BIOLOGICAL SCIENCE
(BOTONY & ZOOLOGY)

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PAPER - II

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BIOLOGICAL SCIENCE

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UNIT I

PLANT PHYSIOLOGY

The description of structure and variation of living organisms over a period of time, ended up as two, apparently irreconcilable perspectives on biology. The two perspectives essentially rested on two levels of organisation of life forms and the related phenomena. One described at organismic and above level of organisation while the second described at cellular and molecular level of organization. The first resulted in ecology and related disciplines. The second resulted in physiology and biochemistry. Description of physiological processes in flowering plants as an example, is what given in the chapters in this unit. The processes of mineral nutrition in plants, photosynthesis, transport, respiration and ultimately plant growth and development are described in molecular terms but in the context of cellular activities and even at organism level. Wherever appropriate, the relation of the physiological processes to the environment is also discussed.

Chapter 1

Transport in plants

Have you ever wondered how water reaches the top of tall trees, or for that matter how and why substances move from one cell to the other, whether all substances move in a similar way, in the same direction and whether metabolic energy is required for moving substances/ Plants need to move molecules over long distances, much more than animals do. Besides, they also do not have a circulatory system, in place. Water taken up by the roots has to reach all parts of the plant, up to the very tip of the growing stem. The photosynthates or food synthesised by the leaves have also to be moved to all parts including the root tips embedded deep inside the soil. Movement across short distances, say within the cell, across the membranes and from cell to cell within the tissue has also to take place. To understand some of the transport processes that take place in plants, one would have to recollect one's basic knowledge of plant cell structure and the anatomy of the plant body. We also need to revisit our understanding of diffusion, besides gaining some knowledge about chemical potential and ions.

When we talk of the movement of substances, we first need to define what kind of movement we are talking about, and also what substances we are looking at. In a flowering plant the substances that would need to be transported are water, mineral nutrients, organic nutrients and plant growth regulators.

Over small distances substances move by diffusion and by cytoplasmic streaming supplemented by active transport. Transport over longer distances proceeds through the vascular system (the xylem and the phloem) and is called **translocation.**

An important Aspect that needs to be considered is the direction of transport. In rooted plants, transport in xylem (of water and the phloem) is essentially unidirectional, from roots to the stems. Organic and mineral nutrients however, undergo multidirectional transport.

Organic compounds synthesised in the photosynthetic leaves are exported to all other parts of the plant including storage organs. From the storage organs they are later re-exported.

The mineral nutrients are taken up by the roots and upwards into the stem, leaves and the growing regions. When any plant part undergoes senescence, nutrients may be withdrawn from such regions and moved o the growing parts. Hormones or plant growth regulators and other chemical stimuli are also transported, though in very small amounts, sometimes in a strictly polarised or unidirectional manner from where they are synthesised to other parts. Hence, in a flowering plant there is a complex traffic of compounds (but probably very orderly) moving in different directions, each organ receiving some substances and giving out some others.

1.1 Means of Transport

1.1.1 Diffusion

Movement of **diffusion** is passive, and may be from one part of the cell to the other, or from one to cell to cell, or over short distances, say, from the inter-cellular spaces of the leaf to the outside. No energy expenditure takes place. In, diffusion, molecular move in a random fashion, the net result being substances moving from regions of higher concentration to regions lower concentrations. Diffusion is a slow process and is not dependent on a 'living system'. Diffusion is obvious to gases and liquids, but diffusion in solids rather than of solids is more likely. Diffusion is very important to plant since it is the only means for gaseous movement within the plant body.

Diffusion rates are affected by the gradient of concentration, the permeability of the membrane separating them temperature and pressure.

1.1.2 Facilitated Diffusion

As pointed out earlier, a gradient must already be present for diffusion to occur. The diffusion rate depends on the size of the substances; obviously smaller substances diffuse faster. The diffusion of any substance across a membrane also depends on its solubility in lipids, the major constituent of the membrane. Substances soluble in lipids diffuse through the membrane faster. Substances that have a hydrophilic moiety find it difficult to pass through the membrane; their movement has to be facilitated. Membrane, Proteins provide sites at which such molecules cross the membrane. They do not set up a concentration gradient: a concentration gradient must already be present for molecules to diffuse even if facilitated by the proteins. This process is called **facilitated diffusion**.

In facilitated diffusion, special proteins help move substances across membranes without expenditure of ATP energy. Facilitated diffusion cannot cause net transport of molecules from low to a high concentration- this would require input of energy. Transport rate reaches a maximum when all of the protein transporters are being used (saturation). Facilitated diffusion is very specific: it allows cell to select substances for uptake. It is sensitive to inhibitors which react with protein side chains.

The proteins form channels in the membrane for molecules to pass through. Some channels are always open; others can be controlled. Some are large, allowing a variety of molecules to cross. **Porins** are proteins that form huge pores in the outer membranes of the plastids, mitochondria and some bacteria, allowing molecules up to the size of small proteins to pass through.

Figure 1.1. shows an extracellular molecule bound to the transport protein; the transport protein then rotates and releases the molecules inside the cell, e.g., water channels- made up of eight different types of **aquaporins.**

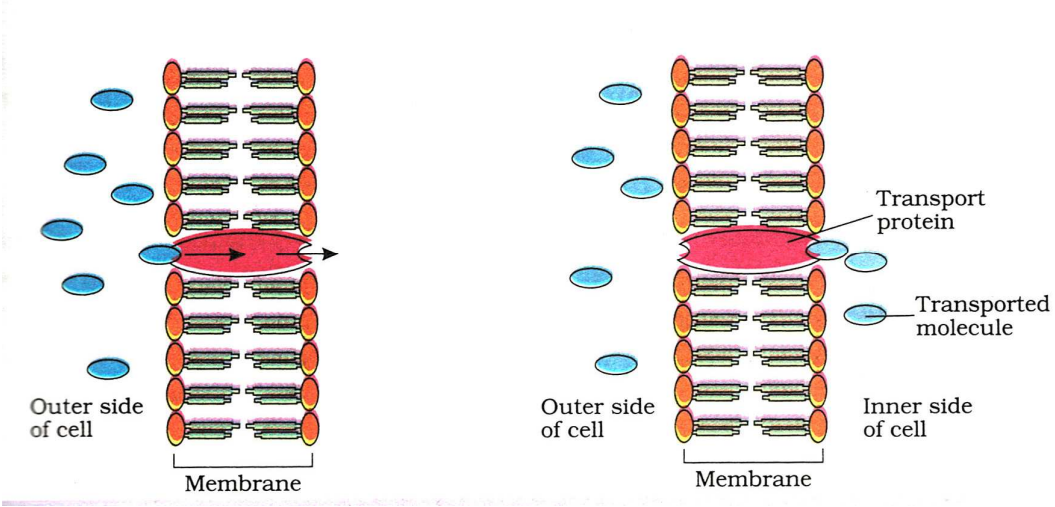


Figure 1.1 Facilitated diffusion

1.1.2.1 Passive symports and antiports

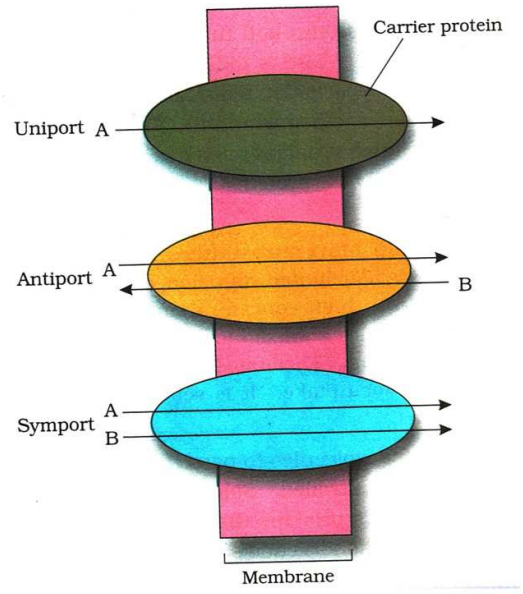


Figure 1.2 Facilitated diffusion

Some carrier or transport proteins allow diffusion only if two types of molecules move together. In a **symport,** both molecules cross the membrane in the same direction; In an **antiport,** they move in opposite direction s (Figure 1.2). When a molecule moves across a membrane independent of other molecules, the process is called **uniport.**

1.1.3 Active Transport

Active transport uses energy to pump molecules against a concentration gradient. Active transport is carried out by membrane-proteins. Hence different proteins in the membrane play a major role in both active as well as passive transport **Pumps** are proteins

that use energy to carry substances across the cell membrane. These pumps can transport substances from a low concentration to a high concentration ('uphill' transport). Transport rate reaches a maximum when all the protein transporters are being used or saturated. Like enzymes the carrier protein is very specific in what it carries across the membrane. These proteins are sensitive to inhibitors that react with protein side chains.

1.2 Plant-Water Relations

The absorption of water by plant cells followed by its movement from one cell to another cell and to different parts of the plant and finally its movement between the plant and its environment constitute the plant water relations of plants.

Water is a vital component for all the metabolic activities of plants. Its content in the plant body mainly depends upon the level of metabolism. Water is an excellent solvent and helps in the uptake and distribution of mineral nutrients and others solutes required for growth and development. Water plays a key role in several reactions of respiration and act as a source for the release of oxygen during photosynthesis.

1.2.1 water Potential

To comprehend plant–water relations, an understanding of certain standard terms is necessary. **Water potential** (Ψw) is a concept fundamental to understanding the water movement. **Solute potential** (Ψs) and **pressure potential** (Ψp) are the main components that determine water potential.

Water molecules possess kinetic energy. In liquid and gaseous form they are in random motion that is both rapid and constant. The greater the concentration of water in a system, the greater is its kinetic energy or 'Water potential'. Hence, it is obvious that pure water will have the greatest water potential. If two systems containing water are in contact, random movement of water molecules will result in the net movement of water molecules from the system with higher energy to the one with lower energy. Thus water will move from the system containing water at higher water potential to the lower water potential. This process of movement of substances down a gradient of free energy is called diffusion. Water potential is denoted by the Greek symbol psi or and is expressed in pressure units such as Pascals(Pa). By convention, the water potential of pure water at standard temperatures, which is not under any pressure, is taken to be zero.

If some solute is dissolved in pure water, the solution has fewer free water molecules and the concentration of water decreases, reducing its water potential. Hence, all solutions have a lower water potential than pure water; the magnitude of this lowering due to dissolution of a solute is called **solute potential** or Ψs. Ψs is always negative. The more the solute

molecules, the lower (more negative) is Ψs. For a solution at atmospheric pressure (water potential) Ψw = (solute potential) Ψs.

If a pressure greater than atmospheric pressure is applied to pure water or a solution. Its water potential increases. It is equivalent to pumping water from one place to another. Can you think of any system in our body where pressure is build up? Pressure can build up in a plant system when water enters a plant cell due to diffusion, causing a pressure to build-up against the cell wall. This makes the cell **turgid** (see section 1.2.2). The magnitude of increment in water potential in such turgid cell is called pressure potential. It is usually positive, though in plants negative potential or tension in the water column in the xylem plays a major role in water transport up a stem. Pressure potential is denoted as Ψp.

Water potential of a cell is affected by both solute and pressure potential. The relationship between them is as follows:

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

1.2.2 Osmosis

Movement of water from a region of higher water potential to a region of lower water potential through a selectively permeable membrane is called osmosis. The selectively permeable membrane allows all kinds of solvents and certain solutes through it.

The important driving force of osmosis is the free energy difference between the two regions of water. In practice this force is expressed as a chemical potential gradient between the two regions (or systems) or more appropriately as a water potential grading.

Significance of osmosis in plants

1. Osmosis helps in absorption of water by plant

2. Movement of water from one cell to another is due osmosis

3. Building resistance of plants to drought and frost

4. Opening and closing of stomata is brought about by osmosis.

Osmosis can be demonstrated by popular experiments named, Thistle funnel experiment (Fig 1.3) and potato osmoscope experiment. Thistle funnel is a glass apparatus having a lower broad opening and an upper stem with a narrow opening. An egg membrane is fixed to the broad mouth of the thistle funnel, which acts as the selectively permeable membrane. The funnel is filled with sucrose solution up to a certain level in the tube. The inverted thistle funnel is immersed in a beaker containing water. After some time the level of the solution in the tube of the funnel due to the movement of water

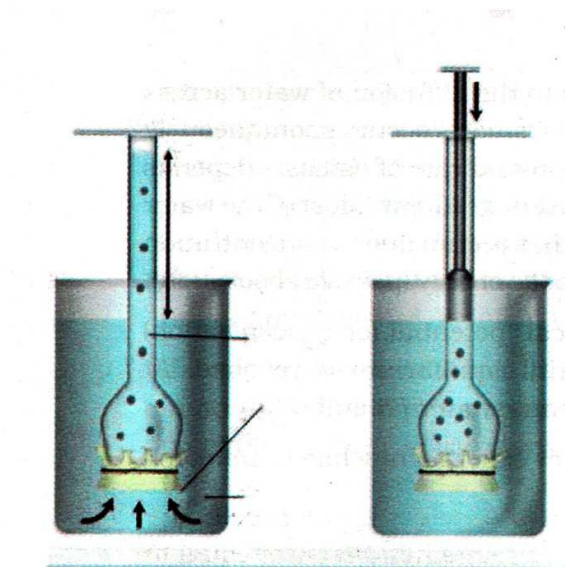


Figure 1.3 Osmosis by Thistle funnel

According to thermodynamic principles, energy flows from higher to lower levels (or More appropriately water from higher potential to low water potential) and therefore water movement takes place from the pure water in the beaker to the solution taken in the thistle funnel through selectively permeable membrane. The direction and rate of water movement depends upon the water potential gradient differences between the two systems, the larger the differences the more the rate of flow between the two systems. The two systems may reach an equilibrium (no net movement of water between the two systems) after some time when the increased level of solution in the thistle funnel develops a pressure known as 'hypostatic pressure', Which balances the force due to the water movement into the solution.

1.2.3 Plasmolysis

When cell is placed is hypertonic solution (higher solute content than the cell), the water potential gradient favours the loss of water from the cell. This is due to the fact that the water potential of external solution is less than the water potential of the cell s referred to as exosmosis. Since cell wall is relatively rigid, the protoplast pulls away from the wall as it shrinks due to water loss. The shrinkage of protoplast of the cell due to loss of water is called plasmolysis. The condition where the cell shows signs of plasmolysis is known as incipient plasmolysis (fig 1.4). In this condition, the protoplast is separated from cell wall only near the corners of the cell. When the cell is completely plasmolysed (complete shrinkage of protoplast away from the cell wall), the turgor pressure and wall pressure remain zero. Thus in completely plasmolysed cell only osmotic potential (π) contributes the water potential. This can be expressed by the following equation.

$$\Psi = \pi + 0 \quad \text{or} \quad \Psi = \pi$$

If a plasmolysed cell is again placed immediately in a hypotonic solution, it will quickly regain its water loss and trugor by osmosis. This process is called deplasmolysis.

If a cell placed in an isotonic solution (solute concentration of the solution is equal to that of the cell), no net diffusion of water takes place.

While plasmolysis is an interesting phenomenon illustrating some of the properties of the plant cell, it does not normally occur in nature with possible exception of extreme water

stress or saline environments. The salting of pickles and the addition of sugar to jams and jellies plasmolyse bacterial and fungal spores, which spoil the food stuffs.

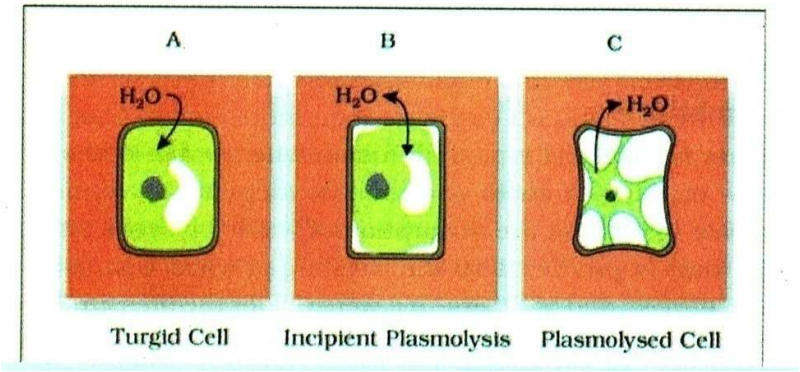


Figure 1.4 plant cell undergoing plasmolysis

1.2.4 Imbibition

The adsorption of water by hydrophilic colloids is called imbibition. An example of plant material which exhibits imbibition is dry seeds before germination.

Different substances have different imbibing capacities. Proteins have very high imbibing capacities followed by starch and cellulose the least. That is why proteinaceous pea seeds swell more on imbibition than starchy wheat seeds.

Imbibition of water increases the volume of the imbibant due to which pressure is created which is known as imbibitional pressure. This process can be tremendous magnitude. This fact becomes amply clear by the splitting of rocks done by interesting dry wooden stakes in the crevices of rocks and soaking them in water.

1.3 Long Distance transport of Water

Long distance transport of substances within a plant cannot take place by diffusion alone. Diffusion is a slow process. It can account for only short distance movement of molecules. For example, the movement of a molecule across a typical plant cell (about 50 um) takes approximately 2.5s. At this rate, can you calculate how many years it would take for the movement of molecules over a distance of 1m within in a plant by diffusion alone?

In large and complex organisms, substances often have to be moved across very large distances. Sometimes the sites of production or absorption and sites of storage are too far from each other; diffusion or active transport would not suffice. Special long distance transport systems become necessary so as to move substances across long distances and at a much faster rate. Water and minerals, and food are generally moved by a **mass** or **bulk** flow system. Mass flow is the movement of substances in bulk or en masse from one point

to another as a result of pressure differences between the two points. It is a characteristic of mass flow that substances, whether in solution or in suspension, are swept along at the same pace, as in a flowing river. This is unlike diffusion where different substances move independently depending on their concentration gradients. Bulk flow can be achieved either through a positive hydrostatic pressure gradient (e.g., Suction through a straw).

The bulk movement of substances through the conducting or vascular tissues of plants is called **translocation.**

Do you remember studying cross section of roots, stems and leaves of higher plants and studying the vascular system? The higher plants have highly specialised vascular tissues-xylem and phloem. Xylem is associated with the translocation of mainly water, mineral salts, some organic nitrogen and hormones from the roots to the aerial parts of the plants. Phloem translocates a variety of organic and inorganic solutes, mainly from the leaves to other parts of the plants.

1.4 Transpiration

Transpiration is defined as the loss of water in the form of vapour from the living tissues of the aerial parts plants. It plays a significant role in the concept of SPAC (Soil-Plant Atmospheric Continuum). The water absorbed by the plant from the soil through the root system is lost into the atmosphere through the shoot system which in turn gets back to the soil through rains.

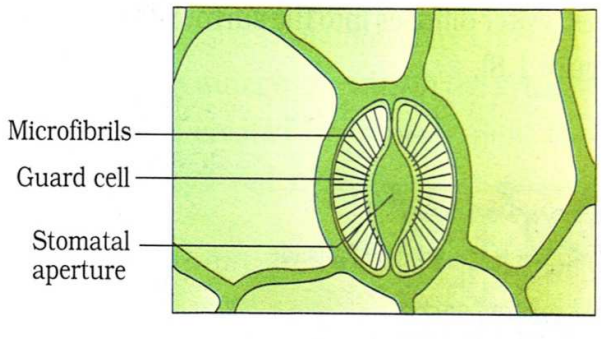


Figure 1.5 A stomatal aperture with guard cells

The phenomenon of transpiration occurs mostly through stomata (singular-stoma) located on leaves (Figure 1.5). It is also occurs through cuticle and lenticels in insignificant quantities. You have studied the basic structural details of stomatal apparatus in your previous class (chapter 12). Stomata in most plants are **photoactive stomata**. They open during the day and close during the night. But in succulent plants (e.g. Cacti, Bryophyllum) it is noted that transpiration occurs at night through **scotoactive stomata** that open during the night and remain closed during the day time.

Can you guess why?

Besides transpiration, exchange of oxygen and carbon dioxide also occurs through stomata. Usually the lower surface of a dorsiventral (often dicotyledonous) leaf has a greater number of stomata while in an isobilateral (often monocotyledonous) leaf they are nearly equal on both surfaces.

Transpiration is affected by several external factors: temperature, light, humidity and wind speed. Plant factors that affect transpiration include number and distribution of stomata, per cent of opened stomata, water status of the plant, canopy structure, available soil water, root / shoot ratio etc.

Opening and closing of stomata

The immediate cause of opening or closing of the stomata is a change in the turgidity of the guard cells. The inner wall of each guard cells. Stomatal movements are closely associated with metabolic changes and solutes levels of guard cells. It is explained by K+ pump hypothesis.

The **K+ pump hypothesis** or Active proton concept was proposed by Levitt (1974) and later elaborated by Bowling (1976). The opening and closing mechanism is described in a step wise manner.

Opening mechanism

1. In the presence of light, the starch already present in the guard cells is converted into malic acid.

2. Malic acid dissociates into malate ions (anions) and H+ ions.

3. An effulex of H+ and an influx of K+ ions take place from the subsidiary cells. The outwards movement of H+ ions from the guard cells require metabolic energy in the form of ATP, hence active transport. Where as the movement of K+ ions occurs in exchange for H+ ions.

4. As K+ ions migrate into guard cells, in excess of H+ ion exchange, they are suitably balanced by the intake of cl- ions.

5. The two anions (malate and chloride) combine with K+ ions in the guard cells resulting in potassium malate and potassium chloride which are automatically active.

6. These salts increase the concentration of guard cells, thus decreasing the water potential values (values become more negative). Therefore a water potential gradient is established between guard cells and its surrounding epidermal cells.

7. Due to water potential gradients, water moves from surrounding epidermal cells (High) into the guard cells (Low) resulting in increased turgidity due to endosmosis.

8. This endodermis into guard cells makes the outer wall more convex and drawing the inner walls of guard cells apart and thus finally opening the pore. In monocots, when the guard cells expand by water intake, their ends with thin walls bulge outwards and draw the middle thick walls apart. This results in stomatal opening.

Closure Mechanism : 1. In the dark, K+ uptake is inhibited by CO2 and abscissic acid (ABA- a plant hormone) by changing the membrane permeability.

2. The K+ and Cl- ions, move passively out of the guard cells, into the surrounding epidermal cells along the electrochemical gradients.

3. The guard cells also dispose of accumulated malate, partly by consumption during Krebs cycle of respiration and partly by releasing it to other epidermal cells.

The efflux results in the loss if turgidity within the guard cells, due to exosmosis, the thin inner walls of the guard cells return to their original position resulting in the closure of the pore.

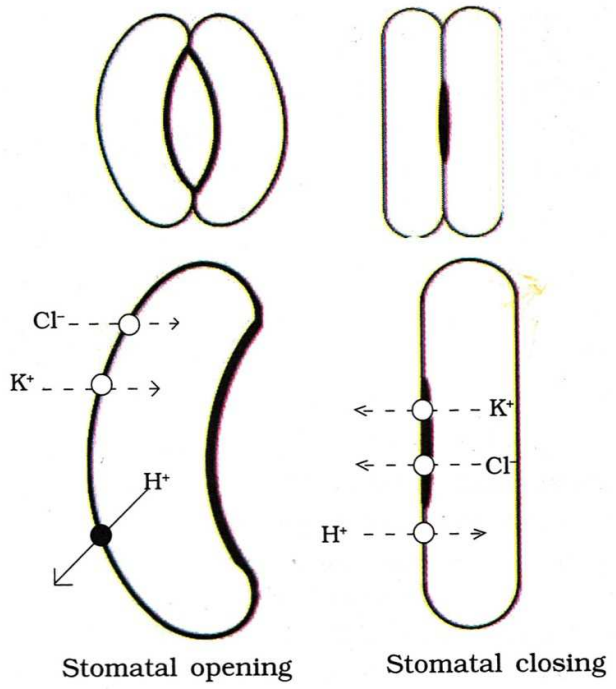


Figure 1.6 Stomatal opening and closing

1.6 Phloem Transport: Flow from Source to Sink

Food, primarily sucrose, is transported by the vascular tissue phloem from a source to a sink. Usually the source is understood to be that part of the plant which synthesises the food, i.e., the leaf, and sink, the part that needs or stores food. But, the source and sink may be reversed depending on the season or the plant's needs. Sugar stored in roots may be mobilised to become a source of food in the early spring when the buds of trees act as sink; they need energy for growth and development of the photosynthetic apparatus. Since the source- sink relationship is variable, the direction of movement in the phloem can be upwards or downwards, i.e., **bi-directional**. This contrasts with that of the xylem where the

movement is always **unidirectional,** i.e., upwards. Hence, unlike one-way flow of water in transpiration, food in phloem sap can be transported in any required direction so long as there is a source of sugar and a sink which is able to use, store or remove the sugar. Phloem sap is mainly water and sucrose, but other sugars, hormones and amino acids are also transported or translocated through phloem. An experimental set-up suggested by Munch as shown in Figure 1.7 helps in understanding bi-directional phloem transport in plants.

1.6.1 The Pressure Flow or Mass Flow Hypothesis

The accepted mechanism used for the translocation of sugars fro source to sink is called the pressure flow hypothesis. (see Figure 1.7). As glucose is prepared at the source (by photosynthesis) it is converted to sucrose (a dissacharide). The sugar is then moved in the form of source into the companion cells and then into the living phloem sieve tube cells by active transport. This process of loading at the source produces a hypertonic condition in the phloem. Water in the adjacent xylem moves into the phloem by osmosis.

As osmotic pressure builds up, the phloem sap moves to areas of lower pressure. At the sink osmotic pressure must be reduced. Again active transport is necessary to move the source out of the phloem sap and into the cells which will use the sugar- converting it into energy, starch, or cellulose. As sugars are removed, the osmatic pressure decreases and water moves out of the phloem.

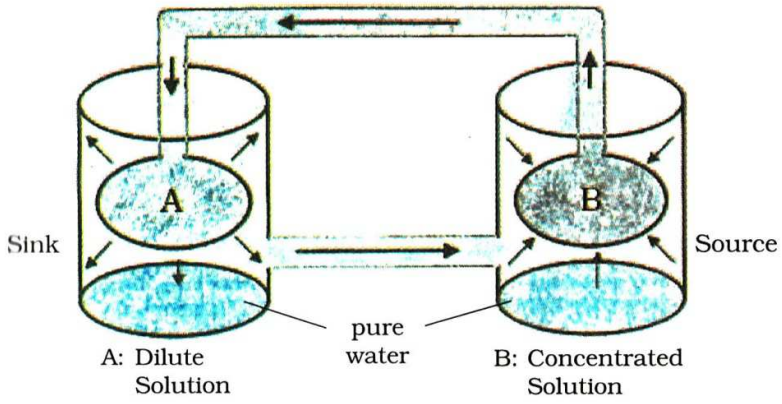


Figure 1.7 Illustration of mass flow by Munch

To summarise, the movement of sugars in the phloem begins at the source, where sugars are loaded (actively transported) into a sieve tube. Loading of the phloem sets up a water potential gradient that facilitates mass movement in the phloem.

Phloem tissue is composed of sieve tube cells which form long columns with holes in their end walls called sieve plates. Cytoplasmic strands pass through the holes in the sieve plates, forming continuous filaments. As hydrostastic pressure in the phloem sieve tube increases, pressure flow begins, and the sap moves through the phloem. Meanwhile, at the sink,

incoming sugars are actively transported out of the phloem and removed as complex carbohydrates. The loss of solutes produces a high water potential in the phloem and water passes out, returning eventually to xylem.

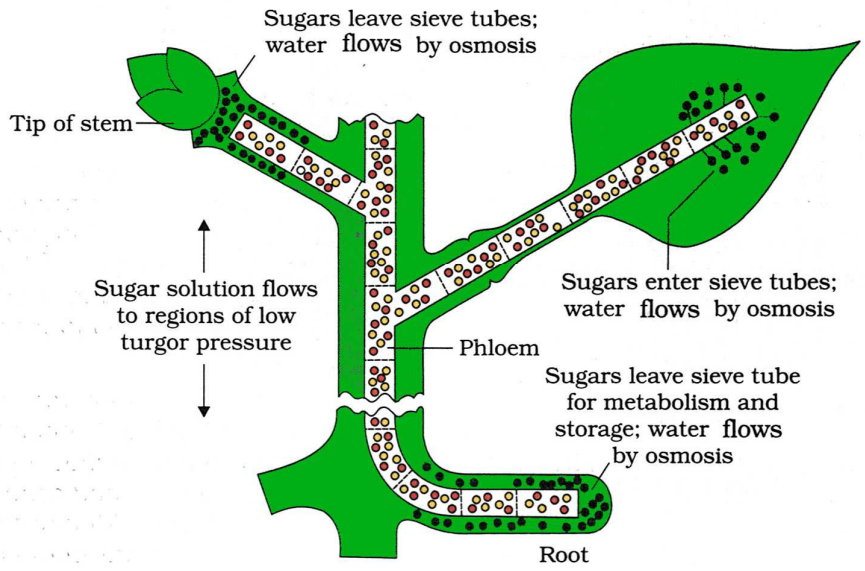


Figure 1.8 Diagrammatic presentation of mechanism of translocation

SUMMARY

Plants obtain a variety of inorganic elements (ions) and salts from their surroundings, especially from water and soil. The movement of these nutrients from the environment into the plant as well as from one plant cell to another plant cell membrane can be through diffusion, facilitated transport or active transport. Water and minerals absorbed by roots are transported by the Xylem and the organic material synthesized in the leaves is transported to other parts of plants through phloem.

Passive transport (diffusion, osmosis) and active transport are the two modes of nutrient transport across cell membranes in living organisms. In passive transport, nutrients move across the membrane by diffusion, without any use of energy as it is always down the concentration gradient and hence entropy driven. Diffusion of substances depends on their size, solubility in water or organic solvents. Osmosis is a special type diffusion of water across a semi-permeable membrane which depends on pressure gradient and concentration gradient. In active transport, energy in the form of ATP is utilized to pump molecules against a concentration gradient across membranes. Water potential is the potential energy of water which helps in the movement of water. It is determined by solute potential and pressure potential. The behaviour of the cells depends on the surrounding solution. If the

surrounding solution of the cell is hypertonic, it gets plasmolysed. The absorption of water by seeds and dry wood takes place by a special type of diffusion called imbibitions.

In higher plants, there is a vascular system, xylem and phloem, responsible for translocation. Water, minerals and food cannot be moved within the body of a plant by diffusion alone. They are, therefore, transported by a mass flow system – movement of substances in bulk from one plant to another as a result of pressure differences between the two points.

Water absorbed by roots hairs move deeper into the root by two distinct pathways, apoplast and symplast. Various ions and water from soil can be transported upto a smaller height in stems by root pressure. Transpiration pull is the most acceptable factor to explain the transport of water. Transpiration is the loss of water in the form of vapours from the plant parts through stomata. Temperature, light humidity, wind speed and number as well as distribution of stomata affect the rate of transpiration. Excess water is also removed through the tips of leaves of plants by guttation.

Phloem is responsible for transport of food (primary sources) from the source to the sink. The translocation in phloem is bi-directional: the source –sink relationship is variable. The translocation in phloem is explained by the pressure –flow hypothesis.

GLOSSARY

Apoplast: The path of water within the plant system that moves without crossing membranes, i.e., through intercellular spaces or through the space between cell wall and plasma membrane or through the non-living xylem tissue. Hence apoplastic path of water is considered to be non-living path.

Diffusion: The movement of solute particles from a region of their higher concentration to the region of their lower concentration without having any membrane. This is a passive downhill movement which is driven by concentration gradient and not by any metabolic energy.

Flaccid cell: A cell when kept in hypertonic solution tends to loose water through osmosis and such a cell is said to be flaccid cell.

Guttation: The loss of water from the leaves of plants in liquid form.

Hypertonic solution: When two solutions are separated by a membrane, the solution whose concentration is higher is called hypertonic solution.

Hypotonic solution: When two solutions are separated by a membrane, the solution whose concentration is lower is called hypotonic solution.

Imbibition: The phenomenon of adsorption of solids such a water adsorption by dry seeds.

Incipient palsmolysis: The stage of the cell at the beginning of plasmolysis wherein the membrane separation can be noticed at the corners.

Osmosis: The physical phenomenon of diffusion of solvent from a region of lower concentrated solution to a region of higher concentrated solution through a semi-permeable membrane.

Plasmolysis: The phenomenon of shrinkage of protoplast due to osmostic diffusion of water from the cells into surrounding environment.

Root pressure: The positive pressure build up in the xylem of roots due to absorption of water and ions from the soil.

Symplast: The path of water movement in the plant systems that crosses the membrane.

Turgid cell: The state of a normal living cell in which the plasma membrane remains appressed against the cell wall wherein the turgor pressure and wall pressure are at their maximum values but in opposite direction.

QUESTIONS

Very Short Answer Type Questions

1. what are porins?

2. Define water potential

3. Differentiate osmosis from diffusion.

4. Compare transpiration and evoparation.

5. Explain why xylem transport is unidirectional while that in phloem is bidirectional?

6. Does transpiration occur at night? Give an example.

7. How does ABA bring about the closure of stomata under water stress conditions?

Short Answer Type Questions

1. Define and explain Water potential.

2. What is meant by plasmolysis? How it is practically useful to us?

3. Explain pressure flow hypothesis.

4. Explain the mechanism of opening of stomata.

Excercises

1. Compare facilitated diffusion and simple diffusion.

2. Compare imbibing capacities of pea and wheat seeds.

3. Why do stomata close under water stress conditions?

Chapter 2

Mineral Nutrition

2.1 Introduction

The basic needs of all living organisms are essentially the same. All living organisms require macromolecules, such as carbohydrates, proteins and fats, and water and minerals for their growth and development.

This chapter focuses mainly on inorganic plant nutrition, wherein you will study the methods to identify elements essential for the growth and development of plants and the criteria for establishing the essentiality. You will also study the role of the essential elements and major deficiency symptoms, as well as the mechanism of absorption of these essential elements. This chapter also introduces you briefly to the significance and the mechanism of biological nitrogen fixation.

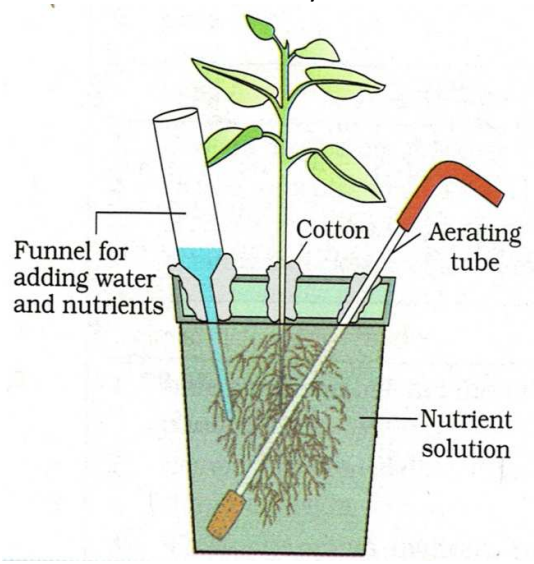


Figure 2.1 Diagram of a typical set-up for nutrient solution culture

In 1860 Julius Von Sachs, a prominent German botanist, demonstrated for the first time that plants could be grown to maturity in a defined nutrient solution in the complete absence of soil.

The technique of growing plants in a specified nutrient solution is known as hydroponics.

2.2 Essential Mineral Elements

Most of the minerals present in soil can enter plants through roots. In fact, more than sixty elements are found in different plants. Some plant species accumulate selenium, some others gold, while some plants growing near nuclear test sites take up radioactive strontium. There are techniques that are able to detect the minerals even at a very low concentration (10-8 g/mL). The question is, whether all the diverse mineral elements present in a plant, for example gold and selenium as mentioned above, are really necessary for plants? How do we decide what is essential for plants and what is not?

2.2.1 Criteria of Essentiality

The criteria for essentiality of an element are given below:

(a) The element must be absolutely necessary for supporting normal growth and reproduction. In the absence of the element the plants do not complete their life cycle or set the seeds.

(b) The requirement of the element must be specific and not replaceable by another element. In other words, deficiency of any one element cannot be met by supplying some other element.

(c) The element must be directly involved in the metabolism of the plant. Based upon the above criteria only a few elements have been found to be absolutely essential for plant growth and metabolism. These elements are further divided into two broad categories based on their quantitative requirements.

(i) Macronutrients

(ii) Micronutrients

Macronutrients are generally present in plant tissues in large amounts (in excess of 10 mmole Kg-1 of dry matter). They include carbon, hydrogen, oxygen, nitrogen, phosphorous, sulphur, potassium, calcium and magnesium. Of these, the frame work elements- carbon, hydrogen and oxygen are mainly obtained from Co_2 and H_2o, while the others are absorbed from the soil as mineral nutrients.

Micronutrients or trace elements are needed in very small amounts (less than 10 m mole Kg-1 of dry matter).These include iron, manganese, copper, molybdenum, Zinc, boron, chlorine and nickel.

In addition to the 17 essential elements named above, there are some beneficial elements such as sodium, silicon, cobalt and selenium. They are required by higher plants.

Essential elements can also be grouped into four broad categories on the basis of their diverse functions. These categories are:

(i) Essential elements that are components of biomolecules and hence are structural elements of cells (e.g., carbon, hydrogen, oxygen and nitrogen etc.).

(ii) Essential elements that are components of energy – related chemical compounds in plants (e.g., magnesium in chlorophyll and Phosphorous in ATP).

(iii) Essential elements that activate or inhibit enzymes. For example Mg^{2+} is an activator for both ribulose bisphosphate carboxylase and oxygenase and Phosphorenol pyruvate carboxylase, both of which are critical enzymes in photosynthetic carbon fixation; Zn^{2+} is an activator of alcohol dehydrogenase and Mo of nitrogenase during nitrogen metabolism. Can you name a few more elements that fall in this category? For this, you will need to some of the biochemical pathways which you will study in the later chapters.

(iv) Some essential elements can alter the osmatic potential of a cell. Potassium plays an important role in the opening and closing of a stomata. You may recall the role of minerals as solutes in determining the water potential of a cell.

2.3 Metabolism of Nitrogen

As nitrogen is the essential mineral element required in the largest quantity by plants in general, its metabolism is discussed here.

2.3.1 Nitrogen Cycle

Apart from Carbon, Hydrogen and Oxygen. Nitrogen is the most prevalent element in living organisms. Nitrogen is a constituent of amino acids, proteins, hormones, chlorophylls and many vitamins. Plants compete with microbes for the limited nitrogen that is available in soil. Thus, nitrogen is a limiting nutrient for both natural and agricultural eco-systems. Nitrogen exists as two nitrogen atoms joined by a very strong triple covalent bond (N=N). The process of conversion of molecular nitrogen (N_2) to ammonia or nitrogen oxides, nitrites and nitrates is termed as **nitrogen-fixation**. In nature, lightning and ultraviolet radiation provide enough energy to convert nitrogen to nitrogen oxides (NO, NO_2, NO_3). Industrial combustions, forest fires, automobile exhausts and power-generating stations are also sources of atmospheric nitrogen oxides.

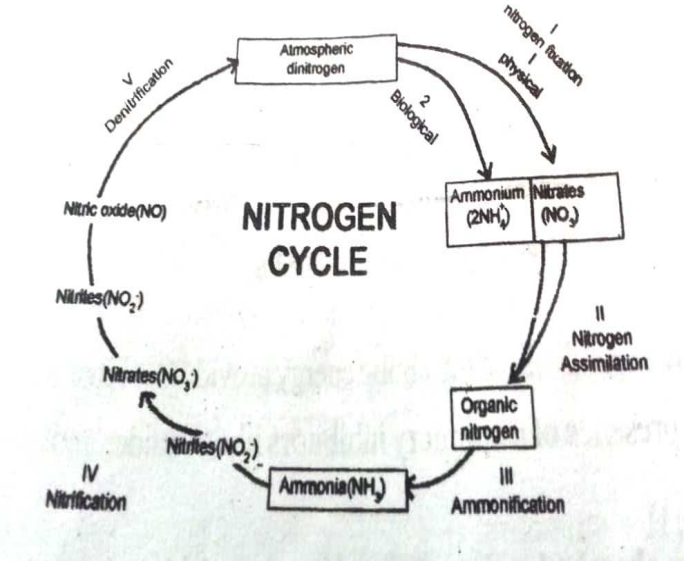


FIG : 2.2 Nitrogen cycle

The nitrates and ammonia thus formed are absorbed by plants and converted into amino acids, proteins, enzymes, nucleic acids, pigments and hormones etc., in the plant body. These constitute the organic form of nitrogen. When plants are eaten by animals this organic nitrogen is passed on into animal body. The process of absorbing nitrates, ammonia and chemically binding the nitrogen with other elements to produce organic nitrogen in plants and thereby into animals constitutes nitrogen **assimilation.**

Decomposition of organic nitrogen of dead plants and animals into ammonia is called **ammonification.** Some of this ammonia volatilises and re-enters the atmosphere but most of it is converted into nitrate by soil bacteria in the following steps:

$$2NH_4 + 3O_2 \longrightarrow 2\,NO^-_2 + 2\,H^+ + 2\,H_2O$$
$$2NO^-_2 + O_2 \longrightarrow 2\,NO^-_2$$

Ammonia is firs oxidised to nitrite by the bacteria *Nitrosomonas* and / or *Nitrococcus.* The Nitrate is further oxidised to nitrate with the help of the bacterium *Nitrobacter*. The steps are called **nitrification** (Figure 2.2). The nitrifying bacteria are called Chemoautotrophs.
The nitrate thus formed is absorbed by plants and is transported to the leaves. In leaves, it is reduced to form ammonia that finally forms the amine group of amino acids. Nitrate present in the soil is also reduced to nitrogen by the process of **denitrification.** Denitrification is carried out by the bacteria *pseudomonas* and *Thiobacillus.*

2.3.2. Biological Nitrogen Fixation

Very few living organisms can utilise the nitrogen in the form N2, available abundantly in the air. Only certain prokaryotic species are capable of fixing nitrogen. Reduction of nitrogen to ammonia by living organisms is called **biological nitrogen fixation.** The enzyme, nitrogenise, which is capable of nitrogen reduction is present exclusively in prokaryotes . Such microbes are called N2-fixers.

$$N \equiv N \xrightarrow{\text{Nitrogenase}} NH_3$$

The nitrogen fixing microbes can be free- living or symbiotic. Examples of free- living nitrogen fixing aerobic microbes are *Azotobacter* and *Beijernickia* while *Rhodospirillum* is anaerobic and *Bacillus* free- living. In addition, a number of cyanobacteria such as *Anabaena* and *Nostoc* are also free- living nitrogen-fixers.

Symbiotic nitrogen fixation

Several types of symbiotic nitrogen fixing associations are known. The most prominent among them is the legume-bacteria relationship. Species of rod-shaped *Rhizobium* has such a relationship with the roots of several legumes such as alfalfa, sweet clover, sweet pea, lentils, garden pea, broad bean, clover beans, etc. The most common association on roots is as nodules. These nodules are small outgrowths on the roots. The microbe, Frankia, also produces nitrogen-fixing nodules on the roots of non-leguminous plants (e.g., Alnus). Both *Rhizobium* and Frankia are free- living in soil but as symbionts can fix atmospheric nitrogen.

Uproot any one plant of a common pulse just before it flowers. You will see near-spherical outgrowths on the roots. These are nodules. If you cut through them you will notice that the central portion is red or pink. What makes the nodules pink? This is due to the presence of leguminous haemoglobin or leg-haemoglobin.

Nodule Formation

Nodule formation involves a sequence of multiple interactions between *Rhizobium* and roots of the host plant. The principle stages in the nodule formation are summarised as follows:

Rhizobia attracted by the sugars, aminoacids etc., released by the host legume, multiply and colonise the surroundings of roots and get attached to epidermal and root hair cells. The root-hairs curl and the bacteria invade the root-hair. An infection thread is produced, carrying the bacteria into the cortex of the root. Bacteria initiate nodule formation in the cortex of the root. Then the bacteria released from the thread into the cortical cells of the host stimulate the host cells to divide. Thus leads to the differentiation of specialised nitrogen fixing cells. The nodule thus formed establishes a direct vascular connection with the host for exchange of nutrients. These events are depicted in Figure 2.3.

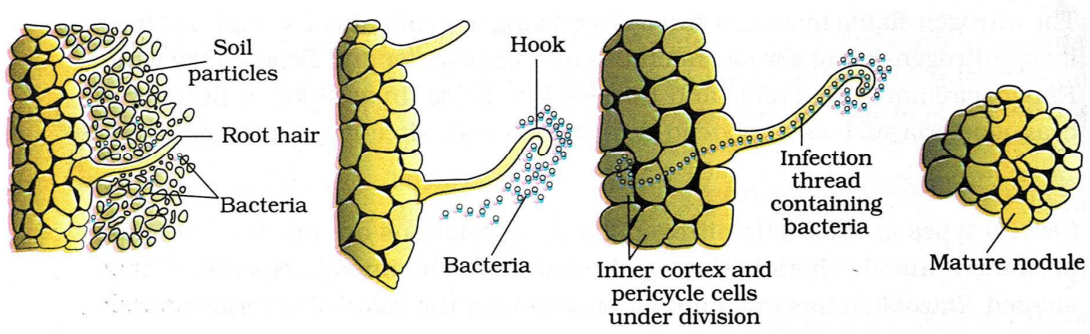


Figure 2.3 Development of root nodule in soyabean

The nodule contains all the necessary biochemical components, such as the enzyme nitrogenase and leg haemoglobin. The enzyme nitrogenase is a Mo-Fe protein and catalyses the conversion of atmospheric nitrogen to ammonia (Figure 2.4) the first stable product of nitrogen fixation. The reaction is as follows:

$$N_2 + 8H^+ + 8e^- + 16\ ATP \longrightarrow 2NH_3 + H_2 + 16\ ADP + 16\ Pi$$

The enzyme nitrogenase is highly sensitive to the molecular oxygen; it requires anaerobic conditions. The nodules have adaptations that ensure that the enzyme is protected from oxygen. To protect these enzymes, the nodule contain an **oxygen scavenger** called leg-haemoglobin. It is interesting to note that these microbes live as aerobes under free-living conditions (where nitrogenase is not operational), But during nitrogen-fixing events, they adapt to anaerobic conditions, thus protecting the nitrogenase enzyme. You must have noticed in the above reaction that the ammonia synthesis by nitrogenase requires a very high input of energy (8 ATP for each NH_3 produced). The energy required, thus, is obtained from the respiration of the host cells.

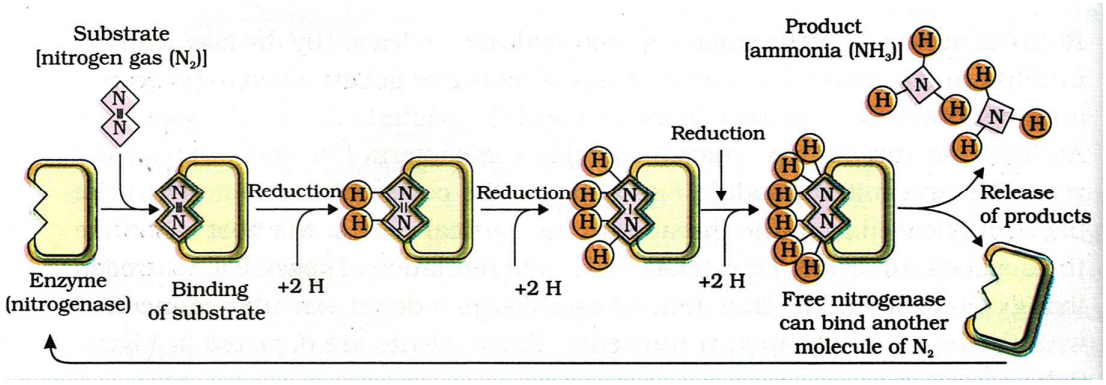


Figure 2.4 steps of conversion of atmospheric nitrogen to ammonia by nitrogenase enzyme

Fate of Ammonia : At physiological PH, the ammonia is protonated to form NH4+ (ammonium) ion. While most of the plants can assimilate nitrate as well as ammonium ions, the later is quite toxic to plants and hence cannot accumulate in them. Let us now see how

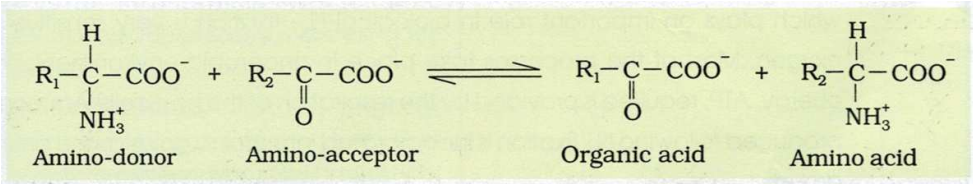
the NH4+ is used to synthesise amino acids in plants. There are two main ways in which this can take place:

(i) Reductive amination : In these processes, ammonia reacts with α– ketoglutaric acid and forms glutamic acid as indicated in the equation given below:

α-Ketoglutaric acid+ NH4+ + NADPH $\xrightarrow[\text{dehydrogenase}]{\text{Glutamate}}$ Glutamate + H2O + NADP

(ii) Transamination : It involves the transfer of an amino group from an amino acid to the keto group of a keto acid. Glutamic acid is the main amino acid from which the transfer of NH2, the amino group, takes place and other amino acids are formed through transamination. The enzyme **transaminase** catalyses all such reactions . For example,

Equation



The two most important amides – asparagines and glutamine- found in plants are a structural part of proteins. They are formed from two amino acids, namely aspartic acid and glutamic acid , respectively by addition of another amino group to each. The hydroxyl part of the acid is replaced by another NH_2^- radicle. Since amides contain more nitrogen the amino acids, they are transported to other parts of the plant via xylem vessels. In addition, along with the transpiration stream, the nodules of some plants (e.g., Soyabean) export the fixed nitrogen as ureides. These compounds also have a particularly high nitrogen to carbon ratio.

SUMMARY

Plants obtain their inorganic nutrients from air, water and soil. Plants absorb a wide variety of mineral elements. Not all the mineral elements that are absorbed are required by plants. Out of the more than 65 elements found in plants, less than 21 are defined as essential and are beneficial for normal plant growth and development. The elements required in large quantities are called macronutrients while those required in less quantities or in traces are termed as micronutrients. These elements are either essential constituents of proteins, carbohydrates, fats, nucleic acids etc., and / or take part in various metabolic processes.

Nitrogen is very essential for the sustenance of life. Plants can not use atmospheric nitrogen directly. But some of the plants in association with N_2- fixing bacteria, especially roots of legumes, can fix this atmospheric nitrogen into biologically usable forms. Nitrogen fixation

requires a strong reducing agent and energy in the form of ATP, N_2- fixation is accomplished with the help of nitrogen-fixing microbes, mainly Rhizobium. The enzyme nitrogenase which plays an important role in biological N_2-fixation is very sensitive to oxygen. Most of the processes take place in anaerobic environment. The energy, ATP, required is provided by the respiration of the host cells. Ammonia produced following N_2 fixation is incorporated into amino acids as the amino group.

GLOSSARY

Ammonification: Accumulation of nitrogen in the form of ammonia in soil mainly due to decomposition of organic organic nitrogen by microorganisms.

Chemoautotroph: An organism that derives its carbon source while the energy is obtained from the oxidation of chemical substances.

Denitrification: The process of convertion of usable form of nitrogen (nitrate) into unusable form (molecular nitrogen).

Leg-haemoglobin: The red pigment produced in legume root nodules that removes oxygen from the vicinity of Nitrogenase enzyme. It acts as Oxygen scavenger.

Photoautotrophs: An organism which derives its carbon from organic sources and energy from sunlight.

QUESTIONS

Very Short Answer Questions

1. How do you categorize a particular essential element as a macro or micronutrient?

2. Out of the17 essential elements which elements are called non-mineral essential elements?

3. Which element is regarded as the 17 th essential element?

4. Name the essential elements present in nitrogenase enzyme.

5. Write the balanced equation of nitrogen fixation.

Short answer Type Questions

1. Explain the steps involved in the formation of root nodule.

2. Write in brief how plants synthesize amino acids.

Excercises

1. Which enzyme is activated by the 17 the essential element?

2. What acts as oxygen scavenger in legume – root nodule combination?

Chapter 3

Enzymes

Almost all enzymes are proteins. These are some nucleic acids that behave like enzymes. These are called ribozymes (eg. 23s r RNA). One can depict an enzyme by a line diagram. An enzyme like any protein has a primary structure, i.e., amino acid sequence of the protein. An enzyme, like any protein, has a secondary and the tertiary structure too. When we look at a tertiary structure of the protein chain folds upon itself, the chain criss-crosses itself and hence, many crevices or pockets are made. One such pocket is the 'active site'. An active site of enzyme is a crevice or pocket into which the substrate fits. Thus enzymes, through their active site, catalyse reactions at a high rate. Enzyme catalysts differ from inorganic catalysts in many ways, but one major difference needs mention. Inorganic catalysts work efficiently at high temperatures and high pressures. While enzymes get damaged at high temperature (say above 40 C). However, enzymes isolated from organisms which normally live under extremely high temperature (e.g., hot vents and sulphur springs), are stable and retain their catalytic power even at high temperatures (up to 80-90 C). Thermal Stability is thus an important quality of such enzymes isolated from thermophilic organisms.

3.1 Chemical Reactions

How do we understand these enzymes? Let us first understand a chemical reaction. Chemical compounds undergo two types of changes. A physical change simply refers to change in shape without breaking of bonds. This is a physical process is a change in the state of matter; e.g., When ice melts into water, or when water becomes a vapour. These are also physical processes. However, when bonds are broken and new bonds are formed during transformation, it is called chemical reaction. For example:

$$Ba(OH)_2 + H2S0_4 \longrightarrow BaS0_4 + 2\ H_20$$

Catalysed reactions proceed at rates vastly higher than that of uncatalysed ones. When enzyme catalysed reactions are observed, the rate is vastly higher than the same but uncatalysed reaction. For example

$$C0_2 + H_20 \xrightleftharpoons{\text{carbonic anhydrase}} H_2C0_3$$

Carbon dioxide water Carbonic acid

In the absence of any enzyme this reaction is very slow, with about 200 molecules of H_2C0_3 being formed in an hour. However, by using the enzyme present within the cytoplasm, called carbonic anhydrase, the reaction speeds dramatically with about 600,000 molecules being formed every second. The enzyme accelerates the reaction rate by about 10 million times. The power of enzymes is incredible indeed!

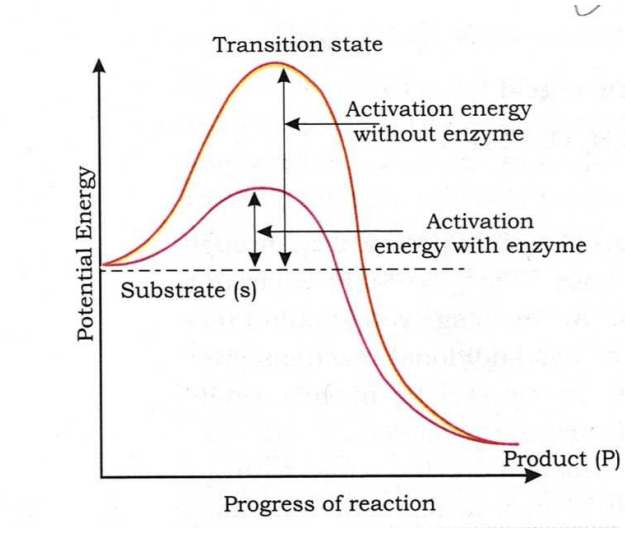
3.2 How do Enzymes bring about such High Rates of Chemical Conversions?

To understand this we should study enzymes a little more. We have already understood the idea of an 'active site'. The chemical or metabolic conversion refers to a reaction. The chemical which is converted into a product is called a 'substrate'. Hence enzymes, i.e. proteins with three dimensional structures including an 'active site', convert a substrate (S) into a product (P). Symbolically, this can be depicted as;

$$S \longrightarrow P$$

It is now understood that the substrate 'S' has to bind the enzyme at its 'active site' within an a given cleft or a pocket. The substrate has to diffuse towards the 'active site'. There is thus, an obligatory formation of an 'ES' complex. E stands for enzyme. This complex formation is transient phenomenon. During the state where substrate is bound to the enzyme active site, a new structure of the substrate called transition state structure is formed. Very soon, after the expected bond breaking /making is completed, the product is released from the active site. In other words, the structure of substrate gets transformed into the structure of product(S). The pathway of this transformation must go through the so-called transition state structure. There could be many more 'altered structural states' between the stable substrate and the product. Implicit in this statement is the fact that all

other intermediate structural states are unstable. Stability is something related to energy status of the molecule or the structure. When we represent this pictorially through a graph it looks like what is given in Figure 3.1.



The y-axis represents the potential energy content. The x-axis represents the progression of the structural transformation or states through the 'transition site'. You would notice the energy level difference between S and P. If 'P' is at a lower level than 'S', it is an exothermic reaction. One need not supply energy (by heating) in order to form the product. However, whether it is an exothermic or spontaneous reaction or an endothermic or energy state or transition state.

Figure 3.1 Concept of activation energy

The difference between average energy content of 'S' and that of this transition state is called 'activation energy'. Enzymes eventually bring down this energy barrier, making the transition of 'S' to 'P' more easy.

3.3 Nature of Enzyme Action

Each enzyme (E) has a substrate (s) binding site in its molecule so that a highly reactive enzyme-substrate complex (ES) is produced. This complex is short –lived and dissociates into its product(s) P and the unchanged enzyme, with an intermediate formation of the enzyme –product complex (EP).

The Formation of the ES complex is essential for Catalysis.

$$E + S \longrightarrow (ES) \longrightarrow (EP) \longrightarrow E+P$$

Formation of (ES) complex has been explained with 'Lock and Key' hypothesis by Emil Fischer (1884) and much later with 'induced –Fit' hypothesis by Daniel E. Koshland (1973).

The catalytic cycle of an enzyme action can be described in the following steps:

1. First, the substrate binds to the active site of the enzyme, fitting into the active site.
2. The binding of the substrate induces the enzyme to alter its shape, fitting more tightly around the substrate.
3. The active site of the enzyme, now in close proximity to the substrate, breaks the chemical bonds of the substrate and the new enzyme-product complex is formed.

4. The enzyme releases the products of the reaction and the free enzyme is ready to bind to another molecule of the substrate and runs through the catalytic cycle once again.

3.4 Factors Affecting Enzyme Activity

The Activity of an enzyme can be affected by a change in the conditions which alter the tertiary structure of the protein. These include temperature, PH, change in substrate concentration or binding of specific chemicals that regulate its activity.

Temperature and PH

Enzymes generally function in a narrow range of temperature and PH (Figure 3.2). Each enzyme shows its highest activity at a particular temperature and PH, called the optimum temperature and optimum PH. Activity declines both below and above the optimum value. Low temperature preserves the enzyme in a temporarily inactive state whereas high temperature destroys enzymatic activity because proteins are denatured by heat.

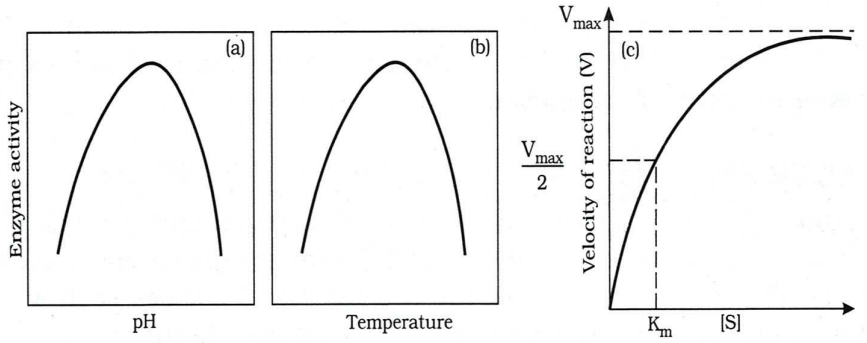


Figure 3.2 Effect of change in : (a) pH. (b) Temperature and (c) Concentration of subtract on enzyme activity.

Concentration of Substrate

With the increase in substrate concentration, the velocity of the enzymatic reaction rises at first. The reaction ultimately reaches a maximum velocity (V max) which is not exceeded by any further rise in concentration of the substrate. This is because the enzyme molecules are fewer than the substrate molecules and after saturation of these molecules, there are no free enzyme molecules to bind with the additional substrate molecules (Figure 3.2). Substrate concentration required to cause half the maximal reaction rate is termed as Michaelis-Menten constant (Km). Km Values represent approximate inverse measures of the affinity of the enzyme for a given substrate.

The activity of an enzyme is also sensitive to the presence of specific chemicals that bind to the enzyme. When the binding of the chemical shuts off enzyme activity, the process is called inhibition and the chemical is called an inhibitor.

3.5 Classification and Nomenclature of Enzymes

Thousands of enzymes have been discovered, isolated and studied. Most of these enzymes have been classified into different groups based on the type of reactions they catalyse. Enzymes are divided into 6 classes each with 4-13 subclasses and designated accordingly by a four- digit number.

Oxidoreductases/ dehydrogenases: Enzymes which catalyse oxidoreduction between two substrates S and S .

E.g : Malate + NAD+ $\xrightarrow{\text{Malate dehydrogenase}}$ Oxaloacetate + NADH+ H+

Transferases: Enzyme catalysing hydrolysis of a group, G (other than Hydrogen) between a pair of substrate S and S.

E.g: Glucose + ATP $\xrightarrow{\text{Hexokinase}}$ Glucose-6-phosphate +ADP

Hydrolases: Enzymes catalysing hydrolysis of ester, ether, peptide, glycosidic, C-C, C-halide or P-N bonds.

E.g: Fructose-1,6- bisphosphate + H20 $\xrightarrow{\text{Fructose 1,6 – bisphosphotase}}$ Fructose-6-phosphate + Inorganic phosphate

Lyases : Enzymes that catalyse removal of groups from substrates by mechanisms other than hydrolysis leaving double bonds.

E.g: Arginosuccinic acid $\xrightarrow{\text{Arginosuccinase}}$ Arginine + Fumaric acid

Isomerases: Includes all enzymes catalysing inter- conversion of optical, geometric or positional isomers.

E.g: Ribose -5 - phosphate $\xrightarrow{\text{Ribose-5- phosphate isomerase}}$ Ribulose -5- Phosphate

Ligases: Enzymes catalysing the linking together of 2 compounds, e.g., enzymes which catalyse joining of C-0, C-S, C-N, P-0 etc, bonds.

E.g: Glutamic acid + NH3 + ATP $\xrightarrow{\text{Glutamine Synthetase}}$ Glutamine + ADP + ip

The above classification provides for a four digit code to identify individual enzymes. While instance, Glucose -6- phosphotransferase has the enzyme code (E.C) 2.7.1.2 . The first digit of the code indicates the major class of the enzyme. While the second and the third digits indicate sub- class and sub- subclass respectively. The last digit of the code is the serial number of the enzyme in a particular sub- subclass.

3.6 Co-factors

Enzymes are composed of one or several polypeptide chains. However, there are a number of cases in which non-protein constituents called co-factors are bound to the enzyme to make the enzyme catalytically active. In these instances, the protein portion of the enzyme is called the apoenzyme. Three kinds of cofactors may be identified: Prosthetic groups, co-enzymes and metal ions.

Prosthetic groups are organic compounds and are distinguished from other cofactors in that they are tightly bound to the apoenzyme. For example, in peroxidase and catalase, which catalyze the breakdown of hydrogen peroxide to water and oxygen, haem is the prosthetic group and it is a part of the active site of the enzyme.

Co-enzymes are also organic compounds but their association with the apoenzyme is only transient, usually occurring during the course of catalysis. Furthermore, co-enzymes serve as co-factors in a number of different enzyme catalyzed reactions. The essential chemical components of many coenzymes are vitamins, e.g., both coenzyme Nicotinamide adenine dinucleotide (NAD) and NADP contain the vitamin niacin.

A number of enzymes require metal ions for their activity which form coordination bonds with side chains at the active site and at the same time form one or more coordination bonds with the substrate, e.g., Zinc is a cofactor for the proteolytic enzyme carboxy peptidase.

Catalytic activity is lost when the co-factor is removed from the enzyme which testifies that co-factors play a crucial role in the catalytic activity of the enzyme.

SUMMARY

Enzymes are proteins which catalyse biochemical reactions in the cells. Ribozymes are nucleic acids with catalytic power, Proteinaceous enzymes exhibit substrate specificity, require optimum temperature and Ph for maximal activity. They are denatured at high temperatures. Enzymes lower activation energy of reactions and enhance greatly the rate of the reactions.

GLOSSARY

Activation energy: Energy that is required for the substrate to get converted to product.

Active site: A cleft or pocket in the 3- D structure of a catalytic protein into which the substrate fits in.

Apoenzyme: The protein part of a holoenzyme.

Co-enzyme: An organic co-factor that is loosely bound to the apoenzyme.

Co-factor: non-protein part of a holoenzyme. It could be a metal ion or an organic compound.

Enzyme: A protein with catalytic property.

Enzyme code: a systematic code number, indicating scientific and specific enzyme activity as per IUB system. It is four digit code.

Prosthetic group: An organic co-factor that is tightly bound to the apoenzyme.

Substrate: The substance which is converted to product in a enzyme catalyzed reaction.

Thermophiles: Organisms that survive in hot water springs i.e., at high temperature.

QUESTIONS

Very Short Answer Type Questions

1. Who proposed 'Lock and Key' hypothesis.

2. Who proposed Induced fit hypothesis.

3. Distinguish between apoenzyme and cofactor.

Short Answer Type Questions

1. Explain the importance of (ES) complex formation.

2. Explain different types of cofactors.

Excercises

1. what are isoenzymes?

2. What is turnover number? What is the fastest acting enzyme?

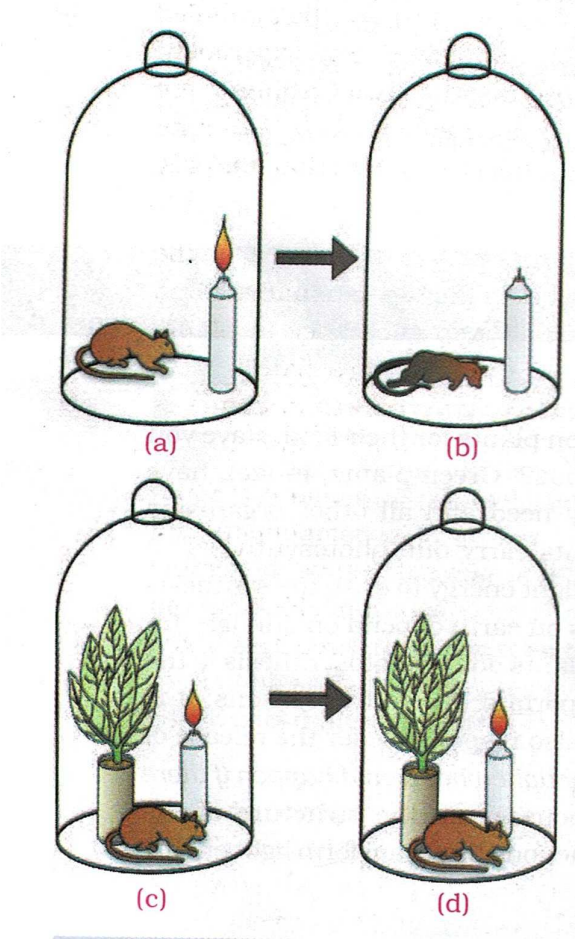
Chapter 4

Photosynthesis in Higher plants

All animals including human beings depend on plants for their food. Have you ever wondered from where plants get their food? Green plants, in fact, have to make or rather synthesize the food they need and all other organisms depend on them for their needs. Green plants carry out 'photosynthesis', a physico-chemical process by which they use light energy to drive the synthesis of carbohydrates. Ultimately, all living forms on earth depend on sunlight for energy. The use of energy from sunlight by plants doing photosynthesis is the basis of life on earth. Photosynthesis is important due to two reasons: it is the primary source of all food on earth. It is also responsible for the release of oxygen into the atmosphere. Have you ever thought what would happen if there were no oxygen to breath? This chapter focusses on the structure of the photosynthetic machinery and the various reactions that transform light energy into chemical energy.

4.1 What do we know?

Let us try to find out what we already know about photosynthesis. Some simple experiments you may have done in the earlier classes have shown that chlorophyll (green pigment of the leaf), light and co2 are required for photosynthesis to occur.



You may have carried out the experiment to look for starch formation in two leaves – a variegated leaf or a leaf that was exposed to light. On testing these leaves for starch it was clear that photosynthesis occurred only in the green parts of the leaves in the presence of light.

Another experiment you may have carried out is the half-leaf experiment, in which a part of a leaf is enclosed in a test tube containing some KOH soaked cotton (which absorbs CO_2), while the other half is exposed air. The setup is then placed in light for some time. On testing for starch later in the two halves of the leaf, you must have found that the exposed part of the leaf tested positive for starch while the portion that was in the test tube, tested negative. This showed that CO_2 is required for photosynthesis. Can you explain how this conclusion could be drawn?

Figure 4.1 Priestly experiment

4.2 Early experiments

It is interesting to learn more about those simple experiments that led to a gradual development in our understanding of photosynthesis.

Joseph priestly (1773-18040 in 1770 performed a series of experiments that revealed the essential role of air in the growth of green plants. Priestly, you may recall, discovered oxygen in 1774. Priestly observed that candle burning in a closed space – a bell jar soon gets extinguished (figure 4.1 a, b, c, d). Similarly, a mouse would soon suffocate in a closed space. He concluded that the both the burning candle or the animal that breath air, somehow, damage the air. But when he placed a mint plant in the same bell jar, he found that the mouse stayed alive and the candle continued to burn. Priestly hypothesised as follows: plants restore to the air whatever breathing animals and burning candles remove.

Can you imagine how priestly would have conducted the experiment using a candle and a plant? Remember, he would need to rekindle the candle to test whether it burns after a few days. *How many different ways can you think of lighting the candle without disturbing the set-up?*

In 1854 Julius von Sachs provided evidence for the production of glucose during the growth of plants. Glucose is usually stored as starch. Sach's later studies showed that the green substance in plants (chlorophyll as we know it now) is located in special bodies (later called chloroplast) within plant cells. He found that the green parts in plants is where glucose is made, and that the glucose is usually stored as starch.

Now considered the interesting experiments done by T.W Engelmann (1843- 1909). Using a prism Engelman split light into its spectral components and then illuminated a green alga, *Cladophora,* placed in a suspension of aerobic bacteria. The bacteria were used to detect the sites of O_2 evolution. He observed that the bacteria accumulated mainly in the region of blue and red light of the split spectrum. A first action spectrum of photosynthesis was thus described. It resembles roughly the absorption spectra of chlorophyll a and b (discussed in section 4.4).

A milestone contribution to the understanding of photosynthesis was that made by a microbiologist, Cornelius Van Niel (1897- 1985), who, based on his studies of purple and green bacteria, demonstrated that photosynthesis is essentially a light-dependent reaction in which hydrogen from a suitable oxidisible compound reduces carbon dioxide to carbohydrates. This can be expressed by:

$$2H_2A + CO_2 \xrightarrow{\text{light}} 2A + CH_2O + H_2O$$

In green plants H2O is the hydrogen donor and is oxidised to O_2. Some organisms do not release O_2 during photosynthesis. When H_2S is the hydrogen donor for purple and green sulphur bacteria, The 'oxidation' product is sulphur or sulphate depending on the organism, and not O_2. Hence, Niel inferred that the O_2 evolved by the green plant comes from H_2O, not from carbon dioxide. This was later proved by using isotopic techniques. The correct equation that would represent the overall process of photosynthesis, is therefore:

$$6CO_2 + 12\ H_2O \xrightarrow{\text{light}} C_6H_{12}O_6 + 6H_2O + 6O_2$$

Where $C_6\ H_{12}\ O_6$ represents glucose. The O_2 released is from water; this was proved using radio isotopic techniques (^{18}O, a heavy isotope). Note that this is not a single reaction but a description of a multistep process called photosynthesis. *Can you explain why twelve molecules of water as substrate are used in the equation given above?*

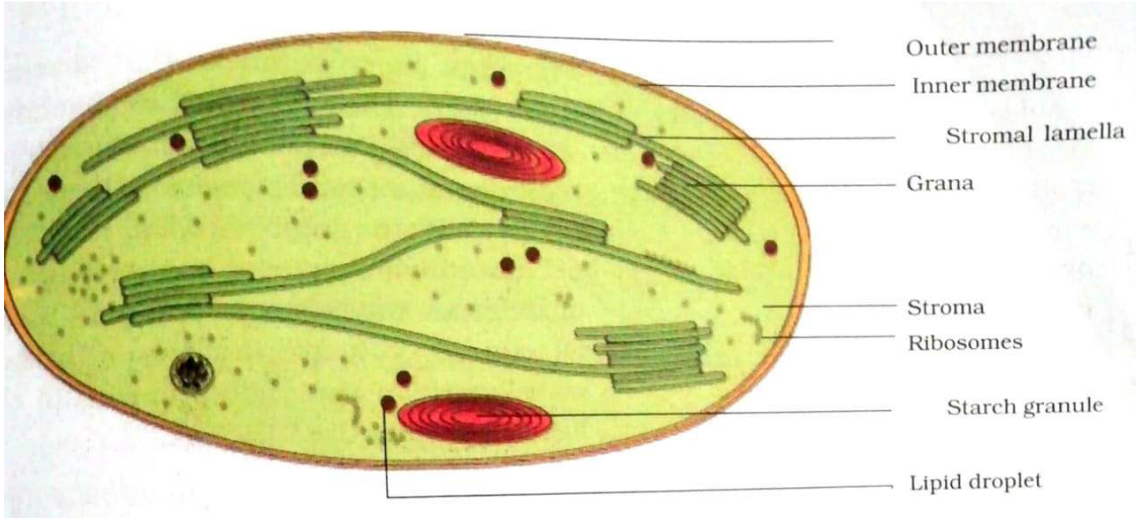


Figure 4.2 Diagramatic representation of an electron micrograph of a section of chloroplast

4.3 What is the site of Photosynthesis?

You would of course answer: in 'the green leaf' or you may add, 'in the chloroplast' based on what you earlier read in chapter 9 of the first year book. You are definitely right, photosynthesis does take place in the green leaves of plants but it does so also in other green parts of the plants. Can you name some other parts where you think photosynthesis may occur?

You have studied the structure of chloroplast earlier. Within the chloroplast there is membranous system consisting of grana, the stroma lamellae and the fluid stroma (Figure 4.2). There is a clear division of labour within in the chloroplast. The membrane system is responsible for the synthesis of ATP and NADPH. In stroma, enzymatic reactions incorporate CO2 into the plant, leading to the synthesis of sugar, which in turn forms starch. The former set of reactions, since they are directly light driven but are dependent on the products of light reactions (ATP and NADPH) and are called, by convention, dark reactions. However, this should not be taken to mean that they occur in darkness or that they are not light-dependent.

4.4 How many pigments are Involved in Photosynthesis?

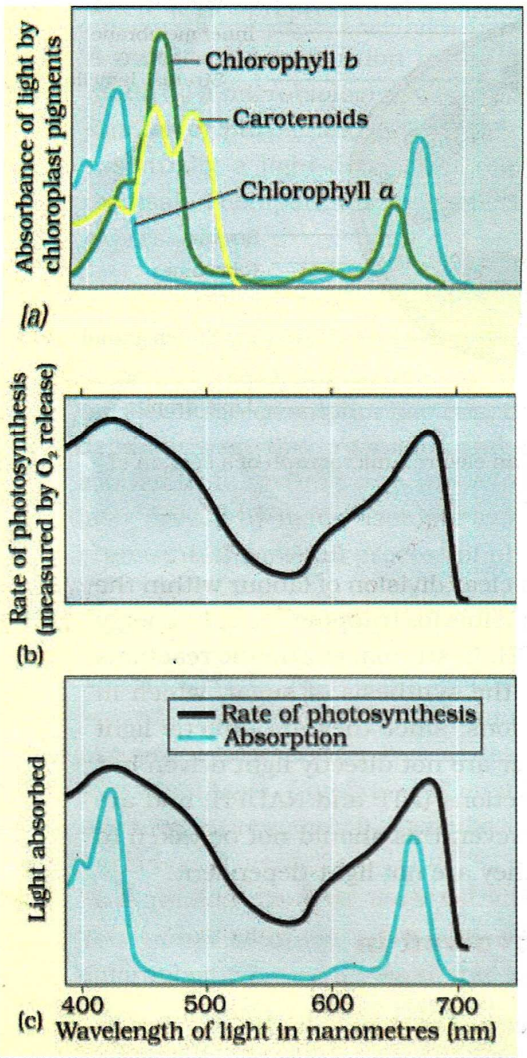


Figure 4.3a Graph showing the absorption spectrum of chlorophyll *a*, *b* and the carotenoids

Figure 4.3b Graph showing action spectrum of photosynthesis

Figure 4.3c Graph showing action spectrum of photosynthesis superimposed on absorption spectrum of chlorophyll *a*

Have you ever wondered why and how there are so many shades of green in leaves- even in the same plant? We can look for an answer to this question by trying to separate the leaf pigments of any green plant through paper chromatography. A chromatographic separation of the leaf pigments shows that the colour that we see in leaves is not due to a single pigment but due to four pigments: Chlorophyll a (bright or blue green in chromatogram), chlorophyllb (yellow green), Xanthophylls (yellow) and caroteins (yellow - orange). Let us now see what roles the various pigments play in photosynthesis.

Pigments are substances that have an ability in absorb light at specific wavelengths. Can you guess which is the most abundant plant pigment in the world? Let us study the graph showing the ability of chlorophyll a pigment to absorb lights of different wavelengths (Figure 4.3 a). Of course, you are familiar with the wavelength of the visible spectrum of light as well as the VIBGYOR.

From Figure4.3a can you determine the wavelength (colour of light) at which chlorophyll a shows the maximum absorption? Does it show another absorption peak at any other wavelengths too? If yes, which one?

Now look at Figure 4.3b showing the wavelength at which maximum photosynthesis occurs in a plant. You can see that the wavelengths at which there is maximum absorption by chlorophyll a i.e., in the blue

and the red regions, also shows higher rate of photosynthesis. Hence, we can conclude that chlorophyll a is the chief pigment associated with photosynthesis. But by looking at Figure 4.3c can you say that there is a complete one-to-one overlap between the absorption spectrum of chlorophyll a and the action spectrum of photosynthesis?

These graphs, together, show that most of the photosynthesis takes place in the blue and red regions of the spectrum: some photosynthesis does take place at the other wavelengths of the visible spectrum. Let us see how this happens. Though chlorophyll is the major pigment responsible for trapping light, other thylakoid pigments like chlorophyll b, xanthophylls and caroteins which are called **accessory pigments** also absorb light and transfer the energy to chlorophyll a. Indeed, they not only enable a wider range of wavelength of incoming light to be utilised for photosynthesis but also protect chlorophyll a from photo-oxidation.

4.5 What is Light Reaction?

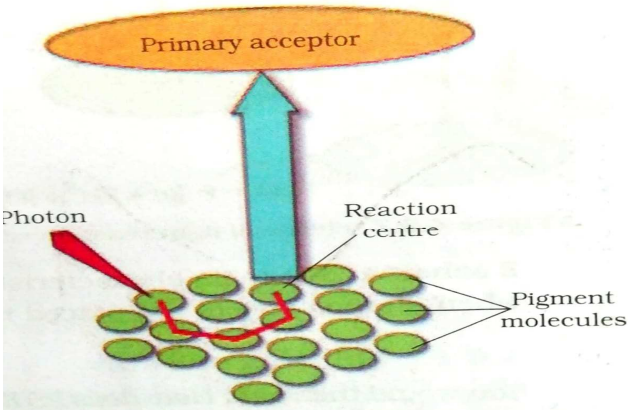


Figure 4.4 The light harvesting complex

Light reactions or the 'photochemical' phase include light absorption, water splitting, oxygen release, and the formation of high energy chemical intermediates, ATP and NADPH. Several complexes are involved in the process. The pigments are organised into two discrete photochemical **light harvesting complexes (LHC)** within the **photosystem I (PSI)** and **Photosystem II (PSII)**. These are named in the sequence of their discovery. The LHC are made up of hundreds of pigment molecules bound to proteins. Each photosystem has all the pigments forming a light harvesting system also called **antennae** (Figure 4.4). These pigments help to make photosynthesis more efficient by absorbing different wavelengths of light. A special chlorophyll a forms the **reaction centre**. The reaction centre is different in both the photosystems. In PSI, the reaction centre, chlorophyll a, has an absorption peak at **P700**. While in PSII, it has absorption maxima at 680 nm and is called **P680.**

4.6 The Electron Transport

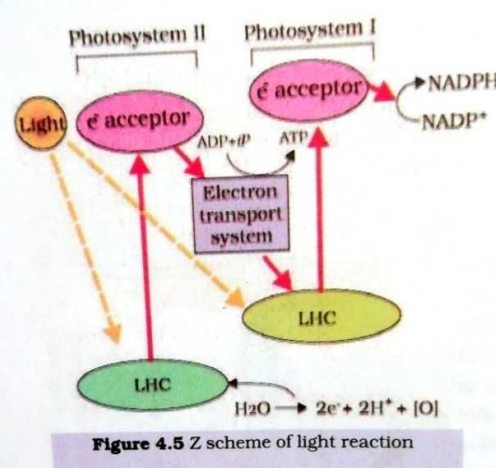


Figure 4.5 Z scheme of light reaction

In Photosystem II the reaction centre chlorophyll a absorbs 680nm wavelength of red light causing electrons to become excited and jump into an orbit farther form the atomic nucleus. These electrons are picked up by an electron acceptor which passes them to an **electron transport system consisting of cytochromes** (Figure 4.5). This movement of electrons form pheophytin to P_{700} is downhill, in terms of an oxidation- reduction or redox potential scale. The electrons are not used up as they pass through the electron transport chain, but are passed on to the pigments of photosystem PSI. Simultaneously, electrons in the reaction centre of PSI are also excited when they receive red light of wavelength 700 nm and are transferred to another accepter molecule that has a greater redox potential. These electrons then are moved downhill again, this time to a molecule of energy-rich $NADP^+$. The addition of these electrons reduces $NADP^+$ to NADPH+ H^+ . This whole scheme of transfer of electorns starting from the PSII, uphill to the acceptor, down the electron transport chain to PSI, excitation of electrons transfer to another acceptor, and finally downhill to NADP+ causing it to be reduced to NADPH + H^+ is called the **Z Scheme,** due to its characteristic shape(Figure 4.5). This shape is formed when all the carriers are placed in a sequence on a redox potential scale.

4.6.1 Splitting of Water

You would then ask, *How does PSII supply electrons continuously?* The electrons that were moved from photosystem II must be replaced. This is achieved by electrons available due to splitting of water. The splitting of water is associated with the PSII; water is split into protons, oxygen and electrons. This creates oxygen, one of the net products of photosynthesis. The electrons needed to replace those removed from photosystem I are provided by Photosystem II.

$$2\ H_20 \longrightarrow 4\ H^+ + O_2 + 4e^-$$

We need to emphasise here that water splitting comples (Oxtgen Evolving Complex OEC) is associated with the PSII, which itself is physically located on the inner side of the membrane of the thylakoid. Then, *where are the protons and O2 formed likely to be released – in the lumen? Oron the outer side of the membrane?*

4. 6. 2 Cyclic and Non - Cyclic Photo-Phosphorylation

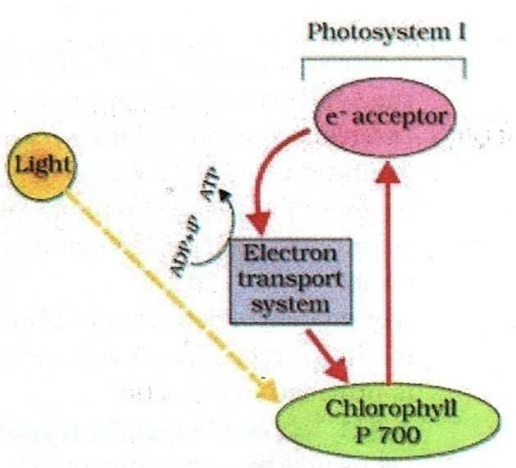


Figure 4.6 Cyclic photophosphorylation

Living organisms have the capability of extracting energy from oxidisable substances and storing this in the form of bond energy. Special substances like ATP carry this energy in their chemical bonds. The process through which ATP is systhesised by cells (in mitochondria and chloroplasts) is named phosphorylation. Photo-phosphorylation is the systhesis of ATP from ADP and inorganic phosphate in the presence of light. When the twp photosystems work in a series, first PSII and then PSI, a process called non-cyclic photo-phosphorylation occurs. The two photosystems are connected through an electron transport chain, as seen in the Z scheme. Both ATP and NADPH+H^+ are synthesised by this kind of electron flow (Figure 4.5).

When only PSI is functional, the electron is circulated within the photosystem and the photophosphorylation occurs due to the cyclic flow of electrons(Figure 4.6). A possible location where this could be happening is in the stroma lamellae. While the membrane or lamellae of the grana have both PSI and PSII, the stroma lamellae membranes lack PSII as well as NADP reductase enzyme. The excited electron does not pass on to $NADP^+$ but is cycled back to the PSI complex through the electron transport chain (Figure 4.6). The cyclic flow hence, results only in the synthesis of ATP, but not of NADPH+H^+. Cyclic photo phosphorylation also occurs when only light of wavelengths beyond 680 nm are available for excitation.

In green plants, cyclic photo-phosphorylation is an additional source of ATP required for chloroplast activities over and above that is required in the Calvin cycle.

A great deal of detailed about photosynthetic electron transport has been worked out in the recent past. A modern scheme of photosynthetic electron transport is provided in Fig 4.6a.

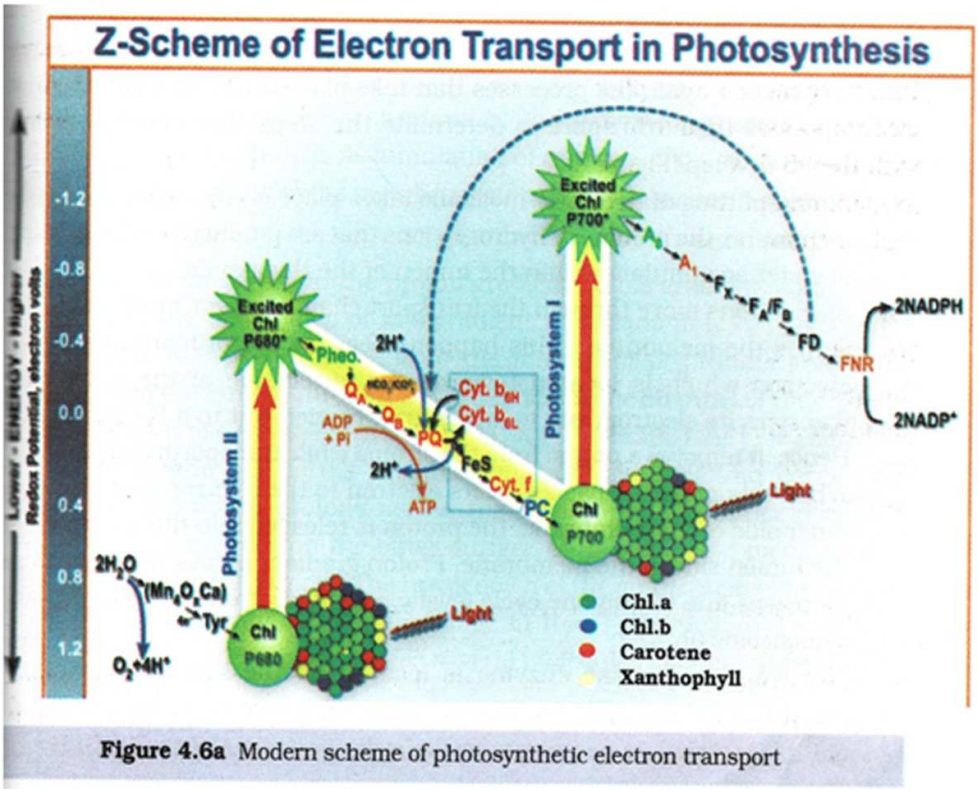


Figure 4.6a Modern scheme of photosynthetic electron transport

4.6.3 Chemiosmatic Hypothesis

Let us now to try to understand how actually ATP is synthesized in the chloroplast. The chemiosmatic hypothesis has been put forward to explain the mechanism. Like in respiration, in photosynthesis too. ATP synthesis is linked to development of a proton gradient across a membrane. This time these are membranes of the thylakoid. There is one difference though. Here the proton accumulation is towards the inside of the membrane, i.e., in the lumen while in respiration, protons accumulate in the intermembrane space of the mitochondria when electrons move through the ETS (Chapter 5).

Let us understand what causes the proton gradient across the membrane. We need to consider again the processes that take place during the activation of electrons and their transport to determine the steps that cause a proton gradient to develop (Figure 4.7).

(a) Since splitting of the water molecule take place on the inner side of the membrane, the protons or hydrogen ions that are produced by the splitting of water accumulate within the lumen of the thylakoids.

(b) As electrons move through the transport chain, protons are transported across the membrane. This happens because the primary acceptor of electron which is located towards the outer side of the membrane transfers its electron, not to an electron carrier, but to a H carrier (PQ). Hence, it removes a proton from the stroma while transporting an electron. When this molecule passes on its electron to the electron carrier on the inner side of the

membrane, the proton is released into the inner side or the lumen side of the membrane. Proton gradient across the membrane increases due to quinine cycle (cyclic flow of electrons between PQ and cytochrome b).

(c) The NADP reductase enzyme is located on the stroma side of the membrane. Along with the electrons that come from the acceptor of electrons of PSI, protons are necessary for the reduction of NADP+ to NADPH + H^+. These protons are also removed from the stroma.

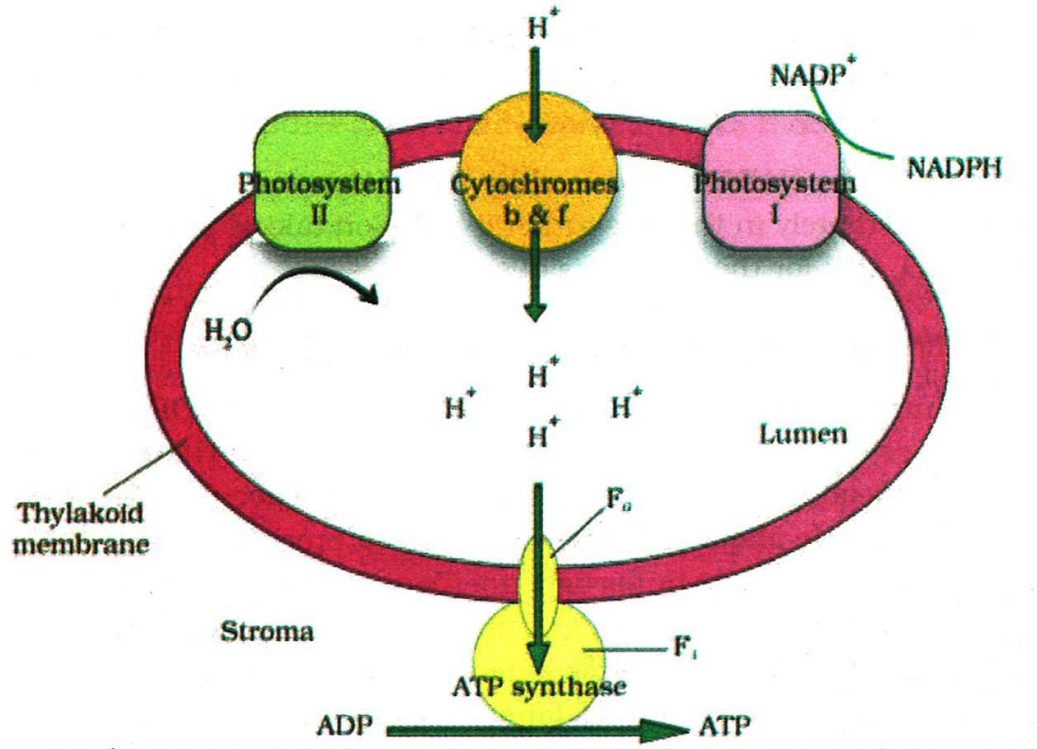


Figure 4.7 ATP synthesis through chemiosmosis

Hence, within the chloroplast, protons in the stroma decrease in number, While in the lumen, there is accumulation of protons. This creates a proton gradient across the thylakoid membrane as well as a measurable decrease in PH in the lumen.

Why are we so interested in the proton gradient? This gradient is important because it is the breakdown of this gradient that leads to release of energy. The gradient is broken down due to the movement of protons across the membrane to the stroma through the trans membrane channel of the F_o is embedded in the membrane and forms a trans membrane channel that carries out facilitated diffusion of protons across the membrane. The other portion is called F_1 and protrudes on the outer surface of the thylakoid membrane on the

side that faces the stroma. The breakdown of the gradient provides enough energy to cause a conformational change in the F_1 particle of the ATPase, which makes the enzyme synthesise several molecules of energy-packed ATP.

Chemiosmosis requires a membrane, a proton pump, a proton gradient and ATPase. Energy is used to pump protons across a membrane, to create a gradient or a high concentration of protons within the thylakoid lumen. ATPase has a channel that allows diffusion of protons back across the membrane ; this releases enough energy to activate ATPase enzyme that catalyses the formation of ATP.

Along with the NADPH produced by the movement of electrons, the ATP is used immediately in the biosynthetic reaction taking place in the stroma for fixing CO_2 and for the synthesis of sugars.

4.7 Where are the ATP and NADPH used?

We learnt that the products of light reaction are ATP, NADPH and O_2. Of these O_2 diffuses out of the chloroplast while ATP and NADPH are used to drive the processes leading to the synthesis of food, more accurately, sugars. This is the **biosynthetic phase** of photosynthesis. This process does not directly depend on the presence of light but is dependent on the products of the light reaction, i.e., ATP and NADPH, besides CO_2 and H_2O. You may wonder how this could be verified; it is simple: immediately after light becomes unavailable, the biosynthetic process continues for some time, and then stops. If then, light is made available, the synthesis starts again.

Can we, hence say that calling the biosynthetic phase the **dark reaction** *is a misnomer? Discuss the amongst yourselves.*

Let us now see how the ATP and NADPH are used in the biosynthetic phase. We saw earlier that CO_2 is combined with H_2O to produce $(CH_2O)n$ or sugars It was of interest to scientists to find out how this reaction proceeded and to find out what is the first product formed when CO_2 is taken into a reaction or fixed. Just after World War II, among the several efforts to put radioisotopes to beneficial use, the work of Melvin Calvin is exemplary. The use of radioactive ^{14}C by him in algal photosynthesis studies led to the discovery that the first CO_2 fixation product was a 3 carbon organic acid. Calvin also contributed to working out the complete biosynthetic pathway; hence it was called **Calvin cycle**. The first product identified was **3-phosphoglyceric** acid or, in short, **PGA**. *How many carbon atoms does it have?*

Scientists also tried to know whether all plants have PGA as the first product of CO_2 fixation, or whether any other product was formed in other plants. Experiments conducted over a wide range of plants led to the discovery of another group of plants, where the first stable product of CO_2 fixation was again an organic acid, but one which had 4 carbon atoms in it. This acid was identified to be **oxaloacetic acid** or OAA. Since then CO_2 assimilation during photosynthesis is said to be of two main types: those plants in which the first product of CO_2

fixation is a C_3 acid (PGA), i.e., the **C_3 pathway**, and those in which the first product is a C_4 acid (OAA), i.e., the **C_4 pathway.** These two groups of plants showed other associated characteristics that will discuss later.

4.7.2 The Calvin Cycle

Melvin Calvin and his associates Andrew Benson and James Bassham discovered this pathway in Chlorella and hence named as ' Calvin cycle'.

Calvin cycle is also called **Photosynthetic Carbon reduction** Cycle (PCR – cycle) or **Reductive Pentose Phosphate Pathway** (RPP-cycle).

The technique involved in the discovery is already discussed above. The entire pathway is discussed under three phases: - 1. Carboxylation, 2. Reduction, 3. Regenaration.

1. Carboxylation phase

1. In this phase three molecules of CO_2 are accepted from the atmosphere by three molecules of the primary CO_2 acceptor, **ribulose 1, 5 bisphosphate (RuBP),** which is a five carbon compound, to form six molecules of 3- phosphoglyceric acid (3 PGA)in the presence of water. This carboxylation reaction is catalysed by an enzyme called *RUBP carboxylase/ oxygenase*, abbreviated as RUBISCO. This enzyme has the credit of being the most abundant protein in the plant kingdom and accounts for 25-50% of soluble proteins in leaves.

3 RuBP + 3 CO_2 + 3 H_2O ⟶ **6** 3- phosphoglyceric acid

(5C) (3C)

The first stable compound of this pathway (3PGA) is a three carbon compound, hence the pathway is called C3 pathway. The plants which undergo this pathway only are called C3 plants.

2. Reduction Phase

2. Six molecules of 3-PGA react with six molecules of ATP (which was formed in light phase) resulting in six molecules of 1,3 –bisphospho-glyceric acid. This reaction is catalysed by *phosphoglycerokinase*. Six ADP molecules are generated in this reaction.

6 3-PGA + **6** ATP ⟶ **6** 1,3 -bis PGA + **6** ADP

(3C) (3C)

Six molecules of 1,3 – bisphosphoglyceric acid are reduced to six molecules of glyceraldehydes -3- phosphate.

6 1, 3 – bis PGA + **6** NADPH + H+ ⟶ **6** G –3P + **6** $NADP^+$ + **6** H2PO4.

(3C) (3C)

The enzyme catalysing this reaction is *glyceraldehydes – 3 – bisphosphate dehydregenase*. The six molecules of glyceraldehyde - 3 – phosphate formed are collectively termed as **"G-3 P- Pool".**

1/6 th of the triose phosphate (one molecule of G -3P) is exported out from the chloroplast into the cytosol for the synthesis of hexose and then sucrose. The remaining 5/6 th of triose phosphates (5 molecules of G – 3P) is used for the regeneration of the CO_2 acceptor (ribulose 1, 5 – bisphosphate).

During Calvin cycle, for the uptake of 3 molecules of CO_2 there is one triose phosphate (G -3P) available for export from the chloroplast. Hence an uptake of 6 molecules of CO_2 accunts for two triose phosphates (G -3P) being exported to cytosol, ultimately forming a hexose molecule. Thus, it can be summarized that the actual synthesis of hexoses (for storage) occurs in the cytosol.

The main end products of the Calvin cycle are the disaccharide, sucrose (glucose-fructose) and starch. Sucrose is synthesized in the cytosol from where it is transported away in phloem and used or stored (after converting into starch) in other tissues. Starch is also synthesized in chloroplasts when sucrose synthesis does not keep pace with the calvin cycle.

3. Regeneration phase

In order to maintain the continuity of CO_2 reduction, it is of vital importance to regenerate the RuBP molecules (acceptor). This is accomplished by a series of reactions involving many intermediate compounds having four, five, six and seven carbon sugars. The details of regeneration are given below:

Out of the five molecules of glyceraldehydes – 3 phosphate meant for regeneration ($5/6^{t}$ th) of RuBP, two molecules are isomerised to dihydroxy acetone phosphate (DHAP). This reaction is catalysed by *triose phosphate isomerase*.

2 Glyceraldehyde -3- phosphate $\rightleftharpoons$ **2** Dihydroxy acetone phosphate

(3C) (3C)

3. One molecule of glyceraldehydes – 3 – phosphate condenses with one molecule of DHAP obtained in the above reaction and forms one molecule of fructose – 1, 6 – bisphosphate. This reaction is catalysed by the enzyme *aldolase.*

Glyceraldehyde – 3- phosphate + Dihydroxy acetone phosphate $\longrightarrow$ Fructose 1,6 –bisphos-

(3C) (3C) (6C) phate

4. One molecule of fructose 1, 6 – bisphosphate undergoes dephosphorylation to form fructose – 6 – phosphate undergoes dephosphorylation to form fructose – 6 – phosphate

and an inorganic phosphate is released. This reaction is mediated by the enzyme *fructose – 1,6 – bisphosphatase*.

Fructose 1.6 bisphosphate ⟶ Fructose -6 – phosphate + pi

(6C) (6C)

5. Fructose - 6 - phosphate obtained in the above reaction combines with one molecule of glyceraldehydes – 3 – phosphate (G-3p) to form one molecule each of erythrose - 4 - phosphate and xylulose – 5 – phosphate. This reaction is catalyzed by the enzyme *transketolase.*

Fructose – 6 –phosphate + Glyceraldehyde - 3 - phosphate ⟶

(6C) (3C)

Erythrose -4- phosphate + Xylulose – 5 – phosphate

(4C) (5C)

6. A molecue of Erythrose – 4 – phosphate of the previous reaction combines with the DHAP to form one molecule of Sedoheptulose – 1, 7 – bishosphate in the presence of the enzyme *aldolase.*

Erythrose – 4 – phosphate + Dihydroxy acetone phosphate ⟶

(4C) (3C) Sedoheptulose – 1,7,-bisphosphate

(7C)

Subsequently sedoheptulose 1,7 bisphosphate undergoes dephosphorylation to from one molecule of sedoheptulose–7–phosphate. This dephosphorylation is catalysed by *sedoheptulose - 1, 7 bisphosphatase*.

7. One molecule of Sedheptulose – 7 – phosphate combines with one molecule of glyceraldehydes 3 – phosphate (G- 3P) to form one molecule each of Ribose – 5 – phosphate and Xylulose – 5 – phosphate in the presence of enzyme *transketolase*.

Sedoheptulose -7- Phosphate + Glyceraldehyde -3 – phosphate ⟶

(7C) (3C)

Xylulose -5- phosphate + Ribose -5- Phosphate

(5C) (5C)

8. Two molecules of Xylulose -5- phosphate (obtained in reaction nos. (5) and (7)) are converted into two molecules of Ribulose -5- phosphate. This reaction is catalyzed by *Ribulose -5- phosphate epimerase.*

2 Xylulose -5- phosphate ⟶ **2** Ribulose -5- phosphate

(5C) (5C)

9. One molecule of ribose -5- phosphate (obtained in the reaction (7)) is isomerized to one molecule of Ribulose -5- phosphate . This reaction is catalyzed by *Ribose -5- phosphate isomerase.*

Ribose -5- phosphate ⇌ Ribulose -5- phosphate
(5C) (5C)

10. Three molecules of Ribulose -5- phosphate (obtained from reaction nos. (8) and (9)) are further phosphorylated to three molecules of Ribulose – 1,5 – bisphosphate (RUBP) thus regenerating the CO_2 acceptor. Three molecules of ATP are utilized in this reaction, which are obtained from the light phase of photosynthesis. This reaction is catalyzed by *Ribulose -5- phosphate kinase.*

3 Ribulose -5- Phosphate + 3 ATP → 3 Ribulose -1,5-bisphate + 3ADP
(5C) (5C)

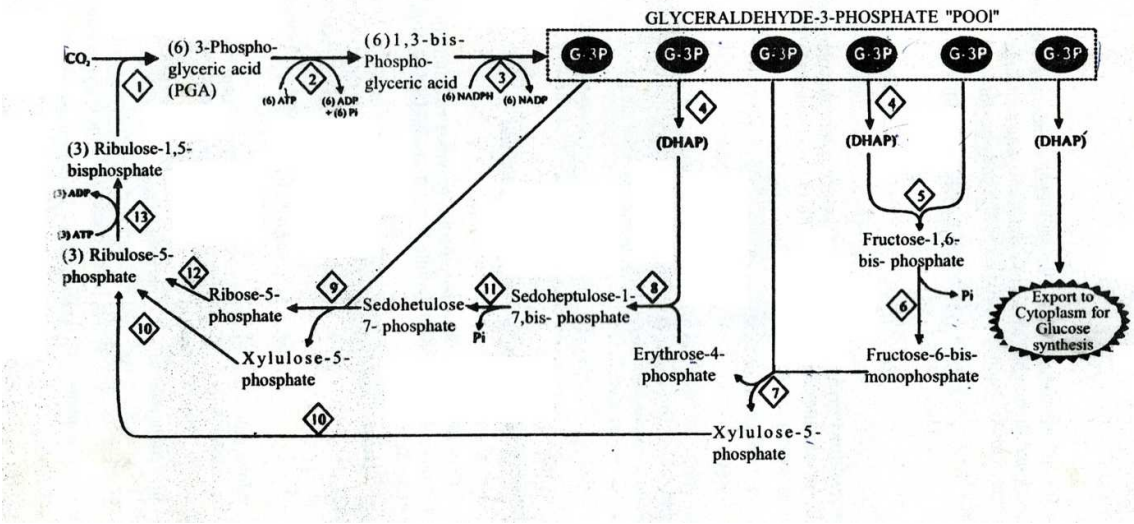


Figure 4.8 Bio-chemical reactions of calvin Cycle (PCR cycle)

Enzymes indicated in numbers are (1) RuBP carboxylase/oxygenase (RUBISCO)(2) Phospho glycerokinase (3) GAP dehydrogenase (4) Triose phate isomerase (5) Aldolase (6) Fructose 1,6-bisphosphatase (7) Transketolase (8) Aldolase (9) Transketolase (10) Ribulose—phosphate epimerase (11) Sedoheptulose -1,7-bisphosphate (12) Ribulose-5phosphate isomerase (13) Ribulose-5-phosphate kinase

Energitics of Calvin Cycle

For reducing three molecules of CO_2 by Calvin Cycle, a total 6 molecules of NADPH and 9 molecules of ATP are required. In order to obtained a hexose sugar, 12 molecules of NADPH and 18 molecules of ATP are required.

4.8 The C4 Pathway

This pathway is called by several names like β- Carboxylation pathway or Hatch and Slack pathway.

Kortschak, Hartt & Burr (1965) found that 3-PGA is not the initial product of photosynthesis in case of sugarcane, instead it was four carbon dicarboxylic acids like malic and aspartic acids. **M.D Hatch and C.R. Slack** confirmed the results of the above scientists while working on sugarcane plants. Because the first stable compound, is a four carbon compound, the pathway is termed as C_4 pathway and plants which undergo this pathway are called C_4 plants**. Prof. V.S.Rama Das**, has done extensive research work on C_4 plants in India.

This pathway occurs in plants of subtropical and tropical regions world, where the temperature ranges between 30^0 C and 45^o C, about 1500 species angiosperms, belonging to 19 families exhibit this pathway (C_4 – plants). C_4 plants are better equipped to withstand drought and are able to maintain active photosynthesis even under conditions of water stress. This suggests that C_4 pathway is biologically more evolved than C_3 pathway.

Structural peculiarity of C4 leaf

The most distinguishable anatomical feature of a C_4 leaf is the presence of a bundle sheath surrounding the vascular bundles. This anatomical specialization of a C_4 leaf is called **"Kranz anatomy"**. Kranz, is a German word, which means 'wreath' in English due to ring like arrangement of bundle sheath cells. While 'agranal chloroplasts" are present in bundle sheath cells, the mesophyll cells of a C_4 leaf have grana in their Chloroplast (**Chloroplast dimorphism**).

Reactions of C_4 pathway

This pathway operates in two photosynthetic cells: Mesophyll and Bundle sheath, thus exhibiting division of labour. Step wise reactions are as follows:

1. Carbondioxide is accepted (actually in the form of bicarbonate ions = HCO_3^-) by a primary carbon acceptor called phosphoenol pyruvic acid (PEP) to form Oxaloacetic acid (OAA), which is a four carbon dicarboxylic acid . This reaction occurs in the cytosol of the mesophyll cell and it is catalysed by the enzyme *PEP – Carboxylase* (PEP case). OAA, thus formed in the cytosol now enters into the chloroplast of the mesophyll cell.

CO_2 + phosphoenol Pyruvic acid + H_2O ⟶ Oxaloacetic acid +H_2PO_4

(3C) (4C)

2. Oxaloacetic acid ids reduced to malic acid, which is also a four carbon dicarboxylic acid, by using the light generated NADPH. This reaction is catalysed by the enzyme *Malic dehydrogenase.*

Oxaloacetic acid + NADPH ⟶ Malic acid + NADP+

(4C) (4C)

In some C4 plants Oxaloacetic acid is converted into aspartic acid by transamination. However the path of carbon in malate formers is only discussed below.

3. Malic acid formed in the chloroplast of mesophyll cells is now transported to the chloroplast of bundle sheath cell. Malic acid now undergoes oxidative decarboxylation to form Pyruvic acid, a three carbon organic acid. This reaction is catalysed by *Malic enzyme*. The oxidation of Malic acid during this reaction is associated with the reduction of $NADP^+$ into $NADPH+ H^+$.

$$\text{Malic acid} + NADP^+ \longrightarrow \text{Pyruvic acid} + NADPH + H^+ + CO_2$$

(4C) (4C)

4. The CO_2 generated in the above reaction ids utilized in Calvin cycle (PCR cycle) to synthesize sugars.

5. The pyruvic acid produced in bundle sheath cell during step (3) moves to chloroplast of mesophyll cell. Here it is phosphorylated back to phosphoenol pyruvate (PEP). The enzyme *pyruvate dikinase* catalyses this reaction. Two molecules of ATP help in phosphorylation. PEP then enters into cytosol of the mesophyll cell.

$$\text{Pyruvic acid} + 2ATP + Pi \longrightarrow \text{Phosphoenol pryuvate} + 2AMP + 2\,PPi$$

(3C) (3C)

During this pathway there are **two carboxylations** (one each in mesophyll and bundle sheath) followed by **one decarboxylation** in bundle sheath cell. To synthesize one molecule of glucose, C_4 plants utilize **30 ATP and 12 NADPH**. In this pathway, 12 molecules of ATP are utilized in excess to build the CO_2 concentration levels in bundle sheath cells.

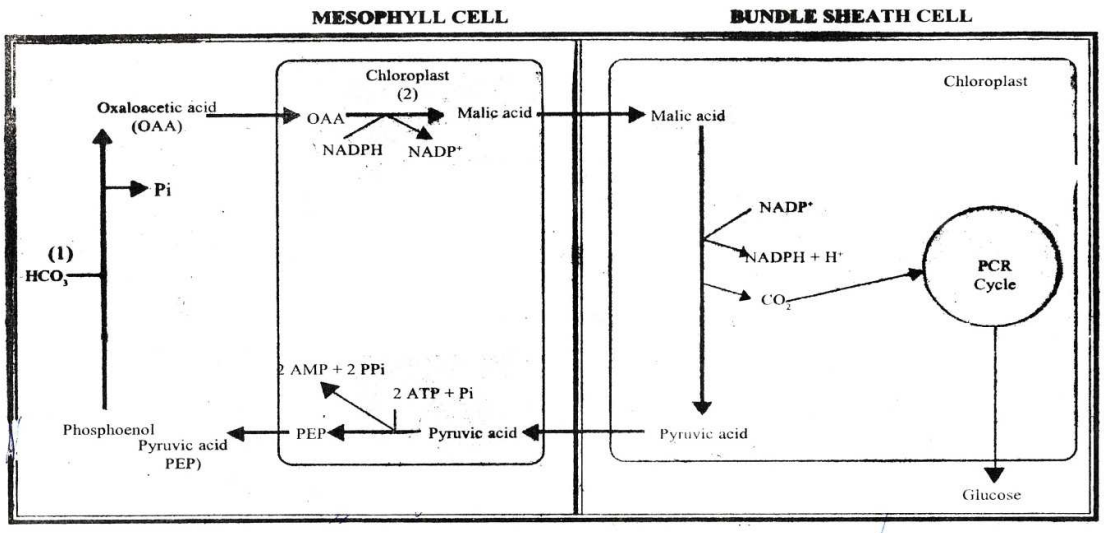


Fig 4.9 Hatch and Slack Pathway (C_4 Cycle)
Enzymes : (1) PEP - carboxylase (2) Malic dehydrogense
(3) Malic enzyme (4) Pyruvate dikinase

Crassulacian Acid Metabolism (CAM) is an alternative to the C_3 and C_4 pathways of CO_2 fixation found in dry and hot climates. eg., Cacti. Crassulaceae is one of the family of such plants.

CAM pathway is similar to C_4 pathway in that CO_2 is trapped by highly efficient PEP Carboxylase. However, instead of having two kinds of photosynthetic cells. CAM plant has only one kind of photosynthetic cell in which CO_2 is fixed during night and used to make glucose during day. In C_4 plants, CO_2 fixation and Calvin cycle are separated in space, while in CAM plants they are separated in time.

4.9 Photorespiration

Let us try and understand one more process that creates an important difference between C_3 and C_4 plants – Photorespiration. To understand the photorespiration we must know a little more about the first step of the Calvin pathway- the first CO_2 fixation step. This is the reaction where RUBP combines with CO_2 to form 2 molecules of 3 PGA that are catalysed by RUBIsCO.

$$RuBP + CO_2 \xrightarrow{RuBisCo} 2x\ 3\ PGA$$

RuBisCO is the most abundant enzyme in the world (Do you wonder why?). It is characterised by the fact that its active site can bind to both CO_2 and O_2 – hence the name . Can you think how this could be possible? RuBisCO has a much greater affinity for CO_2 than for O_2. Imagine what would happen if this were not so! This binding is competitive. It is the relative concentration of O_2 and CO_2 that determines which of the two will bind to the enzyme.

In C_3 plants some O_2 does bind to RuBisCO, and hence CO_2 fixation is decreased. Here the RuBP, instead of being converted to 2 molecules of PGA, binds with O_2 to form one molecule of phosphoglycerate and phosphoglycolate in a pathway called photorespiration. In the photorespiratary pathway, there is the synthesis neither of sugars, nor of ATP. Rather there is a release of CO_2 withthe utilisation of ATP. In the photorespiratory pathway there is no synthesis of ATP or NADPH. Therefore, photorespiration is a wasteful process.

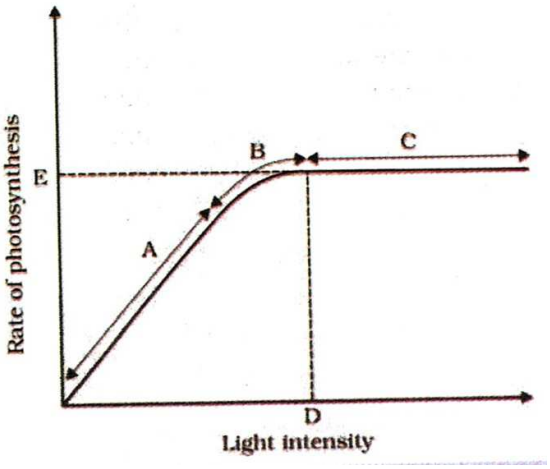
In C_4 plants photorespiration does not occur. This is because they have a mechanism that increases the concentration of CO_2 at the enzyme site. This takes place when the C_4 acid from the mesophyll is broken down in the bundle sheath cells to release CO_2. In turn, this ensures that the RuBisCo functions as a carboxylase, minimising the oxygenase activity.

Now that you know that the C_4 plants lack photorespiration, you probably can understand why productivity and yields are better in these plants. In addition these plants show tolerance to higher temperatures.

4. 10 Factors affecting Photosynthesis

An Understanding of the factors that affect photosynthesisis necessary. The rate of photosynthesis is very important in determining the yield of plants including crop plants. Photosynthesis is influenced by several factors, both internal (plant) and external. The plant factors include the number, size age and orientation of leaves, mesophyll cells and chloroplasts, internal CO_2 concentration and the amount of chlorophyll. The plant or internal factors are dependent on the genetic predisposition and the growth of the plant.

The external factors Include the availability of sunlight, temperature, CO_2 concentration and water. All these factors simultaneously affect the rate of photosynthesis. Though several factors interact and simultaneously affect the photosynthesis or CO_2 fixation, usually one factor is predominant or is the one that limits the rate. Hence, at any point the rate of photosynthesis is determined by the factor available at sub-optimal levels.



When several factors affect any (bio) chemical process, Blackman's (1905) **Law of Limiting Factors** comes into effect. This states the following:

"If a process (like photosynthesis) is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the factor that is present in a relative minimal value."

Figure 4.10 Graph showing effect of light intensity on the rate of photosynthesis

For example, despite the presence of a green leaf and optimal light and CO_2 conditions, a plant may not photosynthesise if the temperature is very low. But if given the optimal temperature the leaf will start photosynthesising.

SUMMARY

Green plants make their own food by photosynthesis. During this process carbon dioxide from the atmosphere is taken in by leaves through stomata and used for making carbohydrates, principally sucrose and starch. Photosynthesis takes place only in the green parts of the plants, mainly the leaves, within the leaves, the mesophyll cells have a large number of chloroplast, the membranes are sites for the light reaction, while the chemosynthetic pathway occurs in the stroma. Photosynthesis has two light stages: the light reaction and the carbon fixing reactions. In the light reaction the light energy is absorbed by the pigments present in the antenna and funnelled to special chlorophyll a molecules called reaction centre chlorophylls. These are two photsystems, PSI and PSII . PSI has a 700 nm absorbing chlorophylla P700 molecule at its reaction centre, while PSII has P680 reaction centre that absorbs red light at 680 nm. After absorbing light chlorophylls are exited and electrons are transferred through PSII and PSI and finally to NAD forming NADH. During this process a proton gradient is created across the membrane of the thylakoid. The breakdown of the proton gradient due to movement through the F0 part of the ATPase enzyme releases enough energy for synthesis of ATP. Splitting of water molecules associated with PSII resulting in the release of O_2, protons and transfer of electrons to PSII.

In the carbon fixation cycle, CO_2 is added by the enzyme, RuBisCO to a 5-carbon compound RuBP that is converted to 2 molecules of 3-carbon PGA. This is then converted to sugar by the Calvin cycle, and the RuBP is regenerated. During this process ATP and NADPH synthesised in the light reaction are utilised. RuBisCO also catalyses a wasteful oxygenation reaction in C_3 plants: photorespiration.

Some tropical plants show a special type of photosynthesis called C_4 pathway. In these plants the first product of CO_2 fixation that takes place in the mesophyll is a 4-carbon compound. In the bundle sheath cells the Calvin pathway is carrie4d out for the synthesis of carbohydrates.

GLOSSARY

Absorption spectrum: Graph showing light absorption by photosynthetic pigments as a function of wavelength of light.

Action Spectrum: Graph showing rate of photosynthesis as a function of wavelength of light.

Assimilatory power: Chemical energy generated as a result of light reaction. It is in the form of ATP, NADPH. Also known as reducing power.

CAM: Crassulacean Acid Metabolism, an alternative pathway of CO_2 fixation found on plants living in dry and hot climates eg., Cacti.

Chemiosmosis: proposed by Mitchell, explains ATP generation from ADP and Pi driven by proton gradient across membrane in mitochondria and chloroplasts.

Dark Reaction: Phase of photosynthesis not directly light driven, but dependent on the products of light reaction. Now more appropriately known as biosynthetic phase.

Kranz Anatomy : Typical of C_4 leaf with particularly large cells around vascular bundles i.e., wreath like bundle sheath.

Law of limiting factor: In a process participated by a number of separate factors, the rate of the process is limited by the factor which is present in minimal value.

Light harvesting Complex (LHC): A group of molecules, associated with reaction centre of a photosystem, collecting light energy to be transferred to the reaction centre eventually.

Light reaction: Phase of photosynthesis driven directly by light.

Photolysis of H_2O: Splitting of water by photosystem II in the presence of light.

Photorespiration: Light dependent release of CO_2 and uptake of O_2 by the green tissue of particularly C_3 plants.

Reaction centre: Special chlorophyll which routinely undergoes photo oxidation, designated as P_{700} in photosystem I and P_{680} in photosystem II.

Redox potential: The tendency of a molecule or atom to give or take up electrons.

RUBISCO: Enzyme that catalyses addition of CO_2 to RUBP. The most abundant protein on earth.

Z-Scheme: Schematic representation of photosynthetic electron transport from H_2O to $NADP^{+,}$ in line with redoxpotential gradient. First proposed by Hill and Bendall.

Questions

Very Short Answer Type Questions

1. Where does photolysis of water occur?

2. Distinguish between Action spectrum and Absorption spectrum.

3. How many molecules of ATP and NADPH are needed to fix a molecule of CO_2 in C_3 plants?

4. What is the primary acceptor of CO_2 in C_4 plants?

5. Explain the term ' Energy currency'.

6. What is the first stable compound formed in a Calvin cycle?

7. Define a law of limiting factors proposed by Blackman.

Short Answer Type Questions

1. Explain the structure of the Chloroplast? Draw a neat labelled diagram.

2. Write any four differences between C_3 and C_4 Plants.

Exercises

1. Why photorespiration does not occur in C4 plants?

2. By looking at a plant externally, Can you tell whether a plant is C_3 or C_4? Why and How?

3. Why do you believe Chloroplast and Mitochondria to be semi- autonomous organelle.

Chapter 5

Respiration in Plants

Respiration is a catabolic, enzyme mediated oxidative process by which the C-C bonds of food materials like carbohydrates (common respiratory substrate), fats, aminoacids and organic acids are broken down to release considerable amount of energy, hence 'exerganic process'.

Catabolism is the degrative phase of metabolism in which relatively large and complex nutrient molecules (eg., Carbohydrates ,lipids and proteins) are degraded to yield smaller ,simpler molecules. Catabolismis accompained by the release of chemical energy inherent in the structure of organic nutrient molecules.

$C_6H_{12}O_6 + 6O_2 \longrightarrow 6\ CO_2 + 6H_2O + 686$ K.Cal

Mitochondria (Fig 5.1) are the cell organelles associated with the process of respiration (aerobic). You already studied the structure of mitochondria in cell Biology unit of first year. However a brief account of mitochondrial structure is essential to understand the respiratory mechanism. Mitochondria are limited by a smooth outer membrane and contain a highly invaginated inner membrane, which has a special role in **energy transduction**. The space between these two membranes is called the **inter membrane space** (peri mitochondrial space**).** The invaginations of inner membrane are called **mitochondrial crests** or **cristae**. The inner membrane is selectively permeable to a few molecules like water, O_2 and CO_2. However, its permeability to protons is very low, or absent. Which is a significant factor for ATP synthesis.

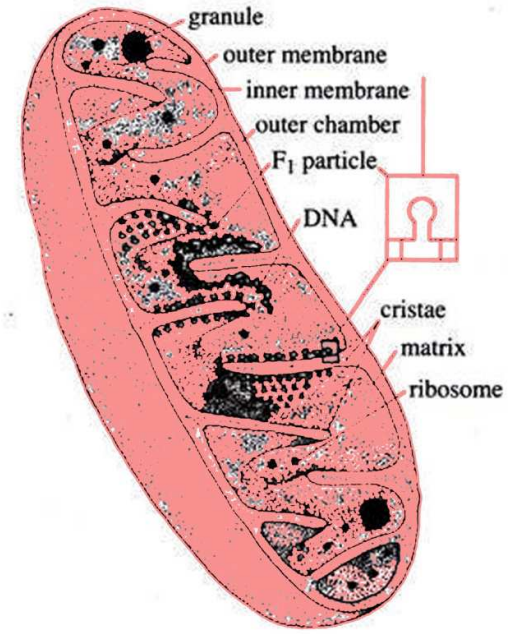


Fig. 5.1 : Three-dimensional diagram of a mitochondrion cut longitudinally. The cristae are folds of the inner membrane and on their matrix side they have the F_1 particles. The inset shows an F_1 particle with the head piece and stalk.

The stalked particles on the mitochondrial crests are called as **Fo – F1 complexes**, which were previously referred to as oxysomes or elementary particles (Fig.5.1). The inner chamber of mitochondria is filled with a dense and granular 'mitochondrial matrix', which contains 70% of respiratory enzymes, 70S type of ribosomes , circular DNA, RNA and several ions.

Respiration being an exerganic process it releases energy, which is either lost in the form of heat or temporarily packed within a chemical compound called **adenosine tri phosphate** or **ATP** (Energy currency of a cell). The advantage of ATP is the that stores energy to be immediately released whenever needed and can move within a cell from one place to another.

Types of Respirations

Essentially there are two types of respiratory mechanisms with reference to the involment of Oxygen. **1. Anaerobic respiration** – which occurs in the absence of oxygen. **2. Aerobic respiration** – which occurs in the presence of oxygen.

5.1 Anaerobic respiration

During the process of respiration, oxygen is utilised, and carbon dioxide, water and energy are released as products. The combustion reaction requires oxygen. But some cells live where oxygen may or may not be available. Can you think of such situations (and organisms) where O2 is not available? There are sufficient reasons to believe that the first cells on this planet lived in an atmosphere that lacked oxygen. Even among present-day living organisms, we know of several that are adapted to anaerobic conditions. Some of these organisms are facultative anaerobes, while in others the requirement for anaerobic condition is obligate. In any case, all living organisms retain the enzymatic machinery to partially oxidise glucose without the help of oxygen. This breakdown of glucose to pyruvic acid s called **glycolysis.**

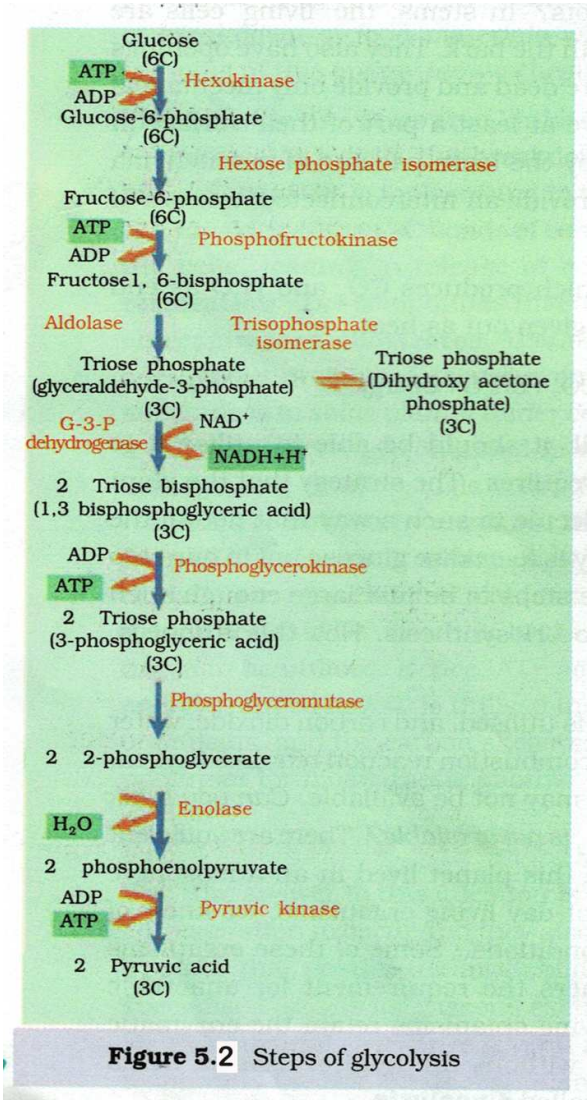


Figure 5.2 Steps of glycolysis

5.1.1 Glycolysis

The term glycolysis has originated from the Greek Words, *glycos* for sugar and *lysis* for splitting. The scheme of glycolysis was given by Gustav Embeden , Otto Materhof, and J. Parnas, and is often referred to as the EMP pathway. In anaerobic organisms, it is the only process in repiration. Glycolysis occurs in the cytoplasm of the cell and takes place in all living organisms. In this process , glucose undergoes partial oxidation to form two molecules of pyruvic acid. In plants, this glucose is derived from sucrose, which is the end product of hphotosynthesis, or from storage carbohydrates. Sucrose is converted into glucose and fructose by the enzyme invertase, and these two monosaccharides readily enter the glycolytic pathway. Glucose and fructose are phosphorylated to give rise to glucose-6-phosphate by the activity by the activity of enzyme hexokinase. This phosphorylated form of glucose then isomerises to produce fructose-6-phosphate. Subsequent steps of metabolism of glucose and fructose are the same. The various steps of glycolysis are depicted in figure 5.2. In glycolysis a chain of ten reactions , under the control of different enzymes, takes place to produce Pyruvate from glucose. While studying the steps of glycolysis, please note the steps at which utilisation or synthesis of ATP or (in this case) NADPH + H^+ take place.

ATP utilised at two steps: first in the convertion of glucose into glucose -6-phosphate and second in the convertion of fructose-6-phosphate to Fructose 1,6-bisphosphate.

The fructose1,6-bisphoaphate is split into dihydroxyacetone phosphate and 3-phospho glyceraldehydes (PGAL). We find that there is one step where NADPH + H+ is formed from NAD+; this is when 3-phosphoglyceraldehyde (PGAL) is converted to 1,3-bisphospho glycerate(BPGA). Two redox-equivalents are removed (in the form of two hydrogen atoms) from PGAL and transferred to a molecule of NAD+. PGAL is oxidised and with inorganic phosphate to get converted into BPGA. The conversion of BPGA to phosphoglyceric acid

(PGA), is also an energy yielding process; this energy is trapped by the formation of ATP. Another ATP is synthesised during the conversion of PEP to pyruvic acid. *Can you then calculate how many ATP molecules are directly synthesised in this pathway from one glucose molecule?*

Pyruvic acid is then the key product of glycolysis. What is the metabolic fate of pyruvate? This depends on the cellular need. There are three major ways in which different cells handle pyruvic acid produced by glycolysis. These are lactic acid fermentation, alcoholic fermentation and aerobic respiration. Fermentation takes place under anaerobic conditions in many prokaryotes and unicellular eukaryotes. For the complete oxidation of glucose to CO_2 and H_2O, however, organisms adopt Kreb's cycle which is also called as aerobic respiration. This requires oxygen supply.

5.1.2 Fermentation

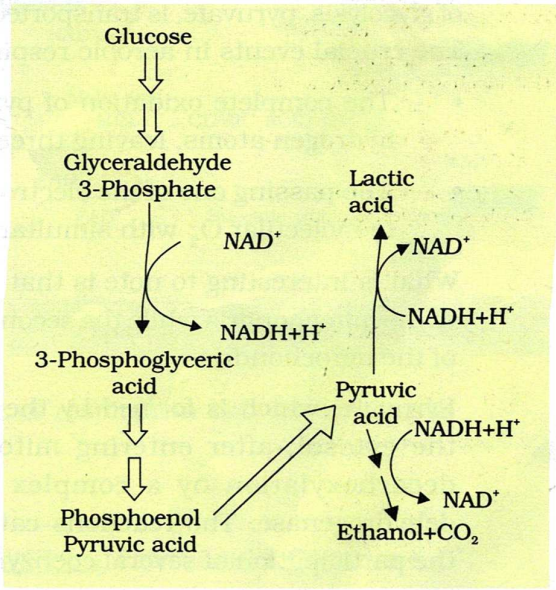


Figure 5.3 Major pathways of anaerobic respiration

In fermentation, say by yeast, the incomplete oxidation of glucose is achieved under anaerobic conditions by sets of reactions where pyruvic acid is converted to CO_2 and ethanol. The enzyme, pyruvic acid decarboxylase and alcohol dehydrogenase catalyse these reactions. Other organisms like some bacteria produce lactic acid fron pyruvic acid. The steps involved are shown in Figure 5.3. In animal cells also, like muscles during exercise when oxygen is inadequate for cellular respiration, pyruvic acid is reduced to lactic acid by lactate dehydrogenase. The reducing agent is NADPH + H^+ which is reoxidised to NAD^+ in both the processes.

In both lactic acid and alcohol fermentation not much energy is released; less than seven per cent of the energy in glucose is released and not all of its trapped as high energy bonds of ATP. Also, the processes are hazardous – either acid or alcohol is produced. What is the net ATPs that is synthesised (Calculate how many ATP are synthesised and deduct the number of ATP utilised during glycolysis) When one molecule of glucose is fermented to alcohol or lactic acid? Yeasts poison themselves to death when the concentration of alcohol reaches about 13 per cent. How do you think alcoholic beverages with alcohol content greater than this concentration are obtained?

What then is the process by which organisms can carry out complete oxidation of glucose and extract the energy stored to synthesise a large number of ATP molecules needed for

cellular metabolism? In Eukaryotes these steps take place within the mitochondria and this requires O2. Aerobic respiration is the process that leads to complete oxidation of organic substances in the presence of oxygen, and release CO2, water and a large amount of energy present in the substrate. This type of respiration is most common in higher organisms. We will look at these processes in the following section.

5.2 Aerobic Respiration

For aerobic respiration to take place within the mitochondria, the final product of glycolysis, pyruvate, is transported from cytoplasm into mitochondria. The crucial events in aerobic respiration are:

- The complete oxidation of pyruvate by the stepwise removal of all the hydrogen atoms, leaving three molecules of CO_2.
- The passing one of the electrons removed as part of the hydrogen atoms to molecular O_2 with simultaneous synthesis of ATP.

What is interesting to note is that the first process takes place in the matrix of the mitochondria while the second process is located on the inner membrane of the mitochondria.

Pyruvate, which is formed by the glycolytic cayabolism of carbohydrates in the cytosol, after entering mitochondrial matrix, undergoes oxidative decarboxylation by a complex set of reactions catalysed by pyruvic dehydrogenase. The reactions catalysed by pyruvic dehydrogenase require the participation of several coenzymes, including NAD^+ and Coenzyme A.

$$\text{Pyruvic acid} + \text{CoA} + \text{NAD} \xrightarrow[\text{Pyruvate dehydrogenase}]{\text{Mg+}} \text{Acetyl CoA} + CO_2 + \text{NADH} + H^+$$

During this process, two molecules of NADH are produced from the metabolism of two molecules of pyruvic acid (Produced from one glucose molecule during glycolysis).

The acetyl CoA then enters a cyclic pathway, tricarboxylic acid cycle, more commonly called Krebs cycle after the scientists Hans Krebs who first elucidated it.

5.2.1 Tri Carboxylic acid cycle (Krebs cycle)

The TCA cycle starts with the condensation of acetyl group with oxaloacetic acid (OAA) water to yield citri acid (Figure 5.4). The reaction is catalysed by the enzyme citric synthase and a molecule of CoA is released. Citrate is then isomerised to isocitrate. It is followed by two successive steps of decarboxylation, leading to the formation of α-ketoglutaric acid and succinyl CoA. In the remaining steps of the citric acid cycle, Succinyl-CoA is oxidised to OAA, allowing the cycle to continue. During the conversion of succinyl-CoA to succinic

acid a molecule of GTP is synthesised. This is a substrate level phosphorylation. In a coupled reaction GTP is converted to GDP with the simultaneous synthesis of ATP from ADP. Also there are three points in the cycle where NAD^+ is reduced to $NADH^+ + H^+$ and one point where FAD^+ is reduced to $FADH_2$. The continued oxidation of acetyl CoA via the TCA cycle requires the continued replenishment of oxaloacetic acid, the first member of the cycle. In addition it also requires regenaretionof NAD^+ and FAD^+ from NADH and $FADH_2$ respectively. The equation for this phase of respiration may be written as follows:

Condensation:

Oxaloacetic acid + Acetyl CoA $\xrightarrow{\text{citric synthatase}}$ Citric acid + Coenzyme A

Dehydration:

Citric acid $\xrightarrow{\text{aconitase}}$ Cis-aconitic acid + H_2O

Hydration:

Cis-aconitic acid + H_2O $\xrightarrow{\text{aconitase}}$ Isocitric acid

Oxidation I:

Isocitric acid + NAD^+ $\xrightarrow{\text{Isocitric dehydrogenase}}$ Oxalosuccinic acid + NADH

Decarboxylation:

Oxalosuccinic acid $\xrightarrow{\text{oxalosuccinic decarboxylase}}$ α-ketoglutaric acid + CO_2

Oxidative decarboxylation (Oxidation II):

A-ketoglutaric acid + NAD+ + CO_2 $\xrightarrow{\text{Oxalosuccinic dehydrogenase}}$ Succinyl CoA + NADH + CO_2

Cleavage:

Succinyl CoA + ADP + Pi $\xrightarrow{\text{succinic thiokinase}}$ Succinic acid + ATP + CoA

Oxidation III:

Succinic acid + FAD $\xrightarrow{\text{Succinic dehydrogenase}}$ Fumaric acid + $FADH_2$

Hydration:

Fumaric acid + H_2O $\xrightarrow{\text{Fumarase}}$ Malic acid

Oxidation IV:

Malic acid + NAD^+ $\xrightarrow{\text{Malic dehydrogenase}}$ Oxaloacetic acid + NADH

Summary equation for the oxidation of pyruvic acid in mitochondrion is:

Pyruvic acid +4 NAD^+ + FAD + $2H_2O$+ ADP + Pi $\xrightarrow{\text{Mitochondrial matrix}}$ $3CO_2$ + 4 $NADH^+$ + $4H^+$ + $FADH_2$ + ATP

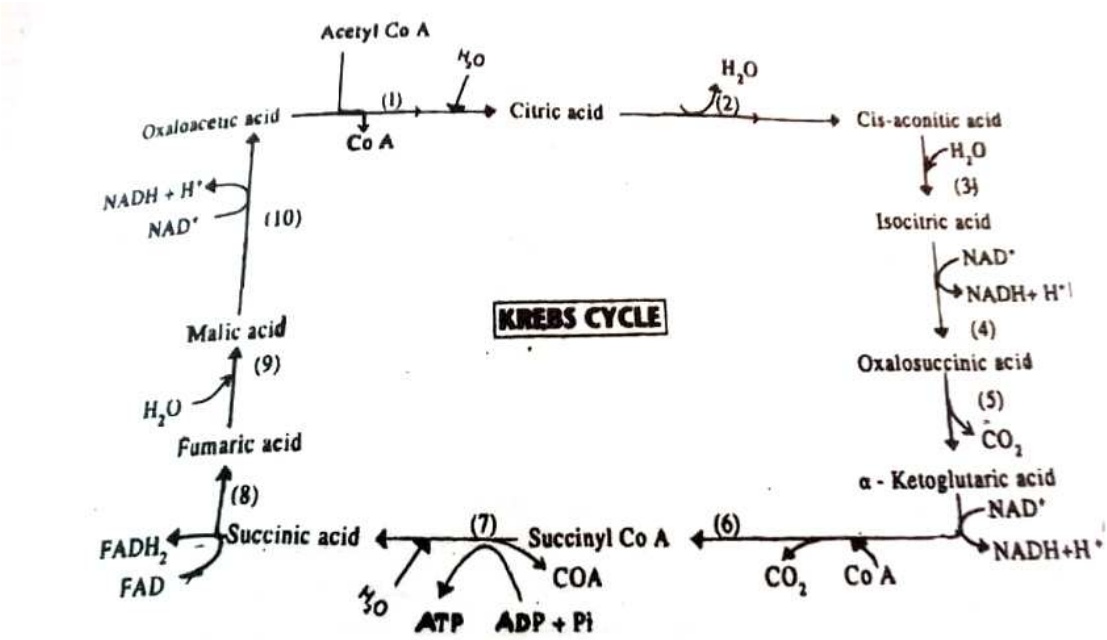


Fig 5.4 Biochemical reactions of Krebs cycle

1. Citric Synthetase, 2. Aconitase, 3. Aconitase, 4. Isocitric dehydrogenase, 5. Oxalosuccinic decaroxylase, 6. α–Ketoglutaric dehydrogenase, 7. Succinic thiokinase, 8. Succinic dehydrogenase, 9. Fumerase, 10. Malic dehydrogenase

When have seen till now that glucose has been broken down to release CO_2 and eight molecules of NADH + H^+; two of $FADH_2$ have been synthesised besides just two molecules of ATP. You may be wondering why we have been discussing respiration at all – neither O_2 has come into the picture nor the promised large number of ATP has yet been synthesised. Also what is the role of the NADH + H^+ and $FADH_2$ that is synthesised . Also what is the role of the NADH + H+ and $FADH_2$ that is synthesised? Let us now understand the role of O_2 in respiration and how ATP is synthesised.

5.3.2 Electron Transport Sytem (ETS) and Oxidative phosphorylation

The following steps in the respiratory process take place in order to release and utilise the energy stored in NADH + H^+ and $FADH_2$. This is accomplished when, they are oxidised through the electron transport system and the electrons are passed on to O_2 resulting in the formation of H_2). The metabolic pathway through which an electron passes from one carrier to another is called the electron transport system (ETS) (Figure 5.5) and it is present in the inner mitochondrial membrane. Electrons from NADH produced in the mitochondrial matrix during the citric acid cycle are oxidised by an NADH dehydrogenase (complex I), and electrons are then transferred to ubiquinone located within the inner membrane. Ubiquinone also receives reducing equivalents via $FADH_2$ (complex II) that is generated during oxidation of succinate in the citric acid cycle. The reduced Ubiquinone (ubiquinol) is then oxidised with the transfer of electrons to cytochrome $_c$ via cytochrome bc_1 complex (Complex III). Cytochrome c is a small protein attached to the outer surface of the inner membrane and acts as a mobile carrier for the transfer of electrons between complex IV refers to cytochrome c oxidase complex containing cytochromes a and a_3 and two copper centres.

When the electrons pass from one carrier to another via complex I to IV in the electron transport chain, they are coupled to ATP synthase (Complex V) for the production of

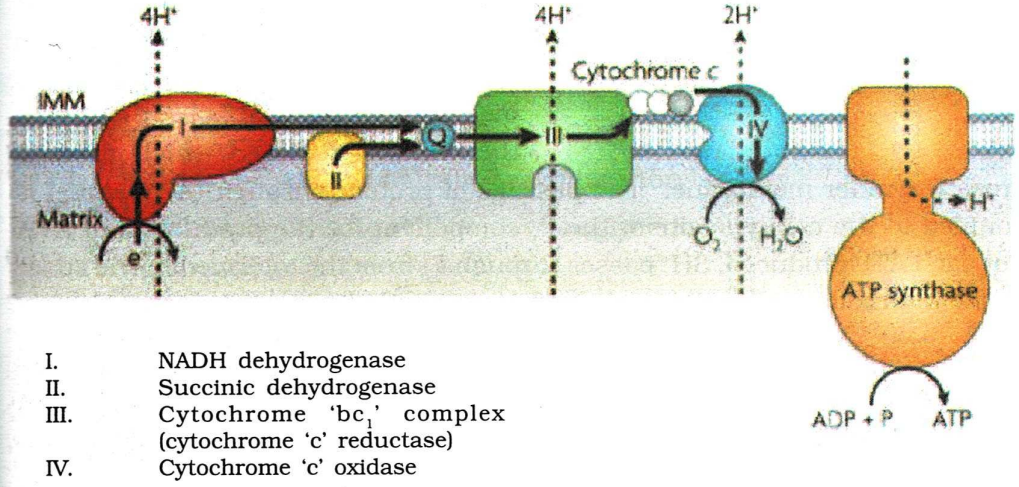


Figure 5.5. Respiratory Electron Transport and Oxidative Phosphorylation

ATP from ADP and inorganic phosphate. The number of ATP molecules synthesised depends on the nature of the electron donor. Oxidation of one molecule of NADH gives rise to 3 molecules of ATP, while that of one molecule of $FADH_2$ produces 2 molecules of ATP. Although the aerobic process of respiration takes place only in the presence of oxygen, the role of oxygen is vital, since it drives the whole process by removing hydrogen from the system. Oxygen acts as the final hydrogen acceptor. Unlike photophosphorylation where is the light energy that is utilised for the production of

proton gradient required for phosphorylation, in respiration it is the energy of oxidation reduction utilised for the same process. It is for this reason that the process is called oxidative phosphorylation.

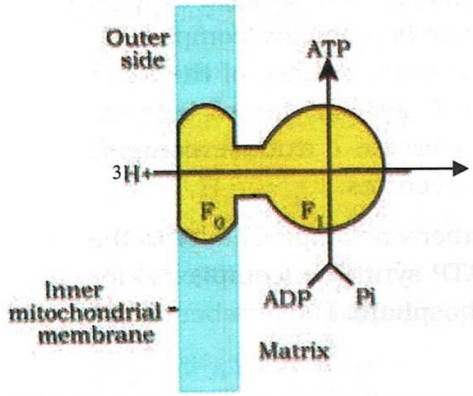


Figure 5.6 Diagramatic presentation of ATP synthesis in mitochondria

You have already studied about the mechanism of membrane-linked ATP synthesis as explained by chemiosmatic hypothesis in the earlier chapter. As mentioned earlier, the energy released during the electron transport system is utilised in synthesising ATP with the help of ATP synthase (complex V). This complex consists of two major components, F_1 and F_0 (Figure 5.6) The F_1 headpiece is a peripheral membrane protein complex and contains the site for synthesis of ATP from ADP and inorganic phosphate. F_0 is an integral membrane protein complex that forms the channel through which protons cross the inner membrane. The passage of protons through the channel is coupled to the catalytic site of the F_1 component for the production of ATP. For each ATP produced, 3 H^+ passes through F_0 from the intermembrane space to the matrix down the electrochemical proton gradient.

5.4 The Respiratory Balance Sheet

It is possible to make calculations of the net gain of ATP for every glucose molecule oxidised; but in really this can remain only a theoretical exercise. These calculations can be made only on certain assumptions that:

- There is a sequential, orderly pathway functioning, with one substrate forming the next and with glycolysis. TCA cycle and ETS pathway following one after another.
- The NADH synthesised in glycolysis is transfer into the mitochondria and undergoes oxidative phosphorylation.
- None of the intermediates in the pathway are utilised to synthesise any other compound.
- Only glucose is being respired – no other alternative substrates are entering in the pathway at any of the intermediary stages.

But these kind of assumptions are not really valid in a living system; all pathways work simultaneously and do not take place one after another; substrates enter the pathways and are withdrawn from it as and when necessary; ATP is utilised as and when needed; enzymatic rates are controlled by multiple means. Yet, it is useful to do this exercise to

appreciate the beauty and efficiency of the living system in extracting and storing energy. Hence, there can be a net gain of 36 ATP molecules during aerobic respiration of one molecule of glucose.

Now let us compare fermentation and aerobic respiration:

- Fermentation accounts for only a partial breakdown of glucose where as in aerobic respiration glucose completely degraded to CO_2 and H_2O.
- In fermentation there is a net gain of only two molecules of ATP for each molecule of glucose degraded to pyruvic acid where as many more molecules of ATP are generated under aerobic conditions.
- NADH is oxidised to NAD^+ rather slowly in fermentation; however the reaction is vigorous in the case of aerobic respiration.

Balance Sheet of ATP production in aerobic oxidation of glucose.

(1) Glycolysis:

1. ATP produced by substrate level phosphorylation

Bisphosphoglyceric acid to phosphoglyceric acid : 2x1 = 2 ATP

Phosphoenol pyruvic acid to pyruvic acid : 2x1 = 2 ATP

ATP consumed : for the phosphorylation of glucose

and fructose-6-phosphate : - 2 ATP

Net gain of ATP : +2 ATP

2. ATP from NADH generated in glycolysis:

G-3-P to BPGA (2 NADH, each worth 2 ATP) : 2x2 = 4 ATP

ATP gain from glycolysis in the presence of O_2 : [(a)] 6ATP

(2) Oxidative decarboxylation of pyruvic acid

Pyruvic acid to acetyl CoA

(2NADH, each worth 3 ATP) : [(b)]2x3= 6 ATP

(3) Krebs Cycle

1. ATP produced in substrate level phosphorylation:

Succinyl CoA to Succinic acid : 2x1 = 2 ATP

2. ATP from NADH: Isocitric acid to Oxalosuccinic acid : 2x3 = 6 ATP

α-ketoglutaric acid to succinyl CoA : 2x3 = 6 ATP

Malic acid to Oxaloacetic acid : 2x3 = 6 ATP

3. ATP from $FADH_2$: Succinic acid to Fumaric acid : 2x2 = 4 ATP

ATP value of kreb's cycle : (C) 24 ATP

Net gain of ATP in aerobic respiration per mole glucose

(a+b+c) : 36 ATP

5.5 Amphibolic Pathway

Glucose is the most favoured substrate for respiration. All carbohydrates are usually first converted into glucose before they are used for respiration. Other substrates can also be respired, as has been mentioned earlier, but then they do not enter the respiratory pathway at the pathway at the first step. The points of entry of different substrates in the respiratory pathway are given in Figure 5.7. Fats would need to be broken down into glycerol and fatty acids first. If fatty acids were to be respired they would first be degraded to acetyl CoA and enter the pathway. Glycerol would enter the pathway after being converted to PGAL. The proteins would be degraded by proteases and the individual amino acid s (after deamination), depending on their structure, would be enter the pathway at some stage within the Kreb's cycle or even as pyruvate or acetyl Co A.

Since respiration involves the breakdown of substrates, the respiratory process has traditionally been considered a catabolic process and the respiratory pathway as a catabolic pathway. But is this understanding correct? We have discussed above, at which points in the respiratory pathway different substrates would enter if they were to be respired and used to derive energy. What is important to recognise is that it is these very compounds that would be withdrawn from the respiratory pathway for the synthesis of the said substrates.

Hence, fatty acids would be broken down to acetyl Co A before entering the respiratory pathway when it is used as a substrate. But when the organism needs to synthesise fatty acids, acetyl Co A would be withdrawn from the respiratory pathway for it. Hence, the respiratory pathways comes into the picture both during the breakdown and the synthesis of protein too, respiratory intermediates from the link. The breaking down process within the living organism constitute catabolism, while synthesis is anabolism. Because the respiratory pathway is involved in both anabolism and catabolism, it would be better to a catabolic one.

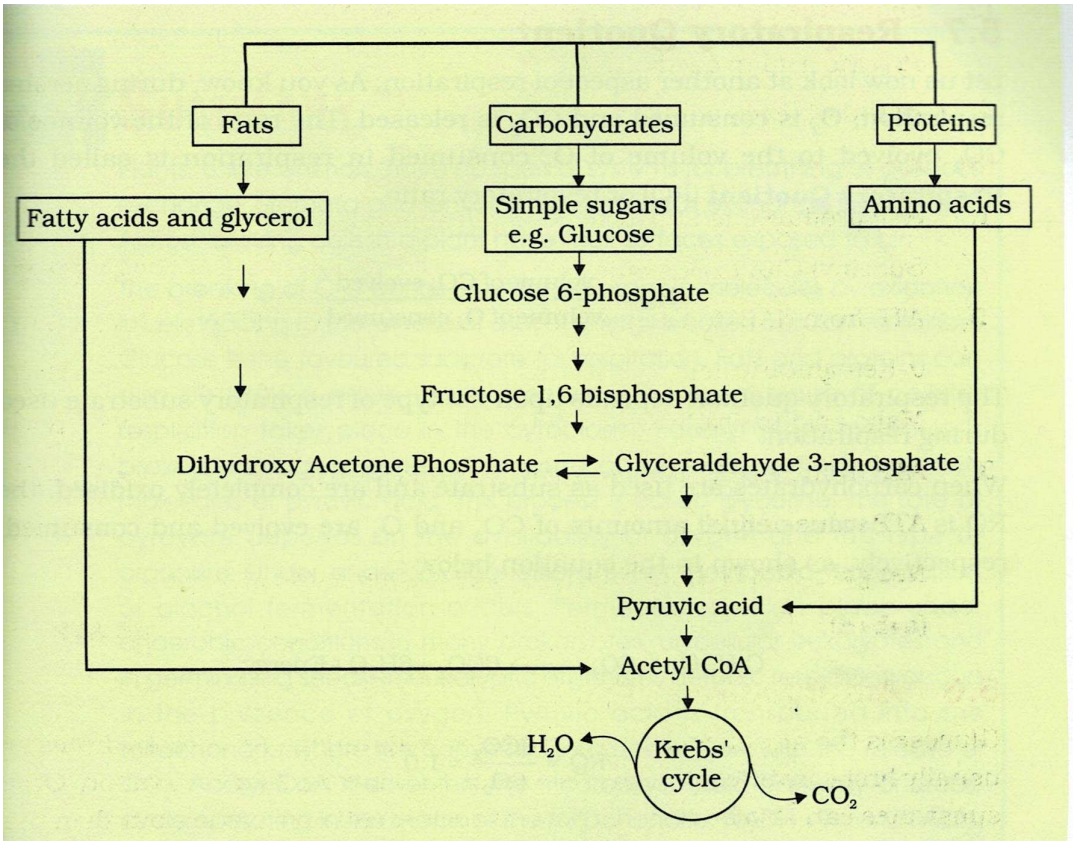


Figure 5.7 Interrelationship among metabolic pathways showing respiration mediated breakdown of different organic molecules to CO_2 and H_2O

5.6 Respiratory Quotient

Let us now look at another aspect of respiration. As you know, during aerobic respiration, O_2 is consumed and CO_2 is released. The ratio of the volume of CO_2 evolved to the volume of O_2 consumed in respiration is called the **Respiratory Quotient** (RQ) or respiratory Ratio.

$$RQ = \frac{\text{volume of } CO_2 \text{ evolved}}{\text{volume of } O_2 \text{ consumed}}$$

The respiratory quotient depends upon the type of respiratory substrate used during respiration.

When Carbohydrates are used as Respiratory substrates and are completely oxidised , the RQ is 1, because equal amounts of CO_2 and O_2 are evolved and consumed , respectively.

When proteins are respiratory substrates the ratio would be about 0.9.

When fats are used in respiration, the RQ is less than 1.

What is the important to recognise is that in living organisms respiratory substrates are often more than one; pure proteins or fats are never used as respiratory substrates.

SUMMARY

Plants unlike animals have no special systems for breathing or gaseous exchange. Stomata and lenticels allow gaseous exchange by diffusion. Almost all living cells in a plant have their surface exposed to air.

The breaking of C-C bonds of complex organic molecules by oxidation in cells leading to the release of a lot of energy is called cellular respiration. Glucose is the favoured substrate for respiration. Fats and proteins can also be broken down to yield energy. The initial stage of cellular respiration takes place in the cytoplasm. Each glucose molecule is broken, through a series of enzyme catalysed reactions, into two molecules of pyruvic acid. This process is called glycolysis. The fate pyruvate depends on the availability of oxygen and the type of organism. Under anaerobic conditions either lactic acid fermentation or alcoholic fermentation occurs. Fermentation takes place under anaerobic conditions in many prokaryotes, unicellular eukaryotes and in germinating seeds. In eukaryotic organisms aerobic respiration occurs in the presence of oxygen. Pyruvic acid is transported into the mitochondria where it is converted into acetyl CoA with the release of CO_2, Acetyl CoA then enters the tricarboxylic acid pathway or Kreb's cycle operating in the matrix of the mitochondria. NADH+ H^+ and $FADH_2$ are generated in kreb's cycle. The energy in these molecules as well as that in the NADH + H^+ synthesised during glycolysis are used to synthesise ATP. This is accomplished through a system of electron carriers called electron transport system (ETS) located on the inner membrane of the mitochondria. The electrons, as they move through the system, release enough energy that is trapped to synthesise ATP. This is called Oxidative phosphorylation. In this process, O2 is the ultimate acceptor of electrons and it gets reduced to water.

The respiratory pathway is an amphibolic pathway as it involves both anabolism and catabolism. The respiratory quotient depends upon the type of respiratory substances used during respiration.

GLOSSARY

ATP: Adenosine triphosphate, known as energy currency of the cell. On hydrolysis one molecule of ATP yields 7.6 K. Cal of energy.

Cellular respiration: Breakdown of food materials within a cell to release energy as ATP.

Aerobic respiration: Respiration taking place in the presence of oxygen.

Fermentation: Enzymatic conversion of sugars to usually, ethyl alcohol by microbes/ microbial enzymes under anaerobic conditions.

Glycolysis: A cytosolic process in which glucose is broken down to two molecules of pyruvic acid.

Kreb's cycle: a cycle of reactions which oxidises acetyl CoA. Also called citric acid cycle or TCA cycle.

Oxidative Phosphorylation: Synthesis of ATP from ADP and pi coupled to electron transport from substrate to molecular oxygen.

Respiratory Quotient: Ratio of the volume of CO_2 liberated to the volume of O_2 absorbed during respiration.

QUESTIONS

Very short Answer Type Questions

1. Explain the economic importance of fermentation.

2 What is the common pathway for aerobic and anaerobic respirations?

3. Which substance is known as the connecting link between glycolysis and kreb's cycle.

4. Define Respiratory Quotient.

5 what is the specific role of F_o-F_1 particles in respiration?

Short Answer Type Questions

1. Pyruvic acid is the end product of glycolysis. What are the three metabolic fates of pyruvic acid under aerobic and anaerobic conditions.

2. Why is the respiratory pathway referred to as an amphibolic path way? Explain.

3. Do you know of any step in kreb's cycle where there is a substrate level phosphorylation? Expalin.

EXERCISES

1. What is oxidative phosphorylation?

2. Define RQ. What is its value for fats?

3. In a way green plants and cynobacteria have synthesised all food on earth. Comment.

Chapter 6

Plant growth and development

You have already studied the organisation of a flowering plant in the first year Intermediate course. Have you ever thought about how structures like roots, stems, leaves, flowers, fruits and seeds arise and that too in an orderly sequence? You are, by now, aware of terms like seed, seedling, plantlet and mature plant. You have also seen that trees continue to increase in height and girth over a period of time. However, the leaves, flowers and fruits of the same tree not only have limited dimensions but also appear and fall periodically and sometime repeatedly. Why does the vegetative phase precede flowering in a plant? All plant organs are made up of a variety of tissues; is there any relationship between the structure and the function of these be altered? All cells of a plant are descendents of the zygote. The question is, then, how do cells have different structural and functional attributes? Development is the sum of two processes: growth and differentiation. To begin with, it is essential to know that the development of a mature plant from a zygote (fertilized egg) follow a precise and highly ordered succession of events. During this process a complex body organisation (Figure 6.1) is formed whereby roots, leaves, branches, flowers, fruits and seeds are produced and eventually die (Figure 6.1). In this chapter, you will also study some of the factors which govern and control these developmental processes. These factors are both intrinsic (internal) and extrinsic (external) to the plant.

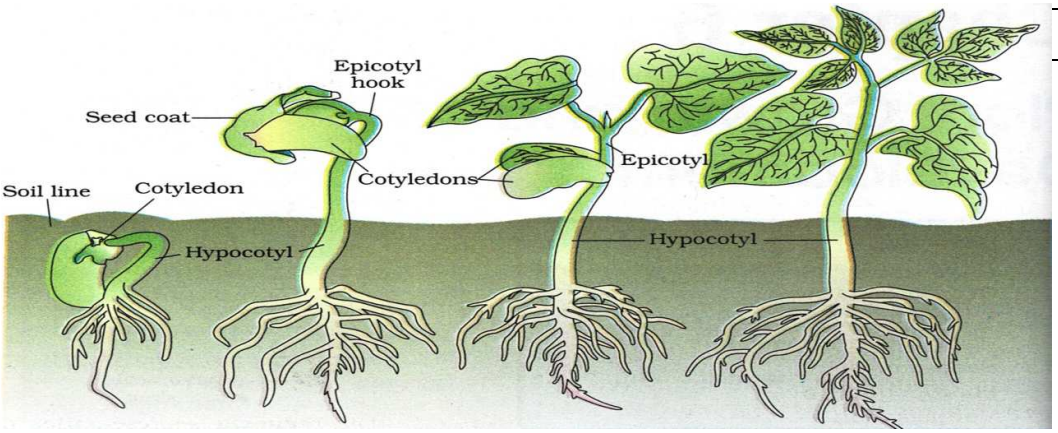


Figure 6.1 Germination of seedling development in bean

6.1 Growth

Growth is regarded as one of the most fundamental and conspicuous characteristics of a living being. What is growth? Growth can be defined as an irreversible permanent increase n size of an organism or its parts or even of an individual cell. Generally, growth is accomplished by metabolic processes (both anabolic and catabolic), that occur at the expense of energy. For example, expansion of a leaf is growth. How would you describe the swelling of piece of wood when placed in water?

6.1.1 Phases of Growth

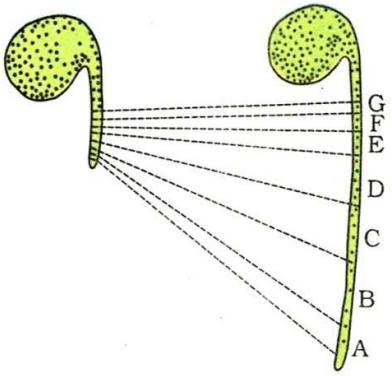


Figure 6.2 Detection of zones of elongation by the parallel line technique. Zones A, B, C, D immediately behind the apex have elongated most.

The period of growth is generally divided into three phases, namely meristamatic, elongation and maturation (Figure 6.2). Let us understand this looking at the root tips. The constantly dividing cells, both at the root apex and the shoot apex, represent the meristematic phase of growth. The cells in this region are rich in protoplasm and possess large conspicuous nuclei. Their cell walls are primary in nature, thin and cellulosic with abundant plasmodesmatal connections. The cells proximal (just next, away from the tip) to the meristematic zone represent the phase of elongation. Increased vacuolation, cell enlargement and new cell wall deposition are the characteristics of the cells in this phase. Further away from the apex, i.e., more proximal to the phase of elongation, lies the portion of axis which is undergoing the phase of maturation. The cells of this zone attain their maximal size in terms of wall thickening and protoplasmic modifications. Most of the tissues

and cell types you have studied in chapter 12 of First year Intermediate course represent this phase.

6.1.2 Growth rates

The increased growth per unit time is termed as growth rate. Thus, rate of growth can be expressed mathematically. An organism, or a part of the organism, can produce more cells in a variety of ways.

Growth curve is obtained by plotting growth rate of a plant against time. Growth curve resembles the shape of 'S' and so it is named as sigmoid curve (Figure 6.3).

The growth curve consists of three phases of growth. They are:

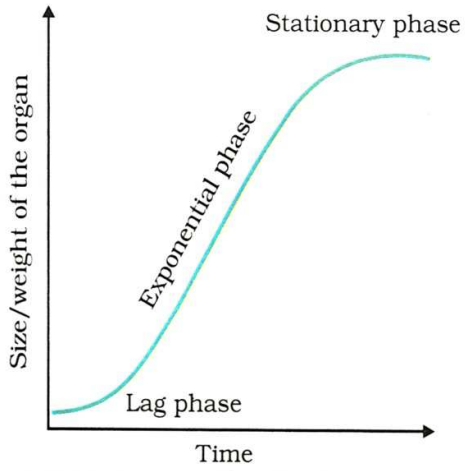


Figure 6.3 An idealised sigmoid growth curve typical of cells in culture, and many higher plants and plant organs

(i) Lag phase :- This is the first phase of growth. In this, period, growth is slow but steady. Since new cells are from meristematic zones, this phase is also called formative phase.

(ii) Log phase :- This is the second phase of growth. This is a period of maximum growth. In this phase, plant shows rapid increase in its height. It is called logarithemic phase orexponential phase.

(iii) Senescent phase:- This is the third phase during which plant exhibits declining growth activity. This phase corresponds to maturity of cells. The log phase is followed by senescent phase.

The total period of time during which all the three phases of growth occur is called grand period of growth. Hence growth curve is also called as grand period curve.

6.2 Plant growth regulators

Plant growth regulators (PGRS) are small, simple molecules of diverse chemical composition. They could be indole compounds (indole-3-acetic acid, IAA); adenine derivatives (N6-furfurylamino purine, kinetin), derivatives of carotinoids (abscisic acid, ABA); terpenes (gibberellic acid, GA3) or gases (ethylene, C2H4). Plant growth regulators are variously described as plant growth substances, plant hormones or phytohormones in literature.

PGRs can be broadly divided into two groups based n their functions in a living plant body. One group of PGR is involved in growth promoting activities, such as cell division, cell

enlargement, pattern formation, tropic growth, flowering, fruiting and seed formation. These PGRs are also called plant growth promoters, e.g., auxins, gibberellins and cytokinins. The PGRs of the other group play an important role in plant responses to wounds and stresses of biotic and a biotic origin. They are also involved in various growth inhibiting activities such as dormancy and abscission. The PGR, absciscic acid belongs to this group. The gaseous PGR, ethylene, could fit in either of the groups, but it is largely an inhibitor of growth activities.

6.2.1 The Discovery of Plant Growth Regulators

Interestingly, the discovery of each of the five major groups of PGRs has been accidental. It all started with the observation of Charles Darwin and his son Francis Darwin, that the coleoptiles of canary grass responded to unilateral illumination by growing towards the light source(phototropism). After a series of experiments, it was concluded that the bending of the entire coleoptiles (Figure 6.4). Auxin was isolated by F.W Went from the tips of coleoptiles of oat seedlings. The 'bakane' (foolish seedling) disease of rice seedlings is caused by a fungal pathogen Gibberella fusikuroi. E.Kurasawa reported the appearance of symptoms of the disease in uninfected rice seedlings when they were treated with sterile filtrates of the fungus. The active substances were late identified as gibberellic acid.

F.Skoog and his co-workers observed that from the intermodal segments of tobacco stems the callus (a mass of undifferentiated cells) proliferated only if, the following: extracts of vascular tissues, yeast extract, coconut milk or DNA. Skoog and Miller later identified and crystallised the cytokinesis promoting active substance that they termed kinetin.

During the mid-1960s, three independent researches reported the purification and chemical characterisation of three different kinds of inhibitors: inhibitor-B, Absission II and dormin. Later all the three were proved to be chemically identical. It was named abscisic acid (ABA).

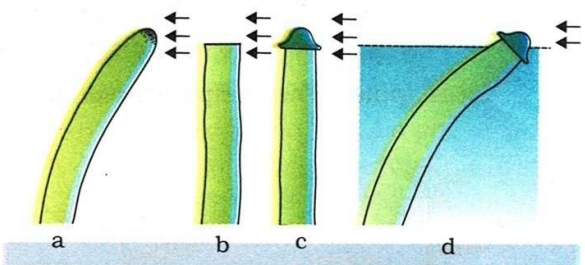


Figure 6 4 Experiment used to demonstrate that the tip of the coleoptile is the source of auxin. Arrows indicate the direction of light

Experiment used to demonstrate that the tip of the coleoptiles is the source of auxin.

Cousins confirmed the release of volatile substance from ripened oranges that hastened the ripening of stored unripened bananas. Later this volatile substance was identified as ethylene, a gaseous PGR. Let us study some of the physiological effects of these five categories of PGRs in the following section.

6.2.2 Physiological Effects of Plant Growth Regulators

6.2.2.1 Auxins

Auxins (from Greek 'auxein': to grow) was first isolated from human urine. The term 'auxin' is applied to indole-3-acetic acid (IAA),and to other natural and synthetic compounds having certain growth regulating properties. Auxins are generally produced by the growing apices of the stems and roots, from where they migrate to the regions of their action. Auxins like IAA and indole butyric acid (IBA) have been isolated from plants. NAA (Naphthaleneacetic acid) and 2, 4-D (2,4-dichlorophenoxy acetic acid) are synthetic auxins. All these auxins have been used extensively in agricultural and horticultural practices.

Physiological Effects of Auxins

Auxins are characterised by their capacity to stimulate

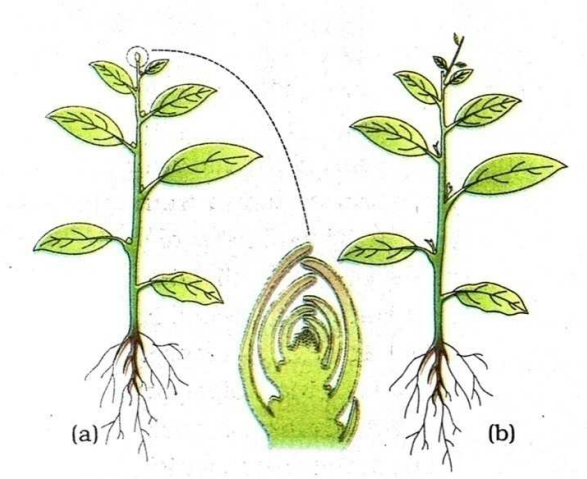


Figure 6 5 Apical dominance in plants : (a) A plant with apical bud intact (b) A plant with apical bud removed Note the growth of lateral buds into branches after decapitation.

1. Auxins helps to initiate rooting in stem cuttings, an application widely used for plant propagation in horticulture.

2. Auxins promote flowering e.g. in pineapples.

4. They also prevent fruit and leaf drop at early stages but promote the abscission of older mature leaves and fruits.

4. In most higher plants, the growing apical bud inhibits the growth of lateral (axillary) buds, a phenomenon called apical dominance. Removal of shoot tips (decapitation) usually results in the growth of lateral buds (Figure 6. 5). This is widely applied to tea plantations and hedge-making. Can you explain why?

5. Auxins also induce parthenocarpy, e.g., in tomatoes.

6. They widely used as herbicides. 2,4-D, widely used to kill dicotyledonous weeds. Does not affect mature monocotyledonous weeds.

6.2.2.2 Gibberellins

Gibberellins are another kind of promotery PGR. There are more than 100 gibberellins reported from widely different organisms such as fungi and higher plants. They are denoted as GA1, GA2, GA3 and so on. However, gibberellins to be discovered and remains the most

intensively studied form. All GAs are acidic. They produce a wide range of physiological responses in plants.

1. Their ability to cause an increase in the length of axis is used to increase the length of grapes stalks.

2. Gibberellins cause fruits like apple to elongate and improve their shape.

3. They also delay senescence. Thus, fruits can be left on the tree longer so as to extend the market period.

4. GA3 is used to speed up the malting process in brewing process.

5. Spraying sugarcane crop with gibberellins increase the length of the stem, thus increasing the yield by as much as 20 tonnes per acre.

6. Gibberellins also promote bolting (internode elongation just prior to flowering) in beet, cabbages and many plants with rosette habit.

6.2.2.3. Cytokinins

Cytokinins have specific effects on cytokinesis, and were discovered as kinetin (a modified form of adenine, a purine) from the autoclaved herring sperm DNA. Kinetin does not occur naturally in plants. The search for natural substances with cytokinin-like activities to the isolation of zeatin from corn-kernels and coconut milk. Since the discovery of zeatin, several naturally occurring cytokinins, and some synthetic compounds with cell division promoting activity have been identified. Natural cytokinins are synthesized in regions where rapid cell division occurs. For, example root apices, developing leaves, lateral shoot growth and adventitious shoot formation. Cytiokinins help to overcome the apical dominance. They promote nutrient mobilisation which helps in the delay of leaf senescence.

6.2.2.4 Ethylene

Ethylene is a simple gaseous PGR. It is synthesized in large amounts by tissues undergoing senescence and ripening fruits. Effects of ethylene on plants include horizontal growth of seedlings, swelling of the axis and apical hook formation in dicot seedlings. Ethylene promotes senescence and abscission of plant organs, especially of leaves and flowers. Ethylene is highly effective in fruit ripening. It enhances the rate of respiration is called respiratory climactic. Ethylene breaks seed and bud dormancy, initiates germination in peanut seeds and sprouting of potato tubers. Ethylene is used to initiate flowering and for synchronising fruit-set in pineapples.

6.2.2.5 Abscisic acid

As mentioned earlier, abscisic acid (ABA) was discovered for its role in regulating abscission and dormancy. But like other PGRs, it is also has other wide ranging effects on plant growth and development. It acts as a general plant growth inhibitor and an inhibitor of plant metabolism. ABA inhibits seed germination. ABA stimulates the closure of stomata in the epidermis and increase the tolerance of plants to various kinds of stresses. Therefore, it is called the stress hormone. ABA plays an important role in seed development, maturation and dormancy. By inducing dormancy, ABA helps seeds to withstand desiccation and other factors unfavourable for growth. In most situations, ABA acts as an antagonist to GAs.

6.3 Seed Dormancy

Germination of seeds can be defined as the process by which the seedling comes out from the seed. A seed may remain viable but unable to germinate or grow for several reasons. These can be classified into external and internal conditions. An internal situation that is easy to understand is an embryo that has not reached morphological maturity capable of germination (e.g. Ranunculus). Only time will allow this maturity to develop of seeds of wild plants is often limited in this or some other internal way, but seeds of many domestic plants may be limited only by lack of moisture or worm temperatures. To distinguish between these two different situations, seed physiologists have used two terms: Quiescence is the condition of a seed when it is unable to germinate only because of favourable external conditions normally required for growth are not present and dormancy is the condition of a seed when it fails to germinate because of internal conditions, even though external conditions (e.g., temperature, moisture and atmosphere) are suitable. The dormancy of seeds may be caused by hard seed coats that prevent uptake of oxygen or water (e.g., Fabaceae). Such type of dormancy caused by hard seed coats can be broken by scarification, a method by which the hard seed coat ruptured or weakened. Seeds of certain plants (e.g., tomato) contain chemical compounds which inhibit their germination. Many seeds (e.g., ploygonum) will not germinate until they have been exposed to low temperatures moist conditions in the presence of oxygen for weeks to months. Rarely, moist seeds respond to high temperatures and several seeds respond best when daily temperatures alternate between high and low. The practice of layering the seeds during winter in layers of moist sand and peat is called stratification or prechilling.

6.4 Photoperiodism

It has been observed that some plants require periodic exposure to light to induce flowering. It is also seen that such plants are able to measure the duration of exposure to light. For, example, some plants require the exposure to light for a period exceeding a well defined critical duration. While others must be exposed to light for a period exposure less than this critical duration before flowering is initiated in them. The former group of plants are called long day plants while the later ones are termed short day plants. The critical

duration is different for different plants. There are many plants, however, where there is no such correlation between exposure to light duration and induction of flowering responses and such plants are called day-neutral plants (figure 6.6). It is now known that only the duration of light period but the duration of dark period also is of equal importance. Hence, it can be said that flowering in certain plants depends not only on a combination of light and dark exposures but also their native durations. This response of plants to periods of day/night is termed photoperiodism. It is also interesting to note that while shoot apices modify themselves into flowering apices prior to flowering, they (i.e., shoot apices of plants) by themselves cannot perceive photoperiods. The site of perception of light /dark duration is the leaves. It has been hypothesised that there is a hormonal substance(s) that is responsible for flowering. This hormonal substance migrates from leaves to shoot apices for inducing flowering only when the plants are exposed to the necessary inductive photoperiod.

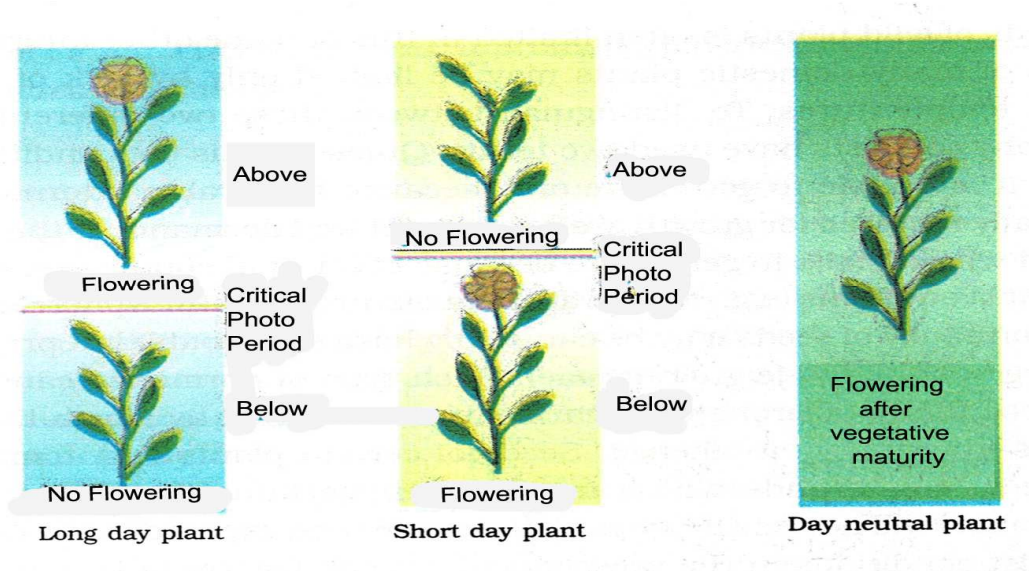


Figure 6.6 Photoperiodism : Long day, short day and day neutral plants

SUMMARY

Growth is one of the most conspicuous events in any living organism. It is an irreversible increase expressed in parameters such as size, area, length, height, volume, cell number etc. It conspicuously involves increased protoplasmic material, in plants, meristems are the sites of growth. Root and shoot apical meristems sometimes along with intercalary meristems, contribute to the longitudinal growth of plant axes. Growth is indeterminate in higher plants. Following cell division in root and shoot apical meristem cells, growth can be arithmetic or geometrical. Growth may not be generally is not sustained of a high rate

throughout the life of cell/tissue/organ/organism. One can define three principle phases of growth- the lag, the log and the senescent phase.

Plant growth and development are under the control of both intrinsic and extrinsic factors. Intercellular intrinsic factors are the chemical substances, called plant growth regulators (PGR). There are diverse groups of PGRs in plants. They belonging to five main groups: auxins, gibberellins, cytokinnins, abscisic acid and ethylene. These PGRs are synthesised in various parts of the plant. PGRs have diverse physiological effects on plants. Diverse PGRs also manifest similar effects. PGRs may act synergistically or antognistically. Plant growth and development is also affected by light, temperature, nutrition, oxygen status, gravity and other such external factors.

Flowering in some plants is induced only when the plant is exposed to a certain duration of photoperiod. Depending on the nature of photoperiod requirements, plants are called short day plants, long day plants and day-neutral plants.

Glossary

Apical Dominance: It is the phenomenon in which a growing apical bud inhibits the growth of lateral (Axillary) buds. Auxin synthesized in apical meristem is responsible for apical dominance.

Bolting: It is the sudden elongation of internodes which is followed by flowering.

Ethephon: It is an ethylene releasing chemical formulation.

Growth: It is an irreversible, permanent increase in size of an organism or its parts or even of an individual cell.

Phototperiodism: The flowering response of plants to periods of day/ night is termed photoperiodism.

Respiratory climatic: The rise in the rate of respiration during the ripening of the fruits is known as respiratory climatic. Ethylene is responsible for it.

Sigmoid curve: 'S' shaped growth curve obtained by plotting the parameter of growth against time is called sigmoid or 'S'- curve.

QUESTIONS

Very Short Answer Type Questions

1. Define respiratory Quotient.

2. What is apical dominance?

3. What is meant by bolting?

4. What is ethephon?

5. What is Respiratory climatic.

6. Which of the PGRs is called stress hormone?

7. Define the terms quiescence and dormancy.

Short Answer Type Questions

1. Write a notes on agricultural/ horticultural applications of auxins.

2. Write the physiological responses of gibberellins in plants.

3. Write any four physiological effects of cytokinins in plants.

4. What are the physiological processes that are regulated by ethylene in plants?

EXCERSISES

1. Life plays an important role in the life of all organisms. Name any three physiological processes in plants which are influenced by light.

2. Would a defoliated plant respond to photoperiodic cycle? Why?

UNIT II

MICROBIOLOGY

Chapter 7 : Bacteria

Chapter 8 : Viruses

Microbiology (Greek, micros (small, bios (=life), logos (=study) is a branch of biology that deals with the scientific study of microorganisms, (simply, microbes) that are to small to be seen with the naked eye and is concerned with the structure, function, classification and ways of controlling and using their activities.

As already introduced to your first year book, microbes includes group of simple life forms like a protozoa, microscopic Algae and Fungi (Yeasts and moulds), Bacteria and viruses. These are omnipresent in vast numbers and are described as **ubiquitous**. You will study more about bacteria and viruses in the next two chapters.

The work done by **Leeuwenhoek** (1632-1723) and later in the 19th century by **Louis Pasteur** (1822-1893) and **Robert Koch** (1843-1910) formed the foundation for this subject. **Louis Pasteur** developed the techniques of pasteurization and preparation of vaccines.

Microorganisms play a prime role in every field of human endeavour. They affect human life in several ways.

Chapter 7

Bacteria

Bacteria are most abundant organisms on Earth. The credit for observation and description of Bacteria goes to Anton van Leeuwenhoek (1674). Later Ehrenberg (1829) coined the term Bacteria for these microscopic creatures. In 1850s Louis Pasteur's work showed that bacteria are chemical factories capable of bringing about significant changes in nature. He did extensive research work on bacteria and regarded as the father of bacteriology. In 1870s Koch's experiments established their link to infectious disease – the germ theory of diseases. Due to the efforts of several enthusiastic scientists in the last two centuries to understand the bacterial world, the study of bacteria is now recognised as Bacteriology- a new branch of biology.

Can u recall the position of bacteria in Whittaker's five kingdom classification?

Bacteria are found everywhere. They are found in soil, water, air and on or

inside living organisms. They also occur in a variety of foods. They can withstand extreme cold, heat and drought conditions. They are thus met within much unexpected media-like arctic snow, volcanic ash, hot water and sulphur springs etc. In terms of sheer numbers they greatly exceed every other group of organisms on the plane. Some bacteria are deadly parasites of plants, animals and human beings. Some bacteria make mutually beneficial symbiotic association with plants. Escherichia coli (E. coli) is an common inhabitant of human intestine.

can you recollect and name any such symbiotic bacterium and name the special structures in which it is accommodated on roots of plants?

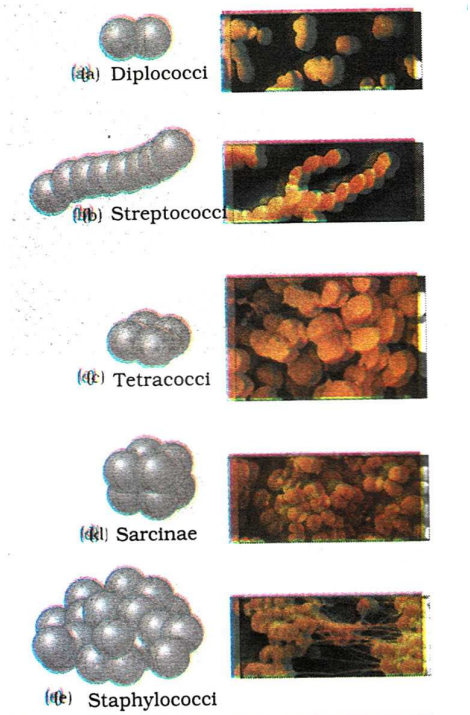


Figure 7.1 Arrangements in Coccal Forms of Bacteria

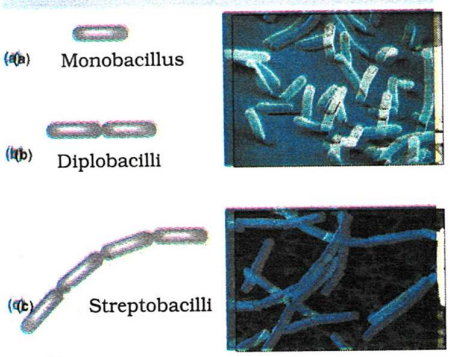


Figure 7.2 Arrangements of Bacillus forms

7.1 Morphology of Bacteria

7.1.1 Size

Bacteria are very small organisms which are barely visible under the light microscope. Their size varies according to the species .Majority of the bacteria are in the range 2.0to 5.0um in length and 0.5 to 1.0um in breadth.

7.1.2. Shape

Bacteria vary in their shape which is usually constant for each species. According to their shape, the bacteria are differentiated into the following types :

1. Cocci (singular: Coccus): Spherical shaped bacteria are called Cocci. Based on the number and arrangement of cells, cocci are divided into six types as follows :

a. Monococcus : A single spherical bacterium.

b. Dipllococcus : A pair of spherical bacteria.

c.Tetracoccus : A group of four spherical bacteria.

d. Streptococcus : A chain of spherical bacteria arranged in a single row.

e. Staphylococcus : A group of cocci bacteria forming irregular shapes.

f. Sarcinae : Cocci arranged in cubes of eight.

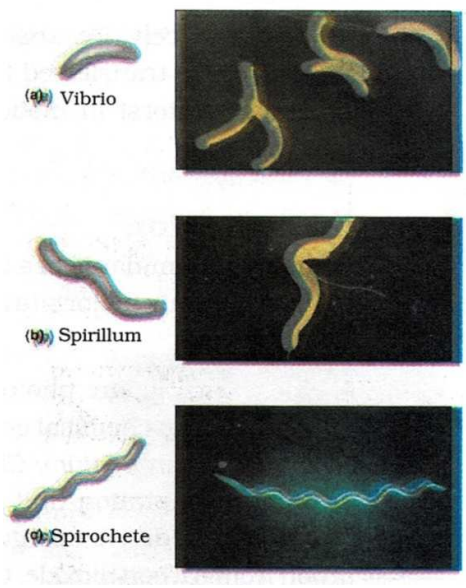


Figure 7.3 Spiral forms of bacteria

2. Bacilli (singular: Bacillus) : Rod shaped bacteria are called 'bacilli'. The bacilli are of three types.

a. Monobacillus: A single rod shaped bacterium.

b. Diplobacillus: Rod shaped bacteria arranged in pairs.

c. Streptobacillus : A chain of rod shaped bacteria.

3. Vibrio (Singular : Vibrio) : Comma shaped bacteria are called ' Vibrios'.

4. Sprilla (singular: Spirillium): Spiral Shaped bacteria are called 'spirilla'.

A few bacteria are known to change their shape depending upon the type of environment and nutrients available. Such bacteria are known as 'Pleomorphic bacteria'. This phenomenon is called 'pleomorphism'. E.g: Acetoacter.

7.2 Bacterial cell structure

With the development of the electron microscope in the 1940s, bacteria revealed themselves as more than simple spheres and rods. Scientists uncovered a wealth of **microscopic** and sub- microscopic details of bacteria showed how very minute bacteria can be as complex as very large visible organisms. Recollect the prokaryotic cell structure you have already studied in your first year. However we will discuss two variant components of bacteria cell here. These are :

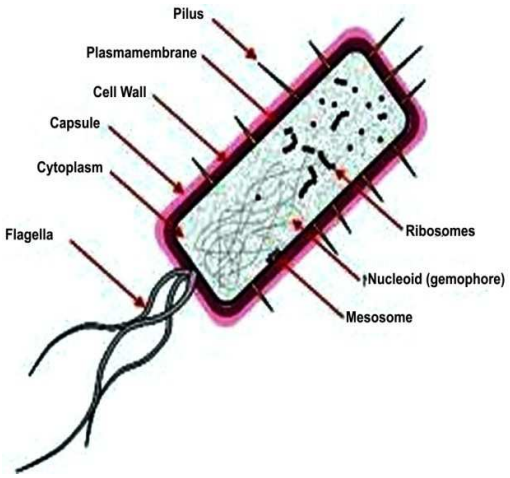


Figure 7.4 Cell structure of Bacteria

7.2.1 Flagella

Some species of bacteria are endowed with flagella and show rapid wavelike movement. One advantage of motility is that it enables a bacterium to move toward a favourable environment or away from an adverse one.

Bacteria cells have four arrangements of flagella: **Monotrichous** (a single polar flagellum), **amphitrichous** (a single flagellum at each end of the cell), **lophotrichous** (two or more flagella at one pole of the cell), and **peritrichous** (flagella distributed over the entire cell).

7.2.2 Plasmids

In addition to the "bacterial chromosome", or **genophore** which is the main genetic material, bacteria often contain small circular ,double stranded DNA molecule called **Plasmids.** Plasmids usually contain fewer genes than the genophore and often confer protective traits such as resistance to drugs and production of toxins and enzymes. They may be gained or loss without harming the bacterial cell. Because plasmids can be readly manipulated in the laboratory and transferred from one bacterial cell to another, these are used as agents (vectors)in modern genetic engineering technique (described in unit v).

7.3 Nutrition

The main determinants of a bacterial nutritional type are its sources of carbon and energy. Four major nutritional groups of bacteria are described following this criterion.

1. **Photoautotroph's**: These bacteria like green plants possess the chlorophyll and obtain the energy from sunlight and carbon from atmospheric carbon dioxide. E.g: Purple sulphur bacteria – Chromatium
   Green sulphur bacteria – Chlorobium.
2. **Photoheterotrophs**: These bacteria obtain energy from sunlight but carbon is derived from organic sources. E.g: purple non- sulphur bacteria- Rhodospirillum, Rhodomicrobium.
3. **Chemoautotrophs**: They have an unusual nutritional adaptation that requires neither sunlight nor organic nutrients. These bacteria derive energy from oxidation of inorganic substances and carbon from carbon dioxide. These bacteria such as Nitrosomonas, Nitrobacter, Baggiotoa and Methanogens play an important role in recycling inorganic nutrients.
4. **Chemoheterotrophs** : This group of bacteria derive both carbon and energy from organic compounds. Processing these organic molecules by respiration or fermentation releases energy in the form of ATP. They are categorized into two groups based on how they obtain their organic nutrients. **Saprophytes** are free living microorganisms that fed primarily on organic detritus from dead organisms (E.g: Bacillus Sps). **Parasites** derive nutrients from the cells or tissues of a host (E.g: Xanthomonas, Salmonella).

Table 7.1 Nutritional classification of bacteria

Type	Energy source	Carbon source
1.photoautotrophs	Light	Co_2
2.Photoheterotrophs	Light	Organic compounds
3.Chemoautotrophs	Electrons from inorganic compounds	Co2
4.Chemoheterotrophs	Electrons from organic compounds	Organic compounds

Bdellovibrio bacteriovorous grows as a parasite on some harmful bacteria, and their abundance is supposed to be responsible for the microbial purity of Ganges waters.

7.4 Reproduction

Do you remember that we studied in first year that Bacteria reproduces commonly by a kind of cell division called binary fission? In this process, the dividing bacteria cell produces two genetically identical daughter cells. The interval time between successive binary fissions of a cell is known as generation time (or doubling time) which may be as little as 20 minutes in some bacteria.

7.4.1 Sexual Reproduction

True sexual reproduction is absent in bacteria. However, the exchange of genetic material (genetic recombination) which is the sexual reproduction takes place in three ways. They are :

7.4.1.1 Conjugation

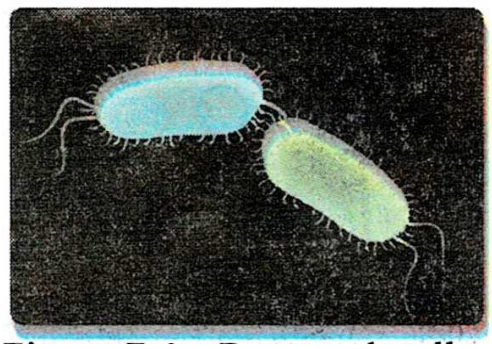


Figure 7.5 Bacterial cells in conjugation.

The transfer of genetic material through direct cell to cell contact is known as conjugation. It was first reported by **Lederberg and Tatum (**1946) in *Escherichia coli.*

In some of the E.coli cells in addition to the DNA of the Nucleoid, another DNA strand occurs in the cytoplasm. This is called plasmid (F plasmid) or F factor. E. coli cells having F plasmid are called **F+** cells or **donor** cells and the cells without F plasmid are called **F-** cells or **acceptor Cells**.

F+ cells have sex pili. During conjugation the F+ and F- cells come close together, contact physically and bind to each other with the help of sex pili. A conjugation tube is established between F+ and F- cells. The F plasmid in F+ cell replicates and forms a copy of it, which

moves into the F- cell through the conjugation tube. The F- cell becomes the F+ cell as it receives the F plasmid . Thereafter the two cells (conjugants) separate from each other.

7.4.1.2 Transformation

This mode of bacterial reproduction was discovered by **Frederick Griffith** (1928) in *Streptococcus pneumonia.* Transformation is the uptake of a naked DNA molecule of the fragments of a bacterial cell and the incorporation of this DNA molecule into the recipient chromosome in a heritable form.

7.4.1.3 Transduction

The transfer of genetic material from one bacterium to another through bacteriophase is known as transduction. It was discovered in 1951 by **Lederberg** and **Zinder** in *Salmonella typhimurium.-*

7.5 The importance of Bacteria to Humans

Bacteria are known to cause plant, animal and human diseases . yet many bacteria are directly or indirectly beneficial to humans. Hence, they are considered both as **'Friends and foes of man'.**

Some bacteria that cause human diseases are :

Clostridium tetani	Tetanus
Clostridium botulinum	Botulism
Vibrio cholera	Cholera
Salmonella typhi	Typhoid
Corynebacterium diphtheria	Diphtheria
Mycobacterium tuberculosis	Tuberculosis
Diplococcus pneumonia	Pneumonia
Mycobacterium leprae	Leprosy
Neisseria gonorrhoea	Gonorrhoea
Treponema pallidum	Syphilis

Bacteria also cause certain plant diseases like **Blight of rice** (Xanthomonas oryzae), **Citrus canker** (X.Oxonopodis pv. Citri) and **Crown gall** of apples and pear (Arobacterium tumifaciens).

Microbes are now used in extracting valuable metals like Uranium from rocks. The process is known as Bio-mining. The use of microbes in mining reduces the cost of production by more than 50%. DNA components from bacteria are used as **Biosensors** that can detect biologically active toxin pollutants. They also find application in medical diagnostics, food and fermentation operations. The most important development in Biotechnology depends on the possibility of altering the genetic makeup of bacteria through **genetic engineering** about which you will study in chapter 11 and 12. Some of the more common uses of bacteria are presented in detail in chapter 14.

Summary

Bacteria are an important group of microbes which are omnipresent. Though small, they are endowed with the ability to perform certain acts of living things. i.e., they take food, grow and reproduce. Some bacteria are deadly parasites while few form symbiotic associations with plants and animals and humans. The cell wall provides shape and the protection to bacteria. Bacteria occur in several shapes – cylindrical (Bacillus), Spherical (Cocci), Spiral (Spirillum). Bacilli and cocci also occur in a number of cellular arrangements. One or more flagella occur on many bacilli and provide for cellular motility. Saprophytic and parasitic bacteria have biomedical importance. Bacteria normally reproduce by binary fission. Bacterial plasmids can be manipulated in the laboratory and are used as vectors in r DNA technology. Genetic exchange among bacteria takes place by conjugation, transformation and transduction. Conjugation is genetic transfer through direct contact between two bacterial cells and is facilitated by conjugative plasmids. Some bacteria can take up DNA from their environment and incorporate it into their genome to undergo transformation. Transduction is mediated by bacteriophages.

Glossary

Antibiotic: An antimicrobial agent produced through naturally by a bacterium or fungus.

Germ theory of disease: The principle that microorganisms cause disease.

Pasteurization: The process of mild heating to kill particular spoilage microorganisms or pathogens.

Symbiosis: The living together of two different organisms or populations.

Vaccine: Is a biological preparation that improves immunity to a particular disease.

Questions

Very short answer type questions

1. Define Microbiology.
2. What are pleomorphic bacteria? Give an example.
3. What is sex pilus?
4. What is genophore?
5. What is Plasmid?
6. What is the significance of plasmid?
7. Name the Bacteria which is common inhabitant in human intestine.
8. What is conjugation?
9. In organism conjugation was discovered?
10. What is transformation?
11. In which organism transformation was discovered?
12. What is transduction?
13. Who discovered transduction? In which organism transduction was discovered?

Short Answer type Questions

1. How are bacteria classified on the basis of morphology?
2. How are bacteria classified on the basis of number of distribution of flagella?
3. Write briefly about Chemo heterotrophs and their significance.
4. Explain the conjugation in bacteria.

Excersises

1. Why Anton Van Leeuwenhoek named bacteria as animalcules after their discovery?

2. When root nodules of legumes are removed, which process may be affected?

3. A number of repeated divisions has taken place in a monococcus bacterium in the same plane. What type of Cocci result?

Chapter 8

Viruses

Viruses are a unique group of **" biological entities"** known to infect every type of cell, including those of bacteria ,algae, fungi, protozoa, plants and animals.

Remember that you have already gone through an introductory account of viruses in your first ear course.

Viruses are very different from other organisms. They are not composed of cells and can not be seen under a light microscope. A virus particle contains a single type of nucleic acid, either **DNA** or **RNA**. The nucleic acid is enclosed in a protein coat and the coat is sometimes covered in an envelope the life of lipids, proteins and carbohydrates. Viruses do not exhibit most of the life processes of a cell, but maintain genetic continuity through multiplication and undergo mutations. Thus, they are certainly more than inert lifeless molecules. Viruses are best described as **infectious particles.**

Viruses are **obligate intracellular parasites** i.e. they can not multiply unless they invade a specific host cell and instruct its genetic and metabolic machinery to make and release daughter or progeny viruses. In this process, they destroy the host cells causing serious damage and diseases in humans, plants and animals. The study of viruses is known as **Virology.**

8.1 Discovery

Viruses have been victimizing mankind from ancient time, causing many diseases in humans and also in economically useful plants and animals. As identifiable agent responsible for these diseases was not known even after the proposition of the **germ theory of disease.** **Edward jenner** while trying to understand the nature of small pox disease developed the vaccine without understanding the true nature of the infectious agent

involved. **Louis Pastuer (1865)** developed the vaccine for rabies. He called the disease agent as Virus (=poison).The actual credit of discovering the viruses goes to **Iwanowski (1892**). He demonstrated that the agents responsible for causing the mosaic disease of tobacco were " **filterable agents**", indicating the existence of causing agents are smaller than bacteria. **Martinus Beijerink** (1898) Established that the filterable infective agent was diffusible like a liquid. He called the infective agent a ' **Contagium vivm fluidum'** (=living infectious fluid).

W.M Stanley (1935) purified the sap and announced that the virus causing mosaic disease in tobacco could be crystallized. It was named Tobacco Mosaic Virus (TMV). **Fraenkel Conrat** (1956) confirmed that the genetic material of the TMV is **RNA**. Utilizing the technique of ultracentrifugation, X-ray crystallography and electron microscopy, a number of new viruses were reported and their ultrastructure was elucidated.

8.2 Classification of viruses

At present the **International Committee on Taxonomy of Viruses (ICTV)** regulates the norms of classification and nomenclature of Viruses. The ICTV scheme has only the three heirarchial levels – the Family names (including some sub-families), **Genus** and **Species.** The family name ends with the suffix 'viridae' while the genus names with the 'virus' and the species names are common in English expressions describing their nature. Viruses are named after the disease they cause (Polio virus).

8.3 Structure of Viruses

Viruses range in size from 300 nanometers (nm) as in TMV to 20 nm as in **parvoviruses**. Viruses may be classified into several different morphological types. Helical viruses resemble long rods that may be rigid or flexible like rabies virus and tobacco mosaic virus. Many animal, plant, bacterial and polio viruses are polyhedral (many-sided). The capsid of some viruses is covered by an envelope and such enveloped viruses roughly spherical. Influenza virus is an enveloped virus. Bcteriophases, the viruses which infect bacteria, have complicated structures are called complex viruses. They have 'polyhedral symmetry' in the 'head' and 'helical symmetry' in the tail sheath.

All viruses consists of two basic components: a core of nucleic acid that forms the genome and the surrounding coat of protein is known as capsid. The capsid gives shape to the virus and provides a protective covering for the genome. It is made up f protein subunits called capsomeres. The number of capsomeres is characteristic for each type of virus.

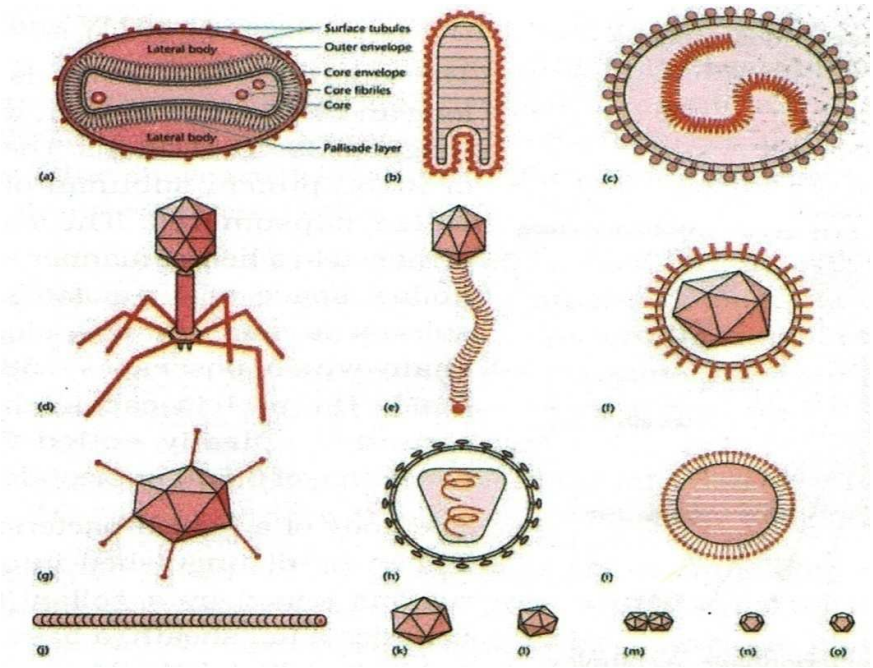


Figure 8.1 (a) Vaccinia virus (b) Rabies virus (c) MumpsVirus (d) Bacteriophage (e) Lambda Phage (f) Rubella virus (g) Adeno virus (h) HIV Virus (i) Influena Virus (j) TMV (k) Papiloma virus (j) Papova virus (m) Turnip Yellow Mosaic Virus (n) Wound tumor Virus

A virus contains its genetic information in their a double stranded (ds) DNA, or single stranded (ss)DNA, i.e., ds DNA or ssDNA. In general, viruses that infect plants have SSRNA and viruses that infect animals have ds DNA. Bacteriophages are usually ds DNA viruses. Viral nucleic acid molecules are either circular or linear. Most of viruses have a single nucleic acid molecule but a few have more than one (e.g. HIV- which has identical molecules of RNA representing its genomic copies.

Let us look at the detailed structure of two typical viruses – the TMV and T_4 phase.

The tobacco mosaic virus (TMV) to be crystallized first. Franklin et. al (1957) have described the structure of TMV. It is rod shaped virus. It is about 300 nm long and 18 or 19 nm in diameter with molecular weight of 39×10^6 daltons. The capsid is made up of 2,130 protein subunits of identical size. They are called capsomeres. Inside the protein capsid there is a single stranded RNA molecule which is also spirally coiled to form helix.

The body of a typical bacteriophase such as T_4 can be distinguished into head and tail regions joined by a collar. The head is hexagonal pyramid and it enclosing the folded double stranded DNA (ds DNA). The tail region includes a tail sheath, a base plate, 6 tail pins and 6 tail fibres. The tail sheath aids in injecting viral DNA into the host cell.

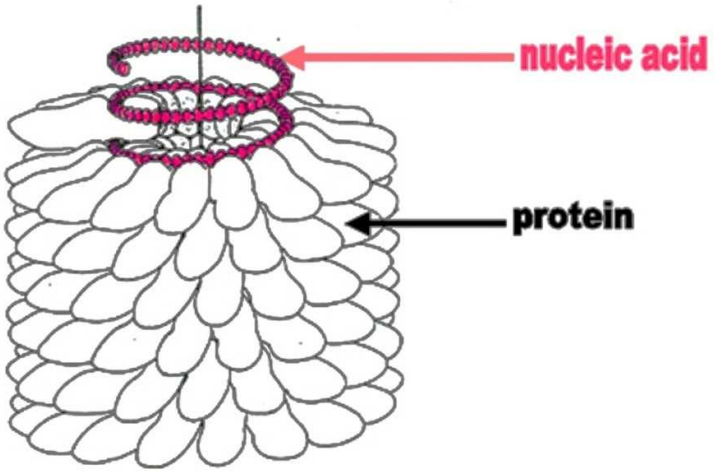
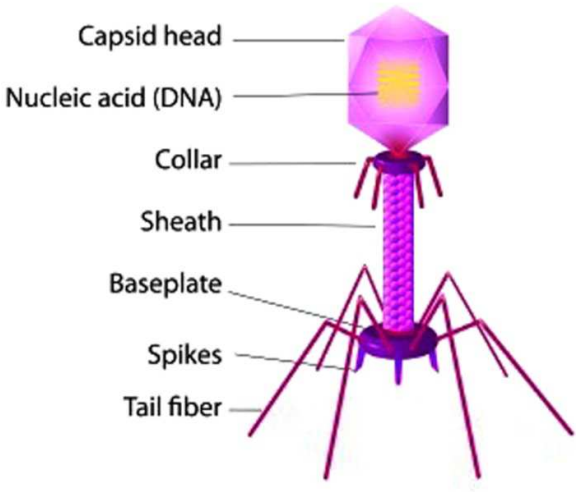


Figure 8.2 Tobacco Mosaic Virus –Helical Symmetry

8.4 Multiplication of Bacteriophages

For a virus to multiply or reproduce or replicate , it must invade a host cell and take over the host's metabolic machinery. Though the method by which a virus enters and exits a host cell may vary, the basic mechanism of viral multiplication is similar for all viruses. The best understood viral life cycles are those of the bacteriophases. Phages can multiply by two alternative mechanisms: the lytic cycle and he lysogenic cycle. The lytic cycle ends with the 'lysis' or death of the host cell, where as the host cells remains alive in the lysogenic cycle.



8.4.1 Lytic cycle

All the T- even phages (T2,T4,T6) exhibit lytic cycle and the phages are called virulent phages. This cycle can be differentiated into several phases, namely i. Adsoption ii. Penetration iii. Latent period iv. Lysis.

i. Adsorption : Attachment of the virus to the surface of host bacterium is called adsorption. The phages get attached to the surface of the host at specific sites with the help of tail fibres.

Figure 8.3 Bacteriophase – A complex Virus

ii. Penetration : After adsorption, the phages are able to release an enzyme called lysozyme which hydrolyses the bacterial cell wall. The injection of the phase nucleic acid into the host cell is known as penetration. The protein shell remains outside the host cell, which is reffered to as '**ghost'**. Soon after phase DNA injection , the host DNA degrades and the cell is forced to make viral constituents.

iii. Latent period: The first half of the latent period is called 'the eclipse' because the host cells do not contain any compelete infective viruses. During the second half of the latent period, called 'maturation period', the synthesis of complete new viruses occur. Usually coat proteins are synthesized first and the nucleic acid later.

iv. Lysis: At the end of latent period, the host cell wall ruptures or bursts and all the virions are released . This is known as 'lysis'. The cause of lysis is assumed to be due to the presence of 'lysozyme' enzyme, capable of dissolving the cell wall. The number of newly synthesized phase particles released from a single cell is reffered to as 'burst size'.

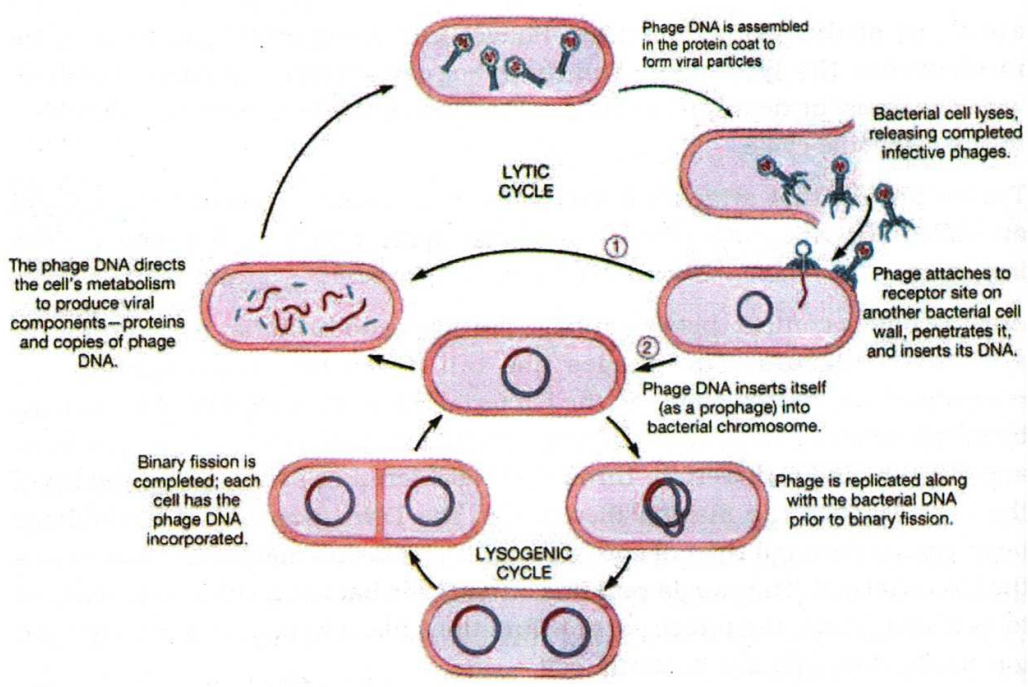


Figure 8.4 Viral Multiplication cycles

8.4.2 The Lysogenic cycle

Some bacteriophages such as Lambda phages do not cause lysis and death of the host cell when they multiply. Instead, the phage DNA upon penetration into an E. coli cell gets integrated in to the circular bacterial DNA, becomes part of it remains latent (inactive). Such phages are called temperate phages. The inserted Phase DNA is now called prophase.

Every time the bacterial genetic material replicates, the prophase also undergoes replication. The prophage remains latent within the progeny cells. However in some rare spontaneous events the phage DNA separates from the bacterial genetic material , leading to the initiation of the lytic cycle.

8.5 Viral diseases in Plants

Viruses, being obligate parasites, cause many plant diseases while growing inside them. Plant diseases caused by viruses are mostly Systemic in nature (affect the whole plant). The leaves generally exhibit characteristics symptoms. The usual symptoms of viral diseases are chlorosis (e.g. peach yellowing disease), mosaic (e.g. tobacco mosaic disease) , Vein clearing (e.g. Bhending vein clearing , malformation (e.g. swollen shoot of cocoa) and breaking of flowers (e.g. tulip mosaic break) etc.

Viriods

Some virus like infectious agents possess only the nucleic acid without protein coat . They are known as 'Viroids' .

e.g. potato spindle tuber virus.

Prions

These are some infectious agents which possess only proteins but not not nucleic acids . such agents are called 'prions'.

e.g. Mad cow disease of cow.

8.6 Viral diseases in Humans

Viruses cause wide spread diseases in humans such as common cold, hepatitis ,chickenpox, influenza, herpes, warts, polio etc. Although most viral infections do not result in death, some such as Rabies, AIDS and Ebola have high mortality rate, others such as polio and neonatal rubella can lead to long term debility (physical weakness).

Do you know, some viruses such as Epstein –Bar virus and Human papiloma virus cause cancers in animals and Humans.

Chronic hepatitis B (caused by Hepatitis B virus) may lead to cancer. Such cancer causing viruses are called Oncoviruses.

SUMMARY

Viruses are very small, infectious, obligate intracellular parasites. They are acellular and lack Metabolism of their own. Structurally they are very simple, with a nucleic acid core

protected by a protein capsid. The complete virus particle is called a virion and its genome comprises of either DNA or RNA. Viruses can be crystallized. Viruses occur in several morphological forms. Viruses are grouped according to their shaped properties and classified by ICTV system of classification. Viruses multiply in host cells which they attack. The viral genome is replicated and directs the synthesis of other virion components by cellular systems of other virion components.

Viruses that attack bacteria are called bacteriophages. Virulent phages follow lytic cycle of replication where as temperate phages follow lysogenic cycle of replication. In this process viruses can cause cancer and they are called oncogenic viruses. There exist infectious agents that are simpler than viruses-viroids and prions which also can cause diseases.

Glossary

Burst Size: The number of newly synthesized bacteriophge particles released from a single affected host cell.

Latent infection: A condition in which a pathogen remains in the host for long periods without causing any disease symptoms.

Questions

Very Short Answer Type Questions

1. What is the shape of T4 phage?
2. What is the genetic material in T4 phase?
3. What is the shape of TMV?
4. What is lysozyme?
5. Define 'lysis'.
6. What is prophase?
7. Give an example to prophase?

Short Answer Questions

1. Explain the chemical structure of viruses?
2. Explain the structure of TMV.
3. Explain the structure of Bacteriophase.
4. Explain the lytic cycle in viruses.

Excersises

1. Viruses are bridges between living and non-living components .Explain.

2. How can you assume that viruses are smaller than bacteria?

3. All Viruses can be seen only under electron microscope. Will you agree? If not why?

UNIT III

GENETICS

Chapter 9: Principles of inheritance and variation

The work of mendel and others who followed him gave us an idea of inheritance patterns. However the nature of those 'factors' which determine the phenotype was not very clear. As these' factors' represent the genetic basis of inheritance. understanding the structure of genetic material and the stuructural basis of genotype and phenotype conversion became the focus of attention in biology for the next century. The entire body of molecular biology was a consequent development with major contributions from Watson, Crick, Nirenberg, Khorana, Korenbergs (father and son), Benzer, Monod, Brenner, etc. In this unit the basic principles of inheritance have been examined and explained.

CHAPTER 9

Principles of Inheritance and Variation

Have you ever wondered why an elephant always gives birth to a baby elephant and not some other animal? Or why a mango seed forms only a mango plant and not any other plant?

Given that they do, are the offspring identical to their parents? Or do they show differences in some of their characteristics? Have you ever wondered why siblings sometimes look very similar to each other? Or sometimes even so different?

These and several questions are dealt with, scientifically, in a branch of biology is known as Genetics. This subject deals with the inheritance, as well as the variation of characters from parents to offspring. Inheritance is the process by which characters are passed on from parents to progeny; it is the basis of heredity. Variation is the degree by which progeny differ from their parents as well as among themselves.

Humans knew from as early as 8000- 1000 B.C that one of the cause of variation was hidden in sexual reproduction. They exploited the variations that were naturally present in the wild populations of plants and animals to selectively breed and choose organisms that possessed desirable characters. For example, through artificial selection and domestication from ancestral wild cows, we have well- known Indian breeds. E.g., sahiwal cows in Punjab and 'Ongole' bulls in Andhra Pradesh.

9.1 Mendel's Experiments

Gregor Johann Mendel (1822-1884) was an Austrian Monk who worked at Altburnn Monastery near Burnn, now in Czechoslovakia. His experiments on garden pea, *Pisum sativum* were carried out during the years 1856 to 1864 to study the mechanism of inheritance.

Mendel was not the first to perform hybridization experiments, but he was the first to consider their results in terms of single traits. He designed appropriate experiments and actually counted and classified the progeny that resulted from the crosses. Then he compared the proportions with mathematical models. His experiments were elegant and his conclusions constitute the foundation 0f the modern sciences of genetics.

The results obtained from the experiments were presented in a paper entitled "Experiments in plant Hybridisation" which was published in 1866.

The results of Mendel's hybridization experiments remained virtually unknown for 34 years after their publication. In 1900 Mendel's paper was discovered by three scientists: Hugo de Vries, Carl Correns and Erich Von Tschermark. All the three investigators confirmed Mendel's principles independently from their own studies.

Selection of experimental plant

Mendel selected garden pea plant (*Pisum sativum*), a member of Fabaceae for his hybridization experiments because of the following reasons.

1. It is an annual plant that has well defined characteristics.
2. It can be grown and crossed easily.
3. It has bisexual flowers containing both female and male parts.
4. It can be self- fertilized conveniently.
5. It has a short life cycle and produces large number of offsprings.

Mendel conducted artificial pollination / Cross pollination experiments using several true-breeding pea lines. A truebreeding line is one that, having undergone continuous self-pollination, shows the stable trait inheritance and expression for several generations. Mendel selected 14 true breeding pea plant varieties, as pairs which were similar except for one character with contrasting traits. Some of the contrasting traits selected were smooth or wrinkled seeds, yellow or green seeds, smooth or inflated pods, green or yellow pods and tall or dwarf plants (9.1).

Table 9.1 Contrasting traits studied by Mendel in pea

S.No	Characters	Contrasting Traits
1	Stem height	Tall/dwarf
2	Flower colour	Violet/White
3	Flower position	Axial/ terminal
4	Pod shape	Inflated/ Constricted
5	Pod Colour	Green/ Yellow
6	Seed Shape	Round / Wrinkled
7	Seed colour	Yellow / Green

9.2 Inheritance of one gene (Monohybrid Cross)

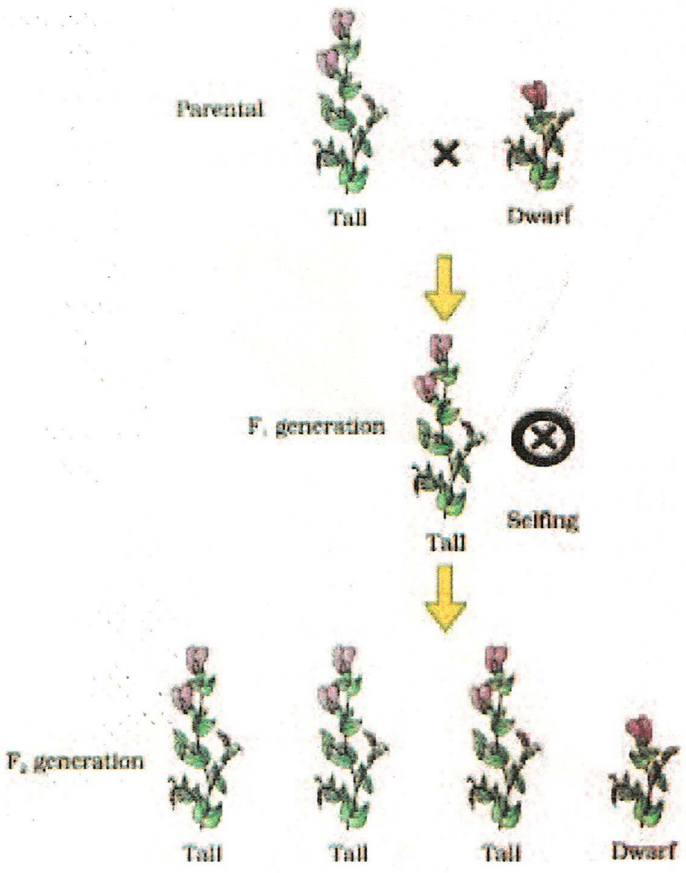


Figure 9.1 Diagrammatic representation of as monohybrid cross.

In one experiment when a tall plant and a dwarf plant were crossed, all the hybrids produced in the F1 generation (First filial generation) were tall. The dwarf trait did not appear in the F1 progeny. When F1 plants were selfed and F1 generation progeny were classified, the dwarf trait appeared again.

Careful analysis of the results showed that about three-fourth of the plants were tall and one-fourth were dwarf. Out of a total of 1064 F2 plants, 787 were tall and 277 were dwarfs. So a ratio of 3.1 can be noticed. In this experiment parents differing in only one trait were crossed hence this is called a monohybrid cross and the ratio between tall and dwarf plants (3:1)

In the above cross parents carrying similar genes such as TT or tt are called homozygous plants and those with unlike genes such as Tt are called Heterozygous plants. A Homozygote has two similar type of alleles. TT or tt and will produce genetically uniform (one kind only) Monohybrid cross

types of gametes. A heterozygote has two dissimilar types of alleles (Tt) and will produce genetically different types of gamates – some with 'T' and some with 't'.

A gamete is conventionally represented by writing the allelic symbol inside a circle such as 

The progeny obtained in F1 generation were heterozygous. In F2 generation, a phenotypic ratio of 3:1 and a genotypic ratio of 1:2:1 is noticed between tall and dwarf plants. (1=TT, 2=Tt ,1=tt)

Phenotype : The physical appearance of a trait is called phenotype.

Genotype : The genetic makeup of an individual is called genotype.

Contrasting Characters : The alternative form of a character

Alleles: The two alternative forms of a gene are called alleles. One of the alternative forms of a gene that occurs at a given locus in a chromosome is the allele. These alleles occur on the identical loci in homologous chromosomes. For example the alleles responsible for tallness is "T" and the allele responsible for dwarfness is "t".

Gene locus: The location of a particular gene on a given chromosome is called gene locus.

Mendel observed that in F1 hybrids the character of tallness dominated or conceals the dwarf character. The character which is expressed in F1 generation is called dominant trait and that which remained unexpressed is called recessive trait. Such pair of contrasting traits are called alleolomorphs and the genes controlling them are called alleles (T or t).

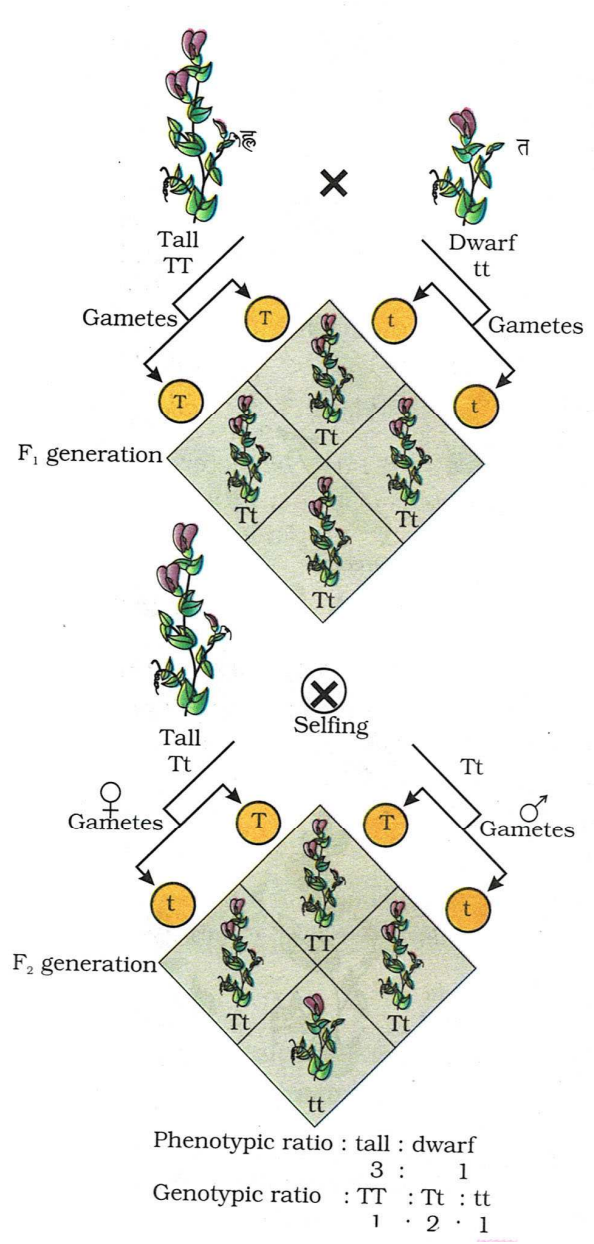


Figure. 9.2 A punnett square used to understand a typical monohybrid cross between true-breeding tall plants and true breeding dwarf plants conducted by mendel.

9.2.1 Back cross and Test cross

Back cross: When F1 individuals are crossed with any one of its parents or organisms that are phenotypically and genotypically similar to the parents, it is called Back cross. If the F1 hybrid is crossed with the parent having dominant traits no recessive individuals are obtained in the progeny.

Test cross: When the F1 individuals are crossed with the recessive parent or organism similar in phenotype and genotype to the recessive parent, it is called Test cross. It is used to test whether an individual is homozygous (pure) or Heterozygous (Hybrid). A monohybrid test cross gives a phenotypic ratio of 1:1 and a dihybrid test cross gives a ratio of 1:1:1:1.

Based on his observations on monohybrid crosses, Mendel proposed two general based rules are called the Principles or Laws ofDominance and the Second Law orLaw of Segregation.

9.2.1. Law Of Dominance

(i) Characters are controlled by discrete units called factors.
(ii) Factors occur in pairs.
(iii) In a dissimilar pair of factors pertaining to a character one member of the pair dominates (dominant) the other (recessive).

The law of dominance is used to explain the expression of only one of the parental characters in a monohybrid cross in the F1 and the expression of both in the F2. It also explains the proportion of 3:1 obtained at the F2.

9.2.2 Law of segregation or Law of purity of gametes

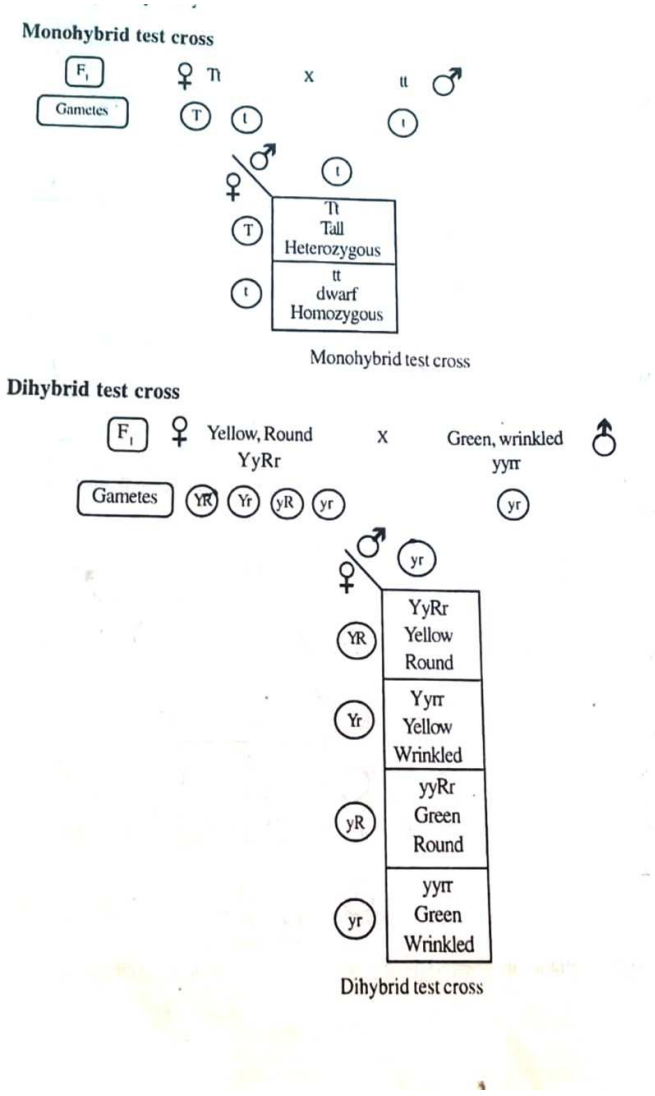


Figure 9.3 Diagramatic representation of a test cross

Mendel Law of Segregation states that "the two alleles of a gene when present together in a heterozygous state, do not fuse or blend in any way, but remain distinct and segregate during meiosis or in the formation of gametes so that each meiotic product or gamete will carry only one of them.

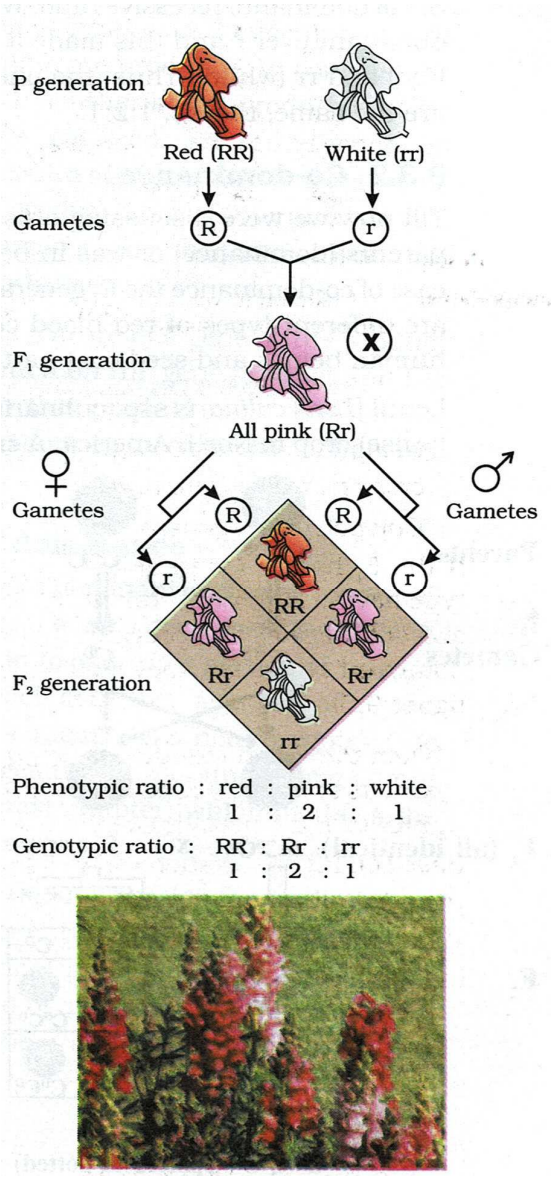
This law is based on the fact that the alleles do not show any blending and that both the characters are recovered as such in the F2 generation though one of these is not seen at the F2 stage. Though the parents contain two alleles during gamete formation, the factors or alleles of a pair segregate from each other such that a gamete receives only one of the two factors.

Of course, a homozygous parent produces all gametes that are similar while a heterozygous one produces two kinds of gametes in equal proportion, each having one allele, segregation in all organisms reproducing by normal sexual method.

9.3 Deviation from Mendelian concept of dominance

9.3.1 Incomplete Dominance

When experiments on peas were repeated using other traits in other plants, it was using other traits in other plants, it was found that sometimes the F1 had a phenotype that did not resemble either of two parents and was in between the two. The inheritance of flower colour in the dog flower (snapdragon or *Antirrhinum* sp.) is a good example to understand incomplete dominance. In a cross between true- breeding (homozygous) red-flowered (RR) and truebreeding (homozygous) white-flowered plants (rr), the F1 (Rr) was pink (Figure 9.4). When the F1 was self-pollinated, the F2 resulted in the following ratio 1 (RR) Red: 2(Rr) pink: 1 (rr) white. Here the genotypic ratios were exactly as we would expect in any mendelian monohybrid cross, but the phenotypic ratio had changed from the 3:1 of dominant: recessive ratio. What happened was that R was not completely dominant over r and this made it possible to distinguish Rr as pink from RR(red) and rr (white). Thus, the phenotypic and genotypic ratios in F2 progeny are the same, that is, 1:2:1.



9.4 Inheritance of two genes

In another experiment Mendel crossed a homozygous pea plant having yellow round seeds (YYRR) with a homozygous pea plant having green wrinkled seeds (yyrr). All the F1 hybrids were found to have yellow round seeds. such a cross involving two parental plants differing in two characters is called a dihybrid cross.

Figure 9.4 Results of Monhybrid cross in the plant Snapdragon.

Where one allele is incompletely dominant over the other side. When the F1 hybrids were allowed to undergo self pollination. The F2 generation progeny was showing four kinds of phenotypes in a definite pattern. From a total of 556 seed obtained, 315 were yellow and round, 101 were yellow and wrinkled, 108 were green and round, 32 were green and wrinkled. These results were found to fit very nearly a phenotypic ratio of 9:3:3:1 and the genotypic ratio of 1:2:2:4:1:2:1:2:1. When second generation progeny is raised from the first generation, the genes combine independently irrespective of their association.The F2 progeny showed not only the parental combination i.e. yellow round and green wrinkled phenotypes, but also the new combination i.e. yellow wrinkled and green round phenotypes. These new combinations are produced due to genetic recombination among the factors by independent assortment. This recombination is called **Mendalian recombination.**

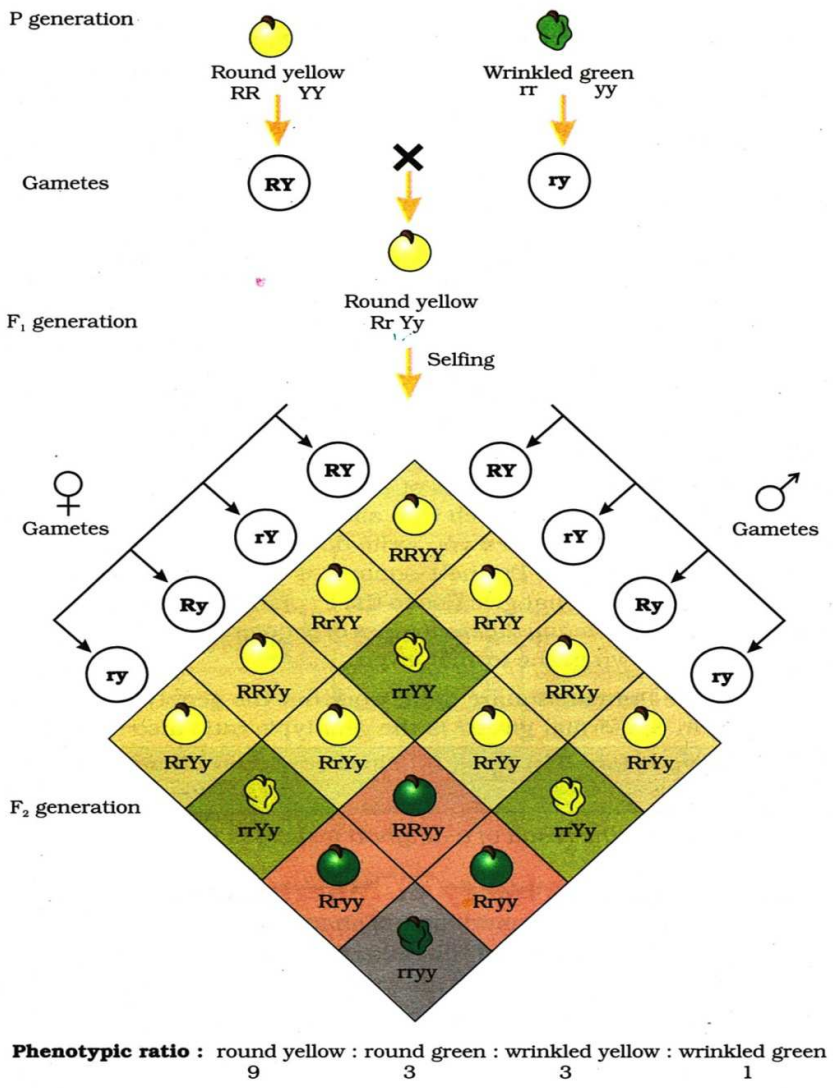


Figure 9.8 Results of a dihybrid cross

Law of Independent Assortment

Based on the results of the dihybrid crosses in a pea plant. Mendel postulated his second law known as the "**Law of independent assortment**" which states that "When the plants differ from each other in two or more pairs of contrasting characters or factors, then the inheritance of one pair of factors is independent to that of the other pair of factors".

9.5 Chromosomal Theory of Inheritance

In 1900, three scientist (**de veries, correns and von Tschermark**) independently rediscovered Mendel's results on the inheritance of characters. Also, by the time due to advancements in microscopy that were taking place, scientists were able to carefully observed cell division. This led to the discovery of structures in the nucleus that appeared to double and divide just before each cell division. These were called chromosomes (colored bodies, as they were visualised by staining. By 1902, the chromosome movement during meiosis had been worked out. **Walter Sutton** and **Theodore Boveri** noted that the behaviour of genes predicted by Mendel and they used chromosome movement (Figure 9.6) to explain Mendel's laws. Recall that you have studied the behaviour of chromosomes during mitosis (equational division) and during meiosis (reduction division). The important thing to remember is that chromosomes as well as genes occur in pairs. The two alleles of a gene pair are located on homologous sites on homologous chromosomes. During Metaphase of meiosis I, the two chromosome pairs can align at the metaphase plate independently of each other (Figure 9.6). To understand this, compare the chromosomes of four different colours in the left and right columns. In the left column (possibility I) orange and green are segregating together. But in the right hand column (possibility II) the orange chromosome is segregating with the red chromosomes. Sutton and Boveri argued that the pairing and separation of a pair of chromosomes would lead to the segregation of a pair of factors they carried. Sutton united the knowledge of chromosomal segregation with Mendalian principles and called it the **Chromosomal theory of inheritance.**

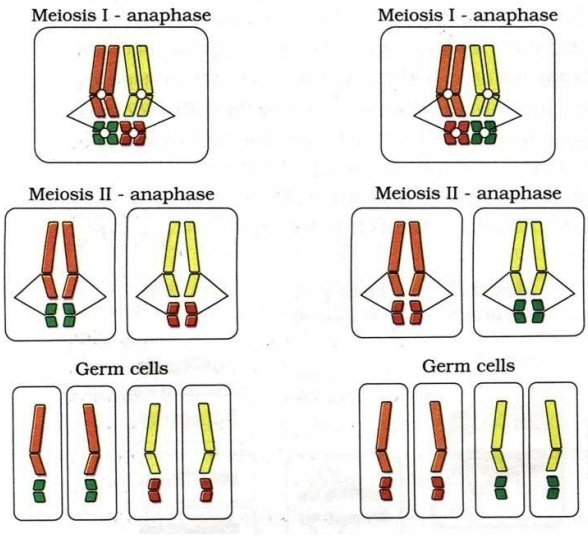


Figure 9.6 Independent assortment of chromosomes

9.6 Linkage and Recombination

You have learn in the previous that heredity and variations are caused due to genes present inside the chromosomes. The law of independent assortment explains that genes combine independently into different groups irrespective of their association with any particular character.

However, in 1906 **Bateson** and **punnett** discovered that in some cases the pair of contrasting characters do not separate and remain together for a number of generations. This is because of the presence of large number of genes in a single chromosome in sequence. Therefore the genes are located very close to one another and tend to be inherited together. This type of coexistence of two or more genes in the same chromosome is called linkage and was discovered by **T.H. Morgan** in 1910.

The number of genes in a chromosome depends on its length. Larger chromosomes generally possess more number of genes than shorter chromosome. The degree of linkage between the genes depends upon the distance between their loci. Closer genes are linked strongly while the farther genes are linked weakly.

Significance of Linkage: It reduces the possibility of variability in gametes unless crossing over occurs. So genetic integrity of organisms is maintained to a large extent for several generations.

Summary

Genetics is a branch of biology which deals with the principles of inheritance and its practices. Progeny resembling the parents in morphological and physiological features has attracted the attention of many biologists. Mendel was the first to study this phenomenon systematically. While studying the pattern of inheritance in pea plants with contrasting characters. Mendel proposed the principles of inheritance. Which are today referred to as 'Mendel's Laws of Inheritance'. He proposed that the 'factors' (later named as genes) regulating the characters are found in pairs known as alleles. He observed that the expression of the characters in the offspring follow a definite pattern in different generations –first generations(F1),second (F2) and so on. Some characters are dominant over others. The dominant characters are expressed when factors are either in homozygous or in heterozygous condition (law of dominance). The recessive characters are only expressed in homozygous conditions. The characters never blend in heterozygous condition. A recessive character that was not expressed in heterozygous condition may be expressed again when it becomes homozygous. Hence, characters segregate during formation of gametes (Law of Segregation). Not all characters show true dominance. Some characters show incomplete dominance. When Mendel studied the inheritance of two characters together, it was found that the factors independently assort and combine in all permutations and combinations(Law of Independent Assortment). Different combinations

of gametes are theoretically represented in a square tabular form known as 'Punnet Square'. The factors (now known as genes) on chromosomes regulating the characters are called the genotype and the physical expression of the characters is called phenotype. After knowing that the genes are located on the chromosomes, a good correlation was drawn between Mendel's laws and segregation and assortment of chromosomes during during meiosis. Mendel's laws were extended in the form of 'chromosomal theory of Inheritance'. Later, it was found that Mendel's Law of independent assortment did not hold true for the genes that were located on the same chromosomes. These genes are called 'linked genes'. Closely located genes assorted together, and distantly located genes, due to recombination, assorted independently.

GLOSSARY

Alleles: The alternative forms of a gene.

Back cross: If the F1 progeny (or heterozygote) are mated back to one of their parents (or individuals with genotype identical to that of either of their parents), the mating is termed backcross.

Dihybrid cross: The cross made between individuals differing in two characters.

Dominance: It is the phenomenon where character is expressed phenotypically in both homozygotes and heterozygotes.

Gene: It is the unit of inheritance and contains the information required to express a character.

Genotype: It is the genetic makeup of an individual.

Heterozygote: An individual having two different alleles for a single character. Consequently it will produce two different types of gametes with reference to a gene.

Homozygote: An individual having two similar or identical alleles for a single character. Hence it will produce only one kind of gametes with reference to a gene.

Incomplete dominance: It is the condition when one allele of a gene is not completely dominant over the other allele and results in the heterozygotes having phenotype different from the dominant and recessive homozygotes.

Linkage: is the presence of two or more genes on a chromosome as a result of which the genes tend more often to be inherited together.

Monohybrid cross: The cross made between two individuals differing in one character.

Phenotype: The physical or external appearance of a character. Sometimes it may require special tests for its identification such as determination of respiratory quotient or serological tests for blood grouping.

Recessive: The character which is not expressed phenotypically in heterozygous condition.

Test cross: The cross between F1 progeny (or heterozygotes) and their recessive homozygous parent (or individuals with recessive homozygous genotype).

QUESTIONS

Very Short Answer Type Questions

1. What is the cross between the F1 progeny and the homozygous recessive parent called?
2. Explain the terms phenotype and genotype.
3. What is the genetic nature of wrinkled phenotype of pea seeds?
4. Define True Breeding.
5. What is Linkage?
6. What is crossing over?

Short Answer Type Questions

1. Mention the advantages of selecting pea plant for experiment by Mendel.
2. Differentiate between the following:
   (a) Dominant and Recessive
   (b) Homozygous and Heterozygous
3. Explain the law of Dominance using a monohybrid cross.
4. Define and design a test-cross.
5. Explain Incomplete Dominance.

Excersises

1. What will be the phenotypic ratio in the offsprings obtained from the following crosses.

a) Aa aa b) AA aa c) Aa Aa d) Aa AA

Note: Gene 'A' is dominant over gene 'a'

UNIT V

MOLECULAR BIOLOGY

Chapter 10 : Molecular Basis of Inheritance

Molecular biology is relatively a young discipline among life sciences. It is about the study of macromolecules and their mechanisms in living things such as gene replication, mutation and expression. Warren Weaver recognized quite early the importance of the physical and chemical approaches to biology, and introduced the term Molecular Biology to uncover the minute details of certain life processes. The Field of Molecular biology arose from the convergence of work by geneticists, physicists and structural chemists on the structure and function of the gene. The classifical period of Molecular Biology began in 1953 with James Watson and Francis Crick's discovery of the double helical structure of DNA.

Chapter 10

Molecular Basis of Inheritance

In the pevious chapter, you learnt about inheritance patterns and the genetic basis of such patterns. During Mendel's time, the nature of those 'factors' regulating pattern of inheritance was not clear. Over the next hundred years, the nature of the putative genetic material was investigated , culminating in the realisation that DNA – deoxyribonucleic acid – is the genetic material, at least for the majority of organisms. In First year you learnt that nucleic acids are polymers of nucleotides.

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are the types of nucleic acids found in living systems. DNA acts as the genetic material in most of the organisms. Though RNA also acts as a genetic material in some viruses, it mostly functions as a messenger and has a additional roles as well. It functions as adapter, structural and in some cases as a catalytic molecule. You have already learnt the structures of nucleotides and the way these monomer units are linked to form nucleic acid polymers. In this chapter we are going to discuss the structure of of DNA, its replication, the process of making RNA from DNA (transcription), the genetic code that determines the sequences of amino acids in proteins, the process of protein synthesis (translation) and the elementary basis of their regulation. The determination of the complete nucleotide sequence of the human genome during last decade has heralded a new era of genomics.

Let us begin our discussion by first understanding the structure of the most interesting molecule in the living system, that is, the DNA. In subsequent sections, we will learn why it is the most abundant genetic material and its relationship with RNA.

10.1 The DNA

DNA is mainly found in the chromosomes and also in small quantities in the nucleolus, mitochondria, chloroplast an in the cytoplasm of bacteria.

10.1.1. Structure

DNA usually exhibits the double helix model, which was discovered by J.D. Watson and F.H.C. Crick (1953), for which they were awarded a Nobel prize in 1962. Their discovery was based on the earlier investigations made by Erwin Chargaff and Franklin and Wilkins by employing x-ray diffraction technique (Crystallography).

According to the double helix model. The DNA is composed of two two polynucleotide strands which are spirally coiled around one another. These two strands are ANTIPARALLEL to each other and both the strands are held together by hydrogen bonds between one purine in one strand and a pyramidine on the opposite strand. The presence of these hydrogen bonds were first observed by L.C. Pauling (1954), Adenine (A) is bonded to Thymine (T) by two hydrogen bonds (A=T), Guanine (G) is bounded to cytosine (C) by three hydrogen bonds (G=-C).

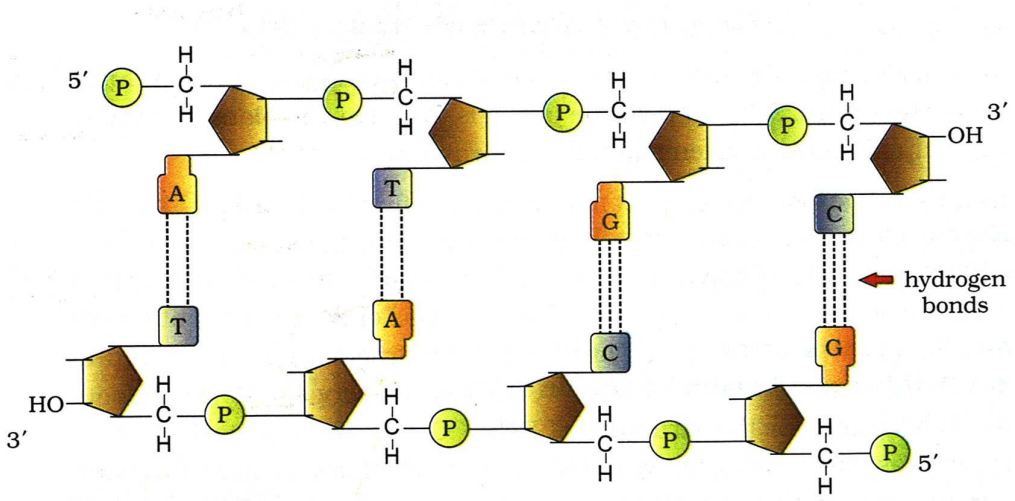


Figure 10.1 Schematic representation of Double strand polynucleotide chain

The amount of Adenine (A) is always equal to Thymine (T) and amount of Guanine is always equal to cytosine (C). The base ratio may vary from species to species, but it is constant for a given species. Erwin Chargaff discovered that purine (A, G) exist in 1:1 ratio.

The diameter between two strands of DNA is $20A^0$ / 2.0 nm. The length of DNA depends up on the number of nucleotides. The two strands of DNA are coiled to form a twisted ladder structure. The coiling occurs at an angle of 360^0. The coiling leads to the formation of "turns" or "helices". Each turn accommodates 10 base pairs or 10 nucleotides. The length of each turn is 34 A^0 or 3.4 nm and the inter nucleotide distance id 3.4 A^o or 0.34 nm.

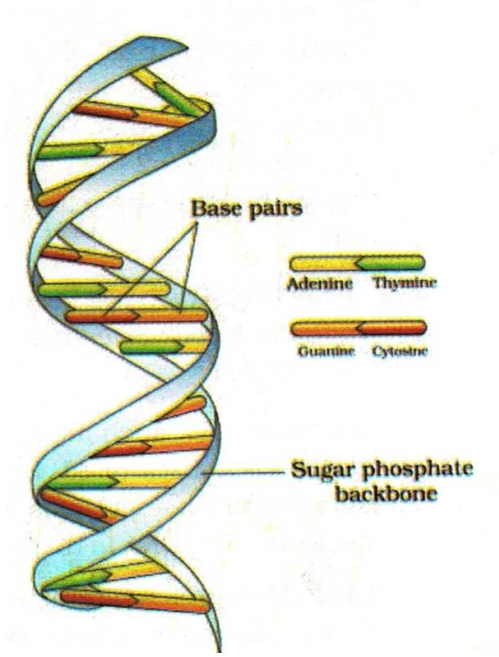


Figure 10.2 DNA Double helix

The proposition of double helix structure for DNA and its simplicity in explaining the genetic implication became revolutionary. Very soon Francis Crick proposed the Central dogma in molecular biology, which states that genetic information flows from

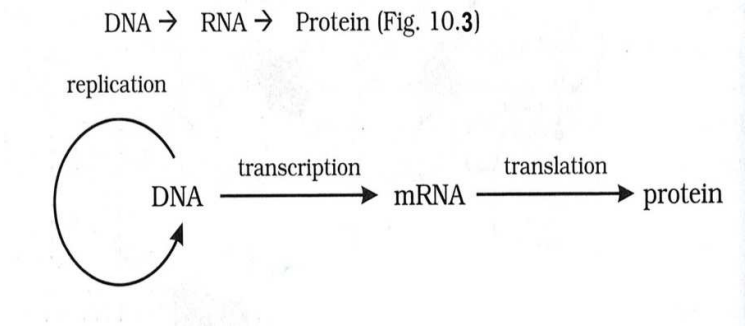


Figure 10.3 central dogma

10.2 The Search for Genetic Material level.

Even though the discovery of nuclein by Meischer and the proposition for principles of inheritance by Mendelwere made almost at th same time, it took a longtime to discover and prove that DNA acts as a genetic material. BY 1926, the quest to determine the mechanism for genetic inheritance had reached the molecular level. Previous discoveries by Gregor Mendel. Walter Sutton, Thomas Hunt Morgan and numerous other scientists had narrowed the search to the chromosomes located in the nucleus of most cells. But the question of what molecule was actually the genetic material, had not been answered.

Transforming Principle

In, 1928 Frederick Griffith, in a series of experiments with *Streptococcus pneumonia* (bacterium responsible for Pneumonia), witnessed a miraculous transformation in the bacteria. During the course of his experiment, he found that a living organism (bacteria) could change in physical form.

When Streptococcus pneumonia (pneumococcus) bacteria were grown on a culture plate, some produced smooth shiny colonies (S) while others produced rough colonies(R). This is because the S strain bacteria have a mucous (polysaccharide) coat, while R strain does not . Mice infected with the S strain (Virulent) die from pneumonia infection but mice infected with the R strain do not develop pneumonia.

S strain injected into mice Mice die (Living S strain cells recovered from the body of mice)

R strain injected into mice Mice live (Living R strain cells recovered from the body of mice)

Griffith was able to kill bacteria by heating them. He observed that heat killed S strain bacteria injected into mice did not kill them. When he injected a mixture of heat killed S and live R bacteria , the mice died. Moreover, he recovered living S strain bacteria from the dead mice.

S strain (heat killed) Injected into mice Mice live (S or R strain cells not found in the body of mice)

S strain (heat killed) + R strain (live) Injected into mice Mice die (Living S strain cells found in the body of dead mice)

He concluded that the R strain bacteria had somehow been transformed by the heat killed S strain bacteria. Some 'transforming principle', transferred from heat killed S strain, had enabled the R strain to synthesize a smooth polysaccharide coat and become virulent . This must be due to the transfer of the genetic material. However, the biochemical nature of genetic material not defined from his experiments.

Biochemical characterisation of transforming principle

Prior to the work of **Oswald Avery**, **Colin Macleod** and **Maclyn McCarthy** (1933-44), the genetic material was thought to be a protein. These scientists worked to determine the Biochemical nature of 'transforming principle' in Griffith's experiment.

They purified biochemicals (proteins, DNA, RNA etc,) from the heat killed S cells to see which ones could transform live R cells into S cells. They discovered that DNA alone from S bacteria caused R bacteria to become transformed.

They also discovered that protein- digesting enzymes (proteases) and RNA-digesting enzymes (RNases) did not affect transformation, so the transforming substance was not a protein or RNA. Digestion with DNase did inhibit transformation, suggesting that the DNA caused the transformation. They concluded that DNA is the hereditary material, but not all biologists were convinced.

Can you think of any difference between DNAs and DNase ?

10.3 RNA World

From the foregoing discussion, an immediate question becomes evident – which is the first genetic material? It shall be discussed in detail elsewhere under chemical evolution, but briefly, we shall highlight some of the facts.

RNA was the first genetic material. There is now enough evidence to suggest that essential life processes (such as metabolism, translation, splicing, etc.), evolved around RNA. RNA used to act as a genetic material as well as catalyst (there are some important biochemical reactions in living systems that are catalysed by RNA catalysts and not by protein enzymes). Catalytic RNA or RNA enzymes are known as **Ribozymes.**

But RNA, being a catalyst, was reactive and hence unstable. Therefore, DNA has evolved from RNA with chemical modifications that made it more stable DNA, being double stranded and having complementary strands, Further resists changes by evolving a process of repair.

10.4 Replication

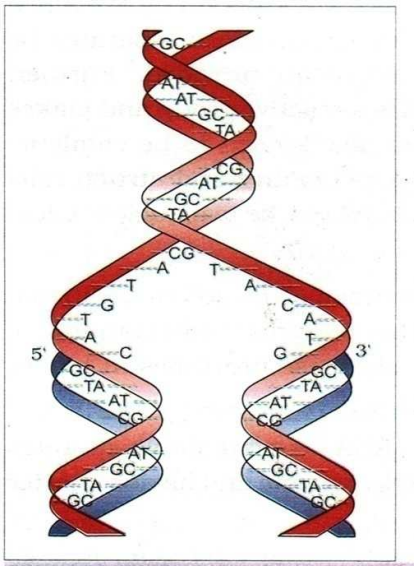


Figure 10.4 Watson-Crick model for semiconservative DNA replication

While proposing the double helical structure for DNA, Watson and Crick had immediately proposed a scheme for replication of DNA. To quote their original statement that is as follows:

"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material" (Watson and Crick, 1953).

The scheme suggested that the two strands would separate and act as a template for the synthesis of new complementary strands. After the completion of replication, each DNA molecule would have one parental and one newly synthesized strand. This scheme was termed as **semi conservative** DNA replication (Figure10.4).

10.5 Transcription

The process of copying genetic information from one strand of DNA into RNA is termed **Transcription**. Here also, the principle of complementarity governs the process of transcription, except that the adenosine now base pairs with **Uracil** instead of **thymine**. However, unlike in the process of replication in which once set in, the total DNA of an organism gets duplicated, in transcription only a segment of DNA and only one of the strands is copied into RNA. This necessitates defining the boundaries that would demarcate the region and the strand of DNA that would be transcribed.

10.5.1 Transcription unit

A transcription unit in DNA is defined primarily by the three regions in the DNA:

(i) A promoter
(ii) The Structural gene
(iii) A Terminater

There is a convention in defining the two strands of the DNA in the structural gene of a transcription unit. Since the two strands have opposite polarity and the **DNA –dependent RNA polymerase** (or transcriptase)also catalyses the polymerization in only one direction., that is , 5' ---- 3' , the strand that has the polarity 3'------ 5' act as a template, and also reffered to as **template strand.** The other strand, which has the polarity(5'----3') and the sequence same as RNA (except **thymine** at the place of **Uracil**), is displaced during transcription. Strangely, this strand (which, does not code for anything) is reffered to as the **Coding strand..** All points while defining a transcription unit are made with reference to the coding strand. To explain the point, a hypothetical sequence from a transcription unit is represented below:

3' – ATGCATGCATGCATGCATGCATGC – 5' Template Strand

5' – TACGTAGCTACGTACGTACGTACG – 3' Coding Strand

Can you now write the sequence of RNA transcribed from the above DNA?

The **promoter** and **terminater** flank the **structural gene** in a transcription unit. The promoter is said to be located towards 5' end (upstream) of the structural gene (the reference is made with respect to the polarity of coding strand). It is a DNA sequence that provides the binding site for RNA polymerase , and it is the presence of a promoter in a transcription unit that also defines the template and coding strands. By switching its position with that of the terminater, the definition of coding and template strands can be reserved. The terminater is located towards 3' end (downstream) of the coding strand and it usually defines the end of the process of transcription (Figure 10.5). There are additional regulatory sequences that may be present further upstream or downstream of the

promoter. Some of the properties of these sequences shall be discussed while dealing with the regulation of gene expression.

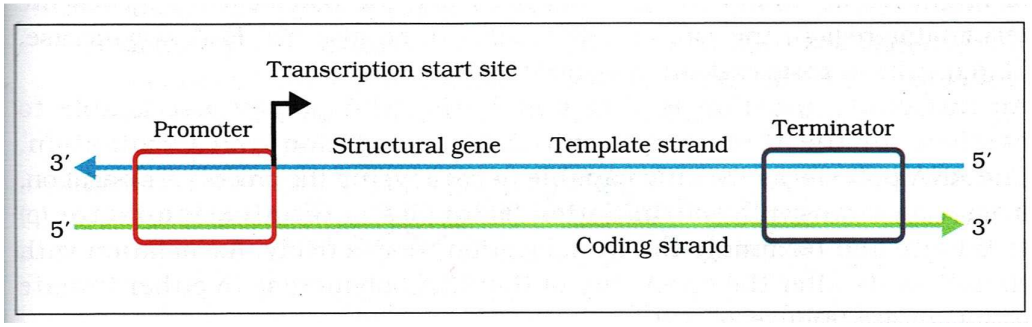


Figure 10.5 Schematic structure of a transcription unit

10.5.2 Transcription Unit and The Gene

A gene is defined as the functional unit of inheritance. Though there is no ambiguity that the genes are located on the DNA. It is difficulty to literally define a gene in terms of DNA sequence. The DNA sequence coding for tRNA or rRNA molecule also define a gene. However by defining **cistron** as a segment of DNA coding for a polypeptide, the structural gene in a transcription unit can be said to be **monocistronic** (mostly in eukaryotes) or **polycistronic** (mostly in bacteria or prokaryotes). In eukaryotes, the monocistronic structural genes have interrupted coding sequences – the genes in eukaryotes are split. The coding sequences or expressed sequences are defined as **exons**. Exons are said to be those sequence that appear in mature or processed RNA. The exons are interrupted by **introns.** Introns or intervening sequences do not appear in mature or processed RNA. The split- gene arrangement further complicates the definition of a gene in terms of a DNA segment.

Inheritance of a character is also affected by promoter and regulatory sequences of a structural gene. Hence, sometime the regulatory sequences are loosely defined as regulatory genes, even though these sequences do not code for any RNA or protein.

10.5.3 Types of RNA and the process of Transcription

In bacteria, there are three types of RNAs: mRNA (messanger RNA), tRNA (transfer RNA), and rRNA (ribosomal RNA). All three RNAs are needed to synthesise a protein in a cell. The mRNA provides the template, tRNA reads the genetic code and brings aminoacids and rRNAs play a structural and catalytic role during translation. There is single DNA – dependent RNA polymerase that catalyses transcription of all types of RNA in bacteria. RNA polymerase binds to the promoter and initiates transcription (**Initiation)**. It uses nucleoside triphosphates as substrate and polymerases in a template dependent fashion following the rule of complementarity. It some how also facilitates opening of the helix and continues

elongation. Only a short stretch of RNA remains bound to the enzyme. Once the polymerase reaches the terminater region, the nascent RNA falls off, So also the RNA polymerase This results in **termination** of transcription.

An intriguing question is that how is the RNA polymerase is able to catalyse all the three steps. Initiation, elongation and termination . RNA polymerase is only capable of catalysing the process of elongation. It associates transiently with **initiation – factor** and **termination – factor** to initiate and terminate the transcription, respectively. Association with these factors alter the specificity of the RNA Polymerase to either initiate or terminate (Figure10.6).

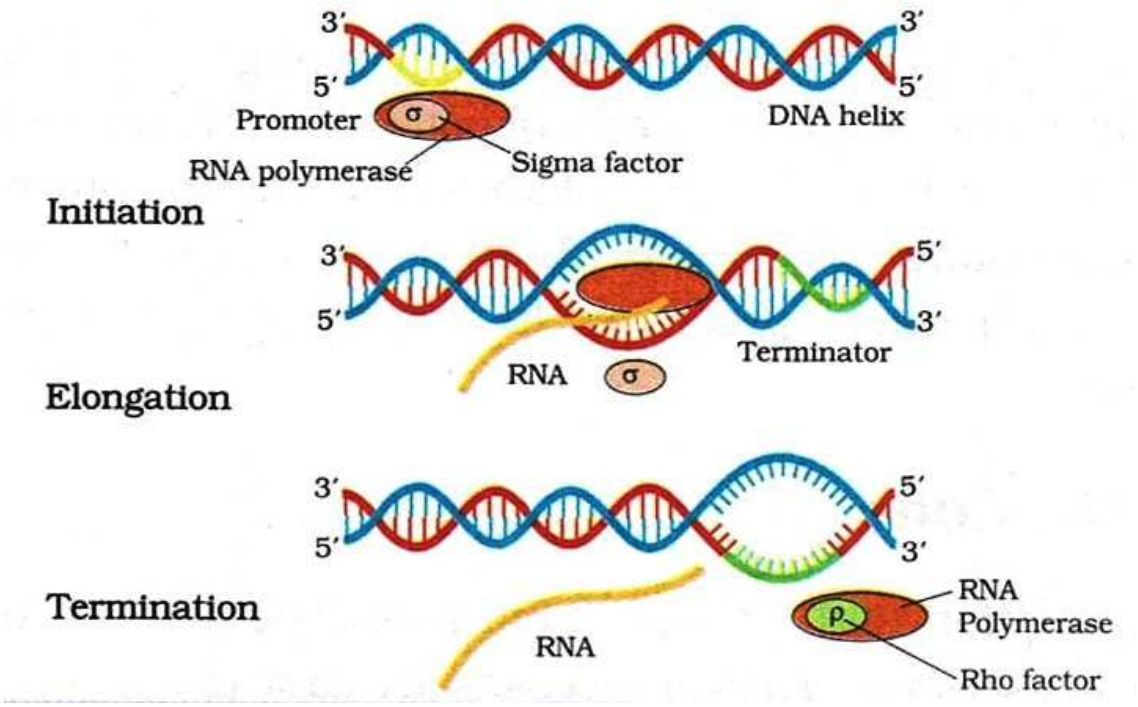


Figure 10.6 Process of Transcription in Bacteria

In bacteria, since the mRNA does not require any processing to become active, and also since transcription and translation take place in the same comportment (there is no separation of cytosol and nucleus in bacteria), many times the translation can began much before the mRNA is fully transcribed. Consequently, the transcription and translation can be coupled in bacteria.

In eukaryotes, there are two additional complexities-

(i) There are at least three RNA polymerases in the Nucleus (in addition to the RNA polymerase found in the organelles). There is a clear cut division of labour. The RNA polymerase I transcribes **rRNAs** (28s, 18S and 5.8S), the RNA polymerase II transcribes the precursor of mRNA, the **heterogenous nuclear RNA (hnRNA**),

where as the RNA polymerase III is responsible for transcription of **tRNA, 5sr RNAs** and **snRNAs (small nuclear RNAs).**

(ii) The second complexity is that the primary transcripts contain both exons and introns and are non- functional. Hence , they are subjected to a process called **Splicing** where the introns are removed and exons are joined in adefined order. Hn RNA undergoes additional processing called **capping** and **tailing**. In capping an unusual nucleotide (methyl guanosine triphosphate) is added to the 5' – end of hnRNA. In tailing, adenylate residues (200-300) are adddat 3'- end in a template independent manner. It is the fully processed hnRNA, now called mRNA, that is transported out of the nucleus for translation.(Figure10.7)

The significance of such complexities is now beginning to be understood. The split- gene arrangements represent probably an ancient feature of the genome. The presence of introns is reminiscent of antiquity, and the process of splicing represents the dominance of RNA- world. In recent times, the understanding of RNA and RNA –dependent processes in the living system have assumed more importance.

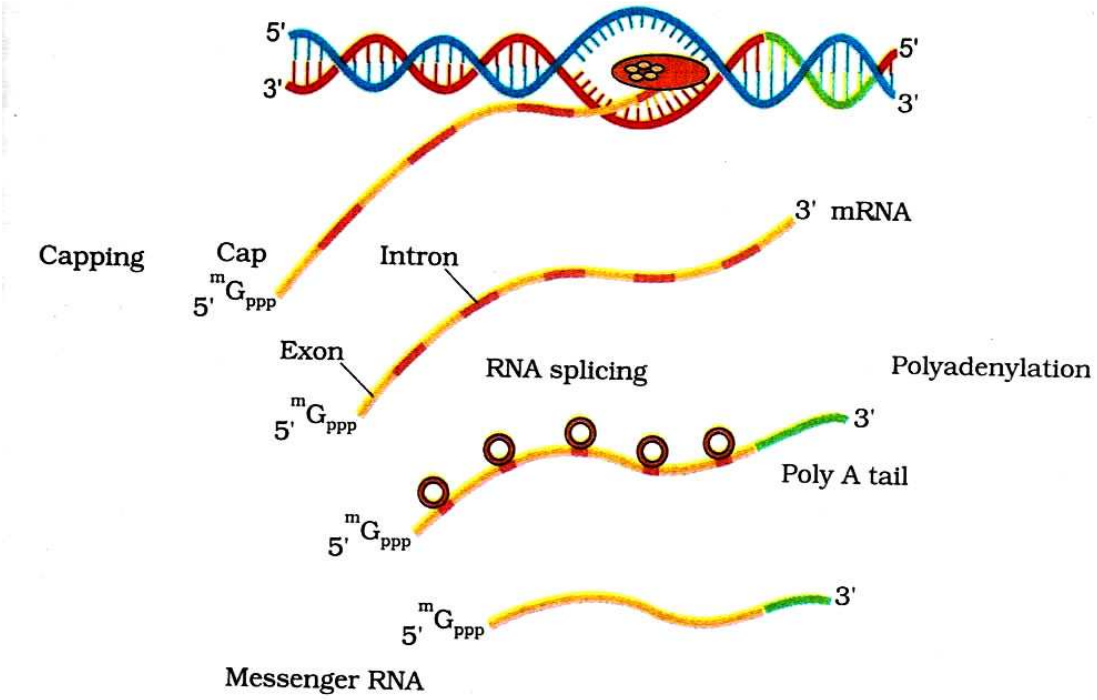


Figure 10.7 Process of transcription in Eukaryotes

10.6 Genetic code

During both replication and transcription, a nucleic acid is copied to form another nucleic acid. Hence, these processes are easy to conceptualise on the basis of complementarity. The process of translation requires transfer genetic information from a polymer of nucleotides to a polymer of amino acids. Neither does any complementarity exist between nucleotides and amino acids, nor could any be drawn theoretically. There existed ample evidences to support the notion that change in nucleic acids (genetic material) was responsible for change in amino acids in proteins. This led to the proposition of a genetic code that could direct the sequence of amino acids during synthesis of proteins.

If determining the biochemical nature of genetic material and the structure of DNA was very exciting, the proposition and deciphering of genetic code were most challenging. In a very true sense, it required involvement of scientists from several disciplines – physics, organic chemists, biochemist and geneticists. It was George Gamow, a physicist , who argued that since there are only 4 bases and if they have to code for 20 aminoacids, the code should constitute a combination of bases. He suggested that in order to code for all the 20 aminoacids, the code should be made up of three nucleotides. This was very bold proposition, because a permutation and combination of 4^3 (4 4 4) would generate 64 codons, generating many more codons than required. Providing proof that the codon was a triplet, was a more daunting task. The chemical method developed by Har Gobind Khorana was instrumental in synthesising RNA molecules with defined combinations of bases(homopolymers such as UUU and copolymers such as UUC,CCA). Marshall Nirenberg's cell free system for protein synthesis finally helped the code to be deciphered. The enzyme polynucleotide phosphorylase (Ochoa's enzyme; named after severo Ochoa) was also helpful in polymerising RNA with defined sequences in a template independent manner (enzymatic synthesis of RNA). Finally a checker board for genetic code was prepared which is given in Table 10.1.

The silent features of the genetic code are as follows:

(i) The codon is triplet, 61 codons code for amino acids and 3 codons do not code for any amino acids, hence they function as stop codons.

(ii) One codon codes for only one amino acid, hence, it is **Unambiguous** and **Specific.**

(iii) Some amino acids are coded by more than one codon, hence the code is **degenerate.**

(iv) The codon is read in m RNA in a contiguous fashion. There are no punctuations.

(v) The code is nearly **universal**: for example , from bacteria to human, UUU would code for phenylalanine (Phe). Some exceptions to this rule have been found in mitochondrial codons, and in some protozoans.

(vi) AUG has dual functions. It codes for Methinone (Met), and also acts as the **initiator** codon.

First position	Second position: U	Second position: C	Second position: A	Second position: G	Third position
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Table 10.1 The codons for the various Amino acids

If following is the sequence of nucleotides in m RNA, predict the sequence of amino acid coded by it (take the help of the checkerboard):

AUG UUU UUC UUC UUU UUU UUC

(There are no gaps in the actual arrangement of the nucleotides)

Now try the opposite. Following is the sequence of amino acids coded by an m RNA. Predict the nucleotide sequence in the RNA:

Met-Phe-Phe-Phe-Phe-Phe-Phe

Do you face any difficulty in predicting the opposite?

Can you now correlate this with two properties of genetic code you have learnt?

10.6.1 tRNA- the Adopter Molecule

From the very beginning of the proposition of code, it was clear to Francis Crick that there has to be a mechanism to read the code and also to link it to the amino acids, because amino acids have no structural specialities to read the code uniquely. Crick postulated the presence of an adapter molecule that would on one hand read the code and on other hand would bind to specific amino acids . The t RNA , then called s RNA (Soluble RNA), was known before the genetic code was postulated , however , its role as an adapter molecule was assigned much later. tRNA has an anticodon loop that has bases complementary to the code, and it also has an amino acid accepter end with which it binds to amino acids, tRNA are specific for each amino acid (Figure 10.15). For initiation , there is another specific tRNA that is reffered to as initiater tRNA. There are no tRNAs for stop codons. In Figure 10.8 , the secondary structure of tRNA has been depicted that looks like a clover – leaf . In actual structure, the t RNA is a compact molecule which looks like an inverted L.

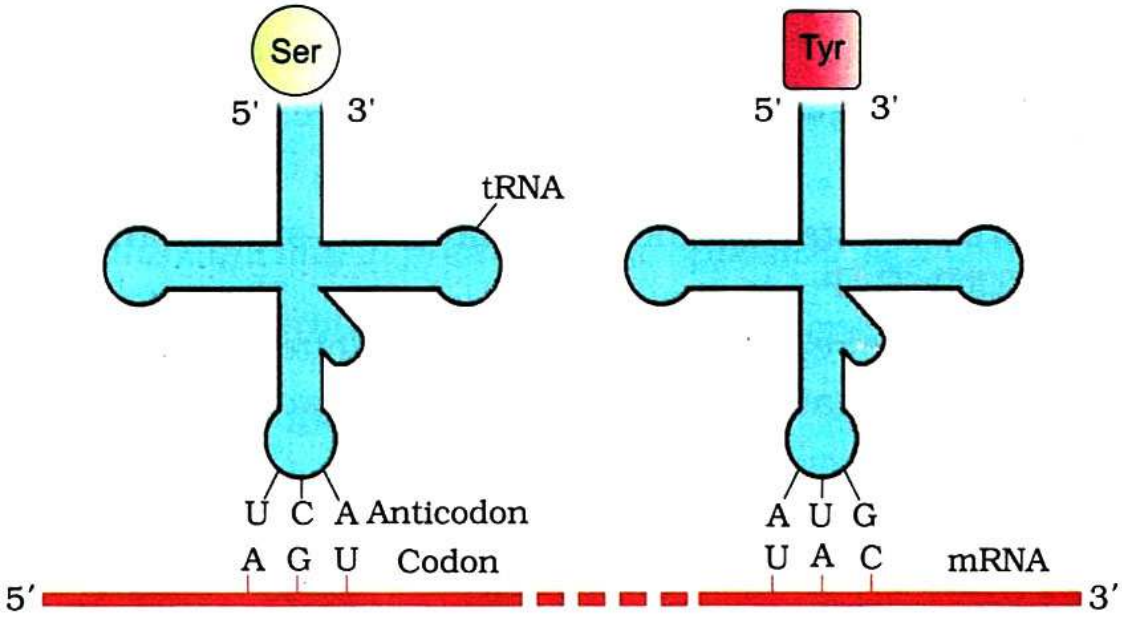


Figure 10.8 t RNA – the adapter molecule

10.7 Translation

Translation refers to the process of polymerisation of amino acids to form a polypeptide chain (Figure 10. 16). The order and sequence of amino acids are defined by the sequence of bases in the mRNA. The amino acids are joined by a bond which is known as a peptide bond. Formation of a peptide bond requires energy. Therefore, in the first phase itself, amino acids are activated in the presence of ATP and linked to their cognate t RNA- a process commonly called as **charging of t RNA** or **aminoacylation of t RNA**, to be more specific. If

two such charged tRNAs are brought close enough , the formation of a peptide bond between them would be favoured energetically . The presence of a catalyst would enhance the rate of peptide bond formation.

The cellular factory responsible for synthesising proteins is the ribosome. The ribosome consists of structural RNAs and about 80 different proteins. In its inactive state, it exists as two subunits; a large subunit and a small subunit. When the small subunit encounters an mRNA, the process of translation of the mRNA to protein begins. There are two sites in the large subunit for subsequent amino acids bind to and thus, be close enough to each other for the formation of a peptide bond. The ribosome also acts as a catalyst (23S rRNA in bacteria is the enzyme- ribosome) for the formation of peptide bond.

A translational unit in mRNA is the sequence of RNA that is flanked by the start codon (AUG) and the stop codon and codes for a polypeptide . An m RNA also has some additional sequences that are not translated and are referred to as **untranslated regions (UTR**). The UTRs are present at both 5' – end (before start codon) and at 3' – end (after stop codon). They are required for efficient translation process.

For, initiation the ribosome binds to the mRNA at the start codon (AUG) that is recognised only by the initiater tRNA. The ribosome proceeds to the elongation phase of protein synthesis. During this stage , complexes composed of an amino acid linked to tRNA sequentially bind to the appropriate codon in mRNA by forming complimentary base pairs with the tRNA anticodon. The ribosome moves from codon to codon along the mRNA. Aminoacids are added one by one , translated into polypeptide sequences dedicated by DNA and represented by m RNA. At the end, **a release factor** binds to the Stop codon, terminating translation and releasing the complete polypeptide from the ribosome.

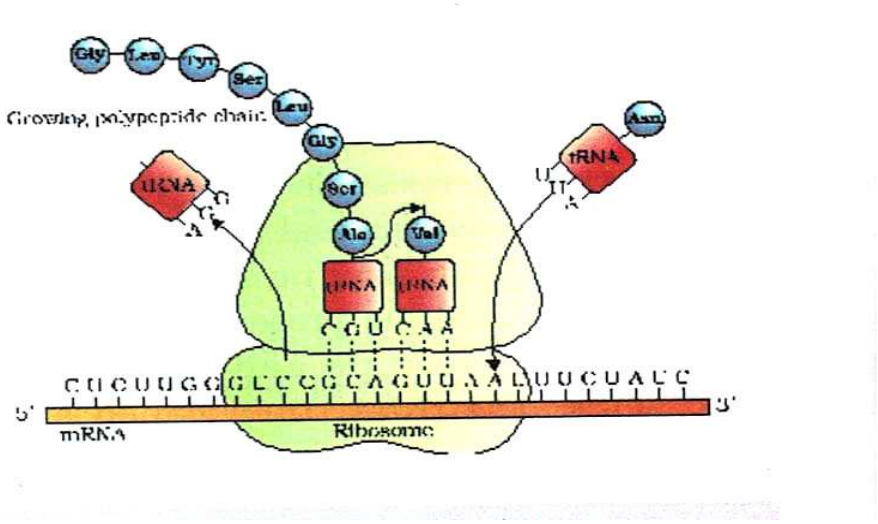


Figure 10.9 Translation

Summary

Nucleic acids are long polymers of nucleotides. While DNA stores genetic information, RNA mostly helps in transfer and expression of information. Though DNA and RNA both function as genetic material. However, RNA was the first to evolve and DNA was derived from RNA. The hallmark of the double stranded helical structure of the DNA is the hydrogen bonding between the bases from opposite strands. The rule is that Adenine pairs with Thymine through two H- bonds, and Guanine with Cytosine through three H-bonds. This makes one strand complimentary to the other. The DNA replicates semiconservatively, the process is guided by the complimentary H-bonding. A segment of DNA that codes for RNA may, the strands of DNA acts as a template to direct the synthesis of complimentary RNA. In, bacteria, the transcribed mRNA is functional, coding sequences, exons, are interrupted by non-coding sequences, Introns. Introns are removed and exons are joined to produce functional RNA by a process called splicing. The messenger RNA contains the base sequences that are read in a combination of three (to make triplet genetic code) to code for an amino acid. The genetic code is read again on the principle of complementarity by t RNA that acts as an adapter molecule. There are specific tRNAs for every amino acid. The tRNA binds to specific amino acid at one end and pairs through H-bonding with codes on mRNA through its anticodons. The site of translation (protein synthesis) is ribosomes, which bind to mRNA and provide a platform for joining of amino acids. One of the rRNA acts as a catalyst for peptide bond formation, which is an example of RNA as an enzyme (ribozyme). Translation is a process that has evolved around RNA, indicating that life began around RNA. Since, transcription and translation are energetically very expensive processes, these have to be tightly regulated.

Glossary

Anticodon: A sequence of adjacent nucleotides in tRNA that is complimentary to the codon in messenger RNA which specifies the amino acid.

Complimentarity: It is the property shared by two nitrogenous bases or two polynucleotide strands which when positioned opposite to each other, can pair by forming hydrogen bonds between A & T or U and between G &C.

Double helix: It is the second order structure formed by two complimentary deoxy polynucleotide chains twisted around each other on mutually opposite orientation and held together by hydrogen bonds.

Genetic code: The set of all possible three- nucleotide combinations in DNA/ mRNA that determine the specific amino acid sequence in a polypeptide chain.

Genomics: It is the field of study dealing with determination of the nucleotide sequence of the genome and its analysis using computer programmes.

Histones: Histones are the chief protein components of chromatin in eukaryotic cells, acting as spools around which DNA winds, and play a role in gene regulation.

hnRNA: Heterogenous nuclear RNA found in the nucleus which becomes mRNA after undergoing processing or maturation.

mRNA: It is the form of RNA that carries information from DNA to the ribosomes and functions as the template for protein synthesis.

Nucleosome : It is the basic unit of DNA pscking into chromatin and consists of a segment of DNA (200 bp) wound around a protein core of 8 histone molecules.

Promoter: It is region of DNA where RNA polymerase binds and initiates transcription.

Replication Fork: It is a site on a DNA double helix where both unwinding of the helix and synthesis of daughter molecules occur.

Nucleosome : It is a group of closely placed structural genes

Questions

Very Short Answer Questions

1. Distinguish between heterochromatin and euchromatin.
2. What is the function of DNA polymerase?
3. Name any Two Viruses which have RNA as a genetic material.
4. What are the components of a nucleotide?
5. What are the components of a transcription unit?
6. What is the difference between exons and introns?
7. What is meant by capping and tailining?
8. What is the function of the codon-AUG?
9. Define stop codon. Write the codons.

Short Answer Questions

1. Write briefly about DNA polymerase.
2. On the diagram of the secondary structure of tRNA shown below indicate the location of the following features:
   a) Anticodon b) Accepter stem c)Anticodon stem d) 5’ end e) 3’ end
3. What are the differences between DNA and RNA.
4. Write the important features of Genetic code?

Excersises

1. Group the following as Nitrogen bases and nucleosides: Adenine, Cytidine, Thymine, Guanosine, Urail and cytosine.

2. If a double stranded DNA has 20 percent of cytosine, calculate the percent of adenine in the DNA.

UNIT V

BIOTECHNOLOGY

Chapter 11 : Biotechnology : Principles and processes

Chapter 12 : Biotechnology and it's applications

Ever since the day of Rene Descartes, the French philosopher, mathematician and biologist of the seventeenth century, all human knowledge, especially naturally natural sciences, were directed to develop technologies which add to the comforts and welfare of human lives. The whole approach to understanding natural phenomena became anthropocentric. Physics and chemistry gave rise to engineering, technologies and industries which all worked for human comfort and welfare. The major utility of the biological world is a source of food , Biological world is as a source of food. Biotechnology, the twentieth century off-shoot of modern biology, changed our daily life as its products brought qualitative improvement in health and food production. The basic principles underlying biotechnological processes and some applications are highlighted and discussed in this unit.

Chapter 11

Biotechnology:

Principles and processes

Biotechnology deals with techniques of using live organisms or enzymes from organisms to produce products and processes useful to humans. In this sense, making curd, bread or wine, which are all microbe-mediated processes, could also be considered as a form of biotechnology. However, the term is used in a restricted sense today, to refer to such of those processes which are genetically modified organisms to produce products useful to human beings on a large scale. Further, many other processes/ techniques are also included under biotechnology. For, example in vitro fertilisation leading to a 'test-tube' baby. Synthesizing a gene and using it, developing a DNA vaccine or correcting a defective gene, are all part of biotechnology.

The European Federation of Biotechnology (EFB) has given a definition of biotechnology that combines both the traditional view and the modern molecular emphasis. The definition given by EFB is as follows:

The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services. This means biotechnology is a science which utilizes properties & uses of micro - organisms or exploits cells and the cell constituents at the industrial level for generating useful products essential to life and human welfare.

11.1 Principles of Biotechnology

The two core techniques that enabled birth of modern biotechnology are:

(i) **Genetic engineering**: This includes techniques to alter the chemistry of genetic material (DNA and RNA), to introduce this material into host organisms and thus change the phenotype of the host organism.

(ii) **Tissue culture**: This involves the growth of only the desired microbe/ eukaryotic cell in large quantities for the manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc. A sterile (microbial contamination- free) environment in chemical engineering processes is essential for this.

The conceptual development of the principles of genetic engineering

sexual reproduction provides opportunities for variations and re-combinations of genes, some of which may be beneficial to the organism as well as the population. Asexual reproduction preserves genetic information, while sexual reproduction permits variation. Traditional hybridisation procedures used in plant and animal breeding very often lead to inclusion and multiplication of undesirable genes, along with the desired genes. The techniques of genetic engineering which include creation of recombinant DNA, use of gene cloning and gene transfer, overcome this limitation and allow us to isolate and introduce only one or a set of desirable genes without introducing undesirable genes into the target organisms.

A piece of DNA, which is somehow transferred into an alien organism, would not able to multiply itself in the progeny cells of the organism. But, when it gets integrated into the genome of the recipient, it may multiply and be inherited along with the host DNA. This is because the alien piece of DNA has become part of a chromosome, which has the ability to replicate. In a chromosome there is a specific DNA sequence called the origin of replication, which is responsible for initiating replicate. Therefore, multiplication of the alien piece of DNA in an organism is not possible unless it becomes a part of chromosome(s) which has a specific sequence known as ‘origin of replication’. Thus, an alien DNA is linked with the origin of replication. So, that this, alien piece of DNA can replicate and multiply itself in the host organism. This process is also called cloning or making multiple identical copies of any template DNA.

Construction of the first artificial recombinant DNA molecule

The first recombinant DNA was constructed by linking a gene encoding antibiotic resistance with a native plasmid (autonomously replicating circular extra-chromosomal DNA) of *Salmonella typhimurium*, Stanley Cohen and Herbert Boyer accomplished this in 1972 by isolating the antibiotic resistance gene by cutting out a piece of DNA from a plasmid which can responsible for conferring antibiotic resistance. The cutting of DNA at specific locations became possible with the discovery of the so-called 'molecular scissors'- restriction enzymes. The cut piece of DNA was then linked with the plasmid DNA. These plasmid DNAs act as vectors to transfer the piece of DNA attached to it. A plasmid can be used as vector to deliver an alien piece of DNA into the host organism. The linking of antibiotic resistance gene with the plasmid vector became possible with the enzyme DNA ligase, which acts on cut DNA molecules and joins their ends. This makes a new combination of circular autonomously replicating DNA created in vitro and is known as recombinant DNA. When this DNA is transferred into *Escherichia coli,* a bacterium closely related to *Salmonella*, it could replicate using the new host's DNA polymerase enzyme and make multiple copies. The ability to multiply copies of antibiotic resistance gene in E. coli was called cloning of antibiotic resistance gene in E. coli.

There are three basic steps in genetically modifying an organism—

(i) Identification of DNA with desirable genes
(ii) Introduction of the identified DNA into the host
(iii) Maintenance of introduced DNA in the host and transfer of the DNA to its progeny.

11.2 Tools of Recombinant DNA Technology

Genetic engineering or recombinant DNA technology can be accomplished only if we have the key tools. i.e., restriction enzymes, polymerase enzymes, ligases, vectors and the host organism.

11.2. 1 Restriction Enzymes

In the year 1963, the two enzymes responsible for restricting the growth of bacteriophage in Escherichia coli were isolated. One of these added methyl groups to DNA, while the other cut DNA. The latter was called restriction endonuclease.

The first restriction endonuclease - Hind II, whose functioning depended on a specific DNA nucleotide sequences was isolated and characterised later. It was found that Hind II always cut DNA molecules at a particular point by recognising a specific sequence of six base pairs. This specific base sequence is known as the recognition sequence for Hind II. Besides Hind II, we know more than 900 restriction enzymes that have been isolated from over 230 strains of bacteria, each of which recognises a different recognition sequence.

The convention for naming these enzymes is that the first letter of the name comes from the genus and the second two letters come from the species of the prokaryotic cell from which they were isolated, e.g., EcoRI comes from Escherichia coliRY13. In EcoRI, the letter 'R' is derived from the name of strain, Roman numbers following the names indicate the order in which the enzymes were isolated from that the strains of bacteria.

Restriction enzymes belong to a large class of enzymes called nucleases. These are of two kinds; exonucleases and endonucleases. Exonucleases remove nucleotides from the ends of the DNA where as endonucleases make cuts at specific positions within the DNA. Each restriction endonuclease functions by 'inspecting' the length of a DNA sequence. Once it finds its specific recognition sequence, it binds to the DNA and cuts each of the two strands of the double helix at specific points in their sugar-phosphate backbones (Figure 11.1). Each restriction endonuclease recognises a specific palindromic nucleotide sequence in the DNA.

Palindromes are group of letters that form the same words when read in both forward and backward directions. For examples, 'MALAYALAM'. The palindrome in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept the same. For example 'the following sequences read the same on the two strands in 5'----- 3' direction. This is also true if read in the 3'---------5' direction.

5' —— GAATTC —— 3'

3' —— CTTAAG —— 5'

commonly most restriction enzymes cut the two strands of DNA double helix at different locations. Such cleavage is generally termed as **Staggered cut.**

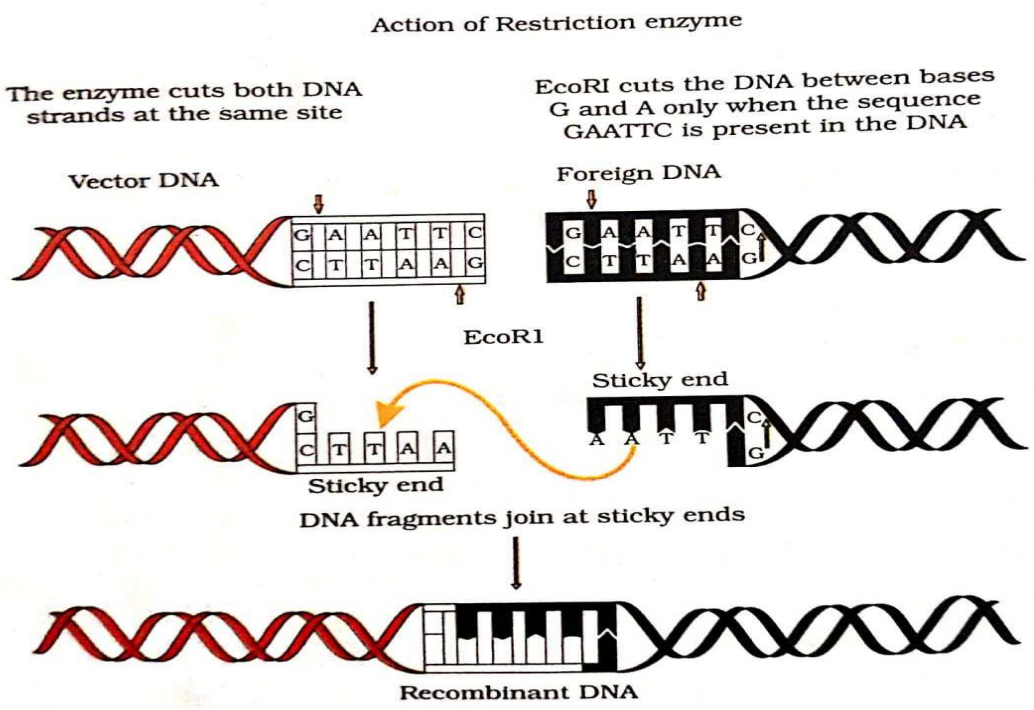


Figure 11.1 Steps in the formation of recombinant DNA by the action of restriction endonuclease enzyme-EcoRI

Restriction enzymes cut the strands of DNA a little away from the centre of the palindrome sites, but between the same two bases on the opposite strands. EcoRI recognises 5'GAATTC3' sites on the DNA and cuts it between G and A. This leaves single stranded portions at the ends. These overhanging stretches are called sticky ends or cohesive ends (Figure 11.1). These are named so because they form hydrogen bonds with their complementary cut counter parts. This stickiness of the ends facilitates the action of the enzyme DNA ligase.

Restriction endonucleases are used in genetic engineering to form 'recombinant' molecule of DNA , which are composed of DNA from different sources/genomes.

When cut by the same restriction enzyme , the recombinant DNA fragments have the same kind of 'sticy-ends' and these can be joined together (end-to- end) using DNA ligases (Figure 11.2).

Which chemical bonds of DNA are cut by restriction endonucleases? Which are formed by DNA ligases?

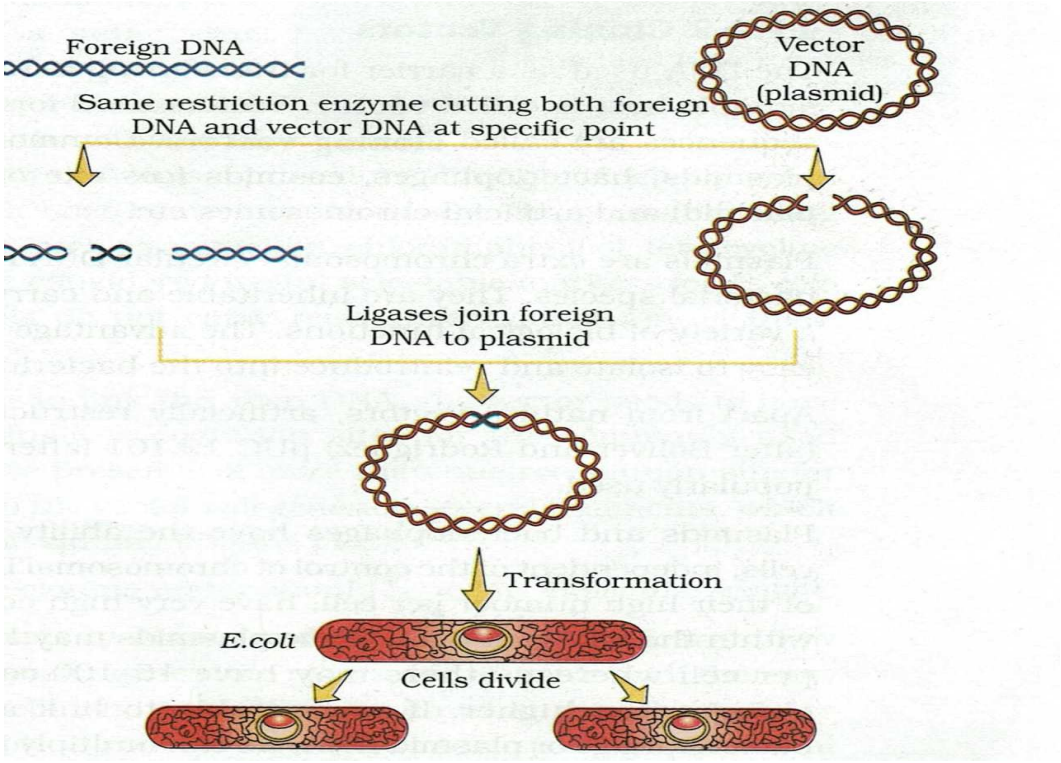


Figure 11.2 Diagramatic representation of recombinant DNA technology

11.2.2 Cloning vectors

The DNA is used as a carrier for transferring a fragment of foreign DNaA into a suitablehost is called vector. Vectors used for multiplying the foreign DNA sequences are called cloning Vectors. Commonly used cloning vectors are called cloning vectors. Commonly used cloning vectors are Plasmids, bacteriophases, cosmids (cos site of phase incorporated into a plasmid) and artificial chromosomes etc.

Plasmids are extra chromosomal circular DNA molecules are found in almost all bacterial species. They are inheritable and carry few genes which determine a variety of biological functions. The advantage of plasmid is that it is very easy to isolate and reintroduce into the bacterium (host).

Apart from natural vectors, artificially restructured plasmids like $P^{BR\ 322}$(after Boliver and Rodriguez) $P^{UC\ 19,\ 101}$ (after University of California) are popularly used.

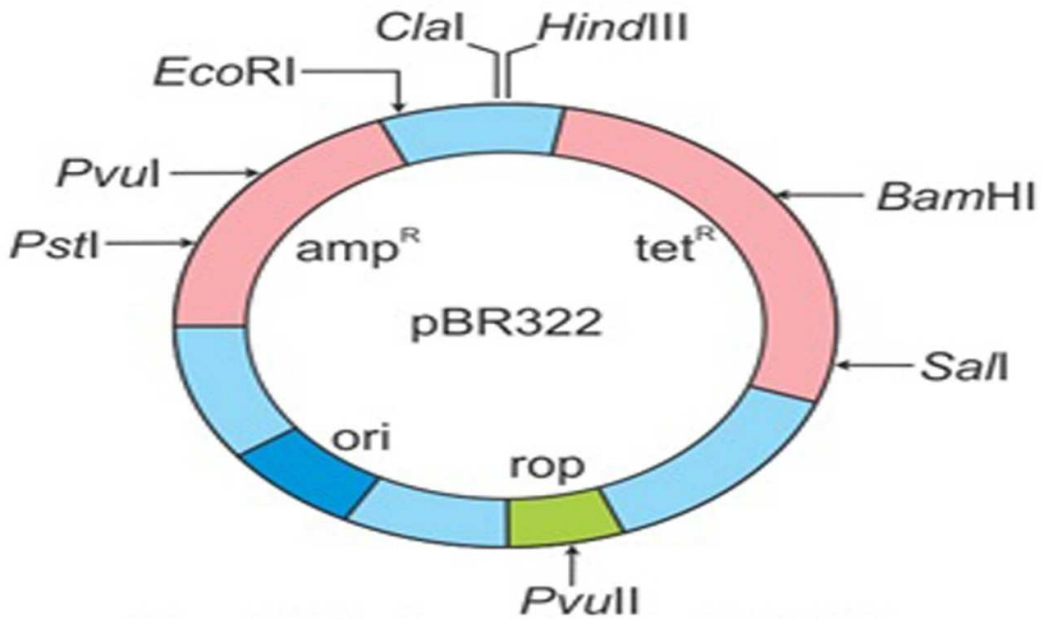


Figure 11.3 E.oli Cloning vector PBR 322 Showing restriction sites (Hind III, EcoR I, BamH I, Sal I, Pvu II, Pst I, ClaI) ori and antibiotic resistance genes (amp ^{R} and tet ^{R}). rop code for the proteins involved in the replication of the plasmid.

Plasmids and Bacteriophases have the ability to replicate within bacterial cells, some plasmids may have only one or two copies per cell where as others may have 15-100 copies per cell. Their numbers can go even higher. If we are able to link an alien piece of DNA with bacteriophage or plasmid DNA, we can multiply its numbers equal to the copy number of the plasmid or bacteriophage. Vectors used at present are engineered in such a way that they help easy linking of foreign DNA and selection of recombinants from non-recombinants.

The following are the features that are required to facilitate cloning into a vector.

i. It must have low molecular weight
ii. It must have a unique cleavage site for the activity of restriction enzymes at single point
iii. It must be able to replicate truly inside a host cell after its introduction (through ori gene-origin of replication)

iv. It must contain genes which provide resistance to antibiotics (tet R for tetracycline resistance, amp R for ampicillin resistance etc). These help in selection of transformed cells from untransfomed cells.

11.2.3 Competent Host (For Transformation with Recombinant DNA)

Since DNA is a hydrophilic molecule, it cannot pass through cell membranes. In order to force bacteria to take up the plasmid, the bacterial cells must first be made 'Competent' to take up DNA. This is done by treating them with a specific concentration of a divalent cation, such as Calcium, which increases the efficiency with which DNA enters the bacterium through pores in its cell wall.

11.3 Processes of Recombinant DNA Technology

Recombinant DNA technology involves several steps in specific sequence such as isolation of DNA, fragmentation of DNA by restriction endonucleases, isolation of a desired DNA fragment, ligation of the DNA fragment into a vector, transferring the recombinant DNA into the host, culturing the host cells in a medium at large scale and extraction of the desired product. Let us examine each of these steps in some detail.

11.3.1 Isolation of the genetic material (DNA)

Nucleic acid is the genetic material of all organisms without exception. In the majority of organisms this is in the form of deoxyribonucleic acid or DNA. IN order to cut the DNA with restriction enzymes, DNA needs to be in a pure form, free from other macro-molecules. Since the DNA is enclosed within the membranes, we have to break the cell open to release DNA along with other macromolecules such as RNA, proteins, polysaccharides and also lipids. The cell wall is digested by treating the bacterial cells /plant tissue with enzymes such as lysozyme (bacteria), cellulase (plant cells), chitinase (fungus) etc. This is followed by the dissolution of all the biological membranes within a cell b detergent lysis (using high power detergents). We know that genes are located on long molecules of DNA interwined with proteins such as histones. The RNA can be removed by treatment with ribonuclease where as proteins can be removed by treatment with protease. Other molecules can be removed by appropriate treatments. Purified DNA ultimately precipitates out after the addition of chilled ethanol. This can be seen as a collection of fine threads in the suspension. DNA that separates out can be removed by spooling.

11.3.2 Cutting of DNA at specific locations

The purified DNA cut into number of fragments by restriction endonucleases. The process of cutting DNA with restriction enzymes is called restriction enzyme digestion. Agarose gel electrophoresis is employed to check the progression of restriction enzyme digestion. The process is repeated with the vector DNA also.

Separation and isolation of DNA fragments

The cutting of DNA by restriction endonucleases results in the fragments of DNA. These fragments can be separated by a technique known as Gel electrophoresis. The DNA fragments separate (resolve) according to their size through the sieving effect provided by the agarose gel. Hence, the smaller the fragment size, the farther away it moves (Figure 11.4).

The separated DNA fragments can be visualised only after staining the DNA with the compound known ethidium bromide followed by exposure to UV raditation (we can not see pure DNA fragments in the visible light and without staining). We can see bright orange coloured bands of DNA in an ethidium bromide stained gel exposed to UV light (Figure 11.5). The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece. This step is known as elution. The DNA fragments purified in this way are used in constructing recombinant DNA by joining them with cloning vectors.

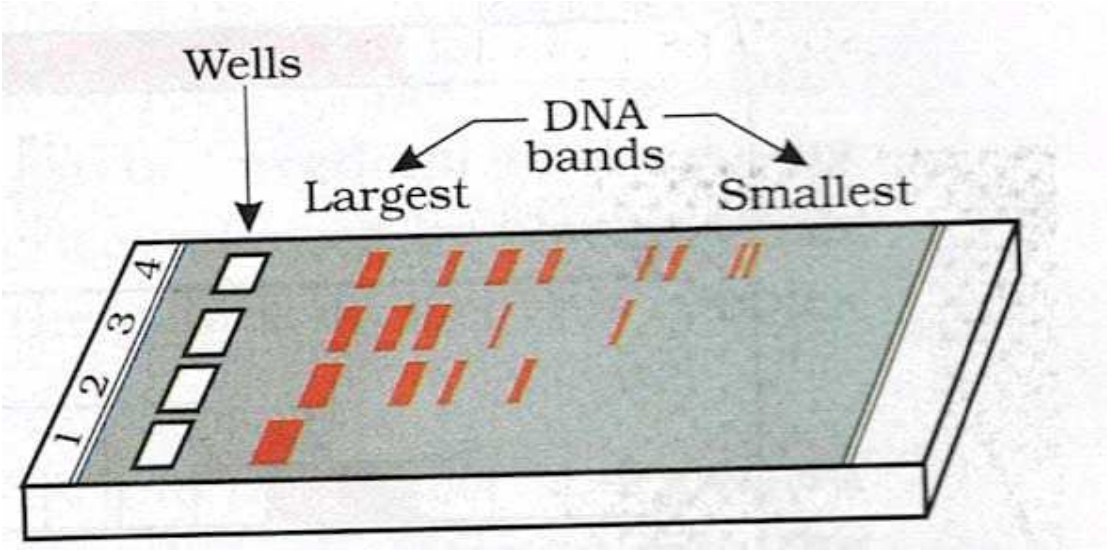


Figure 11.4 A typical agarose gel electrophorosis showing migration of undigested (lane 1) and digested set of DNA fragments (lane 2 to 4)

Insertion of isolated gene into a suitable vector

To isolate a plasmid the bacterial cell is treated with lysozyme to digest the cell wall. Then the bacterial cell is subjected to centrifugation to separate the plasmid.

The joining of DNA involves several processes. After having cut the source DNA as well as the vector DNA with the same restriction enzyme (sticky end ligation technique), the cut 'gene of interest' from the source DNA and the cut vector are mixed and ligase is added. This results in the formation of recombinant DNA (rDNA) or chimaeric DNA.

11.3.3 Amplification of Gene of Interest using PCR

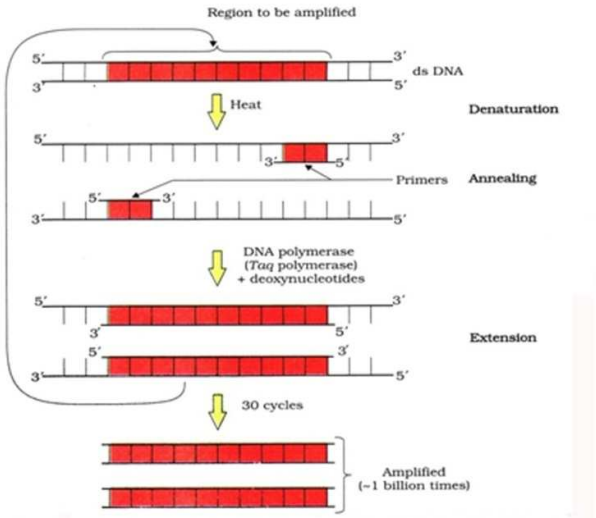


Figure 11.5 Polymerase chain reaction (PCR) : Each cycle has three steps: (i) Denaturation; (ii) Primer annealing; and (iii) Extension of primers

PCR stands for Polymerase Chain Reaction. In this reaction, multiple copies of the gene (or DNA) of interest are synthesised in vitro using two sets of primers (small chemically synthesised oligonucleotides that are complementary to the regions of DNA) and the enzyme DNA polymerase. The enzyme extends the primers, using the nucleotides provided in the reaction and the genomic DNA as template. If the process of replication of DNA is repeated many times, the segment of DNA can be amplified to approximately billion times, i.e., 1 billion copies are made. Such repeated amplification is achieved by the use of a thermostable DNA polymerase such as Taq polymerase (isolated from a bacterium, Thermus aquaticus), which remain active even during the high temperature induced denaturation of double stranded DNA. The amplified fragment, if desired, can now be used to ligate with a vector for further cloning (Figure 11.5).

DNA fingerprint is the pattern of DNA fragments on the gel. Gene amplification is one technique for DNA finger printing.

11.3.4 Insertion of recombinant DNA into the Host Cell/ Organism

The recombinant plasmid or vector obtained is transferred into a suitable bacterial host cell (generally E.coli), by a method known as transformation for the expression of the desired gene. The cells into which the recombinant DNA are inserted are called ‘transformed cells’.

Bacterial cell walls are or not ordinarily permeable to such recombinant vectors, but keeping in dilute solution of calcium chloride renders the bacterial cell wall permeable to the recombinant vectors. Inside the host, these recombinant DNA starts replicating. The transformed cell will begin to grow (on medium) and divide as separate units. The replicated vectors in each cell are passed on to daughter cells giving rise to clones (genetically homogenous population of cells derived from a single cell).

Selection of the transformed host cells :

Depending upon the gene incorporated into the transformed cell, selection of the recombinant clones can be done by two ways: A. Without using probes, B. By using probes (colony hybridization assay).

A. Without using probes:- For example, if antibiotic resistant gene is being cloned the transformed cells are first incubated in a medium without the antibiotic for about an hour, to allow the antibiotic resistant genes to be expressed. Later, these cells are transferred to a medium containing antibiotic. The cells which have expressed the gene will survive and the others die.

B. By Using probes:- When transformed cells are cultured on the nutrient medium, several thousands of cells are produced. All the cells in the culture may not contain the desired gene. In order to select the cells containing the desired gene a method called colony hybridization is employed. In this gene specific probes are used. A Probe is a small fragment of single stranded DNA or RNA which is tagged with a radioactive molecule and is complementary at atleast on one part of desiresd DNA. So that this can search out or locate complimentary DNA sequences from an organism.

11.3.5 Obtaining the Foreign Gene Product

Selected transformed cells must be produced commercially in large quantities. Bio reactors are used for this. In them from one side culture will be added and from other side products will be removed. E.x: Stirring type bio-reactors.

11.3.6 Downstream Processing

After completion of the biosynthetic stage, the product has to be subjected through a series of processes before it is ready for marketing as a finished product. The processes include separation and purification. Which are collectively referred to as downstream processing. The product has to be formulated with suitable preservatives. The formulation has to undergo through clinical trails as in the case of drugs. Strict quality control testing testing for each product is also required. The downstream processing and quality control testing vary from product to product.

SUMMARY

Biotechnology deals with large scale production and marketing of products and processes using live organisms, cells of enzymes. Modern biotechnology using genetically modified organisms was made possible only when man learnt to alter the DNA sequences and construct novel DNA. This key process is called recombinant DNA technology or genetic engineering. This process involves the use of restriction endonucleases, DNA ligase, appropriate plasmid or viral vectors to isolate and ferry the foreign DNA into host

organisms, expression of the foreign gene, purification of the gene product i.e., the functional protein and finally making a suitable formulation for marketing. Large scale production involves use of Bioreactors.

GLOSSARY

Annealing: It is a step in amplification of gene of interest in which two oligonucleotide primers hybridize to each of single stranded template DNA.

Artificial chromosome vectors: These are linear vectors constructed by adding centromere , telomere and origin of replication sites to a DNA fragment.

Bio-reactors: Are large vessels which are used for biological convertion of raw material into specific products.

Competent host: Microbial cells which are capable of taking in DNA molecules from outside and there by undergo transformation.

Culture medium: Consists of nutrients prepared for microbial growth in laboratory.

Questions

Very short Answer Type Questions

1. What are molecular scissors?

2. What is EcoRI?

3. What are cloning Vectors?

4. What is recombinant DNA?

5. What is palindromic sequence?

6. What is the full form of PCR?

7. How it is useful in biotechnology?

8. How can you differentiate between exonucleases and endonucleases?

Short Answer Type Questions

1. Write short notes on restriction enzymes.

2. What are the different methods of insertion of recombinant DNA into the host cell?

EXCERCISES

1. Do eukaryotic cells have restriction endonucleases? Justify your answer.

2. Can you recall meiosis and indicate at what stage a recombinant DNA is made?

CHAPTER 12

Biotechnology and its applications

Biotechnology essentially deals with industrial scale production of pharmaceuticals and biological using genetically modified microbes, fungi, plants and animals. The applications of biotechnology includes therapeutics , diagnostics, genetically modified crops for agriculture, processed food, bioremediation, waste treatment, and energy production. Three critical research areas of biotechnology are:

(i) Providing the best catalyst in the form of improved organism, usually a microbe or pure enzyme.
(ii) Creating optimal conditions through engineering for a catalyst to act, and
(iii) Downstream processing technologies to purify the protein/ organic compound.

Let us now learn how human beings have used biotechnology to improve the quality of human life , especially in the field of food production and health.

12.1 Biotechnological Applications In Agriculture

There are three options that can be used for increasing food production

(i) Agro- chemical based agriculture
(ii) Organic agriculture
(iii) Genetically engineered crop-based agriculture

Around 1960's several countries including India experienced substantial and dramatic increase in agricultural production which was termed as green revolution by William Gaud

(1968), the then director of United States Agency for International Development (USAID). Norman Borlaug to whom the credit of initiating efforts that led to green revolution, is regarded as "Father of Green Revolution". Dr. M.S. Swaminathan and his team were instrumental for the success of green revolution in our country. Green revolution was possible due to a number of factors both economical and scientific, and include use of improved varieties, chemical fertilizers and pesticides, improved irrigational facilities, adoption of better agricultural management strategies, land reforms etc.

Even though Green revolution succeeded in tripling the food supply, it was not enough to feed the growing human population. Increased yields have partly been due to the use of improved crop varieties, but mainly due to the use of better management practices and use of agrochemicals (fertilizers and pesticides). However, for farmers in the developing world, agrochemicals are too expensive, and they show harmful effects on the environment. Further increases in yield with existing varieties are not possible using conventional breeding. Use of genetically modified crops is a possible solution.

With the advent of biotechnology, especially the genetic engineering, a new ray of hope to increase food production and to reduce the use of chemical fertilizers and pesticides has been envisaged which might lead to other type of revolution, the Gene revolution. With the help of genetic engineering, it is possible to break through natural species barriers and systematically transfer genes from one species to another that may not be possible in nature. The gene revolution is likely to provide new plants that would lead to a more environmentally sound agricultural production.

Plants,bacteria, fungi and animals whose genes have been altered by manipulation are called genetically Modified Organisms (GMO). GM plants are useful in many ways. In addition to the production of high yielding and disease resistant varieties, genetic modification has:

(i) Made crops more tolerant to antibiotic stresses (Cold, drought, salt, heat)

(ii) Reduced reliance on chemical pesticides (pest-resistant crops), e.g Bt cotton

(iii) Helped to reduce post harvest losses

(iv) Increased efficiency of mineral usage by plants (this prevents early exhaustion of Fertility of soil)

(v) Enhanced nutritional value of food, e.g., Vitamin A enriched rice.

In addition to these uses, genetic engineering has been used to create tailor made plants to supply alternative resources to industries, in the form of starches, fuels and pharmaceuticals.

Some of the applications of biotechnology in agriculture are the production of pest resistant plants, which could decrease the amount of pesticide used. Bt toxin is produced by a bacterium called Bacillus thuringiensis(Bt for short). Bt toxin gene has been cloned from this bacterium and has been expressed in plants to provide resistance to insects without the need for insecticides: in effect created a bio- pesticide . Examples are Bt cotton, Bt corn, rice, tomato, potato and soyabean etc.

Bt cotton: Some strains of Bacillus thuringiensis produce proteins that kill certain insects such as lepidepterans (tobacco budworm, army worm),coleopterons (beetles) and dipterans(flies, mosquitoes). B. thuringiensis forms protein crystals during a particular phase of growth. These crystals contain a toxic insecticidal protein.

Why does this toxin not kill the Bacillus ?

Actually, the Bt toxin proteins exists as inactive protoxins but once an insect ingests the inactive toxin, it is converted into an active form of toxin due to the alkaline PH of the gut which solubilises the crystals . The activated toxin binds to the surface of of midgut epithelial cells and creates pores that cause cell swelling and lysis and eventually cause the death of the insect.

Specific Bt toxin genes were isolated from Bacillus thuringiensis and incorporated into several crop plants such as cotton (Figure12.1). The choice of genes depends upon the crop and targeted pest, as most Bt toxins are insect group specific. The toxin is coded by a gene named as 'cry'. There are number of them, for example, the proteins encoded by the genes cryIAc and cryIIAb control the cotton bollworms, while that of cryIAb controls corn borer.

Figure 12.1 Cotton boll: (a) destroyed by bollworms: (b) a fully mature cotton boll

12.2 Other Applications Biotechnology

The recombinant DNA technological processes have made immense impact in the area of health care by enabling mass production of safe and more effective therapeutic drugs. Cloned DNAs are utilised in the commercial synthesis of hormones like insulin, interferons and vaccines, antibiotics and other commercial chemicals at lower costs. Further, recombinant therapeutics do not induce unwanted immunological responses as is common in case of similar products isolated from non-human sources. At present, about30 recombinant therapeutics have been approved for human-use the world over. In India, 12 of these are presently being marketed.

Insulin used for diabetes was earlier extracted from pancreas of slaughtered cattle and pigs. Human insulin is now made in bacteria. Yet its structure is absolutely identical to that of the natural molecule. In 1983, Eli Lilly, an American company prepared two DNA sequences corresponding to A and B chains of human insulin and introduced them into plasmids of E.coli to produce insulin chains. Chains A and B were produced separately, extracted and combined by creating disulfide binds to form human insulin. The possibility of production through novel method helped to have continuous supply of insulin and stabilization of its market price.

Gene therapy is a corrective therapy to be taken for hereditary diseases. Here gene inserted into a person's cells and tissues to treat a disease. It involves delivery of a normal gene into the individual or embryo to take over the function of and compensate for the non-functional gene.

Molecular Diagnosis: For effective treatment of a disease, early diagnosis and understanding its pathophysiology is very important. Using conventional methods of diagnosis (serum and urine analysis, etc,) early direction is not possible. Recombinant DNA technology, polymerase Chain Reaction(PCR) and Enzyme Linked Immuno-sorbent Assay (ELISA) are some of the techniques that serve the purpose of early diagnosis.

PCR is now routinely used to detect HIV in suspected AIDS patients, It is being used to detect mutations in genes in suspected cancer patients too. It is a powerful technique to identify many other genetic disorders

ELISA is based on the principle of antigen-antibody interaction. Infection by a pathogen can be detected by the presence of antigens or by detecting the antibodies synthesised against the pathogen.

DNA fingerprinting has successfully helped forensic science in the search of criminals and also solving parentage disputes.

12.3 Transgenic Plants

Plants with desirable characters created through gene transfer methods are called transgenic plants. Though the number of methods have been developed to introduce the cloned genes into plant cells. Ti plasmid of *Agrobacterium tumefaciens* has been widely used as an effective vector of obtaining transgenic plants. Several transgenic plants have been produced to meet specific needs of agriculture and human life. Some of these are given below.

A. Transgenic crop plants having resistance to pathogens and pests:

i. Transgenic papaya resistant to papaya ring spot virus

ii. Bt. Cotton is resistant to insects

B. Transgenic plants suitable for food processing technology:

i. Transgenic tomato 'flavr Savr' is bruise resistant i.e., suitable for storage and transport due to delayed ripening and offers longer shelf-life.

C. Transgenic plants useful for hybrid seed production:

i. Transgenic golden rice obtained from 'Taipe' is rich in vitamin A and prevents blindness.

D. Transgenic plants useful for hybrid seed production:

i. Male sterile plants of *Brassica napus* are produced. This will eliminate the problem of manual emasculation and reduce the cost of hybrid seed production.

E. Transgenic plants tolerant to abiotic stresses caused by chemicals, cold, drought, salt, heat etc.

i. Basmati variety of rice was made resistant against biotic and abiotic stresses.

ii. Round up ready soyabean is herbicide tolerant.

Transgenic plants have been shown to express the genes of insulin, interferon, human growth hormones, antibiotics, antibodies etc. These biochemicals produced by plants are as good as or sometimes better than those produced in bacteria.

Utilization of plants as biofactories o bioreactors for obtaining commercially useful products, specialized medicines, chemicals and antibodies on large scale is described as molecular forming. In the near future this field is expected to revolutionise both the farming as well as biochemical industry.

12.4 Bio-safety and Ethical issues

Despite several advantages, genetic modifications of organisms can have unpredictable results when they are introduced into the natural ecosystem. Some of the apprehensions towards bio-safety issue of genetically engineered crops are:

i. There is fear of transferring allergens from genetically modified food to humans and animals.
ii. Due to molecular forming, there is a risk of changing the fundamental nature o vegetables.
iii. There is a risk of gene pollution, which may result in the development of super weeds.
iv. They may bring about changes in natural evolutionary pattern.

Biopiracy is the term coined to refer to the use of Bio-resources by multinational companies and other organisations without proper authorization from the countries and peoples concerned or without compensatory payment.

Most of the industrialized nations rich financially but poor in biodiversity and traditional knowledge. In contrast, the developing and undeveloped world is rich in biodiversity and traditional knowledge related to bio-resources. Traditional knowledge related to bio-resources can be exploited to develop modern applications and can also be used save time, effort and expenditure during their commercialization.

There has been growing realization of the injustice, inadequate compensation and benefit sharing between developed and developing counties. Therefore, some nations are developing laws to prevent such unauthorised exploitation of their bio-resources and traditional knowledge.

The Indian Parliament has recently cleared the second amendment of the Indian Patents Bill, that takes such issues into consideration, including patent terms emergency provisions and research and development initiatives.

SUMMARY

Biotechnology has given humans several useful products by using microbes, plant, animals and their metabolic machinery. Recombinant DNA technology has made it possible to engineer microbes, plants and animals such that they have novel capabilities. Genetically Modified Organisms have been created by transferring one or more genes from one organisms to another using techniques such as recombinant DNA technology.

Such genetically modified plants have been useful in increasing crop yields, reduce post-harvest losses and make crops more tolerant to stresses. There are crop plants with

improved nutritional value of foods and those which reduced the reliance on chemical pesticides.

Recombinant DNA technological processes have made immense impact in the area of healthcare by enabling mass production of safe and more effective therapeutics.

Gene therapy is the insertion of genes into an individual's cells and tissues to treat hereditary diseases. It does so by replacing a defective mutant allele with a functional one.

The current interest in the manipulation of microbes, plants, and animals has raised serious ethical questions about usage and protection of biodiversity.

GLOSSARY

Bio-remediation: The process of using microbes and plants to break down or recycle environmental pollutions.

'Cry' protein: It is a protein toxin produces by Bacillus thuringiensis that kills certain insects.

DNA finger printing: A technique for the identification of individuals based on small differences in DNA fragment patterns detected by electrophoresis.

Enzyme linked immune-sorbent assay (ELISA): A method to detect a very small amounts of specific proteins utilising antibodies linked to enzymes that catalyse the formation of coloured products.

Transgenic plants: A plant that has been altered to contain a gene from a different species.

QUESTIONS

Very Short Answer Type Questions

1. Expand GMO.
2. Give different types of cry genes.
3. Can a disease be defected before its symptoms appear? Explain the principle involved.
4. What is GEAC.

Short Answer Type Questions

1. List out any four beneficial aspects o transgenic plants.
2. Give a brief account of Bt. Cotton.

Exercises

1. What are transgenic bacteria?
2. What are cry proteins?
3. What is meant by the term bio-pesticide?

UNIT VI

PLANTS, MICROBES AND HUMAN WELFARE

Chapter 13 : Strategies for Enhancement in food Production

Chapter 14 : Microbes in Human welfare

Biology is the youngest of the formalised disciplines of natural science. Progress in Physics and Chemistry proceeded much faster than in Biology .Application of physics and chemistry in our daily life also have a higher visibility than those of biology. However, advances made in the twentieth century and twenty- first century have demonstrated the utility of biological knowledge in furthering human welfare, be it in the health sector or in agriculture. The discovery of antibiotics, synthetic plant-derived drugs and anaesthetics have changed medical practice on one hand and human health on the other hand. Life expectancy of human beings has dramatically changed over the years. Agricultural practices, food processing and diagnostics have brought socio-cultural changes in human communities. These are briefly described in the following two chapters of this unit.

Chapter 13

Strategies for Enhancement

In Food production

With the ever-increasing population of the world, enhancement of food production in a major necessity. You already aware of the economic importance of plants (Unit-IV of first year) as sources of food, medicine and other useful products, Biological Principles as applied to animal husbandry and plant breeding have a major role in our efforts to increase food production. Several new techniques like mutation breeding, tissue culture and r-DNA techniques are going to play a pivotal role in further enhancing food production.

13.1 Plant Breeding

Traditional farming can only yield a limited biomass as food for humans and animals. Better management practices and increase in acreage can increase yield, but only to a limited extent. Plant breeding as a technology has helped increase yields to a very large extent. Who in India has not heard of the **Green Revolution**? The Green Revolution made it possible for our country to not merely meet the national requirements in food production but also helped us even to export it. The Green Revolution was dependent to a large extent on plant breeding techniques for development of high-yielding and disease resistant varieties in wheat, rice, maize, etc.

13.1.1 What is Plant Breeding ?

Plant breeding is the purposeful manipulation of plant species in order to create desired plant types that are better suited cultivation, give better yields and are disease resistant. Conventional plant breeding has been practised for thousands of years, since the beginning of human civilisation: recorded evidence of plant breeding dates back 9000-11,000 years. Many present-day crops are derived from domesticated varieties. Classical plant breeding involves crossing or hybridization of pure lines, followed by artificial selection to produce plants with desirable traits of higher yield, nutrition and resistance to diseases. With advancements in genetics, molecular biology and tissue culture. Plant breeding is now increasingly being carried out by using molecular genetic tools.

If we were to list the traits or characters that breeders have tried to incorporate into crop plants, the first we list would be increased crop yield and improved quality. Increased tolerance to insect pests would be on our list too.

Plant breeding programmes are carried out in a systematic way worldwide in government institutions and commercial companies. The main steps in breeding a new genetic variety of a crop are-

(i) Selection of parents: The foremost aspect of hybridisation is to select homozygous plants with desirable characters are possible. In case the desirable characters are present in heterozygous plants, the parents are grown in isolated places and are self-pollinated repeatedly for several generations, in order to bring homozygosity in the desired traits.

(ii) Emasculation : The majority of crop plants have bisexual flowers. Therefore for making crosses normally self-pollinated crops 'emasculation' is a pre-requisite i.e; removal of anthers from bisexual flowers of female parents, when the flowers are still in bud condition is called 'emasculation'. It prevents self pollination.

In case of large flower buds, emasculation is easily performed by opening the flower buds by means of sterilized forceps and fine needle and then removing anthers without causing Injury to other floral parts. In case of small flowers, which are crowded in dense inflorescences as in bajra, jowar etc, emasculation by the whole inflorescence is dipped in

hot water at 45-50^0 C for different periods of 1-10 minutes. The gynoecium can withstand higher temperatures but anthers get killed.

(iii) Bagging: After emasculation is done, the female flower is enclosed in a polythene bag to prevent any other pollen grains falling on the stigamatic surface. Thus bagging prevents the undesired cross pollination.

(iv) Artificial cross pollination: Pollen grains are collected from the male parent with the help of a brush or blotting paper and these are transferred carefully to the surface of the stigma and thus cross pollination is affected artificially. The flowers are immediately enclosed in a polythene bags. Labels carrying the details of the parents, date of pollination etc. are tagged to the plants.

Seeds and fruits are formed after fertilisation. They are collected and preserved. In the next season, these seeds are sown to raise the F1 generation. Self pollination occurs in F1 plants and plants of F2 generation develop. The plants possessing desirable characters are selected and developed by different methods. Seeds are multiplied and finally released to farmers for cultivation.

India is mainly an agricultural country. Agricluture accounts for approximately 33 per cent of India's Gross Domestic Product (GDP) and employs nearly 62 per cent of the population. After independence, one of the main challenges facing the country was that producing enough food for the increasing population. As only limited land is fit for cultivation. India has to strive to increase to increase yields per unit area in existing farm land. The development of several high yielding varieties of wheat and rice in the mid-1960s as a result of various plant breeding techniques led to a dramatic increase in food production in our country. This phase is often referred to as Green Revolution. Figure 13.1 represents some Indian hybrid crops of high yielding varieties.

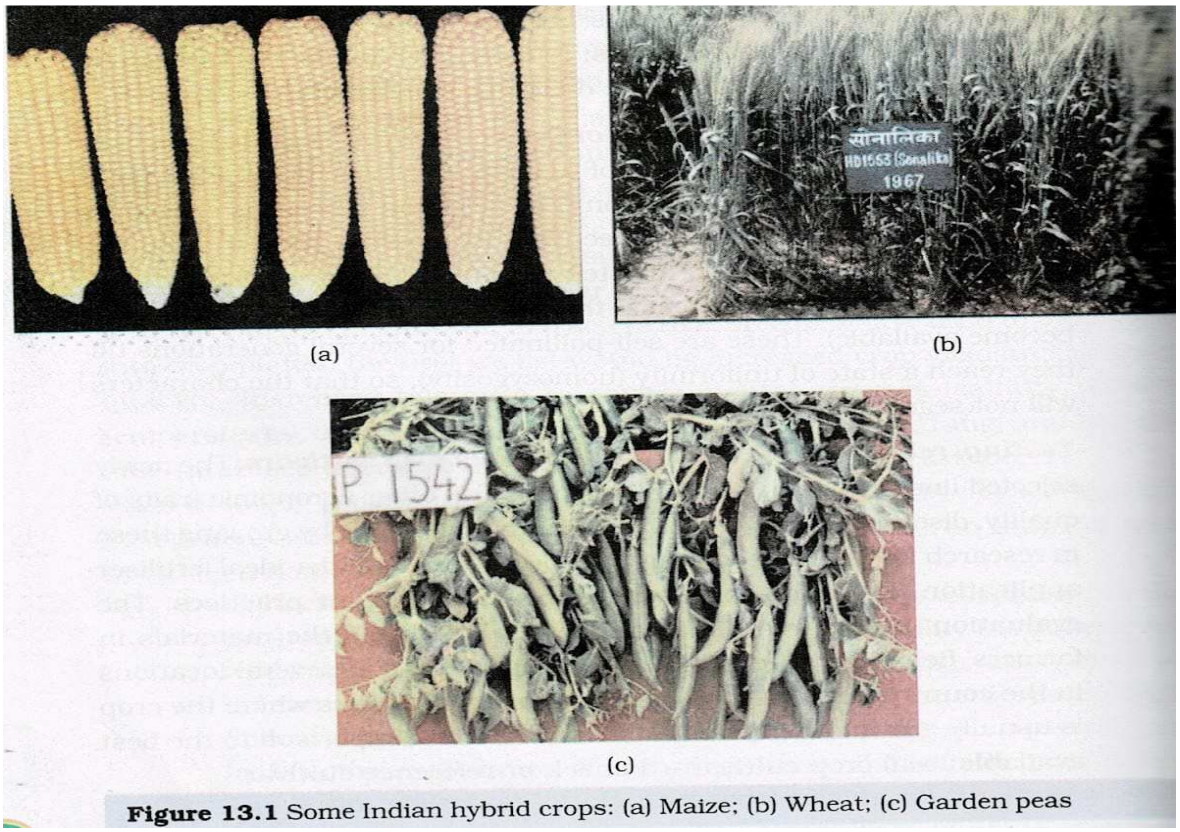


Figure 13.1 Some Indian hybrid crops: (a) Maize; (b) Wheat; (c) Garden peas

Wheat and Rice: During the period 1960 to 2000, wheat production increased from 11 million tonnes to 75 million tonnes while rice production went up from 35 million tonnes to 889.5 million tonnes. This was due to the development of semi-dwarf varieties of wheat and rice. Nobel Laureate Norman E. Borlaug, at International Centre for Wheat and Maize Improvement in Mexico, developed semi-dwarf wheat. In 1963, several varieties such as *Sonalika* and *Kalyan Sona*, which were high yielding and disease resistant , were introduced all over the wheat –growing belt of India. Semi-dwarf rice varieties were derived from IR-8, developed at International Rice Research Institute (IRRI), Philippines) and Taichung Native-1 (from Taiwan). The derivatives were introduced in 1966. Later better-yielding semi-dwarf varieties *Jaya* and *Ratna* were developed in India.

13.2 Single Cell Protein (SCP)

Conventional Agricultural production of cereals, pulses, vegetables, fruits, etc., may not be able to meet the demand the of food at the rate at which human and animal population increasing. The shift from grain to meat diets also creates more demand for cereals as it takes 3-10 kg of grain to produce 1 kg of meat by animal forming.

Can you explain the above statements in the light of your knowledge of food chains?

Table 13.1 some examples of SCP producing organisms

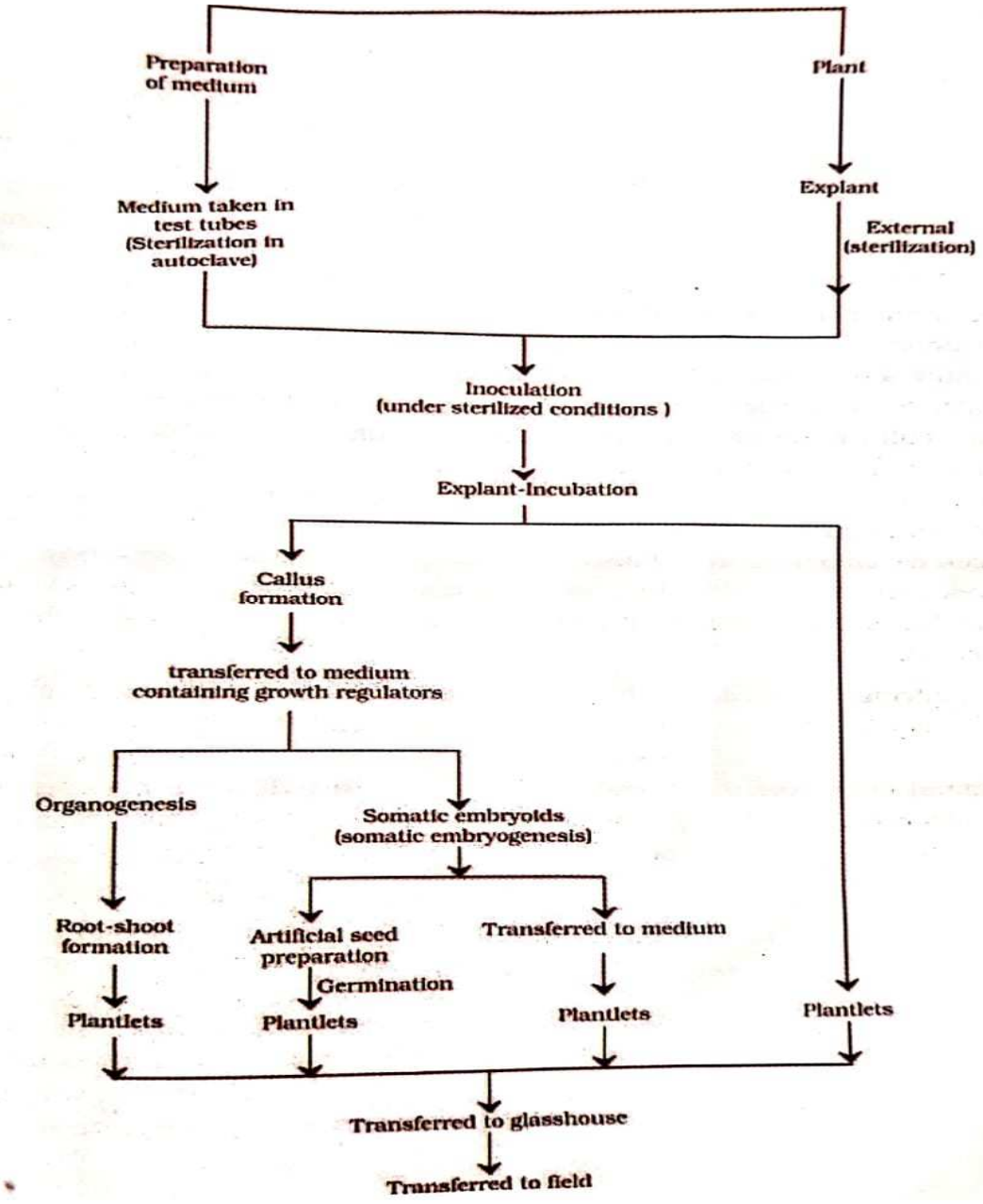
Algae	Fungi	Bacteria
1. Spirulina maxima	1. Candida utilis (Torula yeast)	1. Bravibacterium Ketoglutanicum
2. Chlorella Pyrenoidosa	2. Saccharomyces Cerevisiae (Bakers yeast)	2. Methylophilus Methylotropus
3. Scenedesmus Acutus	3. Chaetomium Cellulolyticum	

More than 25 percent of human population is suffering from hunger and malnutrition. One of the alternate sources of proteins for animal and human nutrition is **Single Cell Protein (SCP).**

13.3 Tissue Culture

As traditional breeding techniques are inadequate to keep pace with demand and to provide sufficiently fast and efficient systems for crop improvement, another technology called tissue culture was developed. What does tissue culture mean? It was learnt by scientists, during 1950s, that whole plants could be regenerated form explants, i.e., any part of a plant taken out and grown in a test tube under sterile conditions in special nutrient media. This capacity to generate a whole plant from any cell is called totipotency.

Flow chart showing plant Tissue Culture Technique



It is important to stress here that the nutrient medium must provide a carbon source and also inorganic salts, vitamins, amino acids and growth regulators like auxins, cytokinins etc. The culture medium is rich in nutrients and therefore attracts the growth of microorganisms, thereby resulting in the contamination and spoiling of the medium. Therefore, the culture medium is sterilized to kill the microorganisms. Sterilization is carried out in a steam sterilizer called the autoclave. The culture medium is autoclaved for 15 min, at 1210 c& 15 pounds of pressure. The transfer of explants onto the sterilized nutrient culture medium is called inoculation and is carried out in an aseptic environment.

The cultures are incubated for 3 to 4 weeks during which period the cells of the explants absorb the nutrients, grow and undergo repeated divisions to produce a proliferating undifferentiated mass of cells known as 'callus' (Fig.13.2a). sometimes shoots or roots may be produced directly. The explants or callus cultured on different combinations of auxins and cytokinins will produce shoots or roots and this process is called 'organogenesis' (Fig.13.2b & c). Alternately, embryo like structures develop from the callus and this phenomenon is known as somatic embryogenesis, the embryo-like structures which develop from the callus are called embryoids. Since these embryoids develop from somatic tissue they are also referred to as 'somatic embryos'. Sometimes, the explants produces the embryoids directly without callus formation.

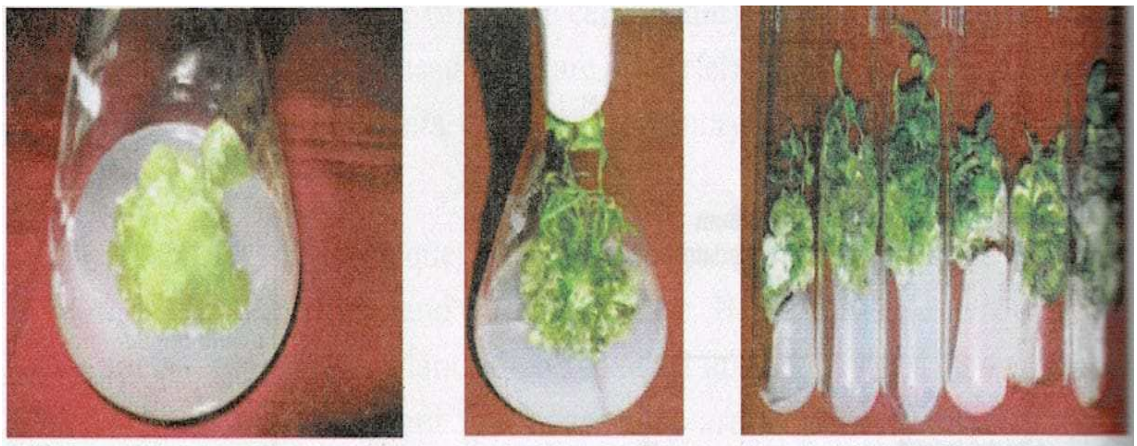


Figure 13.2 Tissue culture

13.3.1 Applications of tissue culture

By applying this technique, it is possible to produce a large number of plants in a very short time and in limited space. Hence the technique is called micro-propagation. Plants thus produced are genetically identical to the original or source plant and hence they are called somaclones. Many economically important plants like tomato, banana, apple, teak, eucalyptus, bamboo etc., have been produced on a commercial scale by the use of this method.

Another important application of the method is the recovery of healthy plants from diseased plants. Although the plant is infected with virus, the meristem (Apical and axillary) is free of virus. Hence, one can remove the meristem and grow it in vitro to obtain virus-free plants. Scientists have succeeded in culturing meristems of banana, sugarcane, potato, etc.

Scientist have even isolated single cells or protoplasts(naked cells) from plants after digesting their cell walls which act as physical barriers , using cell wall hydrolysing enzymes like cellulose and pectinase. Isolated protoplast from two different varieties of plants – each having a desirable character- can be fused to get hybrid protoplasts, which can be further cultured to form a novel plant. These hybrids are called somatic hybrids while the process is called somatic hybridization. Some plants show physical or chemical incompatability in normal sexual crosses. Somatic hybridization technique provides the opportunity for bypassing the conventional breeding barriers through direct transfer of cytoplasmic and nuclear genomes to plant cells and this facilitates the introduction of desirable traits. Imagine a situation when a protoplasts of tomato is fused with that of potato, and then they are grown to form new hybrid plants combining tomato and potato characteristics. Well, this plant has been achieved resulting in the formation of pomato: unfortunately this plant did not have all the desired combination of characteristics for its commercial utilisation.

SUMMARY

Plant breeding may be used to create varieties which are resistant to pathogens and insect pests. This increases the yield of the food. This method has also been used to increase the protein content of plant foods and thereby enhance the quality of food. In India, several varieties of different crop plants have been produced. All these measures enhance the production of food. Techniques of tissue culture and somatic hybridisation offer vast potential for manipulation of plants in vitro to produce new varieties.

GLOSSARY

Germplasm(collection): The entire collection of plants/seeds, having all the diverse alleles for all genes in a given crop is called germplasm collection.

Green Revolution: It is the dramatic increase in food production due to plant breeding techniques.

Hybridisation: The process of producing new crop varieties by crossing two genetically different parents.

Protoplasts: cells whose cell walls have been removed by enzymatic digestion.

Somaclones: Plants grown through tissue culture which are genetically identical withthe original(source) plant are called somaclones.

Somatic hybridization: The process of developing hybrid plants by the fusion of isolated protoplasts.

Totipotency: The ability of a cell (or) an explants to regenerate into a complete plant is called totipotency.

QUESTIONS

Very Short Answer Type Questions

1. What is green Revolution?

2. Give two examples of fungi used in SCP production.

3. What is protoplast fusion?

4. What is emasculation?

5. Name two semi-dwarf varieties of rice developed in India.

Short Answer Type Questions

1. What is meant by germplasm collection? What are its benefits?

2. The culture medium (nutrient medium) can be referred to as a 'highly enriched laboratory soil'. Justify the statement.

3. Give two examples of biofortified crops. What benefits do they offer to the society?

EXCERCISES

1. Which part of the plant is best suited for making virus-free plants and why?

2. Find out what are the various components of the medium used for propagation of an explants in invitro?

Chapter 14

Microbes in Human Welfare

Besides macroscopic plants and animals, microbes are the major components of biological systems on this earth. You have studied about the diversity of living organisms in First year Do you remember which kingdoms among the living organisms contain microorganisms? Which are the ones that are only microscopic? Microbes are present everywhere – in soil, water, air inside our bodies and that of other animals and plants. They are present even at sites where no other life-form could possibly exist- sites such as deep inside the geysers (thermal vents) where the temperature may be as high as 1400C, deep in the soil, under the layers of snow several metres thick, and in highly acidic environments. Microbes are diverse – protozoa, bacteria, fungi and microscopic plant viruses, viroids and also porins that are proteinacious infectious agents.

Microbes like bacteria and many fungi can be grown on nutritive media to form colonies (Figure 14.1), that can be seen with the naked eye. Such cultures are useful in studies on micro-organisms.

In Unit II you have read that microbes cause a large number of diseases in humanbeings, animals and plants. But this should not make you think that all microbes are harmful. Several microbes are useful to man in diverse ways. Some of the most important contributions of microbes to human welfare are discussed in this chapter.

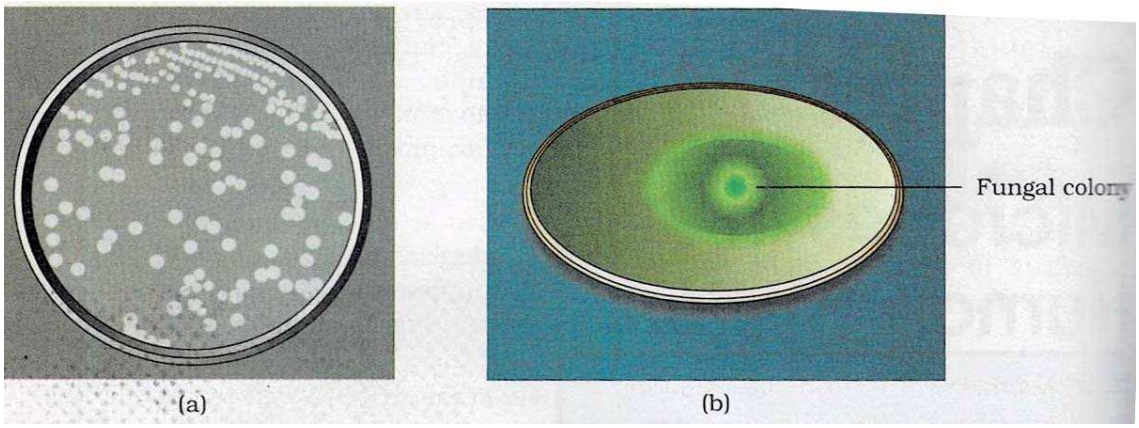


Figure 14.1 (a) colonies of bacteria growing in a petri dish;

(b) Fungal colony growing in a petri dish

14.1 Microbes in Household Products

You would surprised to know that we use microbes or products derived from them everyday. A common example is the production of curd from milk. Micro- organisms such as *Lactobacillus a*nd others, commonly called **lactic acid bacteria (LAB)**, grow in milk and cover it to curd. During growth, the LAB produce acids that coagulate and partially digest the milk proteins. A small amount of curd is added to fresh milk as inoculums or starter contain millions of LAB, which at suitable temperatures multiply, thus converting milk to curd. Which also improves its nutritional quality by increasing vitamin $B_{12.}$ In our stomach too, LAB play a very beneficial role in checking disease- causing microbes. The use of such friendly bacteria for therapeutic purposes and for the betterment of human health has led to the concept of **probiotics.**

The dough which is used for making foods such as dosa and idli is also fermented by bacteria. The puffed appearance of dough is due to the production of CO_2 gas. Can you tell which metabolic pathway is taking place resulting in the formations came from? Similarly the dough which is used for making bread is fermented using baker's yeast *(Saccharomyces cerevisiae*). A number of traditional drinks and foods are also made by fermentation by the microbes. Cheese is one of the oldest food items in which microbes were used. Different varieties of cheese are known by their characteristic texture, flavour and taste, the specifically coming from the microbes used. For example, the large holes in "Swiss cheese' are due to the production of a large amount of CO_2 by a bacterium named *Propionibacterium sharminii.* The 'Roquefort cheese' is ripened by growing a specific fungi on it, which gives it a particular flavour.

14.2 Microbes in Industrial products

Even in industry, microbes are used to synthesise a number of products valuable to human beings. Beverages and antibiotics are some examples. Production on an industrial scale requires growing microbes in very large vessels called **fermentors** (Figure 14.2)

14.2.1 Fermented Beverages

Figure 14.2 Fermentors

Figure 14.3 Fermentation Plant

Microbes, especially yeasts, have been used from time immemorial for the production of beverages like wine, beer, whisky, brandy and rum. The same yeast Saccharomyces cerevisiae, used for bread- making and commonly called brewer's yeast, is used for fermenting malted cereals and fruit juices, to produce ethanol. Do you recollect the metabolic reactions which result in the production of ethanol by yeast? Depending on the type of the raw material used for fermentation and the type of processing (with or without distillation) different types of alcoholic drinks are obtained. Wine and beer are produced without distillation whereas Whisky, brandy and rum are produced by the distillation of the fermented broth. The Photograph of a fermentation plant is shown in Figure 14.2.

14.2.2 Antibiotics

Antibiotics produced by microbes are regarded as one of the most significant discoveries of the twentieth century and have greatly contributed towards the welfare of human society. Anti is a Greek word that means "against' and bio means 'life'; together they mean 'against life' (in the context of disease causing organisms); Where as with reference to human beings, they are 'pro life' and not against. Antibiotics are chemical substances which are produced by some microbes and can kill or retarded the growth of other (disease-causing) microbes.

You are familiar with the commonly used antibiotic. **Pencillin.** Do you know that pencillin was the first antibiotic to be discovered, and it was a chance discovery? **Alexander Flemming** while working on ***Staphylococci*** bacteria. Once observed a mould growing in one of his unwashed culture plates around which Staphylococci could not grow. He found out that it was due to chemical produced by the mould and he named it pencillin after the mould *Pencillium notatum*. However, its full potential as an effective antibiotic was established only much later by Ernest Chain and Howard Florey. This antibiotic was extensively used to treat American soldiers wounded in world War II. Fleming, Chain and Florey were awarded the Nobel Prize in 1945 for this discovery.

After pencillin, other antibiotics were also purified from other microbes. Can you name some other antibiotics and find out their sources? Antibiotics have greatly improved our capacity to treat deadly diseases such as the Plague, Woughing cough (kali khansi), diphtheria (gal ghotu) and leprosy (kushi rog), which used to kill millions all over the globe. Today, we cannot imagine a world without antibiotics.

14.2.3 Chemiclas, Enzymes and other Bioactive Molecules

Microbes are also used for commercial and industrial production of certain chemicals like organic acids, alcohols and enzymes. Examples of acid producers are *Aspergillus niger* (a fungus) of citric acid. *Acetobacter aceti* (a bacterium) of acetic acid; *Clostridium botylicum* (a bacterium) of butyric acid and *Lactobacillus (*a bacterium) of lactic acid.

Yeast (*Saccharomyces cerevisiae*) is used for commercial production of ethanol. Microbes are also used for production of enzymes. Lipases are used in detergent formulations and are helpful in removing oil stains from Laundry. You must have noticed that bottled fruit juices bought from the market are clearer than those made at home. This is because bottled juices are clarified by the use of pectinases and proteases. Streptokinase, produced by the bacterium *Streptococcus* and modified by genetic engineering is used as a 'clot buster' for removing clots from the blood vessels of patients who have undergone myocardial infection leading to heart attack. Another bioactive molecule, cyclosporine A, that is used as an immunosuppressive agent in organ-transplant patients, is produced by the fungus

Trichoderma polyspermum. Statins produced by the yeast *Monascus purpureus* have been commercialised as blood- cholesterol lowering agents. They act by competitively inhibiting the enzyme responsible for the synthesis of cholesterol.

14.3 Microbes in Sewage Treatment

We Know the large quantities of waste water are generated everyday in cities and towns. A major components of this waste water is human excreta. Municipal waste-water is also called sewage. It contains large amounts of organic matter and microbes, many of which are pathogenic. Have you ever wondered where this huge quantity of sewage or urban waste water is disposed off daily? This cannot be discharged into natural water bodies like rivers

and streams directly – you can understand Why. Before disposal, hence, sewage is treated in sewage treatment plants (STPs) to make it less polluting. Treatment of waste water is done by heterotrophic microbes naturally present in the sewage. This treatment is carried out in two stages:

Figure 14.4 Secondary treatment

Primary treatment: The treatment involves physical removal of particles- large and small- from the sewage through filtration and sedimentation. These particles are removed in stages: initially, floating debris is removed by sedimentation. All solids that settle from the **primary sludge**, and the effluent from the primary settling tank is taken for secondary treatment.

Secondary treatment or Biological treatment : The primary effluent is passed into large aeration tanks (Figure 14.4) where it is constantly agitated mechanically and air is pumped into it. This allows vigorous growth of useful aerobic microbes into flocs (masses of bacteria associated with fungal filaments to form mesh like structures). While growing, these microbes consume the major part of the organic matter in the effluent. This significantly reduces the BOD (**biological oxygen demand**) of the effluent. BOD refers to the amount of the oxygen that would be consumed if all the organic matter in one litre of water were oxidised by bacteria. The sewage water is treated till the BOD is reduced. The BOD test measures the rate of uptake of oxygen by micro-organisms in a sample of water and thus, indirectly, BOD is a measure of the organic matter present in the water. The greater the BOD of waste water, the more is its polluting potential.

Figure 14.5 An aerial view of a sewage plan

Once the BOD of sewage or waste water ids reduced significantly, the effluent is then passed into a settling tank where the bacterial 'flocs' are allowed to sediment. This sediment is called **activated sludge.** A small part of the activated sludge is pumped back into the aeration tank to serve as the inoculum. The remaining

major part of the sludge is pumped into large tanks called **anaerobic sludge digesters**. Here other kinds of bacteria, which grow anaerobically, digest the bacteria and the fungi in the sludge. During this digestion, bacteria produce a mixture of gases such as methane, hydrogen sulphide and carbon dioxide. These gases form **biogas** which can be used as a source of energy as it is inflammable.

The effluent from the secondary treatment plant is generally released into natural water bodies like rivers and streams. An aerial view of such a plant is shown in Figure 14.5.

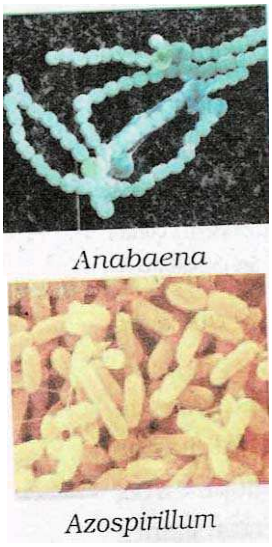
You can appreciate how microbes play a major role in treating millions of gallons of waste water everyday across the globe. This methodology has been practised for more than a century now, in almost all parts of the world. Till date, no man- made technology has been able to rival the microbial treatment of sewage.

You are aware that due to increasing urbanisation. Sewage is being produced in much larger quantities than ever before. However the number of sewage treatment plants has not increased enough to treat such large quantities. So the untreated sewage is often discharged directly into rivers, leading to their pollution and an increase in water-borne diseases.

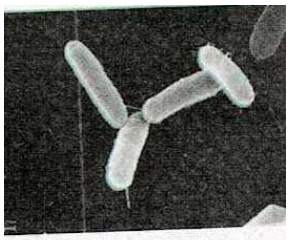
The Ministry of Environment and Forests has initiated **The Ganga Action Plan** and **The Yamuna Action Plan** to save the major rivers of our country from pollution. Under these plans, it is proposed to build a large number of sewage treatment plants so that only treated sewage may be discharged in the rivers. A visit to a sewage treatment plant situated in any place near you would be a very interesting and educating experience.

Besides the above, microbes can be remove toxic substances that are accidentally released into the environment such as oil or chemical spills and lands with toxic wastes. This process is known as **Bioremidiation.**

14.6 Microbes as Biofertilizers



With our present day life styles, environmental pollution is a major cause of concern. The use of the chemical fertilizers to meet the ever- increasing demand of agricultural produce is one of the important cause of this pollution. Of, course, we have now realised that there are problems associated with the overuse of chemical fertilisers and there is immense pressure to switch to organic farming- the use of biofertilisers. Biofertilisers are organisms that enrich the nutrient quality of the soil. The main sources of biofertilisers are bacteria, fungi and cyanobacteria. You have studied about the nodules on the roots of leguminous plants formed by the symbiotic association with Rhizobium. These bacteria fix atmospheric



Azotobacter

nitrogen into organic forms, which is used by the plant as a nutrient. Other bacteria can fix atmospheric nitrogen while free-living in the soil (Examples Azospirillum and Azobacter), thus enriching the nitrogen content of the soil.

mycorrhiza

Fungi are also known to form symbiotic association with plants (mycorrhiza). Many members of the genus Glomus form mycorrhiza. The fungal symbiont in these associations show other benefits also, such as resistance to root- borne pathogens, tplerence to salinity and drought, and an overall increase in the plant growth and development. Can you tell what advantage the fungus derives from this association?

Cyanobacteria are autotrophic microbes widely distributed in aquatic and terrestrial environments. Many of them can fix atmospheric nitrogen, eg, Anabaena, Nostoc, Oscillatoria, etc. In Paddy fields cyanobacteria serve as an important biofertiliser. Blue green algae also add organic matter to the soil and increase its fertility. Currently, in our country, a number of biofertilisers are available commercially in the market and farmers use these regularly in their fields to replenish soil nutrients and to reduce dependence on chemical fertilisers.

SUMMARY

Microbes are very important component of life on earth. Not all microbes are pathogenic. Many microbes are very useful to human beings. We use microbes and microbially derived products almost every day. Bacteria are called lactic acid bacteria (LAB) grow in milk and convert it into curd. The dough which is used to make bread is fermented by yeast called Saccharomyces cerevisiae. Certain dishes such as idli and dosa, are made from dough fermented by microbes. Bacteria and fungi are used to impart a particular texture, taste and flavour to cheese. Microbes are used to produce industrial products like lactic acid, acetic acid and alcohol, which are used in a variety of processes in the industry. Antibiotics like pencillin, produced by useful microbes, are used to kill disease- causing harmful microbes. Antibiotics have played a major role in controlling infectious diseases like diphtheria, whooping cough and pneumonia. For, more than a hundred years, microbes have been used to treat sewage (waste water) by the process of activated sludge formation. This helps in recycling of water in nature. Methanogens produce methane (biogas) while degrading plant waste. Biogas produced by microbes is used as a source of energy in rural areas. There is a need today for the aggressive use of biofertilisers in place of chemical fertilisers.

GLOSSARY

BOD (Bio chemical oxygen demand): It is the amount of oxygen that would be consumed if all the organic matter in one litre of water is oxidized by bacteria.

Biofertilizers: organisms that enrich the nutrient quality of the soil.

Fermentors: These are large vessels in which microbes are grown in large numbers on an industrial scale.

Mycorrhiza: These are the fungi living in symbiotic association with plants.

Organic farming: The use of biofertilizers in enriching the nitrogen content of the soil.

Primary Sludge: The solids that settle down during the treatment of waste water forms primary sludge.

Vermicompost: The process of compost formation by earthworms.

QUESTIONS

Very Short Answer Type Questions

1. Why does 'Swiss cheese' have big holes. Name the bacteria responsible for it.

2. Name a microbe used for statin production.

3. Give any two microbes that are useful in biotechnology.

4. Name any two genetically modified crops.

5. Name any two industrially important enzymes.

6. Give an example of a rod shaped virus.

Short Answer Type Questions

1. How is *Bacillus thuringiensis* helpful in controlling insect pests?

2. Which bacterium has been used as clot buster? What is its mode of action?

3. What are biofertilizers? Give two examples and discuss their role as biofertilizers.

EXCERCISES

1. Name the states involved in Ganga action plan.

2. Do you think microbes can also be used a source of energy? If yes, how?

3. Find out the role of microbes in the following and discuss it with your teacher. (a) Single cell protein (SCP) (b) Soil

References:

SCERT – Intermediate Academy Books

NCERT – Intermediate Academy Books

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SCHEME AND WEIGHTAGE

1. The Question paper is to be set for 40 (Forty) marks. The student has to answer for 25 (Twenty five) marks.

2. There will be 8 (Eight) Very Short Answer Questions each carrying 1 (One) Mark. The student has to answer 5 questions from this Section-A.

3. In Section-B there will be 8 (Eight) Short Answer Question each carrying 4 (Four) Marks. The student has to answer 5 questions from this Section-B.

Weightage for the Chapters included in the Syllabus is as follows:

S.No.	Unit	Number of Periods	Weightage of Marks
1.	Plant Physiology	18	10
2.	Micro Biology	06	05
3.	Genetics	04	05
4.	Molecular Biology	07	05
5.	Biotechnology	07	07
6.	Plants, Microbes and Human Welfare	08	08
	Total	**50**	**40**

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WEIGHTAGE OF MARKS

S.No.	Chapter	Number of **Periods**	**Weightage of Marks**
1.	Anatomy	04	05
2.	Taxonomy	02	04
3.	Experiments (Physiology, Ecology)	06	06
4.	Spotter/Slides	06	06
5.	Record and Herbarium	02	3+2 = 05
	Total	20	25

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MODEL QUESTION PAPER

Time: 1 ½ Hours **Max.Marks: 25**

Section – A **5x1=5**

Note: i) *Answer any **five** of the questions briefly*
*ii) Each question carries **one** mark.*

1. Define Osmosis?
2. Name the essential elements present in Nitrogenase enzyme?
3. What is Plasmid.
4. Explain the terms Phenotype and Genotype?
5. What are the components of Nucleotide ?
6. What is the full form of PCR ?
7. What are fermenters?
8. Give any two microbes that are useful in biotechnology?

Section – B **5x4=20**

Note: i) *Answer any **five** questions*
ii) Draw labelled diagrams wherever necessary
i*ii) Each question carries **four** marks.*

9. Explain the importance of ES complex formation?
10. With the help of diagram, explain the structure of chloroplast ?
11. Explain lytic cycle?
12. Mention the advantages of selecting Pea plant for experiment by Mendel ?
13. Write the important features of genetic code.
14. What are cloning vectors? Give an example?
15. What are biofertilizers ? Give Two examples and discuss their role as biofertilizers.
16. What any Four applications of Tissue culture ?

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Unit – I : PLANT PHYSIOLOGY

Very Short Answer Questions (1 Mark)

1. What are porins?
2. Define water potential.
3. Differentiate osmosis from diffusion.
4. Compare Transpiration and Evaporation.
5. Explain why xylem transport is unidirectional while that in phloem is bidirectional?
6. Does transpiration occur at night? Give an example.
7. How does ABA bring about the closure of stomata under water stress conditions?
8. Define Hydroponics.
9. How do you categorize a particular essential element as a macro or micronutrient?
10. Out of the 17 essential elements which elements are called non-mineral essential elements?
11. Name two amino acids in which sulphur is present.
12. Which element is regarded as the 17^{th} essential element?
13. Name the essential elements present in nitrogenase enzyme.
14. Write the balanced equation of nitrogen fixation.
15. Who proposed 'Lock and Key hypothesis'
16. Who proposed Induced fit hypothesis?
17. Distinguish between Action Spectrum and Absorption Spectrum.
18. How many molecules of ATP and NADPH are needed to fix a molecule of CO_2 in C_3 plants?
19. What is the primary acceptor of CO_2 in C_3 plants?
20. What is first stable compound formed in a Calvin cycle?
21. What is the primary acceptor of CO_2 in C_4 plants?
22. Explain the term 'Energy currency'.
23. Explain the economic importance of fermenation.
24. What is the common pathway for aerobic and anaerobic respirations?
25. Which substance is known as the connecting link between glycolysis and Krebs cycle?
26. Define Respiratory Quotient..
27. What is apical dominance?
28. What is meant by bolting?
29. What is ethephon?
30. Define Respiratory Climatic.
31. Which of the PGRs is called stress hormone?
32. Define the terms quiescence and dormancy.

Short Answer Questions (4 Marks)

33. Define and Explain Water Potential.
34. What is meant by plasmolysis? How is it practically useful to us?
35. How does ascent of sap occur in tall tress?

36. "Transpiration is a necessary evil' . Explain.
37. Explain the steps involved in the formation of root nodule.
38. Write in brief how plants synthesize amino acids.
39. Explain the importance of [ES] complex formation.
40. With the help of diagram, explain briefly the process of cyclic photosphorylation.
41. Explain the structure of the chloroplast? Draw a neat labelled diagram.
42. Write any Four differences between C_3 and C_4 plants.
43. Write a note on agricultural/horticultural applications of auxins.
44. Write the physiological responses of gibberellins in plants.
45. Write any four physiological effects of cytokinins i plants.
46. What are the physiological processes that are regulated by ethylene in plants?

Unit – II : MICROBIOLOGY

Very Short Answer Questions (1 Mark)

47. Define Microbiology.
48. What are pleomorphic bacteria? Give an example.
49. What is sex pilus?
50. What is a genophore ?
51. What is a plasmid?
52. What is its significance of a plasmid?
53. Name the Bacteria which is common inhabitant in human intestine.
54. What is conjugation ?
55. Who discovered conjugation?
56. In which organism conjugation was discovered?
57. What is transformation?
58. Who discovered transformation?
59. In which organism transformation was discovered?
60. What is transduction?
61. Who discovered transduction? In which organism transduction was discovered?
62. What is the shape of T4 phage?
63. What is the genetic material in T4 phage?
64. What is the shape of TMV ?
65. What is the genetic material of TMV ?
66. What is lysozyme?
67. What is the function of lysozyme?
68. Define 'lysis'.
69. What is a prophage?
70. Give an example to prophage?

Short Answer Questions (4 Marks)

71. How are bacteria classified on the basis of morphology?
72. How are bacteria classified on the basis of number and distribution of flagella?
73. Write briefly about chemoheterotrophs and their significance.
74. Explain the conjugation in bacteria.
75. Explain the chemical structure of viruses.

76. Explain the structure of TMV.
77. Explain the structure of Bacteriophase.
78. Explain the lytic cycle in viruses.

Unit – III : GENETICS

Very Short Answer Questions (1 Mark)

79. What is the cross between the F1 progeny and the homozygous recessive parent called?
80. Explain the terms phenotype and genotype.
81. What is the genetic nature of wrinkled phenotype of pea seeds?
82. Define True Breeding.
83. What is Linkage ?
84. What is crossing over ?

Short Answer Questions (4 Marks)

85. Mention the advantages of selecting pea plant for experiment by Mendel.
86. Differentiate between the following:
    (a) Dominant and Recessive
    (b) Homozygous and Heterozygous
87. Explain the Law of Dominance using a monohybrid cross.
88. Define and design a test-cross.
89. Explain the following terms
    (a) Co-Dominance (b) Incomplete Dominance

Unit - IV: MOLECULAR BIOLOGY

Very Short Answer Questions (1 Mark)

90. Distinguish between heterochromatin and euchromatin.
91. What is the function of DNA polymerase?
92. Name any Two viruses which have RNA as a genetic material.
93. What are the components of a nucleotide?
94. What are the components of a transcription unit?
95. What is the difference between exons and introns?
96. What is meant by capping and tailing?
97. Define peptide bond.
98. What is the function of the codon- AUG?
99. Define stop codon. Write the codons.

Short Answer Questions (4 Marks)

100. Write briefly about DNA polymerase.
101. On the diagram of the secondary structure of tRNA shown below indicate the location of the following features:
    a) Anticodon b) Acceptor stem c) Anticodon stem d) 5' end e) 3' end
102. What are the differences between DNA and RNA.
103. Write the important features of Genetic code?

Unit – V: BIOTECHNOLOGY

Very Short Answer Questions **(1 Mark)**

104. What are molecular scissors?
105. What is EcoR1?
106. What are cloning vectors?
107. What is palindromic sequence?
108. What is Recombinant DNA ?
109. How does one visualize DNA on an Agarose gel ?
110. What is the full form of PCR?
111. How is PCR useful in biotechnology?
112. How can you differentiate between exonucleases and endonucleases?
113. Expand GMO.
114. Give different types of *cry genes.*
115. Name the nematode that infects the roots of tobacco plants.

Short Answer Questions **(4 Marks)**

116. Write short notes on restriction enzymes.
117. What are the different methods of insertion of reombinant DNA into the host cell?
118. List out any Four beneficial aspects of Transgenic plants'
119. Give a brief account of
    A) Bt. Cotton B) Pest resistant plants

Unit – VI : PLANTS, MICROBES AND HUMAN WELFARE

Very Short Answer Questions **(1 Mark)**

120. Name two semi-dwarf varieties of rice developed in India.
121. Give two examples of wheat varieties introduced in India.
122. What is emasculation?
123. Which two species of sugarcane were crossed for better yield?
124. Why does 'Swiss cheese' have big holes.
125. Name the bacteria responsible for big holes in 'Swiss Cheese'.
126. What are fermentors?
127. Name a microbe used for statin production.
128. Expand BOD ?
129. Give any two microbes that are useful in biotechnology.
129. Name any two genetically modified crops.
130. Which species of *Penicillium* produces Roquefort cheese?
131. Name any two industrially important enzymes.
132. Give an example of a rod shaped virus.
133. In which food would you find lactic acid bacteria?
134. Name the lactic acid producing bacterium.
135. Name any two fungi which are used in the production of antibiotics.
136. Name the scientists who were credited for showing the role of penicillin as an antibiotic.
137. Give Two examples of fungi used in SCP production.

Short Answer Questions **(4 Marks)**

138. What is meant by germplasm collection? What are its benefits?
139. The culture medium (nutrient medium) can be referred to as a 'highly enriched laboratory soil'. Justify the statement.
140. Give few examples of biofortified crops. What benefits do they offer to the society?
141. How is *Bacillus thuringiensis* helpful in controlling insect pests?
142. Which bacterium has been used as a clot buster? What is its mode of action?
143. What are biofertilisers? Give two examples and discuss their role as biofertilisers.

BOARD OF INTERMEDIATE EDUCATION-A.P., VIJAYAWADA
VOCATIONAL BRIDGE COURSE BIOLOGICAL SCIENCE
BOTANY PRACTICALS – (w.e.f. 2019-2020)
MODEL QUESTION PAPER
With effect from IPE March - 2020

Time: 3 Hours **Max.Marks: 25**

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1. Take the T.S. of the given material 'A' and prepare a slide. Identify the material
   With suitable reasons. Draw a neat labeled diagram. **5 Marks**
2. Describe the identifying features of plant material 'B' and identify it up to family
   level, by giving reasons. Draw L.S. of flower, Floral diagram and write floral formula. **4 Marks**
3. Conduct the given experiment 'C'. Comment on the results and their significance. **5 Marks**

4. Identify the Spotters/Slides D, E and F (2 x 3) **6 Marks**
5. Record (3 marks) and Herbarium (2 marks) **5 Marks**

BOARD OF INTERMEDIATE EDUCATION-A.P., VIJAYAWADA
VOCATIONAL BRIDGE COURSE SYLLABUS
BOTANY PRACTICALS – (w.e.f. 2019-2020)
QUESTION BANK

Section - A

Anatomy: Section Cutting of the following plant material and preparation of slide.

Monocot Root eg.Crinum
Dicot Root (Primary) eg. Cicer/Trigonella Seedling
Mono Stem eg. Grass
Dicot Stem (Primary) eg. Tridax

Section - B

Taxonomy: Description of the family. Vegetative and Floral Characters that are necessary for Identification of the Family Fabaceae, Solanaceae,
Liliaceae.

Section - C

Physiology Experiments:

1. Determination of transpiration by Cobalt Chloride method.
2. Study of Plasmolysis (by Rheo discolor peels or by grapes)
3. Determination of Osmotic Potential (by Potato Osmometer)
4. Separation of Chlorophyll Pigments (by Paper Chromatography)

Section - D

Spotters/Slides Identification: (One Spotter from each section)

1. Tuberous Root – eg. Carrot
2. Ephiphytic roots – eg: Vanda.
3. Phylloclade – eg. Opuntia
4. Phyllode – eg. Acacia
5. Rhizome – eg. Zinger
6. Corm – eg. Colocasia
7. Bulb – eg. Onion
8. Offsets – eg. Eichhornia
9. Insectivorus leaf – eg. Nepenthes
10. Hypanthodium – eg. Ficus
11. Spadix – eg. Colacasia
12. Drupe – eg. Mango
13. Hespiridium – citrus

Section – E

14. Nostoc – Vegetative filament
15. Spirogyra Vegetative filament
16. Rhizopus – mycelium
17. Agaricus Basidiocarp

Section – F

18. Funaria plant with saprophyte
19. Marchantia Thallus
20. Selaginella – Plant
21. Cycas – microsporophyll
22. Cycas megasporophyll
23. Pisum plant (pea)
24. Zea (corn) plant

V. Record and Herbarium

Herbarium: 10 Sheets should be prepared by the student out of which two will be Economic importance; two will be of Ecological importance and the remaining six will be of the families included in the syllabus.

INDEX

Human Anatomy and Physiology -I

SUMMARY

The digestive system of humans consists of an alimentary canal and associated digestive glands. The alimentary canal consists of the mouth, buccal cavity, pharynx, oesophagus, stomach, small intestine, large intestine, rectum and the anus. The accessory digestive glands include the salivary glands, the liver and the pancreas. Inside the mouth the teeth masticates the food, the tongue tastes the food and manipulates it for proper mastication by mixing with the saliva. Saliva contains a starch digestive enzyme, salivary amylase that digests the starch and converts it into maltose (disaccharide). The food then passes into the pharynx and enters the oesophagus in the form of bolus, which is further carried down through the oesophagus by peristalsis into the stomach. In stomach mainly protein digestion takes place. Absorption of simple sugars, alcohol and medicines also takes place in the stomach. The chyme (food) enters into the duodenum portion of the small intestine and is acted on by the pancreatic juice, bile and finally by the enzymes in the succus entericus, so that the digestion of carbohydrates, proteins and fats is completed. The food then enters into the jejunum and ileum portions of the small intestine. Carbohydrates are digested and converted into monosaccharides like glucose. Proteins are finally broken down into amino acids. The fats are converted to fatty acids and glycerol. The digested end products are absorbed into the body through the epithelial lining of the intestinal villi. The undigested food enters into the caecum of the large intestine through ileo-caecal valve, which prevents the back flow of the faecal matter. Most of the water is absorbed in the large intestine. The undigested food becomes semi-solid in nature and then enters into the rectum, anal canal and is finally egested out through the anus.

Food is one of the basic requirements of all living organisms. The major components of our food are carbohydrates, proteins and fats. Vitamins and minerals are also required in small quantities. Food provides energy and organic materials for growth and repair of tissues. The water we take in, plays an important role in metabolic processes and also prevents dehydration of the body. Biomacromolecules in food cannot be utilised by our body in their original form. The breakdown of bio-macro molecules into simple substances required for their absorption into cells.The process of conversion of these complex food substances into their simple and absorbable form is called **digestion**. They have to be broken down and converted into simple substances in the digestive system. It is carried out by our digestive system by mechanical and biochemical methods.

I A: Digestive System

Human digestive system consists of the alimentary canal and the associated glands.

1.1.1 Alimentary canal structure

The alimentary canal of man begins with the anterior opening, the mouth, and ends with the posterior opening, the anus. Parts of the alimentary canal between the mouth and the anus include buccal cavity, pharynx, oesophagus, stomach, small intestine and the large intestine in the given order.(Figure 1.1)

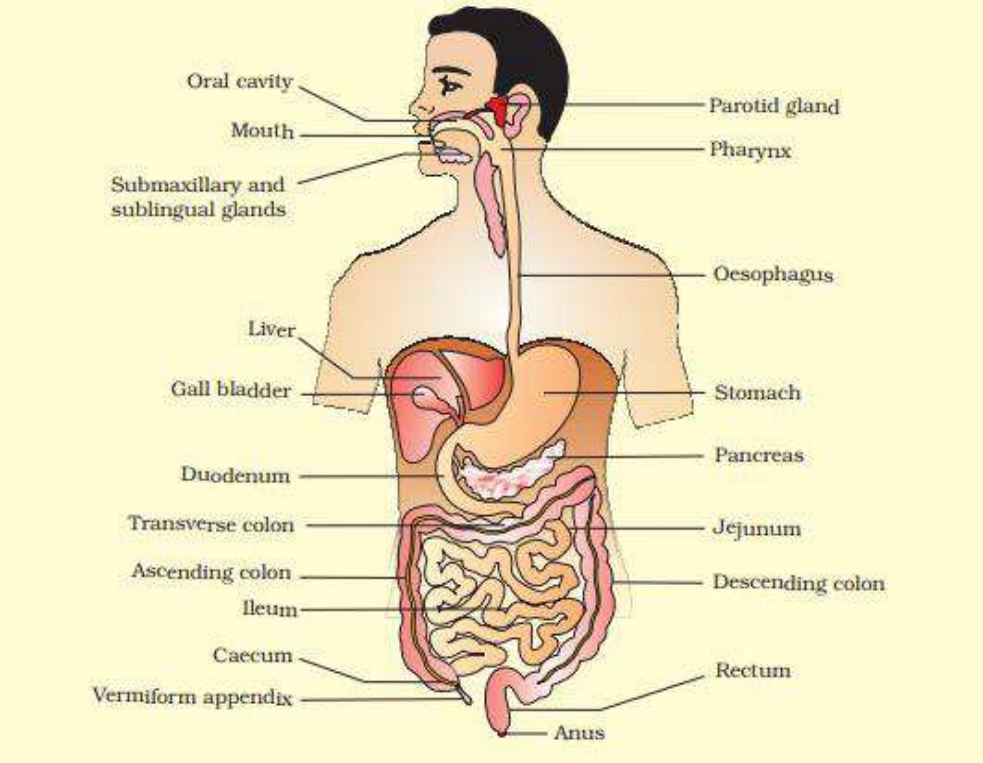


Figure 1.1 The human digestive system

I. Mouth and Buccal cavity

The mouth, bordered by the movable upper and lower lips (labia), leads into the buccal or oral cavity. The palate separates the ventral buccal cavity from the dorsal nasal chamber and facilitates chewing and breathing simultaneously. The anterior bony hard palate is lined by palatine rugae. The posterior soft palate that hangs down into the phaiynx is called uvula. The jawbones bear four kinds of teeth and a tongue occurs at the base of the buccal cavity.

a) Teeth

These are ecto-mesodermal in origin. Teeth of human beings are embedded in the sockets of the jaw bones, hence called **thecodont dentition** . Majority of mammals including human beings form two sets of teeth during their life time, a set of temporary/ milk or deciduous teeth replaced by a set of permanent or adult teeth. This type of dentition is called **diphyodont dentition**. An adult human has 32 permanent teeth, which are of four different types namely, incisors (I), canines (C), premolars (PM), and molars (M), and such a type of dentition is called **heterodont dentition**.(Figure 1.2) The arrangement of different types of teeth in each half of both the jaws in the order I, C, PM, M is represented by the **dental formula**.

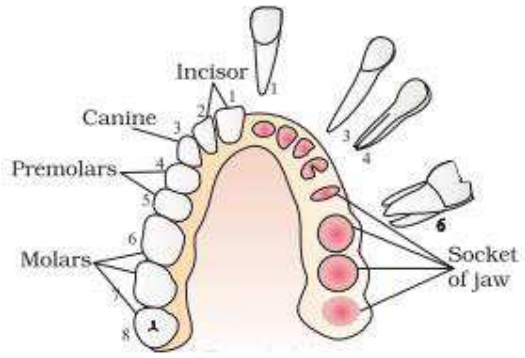


Figure 1.2 Types of teeth in the jaw

In adult humans it is 2123/2123= 32 .

The dental formula of the ‘milk dentition’of a baby is 2102/2102 = 20 teeth. The third molar teeth appear very late (usually 2102at about 21 years of age) and are called **wisdom teeth**. Incisors, the ‘chisel shaped’ teeth are useful in cutting , canines, the dagger like teeth help in tearing, premolars and molars, the cheek teeth, help in grinding the food .

The Structure of a Tooth

Tooth has three parts namely crown (the exposed part), neck (the middle part enclosed by gums) and root (the inner most part embedded in the socket of jaw bone). The bulk of a tooth is formed by a hard material called dentine, which is secreted by odontoblasts, of mesodermal origin. Dentine of the crown is covered by enamel (the hardest substance in the body), which is secreted by ameloblasts of ectodermal origin. A small cavity present inside the tooth is called pulp cavity, which is filled with pulp, and lined by a layer of odontoblasts. Dentine of the root is covered by cementum. The root is fixed in the socket (alveolus) of the jaw bone by cementum and periodontal membrane. The basal parts of teeth are covered by the gums (gingiva).(Figure 1.3)

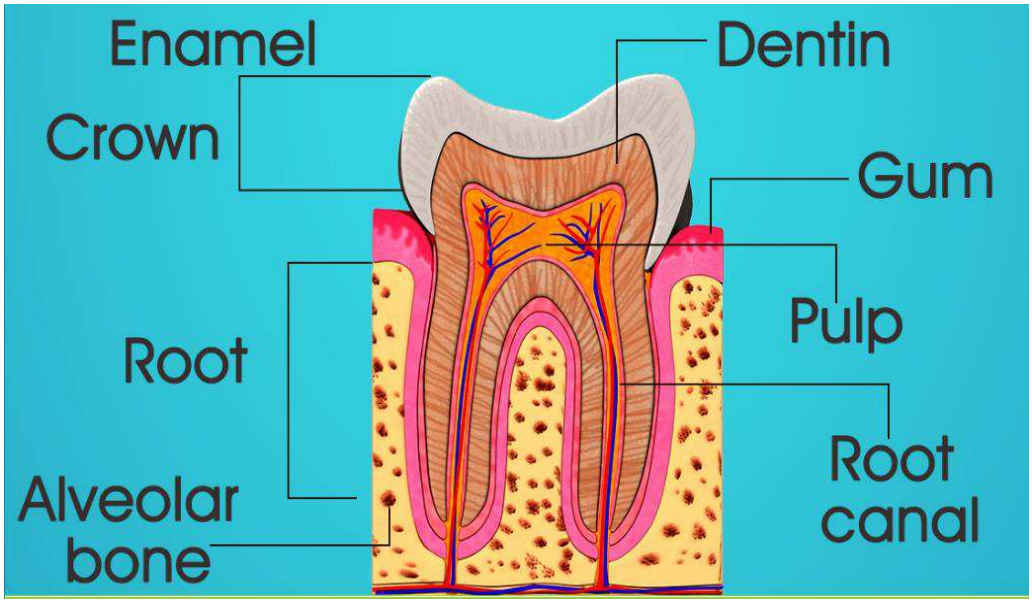


Figure 1.3 L.S. of tooth

b. Tongue

It is a freely movable, muscular sense organ, attached to the floor of the oral cavity by a fold of tissue called frenulum. The upper surface of the tongue has small projections called papillae, some of which bear taste buds. In humans the tongue bears 3 types of papillae namely:

1. fungi form (at the anterior margin and tip of tongue),
2. filiform (on the surface of the tongue) and
3. circumvallate papillae (on the posterior surface/ base of the tongue). The tongue acts as

'universal tooth brush*, and helps in mixing saliva with food, taste detection, deglutition and speaking.

II. Pharynx

The oral cavity leads into a short pharynx which serves as a common passage for food and air. It is divided into nasopharynx (lies above the soft palate), oropharynx (the middle part) and laryngopharynx (the lower part) by the soft palate. Oesophagus and trachea open into the laryngopharynx. The trachea opens into the laryngopharynx through the glottis. A cartilaginous flap called epiglottis prevents the entry of food into glottis during swallowing. Pharynx possesses voluntary muscles to assist in swallowing. Tonsils (lymphoid tissues) present in the pharynx, include i) pharyngeal tonsil or adenoids, ii) a pair of palatine tonsils and iii) a pair of lingual tonsils.

III. Oesophagus

The oesophagus is a thin long tube which extends posteriorly, passing through the neck, thorax and diaphragm and it finally leads into the stomach. A muscular sphincter (gastro-esophageal /cardiac sphincter) regulates the opening of the oesophagus into the stomach.

IV. Stomach

The Stomach is a wide, J-shaped, distensible muscular bag like structure, located in the upper left portion of the abdominal cavity just below the diaphragm. It has three major parts, an anterior cardiac portion into which the oesophagus opens, a middle large fundic region (main body) and a posterior pyloric portion which opens into the first part of the small intestine through the pyloric aperture which is guarded by the pyloric sphincter.(Figure 1.4)

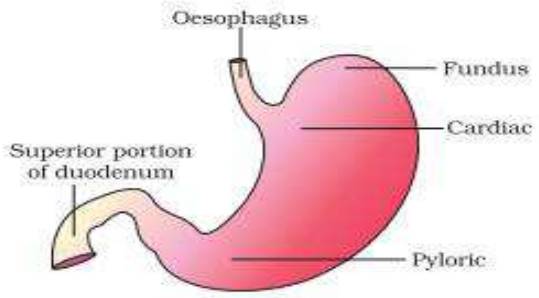


Figure 1.4 Human stomach

IV. Small Intestine

The small intestine is the longest part of the alimentary canal. It is distinguished serially into three regions namely proximal duodenum, middle long coiled jejunum and distal highly coiled ileum. Duodenum receives the hepato-pancreatic duct. Ileum opens in to the large intestine.

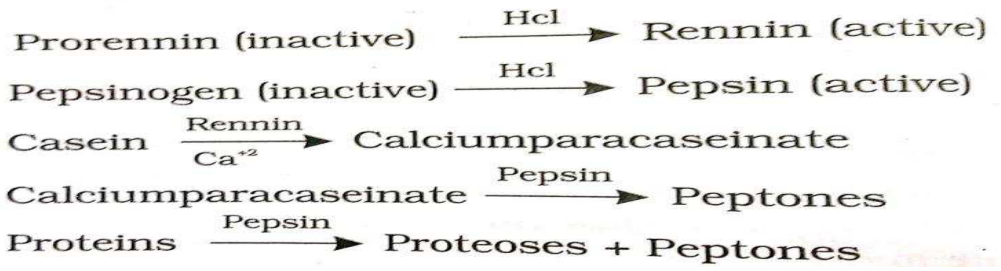
V. Large Intestine

It consists of caecum, colon and rectum. Caecum is a small blind sac which hosts some symbiotic microorganisms. A narrow finger-like tubular projection, the vermiform appendix (abdominal tonsil) which is a vestigial organ, arises from the caecum. The caecum opens into the colon which is divided into - an ascending, a transverse, a descending parts and a sigmoid colon that continues behind into the rectum. Colon shows the external bulged out pouches called haustra. Rectum is a small dilated sac which leads into anal canal that opens out through the anus. It is guarded by an internal anal sphincter formed by smooth muscle' and external anal sphincter formed by a ring of voluntary, striped muscle. There is no significant digestive activity in the large intestine. It is concerned with the absorption of some water, minerals and certain drugs. It secretes mucus which helps in keeping the undigested particles together and lubricating its passage to the exterior.

1.1.2 Digestion the stomach

The stomach stores food for 4-5 hours. The food is mixed thoroughly with the acidic gastric juice of the stomach by the churning movements of its muscular wall and the product is called chyme. The mucus and bicarbonates present in the gastric juice play an important role in the *lubrication* and protection of the mucosal epithelium from ‘excoriation’ by the highly concentrated hydrochloric acid. HC1 provides the acidic pH (1.8) which is optimal for the action of pepsin. It also kills the microorganisms ingested along with food. The proenzymes of gastric juice, the pepsinogen and prorennin, on exposure to hydrochloric acid are converted into the active enzymes, pepsin and rennin respectively. Pepsin converts proteins into proteoses and peptones. Rennin is a proteolytic enzyme found in the gastric juice of infants. It acts on the milk protein, the casein in the presence of calcium ions and converts it into calcium paracaseinate (curdling of milk) and proteoses. Pepsin acts on calcium

paracaseinate and converts it into peptones. Proteoses are a group of compounds formed during protein digestion and they are more complex than peptones. Certain other cells in the wall of the stomach produce bicarbonate, a base, to buffer the acidic contents of the stomach. They also prevent too much acidity in the stomach. The mucus produced by the wall of the stomach forms a physical barrier to prevent HC1 from damaging the wall of the stomach.

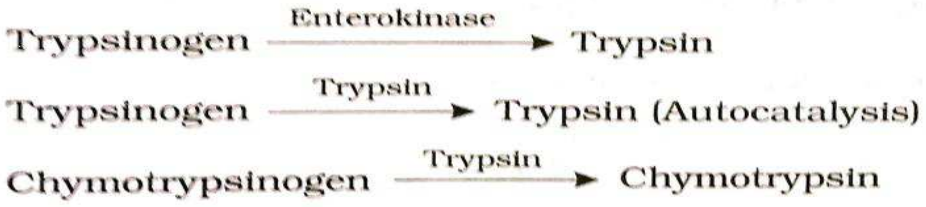


Digestion in the small intestine

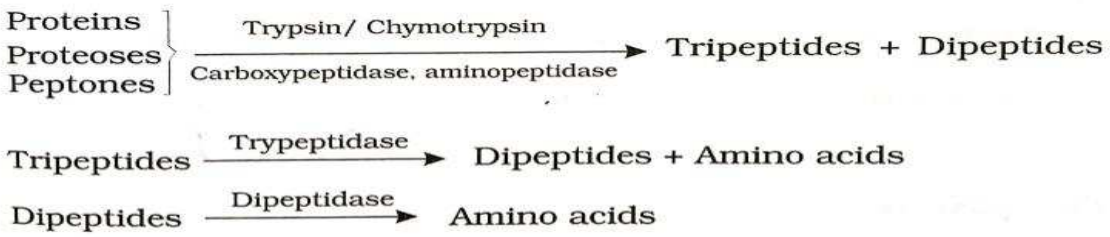
Various types of movements are generated by the muscularis externa layer of the small intestine. These movements help in thorough mixing up of the food with bile, pancreatic juice and intestinal juice in the intestine and thereby facilitate digestion. The mucus along with the bicarbonates from pancreas protects the intestinal mucosa from the acidic medium and provides an alkaline medium (pH7.8) for enzymatic activities. The duodenal cells of the proximal part also produce large amounts of bicarbonate to completely neutralize any gastric acid that passes further down into the digestive tract. All the enzymes of the *pancreatic juice* and succus entericus (mentioned above) act only in alkaline medium.

i. *Digestion of proteins*

Trypsinogen, ehymotrypsinogen and procarboxy peptidases are inactive enzymes. Trypsinogen is activated by the enzyme, enterokinase, secreted by the intestinal mucosa into active trypsin. which in turn activates the other enzymes in the pancreatic juice. Trypsin itself can similarly activate trypsinogen into trypsin (Autocatlysis)



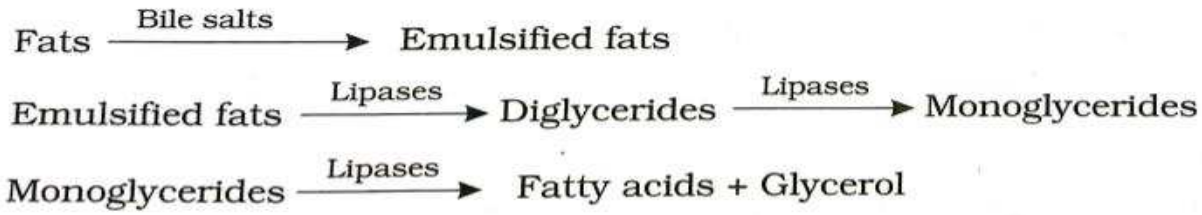
Proteins, proteoses and peptones (partly hydrolysed proteins) in the chime reaching the intestine are acted upon by the proteolytic enzymes of the pancreatic juice and intestinal juice as shown below.



Thus the end products of digestion of proteins namely amino acids are formed in the small intestine.

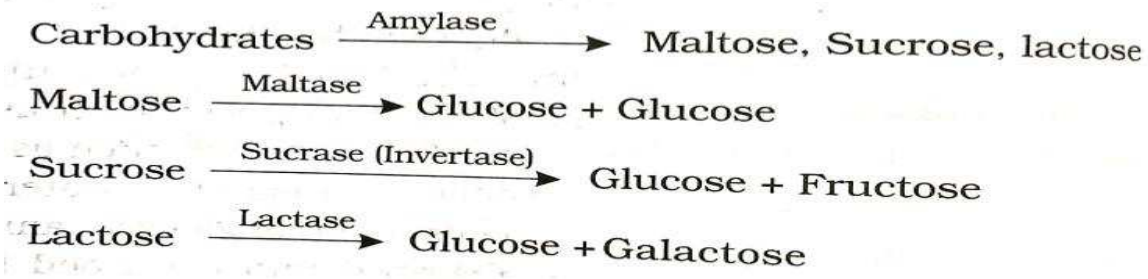
ii. *Digestion of fats*

Bile salts of the bile help in the emulsification of fats i.e. break down of fats into very small micelles. Bile also activates lipases of pancreatic juice (steapsin) and intestinal lipases. These lipases act on emulsified fats and convert them into fatty acids and glycerols.



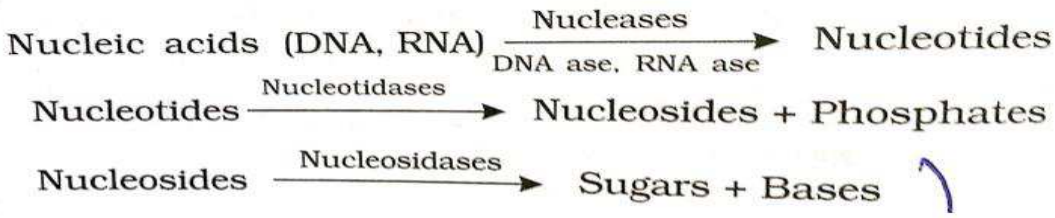
iii. *Digestion of Carbohydrates*

Carbohydrates in the chyme are hydrolysed by the pancreatic amylase into disaccharides. Intestinal disaccharidases act on the 'disaccharides' and convert them into monosaccharides.



iv. *Digestion of nucleic acids*

Nucleases (DNAase, RNAase) of the pancreatic juice act on the nucleic acids to form nucleotides and nucleosides. Nucleotidases and nucleosidases of the intestinal juice covert the nucleotides and nucleosides into pentose sugars and nitrogen bases.



1.1.3 Absorption assimilation of digested food

Absorption is the process by which the end products of digestion pass through the intestinal mucosa into blood or lymph. It is carried out by passive, active or facilitated transport mechanisms. Small amounts of monosaccharides such as glucose and galactose, amino acids and some electrolytes like chloride ions are generally absorbed by simple diffusion. The passage of these substances into the blood depends upon their concentration gradients. However, some of the substances such as fructose and amino acids are absorbed with the help of carrier ions such as Na+. This mechanism is called facilitated transport. Transport of water depends upon the osmotic gradient. Various nutrients such as glucose, amino acids, and some electrolytes such as Na+ are absorbed into the blood by active transport, which occurs against the concentration gradient, by utilizing energy.

Fatty acids and glycerol, being insoluble in water cannot be absorbed into the blood directly. They are first modified into small droplets called micelles, which move into intestinal mucosal

cells. They are reformed into very small protein coated fat globules called chylomicrons, which are transported into the lacteals (lymph capillaries) in the villi by exocytosis (they cannot enter the blood capillaries due to their large size). The lymph vessels ultimately release the absorbed substances into the blood stream through the left subclavian vein via the thoracic duct. These chylomicrons are broken down to fatty acids and glycerol by the action of the enzyme 'lipoprotein lipase' present in the endothelial walls and they diffuse into the adipocytes of the adipose tissue, and liver for storage (as neutral fat / tissue fat).

Absorption of substances takes place in different parts of the gut, like mouth, stomach, small intestine and large intestine. However maximum absorption of the end products of digestion occurs in the small intestine.

Summary of absorption of different parts of digestive system

Certain drugs coming in contact with the mucosa of mouth and lower side of the tongue are absorbed in to the blood capillaries lining them.	Absorption of water, simple sugars, and alcohol etc., takes place.	Principal organ for the absorption of nutrients. The digestion is completed here and the final (end) products of digestion such as glucose, fructose, galactose, amino acids are absorbed into blood through mucosa whereas fatty acids and glycerol are absorbed through the mucosa into lymph of the lacteals.	Absorption of water, some minerals and drugs takes place.

Assimilation

The absorbed substances finally reach the tissues where food materials become integral components of the living protoplasm and are used for the production of energy, growth and repair. This process is called assimilation.

GLOSSARY

Adenoids: A mass of lymphoid tissue present in the nasopharynx also called pharyngeal tonsils.

Brunners's glands:Intestinal glands of the sub mucosa of the duodenum that secrets mucus.

Chyme:Partly digested acidic food found in stomach.

Cystic duct: duct that arises from the gall bladder and joins the hepatic duct to form the common bile duct.

Lacteals :Lymph capillaries of the intestinal villi.

Odontoblasts: Dentine producing the cells of teeth.

Oxyntic cells: Cells of fundic glands of the stomach that secrets HCl and Castle's intrinsic factor.

Parotid glands: The largest of the salivary glands present below the pinnae.

Succus entericus: Intestinal juice screted by Burner's glands and crypts of Lieberkunn.

VERY SHORT ANSWER TYPE QUESTIONS

1	Give the dental formula of adult human being?
2	Explain the terms Thecodont and Dyphiodont?
3	What is Chyme?
4	Name different types of papillae on the tongue of man?
5	What is the hardest substance in the human body? What is its origin?
6	Distinguish between absorption and assimilation?
7	What is auto catalysis? Give one example?

SHORT ANSWER TYPE QUESTIONS

1	Draw a neat labeled diagram of L.S. of Tooth?
2	Describe the process of digestion of proteins in the stomach?
3	What are the functions of the liver?
4	How polysaccharides and disaccharides digested?

I B. Breathing and Exchange of Gases

1.2.1. Structure of respiratory tract

Respiratory system of man includes the following:

I. External nostrils (External Nares)

A pair of external nostrils opens out above the upper lip. They lead into nasal chambers through the nasal passages.

II. Nasal Chambers

They lie above the palate and are separated from each other by a nasal septum. Each nasal chamber can be differentiated into three parts namely;

i. vestibular part (which has hair and sebaceous glands to prevent the entry of dust particles),ii. respiratory part (which is involved in the conditioning the temperature of inhaled air ,it has three thin,' twisted bony plates called turbinals /conchae) and iii.olfactory part (which is lined by an olfactory epithelium).

III. Naso-pharynx

Nasal chambers lead into nasopharynx through a pair of internal nostrils, located above the soft palate. Nasopharynx is a portion of the pharynx, the common chamber for the passage of food and air. Nasopharynx leads into oropharynx and opens through glottis of larynx into the trachea.

IV. Larynx

Larynx is a cartilaginous box which helps in sound production, hence called the voice box- Wall of larynx is supported by nine cartilages. Thyroid, cricoids and epiglottis are the unpaired cartilages, whereas comiculate cartilages (cartilages of Santorin - two small conical nodules of elastic cartilage articulating with the arytenoid cartilages), arytenoids, and cuneiform cartilages are the paired cartilages. Epiglottis is a thin leaf like elastic *cartilaginous flap* attached to the thyroid cartilage to prevent the entry of food into the larynx through the glottis. The *yellow elastic fibres* which connect the thyroid and arytenoid cartilages are called vocal cords/vocal folds. The space between the true vocal cords and the arytenoids cartilages is called rima glottidis.

❖ The mid ventral part of the thyroid cartilage forms the laryngeal prominence called **Adam's apple.**

❖ In males, the vocal cords are thicker, longer, and produce low pitch voice, where as in women and children the vocal cords are usually short and produce high pitch voice.

v. Trachea

Trachea, the wind pipe is a straight tube extending up to the mid-thoracic cavity. The wall of the trachea is supported by 'C' shaped rings of hyaline cartilage. These rings are incomplete dorsally and keep the trachea always open preventing collapse.Internally the trachea is lined by pseudostratified ciliated epithelium.

vi. Bronchi and Bronchioles

On entering the mid thoracic cavity, trachea divides at the level of the fifth thoracic vertebra in to right and left primary bronchi. Each primary bronchus enters the corresponding lung and divides into secondary bronchi

that further divide into tertiary bronchi. Each tertiary bronchus divides and re-divides to form primary, secondary, tertiary, terminal and respiratory bronchioles sequentially. Each respiratory bronchiole terminates in a cluster of alveolar ducts which end in alveolar sacs. Bronchi and initial bronchioles are supported by incomplete cartilaginous rings. The branching network of trachea, bronchi and bronchioles constitute the pulmonary tree' (an upside down tree).

vii. Lungs

Lungs occupy the greater part of the thoracic cavity. Lungs are covered by a double layered pleura, with pleural fluid between them. It reduces friction on the lung surface. The outer pleural membrane is in close contact with the thoracic lining whereas the inner pleural membrane is in contact with lung's surface. *The part starting with external nostrils up to the terminal bronchioles constitute the* conducting part, *whereas the alveoli and their ducts form the* respiratory *or* exchange part *of the respiratory system* The conducting part transports the atmospheric air to the alveoli, clears it from foreign particles, humidifies and also brings the inhaled air to the body temperature. Exchange part is the site of actual diffusion of and between blood and atmospheric air.(Figure 1.5)

The lungs are situated in the thoracic chamber which is anatomically an air-tight chamber. It is formed dorsally by the vertebral column, ventrally the sternum, laterally by ribs and on the lower side by the dome-shaped diaphragm. The anatomical setup of lungs in the thorax is such that any change in the volume of thoracic cavity will be reflected in the lung cavity. Such an arrangement is essential for breathing, as the pulmonary volume cannot be directly altered.

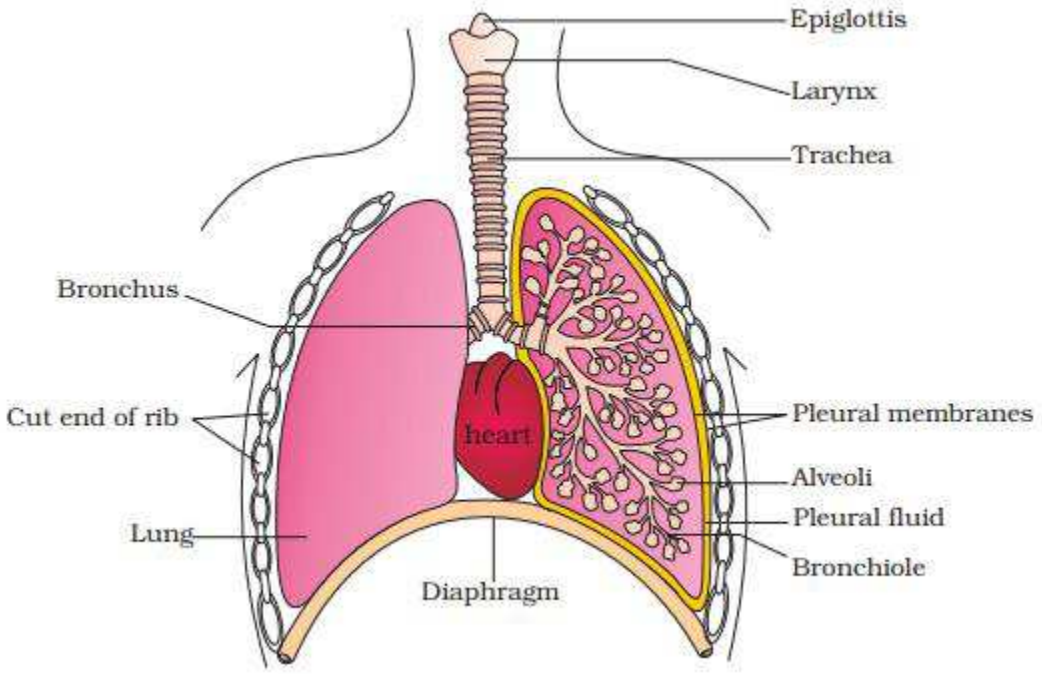


Figure 1.5 Human respiratory system

Respiration in humans involves the following steps:

i. Breathing or pulmonary ventilation by which atmospheric air with 21% of 02 is drawn in and alveolar air rich in CO_2 is sent out.

ii. Diffusion of gases (and) across the alveolar membrane

iii. Transport of gases by blood, between the lungs and tissue cells.

iv. Diffusion of and between the blood in the systemic capillaries and the tissues.

v. Utilization of by the cells for catabolic reactions and resultant production of H2o, and ATP(Cellular respiration).

Mechanism of Breathing

Breathing is a means of maximizing the process of gaseous exchange. The movement of air into and out of the lungs is carried out by creating a pressure gradient between the lungs and the atmosphere. Breathing involves two stages such as inspiration and expiration. Inspiration can occur if the pressure within the lungs (Intra-pulmonaiy pressure) is less than the atmospheric pressure, i.e., there is a negative pressure in the lungs with respect to atmospheric pressure. Similarly, expiration takes place when the intra-pulmonary pressure is higher than the atmospheric pressure. The muscular diaphragm and a specialized set of muscles, the external and internal inter-costal muscles help in generating such gradients.

i. **Inspiration**: Intake of atmospheric air into the lungs is called inspiration. It is an active process , as it takes place by the contraction of the muscles of the diaphragm and the external inter-costal muscles, which extend in between the ribs. The contraction of the diaphragm (phrenic muscles) increases the volume of the thoracic chamber in the anteroposterior axis. The contraction of external inter-costal muscles lifts up the ribs and sternum causing an increase in the volume of the thoracic chamber in the dorso-ventral axis. The overall increase in the thoracic volume causes a similar increase in the 'pulmonary volume'. An increase in the pulmonary volume decreases the intra-pulmonary pressure to less than that of the atmosphere, which forces the air from the outside to move into the lungs, i.e. inspiration.

ii. **Expiration**: Release of alveolar air to the exterior is called expiration. It is a passive process. Relaxation of the diaphragm and the external inter-costal muscles returns the diaphragm and sternum to their normal positions, and reduces the thoracic volume and thereby the pulmonary volume. This leads to an increase in the intra-pulmonary pressure to slightly above that of the atmospheric pressure, causing the expulsion of air from the lungs, i.e. expirarion.(Figure 1.6)

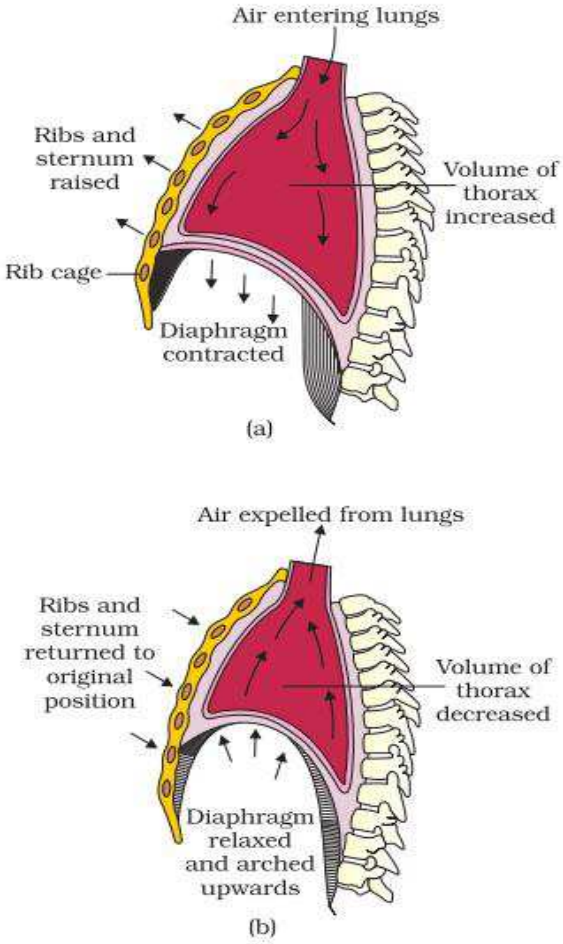


Figure 1.6 Mechanism of breathing showing (a) Inspiration (b) expiration

We have the ability to increase the strength of inspiration and expiration with the help of additional muscles in the abdomen. On an average? A healthy human breaths 12-16 times/minute. The volume of the air Involved in breathing movements, can be estimated by using a spirometer which helps in clinical assessment of the pulmonary functions. Inspiratory Capacity (IC): The total volume of air, a person can inhale after 'normal expiration'. This includes the 'tidal volume' and 'inspiratory reserve volume': IC = TV + IRV. It is about 3000 ml to 3500 ml.

Functional Residual Capacity (FRC): The volume of air that remains in the lungs after normal expiration: FRC=ERV + RV.

Vital Capacity (VC): The maximum volume of air a person can breathe in after 'forced

expiration'. This includes ERV, TV and IRV or the maximum volume of air a person can breathe out after 'forced inspiration': VC=TV+IRV+ERV

Total Lung Capacity (TLC): The total volume of air accommodated in the lungs at the end of 'forced inspiration'. This includes RV, ERV, TV and IRV or vital capacity + residual volume: TLC=VC+RV or TLC= ERV+IRV+TV+RV

Exchange of gases

Alveoli are the primary sites of exchange of gases in the lungs. Exchange of gases also occurs between blood and tissues. O_2 and CO_2 are exchanged in these sites by simple diffusion. The exchange of gases is based on some important factors that can affect the rate of diffusion; these factors include

(i) Partial pressure (ii) solubility of the gases, (iii) thickness of the respiratory membrane, (iv) surface area, (v) distance of diffusion.

The pressure contributed by an individual gas in a mixture of gases is called its partial pressure and is represented as pO_2 for oxygen and pCO_2 for carbon dioxide. The data given below in the table clearly indicates a concentration gradient for oxygen from the alveoli to the blood and from the blood to the tissues. Similarly, a gradient present for in the opposite direction i.e., from the tissues to the blood and from the blood to the alveoli.

Table : Partial pressures (in mm Hg) of oxygen and carbon dioxide at different parts involved in diffusion in comparison to those m the atmospheric air

O_2	159	104	40	95	40
CO_2	0.3	40	45	40	45

As the solubility of CO_2 is 20-25 times higher than that of O_2 the amount of CO_2that can diffuse through the diffusion membrane per unit difference in partial pressure is much higher compared to that of O_2 The diffusion membrane is made up of three major layers namely, the thin *squamous epithelium of the alveolar wall,* the *endothelium of the alveolar capillaries* and the *basement material in between them* As it is a very thin border, it is favourable for diffusion of gases.(Figure 1.7)

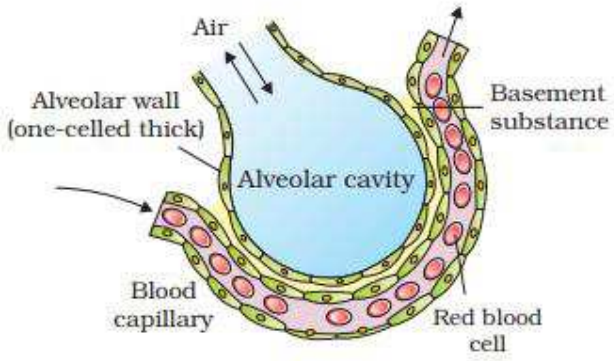


Figure 1.7 A section of alveolus with pulmonary capillary

A. **Pulmonary gas exchange**

Differences in pO_2 and pCO_2 of alveolar air and pulmonary capillaries favour the diffusion of $O_2$from the alveolar air into the blood in the pulmonary capillaries and the diffusion of CO_2in the opposite direction.

B.**Systemic gas exchange**

Difference in $pO_2$and pCO_2 of oxygenated blood in systemic capillaries and tissues favour the diffusion of O_2 from systemic capillaries into tissues and the diffusion of CO2 in the opposite direction.

1.2.2 Transport of gases

Blood is the medium of transport for O_2 and CO_2.

I. Transport of Oxygen

Oxygen is transported from the lungs to the tissues through the plasma and RBC of the blood. 100ml of oxygenated blood can deliver 5 ml of 02 to the tissues under normal conditions.

i. **Transport of oxygen through plasma**: About 3% of 02 is carried through the blood plasma in a dissolved state.

ii. **Transport of oxygen by RBC**: About 97% of O_2 is transported by the RBCs in the blood. Haemoglobin is a red coloured iron containing pigment present in the RBCs. Each haemoglobin molecule can carry a maximum of four molecules of oxygen. Binding of oxygen with haemoglobin is primarily related to the partial pressure of O_2. At lungs, where the partial pressure of O_2 (oxygen tension) is high, oxygen binds to haemoglobin (purplish-bluish-red in colour) in a reversible manner to form oxyhaemoglobin (bright red in colour). This is called oxygenation of haemoglobin.

$$Hb + 4\ \mathbf{O_2} \rightleftarrows Hb(\mathbf{O_2})4$$

At the tissues, where the partial pressure of O_2 is low, oxyhaemoglobin dissociates into haemoglobin and oxygen. The other factors that influence binding of oxygen with haemoglobin are the partial pressure of CO_2, the hydrogen ion concentration (pH) and the temperature.

iii. **Oxygen - haemoglobin dissociation curve**: It explains the relation between percentage saturation of haemoglobin and partial pressure of oxygen. A sigmoid curve is obtained when percentage saturation of haemoglobin with O_2 is plotted against the p O_2. This curve is called 'oxyhaemoglobin dissociation curve' and is highly useful in studying the effect of factors such as pCO_2. H^+ concentration, temperature, etc., on the binding of O_2 with haemoglobin (Figure 1.8).In the alveoli, where there is a high pO_2, low pCO_2, lesser $^{H+}$ concentration (high pH) and lower temperature, the factors are all favourable for the formation of oxyhaemoglobin.In the tissues where low p O_2, high pCO_2, high H^+ concentration (low pH) and higher temperature exist, the conditions are favourable for dissociation of oxygen from oxyhaemoglobin. Under these conditions, oxygen dissociation curve shifts away from the Y-axis (to the right). The effect of pCO_2 and H^+ concentration on the oxygen affinity of haemoglobin is called **Bohr Effect** (increase of carbon dioxide in the blood and decrease in pH results in the reduction of the affinity of hemoglobin for oxygen).

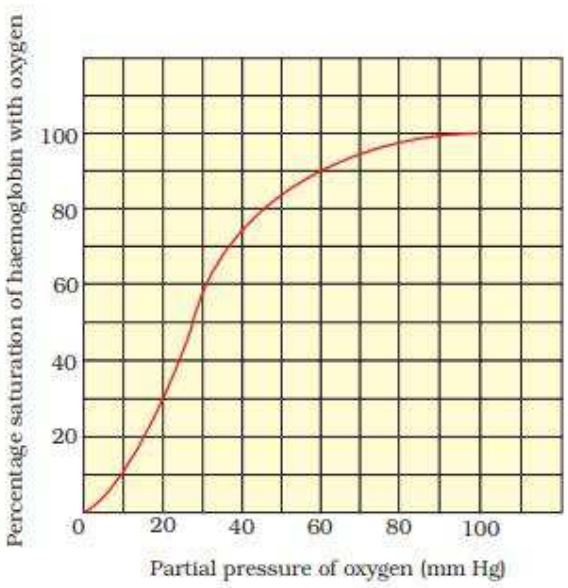


Figure 1.8 Diagram showing oxygen-haemoglobin disassociation curve

NOTE: a rise in pCO_2 and fall in pH decreases the affinity of haemoglobin for oxygen. On the other hand a fall in pCO_2 and rise in pH, increases affinity of haemoglobin for oxygen. *This clearly indicates that* O_2 *gets bound to haemoglobin at the lung surface and gets dissociated at the tissues.*

NOTE: At pO_2 of 100 mmHg. typical in the lungs haemoglobin is saturated to about 97%. At a pO_2°F 40mm Hg which is common in tissues during rest time, haemoglobin is about 75% saturated it means oxyhaemoglobin gives away only about 22% oxygen only to 'resting tissues', it means, only about 1 /5th of the oxygen from blood is unloaded in the tissues. The remaining 4/5th is in the form of 'Reserve' in the blood itself. If the tissues such as skeletal muscles are involved in vigorous exercise, there is more'unl°a<ling tension' in the oxyhaemoglobin and so more oxygen is given away rapidly (up to 62% at 20mmHg.of p02 and the percent of saturation of haemoglobin is only 35%). As mentioned above in a 'resting person', haemoglobin always carries about 70% oxygen (which is still available to tissues, for the asking). Hence oxyhaemoglobin ensures supply of oxygen for survival for 4-5 minutes after the stopping of heart or when breathing is interrupted.

II. Transport of Carbon Dioxide:

CO_2 is transported in three ways.

(i) **In dissolved state**: 7 per cent of C02 is carried in a dissolved state (physical solution) through plasma.

$CO_2 + H_2O \rightleftarrows H_2CO_3$

(ii) **As carbamino compounds**: About 20-25 per cent of C02 combines directly with free amino group of the haemoglobin and forms carbamino- haemoglobin in a reversible manner.

$Hb - NH_2 + CO_2 \rightleftarrows 4Hb - NHCOO + H^+$

This binding of CO_2 is related to the partial pressure of CO_2. pO_2 is a major factor which could affect this binding. When pCO_2 is high and pO_2 is low as in the tissues, binding of more carbon dioxide occurs. When pCO_2 is low and pO_2 is high as in the alveoli, dissociation of CO_2 from carbamino - haemoglobin takes place, i.e., CO_2 which is bound to haemoglobin from the tissues is delivered at the alveoli. Carbamino compounds are also formed by the union of CO_2 with plasma proteins

(iii) **As Bicarbonates**: About 70 per cent of C02 is transported as bicarbonate. RBCs contain a very high concentration of the enzyme, carbonic anhydrase and a minute quantity of the same is present in the plasma too. This enzyme facilitates the following reaction in both the directions.

Carbonic anhydrase Carbonic anhydrase _

CO2+H2O Carbonic anhydrase ▸ H2CO2 ----------► HCO3 +H

At the tissues where partial pressure of CO_2 is high due to catabolism, CO_2 diffuses into the blood (RBC and Plasma) and forms carbonic acid which dissociates into HCO_3~ and H^+. At the alveolar site where pCO_2 is low, the reaction proceeds in the opposite direction leading to the formation of CO_2 and water. Thus CO_2 is mostly trapped as bicarbonate at the tissues and transported to the alveoli where it is released out as CO_2. Every 100 m.l. of deoxygenated blood delivers approximately 4mL of CO_2 to the alveolar air.

GLOSSARY

Alveoli : thin walled ,irregular, highly vascularized bag like structure that form sites of exchange of respiratory gases in lungs.

Bohr Effect:Effect of CO_2 and H^+ on oxygen affinity of heamoglobin.

Carbonic anhydrase: An enzyme present in RBC , that catalyses the formation of carbonic acid from CO_2 and H_2O.

Chloride Shift: Exchange of Chloride and Bicarbonate ions between erythrocytes and plasma.

Glottis: It is an opening of the larynx into pharynx.

Pulmonary Exchange: Exchange of O_2 and CO_2 between pulmonary capillaries and alveoli of lungs.

Systemic Exchange: Exchange of O_2 and CO_2 between systemic capillaries and tissues.

Thyroid cartilage: The largest cartilage that supports the ventral and lateral walls of the larynx.

Vocal cords:A pair of thin strands of yellow elastic fibers that extend between thyroid and arytenoids cartilages.They help production of sound.

VERY SHORT ANSWER TYPE QUESTIONS

1	What are conchae?
2	What is meant by chloride shift?
3	Name the muscles that help in normal breathing movements?
4	What is the effect of pCO_2 on Oxygen transport?
5	Define oxyhaemoglobin curve?
6	Draw a diagram of oxyhaemoglobin disassociation curve?

SHORT ANSWER TYPE QUESTIONS

1	What are the major transport mechanisms of CO_2? Explain?
2	Explain the process of Inspiration and Expiration under normal conditions?

Human Anatomy and Physiology –II

SUMMARY

Vertebrates circulate blood, a fluid connective tissue, in their body, to transport essential substances to the cells and to carry waste substances from there. Another fluid, lymph is also used for the transport of certain substances. Blood comprises of a fluid matrix, plasma and formed elements. Red blood cells, white blood cells and platelets together constitute the formed elements. The spaces between cells in the tissues contain a fluid derived from blood called tissue fluid. This fluid called lymph is almost similar to blood except for the protein content and the formed elements. All vertebrates and a few invertebrates have a closed circulatory system. Human circulatory system consists of a muscular pumping organ, heart, a network of vessels and a fluid, blood. Heart has two atria and two ventricles. Cardiac musculature is auto-excitable. Sino-atrial node (SAN) generates the maximum number of action protentials per minute and therefore, it sets the pace of the activities of the heart. Hence it is called the Pacemaker. The action potential causes the atria and then the ventricles to undergo contraction (systole) followed by their relaxation (diastole). The systole forces the blood to move from the atria to the ventricles and to the pulmonary artery and the aorta. The cardiac cycle is formed by sequential events in the heart which is cyclically repeated and is called the cardiac cycle. A healthy person shows 72 such cycles per minute. About 70 mL of blood is pumped out by each ventricle during a cardiac cycle and it is called the stroke or beat volume. Volume of blood pumped out by each ventricle of heart per minute is called the cardiac output and it is equal to the product of stroke volume and heart rate (approx 5 litres). We have a complete double circulation, i.e., two circulatory pathways, namely, pulmonary and systemic are present. The pulmonary circulation starts by the pumping of deoxygenated blood by the right ventricle which is carried to the lungs where it is oxygenated and returned to the left atrium. The systemic circulation starts with the pumping of oxygenated blood by the left ventricle to the aorta which is carried to all the body tissues and the deoxygenated blood from there is collected by the veins and returned to the right atrium. Though the heart is autoexcitable, its functions can be moderated by neural and hormonal mechanisms.

IIA Body fluids and Circulation

Animals have evolved various methods for transport of substances among body parts Blood is the most commonly used circulatory fluid used by higher vertebrate including human beings. In higher animals in addition to blood **Lymph** is used to transport certain substances.

2.1.1 Lymph and Blood

Lymph: It is the network of thin-walled vessels that arise as blind-ended lymph capillaries in most of the tissues of the body. The lymph capillaries unite to form a tree like structure of increasingly larger lymph vessels, which finally drain lymph into veins in the lower neck region. Lymphatic system is an open circulatory system.

- Lymph capillaries are microscopic, closed-ended tubes that form vast network in the intercellular spaces. The walls are composed of endothelial cells,-with porous junctions, through which interstitial fluid or extracellular fluid **(ECF)**, proteins, microorganisms and absorbed fats can easily enter. Once the extra cellular fluid enters the lymphatic capillaries, it is referred to as lymph.
- The lymph capillaries merge and form large, lymphatic vessels which lead into larger lymph ducts. The walls of the lymph vessels and ducts are similar to those of veins and are provided with valves. The smooth muscles of their walls cause peristaltic waves of contraction pushing lymph towards the neck region.

All the lymph vessels from the lower part of the body eventually empty lymph into the thoracic duct. The thoracic duct of the lymphatic system is the largest lymphatic vessel **(lymph** duct) in the body. It is also known as the left lymphatic duct, chyliferous duct etc., and it empties the lymph into the venous system at the junction of the left internal jugular vein and left **subclavian vein.** Lymph collected from the left side of the head, the left arm, and the parts of the chest region also enters the thoracic duct before it empties into the venous system. Lymph from the right side of the neck, head, right arm, and the right part of the thorax is collected into the right lymphatic duct which empties it into venous system at the junction of the **right sub clavian vein** and internal jugular vein. Thus, lymph ,which was once a part of the blood that came out of the blood vessels to supply oxygen and nutrients to tissues, as the ECF, is finally drained back into the left and right subclavian veins (venous system).

Before lymph is returned to the heart via the venous system, bacteria, viruses etc., are phagocytised by the WBC in the lymph nodes. The lymphatic system also consists of other organs such as spleen, thymus, tonsils etc. MALT and appendix also constitute a part of the lymphatic system.

Formation of lymph

When blood passes through the capillaries, due to high filtration pressure at the arteriolar ends of capillaries, along with plasma, many substances such as glucose, small sized organic molecules, inorganic salts etc. (except the large plasma proteins) are filtered into the extracellular spaces where it is called **'**tissue fluid'/'ECF'**.** This causes an increase in **colloidal osmotic pressure** of the plasma **.** This pressure favours the movement of about 85 percent of the ECF into the capillaries at the venular ends after exchanging oxygen, CO_2, nutrients, other metabolites etc., with the tissues. The remaining 15% of ECF is collected into the lymphatic system through *lymph capillaries.* Henceforth it is called **lymph.**

Composition of lymph

Lymph is similar in its composition to the blood's plasma, except that it contains a much lower concentration of proteins and other nutrients than plasma. Erythrocytes are absent. Lymph contains water, some plasma proteins, electrolytes, leucocytes mostly **lymphocytes,** some coagulation factors, antibodies, enzymes, nutrients etc. *An emulsion of lymph and triglyceride fat (chylomicrons), characteristically present in lacteals is called* **chyle.**

Functions of lymphatic system

1. Lymph returns the absorbed nutrients and wastes from the body parts to the blood.
2. It transports lymphocytes from the lymphatic glands to the blood.
3. It transports *digested fats* which are absorbed through lacteals, present in the intestinal villi, to the blood vascular system.
4. It destroys the invading microorganisms and foreign particles in the **lymph nodes**

Blood circulatory system

Clotting of blood

When a blood vessel is injured a number of physiological mechanisms are activated that promote **hemostasis** (hemo = blood; stasis = standing). Breakage of a blood vessel exposes collagen proteins to the blood. This initiates three separate, but over lapping hemostatic mechanisms - (1) vasoconstriction (2) the formation of a platelet plug, and (3) the production of a web of fibrin proteins (blood clot).

(1) **Vasoconstriction-** When a blood vessel is damaged, the smooth muscles in it's wall contract, which makes the lumen of the vessels narrow, sometimes so strongly that blood flow is completely stopped.

(2) **Platelet-plug formation-** When the endothelium is ruptured, platelets adhere to the collagen and release some secretions. These secretions aggregate other platelets and make them sticky, so that they adhere to those already stuck on the collagen and form a 'platelet- plug'.

(3) **Production of web of fibrin protein-** The third mechanism for hemostasis is the formation of clot. The activator substances from the injured vascular wall, platelets and blood proteins adhering to the injured vascular wall, initiate the clotting process. Clot is a web of fibrin with blood cells trapped in it.

Mechanism of blood clotting

Clotting takes place in three essential steps.

i) Step -1: It involves the formation of a complex of activated substances collectively called, prothrombin activator. It is formed by a complex cascade of chemical reactions that occur in the blood by the involvement of clotting factors in two **pathways.**

(a) Intrinsic pathway: It occurs when the blood is exposed to collagen of injured wall of blood vessel. This activates Factor XII, and in turn it activates another clotting factor, which activates yet another reaction **(cascade fashion),** which results in the formation of the prothrombin activator.

(b) Extrinsic pathway: It occurs when the damaged vascular wall or extra vascular tissue comes into contact with blood. This activates the release of tissue thromboplastin, from the damaged tissue. It activates the Factor VII. As a result of these cascade reactions, the final product formed is the prothrombin activator.

ii) Step -2: The prothrombin activator, in the presence of sufficient amounts of ionic Ca++, causes the conversion of inactive **prothrombin** to active **thrombin (activation of prothrombin).**

iii) Step -3: Thrombin converts the soluble protein fibrinogen into soluble fibrin monomers, which are held together by weak hydrogen bonds. The fibrin stabilizing factor (Factor XIII, released from platelets) replaces hydrogen bonds with covalent bonds and cross links the fibres to form a 'mesh work'. The insoluble mesh work of fibrin fibers spreading in all directions adhere to the damaged surfaces and trap the blood cells and platelets.

Within a few minutes after the clot is formed. It begins to contract so that the fluid is expelled out. This is called clot retraction and the fluid thus formed is the **serum (plasma without fibrinogen).**

Clotting factors

Factor	Name
I	Fibrinogen
II	Prothrombin
III	Thromboplastin
IV	Calcium ions (Ca++)
V	Proaccelerin
VII	Proconvertin
VIII	Antihemophilic Factor / Antihemophilic Globulin **(AHG)**
IX	Plasmathromboplastin component **(PTC)** or Christmas Factor
X	Stuart- Prower Factor
XI	Plasmathromboplastin antecedent **(PTA)**
XII	Hageman's Factor
XIII	Fibrin Stabilizing Factor

* Factor VI is no longer referred.

Anticoagulants

They are as follows:

- **Heparin** is an anticoagulant synthesized by mast cells and basophils. It activates antithrombin, a plasma protein, which combines with thrombin and inactivates it.
- **Coumarins** of plant origin are the precursors of anticoagulants such as warfarin (see

glossary), which are antagonistic to Vit-K and thus prevent the synthesis of the blood clotting factors - **n, VII, IX** and **X,** formed in the **liver.**

- Clotting of blood in test tubes in clinical laboratories and 'Blood Banks' can be prevented by the addition of **citrates** or **oxalates** of sodium or Ethylene diamine tetra acetic acid **(EDTA)**. They bind to calcium ions and thus make Ca++ unavailable for the action in clotting.

2.1.2 Structure of Heart

The heart is **mesodermal** in origin. It is a thick walled, muscular and pulsating organ, situated in the **mediastinum** (the region in the thorax between the two lungs), and with its apex slightly turned to the left. It is of the size of a clinched fist.

The heart is covered by a double walled pericardium which consists of the **outer fibrous pericardium** and the **inner serous pericardium** The serous pericardium is double-layered, formed of an outer **parietal layer** and an inner **visceral layer.** The parietal layer is fused with the fibrous pericardium, whereas the visceral layer adheres to the surface of the heart and forms its outer layer, the **epicardium** The two layers are separated by a narrow pericardial space, which is filled with the **pericardial fluid** This fluid reduces friction between the two membranes and allows free movement of the heart.

The wall of the heart consists of three layers. They are the outer **epicardium** the middle **myocardium** (a thick layer of cardiac muscles), and the inner most **endocardium** (a thin layer of endothelium). The endothelium covers the heart valves also and is continuous with the endothelial lining of the large blood vessels connected to the heart.

External structure

Human heart has four chambers, with two relatively smaller upper chambers, called **atria** and two larger lower chambers called **ventricles** Atria and ventricles are separated by a deep transverse groove called **coronary sulcus** (atrio-ventricular groove). The muscular pouch like projection from each atrium is called **auricular appendix** (auricular appendage). The ventricles are separated by two inter ventricular grooves (anterior and posterior), in which the coronary arteries and their branches are lodged.

Internal structure

i) Atria: Atria are thin walled 'receiving chambers' (upper chambers). The right one is larger than the left. The two atria are separated by thin inter-atrial septum. In the fetal heart, the atrial septum has a small pore called **foramen ovale.** Normally the foramen ovale closes a.t birth, when lungs become functional. It is represented by a depression in the septum between the right and left atria, called **fossa ovalis** (that marks the position of the foramen ovale in the fetus). If, the foramen ovale does not close properly, it is called a **patent foramen ovale.**

The right atrium receives deoxygenated blood from different parts of the body (except the lungs) through three **caval veins** viz. the two precavals (right and left) and a post caval vein. It also receives blood from the myocardium (wall of the heart) through the coronary sinus, whose opening into the right atrium is guarded by the **valve of Thebesius** Opening of the postcaval vein is guarded by the valve of the inferior vena cava or **Eustachian** valve It directs the blood to the left atrium through the foramen ovale, in the foetal stage, but in the adult it becomes rudimentary and non-functional. The openings of the precaval veins into the right atrium have no valves. The left atrium receives blood from each lung through two pulmonary veins, which open into the left atrium. The two left pulmonary veins open by a common aperture in some.

Atria and ventricles are separated by a membranous **atrio-ventricu septum** which possesses left and right atrioventricular apertures. The left and right apertures are guarded by **bicuspid (mitral valve)** and **tricusp** valves respectively.

II) Ventricles: These are the thick walled blood pumping chambers (lower chambers), separated by an interventricular septum. The wall of the left ventricle is thicker than that of the right ventricle. The inner surface of the ventricles is raised into muscular ridges or columns called **columnae cameae/ trabeculae cameae** projecting from the inner walls of the ventricles. Some of these ridges are large and conical, and are called **papillary muscles** whose apices are connected to the **chordae tendineae 'heart strings'.** They are cord-like collagenous processes that connect the **papillary muscles** to the **tricuspid valve and** the **mitral valve** in the heart. They prevent the cusps of the atrioventricular valves from bulging too far into atria during ventricular systole.(Figure no.2.1)

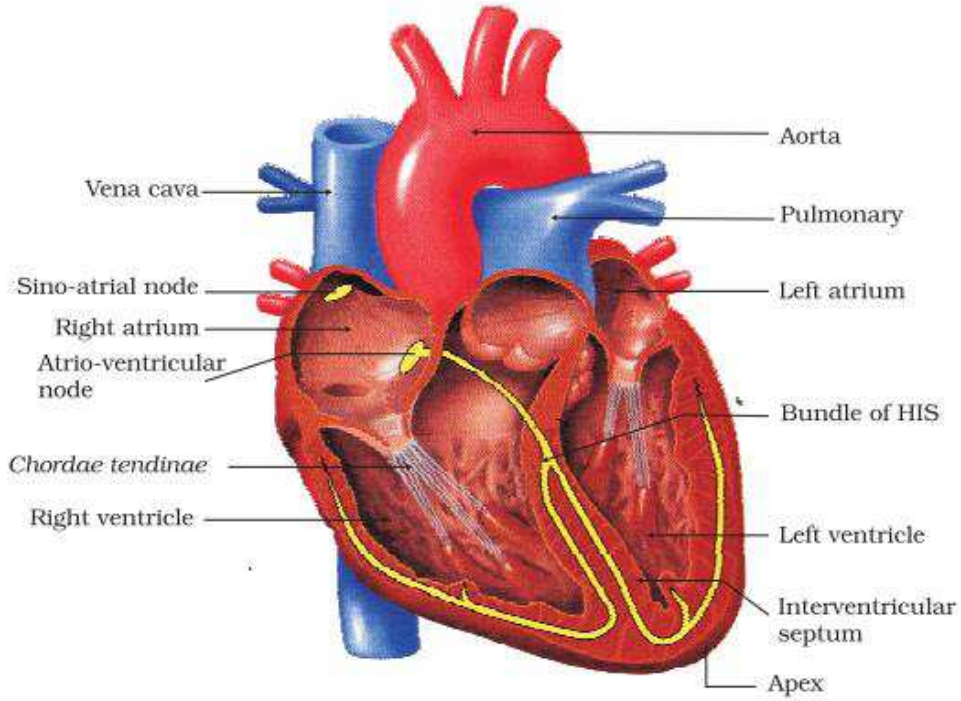


Figure 2.1 Human heart internal structure

Nodal tissue

A specialized cardiac musculature called the nodal tissue is also distributed in the heart. A patch of this tissue called the **sinoatrial node** (SAN) is present in the right upper comer of the right atrium near the openings of the superior venae cavae. Another mass of this tissue, called the **atrioventricular node** (AVN), is seen in the lower left corner of the right atrium close to the atrioventricular septum. A bundle of nodal firbres, called **atrioventricular bundle** (AV bundle/'His' bundle) continues from the AVN into the inter-ventricular septum. It divides into right and left **bundle branches-** These branches give rise to minute fibres called **Purkinje firbres** that extend throughout the ventricular musculature /walls of the respective sides.

SAN consists of specialized **cardiomyocytes.** It has the ability to generate **action potentials** *without any external stimuli* **[myogenic), hence called 'pace maker9. AV NODE** is a **relay point'** that relays the action potentials received from the SA node to the ventricular musculature. SAN can generate action potentials every 0.6 sec. (which means it can initiate 100 beats per minute). Our heart normally beats 70-80 times per minute (on average 72 beats min-1). Autonomous and hormonal coordination systems take charge of increasing or decreasing the rates, depending on the situation.

iii) **Aortic arches:** The **pulmonary arch** arises from the left anterior angle of the right ventricle. Its opening is guarded by the **pulmonary valve** and it carries deoxygenated blood to the lungs. The **systemic arch** (left) arises from the left ventricle and transports oxygenated blood to different parts of the body through its branches. Its opening is guarded by the **'aortic valve'** The pulmonary and aortic velves are madeup of three semilunar flaps, each. A fibrous strand, known as **ligamentum** arteriosum is present at the point of contact of the systemic and pulmonary arches. It is the remnant of the **ductus arteriosus which** connects the systemic and pulmonary arches in the embryonic stage.

2.1.3 Working of Heart

The cardiac events that occur from the beginning of one heart beat to the beginning of the next constitute a cardiac cycle. This cardiac cycle consists of three phases, namely **atrial systole, ventricular systole** and **cardiac diastole.**

To begin with, all the four chambers of the heart are in a relaxed state/ **joint diastole stage**. Blood from the pulmonary veins and venae cavae flows into the respective atria. As the A-V valves are in open condition, blood flows into the left and right ventricles, through the left and right atrioventricular apertures. The semilunar valves of the pulmonary and aortic arches are closed at this stage.

Atrial systole

The SAN now generates an action potential which stimulates both the atria to contract simultaneously causing the **atrial systole**. It lasts about 1 sec. This increases the flow of blood into the ventricles by about 30% . It means atrial systole accounts for about 30 % of the filling of the ventricles, the remaining blood flows into the ventricles before the atrial systole.

Ventricular systole

The action potentials from the SAN reach the AVN from where they are conducted through the bundle of His, its branches and the Purkinje fibres to the entire ventricular musculature. This causes the simultaneous *ventricular systole.* It lasts for about **0.3 sec.** The atria undergo relaxation coinciding with the ventricular systole. Ventricular systole increases the pressure causing the closure of the **AV valves** preventing the **backflow** ' of blood. It results in the production of the first heart sound known as **Lub.** As the ventricular pressure increases further,

the semilunar valves guarding the pulmonary artery and the aorta are forced open. This allows the blood in the ventricles to flow into the **aortic arches** and enter the circulatory pathway.

Cardiac diastole

The ventricles now relax and the ventricular pressure falls causing the closure of the semilunar valves which prevents the back flow of blood. This results in the production of the second heart sound known as **Dup.** As the ventricular pressure declines further, the AV valves are pushed open by the pressure in the atria exerted by the blood, which flowed into them through the larger veins. The blood now once again flows freely into the ventricles. All the heart chambers are now again in a relaxed state (joint diastolic phase). Soon, another cardiac cycle sets in.

NOTE; The human heart beats **72** times per minute normally. Hence the duration of a cardiac cycle is about **0.8 sec.**

Cardiac output

The volume of blood pumped out by each ventricle, for each heart beat, is known as the stroke volume. The volume of blood pumped out by the heart from each ventricle per minute is termed cardiac output.

Cardiac output = **stroke volume** x **No. of beats per minute** = 70ml/ beat x 72 beats/minute = 5040 ml/min. or approximately **5 liters**

Double circulation

The blood pumped by the right ventricle enters the **pulmonary artery**, whereas the left ventricle pumps blood into the **aorta.**The deoxygenated blood pumped into the **pulmonary arch** is passed on to the lungs from where the oxygenated blood is carried by the **pulmonary veins** into the **left atrium** . This pathway constitutes the **pulmonary circulation (lesser circulation).** (Figure 2.2)

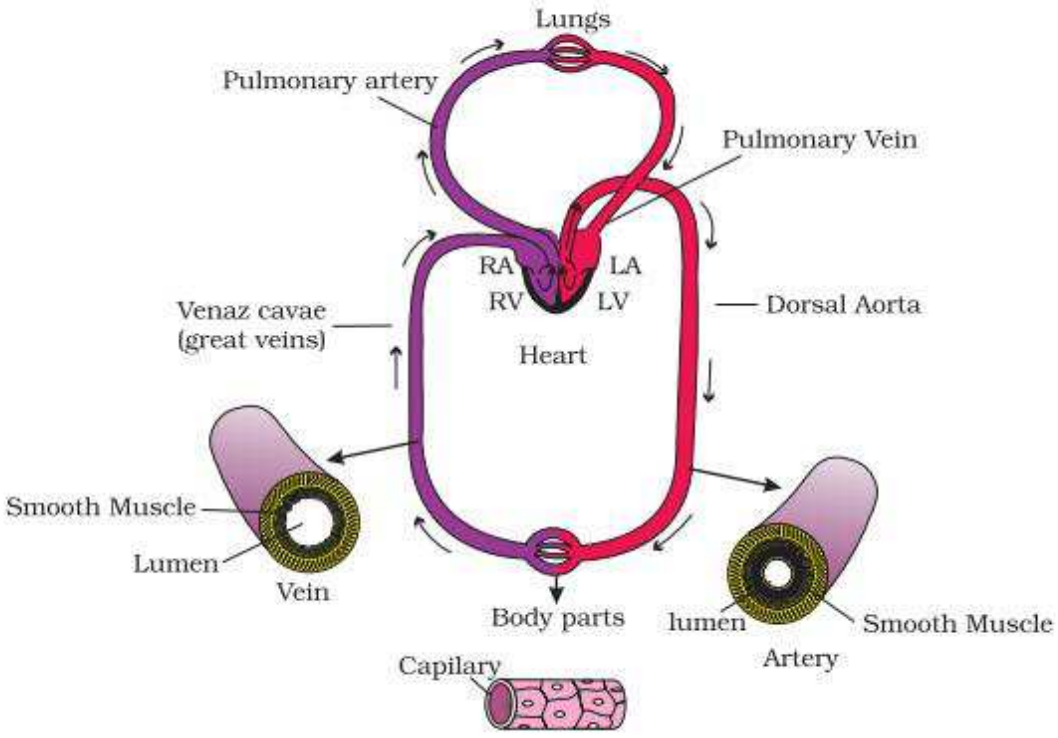


Figure 2.2: Working of human heart

The oxygenated blood entering the aorta is carried by a network of arteries, arterioles and capillaries to the tissues from where the deoxygenated blood is collected by a system of venules, veins and venae cavae and emptied into the right atrium. This is the **systemic circulation (greater circulation**). The systemic circulation provides nutrients, Oa and other essential substances to the tissues and collects C02 and other harmful substances away, for their elimination.

Portal circulation and Coronary circulation

A blood vessel that starts in capillaries and ends in capillaries is called a 'portal vessel'. A system of portal vessel is named after the name of the organ in which it ends in capillaries. In man there is a portal system between the digestive tract and the liver. It is called **hepatic portal system.** The hepatic portal vein carries blood from the gut to the liver before it is delivered to the systemic circulation via the heart. The absorbed foods such as sugars, when

present in excess, are converted into glycogen and stored in the liver cells (glycogenesis). A special coronary system of blood vessels is present in the human body exclusively for the circulation of blood to and from the heart/ cardiac musculature. The coronary circulation in humans includes the right and left coronary arteries and four **cardiac veins/coronary veins.** The coronary veins open into the coronary sinus.

GLOSSARY

Bundle of His: A collection of heart muscle specialized for electrical conduction that transmits electrical impulses from the AV node to the walls of ventricles.

Cardiac arrest: It is the cessation of normal circulation of blood due to sudden stoppage of contraction of heart.

Cascade reaction: A series of chemical or physiological processes that occur in successive stages, each of which is dependent of the preceding one, to produce a culminating effect.

Colloidal osmotic pressure: It is a measurement of pressure exerted within the cardiovascular system by proteins found in blood plasma.

Lacteal: It is the lymphatic capillary in the in the villus of small intestine that absorbs digested food.

Portal venous system: It is a system of blood vessel staring in capillaries in one organ and ending in capillaries in another organ.

Purkinje fibres: These are located in the inner walls of the heart. These fibres are able to conduct cardiac action potential more quickly and efficiently than any other cells in the heart.

VERY SHORT ANSWER TYPE QUESTIONS

1 Sino Atrial Node is called the pacemaker of our heart . Why?
2 Where is the valve of Thebesius in the heart of man?
3 Name the heart sounds. When are they produced?
4 Define cardiac cycle and cardiac output?
5 What is meant by double circulation ? What is its significance ?

SHORT ANSWER TYPE QUESTIONS	
6	Draw a labeled diagram of the L.S.of the heart of man?
7	Distinguish between SAN and AVN?
8	Describe the events in a cardiac cycle briefly?
9	Describe the atria of heart of man?
10	Describe the ventricles of the heart of the man?

II B: Excretory products and their elimination Modes of excretion

Modes of excretion: Animals cannot eliminate free nitrogen, but can eliminate it in the form of nitrogenous end products. The metabolism of excess amino acids, and the nitrogen bases of nucleic acids namely **purines** and **pyrimidines** produce ammonia from which urea or uric acid is formed depending upon the need.

I. **Ammonotelism:** The elimination of ammonia as the chief nitrogenous waste material is termed **ammonotelism.** Ammonia is formed by the oxidative deamination of amino acids. **Deamination** chiefly occurs in the liver of the vertebrate body. The amino group,, separated from the amino acid, combines with hydrogen and becomes ammonia (NH_3). Ammonia is highly toxic and readily soluble in water, hence it should be eliminated from the body quickly and in a very dilute solution. Therefore excretion of ammonia is most common in aquatic species. Many lower invertebrates expel ammonia through the whole body surface by simple **diffusion.** In bony fishes, most of the ammonia is lost as ammonium ions (NH_4) across the epithelium of the gills, with kidneys excreting only minor amounts of nitrogenous wastes. The animals which excrete ammonia are called **ammonotelic animals. .**

II. **Ureotelisin:** Terrestrial adaptation necessitated the production of lesser toxic nitrogenous wastes such as urea and uric acid, for the conservation of water. The elimination of urea as the principal nitrogenous waste material is termed **ureotelism.** It is produced in the vertebrate liver by a metabolic cycle (ornithine cycle) that combines ammonia with carbon dioxide. The circulatory system transports urea to the kidneys for filtration and elimination. Urea is 100,000 times less toxic than ammonia. This permits some animals to transport and store urea safely at higher concentrations (Physiological uremia as seen in cartilaginous fishies). Thus 'urea-excreting' animals require much less water. Some amount of urea may be retained in the medullary fluid of kidneys to maintain the desired osmolarity. Earthworms, cartilaginous fishes, most of the amphibians (which spend most of the time on land) and mammals excrete urea as their chief nitrogenous waste and are called **ureotelic animals.**

III. **Uricotelism:** The elimination of uric acid as the chief nitrogenous waste material is called uricotelism. Uric acid is mainly formed from ammonia mostly in the liver of

sauropsids and in the Malpighian tubules of tracheate arthropods. Uric acid is less toxic than urea and being insoluble in water can be excreted as semisolid paste or pellets with very little water loss. This is a great advantage for animals with little access to water. Tracheate arthropods, land snails, many reptiles and birds excrete uric acid as their major nitrogenous waste, hence they are called **uricotelic animals.**

2.2.1 Human Excretory System

In humans, the excretory system consists of a pair of kidneys, a pair of ureters, a urinary bladder and urethra.(figure 2.3)

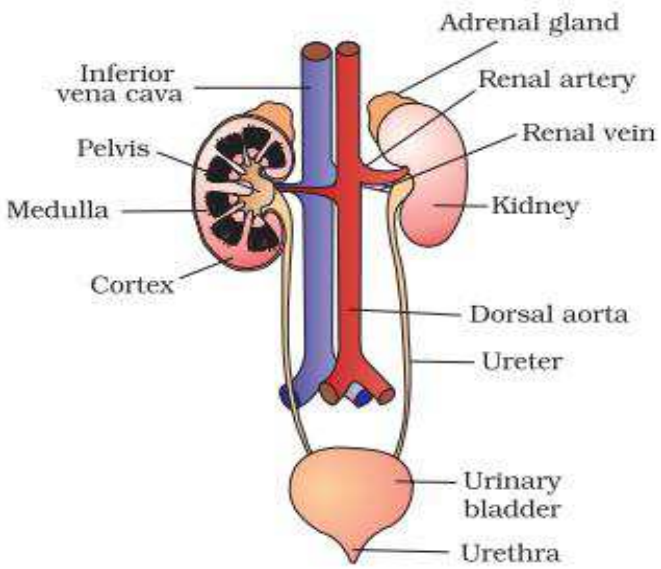


Figure 2.3 Human Urinary system

Kidneys

Kidneys are reddish brown, bean shaped structures, situated on either side of the vertebral column between the levels of the last thoracic and third **lumbar vertebrae,** in a **'retroperitoneal position'.** The right kidney is slightly lower than the left one i due to the presence of liver.

The outer surface of the kidney is convex and the inner surface has a deep notch called **hilum,** the point at which the renal artery and nerves enter and the renal vein and ureter leave. Each kidney is surrounded by a tough, fibrous **capsule** that protects its delicate inner surface.

Internal structure

A longitudinal section of the human kidney shows two distinct regions, the outer **cortex** and the inner **medulla.** The medulla is divided into multiple cone shaped masses of tissue called **renal pyramids.** The renal pyramids are separated by the projections of the cortex called **columns of Bertin.**

The base of each pyramid originates at the border between the cortex and the medulla and terminates in the renal papilla. Renal papillae project into cup like **calyces,** formed by the funnel shaped **pelvis,** which continues out as the ureter. (Figure 2.4)

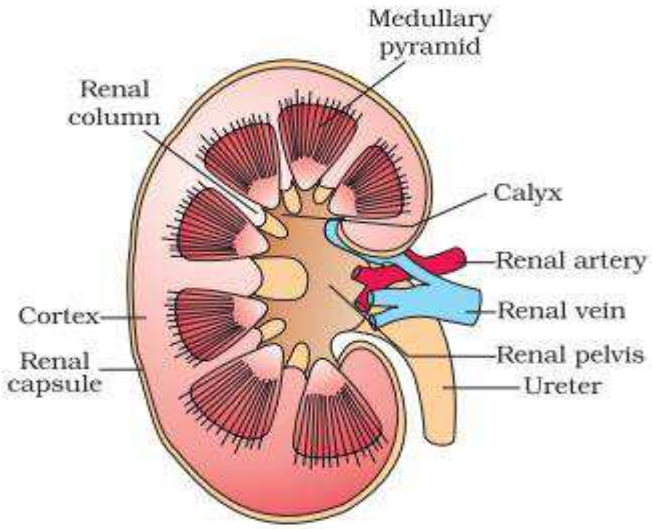


Figure 2.4 L.S. of Kidney

Ureters

These are slender whitish tubes which emerge from the pelvis of the kidneys. Their walls are lined by 'transitional epithelium'. The ureters run downwards and open into the urinary bladder.

Urinary bladder

It is a median storage sac, situated in the lower abdominal cavity. It has thick, muscular, distensible wall lined by 'transitional epithelium'. The neck of the bladder leads into the urethra,

which has an **internal urethral sphincter** (made of smooth muscles) and **external urethral sphincter** (made of striped muscles). Urethra opens near the vaginal orifice in the females and through penis in the males.

Structure of a Nephron

Each kidney has nearly **one million** nephrons which are the 'structural' and 'functional' units. Each nephron has two parts - the **'Bowman's capsule'** and the renal tubule. The Bowman's capsule encloses a tuft of capillaries called **glomerulus,** formed by the **afferent renal arteriole** - a fine branch of the renal artery. Blood from the glomerulus is carried away by an **efferent renal arteriole** of a lesser diameter. The blind end of the tubule forms a double walled cup called Bowman's capsule, which surrounds the glomerulus.

The inner wall of the Bowman's capsule has certain unique cells called **podocytes** which wrap around each capillary. The podocytes are arranged in an intricate manner so as to leave some minute spaces called **'filtration slits** or **'slit pores.** The endothelial cells of the capillaries have numerous pores or '**fenestrations'.** The glomerulus along with the Bowman's capsule constitutes the **Malpighian body** or **renal corpuscle.**(Figure 2.5)

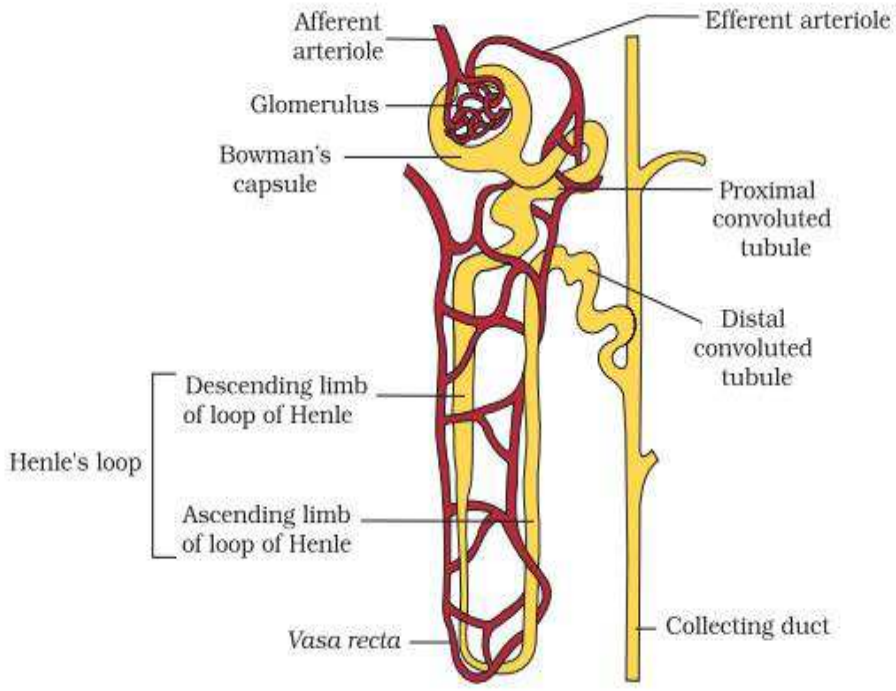


Figure 2.5 Nephron showing blood vessels, collecting duct and tubule

The tubule continues further and forms a highly coiled **proximal convoluted tubule** (PCT). A hairpin shaped **Henle's loop,** which has descending and ascending limbs, is the next part of the tubule. The proximal part of the ascending limb is thin and the distal part is thick. The thick ascending limb continues into the **distal convoluted tubule** (DCT). The DCT continues as the **initial collecting duct** in the cortex. Some initial collecting ducts unite to form a straight collecting duct, which passes through the medullary pyramid. In the medulla, the tubes of each pyramid join and form the duct of Bellini, which finally opens on the tip of the renal papilla. The contents of the duct of Bellini are discharged into the renal pelvis through the renal calyx.

The Malpighian corpuscle, PCT and DCT of a nephron are situated in the cortical region of the kidney, whereas the loop of Henle is in the medulla. In a majority of nephrons, the loop of Henle is too short and extends only very little into the medulla. Such nephrons are called cortical nephrons, in some of the nephrons, the loops of Henle are very long and run deep into the medulla. These nephrons are called juxtamedullary nephrons.

The efferent arteriole emerging from the glomerulus forms a fine capillary network called the peritubular capillaries, around the renal tubule. The portion of the peritubular capillaries that surrounds the loop of Henle is called the vasa recta. The vasa recta is absent or highly reduced in the cortical nephrons. The juxtamedullary nephrons possess well developed vasa recta.

Do you know? After the age of 40 years, the number of functioning nephrons usually decreases, by about 10 percent, every 10 years.

2.2.2 Urine Formation

The formation of urine involves three main processes namely, glomerular filtration, selective reabsorption and tubular secretion-

a) Glomerular nitration: The first step in the formation of urine is the 'filtration' of the blood from the glomerulus into the lumen of the Bowman's capsule and this 'passive' (non-energy consuming process) process is called glomerular filtration The hydrostatic pressure of the blood while flowing in the glomerulus is 60 mmHg. It is opposed by glomerular colloidal osmotic pressure is 32 mmHg (which is exerted by the non-filtered plasma proteins of the blood in the glomerular capillaries) and Bowman's capsular hydrostatic pressure of 18mmHg. The net filtration pressure is 10mm Hg (60-32+18 =10). This causes

the filtration of blood through the 3 layered filtrate membrane formed by the endothelial cells of glomerular capillary together with the basement membrane and podocytes of the Bowman's cup. Blood is filtered through the fine slit pores and fenestrations due to the NFP. Therefore, this process is called 'ultrafiltration'- The filtrate contains almost all the constituents of the plasma, except the proteins. The filtrate thus formed is called ultra-filtrate or 'glomerular filtrate' or 'primary urine', which is hypotonic to the cortical fluid. It passes into the next part of the renal tubule.

NOTE: The amount of filtrate formed by both the kidneys, per minute, is called Glomerular Filtration Rate. The GFR in a healthy individual is approximately 125ml/minute, i.e., 180L per day. Although about 180L of glomerular ultra-filtrate is produced each day, the kidneys normally excrete only 1 to 1.5 L of urine in a 24 hour period. A comparison of these two volumes suggests that nearly 99% of the filtrate is reabsorbed by the renal tubules. This process is called reabsorption/ selective reabsorption.

b) **Selective reabsorption and secretion**: The tubular epithelial cells in different segments of a nephron reabsorb certain substances of the glomerular filtrate either by active or passive mechanisms. About 85% of the filtrate formed is reabsorbed in a constant, unregulated fashion by the PCT and descending limb of Henle's loop (obligatory or mandatory reabsorption) and the reabsorption of the rest of the fluid is 'regulated'. Based on the necessity of re-absorption, the substances of glomerular filtrate can be categorized into high threshold substances' (essential and are efficiently reabsorbed e.g. glucose, amino acids, vitamins, some salts etc.), 'low threshold substances' (absorbed in very little amounts e.g. urea, uric acid etc.) or 'athreshold substances' (actual excretory products and are not reabsorbed at all e.g. creatinine).

During the formation of urine, the tubular cells secrete substances such as H^+, K^+ and NH_3 into the filtrate. Tubular secretion is also an important step in the formation of urine as it helps in the maintenance of ionic and acid- base balance of the body fluids. Mechanism of selective reabsorption and secretion in different parts of a nephron takes place as follows:

i) **In the proximal convoluted tubule**: PCT is lined by simple cuboidal epithelium with brush border', which increases the surface area of absorption. Nearly all the essential nutrients and 70-80% of electrolytes and water are reabsorbed by this segment. Na^+ is

actively transported into the cortical interstitial fluid. This transfer of positive charge drives the passive transport of Cl^-. Glucose, amino acids, and other essential substances are also 'actively transported'. Movement of water occurs by osmosis .PCT also helps to maintain the pH and ionic balance of the body fluids by selective secretion of hydrogen ions ammonia the filtrate and by the absorption of HCO_3- from it.

ii) **In the Henle's loop**: Reabsorption in this segment is minimum. However, this region plays a significant role in the maintenance of high osmolarity of the medullary interstitial fluid.

The descending limb of loop of Henle is permeable to water and almost impermeable to electrolytes, hence reabsorption of water continues as the filtrate moves along the descending limb (passive transport). As a result, the filtrate concentration gradually increases as it moves towards the inner medulla. The ascending limb has two specialized regions, a proximal thin segment in which NaCl diffuses out into the interstitial fluid passively and a distal thick segment in which NaCl is actively pumped out. The ascending limb is impermeable to water. Thus the filtrate becomes progressively more dilute as it moves up to the cortex (towards the DCT).(Figure 2.6)

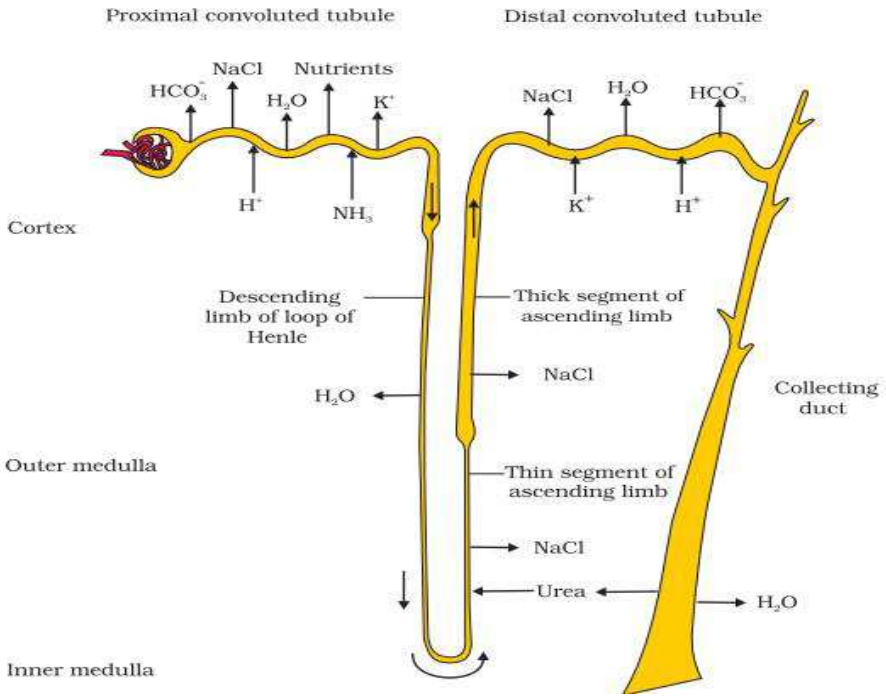


Figure 2.6 Reabsorption and secretion of major substances at different parts of Nephron

iii) In the distal convoluted tubule (DCT)

The cells here are shorter than those in the proximal tubule and lack 'microvilli', indicating that they are not involved much in reabsorption.**'Conditional reabsorption'/ 'facultative reabsorption** of Na^+ and water takes place in this segment. The reabsorption of water is variable depending on several conditions and is regulated by ADH. DCT is also capable of reabsorption of HCO_3^- and selective secretion of H^+ and K^+ ions and NH_3 into the DCT from the peritubular network, to maintain the pH and sodium-potassium balance in the blood.

iv) In the collecting duct (CD)

This long duct carries the filtrate through the medulla to the renal pelvis. Considerable amount of water could be reabsorbed from this region to produce concentrated urine. This segment allows passage of small amount of urea to the medullary interstitum to keep up its osmolarity. It also plays a role in the maintenance of pH and ionic balance of blood by the selective secretion of H^+ and K^+ ions. The renal fluid after the process of facultative reabsorption in the CD, influenced by ADH, constitutes the '**urine**', that is sent out. Urine in the CD is hypertonic to the plasma of blood.

GLOSSARY

Deamination: the removal of an amine group from a molecule.

Duct of Bellini: Any of the large excretory ducts of uriniferous tubules of the kidney that open at the tip of renal papilla into calyx and thus into the renal pelvis.

Glomerular filtration :It is volume of fluid filtered from the renal glomerular capillaries into the Boeman's capsuale.

Osmolarity :The mesure of sulute concentration.

Column of Bertin: It is a medullary extension of the renal cortex in between the renal pyramids.

Renal pelvis:It is the funnel like dilated proximal part of ureter in the kidney.

Renin: It is an enzyme secreted by JG cells .This enzyme catalyzes the conversion of **angiotensinogen** into **angiotensin 1.**

Ultra filtration: It is the process of filtration of water and some constituents of plasma except the proteins.

VERY SHORT ANSWER TYPE QUESTIONS	
1	What are the columns of Bertin?
2	Define glomerular filtration?
3	Name the blood vessels that enter and exit the kidn
4	What are renal pyramids and renal papillae?
5	What is meant by the term osmoregulation?

SHORT ANSWER TYPE QUESTIONS	
1	Draw a neat labeled diagram of the V.S. Kidney ?
2	Describe the internel structure of kidney of man?

Human Anatomy and Physiology –III

SUMMARY

Movement is an essential feature of all living beings. Protoplasmic streaming, ciliary movements, movements of fins, limbs, wings, etc., are some forms exhibited by animals. A voluntary movement which causes the animal to change its place, is called **locomotion**. Animals move generally in search of food, shelter, mate, breeding ground, better climate or to protect themselves. The cells of the human body exhibit amoeboid, ciliary and muscular movements. Locomotion and many other movements require coordinated muscular activities. Three types of muscles are present in our body. Skeletal muscles are attached to skeletal elements. They appear striated and are voluntary in nature. Visceral muscles, present in the inner walls of visceral organs are nonstriated and involuntary. Cardiac muscles are the muscles of the heart. They are striated, branched and involuntary. Muscles possess excitability, contractility, extensibility and elasticity. Muscle fibre is the anatomical unit of muscle. Each muscle fibre has many parallelly arranged myofibrils. Each myofibril contains many serially arranged units called **sarcomere** which are the functional units. Each sarcomere has a central 'A' band made of thick myosin filaments and two half 'I' bands made of thin actin filaments on either side of it marked by 'Z' lines. Actin and myosin are polymerised proteins with contractility. The active sites for myosin on resting actin filament are masked by a protein-troponin. Myosin head contains ATPase and has ATP binding sites and active sites for actin. A motor neuron carries signal to the muscle fibre which generates an action potential in it. This causes the release of Ca^{++} from sarcoplasmic reticulum. Ca^{++} activates actin which binds to the myosin head to form a cross bridge. These cross bridges pull the actin filaments causing them to slide over the myosin filaments and thereby causing contraction. Ca^{++} ions are then returned to sarcoplasmic reticulum which inactivate the actin. Cross bridges are broken and the muscles relax. Repeated stimulation of muscles leads to fatigue. Muscles are classified as Red and White fibres based primarily on the amount of red coloured myoglobin pigment in them. Bones and cartilages constitute our

skeletal system. The skeletal system is divisible into axial and appendicular. Skull, vertebral column, ribs and sternum constitute the axial skeleton. Limb bones and girdles form the appendicular skeleton. Three types of joints are formed between bones or between bone and cartilage – fibrous, cartilaginous and synovial. Synovial joints allow considerable movements and therefore, play a significant role in locomotion.

IIIA Musculo-Skerletal System

The muscle

Muscle is specialised tissue with special properties such as ***excitability***, ***contractility***, and ***relaxation***. About 40-50 percent of total body weight of human adult is contributed by the muscles. The skeletal muscles are primarily involved in bringing in changes in body potures.

3.1.1 Structure of a Skeletal Muscle

Let us examine a skeletal muscle in detail to understand the structure and mechanism of contraction. Each organised skeletal muscle in our body is made of a number of muscle bundles or fascicles. Each fascicle contains a number of cylindrical muscle fibers. The fascicles are held together by a common collagenous connective tissue layer called fascia.

NOTE: Collagen is a fibrous protein constituent of bone, cartilage, tendon, and other connective tissue. It is the chief protein in the human body.

A) *Ultra structure of a Skeletal Muscle Fibre*

Each muscle fibre is lined by the plasma membrane called sarcolemma enclosing the sarcoplasm. Skeletal muscle fibre is a '**syncytium**', as each fiber is formed by fusion of embryonic, mononucleate '**myoblasts**'. Hence, the skeletal muscle cells are multinucleate, with characteristically peripheral nuclei (just below the sarcolemma). The endoplasmic reticulum, also called sarcoplasmic reticulum of the muscle fibers is the store house of calcium ions. A characteristic feature of the muscle fiber is the presence of a large number of parallel filaments called myofilaments or myofibrils, in the sarcoplasm.(figure 3.1)

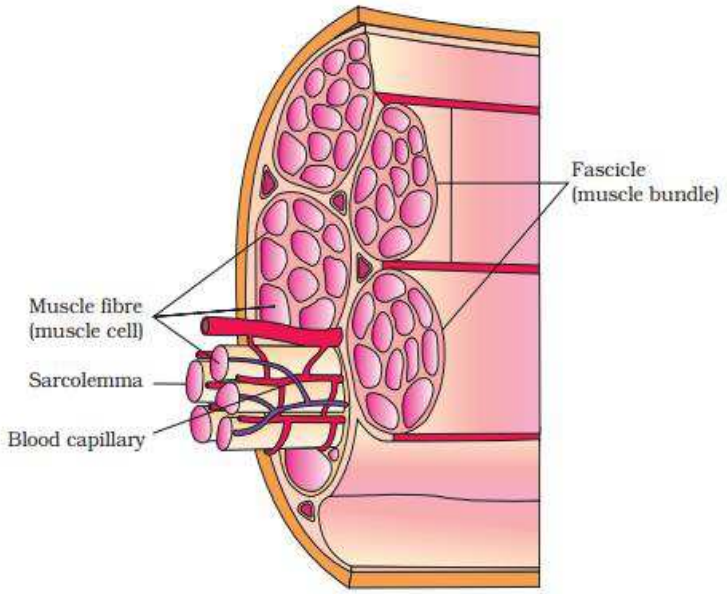


Figure 3.1 Skeletal muscle fiber

i) *Structure of Myofibril*

Each myofibril has alternate dark and light bands in it. A detailed study of the myofibril has established that the striated appearance is due to the distribution pattern of two important proteins - actin and myosin. The light band contains actin and two regulatory proteins called troponin and tropomyosin. The light band is called I-band or Isotropic band. The dark band called A -band or Anisotropic band contains myosin. Both the proteins, actin and myosin, are arranged as rod-like structures, parallel to each other and also to the longitudinal axis of the myofibrils. Actin filaments are thinner compared to the myosin filaments, hence actin and myosin filaments are commonly called thin and thick filaments, respectively. In the centre of each T band is an elastic fiber called 'Z' line (called Z line because of its 'Z' like appearance in electron micrographs) which bisects it. The thin filaments are firmly attached to the Z' line (also called Krause's membrane or Dobie's line). The thick filaments are also held together in the middle of the 'A' band by a thin fibrous membrane called M' line. All the thick filaments are arranged at the same level, thus giving the characteristic striated/striped appearance. The 'A' and 'I' bands are arranged alternately throughout the length of the myofibrils.

ii) Sarcomere

The portion of the myofibril between two successive 'Z' lines is called sarcomere. It is the functional unit of contraction. In the resting /relaxed state, the edges of the thin filaments encroach into the A -band on either side and partially overlap the free ends of the thick filaments. The central part of the A -band/dark band without the thin filaments is called the 'H' zone / 'H' band / Hensen's disc. It is relatively less dark than the edges of the A-band as there are no thin filaments in it. (Figure 3.2)

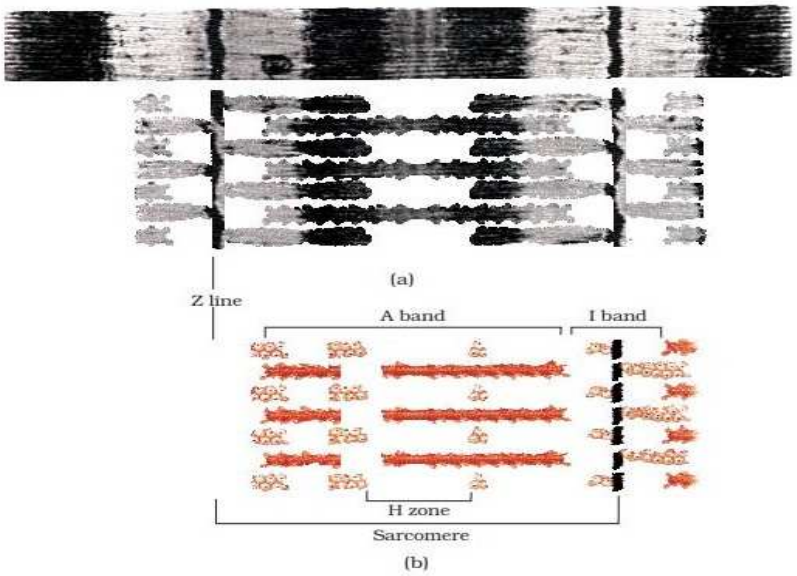


Figure 3.2 Diagrammatic represent of (a) anatomy of sarcomere (b) sarocomere

iii) Triad system

Sarcolemma, in many types of muscles, invaginates into the sarcoplasm and forms transverse tubules (T-tubules) at the level of Z line in the nonmammalian vertebrates. *In the mammals, they penetrate into the junction between the* A *and* I *bands.* Each T tubule is flanked on either side by several terminal cisternae of the sarcoplasmic reticulum. T tubule and the two terminal cisternae at its sides form the triad system. Most of the stored calcium of the sarcocyte is located in the terminal cisternae.

A) Motor Unit

A motor neuron and the set of muscle fibres innervated by all the telodendrites constitute a motor unit. The junction between a motor neuron and the sarcolemma of a muscle fibre is called the

neuromuscular junction or motor- end plate. Sarcomere of a striated muscle can be considered a functional unit of contraction.

3.1.2 Mechanism of Muscle Contraction

Mechanism of muscle contraction is best explained by the '**Sliding Filament Theory**'. It states that contraction of a muscle fibre takes place by the sliding of the thin filaments over / in between the thick filaments. It was proposed by Jean Hanson and Hugh Huxley. The process of muscle contraction can be studied under the following heads:

i) **Excitation of muscle**: Muscle contraction is initiated by A signal sent by the central nervous system (CNS) via a, motor neuron. A neural signal reaching the neuromuscular junction releases a neurotransmitter (acetylcholine) which generates an action potential in the sarcolemma. When the action potential spreads to the triad system through the T tubules, the cisternae of the sarcoplasmic reticulum release calcium ions into the sarcoplasm.

ii) **Formation of Cross bridges**: Increase in the Ca_2^+ level leads to the binding of calcium ions to the subunit Tn-C of the troponin of the thin filaments. This makes troponin and tropomyosin complex to move away from the active sites of actin molecules. Now, the active sites are exposed to the heads of the myosin. Utilizing the energy released from hydrolysis of ATP, the myosin head now binds to the exposed 'active sities' on the actin molecules to form a cross bridge and is released.(Figure 3.3)

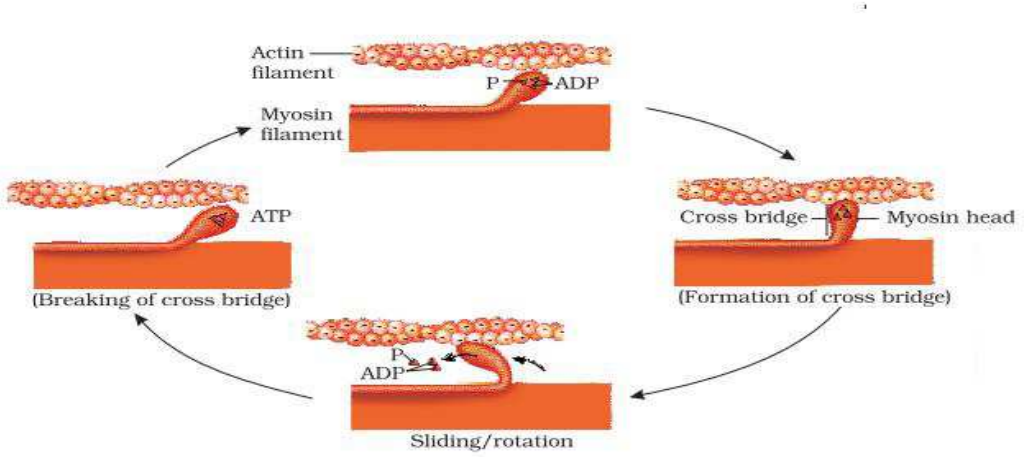


Figure 3.3 stages of cross bridge formation

iii) **Power Stroke**: The cross bridge pulls the attached actin filaments towards the centre of the 'A' band. The 'Z' lines attached to these actin filaments are also pulled inwards from both the sides, thereby causing shortening of the sarcomere, i.e., contraction. During the shortening of the muscle, the T bands get reduced in size/length (Z membranes of the sarcomere are brought closer), whereas the 'A' bands retain their size /length. It is important to note that myofilaments do not actually shorten. As the thin filaments are pulled deep in to the A bands making the H bands narrow, the muscle show the effect-**contraction**.

iv) **Recovery Stroke**: The myosin goes back to its relaxed state and releases ADP. A new ATP molecule binds to the head of myosin and the crossbridge is broken. The new ATP is again hydrolysed by the ATPase of the myosin head and the cycle of cross bridge formation, and breakage is repeated causing further sliding.

v) **Relaxation of Muscle**: When motor impulses stop the Ca_2^+ ions are pumped back into the sarcoplasmic cistemae. It results in the masking of the active sites of the actin filaments. The myosin heads fail to bind with the active sites of actin. These changes cause the return of 'Z' lines back to their original position, i.e., relaxation.(figure 3.3)

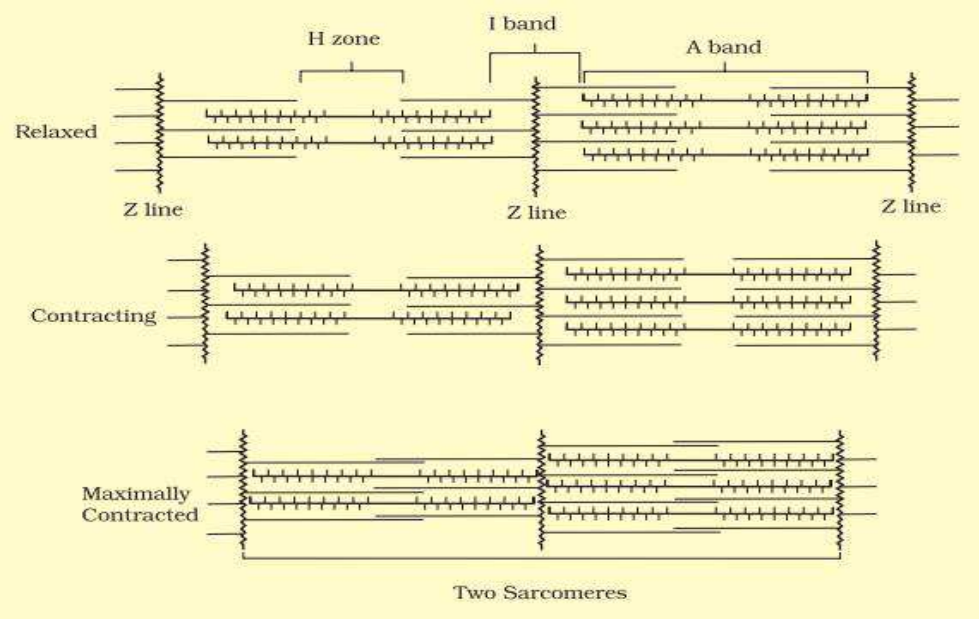


Figure 3.4 Sliding filament theory of muscle contraction

Muscle Fatigue

Repeated activation of the skeletal muscles can lead the accumulation of lactic acid due to anaerobic breakdown of glucose in them, causing fatigue. At this state the skeletal muscle fails to contract temporarily.

Cori Cycle

The lactic acid produced during rapid contractions of skeletal muscles under low availability of oxygen is partly oxidized and a major part of it is carried to the liver by the blood, where it is converted into pyruvic acid (pyruvate) and then to glucose through gluconeogenesis.

The glucose can enter the blood and be carried to muscles and immediately used. If, by this time the muscles have stopped contraction, the glucose can be used to rebuild reserve of glycogen through glycogenesis. This two way traffic between skeletal muscle and liver is called the Cori cycle.

3.1.3 The Skeleton

Skeletal system consists of a framework of bones and a few cartilages. This system has a significant role in maintaining the posture and the movement of body parts. In adult human beings, skeletal system is made up of 206 bones and a few cartilages. It is grouped into two principal divisions - the *axial skeleton* and the *appendicular* skeleton.

Axial skeleton

It comprises 80 bones distributed along the main axis of the body. The skull, vertebral column, sternum and ribs constitute the axial skeleton.

I. The skull

It is composed of two sets of bones -cranial and facial bones (22 bones in all).

Cranium, the brain box, is formed by eight flattened bones. They are a) *frontal bone* (1), b) *Parietals* (2), c) *Temporal bones* (2), d) *Occipital bone* (l), e) *Sphenoid bone* (1) and f) *Ethmoid bone(* 1).(Figure 3.5)

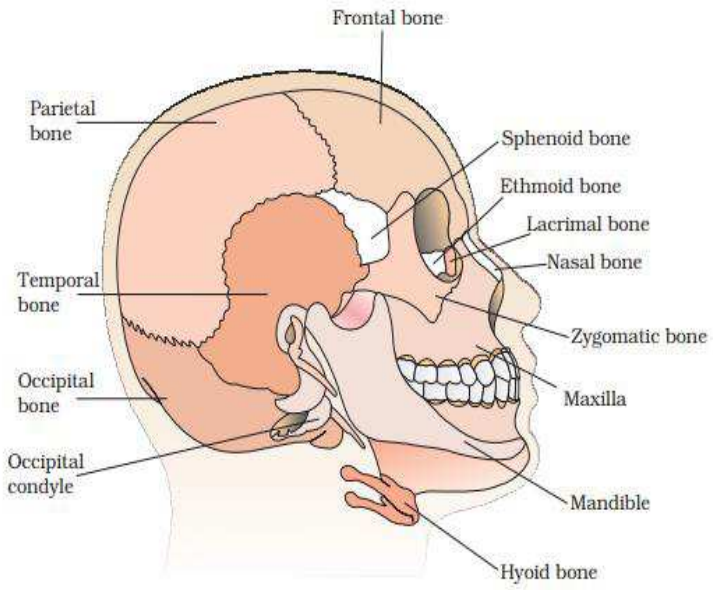


Figure 3.5 Diagrammatic view of human skull

i) Frontal bone: It forms the forehead, anterior part of the cranial floor, and the roof of the orbits.

ii) Parietal bones: They form the major portion of the sides and roof of the cranial cavity. They are joined to the frontal bone by a coronal suture and posteriorly to the occiput by lambdoid suture.

iii) Temporal bones: They form the lateral parts and the floor of the cranium.

iv) Occipital bones : It forms the posterior part and most of the base of the cranium. It has a large opening, the foramen magnum. Medulla oblongata passes out through this foramen and joins the spinal cord. Two occipital condyles are present one on each side of the foramen magnum .

v) Sphenoid bone : It is present at the middle part of the base of the skull. It is the keystone bone of the cranium because it articulates with all the other cranial bones.

vi) Ethmoid bone: It is present on the midline of the anterior part of the cranial floor.

A) **The facial region** is made up of 14 skeletal elements which form the front part of the skull. The bones of the facial skeleton are the *nasals (2), the maxillae (2), the zygomatic bones (2), the mandible (1), the lacrimal bones (2), the palatine bones (2), inferior nasal conchae (2),* and *the vomer* (1)

i) Nasal bones: These are paired bones that form the bridge of the nose.

ii) Maxillae: Two maxillae join together and form the upper jaw. The maxilla bears sockets (alveoli) for lodging the maxillary teeth. The palatine process is involved in the formation of the hard palate.

iii) Zygomatic bones: These are known as cheek bones.

iv) Lacrimal bones: These are the smallest bones of the face

v) Palatine bones: They form the posterior portion of the hard palate.

vi) Nasal conchae: These are scroll like bones that form a part of lateral wall of the nasal cavity. Nasal conchae are 3 pairs, namely superior, middle and inferior.

vii) Vomers: It is a triangular bone present on the floor of nasal cavity.

viii) Mandible (Lower jaw): It is 'U' shaped and is the longest and strongest of all the facial bones. It is the only movable skull bone (except the ear ossicles).

B) ***Skeletal structures associated with sense organs***

i) The nasal cavity is divided into left and right cavities by a vertical partition called the nasal septum.

ii) Orbits: Orbits are bony depressions which accommodate the eyeballs and associated structures.

iii) Ear Ossicles: Each middle ear contains three tiny bones - Malleus (modification of articular), Incus (modified quadrate) and Stapes (modified hyomandibula), collectively called ear ossicles.

C) ***Hyoid Bone***

It is a single U shaped bone present at the base of the buccal cavity between the laiynx and the mandible. The hyoid bone keeps the larynx open.

II Vertebral Column

Our vertebral column is formed by 26 serially arranged units called vertebrae.

Structure of Vertebra

Each vertebra has a central hollow portion (neural canal) through which the spinal cord passes. A typical vertebra consists of the body (centrum), vertebral arch, and many processes for articulation or attachment of muscles. Articulations between the bodies of successive vertebrae are called intervertebral discs.

The vertebral column is differentiated into cervical (7), thoracic (12), lumbar (5), sacral (1-fused) and coccygeal (1-fused) regions starting from the skull.(Figure 3.6)

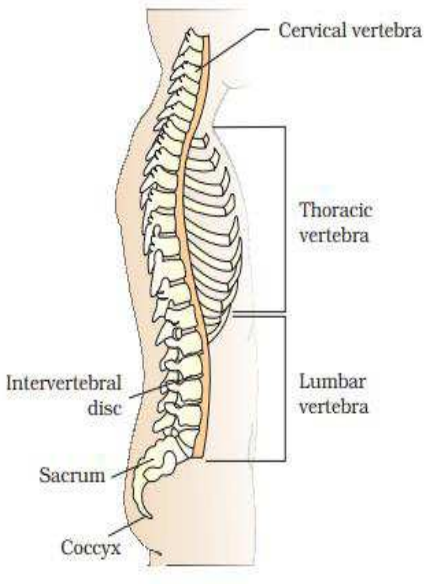


Figure 3.6 Vertebral column (right lateral view)

a) Cervical vertebrae: The first vertebra is called the atlas and the second the axis. Atlas is articulated with the occipital condyles, and has an odontoid canal. Axis has a strong odontoid process that fits in to odontoid canal of the atlas to facilitate rotation of head.

b) Thoracic vertebrae: Thoracic region consists of twelve vertebrae. The heads of ribs namely capitulum and tuberculum articulate with the 'articular facets' of the thoracic vertebrae.

c) Lumbar vertebrae: These are the largest and strongest free vertebrae. They provide surface for the attachment of the large back muscles.

d) Sacrum: It is a triangular bone formed by the fusion of five sacral vertebrae.

e) Coccyx: It is a triangular bone formed by the fusion of four coccygeal vertebrae.

Functions

i) Vertebral column protects the spinal cord in its neural canal

ii) It supports the skull

iii) It serves as the point of attachment for the ribs and musculature of the back.

III Sternum (breast bone)

It is a flat bone on the mid ventral line of the thorax. It consists of three parts. The superior (anterior) part is the manubrium, the middle part is the body, and the inferior (posterior) smallest xiphoid process. The sternum space for the attachment of the thoracic ribs and abdominal muscles.

IV Ribs

Twelve pairs of ribs are present in the human chest. Each rib is a thin flat bone connected dorsally to the vertebral column and ventrally to the sternum. It has two articulation surfaces on its dorsal end, hence called bicephalic. The first seven pairs of ribs are called true ribs (vertebro-sternal ribs). Dorsally, they are attached to the thoracic vertebrae and ventrally connected to the sternum with the help of hyaline cartilages. The remaining ribs are called 'false ribs'. The 8^{th}, 9^{th} and 10^{th} pairs of ribs do not articulate directly with the sternum but join the cartilaginous (hyaline cartilage) parts of the seventh rib. These are called vertebro-chondral ribs. Last 2 pairs (11^{th} and 12^{th}) of ribs are not connected ventrally either to the sternum or the anterior ribs, hence called floating ribs. The thoracic vertebrae, ribs and sternum together form the rib cage.(Figure 3.7)

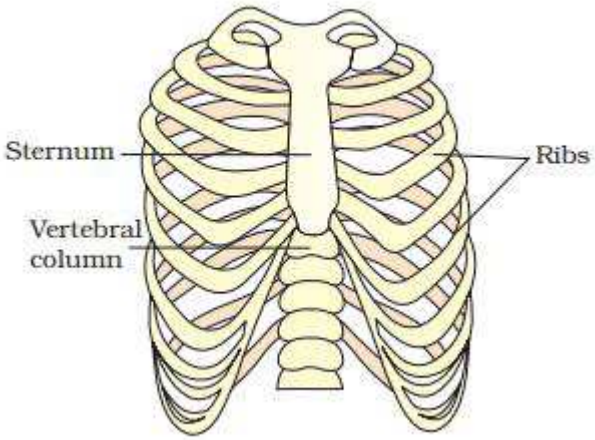


Figure 3.7 Ribs and rib cage

3.1.4 Appendicular Skeleton

The bones of the limbs along with their girdles constitute the appendicular skeleton. The appendicular skeleton is composed of 126 bones.

i) Bones of the Forelimb

Each limb is made of 30 bones. The bones of the hand (forelimb) are humerus, radius and ulna, carpals (wrist bones - 8), metacarpals (palm bones - 5) and phalanges (digits - 14).(Figure 3.8)

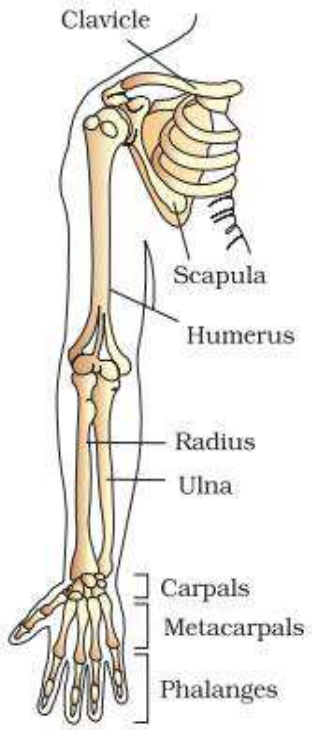


Figure 3.8 Fore limb

ii) Bones of the Hind limb

The bones of the leg are Femur (1) (thigh bone - the longest bone), tibia (1) and *fibula* (1J, tarsals (ankle bones - 7), metatarsals (5) and phalanges (digits- 14) are the bones of the legs (hind limbs). A cup shaped bone called patella (knee cap) (1) covers the knee joint ventrally.(Figure 3.9)

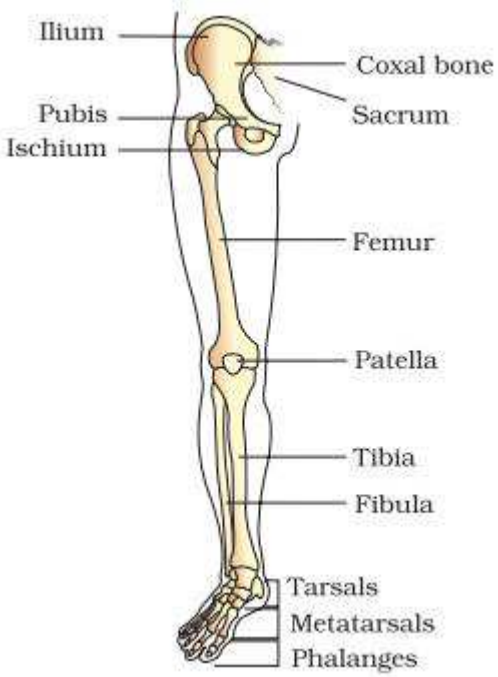


Figure 3.9 Hind limb

RECALL: Bones such as the knee cap are formed in tendons and are called 'sesamoid bones'.

iii) ***Pectoral Girdle***

Pectoral girdle consists of two halves and helps in the articulation of the upper limbs with the axial skeleton. Each half of pectoral girdle consists of a clavicle (collar bone) and a scapula. The scapula is a large triangular, flat bone situated in the dorsal part of the thorax between the second and the seventh ribs. The dorsal, flat, triangular body of the scapula has a slightly elevated ridge called the spine which projects as a flat, expanded process called the acromion process. The clavicle articulates with it. The acromion process articulates with the clavicle and provides space for attachment of the muscles of the upper limb and chest. Below the acromion process is a depression called the glenoid cavity, which articulates with the head of the humerus to form the shoulder joint.

iv) ***Pelvic Girdle***

Pelvic girdle consists of two identical halves called coxal bones/hip bones. Each coxal bone is formed by the fusion of three bones - ilium, ischium and and pubis. At the point of fusion of the above bones is a cavity called acetabulum which the thighbone articulates. The two halves of the pelvic girdle meet ventrally to form the pubic *symphysis* containing fibrous cartilage}

3.1.5 Joints

Joints are essential for all types of movements involving the bony parts of the body. Locomotor movements are no exception to this. Joints are points of contact between bones, or between bones and cartilages. Force generated by the muscles is used to carry out movement through joints, where the joint acts as a fulcrum. The movability at these joints varies depending on different factors.

Types of Joints

Joints are classified into three major structural forms, namely fibrous joints, cartilaginous joints and synovial joints.

A.) **Fibrous joints**: The fibrous joints do not allow any movement (synarthroses). These are composed of dense irregular connective tissue. These are three types- sutures, syndesmoses and gomphoses. a) Sutures are present between cranial bones, e.g., coronal suture present between frontal and parietal bones. b) Syndesmoses e.g.**,**Interosseous membrane between tibia and fibula, c) Gomphoses e.g., Dento- alveolar joint. Sutures and gomphoses are immovable joints (synarthroses). Syndesmose is slightly movable (amphiarphrose).

B.) **In cartilaginous joints**: The bones involved are joined together with the help of cartilages. These are two types- a) Synchondroses e.g., epiphyseal plate present between epiphysis and diaphysis. (b) Symphysis e.g., pubic symphysis of pelvic girdle. Synchondrose is a synarthrose and symphysis is an amphiarthrose.

C.) **Synovial joints**: These are characterized by the presence of a fluid filled synovial cavity between the articulating surfaces of the two bones. The articular surfaces of the bones are covered by hyaline cartilage. All synovial joints are diarthroses (freely movable joints).

Structure of synovial joint

Synovial joint is covered by a double layered synovial capsule. The outer layer consists of dense fibrous irregular connective tissue with more collagen fibres. This layer is continuous with the periosteum and resists stretching and prevents the dislocation of joints. Some fibres of these membranes are arranged in bundles called ligaments. The inner layer of synovial capsule is formed of areolar tissue and elastic fibers. It secretes a viscous *synovial fluid* which contains

hyaluronic acid , phagocytes etc. and acts as a 'lubricant' for the free movement of the joints.(Figure 3.10) Synovial joints include *Ball and socket joint, Hinge joint, Pivot joint, Gliding joint, Condyloid joint, Saddle joint.*

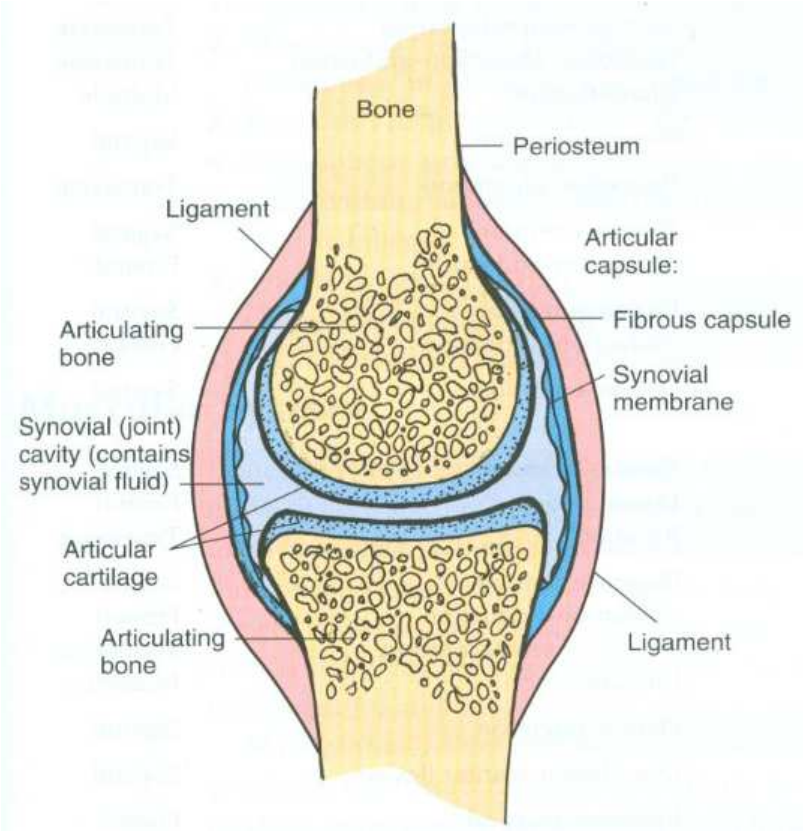


Figure 3.10 Synovial joint

a.) Ball and socket joint: e.g. hip joint (between femur and pelvic girdle), shoulder joint (between humerus and pectoral girdle).

b.) Hinge joint: e.g. elbow joint, knee joint etc

c.) Pivot joint: e.g. atlanto-axial joint between ulna and radius.

d.) Gliding joint: e.g. inter-carpal joints, inter- tarsal joints.

e.) Condyloid joint: e.g. the joints between the carpals and metacarpals, joint between occipital condyles and atlas.

f.) Saddle joint: e.g. joint between the carpal and metacarpal of the thumb.

GLOSSARY

Acromoian process: Point of attachment of clavicle toscapula in pectoral girdle of mammals.

Amphiarthrose: Slightly moveable joints of vertebrates.

Clavicle: Membrane bone of ventral side of the pectoral girdle of many vertebrates.

Cori cycle: The lactic acid produced in the fast working muscle is converted into glucose in the liver.

Fascia: Sheet of connecting tissue enclosing muscles.

Fascicles: bundle of muscle fibers covered by connective tissue sheeth.

Ligaments: connective tissue joining the bone with anther bone.

Manubrium: The first upper bone of the sternum.

Myoglobin: Myoglobin is an iron containing and oxygen binding protein found in muscle tissue of mammals.

Sarcomere: structural and functional unit of myofibril.

Synarthroses: Immovable joints of vertebrates.

VERY SHORT ANSWER TYPE QUESTIONS

1. What is a motor unit with reference to muscle and nerve?
2. What is triad system?
3. Name two cranial sutures and their location?
4. Name the keystone bone of cranium?

SHORT ANSWER TYPE QUESTIONS

1	List out the bones of human cranium?
2	Write a short note on sliding filament theory of muscle contraction?
3	List the bones human fore limbs?
4	List the bones the human leg limbs?
5	Describe the structure of Synuvial joint with the help of neat labeled diagram?

III B Neural Control and Coordination

3.2.1 The Central Neural System (CNS)

The CNS includes the brain and the spinal cord. It develops from neurectoderm.

BRAIN (the living super computer)

It is the site of information processing and control. It is protected in the cranial cavity and covered by three connective tissue membranes called cranial meninges namely, dura mater, arachnoid mater and pia mater. Dura mater is the outer most, thick, double layered membrane which lines the inner surface of the cranial cavity. Arachnoid mater is a thin, webby middle membrane covering the brain. It is separated from the dura mater by a narrow subdural space. Pia mater is a thin, innermost meninx which closely adheres to the brain. Pia mater is separated from the arachnoid membrane by the subarachnoid space. The brain can be divided into three major parts called i.Forebrain,ii.Midbrain and iii.Hindbrain.(figure 3.11)

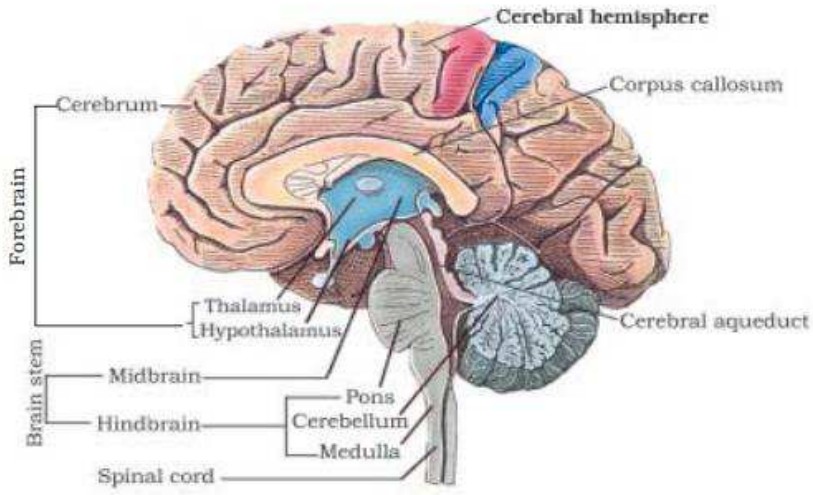


Figure 3.11 Human brain

i) Forebrain (Prosencephalon)

The forebrain consists of i. Olfactory bulb, ii. Cerebrum and iii. Diencephalon.

Olfactory Bulb: Olfactory bulbs receive impulses pertaining to smell from the olfactory epithelium.

Cerebrum: Cerebrum forms the major part of the brain and is longitudinally divided into the left and the right cerebral hemispheres by a deep cleft called longitudinal fissure'. The two hemispheres are internally connected by a transverse, wide and flat bundle of myelinated fibres beneath the cortex, called '**corpus callosum**' (colossal commissure). It brings 'coordination' between the right and left sides of the cerebral hemispheres. The surface of the cerebrum is composed of grey matter and is called the '**cerebral cortex**'. The neuronal cell bodies are concentrated in the cerebral cortex.

The surface of the cerebral cortex shows many convolutions or folds and grooves. The folds are called **gyri** (singular: gyrus), the deepest and shallower grooves between the folds are called fissures and **sulci**, respectively. Gyri and sulci increase the surface area of the cerebral cortex. Cerebral cortex has three functional areas called a)sensory areas, that receive and interpret the sensory impulses b) motor areas, which control voluntary muscular movements c) association areas, which are neither clearly sensory nor motor in function and they deal with more complex 'integrative functions' such as memory and communications. The cerebral medulla consists of mostly myelinated axons (white matter). Each cerebral hemisphere of the cerebrum is divided into four lobes namely frontal, parietal, temporal and occipital lobes.

Diencephalon (Thalamencephalon): The main parts of the diencephalon are the epithalamus, thalamus and hypothalamus.

i) **Epithalamus**: It is the roof of the diencephalon. It is a non-nervous part which is fused with the pia mater to form the anterior choroid plexus. Just behind the anterior choroid plexus, the epithelium of the epithalamus forms a pineal stalk, which ends in a rounded structure called pineal body.

ii) **Thalamus**: It lies superior to the mid brain. It is the major coordinating centre for sensory and motor signalling.

iii) **Hypothalamus** (the thermostat of the body): It lies at the base of the thalamus. The hypothalamus forms a funnel-shaped downward extension called infundibulum', connecting the hypothalamus with the pituitary gland. It also contains several groups of neurosecretory cells, which secrete hormones called hypothalamic hormones.

Hypothalamus controls and integrates the activities of the autonomous nervous system (ANS) and it has osmoregulatory, thermoregulatory, thirst, feeding (hunger) and satiety centres.

Limbic system

The inner parts of the cerebral hemispheres and a group of associated deep structures like amygdala or amygdale, hippocampus etc. form the limbic system. The limbic system along with hypothalamus is involved in the regulation of *sexual behaviour* and *expression of emotional reactions,*

ii) Midbrain (Mesencephalon)

The midbrain is located between the thalamus/hypothalamus of the forebrain and the **pons Varolii** of the hindbrain. The ventral portion of the midbrain consists of a pair of longitudinal bands of nervous tissue called cerebral peduncles or **crura cerebri** (sing: crus cerebrum) *(which connect the cerebral hemispheres with the pons).* The dorsal portion of the midbrain consists of four rounded lobes called **corpora quadrigemina** (Four optic lobes). The two larger anterior optic lobes are called **superior colliculi** and the smaller posterior lobes are called **inferior colliculi**. The superior colliculi and the inferior colliculi are concerned with visual and auditory functions, respectively.

iii) Hindbrain (Rhombencephalon)

The hind brain comprises cerebellum, pons Varolii and medulla oblongata.

Cerebellum ('the little brain')

it is the second largest part of the brain. It consists of two cerebellar hemispheres and a central vermis. Each cerebellar hemisphere consists of three lobes namely anterior, posterior and floccular lobes. It has a branching tree - like core of white mater called **arbor vitae** (the tree of life) surrounded by a sheath of grey matter (cerebellar cortex).

Pons Varolii

It lies in front of the cerebellum below the mid brain and above the medulla oblongata. It consists of nerve fibres which form a bridge between the two cerebellar hemispheres. It is a relay station between the cerebellum, spinal cord and the rest of the brain. Pons has the pneumotaxic centre (involved in the control of the respiratory muscles as it regulates the amount of air a person can take in, each time).

Medulla oblongata

It is the posterior most part of the brain. It extends from the pons Varolii above and continuous with the spinal cord below. It has a very thin, vascular folded structure called posterior choroid plexus. Medulla includes cardiovascular and respiratory centers, the centers for swallowing, vomiting, coughing, sneezing and hiccupping. The midbrain, pons and the medulla oblongata are together referred to as the brain stem .The medulla oblongata passes out of the cranium through the foramen magnum and joins the spinal cord.

Ventricles of the human brain

Human brain consists of four ventricles. The first and second ventricles (lateral ventricles or paracoels) are present in the right and left cerebral hemispheres respectively. The third ventricle (diocoel) occurs in the diencephalon. The two paracoels are connected to the median diocoel individually by the two foramina of Monro' (interventricular foramina). The fourth ventricle (myelocoel) is present in the medulla. The myelocoel and the diocoel are connected by a narrow canal called iter or aqueduct of Sylvius/cerebral aqueduct. The metacoel is continuous with the central canal of the spinal cord.

The ventricles of the brain and the subarachnoid space are filled with Cerebro-spinal fluid (CSF). CSF is an alkaline, colourless fluid which is filtered from the choroid plexuses into the ventricles of the brain.

NOTE: CSF serves as shock absorbing medium. CSF is recycled (flushed) 4 times per day in order to clear out metabolites and toxins.

Spinal Cord

The spinal cord is located in the vertebral canal (neural canal) of the vertebral column. It is protected by the bony arch of each vertebra and the three spinal meninges called dura mater, arachnoid mater and pia mater.

In the adult, it extends from the medulla oblongata to the superior border of the second lumbar vertebra.

It has two conspicuous enlargements, the superior enlargement called '**cervical enlargement**' and the inferior enlargement called '**lumbar enlargement**'. The spinal cord is divided into right and left halves by two groove namely, the anterior (ventral) median fissure and the posterior (dorsal) median sulcus. Inferior to the lumbar enlargement, the spinal cord tapers to a conical portion known as the **conus medullaris**, which ends at the level of the

intervertebral disc between the first and second lumbar vertebrae in the adult. The extension of the conus medullaris as the non-nervous fibrous tissue to the coccyx is called **Jilum terminale**. The internal anatomy of the spinal cord shows H- shaped or butterfly shaped central area of grey matter surrounded by the outer white matter. The grey matter is composed of cell bodies, neuroglia, dendrites and unmyelinated axons and it surrounds a narrow longitudinal cavity called central canal' or 'spinal neurocoel which is the continuation of the fourth ventricle of the brain and is lined by the ependymal epithelium. The grey matter is subdivided into regions called 'anterior and posterior horns' on each side. The white matter consists of bundles of myelinated axons and it is organized into the regions called anterior (ventral) funiculus, posterior (dorsal) funiculus and lateral funiculi, one on each side. The spinal cord acts as a coordinating centre for simple spinal reflexes. It also acts as the *middle man'* between the receptors and the effectors, as it conducts sensory and motor impulses to and from the brain.(Figure 3.12)

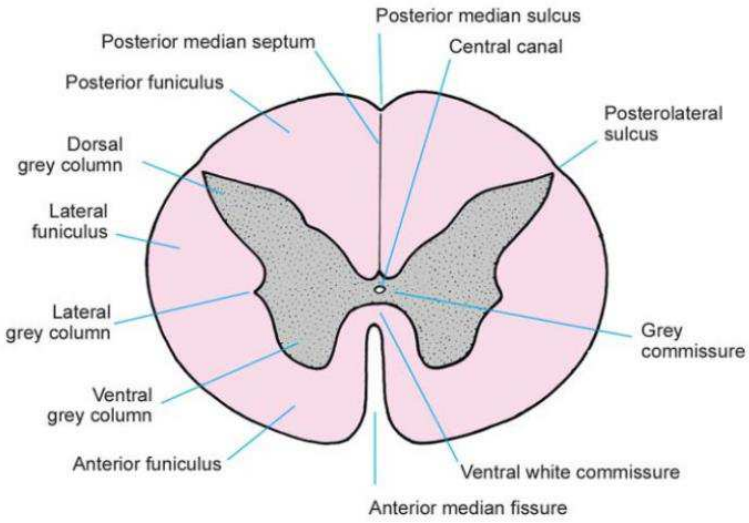


Figure 3.12 T.S. of Spinal chord

Synaptic Transmission

A nerve impulse is transmitted from one neuron to another through junctions called synapses. A synapse is formed by the membranes of a pre-synaptic neuron and a post-synaptic neuron, which may or may not be separated by a gap called synaptic cleft. There are two types of synapses, namely, electrical synapses and chemical synapses. At electrical synapses, the membranes of pre- and post -synaptic neurons are in very close proximity compared to chemical synapses. These synapses are electrically conductive links between two neurons and are also called gap junctions'. Impulse transmission across an electrical synapse is always

faster than that across a chemical synapse and they are in some cases bidirectional conductions.

At a chemical synapse, the membranes of the pre- and post-synaptic neurons are separated by a fluid-filled space called synaptic cleft (a structural gap and a functional bridge).

Chemicals called neuro transmitters are involved in the transmission of impulses at these synapses. The axon terminals contain vesicles (boutons) filled with these neuro transmitters. When an impulse (action potential) arrives at the axon terminal, it depolarizes the membrane opening voltage gated calcium channels. Calcium ions stimulate the movement of the synaptic vesicles towards the membrane where they fuse with the plasma membrane and release their neurotransmitters in the synaptic cleft by exocytosis. The released neuro transmitters bind to their specific receptors, present on the post-synaptic membrane. Acetyl choline is the most common neurotransmitter. Epinephrine, norepinephrine, dopamine, serotonin etc. are either excitatory or inhibitory neurotransmitters. Glycine, GABA (Gamma Amino Butyric Acid) are inhibitory neurotransmitters. The post synaptic membrane has ligand gated channels. They are ion channels which respond to chemical signals (ligands), rather than to changes in the membrane potential). The entry of ions can generate a new potential in the post-synaptic neuron. The new potential developed may be either excitatory or inhibitory. Excitatory post synaptic potentials (EPSPs) cause depolarization, whereas inhibitory post synaptic potentials (IPSPs) cause hyperpolarisation of post synaptic membrane. Post synaptic potentials are graded potentials and summation' of these potentials occurs at the 'axon hillocks'. Summation of inputs from many presynaptic membranes is called spatial summation', whereas summation of successive inputs from a single presynaptic membrane is called 'temporal summation'. Development of action potential depends on which is more? - *The sum of excitatory post synaptic potentials or the sum of inhibitory postsynaptic potentials.* (Figure 3.13)

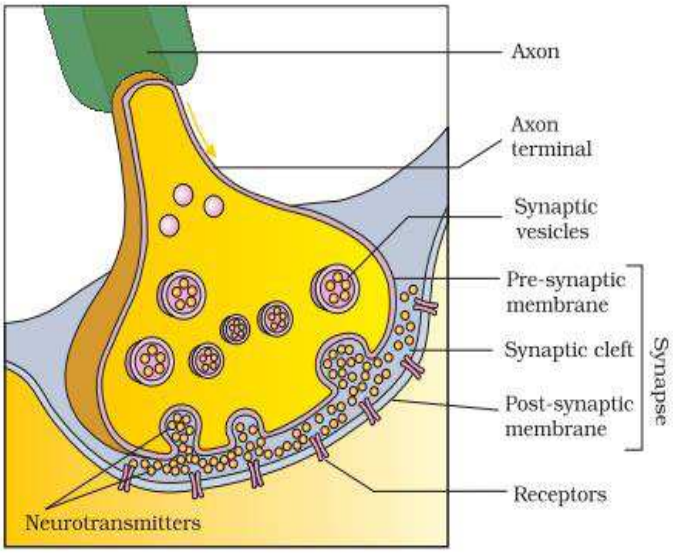


Figure 3.13 Diagram showing synaptic transmission.

Sensory Reception and Processing

In the human body the different types of receptors detect all types of changes in the environment and send appropriate signals to the CNS, where all the inputs are processed and analysed. Then the different controlling centres of the brain send 'motor impulses' to the 'effectors' through motor nerves. This is how you can sense changes in the environment. In the following sections, you will be introduced to some of the important receptors of human body.

1) **Exteroceptors**: They are located at or near the surface of the body, and sensitive to external stimuli like hearing, vision, touch, taste and pain etc.
2) **Interoceptors** (visceroceptors): They are located in the visceral organs and blood vessels and sensitive to internal stimuli.
3) **Proprioceptors**: They are also a kind of interoceptors and they provide information about body position and movement and are located in muscles, tendons, joints and the internal ear.
4) **Thermoreceptors**: They respond to heat (caloreceptors) and cold (frigidoreceptors).

THE EYE

The eye is the organ of vision which is located in the orbit of the skull. Eye consists of accessory structures and the eye ball.

Accessory structures of eye

Eye lids, eye lashes, eye brows, lacrimal appartus and extrinsic eye muscles are the accessory structures of eye. The eye lids, the eye lashes and the eye brows are useful for the protection of the eye. The lacrimal apparatus is a group of structures that produce and drain lacrimal fluid or tears which contains salts, mucus and bactericidal enzyme called lysozyme. There are six extrinsic (extra ocular) eye muscles present attached to the human eye, namely superior, inferior, lateral, medial rectus muscles, and superior oblique and inferior oblique muscles. These muscles aid in the movement of the eye and they receive their innervations from the III, IV and VI cranial nerves.

The eye ball

Anatomically, the wall of the eye ball can be divided into three layers: fibrous tunic, vascular tunic, nervous tunic (retina). It also has a lens which is held in position by suspensory ligaments.

Fibrous tunic

It is the outer coat of the eye ball consisting of the anterior cornea (acts as a sort of ;fixed lens) and the posterior sclera. The cornea is a non-vascular, transparent coat that covers the coloured iris. Cornea is covered by a thin layer called conjunctiva. The sclera, 'white' of the eye, is a coat that covers the entire eye ball. The sclera gives shape to the eye ball, makes it more rigid, and protects its inner parts. At the junction of the sclera and cornea is a channel known as the scleral venous sinus or canal of Schlemm.(Figure 3.14)

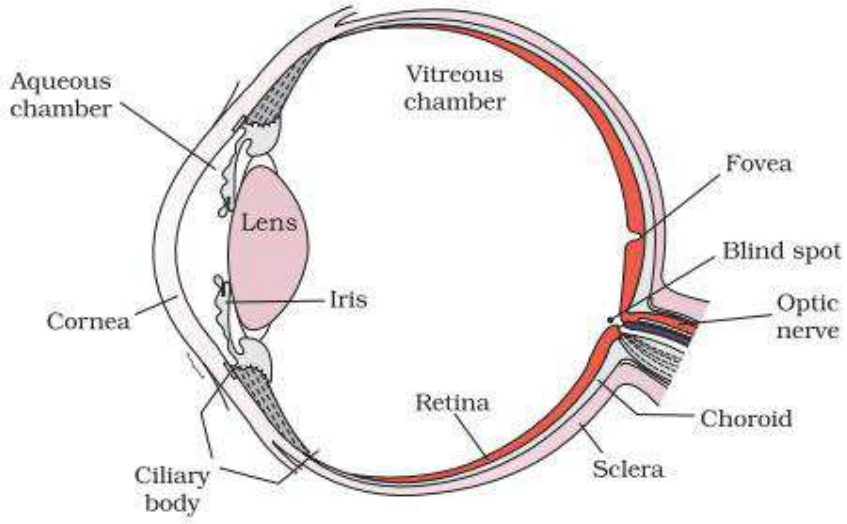


Figure 3.14 Structure of eye.

Vascular tunic

The vascular tunic or uvea is the middle layer of the eye ball. It has three portions: choroid, ciliary body and iris. The choroid is highly vascularised and looks bluish in colour. It is thick in the anterior part to form the ciliary body. The ciliary body is a pigmented and vascularized part that consists of the ciliary processes. The muscle associated with the ciliary body is a circular band of smooth muscle that holds and alters the shape of the lens for near or far vision (eye accommodation power- the process by which the eye changes optical power to focus on an object as its distance varies). The iris is the coloured portion of the eyeball. It is suspended between the cornea and the lens and is attached at its outer margin to the ciliary processes. The aperture in the centre of the iris is called pupil. The principal function of the iris is to regulate the amount of light entering the vitreous chamber of the eye ball through the pupil. The diameter of the pupil is regulated by the muscles of the iris.

Retina (Nervous tunic)

This is the third and inner coat of the eye. It consists of a pigmented epithelium (non-visual portion) and a neural portion (visual portion). Thepigmented epithelium is a sheet of melanin - containing epithelial cells that lie between the choroid and the neural portion of the retina. The neural portion of the retina has three layers of retinal neurons namely: photoreceptor layer (the layer closest to the choroid coat), bipolar cell layer and ganglion cell layer. Photoreceptor layer consists of two types of photoreceptor cells called rods and cones. Rods contain a purplish-red protein called the rhodopsin or visual purple, which contains a derivative of vitamin-A and they are important in twilight (scotopic vision- ***the vision of the***

eye under low light conditions). Cones contain a visual pigment (iodopsin, made of a protein called photopsin) and they are important in daylight (photopic) vision and colour vision. There are three types of cones, each having different sensitivity and they provide '***optimal response***' to red, green and blue colours.

The centre of the posterior portion of the retina is called the macula lutea or yellow spot. A small depression present in the centre of the yellow spot is called fovea centralis, and it contains only cones. Fobea is responsible for sharp, central vision, which is useful while walking, reading, driving etc. The axons of the ganglion cells extend posteriorly and exit the eye ball as the optic nerve. The site of the retina where the optic nerve exits the eye ball is called optic disc or blind spot which is devoid of photoreceptor cells (no image is formed at that spot).(Fifure 3.15)

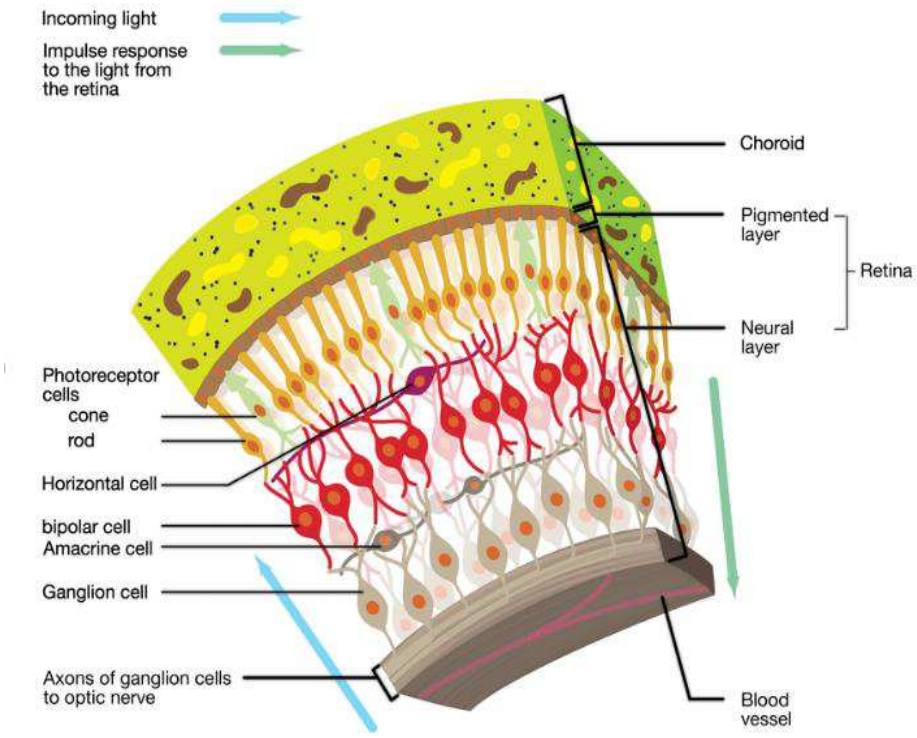


Figure 3.15 Structure of Retina

Lens

A non-vascular and transparent lens is present within the cavity of the eye ball just posterior to the pupil and iris. The lens is held in position by encircling suspensory ligaments. Ciliary muscles control the shape of the lens helping focusing light on the retina.

Chambers of the eye's interior

The interior space of the eye ball is divided by the lens into anterior and posterior chambers. The anterior chamber is filled with aqueous humor (secreted by ciliary processes), which helps in nourishing the lens and the cornea. The posterior cavity lies between the lens and the retina and contains a jelly like substance called vitreous body / vitreous humor. The vitreous body contributes to the intraocular pressure along with aqueous humor and helps to protect the shape of the eye ball.

Mechanism of Vision

The light rays of visible wave length focused on the retina through the cornea and lens generate potentials (impulses) in rods and cones. As mentioned earlier, the photosensitive compounds (photo pigments) in the human eyes are composed of opsin (a protein) and retinal (produced from vitamin A). Light induces dissociation of the retinal from opsin resulting in changes in the structure of the opsin. This causes changes in membrane permeability. As a result action potentials develop in the ganglion cells. These action potentials (impulses) are transmitted by the optic nerve to the visual cortex area of the brain (occipital lobes of the cerebrum), where the neural impulses are analyzed and the image formed on the retina.

THE EAR

The ear is the site of reception of two senses, namely hearing and equilibrium. Anatomically, the ear is divided into three principal regions: **the external ear**, **the middle ear** and **the internal ear.**

External ear (outer ear)

The outer ear consists of the pinna(auricle), external auditory meatus (external auditory canal) and ear drum. The auricle is a flap of elastic cartilage covered by skin which collects vibrations in the air that produce sound. The external auditory meatus is a curved tube that leads inwards and extends up to the **tympanic membrane** (the ear drum), which is a thin, semi transparent partition between the external auditory canal and middle ear. The tympanic membrane is composed of connective tissues covered with skin outside and with mucus membrane inside. There are very fine hairs and ear's wax secreting 'sebaceous glands' called ceruminous glands in the skin of the pinna and the meatus. The combination of hair and cerumen (ear's wax) helps to prevent dust and foreign particles from entering the ear.

The middle ear

The middle ear (tympanic cavity) is a small, air - filled cavity in the temporal bone. It is separated from the external ear by the eardrum and from the internal ear by a thin bony partition that contains two small membrane - covered openings called the oval window (fenestra ovalis) and the round window (fenestra rotunda). The middle ear contains three ossicles called malleus (hammer bone), incus (anvil bone) and stapes (stirrup bone) which are attached to one another in a chain-like fashion. The malleus is attached to the tympanic membrane and its head articulates with the incus. The incus is the intermediate bone in the series of 'ear ossicles' and articulates with the head of the stapes. The stapes is attached to the oval window in the thin bony partition between the middle and inner ear. The ear ossicles transmit sound waves to the inner ear. A Eustachian tube connects the middle ear cavity with the pharynx. The Eustachian tube helps in equalizing the pressures of air on either sides of the ear drum.(Figure 3.16)

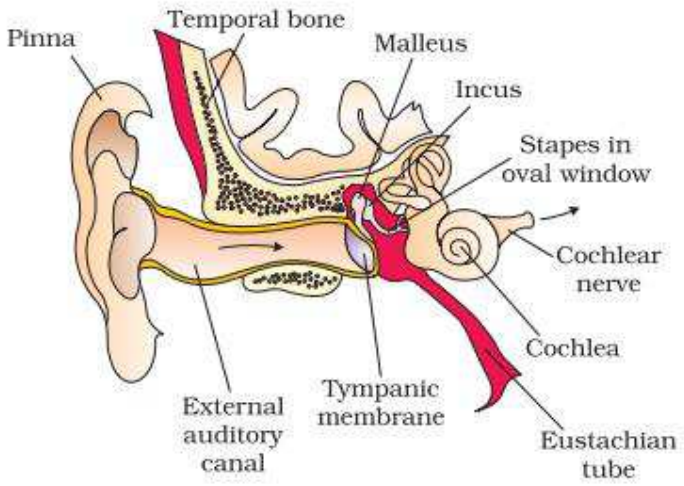


Figure 3.16 Structure of Ear

Inner ear

The fluid-filled inner ear called labyrinth consists of two parts, the bony and the membranous labyrinths. The bony labyrinth is a series of channels which can be divided into three areas: 1) cochlea, 2) vestibule, and 3) semicircular canals. The cochlea is a coiled (watch spring like) portion of the labyrinth. Infact the cochlea is three tubes in one*. The three tubes are namely scala vestibuli, scala media and scala tympani (cochlear duct). The scala vestibuli and scala media are separated by a membrane called Reissner's membrane, the scala media and scala tympani are separated by another membrane called basilar membrane. Scala vestibuli and scala tympani are filled with perilymph. However the scala media is filled with

endolymph. At the base of the cochlea, the scala vestibuli ends at the 'oval window', while the scala tympani terminate at the 'round window' which leads to the middle ear. The cochlear epithelium forms a sensory ridge called "**organ of Corti**" on basilar membrane. The organ of Corti contains hair cells' that act as 'auditory receptors'. The hair cells are present in rows on the internal side of the organ of Corti. These cells are innervated by the nerve fibres of the cochlear branch of the VIII cranial nerve. The basal end of the hair cell is in close contact with the afferent auditory nerve fibres. A large number of processes called stereocilia are projected from the apical part of each

hair cell. Above the rows of the hair cells is a thin elastic membrane called **tectorial membrane**.

There are three semicircular canals, each one lies approximately at right angles to the other two. The base of each canal is swollen and is called ampulla, which contains a projecting ridge called crista (which has hair cells') - The semicircular canals provide a sense of 'angular acceleration' perceiving rotation of the body (direction of body movement or turning of head to sides). The vestibule is the oval central part of the bony labyrinth located between the cochlea and the semicircular canals. The membranous labyrinth in the vestibule consists of two sacs called the saccule and the utricle (collectively called the otolith organ)- The saccule and the utricle contain a projecting ridge called macula- It has receptors for gravity .Saccule and utricle provide a sense of linear acceleration. The saccule perceives vertical movement (as when you are going in a lift -moving upwards or downwards). The utricle perceives horizontal movement (as when you are going in a car - moving forwards or backwards).

The semi-circular canals and the otolith organ together constitute the "vestibular apparatus" The semi-circular canals, the saccule and the utricle are innervated by the vestibular branch of VIII cranial nerve.It is concerned with equilibrium (maintenance of balance of the body and posture). Vestibular branch of the auditory nerve carries these impulses to the brain.

Mechanism of hearing

The external ear receives sound waves and directs them to the ear drum. The ear drum vibrates in response to the sound waves and these vibrations are transmitted through the ear osssicles to the oval window. The vibrations are passed through the oval window on to the fluid of cochles, where they generate waves in peri and endolymphs. These waves ripple in the basilar membrane. These movements of the basilar membrane bend the hair cells,

pressing them against the tectorial membrane. As a result nerve impulses are generated in associated afferent neurons. These impulses are transmitted by the afferent fibres via the auditory nerves to the auditory cortex of the brain, where the impulses are analyzed and the sound is recognised.

GLOSSARY

Amygdala : the part of the telencephalon located inthe temporal lobe of the brain. It is involved in the memory, emotion and fear. Essentially acting as 'brain's warning' center.

Brain stem: the region of the brain that consists of mid brain, pons and medulla.

Blind spot: The place of retina which has no photoreceptors.

Cochlea: A spirally coiled part of the internal ear in which the organ of corti is present.

Gyrus: The elevated portion of the cerebral surface.

Hippocampus: It is the part of the cerebral hemispheres in the basal medial part of the temporal lobe. This part of the brain is important for learning and important for converting short term memory to permanent memory.

Iris: it is the shelf like diaphragm of choroid of the eye.

Organ of Corti: The hearing apparatus that is present in the middle canal of the cochlea.

Sulcus: The depression between gyri of cerebrum.

VERY SHORT ANSWER TYPE QUESTIONS

1. Name the cranial meninges covering the brain of the man?
2. What is corpus callosum?
3. What do you know about arbour vitae?
4. What is organ of corti?
5. Distinguish between the blind spot and the yellow spot?

SHORT ANSWER TYPE QUESTIONS

1 Draw a labeled diagram of T.S. of the spinal cord of man?

2 Give an account of the Retina of the human eye?

3 Give an account of synaptic transmission?

Human anatomy and physiology – IV

SUMMARY

There are special chemicals which act as hormones and provide chemical coordination, integration and regulation in the human body. These hormones regulate metabolism, growth and development of our organs, the endocrine glands or certain cells. The endocrine system is composed of hypothalamus, pituitary and pineal, thyroid, adrenal, pancreas, parathyroid, thymus and gonads (testis and ovary). In addition to these, some other organs, e.g., gastrointestinal tract, kidney, heart etc., also produce hormones. The pituitary gland is divided into three major parts, which are called as pars distalis, pars intermedia and pars nervosa. Pars distalis produces six trophic hormones. Pars intermedia secretes only one hormone, while pars nervosa (neurohypophysis) secretes two hormones. The pituitary hormones regulate the growth and development of somatic tissues and activities of peripheral endocrine glands. Pineal gland secretes melatonin, which plays a very important role in the regulation of 24-hour (diurnal) rhythms of our body (e.g., rhythms of sleep and state of being awake, body temperature, etc.). The thyroid gland hormones play an important role in the regulation of the basal metabolic rate, development and maturation of the central neural system, erythropoiesis, metabolism of carbohydrates, proteins and fats, menstrual cycle. Another thyroid hormone, i.e., thyrocalcitonin regulates calcium levels in our blood by decreasing it. The parathyroid glands secrete parathyroid hormone (PTH) which increases the blood Ca_2^+ levels and plays a major role in calcium homeostasis. The thymus gland secretes thymosins which play a major role in the differentiation of T-lymphocytes, which provide cell-mediated immunity. In addition, thymosins also increase the production of antibodies to provide humoral immunity. The adrenal gland is composed of the centrally located adrenal medulla and the outer adrenal cortex. The adrenal medulla secretes epinephrine and norepinephrine. These hormones increase alertness, pupilary dilation, piloerection, sweating, heartbeat, strength of heart contraction, rate of respiration, glycogenolysis, lipolysis, proteolysis. The adrenal cortex secretes glucocorticoids and mineralocorticoids. Glucocorticoids stimulate gluconeogenesis, lipolysis, proteolysis, erythropoiesis, cardio-vascular system, blood pressure, and glomerular filtration rate and inhibit inflammatory

reactions by suppressing the immune response. Mineralocorticoids regulate water and electrolyte contents of the body. The endocrine pancreas secretes glucagon and insulin. Glucagon stimulates glycogenolysis and gluconeogenesis resulting in hyperglycemia. Insulin stimulates cellular glucose uptake and utilisation, and glycogenesis resulting in hypoglycemia. Insulin deficiency and/or insulin resistance result in a disease called diabetes mellitus. The testis secretes androgens, which stimulate the development, maturation and functions of the male accessory sex organs, appearance of the male secondary sex characters, spermatogenesis, male sexual behaviour, anabolic pathways and erythropoiesis. The ovary secretes estrogen and progesterone. Estrogen stimulates CHEMICAL COORDINATION AND INTEGRATION 341 growth and development of female accessory sex organs and secondary sex characters. Progesterone plays a major role in the maintenance of pregnancy as well as in mammary gland development and lactation. The atrial wall of the heart produces atrial natriuretic factor which decreases the blood pressure. Kidney produces erythropoietin which stimulates erythropoiesis. The gastrointestinal tract secretes gastrin, secretin, cholecystokinin and gastric inhibitory peptide. These hormones regulate the secretion of digestive juices and help in digestion.

IV A – Endocrine system and chemical co-ordination

4.1.1 List of endocrine glands

Some higher invertebrates possess very simple endocrine system that produces a few hormones, whereas a well developed endocrine system that produces a large number of hormones occurs in the vertebrates. The human endocrine system is described here.

Human Endocrine System

The endocrine glands of human beings are well studied than those of any other animal because of their role in the maintenance of human health. The endocrine glands and the hormone producing diffused tissues / cells located in different parts of the human body constitute the endocrine system. The organized endocrine tissues / organs in the human body include **pituitary**, **pineal**, **thyroid**, **parathyroid**, **adrenal**, **pancreas**, **thymus** and **gonads (testes and ovaries)**. Besides these, some other non-endocrine organs such as gastrointestinal tract, liver, kidney, and heart also produce hormones. A brief account of the structure and functions of all the major endocrine glands and hypothalamus is given below.(Figure 4.1)

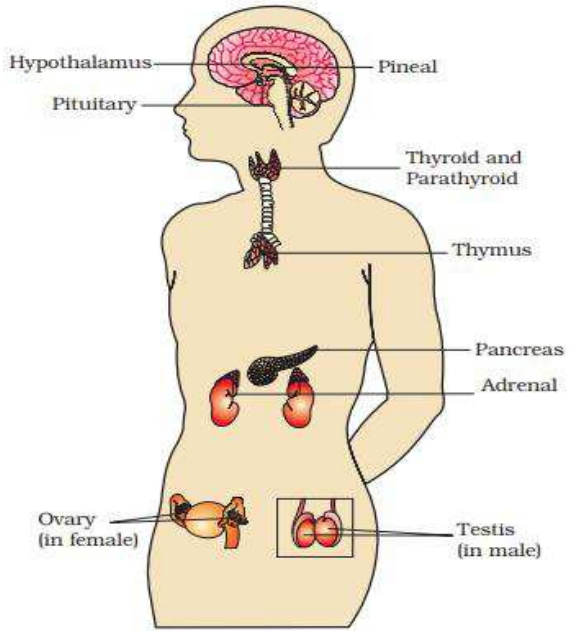


Figure 4.1 Location of endocrine glands

Hypothalamus

(Greek: Hypo = under; Thalamus = chamber)

The hypothalamus is located below the thalamus, constituting the floor of the diencephalon, a part of the fore brain.It connects the neural and endocrine systems as it is closely tied to the pituitary gland. It responds to the sensory impulses received from different receptors by sending out appropriate neural or endocrine signals.It regulates a wide range of body functions. It contains several groups of neurosecretory cells called nuclei' which produce hormones called neurohormones. They are transported to the neurohypophysis through the axons of the hypothalamo-hypophysial tract.The two types of hormones produced by the hypothalamus are 1) the releasing hormones (which stimulate secretion of pituitary hormones), and 2) the inhibiting hormones (which inhibit secretions of pituitary hormones).

For example, a hypothalamic hormone called growth hormone releasing hormone (GHRH/Somatocrinin) stimulates the synthesis and release of **Somatotropin** (growth hormone) by the pituitary. On the contrary the **growth hormone-inhibiting hormone** (GHIH) or somatostatin from the hypothalamus inhibits the release of growth hormone from the pituitary. These hormones originating in the hypothalamic neurons pass through axons and are released from their nerve endings. They reach the anterior pituitary through a portal circulatory system called **hypophysial portal system** and regulate the functions of the anterior pituitary. The posterior pituitary is under the direct neural regulation of the hypothalamus.

Pituitary Gland

The pituitary gland, also called '**hypophysis**', is a small unpaired round body. It is located immediately beneath the hypothalamus, attached to it by a stalk, called the infundibulum. It lies in a bony depression called **sella turcica** of the sphenoid bone. It is divided anatomically into anterior pituitary or adenohypophysis and posterior pituitary or neurohypophysis. Between these two is a small zone called the pars intermedia.(Figure 4.2)

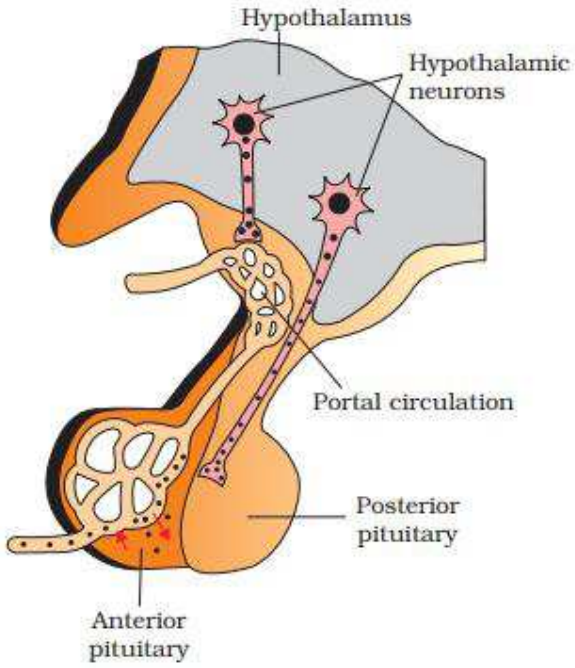


Figure 4.2 Diagrammatic representations of pituitary and its relationship with hypothalamus

Origin of the pituitary

Embryologically the two portions of the pituitary have their origins from two different sources (dual origin). The anterior pituitary (adenohypophysis) develops from the **Rathke's pouch** which is an embryonic outgrowth from the roof of the oral region. An outgrowth from the floor of the hypothalamus gives rise to the infundibular process which becomes the neurohypophysis.

Anterior pituitary /Adenohypophysis

The anterior pituitary produces six important peptide hormones. The hormones of the anterior pituitary play major roles in the control of metabolic functions throughout the body.

1. **Growth Hormone (GH)**: It is also called somatotropin. It stimulates mostly the cells of the liver to produce insulin-like growth factor (IGFs).They stimulate cell division in the epiphyseal plates leading to 'elongation of bones'. They also promote growth of the entire body by accelerating protein synthesis, cell division and cell differentiation. They also decrease the catabolism of proteins.
2. **Prolactin**: It is also called lactogenic hormone / luteotropic hormone (LTH): It causes enlargement of the mammary glands of the breasts and prepare them for the production of milk. In women the major action of prolactin is to initiate and sustain lactation.

Prolactin also helps in maintaining the corpus luteum of the ovary, which is the source of the female sex hormone progesterone, and thus it helps to sustain pregnancy.

3. **Thyroid Stimulating Hormone (TSH) / (Thyrotropin**): It stimulates the synthesis and secretions of thyroid hormones from the thyroid gland.
4. **Adrenocorticotropic Hormone / Corticotropin ACTH**: Controls the synthesis and secretion of steroid hormones called glucocorticoids, by the adrenal cortex.
5. **Follicle Stimulating Hormone (FSH)**: It stimulates growth and development of the ovarian follicles in females. In males FSH, along with the androgens, regulates spermatogenesis.
6. **Luteinizing hormone (LH):** In males it stimulates the interstitial cells of Leydig of the testes for the synthesis and secretion of hormones called androgens ('testosterone' is the chief androgen). In females, this hormone stimulates 'ovulation' from the fully mature Graafian follicles. Besides this, it stimulates the ovaries to produce 'estrogens' and progesterone. It maintains the corpus luteum formed from the remnants of the Graafian follicles, after ovulation.

Pars intermedia secrete only one hormone called melanocyte stimulating hormone (MSH). In human beings, pars intermedia is almost merged with pars distalis and the role of MSH is not significant, excepting that it can darken the hair by increasing deposition of melanin in the developing hair shaft. In human fetal life it produces, MSH which regulates pigmentation in the melanocytes of the lower vertebrates and its role in man is not significant.

Posterior pituitary / Neurohypophysis: It stores and releases two hormones called oxytocin and vasopressin /ADH, which are actually synthesized by the hypothalamus and are called neuro-secretions. They are small chain peptides. Oxytocin works on the smooth muscles of the body and induces their contraction. In a female it stimulates powerful contractions of the uterus during child birth and ejection of milk from the mammary glands. Vasopressin affects the kidney and stimulates reabsorption of water and electrolytes by the DCT and the collecting duct from the nephric filtrate. Thus urine becomes hypertonic and diuresis (increased excretion of the urine) is prevented. Hence, it is also called 'anti-diuretic hormone (ADH)

Pineal Gland

The pineal gland or epiphysis cerebri is located on the dorsal surface of the diencephalon. The pineal secretes a hormone called **melatonin**. It plays a very important role in the

regulation of 24 hour (diurnal / circadian) rhythms of our body. For instance, it helps in maintaining the normal rhythms of the sleep-wake cycle, body temperature etc. Besides this, melatonin influences metabolism, pigmentation, menstrual cycle and defense capability of our body. In some animals it stimulates gonads while in others it inhibits the gonads.

Thyroid Gland

It is the largest endocrine gland and is endodermal in origin. It lies close to the body surface, in the neck near the junction of larynx and the trachea. The thyroid consists of two lobes connected by a thin flap of connective tissue called isthmus. The lobes lie on either side of the trachea and extend upwards along the sides of the larynx. The complete thyroid gland somewhat resembles a butterfly with its wings spread or H shape.(Figure 4.3)

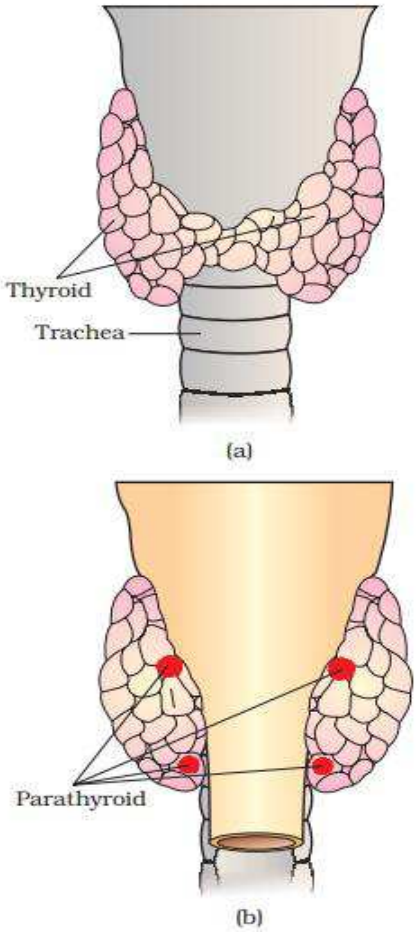


Figure 4.3 Diagrammatic view of the position of Thyroid and Parathyroid (a) Dorsal side (b) Ventral side

Thyroxine

The thyroid gland is composed of follicles and stromal tissues. Each thyroid follicle is composed of follicular cells, enclosing a cavity. These follicular cells produce two major hormones, namely tetra-iodothyronine or thyroxine (T_4) and tri-iodothyronine (T_3). Iodine is necessary for the normal rate of hormone synthesis in the thyroid. Thyroxine (T_4) and Triiodothyronine have the same endocrine functions.

Thyroid hormones (calorigenic hormones) play an important role in the regulation of the basal metabolic rate (BMR), the rate of consumption of oxygen per unit weight of the body, at rest. Thyroid hormones increase the basal metabolic rate by stimulating the use of cellular oxygen to produce ATP. As cells produce and use more ATP, more heat is given off and the temperature of the body rises. In this way, they maintain normal body temperature of a homeotherm. These hormones stimulate the process of erythropoiesis. Thyroid hormones control the metabolism of carbohydrates, proteins and fats. Maintenance of water and electrolyte balance is also influenced by thyroid hormones.

Calcitonin ,

The parafollicular cells of the thyroid produce a polypeptide hormone called Thyrocalcitonin (TCT). It plays an important role in maintaining proper levels of calcium (Ca^{2+}) and phosphates in the blood. It decreases blood calcium by acting on bones and thus it counters the effect of parathormone. Higher calcium content in the plasma stimulates the secretion of calcitonin (feedback control mechanism).

Parathyroid Glands

In humans there are four parathyroid glands, located on the back side of the thyroid gland. Each lobe of thyroid has one pair of parathyroids, of endodermal origin. The parathyroid glands secrete a hormone called parathyroid hormone (PTH) / parathormone. The secretion of PTH is controlled by the levels of Ca^{2+} in the circulatory fluids. This hormone augments the Ca^{2+} levels in the blood. 'Thus it is a hypercalcemic hormone, it stimulates the process of bone restoration (dissolution/demineralization of the bone) by stimulating the osteoclasts to dissolve calcium phosphate of the matrix of bones releasing calcium into blood. PTH also stimulates reabsorption of calcium ions by renal tubules and increases the absorption of Ca2+ from the gut. Together with thyrocalcitonin (TCT), it plays a significant role in calcium balance in the body. It promotes the activation of vitamin D into the active form, the horomone called '**calcitriol**'.

Thymus Gland

The thymus gland is a lobular structure located just above the heart and the aorta, underneath the breast bone. This gland plays a significant role in strengthening the immune system. It secretes peptide hormones called **thymosins**. Thymosins play a major role in the differentiation of T- lymphocytes, which provide cell-mediated immunity. In addition, thymosins also promote the production of antibodies to provide humoral immunity.

Adrenal Glands

One pair of adrenal glands is present in the body of a human being, located at the anterior part of each kidney. It is also called suprarenal gland. Adrenal gland is composed of two types of tissues. The peripheral tissue is the adrenal cortex (mesodermal in origin) and the centrally located tissue is called adrenal medulla (ectodermal in origin).(Figure 4.4)

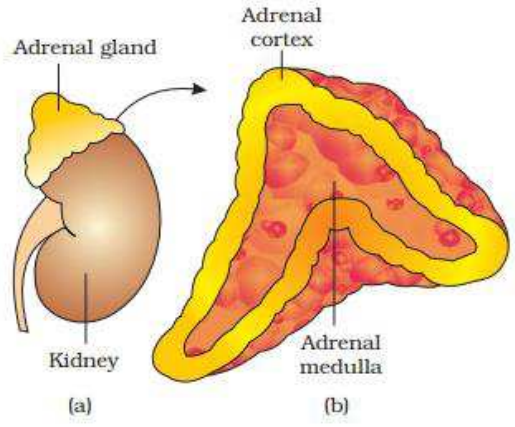


Figure 4.4 Diagrammatic representation of (a) Adrenal gland on kidney (b) Section showing two parts of adrenal gland

a. **Adrenal Cortex**: Adrenal cortex is the peripheral tissue of the adrenal gland. It is differentiated into three zones. The outer zone is called zona glomerulosa. the middle one is known as zona fasciculata and the inner one, the zona reticularis. The adrenal cortex secretes many hormones commonly called corticoids (steroid hormones).

Zona glomerulosa secretes mineralo-corticoids (aldosterone is the chief mineralo-corticoid). Aldosterone regulates the balance of water and electrolytes in the body.

Aldosterone, whose secretion is activated by 'angiotensin II' when the blood pressure falls , acts mainly on the renal tubules and stimulates the reabsorption of Na^+ and water from renal tubules and excretion of K^+ and phosphate ions. Thus aldosterone helps in the maintenance of electrolytes, body fluid volume, osmotic pressure and blood pressure.

Zona fasciculata secretes glucocorticoids. They are concerned with the carbohydrate metabolism. In the body of humans cortisol is the chief glucocorticoid. The glucocorticoids are 'life saving' hormones.

Glucocorticoids stimulate gluconeogenesis, lipolysis and proteolysis. These corticoids inhibit cellular uptake and utilization of amino acids. Cortisol (also called hydrocortisone) regulates the cardio-vascular system and the kidney functions. Glucocorticoids, specially cortisol, generates antiinflammatory reactions and suppresses immune responses. Cortisol stimulates RBC production. Cortisol is a "stress combat" hormone.

Zona reticularis synthesize sex hormones. Androgens are the male sex hormones and testosterone is the chief androgen. It is concerned with the development of growth of axial, pubic and facial hair during puberty.

a. **Adrenal medulla**: It secretes two hormones called *adrenaline* or *epinephrine* and *noradrenaline* or *norepinephrine*. These are commonly called catecholamines. These hormones are secreted in response to stress of any kind and during emergency situations, hence are called emergency hormones or hormones of fight or flight. These hormones enhance alertness, dilation of pupils, piloerection (involuntary erection of hair on skin), sweating, dilation of the bronchioles etc. These hormones increase the rate of heartbeat, the strength of the contraction of heart and also increases the rate of respiration. Catecholamines also stimulate the breakdown of glycogen, lipids and proteins. Thus the concentration of glucose in the blood increases.

Pancreas

Pancreas is both exocrine and endocrine in function. It is an elongated organ located posterior to the stomach. The exocrine portions of pancreas are known as **acini**. The endocrine portion of the pancreas is just 1 to 2% and consists of 1 to 2 million Islets of Langerhans. The two main types of cells in the Islets of Langerhans are the α-cells and ß-cells. The α-cells produce

the hormone, glucagon, whereas the ß-cells produce insulin. Glucagon is secreted in response to hypoglycemia. Its action is mainly on the liver cells (hepatocytes) and it stimulates glycogenolysis. This increases the sugar level in the blood (hyperglycemia). It also converts amino acids and fatty acids into glucose (gluconeogenesis) which also increases the level of glucose in the blood. This hormone decreases the uptake of glucose and its utilization by the cells. Therefore glucagon is a hyperglycemic hormone Insulin regulates the normal glucose level in the blood. It mainly acts on the liver cells and adipocytes and increases the uptake and utilization of glucose by the body cells. Glucose is taken up by the hepatocytes, skeletal muscles and adipocytes, thus reducing the level of glucose in the blood (hypoglycemia)- Insulin promotes conversion of glucose into glycogen (glycogenesis) in the target cells (hypoglycemic hormone). Both glucagon and insulin maintain the homeostasis of glucose in the blood. Persistent hyperglycemia leads to a complex disorder called diabetes mellitus.

Testes (Male Gonadal Glands)

Testes are the male gonads. Each testis is enclosed in a scrotal sac outside the abdomen. Testis is a cytogenic gland, which produces both sperms and sex hormones. The Leydig cells or interstitial cells of Leydig , lie in the 'inter-seminiferous tubular spaces', they produce androgens, chiefly the testosterone. Male sex hormones are required for the development, maturation and functioning of the male accessory sex organs such as epididymis, vas deferens, seminal vesicles, prostate gland, urethra etc. These hormones control muscular growth, growth of facial and axillary hair, aggressiveness, low pitch voice (masculine voice) etc. Androgens stimulate the process of spermatogenesis. Androgens affect the central neural system, controlling the male sexual behavior (libido / sex drive /sexual urge) and also have an effect on *protein* and ***carbohydrate anabolism.***

Ovaries (Female Gonadal Gland)

Ovaries are the female gonadal organs present in the abdominal cavity. These are cytogenic organs and produce one ovum during each menstrual cycle. Besides this, ovaries act as endocrine glands too producing the female hormones chiefly: estrogen and progesterone. Ovarian follicles and stromal tissues are present in the ovary. The hormone estrogen is produced by the growing follicles of the ovary. After ovulation, the ruptured follicle becomes a 'yellow body' called corpus luteum (which acts as a temporary endocrine gland) and secretes progesterone. After a few days, in the absence of pregnancy, the corpus luteum

stops functioning and becomes the 'corpus albicans'.

Estrogen is responsible for the development and the activity of the female secondary sex organs, development of the growing ovarian follicles, high pitch of voice etc. and the development of the mammary glands. Estrogen also controls the female sexual behaviour.

Progesterone has an important role in preparing the uterus for the implantation of the blastocyst in the wall of the uterus. It inhibits contraction of the uterus. Thus it supports pregnancy. In case of deficiency of this hormone, pregnancy fails to maintain. It stimulates the formation of alveoli (sac like structures which store milk) in the mammary glands and secretion of milk.

4.1.2 Human hormonal disorders

When hormones are secreted in normal quantities, a dynamic effect is exerted in the maintenance of 'homeostasis and regulation of biological processes'. If the production of the hormones is imbalanced by over production or under production, the biological processes are disturbed, and abnormalities occur. Some important disorders caused by hypo or hyper secretion of hormones include dwarfism, gigantism, acromegaly, cretinism, simple goiter, exophthalamic goiter, diabetes (mellitus and insipidus), ***Addison's disease, Cushing syndrome*** etc.

Hypersecretion of growth stimulating hormone (somatotropin), before puberty and completion of ossification, leads to an abnormality called gigantism. This is 'over growth' of the skeleton resulting in abnormal height of the person affected. Hypersecretion of this hormone in adults results in an abnormality called **acromegaly**. This disease is characterized by enlargement of the bones of the jaw, hand and feet, thickened nose, lips, eyelids and wide finger tips and 'gorilla like appearance' of the person affected.

Hyposecretion of GH during childhood retards growth, resulting in a 'pituitary **dwarf** / '**midget**'. The pituitary dwarf is sexually and intellectually a normal individual.

Over activity of the thyroid, cancer of the gland or development of nodules of thyroid lead to hyperthyroidism. In adults abnormal growth causes a disease called **exophthalamic goiter** with characteristically protruded eyeballs.

Hyperthyroidism also affects the physiology of the body (increased metabolic rate). Inadequate supply of iodine in the diet results in hypothyroidism and enlargement of the thyroid gland. This condition is called **simple goiter**.

During pregnancy, due to hypothyroidism, defective development of the growing baby leads to a disorder called cretinism. Physical and mental growth gets severely stunted (thyroid dwarj) due to untreated congenital hypothyroidism[9] (deficiency of thyroid hormones *by birth)*. Stunted growth, mental retardation, low intelligence quotient, abnormal skin, deafness and mutism are some of the characteristic features of this disease. In adult women, hypothyroidism may cause irregular menstrual cycles. In adults the hypothyroidism results in a condition called **myxedema.** Lethargy, mental impairment, intolerance to cold, puffiness of face and dry skin are some of the symptoms of myxedema.

Over activity of parathyroid gland (hyper parathyroidism) causes excess decalcification leading to bone deformities and fractures. Besides this, calcification of kidneys takes place which results into stone formation and urinary problems. Under activity of parathyroid gland (hypo-parathyroidism) leads to **tetany** (prolonged contraction of muscles). The calciuihlion levels in the blood decrease (hypocalcemia).

Under secretion of insulin by the pancreatic gland (hypo-secretion) increases the level of glucose in blood (hyperglycemia). Prolonged hyperglycemia leads to a disease called **diabetes mellitus**, associated with loss of glucose through urine (glycosuria) and formation of harmful compounds called 'ketone bodies'. Insulin therapy is used to treat diabetic patients.

Hyper secretion of insulin leads to decreased level of glucose in the blood (hypoglycemia) resulting in **insulin shock**.

Deficiency of vasopressin causes a disease called **diabetes insipidus**. It does not involve loss of sugar in urine (a difference from diabetes mellitus).

Addison's disease is caused due to hyposecretion of glucocorticoids by the adrenal cortex. This disease is characterised by loss of weight, muscle weakness,

fatigue and reduced blood pressure. Sometimes darkening of the skin in both exposed and nonexposed parts of the body occurs in this disorder. This disorder does not allow an individual to respond to stress.

Cushing's syndrome: It results due to over production of glucocorticoids. This condition is characterized by breakdown of muscle proteins and redistribution of body fat resulting in spindly arms and legs accompanied by a round moon face, buffalo hump on the back and pendulous abdomen.

Wound healing is poor. The elevated level of cortisols causes hyperglycemia, over deposition of glycogen in liver and rapid gain of weight.

GLOSSARY

Acromegaly: An abnormal growth especially of bones of the face due to over secretion of pituitary growth hormone after reaching adulthood.

Catecolholamines: A common name given to hormones namely adrenaline, noradrenaline and dopamine.

Diabetes Insupidus: Excessive urine and extreme thirst as a result of inadequate output of pituitary hormone ADH(Anti diuretic hormone).

Diabetes mellitus: a condition resulting from lack of insulin as a result of which, the body cannot store or oxidise sugar efficiently.

Growth factors: Hormones secreted by non endocrine tissue, which are essential for normal growth of tissues and their repair.

Hepatocytes: Cells of liver.

Melanocytes: Melanin containing cells.

Midget: pituitary dwarf, a condition due to hyposecretion of growth hormone.

Sella turcica: A cavity of the sphenoid bone in the skull in which the pituitary gland is lodged.

VERY SHORT ANSWER TYPE QUESTIONS

1. What is acromegaly?
2. What hormone is called anti-diuretic hormone?
3. Distinguish between diabetes insipidus and diabetes mellitus?
4. What is insulin shok?

SHORT ANSWER TYPE QUESTIONS

1 List out the names of endocrine glands present in human beings and mention the hormones?

2 Give an account of the secretions of Pituitary glands?

3 Write a note on Addison's disease and Cushing's Syndrome?

IV B- Immune system

Basic concepts of Immunology

To understand immunology better, we have to know certain basic concepts such as lines of defence, cells, organs *and* soluble mediators *of the immune system,* antigens, types of immunity, mechanism of immunity *and* immunological disorders.

Lines of Immunity or Lines of defence in the body

Whenever bacteria, viruses, fungi and parasites try to enter the body of an organism, skin, mucous membranes and the enzyme lysozyme of saliva, tears, etc., prevent their entry. This is called the First line of defence. If the microorganisms cross this line and enter the body, the phagocytes, natural killer cells, antimicrobial substances, inflammation, fever, etc., destroy them. This is called the Second line of defence. These two lines of defence are very fast reacting responses but they are not specific. If the microbes cross even this line, then the lymphocytes and antibodies fight against them. It is called the Third line of defence. It is highly specific but takes several days to become fully functional. If all the three lines of defence fail, it results in diseases.

Cells of the immune system

These are mainly of three types, namely (i) Lymphocytes, (ii) Phagocytes and (iii) Auxiliary cells.(Figure 4.5)

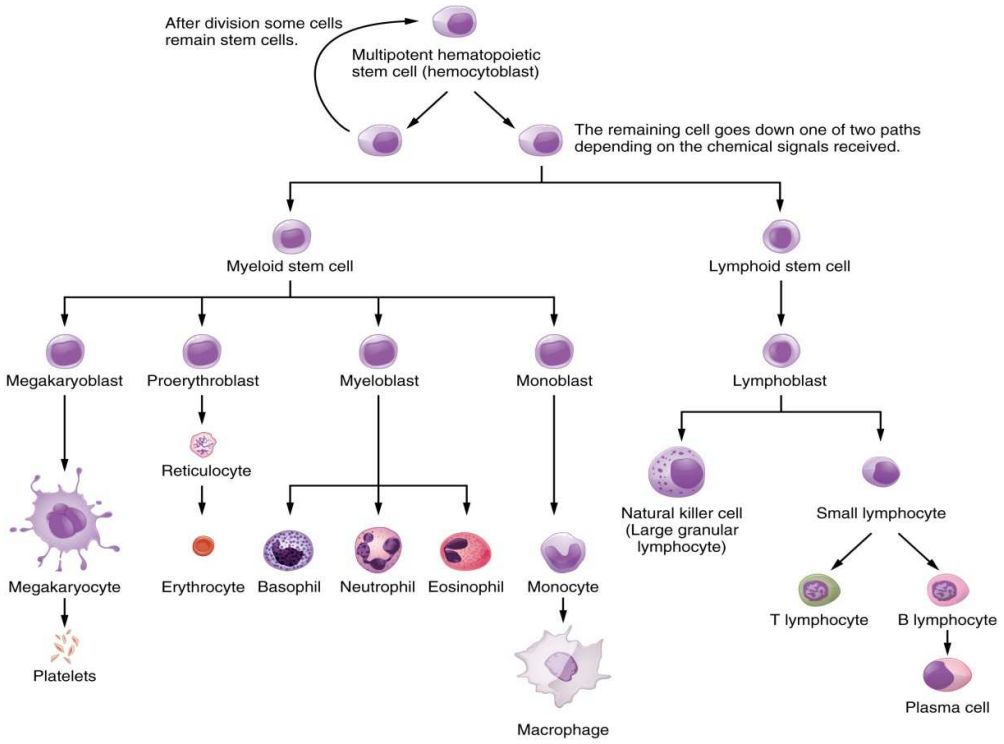


Figure 4.5 Cells of immune system.

1. Lymphocytes

These are the *chief cells* of the *immune system* which are again of three types namely (a) B-cells, (b) T-cells and (c) Large granular lymphocytes (LGLs).

Note: Based on the size, lymphocytes can be divided into small lymphocytes and large lymphocytes. Small lymphocytes include B-cells and T-cells, wheres the large lymphocytes include large granular lymphocytes that consist of Natural killer cells (NK-cells)

(a) B-cells (B-Lymphocytes): The lymphocytes capable of producing antibodies and can capture circulating antigens are called B-cells. They are produced from the 'stem cells' in the bone marrow of adult mammals, liver of foetus and *bursa of Fabricius* in birds. Mature B-cells synthesize various types of antibodies which are displayed on their membrane surfaces. As these antibodies can take antigens, the

mature B-cells are also called immuno-competent B-cells. These mature immuno-competent B-cells reach the secondary lymphoid organs and develop into functional immune cells which later differentiate into long lived memory cells and 'effector' plasma cells. The plasma cells produce antibodies specific to the antigen to which they are exposed. Memory cells store information about the specific antigens and show quick response, when the same type of antigen invades the body later.

(b) T-cells (T-Lymphocytes): The lymphocytes that are not capable of producing antibodies and cannot recognize the free or circulating antigens are known as T-cells. They are produced in the bone marrow, reach the thymus gland and differentiate into mature T- cells or immuno-competent T-cells. in the secondary lymphoid organs, they transform into functional T-cells (cytotoxic T cells) and memory T cells.

Types of T cells

On the surface of T-cells, certain glycoprotein molecules called '*cluster of differentiation* molecules' (CD markers) are present. Based on these protein molecules, the T-cells are of two types, namely $CD4^+$ cells or T_4 cells and $CD8^+$ cells or T_8 cells. Based on their function, the T cells are of two types, namely *T helper cells* and T *cytotoxic cells.*

T helper cells or T_H cells

These are CD4+ cells. They can *recognise* the antigens presented by the APCs. They help the B-cells in producing antibodies, help mononuclear phagocytes ftvJNPs) in phagocytosis and also activate T_c cells to initiate 'cell mediated immunity'. Hence they are involved in both humoral immunity and cell mediated immunity- T cells are also involved in the rejection of 'transplanted tissues /organs. T_H cells also secrete gamma interferon which stimulates cells such as macrophages. The macrophages, inturn secrete cytokines tji^t stimulate T_H cells.

Cytotoxic T cells or T_c cells or Killer T cells

These are $CD8^+$ cells. They can *recognise* the antigens presented by ASCs. As soon as they receive the antigens, they are differentiated into Cytotoxic T Lymphocytes (CTLs) or effector T cells which kill and lyse the virus-infected host cells as well as the tumour cells. Thus, these cells are involved only in cell mediated immunity-

(c) *Large Granular Lymphocytes (LGLs) :* Natural killer cells form the body's most important cells of defense and they destroy the infected cells or altered self cells (such as cancer cells) in an *antibody independent manner (non-specific).* The reaction time ofNK cells is fast as they are always in an active form to identify and destroy even small cancer cells well before they form a tumour, (you *will learn more in the 'mechanism of cell mediated immunity'*

Phagocytes

Based on the types of nuclei present, they are of two types, namely

(a) Mononuclear phagocytes (MNPs) and (b) Polymorph nuclear phagocytes (PMNs)-

(a) Mononuclear phagocytes (MNPs): The monocytes of the blood enter the extra-capillary spaces and transform into histiocytes i_n the connective tissue, Kupffer cells in the liver microglia in the brain, osteoclasts in the bone and synovial cells i_n the synovial fluid, etc.

(b) *Polymorph nuclear phagocytes:;* They are the)granulocytes' of the blood including *neutrophils. basophils* and *eosinophils.* However, in common usage this term is mostly used for neutrophils as they constitute the majority.

Auxiliary cells

The cells that *help lymphocytes* in immune responses are called auxiliary cells. These cells include basophils, mast cells, platelets and antigen presenting cells, Basophils, mast cells and platelets secrete *inflammatory mediators* which cause inflammation in the surrounding tissues. Antigen presenting cells include macrophages.

4.2.1 Vaccination or Immunization

The principle of vaccination or immunization is based on the property of the memory of the immune system. During the process of vaccination, inactivated or weakened pathogens (vaccines) or antigenic proteins of the pathogen are introduced into the body of the host. They initiate the production of appropriate antibodies in the host and also generate memory-B cells and memory T cells. On subsequent exposures, the memory cells recognise that pathogen quickly and overcome the invader with a rapid and massive production of antibodies.

Immunological Disorders

Any situation that results in the impairment of immune system is referred as immundlogical disorder. These are of various types like (a) immunodeficiency disorders, (b) hypersensitivity disorders, (c) auto-immune disorders, (d) graft rejections, etc.

Immunodeficiency disorders

They occur when the immune response of the body is reduced or absent. These are again of two types, namely primary and secondary immunodeficiency disorders. Primary immunodeficiency disorders are caused by the defective genes, e.g. Severe combined immunodeficiency (SCID). The secondary immunodeficiency disorders are caused by various factors like infections, ageing, etc., e.g. HIV/AIDS.

Acquired Immuno Deficiency Syndrome (AIDS)

It is a non-congenital, transmissible, lethal, sexually transmitted disease, caused by Human Immunodeficiency Virus (HIV). It was first reported in 1981 by CDC (Centre for disease control), USA and in the last thirty years, it has spread all over the world, killing more than 25 million people.

Mode of infection

It generally occurs by sexual contact with an infected person, by transfusion of the virus contaminated blood, by sharing the infected needles and from an infected mother to her child through placenta. It is important to note that HIV/AIDS is not spread by mere touch or physical contact. .

Structure of HIV

It is a retrovirus which has an envelope enclosing two ssRNA (single stranded RNA) molecules as the genetic material and two molecules of the enzyme reverse transcriptase. The ssRNA is surrounded by a protein coat, followed by a layer of proteins which is again surrounded by an outer lipid layer that contains a number of glycoproteins such as gp41 and gpl20. These proteins bind to host cell's surface receptors during infection.(Figure 4.6)

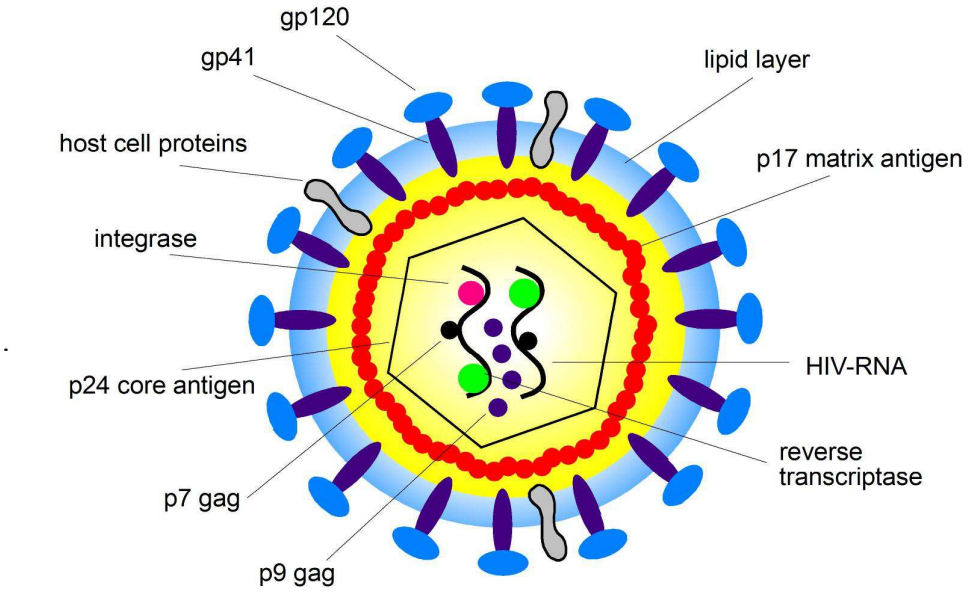


Figure 4.6 Diagram showing HIV virus.

Mechanism

After getting into the body of a person, the HIV enters the T_H cells, macrophages or dendritic cells. In these cells the ssRNA of HIV synthesizes a DNA strand 'complementary' to the viral RNA, using the enzyme reverse transcriptase. The reverse transcriptase also catalyses the formation of the second DNA strand 'complementary' to the first strand forming the double stranded viral DNA. This viral DNA gets incorporated into the DNA of the host cell's DNA by a viral enzyme (integrase) and it is in the

form of a 'provirus'. Transcription of DNA results in the production of RNA, which can act as the 'genome' for the new viruses or it can be translated into viral proteins. The various components of the viral particles are 'assembled' and the HIV are produced. The infected human cells continue to produce virus particles and in this way they act like HIV generating factories. New viruses bud off' from the host cell. This leads to a progressive decrease in the number of T_H cells in the body of an infected person leading to the immunodeficiency in him. Even though HIV attacks cells with CD4 marker, for reasons not known, only T_H cells are destroyed and not the 'macrophages'. The gpl20 molecules on the surface of HIV attach to CD4 receptors of human cells, mostly the T_H cells (gpl20 fits the CD4 marker). Attack on certain types of cells/ tissues only by viruses such as HIV is referred to as 'tissue tropism'.

Symptoms

There is always a time-lag between the first infection and appearance of symptoms. This period may vary from a few months to many years (usually 5-10 years). During this period, the person suffers from bouts of fever, diarrhoea and loss of weight. Due to decrease in the number of T_H cells, the person starts suffering from infections due to bacteria (especially Mycobacterium), viruses, fungi and other parasites such as Toxoplasma. The patients become so immuno-deficient that they are unable to protect themselves against these minor infections, which normal healthy people can easily overcome.

Diagnosis

A widely used diagnostic test for detecing HIV infection is the Enzyme Linked Immuno-Sorbent Assay (ELISA) test. This can be detected within 15 days to 4 months after the exposure to virus. ELISA is only a *screening test* Western blot is used as a more reliable confirmation test for HIV infection.

Treatment of HIV refection and prophylaxis

Treatment of AIDS with 'anti-retroviral drugs' can only prolong the life of the patient but cannot prevent death. Hence, it is better to follow prophylactic methods to avoid HIV infection.

Prophylaxis

Safe sex, proper sterilization of hospital syringes and needles and screening the blood for HIV before transfusion, are some of the important prophylactic methods. In our country the National AIDS Control Organization (NACO) and other non-governmental organization (NGOs) are doing a lot to educate people about AIDS.

Hypersensitivity disorders (Allergies)

The exaggerated response of the immune system to certain antigens present in the environment is called hypersensitivity. There are different types of hypersensitivity reactions of which Type-I hypersensitivity is referred to as allergy* It is due to the release of chemicals such as histamine and serotonin from the mast cells. The antibodies responsible for allergies are of the group Age The substances to which allergy is produced are called allergens- Common examples of allergens are dust mites, pollen, animal dander, etc. Symptoms include sneezing, watery eyes, running nose arid difficulty in breathing. '

For determining the cause of allergy, the patient is exposed to or injected with very small doses of possible allergens, and the reactions are studied. The use of drugs like ate-histamine, adrenalin and steroids quickly control the symptoms of allergy.

GLOSSARY
Barrier: Anything that obstructs free movement.
Bursa of fabricius: a lymphoid organ in birtds taht is connected to the cloaca and is the site of B cell maturation.
Immunosuppressants: Medication which decreases the immune response especially in case tissue transplantation.
Inflammation : a protective response brought about by the mast cells and otrher immunological cells, in an organism to initiate healing process.

Phagocytos: The action of engulfing by phagocytes.

Provirus: A virus genome that is integrated into the DNA of host cell.

Syndrome : A group of symptoms.

Tonsils: the masses of lymphatic tissue present one on either side of the oropharynx.

Wheezing: a wheezing sound during breathing due to respiratory disorder.

VERY SHORT ANSWER TYPE QUESTIONS

1. Differentiate between mature B-cells and functional B-cells?
2. Explain the mechanism of vaccination or immunization?
3. Mention various types of immunological disorders?

SHORT ANSWER TYPE QUESTIONS

1. Write a short notes on B-cells?
2. Explain the mechanism by which HIV multiplies and leads to AIDS?

Unit 5

Human reproduction

SUMMARY

Humans are sexually reproducing and viviparous. The male reproductive system is composed of a pair of testes, the male sex accessory ducts and the accessory glands and external genitalia. Each testis has about 250 compartments called testicular lobules, and each lobule contains one to three highly coiled **seminiferous** tubules. Each seminiferous tubule is lined inside by spermatogonia and Sertoli cells. The spermatogonia undergo meiotic divisions leading to sperm formation, while Sertoli cells provide nutrition to the dividing germ cells. The Leydig cells outside the seminiferous tubules synthesise and secrete testicular hormones called androgens. The male external genitalia is called penis. The female reproductive system consists of a pair of ovaries, a pair of oviducts, a uterus, a vagina, external genitalia, and a pair of mammary glands. The ovaries produce the female gamete (ovum) and some steroid hormones (ovarian hormones). Ovarian follicles in different stages of development are embedded in the **stroma**. The oviducts, uterus and vagina are female accessory ducts. The uterus has three layers namely perimetrium, myometrium and endometrium. The female external genitalia includes mons pubis, labia majora, labia minora, hymen and clitoris. The mammary glands are one of the female secondary sexual characteristics. Spermatogenesis results in the formation of sperms that are transported by the male sex accessory ducts. A normal human sperm is composed of a head, neck, a middle piece and tail. The process of formation of mature female gametes is called **oogenesis**. The reproductive cycle of female primates is called menstrual cycle. Menstrual cycle starts only after attaining sexual maturation (puberty). During ovulation only one ovum is released per menstrual cycle. The cyclical changes in the ovary and the uterus during menstrual cycle are induced by changes in the levels of pituitary and ovarian hormones. After coitus, sperms are transported to the junction of the isthmus and ampulla, where the sperm fertilises

the ovum leading to formation of a diploid zygote. The presence of X or Y chromosome in the sperm determines the sex of the embryo. The zygote undergoes repeated mitotic division to form a blastocyst, which is implanted in the uterus resulting in pregnancy. After nine months of pregnancy, the fully developed foetus is ready for delivery. The process of childbirth is called parturition which is induced by a complex neuroendocrine mechanism involving cortisol, estrogens and oxytocin. Mammary glands differentiate during pregnancy and secrete milk after child-birth. The new-born baby is fed milk by the mother (lactation) during the initial few months of growth.

V A- Human Reproductive System

Human reproduction is a form of sexual reproduction resulting in the conception of child, typically involving sexual intercourse between man and woman. The reproductive events in humans include formation of gametes, transfer of male gametes into female genital tract fertilization that leads to zygote formation. This is followed by the embryonic development. Let us examine male and female reproductive systems in humans.

The Male Reproductive System

The male reproductive system (male genital system) consists of a number of sex organs that are a part of the human reproductive process. The sex organs which are located in the pelvic region include a pair of testes (sing: *testis)* along with accessory ducts, glands and the external genitalia. (Figure 5.1)

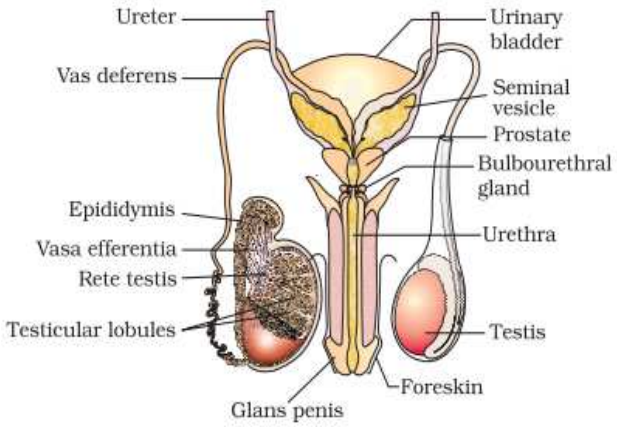


Figure 5.1 Male reproductive system

Testes

The testes **(testicles)** are a pair of oval pinkish male primary sex organs suspended outside the abdominal cavity within a pouch called **scrotum.**

The scrotum helps in maintaining the low temperature of the testes (2-2.5° C lower than the normal internal body temperature) necessary for spermatogenesis. The cavity of the scrotal sac is connected to the abdominal cavity through the **inguinal canal.** Testis is held in position in the scrotum by the **gubernaculums,** a fibrous cord that connects the testis with the bottom of the scrotum and a **spermatic cord,** formed by the vas deferens, nerves, blood vessels and other tissues that run from the abdomen down to each testicle, through the inguinal canal. Each testis is enclosed in a fibrous envelope, the **tunica albuginea,** which extends inward to form septa that partition the testis into I lobules. There are about 250 testicular lobules in each testis. Each lobule contains 1 to 3 highly coiled **seminiferous tubules**. A pouch of serous membrane (peritoneal layer) called **tunica vaginalis** covers the testis. Each seminiferous tubule is lined by the **germinal epithelium** which consists of un-differentiated male germ cells called **spermatogonial mother cells** and it also bears 'nourishing cells' called **Sertoli cells.** The spermatogonia produce the **primary spermatocytes** which undergo meiotic division, finally leading to the formation of

spermatozoa or sperms (spermatogenesis). Sertoli cells provide nutrition to the spermatozoa and also produce a hormone called inhibin, which inhibits the secretion of FSH. The regions outside the seminiferous tubules, called interstitial spaces, contain **interstitial cells** of Leydig or **Leydig cells**. Leydig cells produce androgens, the most important of which is testosterone. Testosterone controls the development of secondary sexual characters and spermatogenesis. Other immunologically competent cells are also present. The seminiferous tubules open into the **vasa efferentia** through the rete testis (a network of tubules in of the testis carrying spermatozoa from the seminiferous tubules to the vasa efferentia. (Figure 5.2)

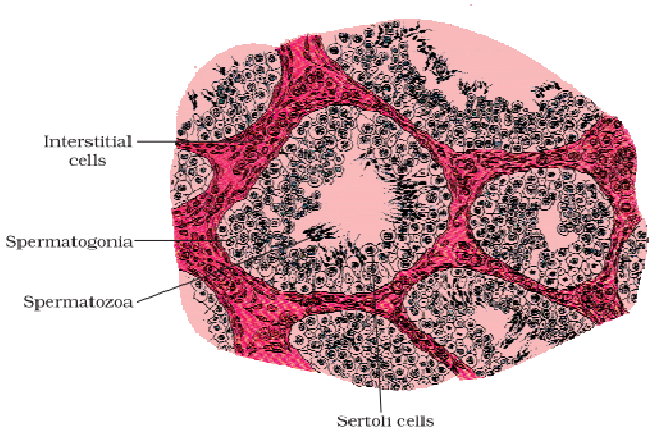


Figure 5.2 Sectional view of seminiferous tubule

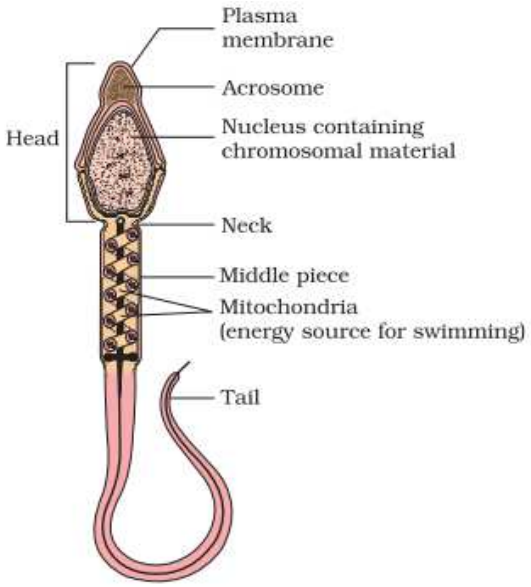


Figure 5.3 Sperm

Epididymis

The vasa efferentia leave the testis and open into a narrow, tightly coiled tube called epididymis located along the posterior surface of each testis. The epididymis provides a storage space for the sperms and gives the sperms time to mature. It is differentiated into three regions caput epididymis, corpus epididymis and cauda epididymis. The caput epididymis receives spermatozoa via the vasa efferentia of the mediastinum testis (a mass of connective tissue at the back of the testis that encloses the rete testis).

Vasa deferentia

The vas deferens or ductus deferens is a long, narrow, muscular tube. The mucosa of the ductus deferens consists of pseudostratified columnar epithelium and lamina propria (areolar connective tissue). It starts from the tail of the epididymis, passes through the inguinal canal into the abdomen and loops over the urinary bladder. It receives a duct from the seminal vesicle. The vas deferens and the duct of the seminal vesicle unite to form a short ejaculatory duct/ductus ejaculatorius. The two

ejaculatoiy ducts, carrying spermatozoa and the fluid secreted by the seminal vesicles, converge in the centre of the prostate and open into the **urethra,** which transports the sperms to outside.

Urethra

In males, the urethra is the shared terminal duct of the reproductive and urinary systems. The urethra originates from the urinary bladder and extends through the penis to its external opening called urethral meatus. The urethra provides an exit for urine as well as semen during ejaculation.

Penis

The penis and the scrotum constitute the male external genitalia. The penis serves as a urinal duct and also intromittent organ that transfers spermatozoa to the vagina of a female. The human penis is made up of three columns of tissue; two upper corpora cavernosa on the dorsal aspect and one corpus spongiosum on the ventral side. Skin and a subcutaneous layer enclose all three columns, which consist of special tissue that helps in erection of the penis to facilitate insemination. The enlarged and bulbous end of penis called **glans** penis is covered by a loose fold of skin (foreskin) called prepuce. The urethra traverses the corpus spongiosum, and its opening lies at the tip of the glans penis **(urethral meatus).**

Male accessory genital glands

The male accessory glands include paired seminal vesicles, a prostate and bulbourethral glands.

Seminal vesicles

The seminal vesicles are a pair of simple tubular glands present postero- inferior to the urinary bladder in the pelvis. Each seminal vesicle opens into the corresponding vas deferens, as the vas deferens enters the prostate gland. The secretion of the seminal vesicles constitutes about 60 percent of the volume of

seminal fluid. It is an alkaline, viscous fluid that contains fructose, proteins, citric acid, inorganic phosphorus, potassium, and prostaglandins. Once this fluid joins the sperm in the ejaculatory duct, fructose acts as the main energy source for the sperm outside the body. Prostaglandins are believed to aid fertilization by causing the mucous lining of the cervix to be more receptive to sperm as well as by aiding the movement of the sperm towards the ovum with peristaltic contractions of the uterus and fallopian tubes.

Prostate gland

Prostate gland is located directly beneath the urinary bladder. The gland surrounds the prostatic urethra, and sends its secretions through several prostatic ducts. In man, the prostate contributes 15-30 percent of the semen. The fluid from the prostate is clear and slightly acidic. The prostatic secretion 'activates' the spermatozoa and provides nutrition.

Bulbourethral Glands

Bulbourethral glands, also called Cowper's glands, are located beneath the prostate gland at the beginning of the internal portion of the penis. They add an alkaline fluid to semen during the process of ejaculation. The fluid secreted by these glands lubricates tw urethra. It is also thought to function as a 'flushing agent' that washes out the acidic urinary residues that remain in the urethra, before the semen is ejaculated.

5.1.2 The Female Reproductive System

The female reproductive system consists of a pair of ovaries along with a *pxtf* of oviducts, uterus, vagina and the external genitalia located in the pelvic region. These parts of the system along with a pair of the mammary glands are integrated structurally and functionally to support the processes of ovulation, fertilization, pregnancy, birth and child care. (Figure 5.4)

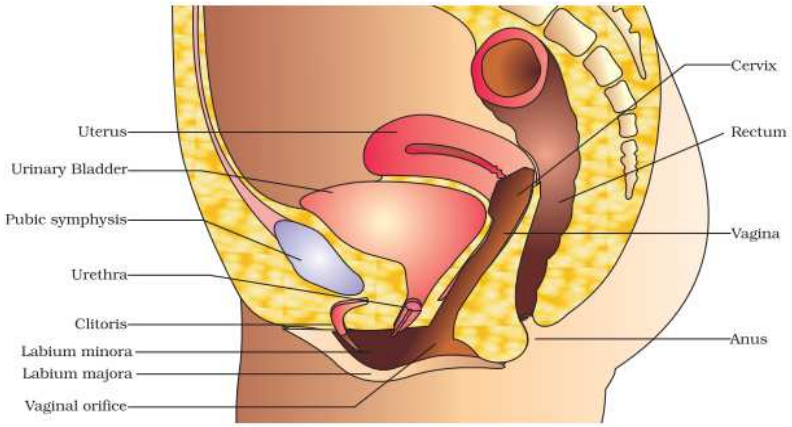


Figure 5.4 Sectional view of female reproductive system

Ovaries

Ovaries are the primary female sex organs that produce the female gametes **(ova)** and several steroid hormones (ovarian hormones). A pair of ovaries is located one on each side of the lower abdomen. The double layered fold of peritoneum connecting the ovary with the wall of the abdominal cavity is known as the **mesovarium.**

The ovaries are covered on the outside by a layer of simple cuboidal epithelium called **germinal (ovarian) epithelium.** This is actually the visceral peritoneum that envelops the ovaries. Underneath this layer there is a dense connective tissue capsule, the **tunica albuginea.** The ovarian **stroma** is distinctly divided into an outer **cortex** and an inner **medulla.** The cortex appears more dense and granular due to the presence of numerous **ovarian follicles** in various stages of development. The medulla is a loose connective tissue with abundant blood vessels, lymphatic vessels, and nerve fibers.

Fallopian tubes (Oviducts)

Each fallopian tube extends from the periphery of each ovary to the uterus, and it bears a funnel shaped **infundibulum.** The edges of the infundibulum possess finger like projections called **fimbriae,** which help in collection of the ovum after

'**ovulation**'. The infundibulum leads to a wider part of the oviduct called **ampulla.** The last part of the oviduct, **isthmus** has a narrow lumen and it joins the uterus. Fallopian tube is the site of fertilization. It conducts the ovum or zygote towards the uterus by peristalsis. The fallopian tube is attached to the abdominal wall by a peritoneal fold called **mesosalpinx.**

Uterus

The uterus is single and it is also called **womb.** It is a large, muscular, highly vascular and inverted pear shaped structure present in the pelvis between the bladder and the rectum. The uterus is connected to the abdominal wall by the peritoneal fold called **mesometrium.** The lower, narrow part through which the uterus opens into the vagina is called the **cervix.** The cavity of the cervix is called **cervical canal** which along with vagina forms the **birth canal**. (Figure 5.5)

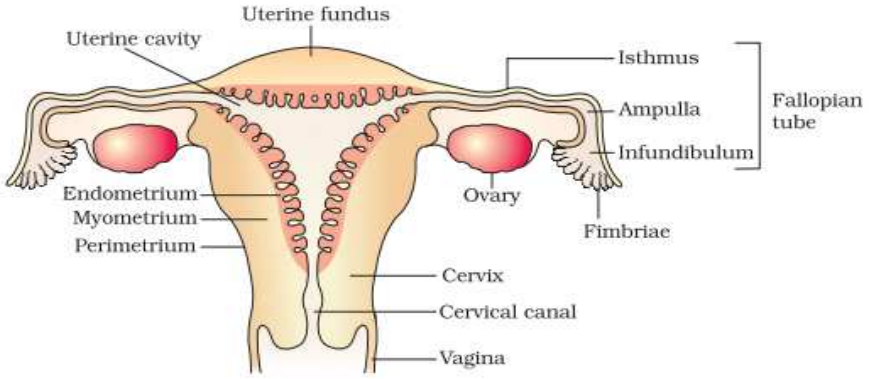


Figure 5.5 Sectional view of uterus

The wall of the uterus has three layers of tissue. The external thin membranous **perimetrium** the middle thick layer of smooth muscle called **myometrium** and inner glandular lining layer called **endometrium.** The endometrium undergoes cyclic changes during menstrual cycle while the myometrium exhibits strong contractions during parturition.

Vagina

The vagina is a large, median, fibro-muscular tube that extends from the cervix to the **vestibule** (the space between the labia minora). It is lined by non-keratinised stratified squamous epithelium. It is highly vascular, and opens into the vestibule by the **vaginal orifice.**

Vulva

The term vulva (vulva=to wrap around) or **pudendum** refers to the external genitals of the female. The vestibule has two apertures- the upper external **urethral orifice** of the urethra and the lower **vaginal orifice** of vagina. Vaginal orifice is often covered partially by a membrane called hymen which is a mucous membrane. Vestibule is bound by two pairs of fleshy folds of tissue called **labia minora** (inner) and larger pair called **labia majora** (outer). **Clitoris** is a sensitive, erectile structure, which lies at the upper junction of the two labia minora above the urethral opening. The clitoris is homologous to the penis of a male as both are supported by **corpora cavernosa** internally. There is a cushion of fatty tissue covered by skin and pubic hair present above the labia majora. It is known as **mons pubis.**

Accessory reproductive glands of female

These glands include Bartholin's glands. Skene's glands and mammary glands**.**

Bartholin's glands

The Bartholin's glands **(Greater vestibular glands)** are two glands located slightly posterior and to the left and right of the opening of the vagina. They secrete mucus to lubricate the vagina and are homologous to the bulbourethral glands of the male reproductive system.

Skene's glands

The Skene's glands **(Lesser vestibular glands)** are located on the anterior wall of the vagina, around the lower end of the urethra. They secrete a lubricating fluid when stimulated. The Skene's glands are homologous to the prostate gland, of the male reproductive system.

Mammary glands

A functional mammary gland is characteristic of all female mammals. The mammary glands are paired structures **(breasts)** that contain glandular tissue and variable amount of fat. The glandular tissue of each breast is divided into 15-20 **mammary lobes** containing clusters of cells called **alveoli-** The cells of the alveoli secrete milk, which is stored in the cavities (lumens) of the alveoli. The alveoli open into mammary tubules. The tubules of each lobe join to form a **mammary duct.** Several mammary ducts join to form a wider **mammary ampulla** which is connected to **lactiferous duct** through which milk is sucked out by the baby.

5.1.3 Fertilisation

The motile sperms swim rapidly, pass through the cervix, enter the uterus and finally reach **ampullary-isthmic junction** of the fallopian tube. The ovum released by the ovary is also transported to the fallopian tube where fertilisation (also called **conception** in humans) takes place.

The oocyte-cumulus complex of the female is surrounded by a zona pellucida, corona radiata, and cumulus layer. When a motile sperm reaches the ovum, it makes its way through corona radiata and zona pellucida. In this process, the enzyme **hyaluronidase** released by the acrosome of a sperm (it is a corona penetrating enzyme) plays an important role in dissolving the **hyaluronic acid** in the ground substance of the follicle cells. The enzyme **acrosin** released from the acrosome as an effect of **acrosome reaction,** dissolves/digests the zona pellucida (lysis of the zona pellucida to facilitate penetration of the sperm). Though several

sperms penetrate through the zona pellucida into the perivitalline space, only one sperm enters the ovum **(monospermy).** This induces the completion of the **meiosis-** II of the secondary oocyte (ovum). The second meiotic division is also unequal and results in the formation of a **second polar body** and an **ovum (ootid).** The fusion of gametes is called **syngamy** or **amphimixis.** The nuclear union results in the formation of **synkaryon (zygotic nucleus).** Corona radiata disappears after fertilisation.

Cleavage

The first phase of embryonic development, the cleavage is holoblastic (because of microlecithal condition of egg), radial, indeterminate and unequal. The mitotic division (cleavage) starts as the zygote moves through the isthmus of the oviduct towards the uterus. The daughter cells are called blastomeres.

Morula

The embryo with 8-16 blastomeres looks like a 'mulberry' and is called the **morula.** Morula is a solid mass of cells. The morula develops in the fallopian tube and reaches the uterus for further development. It is still surrounded by the zona pellucida. Due to unequal cleavage smaller and larger blastomeres are formed. The morula passes through a process called compaction < Now the embryo has a superficial flat cell layer and inner cell mass. The outer/ supeficial layer becomes the trophoblast 0r trophectoderm. The trophoblast serves to attach the embryo to the uterine wall by forming trophoblastic villi. The inner cell mass constitutes formative cells. The inner cell mass gives rise to the embryo proper and is, therefore, also called the embryoblast. This is the first sign of cell differentiation (cells becoming different) in the *t* human embryo.

Blastocyst

Some fluid now passes into the morula from the uterine cavity, and partially separates the cells of the inner cell mass from those of the trophoblast. As the

quantity of the fluid increases, the morula acquires the shape of a cyst. The cells of the trophoblast become flattened, and the inner cell mass comes to be attached to the inner side of the trophoblast on one side only. The morula now becomes a blastocyst.

The cavity of the blastocyst is the **blastocoel** or **segmentation cavity** or **primary body cavity.** The side of the blastocyst to which the inner cell mass is attached is called the **embryonic** or **animal pole,** while the opposite side is the **abembryonic pole.** The cells of the trophoblast above the region of inner cell mass are called **cells of Rauber.**

Implantation

The zona pellucida present around the blastocyst gradually disappears and the cells'of the trophoblast stick to the uterine endometrium. The trophoblast invades the endometrium of the uterus. The blastocyst gets implanted into the uterine mucosa till the whole of it comes to lie within the thickness of the endometrium. This is called **interstitial implantation.** In humans, implantation begins on the 6^{th} day after fertilisation. The process of implantation is aided by proteolitic enzymes produced by the trophoblast. The uterine mucosa also aids the process. (Figure 5.6)

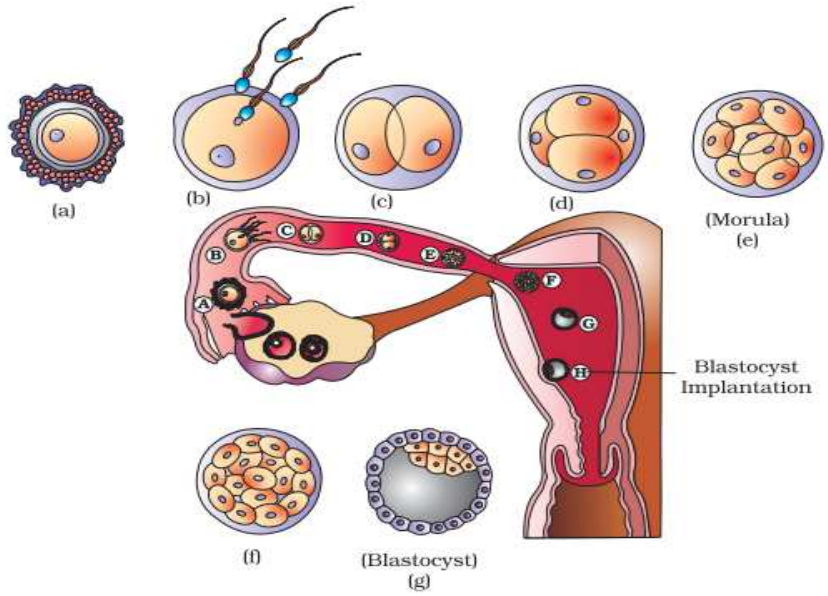


Figure 5.6 Diagrammatic representation of growing embryo

After the implantation of the embryo, the uterine endometrium is differentiated into what is called **decidua.** The portion of the decidua where the placenta is to be fomed (i.e. deep to the developing blastocyst) is called the **decidua basalis.** The part of the decidua that separates the embryo from the uterine lumen is called the **decidua capsularis.** The part lining the rest of the uterine cavity is called the **decidua parietalis.** At the end of pregnancy the decidua is shed off, along with the placenta and membranes.

Formation of Bilaminar Embryonic Disc

Implantation of the blastocyst is completed by the end of the second week. The inner cell mass forms into a disc called **embryonic disc.** Soon, the 'cells of Rauber' disappear exposing the embryonic disc. From the lower part of inner embryonic disc, some cells get separated by **delamination** and eventually form a layer of cells on the inner surface of the embryonic disc i.e. on the surface facing the cavity. This cell layer develops into the **hypoblast,** which is the future extra embryonic endoderm. The remaining part of the embryonic disc is called **epiblast.** Now the embiyonic disc is called **bilaminar embryonic disc.**

The cells of the hypoblast increase in number and spread along the inner surface of the trophoblast. This hypoblast layer below the trophoblast finally encloses a cavity called **yolk sac** or **umbilical vesicle.** Meanwhile, the thickness of the embryonic disc increases towards the caudal end. Gradually the embryonic disc becomes oval.

Gastrulation

Gastrulation involves proliferation, differentiation and movement of cells within the embryo. Along the longitudinal axis of the embryonic disc, a **primitive streak** is formed. A longitudinal furrow known as **primitive groove** forms along the middle of the primitive streak. On either side of it are the **primitive folds.** Anteriorly primitive streak has a shallow **primitive pit.** The anterior end of the primitive streak becomes thickened. This thickened part of the streak is called the **primitive knot** or **primitive node** or **Hensen's node.**

Trilaminar Embryo - Formation of Primary Germ Layers Ingression, the future endodermal cells from the epiblast, replaces the hypoblast and forms the **endoderm** of the embryo. The future mesodermal cells converge towards the primitive folds, **involute** through the primitive groove and reach between epiblast and endoderm. The remaining epiblast is now known as the **ectoderm.** This invasion of the epiblast cells into the space between the epiblast and hypoblast is called **gastrulation.** Thus, the ectoderm, mesoderm and endoderm are all derived from the epiblast. The process of gastrulation converts the bilaminar embryonic disc into a **trilaminar embryonic disc.**

Extraembryonic Membranes

During the development of the human embryo, as in all other amniotes four extraembryonic or *foetal membranes* are formed. They are **chorion, amnion, allantois,** and **yolk sac.** From the blastodisc, amniotic folds called head fold, tail fold and lateral fold are developed as **somatopleure.** As the folds fuse they are differentiated into outer chorion and inner amnion. Between the amnion and the

embryo, there is an **amniotic cavity** filled with amniotic fluid. Amnion protects the embryo as the amniotic fluid acts as a shock absorber and also prevents the embiyo from desiccation. The chorion develops a rich supply of blood vessels and forms an intimate association with the endometrium of the uterus.

Allantois and yolk sac are derived from the **splanchnopleure-** Allantois is formed from the hind gut as an evagination. It stores the waste materials. Allantois and chorion are fused to form **chorio allantoic membrane** which constitutes the placenta. Yolk sac encloses a fluid filled cavity. It is connected to the mid gut. It has no nutritive role.

After gastrulation is complete and any extraembryonic membranes are formed, the next stage of embryonic development begins with **organ formation-**

Organogenesis

During organogenesis, regions of the three embryonic germ layers develop into the rudiments of organs. Whereas gastrulation involves mass movements of cells, organogenesis involves more localized changes.

Formation of the Notochord and Neural Tube

The chorda mesodermal cells converge and involute through the Hensen's node and extend forwards as **notochordal process-** This is later transformed into a solid rod - the notochord, the embryonic axial skeleton which is replaced by the vertebral column. The notochordal mesoderm induces the overlying ectodermal cells to form the **neural plate-** This is a good example of induction. At first the neural plate is oval but later elongates over the underlying notochord along the longitudinal axis of the embryo. The plate invaginates towards the notochord to form a **neural groove,** which deepens progressively to form a tube by fusion of the lateral neural folds. The process of formation of **neural tube** is referred to as **neurulation-**

Differentiation of Mesoderm and Formation of Coelom

The intra embryonic mesoderm spreads in all directions between the outer ectoderm and inner endoderm. The longitudinal column of mesoderm adjacent to the notochord and neural tube on either side is called epimere- The mesoderm around the gut is the hypomere. The mesoderm in between these two is the **mesomere** The epimere becomes segmented into cubical blocks called **somites** or **metameres.** Each somite differentiates into **myotome, sclerotome** and **dermatome.** The sclerotome forms the vertebral column. The dermatome forms the dermis of the skin and other connective tissues. The myotome forms the voluntary muscles of the body. The mesomere forms the urinogenital organs and their ducts. The hypomere splits into outer **somatic** and inner **splanchnic mesodermal layers. Intra embryonic coelom** is formed between these two layers. It gives rise to pericardial, pleural, peritoneal cavities etc.

Placenta formation

After implantation, finger-like projections appear on the trophoblast called **chorionic villi** which are surrounded by the uterine tissue. The chorionic villi and uterine tissue become interdigitated with each other and jointly form a structural and functional unit called placenta between the developing embryo (foetus) and the mother. The maternal and foetal blood do not mix with each other. They are separated by the **placental membranes.**

The placenta consists of two essential portions: a **maternal part of the placenta** derived from the endometrium of the uterus, and **foetal membranes of the foetal part** of the placenta. The maternal components of the placenta are : Uterine epithelium, Uterine connective tissue and Uterine capillary endothelium. The foetal components of the placenta are foetal capillary endothelium, foetal connective tissue and foetal chorionic epithelium.

The placenta of humans is called **chorioallantoic placenta** as allantois also fuses with the chorion in the process of vascularisation. Placenta is **discoidal** as the villi

are restricted to the dorsal surface of the blastodisc. Placenta is **.haemochorial** as the maternal blood comes into direct contact with the foetal chorion. During parturition the placenta is cast off with loss of embryonic membranes and the encapsulating maternal tissues **(decidua)** causing extensive haemorrhage and thereby bleeding. So, it is also called as **deciduate placenta**. (Figure 5.7)

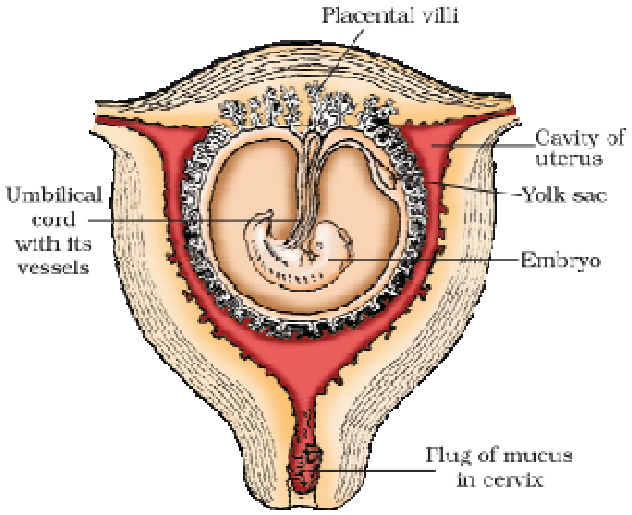


Figure 5.7 The human foetus with the uterus

Functions of Placenta

The placenta facilitates the supply of oxygen and nutrients to the embryo and also removal of carbon dioxide and excretory/waste materials produced by the embryo. The placenta is connected to the embryo through an umbilical cord which helps in the transport of substances to and from the embryo.

Progesterone secreted by the placenta is essential for the maintenance of pregnancy after the 4^{th} month (when the corpus luteum degenerates). Oestrogens (mainly estradiol) produced by the placenta reach maternal blood and promote uterine growth and development of the mammary glands. Human chorionic gonadotropin (hCG) produced by the placenta is similar in its actions to luteinizing hormone. Gonadotropins are excreted through maternal urine where their presence is used as

a test to detect a pregnancy in its early stages. Somatomammotropin has an anti-insulin effect on the mother leading to increased plasma levels of glucose and amino acids in the maternal circulation. In this way it increases the availability of these materials to the foetus.

Pregnancy

Pregnancy is the intra uterine development of the embryo or foetus (also called gestation period). Human pregnancy averages 266 days (38 weeks) from fertilization of the egg or 40 weeks from the start of the last menstrual cycle. Although pregnancy begins with implantation, the process leading to pregnancy occurs earlier aS a result of the female gamete, or oocyte, merging with the male gamete, spermatozoon.

Timetable of some events during pregnancy

Human gestation can be divided for convenience into three trimesters of about three months each. The first trimester is the main period of organogenesis, the development of the body organs. In human beings, after one month of pregnancy, the embryo's heart is formed. The first sign of growing foetus may be noticed by listening to the heart sound carefully through the stethoscope. By the end of the second month of pregnancy, the foetus develops limbs and digits. By the end of 12 weeks (first trimester), most of the major organ systems are formed, for example, the limbs and external genital organs are well-developed. The first movements of the foetus and appearance of hair on the head are usually observed during the fifth month. By the end of 24 weeks **(second trimester)** the body is covered with fine hair, eye-lids separate, and eyelashes are formed. By the end of nine months of pregnancy **(third trimester)**, the foetus is fully developed and is ready for delivery.

Parturition

Childbirth begins with **labour**. a series of strong, rhythmic uterine contractions that push the foetus and placenta out of the body. This process of delivery of the foetus (childbirth) is called **parturition-** The signals for parturition originate from the fully developed foetus and the placenta which induces mild uterine contractions called **foetal ejection reflex-** This triggers release **of oxytocin** from the maternal pituitary. Oxytocin acts on the uterine muscle and causes stronger uterine contractions. This leads to expulsion of the baby out of the uterus through the birth canal (parturition). Soon after the infant is delivered, the placenta along with decidua is also expelled out of the uterus.

Lactation

One aspect of postnatal care unique to mammals is **lactation,** the production of mother's milk. **Mammary glands** of the female undergo differentiation during pregnancy and starts producing milk towards the end of pregnancy by the process called **lactation-** This helps the mother in feeding the newborn. The milk produced during the initial few days of lactation is called **colostrum,** which contains several antibodies absolutely essential to protect the newborn babies from initial sources of infections. **Breast-feeding** during the initial period of infant growth is recommended by doctors for bringing up a healthy baby.

GLOSSARY
Androgens: Androgens are the generic term for any natural or synthetic compound, usually asteroid hormone that stimulate or controls the development and maintenance of male characteristics in vertebrates. **Foetus**: It is a developing mammal or other viviparous vertebrate after the embryonic stage and before birth. **Follicle**: A small spherical group of cells containing a cavity.

Formative cells: The embryonic cells which are capable of producing new cells.

Ingression: The inward migration of future endodermal cells from the epiblastduring gastrulation.

Involution: The inward growth and curling inward of group of cells, as in the formation of gastrula from a blastula.

Oogonium: A primordial ooctye during fetal development.

Umbilical cord: In placental mammals, the umbilical cord is the connecting cord from the developing embryo or foetus to the olacenta.

VERY SHORT ANSWER TYPE QUESTIONS

1. What are the functions of sertoli cells of the seminiferous tubules and the leydig cells in man?
2. Name the copulatory structure of man? What are the three columns of tissues in it?
3. Define spermeogenisis and spermiation?
4. Define gestation period?
5. What is implantation with reference to embryo?
6. Write two functions of each of testis and ovary?
7. Draw a neatly labeled diagram of sperm?
8. Distinguish between involution and ingression?

SHORT ANSWER TYPE QUESTIONS

1. Describe microscopic structure of testis of man?
2. Describe microscopic structure of ovary of woman?
3. Describe the Graafian follicle in women?
4. Draw a labeled diagram of the male reproductive system?
5. Draw a labeled diagram of the female reproductive system?
6. Describe the structure of seminiferous tubule?

VB- Reproductive Health

5.2.1 Sexually transmitted diseases

Disease or infections which are transmitted through sexual contact are collectively called sexually transmitted diseases (STD) or venereal diseases (VD) or reproductive tract infections (RTI). Most common STDs and their causative organisms are shown in the table below.

S.No.	Name of the disease	Causative organism
1	Gonorrhea	*Neisseria gonorrhoeae* (bacteria)
2	Syphilis	*Treponema pallidum* (bacteria)
3	Genital herpes	Herpes simplex virus (HSV)
4	Genital warts, cervical cancer	Human papilloma virus(HPV)
5	Trichomoniasis	*Trichomonas veginalis* (protozoan parasite)
6	Chlamydiasis	*Chlamydia trachomatis* (Bacteria)
7	Hepatitis -B	HBV
8	HIV/AIDS	Human immunodeficiency virus(HIV)

5.2.2 Birth Control - Need and Methods

In the last century an all-round development in various fields significantly improved the quality of life of the people. However, the increased healthcare facilities along with better living conditions had an explosive impact on the opulation growth. Probable reasons for this growth rate are decline in death rate, maternal mortality rate (MMR) and infant mortality rate (IMR). According to the 2011 census the population growth rate (annual %) is still around 1.64 percent. Such a high growth rate could lead to an absolute scarcity of even the basic requirements i.e. food, shelter and clothing, in spite of significant progress made in those areas. To overcome the problem of population explosion, birth control is the only available solution. People should be motivated to have smaller families by using various contraceptive methods. Statutory raise of marriageable age of the females to 18 and that of males to 21 years, and providing incentives to couples with smaller families are two of the other measures taken by the Government to tackle this problem.

Contraception

The intentional prevention of **conception** (fertilization of an egg by a sperm at the beginning of pregnancy) by natural or artificial means is called **contraception. Contraceptives** prevent pregnancy by interfering with the normal process of ovulation, fertilization, and implantation. There are different kinds of contraceptives that act at different points of the process of conception. An ideal contraceptive should have the following qualities, i) user-friendly, ii) easily available, iii) effective and reversible with no or less side effects and iv) do not affect the sexual life of the user.

A wide range of **contraceptive methods** are presently available which could broadly classified as follows. 1. Natural/Traditional methods, 2. Barriers, 3. Intrauterine devices (IUDs),4. Oral contraceptive pills, 5. Injectables, Implants, Vaginal rings and Skin patches, and 6. Surgical methods.

1. Natural methods

These methods depend on the principle of avoiding chances of sperms meeting the ovum. These are of three types.

i) Periodic abstinence: in this method couples avoid or abstain from coitus from the **10th to the 17th day** of the menstrual cycle (the **fertile period)** when ovulation generally occurs.

ii) Withdrawal or Coitus interruptus: in this method, the male partner withdraws his penis from the vagina, just before 'ejaculation', so as to avoid insemination.

iii) Lactational amenorrhea method: Amenorrhoea means absence of menstruation (missing of menstrual period). Ovulation generally will not occur during the period of intense lactation by the mother following parturition (delivery). This is known as **Lactational amenorrhea.** Some couples utilize the contraceptive benefit of this method.

NOTE: As long as the mother fully breast feeds her child, chances of conception are almost zero. In addition breast feeding offers many benefits to the infant such as enhanced immunity, protection against allergies etc. However, this method has been reported to be effective only up to a maximum of six months following parturition

2. Barrier methods

In barrier methods, sperms are prevented from physically meeting with the ovum by barriers. **Condoms** (male and female condoms)(Figure 5.8&5.8) are popular barriers in use. They are made of thin rubber latex sheath. Condoms are used to cover the penis in the male and *cervix* (neck of the uterus) or vagina in the female to prevent ejaculated semen from entering into the uterus of the female. Nirodh j_S an easily wearable popular brand of condoms for males, widely used in India. Using condom will give an additional benefit of protection from contracting (getting infected by) STDs. Other types of 'reusable' 'female barriers' called diaphragms, cervical caps and vaults (made of rubber) are available, jellies and foams are also useful.

Figure 5.8: Male condom

Figure 8.9 Female condom

3. Intra Uterine Devices (IUDs)

These devices are inserted into the uterus by doctors or trained nurses through vagina. Different types of IUDs such as Nonmedicated IUDs (e.g, Lippes loop) Copper releasing IUD's (Cu T, Cu 7, Multiload 375) and hormone releasing IUDs (Progestasert, LNG-20) are available for contraception. IUDs promote 'phagocytosis' of sperms by white blood corpuscles within the uterus and the copper ions released suppress the motility, viability and fertilizing capacity of the spermatozoa.The hormone releasing IUDs, in addition, make the uterus unsuitable for implantation and the cervix hostile/antagonistic to the sperms. IUDs are ideal contraceptives to females who want to delay and/or have space between children. This is a widely accepted method of contraception in India. (Figure 5.10)

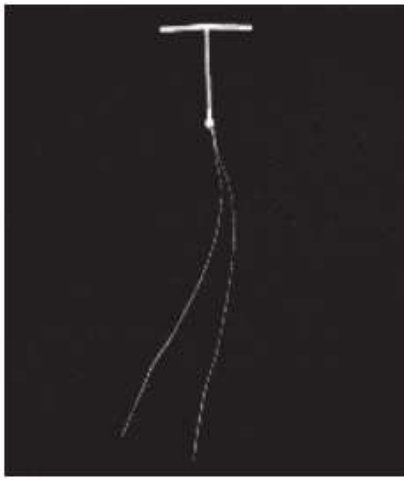


Figure 5.10 Copper T

4 Oral contraceptive pills (OCPs)

Oral administration of small doses of progestogens or progestogen-estrogen combinations in the form of tablets or pills is another popular contraceptive method used by females. Pills have to be taken daily for a period of 21 days starting preferably within the first five days of the menstrual cycle. After a gap of 7 days (during which menstruation occurs) the same course in the same pattern has to be repeated as long as the female desires to prevent conception. The inhibits ovulation, implantation and also alters the quality of cervical mucous and retards entry of sperms. A once a week **pill** by name Saheli developed by CDRI, Lucknow is a non-steroidal oral contraceptive preparation with very few side effects and high contraceptive value.

5 Contraceptive Injections, Drug releasing Implants, Vaginal rings and Skin patches

Progestogens alone or In combination with estrogen can also be used by females in the form of injections, implants, vaginal rings and skin patches. An injection of **depot medroxyprogesterone acetate** (DMPA) provides protection against pregnancy for three months. A contraceptive **implant** is a single rod about the size of a match stick. A health care provider inserts the implant under the skin with a special applicator. It prevents pregnancy for a period of three years. **Vaginal ring** is a flexible, plastic ring to be inserted into vagina. It releases small doses of estrogen and progestogen Which prevent conception. Small **skin patches** loaded with contraceptive hormones are worn on the skin. These patches slowly release **estrogen** and **progestogen** into blood stream to prevent pregnancy.

6 Surgical methods

Surgical procedure to prevent pregnancy is also known as **sterilization.** Sterilization procedure in the male is called **vasectomy** and that in the female, **tubectomy.**

i. **Vasectomy** : A small part of the vas deferens on either side is removed or tied up through a small incision on the scrotum. Thus the sperms are prevented from reaching seminal vesicle and so the 'semen' in 'vasectomised' males do not contain sperms.(Figure 5.11)

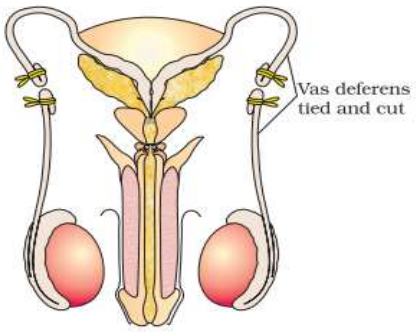


Figure 5.11 Vasectomy

ii. **Tubectomy:** A small part of the fallopian tube on both sides is removed or tied up through a small incision made in the abdomen or through vagina. This will block the entry of ova iftto the fallopian tubes and thus pregnancy is prevented.(Figure 5.12)

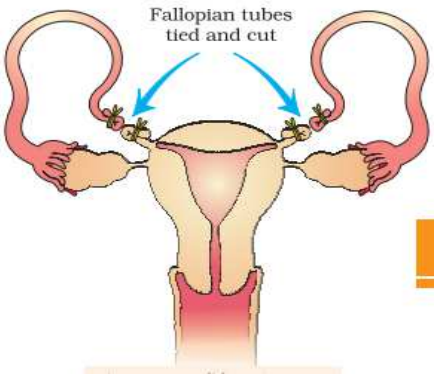


Figure 5.12 Tubectomy

Medical Termination of Pregnancy (MTP)

Intentional or voluntary termination of pregnancy before the full term of gestation is called **Medical termination of pregnancy** (MTP) or induced **abortion.** In this procedure pregnancy is terminated with the help of medications. Government of India made an act in 1971 legalizing MTP with certain restrictions and conditions to avoid its misuse. These restrictions are more important that the administrative machinery can keep a check on indiscriminate and illegal female foeticides. Legalizing MTP is still an issue of serious debate in many countries because of ethical, religious and social issues involved.

5.2.3 Assistant reproductive technology -Surrogacy

In the instances where the women have problems with conceiving or providing suitable environment for the development of the embryo in her uterus, **surrogacy** is suggested. The ovum of the wife/donor and the sperm of the husband/male donor are fertilized and the zygote is transferred into the womb of a **surrogate mother** (a woman who provides her uterus for the development of some other person's embryo and carries the baby until delivery).

GLOSSARY
Amenorrhea: absence of menstrual period/ menstruation in women of reproductive age.
Amniotic sac: it an extra embryonic sac filled with amniotic fluid.
Blastomere : Any of the cells resulting from cleavage of fertilized zygote during early embryonic development.
Coitus: sexual union between a male and a female involving insertion of penis into the vegina.
Foeticide: the destruction of foetus in the uterus.
Implantation: it is the process of early embryo becoming fixed in the wall of uterus.
In vitro: in an artificial environment outside the living organism's body.

In vivo: Within the living organisms.

Intra Uterine device (IUD): A small T- shaped device inserted into uterus to prevent pregnancy.

VERY SHORT ANSWER TYPE QUESTIONS

1. What are measures one has to take to prevent contracting STDs?
2. "It is true that MTP is not meant for population control". Then why did the Government of India legalized MTP?
3. Mention the advantage of lactational amenorrhea?

SHORT ANSWER TYPE QUESTIONS

1. Briefly describe the common sexually transmitted diseases in human beings?
2. Describe the surgical methods of contraception?

GENETICS

SUMMARY

Genetics is a branch of biology which deals with principles of inheritance and its practices. Progeny resembling the parents in morphological and physiological features has attracted the attention of many biologists. Mendel was the first to study this phenomenon systematically. While studying the pattern of inheritance in pea plants of contrasting characters, Mendel proposed the principles of inheritance, which are today referred to as 'Mendel's Laws of Inheritance'. He proposed that the 'factors' (later named as genes) regulating the characters are found in pairs known as alleles. He observed that the expression of the characters in the offspring follow a definite pattern in different–first generations (F1), second (F2) and so on. Some characters are dominant over others. The dominant characters are expressed when factors are in heterozygous condition (Law of Dominance). The recessive characters are only expressed in homozygous conditions. The characters never blend in heterozygous condition. A recessive character that was not expressed in heterozygous condition may expressed again when it becomes homozygous. Hence, characters segregate while formation of gametes (Law of Segregation). Not all characters show true dominance. Some characters show incomplete, and some show co-dominance. When Mendel studied the inheritance of two characters together, it was found that the factors independently assort and combine in all permutations and combinations (Law of Independent Assortment). Different combinations of gametes are theoretically represented in a square tabular form known as 'Punnett Square'. The factors (now known as gene) on chromosomes regulating the characters are called the genotype and the physical expression of the characters is called phenotype. After knowing that the genes are located on the chromosomes, a good correlation was drawn between Mendal's laws : segregation and assortment of chromosomes during meiosis. The Mendel's laws were extended in the form of 'Chromosomal Theory of Inheritance'. Later, it was found that Mendel's law of independent assortment does not hold true for the genes that were located on the same chromosomes. These genes were called as 'linked genes'. Closely located genes assorted together, and distantly located genes, due to recombination, assorted independently. Linkage maps, therefore, corresponded to arrangement of genes

on a chromosome. Many genes were linked to sexes also, and called as sex-linked genes. The two sexes (male and female) were found to have a set of chromosomes which were common, and another set which was different. The chromosomes which were different in two sexes were named as sex chromosomes. The remaining set was named as autosomes. In humans, a normal female has 22 pairs of autosomes and a pair of sex chromosomes (XX). A male has 22 pairs of autosomes and a pair of sex chromosome as XY. In chicken, sex chromosomes in male are ZZ, and in females are ZW. Mutation is defined as change in the genetic material. A point mutation is a change of a single base pair in DNA. Sickle-cell anemia is caused due to change of one base in the gene coding for beta-chain of hemoglobin. Inheritable mutations can be studied by generating a pedigree of a family. Some mutations involve changes in whole set of chromosomes (polyploidy) or change in a subset of chromosome number (aneuploidy). This helped in understanding the mutational basis of genetic disorders. Down's syndrome is due to trisomy of chromosome 21, where there is an extra copy of chromosome 21 and consequently the total number of chromosome becomes 47. In Turner's syndrome, one X chromosome is missing and the sex chromosome is as XO, and in Klinefelter's syndrome, the condition is XXY. These can be easily studied by analysis of Karyotypes.

6.1 Multiple alleles and human blood groups

Generally a gene has two alternative forms/versions called **alleles.** They are present at the same locus in a pair of homologous chromosomes. Two alleles of a gene can form three genotypes in a diploid organism. Sometimes a gene may have more than two alleles. When more than two allelic forms occur at the same locus on the homologous chromosomes of an organism, they are called **multiple alleles.** When more than two alleles exist in a population of a specific organism, the phenomenon is called **multiple allelism.** As mentioned above 'multiple alleles' cannot be observed in the genotype of a diploid individual, but can be observed in a population. The number of genotypes that can occur for multiple alleles is given by the expression **n (n+l)/2,** where, n = number of alleles. A well known example of multiple allelism in man is the expression of **ABO blood types** by three alleles of a single gene which can produce six genotypes.

ABO Blood Types

The ABO blood group system was proposed by **Karl Landsteiner.** He was awarded the Nobel Prize in Physiology or Medicine in 1930 for his work. The phenotypes (blood types) **A, B, AB** and **O types** are characterized by the presence or absence of 'antigens' on the plasma membrane of the RBCs. The A and B antigens are actually carbohydrate groups **(sugar polymers)** that are bound to lipid molecules (fatty acids) protruding from the membrane of the red blood cell. They are also called **isoagglutinogens** because they cause blood cell agglutination in the case of incompatible blood transfusions. 'Blood type A' persons have **antigen A** on their RBCs and **anti-B** antibodies in the plasma. 'Blood type B' persons have **antigen B** on their RBCs and **anti-A** antibodies in the plasma. 'Blood type AB' persons have antigens 'A' and 'B' on the RBCs and no antibodies in the plasma. 'Blood type O' persons have no antigens on their RBCs and both 'anti-A' and 'anti-B' antibodies are present in the plasma.

Bernstein discovered that these phenotypes were inherited by the interaction of three 'autosomal alleles' of the gene named **I,** located on **chromosome 9.** $\mathbf{I^A}$**,** $\mathbf{I^B}$ and **i** (or **1°)** are the three alleles of the gene **I.** The antibodies 'anti-A' and 'anti-B' are called **isoagglutinins** (also called **isohaemagglutinins)** which are usually **IgM** type. The isoagglutinins of an individual cause agglutination reactions with the antigens of another individual. The alleles I^A and I^B are responsible for the production of the respective antigens 'A' and 'B'. The allele i does not produce any antigen. The alleles I^A and I^B are dominant to the allele **i,** but **co-dominant** to each other **(**$\mathbf{I^A = I^B > i}$**).** A child receives one of the three alleles from each parent, giving rise to six possible genotypes and four possible blood types (phenotypes). The genotypes are The phenotypic expressions of and H are 'A' - type blood, the phenotypic expressions of $\mathbf{I^BI^B}$ and $\mathbf{I^Bi}$ are 'B'-type blood, and that of $\mathbf{I^AI^B}$ is 'AB'-type blood. The phenotype of **ii** (I°I°)is 'O'-type blood.

Table 1: Genetic control of the human ABO blood grouping

Genotype	Antigens present on red blood cells	ABO blood group phenotype	Antibodies present in blood plasma	Blood types that can be tolerated	Blood types that can accept blood for transfusion
I^AI^A	A	Type A	Anti-B	A & O	A & AB
I^Ai	A	Type A	Anti-B	A & O	A & AB
I^BI^B	B	Type B	Anti-A	B & O	B & AB
I^Bi	B	Type B	Anti-A	B & O	B & AB
I^AI^B	A & B	Type AB	Neither anti-A nor anti-B	A,B, AB & O	AB only
ii/I^OI^O	Neither A nor B	Type O	Anti-A & anti-B	O only	A,B, AB & O

Blood Typing

The ABO phenotype of any individual is ascertained by mixing a blood sample with an **antiserum** containing 'anti-A' or 'anti-B' antibodies. If a clump is formed with 'anti-A' serum, the type of blood can be 'A' or 'AB'. If a clump is formed with 'anti-B' serum, the type of blood can be 'B' or 'AB'. If a clump is formed with both 'anti-A' and 'anti-B' antibodies, the type of blood is 'AB' and if no clump is produced with either of the antibodies, the type of blood is 'O'.

Importance of ABO groups in blood transfusion

While transfusing blood, the type/types of antigens of the donor and the type/types of antibodies of the recipient are to be taken into consideration. The antibodies of the donor and the antigens of the recipient are relatively of less importance. The RBCs of an 'O group' have no antigens and so agglutination does not occur with any other group of blood. So, persons with 'O group blood' are called **universal donors.** The plasma of persons with 'AB group' blood has no antagonistic antibodies. This does not cause agglutination of RBC received from persons of other groups of blood. So people with 'AB group' blood are called **Universal recipients.** Can you think of how an AB group person can be considered a 'universal donor' with reference to transfusion of plasma?

NOTE : Type AB^+ is the **universal recipient.** Although those with AB blood type may be referred to as universal recipients, in actuality, type **AB^+** blood is that of the universal recipient, whereas type **AB** is not. This is an important distinction to make. Because **A', A^+, B", B^+, AB , AB^+, O"** and **0^+** individuals can all receive blood from donors of type **O"** blood, an individual with **type O"** blood is deemed a **universal donor.**

O^+ is not the universal donor blood type.

Rh Blood Types

Karl Landsteiner and **Alexander S. Wiener** discovered another antigen called **Rh-antigen** or **Rh factor** on the surface of RBCs of **Rhesus monkeys** *(Macaca mulatto)* and later in human beings. But the term 'Rh factor' strictly refers only to the most immunogenic **D antigen** of the Rh-blood group system. The persons having D antigen are called 'Rh positive (Rh^+)' and those without D

antigen are called Rh negative (Rh). Rhesus factor in the blood is an inherited dominant trait. **Anti-D** antibodies are absent in the plasma of any person normally (naturally). However, if an 'Rh negative' person is exposed to Rh positive blood cells (erythrocytes) for the first time, 'anti-D antibodies' are formed in that person's blood. On the other hand, an Rh positive person can receive Rh negative blood without any consequent effects/complications.

Genetic control of Rh system *Fisher and Race hypothesis*

Unlike the A-B-O blood types where all the alleles occur on one pair of loci on chromosome pair 9, the Rh factor involves three different pairs of alleles located on three different loci on **chromosome pair 1.** The Fisher-Race system, which is more commonly in use today, uses the **CDE nomenclature.**

In the above figure, 3 pairs of Rh alleles (Cc, Dd and Ee) occur at 3 different loci on homologous chromosome pair-1. The possible genotypes will have one C or c, one D or d, and one E or e from each chromosome. For example: **CDE/cde; CdE/cDe; cde/cde; CDe/CdE,** etc. All genotypes carrying a dominant 'D allele' will produce 'Rh-positive' phenotype and double recessive genotype 'dd' will give rise to 'Rh-negative' phenotype.

Wiener hypothesis

Wiener proposed the existence of eight alleles **(R^1, R^2, R°, R*, r, r\ r*\ r^y)** at a single Rh locus. All genotypes carrying a dominant 'R allele' **(R^1, R^2, R°, R^2)** will produce 'Rh-positive' phenotype and double recessive genotypes rr (or **rr\ rr", rr^y)** will give rise to 'Rh-negative' phenotype.

Erythroblastosis foetalis

Erythroblastosis foetalis/Haemolytic Disease of the Newbom/Haemolytic disease of the foetus and newborn (HDN/HDFN) is an alloimmune condition that develops in an Rh positive foetus whose father is Rh positive and mother is Rh negative. The genetic consequence in this marriage is the **Rh incompatibility** between the mother (Rh") and the growing foetus (Rh^{+}). The foetal blood cells may pass through the ruptured placenta at birth into the Rh nagative metemal blood. The mother's immune system recognizes the Rh antigens and gets sensitized. The sensitized immune system in the mother produces Rh antibodies. The majority of Rh antibodies are of the **IgG** type which are small in size. So, they can pass through the placental barrier and enter the foetal blood circulation. Generally the first child of this 'genetically incompatible marriage' is unaffected because foetus is delivered by the time the mother gets sensitized and antibodies accumulate and cross through the placenta into the foetus. During the second pregnancy, if the second child is Rh positive, these antibodies cross the placental border and enter the foetal blood circulation. The blood cells of the Rh positive foetus are destroyed, causing HDN.

Haemolytic anaemia and **jaundice** are the symptoms of this disorder in the affected neonatal babies. To compensate the haemolysis of more and more number of RBCs, there is a rapid production of RBCs, not only from the bone marrow, but also from spleen and liver. Now, many large and immature cells in **erythroblast stage** are released into circulation. Because of this, the disease is called **erythroblastosis foetalis.**

6.2 Sex Determination

The-mechanism of sex determination has always been a puzzle to geneticists. In fact, the cytological observations made in a number of insects led to the development of the concept of genetic/chromosomal basis of sex determination.

Sex Chromosomes

In most of the animals a pair of chromosomes is responsible for the determination of sex. These two chromosomes are called **sex chromosomes** or **allosomes.** The chromosomes other than the sex chromosomes are called **autosomes.** The first indication that sex chromosomes were distinct from the other chromosomes came from the experiments conducted by **Henking.** He could trace a specific nuclear

structure all through spermatogenesis in wasps, and it was also observed by him that 50 percent of the spermatozoa received this structure after spermatogenesis, whereas the other 50 percent did not receive it. Henking gave the name **X-body** to this structure, but he could not explain its significance. Further investigations by other scientists led to the conclusion that the X-body of Henking was in fact a chromosome and that is why it was given the name **X-chromosome. Stevens** and **Wilson** first identified **Y-chromosome** as a in the **mealworm,** *Tenebrio molitor*. They revealed that the chromosomal basis of sex depended on the presence or absence of the Y-chromosome.

NOTE : if the two sex chromosomes are similar *(XX),* the individual is described as **homogametic.** Gametes produced from it are similar. If the two sex chromosomes are different **(XY)** or it contains only one sex chromosome **(XO),** the individual is described as **heterogametic.**

Gametes formed from it are dissimilar. The reproductive organs that **produce** gametes form the 'primary sexual characters'. The various explanations given for sex determination are chiefly - **Chromosomal theory** of sex determination, **ii. Genic balance theory** of sex determination and **iii. Haplodipoidy method**

Heterogametic Sex Determination

Heterogametic sex refers to the sex of a species in which the sex chromosomes are not similar. The process of sex determination by allosomes is called **genetic** or **chromosomal sex determination-** In the heterogametic sex determination, one of the sexes produces 'similar' gametes and the other sex (heterogametic sex) produces dissimilar/unlike gametes. The sex of the young one is determined at the time of **syngamy (fertilization)-** It depends on which gamete of the two dissimilar gametes unites with the other gamete produced by the 'homogametic parent'.

Male Heterogamety

In this method of sex determination, the males (heterogametic) produce dissimilar gametes while females (homogametic) produce similar gametes. Male

heterogamety is of two kinds, **XX - XO type** and **XX - XY type**

XX - XO type

In some insects such as bugs, grasshoppers and cockroaches, females are with two X-chromosomes and males are with one X-chromosome in each somatic cell. **McClung** discovered this type in grasshoppers. The unpaired X-chromosome determines the male sex. The karyotype of the female (homogametic) is **AAXX** and that of the male (heterogametic) is **AAXO-** All the ova contain 'AX' complement of chromosomes and the sperms are of two types. One half of the sperms have 'AX' complement and the other half have 'A' complement of chromosomes. The sex of the offspring depends on the type of sperm that fertilizes the ovum. (Figure 6.1)

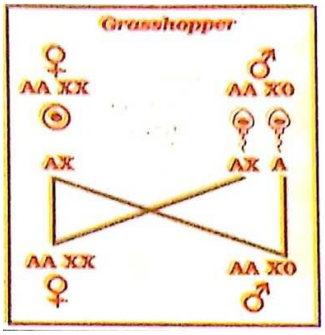


Figure 6.1 Sex determination in Gasshoopper

XX - XY type

In human beings and some insects such as *Drosophila,* both females and males have the same number of chromosomes. The karyotype of the female is **AAXX** and that of the male is **AAXY.** Females are 'homogametic' with 'XX' chromosomes. (Figure 6.2)

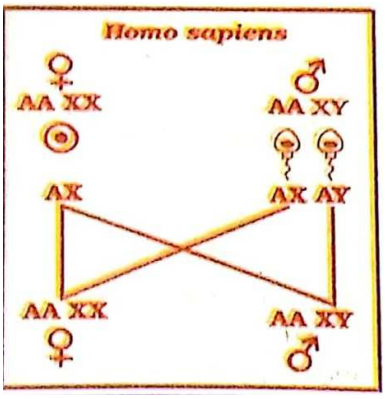


Figure 6.2 Sex determination in Human

They produce similar ova having one X-chromosome each. Males are 'heterogametic' with X and Y-chromosomes. They produce two kinds of sperms; one half of them with X-chromosome and the other half with Y-chromosome. The sex of the offspring depends on the fertilizing sperm. The XX - XY type is also found in most other mammals •

Female Heterogamety

In this method of sex determination, the males produce 'similar gametes' while females produce 'dissimilar gametes'. Female heterogamety is of two kinds, **ZO - ZZ type** and **ZW - ZZ type.**

ZO - ZZ type

In moths and some butterflies, female is heterogametic with one Z-chromosome (ZO) and male is homogametic with two Z-chromosomes (ZZ). The karyotype of female is **AAZO** and mal^ is **AAZZ.** Females produce two kinds of ova, half of them with a Z-chromosome and the other half with no sex chromosome. Males produce similar type of sperms. The sex of the offspring depends on the type of ovum that is fertilized. (Figure 6.3)

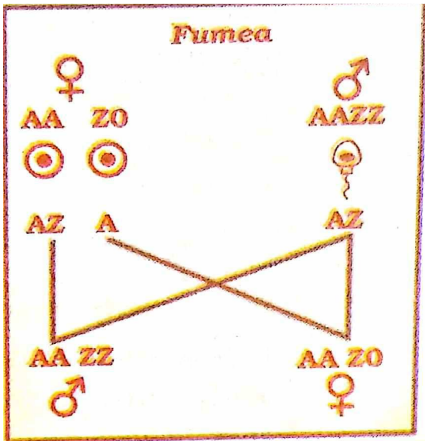


Figure 6.3 sex determinations in Fumea

ZW - ZZ type

In birds, reptiles, some fishes, etc., the females are heterogametic with **ZW-** allosomes and males are homogametic with **ZZ** - allosomes. The karyotype of the female is **AAZW** and that of the male is **AAZZ.** All sperms are similar with the **allosome- Z.** Ova are of two different kinds; one half of the ova are with the **allosome- Z** and the other half with the **allosome-W.** The sex of the offspring depends on the type of ovum that is fertilized.(Figure 6.4)

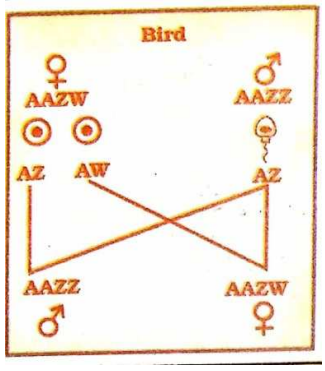


Figure 6.4 Sex determination in Bird

Sex Determination in Humans

It has already been mentioned that the sex determining mechanism in case of humans is **XX - XY type.** Out of 23 pairs of chromosomes present, 22 pairs are

exactly same in both males and females; these are the autosomes. A pair of X-chromosomes is present in the female, whereas the presence of an X and Y-chromosome are determinant of the male characteristic. During spermatogenesis among males, two types of gametes are produced. 50 percent of the total sperm produced carry the X- chromosome and the rest 50 percent has Y-chromosome besides the autosomes. Females, however, produce only one type of ovum with an X- ' chromosome. There is an equal probability of fertilisation of the ovum by the sperm carrying either X or Y-chromosome. In case the ovum is fertilised by a sperm carrying X-chromosome, the zygote develops into a female and the j fertilisation of ovum with Y-chromosome carrying sperm results into a male offspring. Thus, it is evident that it is the genetic makeup of the sperm that determines the sex of the child. It is also evident that in each pregnancy there is always 50 per cent probability of either a male or a female child.

Genic Balance Theory

By studying the Chromosomal theory of sex determination, it may appear at first glance that some genes carried on the sex chromosomes, for example, X and Y are entirely responsible for determining the sex of the offspring. But this is not always true.

The **Genic Balance Theory** of determination of sex was proposed by **Bridges** while working on *Drosophila*. This theory was devised to explain the mechanics of sex determination in *Drosophila melanogaster*. According to his concept, the sex of an individual is determined by the 'balance' between the genes for femaleness located on the X-chromosome and those for maleness located on the 'autosomes'. Hence, the sex of an individual is determined by the ratio of number of its X chromosomes and that of its autosomal sets, the 'Y' chromosome taking no part in the determination of the sex. The ratio is termed as **sex index** and is expressed as follows.

Sex index = Number of X chromosomes / Number of sets of autosomes (X/A)

Bridges studied the offspring resulting from the non-disjunction of X-chromosomes during meiosis in females. **Non-disjunction** (not coming apart) is the failure of paired chromosomes to segregate or separate during the anaphase stage of the first or second meiotic divisions. The result is the production of aberrant gametes with abnormal numbers of chromosomes. There were various types of gametes such as the ones which contained one extra X-chromosome **(AXX)** and some others contained one chromosome less **(AO).** Syngamy of such aberrant gametes and normal gametes produces zygotes with **aneuploid** karyotypes such as **2n+l, 2n-l** etc. AAXO male is produced when an unusual ovum (AO) in the female is fertilized by a sperm with AX chromosome complement.An 'AAXXY female' is produced when an unusual ovum with AXX and a sperm with AY fuse.

Table 2: Chromosomal complements and Sexual phenotypes in Drosophila

Sex Chromosome Complement	Haploid Sets of Autosomes	X : A Ratio	Sexual Phenotype
XX	AA	1.0	Female
XY	AA	0.5	Male
XO	AA	0.5	Male
XXY	AA	1.0	Female
XXX	AA	1.5	Metafemale
XXXY	AA	1.5	Metafemale
XX	AAA	0.67	Intersex
XO	AAA	0.33	Metamale
XXXX	AAA	1.3	Metafemale

Bridges found that the 'AAXXY flies' were 'fertile , females' and 'AAXO llies' were 'sterile males'. It is important to note that the ^presence of Y chromosome i in the 'AAXXY flies' did not cause maleness, and its absence in the 'AAXO flies' did not produce femaleness.

When Bridges crossed triploid females **(AAAXXX)** with normal diploid males **(AAXY),** he obtained normal diploid females, males, triploid females, intersexes, super males and super females. The occurrence of triploid intersexes from such a cross clearly established that autosomes also carry genes for sex determination.Bridges realized that the critical factor in determining sex is the

ratio of X chromosomes to the number of haploid sets of autosomes (A) present. He concluded that Y-chromosome in *Drosophila* lacks male determining factor, **TDF** (encoded by the **SRY gene - Sex-determining Region Y).** However, the [Y-chromosome of *Drosophila* is required for **male fertility.** In 'XO males', r sperms develop but are non-motile.

NOTE : Females have a ratio equal to **1.0.** If the ratio is **>1.0,** such females are called **super females/meta females,** and they are 'infertile'. Normal males have a ratio of X-chromosomes to sets of autosomes of **0.5.** When the ratio is < **0.5** the males are called **super males/** *i* **meta males,** and they are infertile. If the ratio is **between 0.5 and 1.0, the** flies are called **intersexes,** which are larger, with morphological abnormalities and rudimentary bisexual gonads. The investigations on ^{v} *Drosophila* by **Bridges** showed that female determiners are located on **the** X-chromosomes and the male determiners are on the **autosomes.**

Barr Bodies

In mammals, males are heterogametic (XY) and females, homogametic (XX). As the female has two copies of the X chromosome, **Dosage Compensation** Is achieved by the random inactivation of one of the two 'X' chromosomes. The extra X-chromosome undergoes **heterochromatinisation** and becomes inactive during early embryonic development. The descendant cells inherit the same inactivated X chromosome. The heterochromatinized X-chromosome appears as a darkly-staining body attached to the nuclear membrane. This phenomenon was first described by **Murray L. Barr** and the **heterochromatinised X chromosomes** are now called **Barr Bodies.**

Barr and **Bertram** observed chromatin bodies in the inter phase nerve cells of female cats that were not present in cells of the male. In humans, this body can be easily demonstrated in female cells derived from the buckle mucosa (cheek cells) or in fibroblasts (undifferentiated connective tissue cells), but not in similar male cells. A Barr body appears as a small drumstick like projection (drumstick appendage) on one of the lobes of some neutrophils in females . With this

technique, the sex of human embryos can be distinguished at early stages of development. This cellular characteristic generally seems to apply to all mammals.

X-inactivation (also called **Lyonisation,** proposed by **Mary Lyon** and **Liane Russell)** is a process by which one of the two copies of the X-chromosome present in the body cells of female mammals is inactivated. The inactive X- chromosome is 'silenced' by making it a **transcriptionally inactive** structure called **heterochromatic body.** As female mammals have two X-chromosomes in each cell, X-inactivation in their cells causes them not to have twice as many products of the X-chromosomal genes as those of the males which possess a single copy of the X-chromosome in each cell **(Dosage Compensation).**

> **NOTE** : Regardless of how many X-chromosomes a somatic cell possesses, all but one of them appears to be inactivated and can be seen as **Barr bodies**. For example, no Barr body is seen in the somatic cells of Turner females 45, XO; one is seen in Klinefelter males 47,XXY; two in 47,XXX females; three in 48,XXXX females; and so on. Therefore, the number of Barr bodies follows an **N-l rule (N minus one rule),** where **N** is the total number of X-chromosomes present.

Haplodiploidy in honeybees

Haplodiploidy is a mechanism of sex determination that is common in the hymenopteran insects such as honey bees, ants, and wasps. In this system, the sex of the offspring is determined by the number of sets of chromosomes, it receives. Fertilized eggs develop into females, and unfertilized eggs develop **(parthenogenesis)** into males (drones). This means that the males have half the number of chromosomes (haploid) and the females have 'double' the number (diploid), hence the name 'haplodiploidy' for this system of sex determination.

In *Apis mellifera*, drones are haploid with 16 chromosomes, queen and worker bees are diploid with 32 chromosomes in each somatic cell. Males produce sperms by mitosis. As per this type, males have no father and they cannot have sons, but they do have grandfathers and grandsons. Female that feeds on royal jelly becomes the fertile queen. Other females that feed on honey and pollen become

sterile females called worker bees. If a queen bee mates with one drone, her daughters share *3/4* of their genes with each other and not ½ as in the XY and ZW systems.

6.3 Genetic disorders

A number of disorders in human beings have been found to be associated with the inheritance of changed or altered genes or chromosomes. Genetic disorders may broadly be grouped into two categories - **Mendelian disorders** and **Chromosomal disorders.**

Mendelian disorders

Mendelian disorders are genetic diseases showing **Mendelian pattern of inheritance,** caused by a single mutation in the structure of DNA, which causes a single basic defect with pathologic consequences, in some cases. Mendelian disorders are also called **monogenic diseases.** Monogenic diseases run in families and can be dominant or recessive and autosomal or sex linked (allosomic). The pattern of inheritance of Mendelian disorders can be traced in a family with the help of pedigree charts and their analyses.(Figure 6.5)

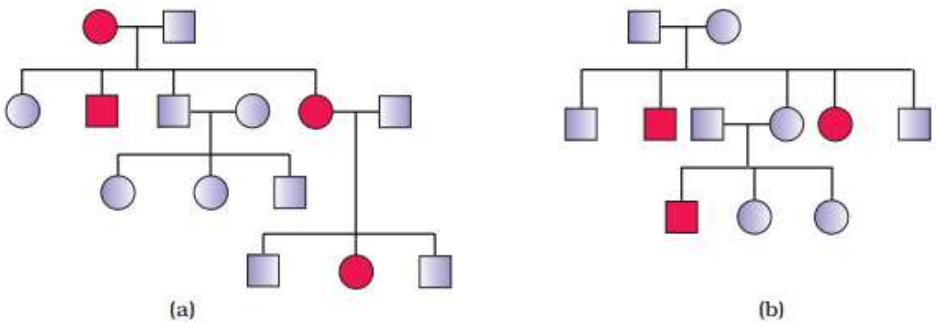


Figure 6.5 Pedigree chart (a) Autosomal dominant trait (b) Autosomal recessive trait

The most common and prevalent Mendelian disorders are Haemophilia, Cystic fibrosis, Sickle-cell anaemia, Colour blindness, Phenylketonuria, Thalassemia, DMD, Albinism, etc.

Haemophilia

Haemophilia A (caused by deficiency of clotting factor VIII) and **Haemophilia B** (caused by deficiency of clotting factor IX) are X-linked recessive disorders that impair the body's ability to control clotting or coagulation of blood. **Haemophilia C** is an autosomal recessive disorder involving lack of the functional clotting 'factor XI'. Haemophilia is also called **bleeder's disease.** Haemophilia A and B follow the characteristic **crisscross pattern of inheritance** like that of colour-blindness. In this disease, a single protein that is a part of the

cascade of reactions involved in the clotting of blood is affected. Haemophilia is more likely to occur in males than in females. This is because females have two X-chromosomes while males have only one, and so the defective gene on the X will certainly express in the male who carries it. As it is caused by a recessive allele on the X chromosome, a female human being has to be 'double recessive' to express haemophilia. Because the chance of a female having two defective copies of the gene (alleles) is very remote, the females are mostly **asymptomatic carriers** of the disorder. The 'allele' is typically passed on from an affected father to 50% of his grand sons through his 'carrier daughters'. The family pedigree of **Queen Victoria** shows a number of haemophilic descendents, as she was a carrier for the disease.

Sickle-cell anaemia

Sickle-cell anaemia is an autosomal recessive genetic blood disorder characterized by red blood cells that assume an abnormal, rigid, sickle shape in hypoxia conditions (at high altitudes or under physical stress, for instance). Sickled cells may clump and clog small blood vessels, often leading to other symptoms throughout the body, including physical weakness, pain, organ damage, and even paralysis.

This disease is controlled by a single pair of alleles, Hb^A and Hb^s found on the chromosome 11. The homozygous individuals for sickle-cell anaemia **(Hb^8 Hb^8)** express the diseased phenotype. Heterozygous individuals **(Hb^A Hb^8)** appear 'unaffected' but they are still, carriers of the disease. Even though two sickle cell alleles are necessary to cause sickle cell anaemia, one dose can affect the phenotype. Persons 'heterozygous' to sickle cell trait can usually lead a healthy life but in prolonged periods of reduced oxygen content in the blood may suffer from symptoms of SCD as both normal and sickle cell haemoglobin are formed in them.(Figure 6.6)

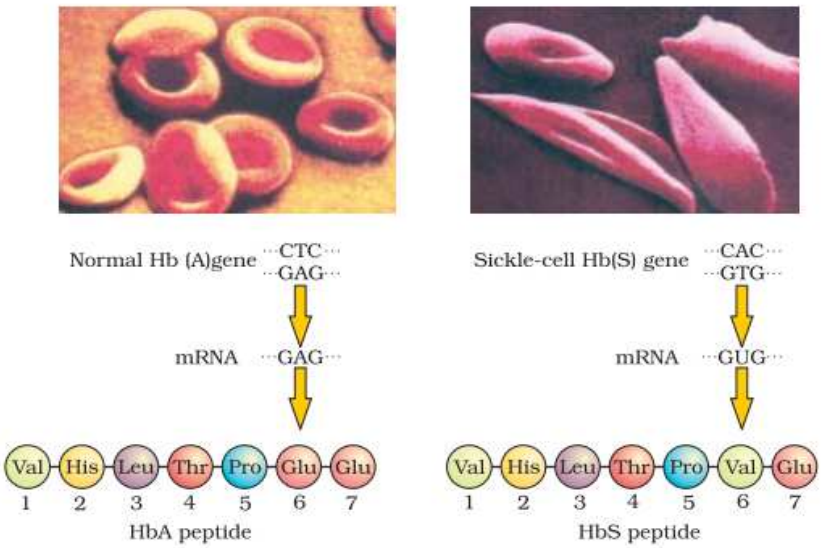


Figure 6.6 Micrograph of the red blood cells and the amino acid composition of the relavent portion of ß-chain of haemoglobin (a) from a normal individual and (b) from an individual with Sickle cell anaemia

Sickle cell anaemia is caused by a **point mutation** in the DNA that codes for the beta globin polypeptide chains of the haemoglobin molecule, causing the replacement of the **glutamic acid** in the sixth position by **valine.** The heterozygous individuals are relatively resistant to the most severe effects of malaria such as those of 'falciparum malaria* also (although they are not resistant to malaria infection) - an effect called **heterozygote advantage.** The heterozygous individuals carry the deleterious alleles in their genomes **(genetic load).**

Phenylketonuria [PKU]

Phenylketonuria was discovered by **A. Foiling.** This is an autosomal recessive, metabolic genetic disorder caused by a mutation in the gene **(PAH- Phenylalanine hydroxylase gene)** located on the chromosome 12 for the hepatic enzyme 'phenylalanine hydroxylase', rendering it nonfunctional. The affected individual lacks the above mentioned enzyme that converts the amino acid **phenylalanine** into **tyrosine.** When phenylalanine hydroxylase's activity is reduced, phenylalanine accumulates and is converted into **phenylpyruvate** and other derivatives. Accumulation of these substances in the brain causes mental retardation. Adherence to a low phenylalanine diet prevents major mental retardation.

Colour blindness

Colour blindness (colour vision deficiency) is a sex-linked recessive disorder. It is the inability or decreased ability to see certain colours or perceive differences between some colours. This phenotypic trait is due to mutation in certain genes located on X-chromosome.The most common inherited forms of colour blindness are Protanopia (red colour bindness), Deuteranopia (green colour blindness) and Tritanopia (blue colour blindness - autosomal).

The son of a woman who carries the allele has a 50 percent chance of being colour-blind. The mother herself is not colour-blind because the allele is recessive. That means its effect is suppressed by her matching dominant normal allele. A daughter will not normally be colour blind, unless her mother is 'colour-blind' or a 'carrier' and her father is colour-blind. The Ishihara colour test, which consists of a series of pictures coloured spots, is most often used to diagnose red-green colour blindness.

Chromosomal disorders

Chromosomal disorders are caused by errors in the 'number' or 'structure' of chromosomes. Chromosomal anomalies usually occur when there is an error in cell division. Aneuploidy is a chromosomal aberration where there is a gain or loss of one or more chromosomes in a 'set'. It is caused by non- disjunction of chromosomes- The result of this error is origin of cells with a deviation from the normal number of chromosomes - aneuploidy.

NOTE : Monosomy (2n-l) is a chromosomal aberration where one chromosome is lost from a pair. Monosomy 23 (loss of an X chromosome) causes a syndrome called Turner's syndrome in human females. Trisomy (2n+l) is a chromosomal aberration where one chromosome is added to the already existing homologous pair. When a chromosome is added to the 21st pair of autosomes, it is called trisomy 21. Trisomy 21 in man causes a syndrome called Down syndrome. Trisomy of sex chromosomes is due to the addition of one sex chromosome. It causes a syndrome called Klinefelter's syndrome.

A. ***Allosomal disorders***

Klinefelter's syndrome

This genetic disorder is caused by trisomy 23rd pair. The karyotype is 47, XXY. A Klinefelter male possesses an additional X-Chromosome along with the normal XY. The principal effects include hypogonadism and reduced fertility. At the same time, feminine sexual development is not entirely suppressed. Slight enlargement of the breasts (gynecomastia) is common, and the hips are often rounded. The somatic cells of a Klinefelter male exhibit Barr bodies in their nuclei.

> *NOTE :* Karyotype *47,* XXY. Note the convention used in designating these chromosome compositions: the number states the total number of chromosomes present, and the symbols after the comma indicate the deviation from the normal diploid number.

Turner's syndrome

The Karyotype is 45, X. It is due to monosomy 23rd pair, where one X- chromosome is lost. Turner female does not show Barr bodies in her somatic cells. The symptoms are short stature, gonadal dysgenesis, webbed neck and broad shield like chest with widely spaced nipples.

B. ***Autosomal disorders***

Most of the following disorders are common in children bom to woman who conceive babies rather late in their reproductive phase.

Down syndrome (Trisomy 21)

Down syndrome is a genetic condition that causes delays in physical and intellectual development. The cause of this genetic disorder is the presence of an additional copy of the chromosome numbered 21 (trisomy of 21st set). The karyotype is designated as Trisomy 21 (47, XX, +21). The affected individual is short statured with small round head, furrowed tongue and partially open mouth. Physical, psychomotor and mental development is retarded.

Edwads syndrome (Trisomy 18)

Edwards syndrome (47, XX, +18) is a chromosomal abnormality characterized by the presence of an extra copy of the genetic material on the 18th chromosome, either in whole (trisomy 18) or in part (such as due to translocations). Edwards syndrome occurs in all human populations but is more prevalent in the female offspring. The majority of people with the syndrome die during the foetal stage; infants who survive experience serious defects (cardiac abnormalities and kidney malfunction) and commonly live for short periods of time.

Patau syndrome (Trisomy 13)

Patau syndrome, is a chromosomal condition associated with severe intellectual disability and physical abnormalities in many parts of the body. Most cases of trisomy 13 (47, XX, +13) result from having three copies of chromosome 13 in each cell in the body instead of the usual two copies. Individuals with trisomy 13 often have heart and kidney defects, brain or spinal cord abnormalities, very small or poorly developed eyes (microphthalmia), cleft palate etc. Due to the presence of several life- threatening medical problems, many infants with trisomy 13 die within their first days or weeks of life.

Cri-du-Chat syndrome (5p minus syndrome)

Cri-du-chat syndrome (cat-cry) is due to a partial deletion of the short arm of chromosome 5, also called 5p monosomy. It might be considered a case of partial monosomy, but since the region that is missing is so small, it is better referred to as 5p segmental deletion. The karyotype is 46, XX, 5p". It is a French term referring to the characteristic cat-like cry of the affected children due to problems with the larynx and nervous system. Such infants are mentally retarded, have a small head with unusual facial features. They die in infancy or early childhood.

Chronic Myelogenous (Myeloid) Leukemia (CML)

In certain cancers such as Chronic myelogenous leukemia (also called Chronic granulocytic leukemia), a piece of the chromosome 9 and a piece of the chromosome 22 break off and ‘switch places’ (exchange places) with each other (reciprocal translocation)- This results in the formation of an abnormally short chromosome 22 and abnormally long chromosome 9. The short 22nd chromosome is called Philadelphia chromosome produced by translocation

which is also called Philadelphia translocation. The karyotype is 46, XX, t(9 ; 22). The Philadelphia chromosome results in the production of an abnormal enzyme called a tyrosine kinase. Along with other abnormalities, this enzyme causes uncontrolled cell cycle progression leading to the cancer called chronic myelogefious leukemia.

GLOSSARY

Agglutinogens : An antigen that stimulates the production of a pertcular agglutinin, such as an antibody.

Aneuploid: Having a chromosome number that is not an exact multiple of haploid number of the species.

Antiserum: It is a blood serum containing antibodies against a specific antigen, used to treat or provide immunity to a disease .

Erythroblast: An immature form of red blood cell, normally found in bone marrow.

Haemolytic anaemia: It is a form of anaemia due to heamolysis, the abnormal breakdown of RBC.

Isoagglutinin: An isoantibody normally present in the serum of an individual that causes the agglutination of RBC of another individual of the same species.

VERY SHORT ANSWER TYPE QUESTIONS

1 What are multiple alleles?
2 What are the antigens causing 'ABO' blood grouping in humans?
3 What is erythroblastosis foetalis?
4 What are Barr bodies?
5 What is Klinfelter's syndrome?
6 What is Down syndrome ?
7 What is Turner's syndrome?

SHORT ANSWER TYPE QUESTIONS

1 How is sex-determined in human beings?
2 Describe erythroblasosis foetalis?
3 Describe the genetic basis of ABO blood grouping?
4 Mention any two autosomal genetic disorders with their symptoms?

Organic Evolution

SUMMARY

The origin of life on earth can be understood only against the background of origin of universe especially earth. Most scientists believe chemical evolution, i.e., formation of bio-molecules preceded the appearance of the first cellular forms of life. The subsequent events as to what happened to the first form of life are a conjectured story based on Darwinian ideas of organic evolution by natural selection. Diversity of life forms on earth has been changing over millions of years. It is generally believed that variations in a population result in variable fitness. Other phenomena like habitat fragmentation and genetic drift may accentuate these variations leading to appearance of new species and hence evolution. Homology is accounted for by the idea of branching descent. Study of comparative anatomy, fossils and comparative biochemistry provides evidence for evolution. Among the stories of evolution of individual species, the story of evolution of modern man is most interesting and appears to parallel evolution of human brain and language.

7.1.1 Introduction of Origin of life

Evolution is the branch of biology that deals with the origin of life and the diversity of organisms on the earth through ages. The literal meaning of evolution is unfolding or rolling out. The word 'organic evolution' was coined by Herbert Spencer. Evolution is a continuous process of development of more complex organization from simple level. According to Dobzhansky "Nothing in Biology makes sense except in the light of Evolution".

Life had a beginning. When did life begin on the earth? What is the mechanism involved in the origin of the life? Since the origin of living organisms, did they change through time? - are some of the fascinating questions that confront our minds. Many theories have been proposed to explain the origin of life. But most of them are of only historical importance. Some of them are discussed here.

I. Theory of Special creation: It is purely a mythological belief. This theory states that living organisms on the earth were created by a Divine Power.

II. Cosmozoic theory or Panspermia: According to this theory, life might have existed all over the universe (cosmos) in the form of resistant spores called cosmozoa or panspermia. They might have reached the earth accidentally.

III. Theory of Spontaneous generation or Abiogenesis: According to this theory, life originated from non-living or decaying and rotting substances like manure, dew, etc. Aristotle, Thales, Plato and Von Helmont believed in this idea of abiogenesis.

IV. Theory of Catastrophism: This theory was proposed by George Cuvier.

According to this theory, earth was subjected to periodic catastrophes. At the time of a catastrophe all the existing organisms would die and new organisms would be created after every catastrophe.

V. Theory of Biogenesis: According to this theory, living organisms originate from pre-existing organisms. Louis Pasteur confirmed this theory by his swan-neck flask experiment.

VI. Theory of Origin of life or Coacervate theory: This theory was proposed by A.I. Oparin of Russia and supported by J.B.S. Haldane of England. According to this theory, primitive organisms evolved spontaneously from the inorganic substances as a result of physical forces such as lightning, ultraviolet radiation, volcanic activities, etc. Thus origin of life is a phenomenon of 'chemical evolution' that led to 'biological evolution'.

Chemical evolution: This includes the origin of the earth and the primitive atmosphere, formation of early simple organic molecules which later formed the complex organic molecules. The earth originated approximately 4.5 to 5 billion years ago. At that time, the temperature of the earth was estimated to be around 5000°C to 6000°C and it slowly cooled down over millions of years. During this process, light elements such as helium, hydrogen, nitrogen, carbon, etc., flowed to the surface and formed the primitive atmosphere. This was hot with abundant hydrogen and absence of free oxygen. Thus, it was a reducing atmosphere. The elements combined and formed compounds such as ammonia, methane, etc. The water vapour in the atmosphere condensed into droplets of rain, which ran over the land as streams, rivers and finally collected to form oceans. Ammonia, methane, etc., were

washed down to the oceans along with the rain water. Mineral-rocks were also dissolved leading to the accumulation of minerals in the sea water. Highly active radicals such as $CH^{\cdot}$ and $CH_2^{\cdot}$ are condensed to form a variety of hydrocarbons. They reacted with ammonia and water to produce various simple organic molecules like sugars, amino acids, fatty acids, purines and pyrimidines which later formed nucleosides and nucleotides- All these reactions occurred in the ocean, which was described as the hot dilute soup or prebiotic soup by J.B.S. Haldane- In the prebiotic soup, these simple organic molecules produced complex polymers like polysaccharides, proteins, fats and nucleic acids- Nucleic acids and proteins combined to form nucleoproteins.

7.2 Biological evolution

This includes (i) the formation of protobionts, (ii) the origin of living organisms from them and their diversification.

(i) **The formation of Protobionts**: From the complex organic molecules, large colloidal aggregates called coacervates (babble like droplets) were synthesized due to intermolecular attractions. Some type of chemical] organisers (considered free genes) developed to give these droplets 'the ability to take-in molecules from the surroundings'. Later they acquired] lipid membranes. Some of the proteins inside them acquired the properties of enzymes resulting in the fast multiplication of molecules.

(ii) **The origin of living organisms**: The 'Free Genes' started absorbing organic molecules from the prebiotic soup and evolved into anaerobic heterotrophs approximately three to four billion years ago. They obtained their energy by the fermentation of some organic molecules. The earliest living organisms had clumps of nucleoproteins containing one or two DNA molecules and were similar to the present day prokaryotes. During the course of evolution these early prokaryotes acquired the carbohydrate-synthesis catalysing enzymes. Thus early chemo- autotrophic organisms (e.g. iron and sulphur bacteria) which can thrive at high temperatures evolved. These organisms could take carbon dioxide into their bodies and used the chemical energy to create carbohydrates and sugars. Meanwhile some bacteria synthesised bacterial chlorophyll (e.g. purple and green sulphur bacteria) from the magnesium porphyrin of ocean waters. This pigment trapped the - solar energy and fixed the CO_2. These were

the anoxygenic photo- autotrophic organisms. Later the bacterial chlorophyll evolved into true chlorophyll as in cyanobacteria and plants. As a result, oxygenic photo-autotrophic organisms (like blue green algae) evolved. It resulted in the increase of large quantity of oxygen in the atmosphere about two billion years ago. These events transformed the reducing atmosphere into modem oxidising atmosphere. With the availability of free oxygen, finally aerobic mode of respiration evolved. Later, eukaryotes evolved probably by two processes, a) Prokaryotes lived in the ancestral eukaryotes symbiotically and evolved into organelles such as mitochondria and plastids. b) The endomembrane system of eukaryotes might have evolved by the infolding of plasma membrane of the ancestral prokaryotes.

Experimental verification of chemical origin of life

Harold Urey and Stanley Miller successfully proved the chemical origin of life that was explained by Oparin, with their simulation experiment. They tried to replicate the primordial conditions in the laboratory. They sealed a mixture of water vapour, methane, ammonia and hydrogen (simulation of primordial atmosphere) in a spark chamber which was provided with electrodes for electric discharge (simulation of lightning energy). It was connected to another flask with the provision for boiling. The spark Chamber was connected on the other end to a condenser tube (simulation of rain and Haldane's soup). After some days, they noticed simple amino acids such as glycine, alanine and aspartic acid in the aqueous solution. Later in similar experiments, formation of all types of amino acids, even adenine and other nitrogen bases were noticed. (Figure 7.1)

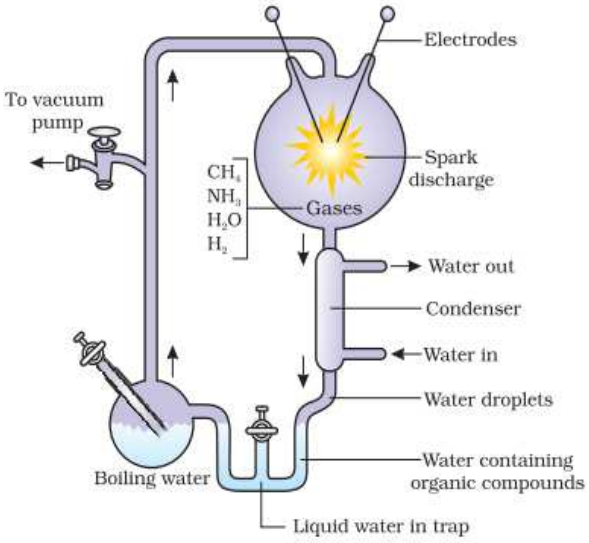


Figure 7.1 Urey and Miller Experiment

7.2 Evidences for Biological Evolution

The theories that explain the evolution are hypothetical. There is no practical proof for them. In fact, it is not possible for anybody to observe even a single change in favour of evolution that occurs in the body of organisms as our life span is too short to notice such slow changes. Hence scientists collected evidences from different branches of biology. Some of them are

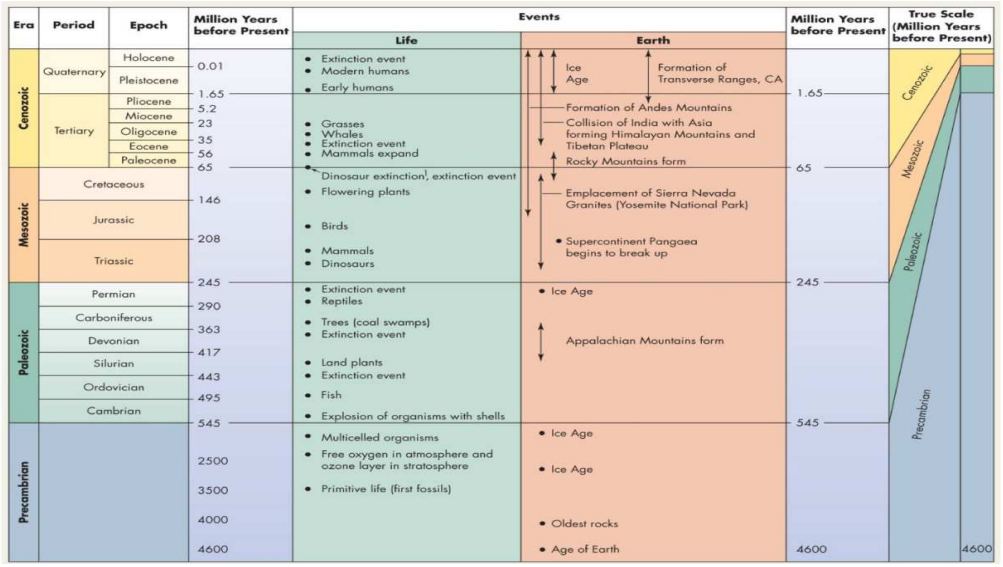
a. Evidences from palaeontology,
b. Evidences from embryology,
c. Evidences from comparative anatomy
d. Evidences from cell and molecular biology

a) **Evidences from Palaeontology**: Palaeontology (Gr. Palaios - old, on-existing, logos - to study) is the study of prehistoric life through fossils. I Fossils are the remnants of plants or animals that were preserved in I the layers of the earth and have been excavated from the soil. They are I of various types like moulds, casts, petrifications, traces, coprolites I (fossilized faecal matter), actual remains of animals, etc. They support! the idea that life has gradually evolved on the earth. The biologists and palaeontologists have found the fossils of many transitional forms j (connecting links) which link all the major groups of

vertebrates e.g.] Eusthenopteron between fishes and amphibians, Seymouria betweenl amphibians and reptiles, Archaeopteryx between reptiles and birds, Cynognathus between reptiles and mammals, etc.

Geological Time Scale: The earth contains different layers of sediments of j which, the bottom layer is the oldest layer and the upper layer is the most recent layer. Based on the age of rocks, a time scale was prepared and it is: called the geological time scale, it depicts the different stages of the evolution of life on the earth over the past millions of years. It consists of five Eras] which are divided into Periods that may be further subdivided into Epochs. These Eras, Periods and Epochs depict the time of origin and dominance of certain groups of animals during certain Eras or Periods. It provides the most direct evidences for the concept of evolution.

Table 1. Geological time scale



Evidences from embryology: The study of the formation and early development of an organism is called embryology. Ernst Haeckel is considered the 'Father of Embryology' and Von Baer is considered the Father of Modem Embryology. When the embryos of different animals are observed, we find a fundamental similarity which tells us that there is a relationship among the animals. Embryology provides evidences from i) the observations of Von Baer, ii) sequence of the developmental stages of some animals, and iii) the biogenetic law

i) The observations of Von Baer: Von Baer studied the embryology of fish, salamander, tortoise, chick and man. He observed that the early embryos of the above animals resemble each other closely. But these embryos differ in the final stages due to the formation of specialized characters. It indicates that the above animals have a common ancestor.

ii) Sequence of the developmental stages: The life of all the multicellular organisms begins with a single celled stage, the zygote. It undergoes cleavages to produce the first stage embryo, the morula, which) develops into a single layered second stage embryo, the blastula. It develops into the third stage embryo, the gastrula, which finally develops into adult. During this process, the zygote represents the unicellular stage, morula and blastula stages represent the colonial protozoan stages, whereat the gastrula stage represents the cnidarian stage. The developmen of the embryos of different organisms differs after the gastrula stage! This sequence of embryos shows that every multicellular organisn passes through the above stages representing their common ancestry

iii) Biogenetic Law or Theory of recapitulation: it was proposed by Ernst Haeckel. It states that ontogeny repeats phylogeny which means the developmental history of an organism repeats the evolutionary history of its ancestor, e.g. 1) Tadpole larva of frog resembles fish both externally and internally. It possesses a tail, gills and two chambered heart like that of a fish. Later it metamorphoses into adult frog 2) Caterpillar larva of butter fly recapitulates its closest ancestor, the annelid in body form 3) In the embryos of birds and mammals, the heart is initially two chambered as in fish, then three chambered as in amphibians, and after that an incompletely divided four chambered heart as in reptiles and finally four chambered (as seen in birds and mammals).

c. Evidences from comparative anatomy: When we compare the anatomy of different animals, we find some similarities among them. For example, the fore limbs of different vertebrates are similar in origin and internal structure. All these indicate that there is a relationship among the organisms. These relationships can be studied under i)^{M} &0mologous organs, ii) Analogous organs, iii) Vestigial organs, iv) Atavistic organs and v) Connecting links.

Homologous organs: The organs which have similar structure and origin but not necessarily the same function are called homologous organs. The evolutionary pattern that describes the

occurrence of similarity in origin and internal structure is called homology. Such organs show adaptive radiation, hence 'divergent evolution', e.g. the appendages of vertebrates such as the flippers of whale, wings of bat, forelimbs of horse, paw of cat and hand of man, have a common pattern in the arrangement of bones even though their external form and functions may vary to suit their mode of life. It explains that all the vertebrates might have had a common ancestor. Do you know? When the animals (of a species) of a certain habitat enter into different habitats, they evolve into different forms. This phenomenon is called adaptive radiation and it indicates divergent evolution, e.g. In Darwin's finches of Galapagos islands, many forms with variations in beak pattern were formed from the original seed-eating birds believed to have come from the main land, the South America. (Figure 7.2)

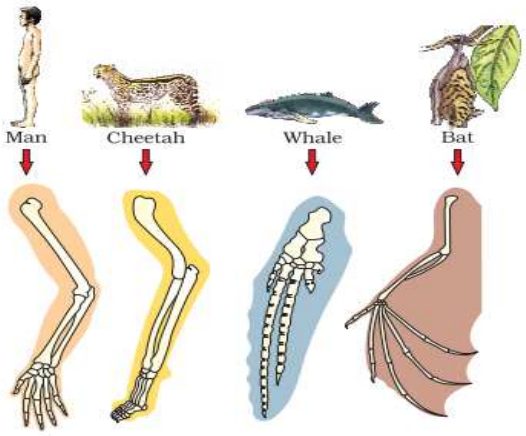


Figure 7.2 Homologous organs

Analogous organs: The organs which have dissimilar structure and origin but perform the same function are called the analogous organs. Analogous organs suggest 'convergent evolution', e.g. wings of a butterfly and wings of a bird.(Figure 7.3)

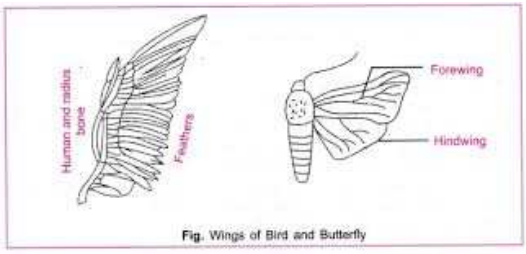


Figure 7.3 Analogous organs

Do you know? When different animals live in the same habitat, they tend to show similarity in body form, e.g. shark and whale have the same body form as both live in aquatic environment. Earthworm and snake have the same body form as both live in burrows. This type of evolution is called convergent evolution.

iii) Vestigial organs: The organs which were functional in the ancestors but non-functional and reduced in the descendants are called vestigial organs. Presence of vestigial organs is the most convincing evidence in favour of organic evolution and also supports the concept of disuse proposed by Lamarck, e.g. Hind limbs in python, hind limbs and pelvic girdle in whale, wings of flightless birds, vermiform appendix, coccyx, plica semilunaris (vestigial nictitating membrane), auricular muscles that move the pinna, hair on the body, mammary glands in males, etc., in human beings. I

iv) Atavistic organs: Sudden appearance of some vestigial organs a a better developed condition as in the case of the tailed humJ baby is called atavism. Such organs are called atavistic orgaw they strongly support the concept of organic evolution.

v) Connecting links: The organisms which possess the characters of two different groups between which they are transitional are called connecting links- They clearly explain the path of evolution, e.g. Peripatus between annelida and arthropoda, prototherians between reptilia and mammalia, etc.

d. **Evidences from cell and molecular biology**: The field of cell and molecular biology provides the most detailed and convincing evidence in favour of biological evolution. They are studied under three headings, namely i) Fundamental unity of life, ii) Blood precipitation (serological) tests and iii) Biochemical recapitulations

i) Fundamental unity of life: In all the living organisms, the structural and functional units are the cells. Every eukaryotic cell contains all the kinds of cell organelles such as Golgi complex, mitochondria, E.R., ribosomes, lysosomes, nucleus, chromosomes, D.N.A. and R.N.A. In all the living organisms, mitochondria help in energy production and storage, ribosomes help in protein synthesis; DNA has the same four types of nucleotides; all the proteins are synthesized from different combinations of the same 20 types of amino acids, the

genetic code is virtually the same everywhere; different types of biochemical substances such as enzymes, hormones, respiratory pigments, etc., and different types of biochemical reactions that occur in all the living organisms are the same, indicating some relationship among all the organisms on the earth.

ii) Blood precipitation tests: They are also called serological tests and were first conducted by H.F. Nuttal. He first injected a small amount of human serum into a rabbit. As our serum proteins are foreign to rabbit, antibodies are produced in that rabbit. The serum of the rabbit was collected, and it is called anti human serum. When it is mixed with the serums of an anthropoid ape, a monkey and a dog in separate test tubes, within a short time, a thick precipitate is formed with the serum of the anthropoid ape, moderate precipitate with that of the monkey and no precipitate with that of the dog. It indicates that the anthropoid ape is more closely related to man than to monkey and dog. These tests also proved that whale is closely related to pig than to dolphin. Is it not interesting to see that evolutionary relationships can be proved by such simple tests?

iii) **Biochemical recapitulations**: Animals recapitulate the biochemical aspects of their ancestors, e.g. a) An adult frog excretes urea but its tadpole excretes ammonia as the fishes do. b) The embryo of a bird excretes ammonia during the first four days of development as the fishes do, then urea in the next nine days as the amphibians do and finally uric acid as the reptiles and birds do. c) Even the mammaliayuembryo first excretes ammonia then uric acid and finally urea.)

7.2 Theories of evolution

Various theories have been proposed to explain the process of evolution, but the theories that explain the scientific basis of organic evolution are Lamarckism, Mutation theory of de Vries, Darwinism and Modern synthetic theory (Neo-Darwinism).

Lamarckism

It was explained by Jean Baptiste de Lamarck (1774- 1829), a French Biologist in his book Philosophic Zoologique. It deals with the influence of environment on organisms, use or disuse of organs and the inheritance of acquired characters. According to him, whenever the

environment of certain organisms undergoes some changes, it forces the organisms to use certain organs more and put certain other organs to disuse. The organs that are used more will increase in size and those not used continuously will degenerate. Such characters that are developed during the life time of an organism are called acquired characters. They are passed on to the next generation (Inheritance of acquired characters).

Examples: Elongation of neck and forelimbs in giraffe (use), absence of limbs in snakes (disuse). Objections: The main objection for Lamarckism came from Augustus Weismann. He conducted decaudalisation experiments on rats and proposed that the bodies of all the living organisms possess somatoplasm and germplasm. If any change occurs in the somatoplasm, it will not be transferred to the next generation but if any change occurs in the germplasm, it will be inherited to the next generation, e.g. 1) Well-developed muscles of athletes are not inherited to their children. 2) Making perforations to pinna for wearing ornaments has been in practice in India for the past several centuries. However no girl child is born with ready-made perforations in their pinna.

Neo-Lamarckism: The contributions of Lamarck paved the way for the gradual acceptance of the concept of biological evolution (not creation) and stimulated countless studies later. Hence it is modified to make it more acceptable. The followers of Lamarck (Neo-Lamarckists) like Cope, Osborn, Packard, Spencer, etc., tried to explain the Lamarck's theory on a more scientific basis. They considered that adaptations are universal. Organisms acquire new structures due to their adaptations to the changed environmental conditions. They argue that external conditions stimulate the somatic cells to produce certain 'secretions' which reach the sex cells through the blood. Such variations can be inherited by the offspring. Paul Kammarer observed the development of normal eyes and skin colour in the cave dwelling salamander, Proteus anguinus, when exposed to daylight. This somatic character was ^passed on to the next generation.

Darwinism

It is an evolutionary theory proposed by Charles Robert Darwin (1809-1882), an English Naturalist. He went on a voyage for five years on a world survey-ship of the British Government, H.M.S. Beagle and explored the fauna and flora of a number of continents and

islands.

He was much influenced by three publications namely 'An Essay on the Principles of Populations' of Thomas I Mai thus, which states that animals increase in geometric progression, whereas their food sources increase in Arithmetic progression, the book written by Sir Charles Lyell entitled 'Principles of Geology' that explains the phenomenon of gradualism (earth has changed slowly l and gradually through ages) and uniformitarianism (the Imuidamental laws that operate today on the earth are in I the same way as they did in the past) and the paper titled 'On the tendency of varieties to depart from original types' of Alfred Russell Wallace. He published his theory in his book titled 'The Origin of Species by means of Natural Selection'.

Theory of Natural Selection: Darwin's theory of Natural Selection does not explain what exactly evolution is, but explains how evolution might have occurred in nature. He believed that evolution is a gradual, rather than a sudden biological event. His theory was based on several facts, observations and inferences. They are

i. Prodigality of production or over production: Every organism tends to increase its population in large proportions. For example, Paramecium divides by binary fission at the rate of three to four times a day. At this rate, the volume of all the Paramecia equals to 10,000 times that of the earth at the end of the 9000^{th} generation. Salmon fish produces 28 million eggs and starfish one million eggs in a season. If all of them hatch and the larvae grow to reproductive age, all the seas will be filled with them in a few generations. Even the slowest breeder, the elephant can produce 19 million descendants at the end of the 800^{th} generation in the absence of any check.
ii. Constancy in population: However, such an abnormal increase is not noticed in the population of any species in nature as the offspring die in large number before reaching the reproductive age. It is true that the food and the other sources do not increase in the same proportion as that of the population.
iii. Struggle for existence: As the food sources are limited, severe competition exists among the members of a population. Darwin called it struggle for existence which is of three types as:

    1. Intraspecific struggle: The competition found among the individuals of the same

species is called intraspecific struggle. It is for food, shelter and mate. It is the most severe check on the rate of reproduction.

2. Interspecific struggle: The competition found among the animals of different species is called interspecific struggle. Most of the species have the same type of food habits. Hence, competition exists among them chiefly for food and shelter.
3. Struggle with the environment: All living organisms struggle with the adverse environmental conditions such as cyclones, floods, earthquakes, tsunamis, volcanic eruptions, etc.

iv. Universal occurrence of variations: No two organisms are exactly similar. They show variations. Even the offspring of the same parents are different. These variations may be harmful or useful or neutral. The useful variations help an organism to overcome struggle. Such variations are passed on to the next generation.

v. Natural selection: According to Darwin, the individuals possessing harmful variations are reproductively less successful. The organisms with beneficial variations would increase the ability to reproduce and leave more fertile offspring. They are said to be the 'fittest organisms' and they only can survive. The organisms with less reproductive success are not represented in future generations, however fit they may be in the struggle for existence. This is called the Natural Selection. Herbert Spencer called this phenomenon 'survival of the fittest'.

vi. Origin of species: Darwin concluded that 'the struggle for existence resulting in the survival of the fittest' allows successive generations to become better adapted to the environment. All the variations selected by Nature accumulate from generation to generation. 'Such an accumulation over a long period of time brings so many changes in an organism that it does not interbreed any more with the original parental species. Such a reproductively isolated organism is considered a 'new species'.

Objections to Darwinism: Though Darwinism is considered the best explanation for the organic evolution; some objections were raised against it. Some of them are

i) It failed to explain the mechanism by which variations occur. Thus Darwin faced the criticism 'DARWINISM EXPLAINS THE SURVIVAL OF THE FITTEST BUT NOT THE ARRIVAL OF THE FITTEST'.
ii) It could not explain the occurrence of vestigial organs, over specialisation of some

organs like large tusks in extinct mammoths, oversized antlers in the extinct Irish deer, etc.

iii) It focused on small, fluctuating variations which are mostly non- heritable.

iv) It did not distinguish between somatic and germinal variations.

v) Darwin did not consider the importance of macro-variations and considered them 'sports of nature'.

Experimental verification of Natural Selection - Industrial melanism

An important practical proof for the operation of Natural Selection is the classical case of industrial melanism, exhibited by peppered moth - *Biston betularia*. These moths were available in two colours, grey and **black**. Prior to industrial revolution, the grey moths were abundant. During the industrial revolution, the black forms were more and the grey forms were less in the industrial cities like Birmingham. Biologists proposed that with the industrial revolution, more soot was released due to the burning of coal, which resulted in the darkening of the barks of trees. Grey moths on the dark bark were easily identified and predated more by birds. Hence the number of grey moths decreased and that of the black moths increased in the population. It means Nature offered 'positive selection' pressure to the black (melanic) forms. Bernard Kettlewell a British ecologist, tested this hypothesis experimentally. He collected both the grey and the black forms of Biston betularia for his experiment. He released them in two sets of equal numbers; one set in Birmingham,polluted urban area, and the other set in Dorset, an unpolluted rural area. After a few days he recaptured them. Of those moths recaptured from Birmingham, there were more black forms. Among those recaptured from Dorset there were more grey forms. The reason for such a difference is: the melanic forms could not be easily spotted by predator birds as their body colour merged with the dark colour of the bark of trees in Birmingham area. In the rural areas (Dorset) the grey forms had better survival chance as their body colour merged with the light coloured surroundings. This explains the differential survival of the moths due to Natural Selection. It will be interesting to know that there was a reversal in the selection process after the introduction of pollution check laws in the urban areas.

Mutation Theory

It was proposed by Hugo de Vries, a Dutch botanist who coined the term 'mutation'. Mutations are sudden, random inheritable changes that occur in organisms. He found four different forms in Oenothera lamarckiana (commonly called 'evening primrose') such as O. brevistylis-small style, O. levifolia-smooth leaves, O. gigas-the giant form, O. nanella-the dwarf form (mutant varieties). T. H. Morgan studied the inheritance pattern of mutations in Drosophila melanogaster. Darwin called mutations (large variations) sports of nature or saltations, whereas Bateson called them discontinuous variations.

Salient Features of Mutation theory

1) Mutations occur from time to time i_n naturally breeding populations.
2) They are discontinuous and are not accumulated oyer generations.
3) They are full-fledged, and so there are no 'intermediate *forms'.
4) They are subjected to Natural Selection.

7.5 Speciation

The process by which one species evolves into one or more different species is called speciation. Evolution of new species in a single lineage is called anagenesis or phyletic evolution. On the other hand, if one species diverges to become two or more species, it is called cladogenesis or divergent evolution. If speciation takes place due to geographical isolation, it is called allopatric speciation. If speciation takes place in the organisms which live in the same habitat, capable of interbreeding, but do not interbreed due to some isolation mechanisms is called Sympatric speciation.

GLOSSARY

Actual remains: Fossils of the actual animals or animal parts trapped in amber, ice e.t.c., eg. Woolly mammoth in ice of Sibarian snow lands, insects in amber.

Archipelago: A group of many islands in a large body of water.

Catastrophe: A sudden violent natural calamity like an earthquake, tsunami, volcanic eruption.

Gene pool: The sum total of all the genes present in an sexually reproductive population during a given period of time.

Mould: Fossilized impressions made in the sediment or a negative image of an organism. When an organism buried in sediment the organic matter completely decomposes over aperiod of time, leaving behind a hollow area with the organisms shape.

Ontogeny: Developmental history of an individual.

Phylogeny: Ancestral history of an individual.

Simulation: The act of creating conditions similar to those believed to be present actually.

Traces: Fossilized nests, burrows, footprints e.t.c.,

VERY SHORT ANSWER TYPE QUESTIONS

1 What is panspermia?

2 Define prebiotic soup?

3 Define biogenetic law giving an example?

4 What is common between Darwinism and Lamarckism?

5 Mention names of any four connecting links that you have studied?

6 Define atavism with examples?

7 Distinguish between Allopatric and Sympatric speciation?

SHORT ANSWER TYPE QUESTIONS
1. Distinguish between homologous and analogous oragans? 2. Write short notes on theory of mutations? 3. Explain the Darwin's theory of evolution with industrial melanism as an experimental proof?

Applied Biology

SUMMARY

Animal husbandry is the practice of taking care and breeding domestic animals by applying scientific principles. The ever-increasing demand of food from animals and animal products both in terms of quality and quantity has been met by good animal husbandry practices. These practices include (i) management of farm and farm animals, and (ii) animal breeding. In view of the high nutritive value of honey and its medicinal importance, there has been a remarkable growth in the practice of bee-keeping or apiculture. Fishery is another flourishing industry meeting the ever-increasing demand for fish, fish products and other aquatic foods. Plant breeding may be used to create varieties, which are resistant to pathogens and to insect pests. This increases the yield of the food. This method has also been used to increase the protein content of the plant foods and thereby enhance the quality of food. In India, several varieties of different crop plants have been produced. All these measures enhance the production of food. Techniques of tissue culture and somatic hybridisation offer vast potential for manipulation of plants in vitro to produce new varieties.

8.1 Animal Husbandry

The strategies adopted for enhancing food production are bound to play a major role in meeting the requirement of food for the ever increasing world's population in the near future. The biological principles that are applied to animal husbandry will become crucial in our efforts to increase the food production.

Animal husbandry is the agricultural practice of breeding and raising livestock (all domesticated animals reared for the benefit of man). It includes buffaloes, cows, pigs, horses, cattle, sheep, camels, goats etc. However the term livestock is often used for farm animals. If extended, it also includes poultry farming and fisheries. Since time immemorial, animals like bees, silk-worm, prawns, crabs, fishes, birds, pigs, cattle, sheep, goats and camels have been

used by humans for products such as honey, silk, meat, pork, milk, hydes, wool, etc. Dairy farms, Dairy industry, Poultry and Aquaculture have been providing employment and additional source of income, mainly in rural areas.(Figure 8.1)

Figure 8.1 Ongole cattle breed

Even though the estimated world's livestock population in India and China together is more than 70%, their contribution to world's farm produce is only 25%, i.e. the productivity per unit is very low. The average annual milk yield is about 170 liters per cow in India. Contrary to it, the average annual milk yield is about 4,100 liters per cow in Netherlands. Because of its low productivity the Indian cow is known as 'teacup cow'. So, newer technologies have to be applied to achieve improvement in quality and productivity. Modem methods of breeding, MOET (multiple ovulation and embryo transfer) and production of transgenic animals must be taken up on a large scale in addition to conventional practices and care.

Cattle population in India consists of three groups of breeds. They are:

1. Milch breeds which yield higher quantity of milk and the bullocks are not useful in farms or transport. The superior milch buffalo breed is MURRAH.
2. Draught breeds in which cows are poor in milk production and the bullocks are excellent draught animals.
3. Dual purpose (general utility)

Management of Farm and Farm Animals

A professional approach to farm management gives the much needed boost to our food production and it certainly has an edge on traditional practices. The farm management ensures optimum quality feed, breeding and caring. It provides good infrastructure and takes

care of sick animals.

Dairy Farm Management

Breeding, feeding and management of milch animals. production, processing and marketing of their milk and milk products on economic basis constitute dairying. Commercial dairy systems house five to hundreds of hybrid cows or Murrah hybrid buffaloes. The different kinds of products that can be made with milk from a dairy farm are butter, ghee, curds, butter milk, cheese, paneer, etc.

Dairying provides an occupation and income in all seasons to the farmers. Milk is considered an indispensable food for l/4th of the population which includes infants and children. In recent years dairying has assumed new dimensions with emphasis on increased production of quality milk through technical innovation and modem management methods. Indian dairy industry had witnessed fantastic growth, since the inception of the programme/ project Operation Flood' by National Dairy Development Board (NDDB).

In dairy farm management, we deal with processes and systems that increase yield and improve quality of milk. The following are essential components.

1. Selection of good breeds having high yielding potential, combined with disease resistance is important as milk yield primarily depends on the quality of breeds in the farm. Examples include high yielding hybrid cows of the exotic breeds like Holstein-Friesian, Jersey, Ayrshire and Brown Swiss. Holstein- Friesian cow yields up to 30 litres of milk/ day.
2. Proper housing with adequate water, ventilation, suitable temperature, etc., is needed to enhance the yield potential.
3. Feeding of cattle with special emphasis on quality and quantity of fodder should be done in a scientific manner.
4. While milking, storage and transport, cleanliness and hygiene are of paramount importance. As these processes are mechanised, direct contact of the produce with the handler is reduced.
5. A regular visit by a veterinary doctor is necessary.
6. Proper record keeping and inspections would help to identify and rectify the problems at the earliest.

8.2 Bee-Keeping

In the insect world, the economically important honeybees exemplify colonial living, reciprocal communication, division of labour and polymorphism (caste differentiation). Bee-keeping or apiculture is the maintenance of hives of honey bees for the production of honey and wax. Bee-keeping is an age-old cottage industry.

The two species of honey bees widely used in bee keeping in India are:

1. *Apis mellifera* (European bee): A favourite for apiculture: yields large quantities of quality honey.
2. *Apis cerana indica* (Indian or Astan hive bee): It is wild and is also domesticated.(Figure 8.2)

Figure 8.2 Honey bee

A BEE COLONY: Queen (fertile female), drones (haploid males) and workers (sterile females) are the three castes in a beehive.

QUEEN: It is the largest individual in the colony; It is a fertile, diploid female, one per bee hive and the egg layer of the colony. She lives for about five years and her only function is to lay eggs. The queen bee during its nuptial flight receives sperms from a drone and stores in the spermathecae and lays two types of eggs, the fertilised and unfertilised. All fertilised eggs develop into females. All the larvae developing from the fertilised eggs are fed with the royal jelly (vitamin and nutrient rich secretion from the glands in the hypopharynx of the nurse workers) for the first 4 days only. Afterwards royal jelly is fed only to the bee that is bound to develop into next queen, whereas the other larvae fed on bee bread (honey and pollen) become workers (sterile females).

DRONES ***(haploid, fertile males)***

These are robust, large winged, small numbered, short lived and fed with bee bread by nurse workers. They are developed from the unfertilized ova by arrhenotoky (male parthenogenesis).

WORKER BEES

They are multifaceted sterile females which develop from the fertilised eggs and perform diverse functions. They live for two or three months. They are the smallest in size among the 3 castes. Worker bees secrete wax, build hexagonal cells of the honey-comb, gather nectar from flowers, manufacture and store honey, gather pollen, make propolis (bee glue-used to seal the cracks in the combs). The scout bees (worker bees) perform a 'waggle dance' after returning to the hive, to inform other bees about source of nectar. Waggle dance (with waggle phase and return phase) informs the other worker bees the direction with reference to sun's position and distance of availability of food (discovered by Nobel Laureate Karl von Frisch). They also guard the hive for which they possess a poisonous sting. They have chewing and lapping type of mouth parts.

Beehive

It is an enclosed structure in which honey bees live and raise their young. Domesticated honeybees live in man-made beehives or 'apiaries'. Bee wax is produced from their abdominal glands (present on 2^{nd} to 5^{th} abdominal segments). Bees use the storage cells to store food (honey and pollen) and brood cells to house the "brood" (eggs, larvae, and pupae).

Economic importance of Honey bees

The bee products like Honey, wax, propolis and bee venom are used in various ways.

1. Honey is a rich source of fructose, water, glucose, minerals and vitamins.
2. Bee's wax is used in the preparation of cosmetics, polishes of various kinds and candles.
3. Propolis is used in the treatment of inflammation and superficial bums.
4. Bee's venom, which is extracted from the sting of worker bees, is used in the treatment of rheumatoid arthritis.

5. Pollination; Bees are the pollinators of our crop plants such as sunflower, Brassica, apple and pear.

Factors /requirements for successful Bee-keeping:

1. Knowledge of nature and habits of honey bees.
2. Selection of suitable location (termed Apiary or Bee yard) for keeping the beehives.
3. Raising a hive with the help of a queen and small group of worker bees.
4. Management of beehives during different seasons
5. handling and collection of honey and bee wax.

8.3 Fishery Management

Fishery is an industry or occupation devoted to the catching, processing for storage in freezers and selling of fish, shellfish or any other aquatic animals for human consumption.

Types of Fisheries

Based on how the fishery resource is obtained, fishery can be categorised broadly into two types 1. Capture and 2. Culture fisheries.

I. **Capture fishery**: in capture fishery the resource is obtained from the natural body of water. Capture fishery is either marine or inland. If the capture is from the sea, it is calied marine fishery. In the sea, if the resource is captured from open waters, it is called offshore fishery, if the resource is captured from coastal waters, it is called inshore fishery. If the capture is from estuaries or fresh water it is called inland fishery.

2. **Culture fishery** (aquaculture): It involves rearing and management of selected aquatic organisms under regulated conditions and their subsequent harvesting after the stipulated time.

Aqua culture can be categorised into certain types based on the medium used in culturing (fresh water, brackish water and mariculture), organisms selected for culturing (prawn, crab, oyster and pisciculture) and the presence or absence of fins in those organisms (fin fish fishery/shell fish fishery). Aquaculture (culture fishery) not only includes the culturing of fish, but also culturing of other aquatic organisms under regulated conditions to achieve

better production. The term Pisciculture is used when the organisms cultured are exclusively fin fishes.

The organisms selected for culture practices must obviously possess certain features to make their culture profitable. They show fast rate of growth, economy of feed, early maturity, disease resistance, high nutritious value, high consumer demand and export value. Indian carps namely ***Catla catla*** (catla), ***Cirrhinus mrigala*** (mrigal), ***Labeo rohita*** (rohu) are extensively used in fresh water pisciculture in India. The common carp, grass carp and silver carp (exotic-Chinese carps) are also used in pisciculture in recent years in India, as growth rate and reproductive potential are high in them. The Indian carps are also harvested from natural fresh water bodies (capture fishery). (Figure 8.3)

Figure 8.3 Indian major carps

Hypophysation *or induced breeding is followed in artificial breeding. Pituitary extracts containing gonadotropins (FSH and LH)* ***orovaprim*** *(a synthetic analogue of gonadotropin releasing agent and Dopamine inhibitor) are injected into brood fish to induce release of spawn for seed production.*

Economic Importance of Fishery

1. ***As Food***

Fish meat, in general, is a good source of proteins, vitamins (A and D), minerals and rich in iodine. Tunas, shrimps and crabs are not only edible but have export value also.

2. ***By-products***

A. Shark and cod liver oils are good sources of vitamins A and D. Oil from Sardine

and Salmon are good sources of omega 3 fatty acids, which have multiple functions (reduce cholesterol, help prevent cancer cell growth etc.,).

B. Fish guano: Fertilizer prepared from 'scrap fish'.

C. Other fish by-products are shagreen, Isinglass (substance obtained from dried swim bladders of mostly cat fish, used in clarification of wines) etc. Fisheries have carved a niche in the Indian economy. We now talk about 'Blue Revolution* as being implemented on lines similar to 'Green Revolution'. In addition to pisciculture, the culture of prawns, crabs and pearl oysters*^ enable us earn foreign exchange worth millions of dollars from their exports.)

8.4 Biotechnological Applications in Medicine

Recombinant DNA technology involves manipulation of genes or microorganisms to produce certain products useful to mankind. These recombinant DNA technological processes have made an immense impact in the area of human health care. They are used in the production of hormones, synthetic vaccines, effective therapeutic products etc. These products like the recombinant therapeutics do not induce unwanted immunological responses. At present about 30 recombinant therapeutics have been approved for human use, the world over.

Genetically Engineered Insulin

You have already learnt that INSULIN is a protein hormone produced from the beta cells of islets of Langerhans of the pancreas and is required to accelerate the uptake of glucose into the body cells and to promote glycogenesis, lipogenesis, protein synthesis, etc. Insulin must be injected or given via an insulin pump. It cannot be administered orally, because it is a protein and is broken down in the stomach before it can be absorbed. Attempts are underway to develop orally administerable insulin.

Prior to the development of humulin, diabetic patients used to be dependent upon animal insulin extracted from the pancreatic islet tissue of slaughtered cattle and pigs. The animal insulin is more expensive, less accessible and causes allergies. They also cause several side effects, due to certain non-self proteins in them, which act as antigens. Currently, genetically engineered E.coli which can be grown in large quantities to produce human (humulin) insulin is available and from which we can make as much insulin as we need. (Figure 8.4)

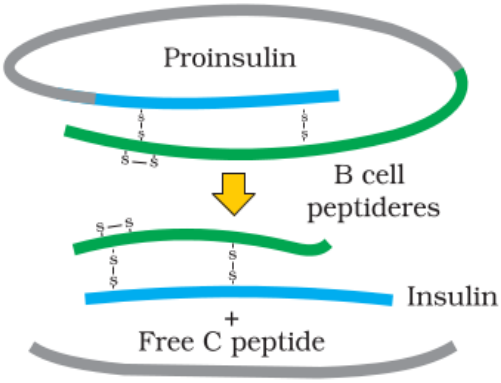


Figure 8.4 Maturation of pro-Insulin into Insulin

Structure of insulin: Human insulin is made up of 51 amino acids arranged in two polypeptide chains -chain A (21 amino acids) and chain B (30 amino acids), which are linked together by disulphide linkages. In mammals, including humans, insulin is synthesised as a pro-hormone (like a pro-enzyme, which needs to be processed before it becomes fully mature and functional hormone) which contains an extra stretch called the c peptide. This c peptide is not present in the mature insulin and is removed during maturation into insulin.

The main challenge for the production of insulin in the laboratory using rDNA technique was getting insulin assembled into its mature form. In 1983, Eli Lilly, an American company, prepared two DNA sequences coding for A and B chains of human insulin and introduced them into plasmids of E.coli to produce insulin chain. The chains A and B were produced separately and combined by creating disulphide bonds to form 'humulin' which is the brand name for a group of biosynthetic human insulin products.

8.5 Vaccines

The term 'vaccine' was coined by Edward Jenner. He immunized a boy against small pox by inoculating him with a relatively less dangerous cow pox virus. The technique of attenuating or weakening of a microbe was developed by Pasteur.

A vaccine is a biological preparation that improves immunity to a particular disease. A vaccine typically contains the disease- causing microorganism, and is often made from weakened or killed forms of the microbe. The toxins or one of the surface proteins of the microorganisms are also used in preparing vaccines.

Vaccines generally prophylactic. Moreover, they may not guarantee total protection from a disease. It may be due to inadequate immune response of the host's immune system. Even if the host develops antibodies, the human immune system may not effectively defend the body from the pathogen immediately. Therefore certain adjuvants are used to boost the immune response. Most often aluminium adjuvants are used.

Use of vaccines in the Afro-Asian countries has reduced the mortality rate. For example smallpox could be eradicated completely in India/World by intensive vaccination programme in an organised manner, by governmental agencies,as it is being done in the case of polio, nowadays. Though there are vaccines available against several pathogens, vaccines against dreaded diseases such as AIDS and Malaria are yet to be developed.

The following are some important Biotechnologically produced vaccines.

1. Attenuated Whole Agent Vaccines

   They contain disabled (made less virulent) live microorganisms. Mostly they are antiviral. Examples: vaccines against yellow fever, measles, rubella and mumps and the bacterial disease such as typhoid.

2. Inactivated Whole Agent Vaccines

   They contain 'killed microbes' (virulent before killing). Examples: vaccines against influenza, cholera, bubonic plague, polio, hepatitis A, rabies and Sabin's oral polio vaccine.

3. Toxoids

They contain toxoids' which are inactivated 'exotoxins' of certain microbes. Examples: the vaccines against Diphtheria and Tetanus. Thus, vaccines are used in the prevention of diseases as they induce artificially acquired active immunity.

Gene Therapy

Gene therapy is the insertion of genes into an individual's cells and tissues to treat a disease, such as a hereditary disease in which a deleterious mutant allele is replaced with a functional one. The biology of human gene therapy is a collection of methods/techniques that allows

correction of a gene defect that has been diagnosed in a child/embryo.

Basic Technique: A normal gene is inserted into the genome to supplement an abnormal gene (mutated gene), which is disease causing. To deliver the normal gene (therapeutic gene) a vector must be used. The vector unloads its genetic material containing the therapeutic human gene' into the target cell. The generation of a functional protein product from the therapeutic gene restores the cell to its normal state.(Figure 8.5)

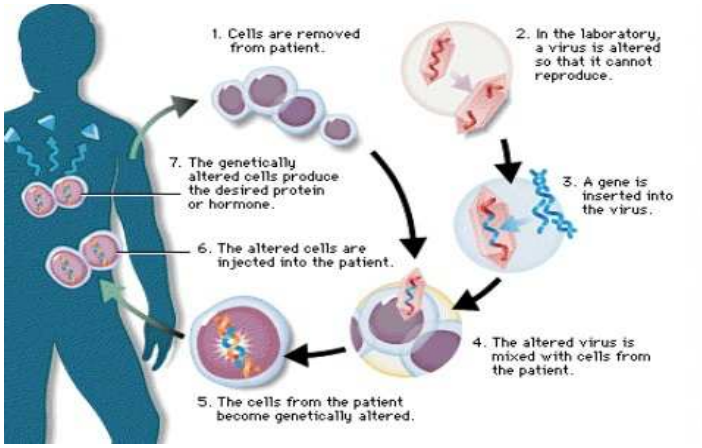


Figure 8.5 Important steps in the process of gene therapy

Types of Gene Therapy: Two basic types of gene therapy can be applied to humans, germ line and somatic line

a. Germ line gene therapy: In this type of therapy, functional genes (normal genes) are introduced into sperms or ova and are thus integrated in to their genomes. Therefore the change or modification becomes heritable. Due to various technical and ethical reasons, the germ line gene therapy remained at the Infant stage' for the time being.

b. Somatic line therapy: In this type of therapy, functional genes are introduced into somatic cells of a patient. The approach is to correct a disease phenotype by treating some somatic cells in the affected person. The changes effected in this type of GT are non-heritable.

Somatic line therapy can be either ex-vivo or in vivo. In ex-vivo, cells are modified outside the body and then transplanted back. In in-vivo, genes are changed in cells, while they are still inside the body.

First Clinical Gene Therapy

The first clinical gene therapy was given in 1990 to a four year old girl with adenosine deaminase (ADA) deficiency. This enzyme is crucial for the immune system to function. ADA deficiency causes severe combined immunodeficiency (SCID). It is caused by the deletion or dysfunction of the gene encoding for the enzyme ADA. In some children ADA deficiency can be cured by bone marrow transplantation, in others it can be treated by an enzyme replacement therapy, but both these approaches are not completely curative.

As a first step towards gene therapy, lymphocytes from the blood of the patient are grown in a culture outside the body. A functional ADA cDNA (using a retroviral vector) is then introduced into these lymphocytes, which are cultured and subsequently returned to the patient. However, as these cells are not immortal the patients require periodic infusion of such genetically engineered lymphocytes. Permanent cure is possible when the gene isolated from marrow cells producing ADA is introduced into the cells at early embryonic stages.

Diseases and Gene Therapy: Scientists are focusing on diseases caused by single-gene defects, such as cystic fibrosis, haemophilia, muscular dystrophy and sickle cell anaemia, wherein scientists are trying to introduce genes directly into human cells. Currently, most gene therapy studies are aimed at cancer and hereditary diseases linked to a genetic defect. In spite of so many efforts, the biology of GT remains complex and many techniques need improvement and perfection. The genetic link of many diseases is to be understood more fully before GT can be used appropriately.

Transfer of Genetic Material: Genetically modified DNA can be introduced into a eukaryotic cell by a process called ‘Transfection*. It can also be introduced by Gene guns or through ‘electroporation’ of the cells. Viral vectors are used to send the genetic material into cells, a process similar to bacterial transduction. In methods in which DNA is directly sent, the DNA to be delivered is protected from damage during transfer /entry into recipient cell by enveloping it in lipid coats (Lipoplexes) or enveloping in complexes of polymers (Polyplexes). Synthetic oligodeoxy nucleosides are used to inactivate genes (Silencing the genes) involved in causing disease.

Transgenic Animals

Animals that have their own genome and had their DNA manipulated to possess and express an extra (foreign) gene are known as transgenic animals. Transgenic rats, rabbits, pigs, sheep, cows and fish have been produced, although over 95 percent of all existing transgenic animals are mice. Transgenic animals are produced for many reasons aiming at benefitting human beings. Let us try and explore some of the common reasons:

i) **Normal physiology and development**: Transgenic animals can be specifically designed to allow the study of how genes are regulated, and how they affect the normal functions of the body and its development,e.g. study of complex factors involved in growth such as insulin-like growth factor. By introducing genes from other species that alter the formation of this factor and studying the biological effects that result, information is obtained about the biological role of the factor in the body.

ii) **Study of disease**: Many transgenic animals are designed to increase our understanding of how genes contribute to the development of disease. These are specially made to serve as models for human diseases so that investigation of new treatments for diseases is made possible. Today transgenic models exist for many human diseases such as cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's (Discussed in unit iii).

iii) Biological products: Medicines required to treat certain human diseases can contain biological products, but such products are often expensive to produce. Transgenic animals that produce useful biological products can be created by the introduction of the portion of DNA (or genes) which codes for a particular product such as human protein (a-1 antitrypsin) used to treat emphysema. Similar attempts were made for treatment of phenylketonuria (PKU) and cystic fibrosis. In 1997, the first transgenic cow, Rosie, produced human protein-enriched milk (2.4 grams per litre). The milk contained the human alpha-lactalbumin and was nutritionally a more balanced product for human babies than what is available in normal milk from untreated cows.

iv) Vaccine safety: Transgenic mice are being developed for use in testing the safety of vaccines before they are used on humans. Transgenic mice are being used to test the safety of the polio vaccine. If successful and found to be reliable, they could replace the use of monkeys to test the safety of batches of the vaccine.

v) Chemical safety testing: This is known as toxicity/safety testing. The procedure is the same as that used for testing toxicity of drugs. Transgenic animals, that carry genes

which make them more sensitive to toxic substances than non-transgenic animals are produced. They are then exposed to the toxic substances and the effects studied. Toxicity testing in such animals will allow us to obtain results in less time. Indian government has setup GEAC (Genetic Engineering Approval Committee) to look into misuse of DNA manipulation and safety of introducing GM-organisms for public services.

8.6 Stem Cells

Stem cells are biological cells found in most, if not all multicellular animals. They are characterized by their ability to go through numerous mitotic cycles while maintaining the undifferentiated state and differentiated into diverse specialized cell types. Stem cells are responsible for development, repair of adult tissues and cancer, when the control over them is lost. Stem cell research has been hailed for the potential to revolutionize the future of medicine with the ability to regenerate damaged and diseased organs. In admission to self renewal, stem cells also exhibit cellular potency.

In mammals there two types of stem cells. They are (A)Embryonic stem cells (B) Adult stem cells.

(A) **Embryonic stem cells**: They are isolated from the inner cell mass of blastocyst. ES cells are pluripotent cells and give rise to the three primary germ layers-*ectoderm, endoderm* and *mesoderm*. When given necessary stimulation, they can develop into more than 200 cell types of the adult body. These cells are immortal i.e. they can proliferate in a culture medium and they are a source of a large number of cells in the undifferentiated state.

(B) **Adult stem cells**: They found in various tissues of children as well as adults. Any cell which is found in the body of a developed animal that has the ability to divide and create another cell like it is called an adult stem cell or somatic stem cell. These cells act as repair system for the body, replenishing adult tissues. Most of the adult stem cells are multipotent.

The red bone marrow is a rich source of adult stem cells. The examples include mesenchymal stem cells, adipocyte stem cells, endothelial stem cells, etc.

Haemopoietic stem cells (HSCs) of red bone marrow are multipotent stem cells. HSCs are

of two types namely myeloid stem cells and lymphoid stem cells that give rise to all the blood cell types.

Myeloid stem cells and lymphoid stem cells are together called secondary stem cells.

Myeloid stem cells: These are non-renewing cells and give rise to erythroid committed progenitor, basophil committed progenitor, eosinophil committed progenitor, granulocyte-monocyte progenitor and Megakaryoblast. Each of these cells give rise to different but specific cell(s).

1. Erythroid committed progenitor (proerythroblast): Erythrocyte is formed through stages like proerythroblast, erythroblast and reticulocyte.
2. Basophil committed progenitor: Gives rise to basophil.
3. Eosinophil committed progenitor: Gives rise to eosinophil.
4. Granulocyte-monocyte progenitor: It gives rise to myeloblast and monocyte committed progenitors. Neutrophil is formed from myeloblast t committed progenitor and monocyte is formed from monocyte committed \ progenitor.
5. Megakary oblast: It forms megakaryocyte which by fragmentation gives' blood platelets.

Lymphoid stem cells: It gives rise to T-cell committed progenitor and B-celt committed progenitor. T-cell committed progenitor differentiates into Tj lymphocyte in Thymus. T-cells may be T-helper or T-cytotoxic cell. B-ceft committed progenitor produces B-lymphocyte in the bone marrow.

Applications Of Stem Cell Therapy

Stem cells, taken from umbilical cord, can be transforming into specialized cells to replace myocytes or neurons or perhaps the entire organism through cell culture. A number of adult stem cell therapies already exist.

8.7 Types of Cancers and causes (Cancer Biology)

Cancer is a leading killer disease in the world. It is basically a condition of failure of cell division control. Unchecked division of cells leads to the formation of ‘neoplasm’ (tumor) and such an abnormal proliferation of cells is called ‘neoplasia’ (formation of a tumor).

Neoplasm may be (1) Benign (harmless e.g. uterine fibroids in a female human being) (2) Pre-malignant (also called 'carcinoma in situ'- in which cells transform into cancerous cells in due course of time) (3) Malignant (cancerous, in which cells invade and destroy surrounding cells forming 'metastasis'). Some neoplasms, however do not form tumors. Oncology is the branch of medicine that deals with tumors, including study of their development, diagnosis, treatment, and prevention. Cancers are caused chiefly due to failure of cell cycle regulation' which is not yet clearly understood.

Normal cells show a phenomenon called contact inhibition* (which means cessation of cell division and cell mobility, when they are in close (physical) contact with each other. Cancer cells (neoplastic cells or tumor cells) lose this property.

As cancer cells actively divide and grow, they starve the normal cells by competing for 'vital nutrients'. Normal cells are joined by 'intercellular adhesion proteins' called 'cadherins' (calcium dependent cell membrane proteins) and they are missing in cancer cells (hence their ability to detach and cause metastasis). Cancer is a disease in which damaged cells do not undergo 'programmed cell death (apoptosis). Cancer cells have abnormal changes on their cell surface /unusual profile of surface antigens,which can be recognized and destroyed by NK cells of the immune system. Another important aspect of tumors is - there is increased growth of blood vessels towards the tumors. When tumors grow in size, diffusion of oxygen and nutrients becomes restricted and so tumors resort to attracting more blood vessels from their surrounding matrix (angiogenesis). If extension of blood vessels can be inhibited, the cancer cells starve and die.

Proto-oncogenes, oncogenes, tumor suppressor genes

Human genome has some 'proto-oncogenes[9] which are normal cellular genes. Due to the effect of certain mutagens,proto-oncogenes mutate into cellular oncogenes' which cause cancers. There are some tumor suppressor genes also in the genome. For example the gene p53 is a gene that codes for a 'protein' (p53 protein) that inhibits the development and growth of tumors. Mutation of proto-oncogenes are dominant (a single copy of the oncogene can lead to cancer). Interestingly, in the case of tumor suppressor genes such as the p53 gene, both copies of the gene in the homologous pair of chromosomes must undergo mutation to allow cancer to develop. The protein p53 plays an important role with reference to the G1 check point in the regulation of cell division cycle. It guards the 'integrity of the DNA' (it is

often called the GUARDIAN ANGEL OF CELL'S GENOME). It stops cell division and helps repair the damaged DNA. If the damage is repaired, the p53 protein allows the cell to enter the 'S' phase. If it is irreparable,it directs the cell to undergo 'apoptosis'. Hence the gene p 53 is called tumor suppressing gene[9]. The retinoblastoma protein (pRB) is also a tumor suppressor protein' that is dysfunctional in several major cancers. Its function is similar to that of p53 protein.

Carcinogens: A carcinogen is a substance that causes cancer. Carcinogens damage the DNA. Several radioactive substances e.g. gamma rays, some 17- V rays, X rays etc. and inhaled asbestos, certain Dioxins . tobacco smoke (which contains many types of carcinogens such as benzopyrene, nitrosamines and inorganic compounds such as chromium, cadmium, arsenic, polonium-210) etc. are carcinogenic. If the DNA damage is too severe to repair, the cells undergo apoptosis. but if the programmed cell death pathway is damaged, the cell becomes a cancer cell.

Types of cancers

There are different types of cancers such as carcinomas (cancers of epithelial tissues/cells which are most common as epithelial cells divide more often), sarcomas (cancers of connective tissues), leukemias (cancers of bone marrow cells resulting in unrestrained production of WBC - a liquid tumor), lymphomas (cancers of the lymphatic system). Certain types of cancers are called 'familial cancers' (cancer that occurs in families; genetic based) and others 'sporadic cancers' (non-hereditary cancers occurring without any family history). Some types of cancers are caused by 'tumor forming RNA viruses' (oncoviruses), e.g. Rous sarcoma virus which causes 'avian sarcoma'.

Detection of cancer

Cancer detection is based on 'biopsy' (removal and microscopic examination of a sample of body tissue from a living organism for diagnostic purposes). It is essentially a histopathological study to detect abnormalities such as cancer. Blood test is performed to detect 'leukemia' (increased leucocyte count, commonly referred to as blood cancer'). The PSA (Prostate Specific Antigen) test is used to screen for early prostate cancer. A mammogram is a low-energy x-ray exam of the breasts to detect breast cancer. X ray, CT scan (Computerized Tomography), MRI (Magnetic Resonance Imaging) etc., are used for

diagnostic imaging of body parts to detect cancer. PET scan detects cancer, and help physicians diagnose breast cancer, lung cancer, colo-rectal cancer, cervical cancer etc. Monoclonal antibody-based biologic drugs are also useful in treating certain cancers (Biotherapy).

Treatment of cancer: Treatment of cancer generally involves

1. Surgical removal of the malignant tumor.
2. Radiation treatment to destroy cancer cells in the tissues adjoining the tumor.
3. Chemotherapy and immunotherapy to destroy cancer cells that might have moved to other parts of the body. Chemotherapy has side effects such as loss of hair due to destruction of 'hair follicle cells' (alopecia),anaemia (due to inhibition of division/destruction of the 'normal' cells of the bone marrow). Anti-cancer drugs such as 'taxol' can stop actively dividing cells. Usually cancer cells avoid detection and destruction by the immune system. Hence cancer patients are given substances known as 'BIOLOGICAL RESPONSE MODIFIERS' such as alpha interferon which actnia^es the immune system of the patient and help in destroying tumors.

8.8 Biomedical Technology

Biomedical technology is the application of principles of biophysics and biochemistry for studying certain biological aspects. It involves-

1. Diagnostic imaging X- ray imaging , Computer Axial Tomography (CAT scan) or Computed tomography (CT scan), Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET scan). Sonography etc.
2. Biomedical procedures to study body's vital functions and their disorders (ECG, EEG)
3. Biochemical procedures to estimate various biochemical components in body fluids (ELISA,Western Blot/Protein immunoblot etc.)

Computerized Axial Tomography (CAT)

Computed Tomography (CT) or Computerized Axial Tomography (CAT) scan is a medical imaging method that employs Tomography (a process of producing

a two dimensional slice through a 3 dimensional object). A large donut shaped X-ray

machine takes X-ray images at many different angles around the body. The X-ray detector of the CT-scanner can see hundreds of different levels of density and tissues in a solid organ. The data is transmitted to a computer which builds up 3-D cross sectional picture of the part of the body and displays the picture on the screen. This recorded image is called ‘tomogram'. The body can be seen on CAT scan as slices from the skin to the central part.

A CAT scanner emits a series of narrow beams through the human body as it moves through an arc, unlike an X-ray machine which sends just one $_t$ radiation beam. The final 3-D picture of a CAT scan is far more superior and detailed than an X-ray one. Sometimes, patients may be given a contrast dye for better resolution.

CAT scans are useful to measure accurately the density of bones in evaluating osteoporosis. CAT scans are performed to analyse the head injuries (blood clots and skull fractures) to know the anatomy of various visceral organs.

MRI (Magnetic Resonance Imaging)

An MRI scan is a diagnostic radiology technique that uses magnetism, rado waves and computer to produce images of body components. It is important to note that MRI does not use ionized radiation, as involved in X-rays , and generally a very safe procedure. MRI is non invasive medical imaging technique that helps physicians diagnose certain anatomical abnormalities or pathological conditions. This technique uses nuclear magnetic resonance of protons to generate proton density images of body parts. Magnetic resonance Imaging uses powerful magnetic field, radio frequency pulses and computer to produce detailed pictures of organs, soft tissues, bones and virtually all other internal body structures. (Figure 8.6)

Figure 8.6 MRI scanner

MRI scanner and procedure

MRI scanner is a giant circular magnetic tube The patient is placed on a movable bed that is inserted into the magnet. Human body is mainly composed of water molecules which contain two hydrogen nuclei / protons, each. The magnet creates a strong 'magnetic field' that makes these protons align with the direction of the magnetic field (protons are not aligned under normal conditions). A second radiofrequency electromagnetic field is then turned on for a 'brief period'. The 'protons' absorb some energy from these 'radio waves'. When this 'second radio frequency emitting field' is turned off, the protons release energy at a radiofrequency which can be detected by the MRI scanner (the protons return to their 'equilibrium state' from the 'energized state at different 'relaxation' rates).

Different types of tissues emit different 'quanta' of energy (in the form of different wave lengths and at different rates). Abnormal tissues, such as tumors, can be detected because the protons in different types of tissues return to their equilibrium state at different rates. Tissues such as bones with less water content [hence, less number of protons) look different in an MRI image. Accordingly there is a contrast between the 'images' of different tissues' based on their water content. Even in the case of the same tissue, 'normal healthy cells' and 'pathological cells' emit different energy waves - hence the difference in the images of the different types of cells.

The information received is processed by a computer, and an image is generated. The image and resolution produced by MRI is quite detailed and can detect tiny changes of structures

within the body. For some procedures, radiocontrast agents, such as gadolinium, are used to increase the accuracy /resolution of the images. After scanning is completed, the computer generated images (tomographs-images of thin slices of body parts) can be transferred to a film (hard copy). A radiologist interprets the images of the body parts and gives his diagnostic opinion.

ECG

ECG may mean electrocardiogram/electrocardiograph, but most commonlyused for electrocardiogram. Electrocardiography is a commonly used, non-invasive procedure for recording electrical changes in the heart. The graphic record, which is called an electrocardiogram (ECG 01 EKG). shows the series of waves that relate to the electrical impulses which occur during each cardiac .cycle. An electrocardiograph is a device which records the electrical activity of the heart muscle (depolarisations and repolarisations).(Figure 8.7)

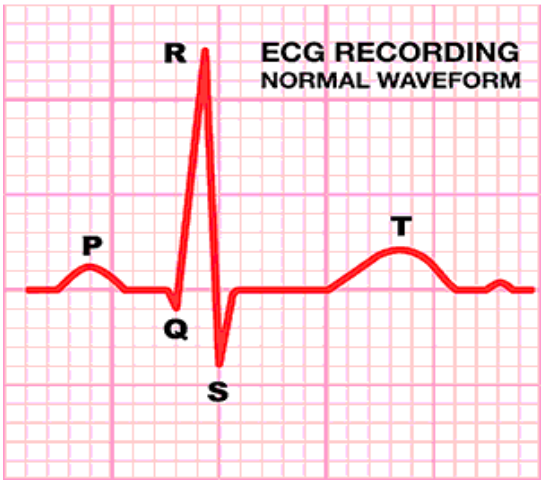


Figure 8.7 ECG

What is electrocardiography ?

Electrocardiography is the technique by which [the electrical activities of the heart are studied. Sensors (electrodes) are placed at specific parts of the body and linked to the pCG machine. ECG is recorded using 12 LEADS'

Obtaining an electrocardiogram typically takes a few minutes, after which the electrodes are removed.

The essential components of an ECG

A normal ECG consists of i. Waves ii. Intervals iii. Segments and

iv. Complexes.

WAVES: ***The waves in a normal record are named P, Q, R, S, and T, in that order.***

A typical ECG tracing of a normal heartbeat (or cardiac cycle) consists of I. a 'P wave, EE. a 'QRS complex' of 'waves', ID. a T wave.

P wave: It represents the 'atrial depolarization or atrial systole'. P wave shows that the impulse is passing through the atria. The normal duration of a P wave is - 0.1 sec.

QRS complex of 'waves': (Ventricular depolarization/ventricular systole)

i. 9 wave is a small negative wave ii. R wave is a tall positive wave iii. S wave is a negative wave. The normal duration of QRS complex of waves is about 0.08- 0.1 sec_k

T wave is a positive wave. It represents the ventricular repolarisation. Its duration is 0.2 sec.

Intervals

i. P-R interval is the interval between the onset of P wave and the onset of Q wave. P-R interval is normally 0.12 - 02 sec.
ii. Q-T interval is the interval between the onset of Q wave and the end of the T wave. It represents the electrical activity in the muscle of the ventricles (ventricular depolarisation).'QT Interval ' is dependent on the heart rate' (the 'faster' the 'heart rate* - the 'shorter' the interval). It lasts for about 0.4 sec.
iii. R-R interval signifies the duration of one 'cardiac cycle' and it lasts for about 0.8 sec. (60/72= 0.8 sec.).

Segments: S-T segment is the time period between the end of the S wave% and the onset of the T wave. It is an 'isoelectric/zero voltage' period.

Clinical Inferences from ECG

1. Enlarged P wave, indicates enlarged atria.
2. Variations in the duration, amplitude and morphology of the QRS complex indicate disorders such as bundle branch block (block of conduction of impulses through the branches of the bundle of His).
3. If the duration of the P-R interval is prolonged, it indicates delay in conduction of impulses from S-A node (pace maker) to the A-V node. P-R interval is prolonged in 'bradycardia' (slow beating of the heart) and shortened in 'tachycardia' (fast beating of the heart).
4. Prolonged Q-T interval indicates myocardial infarction (MI) and hypothyroidism. Shortened Q-T interval indicates 'hypercalcemia'
5. Elevated S-T segment indicates myocardial infarction.
6. Tall T wave indicates hyperkalemia; small, flat or inverted T wave indicates hypokalemia.

8.9 ELISA *(Enzyme Linked Immunosorbent Assay*)

It is a biochemical procedure to detect antigens' or 'antibodies' in a given sample. All microbes have at least an antigen that is unique. This antigen can be purified and used to generate specific monoclonal antibodies'- Thus MABs are made available for this procedure. Both the antibodies and the purified antigens provide effective diagnostic tools. It is a method used mainly to detect the presence of specific antibodies or antigens in a sample of serum, urine etc. It is the first and most basic 'tool' to determine if an individual is positive or negative for a particular pathogen such as HIV. It is a useful tool both for determining serum antibody concentrations (such as the antibodies produced in a person infected by pathogens such as HIV) and also for detecting the presence of specific antigens and hormones such as human chorionic gonadotropins (hCGs).

Requirements for ELISA

i. a ***microtitre plate***

ii. a purified antibody (for detecting an antigen)

iii. a purified antigen (for detecting an antibody)

iv. an enzyme that catalyses the production of colour from a chromagenic substance (mostly peroxidase or alkaline phosphatase or beta galactosidase).

v. a buffer (wash) fluid to remove unbound substances in the well.

vi. a substrate

vii. a spectrophotometer to measure the intensity of the colour of the substrate.

ELISA is a fundamental tool of clinical immunology, and the purpose of an ELISA is to determine if a particular protein is present in a sample, such as serum of a person affected by a pathogen and if so, how much.

ELISA is of two types

I. Direct ELISA - ELISA used to detect antigens

II. Indirect ELISA - ELISA done to detect antibodies Direct EUSA:

Direct ELISA is performed by using 'primaiy antibodies' (antibodies against the 'antigen' of interest) attached/adsorbed on the solid surface of the 'well' on the titre plate. This antibody has affinity for the antigen of interest (antigen for whose detection this test is being conducted). It is of two types.

I) Sandwich Assay: In this type, 'Enzyme coupled antibodies' are used. It measures the amount of antigen sandwiched' between two layers of antibodies (primary antibodies)-

The antigen to be measured should contain at least 2 antigenic sites (multivalent antigen-having several attachment sites for an antibody). Sandwich assays are used to quantify multivalent antigens such as proteins or polysaccharides.

In the illustration given you can notice how an antigen is sandwiched between two antibodies (primary antibodies) The second antibody which is tagged with the enzyme attaches to the primary antibody. When a substrate is added the enzyme acts on it and changes the colour. It happens only if the antigen is present in it. Otherwise there will be no change in colour.

II) Competitive Assay: The common 'pregnancy test' using Human Chorionic Gonadotropin (hCG), as an indicator of pregnancy is an example for which 'Competitive ELISA is conducted.

Protocol (plan of the experiment/assay)

1. Adsorption (the process by which molecules of a substance, collect on the surface of another substance) of the primary antibodies.
2. Adding the 'test sample' (blood, urine) to the TEST SYSTEM
3. If there are hCG molecules in the sample they attach to the adsorbed antibodies.
4. Now a purified hCG linked to an enzyme is added to the TEST SYSTEM.
5. Then the substrate (chromogenic substance) is added.
6. The change in the colour of the solution is measured, if there is any change.

Indirect ELISA

It is used to detect antibodies. The blood of the person undergoing the 'assay' (for example the HIV test) is allowed to clot and the cells are centrifuged out to obtain the clear serum with antibodies (called primary antibodies).

Protocol

1. It is used to detect 'antibodies'.
2. A known 'antigen' is added to the 'well' (adsorbed)
3. Patient's antiserum (serum with specific antibodies) is added
4. The 'antibodies' in the patient's 'antiserum' (primary/ complementary antibodies) bind to the antigens coated on the surface of the 'well'.
5. Enzyme linked antihuman serum globulins (anti HISGs) are added. They bind to the antibody which is already bound to the antigen.
6. Enzyme's substrate is added and the reaction produces a viable colour change which can be measured by a spectrophotometer.

If there are no "anti HIV antibodies " in the semm sample, there is no binding of primary antibodies to the antigens and so 'enzyme linked secondary antibodies' do not bind to the primary antibodies. There cannot be any enzymatic action and so no colour change is observed. The test is said to be 'Negative' and the person is described 'HIV negative'.

However ELISA can be "False positive "under certain circumstances and so it cannot be used as a confirmation test for the presence of HIV antibodies. Similarly Falsenegatives also can occur during the "window period" between infection and development of antibodies against the virus.

GLOSSARY

Apoptosis: A form of cell death in which a programmed sequence of events leads to the elimination of cells without releasing harmful substances into the surrounding area.

Assay: It means to assess- chemical testing done to determine the composition of substance or the concentration of its components.

Conventional practices: Practices which are in accordance with established norms and conventions and requirements.

Hyperkalemia: It refers to the condition in which the concentration of the electrolyte potassium (K^+) in the blood is elevated.

Hypokalemia:It refers to the condition in which the concentration of electrilytr potassium (K^+) is low.

Insulin pump: it is a medical device used for the administration of the insulin in the treatment for diabetes mellitus.

Milch animal: Domestic animal suitable for milk production.

P53 protein: it is a tumour suppressing protein which is crucial in multicellular organisms.

Serum: A clear liquid that separates from blood when it is allowed to clot completely, and is therefore blood's plasma from which fibrinogen has been removed during clotting.

VERY SHORT ANSWER TYPE QUESTIONS

1. What factors constitute dairying?
2. Distinguish between a drone and worker in honey bee colony?
3. Define term fishery?
4. List out any two Indian major carps?

5. Define the term ‘Vaccine’?
6. Define the term ‘transgenic animal’?

SHORT ANSWER TYPE QUESTIONS

1 Define term ‘breed’ what are the objective of animal breeding?

2 Explain in brief about Queen bee?

3 Honey bees are economically important-Justify ?

4 Explain in brief the structure of insulin?

5 Explain the different types of cancers?

6 Write about the procedure in MRI?

7 Write briefly about the different waves and intervals in an ECG?

8 Discus briefly the process of indirect ELISA?

BOARD OF INTERMEDIATE EDUCATION, A.P, VIJAYAWADA
REVISED **SYLLABUS** FOR VOCATIONAL BRIDGE COURSE
ZOOLOGY 2ND YEAR (w.e.f. 2018-19)

UNIT-I: Human Anatomy and Physiology-I

I-A: Digestion and Absorption

1.1.1 Alimentary canal structure

1.1.2 Digestion in stomach and small intestine

1.1.3 Absorption and assimilation

I-B: Breathing and exchange of gases

1.2.1 Inspiration and expiration-structure of respiratory tract

1.2.2 Transport of respiratory gases

UNIT-II: Human anatomy and Physiology-II

II-A: Body fluids and circulation

2.1.1 Lymph and blood

2.1.2 Structure of Heart

2.1.3 Working of Heart

II-B: Excretory products and their elimination

2.2.1 Excretory system-structure of kidney and nephron

2.2.2 Urine formation

UNIT-III: Human anatomy and Physiology-III

III-A: Musculo-skeletal system

3.1.1 Structure of skeletal muscle

3.1.2 Mechanism of muscle contraction

3.1.3 Skeleton – Skull-vertebral column- sternum

3.1.4 Fore limbs-Hind limbs- Pectoral and Pelvic girdles

3.1.5 Joints-Synovial joint –Ball and Socket-Pivotal-Hinge

III-B: Neural control and Coordination

3.2.1 Central Nervous system-Synaptic transmission-structure of Eye and Ear

UNIT IV: Human anatomy and Physiology-IV

IV-A: Endocrine system and chemical coordination

4.1.1 List of endocrine glands and detailed study of Pituitary, Thyroid, Adrenal and pancreas glands and their secretions

4.1.2 Hormonal disorders – Addison's disease and Cushing's syndrome-mechanism of hormonal action

IV-B: Immune system

4.2.1 Vaccination

4.2.2 Immunological disorders- HIV, hypersensivity

UNIT-V: Human Reproduction

V-A: Human reproductive system

5.1.1 Male Reproductive system

5.1.2 Female reproductive system

5.1.3 Fertilization

V-B: Reproductive Health

5.2.1 Sexually Transmitted Diseases

5.2.2 Birth control-need and methods

5.2.3 Assistant reproductive technology –surrogacy

UNIT-VI: Genetics

6.1 Multiple alleles and human blood groups

6.2 Sex determination

6.3 Mendelian disorders (any 4)

Chromosomal disorders (any 4)

UNIT-VII: Organic Evolution

7.1 Introduction of origin of life

7.2 Biological Evolution

7.3 Evidences of biological evolution

7.4 Brief introduction of theories of evolution (Lamarkism,Darwinism and Mutation theory

7.5 Speciation

UNIT-VIII: Applied Biology

8.1 Animal husbandry-Management-dairy farm management

8.2 Bee-keeping

8.3 Fishery management

8.4 Biotechnological application in medicine- Insulin

8.5 Vaccines – gene therapy-transgenic animals

8.6 Stem cells

8.7 Types of Cancers and Causes

8.8 Biomedical technology-CAT-MRI-ECG

8.9 ELISA

BOARD OF INTERMEDIATE EDUCATION, A.P, VIJAYAWADA

QUESTION BANK FOR VOCATIONAL BRIDGE COURSE

ZOOLOGY 2ND YEAR (w.e.f. 2018-19)

UNIT-I: Human Anatomy and Physiology-I

I-A: Digestion and Absorption

Very short answer questions

1 Give the dental formula of adult human being?
2 Explain the terms Thecodont and Dyphiodont ?
3 What is Chyme?
4 Name different types of papillae on the tongue of man?
5 What is the hardest substance in the human body? What is its origin?
6 Distinguish between absorption and assimilation ?

Short Answer questions

1 Draw a neat labeled diagram of L.S. of Tooth?
2 Describe the process of digestion of proteins in the stomach?
3 What are the functions of the liver?

I-B: Breathing and exchange of gases

Very short answer questions

1 What are conchae?
2 What is meant by chloride shift?
3 Name the muscles that help in normal breathing movements?

Short Answer questions

1 What are the major transport mechanisms of CO_2? Explain?
2 Explain the process of Inspiration and Expiration under normal conditions?

UNIT-II: Human anatomy and Physiology-II

II-A: Body fluids and circulation

Very short answer questions

1 Sino Atrial Node is called the pacemaker of our heart . Why?
2 Where is the valve of Thebesius in the heart of man?
3 Name the heart sounds. When are they produced?
4 Define cardiac cycle and cardiac output?
5 What is meant by double circulation ? What is its significance ?

Short Answer questions

6 Draw a labeled diagram of the L.S.of the heart of man?
7 Distinguish between SAN and AVN?
8 Describe the events in a cardiac cycle briefly?

II-B: Excretory products and their elimination

Very short answer questions

1 What are the columns of Bertin?
2 Define glomerular filtration?
3 Name the blood vessels that enter and exit the kidney?

Short Answer questions

1 Draw a neat labeled diagram of the V.S. Kidney ?
2 Describe the internel structure of kidney of man?

UNIT-III: Human anatomy and Physiology-III

III-A: Musculo-skeletal system

Short answer questions

1 List out the bones of human cranium?
2 Write a short note on sliding filament theory of muscle contraction?
3 List the bones human fore limbs?
4 List the bones the human leg limbs?
5 Describe the structure of Synuvial joint with the help of neat labeled diagram?

III-B: Neural control and Coordination

Short answer questions

1 Draw a labeled diagram of T.S. of the spinal cord of man?
2 Give an account of the Retina of the human eye?

UNIT IV: Human anatomy and Physiology-IV

IV-A: Endocrine system and chemical coordination

Short answer questions

1 List out the names of endocrine glands present in human beings and mention the hormones?
2 Give an account of the secretions of Pituitary glands?
3 Write a note on Addison's disease and Cushing's Syndrome?

IV-B: Immune system

Short answer questions

1 Explain the mechanism by which HIV multiplies and leads to AIDS?

UNIT-V: Human Reproduction

V-A: Human reproductive system

Very short answer questions

1 What are the functions of sertoli cells of the seminiferous tubules and the leydig cells in man?

2 Define spermeogenis and spermiation?
3 Name the copulatory structure of man?
4 What are three columns of tissues in it?

Short answer questions

1 5.2.1

V-B: Reproductive Health

Very short answer questions

1 What are measures one has to take to prevent contracting STDs?
2 "It is true that MTP is not meant for population control". Then why did the Government of India legalized MTP?

Short answer questions

1 Briefly describe the common sexually transmitted diseases in human beings?
2 Describe the surgical methods of contraception?

UNIT-VI: Genetics

Very short answer questions

1 What are multiple alleles?
2 What is erythroblastosis foetalis?
3 What is Down syndrome ?
4 What is Turner's syndrome?

Short answer questions

1 How is sex-determined in human beings?
2 Descibe erythroblasosis foetalis?
3 Describe the genetic basis of ABO blood grouping?
4 Mention any two autosomal genetic disorders with their symptoms?

UNIT-VII: Organic Evolution

Very short answer questions

1 What are panspermia?
2 Define biogenetic law giving an example?
3 What is common between Darwinism and Lamarckism?
4 Mention names of any four connecting links that you have studied?
5 Distinguish between Allopatric and Sympatric speciation?

UNIT-VIII: Applied Biology

Short answer questions

1 Define term breed "what are the objective of animal breeding?
2 Explain in brief about Queen bee?
3 Honey bees are economically important-Justify ?
4 Explain in brief the structure of insulin?
5 Explain the different types of cancers?
6 Write briefly about the different waves and intervals in an ECG?
7 Discus briefly the process of indirect ELISA?

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WEIGHTAGE OF MARKS FOR VOCATIONAL BRIDGE COURSE

ZOOLOGY 2ND YEAR (w.e.f. 2018-19)

S.No of	Topics	No. periods	Weightage Marks
1	**UNIT-I: Human Anatomy and Physiology-I** **I-A: Digestion and Absorption** **I-B: Breathing and exchange of gases**	**08**	**06**
2	**UNIT-II: Human Anatomy and Physiology-II** **II-A: Body fluids and circulation** **II-B: Excretory products and their elimination**	**08**	**05**
3	**UNIT-III: Human anatomy and Physiology-III** **III-A: Musculo-skeletal system** **III-B: Neural control and Coordination**	**06**	**04**
4	**UNIT IV: Human anatomy and Physiology-IV** **IV-A: Endocrine system and chemical coordination** **IV-B: Immune system**	**06**	**04**
5	**UNIT-V: Human Reproduction** **V-A: Human reproductive system** **V-B: Reproductive Health**	**08**	**06**
6	**UNIT-VI: Genetics**	**06**	**05**
7	**UNIT-VII: Organic Evolution**	**04**	**02**
8	**UNIT-VIII: Applied Biology**	**04**	**08**
	Total	**50**	**40**

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MODEL QUESTION PAPER FOR VOCATIONAL BRIDGE COURSE

ZOOLOGY 2ND YEAR (w.e.f. 2018-19)

Time: 1 ½ Hours

Max.Marks:25

Section-A 5x1=5

Note: 1) Answer any five of the following

2) Each question carries one mark

1. What is chime?
2. What is meant by chloride shift?
3. What is meant by double circulation?
4. Name the copulatory structure of man? What are three columns in it?
5. What are the methods one has to take to prevent contracting STDs?
6. What are multiple allels?
7. Define biogenetic law?
8. Distinguish between allopatric and sympatric speciation ?

SECTION-B 5x4=20

Note: 1) Answer any five of the following

2) Each question carries four marks

3) Draw a neat labeled diagram wherever necessary

9. Describe the process of digestion of proteins in the stomach?
10. Draw a neat labeled diagram V.S. of kidney?
11. Write a short note on sliding filament theory in muscle contraction?
12. Explain the mechanism by which HIV multiplies and leads to AIDS?
13. Briefly describe common STDs in human beings?
14. Describe erythroblasosis foetalis?
15. “Honey bees are economically important”. Justify?
16. Explain the different types of cancers?