptiles, mammals. Internal fertilization is an adaptation that enables sperm to reach an egg even when the environment is dry. The male copulatory organ delivers sperm and the female reproductive tract often has receptacles for storage and delivery of sperm to mature eggs. Internal fertilization is typically associated with the production of fewer gametes than external fertilization but results in the survival of a higher fraction of zygotes. Internal fertilization often provides greater protection to the embryos. Many animals show parental care. Internally fertilized eggs of birds and other reptiles possess shells and internal membranes that protect against water loss and physical damage. Some retain the embryo for a portion of any development within the female reproductive tract.

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Significance of asexual reproduction and sexual reproduction

Asexual reproduction and sexual reproduction are important biological processes to ensure existence of a species.

Asexual reproduction allows rapid multiplication of individuals from single parents. Hence no time or energy is needed to be spent in searching mates for the reproduction. In asexual reproduction, there is no or very little genetic variation within a population as the produced offspring are genetically identical to each other and to the single parent. Therefore, asexual reproduction would be more advantageous in stable, favorable environments as it can propagate successful genotypes specifically. However, any mutation in the parent cell, can cause harmful effects on the survival ability of the offspring in changing environments. If there is a harmful mutation in the organisms, environment changes could be deadly to all the individuals in the population.

Unlike asexual reproduction, sexual reproduction results in the production of a unique offspring by combining genetic materials from two parents. Meiotic recombination during sexual reproduction helps produce varied genotypes. Unique gene combinations formed during sexual reproduction would be advantageous as this can enhance reproductive success and survival of a species in changing environments. Beneficial gene combination arising through recombination may speed up adaptation. During sexual reproduction, shuffling of genes might allow a population to clear sets of harmful genes more readily which would enhance the survival of the species.

Structure and Function of the Human Male Reproductive System

Main internal reproductive structures of a male consist of gonads (testes) which produce sperm and reproductive hormones, epididymis which store mature sperms, accessory glands that secrete products required for sperm movement and ducts that transport the mature sperm and glandular secretions. The external reproductive organs of a male are the scrotum and penis.

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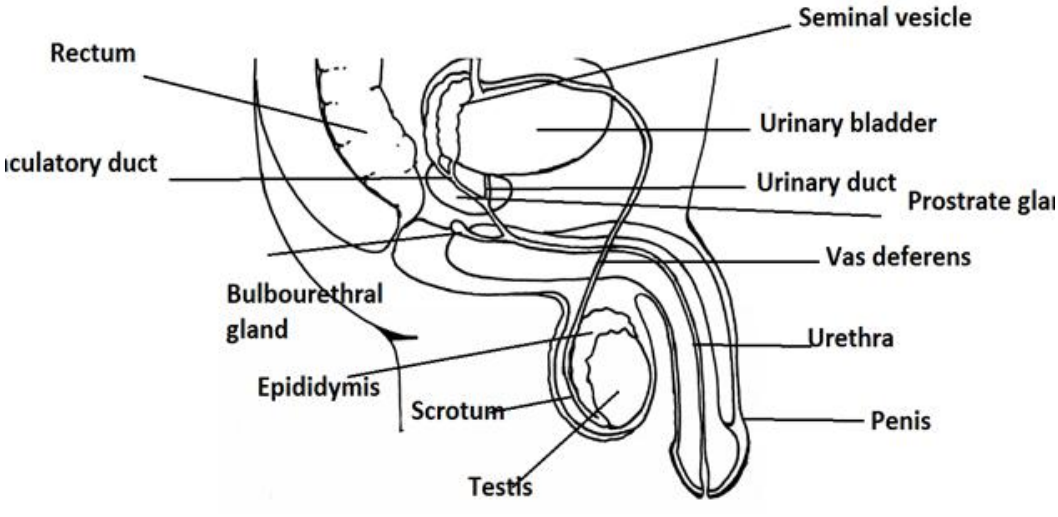


Figure 5.22: The gross structure of male reproductive system. (For orientation, some non-reproductive structures are also labelled)

Scrotum: Scrotum is a pouch formed by a fold of the body wall. Scrotum is divided into two compartments. Each compartment contains one testis, one epididymis and part of the spermatic cords which suspend the testis in the scrotum.

- **Testes:** The testes are found within the scrotum which allows the maintenance of the testis temperature 2ºC below the core body temperature. Sperms are produced properly only when the testes are cooler than the rest of the body. The testes develop in the abdominal cavity and descend into the scrotum just before birth. The testes are cooled by their position outside the abdominal cavity and the thin covering of the scrotum. Each testis contains many lobules. Within each lobule there are highly coiled (convoluted) loops called **seminiferous tubules.** Sperms are produced within these tubules. Different cells that undergo spermatogenesis are surrounded and connected to special type of supporting cells **(Sertoli cells)** located in the seminiferous tubule. Sertoli cells extend from the wall of the seminiferous tubules to the lumen. These cells secrete the hormone inhibin and nourish and provide attachment for cells that are in different stages of spermatogenesis. Groups of cells called Leydig cells (or interstitial cells) are present in the connective tissue between the seminiferous tubules. **Leydig cells** secrete the hormone testosterone and other androgens after puberty which promote spermatogenesis in the tubules. The seminiferous tubules combine to form a single tubule (duct) at the upper part of the testes.

- **Epididymis:** Epididymis is the tightly packed mass that is formed by the repeatedly folded long duct originated from combined seminiferous tubules in the testis. From the seminiferous tubules, the sperm pass into the epididymis. Since it is very long (about 6 m

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in length), it takes about 3 weeks for sperm to travel the length of this duct. During this time the sperms become matured and motile. Matured sperms are stored within the epididymis until ejaculation.

- **Vas deference, ejaculatory duct, urethra and penis:** During ejaculation, sperm are propelled form each epididymis through a muscular duct, the **vas deferens**. Each vas deferens (one from each epididymis) extends around and behind the urinary bladder, where it joins a duct from a seminal vesicle, forming a short **ejaculatory duct.** The ejaculatory ducts open into the urethra which is the passageway for both urinary excretion and delivery of sperms in semen into the female reproductive tract. The urethra runs through the penis and opens to the outside at the tip of the penis which contains erectile tissues derived from modified veins and capillaries.

Spermatogenesis

Spermatogenesis is the process of male gamete formation which includes formation of spermatocytes from a spermatogonium, meiotic division of the spermatocytes, and transformation of the four resulting spermatids (from each spermatocyte) into spermatozoa (sperm). Spermatogenesis occurs within the seminiferous tubules of the testes. The time taken to produce mature sperm cells from a specific spermatogonia in a seminiferous tubule is about seven weeks from start to finish.

The formation and development of sperms is continuous and inexhaustible in adult human males. Cell division and maturation during gametogenesis occur throughout the seminiferous tubules. Hundreds of millions of sperm are produced each day by spermatogenesis. In contrast to oogenesis (discussed later), in spermatogenesis all four cells produced during meiosis of each spermatocyte develop into mature gametes. Spermatogenesis starts at puberty and occurs throughout life. Spermatogenesis produces mature sperms from precursor cells in a continuous sequence.

Main steps in Spermatogenesis

- The stem cells (2n) that give rise to sperms (Spermatogonial stem cells) arise from mitotic division and differentiation of primodial germ cells in the embryonic testes. These stem cells are situated near the edge of the seminiferous tubules. In mature testis, their progeny moves inward as they pass through the different stages of maturation.
- In mature testes, the stem cells divide mitotically to form Spermatogonia (2n), which in turn generate primary spermatocytes (2n) by mitosis.
- Each primary spermatocyte gives rise to four spermatids (n) through meiosis (meiosis 1 and II), reducing the chromosome number from diploid (2n=46 in humans) to haploid (n=23).
- These spermatids differentiate into sperms with its characteristic head, midpiece and tail.
- The sperms are released into the fluid-filled lumen of the tubule and they travel along the tubule into the epididymis, where they become mature and motile.

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After puberty, Leydig cells present between the seminiferous tubules secrete the hormone testosterone which promotes spermatogenesis. Different cells that undergo spermatogenesis are surrounded and connected to special type of supporting cells called Sertoli cells. These cells extend from the wall of the seminiferous tubules to the lumen. These cells secrete inhibin hormone and nourish and provide attachment for cells that are in different stages of spermatogenesis.

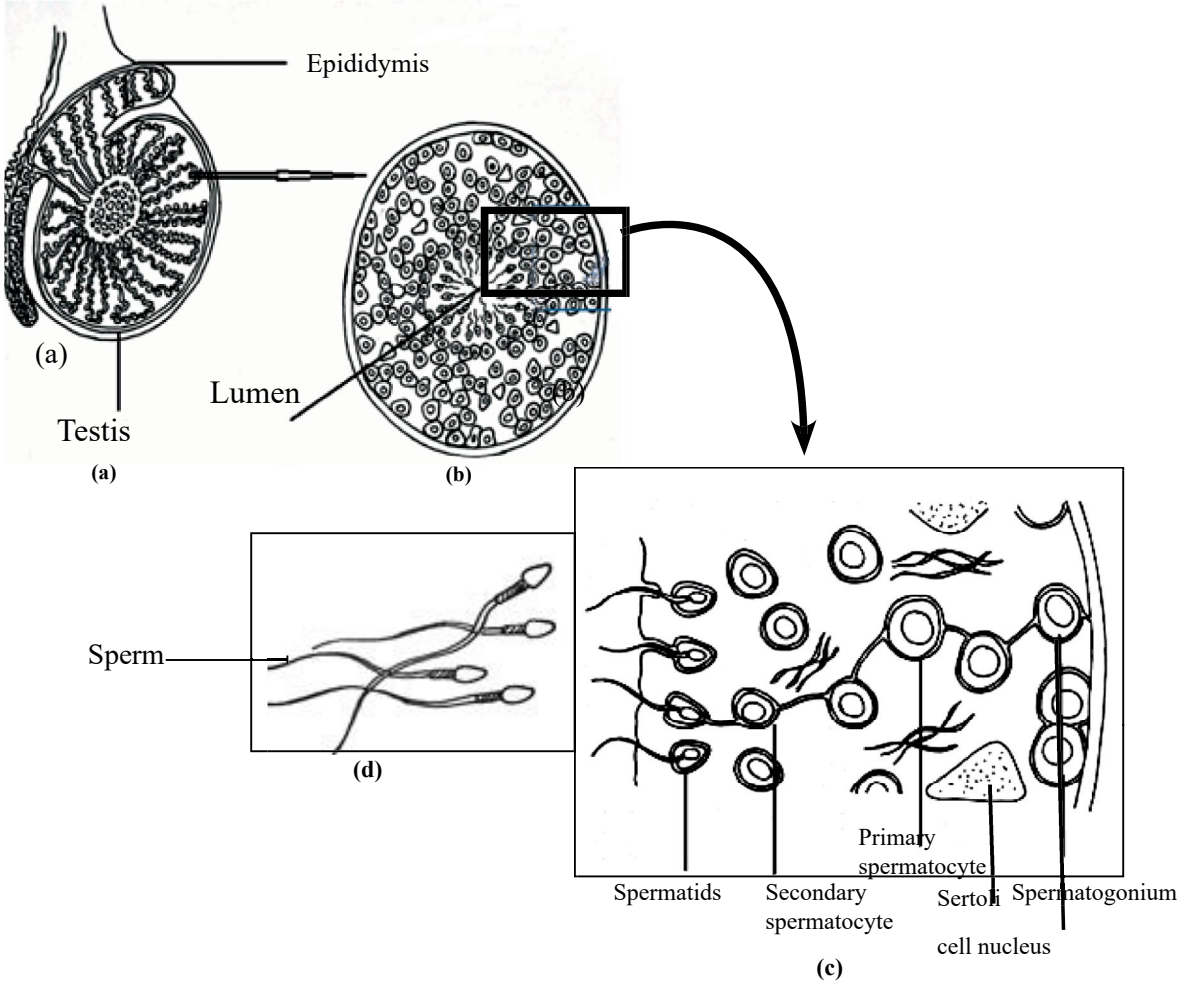
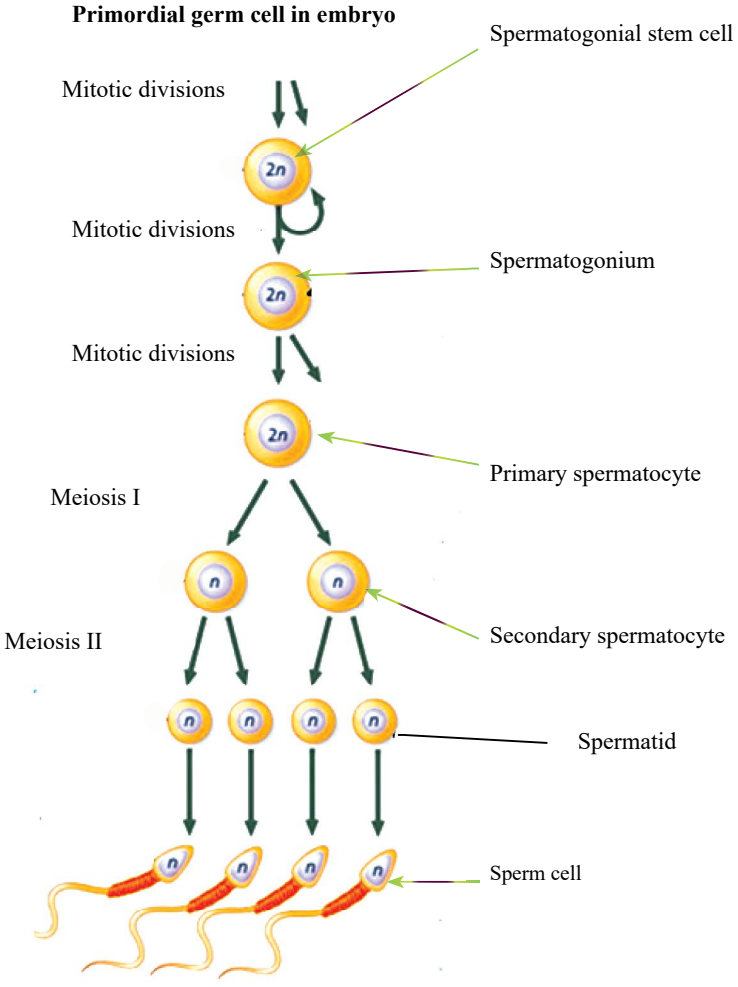


Figure 5.23: (a) T.S of testis (b) T.S of Seminiferous tubule (c) spermatogenesis (d) mature sperm released into lumen of seminiferoud tublue

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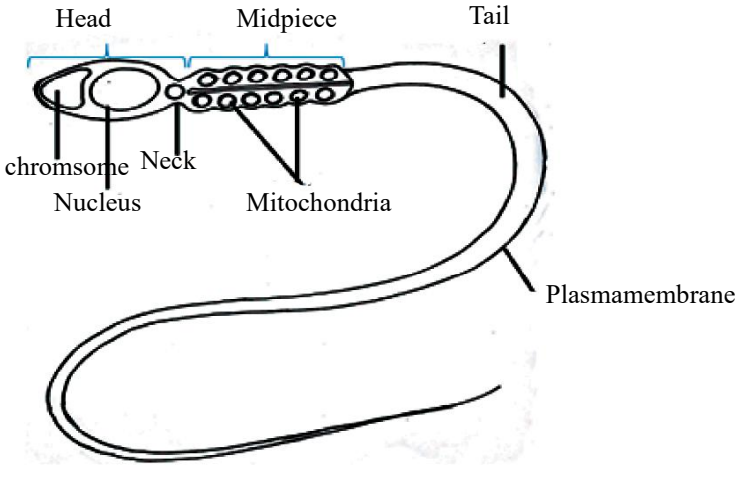


Figure 5.24: -Basic structure of the sperm

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Each sperm is made up of three main parts, the head, midpiece (body) and tail.
Head of the sperm contains the haploid nucleus carrying the paternal genetic material. A special vesicle called the acrosome is present at the anterior end of the head. It contains hydrolytic enzymes such as trypsin and hyaluronidase that help the sperm to penetrate the outer layers of the ovum. Midpiece of the sperm contains many mitochondria that provides ATP necessary for the movement of the tail.

The tail of the sperm contains a long flagellum with typical 9+2 arrangement of microtubules. It is produced by the centriole found near the base of the nucleus. The tail enables the sperm to swim along the female reproductive tract towards an ovum.

Semen

The semen is the fluid that contains a mixture of sperms and the secretions of three sets of accessory glands. The semen is discharged from the urethra during ejaculation. Usually a normal ejaculate contains about 2-5 mL of semen and the sperm count in the semen may be in the range of 40-100 million/mL. Normally, the sperms comprise less than 10% of the final ejaculate. Main fraction of the semen is made up of secretions from seminal vesicles and the prostate gland.
The semen contains several components such as mucus, enzymes, prostaglandins, ascorbic acid, citrate and fructose which promote the survival of sperm. The semen provides a liquid medium for the sperm movement and also helps to neutralize the acidity in the female reproductive tract. The life expectancy of a sperm is about 48-72 hours after ejaculation.

Accessory glands associated with the male reproductive system

There are three sets of accessory glands that produce secretions necessary for sperm survival and movement. They are seminal vesicles, prostate gland and bulbourethral glands.

- **Seminal vesicles:** They are a pair of two small pouches that produce a thick, yellowish fluid that is expelled during ejaculation. Seminal fluid is alkaline to protect the sperm in the acidic environment of the vagina. It contains mucus, fructose (that provides most of the sperm's energy), a coagulating enzyme (helps semen coagulates after ejaculation), ascorbic acid and local regulators called prostaglandins. This fluid contributes to about 60% of the volume of semen. Each seminal vesicle opens into a short duct that joins the corresponding vas deference to form an ejaculatory duct.

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- **Prostate gland:** This gland is found below the urinary bladder. It secretes a thin, milky fluid directly into the urethra through small ducts. This milky secretion contains coagulants, anticoagulant enzymes and citrate which is a sperm nutrient. This fluid contributes to about 30% of the volume of semen.

- **Bulbourethral glands:** They are a pair of small glands found along the urethra below the prostate. These glands secrete a clear alkaline mucus that is able to neutralize any acidic urine remaining in the urethra and lubricates the lining of the urethra.

Hormonal control of the male reproductive system

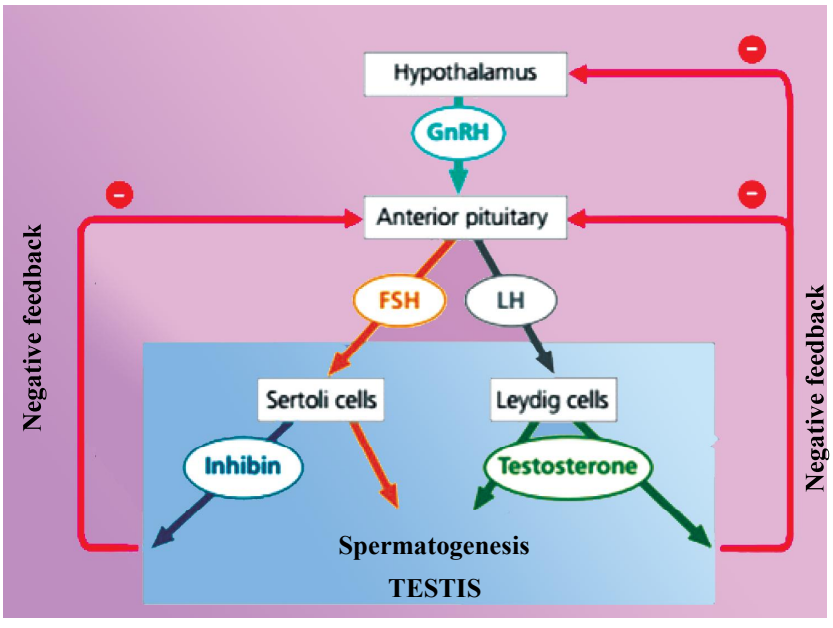


Figure 5.25: Hormonal control of the male reproductive system

- In males, in response to the release of GnRH from the hypothalamus (just before puberty), results in the release of FSH and LH by the anterior pituitary. Rising levels of the FSH and LH hormones at puberty promotes mature functioning of the male reproductive organs. The hormones regulate the development, growth, pubertal maturation and reproductive processes of the body.

- FSH and LH hormones direct spermatogenesis by acting on different types of cells in the testis.
  - FSH stimulates Sertoli cells to nourish the developing sperm.
  - LH causes Leydig cells to produce testosterone and other androgens to promote spermatogenesis.
- Two negative-feedback mechanisms control sex hormone production in males.

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- Testosterone inhibits GnRH, FSH and LH in blood through its effect on the hypothalamus and anterior pituitary.
- Additionally inhibin produced by Sertoli cells, acts on anterior pituitary gland to reduce FSH secretion.

- These negative-feedback circuits maintain testosterone and other androgen levels in the normal range.

Structure and Function of the Human Female Reproductive System

Main internal structures of the female reproductive system consists of two ovaries (female gonads), two oviducts, uterus and vagina.

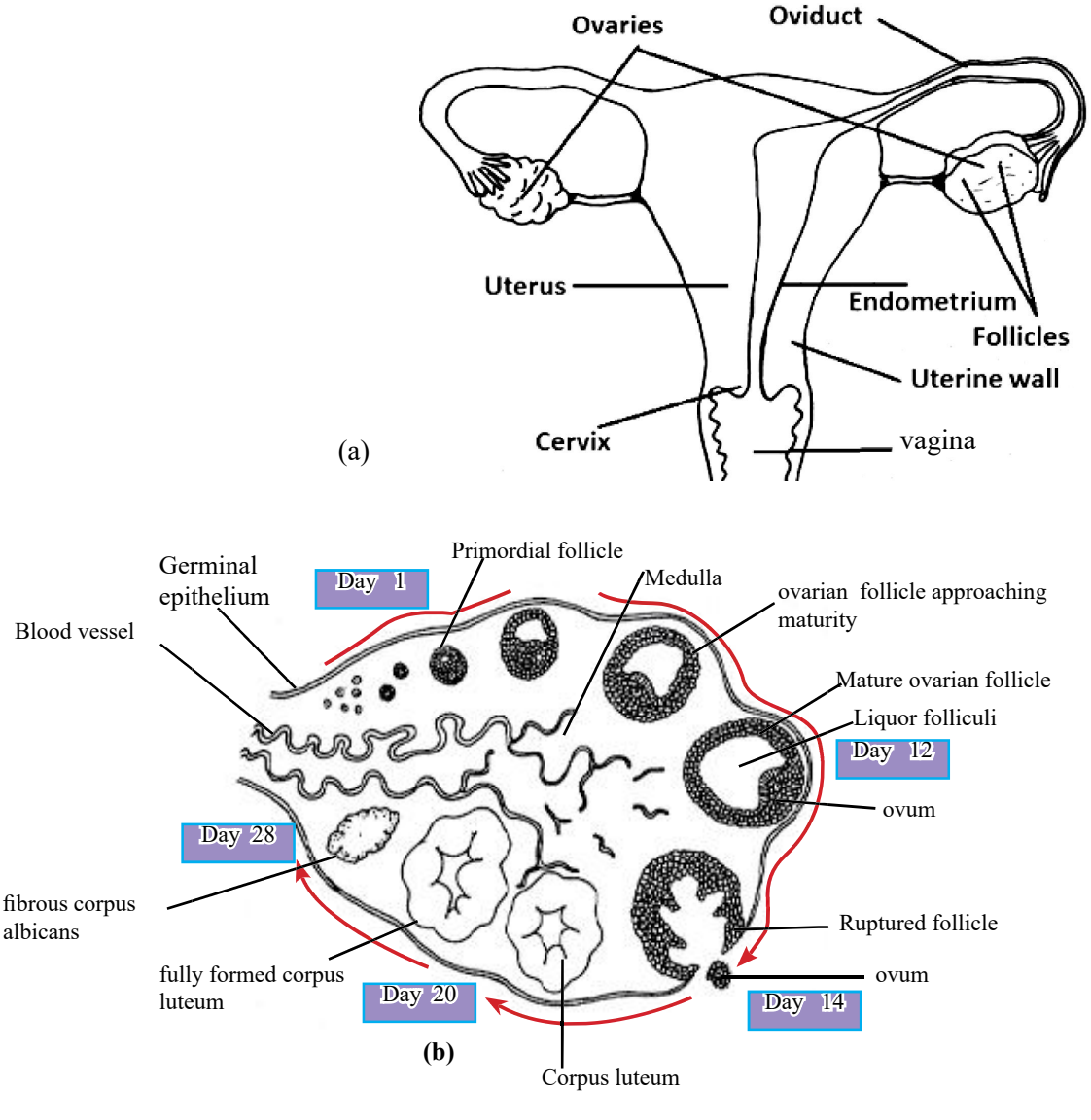


Figure 5.26: (a) The gross structure of the female reproductive system

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Ovaries: The female gonads are the two ovaries. They are found on either side of the uterus and are held in place in the abdominal cavity by ligaments. In the ovary, female gametes are stored and developed prior to ovulation. Ovaries also produce female sex hormones that are needed for physiological changes during the reproductive cycle.

The ovaries have two tissue layers: outer the cortex and inner the medulla. The outer layer of each ovary consists of connective tissues covered by germinal epithelium. The outer layer contains ovarian follicles in various stages of maturity. Each follicle consists of an oocyte, which is a partially developed egg surrounded by support cells. Support cells protect and nourish the oocyte during its development. The ovum is discharged from the ovary at secondary oocyte stage of oogenesis with the first polar body. This secondary oocyte divides into the mature ovum (egg) and a second polar body if a sperm penetrates the secondary oocyte. Human ovum is a round cell with 23 maternal chromosomes and relatively large cytoplasm and is surrounded by numerous supporting cells. In addition, there is a clear layer present between plasma membrane of the ovum and the supporting cells.

Oviducts: The oviduct or the fallopian tube extends from the uterus toward a funnel like opening at each ovary. The dimensions of this tube vary along its length (near the uterus is narrow as a human hair). After ovulation, cilia on the inner epithelial lining of the oviduct help collect the egg by drawing fluid from the body cavity into the oviduct. Then with the help of wave like contractions of the oviduct the cilia convey the egg down the oviduct to the uterus.

Uterus: The uterus or the womb is a thick, pear shaped chamber. Its walls are muscular, which allows it to expand during pregnancy to accommodate the fetus and its inner lining (endometrium) is highly vascularized. The distal end of the uterus narrows to form a neck, called the cervix that opens into the vagina.

Vagina: This is a muscular but elastic chamber with a stratified epithelium. The vagina connects external and internal organs of reproduction. It is the site where sperm is deposited and also serves as the birth canal.

Oogenesis

In the human female, the development of mature oocytes takes a long time. Immature eggs are formed in the ovary of a developing female embryo. But these eggs complete their development many years or even decades later. In oogenesis, cytokinesis during meiosis is unequal, with almost all the cytoplasm segregated to a single daughter cell. This large cell develops to become the egg. The other products of meiosis, which are smaller cells are known as polar bodies. These polar bodies eventually degenerate. Unlike spermatogenesis, the mitotic divisions that occur during

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oogenesis in human females are thought to be complete before birth and the production of mature gametes ceases at about the age 50 years. Unlike in spermatogenesis, during oogenesis there are also long interruptions.

Main steps in oogenesis

- Oogenesis begins in the female embryo with the mitotic division of primordial germ cells that give rise to oogonia.
- Then oogonia divide by mitosis to form cells that begin meiosis, but stops at prophase I before birth.
- Each of these developmentally arrested cell is called a primary oocyte. Each primary oocyte is found within a small follicle, a cavity lined with protective cells. At birth, the ovaries together contain about 1-2 million primary oocytes. Out of these about 500 fully mature between the puberty and the menopause.
- Beginning at puberty, follicle stimulating hormone (FSH) periodically stimulates a small group of follicles to resume growth and development. Out of these only one follicle fully matures each month. During this time the primary oocyte within the follicle completes meiosis I and produces a secondary oocyte and the first polar body. Then the meiosis II starts, but stops at the metaphase.
- The secondary oocyte arrested in meiosis II is released at ovulation (with the first polar body), when its follicle breaks open.
- If a sperm penetrates the secondary oocyte, meiosis II completes and the secondary oocyte divides into the mature ovum and a second polar body. Both meiotic divisions involve unequal cytokinesis, with the smaller cells becoming polar bodies which will degenerate eventually. If a sperm penetrates, there is a single mature egg (ovum) containing a sperm head at the end of oogenesis. The fusion of the haploid nuclei of the sperm and the ovum is called fertilization.
- The ruptured follicle left behind after ovulation develops into the corpus luteum. Corpus luteum secretes estradiol and progesterone hormones which help to maintain the uterine lining during pregnancy.
- If the egg is not fertilized, the corpus luteum degenerates and leaves a small ,permanent scar of fibrous tissue called corpus albicans on the ovary surface.
- A new follicle matures during the next cycle.

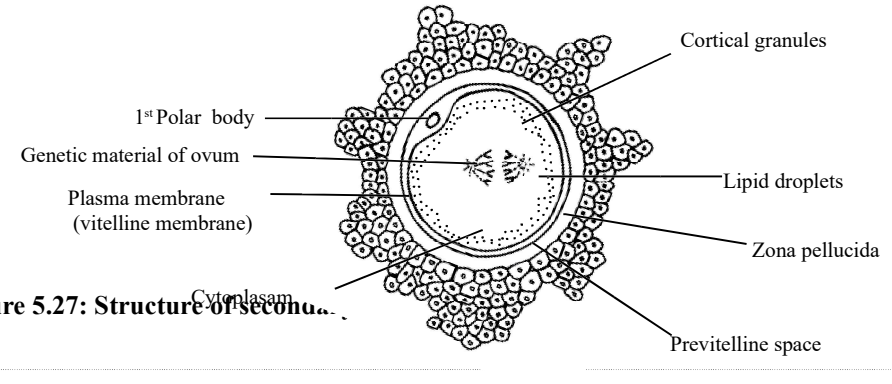


Figure 5.27: Structure of secondary

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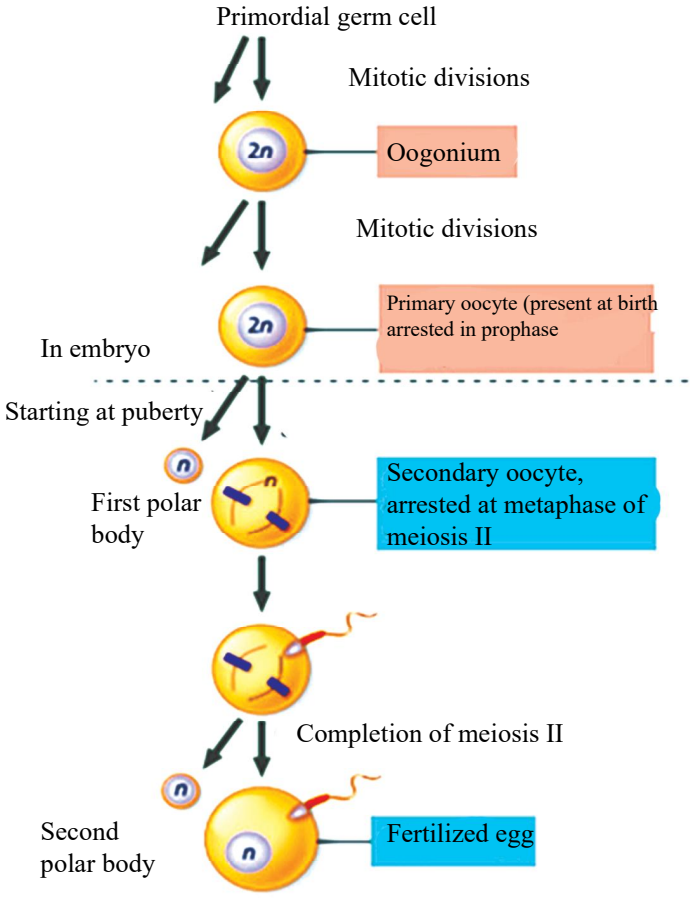
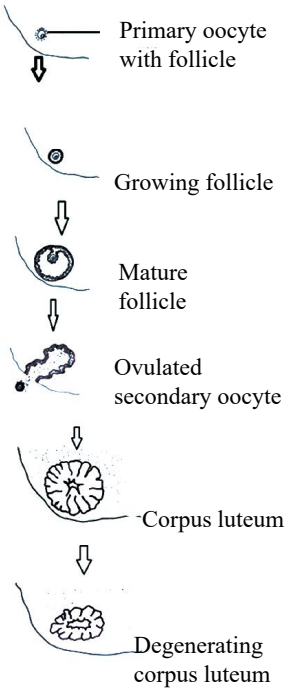


Figure 5.28: The process of oogenesis

Hormonal control of the human female reproductive cycles

Although males produce sperms continuously, females produce ova in cycles. There are two linked reproductive cycles in a human female during their reproductive years; the ovarian cycle and the uterine cycle (or the menstrual cycle). The uterine cycle consists of changes that occur about once a month in the uterus and these cyclic changes in the uterus are controlled by the ovarian cycle, which is cyclic events that occur in the ovaries. Both of these cycles are regulated by hormonal activities which link the two cycles, synchronizing ovarian follicle growth and ovulation with the establishment of a uterine lining which supports the development of embryo.



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1. Ovarian cycle

- Ovarian cycle comprises follicular phase and luteal phase.
- The period during which the follicle grows and the oocyte matures is referred to as the follicular phase. At the beginning of follicular phase, GnRH from the hypothalamus simulates the anterior pituitary to secrete small amounts of FSH and LH.
- FSH stimulates follicle growth, aided by LH.
- Cells of the growing follicle start to make estradiol hormone. Therefore the estradiol level rises slowly during the follicular phase. The low levels of estradiol inhibit the secretion of gonadotropin hormones from the anterior pituitary (negative feedback) so that LH and FSH are kept at relatively low levels in the follicular phase.
- When estradiol secretion by the growing follicle starts to increase sharply, high levels of estradiol stimulate the hypothalamus to increase GnRH secretion which in turn stimulates the anterior pituitary to sharply rise the FSH and LH secretion especially producing a LH surge (through positive feedback mechanism).
- By this time the maturing follicle, containing a fluid-filled cavity has enlarged, forming a bulge at the surface of the ovary. About a day after the LH surge, the follicular phase ends at ovulation. In response to both FSH and the peak in LH level, the follicle and adjacent wall of the ovary rupture, releasing the secondary oocyte which is called ovulation.
- The luteal phase of the ovarian cycle takes place after ovulation. In the luteal phase, LH stimulates the follicular tissue within the ovary to transform into a glandular structure called the corpus luteum.
- The corpus luteum secretes progesterone and estradiol, which then exert negative-feedback on the hypothalamus and pituitary. This feedback reduces LH and FSH secretion to very low levels and thereby prevents the maturation of another egg in the ovary.
- If there is no pregnancy, the low gonadotropin levels at the end of luteal phase promotes disintegration of the corpus luteum.
- Disintegration of corpus luteum leads to the sharp decline in hormones. As a result the negative-feedback of estradiol and progesterone on the hypothalamus and pituitary are removed. This enables the pituitary to produce FSH to stimulate the growth of a new follicles thereby starting the next ovarian cycle.

2. Uterine (menstrual) cycle

Uterine cycle consists of proliferative phase, secretory phase and menstrual flow phase.

- Proliferative phase: Before ovulation, steroid hormones of the ovary stimulate the uterus to prepare for support of an embryo. Growing follicles secrete estradiol that stimulates the endometrium to thicken. This is referred to as the proliferative phase in the uterine cycle. Therefore the follicular phase of the ovarian cycle coordinates with the proliferative phase of the uterine cycle.
- The secretory phase starts after ovulation. After ovulation, estradiol and progesterone that are secreted from the corpus luteum stimulate the maintenance and further development of the lining of the uterus by enlarging the arteries and growth of the endometrial glands.

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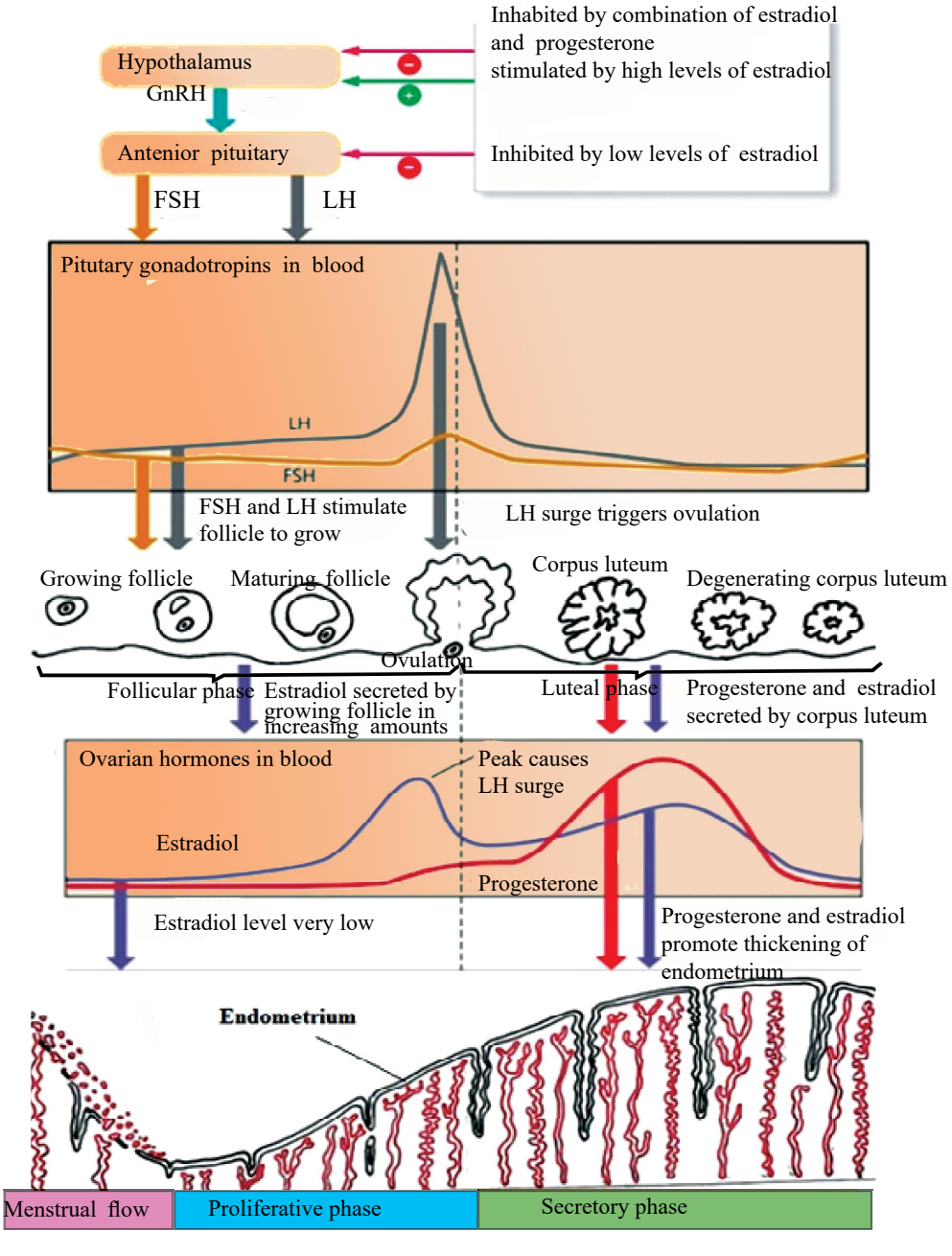


Figure 5.29: The reproductive cycles of human female showing how ovarian cycle and uterine cycle are regulated by changing hormone levels in the blood.

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These glands secrete nutrient fluid that can sustain an early embryo if fertilization occurs. Hence, the luteal phase of the ovarian cycle is coordinated with the secretory phase of the uterine cycle.

- Menstrual flow phase: If implantation of an embryo does not occur, the corpus luteum disintegrates which results in the drop in ovarian hormones. This brings about the end of the secretory phase. As a result, arteries constrict and thereby the uterine lining disintegrates and causes the shedding of endometrial tissues and fluid. This is the menstrual flow phase of the uterine cycle. The cyclic shedding of the blood-rich endometrium from the uterus, a process that occurs in a flow and which lasts for a few days through the cervix and vagina is called menstruation.

Menopause

This is the cessation of ovulation and menstruation in a woman. Usually menopause takes place between the ages of 45 and 55 years. During this time the ovarian supply of oocytes runs out and the estrogen production by the ovary decreases. Ovaries become less responsive to the hormones FSH and LH produced by the anterior pituitary.

Human Development

Growth of a new human being starts when an ovum is fertilized by a sperm usually in the oviduct. During the development of the individual within the mother's uterus, a sequence of events occurs from fertilization to the birth which normally ends in 38 weeks or roughly 9 months. The first 8 weeks of human development is called the embryonic period and thereafter the developing individual is called a fetus.

- **Fertilization/conception and the formation of a human zygote**

  During ovulation a secondary oocyte arrested at metaphase II enters the oviduct. During fertilization a sperm enters the secondary oocyte penetrating the epithelial cells surrounding the oocyte, the glycoprotein layer present between the oocyte's plasma membrane and the surrounding cells. Once the sperm enters the secondary oocyte, meiosis II of the oocyte is completed producing the mature ovum. Subsequently, the two haploid pronuclei of the mature ovum and the sperm fuse to produce a diploid, single cell referred to as the zygote. The fusion of the haploid nuclei of the sperm and the ovum is called fertilization. Fertilization takes place in the upper reaches of the oviduct within 12 to 24 hours after ovulation.

- **Cleavage of the zygote, blastocyst formation and implantation**

  About 24 hours after fertilization, a series of rapid mitotic cell divisions called 'cleavage' take place in the zygote. Cleavage of zygote begins in the oviduct as it moves forward towards the uterus by ciliary and peristaltic movements. Cleavage continues forming a

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solid ball of cells (morula) by the time the embryo reaches the uterus (about 3-4 days after fertilization).

Morula floats within the uterine cavity and gets nutrition by endometrial secretions. About five days after fertilization, a large fluid filled cavity is formed surrounding the ball of cells. With the formation of the cavity, this developing stage is referred to as the blastocyst. Further rearrangements of the cells in the blastocyst results in two distinct structures: the inner cell mass and the trophoblast. Inner cell mass is located internally and eventually develops into the embryo and the membranes enclosing the embryo are called the amniotic sac. The trophoblast which is the outer layer of cells will ultimately develops into the fetal portion of the placenta.

Around 7 days after fertilization, the blastocyst attaches to the endometrium of the mother's uterus. This is called implantation. As the blastocyst implants, the inner cell mass orients towards the endometrium. The trophoblast grows outward and invades the endometrium. This is initiated by the enzyme secreted by the trophoblast to breakdown the uterine lining. Then the trophoblast extends finger like projections into the endometrium. Trophoblast begins to secrete human chorionic gonadotropin (hCG) hormone which has the action similar to LH. The hormone hCG rescues the corpus luteum from degeneration and sustains its secretion of progesterone and estrogen which maintain the uterine lining preventing menstruation.

After implantation, three germ layers are formed in the developing embryo at the end of the gastrulation stage. Extra-embryonic membranes begin to appear which surround the embryo. The placenta is formed by the cells of trophoblast and the adjacent endometrial tissues.

- **Embryonic members / fetal membranes**

  Four new extra embryonic membranes appear after implantation. They are chorion, amnion, yolk sac and allantois. They provide a life support system for further embryonic/ fetal development.

  Chorion becomes the main embryonic portion of the placenta which is the structure for exchange of materials between the fetus and mother. It also protects the embryo/fetus from immune responses of the mother. Chorion produces hCG, an important hormone of pregnancy.

  Amnion is a protective membrane surrounding the embryo/fetus creating a fluid filled cavity which serves as a shock absorber and helps prevent desiccation.

  Yolk sac contributes to the cells that will become blood cells until the fetal liver takes over. It also is the source of primordial germ cells that migrate to the developing gonads.

  Allantois is a small outer-pouching of the yolk sac that serves as an early site for blood formation and is associated with the development of the urinary bladder.

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- **Placenta and umbilical cord**

  During the first 2-4 weeks of embryonic development, the embryo obtains nourishment directly from the endometrium. Eventually, the embryonic trophoblast and the mother's endometrium intermingles and form the placenta. The placenta is a disc shaped organ formed by two parts: embryonic/fetal portion formed by chorionic villi of the chorion and maternal portion formed by the endometrium. The placenta contains both embryonic/ fetal and maternal blood vessels. However maternal and fetal blood vessels do not join and the blood they carry do not normally mix. The placenta mediates the exchange of material (nutrients, respiratory gases, metabolic wastes) between the embryonic/fetal and the mother's circulatory systems. The placenta supplies oxygen and nutrients to the fetus from the maternal blood stream and excrete waste products from the fetus to the maternal blood stream. The placenta also helps to provide immune protection to the developing embryo/fetus. The placenta produces hormones (e.g. hCG, progesterone etc.) needed to sustain the pregnancy.

  Umbilical cord is a flexible cord-like structure containing blood vessels and attaches embryo/fetus to the placenta during gestation. Oxygen poor blood from the embryo/fetus travels to the placenta through the two arteries of the umbilical cord and passes through fingerlike projections (chorionic villi) of the placenta where oxygen and nutrients are acquired. Fetal blood (oxygen rich blood) leaves the placenta through the umbilical vein leading back to the embryo/fetus.

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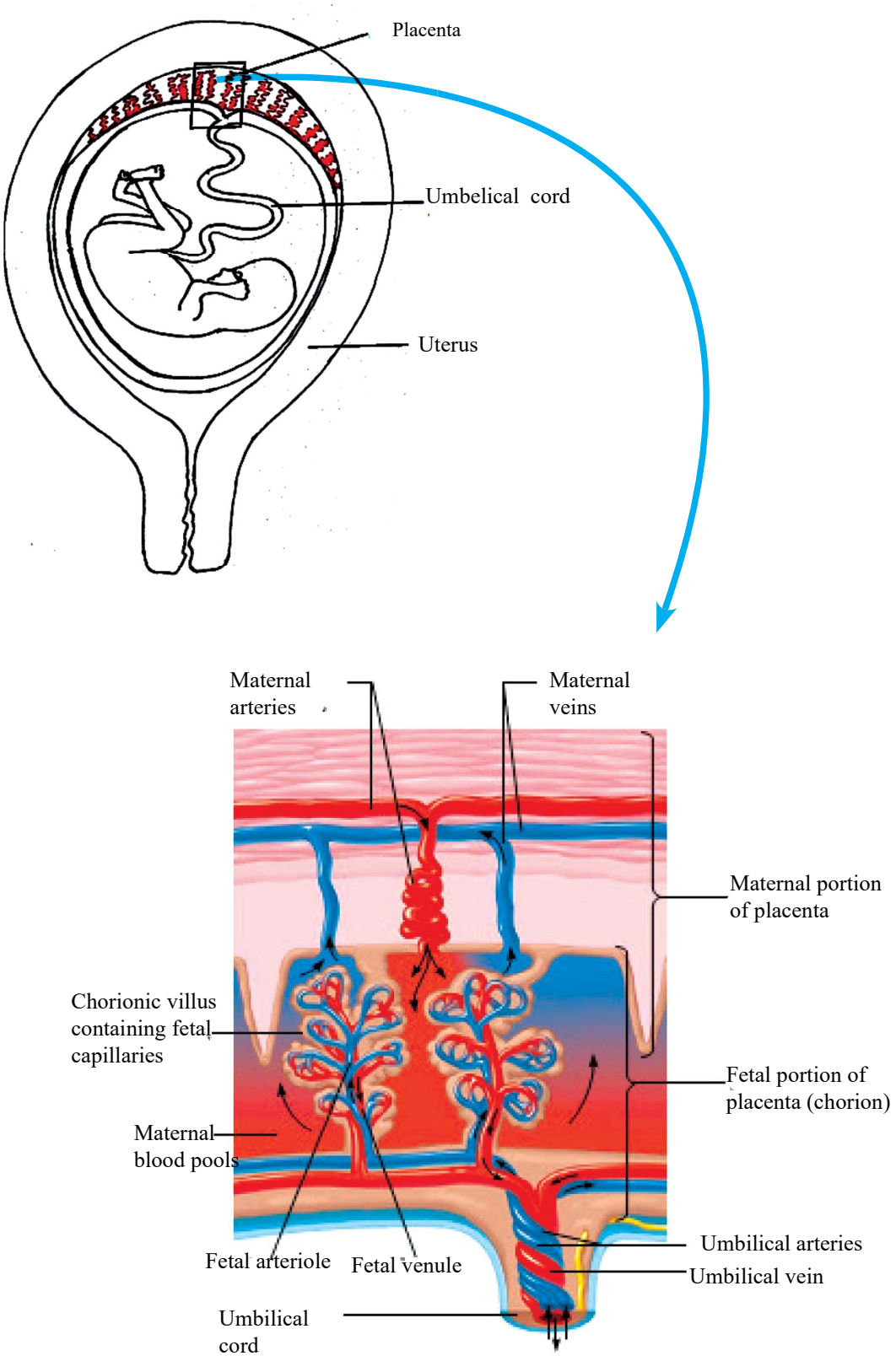


Figure 5.30: Placenta and umbilical cord

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Pregnancy and its duration

Pregnancy or gestation is the condition of carrying one or more developing offspring inside the uterus of a female. Human pregnancy period is usually 38 weeks from fertilization to birth or roughly 9 months (or 40 weeks from the last menstruation to birth). The nine months of pregnancy are divided into three trimesters of about three months each.

During the first trimester, the implanted embryo secretes hormones to regulate the mother's reproductive system and to indicate its presence. The hCG hormone secreted by the embryo, maintains the corpus luteum in the ovary to secrete progesterone and estrogen. Some amount of this hCG passes from the maternal blood to the urine. The presence of hCG in pregnant mother's blood and urine can be easily detected and therefore is used as an early pregnancy detection test. High levels of progesterone brings about rapid changes in the mother. Both ovulation and menstrual cycles stop, the maternal side of the placenta grows, and the breasts and the uterus get larger. Mucus in the cervix of the mother forms a plug which prevents the fetus from infections. Most mothers experience nausea (morning sickness) during the first trimester.

By the second trimester, the level of hCG declines and as a result the corpus luteum deteriorates. But the placenta takes over the production of progesterone and estrogens which helps to maintain the pregnancy. Mother can feel fetal movements. As the fetus grows, mother's abdominal organs become compressed and displaced. In the third trimester of pregnancy this may lead to digestive blockage and frequent urination.

Major fetal changes in each trimester

- **First trimester**

  The first trimester is the most critical stage of development during which the rudiments of all major organ systems appear. This is the main period of organogenesis (the development of the body organs). The heart begins to beat by the 4th week (can be detected at 8-10 weeks). By the 8th week, embryo is said to be the fetus as all the parts of an adult are present in rudimentary form. At the end of the 1st trimester, the fetus is well differentiated and about 5 -7 cm long.

- **Second trimester**

  By the end of second trimester, the fetus assumes distinctively human features. Organ systems are completely developed in this stage. During the second trimester, the fetus grows to about 30 cm in length and is very active so that the mother may feel the fetal movements.

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- **Third trimester**

  The third trimester represents a period of rapid fetal growth. During the early stage of this period, most of the organ systems become fully functional. During the third trimester, the fetus grows to about 50 cm in length and weighs about 3-4 kg. Fetal activity decrease as it fills the space within the uterus.

Maternal immune tolerances of the embryo and fetus

During pregnancy the overall regulation of the mother's immune system changes. These changes allow the mother to keep the embryo in her uterus without rejecting as a foreign body even though half the embryo's genes are inherited from the father and many chemical markers on the surface of the embryo are foreign to the mother.

Process of parturition

Child birth begins with the labor. The labor is a series of strong, rhythmic uterine contractions that push the fetus and placenta out of the body. When labor begins, local regulators (prostaglandins) and hormones (mainly estradiol and oxytocin) induce and regulate further contractions of the uterus. This is a positive feedback mechanism as uterine contractions stimulate secretion of oxytocin which stimulates further contractions of the uterus.

The labor can be divided into three stages. The first stage is the thinning and opening up (dilation) of the cervix. The second stage is the delivery of the baby. In this stage, continuous and strong contractions force the fetus out of the uterus and expell through the vagina. Delivery of the placenta is the final stage of labor.

Lactation

The lactation which is unique to mammals begins as post natal care. Lactation is the secretion and ejection of mother's milk from the mammary glands. Lactation is subjected to nervous and hormonal regulation. The main hormone in promoting milk synthesis and secretion is prolactin. In response to suckling by the new born baby (which initiates nerve impulses from touch receptors in the nipples) and decrease in estradiol and progesterone levels in the mother's blood after birth, the hypothalamus send impulses to the anterior pituitary to secrete prolactin hormone which stimulates the mammary glands to produce milk. Suckling also stimulates the secretion of oxytocin hormone from the posterior pituitary gland and triggers the release (ejection) of milk from the mammary glands. This is a positive feedback mechanism as milk availability encourages continuous suckling, so touch sensation on the nipple and oxytocin release continue further ejecting milk from the mammary glands.

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Composition of human milk and significance of breast feeding

During the first few days after birth, the mammary glands secrete a fluid called 'colostrum' until appearance of true milk. Human milk is a sterile solution that contain nutrients such as lactose, fatty acids, amino acids, minerals, vitamins, and water that are ideal for baby's digestion, brain development and growth. Human milk includes proteins such as casein, lactalbumin and immunoglobulins.

Colostrum and true milk provide nutrients for the baby and contain important antibodies that protect the infant. Several types of white blood cells are also present in the breast milk which help the baby to resist microbial infections. Compared to the true milk, colostrum contains less nutrients (less lactose and no fat) but they are adequate for the early nutritional needs.

Breast feeding supports optimal infant growth, enhances intellectual development and fosters mother-infant relations by establishing early and prolonged contact between them. Compared to cow's milk, the fat, iron and the proteins in the breast milk are more readily metabolized. Lower sodium content of breast milk is more suited to the baby's needs. The baby is less likely to have allergic reactions to mother's milk than the milk from another source.

Birth control methods

The deliberate prevention of pregnancy is called contraception. This can be achieved in several ways. Some contraceptive methods prevent gamete development or releasing gametes. Some contraceptive methods prevent fertilization of gametes. Some methods are available to prevent implantation of an embryo. Unwanted pregnancies are avoided by birth control methods.

Common temporary birth control methods:

- Oral contraceptives for females: Most oral contraceptives contain high concentration of synthetic estrogen and progesterone. High levels of these hormone inhibit GnRH release from hypothalamus and FSH and LH secretions from anterior pituitary through negative feedback. Prevention of LH release blocks ovulation. Inhibition of FSH secretion prevents follicle maturation. Some oral contraceptives contain only high levels of synthetic progesterone (progestin) which thickens cervical mucus so that it blocks entering sperms to the uterus. If fertilization occurs it can interfere with implantation as well.
- Condoms for males: Barrier devices which prevent sperm entry
- IUD (loop) for females: A device placed in the uterus which interferes with fertilization and prevents implantation of a fertilized ovum
- Depo-Provera injection for females: Periodic injection of a synthetic progesterone which thicken cervical mucus and prevents sperm entry. If fertilization occurs it prevents implantation by making the endometrium thin.

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Surgical Sterilization (preventive methods of gamete release)

- Vasectomy for males- Prevents release of sperms
- Tubal ligation for females- Prevents ovum from entering uterus

Abortion

- Abortion is the premature termination of a pregnancy in progress.
- Miscarriage: Spontaneous abortion which occurs naturally.
- Induced abortion is intentionally performed (surgical or nonsurgical). Certain drugs can induce abortion non-surgically within the first 7 weeks after conception. They block progesterone receptors in the uterus thereby preventing progesterone from maintaining the pregnancy.

Table 5.6: Sexually transmitted infections

Infection	Pathogen	Main mode of transmission	Main symptoms
Gonorrhea	*Neisseria gonorrhoeae* bacterium	• Sexual contact, • Mother to child at birth	In males-burning feeling/discomfort when passing urine, yellow discharge with pus from genito-urinary tract.
Syphilis	*Treponema pallidum* bacterium	• Sexual contact, • Mother to child at birth	Accompanied by fever, headache.
AIDS (acquired immunodeficiency syndrome)	HIV(Human immunodeficiency virus)	• Sexual contact • Transfer of body fluids (blood, serum) • Use of unsterilized needles • Mother to fetus/ child :during pregnancy, at birth and during lactation	Sores or painless ulcers on any part of the body (vagina, lips, fingers, nipples), fever, skin rashes Loss of appetite and weight, fever, persistent dry cough, Lymphoma (cancer in lymphatic system), pneumonia and other disease resulting from breakdown of the immune system
Genital herpes	Herpes simplex 2 virus	Sexual contact	Itchy, painful sores around genital area, fever in some cases

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Detecting disorders during pregnancy

- Many development problems and genetic disorders can be diagnosed during the gestation period.
- Ultrasound images can be used to analyze the size and condition of the fetus.
- Amniocentesis and chorionic villi sampling –a needle is used to obtain fetal cells from amniotic fluid or tissue surrounding the embryo. Genetic analysis can be done with these samples.
- Newest method is to use a pregnant mother's blood to analyze the genome of the fetus as the mother's blood contains fetal DNA
- But all detectable disorders are untreatable when the embryo is in the uterus and many cannot be corrected even after birth. However, parents can take informed decisions with the help of these tests.

Infertility

Infertility is the inability to conceive offspring. Both men and women can have reproductive defects leading to infertility. The number of couples facing this problem appears to be increasing in the modern society. Certain forms of infertility are treatable.

Modern reproductive technology for resolving infertility problems

- Some infertility problems are resolved by recent scientific and technogical advances. This includes hormone therapy, surgery and assisted reproductive technology.
- Hormone therapy– Sometimes, hormone therapy can increase sperm production in the infertile male or egg production in the infertile female.
- Surgery: The ducts in the reproductive system that are formed improperly or have become blocked can be corrected surgically to resolve infertility.
- Assisted reproductive technology

  In vitro fertilization (IVF): In vitro fertilization is a series of procedures used to treat infertility problems and assist with the conception of a child. The process involves removal of oocyte(s) from a female ovary and obtaining sperm from a male and combining the oocyte and the sperm to achieve the fertilization under laboratory conditions. The fertilized eggs are incubated until they reach at least 8 cells and then these embryos are transferred to the woman's uterus for implantation and to continue its development. Conventional IVF needs between 50 and 100 thousands of sperm from the male per one oocyte in order to achieve the fertilization. This is due to the fact that in IVF, acrosome reaction has to take place and thousands of sperm cells have to be involved.

  Intra-cytoplasmic sperm injection (ICSI): This is also a type of in vitro fertilization method which is used to address male infertility. If mature sperm are defective or low in number, a whole sperm or a spermatid nucleus is injected directly into the cytoplasm of an oocyte that has been removed from the women's ovary. For insemination, ICSI needs only one sperm per oocyte. Unlike in conventional IVF, the sperm which will be inserted into a particular oocyte is already selected in ICSI. The fertilized egg can then be returned to the woman's uterus for implantation.

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Support and Movement

The structure and functions of the skeletal systems of animals

In the animal kingdom, three major types of skeletons are found. They are hydrostatic skeletons, exoskeletons and endoskeletons.

Hydrostatic skeleton is a fluid filled body cavity which is enclosed by the body wall. In cnidarians, gastrovascular cavity acts as the hydrostatic skeleton. In some animals such as nematodes and annelids, the fluid filled cavity enclosed by the body wall (e.g., pseudocoelom in Nematoda, coelom in Annelida) consists of two muscle layers (longitudinal and circular muscles) which act antagonistically. The combined effect of muscle contraction and fluid pressure aids in locomotion and maintain the shape and form of the animal. In many animals, the spaces between cells are filled with fluid called interstitial fluid which provides support to these cells.

Exoskeleton is a rigid outer covering of the body of the animal which acts as a skeleton. Different types of exoskeletons are seen in the animal kingdom: Chitinous exoskeleton, calcium carbonate exoskeleton and bony plates. Arthropods possess the exoskeleton which is mainly composed of a non-cellular material, chitin. The chitinous exoskeleton is hardened by proteins or calcium carbonate. Exoskeletons that are made up of calcium carbonate are seen in the molluscs. In some reptiles, bony plates serve as the exoskeleton.

Endoskeleton is a hard skeleton which is buried in the soft tissues of the animal. Different types of endoskeletons are seen in the animal kingdom. These include plates of calcium carbonate (in echinodermates), bones and cartilage (in chordates).

Common functions of the skeletal systems in animals

- Support – All skeletons provide a rigid framework for the body and are resistant to compression and tension forces. They help to maintain the shape of body.
- Protection – The skeleton protects the delicate internal organs.
- Movement – Most skeletons are composed of rigid materials which provide a means of attachment for the muscles of the body. Parts of the skeleton operate as levers on which the muscles can pull. When this occurs, movement takes place.

Functions of the human skeletal systems

- Support
- Protection
- Movement

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- Storage and release of calcium under the influence of some hormones (refer competency level 5.7.1)
- Storage and release of phosphates under the influence of some hormones (refer competency level 5.7.1)
- Production of blood cells in the bone marrow

How animals move through water and air?

Swimming: *Different groups of animals swim in different ways. Some animals use their legs as oars to push against the water (e.g. insects and four legged vertebrates). Some animals are jet propelled taking water into the body and squirting it out in bursts (e.g. squids). Fishes swim by moving their body and tail from side to side. Aquatic mammals move by undulating their body and tail up and down (e.g. whales and dolphins). Fusiform body shape is a common adaptation for fast swimming animals.*

Movement through air: *Animals move through air mostly by flying. Gliding downward can occur in some instances. Flying animals use wings to lift the body against the gravity. Wings act as air foil: their shapes alter air currents in a way that helps flying. Fusiform shape of the wings helps to reduce drag force in air.*

The human skeleton

Human skeleton is divided into two main parts: axial skeleton and appendicular skeleton.

- Axial skeleton consists of skull, vertebral column, sternum and ribs.
- Appendicular skeleton consists of girdles (pectoral and pelvic) and limb bones.

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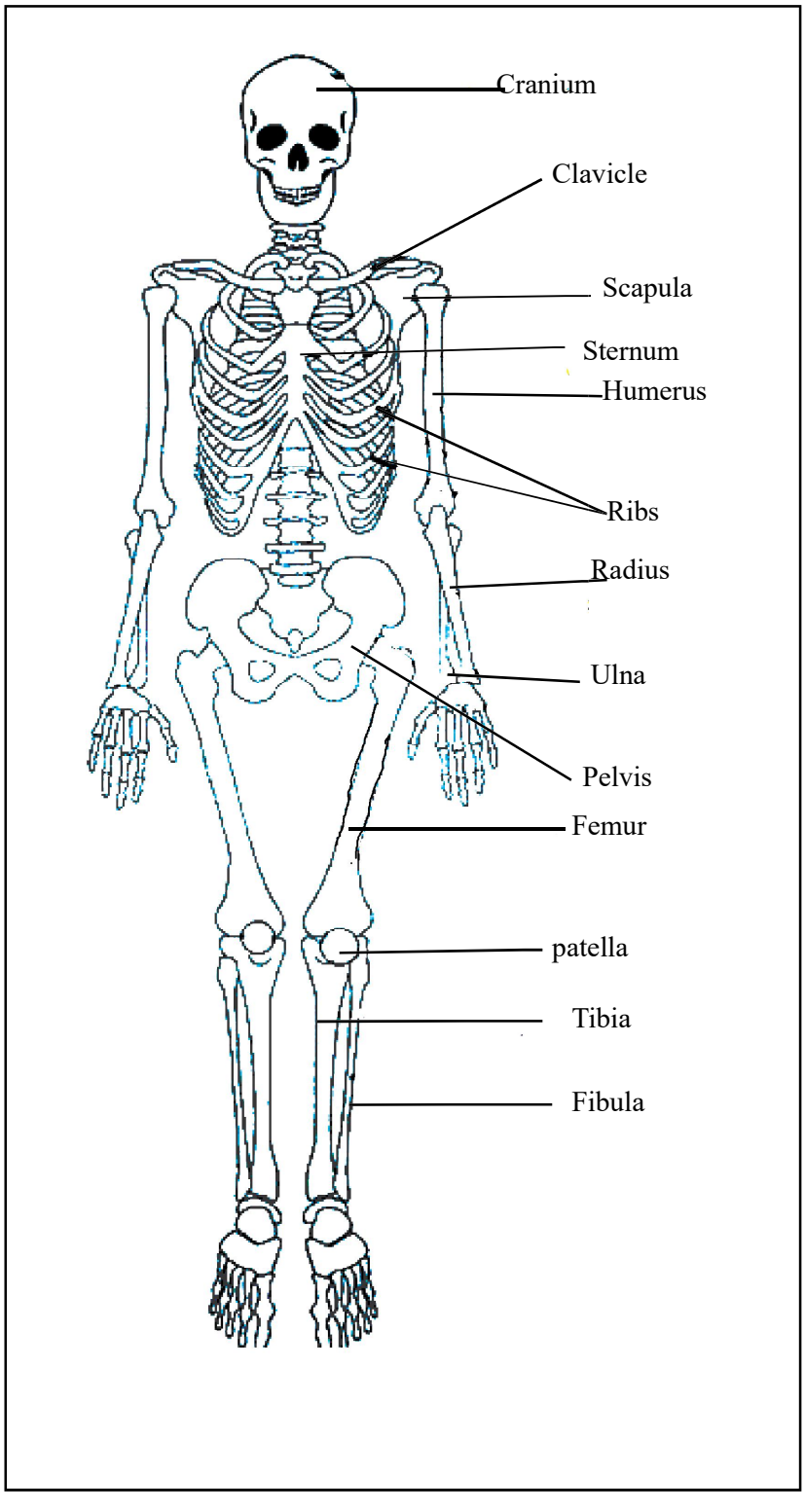


Figure 5.31: The anterior view of the human skeleton

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Organization of the human axial skeletal system

Skull

In humans, the skull rests on the upper end of the vertebral column. The human skull consists of 21 bones which are mostly connected together by ossified joints (sutures). Skull is divided into the cranium (brain case) and the face. The bones in the cranium are the frontal bone, two parietal bones, the occipital bone, two temporal bones, the ethmoid bone and the sphenoid bone. In addition to the frontal bone, thirteen other bones form the skeleton of the face. They are two zygomatic bones (cheek), the maxilla (upper jaw bone), two nasal bones, two lacrimal bones, the vomer, two palatine bones, two inferior conchae and the mandible (lower jaw bone).

Human cranial capacity is nearly 1.5 L. Cranium protects and encloses the brain. It also protects the inner ear, middle ear, olfactory organs and eyes. Bony eye sockets provide attachment to the eye muscles that move them. On the inferior surface of the cranium there is foramen magnum to provide passage to spinal cord. Two smooth rounded knobs (Occipital condyles) on either side of the foramen magnum articulates with the first vertebrae (atlas vertebrae) which permits nodding movements.

In the cranium, soft membranous regions called fontanelles are present which allow slight compressions at birth facilitating parturition. Fontanelles become replaced by bones within 1-2 years of life. Immovable joints (sutures) are present between the skull bones to provide more protection. Several air filled cavities lined by ciliated mucous membrane are present in the skull (in the sphenoid, ethmoid, maxillary and frontal bones). They are called sinuses. They all communicate with the nasal cavity. Sinuses provide resonance to voice and reduce the weight of the skull.

Facial region is situated below the cranium. Some facial bones form the walls of the posterior part of the nasal cavity and form the upper part of the air passages. Maxilla and mandible provide ridges in which teeth are embedded. Upper jaw (maxilla) is fused with cranium. Lower jaw (mandible) is movable. Hard palate (bony) and soft palate (cartilaginous) separate the buccal cavity from nasal cavity. Lower jaw articulates with the cranium. Zygomatic arch (formed from parts of zygomatic bone and temporal bone) provides the surface for muscular attachment for moving the lower jaw. Lower jaw (mandible) contains two processes: Condyloid process which articulates with the temporal bone to form the temporal-mandibular joint; Coronoid process which gives attachment to muscles and the ligaments. At the base of the skull, occipital condyles (1 pair) are present on the two occipital bones to form a hinge joint with atlas vertebrae. Temporal bone contains three processes: zygomatic process (which forms part of the zygomatic arch), mastoid process and styloid process. They provide surfaces for muscle attachment.

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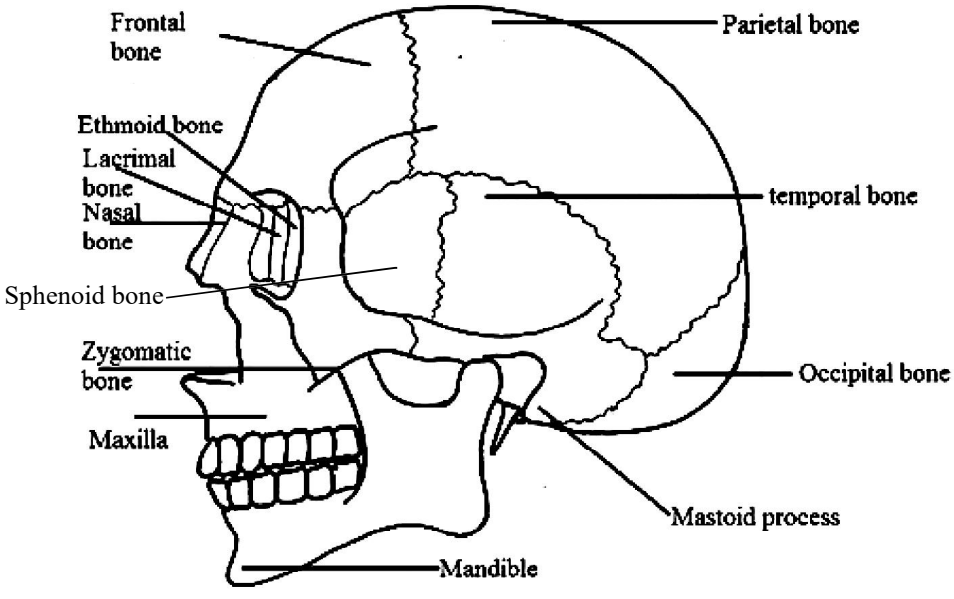


Figure 5.32: The bones of the human skull

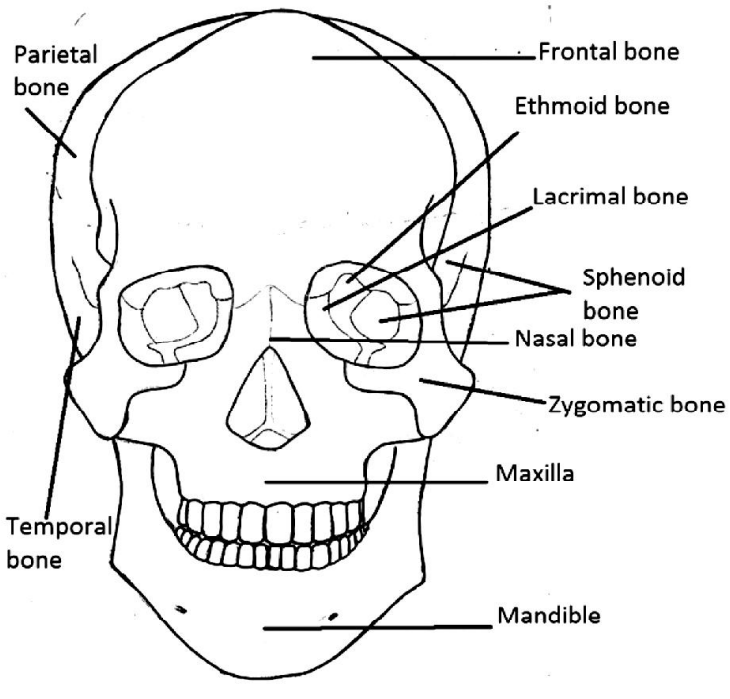


Figure 5.33: The anterior view of the human face (bones in the face)

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Vertebral column

Vertebral column is a strong flexible rod consisting of 26 linearly arranged bones. It consists of 24 separate vertebrae extending downwards from the occipital bone of the skull, the sacrum (formed from 5 fused vertebrae) and coccyx (formed from 4 small fused vertebrae). The vertebral column is divided into different regions. There are 4 distinct regions: cervical spine (formed by 7 vertebrae in the neck), thoracic spine (formed by next 12 vertebrae), lumbar spine (formed by next 5 vertebrae), and the sacrum to which the lowest vertebrae of lumbar spine is articulated; the coccyx is situated at the end.

Curvatures of the vertebral column

In humans, there are four curves in the vertebral column: cervical, thoracic, lumbar and sacral. They can be categorized into two main types: two primary curvatures and two secondary curvatures. Main function of the curvatures is the maintenance of the erect posture.

Primary curvatures: In the foetus, there is only one curvature in the vertebral column. When secondary curvatures are formed the primary curvature is retained only in thoracic and sacral regions which are known as primary curvatures. They are concave anteriorly.

Secondary curvatures: Formed after birth, first cervical curvature develops at about 03 months of birth. Then the child can hold his head upright. Second, lumbar curvature develops when the child is around 7-8 months. Then the child can hold his body upright. These secondary curvatures are convex towards the anterior.

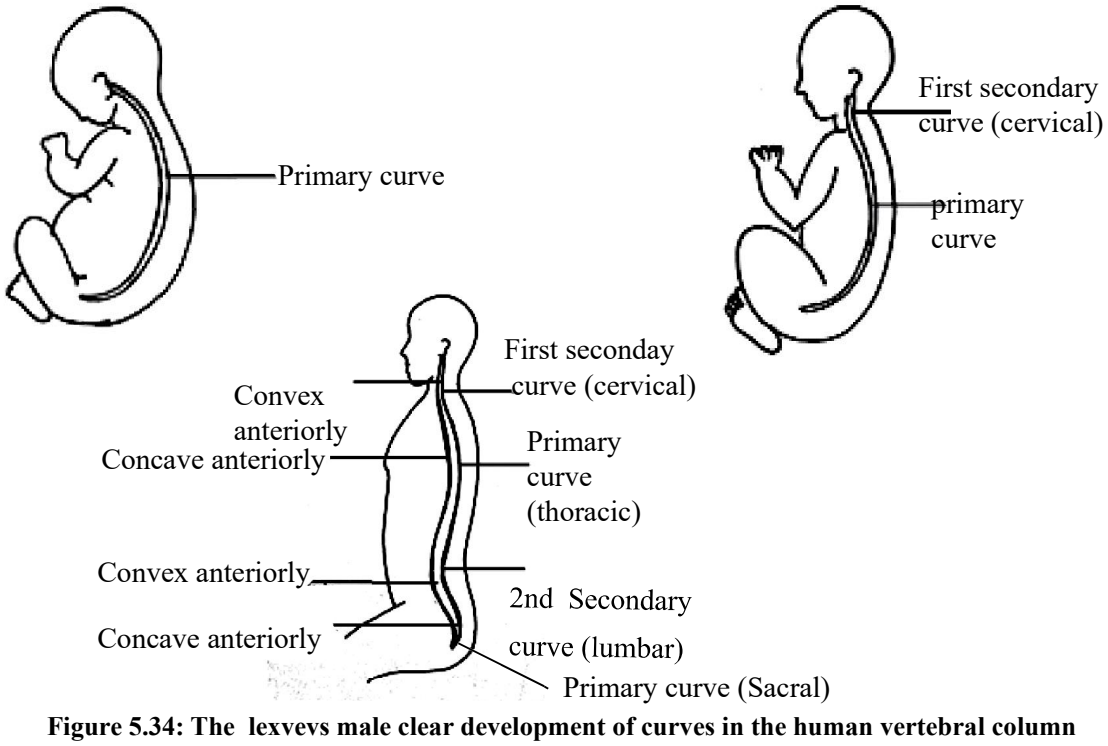


Figure 5.34: The lexvevs male clear development of curves in the human vertebral column

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Types of vertebrae

Structure of a typical vertebra

A lumbar vertebra can be considered a typical vertebra. A typical vertebra consists of the body and the vertebral arch. The body is the largest, broad and flattened part of the vertebrae. The flattened surface of the body of each vertebra articulates with the corresponding surface of the adjacent vertebra so that vertebrae are stacked together in the vertebral column. However the adjacent two vertebrae are not in direct contact with each other as there is a tough pad of cartilage called intervertebral disc between the two vertebrae. The size of the body of the vertebra increases downwards of the vertebral column to support the body weight.

Vertebral arch encloses vertebral foramen which provides passage way for the spinal cord. Processes arising from the neural arch provide surfaces for muscle attachment. Two lateral processes are called transverse processes and the posterior process is called spinous process. The vertebral arch has four articular surfaces: two superior articular surfaces (articulate with the adjacent vertebrae above) and two inferior articular surfaces (articulate with the adjacent vertebrae below).

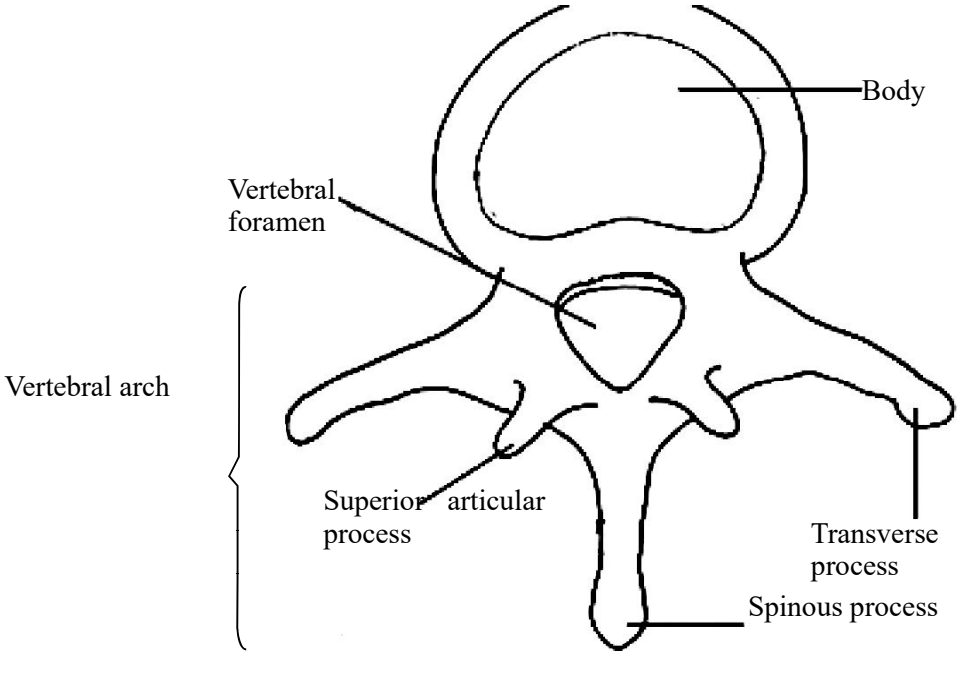


Figure 5.35: The structure of a typical vertebra (lumbar vertebra)

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Region specific vertebral characteristics

Cervical vertebrae: Cervical vertebrae are the first seven vertebrae in the vertebral column. When compared to the other types of vertebra, cervical vertebrae are the smallest. Body of the cervical vertebra is smaller compared to the other vertebrae. In addition, transverse processes of a cervical vertebra have a foramen on each side to provide passage for the vertebral artery. The spinous process of these vertebrae is bifid.

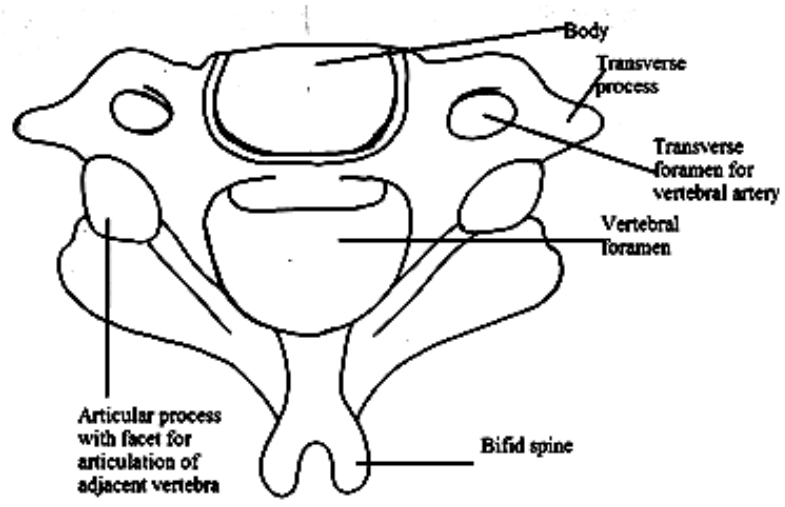


Figure 5.36: The structure of the typical cervical vertebra

The first cervical vertebra is the atlas which is the bone on which the skull rests. It is a ring shaped vertebra with no distinct body or spinous processes. It has two short transverse processes. The atlas contains two flattened facets which articulate with the occipital bone of the skull (condyloid joints), permitting nodding movements. Vertebral foramen of this vertebra is relatively larger to provide the passage of the larger anterior part of the spinal cord.

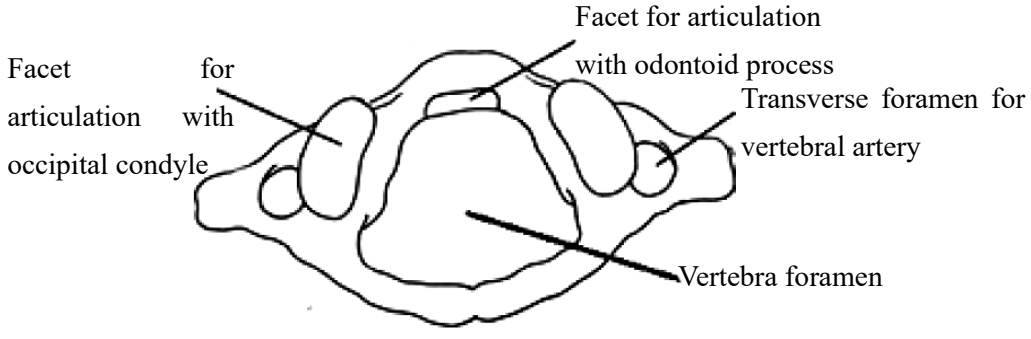


Figure 5.37: The structure of the atlas vertebra

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The second cervical vertebra is the axis. It has a small body with a superior projections called odontoid process which articulates with the atlas vertebra above. The head pivots (turns on side to side) on this joint.

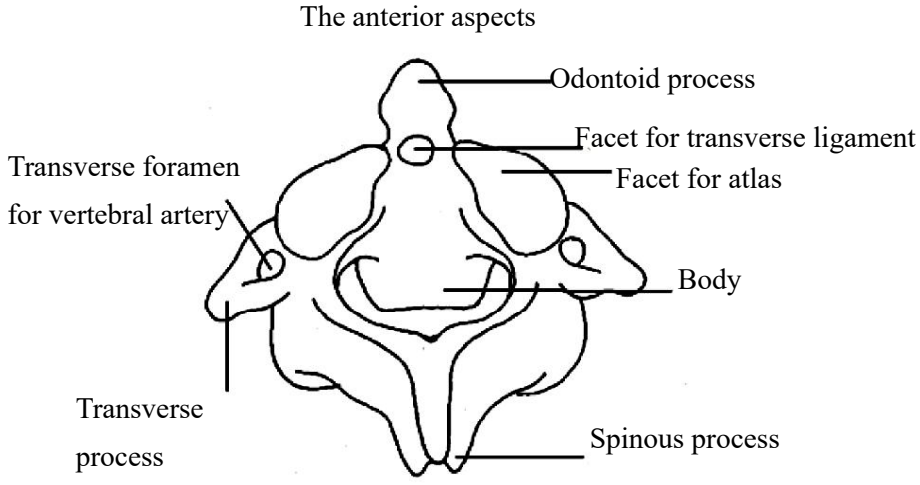


Figure 5.38:The structure of the axis vertebra

Thoracic vertebra: The twelve thoracic vertebrae are larger than cervical vertebrae as this region of the vertebral column has to support more body weight. The body and transverse processes of thoracic vertebrae have facets for articulation with the ribs

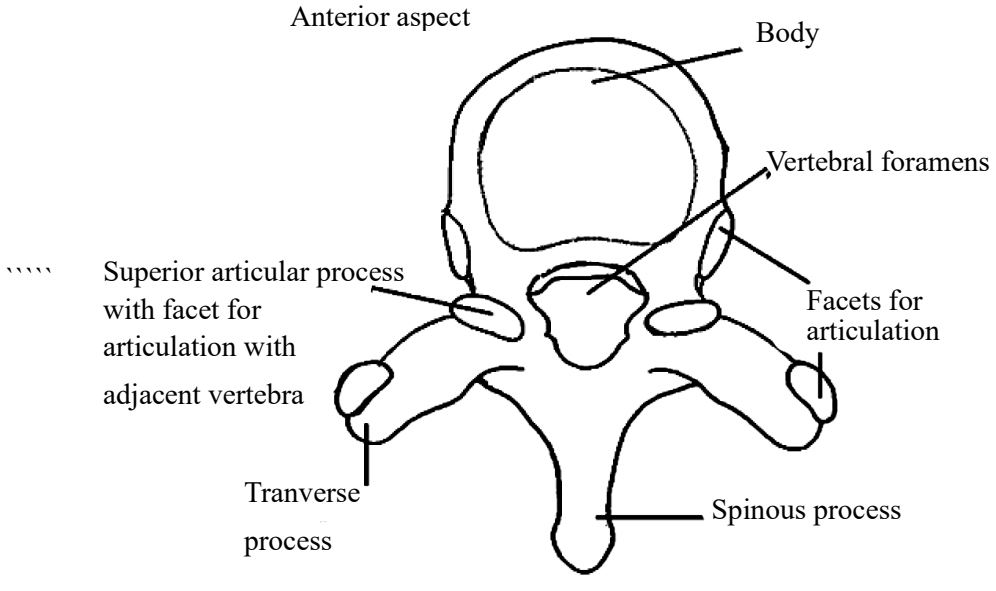


Figure 5.39 :The structure of thoracic vertebra

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Lumbar vertebrae: The five lumbar vertebrae are the largest of the vertebrae because they have to support the weight of the upper body. The size of the body of the lumbar vertebrae is larger compared to the other vertebrae. For attachment of the muscles of lower back, the lumbar vertebrae have relatively large spinal processes.

Sacrum and Coccyx: Sacrum is a triangular shaped large bone consisting of five fused rudimentary vertebrae. It has a concave anterior surface. The upper part articulates with the fifth lumbar vertebrae On each side, sacrum articulates with the pelvic girdle. Inferior tip of the sacrum articulates with coccyx. A series of vertebral foramina are present on each side for passage of nerves. Coccyx consists of fused four terminal vertebrae to form a small triangular bone. The broad base of the coccyx articulates with the tip of the sacrum.

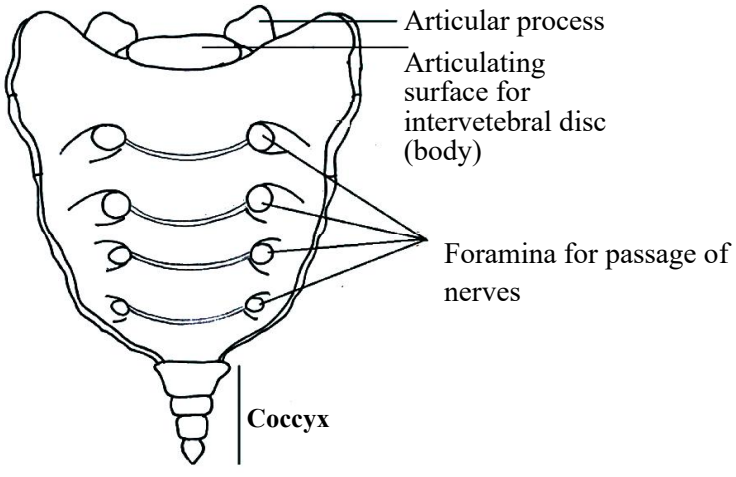


Figure 5.40: The anterior view of the sacrum and coccyx

Common functions of the human vertebral column

The vertebral column helps to maintain the erect posture. It supports the skull and gives attachment to ribs and girdles. It also provides the protection for the spinal cord. Vertebral foramens provide spaces for spinal nerves and blood vessels and lymph vessels. The vertebral column allows flexibility in the body movements. The intervertebral discs act as shock absorbers and protect the spinal cord.

Sternum

Sternum is a long flat bone that forms anterior part of the thoracic cage (which is made up of sternum, ribs and thoracic vertebrae). The uppermost section of the sternum is the manubrium which articulates with the clavicles in the pectoral girdles and the first two pairs of ribs. The body, which is the middle part of the sternum gives attachment to the rest of the ribs. The xiphoid process is the tip of the bone which gives attachment to the diaphragm and muscles of the anterior

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abdominal wall. The sternum provides protection to the organs and blood vessels that lie behind it (heart and lungs) from physical damage. The red bone marrow in the sternum is one of the main sites for production of blood cells.

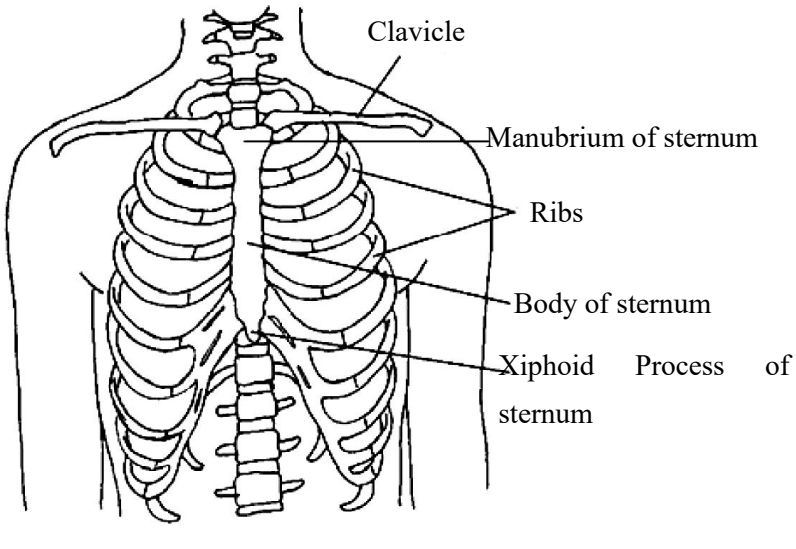


Figure 5.41:The thoracic cage and the location of the sternum

Ribs

The twelve pairs of ribs form the lateral walls of the thoracic cage. They are elongated curved bones. They articulate posteriorly with the thoracic vertebrae of the vertebral column. Anteriorly 7 pairs of ribs articulate with the sternum (true ribs), next 3 pairs articulate with sternum indirectly. In both cases costal cartilages attach the ribs to the sternum. The lowest 2 pairs do not join the sternum (floating ribs). Head of the rib articulates with vertebral bodies, facets of tubercle articulate with transverse process of vertebrae. The thoracic cage which includes the ribs and sternum plays an important role in the mechanism of breathing. Between each ribs intercostal muscles are present which move the rib cage during breathing. The first rib is firmly fixed to the sternum and to the first thoracic vertebra. Therefore it does not move during inspiration. Because it is a fixed point when the intercostal muscle contract they pull the entire rib cage upwards and towards the first ribs. Presence of 12 pairs of ribs and sternum provides protection to the organs such as lungs and heart in the thoracic cavity.

Contribution of human axial skeleton to maintain the upright posture

- Presence of two primary curvatures and two secondary curvatures in the vertebral column. Development of the two secondary curvatures in the vertebral column mainly contribute to maintain the erect posture. (Refer the section on curvatures of vertebral column).

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- The size of the vertebrae (especially the body of the vertebrae) become larger towards the end of the vertebral column as they have to support the weight of the upper body (Refer the section on vertebrae).
- The sacral vertebrae are fused to form a triangular shaped large sacrum to support the weight of the vertebral column and internal organs of the body.
- The two occipital condyles (and the foramen magnum) are located inferiorly at the base of the skull close to the center. In the upright position, this arrangement permits proper balancing of the skull on the vertebral column.

The structure and functions of the human appendicular skeleton

Appendicular skeleton

The appendicular skeleton consists of upper limbs with pectoral (shoulder) girdle and lower limbs with the pelvic girdle. Through the pectoral girdle the upper limb forms the joints with the trunk. Pectoral girdle connects upper limb with the axial skeleton. Pectoral girdle consists of two scapulae (shoulder blades) and two clavicles (collar bones). The lower limb forms a joint with the trunk at the pelvic girdle. Pelvic girdle is formed from two hip bones and it is associated with the sacrum.

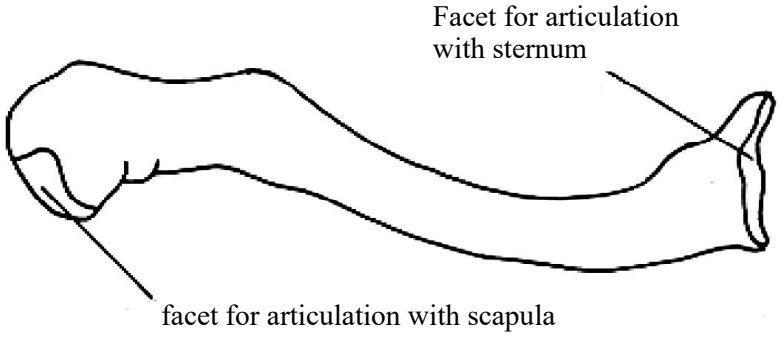


Figure:5.37 -The right clavicle

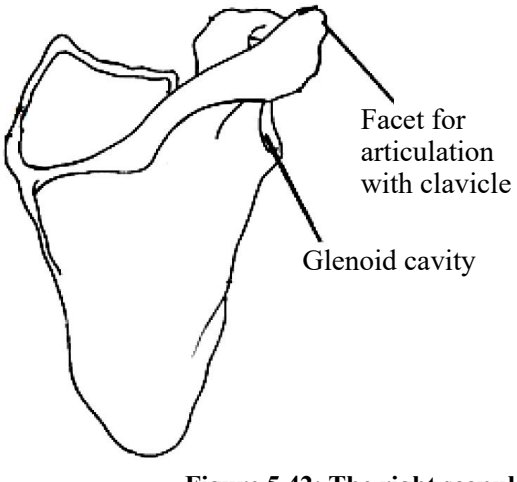


Figure 5.42: The right scapula

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Upper limb

Upper limb consists of humerus, radius, ulna, eight carpal bones, five metacarpal bones and fourteen phalanges. Humerus is the bone of the upper arm.

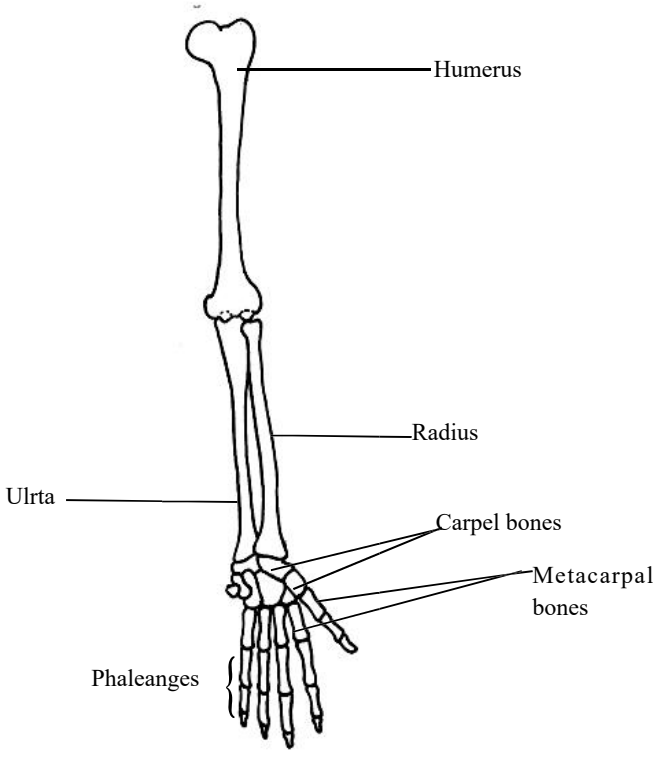


Figure 5.43: Bones of the upper limb

The adaptation of the human upper limb for movement through a wide range

Structure of upper limb is adapted for grasping, weight lifting and movement over a wide range.

Head of the humerus (the bone of the upper arm) forms an incomplete ball and socket joint (shoulder joint) in glenoid cavity of the scapula permitting a vast range of movements. The joint allows for flexion, extension, adduction, abduction, rotation and circumduction.

The distal end of the humerus has two articular surfaces. Through these surfaces, radius and ulna articulate with the humerus at the elbow joint. They articulate with the carpal bones at the wrist joint. Further ulna and radius are articulated with each other at the proximal and distal radio-ulna joints. In addition a fibrous joint connects the bones along their shafts which stabilize their association and maintain their relative position in spite of forces applied from the elbow or wrist. The elbow joint acts as a hinge joint which permits only flexion and extension of the fore arm.

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The carpal bones which are arranged in two rows (proximal row and distal row) are closely fitted together so that there is limited amount of movement between them. Proximal row bones are associated with the wrist joint and distal row bones form joints with metacarpal bones. Wrist joint is present between the distal end of radius and three proximal carpal bones. This arrangement allows pronation (palm down) and supination (palm up) of the lower part of the upper limb. In addition the wrist can be flexed, extended, abducted and adducted.

The proximal ends of metacarpal bones in the palm articulate with carpal bones and their distal ends articulate with phalanges. The joints between metacarpal and phalanges allow movement of the fingers and permit the power grip. Fingers may be flexed extended, adducted, abducted and circumducted with the first finger more flexible than the other. The joint present at the base of the thumb between a specific carpal bone and the first metacarpal bone allows more mobility to the thumb than the other fingers. This leads to opposable nature of the thumb which permits the thumb to move perpendicular to the other fingers. This articulation permits precision grip which is unique to man.

Lower limb

Lower limb consists of femur (thigh bone), tibia (shin bone), fibula, patella (knee cap), seven tarsal bones (ankle bones), five metatarsal bones (bones of the foot) and fourteen phalanges (toe bones).

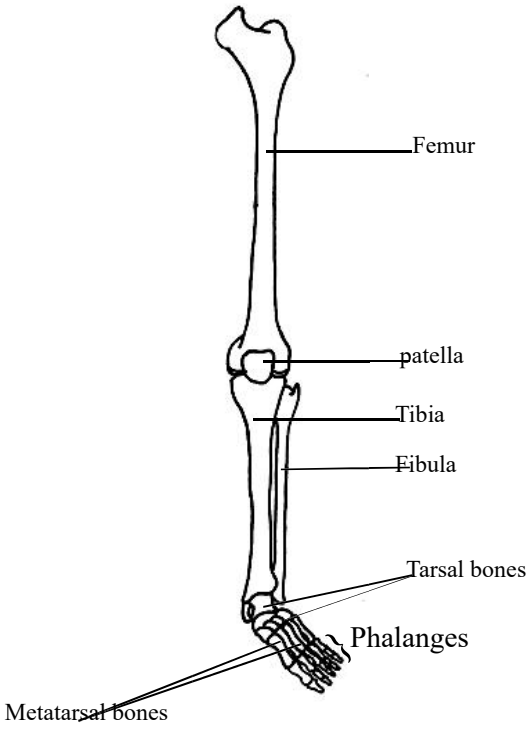


Figure 5.44:Adaptations of the lower limb for the erect posture, bearing of body weight and walking

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Structure of the lower limb is adapted for strength, erect body posture, bearing body weight and walking.

Femur is the longest, heaviest and the strongest bone of the body. Head of the femur forms the hip joint (ball and socket joint) with the acetabulum of the hip bone of the pelvis. This hip joint is very sturdy and powerful as it bears all body weight when standing. The lower limb can be extended, flexed, abducted, adducted, rotated and circumducted at the hip joint.
Lower end of femur articulates with tibia and patella to form the knee joint. Tibia is the medial of the two bones. Possible movements at the knee joint are flexion, extension and a rotatory movement that locks the joint when it is fully extended. When this joint is locked it is possible to stand upright for a long period of time.

Femur transmits the weight of the body through the bones below the knee to the foot. All the lower ends of both tibia and fibula articulate with a specific tarsal bone to form the ankle joint. The ankle joint allows rising in tip toe and lifting toes towards the calf.

The arrangement of bones in the foot supported by associated ligaments and muscles gives the sole of the foot an arched or a curved shape. There are two longitudinal arches and one transverse arch in the foot. Curve running heel to toe is called the longitudinal arch and the curve running across the foot is called the transverse arch. In the upright position, these arches of the foot are important in distributing the weight of the body evenly whether stationary or moving.

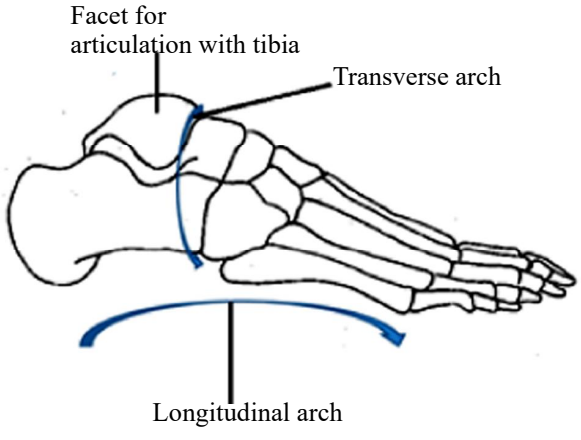


Figure 5.45 :The arches of foot

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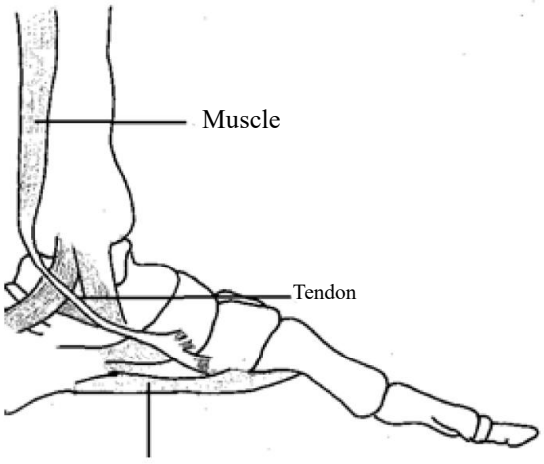


Figure 5.46 :The tendons and ligaments in the foot

Some disorders and abnormalities associated with human skeletal system

Osteoporosis

Osteoporosis is a condition associated with the reduction of bone density due to the exceedance of the bone reabsorption rate over the deposition rate. This gives in fragility to the bone tissue. This condition leads to immobility in joints and may cause fractures, skeletal deformities and bone pain. Factors causing osteoporosis include hormonal imbalances (especially at menopause), calcium deficiency and environmental factors.

Osteoarthritis

Osteoarthritis is a degenerative non-inflammatory disease that causes pain and restricted movements in the affected joints. Articular cartilage at the joints gradually become thinner so that articular surfaces of the bones come in contact and eventually the bones begin to degenerate; the outcome is pain. The cause of osteoarthritis is unknown. But risk factors include excessive use of affected joints, female gender, increasing age, heredity and obesity.

Slipped disc

The bodies of adjacent vertebrae are separated by intervertebral discs which serve as shock absorbers. These intervertebral discs consist of an outer ring of cartilage and a central core of soft gelatinous material. An injury or weakness can cause the inner portion of the intervertebral disc to protrude through the outer ring. This condition is called ‘slipped disc’. This leads to pain and discomfort. If the slipped disc compresses a spinal nerve, there can be numbness and pain along the affected nerve. Slipped disc condition can arise when lifting heavy weights without bending knees.

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Main types of joints in the human skeletal system

Main types of the joints in the human skeletal system are ball and socket joint, hinge joint and pivot joint.

- **Ball and socket joints**

  In these joints, ball shaped head is connected with the cup shaped socket and allows for wide range movements such as flexion, extension, adduction, abduction, rotation and circumduction. There are two ball and socket joints available in the human body: Shoulder joint and hip joint. (Refer upper limb and lower limb)

- **Hinge Joints**

  The articulating ends of the bone fit together in such a way so it looks like a hinge of a door. This allows only restricted movements such as flexion and extensions. Examples for hinge joints are elbow joint, knee joint, ankle joint and joints between the phalanges of the fingers and toes. (Refer upper limb and lower limb)

- **Pivot joints**

  One bone fits into a hoop shaped ligament that holds it close to another bone and allows it to rotate in the ring formed by the ligament. These joints allow a bone or limb to rotate. For example head rotates by the pivot joint formed by the axis vertebrae within the transverse ligament ring and odontoid process of the atlas. (Refer vertebral column)

Skeletal muscle and mechanism of contraction

Features of skeletal muscle tissue

The skeletal muscles are generally attached to the skeletal system and mainly cause voluntary body movements. Skeletal muscle tissue is composed of bundles of long cylindrical cells. These cells are aligned parallel to each other along the length of the muscle. Each cell contain multiple nuclei close to the cell membrane. Inside the cell, bundles of myofibrils containing contractile microfilaments are located longitudinally along the length of the cell. Myofibrils in the muscle cell form repeating sections called sarcomeres. The repeating arrangement of sarcomeres within the skeletal muscle cell gives its striated appearance under the microscope. Sarcomeres are the basic contractile units of the striated muscle cell. Like smooth muscle cells and cardiac muscle cells, skeletal muscle cells show excitability or irritability (ability to receive and respond to stimuli), contractility (ability to contract or shorten), extensibility (ability to stretch or contract) and elasticity (ability to return to its original length after being stretched or contracted). The skeletal muscle is under the voluntary control of the somatic nervous system.

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Structure of the sarcomere, basic mechanism of skeletal muscle movement

Sarcomeres are the repeating contractile units present within a striated muscle cell. The sarcomere is composed of myofibrils containing contractile thick filaments and thin filaments which are made up of specific proteins. The thin filaments (formed mainly from actin protein) attached at the Z line, a dense stripe which forms the borders of the sarcomere. The thick filaments (formed from myosin protein) are fixed (at the M line) in the middle region of the sarcomere. Sarcomeres are found repeatedly between two Z lines in a skeletal muscle cell. At the resting stage of myofibrils, thick and thin filaments are partially overlapped. At the edge of the sarcomere there are only thin filaments while at the center of the sarcomere only thick filaments are present. Such arrangement of thick and thin filaments in the sarcomeres permits the shortening of the skeletal muscle cell during contraction and return to the original state during relaxation. The mechanical function arising from sarcomeres is produced by actin (found in thin filaments) and myosin (found in thick filaments) proteins.

The skeletal muscle contraction is mainly voluntary and under the control of the somatic nervous system. Upon stimulation, individual muscle cells in the skeletal muscle shortens due to the shortening of its sarcomeres, and thus the whole muscle may contract. Converting muscle contraction to movement needs a skeleton to which the muscles attach. Skeletal muscle contractions pull on the tendons attached to the bones. If contraction of the muscle causes the muscle to shorten, the bone and the body part will move. When the nervous stimulation is stopped, the muscles will return to the original length after being contracted.

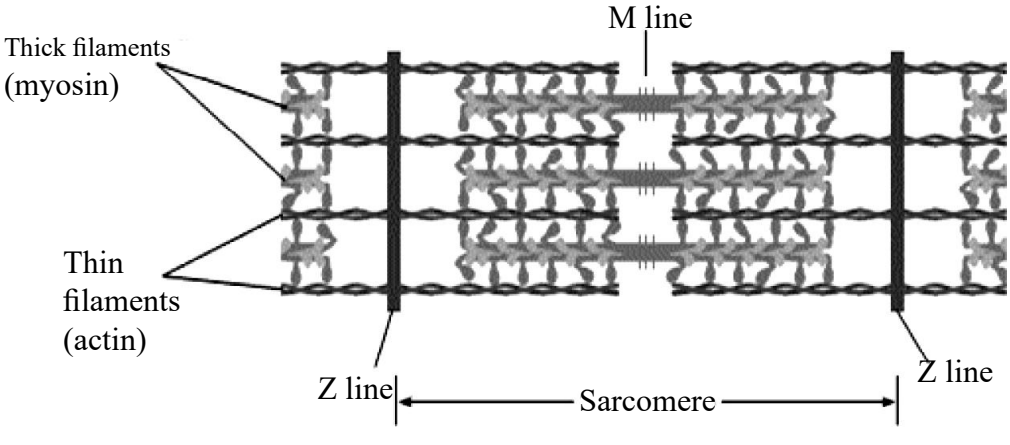


Figure 5.46: The arrangement of a sarcomere

Sliding filament theory is the currently accepted model of striated muscle contraction. According to this theory, when a skeletal (or cardiac) muscle cell contracts, the thick (myosin) filaments and thin (actin) filaments in each sarcomere slide past each other pulling the Z lines at each end of the sarcomere closer to one another shortening the sarcomeres and thus the muscle cell, while the two

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groups of filaments in the sarcomere remain at a relatively constant length. Myosin is the motor protein that does muscle contraction by pulling on thin filaments (actin) in muscle cells. Each myosin molecule is composed of 'tail' region and 'head' region. In the thick filaments, these 'tail' regions are bundled together while the 'heads' are sticking out. The thin filaments are composed of actin molecules which have binding sites for the 'head' region of the myosin molecules. The head region of the myosin can also bind with an ATP molecule when its 'low energy state'.

When the ATP molecule is hydrolyzed to form ADP and phosphate while releasing energy, the myosin head enters into the 'higher energy state'. At this state, the myosin head binds to myosin binding site of actin forming a cross bridge. Thereafter the myosin head returns to its lower energy state by releasing ADP and phosphate, which pulls (slides) the thin filament toward the centre of the sarcomere and so shortening the sarcomere. When a new molecule of ATP binds to the myosin head, the cross bridge is broken, myosin head detaches from actin. A new cross bridge cycle begins again. The contraction of muscles requires many number of repeated cycles of binding and releasing. In each cycle, the myosin head is released from the cross bridge and newly bound ATP is hydrolyzed which promotes binding of myosin again to a new actin molecule. This process occurs along the entire length of every myofibril in the muscle cell. Since in the earlier cycle the thin filament has moved towards the centre of the sarcomere, a new binding site for the myosin head region is exposed in the thin filament. The entire process causes the thick and thin filaments in the muscle cell to slide past each other pulling the Z lines at each end of the sarcomere closer to one another shortening the sarcomere.

Many myosin heads can be found in one thick filament. Within one second, each of these heads can form cross bridges. Ca^{2+} and some other proteins also play a major role in muscle contraction. Myosin can only bind to actin when the binding sites on actin are exposed by the action of calcium ions.

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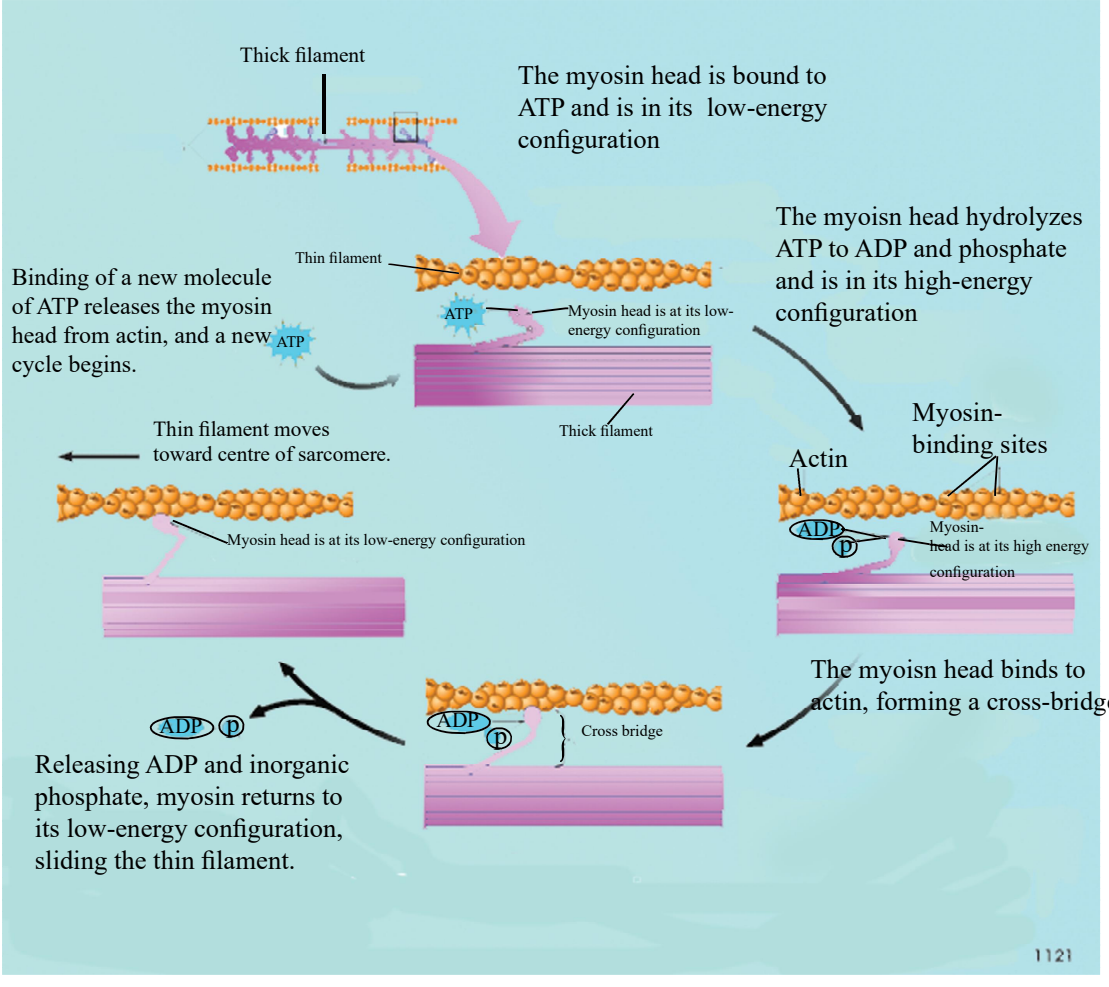


Figure 5.47: Interaction of actin and myosin in skeletal muscle cell contraction

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06 Genetics

Scientific basis of Mendel's experiments

Mendelian Heredity (Mendelism)

Principles in heredity were first formulated by an Austrian Augustinian monk named Gregor Mendel, who is now considered the father of modern genetics. Mendel discovered the basic principles of heredity by breeding garden peas in carefully planned experiments.

His experiments were conducted decades before the concept of chromosomes. The later discovery of chromosomes as the carriers of genetic units supported Mendel's two basic laws of genetics which are now known as **Mendelism.**

Vocabulary in genetics

There are numerous heritable variations among individuals of a population such as brown, green, or blue eyes or black, brown, or blond hair in human population. A heritable feature that varies among individuals of a population, such as hair colour or eye colour is called a **character** in genetics. These heritable variants of a character in an organism, such as brown or blond hair or blue, brown or black eye colour in human are called as **traits**. These traits are transmitted from parents to offspring. Observable traits of an organism is known as phenotype.

Mendel has described about 'heritable factors' in explaining his experimental results. These heritable factors are identified to be **genes** in modern genetics. **Gene** is the basic unit by which genetic information is passed from parent to offspring. It is a DNA sequence residing usually at a specific **locus** on a particular chromosome and contributes to the development of one or more traits by coding for specific proteins or peptides. Locus (Loci in plural) is a fixed position on a chromosome.

There are alternative versions of genes which are called **alleles.** Alleles reside on the same locus of different chromosomes. Alleles vary in their sequence of nucleotides. This change can affect the function of the protein encoded by the gene and thus the phenotype of the organism. Each diploid organism has at least two copies of each gene, residing on the chromosomes received by the two parents. These copies could be identical or could differ from one another. The condition of

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having two identical alleles for a given gene is known as **homozygous** state. Alternatively, having two different alleles for a given gene is referred to as **heterozygous** state.

Phenotype is brought about by the interaction between genotype of the individual with its environment. The genetic make up, or set of alleles, of an organism is known as its **genotype.** An individual's genotype could be either homozygous or heterozygous with respect to a given gene.

At heterozygous state, the allele which determines the organism's phenotype by masking the expression of the other is referred to as the **dominant allele**. The trait produced by the dominant allele is known as the **dominant trait.** The allele which does not exhibit any noticeable effect on the organism's phenotype at heterozygous state is referred as the recessive allele. The trait hidden on the recessive allele is the **recessive trait.** However, they express their trait when they exist in homozygous state.

Mendel tracked only those characters that occurred in two distinct, contrasting phenotypic forms, such as tall stem length vs. short stem length or purple flower colour vs white flower colour. Such traits are referred to as **contrasting traits.**

Mendel used only the **pure breeding** (sometimes called **true breeding**) varieties for his experiments. Pure breeding plants are obtained by self-pollinating over many generations, producing only the same variety as the parent plant. These uniform lines produced from self-fertilization of pure breeding varieties over many generations are called **pure lines.**
During his experiments, Mendel cross-pollinated pure-breeding garden pea plant varieties which shows contrasting **traits**. For example, purple-flowered plants were cross bred with white-flowered plants. Mating or crossing of two pure-breeding varieties with contrasting **traits** is called **hybridization**. Parental generation is referred to as the **P generation** (parental generation). Plant progeny resulted from these hybridization events are referred to as **F_1 generation** (First Filial generation, the word filial from the Latin word for "son"). The progeny that results from the self or cross pollination between these F1 generaion plants are known as **F_2 generation** (Second Filial generation).

An organism that is heterozygous with respect to a single gene of intrest resulting from a cross between parents having homozygous condition for different alleles of specific gene is referred to as a '**monohybrid**'. Breeding experiment conducted between two organisms with heterozygous condition for a specific character is referred to as **'monohybrid cross'.**
An organism that is heterozygous with respect to two genes of intrest resulting from a cross between

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parents having homozygous conditions for different alleles of two specific genes is referred to as a '**dihybrid**'. Breeding experiment conducted between two organisms with heterozygous conditions for two specific characters is referred to as a **'dihybrid cross'.**

Breeding an organism having unknown genotype for a specific dominant trait with an organism having homozygous recessive condition for the same specific trait is called a **test cross.** This is usually done to reveal the unknown genotypes for specific dominant traits in an organism.

Monohybrid Cross

Mendel derived his first law of inheritance by following only a single character in one breeding experiment, such as flower colour. He started by crossing pure breeding parents with contrasting **traits**. All the F_1 progeny produced from pure breeding parents are **monohybrids,** meaning that they are heterozygous for the particular character being followed in the cross.

F_1 hybrid pea plants were then self- or cross-pollinated and F_2 generation was produced to explore the traits resulting from a monohybrid cross (**Figure 6.1**).

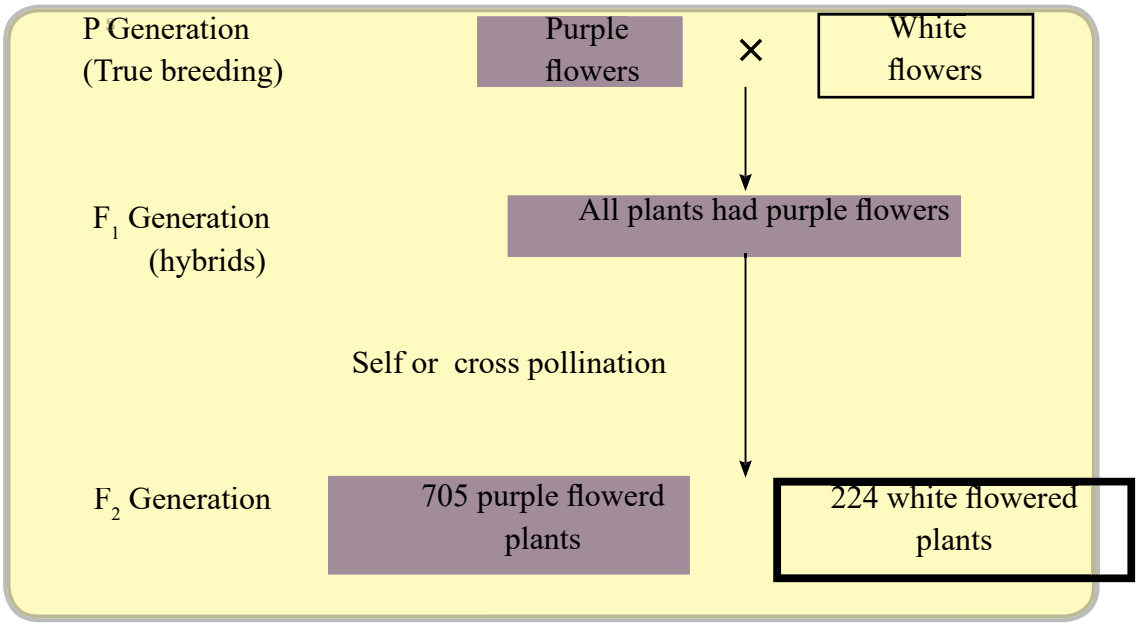


Figure 6.1: Mendel's experiment to investigate the inheritance patterns of a single character over two generations

Mendel, during his experiment, crossed pure breeding purple flowered plants and white flowered plants. Then he allowed the resulting F_1 hybrids to self and cross-pollinate with other F_1 hybrids. Finally, he observed the F_2 generation plants for the colour of the flowers.

During his observation, all F_1 plants produced purple colour flowers. However, in the F_2 generation, both purple and white flowered plants appeared in a ratio of approximately 3: 1.

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Among the heterozygote resulted in the F_1 generation, the “heritable factor” responsible for producing white flowers were suppressed in presence of the "heritable factor" that produces purple flowers. As a result, the heterozygotes were all producing purple colour flowers. Therefore, the ‘heritable factor’ for purple flower colour is dominant to the white (dominant trait). Accordingly, the factor for white flower colour is referred as the recessive trait.

Mendel observed that the same pattern of inheritance consistently occured in six other characters; position of the flower, colour of the seed, shape of the seed, shape of the pod, colour of the pod and the length of the stem.

Mendel’s first law of inheritance: The law of segregation

Mendel’s first law was put forward to explain the 3:1 inheritance pattern observed among the F_2 offspring in his monohybrid experiments using Pea plants.

As per his hypothesis, each ‘heritable character’ is determined by two "heritable factors" which are known as alleles. During the formation of gametes, the alleles for a ‘heritable character’ are separated and get in to each of the gametes formed. This is now known as **Mendel’s law of segregation or Mendel’s first law in inheritance.**

Analyzing genotype and phenotype ratios using Punett square

In Pea plants, Mendel observed two different **traits** based on stem lengths; tall and dwarf. For his experiments, pure breeding tall and dwarf Pea plants were selected for cross pollination. Thereafter the F_1 generation was self pollinated in order to obtain F_2 generation.

During self pollination of F_1 hybrids, gametes carrying different alleles fuse randomly. Such rdom fusion of gametes produces zygotes with four genetic combinations. A Punnett square can be used to illustrate these genetic combinations. A Punnett square is a graphical representation of the possible geneotypes of an offspring arising from a particular cross or breeding event. An example is given figure 6.2.

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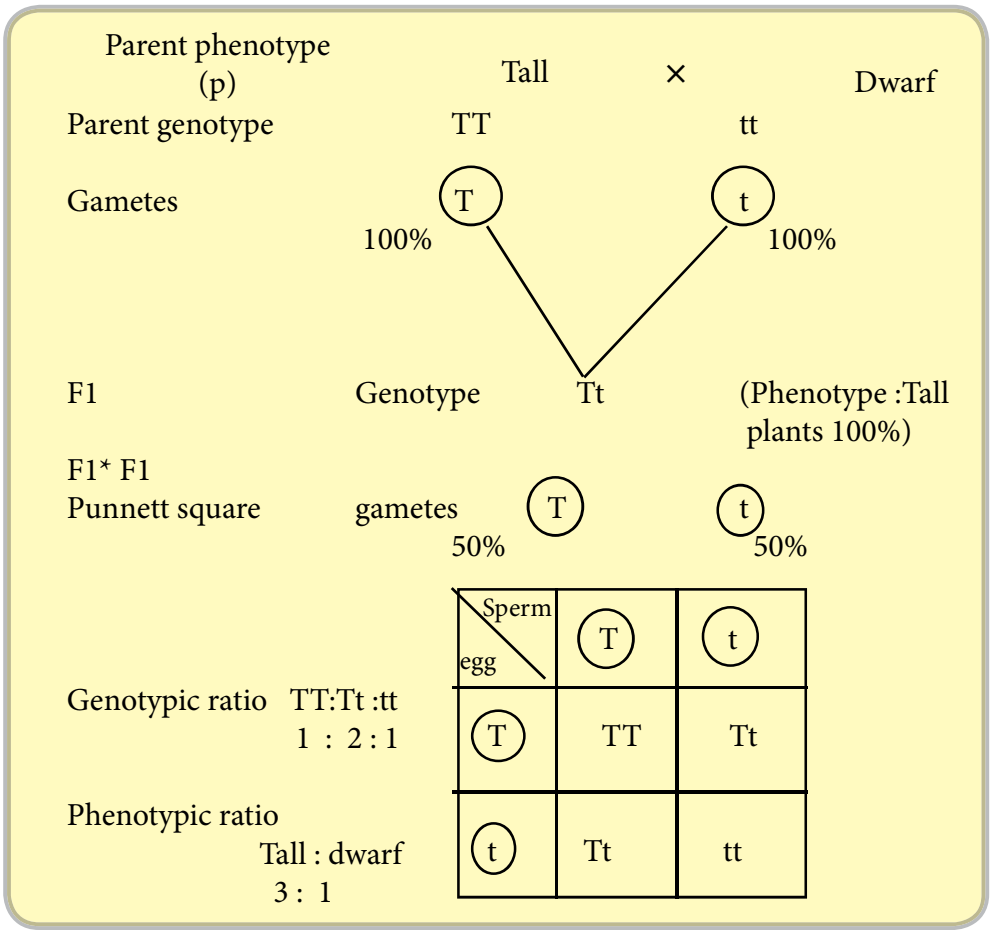


Figure 6.2: Mendel's law of seggregation shown with the aid of the punnett square

Dihybrid Cross

Mendel identified his second law of inheritance by following two characters at the same time, using **dihybrid crosses.** A cross between two homozygous (true breeding) organisms with contrasting traits for two specific characters being followed is known as a **dihybrid cross.** The aim of the Mendel's dihtybrid cross experiment was to find out whether the alleles for one character assort into gametes dependently or independently of the alleles of the other character.

Mendel crossed a true-breeding plant with yellow-round seeds with a true breeding plant with green-wrinkled seeds (Figure 6.3). The cross produced dihybrid F_1 plants, all of which have yellow-round seeds. As shown by the monohybrid crosses, the allele for yellow seeds is dominant (Y) over the allele for green seeds (y) which is recessive. likewise, the allele for round seed is dominant (R), and the allele for wrinkled seed is recessive (r). The F_1 hybrids, are heterozygous for the two characters being followed in the cross (YyRr). The cross between F_1 dihybrids produced the F_2 generation.

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This lead towards two alternative hypothesis for inheritance which predict different phenotypic ratios as shown in **Figure 6.3.**

1. The two characters could be transmitted from parents to offspring as a package. The dominant Y and R alleles or the recessive y and r alleles are passed together, generation after generation. This is called **dependent assortment** of alleles.
   According to this hypothesis only two types of gametes are possible; i.e. YR and yr. Thus the phenotypic ratio of the F_2 generation would be similar to that of a monohybrid cross (3:1)
2. The two characters (seed colour and seed shape) could be transmitted from parents to offspring independent of each other i.e. Y allele could be passed either with R or r allele vice versa. This is called **independent assortment** of alleles.
   This hypothesis predicts four different allelic combinations for a bi-allelic locus and thus four different types of gametes from F_1 generation; i.e, YR, Yr, yR and yr. According to this, both male and female gametes have four possibilities for each. Therefore, during the union of male and female gametes, there are 16 (4 × 4) equally probable ways in which the alleles can combine to produce the F_2 generation. As shown in Figure 3, these combinations would give rise to four different phenotypes with a ratio of 9:3:3:1 (nine Yellow round to three Green-round to three Yellow-wrinkled to one Green-wrinkled).

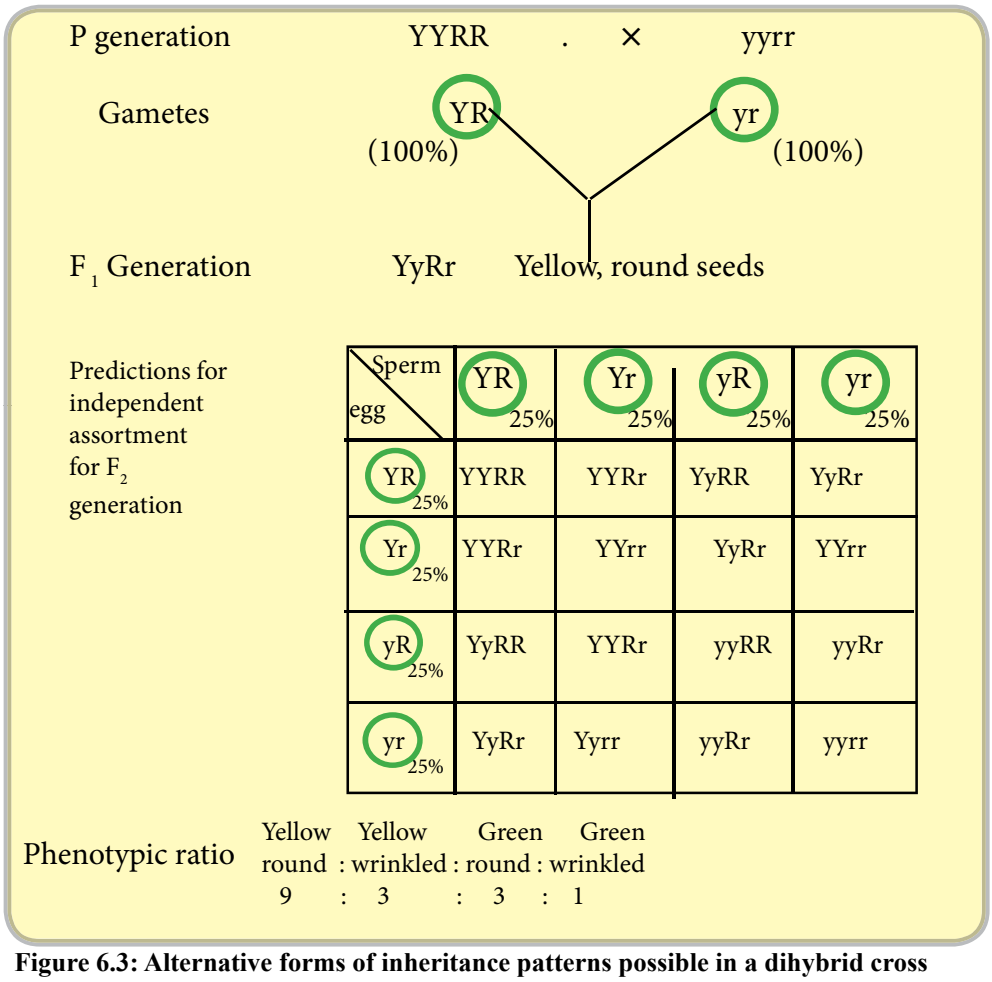


Figure 6.3: Alternative forms of inheritance patterns possible in a dihybrid cross

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Mendel's experiment produced the four distinct phenotypes (Yellow-round, Green-round, Yellow-wrinkled and Green-wrinkled seeds) predicted in the second alternative hypothesis in the ratio of 9:3:3:1. This showed that, the alleles responsible for each trait assorted independently of those of the other.

Mendel's second law of inheritance (The law of independent assortment)

Based on these experiments, Mendel put forward his **second law of inheritance - the law of independent assortment.** The law states that, alleles separate and pair up independently during the formation of gametes. As a result of that, two or more genes assort independently irrespective of the other.

However, according to current knowledge, this condition applies to two circumstances only;

- To genes located on different chromosomes (genes on non-homologous chromosomes)
- To genes located far apart on the same chromosome

Success of Mendel's experiments

Mendel followed a scientific approach in conducting his experiments. The following features in his experiments helped him to unravel the two fundamental principles of heredity.

- Mendel carried out thousands of genetic crosses of any given kind. This allowed his results to closely resemble the probability predictions. Usually, the larger the sample size, the closer the results to the value predicted based on the probability.
- He kept accurate records of his results. These records helped him to trace the patterns which otherwise would go unnoticed.
- He usually followed up each cross for at least two offspring generations (F_1 and F_2). This helped him to uncover some of the traits hidden in the F_1 generation.
- He did a quantitative analysis of the phenotypes of the resulting offspring.

Desirable properties in garden peas for genetic experiments

Garden peas (*Pisum sativum*) carry following desirable properties which makes it a suitable organism to study patterns of inheritance.

- Pea plants are available in many varieties with contrasting traits.
- The generation time is short.
- A large number of offspring is produced from each cross.
- Crossing between the plants could be strictly controlled (self/ cross pollination).

Probability laws and Mendelian inheritance

Mendel's laws of segregation and independent assortment reflect the same rules of probability that applies to tossing coins, rolling dice, and drawing cards from a deck. **Probability** measures

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how likely an event is to occur out of the number of possible outcomes. It is calculated by dividing the number of events of interest by the number of total possible outcomes.

1. The probability scale ranges from 0 to 1.
   An event that is certain to occur has a probability of 1, while an event that is certain not to occur has a probability of 0.
   During allele segregation in a F_1 plant (heterozygous) of a monohybrid cross,
   -probability of each egg carrying the dominant allele = 1/2
   -probability of each egg carrying the recessive allele = 1/2

2. The probabilities of all possible outcomes for an event add up to 1.
   During allele segregation in a heterozygous F_1 plant, probability of all events (having dominant and recessive alleles) = 1/2 +1/2= 1
3. When the occurrence of an event does not affect the occurrence of another event (independent events), the probability of simultaneous occurrence of both events can be obtained by multiplying the probability of one event by the probability of the other event. This is known as the **Multiplication Rule** or **Product rule in Probability.**

   In Mendel's monohybrid crosses, for a F_2 plant to have wrinkled seeds (rr), both the egg and the sperm that come together must carry the r allele.
   The probability that the egg will have an r = 1⁄2
   The probability that the sperm will have an r = 1/2
   The probability of both gametes at fertilization carrying r allele = ½ x ½ = ¼

4. The probability that any one of two or more mutually exclusive events will occur is calculated by adding their individual probabilities. This is the addition rule or sum rule of probability.
   There are two possible mutually exclusive ways for producing F_2 heterozygotes.
   i. The dominant allele come from the egg and the recessive allele from the sperm; the probability of the event = ¼ (according to example in above 3rd sentence)
   ii. The recessive allele from the egg and the dominant allele from the sperm; the probability of the event = ¼ (according to example inabove 3rd sentence)
   So, the probability of getting an F_2 heterozygote = ¼ + ¼ = ½

Prediction of the inheritance patterns in multifactorial crosses

When the pattern of inheritance of two or more characters of an organism is being traced during a genetic cross, it could be called as multifactorial cross. Finding out the outcomes of a multifactorial cross through a Punnett square may be a difficult task. Therefore, applying rules of probability may be useful to predict the outcomes of a multifactorial crossing.

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According to the law of seggeregation, a multifactorial cross can be considered equivalent to multiple independent monohybrid crosses occurring simultaneously.

E.g. 1: Dihybrid cross for seed color and seed shape,

Probabilities for seed color (based on the Punnett square for monohybrid cross)

Seed color	
Genotype	Probability
BB	¼
Bb	½
bb	¼

Seed shape	
Genotype	Probability
RR	¼
Rr	½
rr	¼

B: dominant allele for black colour seed b: recessive allele for brown coloured seeds

R: dominant allele for round shaped seeds r: recessive allele for wrinkled shaped seeds.

The probability of each of the genotypes in the F_2 generation can be determined by using the multiplication rule.

Probability of BbRr = ½ (probability of Bb) × ½ (probability of Rr) = 1/4

Probability of bbRr = 1/4 (bb) × 1/2 (Rr) = 1/8

Probability of bbrr = 1/4 (bb) × 1/4 (rr) = 1/16

E.g. 2: A trihybrid cross for flower color, seed color and seed shape.

Y: Dominant allele for yellow colour petals y: Recessive allele for white coloured petals

B: Dominant allele for black coloured seeds b: Recessive allele for brown coloured seeds

R: Dominant allele for rond seeds r: Recessive allele for wrinkled alleles

YyBbRr x yyBbrr

(YyBbRr): Yellow coloured petals with black round seeds

(yyBbrr): white coloured petals with black round seeds seeds

Flower color	
Genotype	Probability
YY	0
Yy	½
yy	½

Seed shape	
Genotype	Probability
BB	¼
Bb	½
bb	¼

Shape of the seeds	
Genotype	Probability
RR	0
Rr	½
rr	½

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Assume that unknown pea plant is having genotype of TT

The above crossing has resulted 640 plants in the F1 generation. Determine the number of plants exhibits dominant phenotype for at least two characters.

1. Possible genotypes with their individual probabilities for above conditions

   YyBBRr: ½ (probability of Yy) X ¼ (BB) X ½ (Rr)= 1/16

   YyBbRr: ½ X ½ X1/2 = 1/8

   YyBBrr ½ X ¼ X ½ = 1/16

   YyBbrr ½ X ½ X ½ = 1/8

   YybbRr ½ X ¼ X 1/2 = 1/16

   yyBbRr ½ X ½ X ½ = 1/8

   yyBBRr ½ X ¼ X 1/2 = 1/16

2. Probability of exhibiting at least two dominant characters= 1/16 + 1/8 + 1/16 + 1/8 + 1/16 + 1/8 +1/16 = 10/16= 5/8

3. Number of plants expected to exhibit at least two dominant characters = 5/8 × 640= 400 plants

The testcross

This is a deliberate breeding process performed in order to determine the unknown genotypes. The genotype of an individual showing dominant trait may be due either to double dominant genotype or to heterozygous status. This involves the crossing of an organism having unknown genotype for a selected phenotype along with another organism from same species having homozygous recessive condition for same character.

Testcross performed during the monohybrid cross is called as monohybrid testcross. On the other hand, test cross performed during the dihybrid cross is called as dihybrid testcross.

Monohybrid testcross

Let's consider an example for monohybrid testcross. In this example we want to know the genotype of the given tall pea plants. In order to do that, we will cross the tall pea plant with the dwarf pea plant. Since dwarf is a recessive trait the genotype of it will be tt.

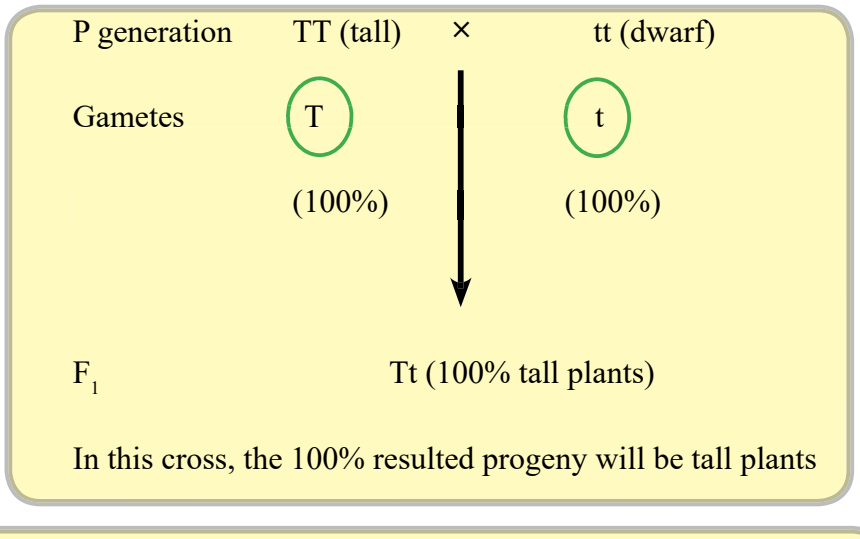
There may be two possible genotypes for tall pea plants;

1. TT
2. Tt

Assume that unknown pea plant is having genotype of TT

Cross between TT and tt will result the following.

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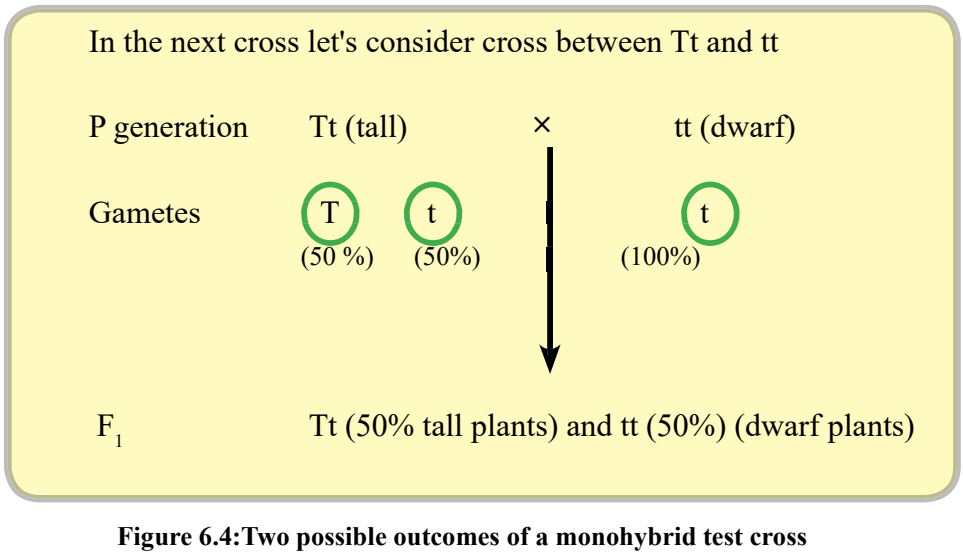


Figure 6.4:Two possible outcomes of a monohybrid test cross

Dihybrid testcross

When an individual with dominant traits for two characters (e.g. RrBb) is crossed with a pure recessive for both characters (wwbb), it is known as a **dihybrid testcross.**

For example, let's consider a cross between plants having red coloured flower petals with black coloured seeds and white coloured flower petals with brown coloured seeds.

The possible genotypes for red coloured petals with black coloured seeds are RrBb/ RRBb/RrBB/ RRBB and for white coloured petals with brown coloured seeds is wwbb.

If the unknown phenotype is RrBb;

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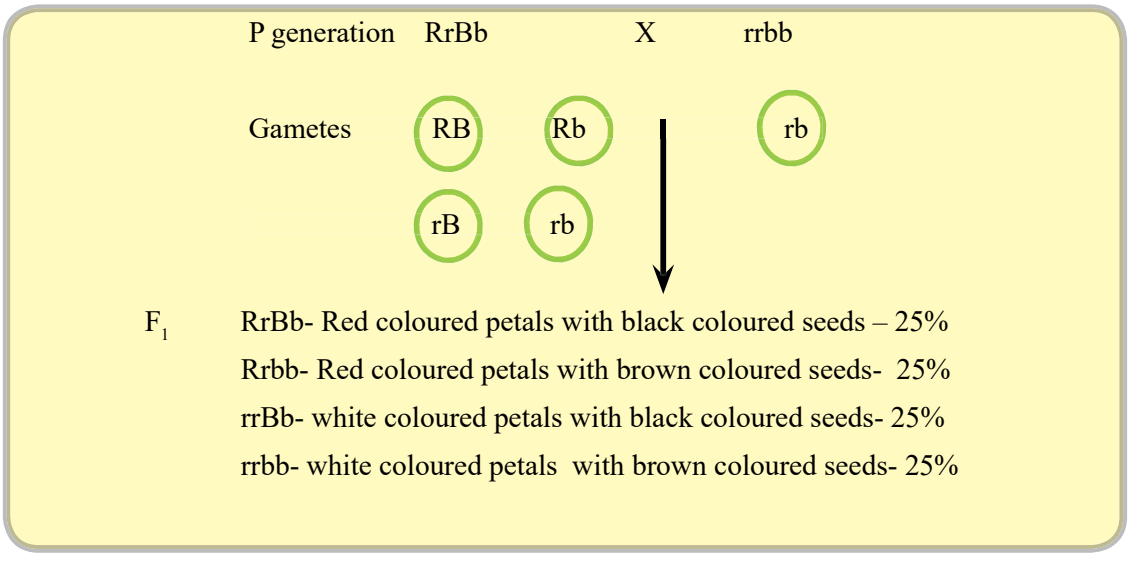


Figure 6.5: (a) Possible outcomes of a dihybrid test cross

In this example, above four phenotypes are possible with 1:1:1:1 ratio.

If the unknown phenotype has the RRBb genotype, the resulting dihybrid cross will produce following phenotypes;

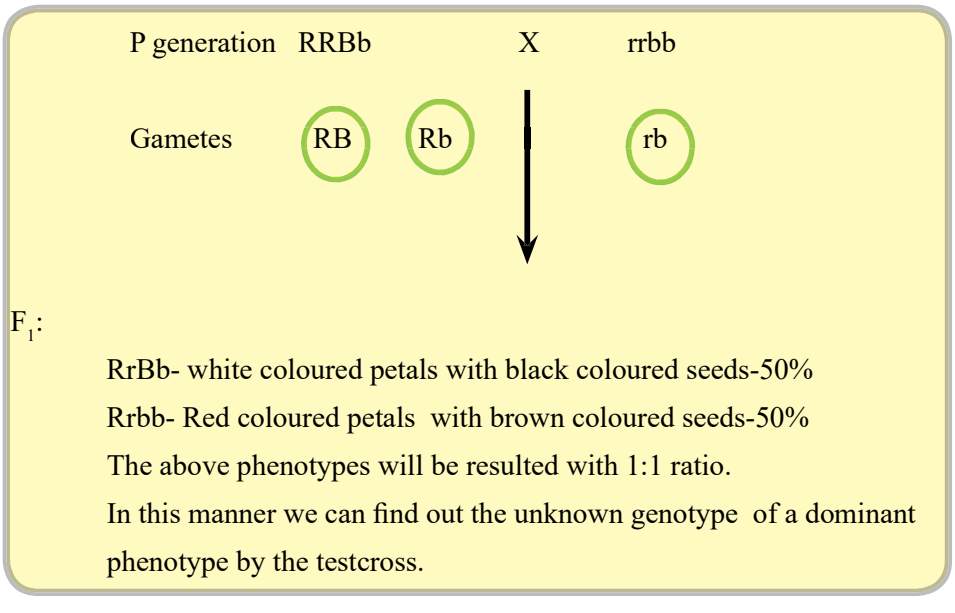


Figure 6.5: (b) Possible outcomes of a dihybrid test cross

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Patterns of inheritance of Mendelian characteristics in humans

Common Mendelian characters

Many human traits follow Mendelian patterns of inheritance. Some common examples are stated below;

Attached or detached earlobe:

The extent to which the earlobe is attached to the head is inherited in the Mendelian pattern. The attached earlobe is a recessive trait. Presence of both copies of the recessive allele (homozygous recessive condition) for ear lobe attachemnt would result in attached ear lobe.

Widow's peak:

The pointed contour of the hairline on the forehead is known as Widow's peak. It is due to a dominant allele, W. Therefore, all individuals who lack a widow's peak must be homozygous recessive (ww).

Dimples on cheek:

Cheek dimples are a genetically transmitted trait found in the muscle of the cheek.

When a person smiles, the shorter muscle on the face pulls up the facial skin. This, in turn, creates a slight depression in the skin, which is called dimple. Dimples often occur on both the cheeks. A single dimple on one cheek is a rare phenomenon. Dimple is a dominant trait and inherited in Mendelian fashion.

Bent thumb (Hitchhiker's thumb) and Straight thumb

Hitchhiker's thumbs is a condition where thumb bend backwards while stretching due to the hyper extensibility of interphalangeal joints. Having the dominant 'S' allele would produce the dominant phenotype of straight thumb. The absence of the dominant alleles would allow the thumb to bend.

Rolling or non-rolling tongue

The ability to roll the lateral edges of the tongue upwards into a tube is known as tongue rolling. The tongue's intrinsic muscles allow some people to form their tongues into specific shapes. Rolling the tongue into a tube shape is a dominant trait with simple Mendelian inheritance.

Pedigree analysis

Diagramatic representation of the inheritence of a particular trait within a given family tree, is called pedigree chart. It is constructed by collecting data for many generations within a given family, so that the pattern of inheritence can be understood.

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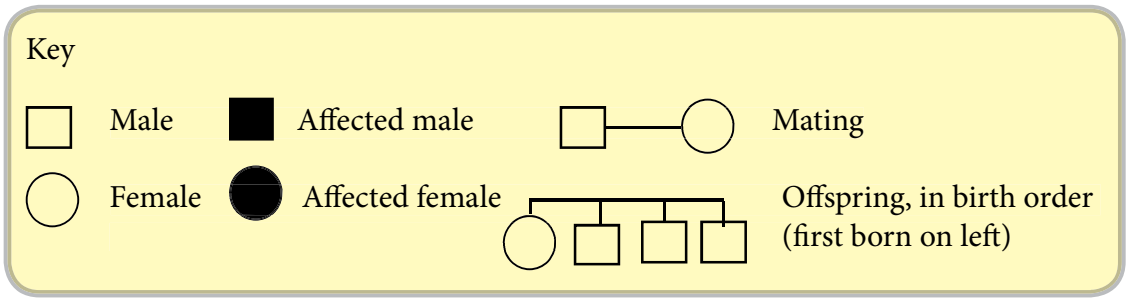


Figure 6.6: Standard Pedigree Symbols

Common Mendelian characteristics in humans analysed with pedigree charts

Widow's peak:

The inheritence of the trait, widow's peak, over three genertations in a particular family is represented in the pedigree chart given below. As shown in figure 6.7, only one grand parent had widow's peak, out of the two pairs. Since widow's peak is a dominant character, grand parents without widow's peak should be homozygous recessive (ww) for the trait. In the next generation some individuals showed the widow's peak, while the others did not. The two grand parents, who express the widow's peak should be heterozygous (Ww) for the condition . Likewise, the two parents of the third generation, who are showing widow's peak should be heterozygous for it, as one of both their parents (1st generation) are homozygous recessive (ww). the third generation with widow's peak can carry either WW or Ww geneotype, as both their parents are having the trait.

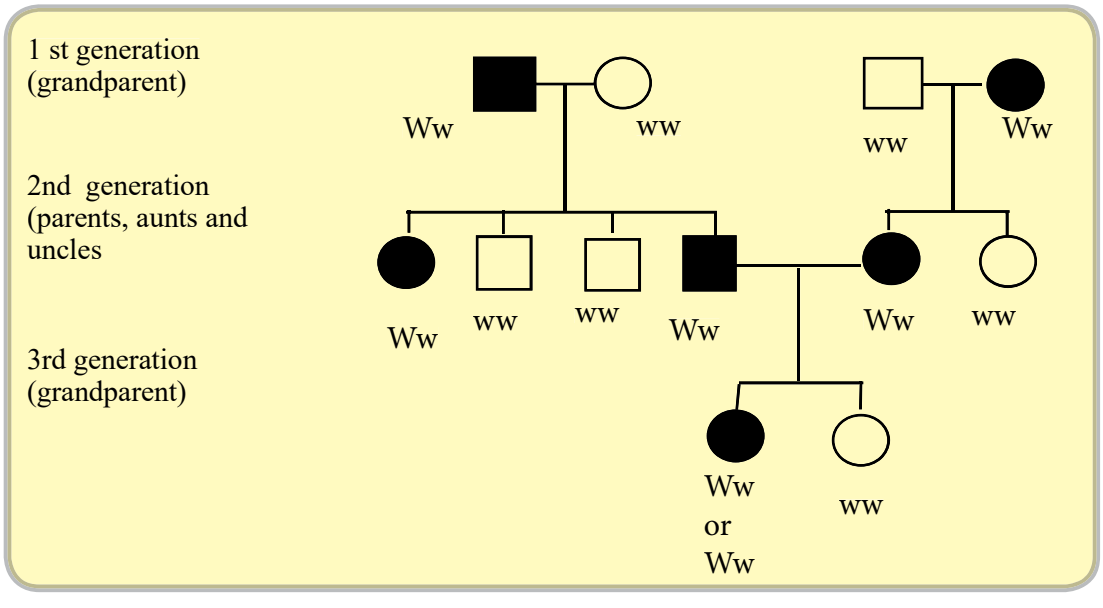


Figure 6.7: Inheritance of Widow's peak

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Attached ear lobe

Attached ear lobe, as explained earlier, is a recessively inherited character. In the pedigree chart given below, the inheritence of the tarit is analysed in the same family that was used to study the widow's peak. The dominant allele, which is causing the free ear lobe is denoted with 'F' while recessive allele is denoted with 'f'.

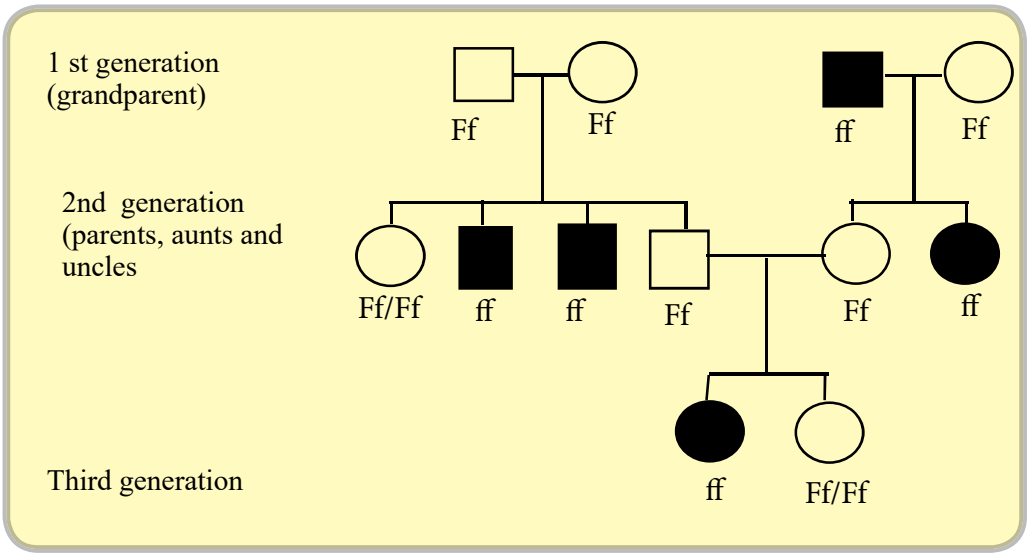


Figure 6.8: The pedigrce chart showing the inheritance pattern of attached lobe

In the first generation, both parents lacking the attachment of ear lobes, resulted mixed progenies with attached and free ear lobes. This tells us that those two parents were heterozygous (Ff), and among the progeny, two males with attached ear lobes carry homozygous recessive alleles (ff) and a male and female with free ear lobe may carry heterozygous (Ff) or homozygous dominant alleles (FF or Ff). Another cross happened at the first generation between a male with attached ear lobe and a female with free ear lobe. This resulted one daughter with attached ear lobe. Therefore, she must possess ff and the other may be FF or Ff. A male from one family and a female from another family, at the second generation expressing free ear lobe phenotype, had resulted progeny having two females, one with attached ear lobes and the other with free ear lobes for the third generation. Therefore, the second generation male and female crossed with free year lobes must be Ff. Third generation female with attached ear lobes must carry ff genotype and the other may carry either FF or Ff.

The probability that another child from the same family will have attached earlobes could be calculated using a monohybrid cross (Ff X Ff). Since homozygous recessive (ff) genotype is causing the condition, the probability is 1⁄4 for each child.

The chance that a child born to the family will have both the widow's peak and attached earlobes can be calculated using probability rules. Assuming that the alleles corresponding to the two

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characters are on different chromosomes, the two pairs of alleles assort independently in this dihybrid cross (WwFf X WwFf).

Thus according to multiplication rule,

Chance of having both widow's peak (WWff/Wwff)

and attached ear lobe = Chance of widow's peak x chance of attached earlo be

= 3/4 x 1/4

= 3/16

Non-Mendelian inheritance

Non-Mendelian inheritance refers to inheritance patterns in which traits do not segregate in accordance with ***Mendel's*** laws of inheritance. Phenotypes that do not appear in ratios predicted by Mendelian genetics are the indicators of Non-Mendelian inheritance.

Examples for non-mendelian patterns

- when alleles are not completely dominant or recessive (incomplete dominance and co-dominance),
- when a particular gene has more than two alleles (polyallelism)
- when a single gene produces multiple phenotypes (pleiotropy)
- sometimes two or more genes are involved in determining a particular phenotype (epistasis and polygenic inheritance)
- gene linkage
- genes which are located in sex chromosomes exhibit a different pattern of inheritance in males and females due to the unequal distribution of genes in their sex chromosomes.

Incomplete dominance

The phenomenon of dominant allele completely masking the recessive phenotype, resulting similar phenotypes for both homozygous dominant zygote as well as heterozygous zygote is called **complete dominance.**

On the other hand, at the heterozygous state, the phenomenon of expressing blend phenotypes from both alleles is called **incomplete dominance.** Degree of expression of each allele in the blend phenotypes may vary based on the nature of the alleles.

In *Mirabilis jalapa* (Four o'clock plant), there are several types of flower colours. When red flowered plants are crossed with white flowered plants, all the F_1 hybrids (heterozygotes) have pink flowers (Figure 6.8). This third, intermediate phenotype results from flowers of the heterozygotes having less red pigment than the red homozygotes.

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When these F_1 pink flowers are self-pollinated or crossed among themselves to raise F_2 generation, they produce red (C^{RR}), pink (C^{RW}) and white (C^{WW}) flowers giving 1:2:1 ratio. This phenotypic ratio is identical with genotypic ratio because heterozygotes are phenotypically intermediate between two homozygous types.

Note: Since, neither allele is dominant, instead of upper- and lowercase letters, a superscript is used to indicate the trait; i.e. C^R for red colour and C^W for white colour.

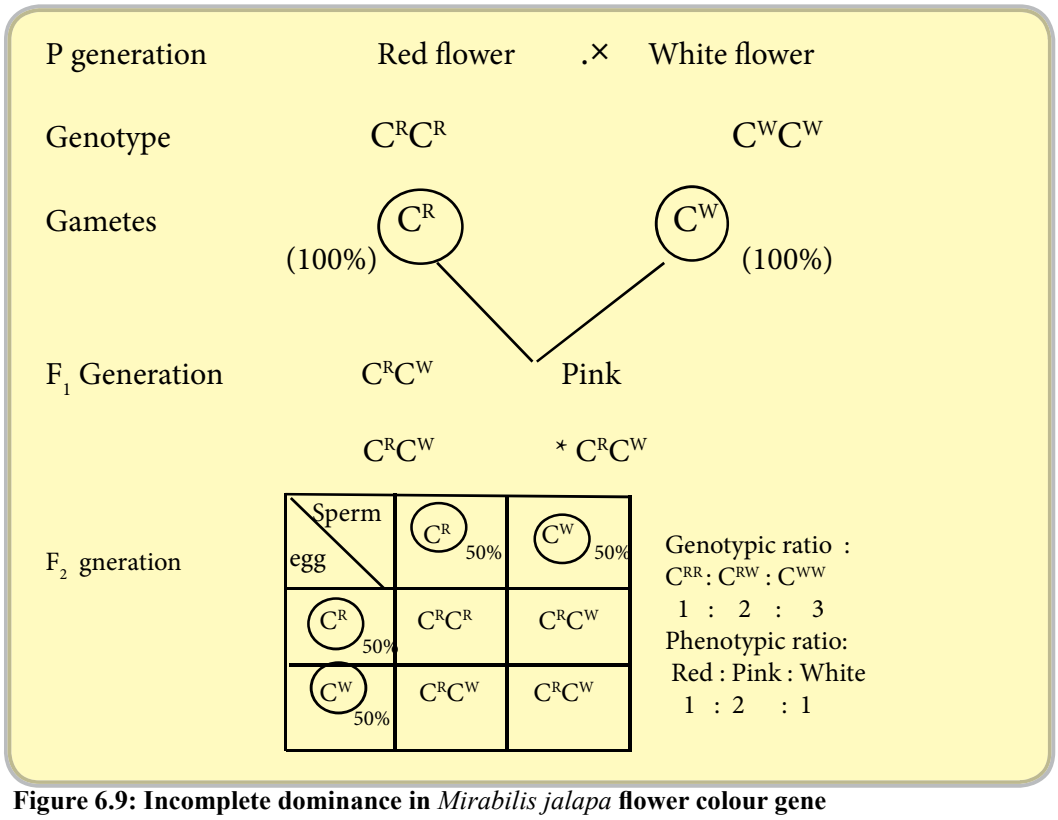


Figure 6.9: Incomplete dominance in *Mirabilis jalapa* flower colour gene

Codominance

In certain traits, at heterozygote state, expression of both alleles contributes equally to the phenotype. Such phenomenon is called **co-dominance.**

For example, a person with AB blood group type has both A and B carbohydrates on the surface of red blood cells at the same time. The two carbohydrates are added to the surface of RBC by enzymes encoded by the I^A and I^B alleles of a single gene. A heterozygous individual would express both carbohydrates (I^AI^B) in an equal manner.

Likewise,

Homozygotes for I^A allele (I^AI^A) will carry only A carbohydrate on RBC.

Homozygotes for I^B allele (I^BI^B) will carry only B carbohydrates on RBC.

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As shown below, the F_1 progeny resulting from the mating of two homozygous individuals for each allele would consist of individuals only of AB blood group. F_2 generation produced by the possible mating with in F_1 progeny or heterozygous individuals for the AB alleles, would produce the three phenotypes, blood type A: AB: B at a ratio of 1:2:1.

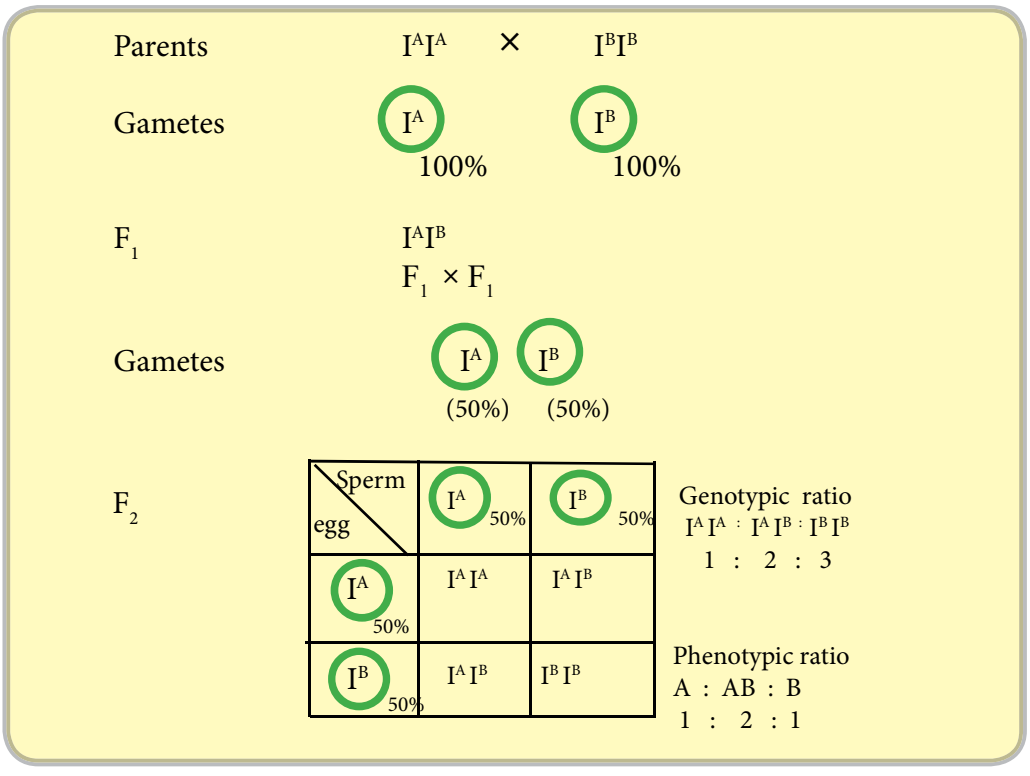


Figure 6.10: Codominance in ABO blood grouping

As shown, the F_2 phenotype ratios in both incomplete dominance and co-dominance are 1:2:1. Hence the two phenomena cannot be differentiated using F_2 phenotypic ratios.

The key to differentiate between incomplete dominance and co-dominance is that in incomplete dominance F_1 generation shows a different phenotype than both the parents whereas in co-dominance F_1 generation shows both the parental traits together.

Polyallelism (Multiple alleles)

Polyallelism refers to the presence of multiple alleles for a single genetic locus, a phenomenon where certain traits are determined by the combination of more than two types of alleles.
E.g. there are three alleles called **I^A, I^B,** and **i** for a single genetic locus which at different combinations determine ABO blood groups in humans.

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In any diploid individual there are only two of the several alleles are present.

As mentioned earlier, the alleles **I^A** and **I^B** code for enzymes that add A and B carbohydrates to the surface of red blood cells. The two alleles are in a co-dominant relationship. However, the allele 'i' results in lack of these carbohydrates on the red cell surface and it is recessive to both I^A and I^B alleles. Therefore, both **I^Ai** and **I^Bi** genotypes will result in dominant phenotypes; i.e. having either the A or B carbohydrates. The **ii** genotype will result in the recessive trait of not having either of the carbohydrates.

Thus, based on the presence of the two carbohydrates, a person's blood group may be one of four types as follows: type A (carbohydrate A present), type B (carbohydrate B present), type AB (both carbohydrates A & B present), or type O (neither of the two carbohydrates present).

F_1 and F_2 generations resulting from a mating between individuals with different blood groups are shown below

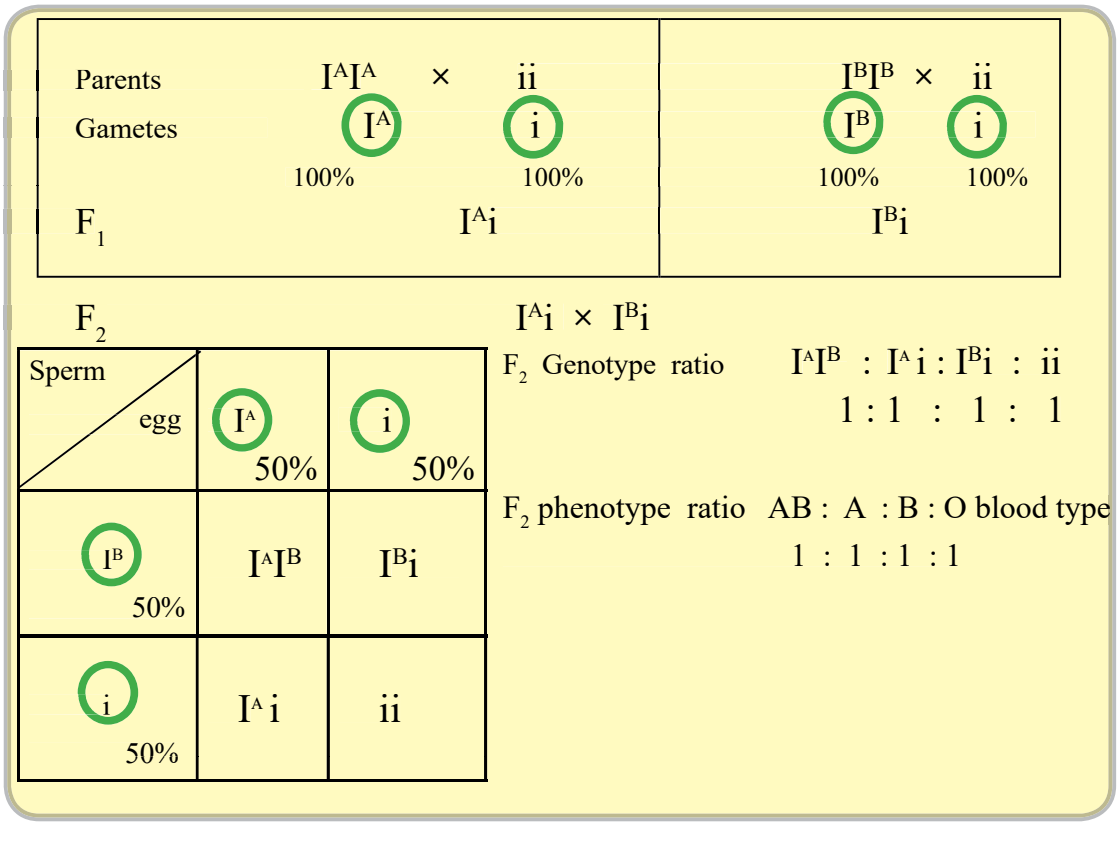


Figure 6.11: Multiple alleles for the ABO blood groups

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Epistatis

Epistasis is the phenomenon resulting from interactions between genes of different loci. The alteration in the phenotypic expression of a gene at one locus is due to the interference of another gene at a different locus.

Epistasis could be categorized into two types; dominant epistasis and recessive epistasis, based on the nature of the gene interaction. This causes deviation from the phenotype ratios of Mendelian principles.

Dominant epistasis

When a dominant allele at a specific lous alters the expression of a separate gene at a different locus, it is referred to as **dominant epistasis.** Dominant epistasis is seen in the plumage colour of house fowls. Consider the following experiment.

A cross between a known homozygous, double dominant, white housefowl and a homozygous double recessive white house fowl results a 100% white F_1 progeny. F_2 generation produced from a cross between these F_1 individuals consists of white and coloured fowls in the ratio of 13:3.

The colour of these two fowl varieties are determined by two separate genes;

- The gene 'C'/ 'c' is responsible for producing colour in the feathers. The dominant 'C' allele produces colour while the recessive 'c' allele result in the absence of pigmentation.
- The gene **'I'** is epistatic to gene **'C'** and suppresses the expression of the colour. The dominant **'I'** allele prevents production of pigments while recessive 'i' allele is unable to prevent colouration.

As a result, the double dominant homozygous (CCII) house fowl is white (dominant **I** allele prevents colouration). The double homozygous recessive house fowl (ccii) is white (recessive c allele couldn't produce colour).

The F_1 generation all consist of heterozygous (CcIi) fowls. Due to the inhibitory effect of the dominant **'I'** allele, all F_1 individuals are white. When these fowls are allowed to interbreed, the F_2 generation genotypes carrying inhibitory **'I'** allele will give rise to white colour plumage, despite the presence of the dominant **'C'** allele. The presence of dominant **'C'** allele in the absence of inhibitory **'I'** allele gives rise to coloured fowls.

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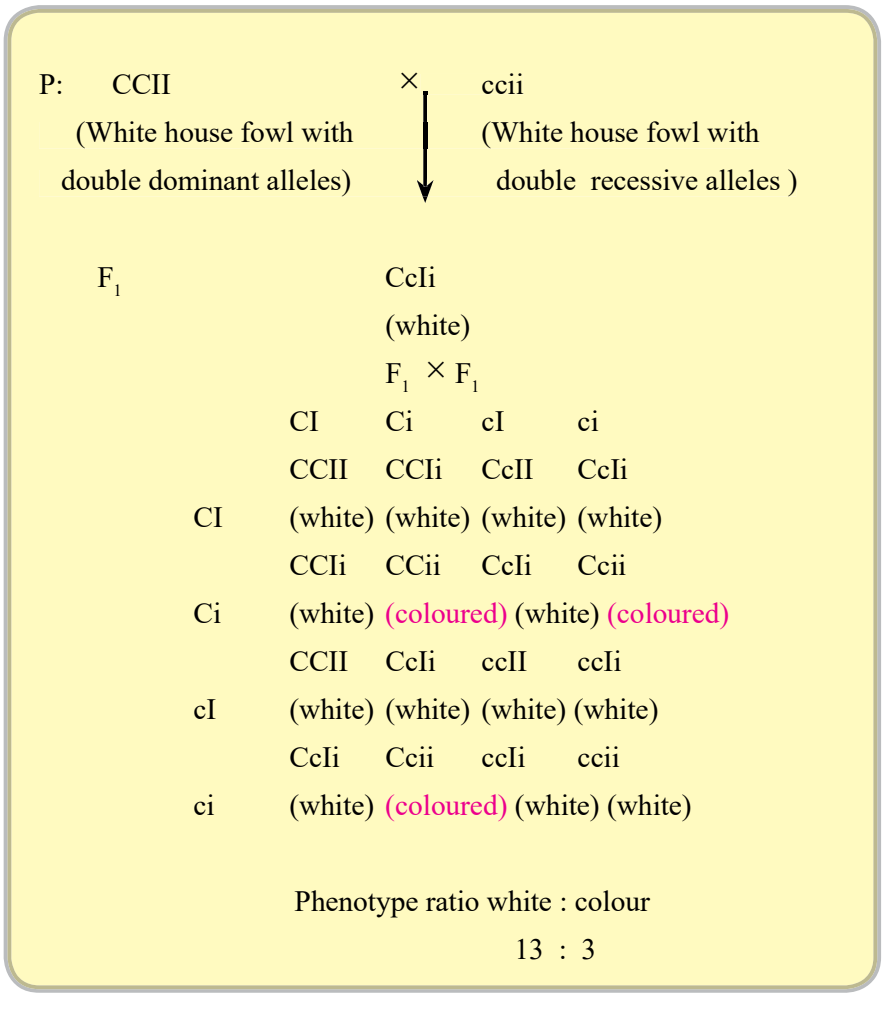


Figure 6.12:An example of dominant epistasis-Plumage colour of house fowl

As per the Mendelian principles, genotype ratios in both F_1 and F_2 generation are expected to be similar to the genotype ratios seen in a normal dihybrid cross. However, due to the effect of epistasis, phenotype ratio would deviate from Mendel's principles for normal dihybrid cross. F_2 phenotypic ratio may get altered from 9:3:3:1 to 13:3.

Recessive epistasis

When a homozygous recessive genotype of a particular chromosomal locus alters/ masks the expression of a separate gene at a different locus, it is referred to as **recessive epistasis.**

A good example for recessive epistasis is found for flower colour in sweet pea (*Lathyrus*) plant. There are purple flowered and white flowered varieties.

A cross between homozygous dominant purple flower (AABB) and homozygous recessive white flower (aabb) strains produced 100% purple colour flowers in F_1 generation. Inter breeding of F_1 plants produced F_2 generation with purple and white flower plants in a ratio of 9: 7.

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The purple colour in sweet pea flower is governed by two dominant genes, A and B. Both A and B alleles are coding for compounds that are necessary for the expression of purple colour. Hence, purple colour will be there only when both the dominant alleles (A and B) are present.

Double recessive genotype at any locus (AAbb, aaBB, Aabb or aabb) results white flowers by masking the expression of purple colour. Thus, double recessive genotype at any of the above locus is epistatic to either homozygous dominant (AA and BB) or heterozygous condition (Aa and Bb) of the other. (AAbb, Aabb, aaBB, aaBb, aabb- White and AaBb, AaBB, AABb, AABB- Purple)(Figuer 6.13)

All of the F_1 generation plants were found to express purple flowers due to the presence of heterozygous condition at both loci (AaBb).

In F_2 generation, plants having genotypes with both **A** and **B** alleles (9/16) express purple flowers, and plants having genotypes with **'aa'** and a **'B'** allele (3/16) or **'A'** allele and **'bb'** alleles (3/16) and **'aabb'** genotype (1/16) produce white flowers, thus only two phenotypic classes are expressed; purple and white. Thus, the normal dihybrid phenotype ratio as per Mendelian principles **9:3:3: 1** is changed to 9: 7 ratios in F_2 generation.

Parents Purple × White Flower

Flower Flower

AABB aabb

↓

F_1 AaBb (Purple Flower)

$F_1 \times F_1$ F_2

	AB	Ab	aB	ab
AB	AABB [P]	AABb [P]	AaBB [P]	AaBb [P]
Ab	AABb [P]	AAbb [W]	AaBb [P]	Aabb [W]
aB	AaBB [P]	AaBb [P]	aaBB [W]	aaBb [W]
ab	AaBb [P]	Aabb [W]	aaBb [W]	aabb [W]

P = Purple Flower, W = White Flower

Figure 6.13: an example of recessive epistasis - flower colour of sweat pea plants

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Polygenic inheritance

Inheritance of a phenotype such as quantitative characters; height, skin colour, intelligence quotient etc. which results from a cumulative expression of two or more genes is called polygenic inheritance.

e.g: Skin colour in humans is determined by many genes. For simplicity only three genes are considered. Each gene (A, B, or C) has a dark-skin allele contributing one "unit" of darkness to the phenotype and being incompletely dominant to the other allele (a, b, or c). Therefore
AABBCC person: very dark skin
aabbcc person: very light skin
AaBbCc person: intermediate between skin colour
Because the dominant alleles from various loci have a cumulative effect, on the skin colour. (Campbell et al, 2015)

Based on the number of genes involved in determining a polygenic character, the phenotypic and genotypic combinations in the progenies may vary. Data for a polygenic character representing a population may result in a **normal distribution curve.** The majority of offspring would be expected to have intermediate phenotypes (skin colour in the middle range).

Genetic linkage

Some genes coding for particular characters are located on the same chromosome and also at a closer distance. Thus, they escape from crossing over and independent assortments occur during the meiotic cell division at gametogenesis and inherit together. This results in deviation from Mendel's law of independent assortment. The above phenomenon is called genetic linkage.
E.g. Inheritance of body colour and wing size in the fruit fly Drosophila

In *Drosophila,* wild-type flies are found to have gray bodies and normal-sized wings. Due to mutation for the above traits body colour becomes black and wings become vestigial. Both characters are determined by genes of autosomal chromosomes.

In this example, the mutant alleles are recessive to the wild-type alleles. The alleles for body colour are indicated as G (gray) and g (black), and those for wing size are indicated as N (normal) and n (vestigial).

To examine the above, wild type flies were crossed with flies which are mutant to both body color as well as wing size and followed by a dihybrid test cross.

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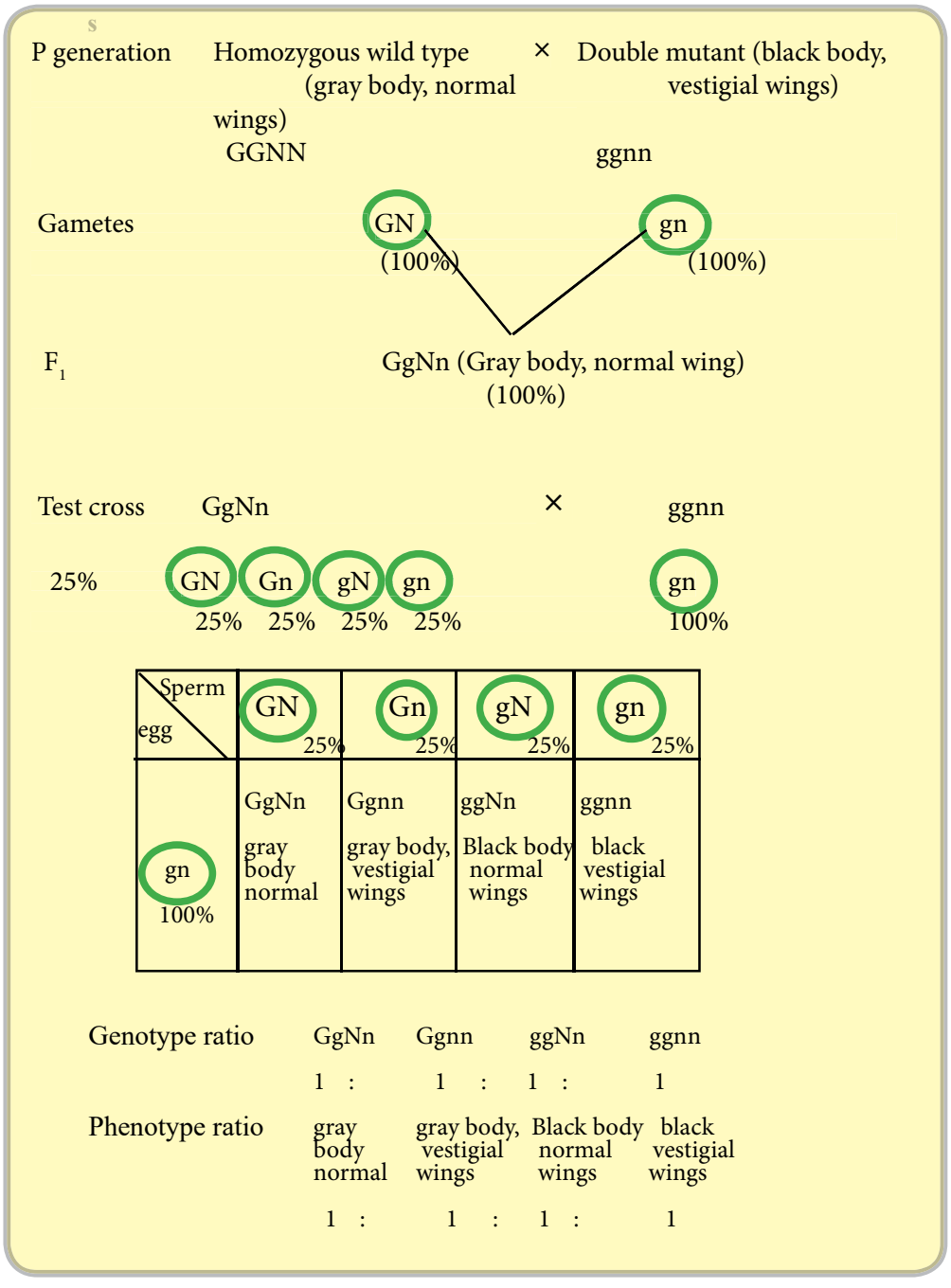


Figure 6.14: Prediction of inheritance of body colour and wing size in the fruit fly *Drosophila* as per Mendelian principles

Most offspring had a parental (P generation) genotype, indicating that the genes for body colour and wing size are genetically linked on the same chromosome.

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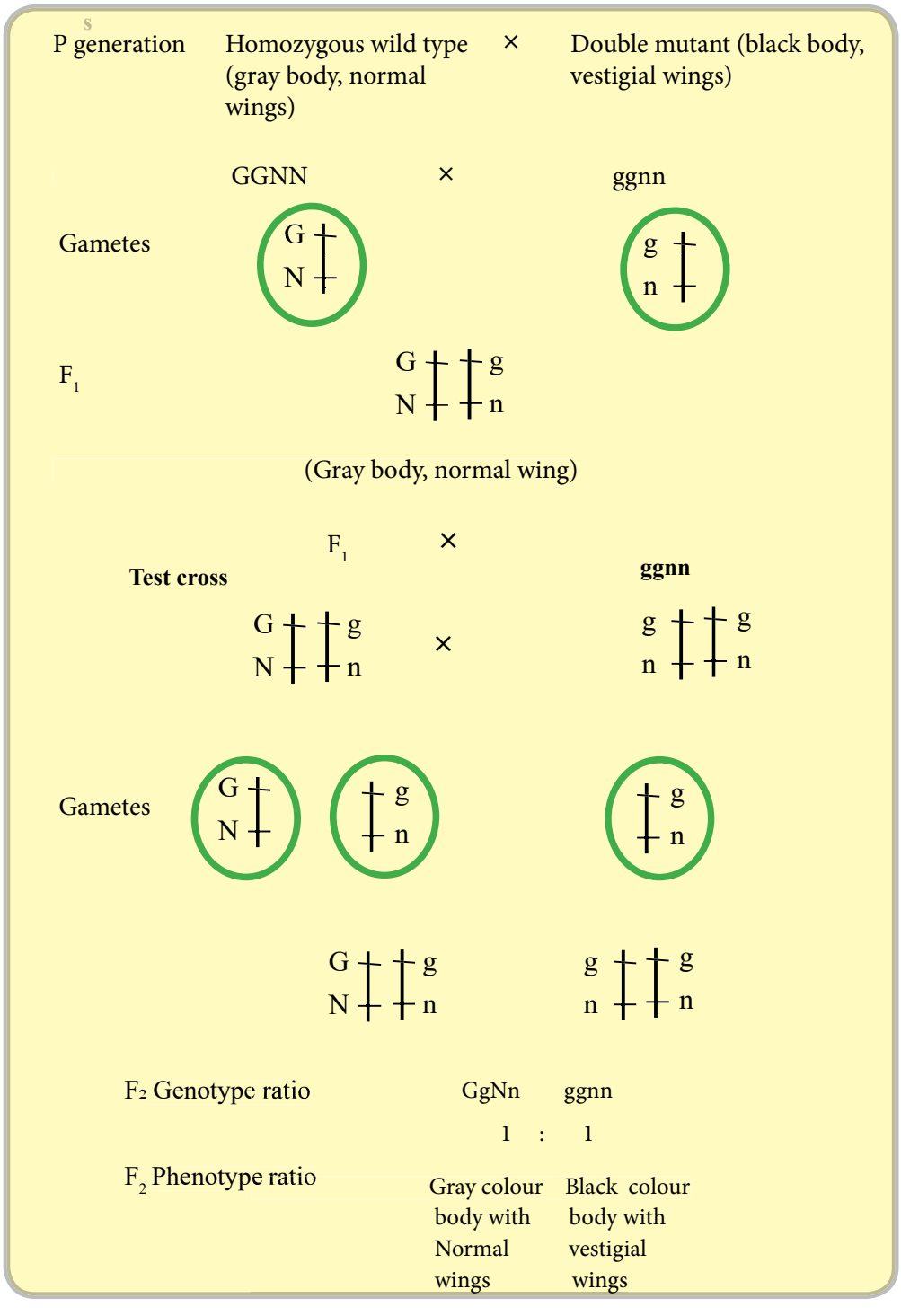


Figure 6.15: Inheritance of body colour gene and wing size gene in the fruit fly *Drosophila*

Though, the genes for body colour and wing size are linked, in some occassions, they get assorted independently due to crossing over. Therefore, the above test cross may results recombinant offsprings in lower frequency. for example, in Morgan's experiment recombinant phenotypes Gray colour body with vestigial wings (Ggnn) and Black colour body with normal wings (ggNn) were observed in lesser numbers.

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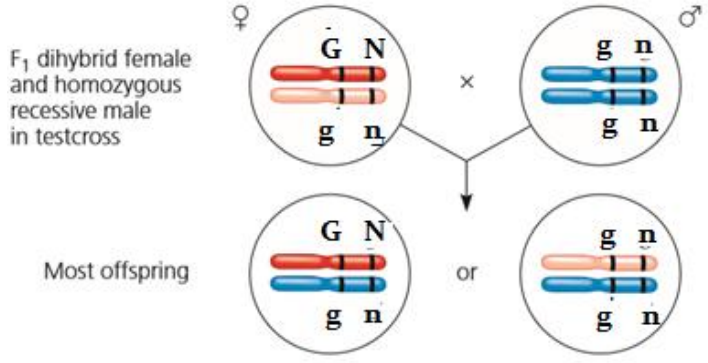


Figure 6.16: Linkage of genes responsible for body colour and wing size in the fruit fly Drosophila

The production of a relatively small number of offspring with non-parental phenotypes indicated occasional breaks in the genetic linkage. This is due to the crossing over occurs between the homologous chromosomes.

Human sex determination

Sex is determined by the expression of sex chromosomes. In humans, each individual carries 22 pairs of autosomal chromosomes and one pair of sex chromosomes. Type of sex chromosomes expressing male traits are named Y chromosome and the other as X chromosome. Comparatively X chromosome is bigger than Y chromosome. They both code for different traits except their homologous regions. When X and Y chromosomes pair up, they remain homologous only in specific regions. On the other hand when chromosome X, X pair up, they both remain homologous to each other.

On the occurrence of gametogenesis in females, meiosis yields haploid eggs carrying 100% X chromosomes, whereas in males, half the number of the haploid sperms produced carry X and the remaining half Y chromosomes. During the fertilization of male and female gametes, the occasion where both egg and sperm carry X chromosomes results in a female zygote, and on the other hand, an occasions where an egg fuses with a sperm carrying Y chromosome results in a male zygote.

Thus, any mating occasions between male and female organisms of same species leading to fertilization can have fifty percent chance for yielding either male or female zygotes.
Anatomical sex signs develop in humans based on the expression of XX and XY chromosomal combination.

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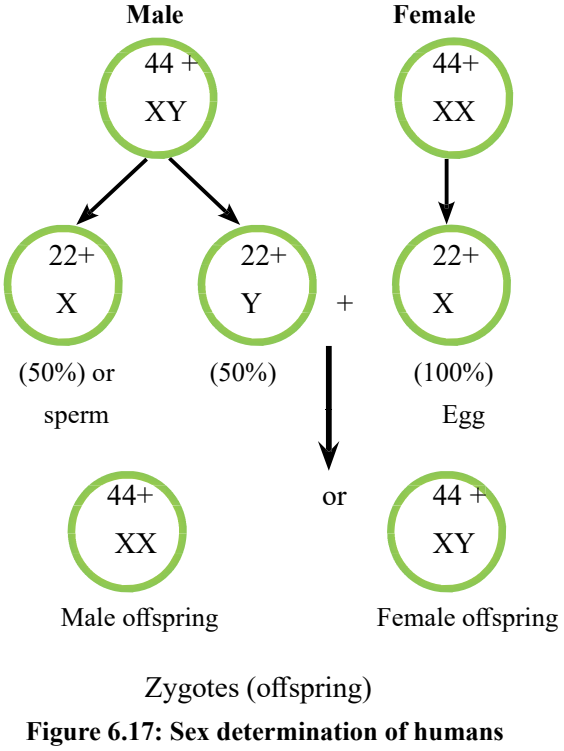


Figure 6.17: Sex determination of humans

Human sex linked characteristics

Certain characters of humans are carried on the genes located on the sex chromosomes. Those genes located on the sex chromosomes are called sex linked genes and the characters expressed by them are called sex linked characters.. Characters expressed by or carried on the X chromosome are called X- linked characters and the genes expressing or carrying those characters are called X- linked genes. On the other hand, characters which are expressed by or carried on the Y chromosome are called Y linked genes and the genes expressing or carrying those characters are called Y linked genes. Y chromosome carry only few genes other than those related to the sex.

Some disorders carried on the Y-linked genes are transferred and expressed only through male progeny.

e.g. absence of certain Y-linked genes causes inability to produce normal sperm.

In addition to sex related characters, X chromosomes carry many other characters which are not relevant to individual sex.

e.g.

Red green colour blindness: *An X-linked recessive disorder characterized by the difficulty in perceiving differences between red and green colours.*

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Haemophilia: *An X-linked recessive disorder where one or more of the proteins required for blood clotting are absent. Haemophilic person run the risk of severe bleeding during injuries due to the delay in clot formation. Campbell et al, 2015)*

Inheritance of X-linked genes

The inheritance of sex linked characters or genes differ for male and female due to the XX genotype of female and the XY genotype of male. During the fertilization, X chromosome from both of their biological parents result a female zygote and on the other hand, X chromosome from the female parent and Y chromosome from the male parent results a male zygote. Thus, for females, X-linked recessive disorders are expressed only at their homozygous genotype. However in males, due to the presence of only one X chromosome, have only one recessive X-linked allele. Therefore, having a recessive X-linked allele with disorder is sufficient for expression.

Pleiotropy

In some occasions, expression of a single gene affects the expression of multiple traits which are not related to each other. The above phenomenon is called as Pleiotropy. Pleiotropic alleles are responsible for the multiple symptoms associated with certain hereditary diseases in humans, such as cystic fibrosis and sickle-cell disease.

Sickle-cell disease

Sickle cell disease is caused by an alteration in the haemoglobin protein of red blood cells. A single gene mutation is responsible for the above condition. In homozygous recessive individuals, all the haemoglobins are of the sickle-cell variety. People living in high altitudes or under physical stress experience low oxygen content in their blood. Low oxygen content in the blood may induce the sickle-cell haemoglobin proteins to get accumulated and results sickle shape in red blood cells. Sickle cells may clump and clog small blood vessels causing tissue and organ damage in several body parts. This may result renal failure, heart failure and thrombosis.

Cystic fibrosis

Cystic fibrosis is a disease condition causing thicker and stickier mucus than its normal nature. As a result, mucus get accumulated in the pancreas, lungs, digestive tract, and reproductive organs which cause lung infections, respiratory failure, poor digestion, and infertility.

The thickening of mucus is due to the excess chlorine secretion of defected chloride channels of the plasma membrane. The defect in the trans-membrane chloride channel occurs as a result of the Cystic Fibrosis Trans-membrane Regulator (CFTR) protein. The altered CFTR protein is due to the mutation of CFTR gene. This is identified as autosomal recessive disorder.

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Epigenetics

Study of occurence of certain phenotypes of certain characters controlled by factors other than their DNA sequence or genetic code is called epigenetics. This is due to 'switching on' and 'switching off' of certain genes by modifying nucleotides of a DNA sequence by methylation and demethylation, where methyl groups are added to wild type DNA sequence or else removed from a methylated DNA sequence. The above random occasions result different modified expression for a single DNA sequence.

Epigenetics results due to either inherited signals from parents or signals arising due to the environmental factors. Inheriting epigenetic traits from parents to the children's generation is called epigenetic inheritance. This may get reversed by various external stimuli from the environment. Some epigenetic influences result in inappropriate gene expressions leading to cancers.

Schizophrenia is a mental disorder that occurs due to the genetic defects. In some identical twins, only one of them gets schizophrenia and the other does not get it. This is due to two types of expressions for same DNA sequence, called epigenetics.

Population genetics

Hardy-Weinberg Equilibrium

Hardy-Weinberg Equilibrium is used to assess whether a population is evolving with respect to a particular characteristic/ genetic locus. The genetic makeup of a trait in a population would remain unchanged, if they are not evolving at that genetic locus. Therefore, the predicted data for a particular trait of a population can be compared with the actual data obtained from the same population. The comparison of both data as mentioned above may help to determine whether the population evolves or not for the considered trait.

Hardy-Weinberg Equilibrium Principle

In 1908 British mathematician **G.H. Hardy** and German physician **W. Weinberg,** independently showed that, in a population that is not evolving, allele and genotype frequencies will remain constant from generation to generation. This is now considered as key concept in population genetics and referred to as **Hardy-Weinberg Equilibrium Principle.**

To determine whether the allele and genotype frequencies have changed in consecutive generations, a Punnett square can be drawn, considering the combination of alleles in all possible crosses in a population.

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The following example can be used to work out the Hardy Weinberg equilibrium.
A wild flower plant population showing incomplete dominance for the flower colour alleles have distinct phenotypes indicating their genotypes. i.e.

Plants homozygous for the C^R allele (C^RC^R) produce red pigment and have red flowers. Plants homozygous for the C^W allele (C^WC^W) have white flowers .
Heterozygous plants (C^RC^W) produce some red pigment and have pink flowers.

In the population of 500 flowers, there were 800 C^R alleles and 200 C^W alleles. Since the flower colour of the above plant type is determined by pair of alleles, 500 flowers may consist of 1000 alleles for their flower petal's pigmentation.
Therefore,

- allele frequency of C^R alleles (p) = $\frac{800}{1000}$ = 0.8
- allele frequency of C^w alleles (q) = $\frac{200}{1000}$ = 0.2

If the gametes are formed at random, the probability that an egg or sperm contains a C^R or C^W allele is equal to the frequency of each of these alleles in the population. Thus, each egg has an 80% chance of containing a C^R allele and a 20% chance of containing a C^W allele; the same is true for each sperm.

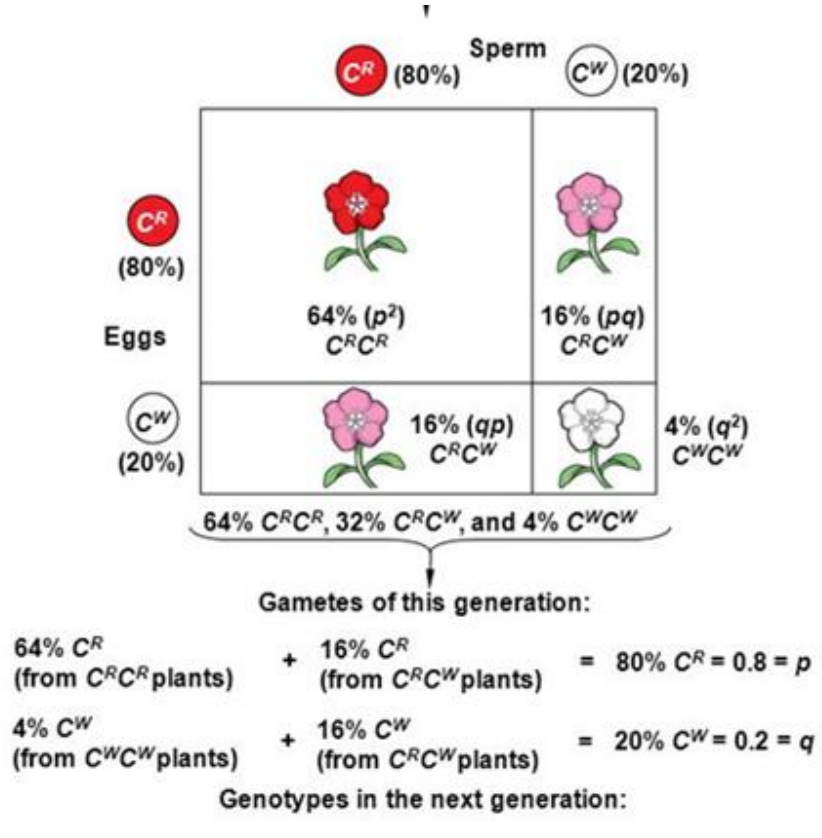


Figure 6.18: The Hardy-Weinberg Principle (Campbell et al, 2015)

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During random fertilization, gametes fuse together randomly. Therefore, rule of multiplication can be applied to calculate the probability for each genotype combination.

According to Hardy-Weinberg equilibrium, if a character is determined by two alleles, the three genotypes will appear in the following proportions;

P_2 = frequency of dominant homozygotes

q_2 = frequency of the recessive homozygotes

2pq = frequency of the heterozygotes

The probability that two C^R alleles will come together, p x p = p^2 = 0.8 x 0.8 = 0.64

Hence the proportion of C^RC^R genotype in the progeny = 64%

The probability that two C^W alleles will come together, q x q = q^2 = 0.2 x 0.2 = 0.04

Hence the proportion of C^WC^W genotype in the progeny = 4%.

C^RC^W heterozygotes can arise in two different ways.

If the sperm provides the C^R allele and the egg provides the C^W allele,

the resulting C^RC^W heterozygotes in the progeny, p x q = 0.8 x 0.2 = 0.16 = 16%

If the egg provides the C^W allele and the sperm the C^R allele,

the resulting C^RC^W heterozygotes in the progeny, q x p = 0.2 x 0.8 = 0.16 = 16%

Thus, the total frequency of heterozygote in the progeny

pq + qp = 2pq = 0.16 + 0.16 = 0.32, or 32%

In the above example, only three kinds of genotypes are possible. When conditions suits for Hardy-Weinberg equilibrium, the sum of frequencies of all three genotypes equals 1. Thus, the equation for Hardy-Weinberg equilibrium can be written as below;

$$P^2 + 2pq + q^2 = 1$$

Conditions for Hardy-Weinberg Equilibrium

The Hardy-Weinberg approach describes a hypothetical population that is not evolving and fulfilling the following conditions.

1. **Absence of mutations.** Mutations result changes in alleles. Insertion, deletion, or substitution of nucleotides result altered alleles. This leads to modified gene pool.
2. **Occurrence of random mating.** Breeding occurs randomly without any influence that causes selectiveness. Mating of closely related individuals may alter the allele frequencies.
3. **Absence of natural selection.** All genotypes of the progeny are expected to survive irrespective to their differences, abilities and the environmental conditions. Variations in survival and reproduction of some genotypes may alter the frequency of alleles.
4. **Size of the population is extremely large.** In small populations, particular genotypes may disappear due to death or infertility. Therefore, larger the population, more likely to favor the Hardy-Weinberg equilibrium.

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5. **Absence of immigration or emigration.** Individuals moving in and out of the populations may cause appearance of new genes and disappearance of existing genes. This is is called gene flow and it may alter allele frequencies.

Naturally, most populations do deviate from Hardy-Weinberg equilibrium except for certain genetic loci. Slowly evolving populations may also do not deviate much from Hardy-Weinberg equilibrium and therefore, they remain as predicted for a non-evolving population.

Evolution and change in gene frequency

Evolution can be explained in terms of changes in allele (gene) frequencies over generations. A species evolves when changes in gene frequencies drive the species into a higher level of adaptation for a specific ecological niche.

Genetic variation within the population is the key to evolution. Mutation will create new alleles and migration will include it into the population to increase variation. Natural **selection will then choose the better adapted individuals based on their phenotypic variations, causing population to evolve.** As a result, after the evolution the population is at a higher adaptive level compared to the level of adaptation they showed before being evolved.

This concept can be illustrated with the evolution of the peppered moth in England during the time of industrialization. The moth had two phenotypic varieties based on their colour; dark and light. Prior to the industrialization of central England, the light-coloured allele was most prevalent. The light-coloured moths had an advantage over the dark coloured ones as they could hide on the white-barked trees to avoid predation from birds.

Due to the pollutants generated parallel to the industrilalization, the light-coloured trees were stained dark. This exposed the light- coloured moths to predation, reducing their numbers. As a result, the light- colour allele became less prevalent. In its place, the dark-colour allele became more predominant, because dark moths could camouflage themselves better on the stained trees and avoid being eaten by their bird predators. The population evolved to a higher adaptive state with the change in gene frequencies (light – colour allele frequency went down while dark – colour allele frequency went up).

Plant and animal breeding

People have intervened in the reproduction and genetic make up of plants and animals since the dawn of agriculture, eight to ten thousand years ago. Early farmers selected the best looking plants and seeds and saved them to plant for the next season. Likewise, the best farm animals were allowed to mate with each other to preserve and improve their desirable traits.

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This phenomenon wherein human beings interfere in the process of reproduction to allow only selective mating to occur, so that offspring with improved characters are produced is called **breeding** (as against natural reproduction).

With the science of genetics became better understood, plant and animal breeders used what they knew about the genes of a plant or an animal to select for specific desirable traits to develop improved plant varieties or animal breeds. The selection for features such as faster growth, higher yields, pest and disease resistance, larger seeds or sweeter fruits in crop plants, colour and pattern of the skin, hair or feathers in animals have now dramatically changed domesticated species compared to their wild relatives.

Importance of plant and animal breeding

In breeding programmes, the attributes, structure and composition of plant and animals are manipulated in such a way to make them more useful to humans. Accordingly, plant and animal breeding has a significant impact of world's agro-economy as discussed below;

Addressing world food and feed quality needs

An estimated 800 million people in the world, including 200 million children, suffer from malnutrition and associated health issues. Plant and animal breeding helps to enhance the value of food by improving their nutritional quality. For example, rice, which is the most widely eaten staple food, lacks many essential vitamins.

Another problem encountered in major food crops is the presence of toxic substances within them such as alkaloids in yam, cynogenic glucosides in cassava (manioc), trypsin inhibitors in pulses, and steroidal alkaloids in potatoes. Plant breeding is useful in reducing these toxic components and making them safer to eat. Plant breeding is also useful in making some plant products more digestible. For example, a high lignin content of the plant material reduces its value for animal feed which can be overcome with the use of breeding techniques.

Addressing food supply needs for a growing world population

It is anticipated that an additional three billion people will be added to the world population within the next three decades. Aligned with this population growth, an expansion in world food supply should be required to meet the projected needs. Unfortunately, land for farming is scarce and therefore more food will have to be produced on less land. This calls for improved and high yielding animal and plant varieties to be developed. In response plant breeding has produced super rice which has 50% more yield compared to the normal rice, super wheat which boost the

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harvest by 20-40% of normal wheat and several high yielding corn, maize and soya bean varieties. The total production of meat and milk has also increased considerably *o*ver the years due to careful use of selective breeding techniques.

Need to adapt to environmental stresses

Weather and soil conditions can have a major impact crop yield. Climate changes and global warming are partly responsible for modifying the crop production environment (e.g., some regions of the world are getting drier and others saltier). To meet the increasing demand for food new cultivars need to be bred which can sustain these adverse conditions. For example, it is necessary to develop new plant types that can resist various biotic (diseases and insect pests) and other abiotic (e.g., salt, drought, heat, cold) stresses in the production environment. In response, genetically modified, pest resistant cotton, maize, and potatoes which carry Bt toxin, salinity tolerant rice varieties, cold tolerant tobacco, potato and strawberry varieties etc. are now available in agricultural industry. Likewise, both crop plants and farm animals (cattle, pig sheep goat etc.) with increased immunity to pathogens have also been produced through various breeding techniques.

Satisfying industrial and other end-use requirements

Consumers are having different requirements based on the texture, colour and composition of a particular food item irrespective of its taste or nutritional value. These diverse demands for the same food can be now successfully met through breeding procedures. For example, potato is a versatile crop used for food and industrial products. Different varieties are being developed by breeders for baking, cooking, fries (frozen), chipping, and for starch. These cultivars differ in size, specific gravity, and sugar content, among other properties. High sugar content is undesirable for frying or chipping because the sugar caramelizes under high heat to produce undesirable browning of fries and chips. Likewise, there is a high demand for seedless fruits such as grapes, melon and strawberries and also for leaner meat. Depending on these end-user requirements, it is possible to develop quality added products using animal and plant breeding techniques.

Developing animal and plant varieties with aesthetic values

Aesthetics is of major importance in horticulture as well as in the industry of ornamental and pet animals. The ornamental plant industry depends to a large extent on the development of new varieties that exhibit new flower/ leaf colours, varying sizes and attractive shapes etc. using plant breeding.

The pursuit of novelty has spurred a similar explosion of types in pet animals as well. Today selective breeding for numerous morphological features and functional abilities have given rise to nearly 400 dog breeds making them one of the most diversified species on earth. Likewise, there

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are close to 50 rabbit breeds, vast number of bird varieties and an extensive range of ornamental fish.

Breeding techniques

Plant and animal breeders use numerous techniques to create new varieties with enhanced features. Many of these techniques have been successfully practiced over centuries even without the knowledge of the underlying genetics. Following section summarizes some of these traditional breeding techniques which has caused significant improvements in agriculture and farming.

Artificial selection

Artificial selection is the earliest form of biotechnology and has been used by humans for thousands of years. It, is a process of selective breeding, where plants or animals with specific traits were selected to breed so that their desired traits could be passed to the next generations to produce a high performing new variety. The method has made a huge impact on agriculture by way of improving plant and animal products before the discovery of more sophisticated technologies like genetic engineering.

The first prerequisite of artificial selection is the availability of variation with respect to the desirable characters. Once a population with a desirable variation is recognized, the best performing individuals for the desired feature are selected. For example, when selecting for fruit size in plants, only those giving the biggest fruits are chosen for the breeding programme and the rest of the population is discarded or rejected. The progeny of the selected individuals is grown further and again screened for the desired feature. This process is repeated sometimes for many generations, until a uniform plant population is attained which has the best-desired characters. Eventually, a new uniform crop variety with the desired characteristic is produced by this successive selection, followed by multiplication of the selected individuals.

The advantage of selective breeding is that it uses the processes of natural selection, but under direct supervision from carefully selected animals or plants with the desired traits. There are no genetic modifications or other forms of tampering that could potentially harm people and the risk to the plant or animal is often minimum.

Crops like corn and wheat are commonly selectively bred in order to obtain the highest yielding plants. Breeding animals with higher protein and lower fat percentages, as well as plants that have higher nutritional values, had been used to create food sources with a higher quality of nutrition. In addition, selective breeding has, effectively removed undesirable traits such as low resistance to disease, in some animals and plants.

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However, selective breeding among animals can take a long time for the process to work. In horse breeding, for example, the given standard to establish a new breed is to have offspring with the desired traits to be produced over the course of 7 generations. This means it may take 25-50 years for the desired traits to become a foundational component of an animal.

Inbreeding and out breeding

Inbreeding

The breeding among genetically similar individuals are known as inbreeding.

Among plant breeders the term “inbreeding” is commonly used to mean self-fertilization, i.e. the fertilization of a flower with its own pollen or with pollen from a different flower on the same plant. This is done to produce an inbred variety, which is exactly the same generation after generation. Many important crops, such as wheat, oats, barley, and tobacco, are produced from seeds which are habitually self-fertilized.

However, in animal breeding the term " inbreeding " is used to refer to the mating of closely related individuals, as, for instance, the mating of father and daughter, brother and sister, or cousins. In both crop plants and farm animals, inbreeding brings uniformity of the required type while preserving the desired characters. Inbreeding is used for developing pure lines in agriculture as well as for research.

As a rule, inbreeding increases homozygosis and thus exposes harmful recessive genes which would have otherwise stay hidden among heterozygotes. Continued inbreeding, therefore, reduces genetic fitness of the population. As a result, the growth and fertility of the inbred population would go down with adverse effect on their productivity. Prevalence of genetic disorders might also increase among the inbred population. This phenomenon of having a reduced genetic fitness in a given population as a result of inbreeding is called **inbreeding depression.**

However, in agriculture and animal husbandry, positive effects of inbreeding will be harnessed as much as possible. To ensure this, only those offspring that are exhibiting the desired trait, without other negative ones, will be used for future breeding. The negatively affected individuals in the progeny are removed or are not be allowed to be bred. Thus, inbreeding is used in agriculture to help accumulation of superior genes.

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Outbreeding

When plants or animals of different breeds (races) are mated with each other, it is known as **outbreeding** or **cross breeding.** This allows the desirable characters of the exotic parent, which the indigenous parent does not have, to be transmitted to the progeny.

For example, cross breeding is carried out by animal breeders to enhance milk and meat production. In India zebu breeds of cows and nondescript cows are crossed with exotic breeds like Holstein Fresian, Brown Swiss and Jersey bulls or their semen, to enhance the milk production potential of the progeny. Likewise crop plants like corn and hemp, are normally cross-fertilized.

Hybrid breeding

When genetically unrelated pure-bred plants or animals in the same species are mated with each other it is known as **hybridization** or **outcrossing.**

Generally, this is carried out with plants and animals who do not share common ancestors on either side of their pedigree up to four to six generations. The offspring of such a mating is known as the **out cross** and will possess stable characteristics and hybrid vigour. **Hybrid vigour,** also called **heterosis,** is the increase in such characteristics as size, growth rate, fertility, and yield in the hybrid organism over those of its parents.

Plant and animal breeders exploit heterosis by mating two different pure-bred lines that have certain desirable traits. The first-generation offspring generally show, in greater measure, the desired characteristics of both parents. This vigour may decrease, however, if the hybrids are mated together; so the parental lines must be maintained and crossed for each new crop or group desired.

In plant breeding, because creating hybrids involves many years of preparation to create pure lines that have to be constantly maintained so that F_1 hybrid seeds can be harvested each year, the seeds then become more expensive. Nevertheless, hybrid seeds have had a tremendous impact on agricultural productivity. Today, nearly all corn and 50% of all rice are hybrids. In the US, the widespread use of corn hybrids, coupled with improved cultural practices by farmers, has more than tripled corn grain yields over the past 50 years from an average of 35 bushels per acre in the 1930s to 115 bushels per acre in the 1990s. No other major crop anywhere in the world even comes close to equaling that sort of success story.

Many cultivars of popular vegetables or ornamental plants are hybrids. In terms of improved plant characteristics, tropical vegetable breeders can point to some rather clear achievements over the last two decades:

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- **Yield improvement.** Hybrids often out yield traditional true breeding varieties (inbred varieties) by 50-100% due to its improved vigor, improved genetic disease resistance, improved fruit setting under stress, and higher female/male flower ratios.
- **Extended growing season**. Hybrids often mature up to 15 days earlier than local true breeding varieties. For many crops, the hybrid's relative advantage over the true breeding is most pronounced under stress conditions.
- **Quality improvement.** Hybrids have helped stabilize product quality at a higher, and more uniform level – this implies improved consumption quality (e.g. firm flesh of wax gourd, crispy taste of watermelon).

Interspecific breeding

In this approach, which is also known as interspecific hybridization, male and female organisms of two different species are mated. The progeny obtained from such a mating are usually different from both the parental species and may be fertile, partially fertile, or sterile.

Plants hybridize much more frequently and successfully than animals do. Pollen from flowering plants disperses widely and may land on flowers of other species allowing natural interspecific breeding to take place. Plant forms are less stringently controlled than animal forms, and so the intermediate form of a plant hybrid is more likely to be physiologically successful.

Often interspecific hybrids are sterile or for some other reason cannot interbreed with the parental species. Occasionally sterile interspecific hybrids can undergo a doubling of their chromosome set and become fertile tetraploids (four sets of chromosomes). For example, the bread wheats that humans use today are a result of two hybridizations each followed by chromosome doubling to produce fertile hexaploids (six sets of chromosomes). In such instances, the hybrids can become new species with characteristics different from either of the parents. Crop yields increase dramatically when hybridization is used to exceed one or more of the parents in size and reproductive potential. For example, boysenberries (Rubus ursinus x idaeus) were developed at Knott's Berry Farm in California. They are a result of a set of crosses between blackberries (*Rubus fruticosus*), European raspberries (*Rubus idaeus*) and loganberries (*Rubus* × *loganobaccus*).
For many fruit crop species, the use of interspecific breeding is increasing, in order to utilize naturally occurring sources of pest and disease resistance, fruit-quality components, etc. within the available germ plasm. For example, winter hardiness of apple was improved by making a hybrid species *Malus* × *domestica* from *Malus* × *asiatica* and *Malus pumifolia.*

However, among animals interspecific breeding is restricted to few species. Common examples include Mule (male donkey x female horse), Hinny (male horse x female donkey) and Liger (male lion x female tiger).

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Note that in mules and hinnies, the common genus the parents belong to is Equus and in liger, its Panthera. Other examples are zebra/donkey cross resulting in an offspring called zonkey, zebra/ horse cross resulting in zorse. The offspring from this cross could develop into adults, but may not develop functional gametes. Sterility is often attributed to the different number of chromosomes the two species have, for example, donkeys have 62 chromosomes, horses have 64 chromosomes.

Genetic principles of breeding techniques

From the beginning of plant and animal breeding, farmers made use of principles of genetics, with or without awareness of these concepts. Following section explains, three most widely used genetic principles applied in animal and plant breeding at present.

Polyploidy

Polyploidy refers to the presence of more than two complete sets of homologous chromosomes per cell nucleus. This is a widely used principle in plant breeding. In plants, polyploidy can be induced artificially using antimitotic agent, colchicine.

One of the most important consequences of polyploidy for plant breeding are the increment in plant organs (‘‘gigas’’ effect) caused by the larger number of gene copies. Polyploid individuals may thus exhibit larger organs compared to their diploid counterparts, such as roots, leaves, tubercles, fruits, flowers and seeds. Polyploid plants also have lower growth rates, and tend to flower later or over a longer period of time than related diploids, which is a desirable feature for ornamental breeding.

In addition, polyploidy often results in reduced fertility due to meiotic errors, allowing the production of seedless varieties such as the triploid watermelon. On the other hand, when the crossing between two species is not possible because of differences in ploidy level, polyploids can be used as a bridge for gene transferring between them. Similarly, the genome doubling in a newly formed sterile hybrid allows the restoration of its fertility.

Genome redundancy (having additional gene copies due to increase ploidy) have other benefits as well. It promotes a ‘‘buffering’’ effect in which the deleterious alleles are masked by the extra copies of wild-type alleles. At the same time, it allows functional diversification of redundant gene copies, in which one member of a duplicated gene pair mutates and acquires a novel function, without compromising essential functions.

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The increment in heterozygosity is another feature that accompanies polyploidy. Higher levels of heterozygosity have been positively related to vigor increment in maize, potato and alfalfa improving the product quality and increasing the tolerance to both biotic and abiotic stresses.

Mutation Breeding

Methods for inducing mutation have the potential of producing new sources of genetic variability for crop breeding. These methods can be employed when it appears there is little, or no, variability for the character to be improved available within the gene pool of the species. This method of inducing desirable mutations in crop plants using either chemical or physical agents was termed **mutation breeding.**

Several agents can be used to cause mutations. This include ionizing radiation such as gamma rays, protons, neutrons, alpha and beta particles and chemicals such as sodium azide and ethyl methanesulphonate. Since the desirable mutations induced by these treatments are found at a very low frequency (0.1% of total mutations), breeders have to screen a large population to select a desirable mutation. In addition, most mutations act in a recessive fashion and are likely to be masked by their dominant allelic counterparts making the screening procedure even harder.

The effectiveness of using induced mutation depends on the breeding system of the plant. Its use in self-pollinated plants is likely to be more successful than in crosspollinated ones. Populations of cross-pollinated plants usually possess stores of genetic variability in the recessive condition and it would not be likely that induced mutation would produce significant amounts of new variability. Further, induced mutation is potentially useful in the improvement of asexually propagated crop plants.

Despite these limitations, mutation breeding efforts continue around the world today. It has improved both morphological and physiological characteristic of both crop and ornamental plants such as flower colours, seed size, crop yield, disease resistance and salinity tolerance, drought tolerance and early maturity. Examples of plants that have been produced via mutation breeding include wheat, barley, rice, potatoes, soybeans, and onions.

Genetic modification

Genetic modification, also called **genetic engineering,** is the direct manipulation of an organism's genes to change the genetic makeup of cells. In this method, genetic material is obtained from one organism showing a desired trait and will be inserted to another second organism using recombinant DNA technology, so that the receiving organism will also show the same desirable trait. Thus, by transferring genes within and across species, improved or novel organisms are produced.

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In traditional plant breeding techniques transfer of genes is limited to the closely related species or genera. For example, traditional breeding techniques could not be used to insert a desired gene from daffodil into rice because the many intermediate species between rice and daffodil and their common ancestor is extinct. With genetic engineering, however, such gene transfers can be done more quickly, more specifically, and without the need for intermediate species. The term **transgenic** or **genetically modified organism (GMO)** is used to describe organisms that have been engineered to express a gene from another species. Advocates for plant biotechnology believe that the genetic engineering of crop plants is the key to overcoming some of the most pressing problems of the 21st century, including world hunger and fossil fuel dependency. Examples of transgenic plant varieties include transgenic papaya that is resistant to ring spot virus, "golden rice," with increased levels of beta-carotene and salinity resistant rice varieties among others.

Natural or artificial breeding: Advantages and disadvantages

Although artificial breeding is extensively practiced today with remarkable economic gains, there are serval drawbacks in the method in comparison to the natural breeding.

The artificial breeding is geared towards producing a uniform set of plants or animals with traits desirable to humans. The development of this uniformity needs compromising the variability within the species. This reduction of genetic diversity will have adverse consequences on the evolutionary fitness of the species leading to low resistance to infections, higher prevalence of congenital anomalies and reduced fertility. For example, when a population of animals or plants with the same genetic traits are attacked by a pathogen to which they are susceptible, the entire population is likely suffer due to the absence of the resistant trait within the gene pool. This limited opportunities for the natural selection to act upon the population is likely to drive them towards a lower fitness plateau.

On the other hand, natural breeding can rule out weaknesses and disabilities which affect survival by allowing natural selection to act upon the species. This will produce fitter and stronger individuals in the long run. However, the natural selection would not guarantee a productivity increase in the consumer perspective, despite the increase in genetic fitness.

As discussed earlier, sometimes inbreeding is practiced as an artificial breeding technique. This would result in an increase in homozygosis which would in turn increase the expression of recessive deleterious mutations that would otherwise stay masked within heterozygotes. This can cause the population to undergo inbreeding depression with adverse effects on the overall fitness.

Sometimes artificial breeding can exhibit negative correlated responses. This refers to the fact that while artificial breeding is improving certain characters in the population, simultaneously and unintendedly it could lead towards deterioration of other characters that are not under direct

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observation. For example, the shape of the skull in some dog breeds has made it difficult for them to eat normal food because of the upper jaw being much shorter than the lower jaw, such as in the case of Boxer or the Bulldog. Likewise, selection for large offspring has resulted in a high fraction of difficult births, sometimes requiring caesarean sections in the Texel sheep, and even almost as a standard way of delivering in the beef cattle breeds Belgian White-and-Blue cattle and the Dutch Improved Red-and-White. This kind of negative responses are difficult to predict in advance and usually only visible after the new breed is established.

Although there are negative impacts artificial breeding is still preferred over natural breeding for the numerous advantages it could bestow upon overall animal and plant productivity as discussed earlier.

Notes:

T**his is to acknowledge that some of the diagrams used in this book have been taken from various electronic sources using internet . This book is not published to make profit and sold only to cover cost.**

The resource book is prepared according to the subject content and learning outcomes of the G.C.E. (A.L) Biology new syllabus which is implemented from 2017.

The content of this Resource book declares the limitation of the G.C.E. (A.L) Biology new syllabus which is implemented from 2017.

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Message from the Director General

The National Institute of Education takes opportune steps from time to time for the development of quality in education. Preparation of supplementary resource books for respective subjects is one such initiative.

Supplementary resource books have been composed by a team of curriculum developers of the National Institute of Education, subject experts from the national universities and experience teachers from the school system. Because these resource books have been written so that they are in line with the G. C. E. (A/L) new syllabus implemented in 2017, students can broaden their understanding of the subject matter by referring these books while teachers can refer them in order to plan more effective learning teaching activities.

I wish to express my sincere gratitude to the staff members of the National Institute of Education and external subject experts who made their academic contribution to make this material available to you.

Dr. (Mrs.) T. A. R. J. Gunasekara
Director General
National Institute of Education
Maharagama.

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Message from the Director

Since 2017, a rationalized curriculum, which is an updated version of the previous curriculum is in effect for the G.C.E (A/L) in the general education system of Sri Lanka. In this new curriculum cycle, revisions were made in the subject content, mode of delivery and curricular materials of the G.C.E. (A/L) Physics, Chemistry and Biology. Several alterations in the learning teaching sequence were also made. A new Teachers' Guide was introduced in place of the previous Teacher's Instruction Manual. In concurrence to that, certain changes in the learning teaching methodology, evaluation and assessment are expected.

The previous Teachers' Instruction Manual contains a line-up of subject matter expected to be learnt, but the newly introduced Teachers' Guide does not accommodate any subject matter. Yet, it provides learning outcomes, a guideline for teachers to mould the learning events, assessment and evaluation. Thus, the need of a resource book, which simply describes the subject content emerges. This book comes to you as a result of an attempt to fulfil that requirement. When implementing the previous curricula, the use of internationally recognized standard textbooks published in English was imperative for the Advanced Level science subjects. Due to the contradictions of facts related to the subject matter between different textbooks and inclusion of the content beyond the limits of the local curriculum, the usage of those books was not convenient for both teachers and students.

As this book is available in Sinhala, Tamil, and English, the book offers students an opportunity to refer the relevant subject content in their mother tongue as well as in English within the limits of the local curriculum. It also provides both students and teachers a source of reliable information expected by the curriculum instead of various information gathered from the other sources.

This book authored by experienced subject teachers and subject experts from the universities is presented to you followed by the approval of the Academic Affairs Board and the Council of the National Institute of Education. Thus, it can be recommended as a material of high standard.

Dr. A. D. Asoka De Silva
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07

Molecular Biology and Recombinant DNA Technology

Nucleic acids are capable in directing their own replication from their monomers. In most organisms, DNA is found as the genetic material. However , some viruses such as influenza virus, consist RNA as their genetic material. Accurate replication of DNA, its transmission from one generation to the other and its ability to store and express hereditary information facilitates DNA for acting as vital genetic material in organisms.

DNA Double Helix Model: James Watson and Francis Crick, based on the X-ray crystallography of a DNA molecule obtained by Rosalind Franklin, proposed the double helix model to describe how the six molecules; de-oxy ribose sugar, phosphate group and four different nitrogenous bases, are organized into DNA and explain its properties. According to this model, DNA is a twisted ladder (spiral staircase) in which the rails are composed of alternating phosphate and sugar molecules forming the back bone. The steps of the ladder are paired nitrogenous bases. A purine is paired with a pyrimidine as per the base pairing rule with either two hydrogen bonds (A=T) or three (G ≡ C). T.H. Morgan and his group, through their experiments concluded that chrosomes are made up of DNA and proteins and genes as regions of chromosomes.

Architecture of Chromosomes

Architecture of chromosomes is the way the DNA molecules are arranged in the nucleoid or nuclear region of the cytoplasm of the prokaryotic cells or in the nucleus of eukaryotic cells.

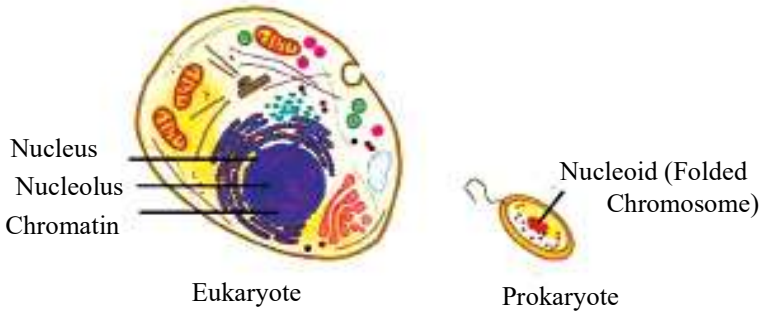


Figure 7.1: DNA is packaged into a nucleus in eukaryotes and a nucleoid in prokaryotes.

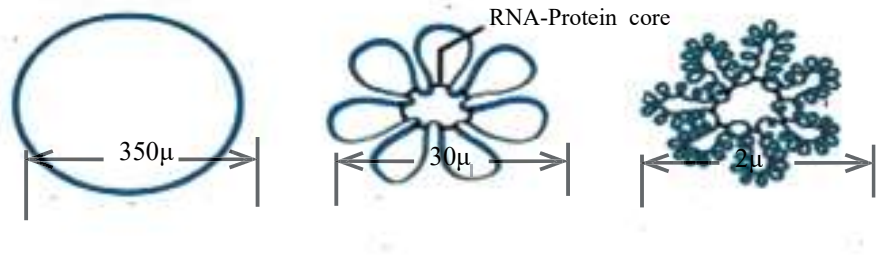
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DNA of both prokaryotes and eukaryotes are called chromosomes. However, true chromosomes are present only in eukaryotes.

Prokaryotic (bacterial) chromosome is a single double stranded circular DNA molecule associated with a few protein molecules. Eukaryotes have several chromosomes, each consisting of a single double stranded linear DNA associated with histones and other protein molecules.

When the size of all chromosomes of an organism are considered, it is an enormous amount of DNA. These facts illustrate that, a cell has a huge problem in having its DNA either in the nucleoid of prokaryotes or the nucleus of eukaryotes. The containment of the genome/DNA in the nucleoid or in the nucleus is called DNA packaging.

Packaging of DNA in prokaryotes is facilitated by the proteins associated with the DNA molecule. The protein molecules cause the DNA to coil (or fold or loop) and supercoil, so the DNA molecule is compacted into the nucleoid. The DNA molecule is initially coiled into loops and these loops then independently supercoil into domains identifiable in electron micrographs. The loops of compacted mass of DNA are bound to a 'core' consisting of RNA and protein. The 'core' also attaches the chromosome to the membrane. The supercoiled DNA can be relaxed by introducing single strand nicks. Since the chromosomes are attached to the membrane and held together by the 'core' acting as a barrier for rotation, the domains can relax and supercoil independently. This is important in the transcription of specific genes. Removal of RNA will lead to loss of independence of the loops.



a) Circular, unfolded Chromosome

b) Folded chromosome 40 to 50 loops

c) Supercoiled, chromosome

Figure 7.2:Folding and supercoiling compact the prokaryotic chromosomes

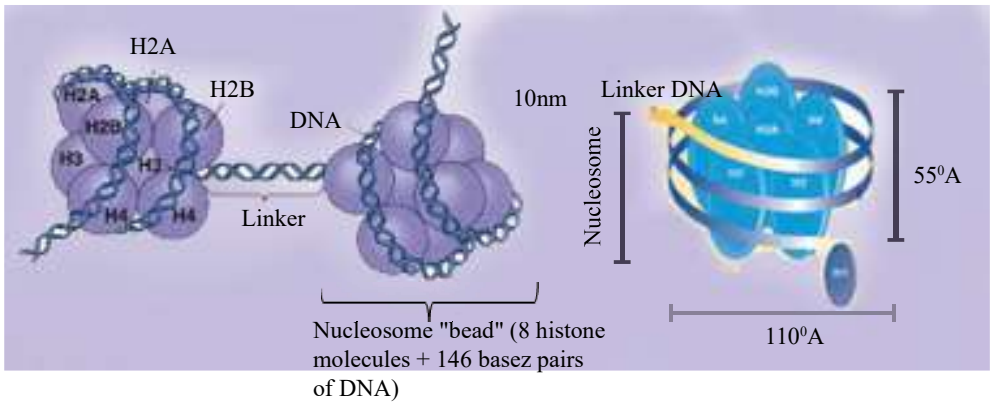
In addition to the chromosomal DNA, certain prokaryotes have extra chromosomal genetic elements called plasmids. They are also circular DNA and are coiled and supercoiled.

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Eukaryotic chromosomes are associated with a large number of proteins called histones that helps to organize the DNA inside the nucleus. This DNA-protein complex is known as chromatin. The chromatin may be lightly packed as in euchromatin or tightly packed as in heterochromatin. Euchromatin is rich in genes and is probably active in transcription. Heterochromatin consists nucleotide sequences which are mostly inactive. They may contribute in gene regulation, epigenetic inheritance and protection from chromosomal integrity.

In the first level, the double helix winds around a complex of eight histone molecules. These are called nucleosomes and they look like beads of a necklace. The adjoining beads of nucleosomes are linked together by a stretch of DNA: linker DNA. (Figure 7.3)

(a)



(b)

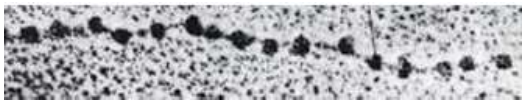


Figure 7.3: a) First level of packing: Forming beads of nucleosomes joined together by linker DNA, b) Electron micrograph showing nucleosome (beads) and linkers (strings)

In the second level, the nucleosomes twist and pack in a spiral fashion to form a chromatin fibre of roughly 30 nm in diameter:30 nm fibres from 10 nm fibres. (Figure 7.4)

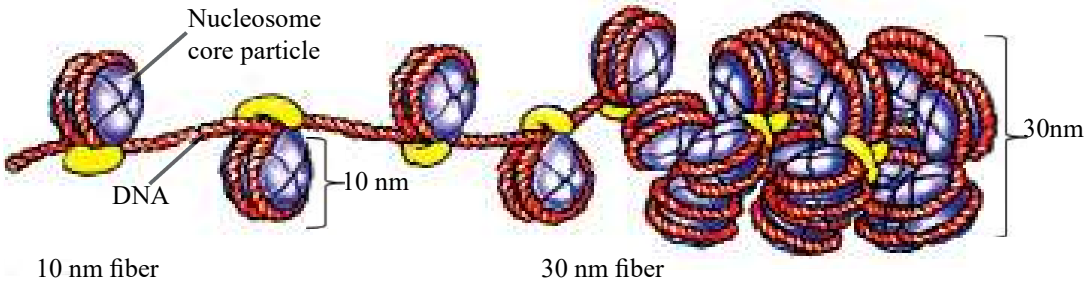


Figure 7.4: Formation of a 30 nm fiber (Cylindrically coiled)

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At the third level, the 30 nm fiber forms loops, called looped domains, attached to a protein scaffold. This structure has a thickness of 300 nm. (Figure 7.5)

Finally, at the fourth level, the looped domains coil, fold and further compact to form the mitotic chromosome. The diameter of a chromatid is about 700 nm. In the metaphase chromosome, the chromatids are already replicated (Figure 7.5).

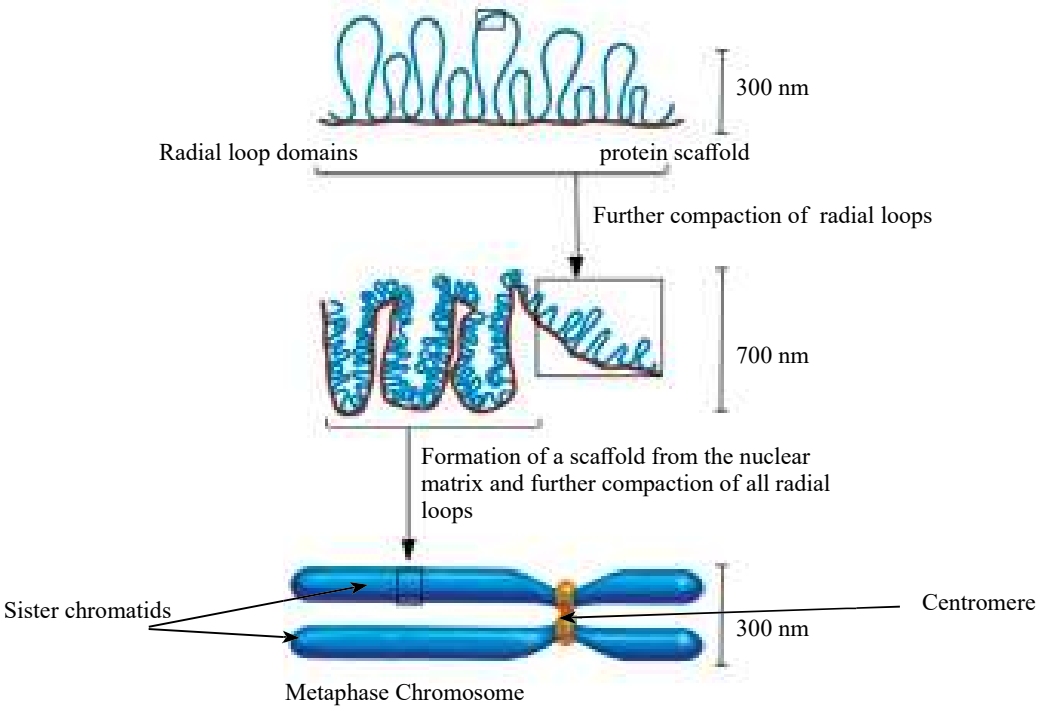


Figure 7.5: Compaction into looped domains on scaffold and into chromatids

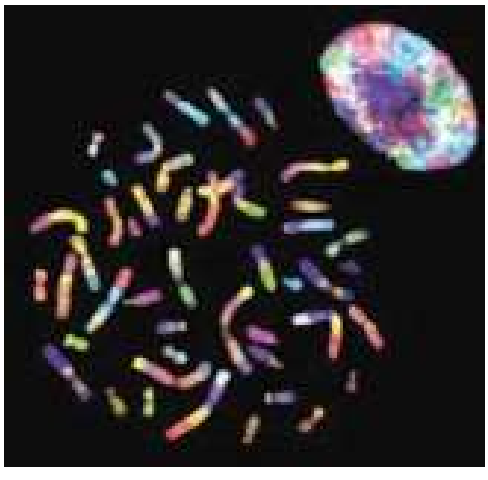


Figure 7.6: Metaphase chromosomes (discrete units) and chromatin in the interphase

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DNA Replication -

This is the process which copies a double stranded DNA molecule to produce two identical copies. Because, DNA replication process is basically similar in prokaryotes and eukaryotes. However, there are differences in enzymes involved. This is because the Eukaryotic nuclear DNA is organized into chromosomes and contain histones in their structure for packaging, and the prokaryotic DNA are usually found as circular molecules, which are supercoiled for packaging.

Importance of DNA replication

- The essential information for life is stored in DNA. Therefore, the new cells produced must receive the DNA from their parent cells as, each cell in the body of a diploid organism should contain the same genetic information as was in the zygote. A multicellular organism grows by addition of new cells.
- Damaged or dead cells are also replaced by new cells.
- In asexual reproduction, the offspring is identical to the parent cell. This is possible only because DNA has the ability to replicate and provide an identical set of genetic information stored in DNA to each daughter cell by mitosis.
- In sexually reproducing organisms, meiosis occurs at some point of life cycle in order to maintain a constant number of chromosomes. DNA replication occurs prior to meiosis.
- DNA replication is a very accurate process, so that it makes identical copies. However, rare errors occur in DNA replication, introducing mutations which results in variation. Variation leads to evolution of organisms.
- Therefore, DNA replication is important in maintenance of the life of an individual organism and continuation of a species.

The process of DNA Replication in prokaryotes

The entire replication process is carefully controlled and coordinated by a number of enzymes and other proteins.

DNA is synthesized on the existing DNA strands of the double helix of the DNA molecule. The parent DNA strands are used as the templates. Thus, the newly synthesized DNA double helix contains one parent DNA strand and a new complimentary strand.

First of all, the tightly packed DNA (supercoiled DNA in prokaryotes and chromatin in eukaryotes) has to relax, so that the DNA replication machinery can have access to the place where the replication begins.

The separation of the double helix occurs at the origin of replication. Origin of replication or 'Ori' is a specific sequence of DNA to which proteins that initiate DNA replication bind. Starting from this, the entire circular DNA is replicated on both directions. Since the enzyme synthesizing the new DNA strand can move only in one direction (5' to 3' direction) one of the new strands can be synthesized continuously while the other will be synthesized as small fragments. The

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small fragments of lagging strand are called okazaki fragments. These are called leading and lagging strands respectively. Replication of large DNA molecules may begin at several origins of replication to speed up the process.

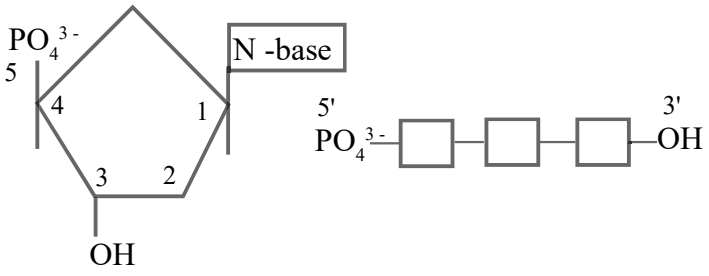


Figure 7.7: 5'- phosphate and 3'- OH of a DNA Molecule

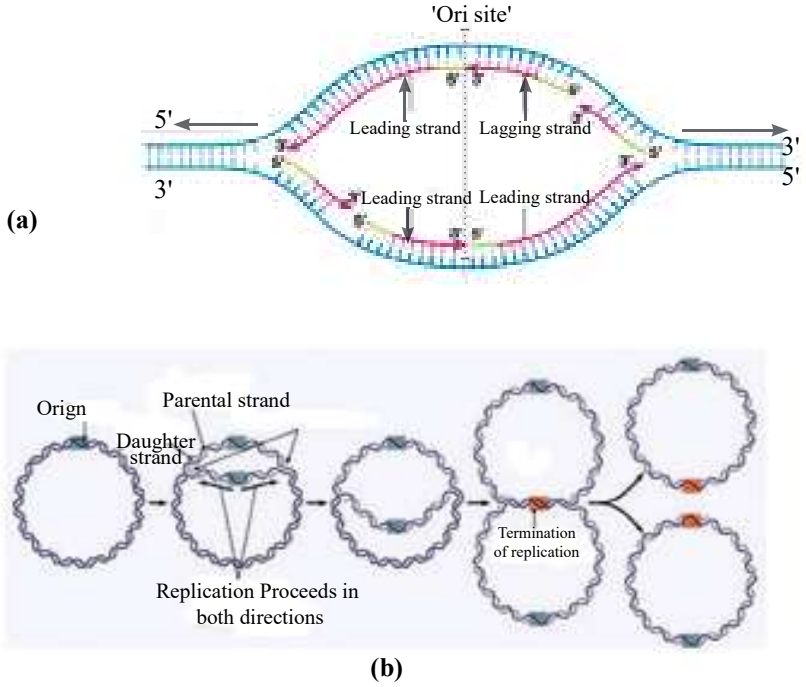


Figure 7.8: (a) Details of DNA a sreplication (b) DNA replication of small circular DNA

Functions of major enzymes and other proteins of replication machinery

A number of d enzymes and other proteins are required in the replication of DNA. These proteins assemble at the orign of replication. The major enzymes involved in the DNA replication are: helicase, topoisomerase, primase, DNA polymerases and DNA ligase. There are several other proteins in the replication machinery including Single Strand Binding (SSB) proteins.

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Helicase: These are the enzymes that unwind the double helix and separate the two strands of a DNA molecule, with the expense of energy as ATP. The separation occurs by breaking hydrogen bonds between the base pairs of the two strands. This is important for exposing the two strands to function as template for new DNA synthesis (replication).

Topoisomerase: These enzymes are working ahead of the direction of DNA synthesis. The untwisting of the strands at one place causes further twisting and strain on other places. Topoisomerase enzymes, introduce breaks on one or both strands of DNA, twist the molecule to relieve the strain and then reseal the cut ends.

Single Strand Binding Proteins (SSB): These protein molecules bind to exposed single stranded DNA to prevent re-pairing of the seperated DNA strands and to stabilize them. If the two strands re-pair, then they cannot serve as templates for new DNA synthesis.

Primase: In order to synthesize a new DNA strand on a template, complementary deoxyribonucleotides should be added one after the other in the correct sequence. This is done by DNA polymerase, but DNA polymerases can add nucleotides only to the 3' end of an already formed nucleic acid chain. For this, a small fragment of nucleic acid chain is sufficient and this fragment is called a primer. Primase is a type of RNA polymerase which can initiate synthesis of RNA on a DNA template by adding ribonucleotides. Primase adds a short RNA primer on the DNA template forming DNA-RNA hybrid to facilitate the action of DNA polymerase (figure 7.9).

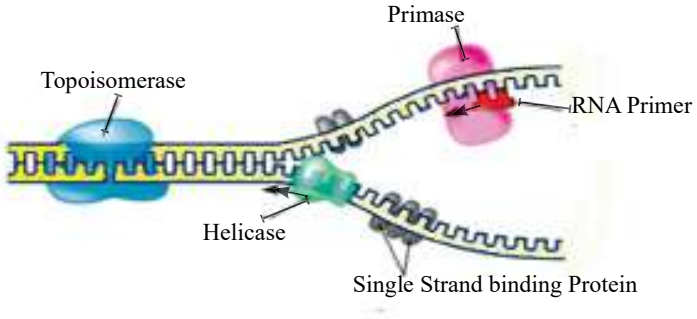


Figure 7.9 : ds DNA replication at the origin of replication forming replication bubble

DNA polymerases: There are several types of DNA polymerases. One type of DNA polymerase initiates DNA polymerization by adding a deoxyribonucleotide to the 3' end of a primer and continues polymerization on 5' to 3' direction elongating the new DNA strand with deoxyribonucleotides with bases complementary to template (figure 7.10).

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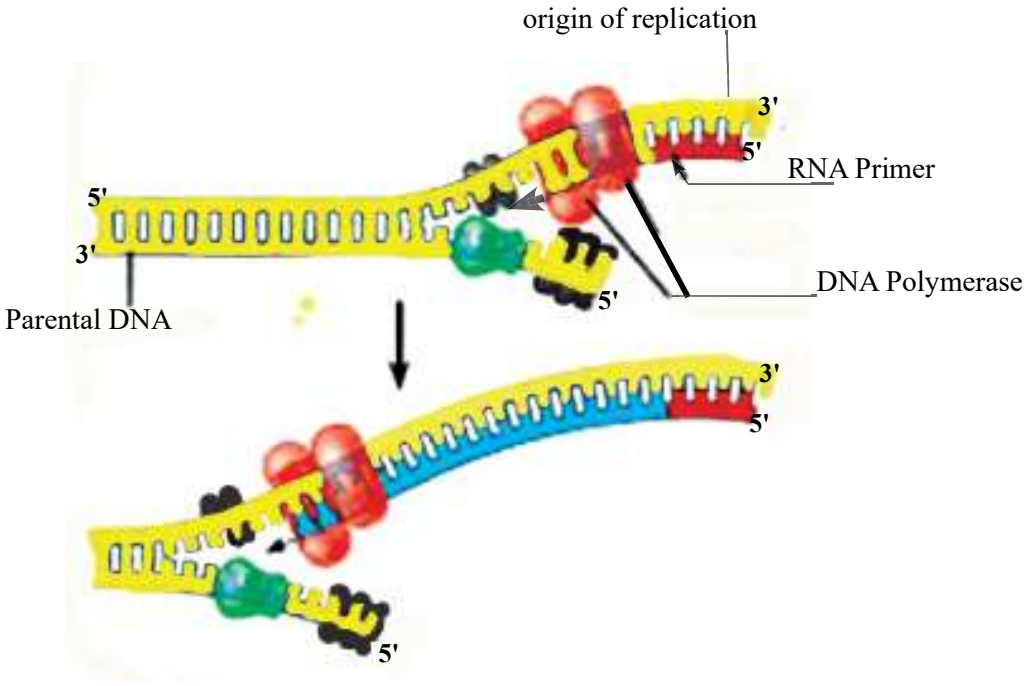


Figure 7.10: DNA Polymerase extends the new strand of DNA starting from 3' end of RNA primer

This DNA polymerase is almost 100% error-free in adding correct complementary nucleotides to growing strand, according to the nucleotide sequence of the parent DNA strands. However one error in 10^5 added nucleotides is possible, has a proofreading mechanism and therefore can correct its own mistakes and the error rate will be reduced by 100,000 times to one in 10^{10}. Therefore, the daughter DNA molecules are almost identical to the parent DNA molecule, and of course to each other.

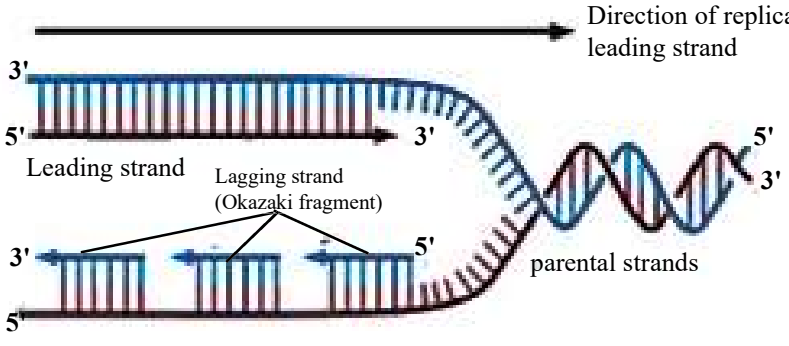


Figure 7.11: How DNA Polymerase solves the problem of antiparallel nature of DNA molecule

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When a wrong nucleotide is added to the growing chain of DNA by DNA Polymerase this mismatch is identified by the same enzyme, stops adding the next nucleotide, removes the incorrect nucleotide exonuclease activity and continues again its polymerase activity. This is called as proofreading activity of DNA polymerase.

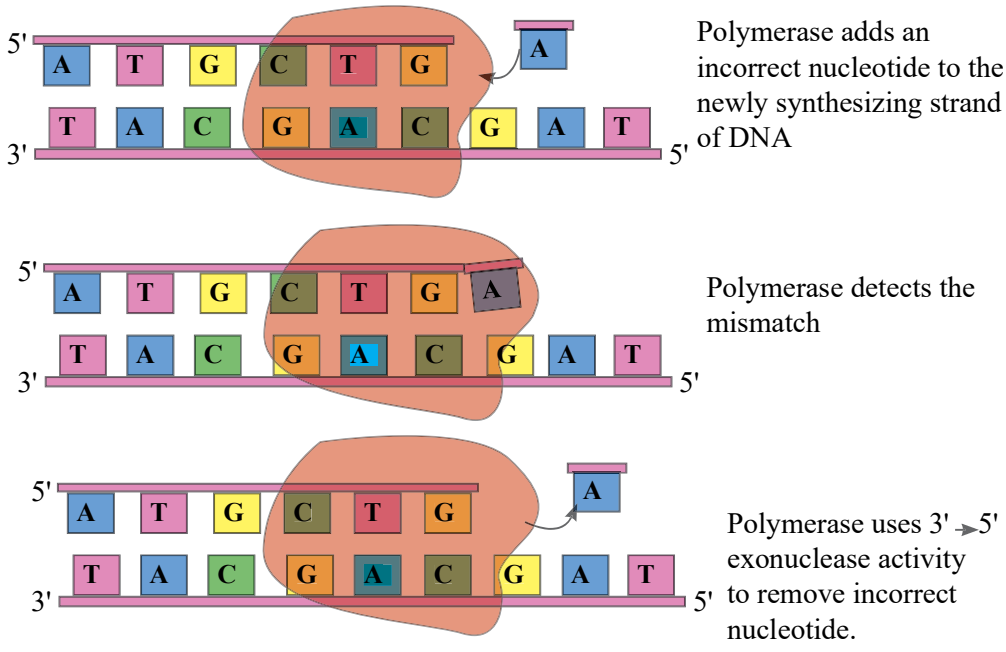


Figure 7.12. Proofreading activity of DNA Polymerase

Another type of DNA polymerase identifies DNA-RNA hybrid, removes ribonucleotides and replaces those with deoxyribonucleotides and so replaces the RNA primer with DNA. Now the DNA segment is incomplete, but the DNA polymerase unable to correct the ends of okazaki fragments, results gaps.

DNA ligase: In DNA synthesis, it joins the newly synthesized adjacent fragments making a complete strand by the formation of a phosphodiester bond. It seals the gaps of the newly synthesized DNA strand.

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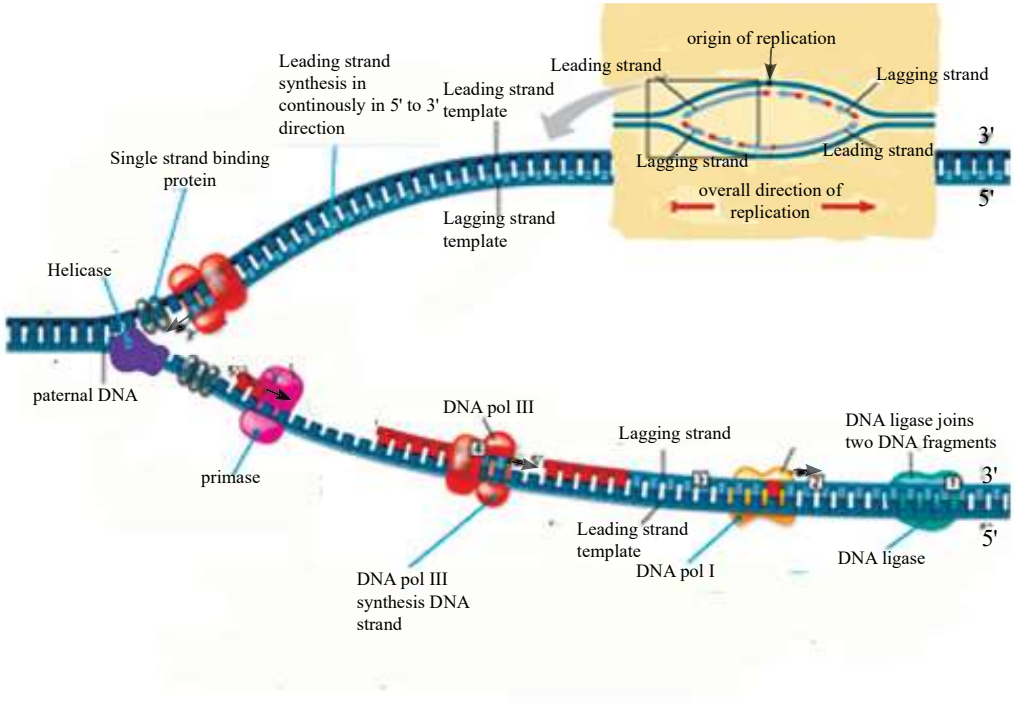


Figure 7.13: Overall process of DNA replication

Overall Process of DNA Replication

Relaxation of tightly wound DNA

Unwinding/ unzipping of the double helix

Stabilization of single stranded DNA

Priming of DNA synthesis by a RNA primer

Extension of new DNA strand - Leading strand: Continuous

- Lagging strand – Discontinuous

Removal of RNA primer and replacing RNA (ribonucleotides) with DNA (deoxyribonucleotides)

Sealing gap between the adjacent nucleotides

Similarities and Differences between Prokaryotic and Eukaryotic DNA Replication:

Eukaryotes share many characteristics of DNA replication of the Prokaryotes. They also use helicase to unwind the dsDNA, and use DNA polymerases for the polymerization reaction. The replication is originated in both types at specific sequences (origin of replication – Ori) to initiate DNA replication, packed DNA is relaxed with topoisomerase and replication process move in the same manner and therefore having leading and lagging strand. RNA primers are formed and replaced. The gaps are sealed by ligase.

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Although the process is superficially similar, there are several significant differences too. The size of the DNA molecule in a eukaryotic chromosome is much larger than that of a circular DNA molecule of a bacterium. Therefore prokaryotes usually have one Ori while an Eukaryotic chromosome has several Ori. The DNA polymerases in eukaryotes and prokaryotes are different from each other in their structure, while having the same functions. The DNA replication of the prokaryotes occurs continuously, whereas in eukaryotes it happens only in the S-phase of cell cycle.

DNA repair and its significance

Damage to DNA can be caused by various chemical and physical agents introducing mismatches in DNA double helix and may lead to a permanent change in the sequence of DNA. This may also be caused by errors in DNA replication that goes unnoticed by proofreading. This is called mutation. A mutation or collection of mutations can make a cell malignant and result in a cancer. Mutations also alter the phenotype, and very often they are either lethal or at least produce unfavorable phenotypes. When the mutations occur in cells producing gametes, they may be passed onto the next generations resulting in variations among offspring.

When such a mismatch is there, the shape of the double helix is distorted. For example, UV radiation can covalently link two adjacent thymine bases, distorting the DNA molecule. One of the two copies of DNA molecules generated by these distorted sequences carries a permanent change in the sequence, a mutation. Such distorted locations are usually recognized and repaired by the DNA repair mechanism of the cell before it becomes permanent, and that reduces the risk of accumulation of mutations. Because DNA repair is very important for the survival of organisms, a large number of DNA repairing enzymes are found in various organisms.

Such enzymes may cut off the mismatched sequences in the damaged strand and replace with correct nucleotides. The cutting (excision) is done by a nuclease and filling the gap, using the correct strand as the template, is done by a type of DNA polymerase. This is called nucleotide excision repair. DNA ligase completes the DNA strand by sealing it with a phosphodiester bond.

Genes and how they work

The nature of prokaryotic and eukaryotic genes

When Gregor Mendel put forward his laws of inheritance in 1860, he used the term hereditary factors to explain what control the characters shown in a phenotype and which are transmitted from generation to generation. At that time, these were imaginary units and where they were located in cellular structure was not known. Today, these physical and functional units of

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heredity have been identified as genes that are located on chromosomes as discrete units. This disclosure began with the development of cytology and the ability to observe the behavior of chromosomes in mitosis and meiosis. Behavior of chromosomes and the behavior of the Mendel's hereditary factors show the same pattern. In eukaryotes, chromosomes occur in pairs in diploid somatic cells, and hence genes also occur in pairs. A pair of chromosomes, coming from the two parents, containing the same genes is called homologous chromosomes. Generally prokaryotes have one chromosome in each cell, and hence may be considered haploid.

Gene
Gene is the fundamental physical and functional unit of heredity.
A gene is comprised of a segment of DNA on a specific site on a chromosome. It specifies the sequence of RNA.

The location or the site of a gene on the chromosome is called a locus (loci in plural). The alteration versions of genes that are located at the same locus on different chromosomes are called alleles of the genes. Prokaryotic genes are housed in loci on the circular DNA molecule, as discrete segments of DNA.

There are many steps in a biochemical pathway and each step is controlled by a gene, and therefore many genes are involved in controlling a particular phenotype. In eukaryotes these genes are scattered among several chromosomes, while in prokaryotes, they are arranged in the same region of the chromosome one after the other as clusters. These clusters are expressed together by a single control region and are transcribed into one mRNA. Such mRNA molecules will be translated into several different peptides. These organized gene clusters in prokaryotes are called operons.

Operon: A group of genes that functions as a single transcription unit. It is comprised of a control region (an operator, a promoter) and structural genes that are transcribed into one mRNA. Coding for several peptides.

In prokaryotes, all DNA segments in the chromosome are functional (either transcribed into mRNA or act as control regions), while in eukaryotes, a large proportion of DNA has no identified function. Such DNA segments that are present in between the genes are referred to as intergenic DNA. There are also sequences within the genes that are transcribed, but are not translated into polypeptides. That means there are coding sequences as well as noncoding sequences in the transcript of the gene. The noncoding sequences within the genes are called introns while the sequences that code for polypeptides are exons. Accordingly the transcript contains exons as well as introns. The transcript is a pre-mRNA and it undergoes processing where introns are excised and exons are joined inorder to produce mRNA.

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Chromosome Theory of Inheritance

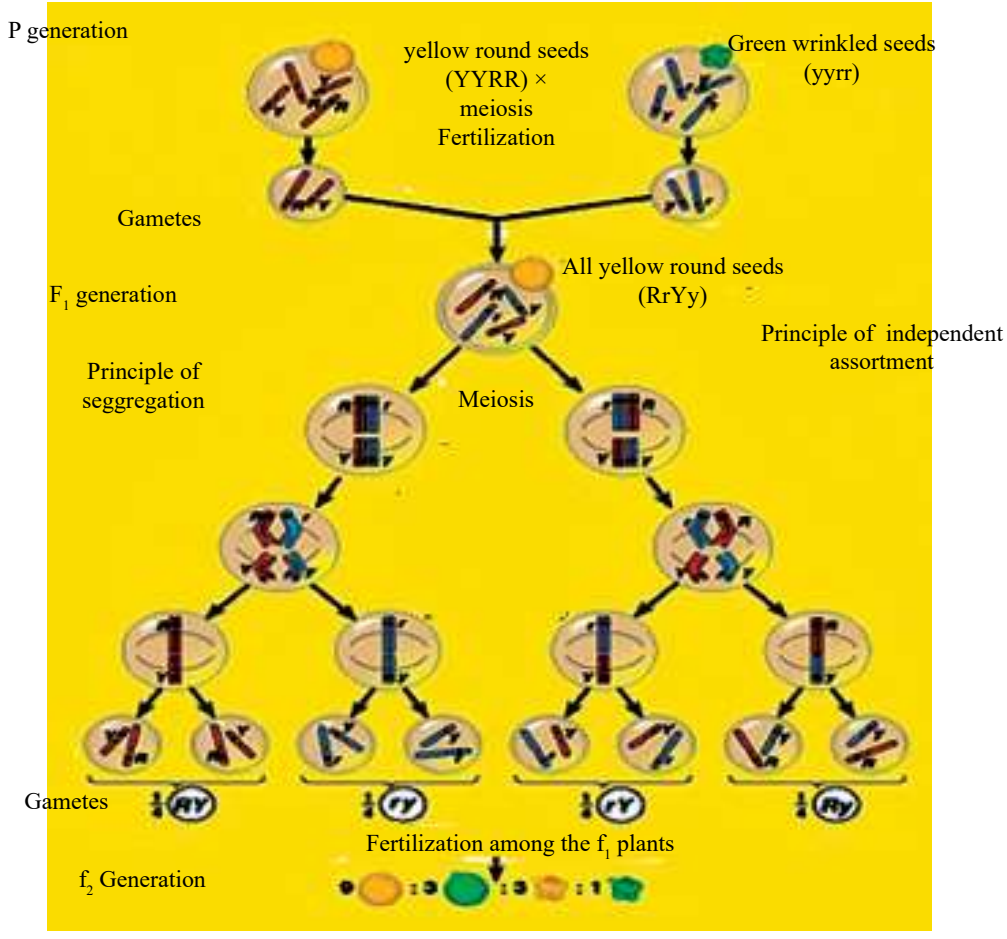


Figure 7.14: Chromosomal basis of Mendel's Laws: How the alleles of genes on homologous chromosomes behave during meiosis

Figure 7.14 shows the parallelism between the behavior of Mendel's hereditary factors and genes and their alleles located on chromosomes. Evidences collected in genetic studies were noted by scientists and several of them independently developed chromosome theory of inheritance. The Mendelian hereditary factors or genes are located on the chromosomes at specific loci. Therefore chromosomes and genes housed on them occur in pairs in diploid cells. The paring of homologous chromosomes in metaphase I occurs at random and therefore this is independent assortment (i.e. no order in arranging maternal and paternal chromosomes). In anaphase I the independently assorted homologous chromosomes separate reducing the number of chromosomes to half. This is segregation. With the independent assortment and segregation of chromosomes, alleles of genes on nonhomologous chromosomes are assorted independently in different combinations in Metaphase I. The alleles segregate after completion of anaphase 1 to form four haploid cells having different allele combinations in equal proportions. The same reasoning can explain the phenotypic ratios observed by Mendel in F_2 generation after random crossing of $F_{I.}$

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Gene Expression

Genes control characters when a gene is at work and, it is said that the gene is expressed. Gene expression is the process by which the information stored in a gene is used to make a functional gene product. The final product of a gene is usually a polypeptide, which after suitable modifications becomes a protein. However several RNAs also function as final gene products. For example, ribosomal RNA (rRNA) and transfer RNA (tRNA) are such RNA molecules, having direct functions.

In examining how genes control characters, it was revealed that (the first suggestion came in 1902 from Archibald Garrod) inherited diseases are caused by the inability to produce related enzymes as a result of inborn errors in metabolism. The symptoms of a hereditary disease condition, known as alkaptonuria is due to inability to make the metabolic enzyme which metabolizes the chemical alkapton. In patients alkapton remains in urea and its oxidation results in black colouration of urine.

The term copying is used in transcription, as in linguistics, since the information written in the nucleotide sequence of DNA by four letters (AGCT) is copied to the same chemical language as a nucleotide sequence of RNA written in four letters (AGCU), with only difference that T is replaced by U. The amino acid sequence in the polypeptide chain is also linear and parallel to the base sequence in the gene or mRNA, but the chemical languages are different: language written in four letters to a language written in 20 letters. This is the reason why the second step is called translation.

The gene expression begins by copying the information stored in a segment of DNA or a gene into sequence of RNA. In synthesis of polypeptide the gene is not directly converted into polypeptide, but involves an RNA molecule to pass the message in DNA to a message in polypeptide. Since the RNA molecule acts as a messenger to communicate the information from DNA to polypeptide, it is called messenger RNA (mRNA). The two steps in polypeptide synthesis are:

1. Transcription - Copying a sequence of DNA into mRNA
2. Translation – Converting the information in mRNA to a sequence of amino acids

Transcription is similar to replication since a DNA strand is acting as a template to make a complementary mRNA strand. The difference in transcription is that the copy is a mRNA molecule and only one DNA strand is copied. The major enzyme catalyzing polymerization is named as RNA polymerase. This is mRNA, because it transmits the message stored in the gene to the place where the polypeptide chain is actually being assembled.

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Message in mRNA is translated to a sequence of amino acids. This process occurs in association with ribosomes, which are present in the cytosol.

In addition to mRNA, other types of RNAs and enzymes are involved in the polypeptide synthesis. The basic mechanism of the process of polypeptide synthesis is similar in prokaryotes and eukaryotes, with some important differences.

Genetic Code

In transcription, each letter of the template will be copied to the corresponding letter in mRNA. Note that the mRNA is complementary to template, and hence it is a copy of the other DNA strand. This seems to be straight forward, one to one copying. On the other hand, language of nucleic acid has four letters (nucleotides), whereas the language of protein has 20 letters (amino acids). If each kind of nucleotide is translated into amino acid, only four amino acids would be specified or coded. Therefore a combination of nucleotides would be needed to code for one amino acid. It has been proven by experiment that amino acids are coded by triplets of nucleotide bases and the protein synthesis is based on a triplet code. Therefore, the genetic code is a triplet code. If three-letter combinations or triplets are considered, $4^3 = 64$ possibilities are there. The three letter words or triplets are read one after the other, and hence are non-overlapping. Since all the words have three letters, no space is required to delimit the words. The genetic code stored in a gene in non-overlapping three-letter word is copied to non-overlapping, three-letter word in complementary mRNA. This is translated by reading three letters at a time identifying an amino acid corresponding to each three-letter word (Figure 7.16). The triplet of nucleotide bases of mRNA or coding for an amino acid formation is called a codon. Therefore the genetic code has 64 codons.

Out of 64, 61 triplets code for 20 amino acids, and the other three are used to signal the “stop” of translation or as ”termination” codons (UAA, UAG and UGA). AUG codon codes for methionine (Met) as “start codon” signaling the protein synthesizing machinery to start translation of mRNA at that codon. Therefore, the first amino acid of all protein is methionine, but this may be enzymatically removed after the translation. Figure 7.16 shows all 64 codons and what each of them codes for. It is easily noticeable that certain amino acids are coded by more than one codon.

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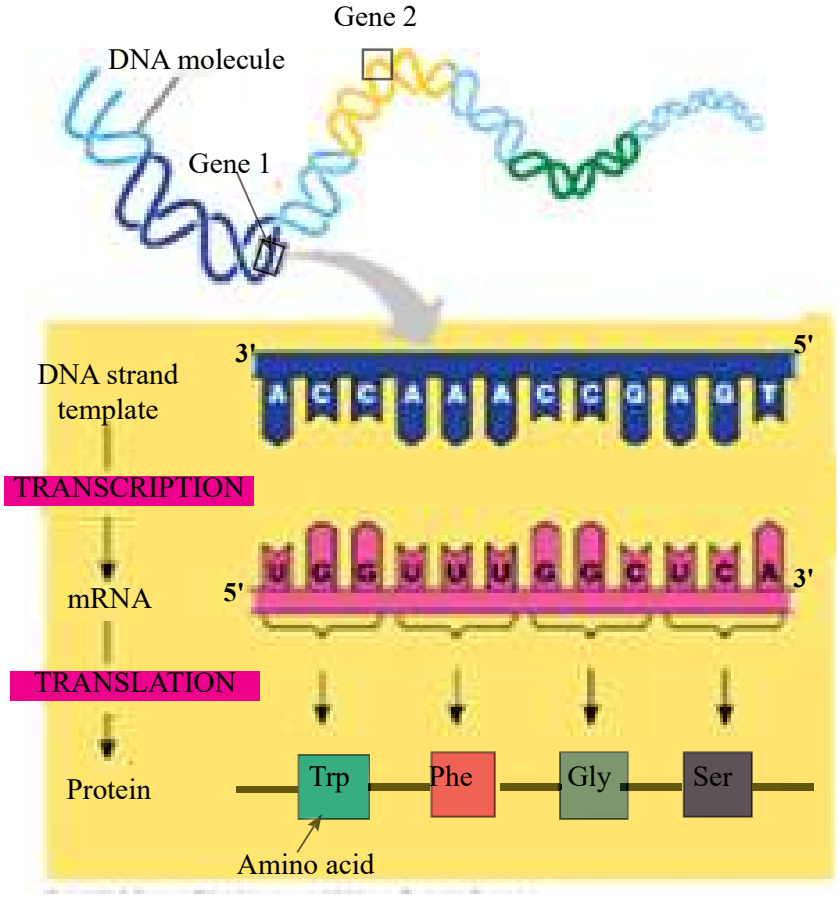


Figure 7.15: The genetic code is copied to mRNA and translated into amino acid in polypeptide chain using triplet codons

To read a message accurately, a starting point and a termination point as well as correct sequence of letters should be identified. This is called a reading frame. The protein synthesizing machinery starts and ends reading at precise positions and reads triplets one after the other in a non-overlapping fashion. Since all words are three letter ones, no spaces between words are required. If the reading starts at a wrong place, entirely wrong message will be read and a wrong polypeptide will be synthesized. If one letter is missing or one letter is added in the reading frame, the wrong message will be read from that point onwards, once again making a wrong polypeptide, if at all. The reading of the message is always from left to right by convention.

Another interesting feature of the genetic code is that its universality. This means nearly all organisms share the same genetic code. Accordingly, a gene isolated from one organism and inserted in to another related or nonrelated organism should express the same protein. This is how human insulin is produced by bacteria. The reading frame for insulin protein will be translated

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in exactly the same way in both human and bacterial cells. A firefly gene can be expressed in tobacco plant, so that the plant emits light.

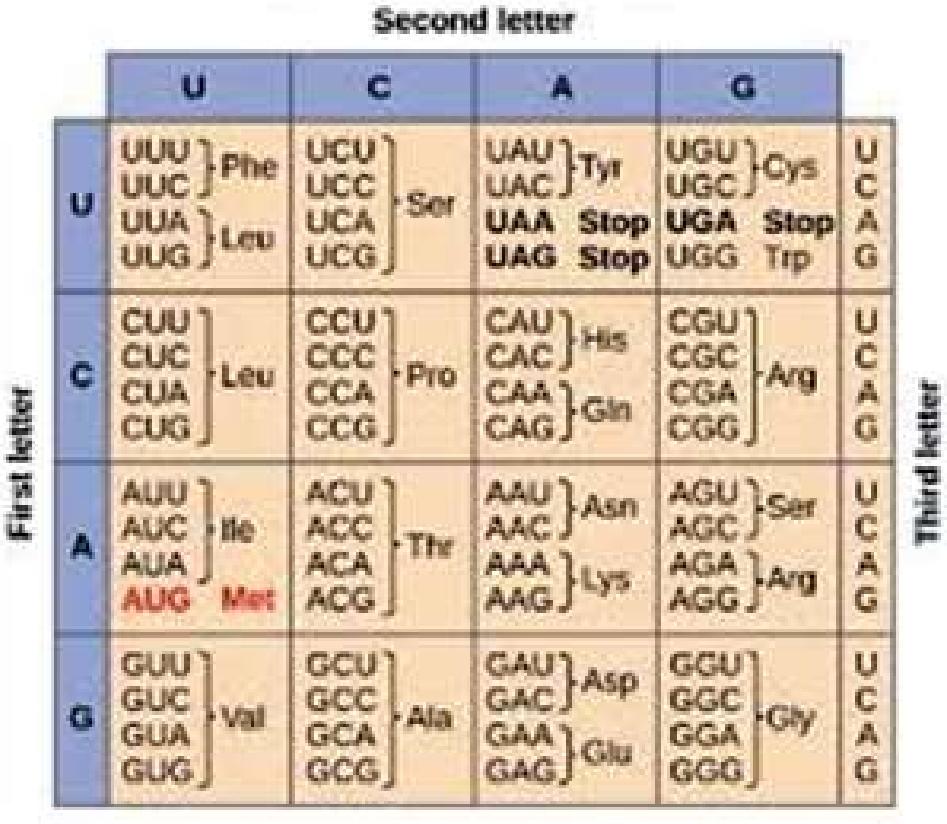


Figure 7.16: The codon table for mRNA

Mechanism of Polypeptide Synthesis

I. Transcription

Transcription is DNA directed RNA synthesis. This is completed in three steps.

1. Initiation: The process of transcription is initiated at a specific site called promoter. The promoter site includes the transcription initiation site and several other nucleotides. Only one strand of the double stranded DNA acts as a template for transcription. This is because only the template strand will have the promoter sequence in the correct orientation, which facillitates the binding of RNA polymerase. The enzyme polymerizing the RNA is RNA polymerase. This enzyme binds to the promoter site in correct orientation. The RNA polymerase then unwinds the two DNA strands and begins the transcription at start point. A component of RNA polymerase has the helicase activity, and hence DNA helicase is not involved in transcription.

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2. Elongation: RNA polymerase enzymes can start adding complementary ribonucleotides against the template DNA. RNA polymerase continues to add nucleotides in 5' to 3' direction until it reaches the transcription termination site. The DNA strands unwinds as the RNA polymerase moves forward, exposing template DNA and allowing pairing with ribonucleotide. The two strands rewind at the other end (figure 7.17)

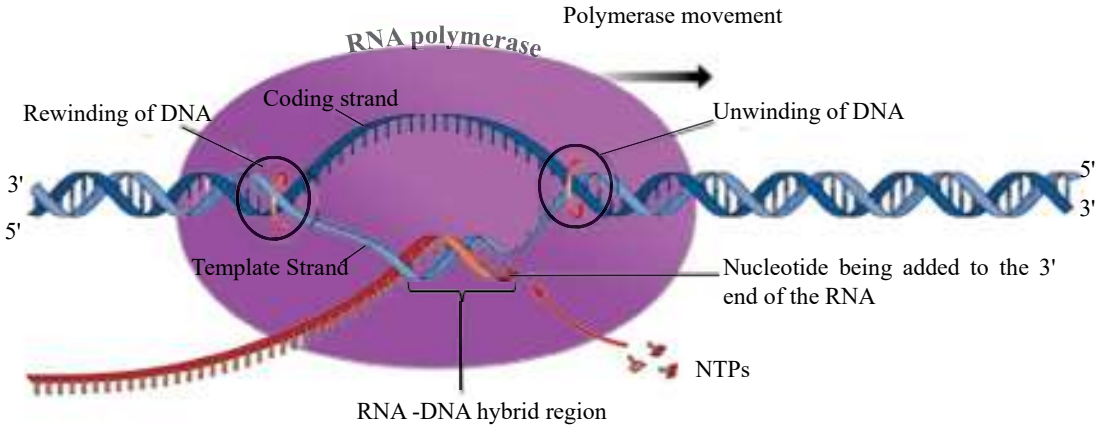


Figure 7.17: Elongation of the newly forming RNA transcript

3. Termination: In prokaryotes, the polymerization continues passing the termination sequence of DNA and RNA polymerase enzyme falls off ending the transcription..

After termination, the newly synthesized pre mRNA in eukaryotes is subjected to RNA processing. The mature RNA leaves the nucleus.

II. Translation

Once the mRNA is in the cytosol, translation process is initiated. Ribosomes read the message written as a sequence of triplet codons in an mRNA and translate it into a sequence of amino acids in a polypeptide with the assistance of transfer RNAs (tRNA). A tRNA attached to the correct amino acid from a pool in the cytosol, transports it to the ribosome which adds the amino acid to the growing end of the polypeptide chain by making a peptide bond. The key players in translation are tRNAs .

A specific tRNA molecule binds a specific amino acid to its one end. Its structure also carries, at a specific location, a triplet of nucleotides, which is complementary to the codon in the mRNA that code for the amino acid it carries. This triplet is known as anticodon and it can base-pair with the codon (figure 7.18). This is how tRNA does the translation, by acting as an adapter molecule between the triplet codon and the amino acid specified by it.

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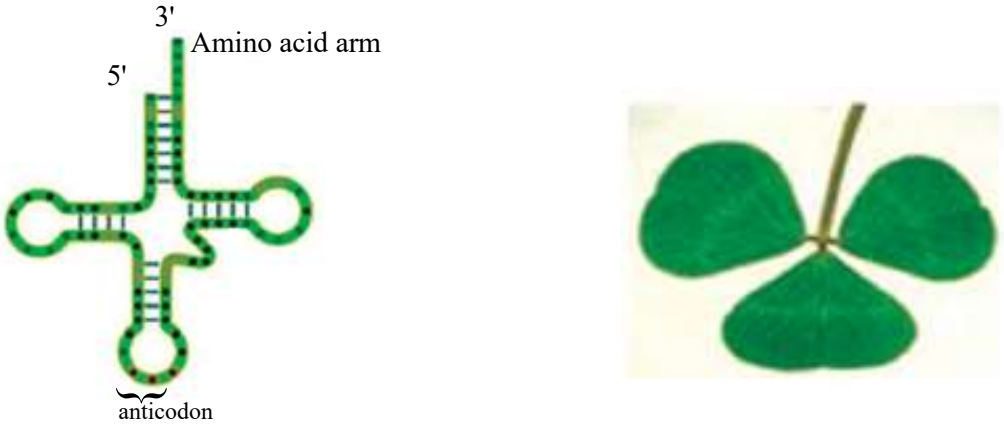


Figure 7.18: Two dimensional model of tRNA showing clover leaf structure

Structure of ribosome

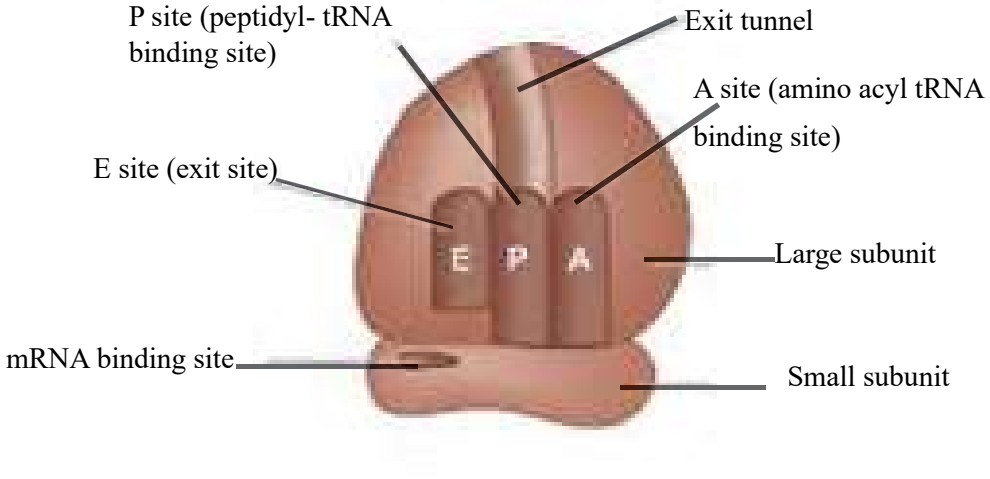


Figure 7.19: Structure of ribosome

Process of translation

The translation is also completed in three stages.

1. **Initiation:** The first step in initiation is binding of small subunit of ribosome to mRNA and to the initiator tRNA which carries methionine as the first amino acid. Then, the two subunits of the ribosome combine to form the functional ribosome. This complex of ribosomal subunits, mRNA and initiator tRNA, is called transcription initiation complex (Figure 7.20). Then the mRNA moves until the AUG start codon, aligns with the P site of the large subunit. Then, the anticodon of the initiator tRNA forms hydrogen bonds with AUG start codon. This signals the initiation of the translation.

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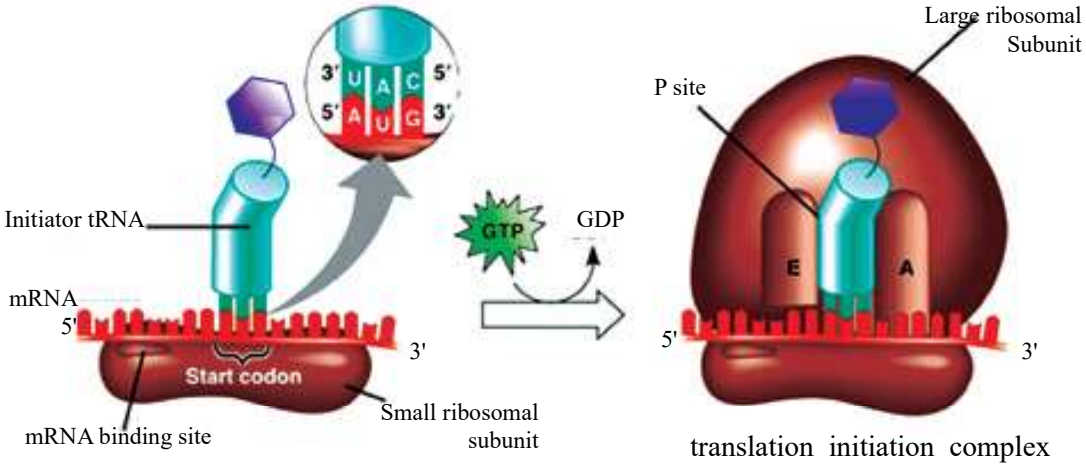


Figure 7.20. Formation of translation initiation complex

2. **Elongation:** In this stage, amino acids are added to the C- terminus of the growing polypeptide chain by peptide bonds, one after the other governed by the triplet codons. Elongation is completed by a three-step cycle. At the end of the initiation stage, P site is occupied with tRNA attached to methionine and A site is empty and is aligned with the next codon. The second tRNA, charged with the corresponding amino acid is brought to the A site, matching the codon and anticodon. This first step in the cycle is the codon recognition. As the second step, a peptide bond is formed between the carboxyl group of the growing polypeptide chain in the P site with the amino group of the amino acid in the A site. An rRNA catalyses this reaction. The third step is the translocation of the mRNA. The mRNA moves codon by codon unidirectionally. In the process, the tRNA with the growing polypeptide chain in the A site is moved to the P site. The released tRNA in the P site will move simultaneously to the E site, from where it is released to the cytosol. The A site is now aligned with the next codon, so that this cyclic process can continue. GTP is used for the energy requirement of the elongation process. See Figure 7.21 for an illustration of elongation stage in translation.

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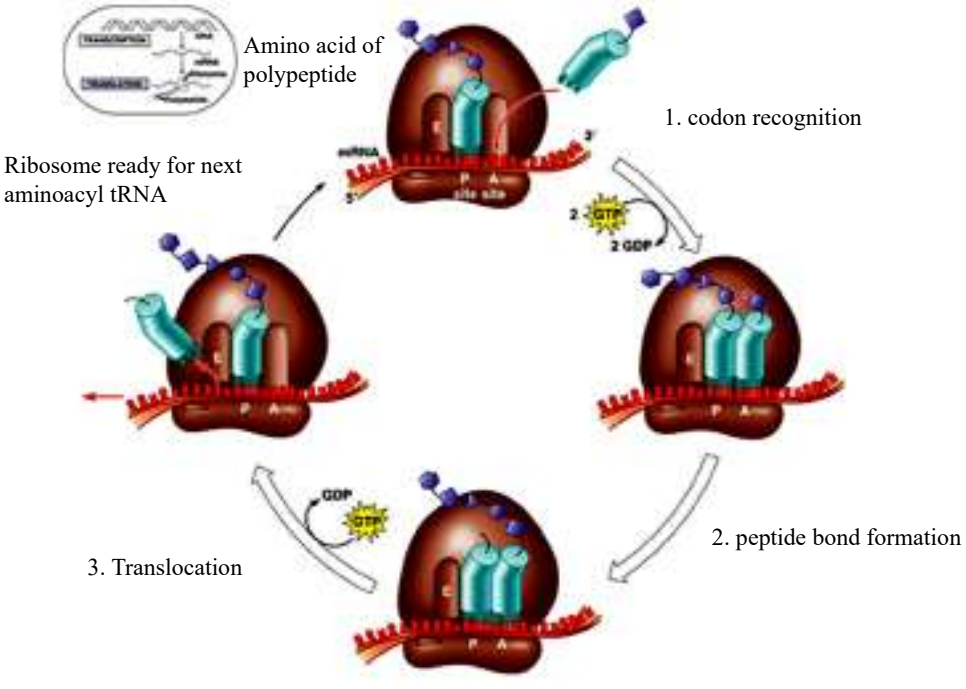


Figure7.21: Three-steps that occur in cyclic manner in elongation stage in translation.

3. **Termination:** When the mRNA moves, it finally aligns one of the stop codons: UAG, UAA or UGA with A site. They do not code for any amino acid, hence there is no tRNA which comes into A site. This releases the completed polypeptide chain to the cytosol. The ribosome and the remainder of the translation assemble then fall apart (Figure 7.22).

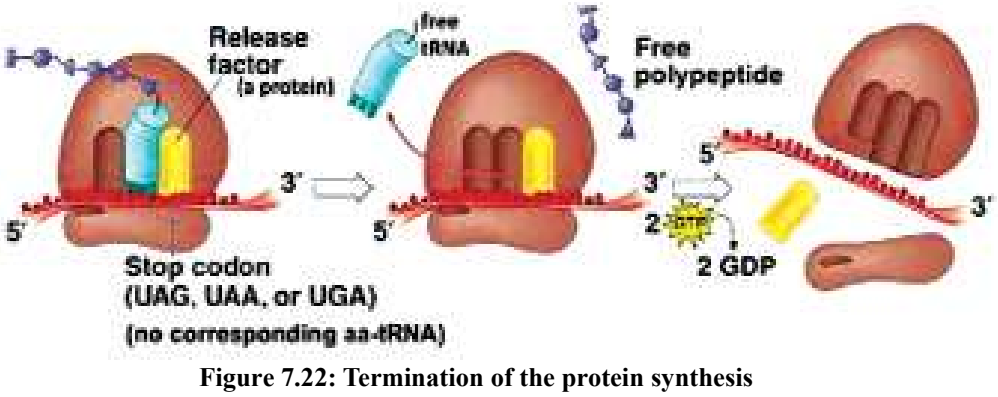


Figure 7.22: Termination of the protein synthesis

Polyribosomes/ Polysomes:

When the mRNA moves away for a sufficient distance, a second ribosome can bind to it. Depending on the length of mRNA, a number of ribosomes can be attached to the mRNA simultaneously. As such, mRNAs that are being translated actively are associated with multiple ribosomes attached to a string of mRNA, forming polyribosomes or polysomes. Forming polyribosomes increases the rate of translation since they allow simultaneous translation by several ribosomes.

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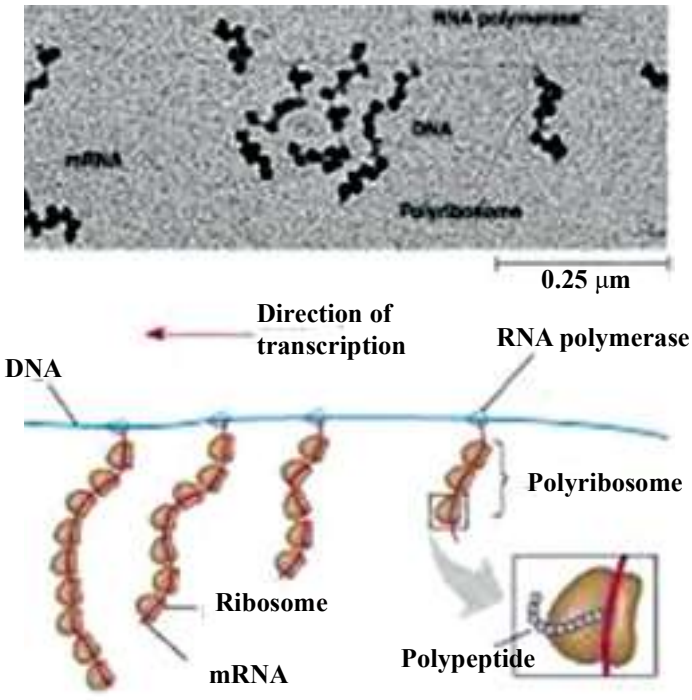


Figure 7.23: Polyribosomes of prokaryotic organism - mRNA is still growing and attached to the DNA

Fate of the proteins

The newly synthesized polypeptide is the primary structure of the polypeptide. This is not functional as a protein as it is. The polypeptide has to assume its functional form by folding (refer unit 02) and sometimes with post-translational modifications.

Certain polypeptides have additional segments than what is required for its function. For example, a short segment of amino acids are present in certain polypeptides to act as signal peptide. Signal peptide guides the polypeptide to a particular location in the cell or to be secreted. This is referred to as protein trafficking. Once the polypeptide is in place, the extra piece of the peptide chain is no longer needed and maybe removed enzymatically.

The post-translational modifications include chemical modification of certain amino acids by attaching sugars (glycoproteins), lipids (lipoproteins), phosphate groups (phosphorylated proteins) and other additions. The first amino acid, methionine, maybe removed enzymatically. Enzymes may also cut the initial polypeptide into two or more pieces which form a functional protein, by joining different combinations. For example, the protein insulin is produced as a single polypeptide, but cut at two places to remove the central piece. The other two polypeptide chains are joined together to form the functional insulin.

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Selective Degradation of Proteins

The amount of a protein in a cell is determined on one hand by the rate of synthesis and on the other by the rate of degradation. The selective degradation of proteins is an essential mechanism in regulation of cellular activities. Certain proteins are degraded in response to specific signals. Faulty or damaged proteins are recognized and rapidly degraded to avoid bad effects due to mistakes in polypeptide synthesis or errors in folding. Some proteins for example regulatory proteins, need to be rapidly degraded after their function . The structural proteins may remain for a longer period.

Mutations

The phenotype of an organism basically depends on the genetic information stored in DNA and the final outcome is a result of the interaction between genetics of the organisms and the effects of the environment.

Alterations in DNA may bring about certain changes in the characters of individuals in a species, which result in phenotypic variation among organisms. These changes occur permanently, and are called mutations.

Mutation
An alteration of the nucleotide sequence of the genome of an organism

Mutations are the sources of variation seen among individuals of a species.

The effect of a mutation may be neutral, beneficial or deleterious. Deleterious mutations may be lethal or at least they are less favourable than the original phenotype. A mutation may also cause complete loss of a function. In rare occasions, the function of a polypeptide may be enhanced due to a mutation. These are beneficial mutations. Entirely new functions may also be resulted by mutations. For example, an enzyme specific to a substrate may change due to mutation in such a way that it changes its specificity to act on another substrate. The product due to the mutation is capable of catalyzing a new biochemical reaction.

There are two major types of mutations based on the scale of alterations in the genetic material; small-scale changes occurring in the sequence of nucleotides in a gene or large-scale changes in the number or the structure of chromosomes. These are known as gene mutations and chromosome aberrations or chromosome mutations, respectively.

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Gene mutations

The permanent alterations of DNA sequence of a gene are called gene mutations. They may be occurred due to rare errors occur during DNA replication. These are called spontaneous mutations. In addition, certain external factors can also cause mutations at a higher rate. Since these factors generate mutations, they are called mutagens. Mutagenic agents can be classified as chemical or physical factors. X-rays and UV rays are examples for mutagenic physical agents.

The mutagenic agents can introduce mutations to replicating DNA in a cell. Mutations are also the cause of carcinogenesis. Therefore, mutagens are carcinogens and carcinogens are mutagens. These chemicals and radiations need to be handled with utmost care.

Types of Gene Mutations

These are small-scale mutations involving only one nucleotide pair or more than one nucleotide pair. If only one pair is altered, these are called point mutations. There are three types of gene mutations. They are:

1. A single nucleotide pair substitution – change one nucleotide pair with another
2. Nucleotide pair insertions – addition of one or more nucleotide pairs
3. Nucleotide pair deletions – removal of one or more nucleotide pairs

The substitution of a pair of nucleotide is a point mutation. Insertions or deletions can be point mutations, or may involve more than one nucleotide pair.

Substitution

Here one nucleotide pair is replaced by another pair (Figure 7.23). There is no change in the lenghth of the gene. Because the same amino acid may be coded by more than one codon. Some substitutions are silent mutations. Substitution of one nucleotide pair of a gene may not have an effect on the polypeptide it codes for. Because the same amino acid may be coded by more than one codons. The third letter in the codon triplet has a wobble, meaning that even if the third letter of a codon is replaced by another letter the same amino acid is coded in the third letter (for example, the codon 5'- GGC-3' on the mRNA will be modified to 5'-GGU-3', if 3'-CCG-5' triplet on the DNA template strand be altered as 3'-CCA-5' by substitution G with A). A substitution may also change one amino acid in the polypeptide. Therefore, the meaning of the primary structure of the polypeptide is changed slightly; hence, these mutations are missense mutations. This substitution of the amino acid with another amino acid may or may not have significant impact on the tertiary or quartnery structure (functional form) of the protein. Occasionaly, this may render the protein a higher activity or even with new properties. However, very often, these changes are either neutral or detrimental, making the protein useless or less efficient.

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It is also, possible that a point mutation converts a codon coding for an amino acid to a stop codon. This causes premature termination of the protein synthesis, and therefore it is called non-sense mutation (figure 7.23). The result is a shorter polypeptide than original, which is usually non functional.

Insertion and deletion

These mutations bring about drastic changes in polypeptides compared to substitutions (note: nonsense mutations due to substitutions may also result in major changes). Insertion or deletion of a nucleotide or a couple of nucleotides will result in shift in the reading frame, reading wrong codons after the point of mutation. Therefore, such mutations are called frameshift mutations (figure 7.23), resulting in extensive missense. The polypeptide may not be functional at all, unless the insertion or deletion occurred is very close to the termination codon. It may also introduce a new stop codon, which was not in the original sequence. If that is the case, the translation terminates causing nonsense mutation. However, if the insertion or deletion is a triplet or a multiple triplet, the reading frame, will be back to the original reading frame immedietly after the point mutation (Figure 7.23). In such cases, one or a small number of amino acids will be added or removed, respectively from the entire sequence. The message will only be slightly changed and the polypeptide may be functional, depending on the importance of the mutated region of the polypeptide in its correct folding.

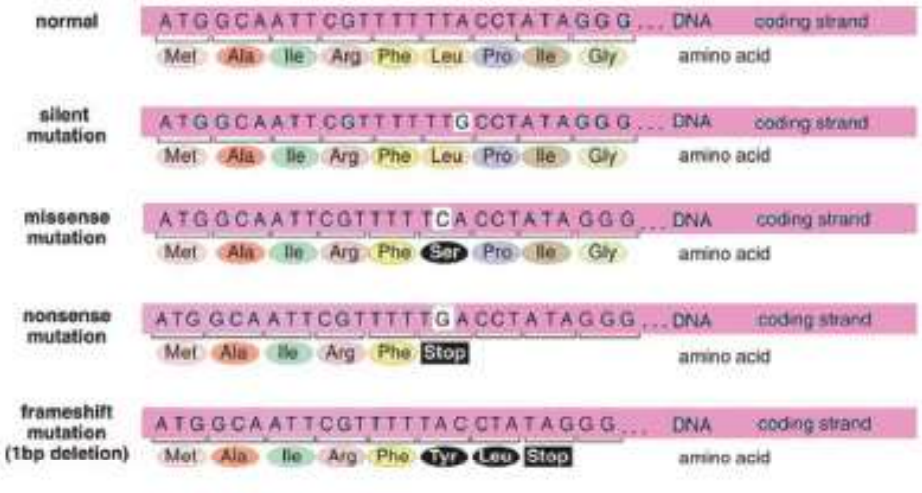


Figure 7.23: Types of Gene mutations

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Chromosome Aberrations/ Chromosome Mutations

Since many genes are involved, most of the chromosomal mutations are lethal, while others are deleterious. Spontaneous abortions or miscarriages in mammals occur due to unusual numbers or structures of chromosomes. Such mutations also bring about various developmental disorders. Beneficial chromosome mutations are extremely rare. In plants, some chromosomal mutations cause beneficial variations.

I. Mutations due to alterations in chromosome structure:

Large chunks of chromosomes, which may include several genes to several hundred genes, may be lost, moved to another chromosome (cut and paste), copied and moved to another chromosome (copy and paste), or the orientation changed in chromosome mutations. These four types of chromosome mutations are called deletion, translocation, duplication and inversion (Figure 7.24).

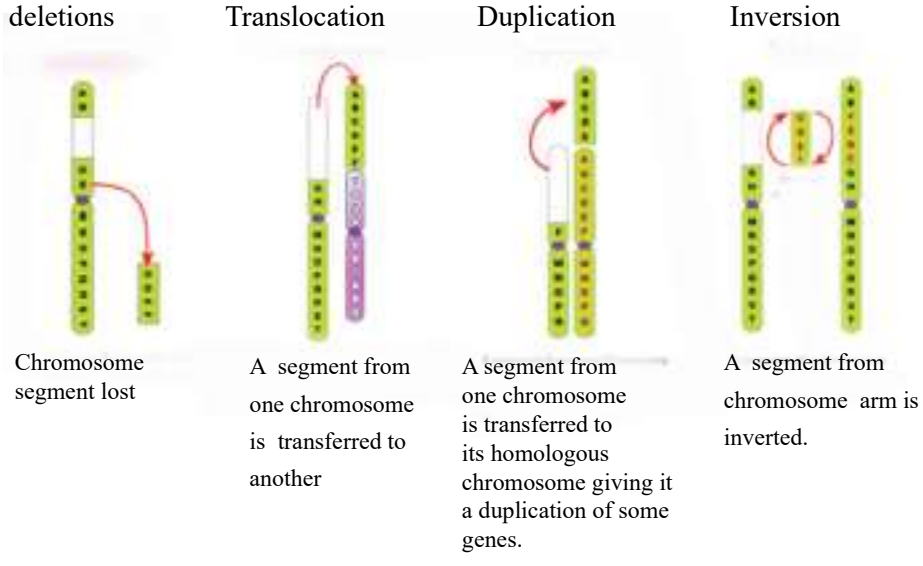


Figure 7.24: Four types of chromosomal mutations

The loss of a part of a chromosome removes several genes, and hence often these changes are lethal. In translocation, there is no loss of total amount of DNA. However, the expression of genes can be altered due to the change in the environment at the new location. The cutting of the chromosome may occur within a gene, and if this happens, that gene cannot function. In duplication, an extra piece of DNA carrying many genes is present in another location of the genome. This situation may also alter the gene expression, usually leading to deleterious impacts on the phenotype. The change of the orientation of a chunk of chromosome or inversions can also result in changes in gene expression; most of these are adverse variations.

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II. Mutations due to alterations in chromosome number:

In addition to the structural changes of the chromosomes, a cell may contain an entire chromosome or even a set of chromosomes in excess to the normal number of chromosomes. A cell may also get one chromosome less than the normal number. When there is one more or one less chromosome in a cell, that situation is referred to as aneuploidy. Here the ploidy level has not changed, but when a complete set of chromosomes are present in excess, then ploidy level is said to be increased, for example to triploid, tetraploid, hexaploid etc.

Aneuploidy is resulted in by the mistakes in meiosis. During meiosis I, the two sets of chromosomes in a diploid cell must separate and move towards the two poles of the cell. However, due to abnormal arrangement of homologous chromosomes, both chromosomes of a pair may migrate to one pole, so the other end has one less chromosomes. The resulting cells or gametes in sexual reproduction also have either one more or one less chromosomes than the haploid number of chromosomes. A similar result is obtained when chromatids of a chromosome do not separate and migrate to the opposite poles of the cell in meiosis II. The inability to separate a pair or pairs of chromosomes in meiosis is called nondysjunction (Figure 7.25). When a gamete with one less chromosomes, unites with a normal gamete, the resulting zygote is an aneuploid with 2n-1 chromosomes. This type of cells is called monosomic, since a specific chromosome has only one of it. A gamete with one more than the usual haploid set of chromosomes may fuse with a normal gamete. The zygote will carry a chromosome in triplicate, and hence 2n+1. This aneuploidy status is called trisomic for that chromosome.

Such abnormalities can take place also in mitosis. Ploidy level can also increase by abnormal separation of chromosomes. A triploid (3n) can result in fertilization of an abnormal diploid egg. If a zygote does not divide after the first mitotic division, it will carry four sets of chromosomes and be developed into a tetraploid (4n).

Animals with higher ploidy level are very rare. Plants on the other hand, can tolerate higher ploidy levels, and often perform better than their diploid individuals.

Examples of plants having higher ploidy levels:
Banana – Triploid (3n); Wheat – hexaploid (6n); Strawberries – Octaploid (8n)

Polyploids are more common in invertebrates than in vertebrates. Among vertebrates, polyploidy is observed in a few fishes and amphibians.

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Polyploids are rather normal compared to aneuploids. Although having higher number of chromosomes than usual, polyploids maintain the genetic balance, whereas genetic balance is lost in aneuploids.

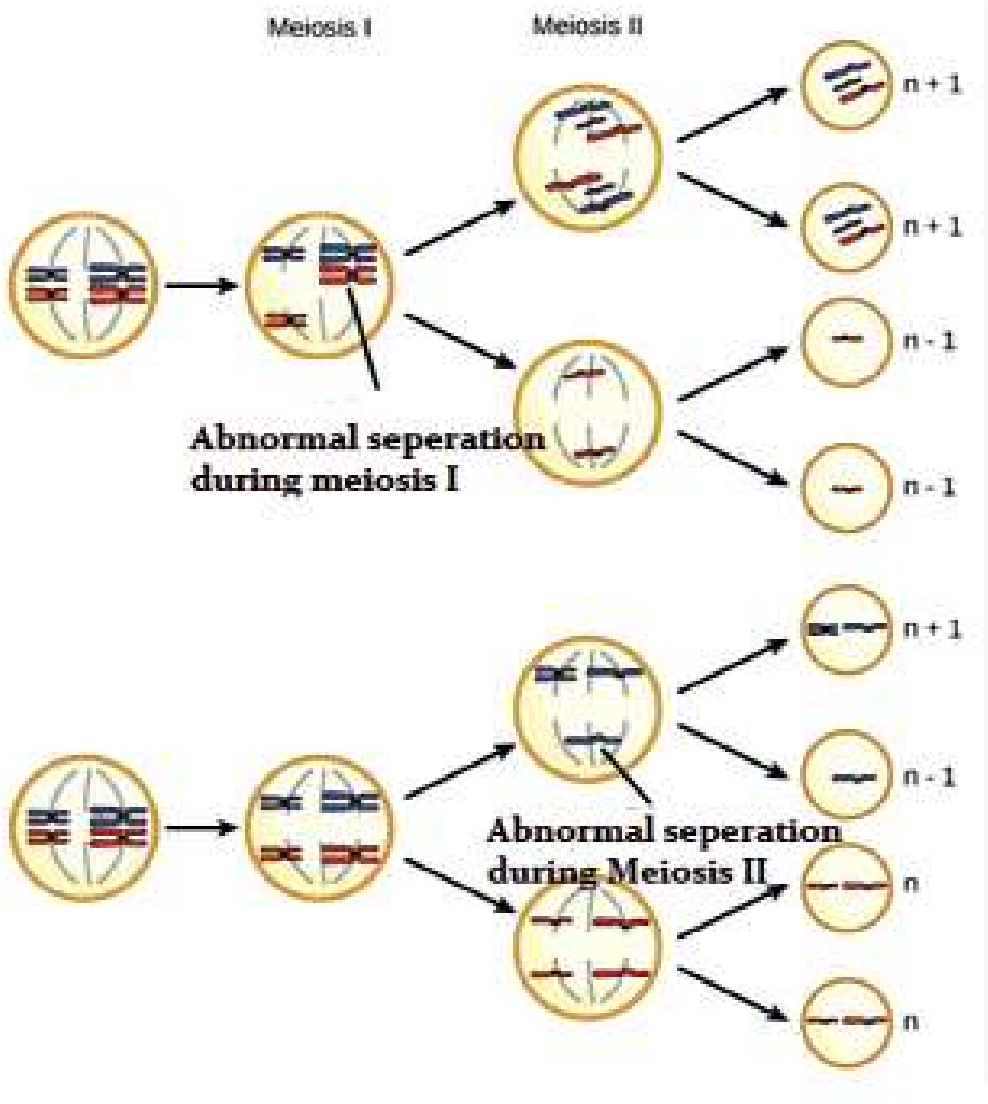


Figure 7.25: Abnormalities in meiotic division causing aneuploidy

Human Genetic Disorders

I. ***due to Gene Mutations***

Two examples of human genetic disorders by gene mutations are described below.

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Colour Blindness

Colour blindness or Colour Vision Deficiency is a genetic disorder, more common in males than in females, due to mutations on one or more genes located in X chromosome. These genes code for proteins that absorb different wavelengths of visible light. These are visual pigments called photopsins, which are categorized as red, green or blue. A person with a normal colour vision can distinguish different colours and their hues because they have all three categories of pigments in the retina. The absorption of different proportions of different wavelengths by the pigments is resolved by the brain as the colour of the object. In human genes coding red and green pigments are located in X chromosomes and gene for blue pigment is on the chromosome 7. Since males have only one X chromosome and the relevant genes are absent in Y chromosome, any defect in one or both of these genes will make the phenotype. In females at heterozygous condition a defective allele in one X chromosome is masked by the good allele in the other. Therefore colour vision deficiency is more common in males (5-8% of males) than in females (less than 1% of females). Colour blindness nearly always affects the perception of red or green colours, because these are the genes that are sex linked.

Sickle Cell Aneamia

Sickle cell aneamia is a genetic disease prevalent in human populations in Africa and other warmer regions of the world. A mutated allele of a gene coding for β -globin subunit of hemoglobin, the oxygen carrying pigment, causes an abnormality of the hemoglobin molecules. The presence of this abnormal hemoglobin in red blood cells changes the shape of the RBCs to curve as a sickle from its disk-shape. Individuals with this disorder have fewer numbers of RBCs, and hence develop aneamia. This is because the sickled RBCs breakdown prematurely. The mutation substitutes glutamic acid at a particular place in the primary structure of the β globin with valine (Figure 7.26), which results in abnormal folding of hemoglobin. The mutated allele is codominant, which means both normal β globin and mutated β globin are produced in individuals who are the heterozygous for this locus. Therefore, they have both good and bad hemoglobin and hence both normal and sickled RBCs are present. They are normally healthy and are the carriers of the mutant allele. Since the mutated alleles causes severely determintal effects in homozygous individuals, normally they should have been eliminated from the human population by natural selection. However, there is prevelence of malaria in warmer countries like in Africa, the heterozygous individuals will survive malaria attacks better than individuals with homozygous wild type alleles. This is because, the malaria parasite can not survive in sickeled RBCs. Therefore, at heterozygous individuals, parasite density remains at a lower level.

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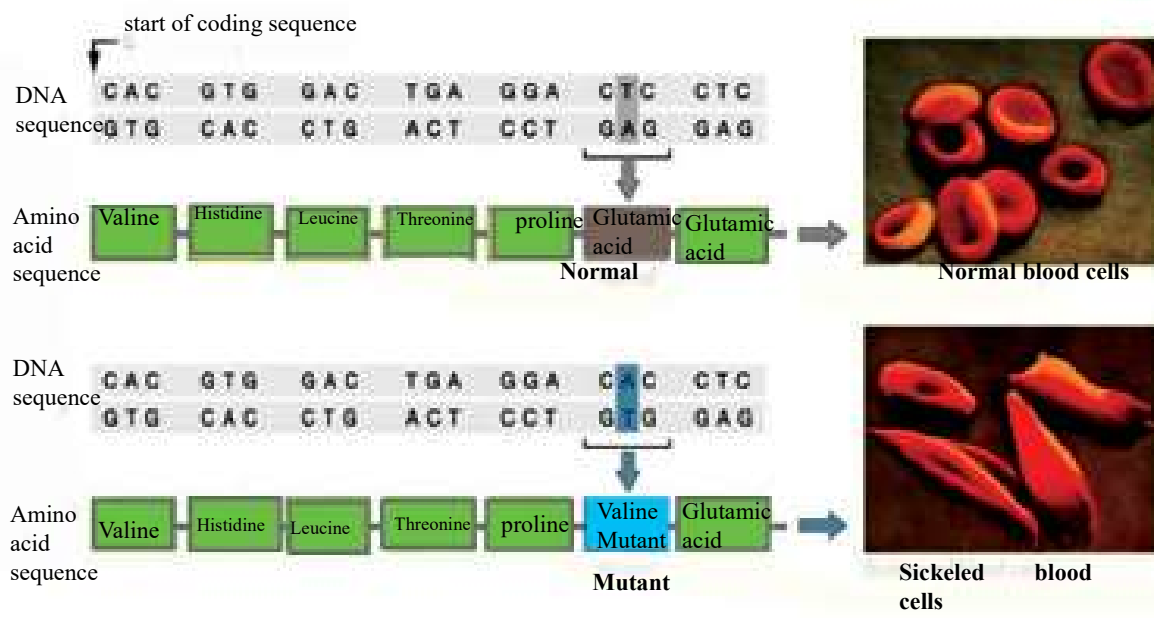


Figure7.26: The molecular basis of sickle cell aneamia

II. Due to Chromosomal Mutations

Chromosomal mutations bring about drastic changes in amount of genetic material or chromosome structure, leading to abortions of the fetus in mammals. If they survive, they show a peculiar set of abnormal characteristics in the phenotype which are called syndromes.

Three genetic disorders of humans due to aneuploidy are described below.

Down syndrome:

Down syndrome is also known as trisomy -21, because the cells of the affected individual contain an extra copy of chromosome 21. The syndrome shows characteristic facial features, short body, heart defects (which can be corrected) and developmental delays. They have a high risk of developing leukemia and Alzheimer disease. Almost all males and half of the females with Down syndrome are sexually under developed and sterile. Their life span is shorter than normal, but can live past middle age with proper medical treatment. However, they have a lower risk of high blood pressure, artherosclerosis (hardening of the arteries), strokes and many types of solid tumors. Despite their abnormalities, many live independently and are employed. The risk of having a baby with Down syndrome increases with the age of the mother. This is caused by nondisjunction in meiosis-I. While Down syndrome is due to trisomy of an autosome, there are human genetic disorders due to aneuploidy in sex chromosomes as well. The aneuploidy conditions in sex chromosomes cause Turner syndrome due to monosomy and Klinefelter syndrome due to trisomy.

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Turner syndrome:

Turner syndrome is due to monosomy in X chromosome. In very rare cases, there are females with only one X chromosome, and hence their genotype is XO. This is the only known viable monosomy in humans. These individuals are phenotypically female, but they are sterile because their sex organs do not mature. When girls affected with Turner syndrome are treated with estrogen replacement therapy, they develop secondary sex characters.

They have a short stature and some may have an extra skin on the neck (webbed neck). Other characters are puffiness or swelling (lymphedema) of the hands and feet, skeletal abnormalities, heart defects, high blood pressure and kidney problems.Most of them have normal intelligence.

Klinefelter syndrome:

This is due to rare condition having an extra X chromosome in the genotype of XXY. Since it carries a Y chromosome, these individuals are males. Although these people have male sex organs, they are sterile. There testes are abnormally small. One out of the two X chromosomes is inactivated. Yet these males may have enlarged breasts and may develop other female body characteristics. They have subnormal (less than normal) intelligence.

XYY trisomy will make males and XXX trisomy will make female in females. They do not show any syndrome and have normal male and female characteristics, respectively. They are fertile and slightly taller than the average.

Genetic Counseling

Genetic counseling is a service which is important for families having genetic disorders or families having a risk of genetic disorders. This service is intended to estimate the risk a couple may have in conceiving a child with genetic disorders and providing necessary advice to avoid such instances. Genetic counseling is a profession that requires, on one side, a good knowledge of human genetics to understand, how traits obeying the rules of simple Mendelian inheritance would behave and on the other side, an ability to provide guidance to minimize the risk of having a child with a genetic disorder. If the family already has such a child, the genetic counselor would also provide advice how to manage the situation and how to plan the birth of next child.

Some of the genetic disorders are multifactorial, which means several factors including multigene inheritance and that is also affected by environment. For example, heart attacks and diabetes may be inherited, but the risk of developing the disease is affected by external environmental factors such as life style and food habits. As such, a clear pattern of inheritance of the disease cannot be traced. The risk of the effect of a trait that follows simple Mendelian laws of inheritance on a child to be conceived can be estimated by studying the history of the family with regard to a

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particular disorder, and hence, becomes the subject of genetic counseling. If the disorder is caused by a dominant allele, it can easily be observed in the potential parents. However, if the allele is recessive, a parent or both the parents with normal phenotype may be homozygous for dominant allele or heterozygous carrier. Tracing the history of the family with regards to the disease using pedigree analysis, would allow estimation of probability of parents being carriers and accordingly would estimate the probability of the risk of having a child with the disorder. The information available through pedigree analysis, sometimes is sufficient to determine exactly the genotype of one or both parents. The genetic counselor explains the situation to the potential parents and guides them to choose the most suitable option in having a child.

Techniques are now available to determine whether the already conceived fetus carries the mutant alleles. For this, the cells of the early fetus are sampled and DNA is sequenced to find out the presence or absence of the mutant allele and, if present, whether the fetus is homozygous or heterozygous. This information is very important to take well informed decision to keep the fetus or to abort it. Legislations of some countries allow abortions of such fetuses which would otherwise be born with genetic disorders. This however is a difficult decision for parents to make. Therefore genetic counselor has a task of guiding the potential parents to make the best decision possible.

Gene Technology

Tools, Techniques and Methods in Gene Technology

This section examines the process of gene technology starting from DNA isolation, through identification of desired DNA sequences to gene technology or recombinant DNA technology. The isolated DNA needs to be cut different pieces of DNA must be joined and at times DNA must be copied *in vitro*. These involve several enzymes acting on DNA. Distinguishing a unique DNA sequence from the rest of the DNA, requires separation of DNA based on the size of the fragments and identification of it. In making genetically modified organisms, this DNA needs to be transferred to a recipient organism using a suitable method. Copying of DNA is possible *in vivo* using cloning and *in vitro* using Polymerase Chain Reaction (PCR). Sequencing of DNA has become a very important technique in many studies in DNA.

Isolation of DNA

Gene technology begins with isolation of the target DNA sequence from the total genome of the donor cells. Purified DNA is required for many applications such as studying DNA structure and chemistry, examining DNA-protein interactions, carrying out DNA hybridizations, sequencing, PCR, performing various genetic studies or gene cloning.

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Since DNA molecules are very long, the entire length of a DNA molecule cannot be isolated, except for shorter DNA molecules such as plasmid DNA and viral DNA. However, breaking of DNA or shearing has to be minimized during the extraction process.

The basic principles and major steps of DNA isolation can be identified as follows.

- **Homogenization or disruption of cells:** DNA is located in the nucleus of the eukaryotic cells and it is concentrated in nucleoid in prokaryotic cells. The first step in DNA isolation is releasing the DNA from the cells by breaking cells or lysis of the cells. Cells can be lysed by mechanical methods such as grinding and homogenization or enzymatically by lysozyme to break bacterial cell wall.
- **Inhibition of DNAse:** When the cells are broken, the DNA may get in touch with DNA degrading enzymes such as Deoxyribonuclease (DNAse). Therefore, DNA must be protected from such enzymes causing shearing. Chelating agents are added to remove metal ions required for nuclease activity.
- **Dissociation of nucleoprotein complexes:** DNA needs to be freed from the proteins that bind to DNA. DNA-protein interactions are disrupted with SDS, phenol, or proteolytic enzymes.
- **Removal of contaminating materials:** All other molecules in a cell are contaminants of DNA. Removal of this contaminants is required for some applications.
- **Precipitation of DNA:** Here the DNA dissolved in aqueous phase is precipitated with cold (0 ^{o}C) ethanol. The precipitate is usually re-dissolved in a buffer. RNA is removed by limited treatment with DNAse free RNAase (ribonuclease).

Enzymes Reacting with DNA

Enzymes are required in cutting, joining and copying of DNA *in vitro*.

1. **Restriction endonucleases:** There are several different types of nucleases in cells having different functions. In gene technology, cutting DNA at precise locations is important. The enzymes that recognize specific sequences of DNA and cuts at or near these sites are called restriction endonucleases. The position where the DNA sequence is cut is called as restriction site or cleavage site (Figure 7.28).

   eg. *EcoRI*- Source: *E. coli*

2. **DNA Ligase:** Cut DNA fragments from different sources are joined together by forming a phosphodiester bond by DNA ligase to obtain recombinant DNA molecules (Figure 7.27). T4 DNA ligase is the most commonly used DNA joining enzyme in gene technology. The source of this enzyme is T4 bacteriophage.

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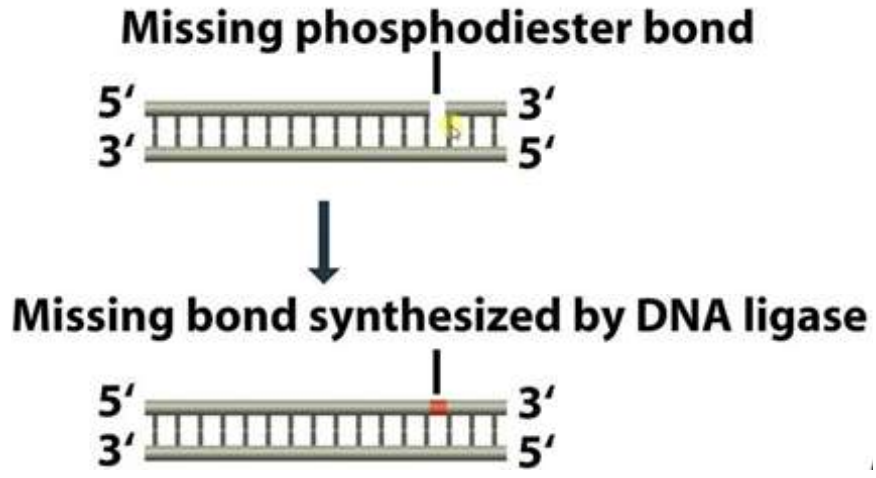


Figure7.27: Formation of a new phosphodiester to fill the gap between adjacent nucleotides

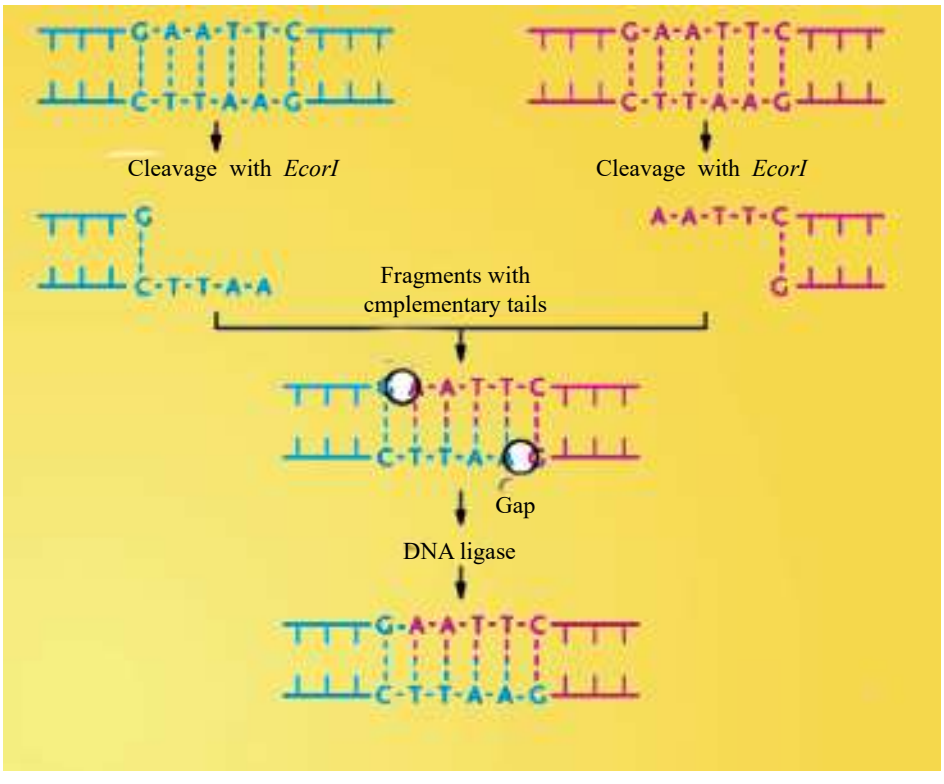


Figure 7.28. Cutting of DNA of different origins with *Eco R I* restriction enzyme and joining fragments of the different DNA by DNA ligase to form a recombinant DNA molecule.

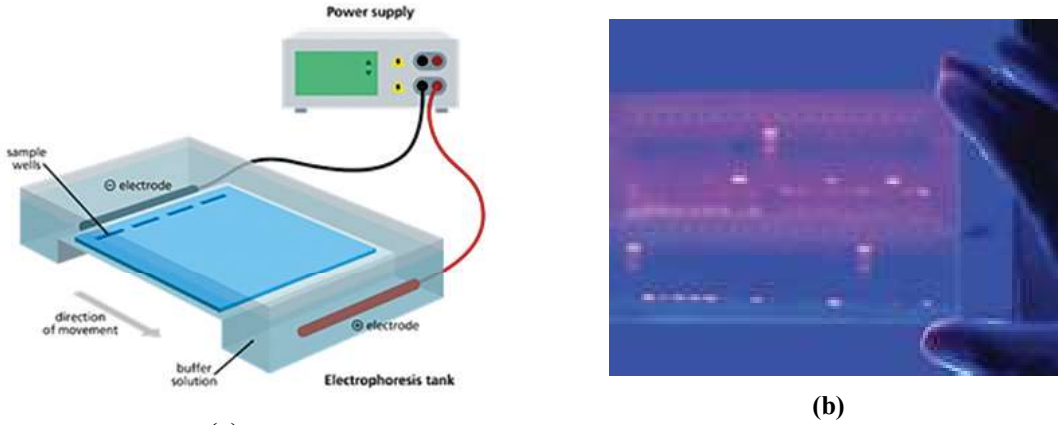
3. **DNA Polymerases**: These are enzymes that add complementary deoxyribonucleotides to the template strand in a growing DNA strand, so that they copy DNA. These are therefore very important in gene technology especially in PCR and DNA sequencing. The most widely used DNA polymerase is Taq DNA polymerase. This is a heat stable enzyme isolated originally from the thermophilic bacterium *Thermus aquaticus.*

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In addition to the enzymes reacting with DNA, enzymes which can make DNA on an RNA template is also very useful in gene technology. These enzymes are called reverse transcriptase, because their action is the reverse of the transcription. This is used to make cDNA (copy DNA or complementary DNA) on an mRNA template.

The DNA shearing or cutting with restriction enzymes makes a mixture of DNA fragments of different sizes. In DNA fingerprinting, using PCR, DNA strands having different sizes are obtained. Therefore in many applications with DNA, separation of DNA molecules becomes important. Separation of fragments having different size on a gel matrix is the most practical way of doing this.

Agarose gel electrophoresis: Electrophoresis is a technique that separates large charged molecules, (such as DNA, RNA and proteins) according to their mobility in an electric field. The speed of a molecule moving in an electric field depends on its net charge and the size. In gel electrophoresis, the molecules are moving through small pores of a matrix of gel. This restricts the movement of the molecules and helps in separation according to the size; larger molecules move slowly compared to smaller molecules. In the case of nucleic acids, the net charge depends on the length of the molecule, and therefore the separation is based on the size. For the separation of DNA, Agarose Gel Electrophoresis is the most frequently used technique. Agarose is purified agar, obtained from a type of seaweed. This forms a polysaccharide matrix. In the Agarose gel electrophoresis equipment, the gel is placed in buffer and a cathode and an anode are placed on either end of the gel (Figure 7.29(a)). When a current is applied using a power supply, the negatively charged DNA molecules migrate towards the anode through the gel. When preparing the gel, wells are formed, and the DNA is loaded into these wells. The separated DNA can be stained by ethidium bromide and be visualized by exposing to UV light (Figure 7.29(b)).



(a) (b)

Figure 7.29: (a) Agarose gel electrophoresis equipment (b) Visualizing separated bands of DNA on a gel using UV light

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Although stains like ethidium bromide show the presence of double stranded DNA band on an agarose gel, those stains cannot differentiate a band with a specific nucleotide sequence from the others. In order to identify such a band among many other bands, a DNA probe is used.

DNA Probes and hybridization: A DNA probe is a fragment of single stranded labeled DNA used to detect the presence of complementary nucleic acid sequences by hybridization. Labeling is a modification of the DNA strand in such a way that it gives a signal which enables the detection of that DNA strand. The labeling can be done by, for example, incorporation of a radioactive isotope, or addition of a fluorescent molecule to the structure of the probe. This single stranded DNA stretch can hybridize to complementary single strand of DNA or RNA. Therefore before hybridizing with the probe, the double stranded DNA needs to be denatured to make room for the probe. The denatured bands on the gel need to be transferred to a nitrocellulose or nylon filter membrane by a process called Southern Blotting. The bands are then fixed to the membrane. Then the labeled probe is added to the membrane and allowed to renature. The probes will bind strongly only to the complementary sequences, which are fixed to the membrane. When the membrane is washed, the probe is removed except the probes bound to the bands with target nucleotide sequence. If the probe is radioactively labelled, the band with the target sequence can be identified by autoradiography of the membrane. If the probe is labeled with a fluorescent dye, the band can be identified using UV light.

Recombinant DNA Technology

All organisms on Earth have evolved from a common ancestor and their genetic information is stored in DNA, except in some viruses. At the chemical level, DNA is the same in all organisms. Further, all organisms share the same genetic code, so the same peptide is encoded by a gene whether it is expressed in a bacterium, a plant or an animal. This forms the basis of recombinant DNA technology, where DNA from two or more different species are joined together and inserted into a host to obtain a new genetic combination which is of value to science, medicine, agriculture, industry and environmental applications.

Recombinant DNA molecules:
DNA molecules made by laboratory methods of genetic recombination which brings together DNA from different sources, creating sequences that are not found naturally.

All the techniques mentioned below are needed to make a recombinant DNA molecule (rDNA).

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They are;

- Isolation of DNA from different sources.
- Restriction digestion of isolated DNA with restriction enzymmes.
- Seperation of DNA fragments by gel electrophoresis.
- Identification of the correct fragments with desired nucleotide sequences using probes.
- joining the DNA fragments from multiple sources using DNA ligase.

Inserting DNA molecules to host cells is a difficult step. Cells show resistance to DNA uptake. This is important for the survival of organisms because invading DNA can cause genetic alterations which are usually harmful.

Therefore, a large number of copies of the recombinant DNA molecules are necessary to make sure at least a few host cells will receive a copy. If the desired DNA fragment is a shortone, *in vitro* multiplication can be done by a technique called DNA cloning.

DNA Clonning

In DNA cloning, the DNA replication machinery of a host cell is used to copy the desired DNA. However, inserting a fragment of DNA into a host cell will not be copied, unless an origin of replication (Ori) is present in that. Therefore, in order to replicate the recombinant DNA molecule or DNA of interest, it has to be combined with DNA with Ori and that can be replicated independent of the chromosomal DNA (chromosomal DNA replicates only once in a cell division). In a bacterial host, there are several copies of plasmids and also when infected with a bacteriophage, a large number of copies of viral DNA per bacterial cell are present. As such the DNA molecule of interest can be integrated into these self-replication units, which are called vectors.

Vectors: The vectors are vehicles to carry the DNA of interest into a host for multiplication or cloning.The vectors used in cloning of DNA are called cloning vectors.When the vector is carrying the foreign DNA, it is called a recombinant vector.

Making a recombinant vector also follows the same procedure for making the recombinant DNA molecule. Here the gene of interest has to be cut with a restriction enzyme and the vector (plasmid or the viral DNA) should also be cut with the same enzyme. These two should be mixed and allowed to integrate and ligated using DNA ligase (Figure 7.30). The site in the vector, where the DNA to be cloned is inserted is a called cloning site. In order to be able to use several restriction enzymes to cut DNA (both the vector and the DNA to be cloned), the cloning site contains a sequence which carry sites for many restriction enzymes. Therefore, this is called multiple cloning site. A host cell, usually a bacterial host which can copy the vector, is then transformed by the recombinant vector. The host then will copy the plasmid carrying the DNA of interest. A number of recombinant plasmid is present in each descendent cell of the colony of bacterial host.

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Types of vectors and their differences

Any self-replicating unit in a particular host cell can be used as a vector. In bacteria, plasmids and bacteriophages are used as vectors. Plasmids are present in yeast cells also. Therefore they can also be used as vectors in yeats. The yeast cloning vectors are called yeast artificial chromosomes or YACs. they are plasmids, but are called chromosomes, because they carry sequences of centromeres. They work as chromosomes when they are lineraized. In addition, they also have autonomously replicating sequences (ARS) to enable them to replicate independent of the cell division. All of these vectors carry genes which are not necessary for a vector. These are removed and this space is used to insert the DNA of intrest. The yeast vectors should contain sequence of centromere and autonomously replicating sequences (ARS).

As described above, main purpose of cloning vector is to copy DNA in an *in vivo* system. For this, number of copies in a single host has to be high. So this condition is fullfilled by bacterial plasmids, bacteriophages and YACs. The transformation of cells is a very inefficient process. However, using bacteriophages as vectors overcome this problem, because the vector can be inserted to the host cell by the mechanism of infection of the bacteriophage. An advantage of YACs is that they are large, therefore, large amount of DNA can be copied using them. It has also another advantage, because they operate in the eukaryotic system.

Transformation:
Direct uptake of exogenous DNA from the surroundings of a host through its cell membrane and incorporation, resulting in a genetic alteration

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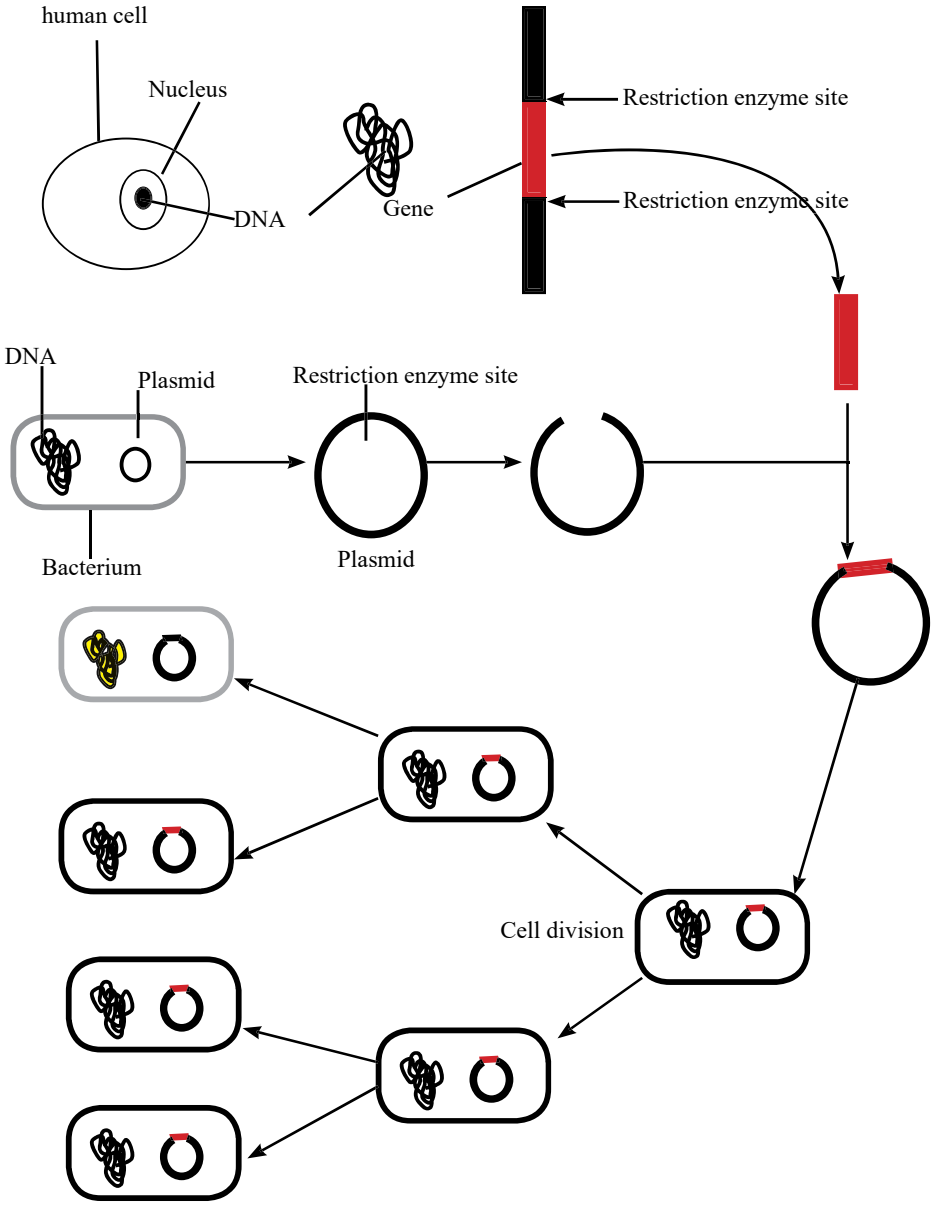


Figure 7.30: Cloning of a gene of interest using a plasmid vector and bacterial host

The copies of the DNA of interest or recombined DNA is retrieved by harvesting the hostcells, releasing the vectors by lysis, isolation of the vectors and cutting the DNA with the same restriction enzymes that was used originally to isolate the fragment of DNA. The recombinant DNA is then isolated after separation and detection on an agarose gel after electrophoresis.

Use of marker genes: The efficiency of the transformation of the host cells with the recombinant plasmid vector is very low. This means, that for one transformed host cell, there are millions or billions of cells that are not transformed. Both transformed and untransformed cells will make

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colonies in a suitable medium, but these cannot be distinguished. Therefore, some sort of marker gene should be engineered into the cloning vector, so that the few colonies originating from the transformed cells can be screened among many untransformed cells. Most common markers are antibiotic resistance genes. The host cell is sensitive to the particular antibiotic, and hence will not grow on a medium containing the antibiotic. Since the vector is carrying the gene for resistance, the transformed cells can grow in the medium with antibiotic. Such markers are called selectable markers, since they allow the growth of only the transformed cells.

There is another problem to be solved. The transformation does not necessarily mean that the insert is there. Not all vectors are recombined with the gene of interest. Therefore, another marker is required to distinguish the colonies with the vector containing the insert from those with vector only.The essential features that should be present in a cloning vector are shown in Figure 7.31.

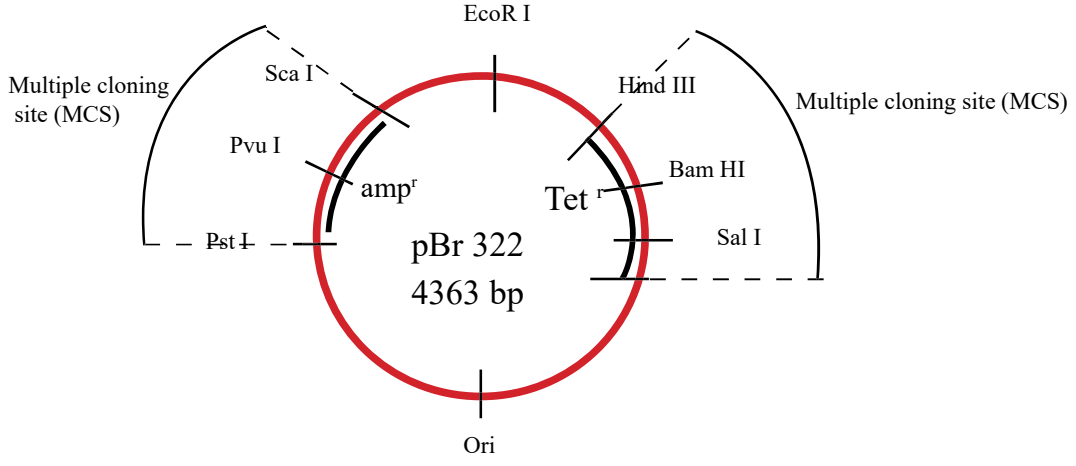


Figure 7.31: An example of a cloning vector (pBR322) showing essential features (ori, multiple cloning site, markers)

DNA Libraries

When a genome is cut into random pieces by shearing using mechanical force or by restriction enzymes, it creates an extremely large number of different sequences, depending on the size of the genome. All these pieces can be integrated into cloning vectors and the recombinant vectors can be used to transform bacterial hosts. These hosts can then be cultivated in a suitable medium for selection of transformed cells and screening for transformed cells with vectors carrying an insert. Since there was no selection for a particular segment of DNA in this case, each transformed cell with an insert may carry a different piece of DNA of the genome selected earlier. When all colonies are isolated and cultivated separately, the collection of these colonies is referred to as a genomic DNA library (Figure 7.32). DNA libraries are, a collection of microbial cultures each propagating a different fragments of a total genomic DNA which are cloned in a population of

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identical vectors.Inserts of each colony can be sequenced separately in order to have a complete sequence of the genome. This is how the elucidation of sequence human genome under the Human Genome Project started.

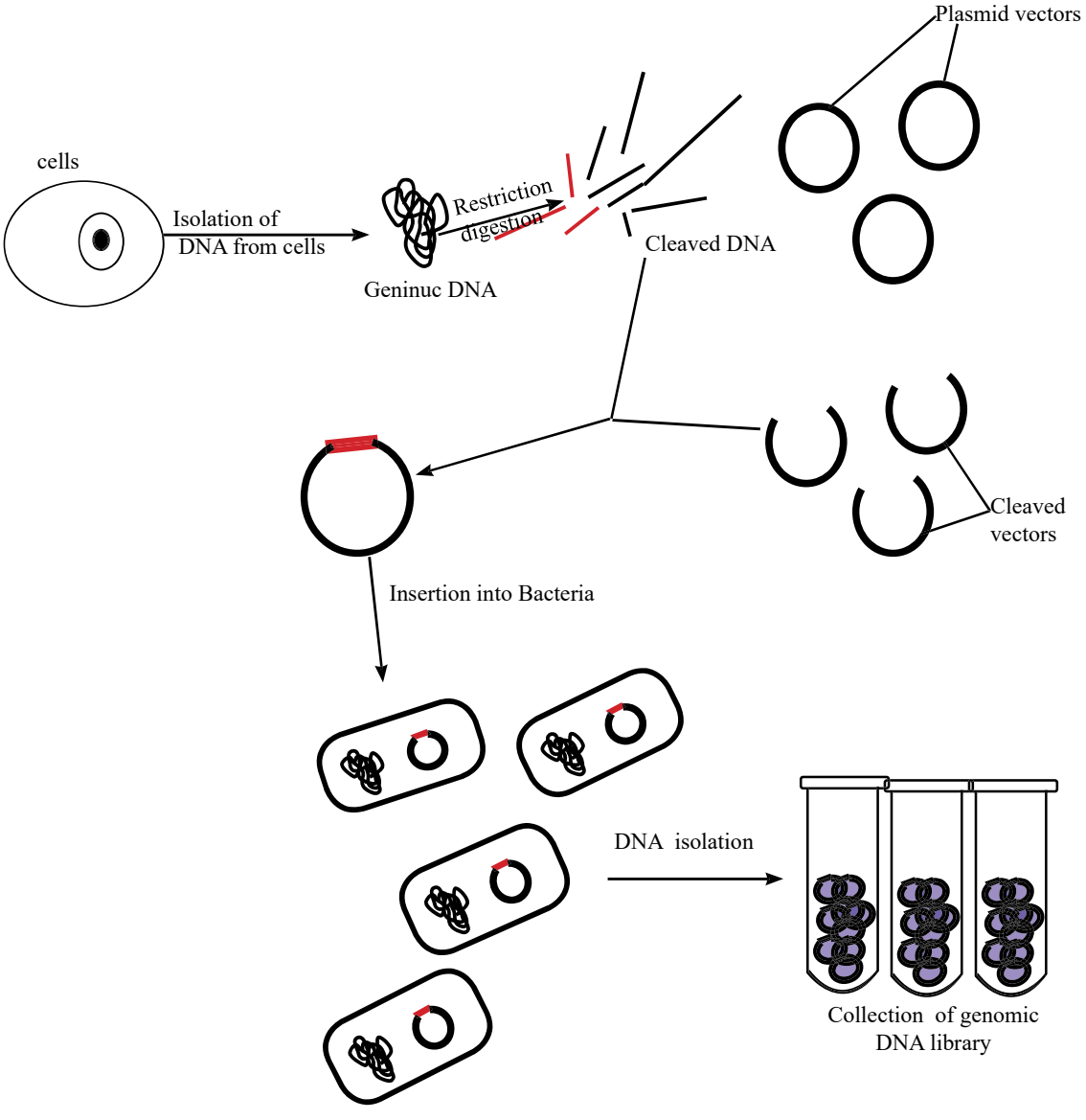


Figure 7.32: Steps in construction of a genomic DNA library

There is another type of DNA libraries. These are called cDNA libraries. These libraries contain complementary DNA obtained by reverse transcription of the mRNAs isolated from cells/ tissues. Collection of mRNAs of a cell is called transcriptome. mRNAs are isolated and reverse transcribed into a DNA strand complementary to the mRNAs. The enzyme used here is reverse transcriptase. The second DNA strand is replicated on the first DNA template using DNA polymerase to obtain double stranded cDNA. The DNA fragments are cloned and similar procedure is followed as for genomic DNA library in order to construct a cDNA library.

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DNA libraries are basically used as sources of DNA fragments for sequencing. cDNA libraries also reflect the pattern of expression of genes.

DNA Delivery Systems

A cell contacting foreign DNA is called a transformed cell. Getting the foreign DNA in a cell can be done using several methods.

- Transformation: In this method a large number of copies of DNA of interest is mixed (e.g. a recombined vector) with host cells. This is based on the ability of a cell to take up DNA from its surroundings through the cell membrane. The efficiency is very low in getting the DNA into the cells. Various treatments can increase the competency (ability to take up DNA from outside) of host cells.
- Transduction: This method is based on the ability of bacteriophages to infect host cells. Viruses infecting plants and animals can also be used as vectors to deliver foreign DNA into plant and animal hosts. The gene of interest is integrated into the modified viral genome and packaged into the protein capsid. This viral particle can pass the recombined DNA as in its normal infection process. The capsid protects the DNA and this method is more efficient than transformation.
- Gene Gun: In this method, small particles of a heavy metal such as gold are coated with a large number of copies of the DNA of interest and the particles are shot at high velocity into the cells to be transformed. The equipment used is the gene gun (Figure 7.33).



Figure 7.33: Gene Gun

Agrobacterium **mediated gene transfer:** *Agrobacterium* is a soil bacterium which can infect plants. Their mode of infection is very special. The infection causes a tumour on the plant, and the bacterium is living inside it. The disease is called crown gall disease. The cells in the tumour or gall are actually genetically transformed by a segment of a plasmid of *Agrobacterium*. This plasmid is calleded Ti (tumour inducing) plasmid (Figure 7.34). A part of this plasmid can actually be transferred to plant genome, and hence called transfer DNA or T-DNA. T-DNA contains genes which induce formation of a tumour and also characters associated with pathogenicity. What is needed for transfer of the DNA is the right and left boarder sequences of the T-DNA. Therefore

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scientists have removed most of the bacterial genes, including virulent genes from T-DNA and have made room for inserting genes of interest into this space between the two border sequences. *Agrobacterium* can deliver this modified T-DNA with inserted gene to plant cells through its infection ability. However plant cells will not become sick because the virulent genes have been removed from T-DNA. This is called disarming the T-DNA.

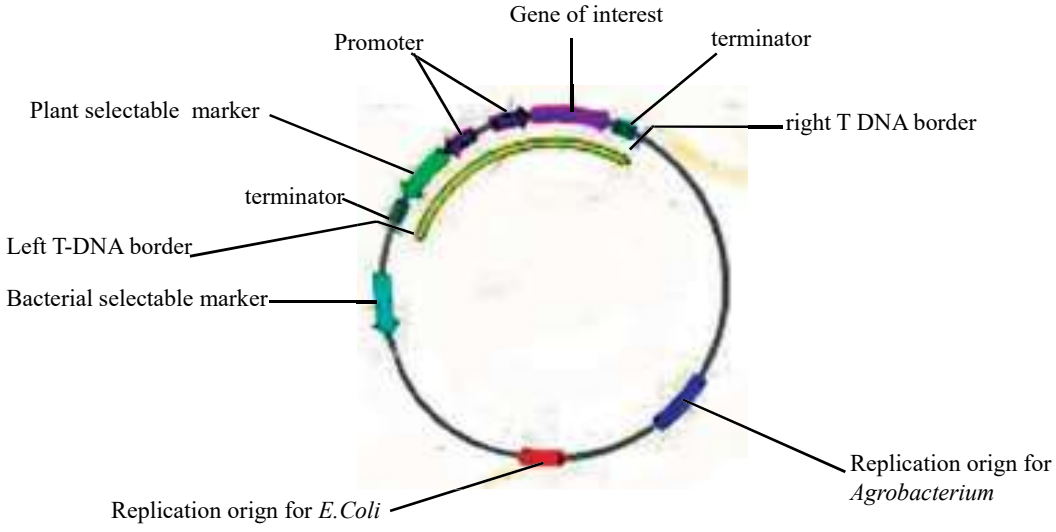


Figure 7.34: Ti Plasmid vector

DNA Analysis

When the morphological characters are used to classify, the number of characters available is limited, so that usually the smallest group identified is a species. When more characters are available, more divisions such as subspecies, strains, varieties etc. become possible. Biochemical properties (i.e. enzyme activities) are useful characters in classification to separate organisms into smaller groups. Since characters are controlled by the combination of the genetics of the organism and its environment, above mentioned characters may vary depending on the environment. Hence if one needs to look how two groups of organisms are similar or different genetically, he has to investigate at the DNA level.

Various techniques for DNA analysis have been developed to facilitate the identification of genetic similarities and differences among organisms and some of these can be used even to identify an individual. These techniques are used in conjunction with techniques such as DNA isolation, gel electrophoresis and use of probes that were discussed above.

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Restriction Maps

As shown earlier, restriction enzymes cut dsDNA into fragments at specific sequences. Depending on the number of restriction sites and where they are located, a number of fragments of varying sizes will be generated. Different restriction enzymes cut at different sites and generate different numbers of fragments having different sizes. A restriction map is a diagram showing the position of each restriction site with respect to each other and the distance between these sites (Figure 7.35).

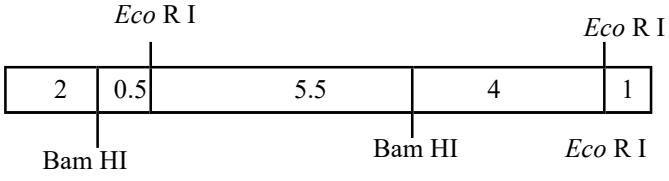


Figure7.35:Restriction map of a small segment of DNA

Restriction maps are very important in construction of cloning vectors: Cloning vectors are cut by restriction enzymes at the cloning site to insert the fragments of DNA from other sources to the cloning site. Restriction map of a commonly used plasmid vector is shown in Figure 7.36.

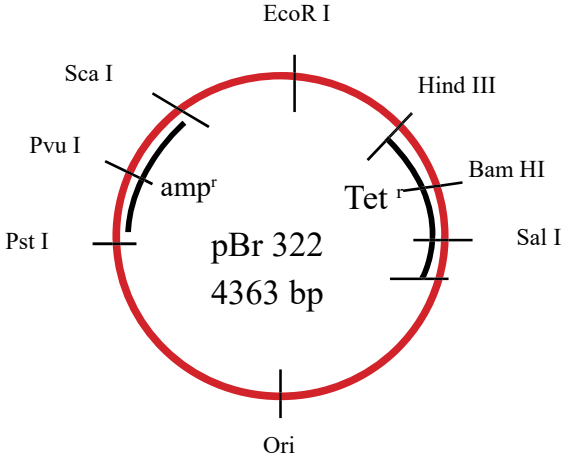


Figure7.36: Restriction map of a pBR322 plasmid vector

DNA Sequencing

A DNA molecule is made of two complementary and antiparallel strands each comprising four bases; adenine, guanine, cytosine and thymine, arranged linearly in a sequence. DNA sequencing is a process to determine the precise order of these bases in a DNA molecule.

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The techniques have been improved greatly since the introduction of DNA sequencing in 1977. The technology of DNA sequencing that was available by the time of obtaining the sequence of entire human genome in 2003, under the human genome project was called first generation sequencing technology. Those methods were time consuming and it was possible to determine the sequence only of short DNA fragments. The technology developed since then to next generation sequencing or second generation sequencing and to the most modern third generation sequencing technology. The most modern technology can sequence strands of lengths of millions of nucleotides, and therefore, the time required for sequencing has reduced greatly. Although the Human Genome Project took 15 years to complete the sequencing of human genome, one can today obtain his/ her genome sequenced in several hours at a cost 1000 US$ (2018).

With the development of the technology of DNA sequencing, its applications also have been widened.

Applications of DNA sequencing

Molecular Biology: Information on base sequence of DNA is important in understanding the functions of DNA. Genes coding for a polypeptide can be located by studying the DNA sequence. Certain domains in the DNA sequence of a gene specify the function of the protein. For example, whether the protein will be a transmembrane protein or whether it is a DNA binding protein. DNA sequencing has revealed the presence of multiple copies of genes in human genome. Although amino acid sequence of a peptide can be determined using an amino acid sequencer, it has now become easier to work out the amino acid sequence via DNA sequence.

Evolutionary biology: DNA is passed from generation to generation. Over time, changes occur and accumulated in the DNA. Therefore the similarities and differences in the sequences of DNA of individuals within a species and among different species reveal their evolutionary relationships. Sequencing of DNA samples obtained from ancient humans remains preserved, for example, as mummies or buried in ice or as fossilized remains has given insight into when the *Homo sapience* evolved, and how they migrated to conquer the world.

Medicine: Certain families inherit some genetic disorders. Sequencing of DNA reveals if a healthy person is a carrier or not. How a particular disease causing allele is distributed among family members is very important in assessing the risk and to plan a management. Similarly diagnosis of cancer is also possible through DNA sequencing. The response to a drug given for cancer can be followed by sequencing DNA carried in the blood of a patient. If a drug is responding, then the DNA sequences related to cancer must be reduced in blood. DNA isolated from a placenta of a fetus is useful in early diagnosis of presence of genetic disorders.

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Forensics: Except for identical twins, it is extremely rare that two individuals share exactly the same sequence of DNA. That means DNA sequencing can identify an individual having the same DNA sequences as DNA material from (blood, hair, sperm, saliva etc) found in a crime scene. Similarly paternity testing is another use of DNA sequencing.

Metagenomics: Microbiome is the totality of microorganisms in a particular habitat including human body and different environments. Conventional methods for studying the microorganisms in a microbiome are based on culturing in pure cultures. However, a large number of microorganisms are unculturable and hence, are largely neglected. Metagenomics is a science in which DNA present in an environment is extracted as community DNA and studying this sample as a whole. Sequencing some specific sequences in this community DNA and analysis using suitable software, will reveal the number of different species and their identity. Some of these are presently known and many others may be new species. Therefore, metagenomics studies are important in ecology, epidemiology and other fields.

DNA Fingerprinting

The unique set of genetic markers of an individual makes its DNA fingerprint or genetic profile. The presence/ absence of markers are nowadays determined by mostly PCR using specific primers for the marker (see below). These markers are called Small Tandem Repeats (STR markers) or Microsatellite DNA. Eukaryotic DNA contains some non-coding sequences where two to six base pairs are repeated tandemly (one after the other) 100 to 1000 times, so that the lengths of these repeats vary. Since they are non-coding, variations do not have an impact on phenotype. These are variable in individuals, so that can be used as makers. The advantages of using STR markers are

- they occur frequently in genome
- easily amplified by PCR
- highly variable polymorphisms and
- a large number of characterized STRs are available.

The method used earlier was probing specific sequences using labeled markers as describes earlier (see DNA Probes and hybridization).

In DNA profiling, a set of markers (probes or PCR primers) is used. A DNA fingerprint cannot be obtained by using one marker, since there are many individuals with the same banding pattern. When more and more markers are used in combination, the probability of finding the same pattern reduced. It has been calculated that, if 13 markers are used the probability becomes between 10 billion to several trillions. Since the world population is in the region of seven billion, it is highly unlikely that two individuals to have the same genetic profile/ fingerprint.

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Applications of DNA Fingerprinting

Criminal identification and victim identification (Figure 7.37) – The fingerprints of the suspects are matched with fingerprints obtained from biological material obtained from a crime scene. The expert's opinion on the identity of the criminal is accepted by the Courts of law.

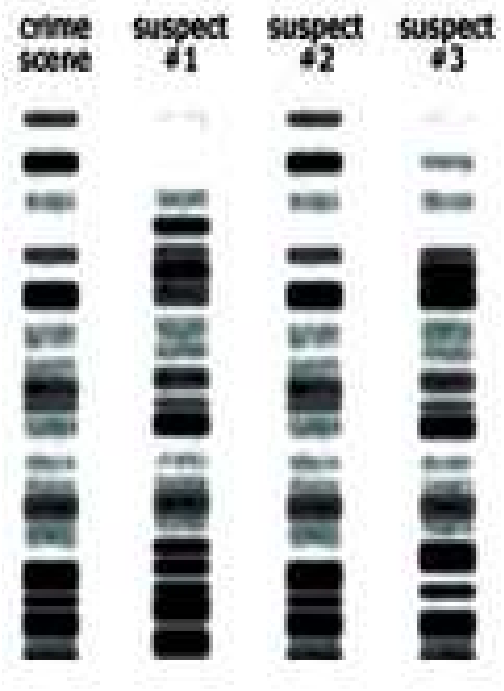


Figure7.37: Comparison of DNA fingerprints of a sample from the crime scene and three suspects.

The profile of suspect #2 matches with that of the crime scene sample.

Paternity testing (Figure 7.38). The DNA fingerprint of a child is never identical to that of either the father or the mother. However, the child shares some markers from the father and others from the mother. Therefore, when the paternity of a child is in question, DNA profiling can be accurately used to confirm or rule out a person as the father of that particular child (Figure 7.38).

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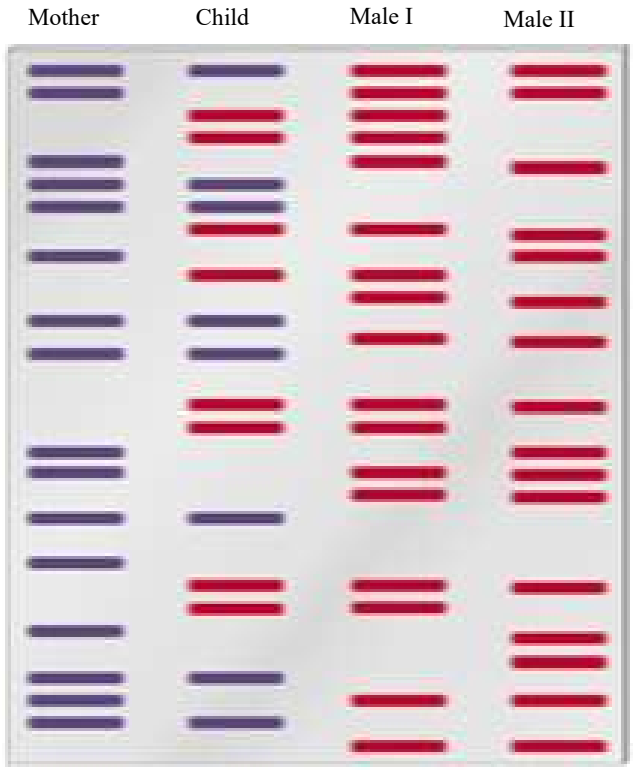


Figure7.38: Use of DNA fingerprinting for paternity testing

Identifying infectious agents: When probes or primers are known to obtain the fingerprint of a pathogenic infectious organism, DNA fingerprinting can detect the presence or absence of this organism in the patient or in food or water.

Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is used to copy sequences of DNA *in vitro,* mimicking the DNA replication. As in replication, a DNA polymerase enzyme is needed to catalyze the reaction of elongation of the new DNA strand. The raw materials needed are the deoxyribonucleotide triphosphate (dNTPs), which are the four deoxyribonucleotides (dATP, dGTP, dTTP and dCTP). A single stranded template DNA is required. Since the DNA polymerase cannot initiate DNA replication, a primer is also needed. In PCR, a primer is a specific sequence of DNA with a small number of nucleotides (oligonucleotide) which is complementary to a sequence at 3' end of the target DNA to be copied. To copy both the strands two primers, each binding to either of the two strands at 3' end, are used. In the cell, the primer is an RNA sequence. In addition to these, Mg^{2+} is also required. These become the ingredients in PCR mix.

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The DNA fragment in which the sequence to be copied is present in dsDNA, and hence it needs to be denatured. Denaturation is done by heating the PCR mix to 95 °C. At this temperature most of the enzymes will denature, and therefore it may be necessary to add DNA polymerase after the denaturation. However, the enzymes of the thermophilic organisms are resistant to high temperature. Therefore, the common heat resistant DNA polymerase used in PCR is Taq DNA polymerase which is obtained from thermophilic bacterium *Thermus aquaticus.* The primer will bind to the complementary sequence of the denatured template DNA. This happens at a lower temperature and this step is called annealing. The annealing temperature will depend on the sequence and the length of the primer. Once the annealing of the primer is achieved, then the primer extension (DNA synthesis) will take place at a different temperature. This is the optimum temperature of the DNA polymerase used. When sufficient time is given, a complementary copy of the template DNA is completed. At the end of this first thermal cycle (denaturation, annealing and extension temperatures), one copy of each strand is produced. However, these are longer than the intended copy of the target DNA sequence (Figure 7.39). After a couple of PCR cycle, an exact copy of the target DNA is synthesized. After this, copies of the target DNA will be produced in an exponential manner (i.e. 2, 4, 8, 16, etc.) after each cycle. A typical PCR will have 35 to 40 cycles. At the end, millions of copies of the desired DNA sequence will be produced from a single template DNA molecule. The PCR product formation in the first three cycles is shown in Figure 7.39.

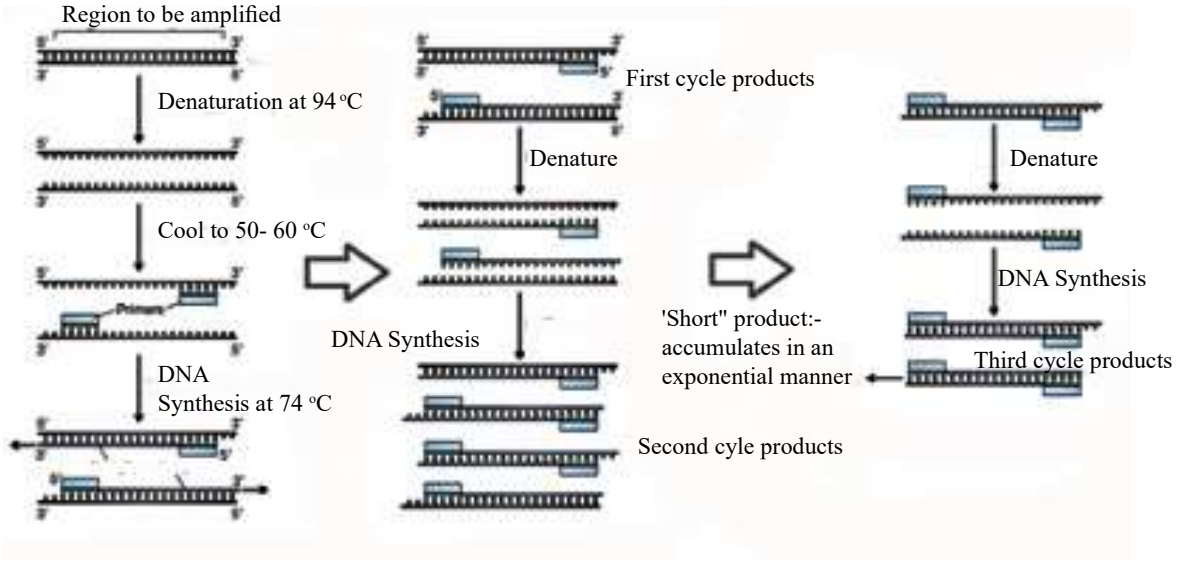


Figure 7.39: PCR products formed at the end of first to third cycles

The repeating cycles are automated and are done in a PCR machine (thermal cycler) (Figure 7.40). PCR mixture is prepared in PCR tubes and these are inserted in to the wells in PCR machine.

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Figure 7.40:. A PCR machine showing the wells where the PCR tubes are inserted

PCR is a rapid method of obtaining a large number of copies of DNA with high accuracy.

PCR is an essential technique in cloning procedure which allows generation of large amounts of pure DNA from tiny amount of template strand and further study of a particular gene.

Applications of PCR

- Analyzing clinical specimens for the presence of infectious agents (e.g. HIV, hepatitis, malaria)
- Analysis of mutations causing genetic diseases. (e.g. cystic fibrosis, sickle cell aneamia, phenylketonuria)
- Used in forensics laboratories. Since PCR can make a large number of copies from a small number of template DNA, it is especially useful because only a tiny amount of original DNA is required (e.g. droplet of blood or a single hair).
- PCR is an essential technique in cloning procedure which allows generation of large amounts of pure DNA from tiny amount of template strand and further study of a particular gene.
- Sequencing of DNA depends on PCR.
- PCR has been used to identify, and to explore relationships among species in the field of evolutionary biology.
- In anthropology, it is also used to understand the ancient human migration patterns. In archaeology, it has been used to spot the ancient human race.
- PCR commonly used by paleontologists to amplify DNA from extinct species or cryopreserved fossils of millions of years old, and can thus be further studied to elucidate on their evolutionary relationships.

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Applications of Genetically Modified Organisms (GMOs)

The host with the extra trait introduced by genetic engineering techniques is called a genetically modified organism (GMO). Some related terms are Genetically Engineered Microorganisms (GEMs), transgenic organisms and living modified organisms (LMOs). The food and feed obtained from GMOs are called Genetically Modified Food (GMF). Since majority of the crop plants, farm animals and pets animals used today are genetically modified by domestication, it is important to note that what is referred to as GMOs in today's context are essentially the organisms derived as a result of rDNA technology.

(Note: It is important to realize that animal cloning is not a gene technology. It does not include any of the above mentioned steps.)

The steps in the process making a genetically modified plant or an animal are as follows.

1. Identification of a suitable gene
2. Isolation and purification of the gene
3. Amplification of the gene by cloning
4. *In vitro* modifications of the gene of interest
5. Amplification of modified gene by cloning
6. Transformation of the recipient cells (microbial cells, cells or protoplasts of plants or fertilized eggs of animals)
7. Screening for expression of the inserted gene
8. Monitoring for stable integration of the modified gene
9. Backcrossing to introduce the new trait to other varieties of crops and breeds of animals

The applications of gene technology can be found in many fields, including agriculture, medicine and industry.

Applications of GMO in Agriculture

With the growing human population and decrease in agriculture land, it becomes obvious that , the crop yield per unit area needs to be increased. In order to achieve an economically sustainable agriculture, a higher crop yield should be obtained with a low cost of production. Improving the quality of foods is also a major concern in agriculture, in addition to the quantity. The green revolution from 1930s to 1960s increased the crop yield by introducing high yielding crops and use of artificial fertilizer and pesticides. However, impact of green revolution is also limited and is now being overcome by the genetically modified plants (GM crops). When a single plant cell is genetically modified, it can be regenerated in a plant, as plant cells are totipotent. When a useful

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trait is introduced into a crop, it can be introduced to other varieties of the same crop by plant breeding.

Most significant contributions in crop yield enhancement by gene technology in agriculture are due to the production of GM crops that are resistant to

- pests and diseases
- herbicides and
- environmental stresses.

In addition, crops with higher nutritional values are also available, e.g. golden rice enriched with vitamin A, increased triglycerides content in Canola oil.

Plants Resistant to Pests: Several GM crops have been engineered with a gene producing a toxic protein that kills the plant eating larval stages of certain lepidopteron and coleopteron insects. Most widely cultivated pest resistant GM plants are cotton, maize, canola and potato. A GM rice variety resistant to lepidopteron insects is also available.
The protein is called Bt toxin, since the protein originally came from a bacterium; *Bacillus thuringiensis*. Different strains of this bacterium produce several different Bt toxins. When larvae feed on plant parts that express Bt toxins, they die due to the ingestion of the toxin.

Bt toxins are not harmful to mammals and are therefore considered safe for human consumption. However Bt maize (Bt corn) is grown mostly for biofuel and for animal feed. Since the toxin is present in the plant tissues, the only insects that are killed are plant pests and therefore Bt crops are also considered safe for beneficial insects. Figure 7.41 show some insect pests of corn and cotton. Bt toxins are natural products and therefore, are biodegradable.

However, the insects, when exposed to the same toxin over the time, develop resistance to that toxin, rendering the GM crop useless. Several solutions to delay the resistance development in insects have been proposed. Since the pollen grains containing the toxin can escape from the field where Bt crop is planted, insects that are not feeding on the crop may also be killed by accidental ingestion of such pollen. Therefore, Bt crops has a potential danger for non-target insects.

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(a)



(b)





(d)

Figure 7.41: Some insect pests of maize: a) Corn earworm, b) European corn borer and c) Corn rootworm. (d) Cotton bollworm

Plants Resistant to Diseases

A well known example of disease resistant crops developed by genetic engineering is the availability of new varieties of papaya resistant to papaya ringspot virus (PRSV). This virus is limiting the success of papaya cultivation worldwide. The same virus also attacks the cucurbits. Squash with resistance to the virus has been successfully developed and cultivated.

Other examples for disease resistant crops are potato varieties resistant to Potato Virus Y (PVY), Potato Leaf Roll Virus (PLRV) and late blight disease.

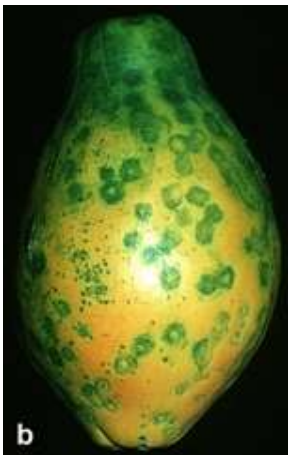


Figure 7.43: Infected papaya fruit with papaya ring spot virus

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Plants Resistant to Herbicides: The herbicide tolerant crops (HTCs) are developed in order to be able to spray a broadspectrum herbicide to control weeds when the crop has been established in the field. When the crop is resistant to the herbicide that is sprayed, it can kill all the weeds without harming the crop. The advantage in this is that the farmers can wait and see if the weeds become a problem and can use herbicide only when it is necessary. This reduces the herbicide usage. However if the same herbicide is used repeatedly, the weeds resistant to that particular herbicide will develop. These are called superweeds. When a crop with resistance to one herbicide is planted after a GM crop with resistance to the same herbicide, leftover seeds of the previous crop may germinate and become a weed which cannot be controlled by the same herbicide. In order to avoid these problems, rotation of crops with tolerance to different herbicides can be practiced.

The best known examples of herbicide tolerant crops (HTCs) are crops modified to tolerate the herbicide glyphosate. These plants are known as "RoundUP Ready" crops because the commercial name of glyphosate is RoundUP.. The examples of commercially available RoundUP Ready crops are cotton, maize, canola, soybean, sugar beet and wheat.

Another type of popular herbicide tolerant crops are called "Liberty Link" and "InVigor" and are resistant to glufosinate. Examples of glufosinate resistant crops include cotton, maize, canola, soybean, sugar beet and rice.

Cotton modified to tolerate Bromoxinol are called BXN Cotton.

GM Plants with other Important Traits

Other agriculturally important traits include improving product quality.

One of the priorities in this area is to increase the nutritional value of crops.

- GM canola varieties with increased triglyceride component and with increased phytase enzyme to breakdown indigestible plant phytate to release phosphorus, GM potato with reduced amylase and increased amylopectin content and soybean with increased oleic acid content in the seed are commercially available.
- A rice variety named Golden rice with increased levels of provitamin A is an interesting example of product quality enhancement. The commercially available variety is modified with genes from *Pantoea ananatis*, a plant pathogenic bacterium containing a yellow pigment.
- Tomato with delayed fruit ripening to increase the flavour and reduce the rate of softening is another interesting development in GM crops. The source of the gene is tomato itself. A part of the gene is copied in reverse direction by changing the orientation of the promoter.
- Non-browning apples due to reduced polyphenol oxidation and maize with increased thermostability of amylase which helps in bioethanol production are other examples.
-

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Among plants that can be genetically modified to tolerate the environmental stresses, only maize and soybean having drought resistance have been commercialized.

Applications in Medicine

Human insulin, vaccines and other therapeutics are being produced using GMOs. These pharmaceuticals produced by GMO are cheaper, since mass production at low cost is possible and are considered safer.

Producing human insulin by genetically engineered *E. coli* is the best known example of the use of genetically modified organisms in producing pharmaceuticals. Insulin extracted from animals had various side effects on diabetic patients. The cost of production was also very high due to the limited amount of source to extract insulin. Since those insulins are not the same as human insulin, they were less effective. Nowadays the entire insulin supply is from *E.coli* genetically engineered by inserting insulin gene from human. Therefore the bacteria derived insulin is exactly the same as its original product.

Hepatitis B vaccine used today is a recombinant vaccine produced in yeast. Engineering plants to produce vaccines in their edible parts is a concept being experimented. The idea is to express an antigenic protein in the plant cells. When the edible part with the antigen (e.g. fruit) is consumed by someone; the person will develop antibodies against the antigen, so that immunity is developed against a particular disease. These are called edible vaccines, and if this becomes successful, low cost, safe vaccines can be produced and the delivery of the vaccine would be painless. Storage will also not be a main problem. Therefore these are more important in less developed regions of the world.

GM mammalian cells grown in cell culture are used to extract factor VIII used to treat haemophiliacs and tissue plasminogen activator (tPA) used to treat heart attack and stroke patients.

After the completion of human genome project, the causes for various genetic diseases are being identified with ease and speed. When the cause is identified, how to correct the mistake in the defective genes can also be worked out. The incorrect gene then can be replaced with the correct one by gene technology. If the problem is the expression of a specific gene, this technology can also influence the expression of that gene. This treatment is called gene therapy or human gene transfer. The DNA is delivered to the target cells extracted from the patient by using a viral vector or as naked DNA using various techniques. The cells with corrected gene are reintroduced into the appropriate tissue of the patient. Although the concept of gene therapy has a long history since 1972, only a few applications are available to date.

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A gene therapy to treat leukemia is the first of that type in the USA. Another example of gene therapy is to replace the mutated beta-globin gene causing sickle cell anemia with the correct gene. During this procedure, bone marrow derived haematopoietic stem cells are extracted from the patient, the normal beta globin gene is inserted into these cells and return the modified cells back into the patient. The corrected bone marrow stem cells will produce normal erythrocytes.
The concept of personalized medicine is being developed, which is based on genetic information of the patient to treat or prevent diseases.
GM insects have been employed to control insect vector born disease. GM mosquitoes have been engineered which do not allow entry of malaria parasite into the gut, therefore breaking the life cycle of the parasite. Release of these mosquitoes into the wild would reduce incidence of malaria. Another example is the making of GM male mosquitoes carrying a male sterile gene. Mass release of these male sterile insects results in mating with females, but not producing offspring. This technology is call 'sterile insect technology' (SIT). In field trials in Brazil, the Aedes aegypti populations have reduced by 95% by the introduction of sterile GM males.

Applications in Industry

Use of GMOs in industry has made it possible to produce novel products with low cost while minimizing environmental impacts. Industries based on organisms or their products run at ambient temperatures and pressures, demanding less energy.

Apart from the booming pharmaceutical industry based on GMOs and GM crop industry, some products originated from GMOs are produced industrially. Some enzymes for food processing and detergents are products of GEMs. Chymosin (rennin or rennet) is the first approved enzyme produced by GMO. This is used in cheese industry to coagulate milk to separate whey. Chymosin has been obtained by extracting it from stomachs of slaughtered calves. Since the supply was limited, the cost was high and it affected dairy industry. The chymosin gene from cattle has been engineered in yeast cells and these recombinant yeasts are the source of chymosin today. The price has gone down considerably and the product is purer and devoid of contaminants of animal origin. Amylomaltase is another enzyme produced by a GM *Bacillus* sp. This enzyme modifies starch to be used as an ingredient in dairy industry.

Aspartame, a strong sweetener, is a food additives produced by GM *E. coli.*

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Concerns of using GMOs

The most significant risk factor in use of GMOs is their possible unintended effects. Since this is a relatively new technology, public is reluctant to accept it readily. However the public also accepts the unlimited potentials of the GMOs. The debate between pro-GMO and anti-GMO groups, organizations and individuals is intensive. Some of the controversial issues about GMOs are mentioned below.

Health Issues

Health Issues

1. Data of some experiments involving rats, mice and other animals showed some health implications after feeding with genetically modified food such as potato, maize, tomato and soybean. The reports include damage to various tissues such as stomach, liver and kidney and even increase in deaths. However, many other scientists question the methodology used in such experiments and they also claim that they could not reproduce the results. Therefore, more independent research is needed to confirm or to reject such claims.

2. Allergy development due to consumption of GM food or inhaling pollen of GM crops is discussed as another health issue. The integration of foreign DNA in the host cells may change gene expression or cause mutations, leading to unpredictable products. Some of them may be allergenic, toxic or even carcinogenic. However, concrete scientific research findings are not available or the findings are doubtful. The technology is now developed so that the insertion can be done at precise locations without disturbing other functions of the host.

3. The possibility of horizontal gene transfer of antibiotic resistant genes used as marker gene is also highlighted as a potential health issue. The GM foods contain such genes and are ingested in large quantities by consumers. Although this is a possibility, all organisms have barriers to horizontal gene transfer and therefore, chance of horizontal gene transfer into humans is very low. Horizontal gene transfer among bacteria is more probable, and hence transfer of antibiotic resistance to pathogenic organisms may have some health related issues. However, the antibiotics used in rDNA technology are not used in chemotherapy.

   On the other hand, the humans as well as all other animals have been eating food of animal or plant origin ever since they were evolved, but there is no evidence to show genes transferred due to eating food.

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Environmental Issues

1. The development of insect tolerant crops may harm the non-target insect by accidentally ingesting the toxin produced in the GM crops. The toxin may be dispersed in pollen and deposit on non-crop plants which the insects feed on. An experiment has shown that Monarch butterfly larvae feeding on milk weed leaves dusted with pollen from GM crops die. However, proponents of GM argue that the amount of pollen dusted on the leaves is very much higher than the amount that could be deposited naturally.

2. Cross pollination will transfer the transgene (the foreign gene) to other non-GM varieties of the same crop as well as wild relatives of the crop. It can therefore, contaminate organic or non-GM farming.

3. When Bt gene is transferred to wild plants, the insects feeding on them will die causing environmental imbalances.

4. When the herbicide resistance genes are transferred to weeds, they cannot be controlled using the same herbicide, and therefore become super weeds.
5. The spread of a foreign gene in naturally growing plants is called gene pollution.

6. Since the herbicide tolerant crops are resistant to a particular weedicide, farmers may tend to over use this herbicide to keep their fields clean. If that is the case herbicide tolerant weeds will develop by frequent exposure of the weeds to the same herbicide. It is however argued that the farmers will spend money just to have a clean field, and they can use a "wait and see approach" in order to spray herbicides only when necessary rather than spraying as a precaution. Rotation of crops with tolerance to different herbicides will avoid development of such super weeds.

7. As GM crops are being accepted by farmers as well as consumers, the extent of land under cultivation will be dominated by GM crops limited to very few varieties. When the crop diversity is reduced to such a small number, the tolerance to environmental impacts become very low and a single environmental event may wipe out entire crop fields, leading to food scarcity.

8. Narrowing down of crop diversity will contribute to loss of genes from the crop gene pool.

Socio-economic Issues

1. The newly developed GM crop varieties will be owned by the developers and patented. Therefore the farmers are compelled to purchase their seeds every year from giant seed companies with a monopoly, spending a lot of money. Poor farmers may not be able afford to buy the seeds, and therefore, there is a risk of widening the gap between the rich and the poor farmers.

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2. There is a growing public concern whether it is ethically correct to patent crops and biological resources including genes that are found in nature. Some crops and products that have been developed traditionally and used by indigenous people have been patented under some biotechnology companies.

3. The consumer has the right to decide whether he/ she purchases GM or non-GM food. To secure this right, the regulatory agencies need to implement a system of labeling to indicate clearly whether the product is GM or not and if it is GM, then what are the changes that have been made. Labeling is mandatory in some countries. However products labeled non-GM have often been found to be contaminated with GM when tested.

4. Biological resources of a country or region with high biodiversity and traditional knowledge are taken away by biotechnology companies without any authorization by the countries and people or without paying compensation for product development. This is called biopiracy.

5. The manipulation of the nature in making GMOs contradicts the beliefs of certain religions.

In order to address potential risks and hazards of GMO, GMF and associated processes, a thorough testing and screening procedures have come into effect. As such the process of producing and commercialization of GMO and GMF are under strict control of several legislations and authorities in order to safeguard the consumer, society and environment. Approval of some GMOs has taken 25 years from the creation to market (e.g. GM Atlantic Salmon which grow twice as faster than non GM).
An example of an international agreement is Cartagena Protocol. Many countries also have their own legislations, such as National Biosafety Framework of Sri Lanka.

Cartagena Protocol on Biosafety

Cartagena Protocol on Biosafety to the Convention on Biological Diversity is an international agreement signed on 15th May, of 2000 in Montreal, Canada as a supplement to the Convention on Biological Diversity (CBD) signed in 1992, at the Rio- Earth Summit and is effective from 1993. Cartagena Protocol on Biosafety came into effect from 11th September, of 2003. It is called Cartagena Protocol on Biosafety since it had been originally scheduled to sign in Cartagena in Colombia. The provisions in CBD were not sufficient to cover many aspects in biodiversity related to the GMOs. The number of signatories to the Cartagena Protocol has exceeded 100. This includes Sri Lanka which gave formal consent to the protocol on 28th April, of 2004.

The aim of the Cartagena Protocol on Biosafety is to protect the biological diversity from the

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potential risks caused by genetically modified organisms or living modified organisms (LMOs) created as a result of modern biotechnology. CBD defined biotechnology as "any technology that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use". The Protocol is based on 'precautionary principle' of CBD so that products of new biotechnologies should follow rigorous control measures to avoid any potential risk to the environment or human health and is applied to transboundary movement, transit, handling and use of LMOs that may have adverse effect on the conservation and sustainable use of biological diversity. This also applies to risks to human health. The provisions of the Protocol are intended to allow developing nations to balance public health against economic benefits. Countries and states may take appropriate actions to limit entering of an LMO to their territory if they feel that there is lack of scientific information to ensure that the LMO is safe on environment or human health. The LMOs maybe intended to be introduced into the environment or to be used as food or feed. The shipment should accompany with appropriate documentation which identify the LMO and whom to contact for further information. Sufficient information should be supplied by the importer or exporter to the importing party to arrive at an informed decision on accepting or rejecting the LMO imports and how to handle them in a safe manner.

The Protocol established a 'Biosafety Clearing House' (BCH) to assist parties to implement the protocol by facilitation exchange of scientific, technical environmental and legal information on and experience with movement of LMOs.

Sri Lanka signed the Protocol in May, 2000 and it is in force in Sri Lanka from July, 2004. Ministry of Environment and Natural Resources was identified as the responsible institution for coordinating the activities related to the Protocol.

National Biosafety Framework of Sri Lanka

The drafting of the National Biosafety Framework of Sri Lanka (NBFSL) was completed in 2005 by Ministry of Environment and Natural Resources (now Ministry of Mahaweli Development and Environment, MoMDE). This is based on a precautionary approach in accordance with Cartagena Protocol on Biosafety with the objective of ensuring that the risks likely to be caused by modern biotechnology and its products will be minimized and biodiversity, human health and environment will be protected in a maximum way regulating the transboundary movements through formulation of relevant policies, regulations, technical guidelines and establishment of management bodies and supervisory mechanisms.

National Biosafety Framework of Sri Lanka is the starting point for a permanent legislative framework on Biosafety in Sri Lanka. Based on the NBFSL two policies have been formulated.

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- National Biosafety, Policy 2005 is one of those. The policy on biosafety sets the overall framework in which adequate safety measures will be developed and put into force to minimize possible risks to human health and the environment while extracting maximum benefits from any potential that modern biotechnology may offer.

- A National Policy on "Access to Biological Resources, sustainable Use and Benefit Sharing" has been formulated in 2013 by MoMDE with the goal of "ensuring conservation and sustainable use of biological resources and the fair and equitable sharing of benefits arising from them" in accordance with Cartagena Protocol on Biosafety and the National Biosafety Framework of Sri Lanka.

However these policies have not yet been enacted by law.

The Target 12 of the National Biodiversity Strategic Action Plan for 2016-2022 of Biodiversity Secretariat of the MoMDE states that "by 2022 biosafety is assured". The actions to be taken in this direction are

(1) strengthening policy on biosafety

(2) implementing biosafety master plan and formulate biosafety legislature

(3) strengthening risk assessment procedures for new technologies

(4) strengthening capacity for risk assessment

(5) developing and implementing legal instruments to protect native biodiversity and indigenous crops from contamination from GMOs and

(6) enhancing Sri Lanka's scientific capacity on biosafety.

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08

Environmental Biology

What is environment ? The environment is the complex of physical, chemical, and biotic factors that act upon an organism or an ecological community and ultimately determine its form and survival

What is environmental biology?

Definition: A scientific study of the origins, functions, relationships, interactions, and natural history of living populations, communities, species, and ecosystems in relation to dynamic environmental processes.

In other words, Environmental biology is the study of different biological components of the environment and its relationship is with each other and with the abiotic components.

Why learn Environmental Biology?

By learning of environmental biology will be able to understand the structure and the functions of the environment we live in. Specifically to:

- understand how biotic and abiotic components of the environment are linked and interact with each other,
- recognize organizational levels of the environment,
- learn about major processes of an ecosystem,
- recognize different components of terrestrial and aquatic ecology,
- understand how human activities are altering ecological systems and
- understand how natural changes in the environment (such as climate change, flooding, erosion etc) affect species and habitats.

Organizational levels of the environment

Several levels of organization are recognized by environmental biologists.

Levels of organization include the individual/ organism , population, community, ecosystem, and

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biosphere.

- Individual/ Organism – The first level is an individual. An individual is any organism or living thing. An organism has its own characters in physiology, evolution and behavior in relation to environmental factors.
- Population –A Group of individuals of the same species, living in the same area and producing fertile offspring through interbreeding.
- Community – A collection of populations of different species living in the same area interacting with each other.
- Ecosystem – An ecosystem is a collection of communities as well as the abiotic factors with which they interact.
- Biosphere – includes the entire portion of Earth that is inhabited by life.

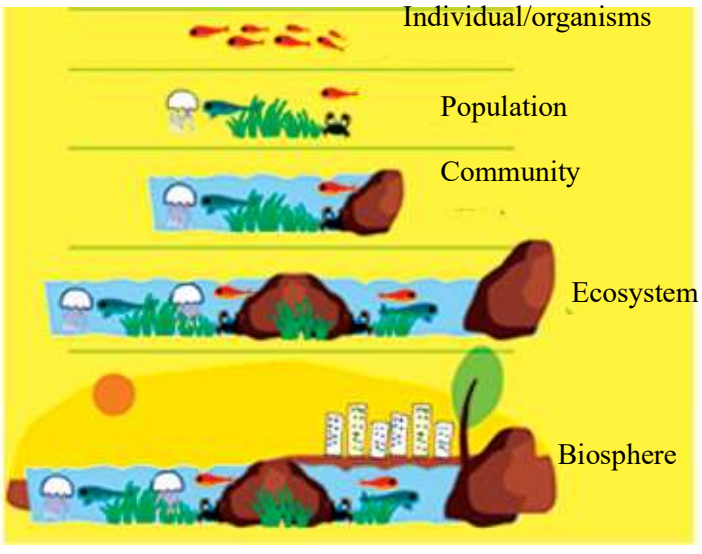


Figure 8.1: Organizational levels of an ecosystem

Major biotic and abiotic components in the environment

All Organisms live in a complex environment that includes several components which are categorized under two major groups:

- Abiotic components – The nonliving components (physical and chemical factors) of the environment such as water, air, light, temperature, nutrients, sunlight, soil. Abiotic resources are usually obtained from the lithosphere, atmosphere, and hydrosphere. Abiotic components limit the geographic range (distribution) and the abundance of species.
- Biotic components – the living components of the ecosystem include plants, animals, fungi, bacteria and protists etc.

Structure and function of ecosystems:

The concept of "Niche" and "habitat"

To understand how an ecosystem work, one should first look into the concepts of "niche" and "habitat."

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Niche

A niche is the role that a particular organism plays in the ecosystem. Each organism plays a particular role in its ecosystem. In other words, a niche is how an organism "makes its living." .This involves how the organism gets its energy, which usually has to do with what an organism eats, and how the organism passes that energy through the ecosystem. An organism's niche also includes how the organism interacts with other organisms, its role in recycling nutrients, tolerance to environment conditions such as temperature, soil moisture, etc. Therefore niche of an organism describes what it needs to live and what it does in a particular ecosystem.

Habitat

The habitat is the physical area where a species lives. Many environment factors are used to describe a habitat. The average amount of sunlight received each day, the range of annual temperatures, and average yearly (annual) rainfall describe a habitat. These and other abiotic factors of the environment such as soil condition will determine the kind of traits an organism must have in order to survive there. A habitat should not be confused with an ecosystem: the habitat is the actual place of the ecosystem, whereas the ecosystem includes both the biotic and abiotic factors of the habitat.

Interactions between abiotic and biotic components of the environment

There are different types of interactions in an ecosystem. They are:

- Biotic-biotic interactions- e.g. competition, feeding relationships, symbiotic relationships between individuals and species.
- Biotic-abiotic interactions- e.g. water uptake of plants from soil
- Abiotic-abiotic interactions-e.g. chemical reactions occurring in the soil.

Feeding relationships within an ecosystem develop food webs and food chains through which energy is passed from one organism to another.

Energy transfer in an ecosystem

The main energy provider for the earth is the sun. Using sunlight the green plants produce their own energy and store in tissues. The source of energy required by all living organisms is the chemical energy of their food. Ultimately, chemical energy in any organism is obtained by the conversion of the radiant energy of sun which are stored in tissues of plants and animals.

Trophic level:

The producers and consumers in ecosystem can be arranged into several feeding groups, each known as trophic level (feeding level). In any ecosystem, producers represent the first trophic level, herbivores represent the second trophic level, primary carnivores represent the third trophic level and top carnivores represent other levels.

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Food chain

A food chain is a linear sequence of organisms through which nutrients and energy pass from one trophic level to another trophic level of an ecosystem beginning with a primary producer. Let's look at the parts of a typical food chain, starting from the bottom (the producers) and moving upward.

primary producers

At the base of the food chain lie the primary producers. The **primary producers** are autotrophs and are most often photosynthetic organisms such as plants, algae, or cyanobacteria. These organisms convert light energy to chemical energy or in another words produce organic matter.

Primary production – is the amount of organic matter produced by autotrophs in a given area during a given period of time. (Unit: $gm^{-2}day^{-1}$ or $kg\ ha^{-1}year^{-1}$)

The organisms that consume (eat) the primary producers are called primary consumers. Primary consumers are usually herbivores (plant-eaters), though they may be algae eaters or bacteria eaters.

The organisms that consume the primary consumers are called secondary consumers. Secondary consumers are generally carnivores (meat-eaters).

The organisms that consume the secondary consumers are called tertiary consumers. These are carnivore-eating carnivores, such as Eagles or big fish species.

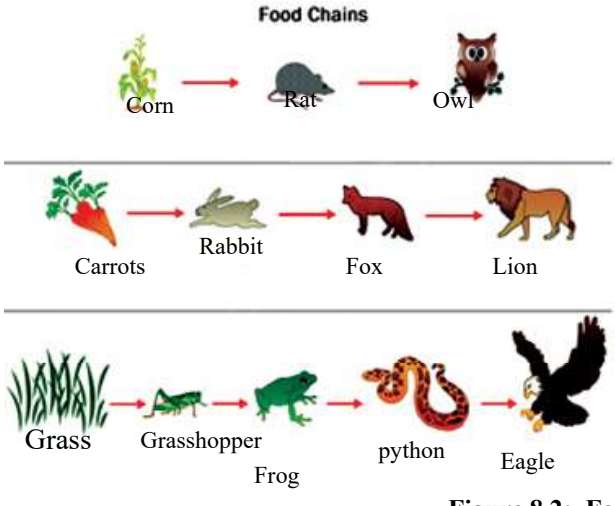


Figure 8.2: Food chains in a terrestrial ecosystem

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Food web

In a natural ecosystem isolated food chains do not exists. The food chains are interconnected with each other to form food webs. The food web is an interconnected feeding relationships in an ecosystem. (Campbell, 10th edition, 2015)

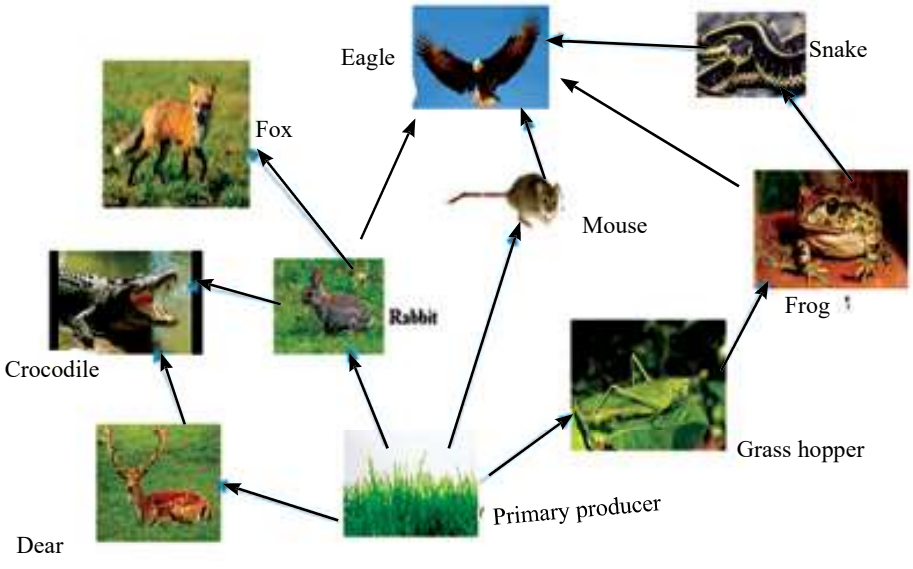


Figure 8.3 : Example for food web in a terrestrial ecosystem

Energy loss along food chains

The flow of energy along a food chain is always unidirectional. At each trophic level in the food chain, a considerable fraction (about 90 %) of the potential energy is lost as heat and respiration. As a result, organisms in each trophic level pass on lesser energy (about 10 %) to the next trophic level than they actually receive. This limits the number of trophic levels in any food chain to four or five. The most ecologically efficient food chain is the shortest one. Longer the food chain the lesser energy is available for top members. Because of this tapering off of available energy in the food chain a pyramid is formed that is known as an ecological pyramid. Shorter food chain has more energy available even at the highest trophic level than that of longer food chain.

Ecological pyramid:

The trophic structure of an ecosystem can be indicated by means of ecological pyramid. The higher the steps in the ecological pyramid the lower will be the number of individuals and the larger their size.

The concept of ecological pyramid was developed by Charles Elton; these pyramids are also known as Eltonian pyramids. The pyramids are a graphical representation which depicts the

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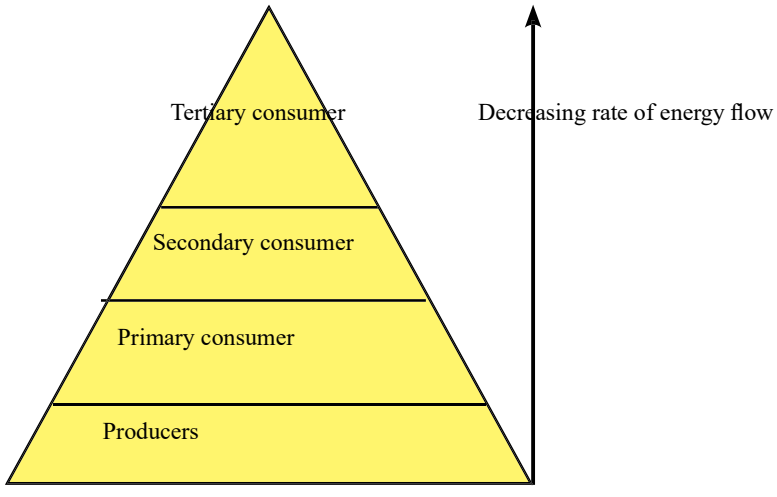
number of organisms, biomass and productivity at each trophic level. All ecological pyramids begin at the bottom with the primary producers and they proceed to various trophic levels such as herbivores consume plants, carnivores prey on herbivores and so on. The highest level is at the top of the food chain. There are three types of ecological pyramids:

- Pyramid of energy
- Pyramid of numbers
- Pyramid of biomass

Pyramid of Energy

Pyramid of energy represents the amount of energy at different trophic levels. The pyramid of energy or the energy pyramid describes the overall nature of the ecosystem. As there is considerable loss of energy during the flow of energy from organism to other, the energy pyramid always upright and vertical.

- This pyramid shows the flow of energy at different trophic levels.
- It depicts the energy is minimum as the highest trophic level and is maximum at the lowest trophic level.
- At each trophic level, there is successive loss of energy in the form of heat and respiration, etc.



Pyramid of Energy

Pyramid of Numbers

The pyramid of numbers depicts the relationship in terms of the number of producers, herbivores and the carnivores at their successive trophic levels. There is a decrease in the number of individuals from the lower to the higher trophic levels. The number pyramid varies from ecosystem to ecosystem. There are three types of pyramid of numbers:

- Upright pyramid of number

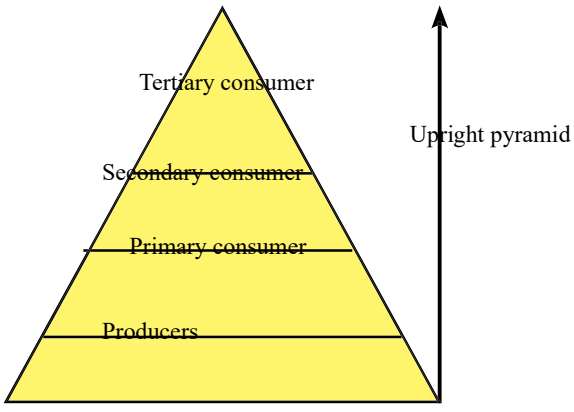
  e.g. Producers → Herbivores → Carnivores

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- Inverted pyramid of number.

  e.g. Host ⟶ Parasites

This type of pyramid number is found in the aquatic and grassland ecosystem, in these ecosystems there are numerous small autotrophs which support lesser herbivores which in turn support smaller number of carnivores and hence this pyramid is upright.



Pyramid of Number

Pyramid of Biomass

The pyramid of biomass is more fundamental. In this pyramid there is a gradual decrease in the biomass from the producers to the higher trophic levels. As the fresh weight of biomass contains a larger amount of water, dry weight of the biomass represents the amount of energy available in the form of organic matter of an organism.

There are two types of pyramid of biomass, they are:

- Upright pyramid of biomass and -This occurs when the larger net biomass of producers support a smaller biomass of consumers.

  Example: Forest ecosystem.
- Inverted pyramid of biomass.

  e.g. In some aquatic ecosystems phytoplanktons supports larger primary consumers.



8.4: Upright pyramid of Biomass

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Cycling of materials in an ecosystem

The materials available for living organisms in the ecosystem is limited. Therefore these materials should be recycled. The material stock in the ecosystem is decreased when the organisms such as animals, plants, etc utilize them. Therefore when the organism is died, they are decomposed by decomposers. Thus those materials will be available for the organisms and absorb them into their body, assimilate and the excess will be removed as waste materials to the environment.

What is a biome?

A biome is a large geographical area which is classified based on the predominant vegetation adapted to that particular environment. The climate and geography of a region determines what type of biome can exist in that region. Each biome consists of many ecosystems whose communities have adapted to the small differences in climate, topography and soil conditions within the biome. Major terrestrial biomes include tropical forest, savanna, desert, chaparral, temperate grassland, temperate broad leaf forest, northern coniferous forest and tundra.

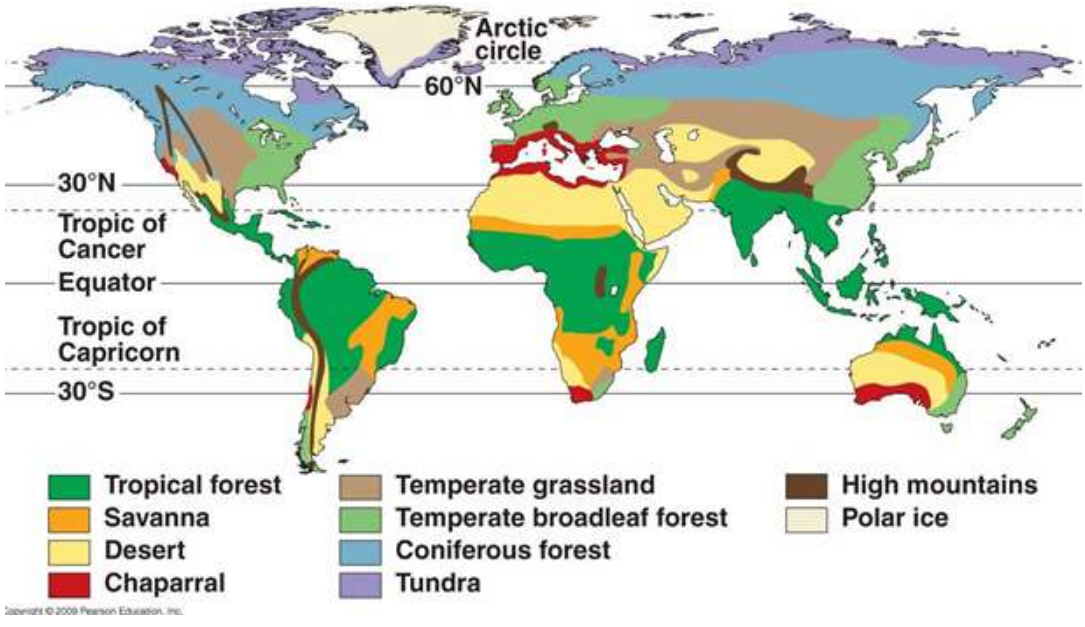


Figure 8.5: World distribution of different types of biomes

1. Tropical forests

This biome is distributed close to the equator and extends to the sub equatorial areas. Therefore both tropical rainforests and tropical dry forests are included.

The average annual rainfall in tropical rain forests is about 2000 -4000 mm and in tropical dry forests it is about 1500- 2000 mm. Seasonal rainfall with a dry season of 6-7 months is prominent in the dry forests and a fairly constant rainy season is prominent in the rainforests.

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Tropical rainforests have an average temperature of 25 -29 °C, whereas in the tropical dry forests it may reach up to 33 °C.

In tropical rainforests an emergent layer, canopy and sub canopy layers are visible. These are followed by an understory layer which consists of shrubs, and large herbaceous plants. The forest floor consists of small herbaceous plants, mushrooms,and a thick layer of dried plant litter. Therefore the vegetation is arranged in several vertical layers and stratification is observed.

Evergreens are prominent in tropical rain forests whereas in dry forests leaves of deciduous species fall in the dry season. Epiphytes are common in this biome. However in dry forests they are less common. Shrubs with thorns as well as succulent plants often occur in dry forests. Tropical forests constitute the higher diversity of plants and animals out of all terrestrial biomes. It has many mammal species and 5-30 million species of arthropods some of which have not been fully described yet. Animals in this biome have adaptations to suit the environment.

Animals in the rainforests show year round activity. Camouflage is common among small animals. Large animals may have markings in their bodies . Birds often migrate to other regions during dry period.

Human involvement in agriculture and development related activities has led to destruction of these forests.



Figure 8.6: A tropical forest

2. Savanna

Savanna is spread close to the equator and subequatorial areas.

The average annual rainfall in this biome is around 300-500 mm. A prolonged dry season of approximately 8-9 months is prominent. The average temperature range is 24- 29 °C. However in subequatorial areas seasonal variations may occur.

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Savannas are characterized by a landscape that supports scattered trees in a tall grass cover. During dry periods the dried grasses catch fire easily. Therefore grasses adapted to withstand drought, naturally grow in savannas. These grasses have a very good underground connectivity. The few herbs found among grass cover contain small leaves to reduce transpiration and thorns to be protected from herbivores.

Seasonal rains encourage a fresh growth of grasses to compensate the loss of biomass due to consumption by herbivores.



Figure 8.7: A Savanna

Many insects (mainly termites), lions, zebras are the common animals in this region. Many animals have effective locomotion for long distance migration and long range vision for hunting. Human settlement from a very early time has been reported in these areas. Due to rearing of cattle and hunting of animals by humans the population of mammals have been decreased. Fires caused by human aid help to maintain the grass cover of this biome in one way but it may also have a negative impact by suppressing the growth of trees.

3. Desert

Desert is a temperate or tropical biome, commonly occurring near 30° north and south latitudes (e.g.Sahara), and in the center of continents (e.g. Gobi desert in north central Asia). The distribution of this biome is determined by the non availability of water such as in areas with annual average rainfall is less than 3000 mm per year.The temperature varies seasonally and daily and exceeds 50 °C in hot deserts while in cold deserts it drops below -30° C.

Deserts have more bare lands. If the vegetation is found they are sparse and widely scattered. Desert plants have several adaptations to withstand the high temperature and scarcity of water.

- Succulent plant body (e.g. Cacti and Euphorbs)
- Most plants have C4 pathway of photosysnthesis
- Deep roots in shrubs
- Ability to tolerate heat and desiccation
- Reduced surface area of leaves
- Presence of spines/ thorns
- Presence of toxins in leaves
-

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Nocturnal animal species are abundant in deserts. Water conservation is a prominent feature, with some species surviving solely on water obtained during the breaking down of carbohydrates in seeds. Animals include, snakes, lizards, beetles, ants, scorpions, rodents and birds.

Since these areas are converted into human settlements and agricultural lands, the natural biodiversity is reduced. Deep wells and well developed systems for transporting water has facilitated human settlement and agricultural lands.



Figure 8.8 : A Desert

4.Chaparral

Chaparral occurs in mid latitude coastal regions such as North America, Chilie, Spain, Southern France and South Africa. Chaparral is best developed in Southern California.

Chaparrals receive an average annual rainfall of 300-500 mm. They experience rainy winters and dry summer. The average temperature is between 10 to 12 °C but can reach up to 40 °C.

The Chaparral biome is typically composed of dwarf forests and shrubs, and interspersed herbaceous vegetation which include grasses and herbs. Chaparral is highly prone to events of catastrophic wildfire. Therefore plants show the some of the following adaptations;

- Seed germination occurring only after a hot fire.
- fire resistant roots.
- Uses of food stored in the fire resistant roots for resprouting after a fire.
- Quick re -sprouting enables usage of nutrients released by the fire.
- Tough evergreen leaves in woody plants to survive in droughts.

Most of the native mammals in chaparrals are browsers. They include deers and goats. Chaparrals are rich in diversity of small mammals, and several species of amphibians, birds, reptiles and insects.

These areas have been reduced and disturbed due to human settlement, urbanization and agricultural conversions. Chaparral is vulnerable to fire caused by human activities.

5. Temperate grasslands

These grasslands occur under temperate climatic regimes that are intermediate to those that support forest and desert. Grasslands in North America are called prairie (they are often called

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steppe in Eurasia and called pampas in Argentina).

Precipitation is highly seasonal with periodic drought. These grasslands have relatively dry winters and wet summers. The average rainfall is 300 to 1000 mm per year.
During winter seasons the average temperature falls below -10 °C and in summer it is about 30 °C.

Grasses are the dominant plant species in these grasslands. The prairie is often divided into three types according to height of the dominant vegetation—tall-grass, mixed-grass, and short-grass. The height of grasses vary from few centimeters to two meters in tall grass pairrie. Fire and droughts are occurred in these grasslands but many plants growing in those areas have adaptations to cope with fire.

Large grazers such as horses and many types of burrowing mammals (prairie dogs in North America) are found in these areas.



The tall-grass prairie is now an endangered natural ecosystem, because it has been almost entirely converted to agriculture and farmlands.

Figure 8.9 : Temperate grassland

6. Temperate broadleaf forest

Distribution of temperate broadleaf forests are at mid latitudes in the northern hemisphere. The average annual precipitation is between 700 to 2000 mm. Significant amount of precipitation is received throughout the year in summer as rain and in winter as snow.

Average temperature during winter is zero while in summer it is up to 35 °C. Summer is hot and humid and allow a favourable time to the growth of trees.

The dominant trees are mostly deciduous. A vertical layering (stratification) can be seen in temperate broadleaf forests. They are closed canopy layer, one or two strata of understory trees, shrub layer and herb layer. There are only few epiphytes can be found.
Many mammals can be seen and they hibernate during winter seasons while the birds species migrate to areas where climate is warmer.

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Forests are disturbed due to logging, clearance for agriculture and for human settlements.

7. Northern coniferous forest

The largest biome on earth is the northern coniferous forest. It is extended as a broad band from upper band of Arctic tundra.

Precepitation: Annual average precipitation is around 300- 700 mm and a periodic drought is also common.

The temperature is -50^0 C in winter and 20 C^0 in summer.

The northern forest is dominated by coniferous trees. e.g. Fir, Pine, spruce are common tree species.

Many conifers are conical in shape and this shape of trees prevents accumulation of snow, thus preventing the breaking down of branches. They have needle like leaves that prevent high transpiration.

The diversity of shrubs and herbs in these forests is less than that of temperate broad leaf forests because of the limitations in precipitation and warm temperature.

Commonly found mammals are brown bears, moose, and siberan tigers. During some period of time dominant plants can be killed by sudden attacks of insects.

These areas are not heavily populated by humans, but logging is a severe threat.



Figure 8.10 : Northern coniferous forest

8. Tundra

This biome covers expansive areas of the arctic region, amounting 20% of earths land surface. Alpine tundra occurs at high altitudes on mountains, while arctic tundra occurs at high latitudes. Most tundras receive very small inputs of water as precipitation, but nevertheless their soil may be moist or wet because there is little evaporation in such cold climates, and deep drainage may be prevented by frozen soil.

The arctic tundra annually receive an average precipitation around 200 to 600 mm and the alpine tundra receives > 1000 mm precipitation respectively.

During the winter time temperature falls below -30 ^{0}C and during the summer temperature it is always less than 10^0C.

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Mostly herbaceous plants can be seen that includes different types of grasses and forbs. Shrubs, mosses, lichens and trees also can be found in tundra. A layer of permafrost which is a permanently frozen layer of soil can be seen. This permafrost layer restrict the growth of the roots of plants.

Large grazing mammals such as Caribou and reindeer (migratory), musk oxen (resident) and predatory wolves, foxes, bears together with many species of migratory birds that nest during summer can be seen in this biome.

This area is sparsely colonized by humans, but heavily used for extraction of minerals and oils.



Figure 8.11 : The tundra

Ecosystems of Sri Lanka

Sri Lanka is in the north-equatorial tropical zone. Therefore it experiences a climate with high rainfall and temperature which permits the country to be blessed with a wide array of terrestrial and aquatic ecosystems. The ecosystems can be broadly classified into groups as follows.

1. Terrestrial ecosystems-
   a. Forests-Lowland rain forests, Dry monsoon forests, Montane forests, Thorn scrubs
   b. Grasslands- Savanna, patana
2. Inland wetland ecosystems- Rivers and streams, Reservoirs, Marshes and swamps, villus
3. Ecosystems is with coastal areas- Lagoons and estuaries, Mangroves, Coral reefs, Sea shore, Sand dunes, Sea grass beds, Salt marshes.

The distribution of major vegetation types may be considered as broadly linked to the local climate (mainly rain fall and temperature), topography, and the edaphic (soil) conditions. A broad classification of major ecosystems of Sri Lanka is given below.

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Table 01:Classifications of eco systems in Sri Lanka

Climatic zone	Parameters T: temperature (^{0}C) R: Rainfall (mm) Alt: Altitude (m)	Forests	Grasslands	Other
Arid zone	T 32-36 R< 1000 Alt <300	Tropical thorn scrubs	Arid	Salt marsh Mangroves Sea shore Dunes Coastal marine, Reservoirs, rivers streams, and riverine Wetland Marshes Villus
Dry zone	T 28-32 R: 1000-1500 Alt< 500	Tropical dry mixed evergreen forests	Damana Talawa Savanna	
Intermediate zone	T 24-28 R 1250-2000 Alt 500-1500	Tropical moist evergreen forests, Tropical sub montane forests	Savanna Dry Patana	
Wet zone	T 16-28 R> 2000 Alt 300-1000 Alt 900-1500 Alt > 1500	Tropical lowland wet ever-green Tropical sub-mon-tane Tropical upper montane	Talawa Dry Patana Wet Patana	

Source; National Atlas of Sri Lanka, 2nd Edition, Survey department of Sri Lanka

Tropical wet low land rainforests:

The tropical wet lowland rainforests are found below 900 m elevation in the south western quarter of the country. The mean annual temperature is about 28 °C, and the mean annual rainfalls varies between 2000 mm- 5000 mm in different locations without any dry period. The humidity is very high.

These forests are characterized by a tall canopy, sub canopy and a sparse shrub layer consisting mostly of the saplings of canopy trees. Sometimes an emergent layer is also seen above the canopy.

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The vegetation is filled with a network of woody lianas that reach to the canopy layer.
A diverse population of epiphytic lichens, mosses, liverworts ferns, orchids are found while many fungi inhabit decaying woods and soil. High endemism of floral and faunal diversity can be seen in these forests. E.g. Sinharaja, Nakiyadeniya and Kanneliya are some examples for wet lowlands rainforests. These forests are disturbed due to expansion of tea cultivation, agriculture, industrial and residential development.

Some tree species found in wet lowland rain forests are;

- S: Hora, T: Ennai (*Dipterocarpus zeylanicus*)
- S: Naa, T: Nagai (*Mesua ferrea*)
- S: Hal, T:Kungiliyam pinai (*Vateria copallifera*)

Some animal species found in the wet lowland rainforests are Purple faced langur, Sri Lanka slender loris, Golden wet zone palm civet.



Figure 8.12 : Tropical wet lowland rain forests

Tropical montane forests

They occur at elevation beyond 1500 m above mean sea level. The average temperature is about

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16 °C and rainfall is about 2000 mm without any dry period. The humidity is high as lowland rain forests.

These forests are characterized by a short canopy of about 13 m, with a dense shrub layer. There are trees with umbrella- shaped rounded crowns and twisted branches having leathery small leaves to accommodate the strong winds that prevail in the montane zone. High density of epiphytes cover the branches and stems of trees. Some plants found in these forests are;

- S: Keena, T: Pongu (*Callophyllum walkeri*)
- S: Walkurudu, T: Kaatu karuwa (*Cinnamomum ovalifolium*)
- S: Gal weralu, E: wild olive (*Elaeocarpus montanus*)

Some animals that inhabit montane rainforests of Sri Lanka are Sri Lanka Yellow eared bulbul, Sri Lanka highland Shrew, Sambar.

Montane forests are distributed in central hills including Knuckles range, Piduruthalagala, Hakgala. Montane forests were greatly affected due to tea cultivation.

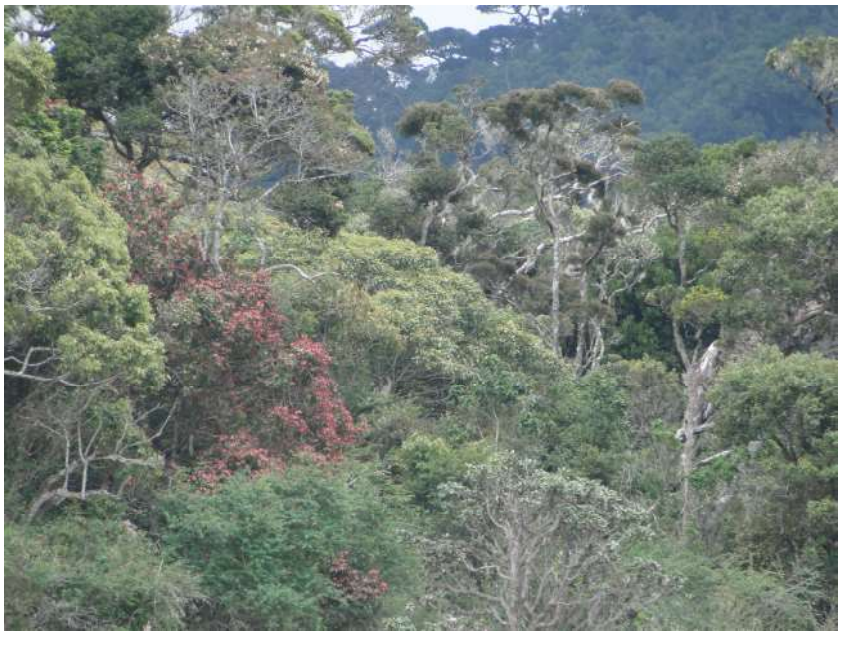


Figure 8.13 : Tropical montane forests

Tropical dry mixed evergreen forests/Dry monsoon forests

These forests have mean annual temperature of about 29 °C and rainfall around 1000 mm- 1500

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mm., most of which falls during the northeast monsoon periods. There is a marked dry spell from May to August. Above forests are distributed in areas below 300m altitude.

These forests are characterized by having sparse canopy, a sub canopy and a well-developed shrub/herb layer. Some plant species naturally found in these forests are

- S: Weera T: Virai (*Drypetes sepiaraia*)
- S: Palu, T: Paalai (*Manilkara hexandra*)
- S: Kaluwara, T: Karun-kaali (*Diospyros ebenum*)

Some of the canopy trees are deciduous during the dry spell of the year. These forests harbor the largest elephant populations in Asia. The mammal fauna includes leopard, bear, deer. Most of the forests are protected as National parks such as Yala National park, Wilpattu National Park, Wasgamuwa National Park, Maduru-Oya national park, Ritigala Strict Nature reserve and many sanctuaries and forest reserves.

These forests are disturbed by 'chena' cultivation, human settlements, extraction of timber, poaching etc.



Figure 8.14: Tropical dry mixed evergreen forests

Tropical thorn scrubs

These are called "scrubs" than "forests" as large trees are sparse and the vegetation is mainly consists of thorny shrubs. They are found in arid lowlands. The mean annual temperature is around 31 °C and rainfall is below 1000 mm with a longer dry period.

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The following plant species are commonly found ;

- S: Gini- andara , T:Vindattai (*Dichrostachys cineria*)
- S: Ranawara, T: Avaram poo (*Cassia auriculata*)
- S: Heeressa, T: Pirandai (*Cissus quadrangularis*)

Due to the dry conditions many fauna species cannot be found. However Deer, Leopard, elephants can be seen in these areas. They are distributed near Hambantota, Yala, Mannar, Puttalam areas and disturbed due to various development pressures.



Figure 8.15 : Tropical thorn scrubs

Savanna

Savannas have a thick grass cover and few scattered trees. These are common on hill slopes of the dry or intermediate zone. The thin layer of soil on hill slopes cannot support the growth of trees and the grass cover of savannas helps to bind soil particles and reduce erosion of soil. Periodic fires are common to this ecosystem as the fried grass cover easily catch fire in dry period of the year. They have trees with fire resistant species.

Some plant species found in Savanna are trees are;

- S: Aralu, T: Kadukkay (*Terminalia chebula*)
- S: Nelli, T: Topu- nelli (*Phyllanthus emblica*)
- S: bulu , T: Adhan- koddai, tanti (*Terminalia bellirica*) and
- have grass species such as
- S: Mana, E: Citronella grass , Vasanai pullu (T) (*Cymbopogon nardus*)
- S: Iluk E: Cogon grass T:Tharpai pullu (*Imperata cylindrica*)

Savannas are seen in Bibile, Monaragala, Mahiyanganaya, Wellawaya areas. These are distributed

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by periodic fires set by villagers for various reasons.

Patana

There are two types of 'Patana' found in Sri Lanka; wet patana grassland and dry patana grassland based on the rainfall pattern and soil of the site.

'Wet Patana' grass lands are found in areas above 1500 m from the sea level with mean annual rainfall of over 2000 mm. Temperature ranges from 5°C to 18° C. Mist, fog and frost are common in these areas and do not experience any dry period.

The grasses do not reach more than one meter height and are called tussock grasses which include the *Chrysopogon nodulibarbis* and *Arundinella villosa*. The animals include a large Sambar and Wild- boare populations and few leopards. Extensive "Wet Patana" are found only in Horton plains.

Dry patana grasslands are found in altitudes between 500 m to 1600 m. They receive a rainfall about 1400 mm to 2000 mm with a definite dry period. Temperature ranges from 18°C to 24° C. The vegetation is made up of grasses that grow up to 1-2 m height such as Pangiri mana (*Cymbopogon nardus*) and *Themeda/* (Pini bara tana)/ *Themeda tremula*.

"Dry Patana" is common on hill tops in in Hantana, Gampola, Welimada, and Haputale .

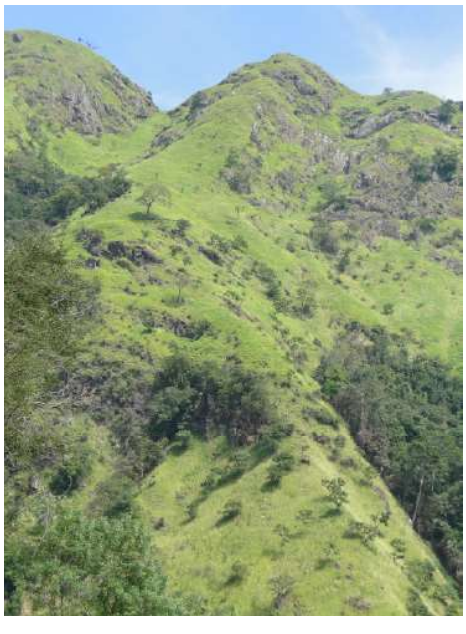


Figure 8.16 : Dry Patana



Figure 8.17 : Wet Patana

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Wetlands

Wetlands are simply habitats with permanent or temporary accumulation of water with associated plant and animals. According to Ramsar Convention, wetlands are defined as areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide not exceed six metres. The wetlands of Sri Lanka, which fit into the Ramsar definition, can be divided into three broad categories:

- Inland fresh water wetlands (e.g. rivers, stream, marshes, swamp forests and villus)
- Coastal wetlands (e.g. lagoons, estuaries, mangroves, sea grass beds, salt marshes and coral reefs)
- Man-made wetlands (e.g. tanks, reservoirs, rice fields and salterns)

Rivers and streams

Sri Lanka has an extensive network of rivers, which drains a total of 103 distinct natural river basins. The river basins originating in the wet highlands are perennial, while many of those in dry zone are seasonal. There is hardly any vegetation to be found in running water.



Figure 8.18 : Streams

Marshes and swamp forests

Inland freshwater marshlands are low lying areas which receive water through surface runoff, ground water seepage or flood water from rivers. These contain peat (partially decomposed organic matter), and water logged sticky clay soil. Many water birds, amphibians and fish species inhabit these areas. Plants that have adapted to grow in shallow stagnant water such as habarala (S) / Semaikilangu (T)- *Colocasia* species, Kekatiya (S)- *Aponogeton* spp, Reeds (E)/ Pan (S) species are abundant in inland marshlands.

Freshwater swamp forests are not common in Sri Lanka. It is a place where forest vegetation is inundated for a short period of time in the year as seen in the Waturana swamp at Bulathsinhala located in the 'kalu ganga basin'.

Villus

Generally villus are the flood plains of the reservoirs. Villu grasslands possess a special link with

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the wildlife in the area, especially elephants and bird populations. The vegetation is dominant with grasses (members of Poaceae) and sedges (members of Cyperaceae). Villu grasslands are located in areas such as Mahawelli flood plains, Wilpattu National park etc.



Figure 8.19 : Villu

Lagoons and Estuaries

Lagoons are coastal wetlands generally separated from the sea by a sand barrier. Eg: Negambo and Bundala lagoons. Estuaries are formed in places where rivers enter the sea and does not contain a sand barrier separating it from seas (e.g. Maduganga/Benthota). The daily tidal fluctuation is a characteristic of these places.

Mangroves

Mangroves ecosystem is an intertidal vegetation that covers fringes of the lagoons and estuaries. They share characteristics usually of saline/brackish water, loose soil and anoxic conditions. The vegetation is also exposed to intense sunlight.

Mangrove plants could be categorized into two groups; true mangroves that occur towards the boundary between sea and land and mangrove associates that occur more towards inland

Common true mangrove species are;

- S: kadol, T: kandal. (*Rhizophora* spp, *Bruguiera* spp,)
- S: Mas athu gas T: kannamaram (*Avicennia marina*)

Common mangrove associates are:

- S: Karan Koku, E: Golden leather fern (*Acrostichum aureu)* S:Katu- ikili E: Holly mangrove (*Acanthus ilicifolius*)

In order to protect from sunlight leaves of mangrove have a thick cuticle to reflect sunrays and/ or cut off excess radiation. Some have salt glands to secrete excess salts that are absorbed by the plants. There are special roots to intake atmospheric oxygen. Seeds of some mangroves germinate while attached to the mother plant (vivipary). This help the seed to establish successfully soon after falling from the tree.

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Mangroves provide a unique habitat mainly for many crustaceans and mollusks.

Mangroves are found in Puttalam, Batticaloa, Trincomalee and Galle.Bentota and Negambo.



Figure 8.20 : Mangroves

Salt marshes

These are marshlands restricted to the arid coastal regions of the country where soil dries up to form crystals of salts during the dry season. Low rainfall, high wind, high temperatures and loose sand blowing with salt are some of the major characteristics found in this ecosystem.

The vegetation has only few plant and animal species . Plants are short, contain fleshy succulent plant bodies One common plant species is *Salicornia* sp. Salt marshes are common in Puttalam, Mannar, Hambantota and Vakarai areas.



Figure 8.21 : Salt Marsh

Sea grass beds

In large lagoon areas with low wave action, the floor of the shallow sea is occupied by sea grasses. These are not grass species but appear like grasses due to the shape of leaves. E.g. Plant species such as *Halodule* spp and *Halophyla* spp. are common sea grasses found in Sri Lanka (especially

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from Kalpitiya to Mannnar. Sea grasses grow in a compact cluster and provides the sea bed a favourable habitat for breeding of many fish species. However, these areas are often disturbed by the fisheries activities as these are the parking areas of fishing boats.



Figure 8.22 : Sea grass bed

Coral reefs

Coral reefs are one of the natural wonders of the world. Coral reefs consist of calcareous structures secreted by a group of marine invertebrates. Coral reefs are famous for their spectacular beauty. They are considered as 'rain forests of the sea' because of their high productivity and high diversity of organisms inhabit them. Coral reefs can be seen in southern coast (Akurala to Tangalle), Gulf of Mannar, etc.

The reefs are habitats for a large number of fish species, invertebrates such as spiny lobsters, sea cucumbers, etc. Marine mammals and reptiles such as dolphins and sea turtles inhabit reefs occassionally.



Figure 8.23 : Coral reefs

Reservoirs

There are no natural lakes in Sri Lanka, but there are numerous ancient irrigation tanks mainly

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scattered in the lowland dry zone. Typical irrigation tanks include 'Parakrama samudra', 'Kala wewa', 'Minneriya wewa and tissawewa.

Aquatic plant species commonly found in the reservoirs are

- S: Manel, E: Water lily (*Nymphaea* spp),
- S: Nelum. T: Tamarai (*Nelumbo nucifera*)
- S: Kekatiya, T: Koddi (*Aponogeton* spp).

Often free floating invasive alien plant species such as *Salvinia* and Japan jabara (S)/ water hyacinth (E) also can be seen in these tanks.



Figure8.24 : Reservoirs

Sea shore

The long sea shore of Sri Lanka varies in nature. The most common sea shore type is sandy sea shores. The sea shore areas share the characteristics of high temperature throughout, and salt spray and high winds especially during the monsoon seasons. Most of the sea shore plants have adapted to these conditions. Examples for these plants are Muhudu Binthamburu (S), Beach Morning Glory (E)- atampu (T) (*Ipomea pescaprae*), Maha rawana revula (S), Ravannan meesai (T)- *Spinifex littoreus* .

The vegetation gradually become stable a distance away from the tide mark, with the stabilization of the soil. In these areas plant species such as wara (S)/ erukkalai (T)- (*Calotropis gigantea*), Wetakeiya (S)/ talai (T)- *Pandanus* spp etc. can be found.

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Figure 8.25 : Sea shore

Sand Dunes

Dunes are characterized by stunted or creping vegetation on large masses of sand. The sand dune structure is determined by wind speed and direction. Dunes are raised beaches of sand and are characteristic of certain coastal areas in the arid zone. Eg: near Mullativu, T



Figure 8.26 : Sand dunes

Biodiversity

Definitions

Biodiversity includes all forms of "life" on earth.

Biodiversity is the variability among living organisms from all sources including terrestrial, marine, and other aquatic ecosystems and their ecological interactions with the environment. Biodiversity is explained under three levels. They are genetic diversity, species diversity and ecosystem diversity.

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a. Genetic diversity

The basic component of biological diversity is the genetic variation that exists both within and among species. This genetic variation is the basis for evolution.

b. Species diversity

This is simply the variation that can be recognized among different species. It includes the number of species (=species richness) and their abundance.

c. Ecosystem diversity is the variety of habitats, living communities and ecological processes in the living world.

Ecosystem diversity is the largest scale of biodiversity, and within each ecosystem, there is a great deal of both species and genetic diversity incorperated.

Ecosystem diversity on a global scale would be the variation in ecosystems in large regions (biomes) such as deserts, forests, grasslands, wetlands and oceans whereas in smaller localized regions it can be explained by means of different ecosystems.

The importance and values of biodiversity

The individual components of biodiversity—genes, species, and ecosystems provide the human society with a wide array of goods and services. Genes, species, and ecosystems of direct, indirect, or potential use to humanity are often referred to as “biological resources”. Genes are used by plant breeders to develop new crop varieties. Many species are used as various foods, medicines, fibers, fuels and industrial products. This include food resources like grains, vegetables, fruits which are obtained from plant resources and meat, fish, egg, milk and milk products from animal resources. The biodiversity products can be harvested and consumed directly without passing through a formal market (non commercial goods). Example: fruits, fish, edible roots, leaves, nuts, flowers, meat, animal product like milk and honey, timber, firewood, fiber, wool, wax, resin, rubber, silk and decorative items, and traditional medicines, etc. Some products can be harvested and available through a formal market (commercial goods). Many industries such as food, textile, leather, silk, paper pulp are based on the direct use of biological resources. The ecosystems provide many services to us, such as air and water purification, erosion prevention and flood control.

Therefore the value of biodiversity is explained through its “goods” and “services” provided to humanity and sustenance of the environment.

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- **Environmental service value**: This is the most important services provided by biodiversity in maintaining critical environmental functions. E.g. Carbon dioxide fixation through photosynthesis, maintaining of essential nutrition cycles, maintaining water cycle and recharging of ground water, soil formation and protection from erosion, regulating climate by recycling moisture into the atmosphere, water purification, pollination, etc.
- **Recreational value**: There is a great aesthetic value provided by biodiversity. Natural landscapes at undisturbed places are a delight to watch and also provide opportunities for recreational activities and hobbies such as bird watching, photography etc. Biodiversity provides inspiration in artistic activities like poetry, painting, dance etc. It promotes eco-tourism, helps to generate revenue by designing of zoological, botanical gardens, national parks etc.
- **Ethical value**: This is the right of all living beings to live on this planet, humans have no right to decide which species should exist since we are just a small part of the greater creation of nature.
- **Educational/Scientific value**: knowledge about biodiversity helps in new scientific discoveries and technological innovations to find solutions to the problems we face today. E.g. learning of other animals like nematodes, rats and primates has helped in understanding human body and development of medicines, knowledge about how animals react before a natural disaster is helpful in disaster management, Interacting with biodiversity is proven to be helpful in developing creativity, relieving stress and development of personality.
- **Social/Cultural/Religious values**: Biodiversity can be important to different societies and communities due to unique reasons E.g. Some wetland sites are sacred to Aborigines of Australia, twenty eight species of trees are sacred for Buddhists, Bulls are considered to be an important part of Hindu culture.



Figure 8.27 : Recreational value of Biodiversity

Loss of biodiversity

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Animal and plant species die off all the time. It's how the biological world rolls. However, things have changed dramatically in recent decades. According to some scientists, Earth is currently in the midst of its sixth mass extinction. The last major extinction occurred some 65 million years ago, when a large asteroid slammed off the coast of Mexico, killing off the dinosaurs and most everything else. Today, scientists say the extinction rate is as much as 1,000 times faster than what should be natural rate would be.This is solely because of the very high and negative impact of humans on biodiversity due to high population and development. Virtually all of Earth's ecosystems have been dramatically transformed through human actions, for example, many mangrove and coral reef areas have been lost. Within groups of conifers, cycads, amphibians, birds, and mammals up to 50% of species are threatened with extinction, according to the IUCN Red List. Within many species groups, such as amphibians, African mammals, and birds in agricultural lands, the majority of species have faced a decline in the size of their population, in their geographical spread, or both.

Threats to biodiversity

Habitat loss/fragmentation: Humans supplant natural ecosystems to grow food, harvest materials, and build our settlements. These actions alter or eliminate the conditions needed for plants and animals to survive. When natural habitats are converted into other human uses such as agriculture or built up area they are no longer able to support the species present in the original habitat. This result in the displacement or destruction of biodiversity. E.g. deforestation, filling of wetlands

Mass scale destruction of Mangrove in lagoons such as Negambo and Puttalam due to establishment of prawn culture destructed the biodiversity of mangroves in these areas.





Figure 8.28 : Habitat loss

When habitats are divided into fragments due to establishment of man built structures such as roads, the animals and plant species are forced to occupy smaller area in a crowded manner which

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is harder for biodiversity to sustain as in previous habitat conditions.

Overexploitation: Harvesting or exploiting biodiversity products in a manner and a rate which it cannot recover within the periods of exploitation leads to danger of biodiversity being completely lost.

E.g. Over collection of indigenous medicinal plants from forests in Sri Lanka for export such as Kotalahimbutu (S)/ - (*Salacia reticulata*). Export of sea cucumber for medicinal purposes from Sri Lankan shores. *Ebony*(E)/Kaluwara (S), Karun-kaali (T)- (*Diospyrus ebanum)* is threatened due to over exploitation during the colonial period. Ebony has a very slow growth rate and take many years to grow. Intense commercial fishing has led to over fishing threatening decline of food fish like Tuna and Cod in world's oceans.

Pollution: Pollution simply means addition of unwanted materials to air, water, soil. Due to extensive use of agrochemicals that wash away with rain water into the water bodies make the water rich in nutrients (eutrophication) resulting in algal blooms. Algal blooms create oxygen-depleted zone in aquatic ecosystems and greatly reduce the populations of fish and other aquatic species.

Uses of synthetic fertilizers for tea in montane areas also has resulted pollution of rivers in many down stream areas affecting the water quality and making it unsuitable for human use

Releasing of Sulphur dioxide (SO_2) and nitrous oxide (N_2O) gasses react with water and make the rain water acidic resulting acid rains. Acid rains caused by air pollution contributes to the death of trees killing many buds, leaves and the seedlings and causing damage to the plant roots.



Figure 8.29 : Water pollution affects aquatic biodiversity.

Introduction of invasive alien species: Invasive alien species are alien (exotic) plants and/or animals whose introduction and spread outside their natural geographic range threaten native biodiversity. Alien invasive species compete against or prey on native species, which can lead

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to their extinction. Once introduced , for a considerable period of time, they may lack natural predators in the new environment. This is a great opportunity for them to reproduces successfully and spread without limits to take over the environment. They can transport disease, out-compete native species, alter food chains, decrease biodiversity, and even change ecosystems properties by altering soil composition or creating habitats that encourage wildfires. E.g. Alien invasive species such as Lantana(E)/ Gandapana (S) / Nayunni (T)-(*Lantana camara*) does not allow germination and seedling growth of many other plants as it produces toxins which are added to soil through leaf litter. Extensive spread of Guinea grass(E)/ Gini- thana (S)/ Ginipullu (T) (*Panicum maximum*) in especially in the dry pathana areas facilitates fire due to its dry biomass during drought seasons.



Figure 8.30 : *Lantana* (Gandapana)

Climate change: Climate change is predicted to be the greatest long-term threat to biodiversity. Increasing temperatures and temperature extremes, increasingly severe droughts, rising sea levels, possible decrease in rainfall, regional flooding and reduced water availability change ecosystems. Many species will not be able to adapt themselves fast enough to keep up with the coming changes driving them to extinction or being endangered. Evidence suggests that the warming of the past century already has resulted in marked ecological changes, such as changes in growing seasons of crop species, distribution ranges, and patterns of seasonal breeding of animals.

Biodiversity hot spots- The areas with a high concentration of endemic species facing exceptional levels of threats have been described by Myers in 1988 as biodiversity hotspots. As a whole Sri Lanka has a high degree of endemism. Sri Lanka (wet zone of Sri Lanka) together with Western Ghats of India is considered as one of the Biodiversity hot spot in South Asian region.

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Extinction of species

- Existing species have to make room for new species either by changing themselves or by becoming extinct. Therefore natural extinction has to be recognized as part of the evolutionary process.
- The rate of evolution has been generally higher than that of extinction. Therefor there has been an increase in Biodiversity over time.
- Extinction is the elimination of the last member of a species from the earth.
- With the growth of human population and civilization, mankind has greatly increased the rate of extinction.
- The earth today is dominated by humans and no ecosystems on earth's surface is free from human influence.
- In general it is estimated that about 5-10% of the species may face extinction within the next 30 years.

Not only a species but also family or genus or a sub species (taxon) can become extinct if there had been a continuous pressure for its survival. The Red Data book published by the International Union for Conservation of Nature (IUCN) provides a list of threatened species and definie extinct and threatened species as follows.

EXTINCT (EX)

A taxon is Extinct when there is no reasonable doubt that the last individual has died. E.g. Dodo (Lived in Mauritius), Wooly Mammoth (Lived in North America), Legume (*Crudia zeylanica*)



Figure 8.31 : *Dodo*

Extinct in the wild (EW): A taxon is extinct in the wild when it is known only to survive in cultivation, in captivity or as a naturalized population (populations) well outside its natural habitat. E.g; Giant tortoise of Seychelles

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Different categories of threatened organisms:

A species is said to be 'threatened' when it is about to leave the world. Threatened organisms are described under three categories. Critically endangered, endangered and vulnerable. The red data book has indicated other categories such as nearly threatened, least concern etc , but these are not considered as a ' threatened species.

CRITICALLY ENDANGERED (CR)

A taxon is critically endangered when the best available evidence indicates that it is facing an extremely high risk of extinction in the wild. Marbled rock frog (E)/Dumbaragalparadiyamadiya (S) and Maha madu (S)/ Ratchatha madupunai (T)- can be listed as one example to represent animals and plants critically endangered in Sri Lanka.

ENDANGERED (EN)

A taxon is endangered when the best available evidence indicates that it is facing a **very high risk** of extinction in the wild. Eg: Etha / Aliya (S)/ Yanai (T), Elephant (E), Wesak Orhid (S/E/T)

VULNERABLE (VU)

A taxon is vulnerable when the best available evidence indicates that it is facing a high risk of extinction in the wild. Punchi Leena (S)/ Dusky-striped, jungle squirrel (E) and Buttercup (E)- are among the vulnerable species found in Sri Lanka .

Endemic species:

An endemic species is a species that is confined to a particular area or country, and not found growing **naturally** anywhere else in the world.

Two examples for plant species endemic to Sri Lanka are:

Dipterocarpus zeylanicus(S: Hora, T: Ennai)

Garcinia quaesita (S: Goraka, T: Gorakappuli)

Examples of two animal species endemic to Sri Lanka are;

Puntius nigrofasciatus (E: Black ruby barb, S: Bulathhapaya, T: Veddiyan)

Loris tardigradus (E: Slender loris, S:Unahapuluwa, T:Thevangu)



Figure 8.32 : Black rubby barb

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Indigenous species: A plant or animal species that occurs in its historically known natural range and that forms part of the natural biological diversity of a particular geographic area.

Examples from Sri Lankan indigenous species are:

Lula(S)/ Snake head (E)/ Viral (T)

Kitul(S)/ Thippilipanai (T)

Exotic (alien) species: A species that has been introduced from another geographic region to an area outside its natural range due to human activities. The introduction of species can be intentional or accidental. The following examples represent examples for intentional and direct introductions. Accidental introductions are indirect introductions often considered as 'contaminations' of direct introductions.

Tilapia (S/E), Japan meen (T) for inland fishery industry.

Rubber (S/T), Rupper (T) for plantation industry.



Figure 8.33: Tilapia

Migratory Species: Migration refers to the act of moving from one place to another in a manner that is seasonally determined and predictable. Migration takes place so that organisms can avoid unfavorable environmental conditions that limit breeding.

Suduredi hora(S)/ Indian fly catcher(E) /Inthianeepidipan (T)

Avichchiya (S)/Indian pitta- (E)/ Aarumanikuruvi (T)



Figure 8. 34 : Indian fly catcher

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Relict species: The remnants of a once widespread species, which are now found in very restricted or isolated areas, due to fact that areas in which these species are found is lost in many parts of the world.

The Tuatara is an example which lives only on a few small islands of New Zealand. - *Ichthyophis* a primitive ancient legless amphibians with a worm like body. *Lingula* found in Tambalagamuwa bay in Trincomalee is an example for a relict species in Sri Lanka.

Flagship species: Flagship species is a species chosen as a symbol or icon to represent an ecosystem in need for conservation. These species are chosen for their vulnerability, attractiveness or distinctiveness in order to bring about support and acknowledgement from the public at large. Thus, the concept of a flagship species is that the publicity given to few key species, will help the conservation of entire ecosystem and all species contained therein.

The Bengal tiger of India, the giant panda of China, Blue magpie of Sri Lanka- (S: Lanka kehibella, T: Neelavudalperumkuyil) are examples.



Figure 35: Blue magpie

Keystone species: These are species that play a very important role in the stability and functioning of a system. If that species is removed the system tends to collapse. e.g. Planktons of a pond

Invasive alien species- : Invasive alien species are alien (exotic) plants and/or animals whose introduction and spread outside their natural geographic range threaten native biodiversity. Invasive alien species take the advantage of 'human disturbances' in the environment to establish and spread themselves. Their capability to tolerate wide range of environment conditions and high reproductive output help them to easily and successfully expand their populations. Although only a small percentage of alien species become invasive they damage biodiversity (ecosystem, species and genetic levels) in everywhere they invade and alter the services and ecosystem

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values of the introduced environment. Therefore invasive alien species are considered as a major cause for depletion of biodiversity and environment degradation. The following examples represent invasive alien animal and plant species in Sri Lanka .

Kalutara Golubella (S)/ Giant African land snail (E) was introduced to Sri Lanka as a contamination of soil brought with some other plants. The soil contained eggs of the snail. Japan jabara (S)/ Water Hyacinth (E)/ Kulavazhai (T) was introduced to the country in nearly 110 years ago as an ornamental plant not knowing that it will become a serious invader afterwards.

Conservation

A principal goal of conservation is to ensure the long term survival of as many species as possible. Species that are in danger of extinction have to be specially protected and steps should be taken to ensure their continued reproduction and survival. Conservation can be done in two ways.

In-situ* conservation*: The species is protected and its reproduction facilitated in its natural habitat. Basically a large enough population and adequate, appropriate, habitat space has to be ensured. E.g. National parks such as Yala and Minneriya national parks, Forest reserves such as Kanneliya, Piduruthalagala

Ex-situ* conservation*: The species is taken out of its natural habitats, and looked after in places where its survival and reproduction are ensured.
Zoological gardens and Botanical gardens of a country play a key role in *ex-situ* conservation.

Global Warming and Climate Change

Definition

Climate change refers to a significant, long-term changes of climate attributed directly or indirectly to human activity, that alters the composition of the global atmosphere in addition to natural climate variability observed over comparable time periods (United Nations Framework Convention on Climate Change /UNFCCC, 2011). In the meantime, the Intergovernmental Panel on Climate Change (IPCC) defined the climate change as “a statistically significant change in the state of the properties of the climate which persists for an extended period, typically decades or longer". This definitions refer to any change in climate over time, whether due to natural variability or as a result of human activity.

Global warming is the increase the average temperature of the Earth’s surface (atmospheric and

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oceanic temperatures) due to enhanced greenhouse effect [or Greenhouse gasses, (GHG)].

Climate change includes warming and the effects arisen due to warming such as melting glaciers, extremes of rains or more frequent drought etc. It can say in another way, global warming is one symptom of much larger problem of climate change.

Contributing factors on global warming and climate change

1. Increase of the emission of CO_2 and other greenhouse gasses (GHG) due to human activities

1.1 Carbon Dioxide (CO_2) is produced by burning of organic matters and it is the most common greenhouse gas to contribute to the global warming and climate change events. Burning of fossil fuel (for running vehicles, electricity generation, industries etc.) is the main cause of the emitting of CO_2 and burning of forests, solid waste are the other main causes contributing the CO_2 emission.

1.2 Methane (CH_4) is another greenhouse gas which has a higher global warming potential. Main emitting sources are anaerobic decomposing (at manure management and waste management), cattle farming, paddy cultivation and enteric fermentation. Even though the CH_4 is a greenhouse gas with high global warming potential, it is less abundant in the atmosphere compared to CO_2.

1.3 Nitrous oxide is (N_2O) another greenhouse gas also with higher global warming potential which is mainly released as by product of fertilizer production and use, other industrial processes, the combustion of certain materials (biomass), nitric acid production, and fossil fuel combustion in internal combustion engines. Nitrous oxide can remain very long time in the atmosphere.

1.4 Manmade industrial gases, namely, Perfluorocarbons (PFCs), Hydrofluorocarbons (HFCs) and Sulfur Hexafluoride (SF_6) are also considered as greenhouse gases with a very high global warming potential.

1.5 Black carbon or the carbon particles which are suspended in lower atmosphere also identified as another cause for global warming. Those particles released as a result of the incomplete combustion of fossil fuels, and other organic matters. These particles are extremely small, ranging from 10 µm 2.5 µm. These Black Carbon have an enormous ability to absorb heart and it cause to increase the air temperature. These particles have ability to absorb heat than CO_2.

Carbon monoxide (CO), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), tropospheric (ground level) ozone (O_3), and nitrogen oxides (NOx) are also considered as gases which have indirect radiative forcing effect. Water vapor, non-methane volatile organic compounds (NMVOCs), and Aerosols are also considered as GHGs.

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2. **Deforestation and decrease of the vegetation cover of the world**

   Deforestation is another main cause for global warming. At present the conversion of tropical forest lands to commercial agricultural plantations like palm oil contributes to the mass deforestation all over the world. Forests remove carbon dioxide from the atmosphere and fix through photosynthesis (carbon sequestration). Deforestation reduce the carbon sequestration capacity, carbon capture on the planet (i.e soil carbon) and increase the atmospheric CO_2 concentration.

3. **Destroying the large quantity of phytoplankton by UV radiation due to the deple tion of the ozone layer.**

   This is also similar to deforestation. Phytoplankton which live in warm seas are very useful to maintain the Oxygen and carbon dioxide balance. Normally the carbon absorption capacity of phytoplankton are higher than terrestrial plants. Though the phytoplankton are unicellular microscopic organism they have been distributed in large area and they were responsible for the 60%-70% of absorption of atmospheric carbon. Due to depletion of the ozone layer UV radiation which comes from sun can destroy this kind of tiny organisms and may cause to reduce the CO_2 absorption capacity of oceans and increase the global temperature.

Effects of the global warming and climate change

1. **Sea level rising**

   Scientists predict an increase in sea levels worldwide due to the melting of two massive ice sheets in polar regions and thermal expansion of water. However, many island nations around the world will experience the effects of sea level rise.

2. **Extreme weather events**

   Occurrence of extreme weather events such as prolong drought, intensive rainfall and its effects such as floods and landslides and storms have been increased for last decades was considered as a negative impact of climate change. It causes loss, damages and disasters.

3. **Less production of foods (Threat to food security)**

   Due to unexpected extreme weather condition the crop production will be getting loss around the world. It may be due to heavy rains or the severe droughts.

4. **Degradation of coral reefs**

   The corals bleaching and degradation due to rises in sea temperature is a severe danger for whole ocean ecosystems and many other species which lives on coral reefs for their survival. According to the recent reports coral populations will collapse by 2100 due to increased temperatures and ocean acidification and its effects

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5. **Increase the insect population.**
   Due to increase of the insect population some diseases such as mosquito-borne malaria and dengue will spread than present. As well the over growth of the insect populations will be a massive threat to food production

6. **Loss of Biodiversity**
   Climate change and global warming may cause reduced biodiversity, Ecosystems structure will be change and some species may move from their present range of occurrence and may survive while some others will not be able to move and could become extinct.

Ozone Layer depletion

Most ozone particles are concentrated in the region of stratosphere in between 10-50 km of the atmosphere, and this layer is identified as the 'Ozone Layer'. This is very much important to protect living things from burning from too much ultraviolet (UV) radiation emitting from the sun

Naturally, the total concentration of ozone in the stratosphere remains relatively constant with the concentration of 300 to 350 Dobson Units (D.U). Ozone depletion is described when levels fall below 200 D.U due to man-made ozone depleting chemicals (ODCs) as in the stratosphere over the South Polar Region. This thinning of the ozone layer refers as Ozone hole.

Contribution factors for the Ozone layer depletion

Ozone depletion occurs when the natural balance between the production and destruction of stratospheric ozone is disturbed or lost. This is mainly due to chlorine and bromine released from man-made compounds called ozone depleting substance such as CFCs, MeBr, Helene and HCFC.

Effects of the Ozone Layer depletion

Depletion of the ozone layer in the stratosphere will increase penetration of solar UV-B radiation which is likely to have profound impact on human health with potential risks of eye diseases, skin cancer and infectious diseases.

It is a known fact that the physiological and developmental processes of plants are affected by UV-B radiation.

UV-B radiation is likely to result in changes in species composition (mutation) thus altering the bio-diversity in different ecosystems. It directly destroyed phytoplankton which form the foundation of aquatic food webs in the sea and it cause to reduce the composition of food web in the sea ecosystem.

UV-B can also cause damage to early development stages of fish, shrimp, crab, amphibians and other animals.

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Desertification

Desertification means "process of land degradation in arid, semi-arid and dry sub-humid areas resulting from various factors, including climatic variations and human activities."(UNCCD, Paris, 1994),

Contribution factors for desertification

The main driving forces of desertification can be separated into climate variations and human activities, according to the UNCCD definition. It is known that human activities have a great influence in the climate change and it has caused desertification

Deforestation is another main factor to decertification because of it is directly influencing to reduce the rainfall, precipitation, soil humidity and water recharge of underground reservoirs

Over-exploitation of water and soil, uncontrolled mining and excessive use of agro-chemical products and as well as poor land management practices also caused to desertification.

Effects of the Desertification

Decrease ecosystems services and reduced biodiversity in affected areas. Decreasing of the vegetation cover induce water scarcity, destroys habitats of animal and plant species and reduces agricultural activities mainly the growth of crop species. It may effect to the food security of the people as well as for the animals. The process of desertification presents a serious impact on the human well-being and health of the people living in the areas affected by droughts and land degradation

Due to the desertification carbon storage capacity of plants and soils will also be reduced in the long run.

Acid rains

Acid rain is one of the most serious global environmental issue which, emerged due to air pollution mainly due to Sulphur dioxide (SO_2) and oxides of nitrogen. These pollutants are released from anthropogenic activities such as combustion of solid waste, fossil fuels in thermal power plants and vehicle engines.

Acid rain is a commonly used term for acid deposition, which includes rain, snow, fog and dry particles that fall from the sky. Uncontaminated precipitation is also slightly acidic and normal rain water may be about a 5.6 on the pH scale because carbon dioxide is dissolved and carbonic

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acid is formed. Acid rain may show a lesser pH.

Contributing Factors of Acid Rains

Mainly caused by the release of sulfur dioxide and nitrogen oxides into the atmosphere due to burning of fossil fuels, where they are converted into sulfuric and nitric acid respectively.

Effects of the Acid Rains

Acid rain directly causes severe damage to building structures and marble statues

Increases the acidity of fresh water ecosystems such as streams, lakes, and marshes and it causes to change the structure and the composition of above fresh eater ecosystems.

Cause to destroy soil organisms and loss the soil fertility.

It cause to leach metals such as copper, aluminum, and some heavy metals such as lead and mercury in the soil and into runoff as well as drinking water

Acid rain causes significant damage to forests. It directly affects trees and other small plants which are important to the ecosystem

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)-1975

The aim of CITES is to ensure that international trade of specimens such as horns and skins of wild animals and whole plant or parts of plants does not threaten their survival. According to CITES, the export of some species (as listed) requires prior permission and presentation of an export permit. An export permit shall be granted only if export of that species will not be detrimental to that species survival. Some examples from Sri Lanka are skin of Sri Lankan Leopard (S: Kotiya, T: Siruththaipuli), plants of *Cycas* - Madu (S) / Madupanai (E).

Conventions related to conservation of the environment

Convention on Biological Diversity (CBD)- 1992

The Convention on Biological Diversity (CBD), known informally as the Biodiversity Convention, addresses all aspects of Biodiversity conservation.

It has three main goals:

- the conservation of biological diversity (or biodiversity)- e.g. conservation of genetic materials and species and ecosystems.
- the sustainable use of components of biological diversity- e.g. imposing limits to control overexploitation.

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- The fair and equitable sharing of benefits arising from genetic resources- e.g. exchange of genetic material, species between countries without conflict.

The Convention on Wetlands (Ramsar Convention)-1971

The Convention on Wetlands, called the Ramsar Convention, provides the framework for the conservation and wise use of wetlands and their resources. In Sri Lanka, there are six wetlands declared as Ramsar sites: Anawilundawa, Bundala, Kumana, Maduganga, Vankalai and Wilpattu.



Figure 8.36 : Anawilundawa wetland

International Convention for the Prevention of Pollution from Ships (MARPOL)- 1973

International Convention for the Prevention of Pollution from Ships (MARPOL) is the main international convention covering prevention of pollution of the marine environment by ships from operational or accidental causes. The objective of this convention is to preserve the marine environment in an attempt to completely eliminate pollution by oil and other harmful substances and to minimize accidental spillage of such substances.

Montreal Protocol- 1989

Montreal Protocol on Substances that Deplete the Ozone Layer is an international treaty designed to protect the ozone layer by phasing out the production of numerous substances that are responsible for ozone depletion.

Kyoto Protocol-1977

The Kyoto Protocol is an international agreement linked to the United Nations Framework Convention on Climate Change (UNFCCC), which commits its Parties by setting internationally binding green house gases (GHGs) emission reduction targets. In 2012 Doha, Qatar, the "Doha Amendment to the Kyoto Protocol" was adopted. During this commitment period (2013-2020)

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parties are committed to reduce GHG emissions by at least 18 percent.

Basel Convention -1989

The Basel Convention on the Control of trans boundary movements of hazardous wastes and their Disposal intends to protect human health and the environment against the adverse effects of hazardous wastes. Its scope of application covers a wide range of wastes defined as "hazardous wastes" based on their origin and/or composition and their characteristics, as well as two types of wastes defined as "other wastes" such as waste containing heavy metals such as Lead (Pb) and Mercury (Hg) and wastes from hospitals which contains contaminants.

Environmental Legislation and Policies in Sri Lanka

The Constitution of the Democratic Socialist Republic of Sri Lanka has provision for the protection, preservation and improvement of the environment. The government of Sri Lanka has formulated many legislation and policies focused on environmental conservation and most of them are revised from time to time to accommodate updates. Legislation is a set of regulations for which stakeholders is given a penalty when it is violated. Policy is a set of practices that is followed by stakeholders and there is no such penalty imposed when it is not practiced.

Fauna and Flora Protection Ordinance and National Environmental Act (NEA) are Some examples for key legislation on environment conservation.

Fauna and Flora Protection Ordinance (FFPO)

The Ordinance, No.2 of 1937, and subsequent amendments, make provisions for the protection of wildlife and flora in the country. The authority responsible for enforcing this law is the Department of Wildlife Conservation (DWLC). Operation and management of National Parks, Strict Natural Reserves, Jungle Corridors and Sanctuaries are conducted under FFPO.

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Notes:

This is to acknowledge that some of the diagrams used in this book have been taken from various electronic sources using internet . This book is not published to make profit and sold only to cover cost.

The resource book is prepared according to the subject content and learning outcomes of the G.C.E. (A.L) Biology new syllabus which is implemented from 2017.

The content of this Resource book declares the limitation of the G.C.E. (A.L) Biology new syllabus which is implemented from 2017.

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G.C.E. (Advanced Level)
Biology
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Message from the Director General

The National Institute of Education takes opportune steps from time to time for the development of quality in education. Preparation of supplementary resource books for respective subjects is one such initiative.

Supplementary resource books have been composed by a team of curriculum developers of the National Institute of Education, subject experts from the national universities and experience teachers from the school system. Because these resource books have been written so that they are in line with the G. C. E. (A/L) new syllabus implemented in 2017, students can broaden their understanding of the subject matter by referring these books while teachers can refer them in order to plan more effective learning teaching activities.

I wish to express my sincere gratitude to the staff members of the National Institute of Education and external subject experts who made their academic contribution to make this material available to you.

Dr. (Mrs.) T. A. R. J. Gunasekara
Director General
National Institute of Education
Maharagama.

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Message from the Director

Since 2017, a rationalized curriculum, which is an updated version of the previous curriculum is in effect for the G.C.E (A/L) in the general education system of Sri Lanka. In this new curriculum cycle, revisions were made in the subject content, mode of delivery and curricular materials of the G.C.E. (A/L) Physics, Chemistry and Biology. Several alterations in the learning teaching sequence were also made. A new Teachers' Guide was introduced in place of the previous Teacher's Instruction Manual. In concurrence to that, certain changes in the learning teaching methodology, evaluation and assessment are expected.

When implementing the previous curricula, the use of internationally recognized standard textbooks published in English was imperative for the Advanced Level science subjects. Due to the contradictions of facts related to the subject matter between different textbooks and inclusion of the content beyond the limits of the local curriculum, the usage of those books was not convenient for both teachers and students.

As this book is available in Sinhala, Tamil, and English, the book offers students an opportunity to refer the relevant subject content in their mother tongue as well as in English within the limits of the local curriculum. It also provides both students and teachers a source of reliable information expected by the curriculum instead of various information gathered from the other sources.

This book authored by experienced subject teachers and subject experts from the universities is presented to you followed by the approval of the Academic Affairs Board and the Council of the National Institute of Education. Thus, it can be recommended as a material of high standard.

Dr. A. D. Asoka De Silva
Director
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09

Microbiology

Nature of microorganisms

Microbiology is the study of organisms that are too small and are not visible clearly to the naked eye or un-aided eye when they exist individually. These organisms are referred to as microorganisms. Microorganisms include bacteria, archaea, cyanobacteria/ Blue green bacteria (BGB), fungi and protists. Mollicutes such as mycoplasmas and phytoplasmas, viruses, viroids and prions are also studied under microbiology.

Microscopic nature of microorganisms

In general, microorganisms are less than 0.1 mm in size and cannot be observed with unaided eye. Therefore, they must be observed with a microscope. Microorganisms and their structural components are measured in micrometers and nanometers.

1 micrometer (μm) = 10^{-6} m

1 nanometer (nm) = 10^{-9} m

Some microorganisms are more readily visible than other because of their larger size.

Ubiquitous nature of microorganisms:

Microorganisms are ubiquitous on earth. They are found in water, soil, air and interior and exterior surfaces of other organisms. Marine and freshwater microorganisms form the basis of food chain in oceans and freshwaters. Some of them do photosynthesis and are primary producers in aquatic environments. Soil microorganisms help recycling of chemical elements between soil, water, air and living organisms. Microorganisms suspended in air as bioaerosols, have the opportunity to travel long distances with the wind current and precipitate. Pathogenic bioaerosols, cause opportunities for disease spreading. Only a minority of microorganisms that associate with other organisms such as plants, animals and human are pathogenic. Majority of them are advantageous or harmless. However, all viruses are harmful to the organisms they attached to. Some microorganisms are capable of inhabiting extreme environmental conditions that are unfavorable or even lethal for other organisms. Such microorganisms are known as extremophiles. Extremophiles have been found inside the Earth's crust, deep sea at high pressures, extreme acidic or extreme basic conditions, hydrothermal vents, frozen sea water and anaerobic conditions. Extremophiles are classified according to the conditions in which they grow.

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Table 9.1: Types of extremophiles

Type of Extremophile	Condition
Thermophiles	high temperatures
Psychrophiles	low temperatures
Acidophiles	acid pH
Alkaliphiles	basic pH
Halophiles	require NaCl
Barophies	high pressure

Some of these extreme environments consist of more than one extreme condition. For example,

- Many hot springs are acidic or alkaline in nature at the same time
- Deep seas are cold and remain in high pressure.

Microorganisms live in such environments are adapted to live with more than one extreme condition.

High growth rate of microorganisms:

Rates of growth and reproduction of microorganisms are high. Microorganisms possess a high surface area/volume ratio due to their smaller size. This means that they have large surface area available for exchange of materials from external environment. As a result, flowing rate of materials in to the inside of cells and the exit of waste materials to the outside of the cells increases and results in high metabolic rate. Therefore, average generation time or the time required to double the population of microorganisms is relatively less.

Morphological, nutritional, and physiological diversity of microorganisms:

Microorganisms possess diverse morphological forms. Bacteria possess diversity in their shapes, basically three distinct shapes; rod shape/ bacillus, spherical shape/ coccus and spiral shape/ spirillum. The coccus bacteria may arranged in different forms; coccus/ monococcus, diplococcus, streptococcus, staphylococcus, tetrads and sarcinae. Bacillus bacteria may arranged in to either diplobacillus or streptobacillus. Spiral bacteria may arranged in to either vibrio or spirillum or spirochete.

Cyanobacteria exhibit a great variety of shapes and arrangements, unicellular to long multi cellular filaments. Multi cellular Cyanobacteria may appear as either filamentous or non-filamentous. Filamentous appear as chains and the non-filamentous appear as clusters or colonies forming spherical, cubical, square or irregular shape. Two morphological varieties are found in viruses based on their symmetry of protein coats; Icosahedral and helical. In fungi, some of them are unicellular and others multicellular, consists of a mass of fine tubular branching threads known as hyphae, collectively form mycelium. Hyphae may be septate or aseptate. Prions are smaller proteinaceous particles. Unicellular protists possess wide range of morphological diversity. Mollecutes are pleomorphic (variable shapes).

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Microorganisms show a diversity of nutritional types. Based on the sources of carbon and energy, nutritional types of microbes are classified. There are four major nutritional types seen among microorganisms; chemoautotrophs, chemoheterotrophs, photoautotrophs and photoheterotrophs. Based on the utilization of O_2(g), microorganisms can be classified in to four physiological groups; obligate aerobes, obligate anaerobes, facultative anaerobes and microaerophiles. Some microbes capable of fixing atmospheric molecular nitrogen, show physiological diversity; free-living nitrogen fixing microbes and symbiotic nitrogen fixing microbes.

Types of microorganisms

1. Bacteria

Bacteria (singular, bacterium) are single-celled (unicellular) prokaryotic organisms. They show different morphological forms and arrangements. The most obvious structural feature of bacteria is the shape of individual cells. There are three basic shapes.

1. Spherical; coccus (plural- cocci)

2. Rod shape; bacillus (plural-bacilli)

3. Spiral shape; (plural- spirilli)

During cell division, cells can remain attached to each other and form different forms of cell arrangements.

1. Different forms of cell arrangement of coccus bacteria (Figure 9.1)

Coccus	Cells divide in one plane. Divided cells detach from each other after cell division
Diplococcus	Cells divide in one plane. Divided cells remain in pairs
Streptococcus	Cells divide in one plane. Divided cells remain attached in chain like pattern
Tetrad	Cells divide in two planes producing 4 cells remain attached together
Sarcinae	Cells divide in three planes and remain attached in groups of 8 cells
Staphylococcus	Cells divide in multiple planes and form grape like clusters of cells

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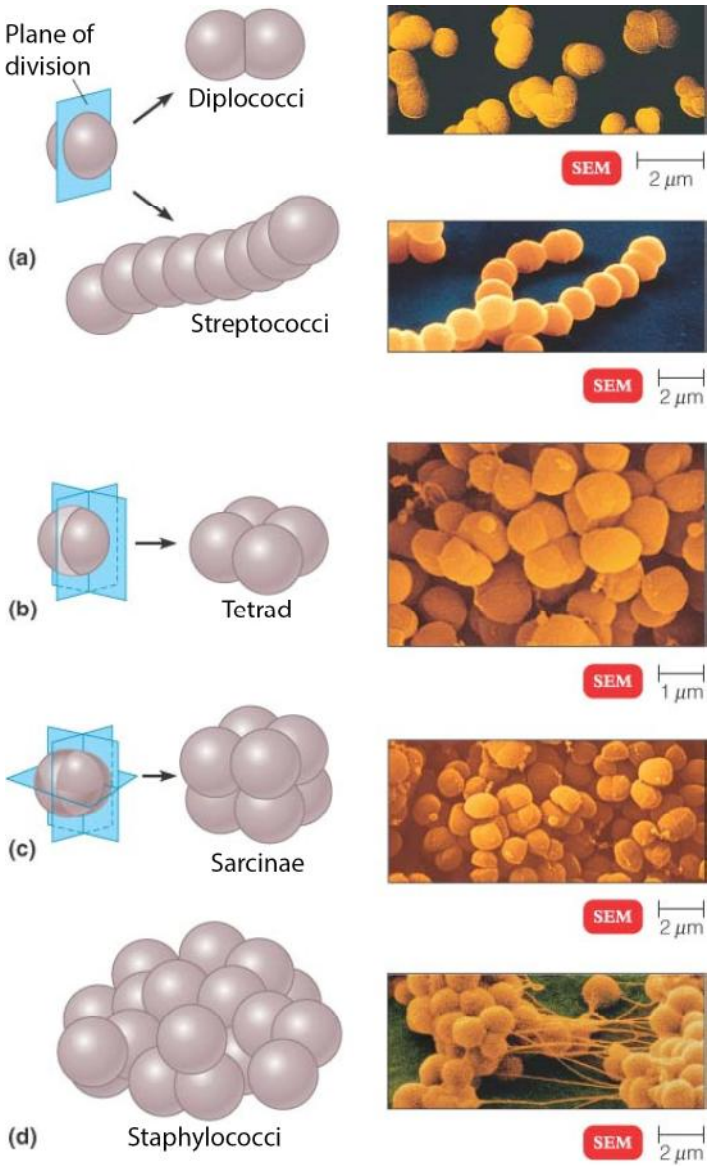


Figure 9.1. Cell arrangement of coccus bacteria. Shown are diagrammatic representations of planes of cell division and various cell arrangements (left) and their scanning electron microscopic (SEM) views (right). Retrieved from Microbiology: An Introduction, 11th edition, by Tortora, Gerard J.; Funke, Berdell R.; Case, Christine L. Pearson Education Ltd (2016).

2. Different forms of cell arrangement of bacillus bacteria (Figure 9.2)

Bacilli divide only across their short axis. Therefore, there are few cell arrangement forms.

Single bacillus	Single rod
Diplobacillus	Remain in pairs after cell division
Streptobacillus	Occur in chains after cell division

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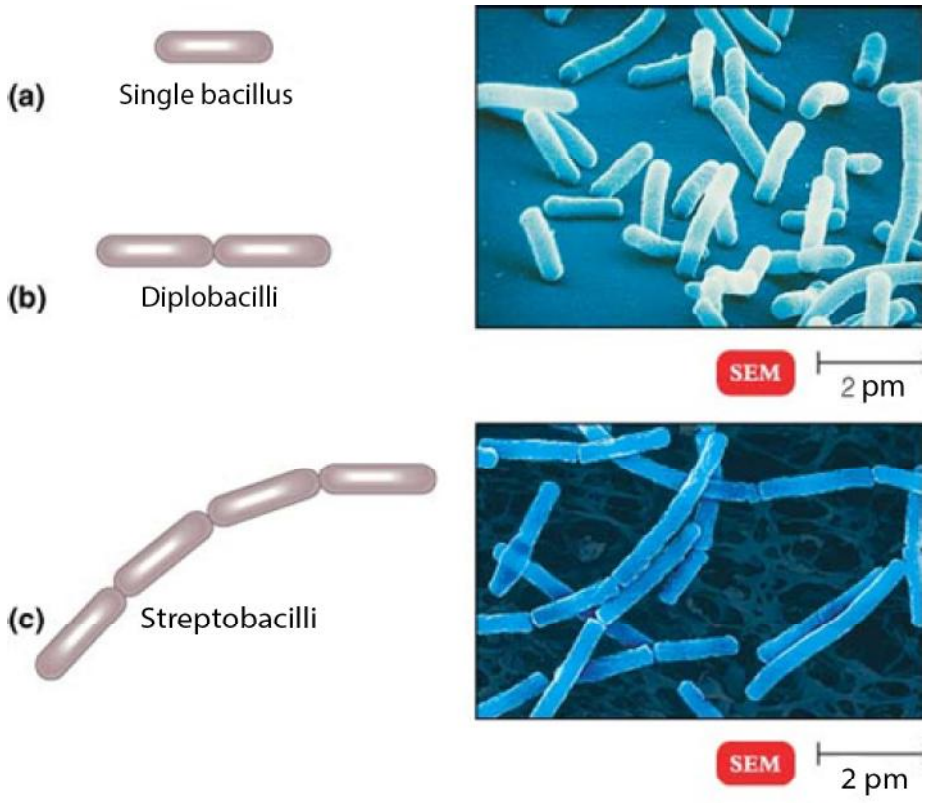


Figure 9.2. Cell arrangement of bacillus bacteria. Shown are diagrammatic representation of (left) and scanning electron microscopic (SEM) views of cells (right). Retrieved from Microbiology: An Introduction, 11th edition, by Tortora, Gerard J.; Funke, Berdell R.; Case, Christine L. Pearson Education Ltd (2016).

3. Different forms of cell arrangement of spiral bacteria (Figure 3)

Spiral bacteria have one or more twists, they are never straight.

Vibrio	Bacteria look like curved rods
Spirillum	Helical shape, like a corkscrew and rigid body
Spirochete	Helical shape, flexible body

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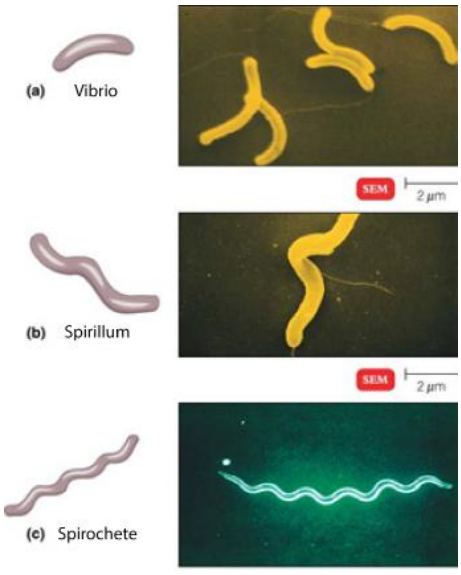


Figure 9.3: Shapes of spiral bacteria. Shown are diagrammatic representation of shapes (left) and scanning electron microscopic (SEM) views of spiral bacteria (right). Retrieved from Microbiology: An Introduction, 11th edition, by Tortora, Gerard J.; Funke, Berdell R.; Case, Christine L. Pearson Education Ltd (2016).

Bacteria show a diversity of nutritional types. Four major nutritional types can be identified among bacteria. They are classified based on the source of energy and carbon.

Nutritional type	Source of energy	Source of carbon	Example
Photoautotrophs	Light	Carbon dioxide (Inorganic Carbon)	Purple sulfur and Green sulfur bacteria
Photoheterotrophs	Light	Organic carbon	Purple non sulfur bacteria
Chemoautotrophs	Inorganic chemicals	Carbon dioxide (Inorganic Carbon)	*Nitrobacter, Nitrosomonas, Thiobacillus thiooxidans*
Chemoheterotrophs	Organic chemicals	Organic carbon	Most bacteria

Microorganisms can be classified into 4 groups on the basis of their tolerance to oxygen.

Physiological Group	Description	Example
Obligate aerobic	These bacteria require oxygen for their survival. They generate energy by oxidative phosphorylation.	*Acetobacter sp*
Obligate anaerobic	They cannot survive in the presence of oxygen. These microorganism generate energy by fermentation	*Clostridium* sp.

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Facultative anaerobic	These microorganisms prefer to grow in the presence of oxygen producing energy by oxidative phosphorylation, but they can also grow in anaerobic environments using fermentation	*Escherichia coli*
Microaerophilic	These microorganisms can grow only in oxygen concentrations lower than those in air	*Lactobacillus* sp.

Some bacteria are able to fix atmospheric nitrogen. They show diversity in nitrogen fixation.

- Free-living nitrogen fixing bacteria: *Azotobacter* sp.
- Symbiotic nitrogen fixing bacteria: *Rhizobium* sp. with legume root

Mostly bacteria undergo asexual reproduction by binary fission, and in some occasion, fragmentation or budding. In rare occasions, bacteria of two strains share a portion of genetic material through the process of 'conjugation'.

Cyanobacteria

Cyanobacteria are named for their characteristic blue-green (cyan) pigmentation. Cyanobacteria also exhibit a great variety of shapes and cell arrangements, unicellular to colonial forms (Figure 9.4).

- **Unicellular form-** Cells separate after cell division. However, in nature majority of unicellular forms stay together by copious secretion of mucilage by daughter cells.
- **Colonial form-** Cells remain attached by walls or held in a common gelatinous matrix forming a colony of cells. Colonies may either be non-filamentous or filamentous. Non-filamentous colonial form- depending on the plane of division and direction there are different arrangements such as spherical, cubical, square or irregular shape. Filamentous colonial form is the result of cell division in a single plane and a single direction forming a chain or thread like structure.

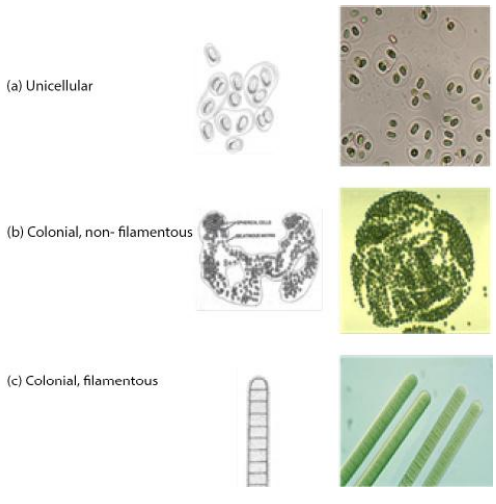


Figure 9.4. Cell arrangement of cyanobacteria. Shown are diagrammatic representations (left) and light microscopic views (left) of cell arrangement.

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Cyanobacteria are photoautotrophs that carryout oxygenic photosynthesis similar to plants and algae. Many of cyanobacteria are capable of fixing atmospheric nitrogen. For examples, *Nostoc* sp. is a free living nitrogen fixing cyanobacteria, *Anabaena -Azolla* symbiotically fix nitrogen with its partner, *Azolla* sp. (water fern). In most cases nitrogen fixation takes place in special cells called heterocyst. Nitrogen fixation is catalyzed by the enzyme called nitrogenase in the heterocyst. Nitrogenase is sensitive to oxygen. Heterocyst carry thick cell wall to protect nitrogenase from oxygen that could diffuse from neighboring photosynthetic cells and from air or water.

Cyanobacteria carry another specialized cell type called akinete. They are thick walled resting spores with stored food. Akinete is resistant to drought and high temperature. Therefore, akinete is able to survive during unfavorable environmental conditions although vegetative cells dries out.

Cyanobacteria reproduce only by asexual methods. Single unicellular and colonial non-filamentous types undergo simple cell division while colonial filamentous and colonial unicellular forms reproduce by fragmentation.

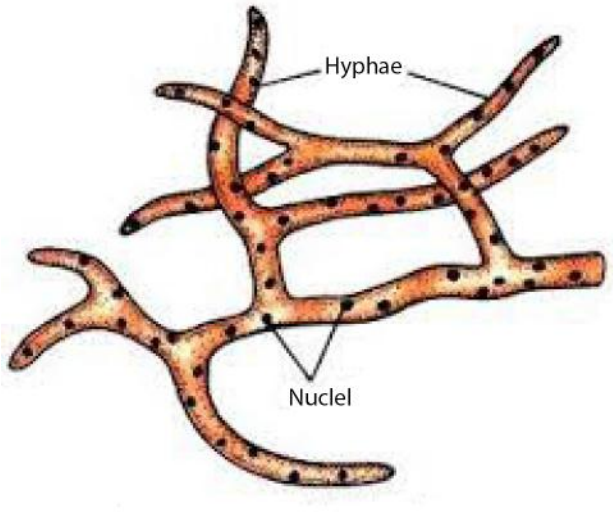


figure 9.5 Fungi fillamentous thallus, branched mycellium

Fungi (singular, fungus) are eukaryotes. They may be unicellular (yeast) or multicellular (molds). Some multicellular fungi form mushrooms. Molds form visible masses called mycelia, which are composed of long filament like structures called hyphae. Many molds contain cross-walls called septa (singular, septum). Septa divide hyphae into distinct single nucleate cell-like units. Some molds do not contain septa in their hyphae resulting in long continuous cells with many nuclei. These are called coenocytic hyphae. The cottony growths sometimes found on bread and fruit are mycelia of molds.

Fungi are chemoheterotrophs and acquire food by absorption. They possess saprophytic mode of nutrition. They play important role in food chain by decomposing dead plant materials by secreting enzymes and thereby recycle vital elements. Parasitic (plant and animal pathogens) and mutualistic (lichens and mycorrhizae) modes of nutrition also found among fungi.

Unicellular fungi reproduce asexually by fission or budding, on the other hand, filamentous fungi (molds) reproduce asexually and/or sexually by producing spores.

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a.



Figure 9.6 (a). Reproduction method of *Penicillium (*spore formation)

b.

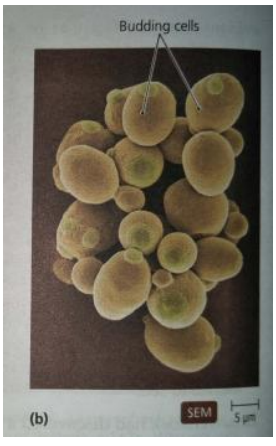


Figure 9.6 b. Reproduction method of Yeast (budding)

Unicellular protists

a.

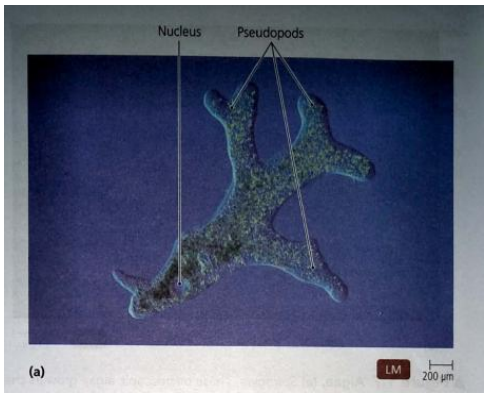


Figure 9. 7 a. *Amoeba*

b.

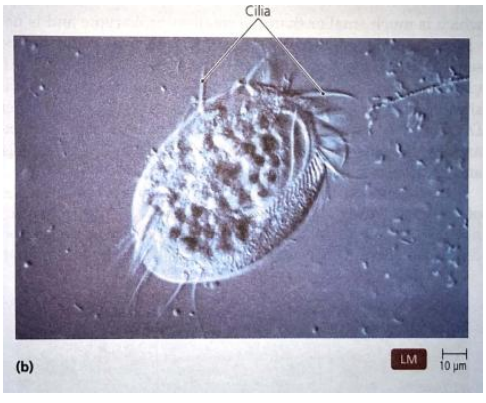


Figure 9. 7 b. *Paramecium*

c.

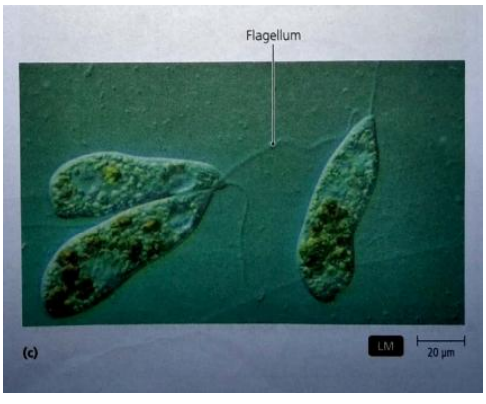


Figure 9. 7 c. *Euglena*

Unicellular Protists are pleomorphic, vary in their shapes and possess locomotive structures such as pseudopods, cilia or flagella. They exist either individually or form colonies. Some join together and form filaments.

Photoautotrophic, heterotrophic and mixotrophic modes of nutrition are found among protists.

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There are aerobic, anaerobic and facultative anaerobic respiratory modes found among protists.

Some algae contribute to the symbiotic interactions in lichens.

They reproduce sexually by gametes and asexually by fission.

Mollicutes

Mollicutes are prokaryotes included in the domain bacteria. Mycoplasma and phytoplasma, are considered unique due to absence of cell walls.

Mycoplasma and Phytoplasma

Mycoplasma are pleomorphic, vary in shape from spherical to filamentous. They are the smallest prokaryotic group of organisms invisible under light microscope. Mycoplasma do not contain flagella. Almost all mycoplasma are parasites of humans and animals. Mycoplasma require high amount of organic growth factors. They reproduce by budding and binary fission and do not produce spores. Mycoplasma are aerobic or facultative anaerobic.

Phytoplasma resembles to mycoplasma in many ways. They are similar in size to mycoplasma. Both can only be seen under electron microscope. Shape varies from spherical to filamentous. Phytoplasma only infect plants and are generally present in the phloem sap. They cannot grow in artificial media. They are transmitted mostly by leafhoppers. Therefore, they reproduce in both leafhoppers as well as plant body. They reproduce by budding and binary fission. They possess aerobic or facultative anaerobic mode of respiration.

Virus

(a) Characteristic features

Viruses are neither prokaryotes nor eukaryotes and do not show any cellular organization. They do not possess any metabolic activity or reproduction when they are out of living host cells. Thus, they are not considered as living organisms. However, once they get in to the host cells, they multiply and cause infection through various metabolic pathways, shows characteristics of living organisms. Since viruses can only multiply within a living host cell, they are obligate parasites. Viruses are very small can only be seen through an electron microscope. Viruses possess simple structures, usually are composed of a central core of a nucleic acid and surrounded by a protein coat called the capsid which is made up of a fixed number of protein subunits called capsomeres. Viruses may have either DNA or RNA as their genetic material. They do not have protein synthesis machinery such as additional RNAs or enzymes for protein synthesis. Therefore, they depend on host cell's protein synthesis machinery. RNA viruses consist of reverse transcriptase enzymes for reverse transcribing RNA in to DNA.

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(b) Morphology and types of viruses

On the basis of capsid architecture two basic morphological symmetries can be identified (Figure 9.8).

1. Helical

2. icosahedron

Based on the above symmetries viruses show four types of morphological forms; helical, polyhedron, complex and enveloped.

- Helical viruses- long rigid or flexible rods. e.g; Rabies virus
- Icosahedron/ polyhedral- icosahedron symmetry. e.g; Adeno virus
- Complex viruses-Exhibits more than one form of symmetry with additional structures. e.g; bacteriophage
- Enveloped viruses. e.g; roughly spherical. Capsid covered by envelopes. e.g; Herpes simplex virus

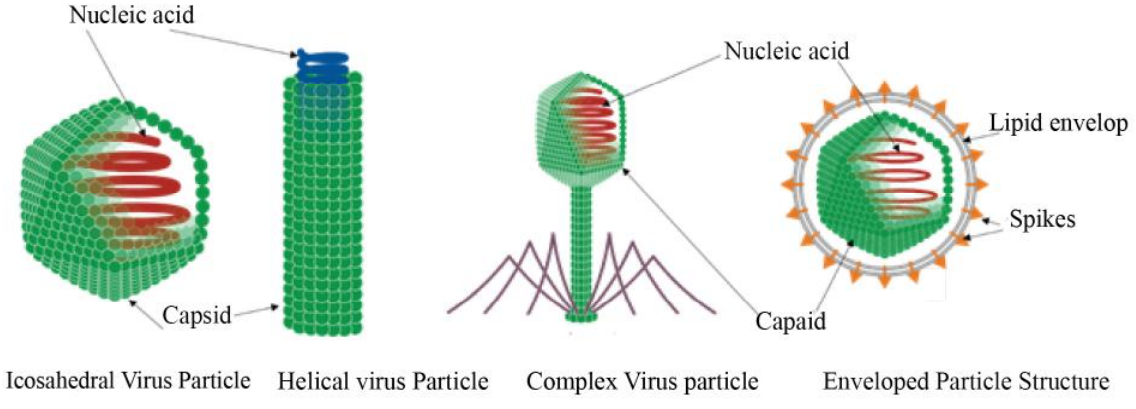


Figure 9.8 Diagrammatic representation of four morphological forms of virus.

Multiplication of viruses

A single virus can give rise to thousands of similar viruses in a single host cell. Therefore, viruses cause serious damages to their host leading to severe diseases in plants, animals and bacteria. Bacteriophages are typical group of viruses that are capable of infecting bacteria. They multiply by two distinct mechanisms; lytic cycle or lysogenic cycle.

Lytic cycle involves with the lysis of the host cell, whereas the lysogenic cycle allows viral DNA incorporating into host DNA and multiply without causing lysis of the host cell.

Lytic cycle of a bacteriophage

There are five distinct steps in the lytic cycle ; attachment, penetration, biosynthesis, maturation and release (Figure 9.9).

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- **Attachment:** The first step is the attachment of virus to a matching receptor site on the bacterial cell.
- **Penetration**: After attachment, bacteriophage injects its DNA into the bacterial cell. This is facilitated by an enzyme which breaks down bacterial cell wall.
- **Biosynthesis:** The next step is the biosynthesis of viral DNA and proteins in the host cytoplasm using host resources. This stage induces degradation of host cell DNA.
- **Maturation and assembly:** Once bacteriophage DNA and proteins are synthesized, DNA and capsid are assembled to form complete virus particles. This is called maturation.
- **Release:** Finally, bacteriophage induce bacterial cell to break open (lyse). Newly produced bacteriophages are released from the host cell. These released bacteriophages can start another lytic cycle in cells in the vicinity.

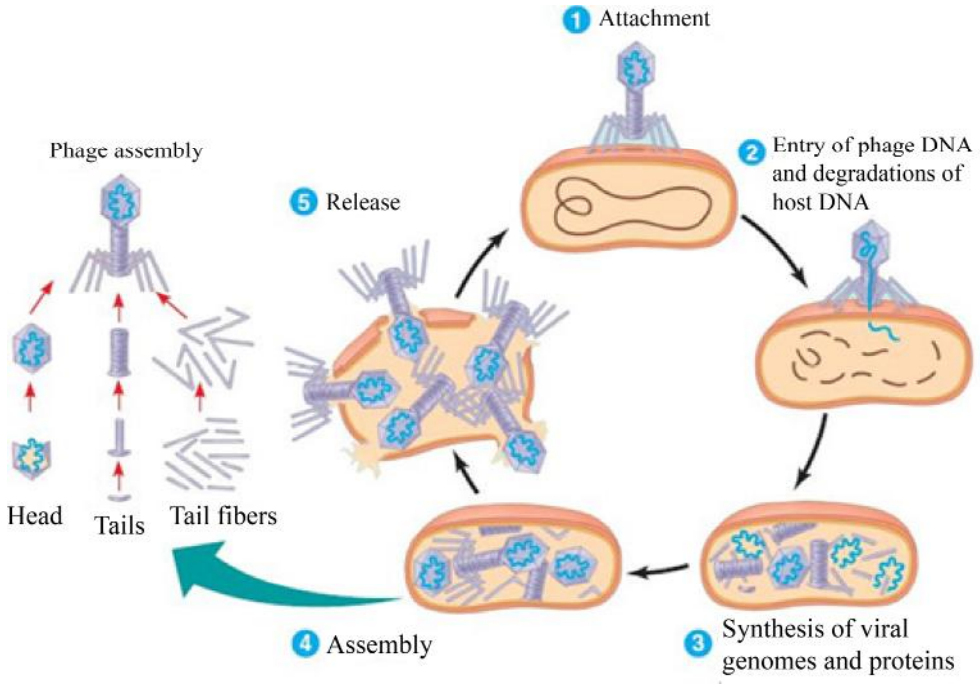


Figure 9.9 Stages of lytic cycle of a bacteriophage

Viroids

Viroids consist only of short piece of naked RNA with no protective layer such as a protein coat. Viroids can only multiply within a living host cell using host cell resources. However, viroids do not contain any gene and only carry signals for their multiplication. Viroids infect plants, but no other life forms till to date.

Prions

Prions are proteinaceous infectious particles. Their size is smaller than virus. Although prions lack nucleic acid they can replicate with the help of a host's gene that encodes the prion protein. They are found as disease causing agents in some birds and mammals. All these diseases are neurological diseases. Eg;

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- Transmissible Spongiform Encephalopathies (TSEs), because large vacuoles develop in the brain giving sponge-like appearance.
- Mad cow disease was one of the serious disease emerged in cattle in 1987.
- Creutzfeldt-Jakob disease (CJD) is one of the human diseases caused by prions.

Human to human disease transmission has been associated with transfusion of infected blood and tissue and organ transplantation. Some TSE infections may be transmitted from cow to human.

Basic laboratory Techniques

For the study of morphology and biochemical properties of microorganisms, it is essential to culture them on artificial media. There are some basic laboratory techniques such as preparation of artificial culture media and sterilization techniques, to be followed in culturing of the microorganism of interest without any contamination. This section describes such basic laboratory techniques.

Methods of sterilization

Sterilization is the process of removal or destruction of all forms of microbial life including endospores.

There are two types of sterilization, physical and chemical.

I. Physical methods of sterilization

Sterilization by moist heat, dry heat, filtering using membrane filters, exposure to UV radiation are some of the physical methods used in sterilization.

- **Moist heat sterilization**

  Here, moist heat is used to destroy the microorganisms present in the desired materials such as culture media, temperature labile reagents/ fluids and various laboratory utensils. This is done by denaturing of proteins by high temperature and pressure.

E.g. autoclaving- In an autoclave, steam with 121 ºC temperature at the pressure of 1 atm/ 15 psi is used for sterilization. Extending the above condition for 15 minutes is sufficient to kill all microorganisms (except prions) and their endospores.

Autoclaving is used to sterilize culture media, solutions, syringes and needles, healthcare instruments and various other items that can withstand high temperatures and pressure. Glassware can also be sterilized with an autoclave if care is taken to ensure that the steam contacts all surfaces.

Pressure cooker also can be used for moist heat sterilization.

- **Dry heat sterilization**

  Here, dry heat is used to destroy the microorganisms present in the desired materials such as glasswares, petridishes, pipettes, inoculation loops, inoculation needles, scalpels, etc.

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1. **Direct flaming**

   It is a simple method of dry heat sterilization. This is used in laboratories to sterilize inoculating loops, inoculating needles and scalpel blades by heating them on the flames of Bunsen burners / hot spirit lamp until they reach red hot.

2. **Incineration**

   It is mostly done in an incineration oven. Incineration is used to sterilize hospital waste. Microorganisms are burned to ash during direct flaming and incineration.

3. **Hot-air sterilization**

   Microorganism are killed by oxidation. Items to be sterilized are heated to about 170 ºC and maintain for 2 hours in a dry air oven. This type of sterilization is often used to sterilize glassware such as Petri plates, flasks, beakers, bottles and glass pipettes.

- **Pasteurization**

  Louis Pasteur found spoilage of beer and wine can be prevented by applying mild heat that kills organisms causing spoilage without seriously damaging the taste, texture and nutritional content of the product. Later, the same principle was applied to milk products, now known as pasteurized milk. The objectives of milk pasteurization are to eliminate pathogenic microorganisms and reduce microbial number which prolongs milk quality under refrigeration.

  High temperature short-time (HTST) pasteurization, which uses temperature of at least 72 ºC for 15 seconds and **low temperature long time (LTLT)** 63 ºC for 30 minutes are the two main methods of pasteurization. Milk can also be sterilized by **ultra-high-temperature (UHT)** pasteurization. Here, milk is heated to about 140 ºC in less than 5 seconds by flashing steam. This milk can be stored for several months without refrigeration.

- **Boiling**

  Boiling the materials such as surgical instruments to 100 ºC. Most of the pathogenic microorganisms are killed at boiling temperature.

- **Filtration- e.g. Membrane filters**

  Filtration is used to sterilize heat sensitive liquids such as solutions containing enzymes, vitamins, antibiotics, vaccines and some culture media. Material to be sterilized is passed through a filter by using vacuum. Filter retains microorganism and the liquid is passed through the filter.

  Membrane filters are widely used to sterilize heat sensitive solutions. Pores of membrane filters are from 0.01 µm to 0.45 µm size, retain almost all the microorganisms including viruses and some large protein molecules.

- **UV Radiation**

  UV Radiation kills microorganisms that falls in to direct exposure, either through destruction or damaging DNA. However, a major disadvantage of UV is that radiation does not penetrate through solid surfaces and coverings such as paper, glass and textile. Therefore, anything to be sterilized should have direct contact with radiation. UV radiation is commonly used to sterilize air in hospital rooms such as operating theaters and nurseries.

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II. Chemical methods of sterilization

Few chemicals such as ethylene oxide and chlorine dioxide (both are gases) are currently used as chemical sterilizing agents. Majority of chemical agents reduce microbial populations to a safe levels or remove vegetative forms of pathogens.

Ethylene oxide kills microorganisms and endospores. It is also highly penetrating. Therefore ethylene oxide is used to sterilize mattresses in hospitals.

Chlorine dioxide has been used to fumigate enclosed building areas contaminated with endospores of Bacillus anthracis. It is most commonly used in water treatment prior to chlorination.

Preparation of culture media

Microorganisms cannot be studied in their natural habitat such as in soil, water or air. Therefore, we need to bring them to the laboratory and provide similar conditions for their growth and reproduction. A nutrient material prepared for providing nutrition and anchorage essential to the growth of microorganisms at laboratory condition is called a culture medium.

Not all microorganism can be grown on a laboratory culture media. They are called non-culturable microorganisms. Some microorganisms grow well on any culture medium whereas other microorganisms require special medium.

Suppose we want to grow a culture of microorganisms present in a certain soil sample. The culture medium should contain necessary nutrients, sufficient moisture and suitable pH. This medium must initially be sterile which means it should not contain any living microorganisms. Therefore, when preparing a culture medium all glassware and liquid nutrient solutions should be sterilized.

Nutrient agar (NA) and potato dextrose agar (PDA) are two general media, commonly used to grow bacteria and fungi respectively. Nutrient agar is made up of peptone, meat extract, sodium chloride, agar and distilled water. Whereas, PDA is made up of potato, glucose, agar and distilled water.

Here, agar is used as a solidifying agent. Agar solidifies at temperatures below 40 ºC which means a culture medium containing agar is a solid medium. For growing microorganisms, solid culture medium is usually contained in Petri dishes or test tubes.

Because most microorganisms appear almost colorless when viewed through a standard light microscope, we have to prepare them for observations. One of the ways this can be done is by staining, means coloring the microorganisms with a dye.

However, before the microorganisms stain, they must be fixed (attached) to the microscopic slide. A simple stain is an aqueous or alcohol solution of a single basic dye. The primary purpose of a simple stain is to highlight the entire microorganisms so that cellular shapes, cell arrangements and basic structures are visible. Some of the simple stains commonly used in the laboratory are methylene blue., crystal violet and safranin.

Microorganisms and diseases

Generally, humans are free of microorganisms at birth. However, during birth, the newborn first get in contact with microorganisms present on the vagina of mother. Usually, these are Lactobacilli. Lactobacilli colonize the intestine of the newborn. After birth, many other microbial populations begin to establish inside or on the surface of the body. These are called normal microbiota of

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human body. However, internal tissues of healthy human body are free of microorganisms.

A part of these microorganisms colonize on the skin, and majority enter the body and colonize on the inner surfaces such as mucous membranes of nose, throat, upper respiratory tract, intestinal tract and genitourinary tract.

A normal healthy body contains a large number of microorganisms. It has been estimated that the human body consists of 1×10^{14} microbial cells for 1×10^{13} total body cells, which means a 10 times more microbial cells than human cells.

Majority of these organisms are generally harmless or even beneficial. For example, minimal colonization of *Escherichia coli* at large intestine prevent colonization of pathogenic bacteria such as Salmonella typhi. *E. coli* in large intestine synthesizes vitamin K and some of the B vitamins that are absorbed into the bloodstream and used by body cells.

Recent interest in the importance of bacteria to human health has led to the study of probiotics. Probiotics are live microbial cultures. e.g. Yoghurt exert a beneficial effect. Serveral studies have shown that ingestion of certain lactic acid bacteria can alleviate diarrhea and prevent colonization by Salmonella enterica during antibiotic theraphy.

Although majority of human microbiota are harmless, some of them may change their interactions with human body under certain conditions and cause infections. Those microorganisms are called opportunistic pathogens. For example, E. coli is generally harmless as long as it remains in the large intestine. However, they may cause diseases, if enter other body parts (urinary bladder- urinary tract infection, lungs- pulmonary infection).

Terms related to infectious diseases

- **Pathogen:** An organisms or entity (non-living entities such as virus and prions) that is capable of causing disease
- **Host:** Organism within which infected pathogens live on or in and multiply.
- **Pathogenicity:** The ability of a pathogen to cause disease in the host by overcoming the defence of a host.
- **Parasite:** An organism or entity living on or in another living organism (host) and obtain nutrients and other resources from the host.

Characteristics of pathogenic microorganisms

- Having optimal growth conditions (e.g. temperature) that corresponds to the body conditions of the host.
- Having structures to adhere to the host cells and protect against host's defense mechanisms. e.g. capsule, pilli
- Produce toxins; endotoxins or exotoxins.
- Having enzymes for invasiveness such as phospholipase, lesithinase, and hyaluronidase.
- Having enzymes such as DNase to alter the host's metabolic processes.

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Virulence and virulent factors

Microbes express their pathogenicity by means of their virulence. Virulence is the degree of pathogenicity of the pathogen. Some pathogens are highly virulent (chicken pox virus) whereas others are less virulent/ non virulent.

Few genes of pathogenic microorganisms express factors which provide them the ability to infect their host and cause disease. Such factors are called virulent factors.

The relationship between a host and a pathogen is dynamic, each modifies the activities and functions of the other. As a result, outcome of such a relationship depends on the virulence of the pathogen and the effectiveness of the host defense mechanisms.

Virulence factors enhance the pathogenicity and allows pathogen to invade and colonize host tissues and disrupt normal body function. Pathogens use two major mechanisms for pathogenicity.

1. Invasiveness

It is the ability of pathogens to invade tissues by overcoming host's defense mechanisms and multiply for colonizing.

Several extracellular enzymes produced by pathogens contribute to invasiveness.

e.g.

- Phospholipase- destroy animal cell membranes.
- Lecithinase- Hydrolyzes the lecithin component of the lipid in the cell membrane.
- Hyaluronidase- destroys the body tissue by breaking down the hyaluronic acid which is cementing substance between cells.

Pathogenic microorganisms do enter passively through various portals or natural openings such as wounds on skin, respiratory, gastrointestinal and genito-urinary tracts.

2. Toxigenecity

Ability of microorganisms to produce biochemical substances known as toxins that disrupt the normal functions of cells. These are proteins or lipopolysaccharides that produce specific harmful effects on the host, thus are called biological poisons. They may be,

- Endotoxins- Endotoxins are lipopolysaccharides. These are thermos-stable toxins which are part of the microbial cell. Toxins are released when the bacteria die and the cell wall breaks apart. All endotoxins cause the same signs of symptoms regardless of the species of pathogen. These symptoms include chills, fever, weakness, generalized aches and sometimes shock and death. Endotoxins are produced only by gram-negative bacteria

  e.g. Lipopolysaccharides of the cell walls of *Salmonella typhi*

- Exotoxins- Exotoxins are produced inside bacterial cells as part of their growth and metabolism and are secreted or released to the surrounding environment after cell lysis. Exotoxins are proteins. Majority of them are enzymes. Due to their catalytic nature even a small amount of toxin is quite harmful. These are thermo-labile protein toxins, being inactivated by boiling. Exotoxins are most commonly produced by gram-positive bacteria and a few gram negative bacteria

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- Exotoxins are classified into three types
  - Neurotoxins- interfere with normal transmission of nerve impulses. E.g., toxins produced by *Clostridium tetani*
  - Enterotoxins- stimulates cells of the gastrointestinal tract in an abnormal way. E.g. Toxins produced by *Vibrio cholera*
  - Cytotoxins- kills host cells by enzymatic attack. E.g. Toxins produced by *Corynebacterium diptheriae*

Important diseases of human caused by microorganisms

Organ	Disease	Causal agent
Skin	Chickenpox Rubella Measles	Herpesvirus varicella-zoster Rubella virus Measles virus
Eye	Conjunctivitis (bacteria/virus)	Haemophilus *influenzae/Adenoviruses*
Nervous system	Bacterial meningitis	*Streptococcus pneumonia* *Haemophilus influenzae* *Neisseria meningitidis*
Nervous system	Tetanus Rabies	*Clostridium tetani* Rabies virus
Cardiovascular system	Rheumatic fever	*Streptococcus pyogenes*
Respiratory system	Tuberculosis Influenza Pneumonia	*Mycobacterium tuberculosis* Influenza virus *Streptococcus pneumoniae*
Digestive system	Hepatitis Food poisoning Cholera Typhoid	*Hepatitis A virus* Staphylococcus aureus Vibrio cholerae Salmonella typhi
Urinary system	Leptospirosis	*Leptospira interrogans*
Reproductive system	Gonorrhea Genital herpes	*Neisseria gonorrhoeae* Herpes simplex virus
Immune system	AIDS	Human immune deficiency virus (HIV)

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Microbial disease control starts from avoidance and prevention of opportunities of getting infection to treatment or curative methods after infection.

Avoidance and prevention of microbial diseases

Good hygienic practices in day to day life is the best method to avoid infectious diseases. Antiseptics, disinfectants and immunization play import role in prevention of infection.

Methods of controlling microbial diseases of human

- **Use of antiseptics and disinfectants**

Antiseptics and disinfectants are chemical substances that are used to kill or reduce microbial population in order to prevent infection. However, such chemicals are not effective against some microorganisms. For example, polio virus, tuberculosis bacterium, spores of bacteria and fungi are not destroyed by most antiseptics and disinfectants.

The major difference between antiseptics and disinfectants is that antiseptics can be safely and directly applied to the human body, whereas disinfectants cannot. Therefore, antiseptics are used in disinfection of living surfaces such as skin. Disinfectants are used in disinfection of non-living surfaces such as operation theatres, bathing areas, sinks, kitchen tops, cutlery, drains etc.

Antiseptics and disinfectants are generally formulated as liquids. Their effectiveness varies with concentration, duration of exposure, temperature and presence of organic matter.

Some examples of antiseptics and disinfectants are given below.

Antiseptics: ethanol, isopropanol, chloroxylenol

Disinfectants: phenol, hypochlorites (calcium hypochlorite and sodium hypochlorite),

- **Use of Antibiotics in controlling microbial diseases**

When the body's defense fails to protect body from the infection or overcome the disease, it has to be treated by chemotherapy with antimicrobial drugs. Antimicrobial drugs kill or interfere with the growth of microorganism without damaging the host. Antibiotics are effective antimicrobial drugs against bacteria.

Some antibiotics affect against a broad range of bacteria and they are termed as broad-spectrum antibiotics, while others affect only against a specific group of bacteria and are called narrow-spectrum antibiotics.

Antibiotics show various modes of action. Some examples are given below.

- Inhibition of cell wall synthesis- Penicillin
- Inhibition of protein synthesis- Erythromycin, Tetracycline
- Disrupting plasma membrane- Daptomycin
- Inhibition of DNA/RNA synthesis- Rifampin

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- **Immunization: Vaccines**

A vaccine is a suspension of weakened pathogens or fractions of organisms that is used to induce immunity. Vaccines are frequently used to control diseases caused by viruses because there is no other control methods once infected. There are several types of vaccines.

1. **Live attenuated vaccines**

   Vaccine contains live pathogens which were deliberately weakened for its pathogenicity. These vaccines, mimic an actual infection. Since the pathogen is active inside the host, such vaccines provide lifelong immunity. More often a booster (secondary) immunization is not required. Examples for live attenuated vaccines are, vaccines for,

   - Measles, Mumps and Rubella (MMR)
   - Chickenpox

2. **Inactivated vaccines**

   Pathogenic microorganism is inactivated or killed in the vaccine. Compared to live attenuated vaccines, inactivated killed vaccines often require repeated booster doses. Examples for inactivated vaccines are vaccines for,

   - Virus diseases such as Rabies, Influenza, Polio
   - Bacterial diseases such as Cholera

3. **Subunit vaccines**

   Subunit vaccines contain only the antigenic fragments of a pathogen that can induce immunity in the recipient. Toxoid vaccines are the best examples for subunit vaccines, those have been used for a long time. Toxoids contain inactivated toxins derived from a pathogen. Examples for Toxoid vaccines are vaccines for Tetanus, Diphtheria, etc. Presently, subunit vaccines are produced using genetic engineering. E.g. Hepatitis-B Vaccines. Subunit vaccines usually require repeated booster dose to obtain full immunity.

Use of microorganisms in industry, agriculture and environment

Microorganisms have been exploited for various purposes long before their discovery. Babylonian and Sumerian civilizations used yeast to make alcohol in as early as 6000 BC. After the discovery of microorganisms, in the latter part of the nineteenth century, pure cultures of microorganisms are being used in the food production. This increased the understanding of microorganisms, their processes and products. At present various industries based on selected microorganisms and their qualities are in operation.

1. **Advantages of using microbial processes over chemical processes**

- Simple nutritional requirements are sufficient for their growth.
- They are able to convert (metabolize) a wide range of raw materials.
- They are able to convert cheap raw materials into industrially important products.
- Due to higher growth rate, they can convert the raw materials into products within a short period of time.
- Their growth conditions can be controlled to obtain desired end products.

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- Reactions can be carried out at low temperatures, energy and pressures compared to the conventional industrial methods.
- They give higher yield with higher specificity when compared to the conventional industrial methods.
- Microbes are amendable to genetic manipulation to obtain desired yield and quality with high efficiency.

Basic principles of metabolic processes of microorganisms for product formations

1. Microbial cells are used as the end products. e.g. single cell proteins
2. Microbial metabolic products are used as end products- they may be either primary end products or secondary metabolites. e.g. primary end products- alcoholic beverages, secondary metabolites- antibiotics.
3. Microbial metabolic processes are used as end products. e.g. bioremediation (heavy metal remediation) , metal extraction (Cu, Fe), retting (production of fibers).
4. Genetically modified microorganisms are used to produce end products. e.g. productions of commercial enzymes (amylase from *Aspergillus niger*), vaccines (hepatitis B), hormones (insulin).

Applications of microorganisms in industries

Industrial microbiology is the large scale production of economically important products using microorganisms and their metabolic processes. Recent technological and biotechnological advances expanded the scope of industrial microbiology. Bacteria, fungi, algae and viruses are used in industry.

In industrial microbiology, microorganisms can be considered as miniature chemical factories, where various energy releasing (catabolic) and energy acquiring (anabolic) chemical reactions take place. Within this factory, raw materials (substrates) are converted into end products, one or more byproducts and wastes. End products can be separated from byproducts and wastes, by purification to obtain a purified industrial products.

Commercial products made by microorganisms and their processes

1. Single cell proteins

- Microbial cells are grown in large scale as food supplement and rich in proteins are called single cell proteins. e.g. Yeast, *Spirulina* sp. and *Chlorella* sp

$$C_6H_{12}O_6 \xrightarrow{\text{Yeast}} 2C_2H_5OH + CO_2$$

2. Alcohol and alcoholic beverages

Microorganisms are involved in the production of almost all alcoholic beverages such as beer, wine, sake, toddy and ethanol. Yeast, *Saccharomyces cerevisiae* ferment sugars into ethanol and carbon dioxide.

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Globally, more than 70% of ethanol is produced by fermentation. Sucrose derived from sugarcane is the most widely used fermentation substrate. In addition, simples sugars derived from plants and dairy waste are also used

e.g. 1. Beer- by the fermentation of cereal grain malt.

2. Wine - produced by the fermentation of grapes or other suitable fruits.

3. Toddy - produced by the fermentation of sap of palms such as Palmyra and coconut.

4. Arrack- produced by fermentation of palm tree sap such as coconut and sugarcane.

3. Production of vinegar

There are two steps in vinegar production.

1. Alcoholic fermentation: sugars in malted grain, sap of palms, sugarcane and fruit juices is fermented by *S. cerevisiae*. Ethanol is subjected to acetic acid fermentation.

2. Acetic acid fermentation: Ethanol derived from alcoholic fermentation undergoes incomplete oxidation and is converted to acetic acid. This process is highly aerobic and involves *Acetobacter sp*. and *Gluconobacter sp.*

$$C_2H_5OH \xrightarrow{\text{Acetic acid bacteria}} CH_3COOH + H_2O$$

4. Dairy products

Dairy products are made by the fermentation of milk. Sugar lactose in milk is fermented by lactic acid producing bacteria into lactic acid. These bacteria are killed during pasteurization, therefore, added externally when making dairy products.

e.g.

- Curd and yoghurt is produced by fermentation of lactose sugar in milk by a mix population of *Lactobacillus bulgaricus, Lactococcus* lactis and *S. thermophilus. L. bulgaricus* add flavor and Streptococcus spp. add creamy texture and flavor.
- Production of cheese- *Streptococcus sp, Penicillium molds*
- Lactic acid is commercially produced by using waste products from cheese and butter industry. *L. bulgaricus* ferment lactose into lactic acid.

5. Organic acids

Majority of commercially produced organic acids are obtained through microbial fermentation. Fermentation substrates such as beat or cane molasses and organisms such as Aspergillus niger are used.

E.g. Citric acids- sucrose fermented by *Aspergillus niger*

6. Metal extraction

Some metals from ore are extracted with the help of microorganisms. This process is called leaching. One of the best example is extraction of copper from lower grades of copper ore for which other extraction methods are unprofitable. Thiobacillus ferrooxidans recover copper

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from the ore that contains iron and sulfur. About 70% of copper in the ore can be recovered by this microbial process. Uranium, gold and cobalt ore are also leached in similar manner using microbial processes.

7. Production of vitamins

Microbes can provide a source of an inexpensive source of vitamins for individual food supplements. E.g. vitamin *B12 – Pseudomonas sp*. and *Propionibacterium* sp

Riboflavin- fermentation by fungi

Vitamin C- *Acetobacter* sp

8. Vaccines

Commercial production of vaccine is done by variety of microbial antigens used in active immunization against various diseases. Some of them are genetically engineered vaccines. E.g. Hepatitis B vaccines.

Commercial production of various antibody preparation used for passive immunization. E.g. anti toxins against tetanus, Botulism toxin and immunoglobulin against rabies.

9. Enzymes

A range of enzymes are commercially produced by microorganisms. For example,

- Amylase: *Aspergillus niger, A. oryzae, Bacillus subtilis*
- Protease: *A. oryzae*
- Lipase: *Rhizopus spp.*
- Invertase: *Saccharomyces cerevisiae*
- Cellulase: *A. niger*

10. Antibiotics

Antibiotics is the most important secondary metabolites of microorganisms. Many antibiotics are still produced by microbial fermentation.

- Tetracycline: *S. aureofaciens*
- Penicillin: *Penicillium chrysogenum*
- Streptomycin: *Streptomyces griseus*

11. Hormones

a. Human insulin

Conventionally, insulin is extracted from animal pancreas. However, this is expensive and often cannot meet the demand. At present, insulin is produced cheaply by using genetically modified *E. coli* and *S. cerevisiae*. This insulin is identical to human insulin.

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b. Human growth hormone

Earlier animal-derived hormones were used as alternatives to human growth hormone, but with very less efficiency. At present this hormone is successfully produced by genetically engineered E. coli in large scale.

12. Retting

Retting is the process of loosening fibers from woody stem or other plant material such as coir. For retting, plant materials are immersed in water for varying periods of time depending on the plant material. Heterogeneous bacterial populations participate in the process under aerobic or anaerobic conditions. Bacteria secrete enzymes, mainly pectinases to facilitate loosening.

13. Biogas production

Anaerobic digestion of organic waste produces various gases, called biogas. The type of biogas produced depends on the substrate biodegraded. Activity of acetogenic bacteria on the organic waste produce carbon dioxide and hydrogen, whereas, activity of methanogenic bacteria produce methane.

14. Biofuel production

Petroleum-based fuel supply is expensive and sometimes uncertain. As a result, much attention has been given to renewable replacement fuels such as ethanol, butanol, biodiesel and biogas etc. In Brazil a large amount of ethanol is produced by microbial fermentation of sugarcane to be used as a source of fuel. Also efforts have been made to produce ethanol and butanol from cellulosic materials such as wood, wastepaper and cornstalks by using genetically modified bacteria. Many researches are going on to produce biodiesel from microalgae.

15. Bakery products

Sugars in bread dough is fermented by S. cerevisiae, bakers's yeast. The primary function of fermentation in bread is to generate carbon dioxide. Bread dough is made with grain flour such as wheat, rye and rice. Dough traps carbon dioxide and rises due to the pressure during baking and form open crumb texture. .

Applications of microorganisms in environment management

Industries and agriculture release various chemicals substances that are not readily degradable in nature. For example, plastic is a synthetic substance that is not biodegraded. Residues of pesticides such as heavy metals, insecticide DDT, herbicide 2,4-D are some other examples of chemicals that are not degraded or very slowly degraded by microorganisms and retain in the soil for long periods and also contaminates groundwater.

1. Bioremediation

Bioremediation is a technology that applies of living organisms to remove, degrade or detoxify pollutants. Bioremediation naturally occurs in the soil. In most occasions microorganisms are used in bioremediation processes. Growth of microorganisms in polluted soils and water can be stimulated to promote biodegradation/ bio-removal of pollutants. Microorganisms with selected properties or genetically modified microorganisms with selected properties can be used to degrade/ remove a specific pollutant from the polluted sites. Bioremediation is currently used to,

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- remediate soil and water contaminated with oil spills, toxic metal waste, hazardous organic waste etc.
- decompose wastewater from food processing and chemical plants.

2. Solid waste treatment

Accumulation of solid waste from house hold (garbage) possesses various environmental and health issues. In waste treatment, waste is degraded by microorganisms either aerobically or anaerobically. Composting, degrades waste aerobically. At the end, waste is converted to a stable material like humus.

Mostly garbage are placed as large compacted landfills or piles, where conditions are mostly anaerobic. In such situations, waste is degraded anaerobically using methanogenic bacteria. Methane gas produced as a byproduct of the degradation process. It may be used to generate electricity or as natural gas.

Applications of microorganisms in agriculture

Microorganisms have various applications in agriculture for improving yield, agronomic characteristics such as improved nitrogen and phosphorus absorption, resistance to pest and diseases and tolerance to drought etc.

1. Bio-fertilizers

Nitrogen and phosphorus are the most limiting nutrients in soil for plant growth and development. Therefore, chemical fertilizers are applied to the soil, to improve bioavailability of these nutrients. However, intensive use of synthetic fertilizers may result environmental problems such as deprivation of soil and water quality. Hence, much attention has been given for microorganisms that can be applied into the cropping systems to improve bioavailability of N and P. These microbial inoculants are called bio -fertilizers.

a. Phosphate solubilizing bacteria and mycorrhizae:

Phosphorus is the most limiting nutrient among all major plant nutrients. Bioavailability of phosphorus in any type of soil is negligible. (Very low amount of phosphorus applied to the soil remain available for plants). Phosphate solubilizing bacteria and Mycorrhizae contributes in improving the solubility of phosphorus in the soil solution. These bacteria and fungi secrete organic acids that dissolve minerals containing phosphorus and chelate cationic partners of the phosphate ions, thereby release phosphorus into the soil solution. At present, commercial formulations of microbial bio-fertilizers are available in the market.

b. Nitrogen fixing microorganism:

Biological nitrogen fixation is a process, where microorganisms convert atmospheric molecular nitrogen into its soluble form. These soluble forms of nitrogen can be assimilated by some plants directly or converted into desired soluble forms of nitrogen. e.g.

- Symbiotic nitrogen fixing
  - *Rhizobium* sp. form intimate relationships with leguminous plants. The fixed nitrogen is released to the soil when plants die, making nitrogen available for other plants. Variousrhizobium inoculations are commercially available.
  - Nitrogen-fixing cyanobacteria, *Anabaena* sp. form symbiotic association with water fern *Azolla*. This system has been successfully used in rice cultivation in many countries.

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- Free living nitrogen fixing
    - Free living nitrogen fixing bacteria such as Azotobacter present in high concentrations at rhizosphere.

c. Plant growth promoting bacteria

Many microorganisms in the plant rhizosphere produce plant growth-promoting substances like auxins (indole-3-acitic acid), cytokinins. These bacteria include,

Pseudomonas putida, P. fluorescens: auxin

Azotobacter sp., Rhizobium sp., B. subtilis, P. fluorescens: cytokinins

Acetobacter sp., Azospirillum sp: Gibberellin

(Names of the above microorganisms are not expected to be memorized by the students)

2. Bio-pesticides/ Bio Control Agents (BCA)

Extensive use of chemical pesticides leads to hazardous side effects to people. These substances or their residues persist in food and the environment. The residual toxicity may affect the non-target organisms. Further, over use of pesticides develop resistance against pesticides among the pests.

Therefore, environmentally friendly and less toxic alternative strategies are required to replace synthetic chemicals as pesticides. Naturally occurring microorganisms are being explored to control pests and diseases. Some microbial formulations are currently commercially available and widely used in many cropping systems. Those include, Entomopathogenic fungi, bacteria and viruses

- .Entomopathogenic fungi: These fungi infect a broad range of insects leading to insect death. They have been formulated into myco-insecticides.
- Entomopathogenic bacteria: *Bacillus thuringiensis*: insecticidal and toxic for many insect larvae. Protein crystals produced by this bacterium are toxic to larvae when ingested. This toxin is called Bt toxin. After ingestion, toxin is dissolved and lyse tissues of the guts of larvae. Majority of biopesticide formulations currently in use are Bt-based.

3. Composting

Composting is a process used to convert plant remains into the equivalent of natural humus by microbial degradation. Degradation of organic matter by a mix population of microbes under warm, moist and aerobic conditions.

Initial activity of thermophilic bacteria on the plant remains increase temperature of the pile to 55-60 °C. As a result, thermophilic bacteria dominate in the degradation process for few days. When temperature decreases over time, thermophilic microbial population is replaced by mesophilic microbial populations. The process can be enhanced by addition of moisture and supply of oxygen through turning the pile. In addition to bacteria, microbes such as fungus, Actinomycetes and protozoa also contribute to the breakdown of organic matter into compost.

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Nature, distribution and role of soil microorganisms

Soil provides an adequate physical and chemical environment for growth of microorganisms in terms of space and nutrients which include minerals, decomposing organic materials, water, gases such as carbon dioxide, oxygen and nitrogen. Within a few centimeter depth of soil, there are different amounts of oxygen, moisture, light and nutrition, increasing the diversity of soil microorganisms.

The top few centimeters of the soil contains the largest community of bacteria. Microbial number declines rapidly with increasing depth. Majority of soil microflora is represented by bacteria. In addition, there are fungi, algae, protozoa and Actinomycetes. Actinomycetes, despite of being a member of domain bacteria, usually it is mentioned separately due to their significances. These microorganisms play a major role in decomposition of complex organic substances and participate in cycling of elements in biogeochemical cycles. Elements are oxidized and reduced by microorganisms for their metabolic requirements.

1. Mineralization

Mineralization is the decomposition of plant and animal residue by using extracellular enzymes of bacteria and fungi. These enzymes break down complex organic materials into simple inorganic materials such as CO_2 and H_2O. This is the major process by which plant nutrients are made available and recycling. Mineralization helps in following ways;

- helps to remove plant and animal debris from the earth surface allowing other organisms to live.
- recycle minerals which are found in limited quantities on the earth.

2. Role of microorganisms in the carbon cycle

- All organisms contain a large amount of carbon in organic compounds such as cellulose, starch, proteins and fat.
- Photosynthesis is the important first step in carbon cycle, in which, the atmospheric inorganic carbon dioxide is reduced/fixed to form organic compounds by photosynthetic organisms. Photoautotrophs such as plants, cyanobacteria, algae and photosynthetic bacteria fix carbon dioxide using energy from sunlight.
- Chemoheterotrophs such as animals and protozoa, depend on organic compounds produced by autotrophs to utilize them as their carbon source.
- Through food chain, carbon fixed from carbon dioxide by autotrophs, transferred from organism at lower trophic levels to the organisms at higher trophic levels.
- Both autotrophs and chemoheterotrophs, release a part of their fixed carbon as carbon dioxide to the atmosphere through respiration. This carbon dioxide is again made available for autotrophs.
- In chemoheterotrophs, undigested food is released to the environment as faeces which is later decomposed by soil microorganisms.

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- Rest of the carbon fixed in organisms, remain within them until they die. Once the organisms are dead, these organic compounds are decomposed and carbon dioxide is returned back to the atmosphere.
- Microorganisms, mainly bacteria and fungi play a major role in organic matter decomposition.
- Microorganisms play another major role in carbon cycle in relation to methane gas- Ocean sediments contain a large amount of methane. However, about 80% of methane generated within ocean is consumed by microorganisms called methanotrophs before it reaches to the atmosphere.
- Despite of the above, methanogenic bacteria in the ocean's depths are constantly producing more methane.

3. Role of microorganisms in the nitrogen cycle

All organisms require nitrogen to synthesize protein, nucleic acid and other nitrogen-containing compounds. About 80% of molecular nitrogen available in the atmosphere. This is not biologically available for organisms. Therefore, it is essential to convert that atmospheric molecular nitrogen into bioavailable forms of nitrogen. Certain groups of microorganisms are able to fix gaseous molecular nitrogen into bioavailable forms of nitrogen such as ammonia, nitrate and nitrite. Therefore, nitrogen available on the earth, organisms and the atmosphere must follow cyclic paths.

There are four key steps in the nitrogen cycle, they are, ammonification, nitrification, denitrification and nitrogen fixation.

- **Ammonification**

More than 90% of organic nitrogen in the soil exist as proteins. Proteins from dead plants and animals are decomposed by extracellular proteolytic enzymes secreted by microorganisms into amino acids. Resulting amino acids are taken into microbial cells and are then subjected to ammonification in which, amino groups of amino acids are converted into ammonia (NH_3). In moist soil, ammonia is solubilized in water to form ammonium ions (NH_4^+). This ammonium ions are utilized by plants and soil microorganisms. Ammonia in dry soil rapidly disappear into the atmosphere.

- **Nitrification**

Nitrification is the process of oxidation of nitrogen in the ammonium ion to produce nitrate. This process is done by nitrifying bacteria living in soil in two stages.

In the first stage, microorganisms such as *Nitrosomonas*, oxidizes ammonium ions into nitrites.

$$NH_4^+ \xrightarrow{\textit{Nitrosomonas}} NO_2^-$$

In the second stage, microorganisms such as Nitrobacter oxidizes nitrites into nitrates.

$$NO_2^- \xrightarrow{\textit{Nitrobacter}} NO_3^-$$

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Plants utilize nitrate as their source of nitrogen. Therefore, microorganisms play essential roles in providing nitrogen in bioavailable forms for plants and animals.

- **Denitrification**

  In the absence of atmospheric oxygen, some microorganism use nitrate and reduces it into N2. This process is called denitrification. Denitrification causes loss of nitrogen into the atmosphere. Therefore, it reduces available nitrogen in the soil. Denitrification frequently occurs in waterlogged soils, where limited oxygen is present. Pseudomonas sp converts nitrate ions into molecular nitrogen through following steps; nitrate to nitrite, nitrite to nitrous oxide and nitrous oxide to nitrogen gas



- **Nitrogen fixation**

  The process of conversion of nitrogen gas to ammonia is termed as nitrogen fixation. Bacteria that are able to fix nitrogen require nitrogenase enzyme. Nitrogenase enzyme is inactivated by oxygen. There are two types of nitrogen fixing microorganisms, free-living and symbiotic.

  - **Free-living nitrogen fixing bacteria-** They are abundantly found in the rhizosphere. Rhizosphere is the soil immediately surrounding plant root. e.g. *Azotobacter sp*. Many cyanobacteria are also able to fix nitrogen. e.g. *Nostoc*. These organisms, exhibit mechanisms to prevent the exposure of nitrogenase enzyme to the atmospheric oxygen; *Azotobacter sp* - high rate of aerobic respiration, Cyanobacteria- heterocyst. Some anaerobic bacteria such as *Clostridium sp* also fixes nitrogen.

  - **Symbiotic nitrogen-fixing microorganism-** They play an important role in agricultural crops such as leguminous crops; soybean, beans, peas and peanuts. These symbiotic nitrogen-fixing bacteria are commonly known as rhizobia. Leguminous plants are specially adapted to facilitate symbiotic nitrogen fixation. Plants form root nodules where, anaerobic conditions and nutrients for bacteria are given. Bacteria fixes nitrogen and make it bio available to be used by the plant. Some non-leguminous plants can also fix nitrogen symbiotically with different microbial combinations. Lichen, a combination of fungus and an algae or cyanobacteria also fixes nitrogen. In rice paddy waters a small free-floating fern, *Azolla* fixes nitrogen symbiotically with *Anabaena sp*.

Interactions of soil microorganisms relevant to plant growth

Microorganisms in the soil directly interact with plants. Interactions in the soil such as rhizosphere, mycorrhizae and endophytes with symbiotic relationship of bacteria, fungi and plant cells. These microorganisms are beneficial to the plants in numerous ways such as nitrogen fixation, increased uptake of water and nutrients such as phosphorous, secretion of plant hormones such as indole acetic acid, increases iron uptake at iron limiting conditions and defense against pathogens. In return plants provide organic compounds essential for the microorganisms.

Rhizosphere

This is a kind of symbiotic interaction between plant roots, and soil surrounding the root surface for about few millimeters. This micro-ecological zone is called rhizosphere. Rhizosphere is considered as the most biodiverse and dynamic habitat on earth.

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Microorganisms in the rhizosphere feed on the compounds exuded by roots such as sugars, amino acids and various aromatic compounds. Microorganisms compete –antagonize using antimicrobial compounds with each other for resources such as nutrients, space, and water in the rhizosphere. Bacteria are the most numerous organisms in the rhizosphere. Three most common genera of bacteria inhabiting rhizosphere are Pseudomonas, Bacillus and Agrobacterium. Root exudates act as chemical signals for bacteria to move towards the root surface. Both pathogenic and symbiotic fungi associate with the rhizosphere.

Mycorrhizae

Mycorrhizae (myco = fungus, rhiza = root) are symbiotic association between plant roots with fungi. All most all land plants have symbiotic association with one or more mycorrhizal fungi. Mycorrhizal fungi extend the surface area over which nutrients and water can be taken up by the plant. They can reach small pores in soil where plant roots cannot reach and uptake nutrients. Most significantly, mycorrhizae increase uptake of immobile nutrients such as phosphorous, zinc and copper. In return, mycorrhiza receive organic carbon from the plant.

Role of soil microorganisms in improving soil quality

Free living soil microorganisms and those associated with root surfaces, play a major role in improving soil quality. Microorganisms are involved in the formation of stable soil-aggregates, which are characteristics of good soil structure in fertile soils. Fungal filaments, Actinomycetes filaments and polysaccharide gums/slimes produced by bacteria are involved in soil aggregation formation.

Microbiology of domestic water and waste water

Contamination routes of drinking water

Drinking water resources can be contaminated with infectious disease causing organisms or chemical pollutants. Since water undergoes a filtering of microorganisms when pass through the soil into deeper layers, generally, water from springs and deep wells are of good quality in microbial point of view. Drinking water can be contaminated by dangerous pathogens when feces enter the water supply. Many diseases are disseminated by the fecal - oral route of transmission, in which, pathogens are shed in human or animal feces, contaminate water, and ingested via drinking. Examples for diseases spread by water (water-borne diseases) are typhoid fever, cholera, diarrhea etc.

Contamination of drinking water by chemical pollutants is a global issue. Industry, domestic and agriculture sectors release a large amount of chemicals that leach from the soil surface to groundwater. Most of these chemicals are resistant to biodegradation. Most freshwaters, such as tanks, often contain excessive amounts of nitrates and phosphates that come from agricultural fertilizer and household chemicals such as detergents. Accumulation of excessive amounts of such nutrients cause eutrophication, and flourish the growth of cyanobacteria and algae. They are toxic to humans. This kind of dense growth of cyanobacteria and algae, is called algal blooms. Various industries also release chemicals that are non-biodegradable. They too can contaminate drinking water supplies.

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Microorganisms as indicators of water quality

Water supplies can be contaminated with pathogenic microorganisms such as Salmonella spp., *Shigella* sp. and *Vibrio* sp. that causes infectious diseases such as typhoid and Cholera. Therefore, it is essential to detect the presence of such microorganisms before the consumption to prevent disease outbreak.

However, it is not practical to test water samples to look for pathogens because, pathogens may present only in small numbers and might not be included in the test sample. On the other hand, since the testing for pathogens take time, discovery of pathogens in the laboratory takes time and, it will be too late to prevent an outbreak of the disease. Therefore, it is important to test water samples routinely for "indicator organisms, which will indicate potential contamination of water supply by pathogens.

One of the major criteria for an indicator organism is that it should be consistently present in human feces in large numbers. This ensures the, presence of indicator organism providing evidence for contamination of water supply with human feces.

Sri Lanka, and many other countries use coliform bacteria as an indicator organism to test drinking water quality. Coliforms are aerobic or facultative anaerobic, gram-negative, non-endospore forming, rod shaped bacteria that ferment lactose to form gas within 48 hours in a lactose broth medium at 35 °C. Coliforms constitute a large portion of human intestinal microflora. They are nonpathogenic line in the intestine. Therefore, presence of coliforms in water indicates fecal contamination. However, there are some coliforms found in plant and soil samples. There are specialized test to distinguish coliforms that has fecal origin from coliforms present in plant and soil samples. Coliform test is routinely carried out by laboratories of the national water supply and drainage board to determine water quality of drinking water.

Water-borne diseases

Pathogenic organisms that are frequently transmitted through water cause infections in the intestinal tract such as typhoid, paratyphoid, cholera, gastroenteritis and dysentery.

Process of drinking water treatment

Drinking water that comes from various water supplies can be polluted at any time. Therefore, water needs to be purified before consumption for our health and safety. The idea of water purification is not to sterilize water, but to make water free of disease-causing organisms. There are three main steps in an urban water treatment plant. They are,

- Sedimentation and coagulation,
- Filtration
- Disinfection.

I. Sedimentation & Coagulation

This is the first step. If water is turbid (cloudy), water is allowed to stand in a holding tank for a period of time to allow settling down of particles suspended in water. This occurs in large reservoirs, where water remains for a holding period during which large particulate matter settles to the bottom. Sedimentation is enhanced by adding alum (Aluminum potassium sulphate) which produces a sticky precipitate. Many microorganisms as well as finely

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suspended matter are removed in this manner.

II. Filtration

After sedimentation and coagulation, water is filtered by passing through beds of fine sand. Filtration removes protozoan cysts and other microorganism. Microorganisms are trapped by surface absorption onto the sand particles. This removes about 99% of bacteria. Some urban treatment plants additionally use activated carbon for the removal of toxic chemicals.

III. Disinfection

The final step in water treatment is disinfection. Water can be disinfected by several methods. Chlorination is one of the most commonly used method. It kills pathogenic bacteria. Disinfection by ozone (O_3) is another method. Ozone is highly reactive. It kills microorganisms by oxidation. Ozone has become a preferred method of disinfection since it leaves no taste or odor and has little residual effect.

Wastewater (sewage) management

Wastewater includes water from household use such as water used for toilets and washing, water from urban drainage systems and from industry. Many developing countries and some developed counties still do not have proper wastewater treatment mechanisms.

- **Principles and main steps in purification of industrial waste water**

I. Primary treatment- Following steps are involved in the primary treatment.

- Large floating materials are screened out
- Removal of sand
- Removal of oil and grease
- Solid matter settles out in sedimentation tanks
- Sludge collected and removed
- No biological activity is used here
- Primary treatment removes 25-35% organic matter

II. Secondary treatment- Following steps are involved in the Secondary treatment.

- The liquid flowing out of primary treatment, enters the secondary treatment stage.
- During this treatment, waste water is aerated to facilitate the growth of aerobic bacteria and rapid microbial oxidation. One of two systems used are, activated sludge and trickling filter.
- In activated sludge, vigorous aeration is done mechanically. In trickling filters, waste water is slowly sprinkled or sprayed over a bed of rocky material and allowed to trickle. In this process, microorganisms grow on the filter bed and oxidize organic matter.
- In secondary treatment 75-95 % of organic material is oxidized.
- The liquid flowing through these systems are then disinfected and allowed to flow

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into natural waters.

- Sludge remaining from both treatments is sent to an anaerobic sludge digester where anaerobic decomposition convert the organic material in the sludge, finally to methane and CO_2 (biogas).
- Digested sludge can be used as fertilizer.

Adverse effects of discharging large amount of waste water into natural water bodies.

- Dissemination of pathogenic microorganisms
- Water pollution due to accumulation of biodegradable material and their decomposition products
- Decomposition which may consume large amounts of oxygen in water affecting aquatic organisms (high BOD – biological oxygen demand)
- Anaerobic decomposition leading to bad smells

Solid waste treatments

Nature of solid wastes

Solid waste include materials such as plant and animal residues, food waste, paper, plastic, polythene and glass. Among these, organic wastes such as plant and animal residues and food wastes are rapidly degradable. However, synthetic materials such as plastic and polythene are not easily degraded and tend to accumulate continuously. Proper management of solid waste, ensure community health and environmental safety. Accumulation of unmanaged waste in big piles in the open environment can lead to soil, air and water pollution which is harmful for the environment and organisms in contact with the polluted environment.

Environmental and hygenic importance of recycling solid wastes

- Open dumping of waste provide breeding grounds for mosquitoes, flies, other insects andrats. These organisms serve as vectors for dangerous diseases such as dengue, chikungunya, various food borne diseases and leptospirosis.
- Contaminated water resources have the risk of spreading water borne diseases such as typhoid, paratyphoid, cholera, dysentery and gastroenteritis.
- Piles of waste at public and residential area often create social issues due to unpleasant smell generated during anaerobic digestion of waste.
- Heavy piles of waste sometimes can be dangerous due to accumulation of methane, generated during anaerobic digestion of waste. Accumulation of methane can cause explosion and fire.
- Groundwater can be polluted by the leachate of waste piles. Leachate means liquid pass through the waste, extracting the dissolved and the suspended matter from the waste.

Therefore, solid waste must be properly managed using appropriate technology which is socially accepted and environment friendly.

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Methods of minimizing problems created by solid wastes (solid waste management)

1. **Sorting and recycling**

   In many countries municipal solid waste is sorted into kitchen waste, plant material, paper, plastic, glass etc. and collected into separate containers. Sorting allows separation of waste that can be recycled such as paper, plastic and glass. Although paper is presumably biodegradable, it is not easily degraded since microorganisms cannot effectively attack papers when the waste is placed into large compacted dumping sites.

2. **Degradation/ decomposition of organic matter**

   Readily degradable organic matter such as kitchen and garden waste is subjected to natural microbial degradation by the process of composting. Resulting compost is used in agriculture and gardening. Compost generate additional income to the waste management authorities such as municipal council. Anaerobic condition in the pile of compost, promote the activity of methanogenic bacteria. The methane gas they produce can be collected to generate electricity.

3. **Sanitary land filling**

   Sanitary landfills are one of the most popular forms of waste disposal, primarily because, they are the least expensive way to dispose the waste. More than four-fifths of municipal solid waste is disposed in landfills. A sanitary landfill is an engineered means of disposal of waste. In a sanitary landfill, waste is spread in layers on a piece of property, usually on marginal or sub marginal land. The objective is to spread the layers and then compact them tightly, greatly reducing the volume of the waste. The waste is then covered by soil. A landfill should not be located in areas with high ground water levels. Much of the waste in a sanitary landfill will decompose through biological and chemical processes that produce solid, liquid and gaseous products.

Microorganisms and food

Why food is spoiled by microorganisms

All food materials used for human consumption are of plant or animal origin. Microorganisms inhabit nearly every niche on earth, and food supply which are of plant or animal origin is no exception. Food have a natural micro biota and also get contaminated with microorganisms in nature during handling and processing. Food materials contain nutrients and water, act as culture media for growth of microorganisms. The nutrients present in the food materials, the same that is required for the growth of many microorganisms. Therefore, food serves as a culture medium such as nutrient broth. Numerous bacteria, yeast and , molds will grow in this broth.

Physical, chemical and biological changes occur in food spoilage

Growth of microorganisms in food changes physical, chemical and biological structures of food and making them unfit for consumption which is termed as food spoilage. Microorganisms growing in food are heterotrophic bacteria and fungi. During this process, they breakdown carbohydrates, proteins and fats, to obtain energy and other requirements for their own growth. Various extra cellular enzymes such as amylase pectinase, cellulase, protease and lipase are secreted by contaminant microorganisms, in order to complete the above process. As a result of this, major constituents of food, undergo chemical, physical and biological changes.

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Chemical changes take place in food

1. Putrefaction

Protein present in the food source is broken down by the proteolytic enzymes secreted by proteolytic microorganisms into Amino acids, Amines, Ammonia and hydrogen sulphide (H S).

2. Fermentation

complex carbohydrates present in the food source are broken down into simple carbohydrates by amylase secreted from microorganisms. Simple carbohydrates (sugars) then converted into carbohydrate food acid, alcohol and gases by enzymes secreted from saccharolytic microorganisms.

3. Rancidity

Lipids present in food source is converted into fatty acids and glycerol by enzymes secreted by lipolytic microorganisms.

Physical changes that may take place in food

1. Softening of food
2. Pigmentation
3. Ropiness
4. Slime or gum formation (polysaccharide)
5. Toxin accumulation

External factors influence on food spoilage

External factors are environmental factors that affect both food and microorganisms.

1. Temperature of storage- Growth of microorganisms is affected by a wide range of temperatures, at low temperature growth is slow, therefore, spoilage is also slow, at ambient temperature growth is high, and therefore, spoilage is also high. However, even at low temperature (e.g. food stored at 4 ^{0}C in the refrigerator getting spoiled) microbial spoilage occurs by psychrophilic bacteria.

2. Relative humidity (RH) of environment- Relative humidity of the storage environment is important, as it relates to the availability of moisture, which is an important factor for food spoilage due to microbial growth. Low moisture containing food should not be stored in high RH environments, as the food will pick moisture from the atmosphere and the microbial growth will be facilitated.

3. Presence and concentrations of gases in the environment- presence or absence of O_2 determine the type of contaminants causing food spoilage. There are both aerobic as well as anaerobic microbes cause food spoilage.

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Internal factors influence on food spoilage

Internal factors are those factors that are present in food itself.

1. pH- Most microorganisms grow best at pH values around 7.0 (6.6 – 7.5) and very few grow below 4.0. In general, molds and yeasts can grow over a wide range of pH from very low to very high (pH 2 - 10). Bacteria in general, grow between pH 5 – 7. Fruits such as limes, orange and banana are likely to be spoiled by molds and yeast. Most animal foods such as beef, chicken, fish, milk are susceptible to bacterial, mold and yeast spoilage.

2. moisture content- Drying is the oldest method of preserving food which is based on reducing moisture content. High moisture containing food such as meat and fish are spoiled by bacteria. Low moisture containing food such as biscuits and bread are spoiled by molds. Dried milk powder or flour which contains very low moisture are not easily spoiled by bacteria or molds. Salt and sugar containing foods (available water is low) are generally spoiled by halophillic bacteria (salted foods), osmophillic and xerophillic molds/ yeast (sugary foods).

3. Nutrient content- Water, source of energy, nitrogen, vitamins and minerals are essential

   nutrients for growth of microorganisms. Nutrient rich food are easily spoiled by microorganisms. e.g.; Milk, meat

4. Biological structure- The natural covering of some foods protect the food from the entry and the damage cause by the spoilage microorganisms. e.g., outer covering of fruits, shell of egg

Food borne pathogens of humans

- Typhoid - *Salmonella typhi*
- Dysentery - *Shigella*
- Cholera - *Vibrio cholera*
- Food poisoning - *Staphylococcus aureus*
- Botulism - *Clostridium botulinum*
- Aflotoxin - *Aspergillus flavus*

Effects of food spoilage on human health

Some microorganisms produce various toxic substances in food, which cause food poisoning or food intoxication when consumed. Illness may result from consuming food spoiled by microorganisms. Microorganisms grow and multiply in food, increasing the numbers of microbial cells, and also produce toxic chemical substances. Ingestion of large numbers of microbial cells and their toxic chemicals by any individual through highly contaminated food, may develop disease. These diseases/ illnesses are of two kinds;

(a) food borne infections- In food borne infections, the contaminating pathogenic microorganisms enter the body of the individual consumed spoiled food, and inside the host, they grow, multiply and also produce toxins which cause symptoms of the characteristic disease.

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e.g. *Salmonella typhi* - typhoid fever

Shigella - dysentery

Vibrio cholerae - cholera

(b) Food intoxication- In food intoxication, the spoiled food contains toxins, produced due to microbial growth. Any individual who consumes such contaminated food containing toxins will develop symptoms of the disease, within a short period of time. e.g;

- Food poisoning - *Staphylococcus aureus*
- Botulism - *Clostridium botulinum*
- Aflatoxins - *Produced by fungi Aspergillus flavus*

In general all the above illnesses are referred to as due to food poisoning.

(c) Viruses also (entero viruses) cause some food borne illnesses.

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Notes:

This is to acknowledge that some of the diagrams used in this book have been taken from various electronic sources using internet . This book is not published to make profit and sold only to cover cost.

The resource book is prepared according to the subject content and learning outcomes of the G.C.E. (A.L) Biology new syllabus which is implemented from 2017.

The content of this Resource book declares the limitation of the G.C.E. (A.L) Biology new syllabus which is implemented from 2017.

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Message from the Director General

The National Institute of Education takes opportune steps from time to time for the development of quality in education. Preparation of supplementary resource books for respective subjects is one such initiative.

Supplementary resource books have been composed by a team of curriculum developers of the National Institute of Education, subject experts from the national universities and experience teachers from the school system. Because these resource books have been written so that they are in line with the G. C. E. (A/L) new syllabus implemented in 2017, students can broaden their understanding of the subject matter by referring these books while teachers can refer them in order to plan more effective learning teaching activities.

I wish to express my sincere gratitude to the staff members of the National Institute of Education and external subject experts who made their academic contribution to make this material available to you.

Dr. (Mrs.) T. A. R. J. Gunasekara
Director General
National Institute of Education
Maharagama.

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Message from the Director

Since 2017, a rationalized curriculum, which is an updated version of the previous curriculum is in effect for the G.C.E (A/L) in the general education system of Sri Lanka. In this new curriculum cycle, revisions were made in the subject content, mode of delivery and curricular materials of the G.C.E. (A/L) Physics, Chemistry and Biology. Several alterations in the learning teaching sequence were also made. A new Teachers' Guide was introduced in place of the previous Teacher's Instruction Manual. In concurrence to that, certain changes in the learning teaching methodology, evaluation and assessment are expected.

When implementing the previous curricula, the use of internationally recognized standard textbooks published in English was imperative for the Advanced Level science subjects. Due to the contradictions of facts related to the subject matter between different textbooks and inclusion of the content beyond the limits of the local curriculum, the usage of those books was not convenient for both teachers and students.

As this book is available in Sinhala, Tamil, and English, the book offers students an opportunity to refer the relevant subject content in their mother tongue as well as in English within the limits of the local curriculum. It also provides both students and teachers a source of reliable information expected by the curriculum instead of various information gathered from the other sources.

This book authored by experienced subject teachers and subject experts from the universities is presented to you followed by the approval of the Academic Affairs Board and the Council of the National Institute of Education. Thus, it can be recommended as a material of high standard.

Dr. A. D. Asoka De Silva
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10

Applied Biology

Aquaculture

Food and agriculture organization (FAO) defines aquaculture as the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants.

Importance of aquaculture

Farming edible aquatic organisms is accepted as one of the best solutions for feeding the ever increasing human population. A healthy diet, high in protein is necessary to ensure that growing population does not succumb to sicknesses and diseases due to lack of essential nutrients. Harvests from wild sources of fish, crustaceans and other aquatic species cannot keep up with the demand presented by the growing human population. Trying to match the demand through commercial fishing would eventually result in over-fishing and the loss of those species entirely. It is accepted that while aquaculture is essential to meet the human demand for fish and fishery products, it also relieves the strain on wild species and allow them to continue to be a significant source of food for humans.

General characteristics of species that could be cultured

- Selected species should withstand the climate of the region in which it is cultured.
- It should grow well (should have a fast growth) in prevailing physical and chemical parameters of water in the area.
- It should be easy to breed (breeding techniques should be available) so that sufficient number of fertilized eggs/early fry could be obtained easily.
- Techniques of incubation of fertilized eggs and rearing techniques of hatchlings/early fry should be available so that the production of sufficient number of young organisms (seed) would be easy under culture conditions.
- If the eggs, larvae, fry, fingerlings, juveniles and adults of the species are hardy it is easier for the hatchery manager/ farmer (handling hardy species is easy).
- Food and feeding habits of each developmental stage of the species should be known (easy to provide nutritionally balanced diet for each developmental stage).
- It should not reproduce in grow-out ponds/tanks.
- If it reaches sexual maturation relatively late, it is advantageous.
- It should accept formulated food and grow well.

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- It should be an efficient converter of economical foodstuffs.
- If it is accidentally released to natural water bodies there should not be adverse environmental impacts.
- It should tolerate high population density & grow well
- Having resistance to common diseases is advantageous.
- It should satisfy consumers by the taste, nutritive value, texture of flesh or appearance/ body color/ color patterns.

Ornamental fish culture

Ornamental fish keeping has been a hobby of humans for centuries of years; initially fish that had some colours were collected from wild and maintained in garden ponds for the joy of watching their aesthetic beauty. Ornamental fishes lure and draw a great attention worldwide through their attractive colouration, shapes and sizes of body and fins, swimming behaviours, ability to live under captive conditions and adaptability to live in little spaces. People keep fish in their homes for variety of reasons such as for decoration, children's education, enjoyment, relaxation of elderly or health affected individuals, prosperity and fortune of home occupants and to collect rare species and even to propagate them. Globally, ornamental fish keeping has emerged as one of the most popular hobbies being second only to the photography.

Species that are commonly used in freshwater ornamental fish culture in Sri Lanka

Guppy (*Poecilia reticulata*)
Black molly *(Poecilia mexicana*)
Swordtail (*Xiphophorous helleri*)
Platy (*Xiphophorous maculatus*)
Angelfish (*Pterophyllum scalarae*)
Discus (*Symhysodon discus*)
Siamese fighting fish (*Betta splendens*)
Kissing gourami (*Helostoma temmincki*)
Goldfish (*Carassius auratus*)
Koi carp *(Cyprinus carpio)*
(see Annexe 1)
(Scientific names are not required to memorize)

Aquarium

An aquarium can be defined as a container that is designed to hold water in which living organisms can be maintained over an extended period of time. Aquaria vary tremendously in size, shape and design ranging from a simple bottle containing some water (e.g. an empty jam bottle is used to keep a male Siamese fighting fish) to a multimillion Litres commercial exhibit tanks.

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Maintenance of a home aquarium

There are activities that should be carried out daily, weekly, fortnightly and monthly in order to maintain environmental conditions (water quality parameters) within the optimum ranges for the fish kept in an aquarium. Provision of correct nutrition and maintenance of water quality within optimum ranges contribute for the well-being and health management of fish kept in an aquarium.

Daily:

1. Feed the fish with a nutritionally balanced diet following a correct feeding regime (suitable daily ration should be offered in 2 or 3 meals)
2. Check on the status of health while feeding and remove the affected individuals for treating in another tank/basin
3. Allowing fish to adapt for the changing intensity of light (to prevent unnecessary stress on fish),
   - Switch on the light of the aquarium several minutes after the room lights have been on or after the day break
   -Switch off the light of the aquarium several minutes before the room lights are switched off or shortly before natural lights fades

Weekly:

1. Fish should not be fed one day per week (not applicable for brood fish, fry and fingerlings)

Fortnightly:

1. Switch off aeration
2. Rake or stir up gently the surface of the rooting medium (under-gravel filter medium)
3. Scrape excess algal growth
4. Allow debris to settle
5. Siphon off the debris along with 20 – 25% of the aquarium water
6. Replace the volume siphoned out with freshwater in which temperature, pH and hardness match with conditions of the water in the aquarium
7. Switch on aeration

Monthly:

1. Take out some water from the aquarium in to a basin /another tank/ bucket
2. Collect the fish carefully using a hand net and introduce them into the basin/ tank/bucket and arrange aeration to it.
3. Rinse rooting medium (under-gravel filter medium)
4. Remove the aeration tube from air lift, scrape off any deposit (algal or calcite) from the opening and clean/scrape off air diffuser stones
5. Introduce the siphoning tube under the filter plates and suck out the accumulated organic debris

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6. Check the terminals of light source
7. Remove dead and dying leaves from plants
8. Prune, thin out and tidy the plants and replace poorly grown plants
9. Rearrange the filter plates and the filter medium
10. Arrange the aeration and fill the aquarium halfway with clean freshwater/aged aerated tap water.
11. Reintroduce the fish with the water and then fill the aquarium to the original level of water using aged clean freshwater

No matter how much time and care is devoted things in an aquarium could go wrong from time to time. Equipment failure, excessive algal growth, poor water quality or occurrence of diseases could be the cause.

Water turning into green frequently, green algae growing on plants on aquarium décor and on the side glasses of the aquarium indicate that the aquarium is receiving too much light. The reduction of intensity and/or duration of light followed by partial water exchange may prevent this situation. Growth of brown algae as brown encrustations on plants on aquarium décor and on the side glasses of the aquarium are indications of insufficient illumination. Blue-green algal “mats” on plants on aquarium décor and on the side glasses of the aquarium indicate a high level of organic pollution. Physical removal of algal mats followed by partial water exchange, a review of the maintenance routine with a view to prevent further accumulation of organic pollutants (whether too much food are offered to fish, inadequate filtration or aeration, overstocking, etc.) are required to correct the situation.

Common diseases of cultured, freshwater ornamental fish

Ornamental fish kept in aquaria are susceptible to numerous diseases. Some of the diseases are infectious and some are non-infectious.

Infectious diseases

Invasion of fish tissues by a disease causing agent (a pathogenic virus, bacterium, fungus, or an obligatory/ oppourtunistic parasite), multiplication of it in/on fish tissues and increasing its’ population may lead to the development of an infectious disease in ornamental fish. However, many infectious diseases could be avoided/prevented through better management practices (BMPs such as maintenance of water quality, correct stocking density of compatible fish with compatible plants, correct feeding regime) to keep the immunity of fish at a higher level and through correct bio-security measures (to prevent contamination by pathogens; Ex: quarantining new fish, plants and other aquarium décor).

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Table 10.1: Common diseases of fresh water ornamental fish species

Disease	Group of the disease causing agent
Bacterial fin rot and gill rot	Bacteria
Heamorrhagic septicaemia	Bacteria
Columnaris disease	Bacteria
External mycosis	Fungi
Fish white spot disease (ich disease)	A Unicellular external, obligatory parasites
Trichodinosis	A Unicellular, external, opportunistic parasite
Gill and skin infestation	Obligatory/oppourtunistic, gill flukes and skin flukes

Environmental impact of ornamental fish culture

Some impacts of ornamental fish culture are beneficial while some could be harmful. The first benefit of ornamental fish culture is species conservation, and production of species that are difficult to obtain from the wild. Breeding and rearing of approximately 90% of freshwater ornamental fish traded globally are done under captive conditions; there is some environmental benefit or elimination of environmental damage via those breeding programs. The golden arrowana, and tiger barb (*Puntius tetrazona*) are two species that have been conserved via ornamental fish production. In addition to the sale of fish to hobbyists, fish are also being reintroduced to habitats in which they have been eliminated.

Invasive ornamental fishes/aquatic plants that are accidentally escaped to natural environment could affect a wide range of native organisms from zooplankton to mammals across multiple levels of biological organizations ranging from the genome to the ecosystem.

With imported live fish non-indigenous disease causing agents may also come in to a country. Haphazard use of broad-spectrum antibiotics and other chemicals as preventive/therapeutic treatments and release of treated water (containing those medications) in to the natural environment may cause antibiotic resistance/ resistance to chemicals used on pathogenic microorganisms including human pathogenic bacteria.

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FOR EXTRA KNOWLEDGE

Bacterial fin rot & gill rot

Aeromonas sp and *Pseudomonas* sp are opportunistically pathogenic bacteria that are commonly found in freshwater in low numbers. If the culture water is organically polluted (due to accumulated faecal matter or excess feed) causing changes in water pH, fish will react by secreting excess mucus on gill surface and the surface of fins. Bacteria will be attracted to mucus, feed on mucus, multiply and increase their population. If bacteria establish on fin edges and begin to obtain nutrition from the tissues of fins, tissues between fin rays will rot away. Similar activities of bacteria on gill tissues will cause primary gill filaments to rot away causing difficulties in gas exchange. Bacterial fin rot and/or gill rot has been recorded in almost all freshwater ornamental fish species reared in Sri Lanka.

Heamorrhagic septicaemia

If a pathogenic *Aeromonas* sp establishes on the body surface and obtain nutrients from epithelial cells of fish, those cells will undergo necrosis (dying) and drop off. If walls of blood capillaries in the skin of fish are damaged, small haemorrhagic wounds (wounds with the appearance of bleeding) may occur on the body surface. Pathogen could then enter the blood stream of fish and develop haemorrhagic septicaemia (entry of bacteria in to internal organs such as liver, spleen and kidney causing internal bleeding and affecting the functions of those vital organs also).

Columnaris disease

Almost all freshwater ornamental fish used in Sri Lankan ornamental fish industry could be susceptible to this disease. Columnaris disease, wrongly termed as mouth fungus disease, is common in most of the guppy farms in Sri Lanka. At the initial stage small white patches appear near mouth and when the affected area enlarges it appears as a fungal infection. Necrotic cells (dead cells) drop off producing wounds in which peripheries are red in colour while the middle area appearing whitish. Affected tissues of caudal fin will gradually die, dead tissues will drop off finally eroding the caudal fin completely up to the caudal peduncle. If the gills are infected, gill filaments will be eroded and fish could die due to breathing difficulties.

External mycosis

Aquatic fungi, *Saprolegnia* sp could produce fungal infections in freshwater ornamental

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fish. These fungi grow naturally on decomposing organic materials. When fungal spores are dispersed, if they drop on a damaged surface of gills, fins or body of fish they will germinate producing fungal hyphae. These fungal hyphae will obtain nutrition from fish tissues allowing the infected areas to undergo necrosis (death). Dead tissues will drop off producing open wounds. Fish eggs, newly hatched-out larvae are highly susceptible to external mycosis. Adult fish that has been living in polluted water at high water temperature also reported to get the disease.

Diseases caused by external, unicellular parasites

Ich disease (fish white spot disease)

Ich disease is common in all species of fish used in freshwater ornamental fish industry of Sri Lanka. Significant mortality of fish is reported in almost all farms during the colder months (December – January) of each year. White spot disease of fish is caused by *Ichthyopthirius*, an obligatory parasite (a unicellular ciliate); theronts (infective stage) swim and get in to the body of fish and occupy gills, fins and body surface of fish. It slowly moves among epidermal and dermal cells of fish, digest cell contents to obtain the required nutrition; as a reaction, some cells of fish will multiply and make a protective layer surrounding each parasite separately producing small nodule like structures. Those nodules could be observed by the naked eye as white spots which are broken when the fully grown parasites (trophozoites) come out from the fish body to complete the life cycle; when a large number of trophozoites comes out, blood capillaries of fish could be damaged resulting haemorrhagic wounds.

Trichodinosis

Trichodina spp are oppourtunistic parasites (unicellular ciliates) that are common in water. Generally, it invades the body of fish latrvae whose immunity is suppressed. it feeds on mucus and when it moves on fish tissues (fins, gills and body surface), fish tissues may be mechanically bruised. Trichodina begins to feed on oppourtunistic bacteria that settle on those wounds and cell debris of fish. Young fish will die due to heavy infestation of these parasites.

Diseases caused by external multicellular parasites

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Gill and skin infestation by flukes

Generally, gill flukes infest gills while the common site of infestation of skin flukes is the skin of fish. Respective flukes attach themselves to the surfaces of gill filaments and skin, feeds on mucus and scraped tissues of gills and skin. As a reaction thick mucus will be secreted by gill and skin tissues, wounded area (due to feeding) would be reddish and highly inflamed. When this occur on gills, it will disturb normal gas exchange; fish will display breathing difficulties (they will keep mouth opened with partially opened opercula) and come to the surface of water or gather near aerating devices. Heavy infestation could cause mortality in fry and fingerlings of fish. Secondary bacterial and fungal infections may contribute for mortality in adult fish also.

Nursery Management and Propagation

Issues in Nursery Management

Nursery is a specialized place for young plants and seedlings to be produced for transplanting in another place. Plant propagation and nursery management can be done in the field, orchard, forest or in a protected environment such as greenhouses, polytunnels or tissue culture laboratories. Main requirement of a nursery is to providing optimum/ favorable conditions for germination and seedling growth, which provides healthy, vigorous, evenly grown plants leading to best transplants.

Growers have to face several issues when they manage plant nurseries. The optimum capacity of a crop could be obtained only by providing optimum environmental conditions to the nursery plants. Some of the issues in nursery management practices are management of light, soil management (soil type, properties of soil and maintenances of soil), pest and disease control, water management (water quality and quantity), nursery structures and environmental controls (protected cultivation versus open air cultivation).

Management of light is a critical factor for rooting cuttings, seed germination, seedling growth as well as in tissue culture facilities. Light can be manipulated by controlling, quality (wave length) and duration (day length, photoperiod).

Soil management is a must to get a quality product. Soil texture, structure, and organic matter content influence the soil condition. Soil condition can be improved via application of fertilizer or manure into soil.

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Pest and disease control is vital to get a quality product and to maintain the plant health. This is achieved via biological, cultural, physical, chemical or combination of these as integrated pest management.

Water management and humidity control is also critical factors, which governs the rooting of cuttings and regulation of plant growth. The type of irrigation used depends on natural conditions of the area, soil type, slope of the land, water availability and the crop to be irrigated. Adequate supply of good quality water is a must in a nursery.

During propagation and nursery management, nutrients are supplied to seedlings. Although there are number of ways to apply fertilizer, the type chosen depends on the form of fertilizer available, cultivated crop requirement and the environmental conditions prevailing. Fertilizer may be applied as either solids or liquids.

Detailed description of nursery structures and protected cultivation is given in next section

In addition to these, other issues faced by growers are;

- Lack of access to modern technology
- Lack of financial incentives to further improve the nursery and cultivation facilities
- Shortage of quality planting/ propagating materials
- Inadequate knowledge on suitable techniques and growth conditions that can be used to grow crops

Methods and Rationale of Protected Cultivation of Crops/ Protected Agriculture

Horticultural crops can be grown in various types of plant growing structures. These structures provide a more favourable environment for plants than the open air/ outdoor cultivation. In simple terms protected cultivation is growing crops under controlled environmental conditions (Fig. 10.1. A). Protected cultivation of horticultural crops will tremendously benefit in terms of enhanced productivity.

Protected cultivation technology is used to protect plants from adverse climatic conditions (wind, heavy rain, mist etc.) by providing optimum conditions to achieve maximum yield and the best quality. This type of cultivation can be done in green house (depending on the covering materials different structures are used such as polytunnels). Protected cultivation is mainly utilized to grow perishable horticultural produce such as, fruits, vegetables and ornamental plant.

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Examples for plants grown in Protected cultivation in Sri Lanka

A simple form of green house is a structure where it is covered with material that allows light to penetrate inside and reach plants. At present with advanced technology, sophisticated modern green houses are constructed in horticulture industry, where the plants' microenvironment is more precisely controlled. Plants grown in green houses including polytunnels are ;

- Vegetables such as bell pepper, tomato, salad cucumber, cauliflower and lettuce
- Fruits- strawberries
- Ornamental plants- Carnations, roses, orchids



Figure.10.1. Photo images of protected cultivation (A) and open-air cultivation (B).

Tissue Culture – Principle and its importance

Tissue culture and micropropagation are two most interesting areas under biotechnology. In general, cell or tissue culture is culture of free living cells or group of similar cells.

More specifically, tissue culture is the ability to establish plant tissues (cells, callus and protoplasts), plant organs (embryos, shoots, roots) in aseptic, *in vitro* culture. The most common benefit of tissue culture is that of cloning or mass production of genetically identical organisms.

The main concept behind plant tissue culture is "totipotent" i.e., a single cell has the genetic programme to grow into an entire new plant. The concept of totipotent was first introduced by the scientists, Matthias Schleiden and Theodor Schwann who postulated the "cell theory" in 1838.

A tissue culture medium generally consists of inorganic salts, organic substances, water and gelling agent. Macro and micronutrients in appropriate ratios make the inorganic salt component. The organic substances include carbon energy source (usually sucrose) plant growth regulators (cytokinins and auxins), vitamins, miscellaneous compounds. Agar is incorporated as the gelling agent. These gels provide physical support for the explant and increase the aeration of the medium. There are several commercial media available for tissue culture (eg. Murashige and Skoog or MS medium, etc.).

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Importance of tissue culture technique:

1. Rapid multiplication of clones
2. Mass propagation of specific clones
3. Genetic uniformity
4. Genotype modifications
5. Ability to produce plants in large numbers in a small space
6. Production of pathogen-free plants
7. All year around production of plants
8. Ability to produce plants which do not produce viable seeds

Examples for tissue-cultured plants are Anthurium (Anthurium andreanum), Banan, Pineapple, Dragon fruit.

Grafting and other propagation methods used in floriculture industry

Seed propagation method/ sexual propagation

The most common and widely used propagation method for plants is seeds. Therefore, production of high quality seeds is a prime importance in the horticulture sector. Growers wish to have high quality seeds with higher percentage of germination and higher viability and free of diseases and pest damages.

Seed germination is the beginning of the next sexual generation of a particular plant. For a seed to initiate germination following conditions must be completed.

1. The seeds must be viable
2. The seed must be subjected to suitable environmental conditions
3. Dormancies existing should be overcome

Anthrium and Orchid plants can be produced using seed propagation methods (At present tissue culture is culture methods).

Vegetative propagation methods used in the Floriculture industry

In nature, some plants can produce sexually via seeds as well as asexually or vegetatively. In vegetatively propagated plants, the new plant is nearly always genetically identical to the parent. The various methods of vegetative propagation include seperation and division, cuttings, layering and grafting. Most of these vegetative propagations methods are used in the floriculture industry.

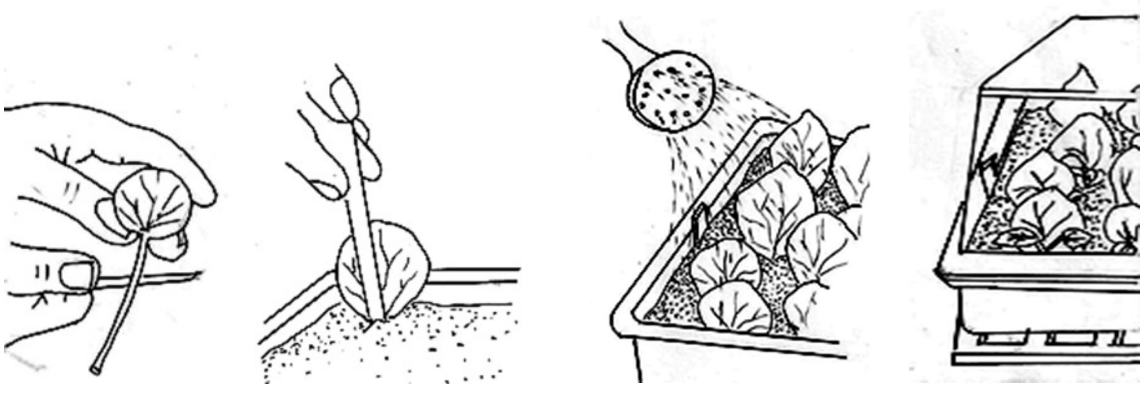
Separation and Division: Separation is a propagation method where naturally detachable structures (eg. Rhizomes, bulbs, and corms) are used. During division, it involves cutting or dividing the plant into sections with both roots and stems (eg. stolons and rooted runners).

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Corms:. Examples; *Alocassia/ Colocasia*??, *Gladiolus*
Runners: (eg., Spider plant/ *Chlorophytum comosum*).
Stolons: (eg. *Cyanodon* grass species, Mentha (mint), Stachys).

Cuttings: Cutting propagation is one of the most important clonal regeneration technique used in horticulture industry (for ornamental plants, fruit trees). Adventitious root formation is a prerequisite in this process. A piece of plant from the stem, leaf, root or leafy bud can generate into a fully developed plant (Fig. 2). The roots arising from stem, leaf, or bud tissue are known as adventitious roots. e.g. Roses, *Ficus*, *Dracaena* sp., African violets, Croton., Although stem and leaf-bud cuttings need only a new adventitious root system to be formed, for root and leaf cuttings, both the new shoot and root systems need to be formed.

Propagation via leaf cuttings: Begonia, African violet, snake plant *(Sansevieria)*,



A B C D

Figure. 10.2. Steps involved during propagation via leaf cuttings. A: Choosing a suitable leaf and trimming the petiole as required, B: Planting the leaf in potting media, C: Watering the leaf cuttings and D: Leaf cuttings with new plantlets in a propagator.

Layering: A method of vegetative propagation, where stems are rooted while attached to the parent plant. Some plants have a natural tendency to regenerate by self-layering, forming adventitious roots from the stem where they touch the soil (strawberry, Cynodon sp.). Layering technique is a good way of getting small number of new plants with relative certainty because the new plant is nourished by it's parent plant until roots are formed and rooted. E.g. Roses, *Hibiscus*

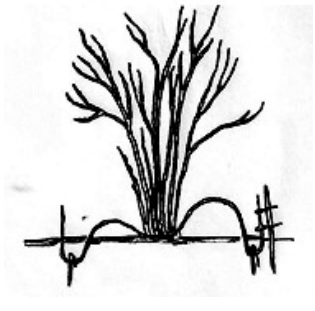


Figure. 10.3. Schematic diagram of a simple layering.

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Grafting: This involves joining two separate plants so that they later function as one healthy plant that has the best characteristics of two parent plants. A root system is provided by one plant (known as root stock or understock) and the desired top part by the other plant (known as scion) e.g. Roses

During a graft union it is vital that the cambium of the scion is placed in close contact with the rootstock cambium (Fig. 10.4). Initially a callus (mass of undifferentiated parenchyma cells) bridge is formed between the scion and rootstock in a successful graft. These callus cells are originated from the scion and the rootstock.

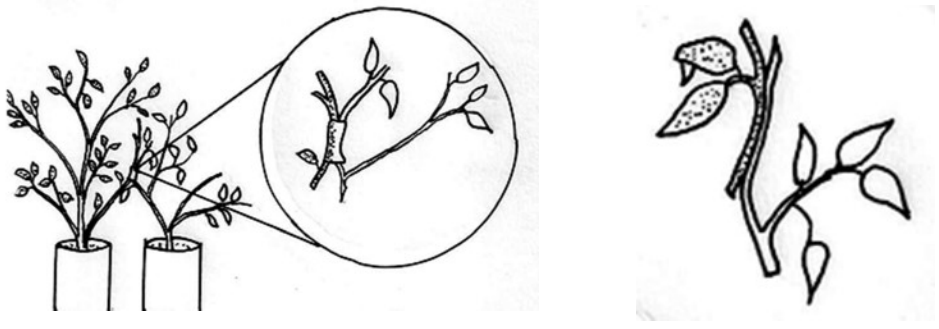


Figure.10.4. Procedure in making a side approach graft. A: Initial joining of the scion and the rootstock which are in separate pots and B: diagram of a completed graft union. Dotted plant indicates the scion and non-dotted one is the root stock

Floricultural practises found in Sri Lanka include cut flowers and Ornamental foliage plants. Anthurium and orchids are examples for cut flowers and Rose, *Dracinia* and Begonia are examples for ornamental foliage plants.

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Food Preservation and Postharvest Losses

Importance of food preservation

Most kinds of foods are readily spoiled unless they are preserved using suitable methods of preservation. food preservation ensures that food could be stored for a particular period. During some seasons over production of certain food occurs and the excess production could be preserved (using suitable methods) and stored to be used later. Food preservation protects the food that is available for human consumption reducing the "food loss".

Food preservation

Food preservation is the process of treating and handling food to stop or greatly slow down spoilage (loss of quality, edibility or nutritive value) caused or accelerated by micro-organisms.

Food could be preserved before undergoing spoilage using suitable techniques which could prevent unnecessary wastage and contribute greatly to meet food requirement of growing human population by,

- eliminating avoidable losses
- making more nutritive food items from low grade raw commodity using proper processing and fortification,
- diverting a portion of food materials presently being fed to animals for human consumption by way of processing and
- fortifying low grade food and organic wastes and by-products into nutritive animal feed.

Principles of Food Preservation

It is essential to prevent contamination of harvested food from damaging agents by hygienic handling, transportation and storage.

Three basic principles of food preservation

- Prevention of entry of microorganisms into food (aseptic).
- Prevention of the growth and activity of microrganisms in food.
- Remove or killing of microorganisms in food.

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Methods of Food Preservation

Food preservation methods aim to prevent contamination in the first place and to remove or reduce the numbers of contaminants. Preservation of food is achieved by application of physical, chemical and/or biological methods. The techniques may be applied separately or in combination. Microorganisms need a source of food and water, and they also need a suitable pH and temperature to grow; one or several of the living conditions needed for the growth of microorganisms have to be removed by the food preservation technique.

Drying

One of the oldest methods of food preservation is drying. Food is dried mostly in the sun and drying reduces water activity of the food sufficiently to prevent or delay microbial growth allowing food to keep for weeks. Most types of grains are dried to increase shelf life. Wheat, corn, oats, rice, rye, and barley are left to dry to prevent spoilage. Hams are a great example of meats preserved through drying in ovens.

Thermal (heat) Treatment

Heating food is an effective way of preserving it because the great majority of harmful pathogens are killed at temperatures close to the boiling point of water. In this respect, heating is a form of food preservation. A preliminary step in many other forms of food preservation, especially forms that make use of packaging, is to heat the food to temperatures sufficiently high to destroy pathogens. In many cases, food is actually cooked prior to their being packaged and stored.

Pasteurization (preserving fresh milk Refer unit 9)

Freezing and chilling

Freezing is perhaps the most common of all modern food preservation methods both commercially and domestically. Commercially freezing is done in different types of freezers under extremely fast freezing or fast freezing (bringing the temperature of fish/meat down to a value between -18 to -30 oC within 15 minutes to 2 hours) to preserve the quality. Frozen products are stored in cold rooms (at -18 to -30 ^{o}C) until distributed in order to prevent the growth and spreading of spoilage microorganisms(the extreme cold prevent microbial growth).

Storage of food (fruits, fruit juices, vegetables, fish, meat, etc.) in refrigerators at low temperatures (chilling at 4 to 7 ^{o}C) reduces the growth and activities of spoilage microorganisms, preserving food for a short period (for 10 to 14 days).

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Salting

Salting also is an older form of preserving raw foods. Salt has an ability to suck the moisture out of food through the process of osmosis. When the food is totally dry from salting, growth of microorganisms are inhibited. Meat is commonly preserved through salting.

Salting and drying

In preparation of dried fish (anchovy, tuna, etc.) fish are first salted and then dried in sun. Addition of salt makes the removal of water fast and the salt inhibits microbial growth on fish even though some moisture is still remaining in flesh.

Addition of Sugar (Sugaring)

Sugar is used in syrup form to preserve fruits and in crystallized form to preserve some food items like ginger and orange peel. Some fruits are glazed with sugar syrup for the fruits to get a superficial, protective coating. Some fresh fruits are cooked with sugar and then dried. High sucrose concentration produces osmotic stress on microorganisms and protects the food from microbial spoilage.

e.g. Jam, sugared toffees made with ash pumpkin

Smoking

Smoking is the process that cooks, flavours and preserves food exposing it to the smoke from burning wood. Smoke is antimicrobial and antioxidant and most often meats and fish are preserved by smoking. The aroma generated by hydrocarbons of burning wood enhances the taste, flavour and adds smokiness to the meat/fish while preserving.

Chemicals

Chemicals inhibit growth and activity of microorganisms while some chemical compounds can kill the microrganisms (e.g. preservation of fruit juice and production of pickels using chemicals such as sodium benzoate, EDTA, acetic acid, and brine solution).

Preservation of Food through radiation

The type of radiation used in processing food materials is limited to radiations from high energy gamma rays, X-rays and accelerated electrons. Processing of food by radiation involves exposure of food to short wave radiation energy to achieve a specific purpose such as extension of shelf-life, insect disinfestation and elimination of food borne pathogens and parasites.
(e.g. packets of spices, ground meat, etc.).

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Postharvest Losses

Food losses that occur along the food supply chain from harvesting of a crop until its consumption is defined as postharvest losses.

Product quality at harvest is influenced by when and how theharvest is done; pre-harvest factors such as source of seed and quality that has been set during growth also contribute for the product quality at harvest. Product quality at harvest could be lost further during harvesting, handling, transporting, storage, domestic processing and distribution.

The postharvest losses can broadly be categorized as,

- weight loss due to spoilage,
- quality loss,
- nutritional loss
- seed viability loss and
- commercial loss.

Postharvest losses in production of cereals (e.g. paddy) fruits and vegetables

During Harvesting - Untimely harvesting

Paddy should be harvested at the best time (considering the variety of paddy). Delayed harvesting of paddy may result quality loss, nutritional loss, seed viability loss, weight loss and commercial loss; those losses could be further increased if paddy fields are flooded due to heavy rains just before harvesting. If paddy is harvested earlier it requires more drying (drying cost), grains with high moisture content are susceptible to mold growth & insect infestation resulting broken grains and low milling yield with significant commercial losses. In addition, depending on the method of harvest, part of the paddy crop may be left and ploughed in to soil which is a commercial loss. Fruits and vegetables also should be harvested at the best time (could vary with the variety) to have maximum quality and to obtain the highest quantity; if harvested too early, the crop will not be ripe/matured enough and if harvested too late it may spoil quickly leading to quality loss and nutritional loss, finally resulting economical losses. Harvesting of fruits and vegetables should be done without allowing physical/ mechanical damages to occur; if damages occur quality and storage life will be reduced as spoilage microorganisms will quickly invade through damaged areas.

During Handling

High shattering loss of paddy seed could occur which is a commercial loss. Harvested paddy should be dried, bagged and stored temporarily without allowing the paddy seed to absorb moisture and greater attacks by insects and rodents.

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. In order to protect the quality, harvested fruits and vegetables should be handled hygienically kept in plastic cartons which could be kept in a shade temporarily. Harvested fruits/vegetables should be sorted to remove damaged, infested, moldy and over-ripe fruits / over-matured vegetables; sorting will reduce spoilage during transport and storage.

Transportation

Poor road infrastructure along with improper and poorly maintained modes of transportation results in large spillage of paddy seed and high contamination. Multiple movements of crop is another major reason for high transportation losses. Sometimes bagged paddy is loaded and unloaded from vehicles several times before it is milled. During each movement some grains are lost as spillage. During handling and transportation of rice, 2 – 10% losses occur in Southeast Asia. Those losses could be minimized using well planned, better mode of transportation with an efficient bulk handling system.

During the transportation of fruits/vegetables arranged at the bottom of stacked crates could be damaged by the

weight of the produce kept above; use of crates that could be stacked without putting the weight on produce at the bottom would solve this issue. Use of straw or something soft in between layers of produce within a crate will reduce the damages due to rubbing. Rough handling of crates containing produce should be avoided to prevent possible damages to the produce. Uncontrolled changes of temperature and excessive shaking could do great damages to the produce during transport. In tropical countries, if transportation is carried out at night time produce could be protected by high temperature of the day time.

Storage

Storage plays a vital role in the food supply chain, although losses occur at each stage of the supply chain from production to consumer level, storage losses are considered most critical in developing countries as maximum losses occur during the storage. Generally, after harvesting/ milling, grains are stored for short or long periods of storage as food reserves, and as seeds for next season. Poor storage infrastructure allows, rice to absorb excess moisture, rice grains with excess moisture to be infested easily by insects pests followed by invasion of spoilage microorganisms, rice to be attacked by rodents and other pests.

Provision of proper storage infrastructure could prevent/reduce the losses that occur during storage of paddy/milled rice.

Uncontrolled changes of temperature could encourage spoilage microorganisms to grow faster spoiling the stored produce (fruits and vegetables). Storage of fruits and vegetables should be done in properly chilled rooms to reduce the rate of spoilage.

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Domestic processing

In domestic processing of different food types at different areas of Sri Lanka following methods are used. The postharvest loss of food processing could be due to early harvesting, improper handling, etc. Postharvest losses that could occur during domestic processing could be reduced by following correct procedures (e.g. harvesting at the right time), hygienic handling to prevent microbial contaminations and proper storage (to prevent attacks of insects, rodents, etc.).

Drying (paddy/rice & other cereals), drying after thermal treatment (jack fruit, etc.), pasteurization (milk), freezing (fish and meat are temporarily stored in freezer compartment of a refrigerator), chilling (fruits, vegetables, etc.), salting (lime, etc.), salting & drying (fish), sugaring (homemade jams), use of chemicals (pickles),

Dengue and filaria

Dengue

Dengue is a vector borne disease which is caused by an arbovirus which is a RNA virus. Epidemics of dengue are reported in tropical and subtropical regions of the world. The virus is transmitted by two species of mosquitoes, they are *Aedes aegypti* and *Aedes albopictus*. The transmission of the dengue virus is dependent on biotic and abiotic factors. The biotic factors include the virus, the vector and the host and the abiotic factors include temperature, humidity and rainfall. The dengue virus is transmitted to human via the biting of an infected female mosquito. The infected humans can be symptomatic as well as asymptomatic and they are the carriers and multipliers of the virus.

The *Aedes* mosquitoes are small to medium sized (approximately 4-7 mm) and it is dark in colour. These mosquitoes have white markings/bands on its body and the arrangement on these bands are different in the body and the legs. They live about 2-4 weeks depending on the environmental conditions.

The life cycle has four stages, they are eggs, larva, pupa and adults. The adult female mosquito lays eggs singly on the inner surface of wet containers above the water level, preferably clear water.

Initially the laid eggs are white in colour and they become shiny black colour within few minutes from deposition of the eggs. These eggs are smooth, long and ovoid in shape and about 1 mm long. The eggs hatch within two days to become a larvae. The larvae rest with an angle to the water surface. If the eggs are not hatched, they can undergo dormant for a period of about six months. The body of larva consists of three major parts i.e. head, thorax and abdomen. After 4-5

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days the larval stage becomes the pupa which is comma shaped and mobile. The pupa becomes the adult mosquito within 1-2 days.

Breeding sites

The female mosquitoes lay eggs in a wide variety of artificial and natural wet containers. They prefer dark coloured surfaces with clear (unpolluted) water. The breeding sites can be found in both indoor as well as outdoor. They include,

- discarded plastic containers- tins, clay pots, yoghurt and ice cream cups, bottles, cans, damaged ceramic items, coconut shells, etc.
- water storage containers such as cement tanks, barrels, etc.
- discarded automobile tyres, and machinery parts,
    - building structures such as roof gutters, concrete slabs etc
    - household/ institutional appliances including refrigerator trays, flower vases, ornamental ponds, squatting pans of wash rooms, etc
    - natural breeding sites such as leaf axils, tree holes, etc.

Symptoms of disease

Dengue can affect infants, young children and adults. It starts with flu-like symptoms. Sudden onset of high fever (40 °C/ 104 °F) could be accompanied by any of the following symptoms. They are severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, or skin rash. These symptoms usually last for 2-7 days after incubation period of 4-10 days from the bite of an infected female *Aedes* mosquito.

Severe dengue (dengue haemorrhagic fever) is a potentially deadly complication due to blood plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment. Warning signs occur 3–7 days after the first symptoms in conjunction with a decrease in temperature (below 38°C/100°F) and include: severe abdominal pain, persistent vomiting, rapid breathing, bleeding from the nose and gums, fatigue, enlargement of liver reduced number of platelets, restlessness and blood in vomit. The next 24–48 hours of the critical stage can be lethal, in which symptoms may progress to massive bleeding, very low blood pressure, shock and death. Proper medical care is needed to avoid complications and risk of shock and death (dengue shock syndrome).

Controlling measures of the vector

The dengue vector controlling methods are mainly aimed at both immature and adult stage of the *Aedes* mosquitoes. Integrated vector management strategy includes environmental, chemical and biological controlling methods.

The most cost effective way is controlling the vector by eliminating its breeding sites. Removal of open source of water through the environmental management could be done by;

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- having continuous water supply to minimize storage of water in cement tanks, barrels, and other containers,
- making mosquito proofing of water storage cement tanks, domestic wells, and over head tanks,
- construction of buildings without roof gutters
- removal of unserviceable roof gutters
- regular cleaning with scrubbing of water storage tanks, flower pots, flower vases, ant traps, refrigerator trays
- proper disposal of solid wastes
- proper storage of used tyres, household and garden utensils.

In addition to environmental management practices, biological controlling methods also can be used to control the vector. They include,

use of fish species which feed on the larval stages of the mosquito. The following fish species could be introduced to water storage tanks, barells, ornamental ponds, etc. to feed on the larval stages of these mosquitoes.

- Guppy (*Poecilia reticulata*)
- Dandi (*Rasbora daniconius*)
- Juvenile stages of Tilapia

The *Bacillus thuringiensis israelensis* (Bti) also can be used to control the dengue vectors. This is a bacterium that produces an endotoxin which is toxic to the larval stages of the mosquitoes.

Another method of mosquito control is thermal fogging which is a form of chemical method. When mosquitoes are exposed to sufficient dosage of this fog they are knoked down and killed.

There are several limitations in vector controlling methods, such as lack of sufficient knowledge in biological controlling methods.

When fish are used to control the vector,

- fish could die in the absence of food in the breeding site and due to changes in water quality parameters such as pH of water in breeding site
- when the domesticated water containers are filled with chlorinated water it may lead to the death of the fish.

when Bti is suggested to control the vector,

- there could be some breeding sites that Bti can't be applied

chemical methods such as fogging,

- could affect health of both humans and animals

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Filaria

Lymphatic Filariasis in Sri Lanka

Filariasis is one of the oldest debilitating disease in the world. This is one of the major leading causes of permanent and long term disability. It does not kill people, but cause permanent disability. Filariasis is a mosquito borne disease transmitted by a thread like nematode, who lives in human lymphatic system. The disease is endemic in developing countries with poor socio-economic status and is often associate with poor environment sanitation. Filaria has been categorized as a neglected tropical disease.

Parasite

- 90% of infections are caused by *Wuchereria bancrofti*
- Human is the exclusive host of *W. bancrofti*

Vector

- In Sri Lanka it is transmitted solely by *Culex quinquefasciatus* mosquito
- *Culex* breeds in polluted water bodies; blocked drains, broken toilet pits, husk pits etc.

The Culex mosquito is small to medium in size (3 to 4 mm in length) and grey-black in colour. The nervures of wings of adult mosquito are beset with brown or blackish scales and the posterior margin of the wings is fringed with bristle and scales. Female lay eggs in the form of egg rafts that floats on the surface of water. After hatching, larva rests keeping its body with an angle to the water surface.

Factors associated with transmission

- Transmission in a community is influenced by
- Number of infected persons (prevalence)
- Density of microfilaria in blood of infected persons
- Density of vector mosquitoes
- Characteristics of the vector (affects development of larvae)
- Frequency of human vector contac

Methods of transmission

Filariasis parasite is introduced to human body by a bite of a female adult mosquito. When the mosquito suck blood from humans, larval stages of parasite falls on to the skin (do not inoculate in to the body as other mosquito borne disease agents). Then these larvae penetrate skin through the bite wound and enter into the human vascular system. From here, the larvae migrate to the lymphatic system Within one year larvae become matured in to adult worms. Adult male and female worms nests and mate in lymphatic vessels and produce large number of tiny, immature

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eggs which hatch into microfilariae. Microfilariae migrate from the lymphatic system and enter the blood stream. Microfilariae live in lungs during day time and travel to peripheral blood during night. From here they transfer to mosquito when it takes a blood meal from human body.
Ingested microfilariae transform into several larval forms and again with another bite enter into human body.

Adult worm lives in lymphatic system for 5-6 years and microfilaria can lives for 1 year.
When adults worms block lymphatic vessels they get distorted and lymph flow does not occur smoothly. Lymph tends to accumulate in dependent parts of the body: legs, hands, testes, penis, and breast causing lymphedema. When microfilaria lives in lungs patients develop dry cough, wheezing, mild fever, weight loss etc.
Fluid accumulation in scrotum manifests as hydrocele. Presence of microfilariae in lungs manifest as occult filariasis. Occult filariasis is characterized by high eosinophilic count with chronic cough and wheezing which worsen at night with dyspnea, chest pain, fever and weight loss.

Clinical manifestations vary from asymptomatic to chronic lymphedema (elephantiasis).
After entering the human body the filarial parasite (L3 larva) develops into an adult worm and remains silent in lymphatics for several years producing microfilaria. These patients are asymptomatic and can only be detected through active surveillance.
Presence of adult parasite in lymphatic system for a longer period gives rise to late manifestations. They are due to dilatation of lymphatic vessels followed by their dysfunction, accumulation of fluid in tissues and increased risk of infection (lymphoedema). With recurrent infections skin of oedematous extremities becomes thickened and later on gives rise to warty, nodular, papillomatous appearance. Lymphoedema is graded according to the state of oedema and appearance of the skin

Controlling measures

- Personal protection from mosquito bites/ prevent mosquito bites by the use of mosquito nets, repellants, long sleeve shirts and trousers.
- Elimination of breeding sites: repair broken septic tanks, cleaning drainages, prevent dumping garbage in to drains, chemical control of aquatic plants in water reservoirs
- Prevent creation of breeding sites
- Use of larvivorous fish such as guppy, nalahandaya in water bodies.
- Screening healthy population with night blood films and detect people who harbour the infection but do not show any symptoms and treat them.
- Monitor vector mosquito population for infectivity

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Limitations of controlling methods

Lack of sufficient kbnowledge among people about the disease, the vector, method of transmission, breeding sites and controlling measures.

Nanotechnology

Nanotechnology is an emerging science involved in designing, building and manipulating minute structures at the nanometer level. A nanometer (nm) is one billionth of a meter (10^{-9} m). Nanotechnology is the creation and use of materials and devices on the same scale as molecules and intracellular structures, typically less than 100 nm in size. The physical and chemical properties of tiny molecules are significantly different than bigger particles as they have a very high surface area to volume ratio.

American Physicist Richard Feynman in 1959 enlightened the world on nanotechnology.

Living organisms are built of cells that are typically 10 µm across. However, the subcellular organelles are much smaller. Proteins are even smaller with a typical size of just 5 nm, which is comparable with the dimensions of smallest manmade nanoparticles. This simple size comparison gives an idea of using nanoparticles as very small probes that would allow us to investigate the cellular machinery without introducing too much interference. Understanding of biological processes on the nanoscale level is a strong driving force behind development of nanotechnology. Nanotechnology has now become a big business with applications in material manufacturing, energy, electronics and engineering. But Applications of nanoparticles in biology and medicine are of particular interest. One of the most important applications of nanotechnology is in medicine. Applications of nanotechnology for improving human health are termed Nanomedicine. Nanotechnology can be applied for prevention diagnosis and treatment of diseases.

Titanium dioxide (TiO_2) and silver (Ag) nanoparticles are used for sterilization of operation theatres and surgical instruments in hospitals. These nanoparticles are able to destroy the microbes. Silver nano lotions are applied in theatres to prevent the entry of microbes. Nano particles are used in the production of antimicrobial coatings and nano - filters. TiO_2 and Silver nano filters prevent the entrance of tiny particles such as viruses. These nano filters are used to examine SARS patients. Nano device sensors are used to monitor blood pressure; blood oxygen levels and hormone concentrations. Nano particles can unclog blocked arteries and detect and eliminate cancer cells. Several nanotechnology enabled drugs are available in the market primarily in areas of cancer treatment. Scientists have developed “smart drugs” using tiny nanoparticles such as gold particles that are introduced into the body to seek out and target special cells such as cancer cells to deliver a cargo that would destroy those that are damaged rapidly and effectively in a silent manner with

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very few side effects.

Spherical nanoparticles consisting of a dielectric core called nanoshells are used in the treatment of cancer. A nanoshell is slightly bigger than a polio virus. Gold nano shells are used in bio imaging enhancements as well.

In the treatment of diabetes a Nano device is attached to the body which can release the required dosage of insulin at required time intervals.

There has been successful application of nano-technology to the treatment and management of pain both in clinical and experimental studies like the fabrication of nano-formulated liposomes to deliver drugs for pain therapy,

Since nanocarrier systems can be easily transferred to the airways, many respiratory diseases can be treated using nanotechnology.
Viva gel is a vaginally applied microbicide in development for prevention of HIV and HSV (Herpes simplex virus). Viva gel is a product of nanotechnology which prevents the entry of HIV and HSV during sexual intercourse.
Nonocomposites are used to replace broken bones and to fill teeth as well.

Stem Cell Therapy

Stem cells are undifferentiated cells which can give rise to cells of the same type. They can divide by mitosis without a limit (or at least for the lifetime of the animal). They are capable of terminally differentiating into other cell types.
Stem cells are required wherever there is a recurring need to replace differentiated cells that cannot themselves divide. The stem cell itself has to be able to divide but it doesn't necessarily have to divide rapidly. In fact, stem cells usually divide at a relatively slow rate.
Stem cells are of two types.

1. Embryonic stem cells
2. Adult Stem cells

1. Embryonic stem cells

Following fertilization of a sperm and an egg cell, the fertilized egg is called a zygote. The zygote divides rapidly. In humans, in around five to seven days after fertilization, the dividing cells create an embryo consisting of a small hollow cluster of approximately 100 cells called a blastocyst. The blastocyst is approximately a seventh of a millimeter in diameter. There is a small cluster of

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around 30 cells tucked inside the blastocyst that form a structure known as the inner cell mass. These cells are the source of embryonic stem cells (ES cells).

ES cells are so special because they can eventually differentiate to form all of the more than 200 cell types that make up the human body. Therefore, ES cells are called pluripotent because they have the potential to develop into a variety of different cell types. Human ES cells (hESCs) are unspecialized cells with two major properties.

- ES cells can self-renew indefinitely to produce more stem cells
- Under the proper growth conditions, hESCs can differentiate into a variety of mature cells with specialized functions

2. Adult stem cells

Adult stem cells are found throughout the body after development. Adult stem cells are present in many tissues. There are many types of adult stem cells specialized for the genesis of different classes of terminally differentiated cells and hence each type of stem cell serves for the renewal of one particular type of tissue.

Eg. Epidermal stem cells for epidermis, Intestinal stem cells for intestinal epithelium, hemopoietic stem cells for blood, neural stem cells for central nervous system

Stem cells can be isolated and cultured in culture media in vitro. When adult stem cells are removed from the body and maintained in culture or are transplanted from one site in the body to another, they generally remain faithful to their origins. This creates practical limitation of using adult stem cells for tissue regeneration.

In contract, ES cells can be kept proliferating indefinitely in culture and yet retain unrestricted developmental potential. If ES cells are put back into a blastocyst, they become incorporated into the embryo and can give rise to all the tissues and cell types in the body, including germ cells. Under the proper growth conditions, human ES cells can differentiate into a variety of mature cells with specialized functions in vitro.

There are ethical concerns of obtaining human embryonic stem cells. Therefore, research on eHSCs is very controversial because of their source- an early embryo.

In late 2006, Scientists demonstrated the successful derivation of pluripotent stem cells from adult tissues by reprogramming human cells taken directly from a volunteer. These are known as induced pluripotent stem cells (iPSCs). Pluripotent stem cells hold promise in the field of

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regenerative medicine because they can propagate indefinitely, as well as give rise to every other cell type in the body (such as neurons, heart, pancreatic, and liver cells), they represent a single source of cells that could be used to replace those lost to damage or disease. Since iPSCs can be derived directly from adult tissues, they not only bypass the need for embryos, but can be made in a patient-matched manner, which means that each individual could have their own pluripotent stem cell line.

Applications of stem cells

There are many potential applications of stem cells from growing healthy tissues to studying them to understand and treat birth defects to genetic manipulation for delivering genes in gene therapy approaches. Creating whole tissues in the laboratory using tissue engineering is another potential application for repairing tissues such as damaged heart muscles and damaged spinal neurons.

Blood stem cells (hemopoietic stem cells) taken from the bone marrow of a healthy immunologically compatible donor can be used to replenish bone marrow of patients with leukemia

Scientists believe that stem cell technologies will play key roles in developing treatments for diseases such as stroke, heart disease, Parkinson disease, Alzheimer disease, diabetes and many more.

The Human Genome project

The human genome has been the focus of biological research for the last two decades and will continue to be the center of attention for many years to come. It is a worldwide effort to identify all human genes of each chromosome along with several other goals. The human genome project is an enormous undertaking in genomics that is providing scientists with exciting insight into human genes, their locations and functions. It was originally a 13 year project (1990-2003) coordinated by the US Department of Energy and the National Institutes of Health. Additional contributions came mainly from UK, Japan, France, Germany, Australia and China.

The aims of the project were to

1. Identify all the genes (approximately 20,000 protein coding genes) in human genome
2. Determine the sequences of the 3 billion chemical base pairs that make up human DNA
3. Store information in data bases
4. Improve tools for data analysis
5. Transfer related technologies to private sector
6. Address, ethical, legal and social issues that may arise from the project.

A major quality assessment of the human genome sequence was published in 2004 indicating over 92% of sampling exceeded 99.99% accuracy which was within the intended goal.

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The Genome Reference Consortium (GRC) which is an international collective of academic and research institutes with expertise in genome mapping is still working on the analyses of the data obtained from the Human genome project.

The sequencing of the human genome holds benefits for many fields, from molecular medicine to human evolution. Improved diagnosis of various diseases, identification of mutations linked to different forms of cancer, the design of medication and more accurate prediction of their effects, gene therapy and control systems for drugs, Study of human evolution and anthropology a are some of them.

Another proposed benefit is the commercial development of genomics research related to DNA based products, a multibillion-dollar industry.

The Human Genome Project, and the other genome projects such as Escherichia coli, yeast, mouse, *Arabidopsis thaliana*, and rice have already been completed. This has therefore opened the way to a comprehensive description of the molecular activities of human cells and the ways in which these activities are controlled. This is central to the continued development, not only of molecular biology and genetics, but also of those areas of biochemistry, cell biology and physiology now described as the molecular life sciences.

The genome projects will have additional benefits that at present can only be guessed at. It is evident that the human genome, in common with the genomes of many other organisms, contains extensive amounts of intergenic DNA. It was thought that most of the intergenic DNA has no function except occasionally it is known that some intergenic DNA acts to control genes nearby. Could the intergenic DNA have a role, but one that at present is too subtle for us to grasp? Though little is known about them, they are thought to have regulatory functions. The first step in addressing this possibility is to obtain a complete description of the organization of the intergenic DNA in different genomes, so that common features, which might indicate a role for some or all of these sequences, can be identified.

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Annexe 1



Angel fish



Black molly



Discus



Fighter fish



Guppy



Koi carp fishes



Kissing Gourami



Platy fish

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Sword tail



Gold fish

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Notes:

This is to acknowledge that some of the diagrams used in this book have been taken from various electronic sources using internet . This book is not published to make profit and sold only to cover cost.

The resource book is prepared according to the subject content and learning outcomes of the G.C.E. (A.L) Biology new syllabus which is implemented from 2017.

The content of this Resource book declares the limitation of the G.C.E. (A.L) Biology new syllabus which is implemented from 2017.

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