# UGZY/BY-08

# BLOCK - 1

# ANIMAL PHYSIOLOGY

Uttar Pradesh
Rajarshi Tandon Open University

# UGZY/BY-08
# Physiology

Block

# 1

## ANIMAL PHYSIOLOGY — I

# PHYSIOLOGY

Physiology is the study of the functions of living organisms and their constituent parts. You will learn in physical and chemical terms, the mechanisms that operate in living organisms at all levels, ranging from the subcellular to the integrated whole organism.

The course on Cell Biology (LSE-01) would have familiarised you with the underlying unity in the functioning of cells. You have learnt in that course that at cellular level there is a striking similarity in molecular mechanisms of plants and animals: be it catabolic pathways or biosynthesis. You would note that the concepts and principles that provide a basis of understanding cellular functions are few for evolution has been both conservative as well as innovative. However, it is remarkable that the similarities are confined to the cellular level and at higher levels of organisation among plants and animals, one witnesses an enormous diversity in their functioning. Nevertheless you will find that at lower levels of organisation, similarities still exist, and differences are more pronounced between higher plants and higher animals.

For example, plants have evolved a unique machinery for synthesising their food and animals being heterotrophic have to depend on them for nutrition. Apart from the mode of nutrition, the processing of ingested food material is distinctly different in plants and animals. Animals have evolved an elaborate digestive system and associated glands. They have a transport system dependent on an efficient pumping mechanism for the distribution of nutrients and other substances, for delivery of respiratory gases and removal of waste material. Plants, although devoid of an active pump (like heart) have indeed a transport system which carries minerals, water, food molecules and other substances to all parts of the plant body. Though the respiratory mechanism is similar at the cellular level, at organismic level plants and animals have evolved separate systems for gas exchange.

One significant physiological difference though is, that plants like lower animals do respond to stimuli, but they possess no system similar to the organised nervous system of higher animals.

The basic principles and mechanism of physiology of animals and plants form the theme of this course on Physiology. It is divided into four blocks. The first and second blocks deal with animal physiology while the third and fourth blocks are concerned with the physiology of plants.

# BLOCK 1 ANIMAL PHYSIOLOGY—I

This block is concerned with the physiology of animals. How they eat, breathe, eliminate their wastes and maintain an internal steady state. For convenience of study, the physiology of animals can be subdivided into a number of processes like respiration, circulation, digestion and absorption, excretion etc. But we must remember that none of these processes work in isolation, they are all interconnected and interdependent. The brain for example, cannot function without a constant supply of blood, carrying oxygen and glucose, provided by the pumping of the heart. Similarly the heart cannot survive for more than a few minutes without oxygen supplied by the lungs via the circulating blood. The lungs in turn cannot function without the commands to respiratory muscles by the brain! Many similar examples can be given at the subcellular level of functioning.

Now that you have a basic knowledge of the functioning at cellular level we thought that it would be more meaningful to study physiology in organism using a comparative approach. By examining how different animals have solved their problems of living within the boundaries of their different environments, we gain an insight into the general principles of physiology that may be overlooked otherwise. We have, therefore, used a comparative approach in our study of the various physiological functions. However, more examples have been chosen from mammals including man to explain physiological functions and much of it applies to other vertebrates. Wherever any facts or observations are peculiar to any species or to man, that particular species has been mentioned.

This block begins with a description of how animals obtain their energy supply, that is, food in Unit 1 — Nutrition, Feeding, Digestion. Various adaptations to obtain food have been discussed. The later part of the unit describes digestion and absorption of food in vertebrates.

The second Unit — Respiration, explains how animals obtain oxygen from their environment, whether in water or in air. The unit reviews the transport of oxygen and carbon dioxide in the blood and the role of respiratory pigments, that have evolved in animals to facilitate the movement of these two gases between the blood and the tissues.

Unit 3 — Circulation, deals with circulation of body fluids and how they are controlled to meet the requirements of the tissues. Major emphasis is given to mammalian circulation as it is the best known.

One of the requirements in regulation of the internal environment in animals is, that appropriate quantities of water and nutrients be retained. To this is related very closely the problem of elimination of toxic wastes that accumulate during metabolism of amino acids and proteins. Unit 4 — Excretion, deals with the mechanisms animals have adopted to eliminate the nitrogen containing end-products of protein catabolism. Structure and functions of the excretory organs of various animals has also been discussed in this Unit.

Unit 5 — Osmotic and Ionic Regulation, considers the osmotic environment and osmoregulatory processes in organs such as kidney, gills and salt glands. It also touches upon their regulation by several hormones, thus emphasising the interconnections between the functioning and regulation of various organ systems.

## Study Guide

Before starting your study of this block you should have studied the course on Cell Biology (LSE-01). We have assumed that you are familiar with the basic concepts of cellular respiration; membrane structure and transport processes in the cell. We have also tried to make physiological principles understandable in terms of basic physics and chemistry. If more information is needed, we suggest that you keep the NCERT Class XI and XII textbooks of physics and chemistry at hand.

Some interesting experiments that have been crucial in explaining a particular concept have been enclosed in boxes throughout the text. These have been included to give you an idea as to how scientists work and come to conclusions. The glossary given at the end of the block will be particularly useful if you happen to be in a habit of reading the units in a differing order from the one given in the block!

## Objectives

After studying this block you will be able to :

- discuss the various components of nutrition, feeding strategies and process of digestion and absorption in animals,
- compare the various modes of respiration in aquatic and terrestrial animals and explain the transport of gases,
- describe the circulation of fluids in animals with particular emphasis on mammals,
- discuss the mechanisms adopted by animals to eliminate their toxic waste materials, and
- discuss the problems faced by aquatic and terrestrial animals while regulating their osmotic and ionic balance.

- distinguish between intracellular and extracellular digestion of proteins, carbohydrates and fats and explain the role of gastrointestinal hormones
- summarise the process of absorption of food from the alimentary canal
- explain energy metabolism in animals relating it to oxygen consumption.

## 1.2 NUTRITION

As we have said earlier all animals are heterotrophs and require food from the environment. What is this food made up of? If the food of a number of different animals is broken down we find that it consists of proteins, carbohydrates, fats, water, minerals and vitamins.

All animals require the above-mentioned broad categories of nutrients although in different amounts. Some of these nutrients are used mainly as fuel (carbohydrates and fats), while others are required principally as structural and functional components (proteins, minerals and vitamins). However, proteins, carbohydrates and fats can all serve as fuel for the body's energy needs, but no animal can survive on fuels alone. Therefore, a balanced diet is needed to meet all the requirements of the body for energy, growth, maintenance, reproduction and physiological regulation. Now let us discuss the importance of these different classes of food in relation to animal nutrition.

### 1.2.1 Proteins

Proteins are continually synthesised in the cells as they are the principal component required for growth. Proteins are composed of amino acids which are derived largely from the diet and partly from the breakdown of protein available in the body.

The terms essential and non-essential amino acids are not very significant because the non-essential amino acids are just as important for the body. May be so important that the body cannot leave them to be supplied externally and so has mechanisms to synthesise them.

Generally all proteins are made from about 20 different amino acids in various combinations. However, it is not necessary to supply all the 20 amino acids. Some can be formed in the body, using other amino acids but others have to be supplied through diet because they are not formed in the body. The amino acids that are synthesised in the body are called **non-essential amino acids** while those that have to be supplied through diet are known as **essential amino acids**.

The requirement of essential amino acids differs from organism to organism. Some bacteria require only one amino acid in sufficient quantities in the growth medium to be able to synthesise the rest. In contrast mammals certainly cannot fulfil their protein requirements by only one amino acid.

How can one determine which amino acid is essential and which is non-essential? The nutritional requirements are determined by deletion experiments i.e. by removing a single nutrient from the diet and then observing the growth and health of the animal. By this method it was found out that 10 amino acids are essential for the growth and well-being of rats (see Table 1.1).

**Table 1.1 : Amino acids classified according to dietary needs of humans and rats**

| Essential | | Non-essential | |
|---|---|---|---|
| Rats | Humans | Rats | Humans |
| Lysine | Phenylalanine | Glycine | Glycine |
| Tryptophan | Lysine | Alanine | Alanine |
| Histidine | Isoleucine | Serine | Serine |
| Phenylalanine | Leucine | Cysteine | Tyrosine |
| Leucine | Valine | Tyrosine | Aspartate |
| Isoleucine | Methionine | Aspartate | Glutamate |
| Theronine | Cystine | Glutamate | Proline |
| Methionine | Tryptophan | Proline | Hydroxyproline |
| Valine | Theronine | Hydroxyproline | Citrulline |
| Arginine | | Citrulline | Histidine |
| | | | Arginine |

b) Why don't domestic cats and dogs need fruit in their diet while humans do?

.....

.....

.....

.....

.....

## 1.3 FEEDING MECHANISMS

All animals have evolved successful methods for extracting their required nutrition from the environment. Thus we find a diversity of feeding mechanisms or strategies according to the nature of food that an animal can obtain. Table 1.6 lists the major feeding methods in animal groups based on the type of food available. It would not be possible to discuss each food gathering device in detail but in a brief discussion we shall consider the basic principles on which the different feeding mechanism operate. From Table 1.6 you will note that taxonomically different animal groups living in the same habitat obtain food in a similar manner. For example, many marine animals (annelids, molluscs, crustaceans) may be **filter feeders** though the organs concerned with the process of filtration may not be anatomically similar.

**Table 1.6 : Feeding methods classified according to type of food**

| Type of food | Method of feeding | Animals using the method |
|---|---|---|
| Small particles | Digestive vacuoles | Amoeba, Radiolarians |
| | Use of cilia | Ciliates, Sponges, Bivalves, Tadpoles |
| | Mucous traps | Gastropods, Tunicates |
| | Tentacles | Sea cucumbers |
| | Filter feeding | Small Crustaceans, Herrings, Baleen Whales, Flamingoes, Petrels |
| Large food masses | Ingestion of inactive masses | Detritus feeders, Earthworm |
| | Scraping, chewing, boring | Sea urchins, Snails, Insects, Vertebrates |
| | Capture and swallowing of prey | Coelentrates, Fishes, Snakes, Bats, Birds |
| Fluid or soft tissue | Sucking plant sap, nectar | Aphids, Bees, Humming-birds |
| | Ingestion of blood | Leaches, Ticks, Insects Vampire bats |
| | Sucking of milk or Similar secretions | Young Mammals, Young Birds |
| | External digestion Uptake from body surface | Spiders Parasites, Tapeworm |
| Dissolved organic solution | Uptake from dilute solution | Aquatic invertebrates |
| Symbiotic supply of nutrients | Intracellular symbiotic algae | Paramecium, Sponges, Flatworms, Corals, Hydras, Clams. |

### 1.3.1 Feeding on Small Particles

Microscopic algae and bacteria can be taken in directly into the cell by the digestive vacuoles. But one of the most successful methods of feeding on small particulate matter is **filter feeding** or **suspension feeding**. Particulate matter includes detritus, living and dead plankton. Most filter feeders use ciliated surfaces to produce currents that draw drifting food particles into the mouth. The animal extracts the suspended food particles by means of structures that act as filters often aided by secretion of mucous which traps the food particles. In sponges, the flagella of the **choanocytes**,

True mastication i.e. chewing of food is found only in mammals. Their teeth are adapted for this specific function. Mammals have basically four types of teeth (Fig. 1.7) each adapted for different type of feeding. Incisors are adapted for biting and cutting and stripping; canines for seizing and piercing; premolars for crushing; and the molars for crushing and grinding. The number and size of these teeth varies according to the type of food eaten.

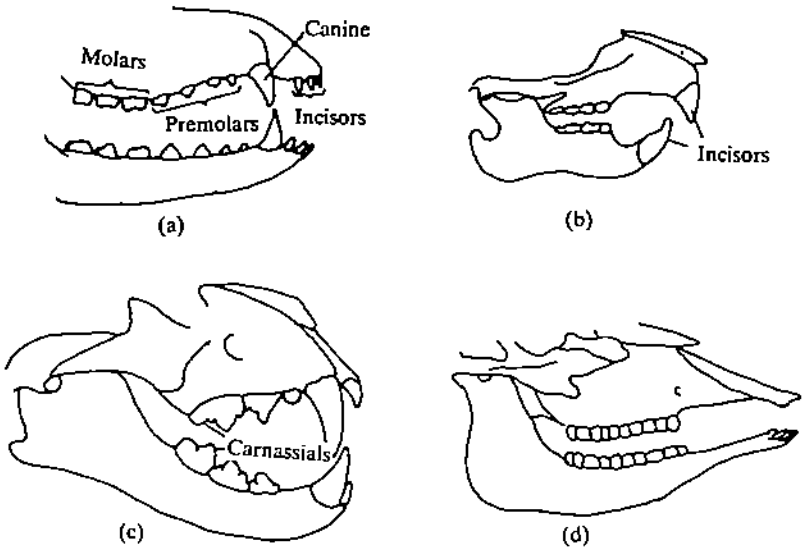



Fig. 1.7 : Mammalian dentition — teeth of (a) generalised mammal, (b) squirrel, (c) African lion, (d) ox

Spiders provide an interesting example of fluid feeding. Their prey are usually larger in size and covered by a hard chitinous covering. Spiders, therefore, pierce the covering by their hollow jaws and pump digestive juices into the prey's body. These liquify the tissues and then the spider sucks the prey empty.

## 1.3.3 Feeding on Liquids

Animals feeding on liquids are generally highly specialised for their feeding habits. Certain protozoa, endoparasites and aquatic invertebrates take up nutrient molecules through their integument from the medium in which they live. For example, endoparasites, which include parasitic protozoa, tapeworms, flukes, certain molluscs and crustaceans are surrounded by host tissue or alimentary canal fluids which are highly nutritive. These parasites lack a digestive system of their own.

All of us are familiar with insects that have well-developed piercing and sucking organs. Mosquitoes, bedbugs and lice and leaches among annelids are some examples. They use anticoagulant to prevent blood from clotting as it leaves the blood vessels ruptured by their piercing or rasping jaws.

**SAQ 2**

a) You must have observed a squirrel, a cow and a dog feeding. What kind of differences would you expect to find in their dentition?

.....

.....

.....

.....

b) Match the type of feeding apparatus in column A with the kind of food in column B.

| Column A | Column B |
|---|---|
| 1) Radula | a) Blood, plant sap |
| 2) Cilia | b) Detritus in mud |
| 3) Mucous Sheets | c) Large chunks of food |
| 4) Sucking mouth parts | d) Algae on rocks |
| 5) Teeth | e) Suspended particles |

## 1.4 DIGESTION

In the earlier sections we considered the nutritional requirements and the various ways used by heterotrophic organisms to obtain nutrition. Whether food is used to give energy or to build the body, the large molecules of food have to be broken down into simpler constituents before they can be used by the body. **The process by which the food is broken down into simpler molecules is known as digestion.** This breakdown is achieved with the aid of enzymes and can take place inside the cell — intracellular digestion or outside the cell — extracellular digestion often in a specialised digestive tract.

Let us first consider intracellular digestion and see how it is different from extracellular digestion.

### 1.4.1 Intracellular Digestion

We all know that unicellular organisms do not have a separate alimentary canal system. All the functions of life are carried out inside a single cell. Food is taken in directly into a cell by phagocytosis/endocytosis and then with the help of enzymes digested in a food vacuole. Fig. 1.8 shows the process of endocytosis in *Amoeba*.

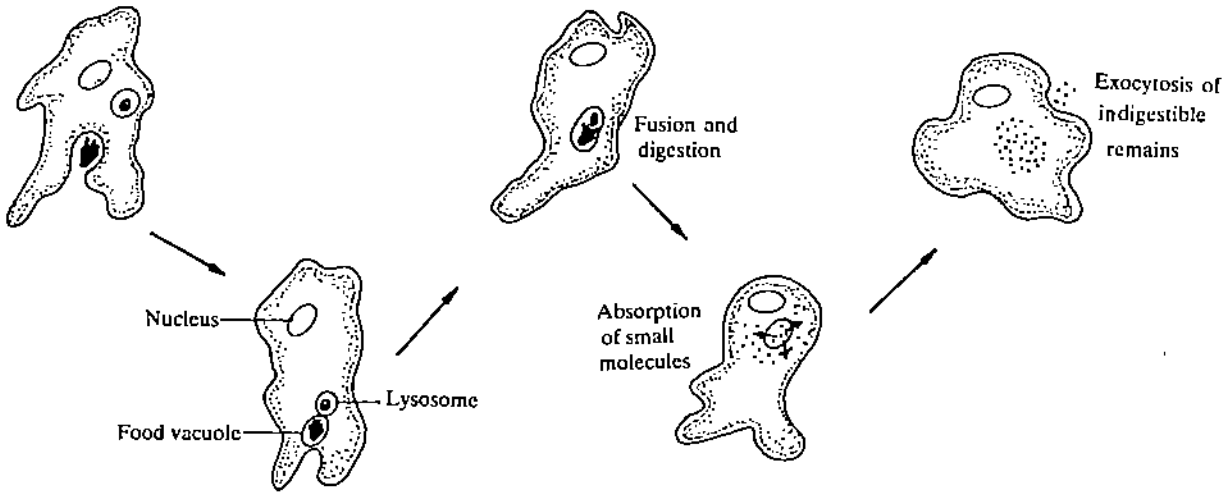



Fig. 1.8 : Digestion in amoeba

Similar intracellular digestion occurs in sponges, some coelentrates, ctenophores and turbellarians. Although the process is called intracellular digestion, the food material is actually separated from the rest of the cellular material by a membrane which it can cross after digestion. In organisms such as cnidarians and platyhelminths, a gut or enteron is present and here along with extracellular digestion where enzymes are secreted into the cavity, intracellular digestion also takes place within the cells that line the cavity. However, in annelids and molluscs more extracellular than intracellular digestion takes place. Digestion is entirely extracellular in nematodes, insects, echinoderms and vertebrates.

### 1.4.2 Digestive Tract

Extracellular digestion takes place in a tubular cavity that extends throughout the length of the organism. All animals after flatworms have a tubular alimentary organisation open at both ends. The development of extracellular digestion freed many animals from feeding continuously on small particles. They could now quickly ingest a few large chunks of food. The overall tubular organisation of the digestive tract also allows the food to travel in one direction passing through regions of digestive specialisation (Fig. 1.9).

In general the digestive system of metazoans is divided into 4 major functional regions of:

- reception
- conduction and storage
- digestion and absorption
- conduction and formation of faeces.

The region for reception is associated with devices for mastication or chewing of food (like teeth); for paralysing the struggling prey (toxic enzymes from saliva); initiating digestion and lubricating the food with mucous.

The oesophagus of chordates and some invertebrates serves to conduct the bolus (mass of chewed food) by peristaltic movement from buccal cavity. In some animals this region has a crop for storage. The crop in birds is also used to ferment mildly or digest food. This is later regurgitated by parent birds for their nestlings. The storage region allows the animals to store food and use it when it is not easily available. For example, leaches take in infrequent large meals of blood and digest it slowly over a month. The herbivore animal spends hours masticating the food it takes in hurridly and stores it in its stomach for further use.

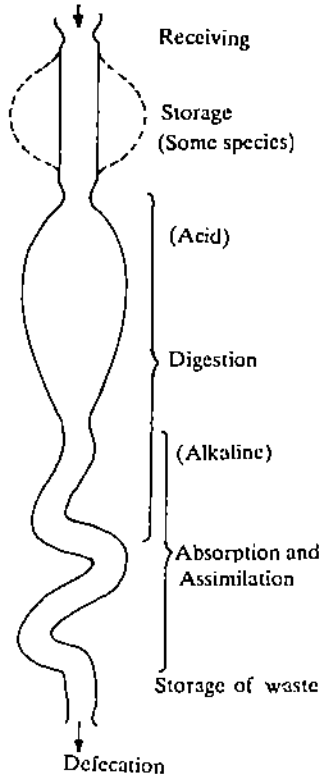



Fig. 1.9 : Generalised digestive tract. One way passage of food allows sequential stages in digestion. Dashed lines represent crop in some animals.

In the third region or digestive region the enzymes reduce the food to a form that can be absorbed by the body of the organism. As the food is digested, the absorbable food is passed to the blood stream and the unabsorbed material is stored briefly in the final section of the alimentary canal where further removal of excess water and consolidation of undigested material into faeces takes place, before it is expelled out of the body. In vertebrates this function is carried out in the large intestine.

In higher vertebrates, each area of the gut is specialised for a certain activity, digestive enzymes are produced in glands as well as in the wall of the gut. Absorption occurs in the intestine predominantly.

## 1.4.3 Digestive Enzymes

Now let us consider the general principles of digestion that are applicable to all animals. We will start with the digestive enzymes that breakdown the large food molecules into smaller soluble component units. This breakdown involves the uptake of water and is called **hydrolysis**. Before reading the following sub-sections, you would find it useful to read Units 9 and 10 of LSE-01 to recapitulate the nature and properties of enzymes in general. However, digestive enzymes differ in the following ways:

a) Digestive enzymes are not as narrowly specific as other enzymes rather they show **group specificity**. For example, enzymes that digest carbohydrates can digest polysaccharides of both animal and plant origin.

b) Even though enzymes performing similar functions in different animals are given same names, they are not identical chemically. For example, trypsin (an enzyme, that hydrolyses proteins) in humans is not identical to that found in fish. Temperature and pH for optimum activity is also different. For example, trypsin from vertebrate pancreas acts best in the pH range of 7–9 but in silkworm the pH range is 6.2–9.

c) Digestive enzymes from pancreas particularly those that digest proteins are secreted in an inactive form.

The three major classes of digestive enzymes are:

i) **Proteases** that hydrolyse peptide bonds in proteins,

ii) **Carbohydrases** that hydrolyse glycosidic bonds in carbohydrate,

iii) **Lipases** that hydrolyse ester bonds in fats

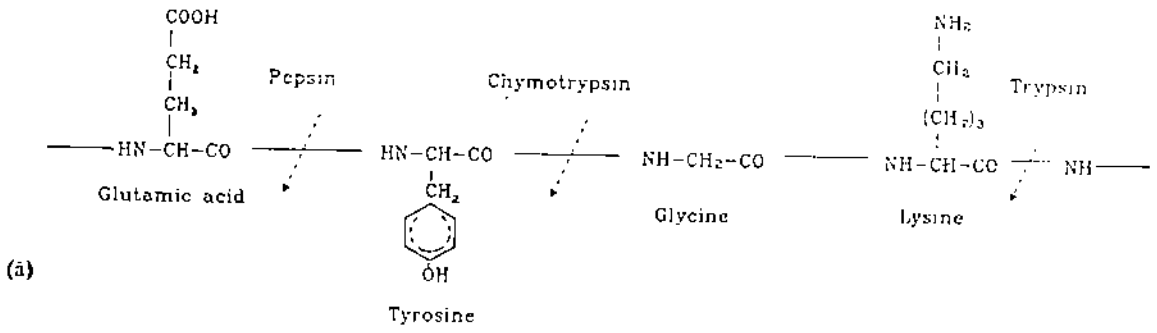
### Protein Digestion

Enzymes that digest proteins are divided into two groups **endopeptidases** and **exopeptidases** according to site of their action in the protein molecule. Endopeptidases confine their attack to the interior of the protein molecule so that the large peptide chain is broken into smaller fragments. This provides many sites for action of exopeptidases that attack only peptide bonds at the end of a peptide chain releasing amino acids, dipeptides and tripeptides. There are several types of endopeptidases and exopeptidases. They are listed in Table 1.7.

**Table 1.7 : Proteases of Animals**

| Extracellular Enzyme<br>Inactive form $\xrightarrow[\text{autocatalyst}]{\text{Activator}}$ Active form | Preferred Peptide Link Attacked |
|---|---|
| **Endopeptidases** | |
| Pepsinogen $\xrightarrow[\text{pepsin}]{\text{HCl}}$ Pepsin | Link to amino group of aromatic amino acid (tyrosine and phenylalanine) |
| Trypsinogen $\xrightarrow[\text{trypsin}]{\text{enterokinase}}$ trypsin | Link to carboxyl end of arginine or lysine |
| Chymotrypsinogen $\xrightarrow{\text{trypsin}}$ chymotrypsin | Link to carboxyl group of aromatic amino acid (tryptophan, tyrosine, phenylalanine) and also bonds adjacent to methionine and leucine when they are present |
| **Exopeptidases** | |
| Aminopeptidase ($Mn^{2+}$, $Mg^{2+}$, $Zn^{2+}$) | Link to terminal amino acid with free amino group |
| Carboxypeptidase ($Zn^{2+}$) (trypsin) | Link to terminal amino acid with free carboxyl group |
| Dipeptidase ($Mn^{2+}$, $Mg^{2+}$, $Zn^{2+}$) | Bonds between pairs of amino acids |

From the table you can see that these exopeptidases and endopeptidases attack specific peptide bonds depending on the chemical group near them. The inactive forms need activators and autocatalysts to convert them into active forms. For example pepsinogen is secreted by the vertebrate stomach. The stomach also secretes HCl which makes the medium acidic (pH 2). This activates pepsinogen into **pepsin.** Pepsin specifically hydrolyses peptide bond between a dicarboxylic and an aromatic amino acid (Fig. 1.10a). In this way short fragments of polypeptide chains are formed. Invertebrates seem to lack pepsin and their main endopeptidase is more like trypsin. Look at Fig. 1.10a again, chymotrypsin also attacks a peptide bond involving aromatic amino acid but on the carboxyl terminal end of the molecule while pepsin attacks on the amino terminal end.



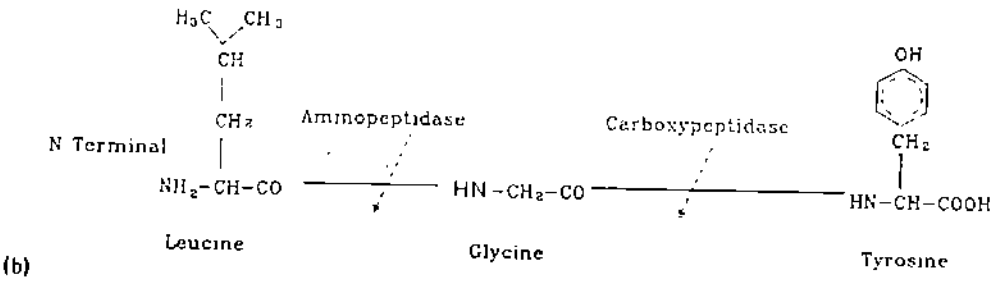




**Fig. 1.10 : Protein digesting enzymes:**
**a) attacking specific peptide bonds in a protein fragment**
**b) breakdown of a tripeptide. However, both exopeptidases cannot attack the same tripeptide**

**Trypsin** is secreted by the pancreas in an inactive form **trypsinogen**. It is activated by enterokinase secreted by the glands in the intestinal wall. As trypsin is formed, it activates more trypsinogen to be converted into trypsin. This is **autocatalytical activation**. Trypsin acts in an alkaline medium between pH 7-9. It breaks a peptide bond next to basic amino acid like arginine or lysine.

The polypeptide fragments are further digested by the exopeptidases. Carboxypeptidase require the presence of zinc ion and trypsin. Other exopeptidases are secreted in active form but need metal ions as cofactors to increase their activity.

Fig 1.10(b) illustrates the action of **aminopeptidase** which removes terminal amino acids having free amino groups and **carboxypeptidase** which removes terminal amino acids possessing a free carboxyl group. In this way these two enzymes remove peptides from each end until a dipeptide fragment consisting of only two amino acids remains. Bonds between these pairs of amino acids are split by **dipeptidases** releasing free amino acid.

The amino acids now, may be absorbed through the cells of the intestinal wall.

### Carbohydrate Digestion

Simple sugars like glucose and fructose can be absorbed and metabolised directly but disaccharides such as sucrose or lactose and polysaccharides such as starch and glycogen have to be broken down to monosaccharides before they can be used in metabolic pathways. **Carbohydrases**, that digest carbohydrates can be grouped into two categories:

i) **Polysaccharases** that split polysaccharides into disaccharides or trisaccharides.

ii) **Glycosidases** that break up the disaccharides or trisaccharides to monosaccharides.

The digestion of carbohydrates, like proteins also occurs in steps. These along with the enzymes responsible for digestion are given in Table 1.8.

**Table 1.8 : Digestion of carbohydrates**

| Polysaccharides $(C_6H_{10}O_5)_x$ | Polysaccharases | Disaccharides $(C_{12}H_{22}O_{11})$ | Glycosidases | Monosaccharide $(C_nH_{2n}O_n)$ |
|---|---|---|---|---|
| Glycogen (animals) | Amylases | Maltose | Maltase | Glucose |
| Starch (Plants) | Amylases | Maltose | Maltase | Glucose |
| Cellulose (Plants & animals) | Cellulases | Cellobiose | Cellobioses | Glucose |
| | | Trehalose (insects and some plants) | Trehalase | Glucose |
| | | Lactose | Lactase | Galactose Glucose |
| | | Sucrose | Invertase | Fructose Glucose |

Between 2–18 per cent of caucasians loose the capacity to produce lactase and between 95–100 per cent oriental and native African races loose the ability to produce lactase as they grow older. They can no longer digest milk which ferments in their gut and produces diarrhoea and related problems. Interestingly yoghurt and cheese do not create any problems as these contain less than 2 per cent lactose due to action of bacteria.

Carbohydrate digestion in vertebrates and invertebrates is very similar. All the enzymes shown in Table 1.8 are not required by all animals. The enzymes present are related to the food habits of the animal. However, amylase and maltase are of universal occurrence. Amylase is secreted in the saliva of man and in larger amounts by the pancreas. Enzyme production in some animals is also influenced by genetic characteristics and enzyme induction. For example, production of maltase and sucrase by the intestinal villi depends on the amount of ingested sugar. If a high maltose or sucrose diet is taken it induces the villi to produce more maltase and sucrase within 2-5 days. Lactase production declines in humans as gut develops after infancy. It ceases in some individuals so that they can no longer hydrolase this

sugar. Now let us consider the digestion of cellulose, the most important structural material of plants and a major component of the diet of herbivores. Very few animals possess the enzyme-cellulases. Then how do animals that feed on plants breakdown this carbohydrate? Cellulases enzymes are synthesised by many bacteria and protistans which live symbiotically in many herbivores and insects. Cellulose digestion is carried on by the help of these **symbiotic microorganisms**. The microorganisms live in the stomach of the ruminants (i.e. cow, sheep, etc.) and breakdown the cellulose. The breakdown products are then utilised by the host. In some invertebrates like silver fish (*Ctenolepisma lineata*) true cellulases have been reported but the insect cannot survive on an only cellulose diet. Some other invertebrates also have some cellulases that partly digest cellulose but none show conclusive evidence of a complete breakdown of cellulose into glucose without the help of symbionts.

Symbiotic flagellates from termites are obligate anaerobic organisms. Because of this sensitivity these flagellates can be removed from termite gut by exposing termites to oxygen at 3.5 atm pressure. The protozoa are selectively killed within half an hour and the termites survive. Such treated termites do not survive when fed on wood though they still have bacteria in the gut. This shows that anaerobic protozoans rather than bacteria, are responsible for cellulose digestion in termites.

**Lipid Digestion**

Digestion of fats is also similar in both invertebrates and vertebrates. **Lipases** are the enzymes that hydrolyse fats. A single lipase can catalyse many steps in the break down of fat. The vertebrate pancreas secrete an enzyme lipase but before it breaks down fat, some detergent-like action is needed to emulsify the fat droplets. Bile salts from the liver, lecithin and cholesterol form **miscelles** and do this job. They reduce the surface tension at the fat-water interphase in a slightly alkaline medium and tiny emulsification droplets of fat are formed. Then the lipase begins to digest the emulsified droplets. The resultant fatty acids and monoglycerates are kept in solution by help of bile salts again and are finally absorbed.

Glycerol is water soluble and easily absorbed and metabolised. Fat like butter is absorbed directly through the intestinal epithelium without hydrolysis.

## 1.4.4 Maintenance of Gut Lining

After studying the digestive enzymes you would wonder why the gut linings are not digested themselves. This is because animals have several mechanisms that protect their gut lining from autodigestion. The mucous membranes of vertebrates secrete a slightly alkaline mucous that lubricates the food and protects the lining cells from corrosive secretions. In addition, the lateral surfaces of exposed epithelial cells are joined by tight junctions that prevent the secretions from penetrating between them. Careful studies have also revealed that the entire lining of the gut is renewed every third day in rats and every 5-6 days in humans. Similar mechanisms are present in invertebrates also. In insects, the fore-gut and hind gut are lined by cuticle. This lining is known as **intima**. Only in the midgut, the epithelial cells are exposed, where most of the digestion occurs. The midgut is lined by a delicate lining the **peritrophic membrane** in some insects. This corresponds to the mucous lining of vertebrates.

## 1.4.5 Coordination of Digestion

You have learnt that digestion is a process in which large food molecules are broken down step by step into their constituents. In primitive metazoans that are continuous feeders, the enzyme producing cells secrete continuously. In higher animals more precise controls are needed to regulate the release of food from stomach to intestine and also the release of digestive enzymes at the proper time. The interplay of nervous and hormonal control is beautifully illustrated when we study the coordination of digestive activity.

In the mammalian mouth, control of salivary gland secretion is entirely nervous; gastric secretions are under hormonal and neural control; and intestinal secretions are slower and are primarily under hormonal control.

Gastrointestinal secretion is largely under the control of **gastrointestinal hormones** secreted by endocrine glands of gastric and intestinal mucosa.

**Gastrointestinal Hormones**

The three main mammalian gastrointestinal hormones are **secretin, gastrin** and **cholecystokinin** (CCK). There are several other hormones, all peptides. The physiology of only three major hormones is listed in Table 1.9.

Table 1.9 : Mammalian gastrointestinal hormones; − denotes inhibition; + stimulation; +++ hormone more important than other two

| | Gastrin | Secretin | Cholecystokinin |
|---|---|---|---|
| Secreted by | Stomach | Duodenum | Duodenum |
| Stimulus for release | Peptides | Acid (HCl) | Amino acids fatty acids |
| | Parasympathetic Nerves | | |
| Effect on : | | | |
| Gastric Motility | + | − | − |
| Gastric HCl Secretion | +++ | − | − |
| Pancreatic secretion | | | |
| bicarbonates | + | +++ | + |
| enzymes | + | + | +++ |

Gastrin secretion is responsible for control of HCl volume; the presence of HCl in turn inhibits further gastrin secretion.

Secretin is released under acidic conditions (low pH); digested fat or bile initiates production of pancreatic juice low in enzymes but rich in salts important in neutralising the acid chyme. CCK is secreted when partially digested proteins or HCl (to a lesser extent) are present. It induces the flow of pancreatic juice rich in enzyme. Fig. 1.11 summaries the action of GI hormones.

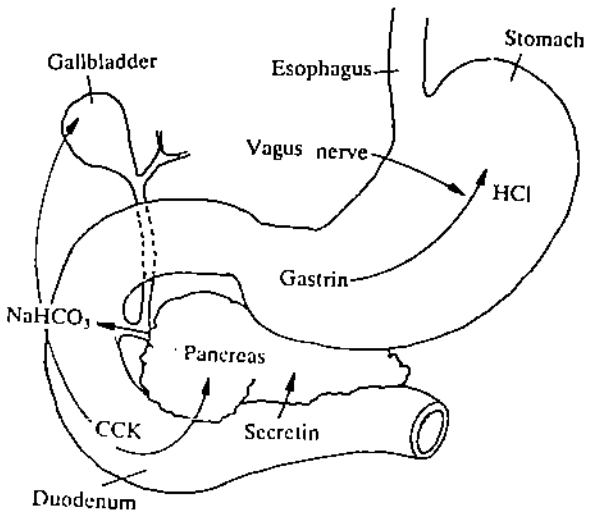



Fig.1.11 : Action of several gastrointestinal hormones. Gastrin is secreted in response to intragastric proteins, stomach distention and stimulation by vagus nerve. Gastrin from the lower stomach stimulates HCl secretion and pepsin from secretory cells. Cholecystokinin (CCK) stimulates pancreas to secrete digestive enzymes and bases to neutralise and digest chyme. It also induces contruction of gall bladder to secrete bile salts. CCK is secreted in response to arrival of amino acids and fatty acids in deodenum from stomach.

These two hormones inhibit stomach motility. Arrival of fat from the stomach initiates the release of CCK by intestinal mucosa. this causes gall bladder to release bile which aids in fat digestion.

**SAQ 3**

a) What are the main advantages of having a digestive tract with a mouth and anus?

.....

.....

b) Choose the correct answer.

Digestion is brought about by

i) acids, ii) enzymes, iii) alkaline solutions, iv) vitamins and minerals

c) Fill in the blanks with appropriate words.

In the process of digestion proteins are converted into ....., carbohydrates to ..... fats to ..... and ..... .

d) Three hormones stimulate the release of digestive materials, ..... stimulates release of gastric juices ..... stimulates release of bicarbonate ion. ..... stimulates release of bile and pancreatic enzymes.

## 1.5 ABSORPTION

The monosaccharides, amino acids and other products of digestion must be passed on to other tissues to be useful for the organism. The process by which the digested material from the alimentary canal enters the blood stream is known as **absorption.**

In intracellular digestion the same cells are concerned with digestion and absorption but in higher multicellular animals there are usually separate tissues and areas of gut for enzyme production, digestion and absorption.

In this section we will mainly be concerned with absorption of amino acids, sugars and fats released during extracellular digestion in vertebrates. In all vertebrates most of the absorption is localised in the intestine. As you already know the wall of the vertebrate intestine is folded and ridged to increase the absorptive surface. These ridges or folds are covered by a velvet like pile of minute absorptive villi (Fig. 1.12). These are highly specialised absorptive organs with a core containing a network of capillaries derived from blood vessels in the gut wall. Each villus also contains a central lymph capillary known as **lacteal** which begins blindly at the tip of the villus and drains into the main lymph channels of the gut wall. Lipids pass mainly into the lacteals while sugars and aminoacids are absorbed directly by the blood capillaries. The villi and intestinal folds contain smooth muscles that contract to bring the villi in contact with the food in the intestine; and also maintaining the circulation in lacteals, lymphatics and small blood vessels.

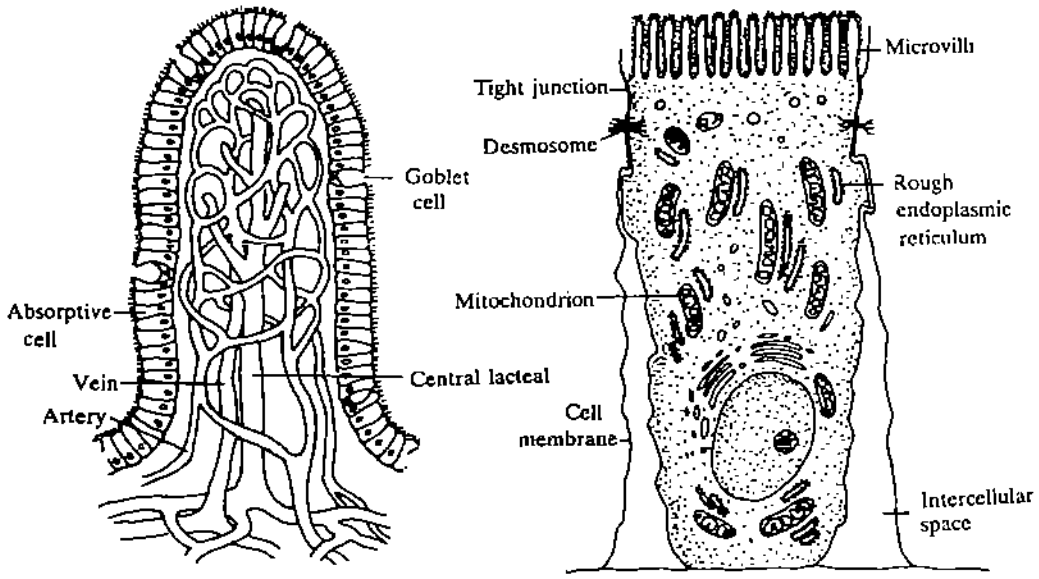



**Fig. 1.12 : Lining of mammalian small intestine**

a) Villus covered with digestive epithelium which consists of absorptive cell and occasional goblet cells.

b) An absorptive cell. The apical surface bears a brush border of microvilli.

Now let us examine the processes involved in actual absorption of the digested food. You could recall from LSE-01 Unit 7 the transport processes across membranes. We can summarise them as follows:

| | |
|---|---|
| **Diffusion** (passive) | A material passes down its electrochemical gradient; at the end of the process the concentration inside the cell is equal to that outside. |

## 1.6 ENERGY METABOLISM

In the preceding sections of the unit, you studied how the products of digestion of food, viz. amino acids, sugars and fatty acids are absorbed and transported to the body tissues. The oxidation of these compounds yields virtually all the chemical energy required by animals and the use of this chemical energy is referred to as their **energy metabolism**. You also learnt in Section 1.2 that generally carbohydrates and fats are the fuel which provide energy but other organic compounds are within wide limits interchangeable in energy metabolism.

How do we measure the rate of metabolism or the actual amount of energy liberated during oxidative metabolism? One way could be to measure the total heat produced by the organism per unit of time. This is the **metabolic rate** of the organism. The metabolic rate can be determined by using the formulation:

rate of energy intake — rate of energy loss per unit time = metabolic rate

Energy intake is the chemical energy content of ingested food over a given period. Energy loss is the chemical energy that remains in faeces and urine produced by the animal over the same period. The energy content of food and wastes is found out by burning them in a **bomb calorimeter** (Fig. 1.15). The material to be tested is placed and burned with the aid of oxygen in a chamber surrounded by a jacket of water. The heat produced is determined by the rise in temperature of the surrounding water. Table 1.10 provides the caloric value of the common food stuffs estimated in a bomb calorimeter and in the body.

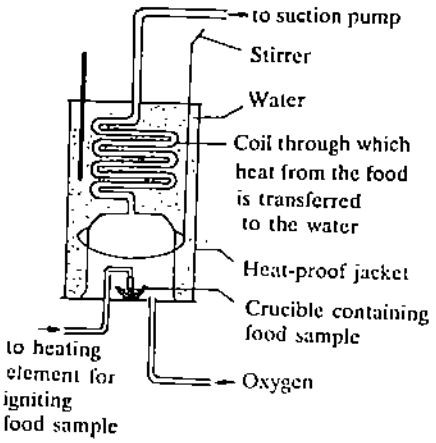



Fig. 1.15 : Bomb calorimeter

These values are essentially average values. During oxidative degradation in animal body, carbohydrates and lipids are fully oxidised to carbon dioxide and water just as in a bomb calorimeter but proteins are not degraded because the major end-product of protein metabolism, is urea which still possess some energy. Accordingly, the value is lower in the body (4.1 kcal/g) as you can see from Table 1.10. Energy derived from one gram of fat is much more than that derived from 1 gram of protein or of carbohydrate.

Table 1.10 : Fuel Content of Food Materials

| Food | Kilocalories per gram | |
|---|---|---|
| | In Bomb Calorimeter | In Body |
| Carbohydrates | 4.1 | 4.0 |
| Lipids | 9.4 | 9.0 |
| Proteins | 5.6 | 4.1 |

The heat produced during the metabolic activities of the body helps in maintaining the body temperature. Generally, warm blooded animals like birds and mammals have specific regulatory mechanisms by which heat production is either increased or

dissipated in relation to the environmental conditions. You will learn more about temperature regulation in Unit 7 of Block 2.

The most commonly used measure to determine metabolic rate is to find the oxygen consumption of the animal. Oxygen consumption is a good indicator of metabolic rate because heat produced for each litre of oxygen used in metabolism is the same irrespective of the food stuff oxidised (see Table 1.11). The highest figure of 5.0 kcal per litre of oxygen is only 10% more than the lowest figure 4.5 kcal/litre of oxygen for proteins. On an average we consider metabolic rate to be equal to 4.8 kcal/litre of oxygen used.

**Table 1.11 : Heat production and respiratory quotient for foodstuff types**

| | Heat produced Kcal | | |
|---|---|---|---|
| | Per litre of $O_2$ consumed | Per litre of $CO_2$ formed | $RQ = \frac{CO_2 \text{ formed}}{O_2 \text{ used}}$ |
| Carbohydrates | 5.05 | 5.05 | 1.00 |
| Fats | 4.75 | 6.67 | 0.71 |
| Proteins | 4.46 | 5.57 | 0.81 |

### Respiratory Quotient

Table 1.11 also shows the ratio of the volume of carbon dioxide evolved to that of the amount of oxygen consumed during oxidation. This is the **respiratory quotient** or **RQ**. It is an important concept in energy metabolism. From the table you can see that RQ is usually between 0.7 and 1.0. However, RQ near 0.7 shows that fat is being metabolised and RQ near 1.0 suggests carbohydrate metabolism. RQ in between 0.7 and 1.0 could indicate either protein or a mixed diet metabolism.

Quite often animals cannot utilise the entire food value because not all the food they consume is fully digested. Also some portion is excreted as urea or ammonia. In general, it has been observed that animals have a higher intake of food than what is indicated by their oxygen consumption data so that their body weight is kept steady. The oxygen consumption per unit weight/per unit time $mm^3O_2/g/hr$ tends to decrease with higher body weight of animals. In other words, small sized animals like mouse, shrew, etc., have a higher metabolic rate than a large sized animal (an elephant) as evidenced by their oxygen consumption. Accordingly smaller animals have a need to feed constantly. This would also mean that an elephant can survive without food for a much longer period of time than a mouse.

### Energy Storage

As we said above, food intake and energy expenditure for animals is approximately equal. If energy expenditure exceeds food intake, then the excess energy is taken up by utilisation of body fat. However, if food intake is excess, then the surplus is stored as fat irrespective of the kind of food eaten.

Excess carbohydrates are changed to fats and accordingly RQ exceeds 1. This is because fats contain relatively less oxygen and the excess oxygen of carbohydrates is used in the metabolism. This reduces the oxygen uptake and the respiratory carbon dioxide, oxygen ratio is increased.

For this reason fat is ideal storage material for energy. It is much lighter and yields twice as much energy as carbohydrates. Migratory birds that may have to fly more than 1000 km non-stop, carry fat as 40% to 50% of their body weight.

Nonetheless, some carbohydrates are important in energy storage. Glycogen a starch-like carbohydrate polymer is stored as granules in the skeletal muscles and liver of vertebrates. During heavy muscular exercise when blood does not deliver sufficient oxygen to meet demands, glycogen provides the energy. It is broken down directly into glucose-6-phosphate, providing fuel for carbohydrate metabolism more directly than does fat.

On the other hand, many animals that do not move about, also store glycogen as excess energy source. For example, clams, oysters and many intestinal parasites like

5) Excess carbohydrates that are not used for metabolism are converted to fats and stored in the body. This increases the weight.

**Terminal Questions**

1) Essential nutrients are materials the animals need to sustain life but cannot synthesise in their cells. The nutrients that are essential differ for each type of animal because the ability to synthesise is genetic and species specific. For example, most vertebrates can synthesise vitamin C but human and some other fruit eating species have lost this synthetic ability. Therefore, vitamin C is essential for us but non-essential for some others.

2)

| **Intracellular** | **Extracellular** |
|---|---|
| Ingestion of small particles | Ingestion of large food masses |
| Digestive enzymes enclosed in a small area. One kind of enzyme acts at one time in digestive vacuole | Usually well-developed digestive tract that allows secreted enzymes to act on food at different parts at the same time, digestion occurs in phases |
| Suited to continuous feeding | Suited to discontinuous feeding |
| | Two openings, a mouth and anus in complex animals so that food passes in one direction |

For more complex freely moving organisms extracellular digestion is more advantageous because they don't have to feed continuously and a division of labour in the digestive tract occurs so that only a few cells are devoted to the digestive processes. Digestion can occur in different phases at the same time.

3) A lining of mucous secreted by the goblet cells and the fact the most digestive enzyme especially protease are present in inactive form.

4) Carbohydrates are hydrolysed to monosaccharides. Proteins are hydrolysed to amino-acids and fats are hydrolysed to the free fatty acids and glycerol.

   i) Amino acids and glucose enter the intestinal epithelial cells by a carrier or cotransporter protein molecules that depend on the action of a sodium ion pump. Free fatty acids or monoglycerides form miscelles with bile salts before entering the intestinal epithelium through diffusion.

   ii) Amino acids and glucose are passed on to the blood stream through another transporter molecule while fatty acids and monoglycerides form chylomicrons which enter the lacteals from where they enter the blood stream.

s know by experience
ility decreases with
temperature. When
ater for boiling, the
rmed at the sides
ling are gases escaping
solution as the
re rises.

Respiratory organs may be of the following types:

- Those that have respiratory surface turned out forming an evagination. These are called **gills**.
- Those that have respiratory surface turned in forming an invagination. These are called **lungs**. Our own lungs are good examples.

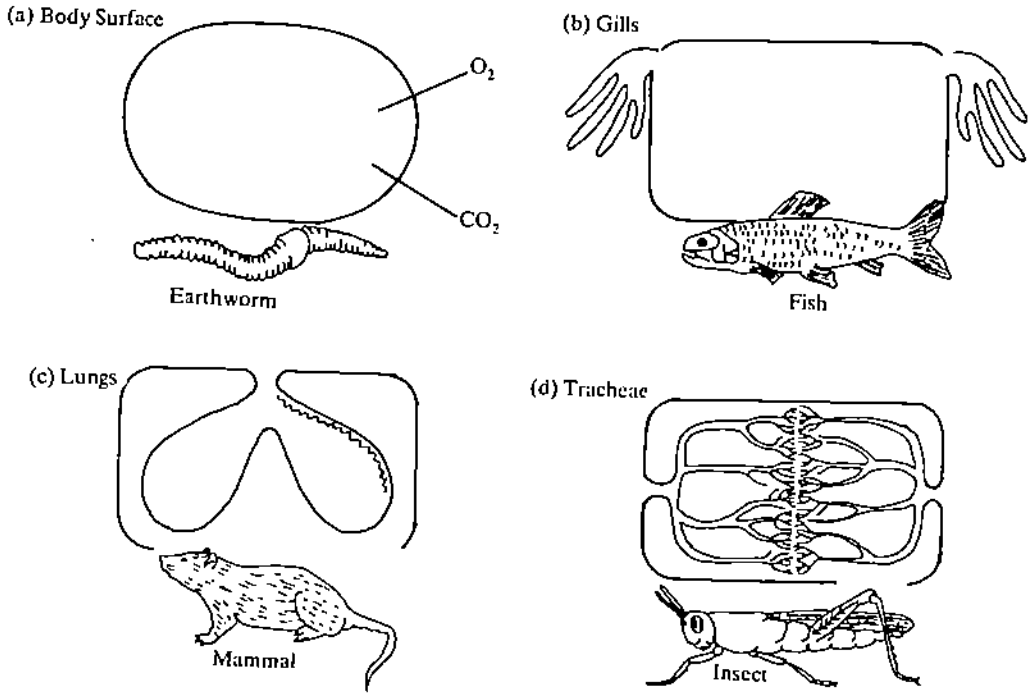



**Fig. 2.1 : Some aquatic animals and animals that maintain moist skins, can exchange gases directly through their integuments (a), which are in contact with water. Gills (b) highly folded external tissues that provide enormous surfaces for exchanging gases with water — are common among larger aquatic animals. Terrestrial animals that exchange gases with the atmosphere do so by means of highly branched internal surfaces that are protected from drying out. Many vertebrates have sac-like lungs (c); insects and some other arthropods have systems of branched tubes called tracheae (d).**

Insects have a special respiratory system. Small openings on the insect's body surface connected to small tubes or trachea that branch and spread throughout the body. The gases diffuse through these branches directly into the cells.

Generally gills are for aquatic breathing and lungs for breathing air. We shall now consider specific kinds of respiratory organs employed by animals that live in water and on land.

**SAQ 2**

Why does diffusion alone suffice to supply oxygen in both *Paramecium* and jelly fish?

.....

.....

.....

.....

.....

## 2.4 GILLS

Gills are highly vascularised extensions of gas exchange membranes. The simplest type may be extention of the body surface as in sea-slugs, sea-stars, and many other

gradient of partial pressure of oxygen between the two compartments; (b) the most efficient gas exchange system is one that ensures the highest possible partial pressure of oxygen in the blood leaving the gill. Now let us suppose that the incoming blood is devoid of all oxygen, and imagine the flow of water and blood is in the same direction i.e. it is concurrent (Fig. 2.4 b) when the two streams come in contact first there is a steep pressure gradient from the water to blood. Oxygen is transferred from the water to blood at a high rate till an equilibrium is reached after which no transfer occurs. Now consider Fig. 2.4a which shows countercurrent flow. When blood which has zero $Po_2$ comes in contact with water for the first time, the water also has low $Po_2$ (since it has been loosing oxygen on its way to this point) but still sufficient for a pressure gradient to be maintained. As the blood moves on it meets with water richer in oxygen and the $Po_2$ of blood increases steadily. At all points along the capillary, $Po_2$ gradient is sufficiently high to permit transfer of oxygen from water to blood. The net effect is that blood leaving the gills in countercurrent exchange has extracted 80 per cent or more of the dissolved oxygen from water.

To move water over the gills, teleosts use combined pumping action of mouth and opercular cover. Water is drawn into the mouth, passes over the gills and flows out through the opercular clefts. valves guard the entrance to the buccal cavity and opercular clefts ensuring a **unidirectional flow of water** (Fig. 2.5). The volume of the buccal cavity can be changed by lowering of the jaw and the floor of the mouth and that of the opercular cavity is changed by the movements of opercular flaps that swing out to enlarge the cavity and swing in to reduce it. Changes in volume of both cavities is synchronised but a pressure differential is maintained across the gills throughout the breathing cycle.

The pressure in the opercular cavity is always slightly lower than the pressure in the buccal cavity providing for a continuous flow of water.

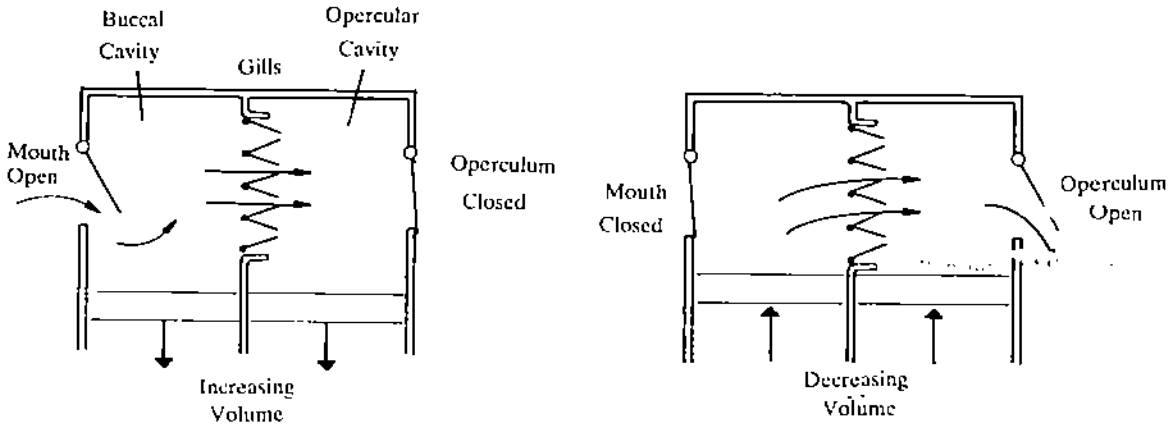



**Fig. 2.5 : Dual pump in fish provides a continuous unidirectional flow of water.**

Some fish do not use pumping action for gill ventilation. It has been known for long that large tunas cannot be kept alive in captivity unless they are put in circular tanks where they can swim continuously. The fish swim with their mouths partly open and there are no visible breathing movements. Water flows continuously over their gills. This is known as **ram ventilation.**

It is now known that many fish breathe by pumping at low speed and change to ram ventilation at high speed. In this case the work of breathing is transferred from the muscles of the opercular pump to the muscles of the body and tail. However, ram ventilation is more economical as far as energy consumption is concerned than opercular pumps at high rates required for fast swimming.

**SAQ 3**

Most fish when taken out of water become asphyxiated, although there is far more oxygen in the air than in water. Apart from the reason that the weight of the gills cannot be supported by them in air, what other factor is responsible for this.

.....

.....

.....

You have seen that respiration in water can be explained through some simple physical principles. Let us now move on to examine respiratory gas exchange in animals that breathe air.

## 2.5 LUNGS

In section 2.2, you studied that air has more oxygen than water. The atmosphere provides a constant supply of oxygen almost everywhere. The greatest disadvantage however, is that air tends to dry out gas exchange membranes. To overcome this, terrestrial animals have to live in extremely moist environments or find some ways to keep their respiratory surfaces moist. The water loss is minimised by turning the respiratory membranes inside the body into lungs in amphibians, reptiles, birds and mammals, and trachea in insects.

Before we proceed to describe terrestrial respiration by lungs let us examine some major advantages of breathing air. If we compare water and air we find that water in equilibrium with atmosphere at 15°C contains only 7 ml of oxygen per litre. In contrast 1 litre of air contains 209 ml of oxygen. Water is much more viscous than air, therefore, aquatic organisms have to expend more energy to move the medium over the respiratory surface. Apart from this oxygen diffuses some 10,000 times more rapidly in air than in water and so can diffuse in lungs over several millimeters while diffusion from water to respiratory surface in fish gill can take place in only minute fraction of a millimeter. The flow of oxygen into the respiratory cavity in terrestrial animals is well regulated according to the oxygen demands of the organism.

Lungs can be simple, characterised by air exchange with surrounding environment by diffusion only. These are called the **diffusion lungs** and are present in small animals such as pulmonate snails, small scorpions, some spiders and some isopods. The other type — **ventilation lungs** are typical of vertebrates. The air passes through a tube into inflatable lungs where gas exchange takes place and oxygen poor, carbon dioxide rich air is then forced out usually through the same tube. This is known as **tidal flow of air.** Ventilation of the lungs can take place in two different ways:

1) By using a pressure pump as in amphibians. Fig. 2.6 shows the process of ventilation in frog. The inflation of lungs depends on positive pressure bucco-pharengeal pump. The nares remain open while glottis is closed (the air does not enters the lungs). The floor of the buccal cavity is raised and lowered periodically (Fig. 2.6a). At irregular intervals the glottis is open and nares are closed. The floor of the buccal cavity is raised forcing air into the lungs. As a result the frog can take in air several times without exhaling and blow itself up to considerable size. The glottis can close and while the air remains inside the lungs the cycle is replated in the buccal cavity.

2) By using a suction pump. Exhalation can be passive and inhalation is aided by muscle contraction or as in mammals, by contraction of muscular dome shaped diaphragm and external intercostal muscles lifting the ribcage. This decreases the pressure in the pleural space so causing the lungs to expand and air flows in.

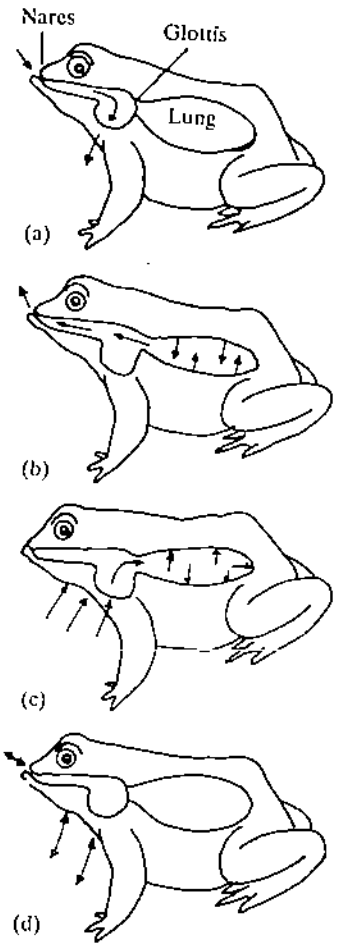



Fig. 2.6 : Breathing cycle in the frog
a) air is taken into the buccal cavity by lowering it.
b) air is permitted to escape from the lungs passing over the buccal cavity.
c) the external nares are closed air is forced into the lungs.
d) while air remains in lungs. The cycle can be repeated.

### 2.5.1 Mammalian Lungs

In this unit we will study mainly mammalian lung as it is the best representative of a respiratory surface adapted for terrestrial respiration. For this purpose, human lung can be taken as a model as shown in Fig. 2.7.

When we breathe, the air enters the wind pipe or trachea which are divided into right and left **bronchi** (Fig. 2.7). These in turn branch repeatedly forming **bronchioles**. The fine branches of the bronchioles lead into **alveolar sacs**, which are clusters of minute sacs, whose diameter ranges between 150 to 300 micron or micrometer. The alveoli have thin walls and capillaries from the pulmonary artery extensively occupy the vascular side of the alveoli (Fig. 2.7). A pair of human lungs contain about 300 million alveoli and the total surface area is about 70 meter square. This area is nearly equal

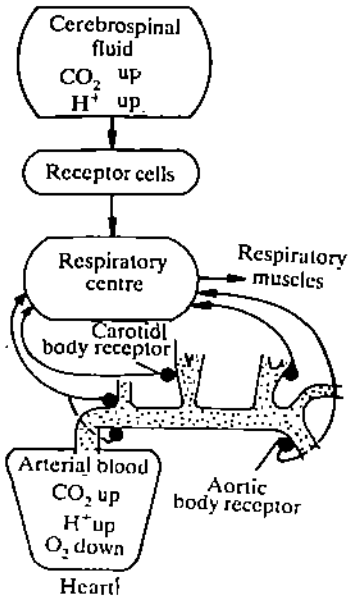



**Fig. 2.10: Action of carbon dioxide receptor cells**

## 2.5.2 Regulation of Respiration

Whenever the need for oxygen in the body increases, ventilation of the respiratory organs also increases. In the same way whenever the level of oxygen in the medium falls, the ventilation must increase or the body should be able to extract more oxygen from the respired air or both these processes must take place.

In lungs of warm-blooded animals i.e. birds and mammals the ventilation is regulated primarily by the amount of carbon dioxide in the lung air. If we add more carbon dioxide to the inhaled air, there is a rapid increase in ventilation, even if carbon dioxide content in inhaled air is increased to that found normally in the lungs (5%), respiratory ventilation volume increases several folds. In higher concentrations, carbon dioxide becomes dangerous. Oxygen on the other hand, has a much smaller effect on ventilation, if we reduce the oxygen concentration from 21% to 18.5% there is virtually no effect.

The rythmic contractions of the diaphragm and of the intercostal muscles are controlled by a respiratory centre which is located in the area of the medulla oblongata and pons of the brain. There are separate neurons for inspiration and expiration that work alternatively.

The respiratory centre is sensitive to increased carbon dioxide levels and increased acidity of the blood. It is peculiar that even though metabolism requires oxygen yet the respiratory centre is not very sensitive to decreased oxygen levels. However, chemo-receptors located in carotid bodies and aortic arches near the heart respond to decreased $Po_2$ levels by increasing the depth and frequency of breathing. Fig. 2.10 depicts the control process graphically.

In contrast aquatic animals that breathe through gills are very sensitive to oxygen levels. Respiratory movement in many species increases if oxygen levels in surrounding medium fall. In amphibia the respiration in tadpoles is regulated by oxygen levels and in adult frogs the mechanism is controlled by carbon dioxide levels.

**SAQ 4**

Mark True or False among the following statements:

i) Lung surfactant increases the surface tension of the alveoli so that they don't collapse.

ii) Tidal volume is the volume of air taken in one breath.

iii) Anatomical dead space is the volume of air present in the trachea, broncheoles etc.

iv) Residual air in the lungs is important for maintaining the carbon dioxide levels in lung.

v) Breathing in mammals is controlled by a part of the brain called cerebrum.

## 2.5.3 Adaptations for Diving and Underwater Swimming

Mammals and birds cannot breathe when swimming underwater, but carbon dioxide continues to be brought to the lungs. As carbon dioxide builds up in the alveolar air, the usual stimulus for breathing is given and the underwater swimmer has to come to the surface to breathe. Many species of mammals for example otter, beavers, seal and whale show adaptations which permit them to stay underwater.

There adaptations are seen best in the sperm whale that dives as deep as 1000 meters for over an hour without any respiratory distress. Physiology of diving is an interesting subject but it is beyond the scope of this unit to discuss it in detail. Briefly we can mention that diving mammals have to conserve their oxygen for as long as possible. For this they have evolved certain adaptations. They are :

i) The heart rate is slowed down along with a fall in blood supply to muscles, skin and visera so that the flow to the brain and heart is maintained. In some mammals receptors in the nasal region when stimulated by water, trigger cardiac slowing.

ii) Diving vertebrate, also have relatively large blood volumes and venous reservoirs.

iii) During a deep dive the high pressure of the water causes the lungs to collapse partially and thus capillary circulation is also restricted there by reducing the solution of air in the blood.

Unlike other mammals that have successfully adapted to underwater, human beings are limited by their respiratory physiology. Thus we see that human divers face several physiological problems. The major one is the increased ambient pressure underwater. For every 10 meters there is an increase by 1 atmosphere (101.3 k Pa) i.e. if one dives 10 m below sea level the pressure is doubled and the amounts of gases dissolved in plasma would also double. The increased levels of oxygen and nitrogen in blood can have serious consequences. **Oxygen toxicity** develops rapidly when the $Po_2$ rises above 2.5 atmosphere because of oxidation of enzymes and other destructive changes that can damage the nervous system and lead to death. For this reason deep sea divers use a mixture of gases instead of pure oxygen. Large amounts of nitrogen dissolved in blood under pressure causes nitrogen narcosis, a condition resembling intoxication. Another danger arises when divers surface too rapidly. The gases expand and their bubbles form emboli in the circulatory system.

Human beings swimming underwater at shallow depths can endanger themselves if they hyperventilate or overbreathe. They can thus reduce the carbon dioxide levels from the alveoli. This will delay the onset of carbon dioxide-respiratory drive and the sensation of the need to breathe. But meanwhile oxygen will continue to be used up and since $Po_2$ receptors are not as sensitive as $Pco_2$ receptors the divers may become unconscious through lack of oxygen and thus drown.

## 2.6 TRACHEAE

Insects that have successfully colonised the terrestrial environment have envolved a respiratory system very different from other land animals — the **tracheal system.** Apart from insects other arthropods, such as millipedes, centipedes, a few archinids and terrestrial crustaceans have tracheae as respiratory organs.

The tracheal system consists of an air filled system of tubes — the tracheal tubes that branch repeatedly until they become fine as capillaries, and are called **tracheoles.** The tracheolar endings are in close proximity to the cells of the body and sometimes they indent into the plasma membrane giving the impression that they actually enter the cells. Tracheae vary in diameter from 1-2 mm and the diameter of tracheoles is between 0.6-0.8 mm. In the larger tracheae, thickenings known as **taenidia** in the cuticular lining prevent the tubes from collapsing. The walls of the tracheae become progressively thinner as they branch so that the tracheolar endings are only 5 mm in diameter. Fig. 2.11 shows the arrangement of tracheal system in some insects.

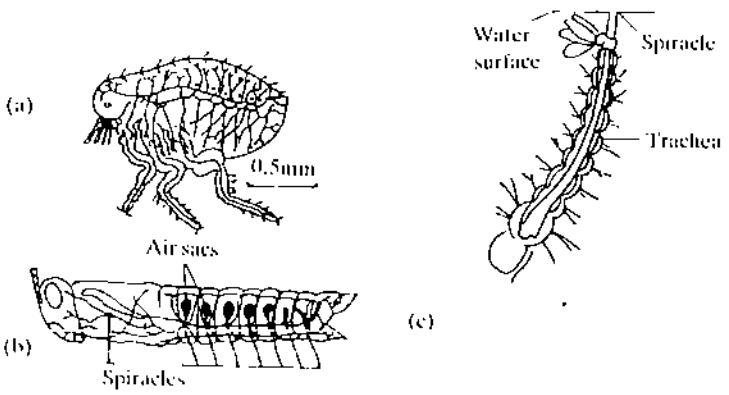



Fig. 2.11 : Some modifications of the tracheal system in insects. (a) The basic pattern, (b) mechanically ventilated air sacs, (c) terminal spiracles in aquatic insects.

The tracheae open to the outside by structures known as spiracles. Usually there are 10 pairs of spiracles. They have closing mechanisms, aided by valves, muscles and filters. Such closing mechanisms also help to conserve water. The tracheoles and tracheolar endings are permeable to water and may contain variable amounts of fluid.

If the amount of haemoglobin that is packed in cells was to be free in plasma, the viscosity of blood would be like syrup. Apart from this, being enclosed in cells provides a more stable environment as reaction of oxygen and haemoglobin is affected by ion concentration and organic compounds in blood.

The structure of haemoglobin was briefly mentioned in Unit 5 of the course in Cell Biology (LSE-01). Let us now consider the structure in more detail.

A group of compounds called porphyrins are widely distributed in plants, animals and bacteria. Porphyrins associate with metals to form metalloporphyrins which form a variety of compounds. For example chlorophyll about which you will read in Block-3 is a magnesium-porphyrin complex and cytochromes which contain porphyrin group play an important role in intracellular oxidation.

When ferrous iron $Fe^{++}$ is added to a porphyrin protoporphyrin IX, ferrous porphyrin or **haem** is formed. The molecular structure is shown in Fig. 2.13.

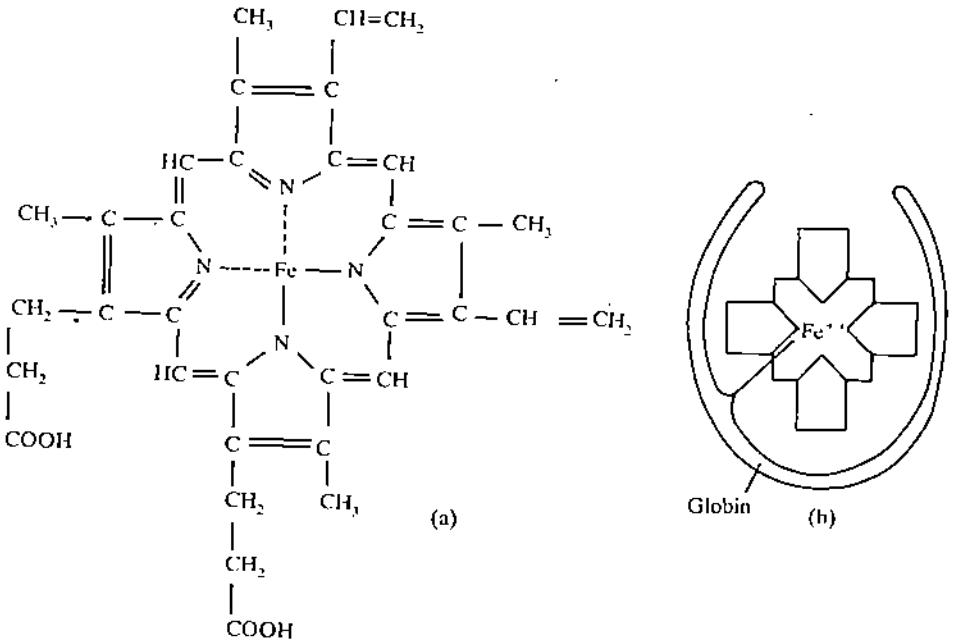



Fig. 2.13 : Chemical structure of haem group (a), Schematic representative of a single submit of haemoglobin (b).

The ferrous iron atom is bound to the four nitrogens of the protoporphyrin ring. The ferrous iron makes two more links, one with the oxygen atom to form. oxyhaemoglobin and the other to the globin portion of the molecule at a single amino acid site (Fig. 2.13b).

The haem group of all haemoglobins is the same and the variations in amino acid sequences of the globin group form the different haemoglobins.

Myoglobin may also have an oxygen storage function especially useful in the functioning of heart. During diastole when coronary blood flow is maximum it stores oxygen and when during systole the coronary arteries are squeezed the stored oxygen is released.

We can build up the structure of haemoglobin by considering a single unit or monomeric form called **myoglobin**. This consists of a single polypeptide chain the globin in which the haem group is embedded. Myoglobin is found in striated muscles of vertebrates and combines with one molecule of oxygen. The vertebrate haemoglobins are tetramers formed by the aggregation of four polypeptide chains each containing a discrete haem group. Thus each haemoglobin can combine with four molecules of oxygen forming **oxyhaeomoglobin** in a reversible reaction and the unoxygenated compound is called **deoxyhaemoglobin.** Two monomers in human haemoglobin are of a type called $\alpha$ and the other two are $\beta$ type. You would recall the unique quaternary structure of haemoglobin from Unit-5 LSE-01.

Nearly all vertebrate haemoglobins are tetramers but invertebrate haemoglobins are more diverse. The most distinctive feature is that the subunits often form large aggregates of relative high molecular weights (Refer to Table 2.3).

In mammalian blood the amount of physically dissolved oxygen is about 0.2 ml of oxygen per 100 ml of blood. The amount found bound to haemoglobin is 20 ml oxygen per 100 ml of blood. The dissolved oxygen is therefore, almost insignificant in animals without respiratory pigments. The only exception is the antarctic fish which

lacks a respiratory pigment altogether. The explanation could be that at low temperature, the metabolic rate is low and oxygen like other gases has higher solubility at low temperature.

## 2.7.2 Oxygen Transport in Blood

All four respiratory pigments are adapted to **load** and **unload** oxygen effectively in the habitats where they have evolved, whether animals live on land where the air contains 210 ml of oxygen per litre or in fresh water containing 8.0 ml/litre or in the sea containing 6.4 ml/litre. The loading unloading reaction can be written as

$$\text{(haemoglobin) Hb} + O_2 \underset{\text{tissue}}{\overset{\text{respiratory surface}}{\rightleftharpoons}} \text{Hb } O_2\text{(oxyhaemoglobin)}$$

The direction of this reaction depends on $Po_2$ of the environment and bond strength or affinity between haemoglobin and oxygen. In the lungs where $Po_2$ is high almost all deoxyhaemoglobin molecules bind to oxygen. Low $Po_2$ in the systemic capillaries promotes unloading. Similarly strong affinity favours loading and weak bonding favours unloading. Haemoglobin has a bond strength which permits 97% of haemoglobin to combine with oxygen when leaving the lung. At the same time the bond is sufficiently weak to permit unloading in tissues. Under normal resting conditions 22% of the oxygen is unloaded. This satisfies the oxygen need of the body simultaneously maintaining a reserve that is utilised during emergency conditions.

Haemoglobin has even a stronger affinity for carbon monoxide than oxygen. The bond is 210 times stronger. Carbon monoxide tends to displace oxygen in haemoglobin and remains attached as the blood passes through the tissues. The transport of oxygen is impaired leading to dangerous consequences and even death.

### Oxygen Dissociation Curves

The oxygen content of blood fully saturated or oxygenated can be calculated. It is known as the oxygen capacity of blood and this varies for different species. In humans the oxygen carrying capacity is 20 ml oxygen per 100 ml blood. The relationship of oxygen carrying capacity to surrounding oxygen concentration can be shown graphically by **oxygen dissociation curves.** These curves are obtained by subjecting blood samples to different partial pressures of oxygen. The per cent oxyhaemoglobin saturation at different partial pressures of oxygen are plotted.

The oxygen dissociation curve (shown in Fig. 2.14) is S-Shaped or a sigmoid curve. From the graph we can see how haemoglobin acts as a carrier of oxygen. Total saturation occurs in the lungs where arterial pressure is above 95 mm Hg and the oxygen is unloaded at low $Po_2$ found in tissues (about 40 mm Hg).

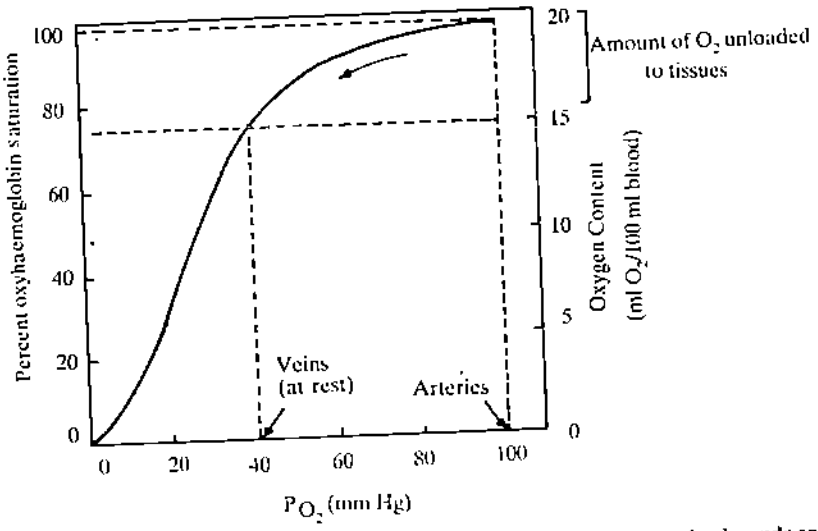



**Fig. 2.14 : Oxygen dissociation curve shows how haemoglobin's oxygen binding capacity depends on partial pressure of oxygen. Note that there is a 22% decrease in per cent oxyhaemoglobin as blood passes from arteries to veins in tissue. This results in unloading of approximately 5 ml of oxygen per 100 ml of blood.**

Also the curve shows that changes in $Po_2$ values from arterial to venous blood result in 97–75 = 22% unloading when resting. During exercise this unloading is increased
. Fig. 2.15 shows the dissociation curve for myoglobin which in contrast is
Myoglobin is described as middleman in the transfer of oxygen from
ondria within muscle cells. This is known as facilitated diffusion of

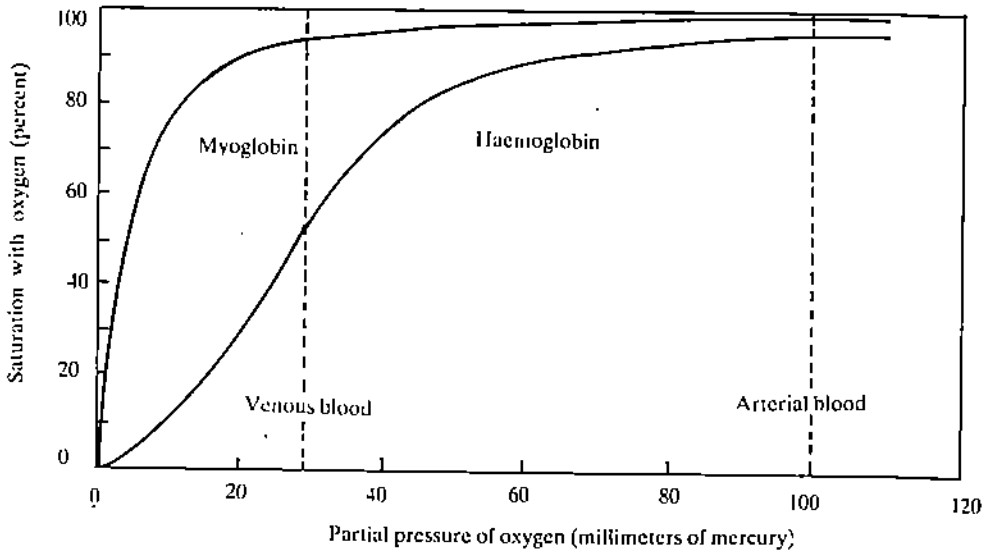



**Fig. 2.15 : A comparison of the dissociation curves for haemoglobin and for myoglobin. At the $Po_2$ of venous blood, the myoglobin retains almost all of its oxygen, indicating a higher affinity than haemoglobin for oxygen. The myoglobin does, however, release its oxygen at the very low $Po_2$ values found inside the mitochondria.**

Fig. 2.15 also indicates that the compound remains oxygenated until quite low levels of $Po_2$ in the surrounding fluids is reached. You can see from the figure that at 20 mm Hg the haemoglobin in the blood is about 30% saturated but the myoglobin in the muscles is above 80% saturated.

In molecular terms how does a S-shaped curve arise? Look at the part of the curve in Fig. 2.14 where $Po_2$ is low. At first the slope is not steep but rises steeply up to a certain point (about 20 mm Hg).

In other words haemoglobin does not take up oxygen at low $Po_2$ but as the oxygenation of the pigment occurs its affinity for more oxygen increases. No such increase in affinity is evident from the myoglobin curve shown in Fig. 2.15. In myoglobin the haem reacts to oxygen independently. In the case of haemoglobin where 4 subunits are present, acquisition of one molecule of oxygen increases the affinity of neighbouring haems for oxygen. This is known as **co-operativity between active sites.** Let us see how this cooperation between active sites modifies the binding of oxygen. It is now known that the haemoglobin can occur in two interchangeable forms, one is called **T (tense)** structure and the other is **R (released)** structure. T structure has relatively low affinity for oxygen and R structure has high affinity for oxygen. The uptake of oxygen by one subunit in T structure changes the whole complex to R structure, which then binds to oxygen one hundred times faster than the first haem group.

Oxygen dissociation curve for a sample of blood is affected by several factors. The most important of them are :

In fever or exercise there is a higher rate of oxygen consumption. Therefore, it is advantageous that haemoglobin delivers $O_2$ more readily at high temperatures.

1) Temperature
2) pH
3) $CO_2$
4) Organic Phosphates

At higher temperature haemoglobin gives up oxygen more readily and the dissociation curve shifts to the right. This is of physiological importance because increased temperature means higher metabolic rate or higher oxygen requirement.

Another important influence is the pH. Increase in carbon dioxide or other acids lowers the pH of plasma and shifts the dissociation curve to the right (Fig. 2.16). At high carbon dioxide concentrations more oxygen is given up at any given oxygen pressure. This effect is known as **Bohr effect** after the Danish scientist who first described it. Therefore, as carbon dioxide enters the blood from respiring tissues it encourages the release of more oxygen. This is an important characteristic be it allows more oxygen to be released to tissue which need it the most. is loaded in the lungs and more unloaded at the tissues due to Bohr ef be the case if only diffusion along the concentration gradient wa

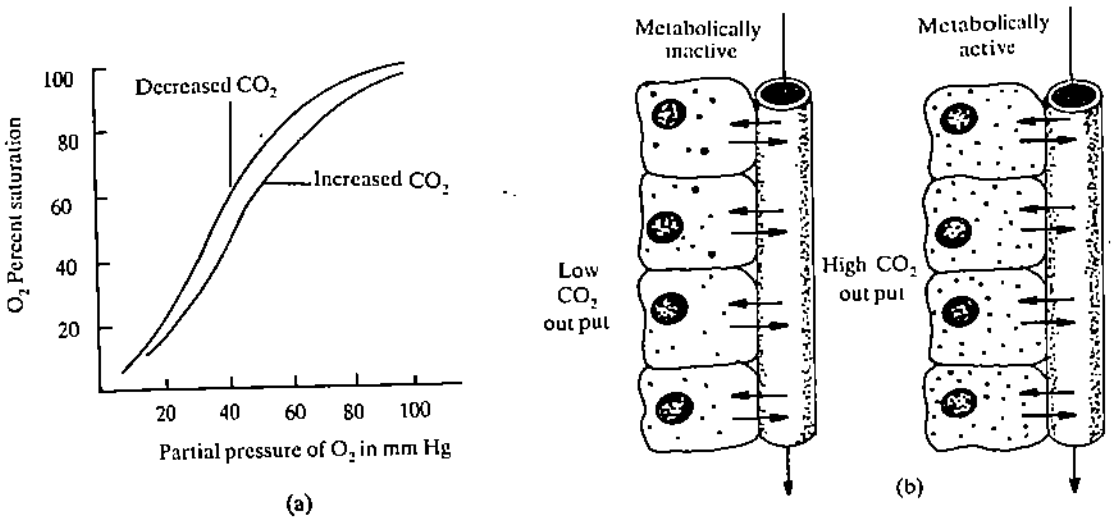



**Fig. 2.16 : Bohr effect. Oxyhaemoglobin surrenders its oxygen more readily in the presence of increasing acidity. (a) The oxygen dissociation curve shifts to the right with increasing acidity; (b) metabolically active cells will receive more oxygen than metabolically less active cells.**

Carbon dioxide lowers the oxygen affinity of haemoglobin even if the pH is kept constant. This effect is due to the binding of carbon dioxide to the terminal amino groups of haemoglobin molecule. This site is not the same site on the molecule where oxygen is bound.

The presence of organic phosphates in the red blood cells helps to explain many peculiarities of the oxygen dissociation curve. Previously the red blood corpuscle was considered to be a bag full of haemoglobin with no metabolism of its own because of the absence of a nucleus. Now we know that it has an active carbohydrate metabolism and RBC has high content of ATP and 2,3-**diphosphoglycerate (DPG)**. 2,3-DPG is a product of glycolysis and it binds to β chain of the globin and reduces oxygen affinity. Experimentally it was shown that pure haemoglobin has greater oxygen affinity than whole blood (the dissociation curve for pure haemoglobin is far to the left of the curve for whole blood). If 2,3-DPG is added to pure haemoglobin solution the oxygen affinity decreases and approaches that of whole blood. The effect of 2,3-DPG is also important in transfer of oxygen from maternal to foetal blood. Foetal haemoglobin is different from adult haemoglobin in having 2 δ chains instead of β chains. Foetal haemoglobin therefore cannot bind to 2,3-DPG and thus has higher affinity for oxygen at a given $Po_2$. This higher oxygen affinity in foetal blood facilitates oxygen transfer from mother to foetus.

The importance of 2,3-DPG within the red blood cells is now recognised in blood banking. Old stored red cells loose then ability to produce 2,3-DPG which means that such cells will not unload their oxygen easily. Modern techniques for storage of blood, therefore, include the addition of energy substrates for respiration and phosphate sources needed for production of 2,3-DPG.

**SAQ 6**

Pick out the true statements from those given below :

i) The haemoglobin of adults contain four identical subunits and a haem group.

ii) When haem and globin fractions are separated the haem group binds, reversibly with oxygen.

iii) Bohr's effect facilitates transfer of oxygen to the tissues because of increased $CO_2$ levels in blood.

iv) 2,3-DPG inhibits the transfer of $O_2$ across the foetal-maternal barrier.

v) In haemoglobin one subunit after attaching to an oxygen facilitates the oxygen binding to others.

## 2.7.3 Carbon Dioxide Transport in Blood

The same transport system that brings oxygen to the tissues must take back carbon dioxide to the environment across the respiratory surface. However, unlike oxygen that is transported exclusively by haemoglobin, carbon dioxide is transported in three ways:

- as dissolved carbon dioxide in plasma (about 8%) and in red cell.
- carbaminohaemoglobin. About 25% of the total blood carbon dioxide is attached to the amino groups in haemoglobin.

$$\text{N}\begin{matrix}\text{H}\\\text{H}\end{matrix} + CO_2 \rightarrow H^+ + \text{Haemoglobin} - NHCOO^-$$

It is then carried to the lungs where haemoglobin releases it in exchange for oxygen.

3) as **carbonic acid** and **bicarbonate** which accounts for most of the carbon dioxide carried by blood.

Carbon dioxide combines with water to form carbonic acid.

$$CO_2 + H_2O \longrightarrow H_2CO_3$$

This reaction occurs spontaneously in plasma at a very slow rate but much more rapidly within the blood cell due to the catalytic reaction of an enzyme, **carbonic anhydrase**. The formation of carbonic acid is favoured by high $Pco_2$ in the capillaries of tissues. Carbonic acid dissociates rapidly in the red blood cells into hydrogen ion ($H^+$) and bicarbonate ion ($HCO_3^-$).

$$H_2CO_3 \longrightarrow H^+ + HCO_3^-$$

The $H^+$ released are buffered by their combination with haemoglobin and $HCO_3^-$ moves out of the cells. The inside of the cell thus gains a net positive charge. This attracts chloride ion ($Cl^-$) which move inside the red blood cells. This exchange of anions as the blood moves through capillaries in tissue is known as **chloride shift** (Fig. 2.17a). The red blood cells are very permeable to both $Cl^-$ and $HCO_3^-$ because the membrane has a high concentration of a special anion carrier protein called band III protein that binds $Cl^-$ and $HCO_3^-$ and transfers them in opposite direction through the membrane.

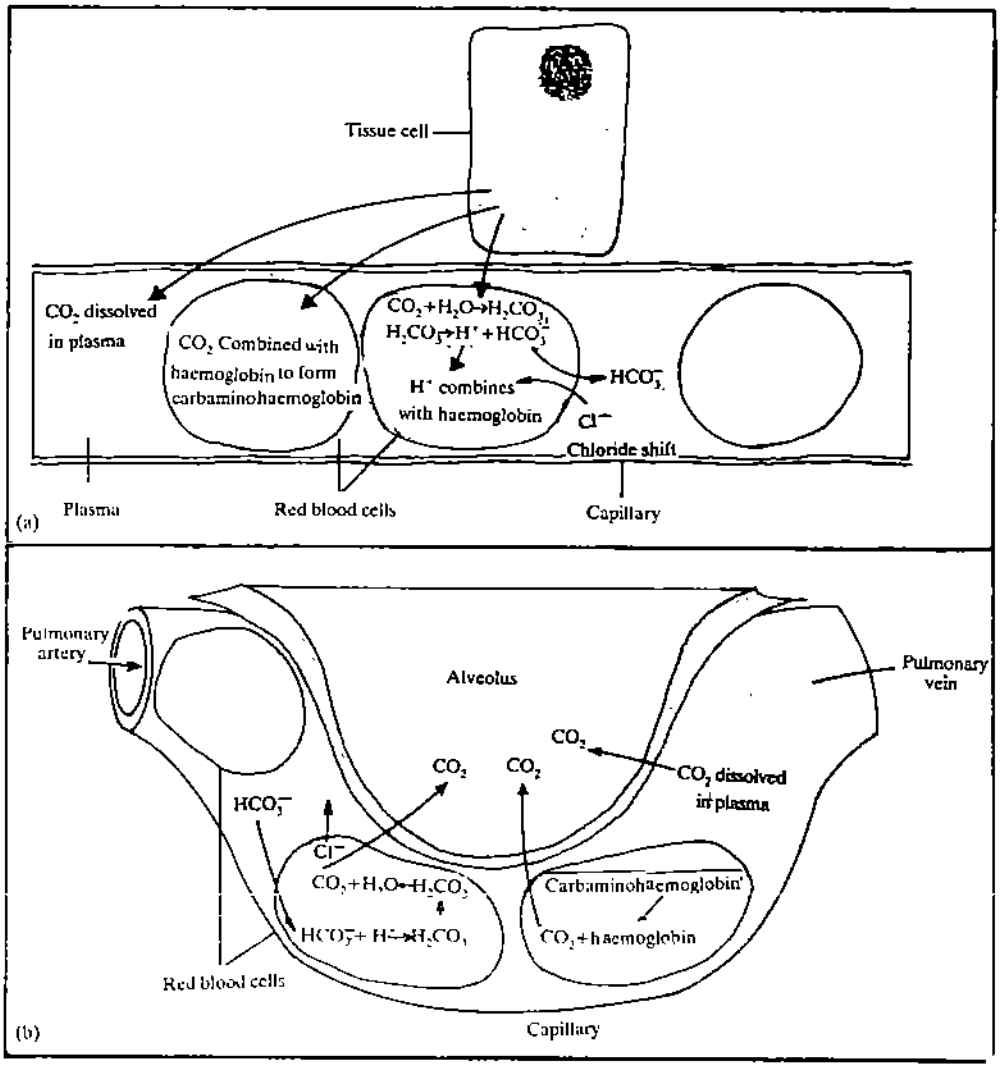



Fig. 2.17 : Carbon dioxide transport in blood (a) in chloride shift, carbon dioxide is transported forms: as dissolved $CO_2$ gas, attached to haemoglobin as carbaminohaemoglobin, an acid and bicarbonate, (b) carbon dioxide is released from the blood as it trave capillaries. A reverse chloride shift occurs and carbonic acid is transformed

The formation of carbonic acid enhances oxygen unloading (Bohr effect) and oxygen unloading in turn improves the ability of blood to form carbonic acid and transport carbon dioxide. When blood reaches pulmonary capillaries deoxyhaemoglobin is converted to oxyhaemoglobin which has a lower affinity for $H^+$. The $H^+$ is released in the red blood cells. This attracts $HCO_3^-$ from plasma which combines with $H^+$ to form $H_2CO_3$

$$H^+ + HCO_3^- \longrightarrow H_2CO_3$$

Under low $Pco_2$ of pulmonary vessels carbonic anhydrase catalyses the formation of carbonic acid to carbon dioxide and water

$$H_2CO_3 \xrightarrow[\text{Low } Pco_2]{\text{Carbonic anhydrase}} H_2O + CO_2$$

Therefore, a **reverse chloride shift** occurs in the pulmonary capillaries to convert carbonic acid, and biocarbonate to $CO_2$ gas which is eliminated in expired breath (Fig. 2.17b).

After having discussed the properties of blood and its role in the transport of respiratory gases we can now proceed to discuss the movement of blood in animals in the next unit on circulation.

## 2.8 SUMMARY

In this unit you have studied that :

- The physiological basis of gas exchange in animals depends on some simple physical principles like solubility of gases, their diffusion rates which in turn depends on the nature of gas, its partial pressure, temperature and presence of other solutes.
- The four basic categories of animal gas exchange mechanisms are diffusion through body surface, gills, lungs and trachea. The design of respiratory surface and the mechanism of breathing are related to the nature of the medium in which the animals live.
- Most aquatic animals exchange gases through gills that are evaginations of respiratory surfaces. Fish gills consist of gill arches with rows of gill filaments. They contain extensive capillary beds in plate-like lamellae. Water flow across the lamellae opposes blood flow, setting up an efficient countercurrent exchange. Diffusion of oxygen in water is slow, therefore, aquatic animals must expend energy to move large volumes of water over the respiratory surfaces.
- The vertebrate lung is a highly branched inpocketing of respiratory surface containing numerous air sacs or alveoli, intimately associated with capillaries and ventilated by a tidal flow of air.
- Gas exchange in lungs takes place in alveoli where partial pressure of oxygen is higher than that in blood hence oxygen diffuses from alveoli to blood. In body tissues the partial pressure of oxygen is low hence oxygen diffuses from blood into tissues. Respiration is regulated by levels of $Pco_2$ and by a respiratory centre in the brain which is sensitive to blood $Pco_2$.
- Insects have evolved a separate system for gas exchange-tracheal system independent of circulatory system.
- Respiratory gases are transported mainly by respiratory pigments in blood. The most well-known pigment is haemoglobin which combines with oxygen to form oxyhaemoglobin. Oxygen dissociation curve depicts per cent oxyhaemoglobin saturation at different values of $Po_2$. Carbon dioxide, pH, temperature and presence of organic compounds in blood influence this curve. A fall in pH decreases the oxygen affinity of haemoglobin for oxygen. This is Bohr effect. Decreased affinity causes unloading of oxygen to tissue. Oxygen affinity also decreases in the presence of 2,3-DPG.
- Carbon dioxide is transported to the lungs by formation of carbonic acid in red blood cells. This reaction is favoured by high $Po_2$ in tissues. Carbonic acid ionises into $H^+$ and $HCO_3^-$. Hydrogen ion is buffered by haemoglobin but anion balance is maintained by chloride shift. Reverse chloride shift occurs in the pulmonary

of limbs and contraction of dorsal hearts are important. In the giant earthworm peristaltic contractions of the dorsal vessel move the blood in the anterior directions and thus fill 5 pairs of lateral hearts (Fig. 3.4).

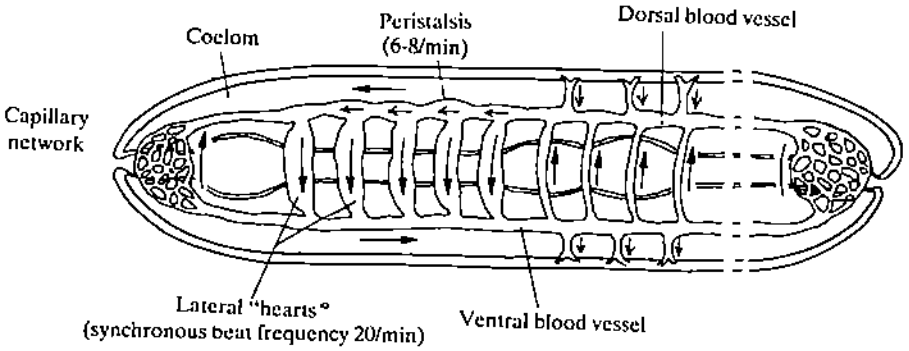



**Fig. 3.4 : Lateral hearts in giant earthworm *Glossoscolex giganteus*.**

Another feature common to all animal circulatory systems is the presence of valves or septa that control the direction of the flow of blood.

Tunicates show an interesting variation. They have open circulatory system and the tube like heart pumps blood by means of peristaltic wave passing from one end to another. There are no valves in the heart. After a series of contractions the heart slows down and stops. After a pause the beat starts in the reverse and blood flows in the opposite direction.

The circulating fluid or blood is carried from the heart in channels and eventually returned to the heart. Vertebrate blood as you know is carried through a system of elastic tubes the **arteries, capillaries** and **veins**. The blood returns to heart without actually leaving this system of tubes. Since blood remains in this closed system it is known as **closed circulation.** In many invertebrates, however, blood is pumped by the heart into a vessel which opens into the open fluid spaces so that the tissues are bathed by the blood which is known as **haemolymph** in this case. Such a system is known as **open circulatory system.**

Open circulation is found in invertebrates e.g. insects, most crustaceans and many molluscs, while annelids cephalopods, echinoderms and vertebrates have closed circulatory systems (Fig. 3.5).

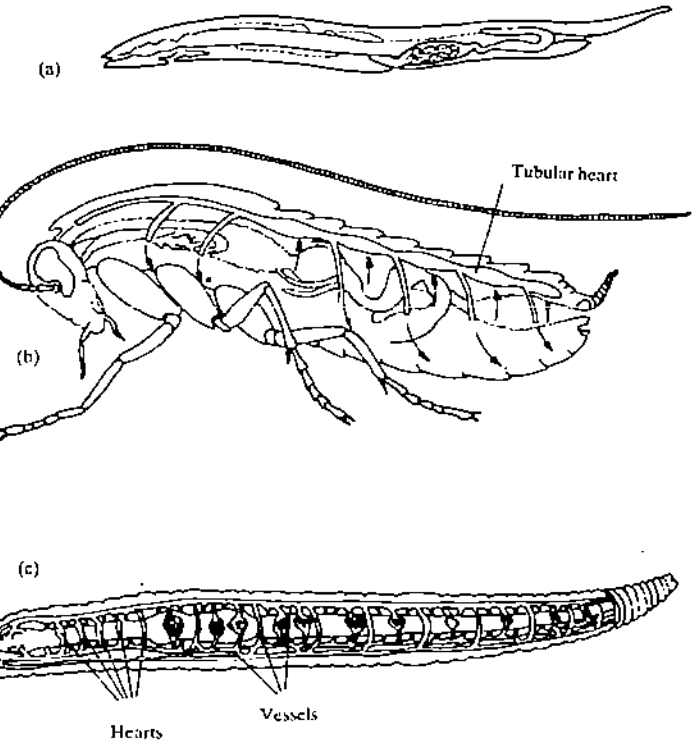



**Fig. 3.5 : In open circulatory systems, pools of blood bathe the body's tissues and organs. (a) Blood in nematodes is moved through the body by muscles that contract as the animal moves. (b) In insects there are tubular hearts too that fill and empty rhythmically, percolating blood over the tissues. (c) Annelid's closed circulatory system. Blood is pushed through a set of hearts into circulatory vessels that take it to the tissues. Another set of vessels take the blood back to the heart.**

In the open system there are no small blood vessels or capillaries connecting the arteries and veins. Nets of minute sinuses or capillaries are found at some points in a blood sinus. They do not form a closed network between arteries and veins and arterial blood sooner or later passes into sinuses and bathes the major organs and

tissue. From these tissue spaces the fluids slowly go back into the open ends of veins or the ostia opening into the heart. In the open circulatory system the organs lie directly into the blood filled spaces or haemocoel.

In general, there is a more complete separation of functions in closed circulatory systems than in open ones. The heart is the main pump that propells the blood through the **arterial system** and maintains a high **blood pressure** in the arteries. The arterial system in turn acts as a pressure reservoir forcing blood through the capillaries. The walls of the capillaries are thin and this allows material to transfer between blood and tissues. Each tissue has many capillaries so that each cell is not far from the capillaries.

Blood leaving the capillaries enters **venules** and **veins** that return the blood to the heart. The **venous system** has low pressure and contains most of the blood and is the large volume reservoir. Blood donors give blood from this reservoir as there is hardly any change in pressure when the blood volume decreases.

A **lymphatic system** has evolved along with the high pressure closed circulatory system to recover fluid loss to the tissue from the blood. You will learn more about the lymphatic system in section 3.5.

### Circulation Patterns

All vertebrates have some similarities in their circulatory systems but as vertebrate life changes from aquatic to terrestrial, the pattern of circulation becomes more complex. Fish and mammals represent two extremes in vertebrate circulation and Fig. 3.6 compares the two. The principal difference is in heart structure, from two chambered in fish to four chambered in mammals.

The fish heart contains two main chambers in series the **atrium** or **auricle** and the **ventricle**. There are two subsidiary chambers (not shown in Figure) the **sinus venosus** which preceeds the atrium and **conus arteriosis** which comes after the heart. These contain valves that prevent backwards flow of blood. Blood makes a single circuit from the heart to the gills where it is oxygenated and then to the dorsal aorta to be distributed to the body from where it returns to the heart by the veins. Such a system has the advantage that all of the blood going to the body has already been oxygenated in the gills. However, there is a disadvantage i.e. the narrow gill capillaries slow down the blood flow resulting in a low blood pressure. This slows the rate of oxygen delivery to the cells and limits the metabolic rate that fish can attain.

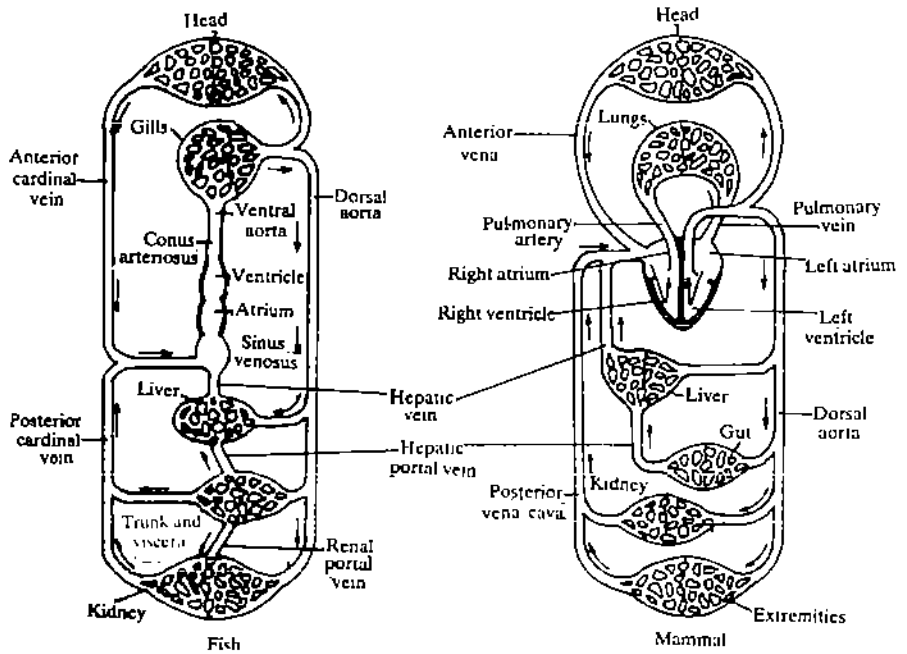



**Fig. 3.6 : Circulatory system pattern in (a) fish and (b) mammals. Rust shows oxygenated blood.**

The evolution of lungs and the need for highly efficient blood distribution necessitates **double circulation** in birds and mammals. One **systemic** circuit with its own pump that sends oxygenated blood to the capillaries of body organs and one **pulmonary** circuit that sends deoxygenated blood to the lungs. By keeping oxygenated and

### 3.4.2 Cardiac Output

Now that you know how blood is pumped out by the heart, let us see how much blood is pumped out with each heartbeat and whether it is related to the oxygen requirements of the animal.

You studied in Unit 1 that small animals have a high rate of oxygen consumption in comparison to bigger animals. Therefore, the heart must supply oxygen at a higher rate too. How is this achieved? Is the size of the heart larger than other animals or is the amount of blood pumped per heartbeat more or, is the rate at which it is pumped more?

Let us first talk about the size of the heart. It is nearly proportional to the body size and makes up approximately 0.6% of the body mass in small as well as large mammals. Differences in the need for oxygen are not reflected in the size of the heart and it is known that the amount of oxygen dissolved in each volume of blood ejected out of the heart is also not dependent on the size of the heart.

Therefore, we can conclude that the pumping frequency must be responsible for supplying adequate amount of oxygen when needed. Heartbeat frequency or **pulse rate** is usually given as the number of heartbeats per minute. The pulse rate for an adult human being at rest is 70 per minute. During exercise this rate increases several times. You will learn more about this later in the unit.

Now, the heartbeat frequency is certainly related to body size. The general rule is, larger the animal, slower the pulse rate. Apart from body size in vertebrates the pulse rate varies with the level of metabolism. Cod fish which is cold-blooded has a pulse rate of 30 per minute, a warm-blooded rabbit of approximately the same weight has a pulse rate of 200 beats per minute.

Among mammals, an elephant weighing 3000 kg has a resting pulse rate of 25 per minute while the tiny shrew of approximately 3 mg has a rate of over 600 beats per minute.

From the above information we can say that animals with a high rate of oxygen consumption get additional oxygen because the rate at which blood is pumped is high. Let us now examine the volume of blood ejected per heartbeat.

The volume of blood ejected from the heart per unit time is called **cardiac output**. In hearts with complete separation of ventricles, cardiac output refers to output of one side only and not of both the ventricles.

The cardiac output can be determined in a number of ways. The most common method is the **Fick's principle**. A. Fick a German physiologist in 1870 described a simple method of calculating cardiac output from measurement of oxygen consumption (or carbon dioxide production) and the difference between oxygen (or carbon dioxide) contents of blood entering and leaving the heart.

$$\text{Cardiac output} = \frac{O_2 \text{ absorbed by lungs}}{\text{arteriovenous oxygen difference}}$$

The volume of blood ejected by each beat of the heart is the **stroke volume**. The mean stroke volume is determined by dividing cardiac output by heart rate. In other words, cardiac output can be calculated if we know the values of heart rate and stroke volume.

Therefore, cardiac output can be increased by increasing either the heart frequency or the stroke volume or both. However, in mammals there may be little change in stroke volume if cardiac output is to be altered, major adjustments are made in the heart rate. The distribution of blood to the various organs of the human body is given in Table 3.2. You can see from the Table that kidneys, liver, heart and brain make up only 5% of the total body weight but receive more than half of the total cardiac output. Stroke volume can also be defined as the difference in volume of blood before and after contraction. Two factors influence stroke volume, one is the hormone adrenaline (epinephrine) which increases contractions thus forcing a larger volume of blood out of the ventricles in a single stroke. The other is the amount of blood present in the ventricles before contraction.

Table 3.2 : Blood flow to major organs of a 70 kg man at rest

| Organ | Organ Mass (kg) | Blood Flow (litre/minute) | Blood Flow (litre/kg/min) |
|---|---|---|---|
| Kidney | 0.3 | 1.2 | 4.0 |
| Liver | 1.5 | 1.4 | 0.9 |
| Heart | 0.3 | 0.25 | 0.8 |
| Brain | 1.4 | 0.75 | 0.5 |
| Skin | 2.5 | 0.2 | 0.08 |
| Muscle | 29.0 | 0.9 | 0.03 |
| Remainder | 35.0 | 0.9 | 0.03 |
| Total | 70.0 | 5.6 | |

If the returning venous blood to the heart is increased, then ventricles will be filled with more blood and following contraction more blood will be ejected out. This relationship between cardiac output and increased venous volume was discovered by the English physiologist Ernest H. Starling. This relationship will be further discussed when we talk of blood flow during exercise.

**SAQ 3**

Tick mark (√) the correct answer :

a) Stroke volume is the

i) volume of blood pumped from the heart
ii) volume of blood pumped into the heart
iii) volume of blood pumped by one ventricle during a single heartbeat
iv) number of heartbeats per minute

b) Cardiac output equals

i) stroke volume multiplied by heart rate
ii) stroke volume divided by heart rate
iii) stroke volume added to the heart rate

c) Fill in the blanks with appropriate words from the text.

Sinoatrial node is the ..... of the heart. The ..... starts at this small piece of muscle and spreads to the ..... from where it spreads to the ..... ..... The impulse is conducted to the ..... of the ventricle from where it then spreads ..... to the ..... of the ventricle. Release of ..... at the ..... slows the heart rate while adrenaline accelerates it.

## 3.5 BLOOD VESSELS

The blood vessels have elastic walls and a layer of smooth muscles in their walls which enables them to change their diameter. There are three main types of blood vessels — arteries, capillaries and veins with characteristic differences among them. Arteries have relatively thick walls that consist of heavy, strong layers of elastic fibres and smooth muscles. As the arteries branch, their diameter becomes smaller and the relative amount of muscle tissue increases in proportion to the elastic tissue. Capillaries are the smallest units, they are single cell thick. Almost all exchange between tissue and blood takes place through the walls of the capillaries. The veins also contain smooth muscles and fibres.

### 3.5.1 Blood Flow

To understand how blood flows in vessels let us first understand the basic physics of flow in tubes and the special properties of blood as a fluid.

The flow of a fluid may be smooth or regular in a straight tube so that each particle moves in a straight line. This is called **laminar flow**. Blood exhibits a similar kind of flow in the blood vessels.

Let us first consider the velocity of flow of blood in the vessels. At any point it is not related to the nearness to the heart but to the total cross-sectional area of that part. This cross-sectional area does not refer to that of a single artery or vein or capillary but to the sum of the cross-sections of all the arteries or capillaries of that area. You would have noted that the velocity of water in a river increases as the river narrows, similarly in circulation the maximum velocity occurs where total cross-sectional area is smallest. The arteries have the smallest cross-sectional area and the capillaries have the largest total cross-sectional area. Fig. 3.9 depicts this relationship.

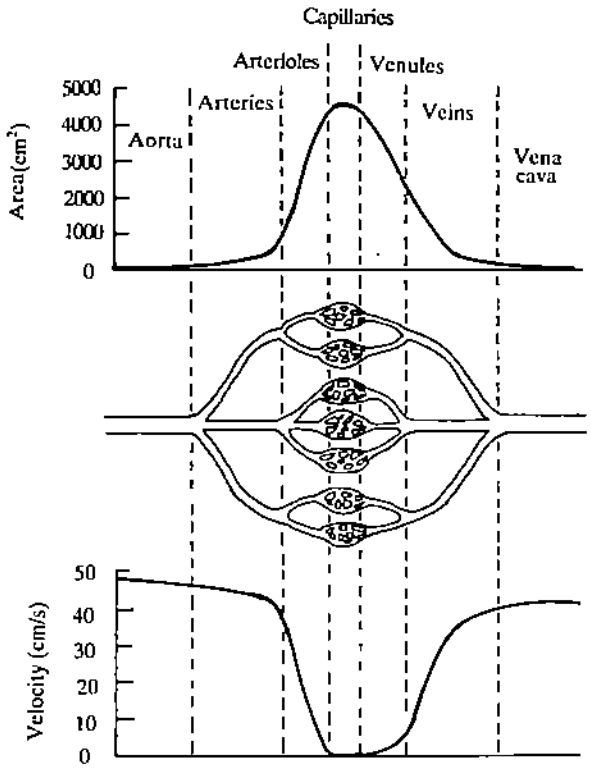



**Fig. 3.9 : Blood velocity is inversely proportional to cross-sectional area at any given point. The velocity is the highest in aorta and falls markedly in capillaries and again increases in vena cava.**

The resistance to flow in a tube results from inner friction in the fluid i.e. the **viscosity**. We all know that water and sugar syrup do not flow at the same rate from a bottle. We can say that water has low viscosity and syrup, a high viscosity. For convenience viscosity of a fluid is expressed relative to the viscosity of water. Blood plasma has a **relative viscosity** of 1.8 mostly as a result of the 7% dissolved proteins. Whole blood is more viscous because of the cells in it, at 37°C, relative viscosity of mammalian blood is between 3 and 4. Therefore, because of the presence of RBC blood behaves as though it is 3-4 times more viscous than water.

However, blood does not behave as expected of a viscous fluid. Its relative viscosity changes with decreasing radius of the blood vessels. In fact in tubes less than 0.3 mm in diameter the relative viscosity of blood approaches that of the plasma, therefore, it flows more easily. In flowing blood, we find that the red cells tend to accumulate in the centre. This accumulation leaves the wall relatively free of cells, therefore, the viscosity in the centre is more than at the sides. Since flow is inversely related to viscosity, flow at the walls will increase slightly and will decrease at the centre slightly.

Another peculiar aspect of blood flow in capillaries is that often the capillary diameter is smaller than RBC and the RBCs easily change shape to pass through the capillary. This gives rise to a very different type of flow — **bolus flow** in which the red cells act as a plug that causes rapid increase in liquid along the walls of the capillary and thus help in the renewal of the diffusible substances in this layer.

### Blood Pressure

We are all familiar with the term high blood pressure. What exactly is blood pressure? By blood pressure we mean arterial blood pressure. During a heartbeat cycle the maximum pressure is referred to as **systolic pressure** and the minimum **diastolic pressure**. The difference between the two is the **pulse pressure**. Blood pressure is

To convert to kilopascals, multiply the blood pressure in mm Hg by 0.1333 k Pa.

generally expressed in millimeters of mercury and as systolic/diastolic, that is 120/80 mm Hg. Blood is 12.9 times less dense than mercury so a blood pressure of 120 mm Hg is equal to 120 × 12.9 = 1550 mm or 155 cm of blood. In other words if the blood vessel was to be opened suddenly the blood would squirt out to a maximum height of 155 cm above the cut.

Pressure generated by the heart decreases due to the flow of blood. Fig. 3.10 shows blood pressures in the circulatory system of man. The blood pressure falls as blood flows from the aorta to the vena cava. The greatest pressure drop takes place in the smallest arterioles which by changing their diameter can regulate the flow of blood to various body organs.

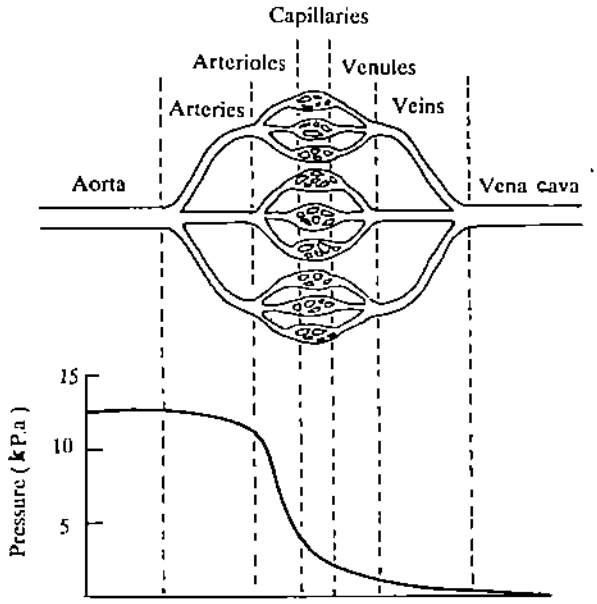



**Fig. 3.10 : Change in blood pressure in different parts of the circulation. The major drop occurs in the arterioles.**

**Box 1**

**William Harvey and the Study of Circulation**

William Harvey's book on hearts and the circulation of blood — first published in Latin in 1628 — provided at a single stroke almost all the major features of circulation as we know them today.

Prior to Harvey's great work, relatively little was known about circulation and most of what was "known" was wrong. Blood had once been thought to be immobile in the body. Later, it was wrongly thought that blood movement is sluggish and that blood erratically changes velocity and direction. It was not even realized that the heart is responsible for the movement of the blood. The beating of the heart and pulsing of the arteries were attributed to "vital spirits" contained within them. It was thought that there are two kinds of blood — one corresponding to what we now recognize as the blood of pulmonary circulation and the other to the blood of systemic circulation.

Harvey combined meticulous anatomical observation with elegant physiological experimentation. Once the structural basis was well understood, he could then turn to the study of function. His comparative observations of heart and blood movements in vertebrates and invertebrates ranged from molluscs and insects to fishes, amphibians, reptiles, birds, and mammals. He estimated the capacities and rates of blood flow through ventricles, and he studied the effects of drugs and of changes of position on the circulation. The valves in veins had been discovered by Fabricius in the sixteenth century, but it took Harvey to explain their function — as he did for the valves of the heart.

Harvey laid the groundwork for our current understanding of much of the circulatory system. The only major step that he missed was the capillaries between arteries and veins. Four years after Harvey's death Marcello Malpighi discovered and described the capillary circulation.

### 3.5.2 Arteries

The arteries deliver blood from the heart. Fig. 3.11 shows the structure of arteries and the different layers of the vessel wall. The thick walls of these blood vessels, except those of the smallest, are supplied by their own capillary network called **vasa vasoram**. The arteries serve four main functions:

1) to act as a conduit for blood between heart and capillaries,
2) to act as a pressure reservoir for forcing blood into small diameter arterioles,
3) to produce a more or less even flow of blood through the capillaries,
4) to control distribution of blood to different capillary networks via selective constriction of the terminal branches.

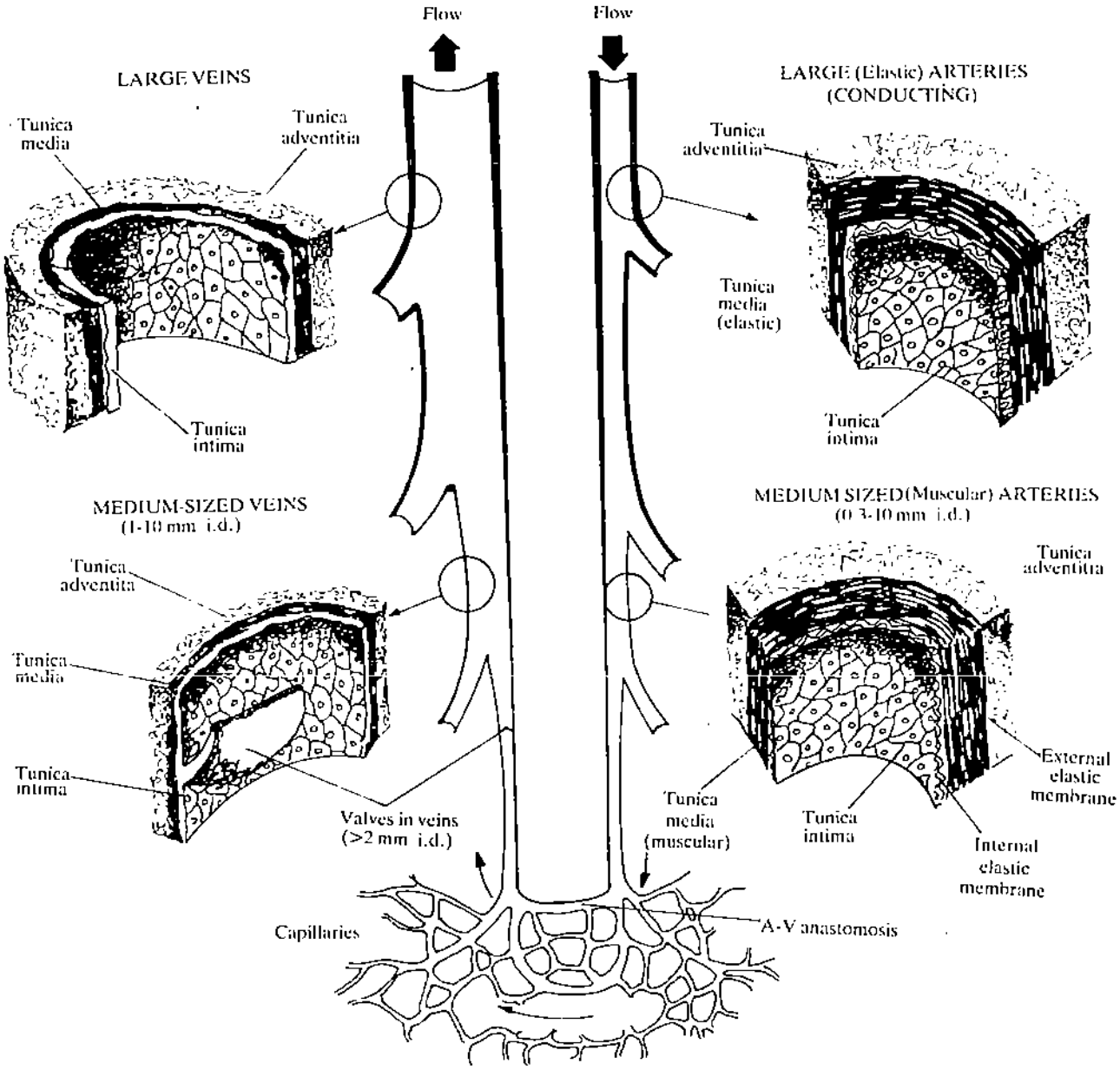



**Fig. 3.11 : Major structures in peripheral blood circulation in mammals. Blood flows from the arteries through the capillaries into the veins (id is internal diameter).**

There is a precise control on arterial blood pressure. The nature of the arterial wall and the volume of blood pumped into the arteries determines the pressure. If any of these are changed, the pressure will also change. Normally, arterial blood pressure varies very little as cardiac output and capillary flow are evenly matched.

The elastic properties of arterial walls vary. Close to the heart the arteries are elastic and dampen the oscillations in pressure and flow generated by the contractions of the heart. As the heart relaxes the pressure in the arteries is maintained by a reduction in vessel volume. If the arteries were rigid tubes then the same pressure fluctuations would be experienced by peripheral vessels as that observed when blood leaves the heart.

### 3.5.3 Veins

The veins bring back the blood from the capillaries to the heart. They form a large volume low pressure system. The vessels have a larger internal diameter. In mammals 50% of the total blood volume is present in the veins and pressure is approximately 10-5 mm Hg. If there is any blood loss, the venous volume is decreased and not the arterial volume. So that arterial blood pressure and capillary blood flow is maintained.

Flow of blood in veins is affected by several factors. Pressure exerted by the diaphragm on the gut and activity of the limbs both help to squeeze the veins of those regions. This squeezing and action of **pocket valves** (that prevent back flow) help the flow of blood towards the heart. Breathing in mammals also helps in drawing the blood from the veins in the head and abdominal cavity.

Smooth muscles in veins also help in regulating blood supply in the venous system. When a person changes his position from sitting to standing the change in the relative position of heart and brain with respect to gravity activates the nerve fibres that are present in the veins of the limb. This causes a contraction of the smooth muscles. The pooled blood is thus, redistributed.

When a person stands immobile for a long period, he can faint because of inadequate venous blood return to the heart. In such cases, cardiac output, arterial pressure and flow of blood to the brain are all reduced. Similar situation occurs in bedridden patients that try to standup after a long period.

### 3.5.4 Capillaries

We said earlier in the unit that most tissues have such an extensive network of capillaries that any single cell is hardly 2-3 cells away from any capillary. The small terminal arteries subdivide to form **arterioles** which divide to form **metarterioles** and then **capillaries**, which rejoin to form the **venules** and veins.

The smooth muscles of the arterioles become discontinuous in the metarterioles and end in a muscle ring the **precapillary sphincter** that controls the blood supply to each capillary bed. Through the precapillary sphincters, the capillary bed can be bypassed altogether and blood can be diverted to areas of greater demand. All capillaries of an animal have the potential to hold 14% of the total blood volume. However, only 30% to 50% of all capillaries are open at a time and thus only 5-7% of the total volume is contained in them.

It is easy to count the number of capillaries in a cross-section of muscle as capillaries run between and parallel to the muscle fibres. The resting muscle of a guinea pig in cross-section of 1 $mm^2$ contains 100 open capillaries through which blood flows. Under maximal exercise, however, 1 $mm^2$ cross-section may show more than 3000 open capillaries! Compare the diameter with an ordinary lead pencil of 3 $mm^2$ cross-section!

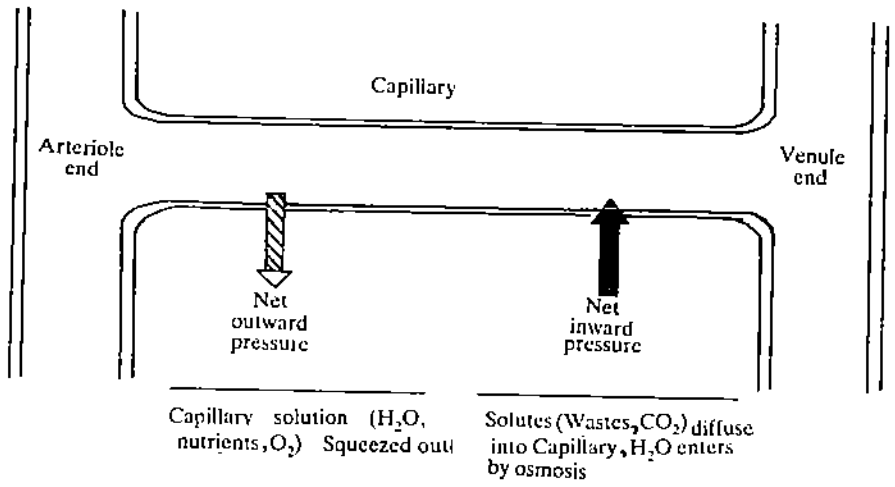



**Fig. 3.12 : Fluid exchange across a capillary wall.**
**Differences in blood pressure and osmotic potential across the walls of a capillary are responsible for the exchange of fluid. On the left, blood leaving an arteriole to enter a capillary is at a relatively high pressure which overcomes the local osmotic potential. Because of this, blood fluids are squeezed out of the capillary (striped arrow). At right, blood leaving a capillary to enter a venule is at a relatively low pressure that does not overcome the local osmotic potential, even though the latter is also relatively low. As a result water enters the capillary by osmosis (solid arrow). In contrast to this exchange of fluid, the exchange of gases, nutrients, and wastes occurs by simple diffusion across the capillary wall.**

Capillaries are made up of a single layer of endothelial cells surrounded by a basal membrane. The walls are thin and fragile but because of their small diameter resist stretching in response to capillary blood pressure. Water and dissolved substances of small molecular weight (gases, salts, sugars, amino acids etc.) can diffuse easily. In addition, fluid is forced out through the walls. Substances of molecular weight more than 70,000 (mostly proteins) do not pass out of the capillary walls. These proteins exert an osmotic effect called the **colloidal osmotic pressure**, which tends to draw water back into the capillary from the surrounding tissue fluid. Another force, the hydrostatic pressure of blood tends to push the water across the endothelial cell layer out of the capillaries. When the hydrostatic pressure within the capillary exceeds colloidal osmotic pressure, fluid is passed out through the capillary wall; when the hydrostatic pressure in the capillary falls below the colloidal osmotic pressure the fluid is drawn in. The hydrostatic pressure in the arterial end of the capillary is higher than the colloidal arterial pressure while at the venous end it is often lower. Therefore, fluid is filtered out at the arterial end and redrawn in at the venous end (see Fig. 3.12).

This amount of fluid forced out and the amount re-entering varies greatly. Usually outflow exceeds inflow and excess fluid remains in the interstitial spaces. This as you already know forms the **lymph**.

### 3.5.5 Blood Flow during Exercise

Whenever we exercise or run we notice that we begin to breathe faster, the heart beats much faster than the usual average of 70 beats per minute. We know that the body at this time needs more oxygen and the heart must supply it to the muscles. There can be two ways of supplying this additional oxygen. The cardiac output can be increased or the amount of oxygen delivered by each volume of blood is increased.

The arterial blood pumped out of the heart is already fully saturated but venous blood normally contains more than half the oxygen present in the arterial blood. Therefore, if more oxygen is extracted from the venous blood it can be supplied to the muscles. The total muscle of a lean person uses about 50 ml of $O_2$ per minute which is supplied by about 1 litre of blood. Arterial blood contains 200 ml of $O_2$/litre and venous blood contains 150 ml of $O_2$/litre. The oxygen extraction therefore, is only 25%. During heavy exercise, blood flow to muscles may be 20 litres per minute or even higher in atheletes and the oxygen extraction increases to 80-90%. In other words, during heavy exercise almost all the oxygen may be removed from the venous blood.

The cardiac output can also be increased to deliver more oxygen. As said earlier, cardiac output can be increased by either increasing stroke volume or heart rate or both. At rest the human heart rate is 70 beats per minute and the stroke volume is 70 ml (from each side) giving a total cardiac output of 5 litres/minute. During exercise the cardiac output is increased about five-folds or more. Most of the increase is due to increase in pulse rate which may become 200 strokes per minute. Stroke volume may also increase beyond 100 ml. In a well-trained athelete the oxygen consumption may be increased as much as 100 times but this becomes possible more due to a three-fold increase in oxygen extraction from the blood.

**SAQ 4**

a) Select the **four** true statements:

   i) Arteries are generally of larger diameter than veins.

   ii) A diameter of about 10 mm is typical of blood capillaries in mammals.

   iii) The arteries near the heart are more elastic and dampen the oscillation in blood flow.

   iv) Whole blood is more viscous than plasma because of presence of blood cells.

   v) Velocity of blood flow is related to the total cross-sectional area of the vessels.

   vi) Viscosity of blood changes with decreasing radius of blood vessels.

   vii) The maximum pressure during a heartbeat is known as diastolic pressure.

b) If osmotic pressure exceeds blood pressure will there be net filtration or net absorption in the capillaries?

## 3.5.6 Lymphatic System

Approximately 99% of the fluid (water) that leaves capillaries at the arteriole end is normally reabsorbed at the venule end. But what about the remaining 1 per cent fluid left in the interstitial spaces? Vertebrate animals have a special system of vessels, the **lymphatic system** (Fig. 3.13), that drains fluid from interstitial spaces in the tissues and returns it to the blood, thus maintaining balance between blood volume and interstitial fluid volume in the body. There are **lymph capillaries** in all parts of the body. Lymph capillaries have blind ends, that is, they are closed at one end. Though the walls of the lymph capillaries are structurally similar to those of blood capillaries their permeability characteristics are different. Their walls are permeable to water and proteins that have been filtered across the capillary walls. This mechanism is important because a few protein molecules leak through the capillary walls and these must be returned to the blood if normal and functionally necessary osmotic differential between blood and interstitial fluid is to be maintained. Large molecules, particularly fat absorbed from the gut and probably high molecular weight hormones reach the blood via the lymphatic system (refer to Unit 1).

Once interstitial fluid enters the lymphatic system, it is called **lymph** which is a pale yellow fluid. Lymph moves through lymph capillaries and lymph vessels as a result of pressure applied by muscle contractions near the vessels and a system of valves that prevent backward flow. Some vertebrate animals (many cyclostomes, fishes and amphibians) have pulsating lymph hearts that move lymph along through the lymphatic system. But human lymph movement is completely passive and depends on the pressure of the contracting muscles adjacent to the vessels. Lymph moves through smaller vessels that unite to form larger vessels. Finally, two major lymph ducts (left thoracic duct and right lymphatic duct) empty into large veins near the heart (Fig. 3.13).

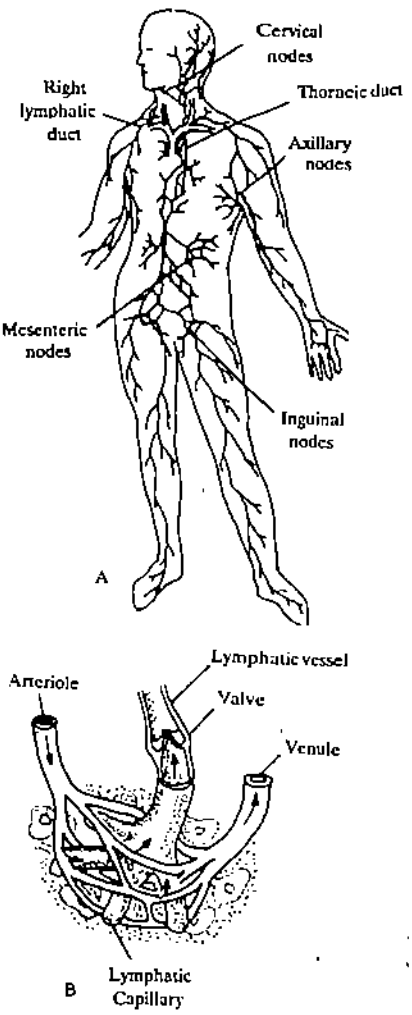



Fig. 3.13 : a) Lymph vessels of the human body.
b) An idealised view of relationship between lymph capillaries, blood and tissues.

In addition, the lymphatic system plays an important role in body's defence system by producing **lymphocytes** and **monocytes** in the **lymph nodes** located along major lymph vessels. Lymph nodes are present in birds and mammals only. The phagocytic cells engulf dead cells, cell debris and foreign objects such as bacteria as the lymph filters through the lymph nodes. In addition to this general phagocytic activity, cells that are present in the lymph nodes as well as in blood, the lymphocytes (white blood cells) are involved in specific defensive responses to infection. Because the lymph nodes are very active in defence responses, they often become swollen and tender during infection. The so called "swollen glands" that often accompany a sore throat are not glands, but active lymph nodes.

Problems with the lymphatic system can interfere with fluid balance in the body. Anything that interferes with the flow of lymph through a lymph vessel causes fluid accumulation in that part of the body drained by the vessel. Not only is fluid drainage impaired, inadequate protein removal alters the osmotic balance between the blood and the interstitial fluid so that even more fluid accumulates in the tissue. Such fluid accumulation is called **oedema**, and it often is visible externally as a swelling in that part of the body. An extreme example of this is a condition caused in the disease filariasis by a parasitic larval nematode *Wuchereria bancrofti* in India. The larval forms are transmitted by mosquito to humans. They invade the lymph nodes and block lymphatic drainage so that portion of the body usually arm or leg and external genitalia become swollen, sometimes to the point of becoming grotesquely mishapen. This condition is known as **elephantiasis** (Fig. 3.14).

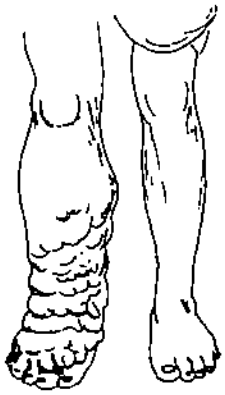


Fig. 3.14 : Swollen and obstructed lymph nodes cause elephantitis.

**SAQ 5**

How are lymph capillaries different from blood capillaries?

.....

.....

.....

.....

.....

## 3.6 HAEMOSTATIC MECHANISMS

When we accidently get hurt or cut our finger, blood starts flowing but within a few minutes it stops flowing too. Several mechanisms help prevent loss of blood from ruptured blood vessels. Damaged blood vessels constrict and so decrease the blood flow; but the most important mechanism,-however, is the closing of the blood vessel at the site of the injury by formation of a plug or **clot** which involves the conversion of liquid blood to jelly which prevents further escape of blood.

The clotting mechanism or **coagulation** has been well studied in mammals especially man, as it is of great medical importance. Let us try to understand the process. When a section of a blood clot is examined under a microscope, it is found to be composed of a tangled mesh of very delicate fibrils among which are entrapped, as in a net, erythrocytes, leucocytes, and many fragmented platelets. The filaments are composed of **fibrin**, an insoluble gel form of the protein **fibrinogen** which is present in the plasma. These filaments may be seen in many places to radiate from centers formed of platelets (Fig. 3.15). If the clot is allowed to stand for a while, it undergoes shrinkage, and as it shrinks, expresses from its meshes a clear, faintly straw coloured fluid, the **serum**. The serum remains fluid idefinitely; it is quite incapable of clotting, for it contains no fibrinogen. Plasma separated from the blood cells by centrifuging, clots in a way similar to that of whole blood and expresses the clear serum. The clot (coagulation) is white, since it contains no cells, but except for this difference it is indentical with that formed in whole blood. **The clotting process is essentially, therefore, a phenomenon of the plasma.** Lymph also clots, though somewhat more slowly and less firmly than does blood or plasma.

**Fig. 3.15 : Human RBC caught in a mesh of fibrin.**

Now we shall study the actual clotting mechanism.

### Clotting Mechanism

Four substances are necessary for coagulation of blood; **prothrombin, thromboplastin, calcium** and **fibrinogen**. Prothrombin gives rise to thrombin, an enzyme. Fibrinogen, prothrombin, and calcium are present in circulating blood. Thromboplastin (a lipid or fat-like compound containing phosphorus) is widely distributed throughout the tissues, the lung and brain being especially rich in this factor. It is absent or present in only small quantities in blood plasma. When blood is shed, thromboplastin is liberated from injured tissue and probably also from the leucocytes of the blood itself. The thromboplastin, acting upon the prothrombin in the presence of calcium in an ionised form, converts, it to active thrombin. Thrombin acts in turn upon the inactive fibrinogen, converting it into insoluble fibrin which is deposited as fine threads to form the framework of the clot. In simplest possible terms the chief factors are summarised in the following scheme:

| | | | | | | |
|---|---|---|---|---|---|---|
| Prothrombin (inactive) in plasma | + | calcium | + | thromboplastin | = | thrombin (active) |
| Thrombin | + | fibrinogen (soluble) in plasma | = | fibrin (Threads) | | |

Blood does not normally clot in the blood vessels because there is not sufficient free thromboplastin to convert the inactive prothrombin into the active thrombin.

The catalytic sequence of events behaves like an **enzyme cascade** with each product of a reaction being responsible for the activation of the next reaction. At least 13 different plasma factors have been recognised (see Table 3.3 in margin). A deficiency of even one factor can delay or prevent clotting. Why has such a complex mechanism evolved? Maybe it is essential to have a number of initial clotting responses to a variety of internal and external stimuli that can cause haemorrhage. At the same time, any ambiguous stimuli would not be able to cause intravascular clotting when no injury occurs.

Coagulation of blood is inhibited by heparin, a mucopolysaccharide that can be isolated from mammalian liver. A haemostatic mechanism is necessary for most animals. In open circulation the contraction of blood vessel to prevent blood loss does not help, but then open systems have low blood pressure and thus decrease the chances of large blood losses.

**Table 3.3 : Blood coagulation factors**

| *Factor (International Committee designation)* | *Synonyms* |
|---|---|
| I | Fibrinogen |
| II | Prothrombin |
| III | Thromboplastin tissue factor |
| IV | Calcium |
| V | Labile factor |
| VI | Now considered identical to factor V |
| VII | Proconvertin |
| VIII | Antihaemophilic factor |
| IX | Christmas factor |
| X | Stuart Prower factor |
| XI | Plasma thromboplastin antecedent |
| XII | Hageman factor |
| XIII | Fibrin-stabilizing factor |

Clotting mechanisms are also seen in invertebrates. The simplest mechanism is the agglutination of blood corpuscles without the involvement of plasma proteins. A cellular meshwork forms which helps to close the wound. Contraction of muscles also helps in this process.

## 3.7 SUMMARY

In this unit you have studied that :

- Plasma, interstitial and intracellular fluids make up the body fluids in animals. These fluids are mostly water but differ from each other in their solute composition. Blood is the fluid transport system which transports nutrients; wastes, heat, regulatory materials and the oxygen carrying pigments in the body.
- Circulatory systems can be divided into two broad categories : Open and Closed. In open circulation the blood is pumped by the heart into spaces where the organs are directly bathed by it. Blood pressure is low. In closed circulation, blood is enclosed in vessels and passes from the arteries into the veins via capillaries. Blood pressure is high and fluid that leaks across capillary wall into interstitial spaces is returned by special vessels forming the lymphatic system to the circulation.
- The heart is a muscular pump that ejects blood into the arterial system. Excitation of the heart starts in a patch of muscle tissue the pacemaker and the contraction spreads to the rest of muscle tissue. The heartbeat consists of rhythmic contraction (systole) and relaxation (diastole) of the whole muscle mass.
- Pulse rate is inversally proportional to body size and animals with high metabolic rates have high pulse rates. Cardiac output is dependent on venous flow and in mammals changes in cardiac output are related to changes in pulse rate rather than stroke volume.
- The arterial system is the pressure reservoir and conduit for blood between heart and capillaries. The elastic arteries dampen the oscillations in pressure and flow caused by the contractions of the heart. Blood to the capillaries is controlled by muscular sphincters at the arteriole end. Blood flow is laminar in the blood vessels. The velocity is maximum in arteries and minimum in capillaries.
- The venous system acts as a conduit for blood between capillaries and the heart and as a blood reservoir. In mammals 50% of the total blood is contained in veins.
- Exchange of materials between blood and tissue takes place through capillary walls. Changes in the composition of extracellular fluid and blood in capillaries causes the fluids to leave the capillaries at the arterial end and to be reabsorbed at the venous end.
- During exercise, the additional supply of oxygen to the muscles is due to increased cardiac output and also due to enhanced oxygen extraction from the blood.
- A system of vessels drains the fluid from the interstitial spaces into the blood vessels. This is the lymphatic system. Lymph contains lymphocytes and monocytes that are essential elements in the body's immune system. These are produced in the lymph nodes.
- Haemostatic mechanisms like contraction of blood vessels and coagulation help to prevent blood loss during injury. Coagulation is a complex process involving an enzyme cascade which results in the final blood clot.

## 3.8 TERMINAL QUESTIONS

1) Summarise in your own words the functions of blood and lymphatic system using the information given in the unit.

.....

.....

.....

2) What are the advantages of a closed circulatory system? In your opinion which is superior — closed or open circulatory system?

.....

.....

.....

3) How does the heart respond to strenuous exercise? Why is the heart rate slow during deep sleep?

.....

.....

.....

4) How are fluids transferred between tissues and capillaries?

.....

.....

.....

5) In closed circulatory systems blood is under some degree of pressure, and if any vessel ruptures a haemorrhage may occur. What is the response of the system to control the situation?

.....

.....

.....

## 3.9 ANSWERS

**Self-assessment Questions**

1) a) Blood is plasma plus blood cells. Plasma is fluid without the blood cells.

   b) In the ion content and protein composition.
   Na and Cl are the predominant ions in extracellular fluid. Protein percentage is less than intracellular fluid. K and Mg are the predominant ions inside the cells.

2) a) iv b) I c) mammals; oxygenated; body organs; pulmonary

3) a) iii, b) i, c) pacemaker; heartbeat; atria; arterioventricular node; tip; upwards; walls acetylcholine; vagus

4) a) iii), iv), v), vi); b) net absorption

3) Lymph capillaries are blind ended, they have no RBCs. They have thinner walls that allow larger molecules like proteins and fats to diffuse through them. Their diameter is larger than blood capillaries.

**Terminal Questions**

1) Refer to section 3.2 and sub-section 3.8.6.

2) Each type is best suited to the environment in which the animal lives but there are certain advantages for animals with closed circulatory system. The blood is transported in vessels therefore at high pressure. It can be delivered much faster to the tissues. Animals with closed system have higher metabolic rate i.e. they can now store energy.

3) During strenuous exercise the heart rate increases, and cardiac output increases, the stroke volume increases only slightly. During deep sleep the metabolic activity is less, therefore, the heart rate slows down.

4) Refer to sub-section 3.5.4.

5) 1) Constriction of vessels at the site of rupture.

   2) Mechanism of clotting which is a series of complex reaction occurring rapidly. The clot in this case prevents further blood loss.

# UNIT 4 EXCRETION

**Structure**



## 4.1 INTRODUCTION

Excretion is the removal of toxic waste products of metabolism from the body. The end-products of metabolism are either eliminated or conserved by an animal depending on its physiology. You have learnt in Block 3 of LSE-01: Cell Biology that carbon, hydrogen, oxygen and nitrogen are the end-products of catabolism. Carbon atoms are eliminated in carbon dioxide, hydrogen in water, and oxygen in carbon dioxide and water. Nitrogen which is the end-product of protein catabolism is found in large quantities. It is highly toxic and needs to be excreted as soon as it is formed, or converted into less toxic form before being eventually excreted. In this unit you will learn how animals get rid of the toxic nitrogen. You will also study the structure and function of the excretory organs of various animals and also the mechanisms that regulate kidney function. In the next unit you shall study about osmoregulation, a process which maintains osmotic and ionic concentration of the body fluids. The processes of excretion and osmoregulation are performed by the same set of organs and maintain homeostasis. In this unit osmoregulatory function of the organs has also been dealt with at some places along with their excretory function.

**Objectives**

After a careful study of this unit, you should be able to :

- explain how ammonia is formed during metabolism
- explain why ammonia is toxic to cells
- explain how ammonia is promptly eliminated in aquatic animals
- discuss pathways of ammonia detoxification in terrestrial animals
- explain how nitrotelism in animals is related to habitat
- discuss anatomical diversity and functional principles of renal organs, and
- explain the importance of renal organs in homeostasis.

## 4.2 NITROGEN EXCRETION

You are aware that nitrogen is a characteristic constituent of amino acids and proteins. Animals receive amino acids in diet and use them to synthesise a variety of functional nitrogenous compounds such as nucleic acids, proteins both enzymic and non-enzymic, some hormones, and neurotransmitters. The amount of amino acids obtained in diet is usually in excess of the need for the synthesis of the functional nitrogenous compounds. The excess of amino acids is either catabolised for the

release of energy or is used for the synthesis of glycogen and fat. When amino acids, proteins or nucleic acids are catabolised, nitrogen-containing excretory end-products are formed. These are **ammonia, urea** and **uric acid.**

Animals are often grouped according to their main excretory products.

i) Those that excrete mainly ammonia ($NH_3$) as the end-product of protein metabolism are called **ammonotelic.**

ii) Those that excrete mostly urea are **ureotelic.**

iii) Those that form mainly uric acid are **uricotelic.**

iv) Those that secrete guanine are **guanotelic.**

Excretion of nitrogen as ammonia, urea or uric acid is closely related to the normal habitat of the animal and to the availability of water. In no animal nitrogen excretion is restricted to a single product. Animals are designated as ammonotelic, ureotelic, uricotelic or guanotelic only to indicate the predominant form in which nitrogen is excreted. The term uricotelism, for example, does not preclude the excretion of ammonia and urea in minor quantities.

The mode of nitrogen excretion may vary within a given group of animals depending on the habitat of the species. For example, South African frog, *Xenopus laevis*, which is aquatic in adult life is ammonotelic whereas many of the truly amphibious frogs and toads (e.g. *Bufo* species) excrete urea as adults. Similarly, the aquatic chelonians (tortoise and turtles) excrete more or less equal proportions of urea and ammonia, the semiaquatic forms are ureotelic and the desert living are uricotelic.

The tadpoles of frogs and toads are ammonotelic, while the adults are ureotelic. Metamorphosis of the tadpole into the adult frog is associated with the induction of all the urea cycle enzymes in the liver. The African lungfish, *Protopterus aethiopicus*, normally lives in water and excretes large quantities of ammonia. In summer, when the ponds dry up, it aestivates in a mud cocoon and becomes ureotelic. With the advent of rains, it shifts its metabolism back to ammonotelism.

You will learn more about ammonotelism, ureotelism and uricotelism in the following section. In Table 4.1 major nitrogenous excretory products in various animal groups are listed.

**Table 4.1 : Major Nitrogen Excretory Products in Various Animal Groups**

| Animal | Major end-product of protein metabolism | Habitat |
|---|---|---|
| Aquatic invertebrates | Ammonia | Aquatic |
| Teleost fish | Ammonia, some urea | Aquatic |
| Elasmobranchs | Urea | Aquatic |
| Crocodiles | Ammonia, some uric acid | Semiaquatic |
| Amphibians, larval | Ammonia | Aquatic |
| Amphibians, adult | Urea | Semiaquatic |
| Mammals | Urea | Terrestrial |
| Turtles | Urea and uric acid | Terrestrial |
| Insects | Uric acid | Terrestrial |
| Land gastropods | Uric acid | Terrestrial |
| Lizards | Uric acid | Terrestrial |
| Snakes | Uric acid | Terrestrial |
| Birds | Uric acid | Terrestrial |

### 4.2.1 Formation of Ammonia

The amino acids which enter the body of the animal through diet are catabolised by a process known as **oxidative transdeamination.** This process is a combination of transamination, deamination and oxidation, and is catalysed by enzymes, **transaminases** and **dehydrogenases** (Fig. 4.1).

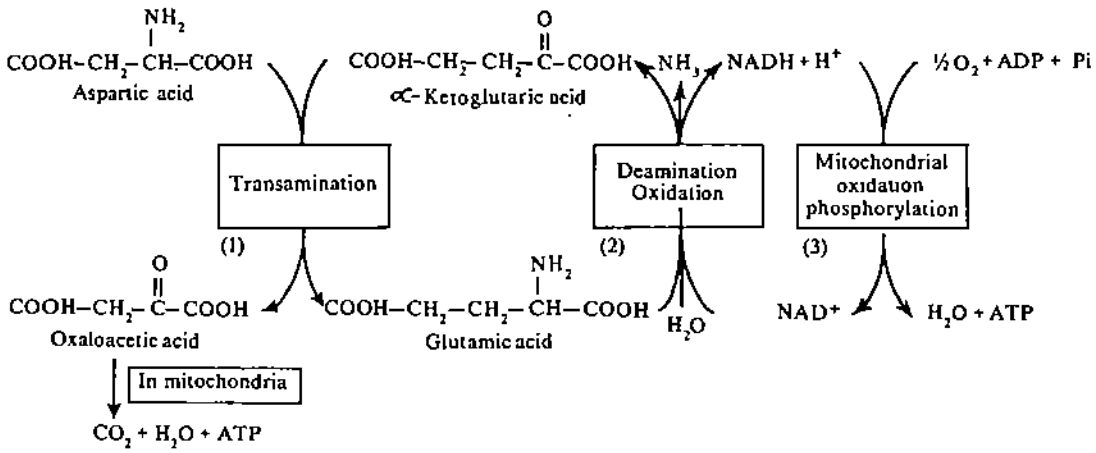



**Fig. 4.1 : Oxidative phosphorylation of amino acids**

You can see in Fig. 4.1 that :

1) The amino group of the amino acid, aspartic acid is transferred to α-ketoglutaric acid converting it into glutamic acid (transamination). During this process the aspartic acid is converted into oxaloacetic acid.
2) In the next step, the amino group of glutamic acid is removed in the form of ammonia (deamination) converting glutamic acid into α-ketoglutaric acid. At the same time, there is also removal of a pair of hydrogen atoms from glutamic acid (oxidation). Thus, the ammonia produced in the reaction actually comes from the amino group of the amino acid (aspartic acid) which has undergone transamination.
3) The hydrogen atoms produced by the oxidation of glutamic acid reduces NAD into NADH + $H^+$.
4) The NADH and the oxaloacetic acid produced are oxidised in the mitochondria to $CO_2$ and $H_2O$ generating ATP in the process.

Ammonia is toxic to cells for the following reasons :

i) Ammonia increases the intracellular pH and hence might affect metabolism adversely through its effects on enzymes.
ii) It withdraws α-ketoglutarate from Krebs' Cycle and NADH from the electron transfer system. This results in lowered cellular concentrations of ATP.
iii) At alkaline pH, the stability of cellular membranes decreases. Ammonium salts also inhibit the active transport of ions through membranes.

Therefore, to prevent ammonia from accumulating to toxic levels in cells, it is either excreted or converted into a less toxic form (detoxification) as soon as it is formed.

### 4.2.2 Ammonotelism

Ammonia diffuses through cell membranes extremely fast because of its high water solubility and small molecular size. Hence, it can be excreted as such only when there is ample water for its rapid removal from the body in the form of a dilute solution. Prompt excretion of ammonia therefore, occurs in aquatic animals, both freshwater and marine, in which there are constant water fluxes occurring between the environment and the body. Freshwater and marine invertebrates and fishes, larval and permanently aquatic amphibians excrete a major portion of their waste nitrogen as ammonia and thus called **ammonotelic** or **ammoniotelic**. The route of ammonia diffusion in these animals is skin, gills or kidneys.

## 4.2.3 Ureotelism

Terrestrial animals with restricted water availability in the environment are faced with the formidable task of water conservation. Since they cannot afford to use liberal quantities of water for excretion, ammonia is converted into a less toxic product. In mammals and semi-terrestrial adult amphibians, the major nitrogenous excretory product is urea, which is less toxic and easily soluble. These animals are therefore called **ureotelic.**

The synthesis of urea ($H_2N.CO.NH_2$) from one molecule of $CO_2$ and two of $NH_3$ occurs in the liver of ureotelic vertebrates by a metabolic pathway known as the **ornithine-urea cycle,** discovered by Kreb and Hensleit in 1932. This is a cyclic pathway that involves five enzyme-catalised reactions (Fig. 4.2).

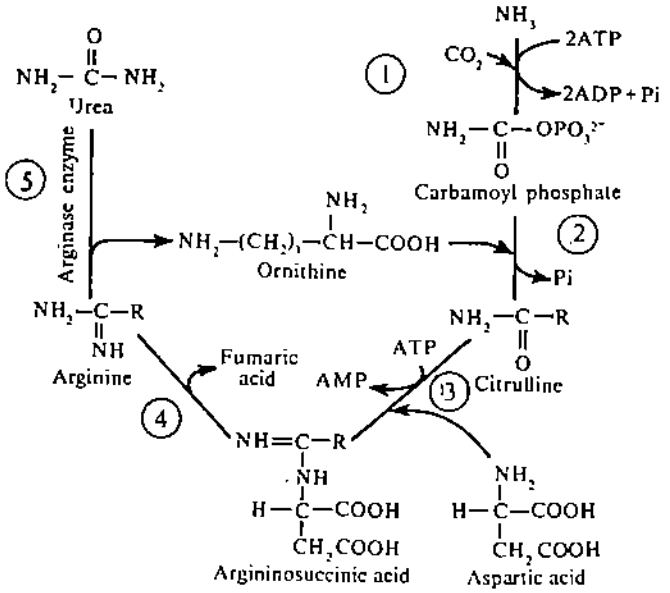



**Fig. 4.2 : Ornithine-urea cycle and the allied reactions**

You can see in Fig. 4.2 that this cycle has five steps :

1) Ammonia, $CO_2$ and ATP are converted into carbamoylphosphate by the enzyme, **carbamoylphosphate synthetase**.
2) **Ornithine carbamoyl-transferase** then makes citrulline from carbamoylphosphate and ornithine.
3) Citrulline is then used in the presence of ATP and aspartic acid for the synthesis of argininosuccinic acid by the enzyme **argininosuccinate synthetase.**
4) Argininosuccinic acid then gives rise to arginine and fumararic acid in the presence of **argininosuccinate lyase.** In the formation of arginine from citrulline, the amino group of asparatic acid is used with the release of the carbon skeleton of the aspertic acid in the form of fumararic acid.
5) Finally, the cycle is completed by splitting arginine into urea and ornithine in the presence of the enzyme **arginase.**

You have seen that nitrogen enters the cycle both as ammonia and the amino group of aspartic acid and leaves as the two nitrogen atoms of urea. Of the two molecules of ammonia required for the synthesis of urea, one enters directly in the form of ammonia and the second in the form of the amino group of aspartic acid. The urea cycle is linked to the tricarboxylic acid cycle (Krebs' cycle) and glutamate dehydrogenase reaction. The price paid by ureotelic animals for the detoxification of two molecules of ammonia into urea is in the form of three molecules of ATP. For further details you may refer to units 11 and 12 (Block 3) of Cell Biology.

### 4.2.4 Uricotelism

In animals which inhabit extremely arid environments, ammonia is converted into uric acid. Uric acid is least toxic, relatively insoluble and is easily precipitated. Hence, it can be excreted in solid form without loss of substantial amount of water. Pulmonate snails, terrestrial insects, squamate reptiles (lizards and snakes) and birds excrete a major portion of their waste nitrogen in the form of semi-solid or solid uric acid and hence are referred to as **uricotelic** animals.

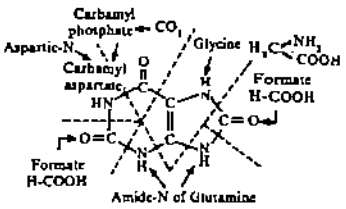



Fig. 4.3 : Uric acid

The synthesis of uric acid from ammonia in these animals occurs by the **inosinic acid pathway**. This pathway was first elucidated by Buchanan and his coworkers in the 1950's in pigeon liver. Uric acid is a member of the purines. While the details of the pathway are beyond the scope of this unit, in Fig. 4.3 the different fragments from which the uric acid molecule is assembled is shown. The dashed lines which divide the uric acid molecule in the Figure indicate the building blocks from which its biosynthesis takes place.

Many insects exhibit a phenomenon known as **storage excretion**. If excretory products are stored in the body instead of being eliminated, no water is expended for their excretion. Since uric acid is non-toxic and highly insoluble, it can be retained in the body for an indefinite period, without any ill-effect. This alternative strategy to the problem of excretion is quite common in cockroaches which store uric acid in fat body and cuticle. The stored uric acid, which may account for as much as 10% of the dry body weight, also provide a nitrogen depot for mobilisation at times of nitrogen deprivation.

### 4.2.5 Guanotelism

Arachnids (spiders and scorpions) excrete mostly guanine and hence are said to be **guanotelic**. Guanine, like uric acid, is relatively non-toxic and insoluble and is excreted in solid form. It is an adaptation to life in arid habitats. It is also a purine and has the same atoms in its ring structure as in uric acid. In fact, guanine is formed as an intermediate during uric acid synthesis in uricotelic animals. In spiders and scorpions, the inosinic acid pathway terminates in guanine formation. The origin of the four nitrogen atoms of guanine is similar to that of uric acid.

**SAQ 1**

Fill in the blank spaces and compare your answers with those given in section 4.7.

a) The amino acids which enter the body of the animal through diet are catabolised by a process known as ..... involving the enzymes transaminase and dehydrogenases.

b) Ammonia withdraws α-ketoglutarate from Krebs' cycle and NADH from the electron transport system. This results in ..... .

c) The synthesis of urea takes place in the liver of ureotelic vertebrates by a metabolic pathway known as the ..... .

d) In the uricotelic animals synthesis of uric acid from ammonia takes place by a pathway known as ..... .

e) Spiders and scorpions excrete mostly ..... and hence they are said to be ..... .

## 4.3 EXCRETORY ORGANS

In the earlier section you were studying about excretory products and their formation. In this section you will study about the organs that eliminate the excretory products.

Excretory organs serve not only to eliminate the nitrogenous waste products, but also to remove accurately regulated amounts of all substances present in excess in the body. Thus they contribute to maintain a steady state or **homeostasis** in animals to over-ride the influence of all those factors in the environment that tend to impose a change.

No special organs of excretion are present in many marine protozoans and sponges. Similarly, the coelenterates, which are mostly marine, and the echinoderms, which are restricted to the sea, are devoid of excretory organs. These marine invertebrates, being isosmotic to their habitat, do not need organs of water and ionic balance. Discharge of nitrogenous wastes and ionic regulation are carried out through the general body surface.

In all the other animal groups, there are discrete organs which function in osmotic regulation, ionic regulation and nitrogen excretion. These are the **contractile vacuoles** of protozoans and poriferans, the **nephridia** of flatworms, roundworms and annelids, the **green glands** of crustaceans, the **coxal glands** of arachnids, the **malpighian** tubules of insects and the **metanephric kidney** of vertebrates. Depending on whether the nephridial tubule is closed at the inner end or opens into the coelom by funnel shaped structure called **nephrostome**, nephridia are respectively termed as **protonephridia** or **metanephridia**. The excretory organs of molluscs, crustaceans and arachnids are actually modified coelomoducts and hence are not true nephridia. With the exception of the contractile vacuoles of protozoans and sponges, the excretory organs are either tubules or aggregates of numerous tubules.

### 4.3.1 Functional Principles

Before studying about the excretory organs, it is important to learn about the basic concepts of osmolarity and membrane permeability. The presence of dissolved solute confers on a solution the property of osmotic pressure. Like other colligative properties, osmotic pressure or osmotic concentration of a solution depends on the number of dissolved particles present per unit volume. While the chemical concentration of a solution is expressed in molarity (moles/litre), osmotic concentration of a solution is expressed in osmolarity (osmoles/litre). For ideal non-electrolytes (e.g., sucrose), a one molar solution is one osmolar. An electrolyte solution, on the other hand, has a higher osmolarity than its molarity. For example, NaCl in the solution dissociates into $Na^+$ and $Cl^-$. Thus, for every molecule of NaCl, one gets two ionic particles in solution, one of $Na^+$ and one of $Cl^-$. Hence, one molar NaCl solution is nearly two osmolar. In the same way, one molar $CaCl_2$ solution is nearly three osmolar. An **osmole** is defined as that amount of a solute which when dissolved in one litre of water has the same osmotic pressure as one mole of an ideal non-electrolyte in one litre of water. If two solutions (solution A and solution B) have the same osmotic concentration, they are said to be **isosmotic** to each other. If solution A has a higher osmolarity than solution B, A is **hyperosmotic** to B or B is **hypoosmotic** to A.

Urine formation by the excretory organs involves (i) the movement of water (solvent) and dissolved molecules of small size (solutes) from the lumen of the excretory organ into the interstitial fluid and blood, and (ii) movement back across the plasma membranes of the intervening cells (cells of the renal organs and blood capillaries).

While a variety of renal organs are encountered in the animal kingdom, the principles of their function are basically similar. In all cases, renal function involves the initial appearance in the tubular lumen of a fluid called **primary urine** which is isosmotic to blood or body fluid from which it is derived. The composition and volume of the primary urine are later altered by the **reabsorption** of useful substances (e.g., glucose) into the blood and by the **secretion** of unwanted substances (e.g., urea) from the blood into the tubular fluid. Both reabsorption and secretion involve active transport. These modifications in the composition and volume of primary urine result in the formation of urine which is the fluid finally excreted from the body.

The mechanisms underlying the formation of primary urine are different in different animals. In many cases, it is by a physical process known as **ultrafiltration** in which the blood is forced by hydrostatic pressure to pass through one or more membranes into the lumen of the renal tubule. The resulting tubular fluid called the **ultrafiltrate** is the primary urine. It is similar to blood in its osmolarity and composition of solutes of small size. But it is devoid of blood cells and proteins of molecular weight larger than 67,000. In principle, this process is similar to the filtration practiced by you in the Chemistry Laboratory to remove suspended particles from a solution. You must have observed that particles larger than the pore diameter of the filter paper are retained in the filter paper and the particles that are small enough to pass through the pores appear in the filtrate by gravitational force. In the present context, the

solution filtered, the filter paper, the gravitational force and the filtrate are analogous to the blood, the porous membranes of the intervening cells, the hydrostatic pressure of the blood and the ultrafiltrate respectively. Only in a few cases (e.g., insects), the primary urine though isosmotic to the haemolymph, is strikingly different from the latter in its composition and is formed by the secretion (active transport) of substances from the haemolymph into the tubular lumen. Whether the primary urine is formed by ultrafiltration or secretion, its volume and composition are subsequently modified by reabsorption and secretion of molecules. In the following sub-section, we will study the structure and function of various excretory organs, before that try to answer the following SAQ.

**SAQ 2**

a) Match the animals given in column A with their excretory organs given in column B.

| A | B |
|---|---|
| i) Protozoans | a) Kidney |
| ii) Worms | b) Contractile vacuole |
| iii) Crustaceans | c) Nephridia |
| iv) Insects | d) Green Glands |
| v) Vertebrates | e) Malpighian tubules |

b) Fill in the blank spaces and compare your answers with those given in section 4.7.

i) Depending on whether the nephridial tubule is closed at the inner end or opens into the coelom by a funnal-shaped structure called nephrostome, nephridia are respectively termed ..... or ..... .

ii) If two solutions have the same osmotic concentration, they are said to be ..... .

### 4.3.2 Contractile Vacuoles

Contractile vacuoles are always found in freshwater protozoans and sponges, but are frequently absent in their marine counterparts. Since a freshwater animal is hyperosmotic to the medium (freshwater) and its surface is permeable to water, it should continually endeavour to pump out the excess water that enters osmotically. When a freshwater protozoan is transferred to dilute sea water, the vacuolar activity decreases. In those marine species that have a contractile vacuole (e.g., some ciliates), vacuolar output increases with dilution of the sea water. The fluid present in the contractile vacuole of freshwater protozoans is hypoosomotic to the surrounding cytoplasm, though still hyperosmotic to freshwater. These observations are consistent with the hypothesis that contractile vacuoles are primarily organs of water balance and function as pumps to remove excess osmotic water. Any role they might play in the excretion of nitrogenous wastes, which is mainly ammonia in protozoans and sponges, is considered to be secondary.

Microscopic observations revealed that in protozoans contractile vacuole exhibits a cyclic functioning. It gradually gets filled with fluid and increases in volume (**diastole**) till it reaches a critical size. Then it moves towards the cell boundary, suddenly expels its contents to the outside and decreases in size (**systole**). The mechanisms of filling and discharge of the vacuole are not yet clearly understood. Electron microscopic studies show that the contractile vacuole contains a single membrane enclosing the lumen. This membrane is surrounded by a layer of densely packed vesicles around which there is a layer of mitochondria which presumably supply energy for the osmotic work done by the vacuole (Fig. 4.4). The vesicles empty their contents into the contractile vacuole by fusion of their membranes. According to current theories, the small vesicles initially contain a fluid isosmotic to the cytoplasm. The fluid later becomes hypoosmotic due to the removal of salts, particularly potassium, by active transport. The hypoosmotic vesicles now fuse and open into the contractile vacuole leading to diastole. It is also believed that contraction of myofibrils present in the vacuolar membrane is probably responsible for the expulsion of the fluid during systole.

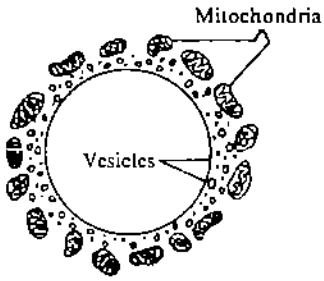



Fig. 4.4 : Contractile vacuole of amoeba

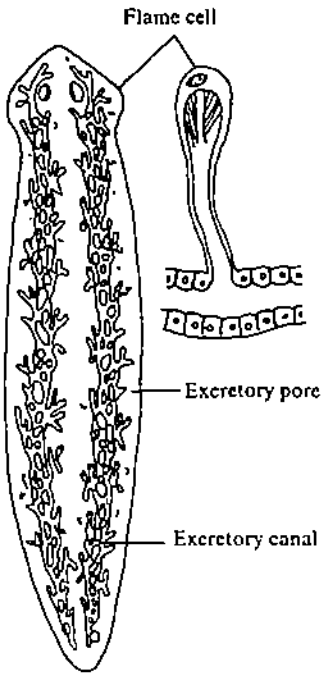



Fig. 4.5 : Excretory system of planerian

### 4.3.3 Nephridia of Worms

As pointed out earlier, nephridia are the excretory tubules in many invertebrates. While all nephridia open to the exterior by an excretory pore known as **nephridiopore**, some have their internal end closed and others open internally into the body cavity by a ciliated funnel called **nephridiostome**. The former type of nephridia are known as **protonephridia** and the latter type are the **metanephridia**.

Protonephridia are more primitive and occur mainly in **acoelomate** and **pseudocoelomate** animals. An animal may have two or more extensively branched protonephridia, each internally terminating in several bulb-like structures called **flame cells** or **flame bulbs**. In the lumen of each flame cell is found a tuft of cilia (Fig. 4.5). Flame cells are common in flatworms and nemertines. In some of the coelomate worms that have protonephridia (e.g., polychaetes), the flame bulbs are replaced by a **solenocytes**. The solenocytes have a single flagellum projecting into the lumen instead of the ciliary tuft as found in the flame cells (Fig. 4.6). It has been suggested that the undulating movements of the ciliary tuft or flagellum generate sufficient negative pressure for filtration and the force required to propel the fluid through the tubules. Flame cell system functions in animals without a circulatory system and pick up substances only from the tissue fluids.

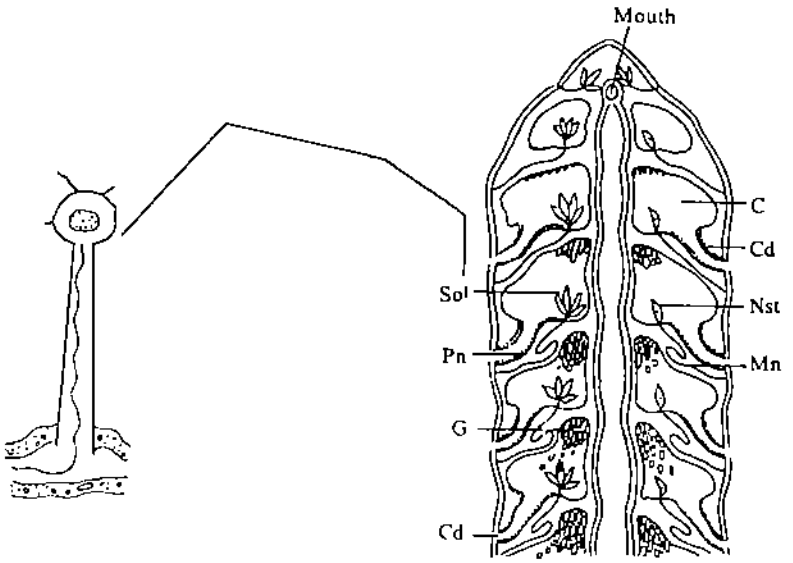



Fig. 4.6 : Excretory system of earthworm

Metanephridia are found in coelomates (e.g., annelids). These animals have evolved a close circulatory system intimately associated with the excretory organs resulting in direct exchange of material between the two systems. In these worms an isosmotic fluid appears initially in the nephridium due to filtration of the coelomic fluid through the ciliary mesh of the nephrostome. Salt is later withdrawn from the fluid as it passes through the extensively looped duct and finally a dilute urine is discharged.

### 4.3.4 Molluscan Kidney

In molluscs, the coelom is represented by the pericardial cavity and the cavities of the kidneys and gonads. The pericardial coelom opens into the renal cavity via the renopericardial duct (Fig. 4.7). Thus the molluscan kidney is a modified coelomoduct of the metanephridial type opening into the pericardial cavity at one end and the mantle cavity at the other. In the molluscs, an initial fluid is formed in the kidney by ultrafiltration of the blood through the wall of the heart. Reabsorption of valuable materials like glucose and secretion of unwanted compounds occur subsequently resulting in the formation of the final urine. There is some evidence that in the abalone, *Haliotis*, glucose reabsorption takes place primarily in the left kidney, while secretion of unwanted compounds occurs in the right kidney. Hydrostatic pressure of the blood provides the filtration pressure and application of back pressure stops urine production.

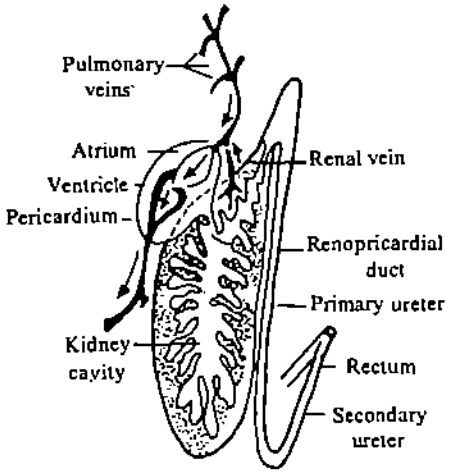



Fig. 4.7 : Kidney and its relationship to the pericardium in the snail

### 4.3.5 Green Glands of Crustaceans

Crustaceans have a pair of renal organs, each of which (Fig. 4.8) consists of an internally closed end-sac with a labyrinth, a long coiled nephridial tubule and a bladder which opens into the excretory pore located at the base of either the antennae where it is called **antennary glands** or the maxillae where it is called **maxillary glands**. The end-sac in many cases is green and hence the name, **green gland**. Marine forms have a shorter tubule than freshwater forms because the freshwater forms have an extra salt-reabsorbing segment in the tubule.

Urine formation in crustaceans involves filtration, reabsorption and secretion. In the end-sac of crayfish antennary gland chloride is reabsorbed from the isosmotic ultrafiltrate during its passage through the tubule so that the final urine excreted is hypoosmotic. Urine concentration is known to vary in crustaceans depending on whether they are freshwater or marine. It is very dilute in the freshwater crustaceans, but it is close to the blood osmolarity in the crustaceans of brackish water. This may be due to the absence of salt-reabsorbing segment in the tubule of the marine forms. The hydrostatic pressure of the blood provides the driving force for filtration.

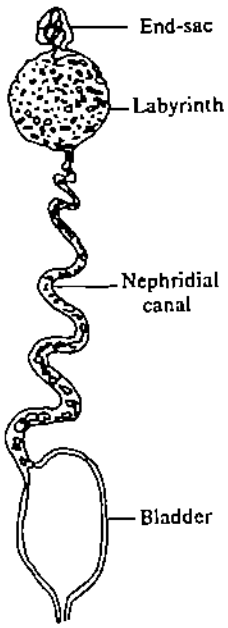



Fig. 4.8 : Structure of the renal organ and urine formation in the crayfish

### 4.3.6 Malpighian Tubules of Insects

Malpighian tubules and hind gut together constitute the excretory system in insects. These are from two to several hundred in number. Each tubule is similar to a protonephridium in that one end is blind and the other opens into the intestine between the midgut and hindgut of the insects. The tubule as a whole is bathed by **haemolymph** (Fig. 4.9).

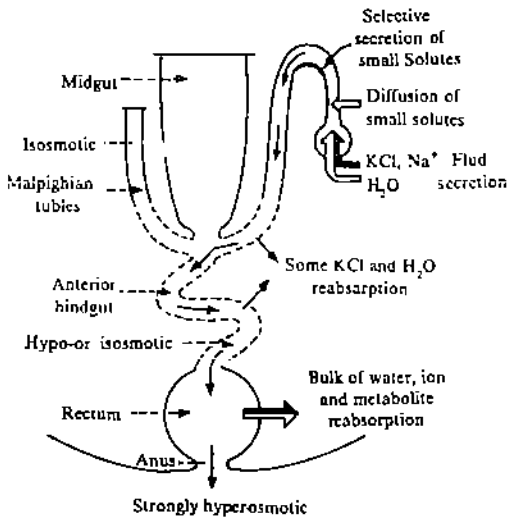



Fig. 4.9 : Function of Malpighian tubules in insects.

The formation of primary urine in malpighian tubules is due to secretion and not due to ultrafiltration. The cells of the malpighian tubules actively transport potassium from the haemolymph and concentrate it in the lumen. This results in the passive osmotic entry of water followed by the diffusion of other low molecular weight substances like salts, sugar, and uric acid. Fig. 4.9 will help you in understanding this phenomena. The rate of urine formation in insects is proportional to the concentration of $K^+$ in the haemolymph. Thus, active transport of $K^+$ is primarily responsible for generating urine flow in the excretory organs of insects. As the potassium-rich isosmotic tubular fluid moves towards the gut, there is secretion of molecules like uric acid and reabsorption of sugars and ions. However, major modification in the composition of the fluid occurs in the hind gut, particularly the rectum, where there is extensive reabsorption of water and physiologically important solutes like $K^+$. Uric acid, which enters the hind gut as water soluble potassium urate, gets precipitated facilitating further withdrawal of water. Insects that live on liquid or wet food, excrete large amounts of hypoosmotic liquid urine. On the other hand, insects that live on dry food, produce extremely dry excreta. To facilitate water reabsorption, the rectum in terrestrial insects show modifications referred to as **rectal glands** or **rectal pads**.

**SAQ 3**

Fill in the blank spaces and compare your answers with those given in section 4.7.

a) A protonephridium has several internally terminating bulb-like structures called ..... .

b) In a molluscs, *Haliotis*, glucose reabsorption takes place primarily in the ..... , while secretion of unwanted compounds occurs in ..... .

c) The urine of the fresh water crustaceans is very dilute compared to the marine crustaceans because the former have ..... ..... in the tubule of green gland.

d) The malpighian tubules of insects are similar to ..... because one end of the each tubule is blind and the other opens into the intestine.

### 4.3.7 Vertebrate Kidney

Typically, all vertebrates have a pair of kidneys, which function on the filtration — reabsorption — secretion principle. Only in a few teleost fishes, the kidney is aglomerular (without a glomerulus) and functions on the absorption — reabsorption — secretion principle as in the malpighian tubules of insects. The functional unit of the vertebrate kidney is the **nephron** or the **uriniferous tubule**. A small fish may have only a few dozen nephrons in its kidneys; a large mammal may have several million

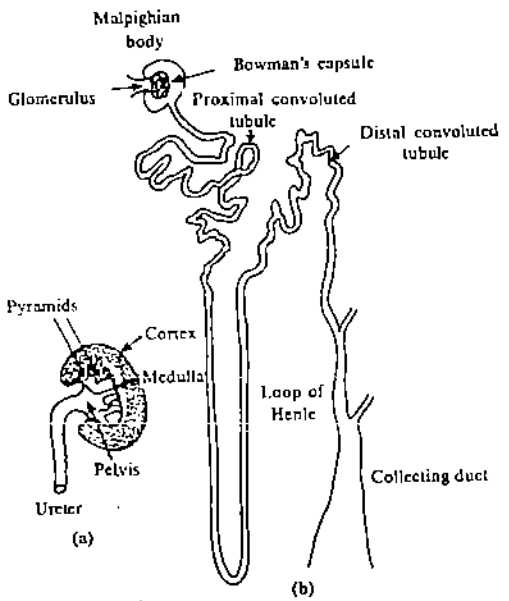



Fig. 4.10 : Schematic diagram of mammalian kidney and nephoron

Blood is brought to the kidney by the renal artery which branches and sub-branches into interlobar arteries and finally into the **afferent arteriole** which gives rise to the capillary network of the glomerulus. An **efferent arteriole** formed by the confluence of the capillaries takes blood away from the Bowman's capsule. The Bowman's capsule latter continues into a long convoluted tubule which is distinguished into the **proximal** and **distal convoluted tubules** respectively. The distal convoluted tubules from different nephrons join to form the **collecting tubule** which carry the urine into the **renal pelvis** from where the ureter starts. The proximal and distal tubules are present in all vertebrates, but in birds and mammals a new U-shaped hair-pin-like segment called **Henle's loop** is present between proximal and the distal tubules. In the following section we shall study structural variations in the vertebrate kidney.

**Structural Variations in Vertebrate Kidney**

The structure of the vertebrate kidney is by no means universal. The mammalian kidney consists of an outer granular **cortex** and an inner striated **medulla**. The granular appearance of the cortex is due to the presence of glomeruli in this region. Medulla looks striated due to the parallel arrangement of blood vessels and tubules of the nephrons. In fishes, amphibians and reptiles, the renal tubules are short, are devoid of Henle's loop and there is no clear cut distinction between cortex and medulla. In birds there is some degree of spatial organisation of the nephrons so that a small central medulla can be discerned from the outer cortex. This division into cortical and medullary regions is most pronounced in the mammalian kidney. It is the presence of Henle's loop that enables the avian and mammalian kidney to produce hyperosmotic urine. The ureteral urine in other vertebrates is either hypoosmotic or at best isosomotic to blood. However, terrestrial reptiles and birds produce a semi-solid or solid urine due to reabsorption of water in the cloaca. In birds and mammals, the ureteral urine itself is hyperosmotic to blood. The maximum urine osmolarity attained by the avian kidney is only about twice that of plasma. The urine concentrating ability of the kidney in mammals is related to the habitat. Desert animals produce highly concentrated urine and freshwater animals produce very dilute urine. The kidneys of some desert mammals can produce a urine which is 25 times more concentrated than the plasma. The beaver which has access to abundant water in the environment, on the other hand, has kidneys with only moderate ability to concentrate the urine.

The ability of mammalian kidney to concentrate urine is closely related to the length of the loop of Henle. Animals that produce the most highly concentrated urine (e.g., sand rat) have very long loops. Those that have a limited ability to concentrate the urine (e.g., beaver) have short loops. On the other hand, mammals with an intermediate ability to concentrate the urine (e.g., man, rabbit) have kidneys with both short and long loops. Since the loop of Henle is a U-shaped tube embedded in the medulla, longer loops would mean thicker medulla. The relative thickness of the renal cortex and medulla in mammals is therefore, correlated with the urine concentration in the animals. In forms which produce low maximum urine concentration, the medulla is much thinner and smaller than in species that produce a highly concentrated urine. This is best appreciated from a comparison of the medullary thickness in rodents from aquatic, general terrestrial and desert environment (Fig. 4.11).

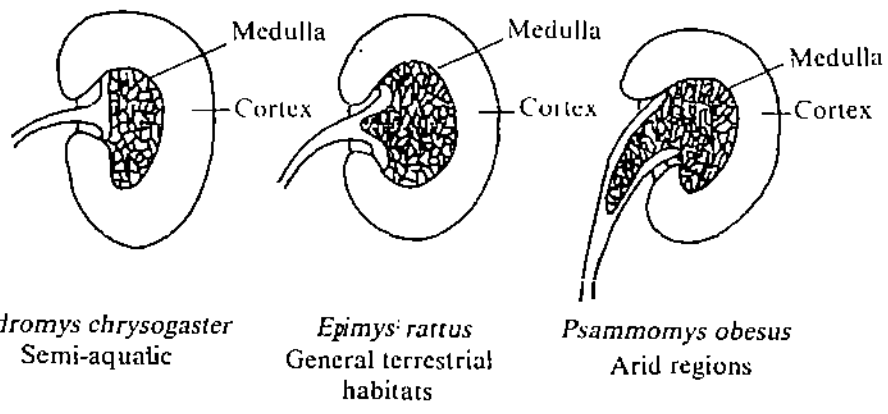



**Fig. 4.11 : Comparison of the kidneys of three rodents from different environments**

While the mammalian kidney has the unquestioned ability of producing a hyperosmotic urine, it does not mean that a mammal always produces a hyperosmotic urine. The urine osmolarity and volume in mammals are related to water intake and ambient temperature. Given the plasma concentration of about 300 milliosmoles per litre, the urine osmotic concentration in humans may vary anywhere between 50 to 1400 milliosmoles per litre. Thus the human kidney has the ability to produce an extremely dilute (hypoosmotic) to a highly concentrated (hyperosmotic) urine depending on the requirements of water conservation in the body. The ranges of urine osmolarity are 50 to 2300 milliosmoles per litre in dog and 50 to 5000 milliosmoles per litre in the desert rat. Urine osmolarity is modulated even in lower vertebrates, though to a limited extent. Thus, a frog in water produces a large volume of highly dilute urine. When exposed to dry air, it puts out a smaller volume of slightly more concentrated urine by reducing glomerular filtration and increasing tubular reabsorption in the presence of **antidiuretic hormone** secreted by the **hypothalamo-hypophyseal system.** However, both in water and dry air, the urine produced by the frog is hypoosmotic to blood; only the degree of hypoosmoticity changes depending on the water status in the body. In the following sections we shall study urine formation in the various parts of vertebrate kidney.

### Glomerular Filtrate

You have studied earlier that a mammalian nephron has a renal corpuscle consisting of Bowman's capsule enclosing glomerulus. Blood entering the glomerulus through the afferent arteriole is filtered into the lumen of the Bowman's capsule to give rise to the ultrafiltrate. The glomerular filtrate is nearly isosmotic to plasma and contains ions and other solutes of small size essentially at the same concentrations as in the plasma. The blood pressure in the glomerular capillaries provides the driving force for filtration.

### Proximal Convoluted Tubule

The glomerular filtrate, which is isosmotic to plasma, enters the proximal convoluted tubule where its volume is reduced by about 80%. This reduction in fluid volume is accomplished by the active reabsorption of NaCl, glucose, amino acids, etc. from the tubular fluid. As these solutes are removed, the tubular fluid becomes hypoosmotic and the interstitial fluid surrounding the tubular wall becomes hyperosmotic. This causes passive diffusion of water out of the tubule to restore isosmotic condition between the tubular fluid and the surrounding cortical interstitium. This reabsorption of water is secondary to solute transport and occurs inevitably irrespective of the water requirements of the body. Hence, it is called the **obligatory reabsorption of water**. Thus, in the proximal convoluted tubule, there is a drastic reduction in fluid volume without any change in its osmolarity.

At the normal plasma glucose concentration of 100 mg per 100 ml, glucose is totally reabsorbed from the ultrafiltrate in the proximal convoluted tubule. If the plasma glucose concentration increases above the normal level, its concentration in the ultrafiltrate also increases correspondingly. If it exceeds, the capacity of the transport mechanism to reabsorb, some glucose appears in the urine as happens in diabetic patients.

### Loop of Henle

The reduced volume of fluid from the proximal tubule, still isosmotic to blood, enters the descending limb of Henle's loop (Fig. 4.10). As the fluid passes down the descending limb, it enters regions where the tubular walls are surrounded by interstitial fluid of increasing osmotic and $Na^+$ concentration. The walls of the descending limb are permeable to water and salts. So, water leaves and sodium enters the tubular fluid so that as it descends the loop of Henle, the fluid becomes progressively more concentrated and hyperosmotic to blood. There is also perhaps

some inward diffusion of urea from a urea-rich interstitial space. As a result of these events, the tubular fluid, at any given level in the descending limb, maintains isosmotic relationship to the surrounding interstitium.

The tubular fluid from the descending loop of Henle passes through the hair-pin bend of the loop, and enters the ascending limb of Henle's loop. The walls of ascending loop do not permit diffusion of water and salts. However, the cells of these walls, particularly of the deeper parts of the ascending limb, actively transport $Na^+$ outward. Recent studies reveal that it is $Cl^-$ which is actively transported out of the ascending limb and $Na^+$ accompanies as the counter-ion. In this part there may also be some active transport of urea out of the tubular fluid. Because of this active transport out of the tubule, the fluid becomes progressively more dilute as it moves up the ascending limb. Since ascending limb is impermeable to water, there is no entry of water into the tubular fluid from the surrounding interstitium. Due to the net withdrawal of solute, the fluid at any given level in the ascending limb is hypoosmotic to the fluid in the interstitium as well as the descending limb.

**Distal Convoluted Tubule**

A hypoosmotic fluid from the ascending loop of Henle enters the distal convoluted tubule. Under conditions of water diuresis, i.e., when there is no need for water conservation, the hypoosmotic tubular fluid passes into the collecting tubule unchanged in its osmolarity. When water is to be conserved by the body, the hypothalamo-hypophyseal system secretes **antidiueretic hormone** (ADH) (also known as **vasopressin**) which makes the walls of the distal convoluted tubule permeable to water. So, when the ADH titer is high in the blood, water diffuses out of the distal tubule and the fluid becomes isosmotic to blood by the time it enters the collecting tubule.

In amphibians and reptiles, where there is no loop of Henle, the fluid from the proximal tubule passes into the distal tubule directly and the final concentration of the ureteral urine may be hypoosmotic or at best isosmotic. Urine in these animals is made hypoosmotic by reabsorption of salt. Under dry conditions, in these animals, the urine is made less hypoosmotic and smaller in volume due to reabsorption of water in the distal tubule under the influence of ADH. ADH is referred to as **vasotocin** (not vasopressin) in lower vertebrates.

**Collecting Tubule**

The fluid that reaches the collecting tubules may be hypoosmotic or isosmotic depending on the water status of the animal. Final adjustments in urine composition are made in the collecting tubules, whose walls are permeable only in the presence of ADH. In the absence of ADH, as it happens during water diuresis, the hypoosmotic fluid that enters the collecting tubule passes through the latter unchanged, so that a dilute urine of relatively large volume is excreted. Under situations demanding water conservation, ADH is secreted and the walls of the collecting tubule become permeable to water. The fluid entering the collecting tubules becomes progressively more concentrated as it passes down making the final urine practically isosmotic to the interstitial fluid and hyperosmotic to blood..

**Vasa Recta**

The accumulation of water reabsorbed from the descending limb of Henle's loop or the collecting tubule would disturb the osmotic concentration gradient in the medulla. The capillary loops of vasa recta, which run parallel to the loop of Henle, remove the excess water and thereby maintain the concentration gradient in the medullary interstitium. The vasa recta loops are freely permeable to water and solutes. As blood passes down the vasa recta, water diffuses out into the interstitial fluid and salt diffuses in. Blood therefore becomes increasingly concentrated as it passes down in the vasa recta towards the renal papilla. When blood ascends towards the cortex, water moves in and salt moves out. The vasa recta also removes any excess salt that might accumulate in the medullary interstitium.

Fig. 4.12 illustrates urine concentration in the various parts of nephron.

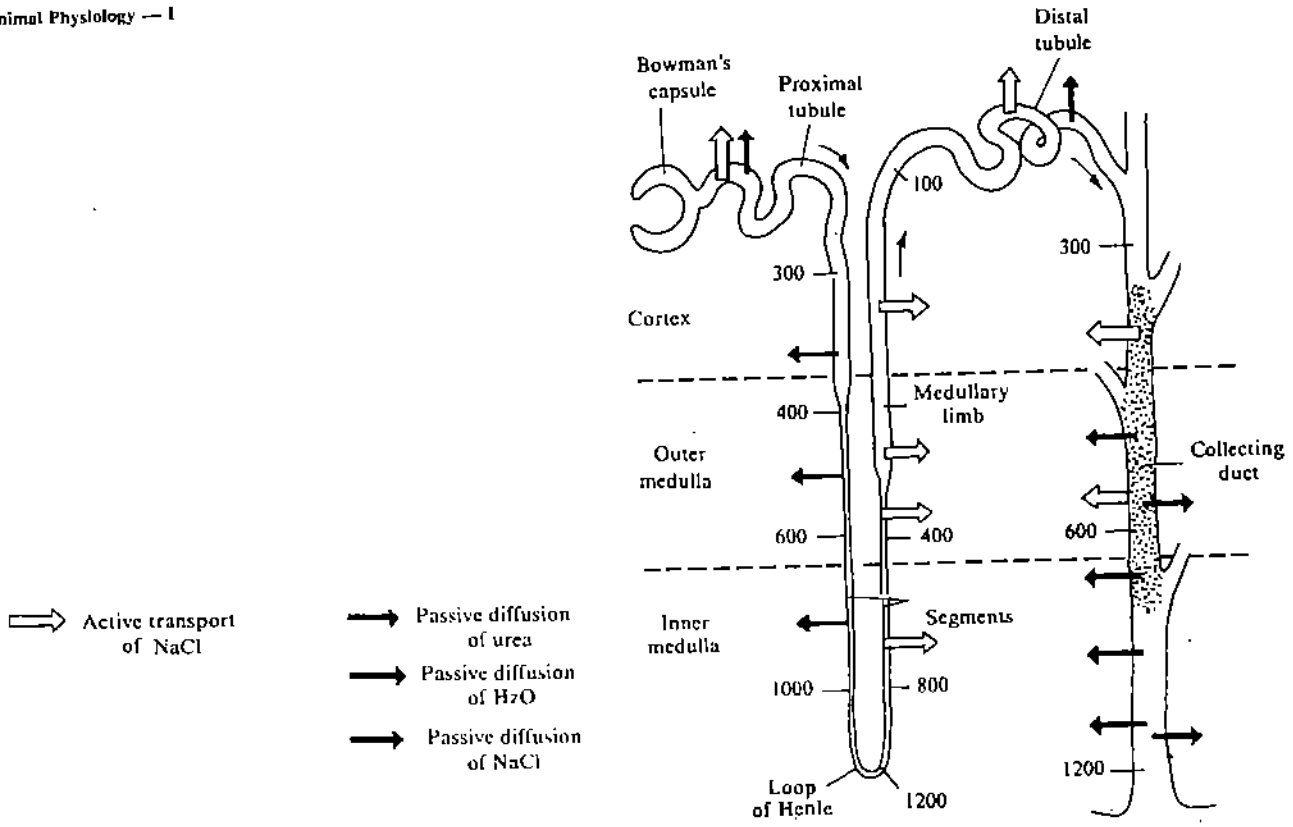



Fig. 4.12 : Urine concentration in the nephron

## 4.4 REGULATION OF KIDNEY FUNCTION

After studying about the structure of kidney and formation of urine in it, we shall now learn how the function of kidney is regulated. You have studied earlier in this unit that urine production is dependent upon the filtration process. Reduction in blood supply to the kidney results in a drop in the net filtration pressure and so, urine production ceases. Under these conditions, a series of events are initiated in the kidney i self to develop a hypertensive state so that there is better flow of blood to the kidney and resumption of urine production. The **autoregulation** of renal blood flow involves a neuroendocrine mechanism related to the **juxtaglomerular apparatus** in the kidney (Fig. 4.13).

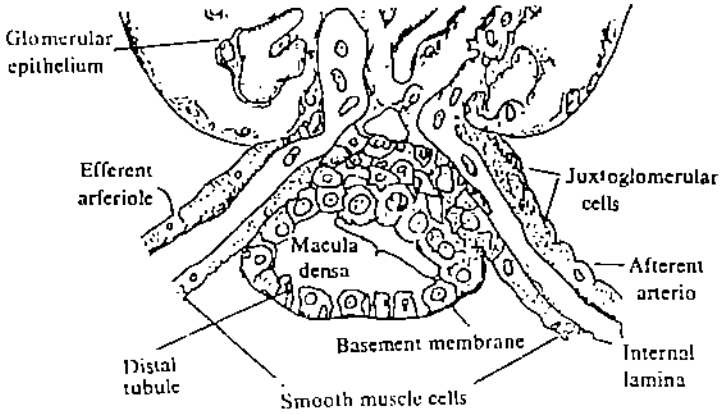



Fig. 4.13 : Structure of the juxtaglomerular apparatus in mammals

When the afferent arterial blood pressure is low, the stretch sensitive receptors of the arterioles initiate nerve impulses which induce the secretion of a proteolytic enzyme called **renin** by the juxtaglomerular cells into the blood. This enzyme causes the release of a decapeptide called **angiotensin-I** from a large globular plasma protein known as **angiotensinogen**. Another proteolytic enzyme from the plasma, the **converting enzyme**, removes two amino acids from angiotensin-I to form an octapeptide called **angiotensin-II**. Angiotensin-II is about 200 times more powerful

than norepinephrine in its vasopressor activity. It increases the blood pressure by two mechanisms. Firstly, it acts on the smooth muscle of the arterioles and causes strong vasoconstriction. Secondly, it stimulates the secretion of aldosterone by the adrenal cortex. Aldosterone enhances the uptake of $Na^+$ by the kidney tubules and hence causes a rise in the plasma $Na^+$ level. This results in an increase in the extracellular fluid volume and consequently an elevation in blood pressure. Angiotensin-II is degraded by an enzyme called **Angiotensinase**, present in plasma. Fig. 4.14 will help you in understanding the regulatory mechanism.

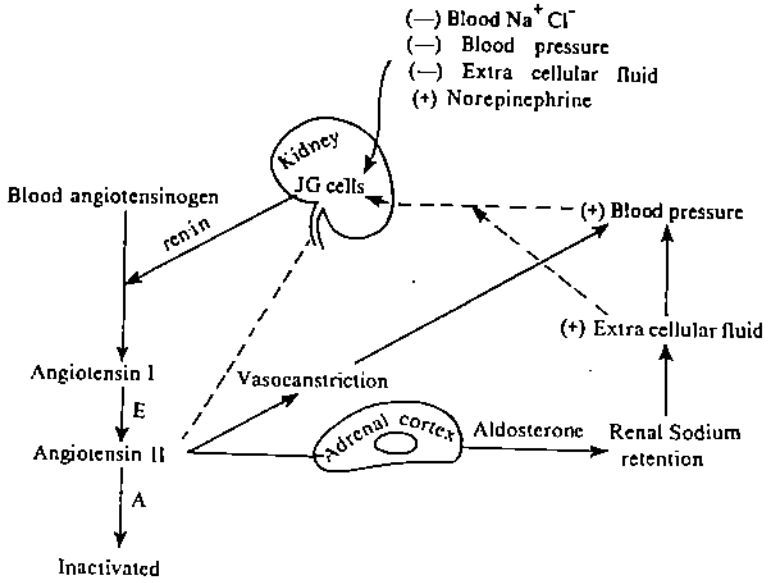



Fig. 4.14 : Renin-angiotensin mechanism for the regulation of kidney functions

**SAQ 4**

Fill in the blank spaces and compare your answers with those given in section 4.7.

a) There is a structural variation in the vertebrate kidney. In fishes, amphibians and reptiles the nephrons wander randomly and hence there is no clear cut distinction between ..... and ..... .

b) The urine concentration ability of the kidney in mammals is related to the habitat. Desert animals produce ..... ..... urine and fresh water animals produce ..... urine.

c) In lower vertebrates ..... is referred to as vasotocin.

d) Angiotensin-I is converted into Angiotensin-II by a proteolytic enzyme called ..... .

## 4.5 SUMMARY

You have studied in this unit that:

- catabolism of proteins gives rise to nitrogen which is highly toxic to the animal. Therefore, animals get rid of the toxic nitrogen in the form of ammonia or convert it into less toxic forms such as urea and uric acid before it is being excreted.
- animals which predominantly excrete ammonia are called ammonotelic, those that excrete urea are known as urotelic and uric acid excreting animals are known as uricotelic.
- the mode of nitrogen excretion in animals is an adaptive character related to the availability of water in the environment.
- a variety of tubular renal organs are present in animals for the elimination of unwanted materials and for osmotic and ionic regulation. Despite their morphological diversity, their functional principles are remarkably similar,
- in an isosmotic primary urine first formed in the tubular lumen by either filtration of blood or active solute transport followed by passive diffusion of water from blood, useful materials are reabsorbed before secretion of the urine.

- in uricotelic animals, the urine is made into a semisolid or solid mass due to water reabsorption in the rectum in insects, in the cloaca of birds and terrestrial reptiles. The ability of the avian and mammalian kidney to produce a hyperosmotic urine is due to the presence of loop of Henle. Desert mammals which produce an extremely hyperosmotic urine have longer loops of Henle than the other animals which produce a relatively less hyperosmotic urine,
- kidney function is hormonally regulated.

## 4.6 TERMINAL QUESTIONS

1) Explain in the space given below why ammonia is toxic to the animal.

.....

.....

.....

.....

.....

2) Explain briefly in the space given below the phenomenon of storage excretion in insects.

.....

.....

.....

.....

.....

3) Explain briefly in the space given below, the formation of uric acid in Malpighian tubules of insects.

.....

.....

.....

.....

.....

4) Explain in the space given below structure of nephron in vertebrates.

.....

.....

.....

.....

.....

5) Explain briefly in the space given below the role of loop of Henle in the formation of urine.

.....

.....

.....

.....

.....

6) Explain briefly how kidney function is regulated by renin angiotensin system.

.....

.....

.....

.....

.....

## 4.7 ANSWERS

**Self-assessment Questions**

1) a) oxidative transdeamination
   b) depletion of cellular concentration of ATP
   c) ornithine cycle
   d) inosinic acid pathway
   e) guanine, guanotelic

2) a) i) b, ii) c, iii) d, iv) e, v) a
   b) i) protonephridia, metanephridia
      ii) isosmotic

3) a) flame cells
   b) left kidney, right kidney
   c) salt reabsorbing segment
   d) protonephridium

4) a) cortex, medulla
   b) highly concentrated, very dilute
   c) antidiuretic hormone (ADH)
   d) converting enzyme

**Terminal Questions**

1) Refer to sub-section 4.2.1
2) Refer to sub-section 4.2.4
3) Refer to sub-section 4.3.5
4) Refer to sub-section 4.3.6
5) Refer to sub-section 4.3.6
6) Refer to section 4.4

# UNIT 5 OSMOTIC AND IONIC REGULATION

**Structure**



## 5.1 INTRODUCTION

In the previous unit you have learnt that ammonotelism, ureotelism and uricotelism are the adaptations of the animals for the removal of toxic nitrogenous wastes and thereby maintain homeostasis. Animals regulate the concentration of water and salts in their body fluids in accordance with their external environment. The process of maintenance of osmotic concentration of the body fluids is called **osmoregulation**. Osmoregulation and excretion are intimately related as the ultimate aim of these processes is to maintain homeostasis. These processes are performed by the same set of organs. Kidney is the major organ of osmoregulation in vertebrates. Gills, integument, salt glands and rectal glands assist kidneys in this endeavour. The osmoregulatory organs of invertebrates are nephridia, antennal glands and malpighian tubules. The cuticle of insects also performs an excellent osmoregulatory function in both aquatic and terrestrial insects. In this unit you shall study about the osmotic environments, osmotic exchanges between animal and the environment, the mechanisms used by various animals to cope up with environmental osmotic extremes and also about role of hormones in osmotic and ionic regulation.

**Objectives**

After reading this unit you shall be able to :

- explain the meaning of osmoregulation
- discuss how aquatic animals cope up with the osmotic problems
- explain how migratory fishes maintain constant osmotic pressure of their body fluids
- discuss the mechanisms the terrestrial animals have evolved to face the extremes of desert conditions
- explain the secret of the survival of kangroo rat in the arid desert,
- discuss role of hormones in regulating water and electrolyte balance in the body fluids.

## 5.2 PROBLEMS OF OSMOREGULATION

You have learnt in the course LSE-01 : Cell Biology that, water, some inorganic salts, nutrient molecules are some of the important components of the body fluids. It is essential for the animals to maintain appropriate concentration of these components for the survival.

It is agreed that life originated in the sea, and during the course of evolution animals spread from oceans into the estuaries, rivers and onto the land. These new environments are osmotically different from that of the sea. Since life originated in the sea, body fluids of the animals are more or less similar to that of seawater in its general composition. Therefore, animals inhabiting marine environment do not have the problem of osmoregulation, because their body fluids are isotonic to their external environment, the sea. But animals spread over to brackish, freshwater, and terrestrial environments have the problem of osmoregulation because their body fluids are hypertonic to their external environment. Therefore, these animals evolved various physiological and behavioural adaptations to cope up with the rigors of the osmotic environments. The osmoregulatory organs of these animals play a vital role in this effort.

An osmoregulatory animal is generally in an osmotic steady state eventhough there may be hourly and daily variations in osmotic balance. The concentration of internal salts and water is maintained relatively constant. The intake and outflow of water and salts are equal. Such osmotic homeostasis is maintained at the cost of metabolic energy, obtained from ATP (Fig. 5.1).

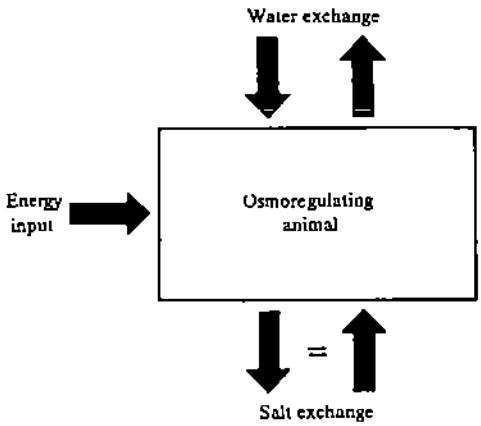



**Fig. 5.1 : Osmotic homeostasis in osmoregulatory animal**

The need for the evolution of efficient osmoregulatory mechanism gave chance for animal speciation and diversification. The resourcefulness of evolutionary adaptations can be realised if we consider the adaptations of arthropods and vertebrates. If they had not evolved suitable osmoregulatory mechanism how they have been so successful in both terrestrial and aquatic environments — the environments that are osmotically hostile and difficult. You will learn about such adaptions in the following sections of the unit.

The osmotic exchanges that take place between an animal and its environment are of two different types :

1) obligatory exchanges and
2) regulated exchanges.

**In obligatory exchange** osmotic exchanges occur mainly in response to physical factors over which the animal has little or no physiological control. Whereas **in regulated exchanges**, the osmotic exchanges are physiologically controlled and it serves to aid in maintaining internal homeostasis. Regulated exchanges generally serve to compensate for the obligatory exchanges.

Animals that maintain osmolarity of their body fluids constant irrespective of the medium in which they live are termed **osmoregulators.** Animals that do not actively control the osmotic condition of their body fluids and instead conform to the osmolarity of the medium in which they live are termed **osmoconformers.** Most vertebrates, except elasmobranchs and hagfishes are strict osmoregulators, maintaining the composition of body fluids within small osmotic range. Marine invertebrates are in osmotic balance with seawater. The concentration of $Na^+$ K , $Ca^{2+}$, $Mg^{2+}$ and $Cl^-$ in their body fluids is close to the concentration of these ions in the seawater, in which they live. This is clearly illustrated in Fig. 5.2. In the following section we shall learn about life in different osmotic habitats.

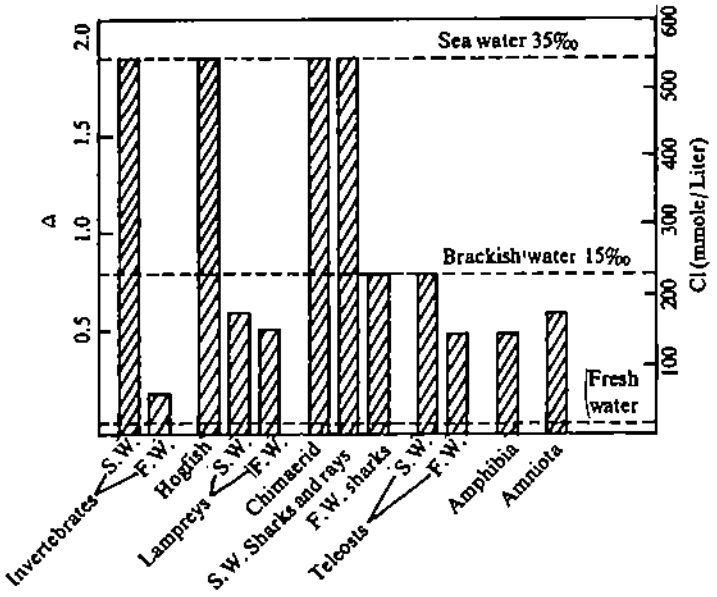



**Fig. 5.2 : Osmolarity of body fluids in different groups of animals in relation to the tonocity of their environments**

**SAQ 1**

Match the terms given in column I with their definitions given in column II and compare your answers to those given in section 5.8

| Column I | | Column II | |
|---|---|---|---|
| a) Obligatory exchange | [ ] | i) | Animals that maintain an internal osmolarity different from the medium in which they live |
| b) Regulated exchanges | [ ] | ii) | Physiologically controlled osmotic exchanges to maintain internal homoeostasis |
| c) Osmoregulators | [ ] | iii) | Animals that do not actively control the osmotic condition of their body fluids, and instead conform to the osmolarity of the medium in which they live. |
| d) Osmoconformers | [ ] | iv) | Osmotic exchanges that occur mainly in response to the physical factors over which the animals have no physiological control. |

## 5.3 OSMOREGULATION IN AQUEOUS ENVIRONMENTS

You are aware that the aqueous environments are of two types: i) Freshwater and ii) Seawater. The osmotic concentration of these environments ranges from several milliosmoles per litre in freshwater lakes to about 1000 milliosmoles per litre in ordinary seawater. It is even more in landlocked saltseas. The whole body and the respiratory surface of the aquatic animals is immersed in these osmotically extreme environments.

The animals which can tolerate a wide range of salinities are called **euryhaline** and those which tolerate only a narrow osmotic range are termed **stenohaline**. In the following sub-section we shall learn about osmotic regulation in freshwater and seawater animals.

### 5.3.1 Freshwater Animals

In Fig. 5.2 you have seen that the body fluids of freshwater animals are hyperosmotic to their aqueous surroundings. This results in two kinds of osmotic problems;

i) due to osmotic gradient water moves into their bodies resulting in swelling of their body.

ii) since the surrounding environment is low in salt content, there is continuous loss of body salts.

Therefore, freshwater animals must prevent net gain of water and net loss of salts. Net gain of water is prevented by producing dilute urine. Freshwater fishes produce copious urine than the marine fishes. The useful salts are largely retained by reabsorption in the kidney tubules. The salts which are passed out in urine are replaced partly from the ingested food. Salts are also extracted from the hypoosmotic surroundings by active transport across the **transporting epithelia**. The transporting epithelia for example are found in the gills of fish and in the skin of amphibians. The active transport of NaCl in gills takes place against a concentration gradient in excess of 100 folds (Fig. 5.3a).

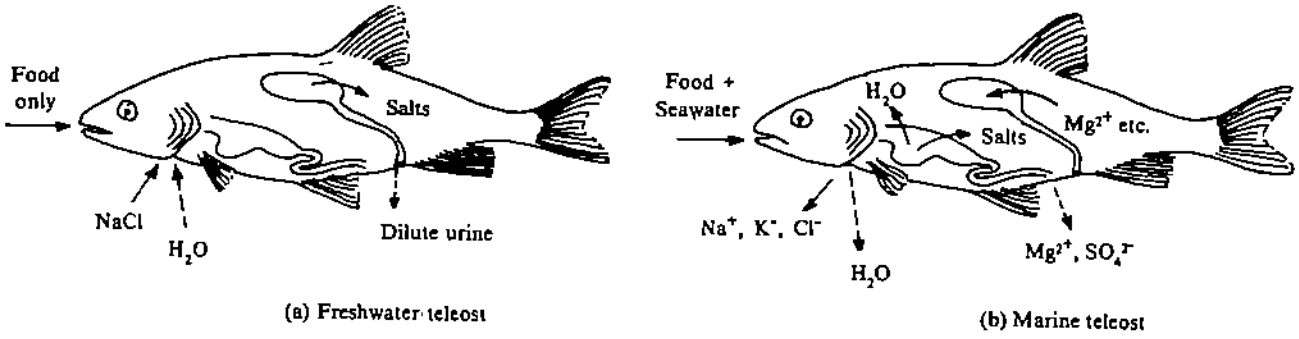



**Fig. 5.3 : Salt and water exchange in (a) freshwater and (b) marine teleosts. Solid arrows indicate active transport, broken arrows indicate passive transport processes.**

In some freshwater animals including fishes, reptiles, birds and mammals water uptake and salt loss are minimised due to the presence of the integument, which is less-permeable to water and salts. Fresh water animals other than reptiles, birds and mammals who have relatively impermeable integument do not drink excess of freshwater, reducing the need to expell excess water as done by their cohabitants having integument permeable to water and salts.

## 5.3.2 Marine Animals

You have learnt through Fig. 5.2 that the composition of body fluids of marine invertebrates, including the ascidians are similar to seawater. Such animals need not expend much energy in regulating the osmolarity of their body fluids. In a few vertebrates too plasma is found isosmotic to their environment. In hagfish (*Myxine*), for example, the concentrations of $Ca^{2+}$, $Mg^{2+}$ and $SO_4^{2-}$ are maintained significantly lower than they are in the seawater, whereas $Na^+$ and $Cl^-$ are maintained higher in the body fluids than the seawater. Like the hagfish, the cartiligenous fishes such as sharks, rays and skates have plasma that is isosmotic to the seawater. However, in these fishes the concentration of inorganic electrolyte is maintained far lower than the seawater. The excess inorganic electrolytes such as NaCl are excreted via the kidneys and also by means of a special excretory organ, the **rectal gland**, located at the end of the alimentary canal.

The body fluids of marine teleosts are hypotonic to seawater, so these fishes lose water to the environment specially across the gill epithelium. To replace the lost volume of water they drink seawater (Fig. 5.3 b). The ingested seawater along with NaCl and KCl is absorbed across the intestinal epithelium. 70 to 80% of it enters the bloodstream. Most of the divalent ions such as $Ca^{2+}$, $Mg^{2+}$ and $SO_4^{2-}$ are expelled through the anus. The excess salt absorbed along with the water is subsequently eliminated from the blood by active transport of $Na^+$ and $Cl^-$ and perhaps $K^+$ across the gill epithelium into the seawater. Divalent salts are also secreted by the kidneys. Gills have special type of secretory epithelium known as **chloride cells.** These cells actively secrete chloride and probably sodium also into the seawater (Fig. 5.4).

The urine of marine teleosts is isotonic to the blood, but rich in those salts ($Mg^{2+}$, $Ca^{2+}$ and $SO_4^{2-}$) that are not secreted by the gills. The net result of the combined

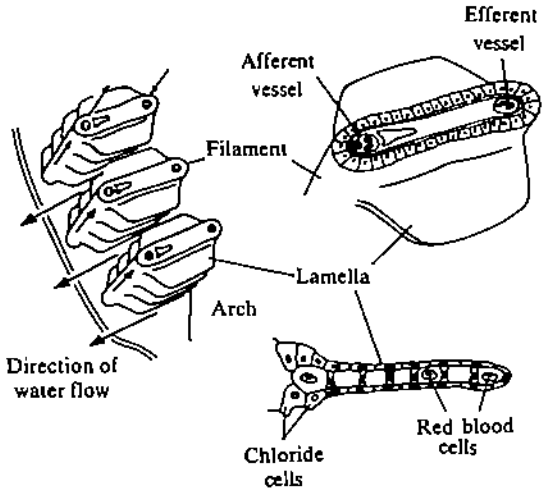



Fig. 5.4 : Chloride cells in the gills

osmotic work of gills and kidneys in the marine teleosts is a net retention of water that is hypotonic to both the ingested seawater and to the urine. Migratory fishes for example, the salmon of North-west pacific make use of this facility to maintain a more-or-less constant plasma osmolarity even though they migrate between marine and freshwater environments.

Marine reptiles for example, iguanas, estuarine sea turtles, crocodiles, sea snakes and marine birds like the marine teleosts do not produce urine which is hyperosmotic to their body fluids. Instead they have specialised organs for the secretion of salts known as **salt glands**, located in the cranium of the animals. In birds these are generally present on the bill below the eyes and in lizards they are near the nose or eyes. In the brackishwater crocodiles salt glands are found in the tongue. Although neither reptilian nor avian kidneys are capable of producing a very hypertonic urine, the salt glands of marine reptiles and birds secrete enough salt to enable them to drink salt water even though their kidneys are unable to produce urine more concentrated than seawater (Fig. 5.5).

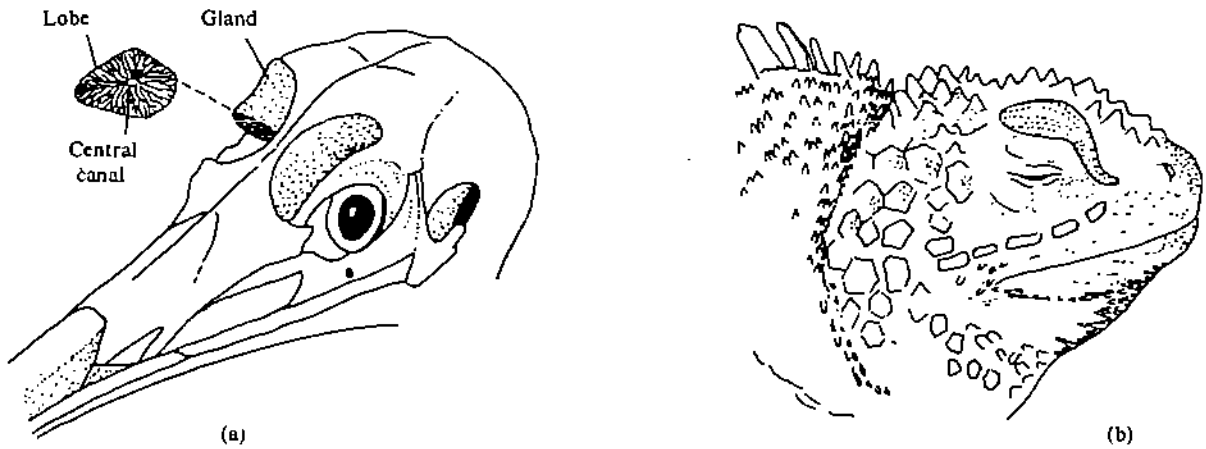



Fig. 5.5 : Salt glands in birds and reptiles

Salt glands, along with the gills of marine teleosts, compensate in these groups for the inability of the sub-mammalian kidney to produce a urine that is strongly hypertonic to their body fluids.

Marine mammals such as sea lions, whales, seals have no external salt-secreting organs. But the kidneys of these animals are capable of producing a very hypertonic urine. Marine mammals do not imbibe sea water. They only ingest the seawater that is present in their food. Another source of water for marine mammals is the metabolic water. You have learnt in Units 11 and 12 of Cell Biology (LSE-01) that metabolic water is obtained from the metabolism of food molecules, during which hydrogen atoms combine with oxygen to produce water.

Human beings, like other mammals cannot drink seawater because their kidneys can remove only up to 6 g of $Na^+$ from the bloodstream per litre of the urine produced. Seawater contains 12 g of $Na^+$ per litre. This causes accumulation of salt in the body and they cannot drink equivalent amount of water for its removal, and would lead to rapid dehydration.

**SAQ 2**

State whether the statements given below are true or false.

i) The animals which can tolerate a wide range of salinities are called euryhaline and those which tolerate only a narrow osmotic range are termed stenohaline.

ii) Osmotic concentration of seawater is about 1000 million osmoles per litre.

iii) Freshwater fishes produce copious urine than the marine fishes.

iv) In freshwater fishes salt is extracted from the hypoosmotic surroundings by active transport in the gills across its transporting epithelia.

v) The body fluids of marine invertebrates are hypotonic to the seawater.

vi) The chloride cells, located in the gills of marine teleost expel the excess salt by active transport.

vii) In marine mammals the excess salt is secreted by the salt glands.

## 5.4 OSMOREGULATION IN TERRESTRIAL ENVIRONMENT

In the earlier section of this unit you have learnt about osmoregulation in aquatic environment. In this section, we shall study how the terrestrial animals cope up with the problems of osmoregulation.

Just like the aquatic animals which are submerged in an aqueous medium, animals in a terrestrial environment can be thought of as submerged in an ocean of air. Unless the humidity of the air is high, animals having a water permeable epithelium will be subjected to dehydration very much as if they were submerged in a hypertonic medium such as seawater. In order to avoid dehydration, the epithelium should be totally impermeable to water. The evolutionary process was not found this to be a feasible solution to the problem of desiccation, since an epithelium that is impermeable to water will be dry and such a type of epithelium will have limited permeability to respiratory gases. This mechanism will not fulfil the respiratory needs of a terrestrial animal. Due to the presence of permeable respiratory epithelia, air-breathing animals lose water across it, which would result in dehydration. Various means have been evolved to minimise the water loss into the air through the respiratory epithelium and also other parts of the body. We shall now learn about them.

### i) Water movement through the integument

The integument of most terrestrial animals is relatively impermeable to water and very little water is lost through the skin (Table 5.1).

Table 5.1 : Evaporative Water Loss of Animals under Desert Conditions

| Species | Water loss ($mg/cm^2/h$) | Remarks |
|---|---|---|
| **Arthropods** | | |
| *Elcodes armata* (beetle) | 0.20 | 30°C; 0% r.h. |
| *Hadrurus arizonensis* (scorpion) | 0.02 | 30°C; 0% r.h. |
| *Locusta migratoria* (locust) | 0.70 | 30°C; 0% r.h. |
| **Amphibian** | | |
| *Cyclorana alboguttatus* (frog) | 4.90 | 25°C; 100% r.h. |
| **Reptiles** | | |
| *Gehydra varegata* (gecko) | 0.22 | 30°C; dry air |
| *Ulta stansburiana* (lizard) | 0.10 | 30°C |

| | | |
|---|---|---|
| **Birds** | | |
| *Amphispiza belli* (sparrow) | 1.48 | 30°C |
| *Phalaenptilus nuttallii* (poorwill) | 0.86 | 35°C |
| **Mammals** | | |
| *Peromyscus eremicus* (cactus mouse) | 0.66 | 30°C |
| *Oryx beisa* (African oryx) | 3.24 | 22°C |
| *Homo sapiens* | 22.32 | 70 kg; nude, sitting in sun; 35°C |

(r.h. stands for relative humidity)

You have seen in the above table that insects lose very little moisture through the integument. It is due to the presence of waxy cuticle which is highly impermeable to water. The wax is deposited on the surface of the exoskeleton through fine canals that penetrate the cuticle (Fig. 5.6).

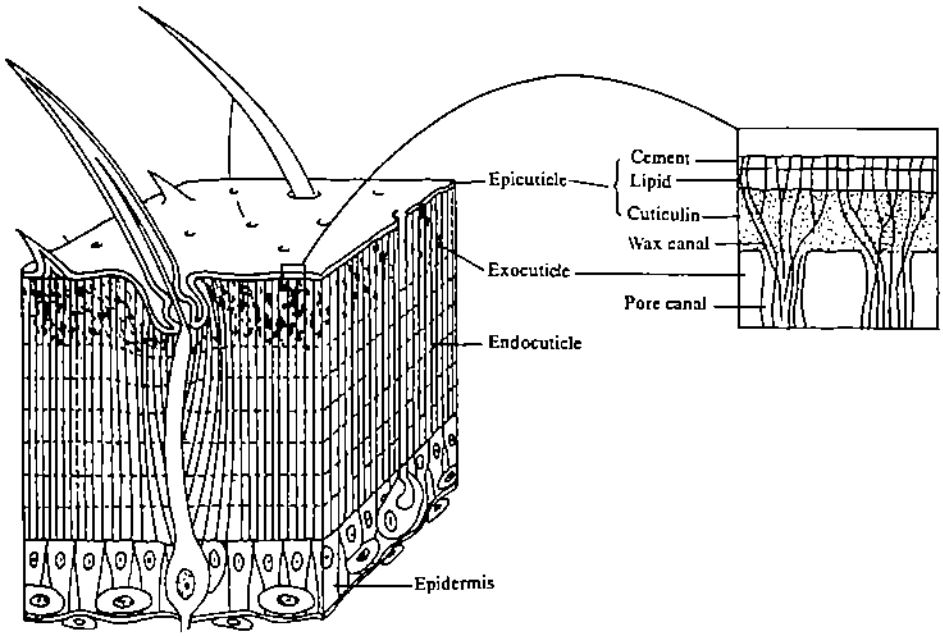



Fig. 5.6 : General features of insect integument

You must have noted in Table 5.1 that water permeability property of vertebrate integument varies widely. Reptiles, some desert amphibians, birds and many mammals have relatively impermeable skins. However, amphibians, as well as mammals that perspire, can become dehydrated at low humidity due to water loss through the integument. Animals with highly permeable skin are simply not able to tolerate very hot, dry environments. Most amphibians stay close to water and replenish their supply of water. These animals avoid desiccation by staying in cool, damp microenvironments during hot, dry times of the day. Toads which may temporarily wander away from a body of water or may have to wait for rains have oversized urinary bladders in which they store water. When necessity arises water will move osmotically from the lumen of the bladder into the interstitial fluid and the blood. The epithelium of the bladder like the amphibian skin is capable of actively transporting $Na^+$ and $Cl^-$ from the bladder lumen into the body to compensate the salts lost during times of excessive hydration. Many anurans have specialised regions of the skin on the abdomen and thighs called **seat patches**. These regions when immersed can take up water at a rate of three times the body weight per day.

ii) **Water loss during air breathing**

You have learnt that water is lost through the respiratory surface. In the terrestrial vertebrates the evaporative loss is reduced, because the respiratory surface in them (the lungs) is internal to the body cavity. Even within the lungs ventilation of the respiratory epithelium by unsaturated air will cause evaporation of the moisture wetting the epithelial surface. In birds and mammals such evaporative loss is

enhanced because of the difference between the body temperature and ambient temperature. Warmer air can hold more moisture when saturated than cool air. Since the expired air is more warmer than the inspired air, water is lost during expiration.

In a number of vertebrates the respiratory loss of water is minimised through a mechanism known as **temporal countercurrent system**. During inspiration cool air entering the lungs via nasal passage gets warmed by the heat of the nasal passage and absorbs moisture from the respiratory epithelium of the lungs. During expiration, the same air loses most of the heat it gained earlier as it warms the cool nasal passage on its way out. As the expired air gives up some of its heat to the tissue of the nasal passage, most of the moisture acquired from the respiratory epithelium condenses on the cool nasal epithelium. With the next inhalation, this condensed moisture again contributes to the humidification of the inspired air, and the cycle is repeated (Fig. 5.7).

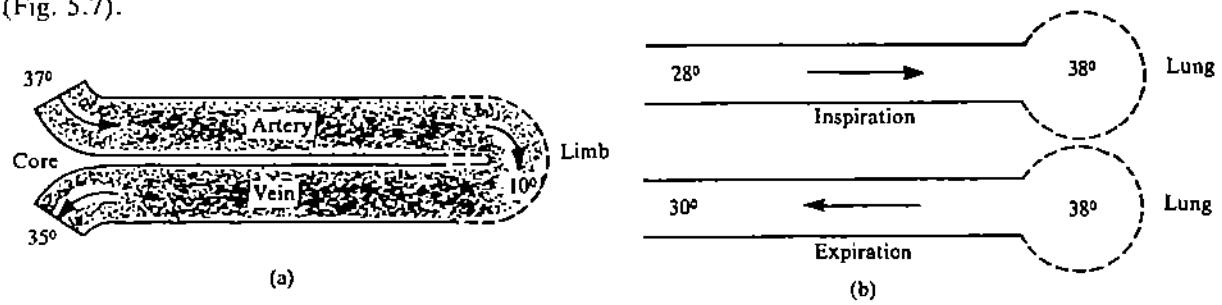



Fig. 5.7 : Temporal countercurrent system

You have learnt that cuticle is highly impermeable and thus in insects there is no water loss through this route. The major route of water loss in terrestrial insects is via the **tracheal system**. You have learnt in Unit 2 that tracheal system consists of air-filled **tracheoles** that supply oxygen to the tissue. The entrance of the tracheoles are known as **spiracles**. The spiracles have muscles and function like valves of the tracheoles. The spiracles remain closed to prevent the water loss. They open periodically for a very short while for leting in oxygen for respiration and close. The leting out of carbon dioxide does not follow the leting in of oxygen. Carbon dioxide is accumulated and expelled out at one burst. Water may be lost only at this moment. The periodic opening and closing of the spiracles is done by the **spiracular muscles.** Certain terrestrial arthropods have the ability to extract water vapour directly from air.

### iii) Water loss during excretion

In terrestrial animals body water is also lost during excretion of nitrogenous wastes. A number of physiological adaptations have taken place to minimise the loss of water associated with this phenomena. You have learnt in the previous Unit that among terrestrial invertebrates, insects are highly effective in conserving water. In terrestrial vertebrates, kidney is the chief organ of osmoregulation and excretion. The loop of Henle is the specialised part of the nephron which produces hyperosmotic urine. Amphibians and reptiles which are unable to produce a hyperosmotic urine, as an adaptive consequence, cease urine production entirely during the period of osmotic stress.

### Kangroo Rat : A Classical Example of Adaptations for Desert Life

Kangroo rat *Dipodomys merriami*, a native of South-West America is a classical example of how small mammals survive in desert. It exhibits all the osmoregulatory adaptations for desert life. It survives in arid conditions without ingesting any free water by the following adaptations:

i) It avoids much of the daytime heat through nocturnal life-style, keeping cool during daylight hours by remaining in a burrow. This conserves water loss through evaporative cooling.

ii) It conserves respiratory moisture by an efficient nasal countercurrent mechanism.

iii) It secretes highly concentrated urine.

iv) The rectum absorbs water from the faeces resulting in dry faecal pellets.

Kangroo rat is not known to drink water. It gets only a trace of free water from the dry seeds it eats. Primarily it depends upon the metabolic water for its survival.

In mammals **aldosterone**, a mineralocorticoid is concerned with electrolyte balance. It is produced in response to **adrenocorticotropic hormone** (ACTH) secreted by the pituittary. ACTH itself is released in response to a **corticotropin releasing factor** (CRF) from the hypothalamus. **Glomerulotrophic hormone** from the pineal gland is also known to stimulate aldosterone secretion. In nonmammalian vertebrates apart from aldosterone, another mineralocorticoid, **cortisol** is known to promote NaCl absorption. The mineralocorticoids increase the tubular absorption of sodium and promote the renal excretion of potassium.

iv) **Angiotensin**

Besides the stimulation of the adrenal cortex by ACTH and glomerulotrophic hormone to release aldosterone, kidneys themselves monitor the levels of sodium by **renin-angiotensin** system. When the sodium level falls, juxtaglomerular cells of the glomerulus release an enzyme renin into the blood. Renin acts on a precursor plasma protein angiotensin, hydrolysing it to the active hormone angiotensin. Angiotensin acts on the adrenal cortex and promotes aldosterone secretion. Aldosterone in turn promotes uptake of sodium by the renal tubules. Aldosterone also acts on the kidney arterioles ensuring a regular blood flow in the organ. In Fig. 5.9 you can see the complex interactions of all the hormones in the maintenance of water and salt balance.

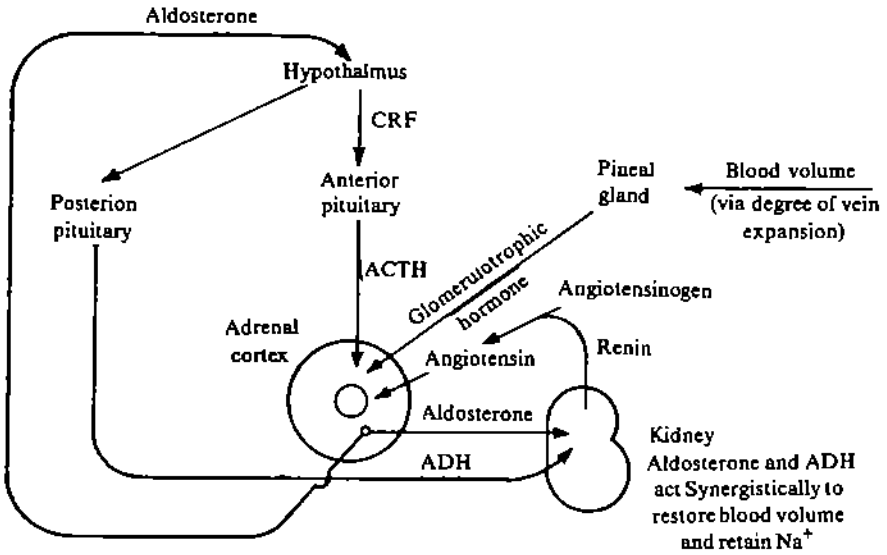



Fig. 5.9 : The hormonal mechanism involved in osmoregulation

**SAQ 4**

Explain briefly role of the following hormones in osmoionic regulation.

i) Antidiuretic hormone : .....

.....

.....

.....

ii) Prolactin : .....

.....

.....

iii) Aldosterone : .....

.....

.....

## 5.6 SUMMARY

You have learnt in this unit that:

- Osmoregulation is a process for the maintenance of osmotic concentration of the body fluids. Animals have adapted various physiological and behavioural mechanisms to cope up with the rigors of the osmotic environments.
- Since the body fluids of freshwater animals are hyperosmotic to their aqueous surroundings water moves into their body due to osmotic gradient and body salts leak out of their body. They prevent net gain of water by producing copius urine. The lost salts are replaced partly from the food they eat and much is extracted from the hypoosmotic surrounding by active transport.
- The body fluids of some marine animals are isosmotic to the seawater. Therefore, they need not expend much energy for regulating the osmolarity of the body fluids. Marine animals specially the teleosts, whose body fluids are hypoosmotic to the seawater, tend to lose water from their body. Therefore, they drink seawater to replace the lost water. The excess salts that enter the body along with the seawater is expelled through the anus, kidneys and also across the gills by active transport. Marine reptiles and birds have salt glands for the secretion of salts that entered the body through the seawater they drink.
- In the terrestrial environment animals face the problem of loss of body water due to the arid condition. Salts are also lost along with the water. Animals inhabiting arid environment avoid loss of water by adopting noctural habits and by staying in cool, damp microenvironments during the hot, dry times of the day. Physiologically they evolved mechanisms such as temporal countercurrent system and production of highly concentrated urine to avoid water loss during respiration and excretion respectively.
- Hormones such as diuretic hormone, antidiuretic hormone, chloride transport stimulating hormone play a vital role in the regulation of water and electrolyte balance in invertebrates. Prolactin, antidiuretic hormone, mineralocorticoids, angiotensin, prostaglandins are the hormones involved in the osmoionic regulation in vertebrates.

## 5.7 TERMINAL QUESTIONS

1) Explain briefly how do migratory fishes cope up with the problem of osmoregulation.

.....

.....

.....

.....

.....

2) Explain briefly the adaptations found in kangroo rats for the arid conditions.

.....

.....

.....

.....

.....

## 5.8 ANSWERS

**Self-assessment Questions**

1) a) iv, b) ii, c) i, d) iii

Dear Student,

While studying these units you may have found certain portions of the text difficult to comprehend. We wish to know your difficulties and suggestions in order to improve the course. Therefore, we request you to fill and send us the following questionnaire which pertains to this block.

## QUESTIONNAIRE

LSE-05
Block-1

Enrolment No. | | | | | | | | | | |

1) How many hours did you need for studying the units?

| Unit Number | | | | | |
|---|---|---|---|---|---|
| No. of hours | | | | | |

2) How many hours (approximately) did you take to do the assignments pertaining to this block?

| Assignment Number | | |
|---|---|---|
| No. of hours | | |

3) In the following table we have listed 4 kinds of difficulties that we thought you might have come across. Kindly tick (√) the type of difficulty and give the relevant page number in the appropriate columns.

| Page Number | Types of difficulties | | | |
|---|---|---|---|---|
| | Presentation is not clear | Language is difficult | Diagram is not clear | Terms are not explained |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

4) It is possible that you could not attempt some SAQs and TQs.
In the following table are listed the possible difficulties. Kindly tick (√) the type of difficulty and the relevant unit and question numbers in the appropriate columns.

| Unit No. | SAQ No. | TQ No. | Type of difficulty | | | |
|---|---|---|---|---|---|---|
| | | | Not clearly posed | Cannot answer on basis of information given | Answer given (at the end of Unit) not clear | Answer given is not sufficient |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |

5) Were all the difficult terms included in the glossary. If not, please list in the space given below.

6) Any Other Suggestion(s)

To,

The Course Coordinator (LSE-05: Physiology: Blocks 1 & 2)
School of Sciences
Indira Gandhi National Open University
Maidan Garhi
New Delhi-110 068

# Notes

# Notes

Uttar Pradesh
Rajarshi Tandon Open University

# UGZY/BY-08
# Physiology

Block

# 2
# ANIMAL PHYSIOLOGY—II

# BLOCK 2 ANIMAL PHYSIOLOGY—II

In the earlier block of this course, you have studied the physiology of nutrition, respiration and circulation, which are closely linked. You also studied how animals maintain their fluid balances in varying environments and the related problems of excretion. The theme in the first block was the relationship between the animal and its environment.

This theme is continued in the second block in the units on Movement (Unit 6) and Temperature Relations in Animals (Unit 7). An animal has to move from place to place in search of mate, food and also to escape from enemies and extreme climatic conditions. You will study the three fundamental mechanisms that generate movements in animals : amoeboid movement, ciliary and flagellar movements and muscle contraction which is the most apparent and dramatic evidence of animal life. Through evidences from electron microscopy, biochemistry and biophysics we now have a better knowledge of how the contractile mechanism of muscles is organised, and how it produces muscle shortening.

You have read in Cell Biology (LSE-01) that temperature alters the rate of metabolism. Active animal life is restricted to a narrow range of temperature, from a low limit of $-1^{\circ}C$ of arctic waters to an upper limit of $50^{\circ}C$, the temperature of some hot springs. Unit 7 discusses the temperature reactions in animals and how animals cope up with this effect.

Unit 8 deals with one of the basic functions of living organisms—reproduction. Reproduction is required for the continuance of species and certain animals like insects and fishes are born and survive just for the sake of reproduction. The queen bee or the queen termite, for example, reproduce throughout its life and do not perform any other function. The salmon in fish survive till they breed and die soon after the breeding season. This unit deals with the functional anatomy of the reproductive organs mainly in vertebrates.

The regulation and control of all physiological processes is discussed in Units 9 and 10 on Communication I and II. The animal body needs to be informed of the external environmental condition as well as internal condition to be able to coordinate all the physiological processes. This function is primarily performed by the nervous system. Unit 9 introduces some fundamental aspects of the nervous system, the structure and function of the basic unit the neuron. The second integrating system is the endocrine system that is dealt with in the last unit of this block.

Both these communication systems use chemical messengers and there is a marked overlap in their functions. Unit 10 emphasises these similarities especially at the biochemical level.

## Study Guide

Before you begin your study of this block we would again emphasise that you should have the Cell Biology units handy especially Unit 15.

Units 8, 9 and 10 in this block are rather long units. You need to devote more time to them.

Experiments that helped to understand the functioning of certain processes have been enclosed in boxes. The glossary at the end of the block explains some key words and would serve to re-enforce some of the concepts.

## Objectives

After studying this block you should be able to :

- describe the physiological process involved in various modes of movement in animals,
- describe the effects of temperature on animals explaining the physiological adaptations used by them to escape the rigors of extreme temperature,
- describe the structure and functions of reproductive organs and the factors responsible for regulation of reproduction,
- describe how nervous system and endocrine system integrate the various physiological functions in animals,
- explain that at cellular level all communication in the body is through chemical messenger.

# UNIT 6 MOVEMENTS

## Structure



## 6.1 INTRODUCTION

In the previous Block you have read about the physiology of nutrition, respiration, circulation, excretion and osmoregulation in animals. In this unit you will learn about physiology of movement in animals. Often we consider movement in connection with locomotion, i.e., an organism moving from place to place. It is a characteristic and fundamental property of animals. However, even animals that remain attached and never move about such as corals, sponges, etc. exhibit variety of movements. There are three basic mechanisms, that animals use to achieve motion. The three basic mechanisms of movements are :

i) **amoeboid movement,** ii) **ciliary movement,** and iii) **muscular movement.**

**Amoeboid movement** derives its name from the locomotion of amoeba. It involves extensive changes in cell shape, flow of cytoplasm and pseudopodial activity. **Ciliary locomotion** is the characteristic way in which ciliated protozoans such as *Paramecium* move. However, cilia are found in all animal phyla and serve a variety of functions. For example, cilia set up currents that effect movement through the water vascular systems of echinoderms. The respiratory tract of air breathing vertebrates is lined by the ciliated cells that slowly remove foreign particles that lodge on their surfaces. The sperms move with the aid of tail, which in principle acts like a cilium. **Muscular movement** is the fundamental mechanism used in the majority of animals for a variety of movements. Muscle has the ability to exert a force by shortening, called **'muscle contraction'.** This force is used for a variety of purposes. In this unit you will read about mechanism of amoeboid movement and ciliary movement, structure of the muscle and the mechanism of muscle contraction.

### Objectives

After reading this unit you shall be able to :

- explain the physiology of amoeboid movement, ciliary and flagellar movement
- elucidate the structure of vertebrate muscle and explain the molecular basis of muscle contraction
- describe the mechanisms that regulate muscle contraction
- differentiate the structure and function of skeletal muscles, cardiac muscles and smooth muscles.

## 6.2 AMOEBOID MOVEMENT

Amoeboid movement is the characteristic of some protozoans, slime molds and vertebrate white blood cells. The movement of these is due to cytoplasmic streaming, change in cell shape and extension of pseudopodia. These changes are easily observed under the microscope, but the mechanisms involved in activating the movement are not well understood.

When an amoeba has to move, it stretches its arm-like extensions, the **pseudopodia,** into the required direction, and its cytoplasm flows into the newly formed pseudopodia. The newly formed pseudopodia gradually extend and enlarge so that the entire cell occupies the space where previously only a small pseudopodium began to form. As the cell moves, new pseudopodia are formed in the direction of the movement, while the posterior parts are withdrawn. It is not known with certainty how the extending and retracting of the pseudopodia takes place. It appears that there is a transaction of the regions of the cytoplasm from fluid-like **sol** to semi solid **gel** state. Now we shall study how amoeboid movement is accomplished by transactions of cytoplasm from sol to gel state.

Under the light microscope, we can find two regions in the cytoplasm of the amoeba; i) the central region, the **endoplasm** which is fluid like sol and ii) the **ectoplasm,** the region of the cytoplasm just beneath the plasma membrane, which is gel-like.

In the phase contrast microscope, we can see that the endoplasm contains abundant particles and membranous organellae, found in constant random motion, indicating their freedom of movement in the sol region of the cytoplasm. Ectoplasm contains a three-dimensional network of cross-linked **actin fibres,** and all other organelle are excluded from the region. This gel region apparently decides the shape of the pseudopodium and may transmit tension from the regions of cellular contraction to the sites of contact with the substratum. It is believed that the ectoplasm contains non-cross-linked actin filaments and probably **myosin filaments** also. As a pseudopodium elongates and the sol-like endoplasm streams into it, the region of the endoplasm near the tip of the pseudopodium apparently transforms into gel-like ectoplasm (Fig. 6.1). Simultaneously, the ectoplasm elsewhere in the cell transforms into sol-like endoplasm, probably by an uncrossing of linking actin fibres. Proteins such as **actin, fimbrin** and **fodrin** are involved in the sol-to-gel transition. They cross-link actin filaments and bundle them to each other. Crosslinking of actin filaments produces a network confining the movement of individual actin molecules and results in the semisolid gel state.

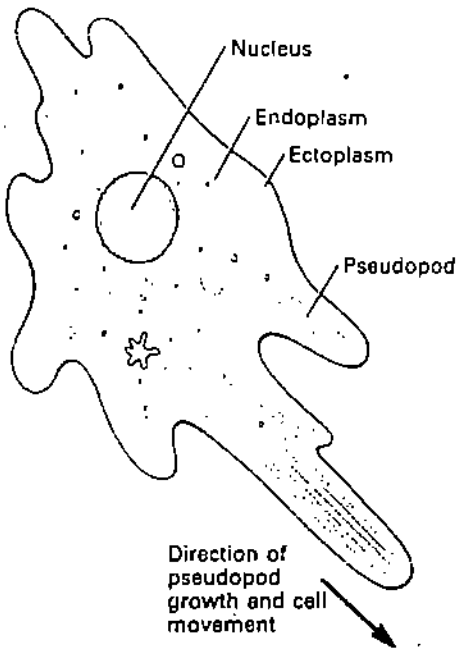



Fig. 6.1 : Schematic diagram of a moving amoeba cell, showing the sol-like endoplasm and the cortical gel-like ectoplasm.

Since actin and myosin are found in all eukaryotic cells, it thus appears that both cytoplasmic streaming and the formation of pseudopodia may depend on the interaction between myosin and actin filaments, similar to that found in muscle contraction. You will learn muscle contraction later in this unit.

It is not known how amoeboid movement is controlled or regulated. Amoebae cannot extend pseudopodia in all directions simultaneously and if they do they would be ripped apart! It has been shown however that the ability of many actin binding proteins to crosslink actin fibres is strongly dependant on both $Ca^{2+}$ concentration and pH. Thus, $Ca^{2+}$ and $H^+$ may regulate the sol-to-gel transition.

In the low molar concentration of $Ca^{2+}$ (= ppm range), when pH is lowered to 6.8, the cytoplasm of amoeba sets as a gel. Conversely, solation of the gel is induced by raising the pH or $Ca^{2+}$ concentration. Studies have implicated involvement of a **gelsolin** or **villin** protein in the gel-to-sol transition, because these proteins fragment actin filaments in the presence of micromolar concentration of ($10^{-6}$ moles) $Ca^{2+}$. It has been suggested that directed growth of pseudopodia is due to differences in $Ca^{2+}$ or $H^+$ concentration among various regions of the cytoplasm; whether this is the case, however remains to be determined. In the next section you will read about ciliary and flagellar movements.

**SAQ 1**

Fill in the blanks and compare your answers with those given at the end of the unit.

a) Amoeboid movement is the characteristic of some ..... and vertebrate .....

b) When an amoeba has to move, it stretches its arm-like extensions known as .....

c) ..... and ..... are the two regions of the cytoplasm of amoebas as can be seen under the light microscope.

## 6.3 CILIARY AND FLAGELLAR MOVEMENTS

Cilia and flagella or their derivatives occur in all animal phyla. They constitute the primary locomotory structures of many protozoa and of several kinds of metazoan cells, for example, sperm, ciliated epithelia of invertebrates and vertebrates. Derivatives of cilia occur in a wide variety of photoreceptor, mechanoreceptor and chemoreceptor cells.

Cilia and flagella have a similar internal structure; the difference lies in their beating patterns which are illustrated in Fig. 6.2. A flagellum like a tail of sperm, beats with a symmetrical undulation that is propagated as a wave along the flagellum (Fig. 6.2a). A cilium, in contrast beats asymmetrically with a fast or dash-like stroke in one direction, followed by a slower recovery motion in which the bending cilium returns to the original position (Fig. 6.2b). In flagellar motion, water is propelled parallel to the long axis of the flagellum; in ciliary motion water is propelled parallel to the surface that bears the cilia.

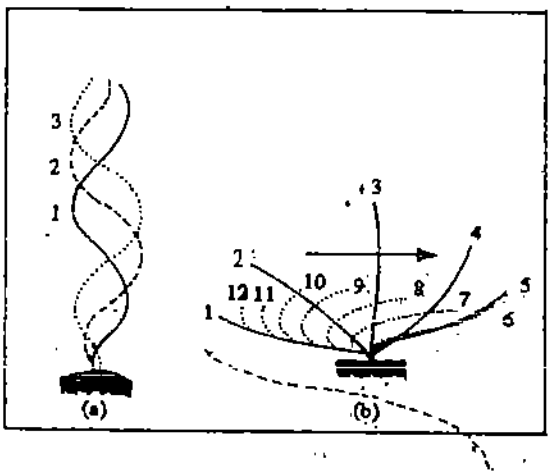



Fig. 6.2 : a) The typical beat of a flagellum propels water parallel to the main axis of the flagellum (arrow).
b) The beating of a cilium (right) propels water parallel to the surface to which the cilium is attached.

## Structure of the Cilia and Flagella

Cilia and flagella are hair-like cell organelles. The diameter (0.2 μm) and internal structure of cilia and flagella is similar to each other but these structures differ in length. Cilia are generally less than 15 μm in length while flagella may be as long as 200 μm. The internal structure and the molecular composition of cilia and flagella have been well studied by electron microscopy and biochemical techniques. In Fig. 6.3 you can see that the covering membrane of the cilium or flagellum is continuous with the plasma membrane of the cell. It is actually the evagination of the plasma membrane. The cilia is attached to the body of the organism by a **basal body** or **kinetosome.**

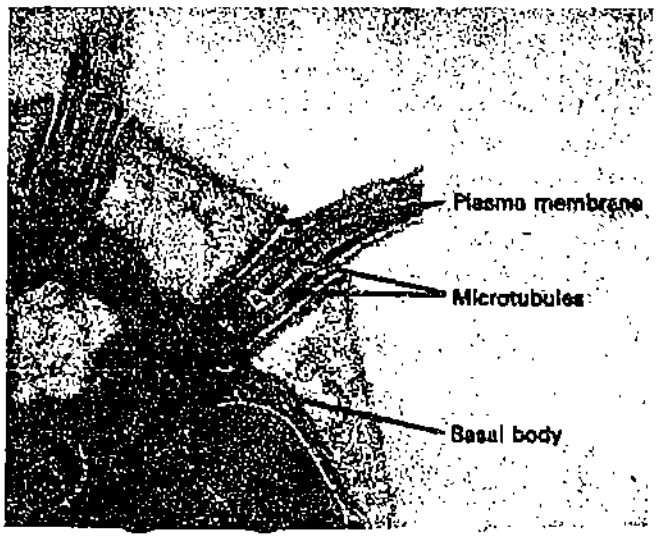



Fig. 6.3 : Electron micrograph of flagella showing the plasma membrane, microtubules and the basal body.

The main internal structures of a cilium are **microtubules**, which extend from the base to the tip. The microtubules are arranged in 9 + 2 configuration, consisting of nine outer doublets surrounding two single central microtubules (Fig. 6.4).

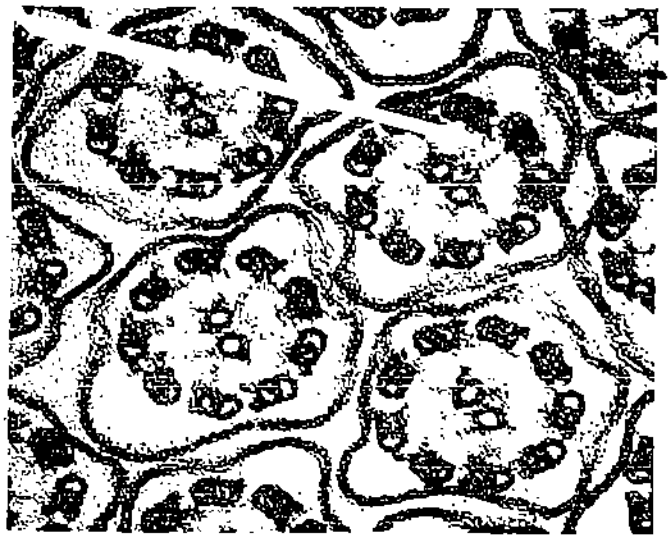


Fig. 6.4 : Cross-sections of the flagella showing the characteristic 9 + 2 arrangement of the filaments in each flagellum.

Each microtubule is a hollow cylinder composed of polymers of the globular proteins-**tubulins.** The outer doublets each consists of a complete tubule (the A tubule) with 13 subunits and an attached incomplete B tubule containing only 10 or 11 subunits (Fig. 6.5). Each A tubule bears two **side arms**, called **dynein arms** that project laterally towards the B tubule of the next doublet. There is a series of radial spokes which extend from the A sub-tubule to the central pair of microtubules. The outer doublets are connected circumferentially by **nexin links** (see Fig. 6.5). The entire array of microtubules and associated arms and links is called the **axoneme.** The nine peripheral doublets merge at the base to form a hollow tube that forms the basal body.

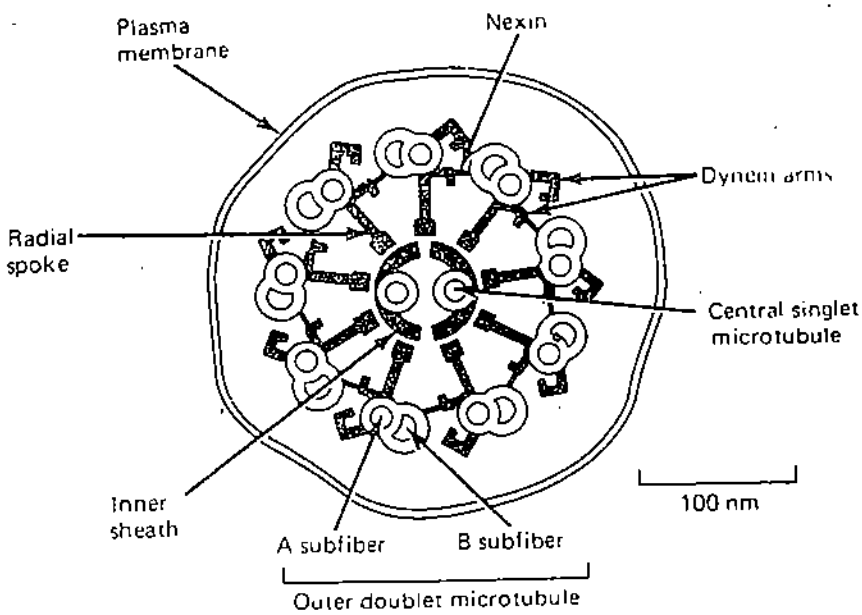



Fig. 6.5 : Diagram showing the internal structure of a cilium in cross-section.

## Mechanism of Movement

Three types of mechanisms have been suggested to explain the mechanism of movement of cilia and flagella. These are :

i) the flagellum moves passively, much like a whip, by forces exerted at its base;

ii) the elements along the inner curvature of a propagating wave contract while the opposite side does not. Such a type of contraction takes places alternately on the inner curvature of a propagating wave on opposite side to bend the cilium or flagellum from side to side, and

iii) the thin filaments of the cilium do not change shape, but move past one another to produce a curvature, similar to the sliding filaments during muscle contraction (Fig. 6.6).

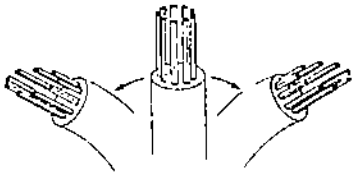


Fig. 6.6 : Diagram of sliding microtubules during beating of a cilium. The membrane of the distal portion of the cilium is removed, exposing the 9 outer doublets. In the straight cilium (center) all of the tubules end at the same point. During beating, the outer doublets slide past one another (left and right) causing a bending of the cilium and a displacement at the end of the tubules.

Electron microscopic studies demonstrate that bending of flagellum occurs when the extending dynein arms attach to the neighbouring B-tubule, inducing sliding movement. The dynein arms seem to "walk" along the cilium, presumably by the attachment of radial spokes to the central microtubule to constrain the sliding. The radial spokes and the nexin links are required to convert the sliding movement into typical bending of the cilium or the flagellum. The energy for the sliding movement is provided by the hydrolysis of ATP (Fig. 6.7).

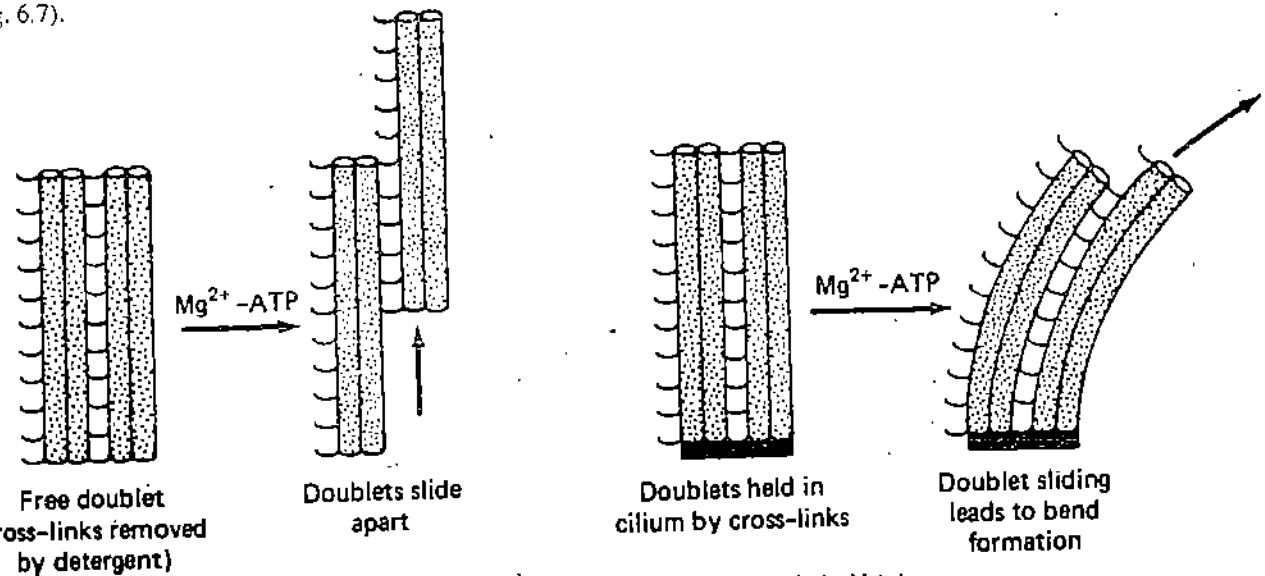



Fig. 6.7 : Diagram illustrating the experimental demonstration of sliding of outer microtubule doublets *in vitro* as well as the bending of the doublets if they are bound together at one end. The requirement of the $Mg^{2+}$ and ATP is also shown.

**SAQ 2**

Fill in the blanks and compare your answers with those given at the end of this unit.

a) The cilia is attached to the body of the organism by a . . . . . .

b) In the cilia or flagella the microtubules are arranged in . . . . . configuration.

c) Dynein arms are present in . . . . . tubules or . . . . . of the cilia or flagella.

## 6.4 MUSCLE AND MOVEMENTS

In the earlier section you have read about amoeboid movement, ciliary and flagellar movement. In this section you will learn how muscles are involved in the movement.

Muscle cells are found in almost all the phyla of the animal kingdom except the phylum protozoa. Contraction and relaxation of these muscles brings about movement in the organisms. In vertebrates there are three types of muscles : skeletal muscles, **cardiac** muscles and **smooth** muscles. Skeletal muscles are attached to the bones in the arms, legs and the spinal cord and produce activities such as walking, movement of head, hands, etc. Cardiac muscles are the muscles of the heart. These are specialised for continuous contractions of the heart, needed in pumping of the blood. Smooth muscles are present in the walls of internal organs such as the large and small intestine, the gall bladder and large blood vessels. Contraction and relaxation of smooth muscles control the diameter of blood vessels and also propel food along the gastro-intestinal tract. Under the microscope the skeletal muscles and the cardiac muscles exhibit transverse light and dark bands alternating with each other. Therefore, the skeletal muscles and the cardiac muscles are also called **striated muscles**. The smooth muscles do not have striations. You will read more about the structure of these three types of muscles in Sub-section 6.4.1.

Skeletal muscles are usually called **voluntary** because, the muscles of the limb and trunk are under control of the will.

Your leg or your hand will move only if you wish to move them. However, some movements like breathing do take place without our control. Smooth muscles are not under the control of the conscious mind, and therefore are called **involuntary**. Their contractions are usually slower than those of skeletal muscles and these movements normally take place without our knowledge. In the next subsection you shall learn about the structure of skeletal muscles.

### 6.4.1 Structure of Vertebrate Skeletal Muscles

Vertebrate skeletal muscles are composed of a large number of long, cylindrical and multinucleated cells, called **muscle fibres** arranged parallel to each other. The fibres contain longitudinally arranged elements called **myofilaments**. The myofilaments are organised into **myofibrils**. The fibres measure between 0.1 to 0.01 mm in diameter and several centimetres long. The myofibrils have characteristic cross striations called **Z-lines**, which are repeated at regular intervals. The region between two Z-lines is called a **sarcomere**, which is a functional unit of a myofibril. Thus a myofibril consists of longitudinally repeating sarcomeres. The Z-lines of adjacent myofibrils are lined up with each other, forming alternating A-bands and I-bands. There is a lighter region in the middle of the A-band called the H-Zone.These bands appear continuous for all the fibrils of a muscle fibre and it is this alignment of banding that gives the fibre its striated appearance (Fig. 6.8).

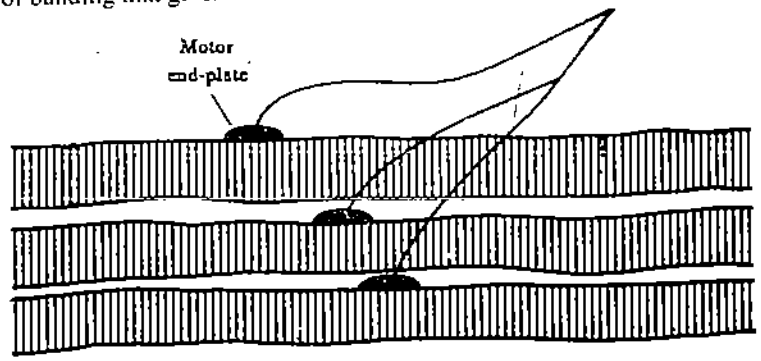



Fig. 6.8 : Schematic diagram of striated (skeletal) muscle and its components.

Myofibrils consist of two kinds of myofilaments. The **thick filaments** and the **thin filaments**. The thick filaments are made up of **myosin** and the thin filaments are of **actin** and the regulatory proteins **troponin** and **tropomysin**. The thick filaments are confined to A-band whereas the thin filaments extend from the Z-line and enter into A-band between thick filaments. The I-band contains only thin filaments. In the region of overlap between thick and thin filaments, the adjacent thick and thin filaments interact with each other by forming cross bridges. The cross bridges are projections of myosin molecules whose interaction with actin molecules generate the force for the muscle contraction.

The muscle fibre is surrounded by a cell membrane called **sarcolemma.** The sarcolemma connects with a complex system of transverse tubules, called **T-system** that runs across the muscle cells near the Z-lines. The T-tubules appear as invagination of the sarcolemma into the interior of the fibre. The muscle fibre is also surrounded by a sleeve-like structure called **sarcoplasmic reticulum,** which is involved in the initiation of muscle contraction (Fig 6.9).

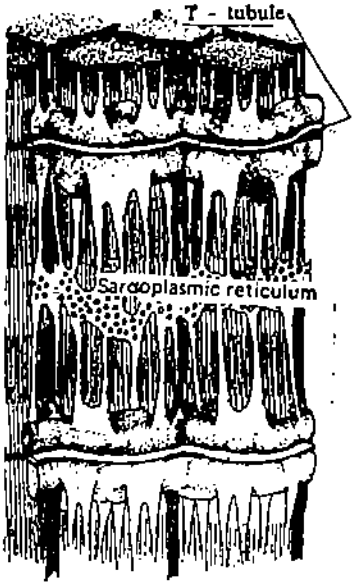



**Fig. 6.9 : Shows the system of T-tubules and the sarcoplasmic reticulum that surrounds the striated muscles.**

## 6.4.2 Mechanism of Muscle Contraction

Contraction is the function of a muscle. It is a physical activity generating force. It causes shortening of the muscle. The widths of the I-bands and H-zone both decrease but the width of the A-band remains constant. Apparently the lengths of the myosin and actin filaments also do not change. This shows that muscle shortens in contraction without either of the kinds of filaments changing length. Instead contraction of the muscle fibre is brought about by sliding of the filaments past each other in their region of overlap. These observations were first made in 1954 by two independent teams, H.E. Huxley and Hanson and A.F.Huxley and Niedergerke, and they proposed it as **"sliding filament model"** which has been experimentally confirmed. In Fig. 6.10 you can note the relation of the myofilaments during shortening of two sarcomers.

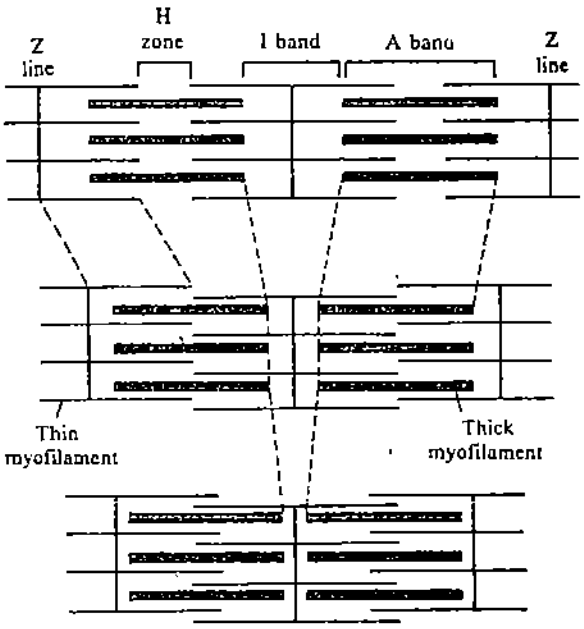



Fig. 6.10 : The sliding-filament hypothesis.

The process of sliding of thick and thin filaments past each other involves the cross bridges which extend from the thick filaments to contact the thin filaments. To understand how these cross bridges generate the force of contraction, it is necessary to study the molecular structure of actin and myosin filaments.

## 6.4.3 Molecular Basis of Muscle Contraction

You have studied in Sub-section 6.4.1 that the thick filaments are composed of protein myosin and the thin filaments contain primarily protein action. Myosin molecules are very large proteins, each consisting of a double-headed globular region joined to a long rod or tail (Fig. 6.11).

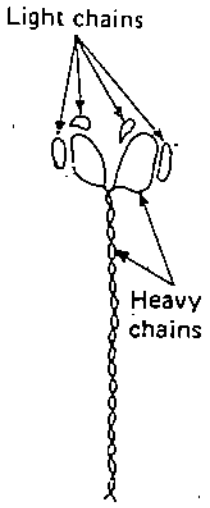



**Fig. 6.11 : Myosin molecule : It consists of two heavy chains and four light chains.**

As you see in Figure 6.11 that a myosin molecule contains two **heavy** chains; part of a heavy chain makes up one head and the other part extends the length of the tail. Myosin heads also contain four smaller **light** chains. Thus, each myosin molecule consists of six polypeptide chains. The tails of many myosin molecules together make up the thick filament. Whereas the globular heads project to the side forming the cross bridges.

The myosin molecules in a thick filament have their head-ends oriented towards the end of the filaments and their tails pointing towards the middle. As a result there is a short bare zone devoid of cross bridges at the middle of the thick filaments (Fig. 6.12).

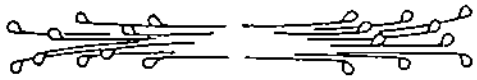


Fig. 6.12 : Polarity of myosin molecules in a thick filament.

The thin filaments which contain actin molecules have a different arrangement. The thin filaments contain two chains of actin molecules wound around each other in a helix (Fig. 6.13).

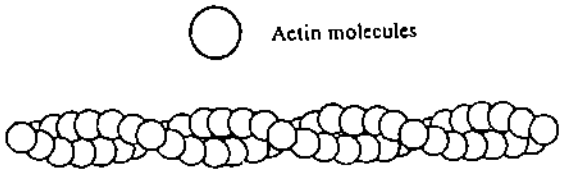



Fig. 6.13 : The thin filament.

Actin molecules of the thin filaments are also oriented in a specific manner. All the molecules on one side of the Z-line have one orientation and all those on the other side have the opposite polarity. Hence, the polarities of both the actin and myosin molecules are reversed on the opposite sides of the middle of a sarcomere. Fig. 6.14 illustrates arrangement of thick and thin filaments in a muscle fibre.

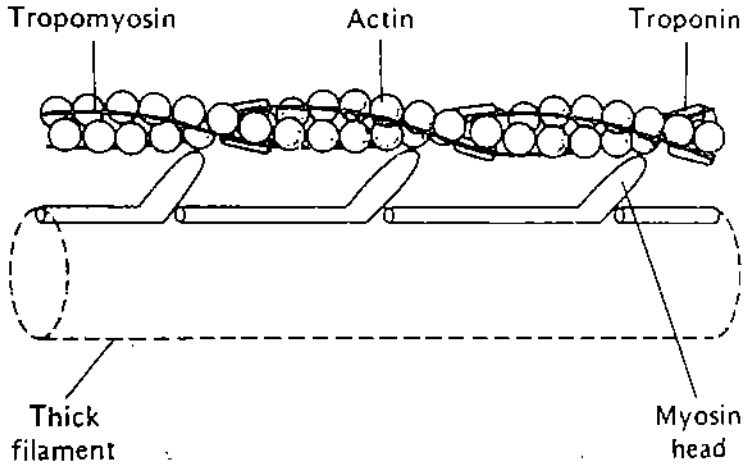



Fig. 6.14 : The thick filament consists of the protein myosin with its heads protruding towards the actin strands.

After learning about the molecular structure of both myosin and actin fibres, we shall now learn about the mechanism of muscle contraction. The immediate source of energy for a muscle contraction is adenosine triphosphate (ATP). The energy required to move myosin and actin filaments past each other comes from the binding and splitting of ATP by the globular heads of myosin molecules. These heads which form the cross bridges, cyclically attach to actin molecules and then swivel, acting as oars that pull the actin and myosin molecules past each other and thereby help in the sliding movement. The globular subunit of myosin has two active sites, one for actin and the other for ATP. In the cross bridging cycle, the globular head binds ATP and splits it to ADP + Pi in the presence of $Mg^{2+}$, but does not release the ADP and Pi. The energy released is stored in the myosin ADP complex. This complex then binds actin, forming actin-myosin-ADP-Pi-complex. In the next step, ADP and Pi are released by myosin and the myosin head changes the conformation, pulling the attached actin towards the middle of the myosin filament. The myosin head then binds to a new ATP, triggering its release from actin. Subsequently, the new ATP is hydrolysed, blocking the myosin head in position to bind another actin molecule (Fig. 6.15).

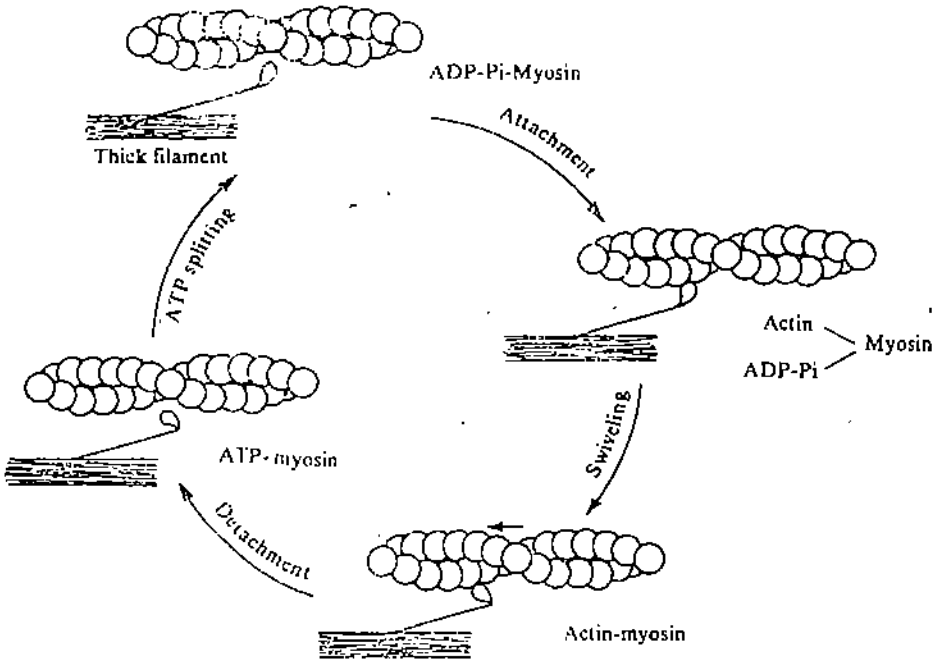



Fig. 6.15 : Proposed molecular events of a single cross bridge cycle. The thick and thin filaments move relative to each other by the swivelling action of the myosin head. The initial state in a relaxed muscle fibre is at the top of the figure.

### 6.4.4 Control of Contraction by Calcium and Regulatory Proteins

You have read in the earlier sections that the thin filaments of myofibril consist of actin and regulatory proteins, **troponin** and **tropomyosin**. Tropomyosin is a long protein coiled along the groove between the two chains of actin filament. Troponin is also found on the actin filaments. It is located at regular intervals along the actin filaments.

At the resting state of the muscle, tropomyosin prevents the interaction of myosin head with the actin filament by blocking the cross bridges binding sites of actin molecules (Fig. 6.16a). The troponin which acts as a controlling protein has a high affinity for calcium ion. It has a binding site for $Ca^{2+}$. When a muscle is stimulated, the calcium ion concentration within the muscle fibre rises abruptly; the calcium ions bind to troponin and bring about conformational changes in both troponin and tropomyosin molecules. This effect induces the movement of tropomyosin to uncover the binding sites and allows cross bridges of the myosin to bind to the actin filament. Thus, calcium ion acts as the physiological regulator of muscle contraction (Fig. 6.16b).

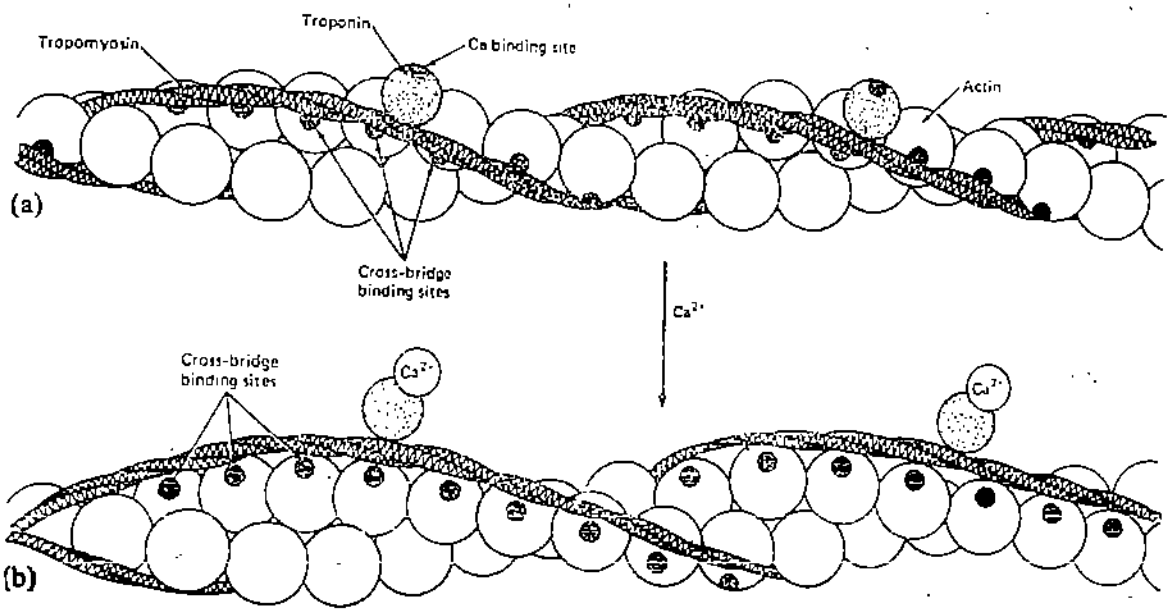



Fig. 6.16 : Actin-linked regulation of contraction in vertebrate skeletal muscle. In the absence of calcium ions, tropomyosin blocks the cross bridge binding sites of actin molecules. Calcium ions bind to troponin inducing the movement of tropomyosin to uncover the binding sites and allowing cross bridges to bind to the thin filaments.

### 6.4.5 Initiation of Muscle Contraction

After studying the mechanisms of muscle contraction you must be interested to know how muscle contraction is initiated.

Muscle contraction is stimulated by nerve impulse. Muscles are associated with nerve endings. The junction of the nerve end and the muscle is called **neuromuscular junction** or **motor end plate**.

You have read in the earlier section that contraction of the muscle is triggered by the presence of $Ca^{2+}$ that bind to troponin. $Ca^{2+}$ are stored in the sarcoplasmic reticulum.

Nerve impulse produces depolarisation of the sarcolemma which propagates rapidly over the entire surface of the fibre and also propagates along T-tubular membranes into the interior fibre. This depolarisation is referred to as **excitation** of the fibre. In resting muscle $Ca^{2+}$ is largely confined to the lateral sacs of the sarcoplasmic reticulum. Depolarisation of tubules induces release of $Ca^{2+}$ from the sarcoplasmic reticulum. $Ca^{2+}$ rapidly diffuses to the adjacent myofilament and bind to troponin. Binding of $Ca^{2+}$ to troponin changes the conformation of both troponin and tropomyosin molecules exposing the myosin binding sites of the actin filament. The globular heads of the myosin molecules then bind to the myosin binding sites on the actin forming cross bridges. The cross bridges move thick and the thin filaments relative to each other resulting in contraction of the fibre. Relaxation of the muscle fibre results from the recovery of $Ca^{2+}$ back into the sarcoplasmic reticulum. This is done by an ATP dependent $Ca^{2+}$ pump. Decrease in the concentration of $Ca^{2+}$ dissociates $Ca^{2+}$ from troponin and then tropomyosin inhibits contraction (Fig. 6.17).

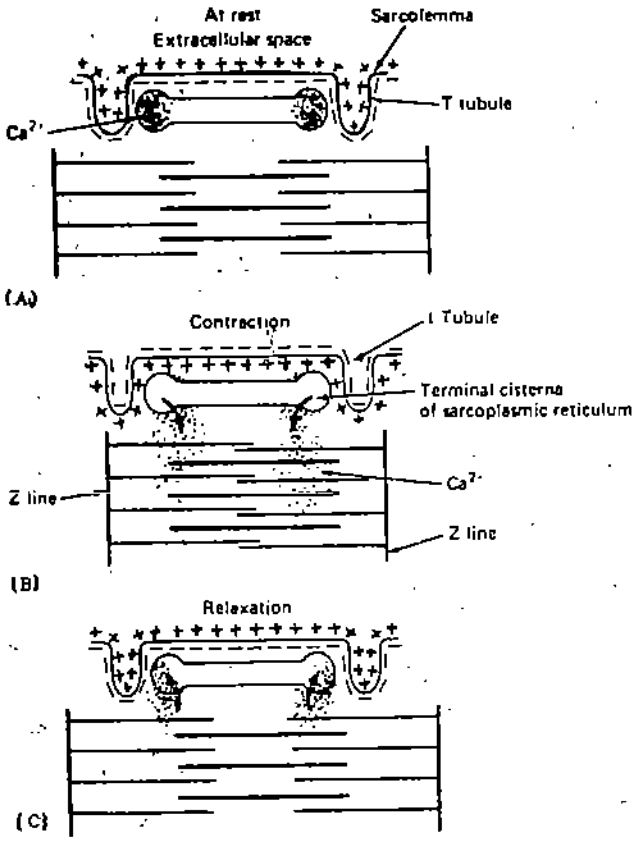



Fig. 6.17 : Diagram showing how calcium release and recovery control the sliding of actin and myosin filaments. (A) T-tubules continuous with the sarcolemma (plama membrane) penetrate the interior of the muscle fibre. (B) When action potentials pass down the T-tubules, calcium ions are released from terminal cisternae of the sarcoplasmic reticulum. Calcium permits cross bridge interaction and sliding of actin and myosin filaments. (C) Relaxation results from the recovery of calcium ions by active transport back into the terminal cisternae.

### 6.4.6 Energetics of Muscle Contraction

You have studied in the above sections that ATP is the immediate source for muscle contraction. It is required for the process of relaxation also. Muscle contains only enough ATP to sustain contraction for a few seconds. You have read in Units 10 and 11 of LSE-01;

Cell Biology that **glycolysis** and **oxidative phosphorylation** are the source of ATP, but these multistep pathways do not increase their rates immediately to supply ATP for muscle contraction. ATP required for the muscle contraction is generated immediately from one of the two energy rich compounds **creatinine phosphates** in vertebrates and **arginine phosphate** in invertebrates. These energy rich compounds are kflown as **phosphogens.** In vertebrate muscle creatinine phosphate rephosphorylates ADP in a reversible reaction.

Creatinin Phosphate + ADP $\rightleftharpoons$ Creatinin + ATP

If ATP becomes depleted, the muscle is unable to keep contracting and this process is known as **muscle fatigue.** Fig. 6.18 illustrates biochemical pathways that produce ATP during vertebrate muscle contraction.

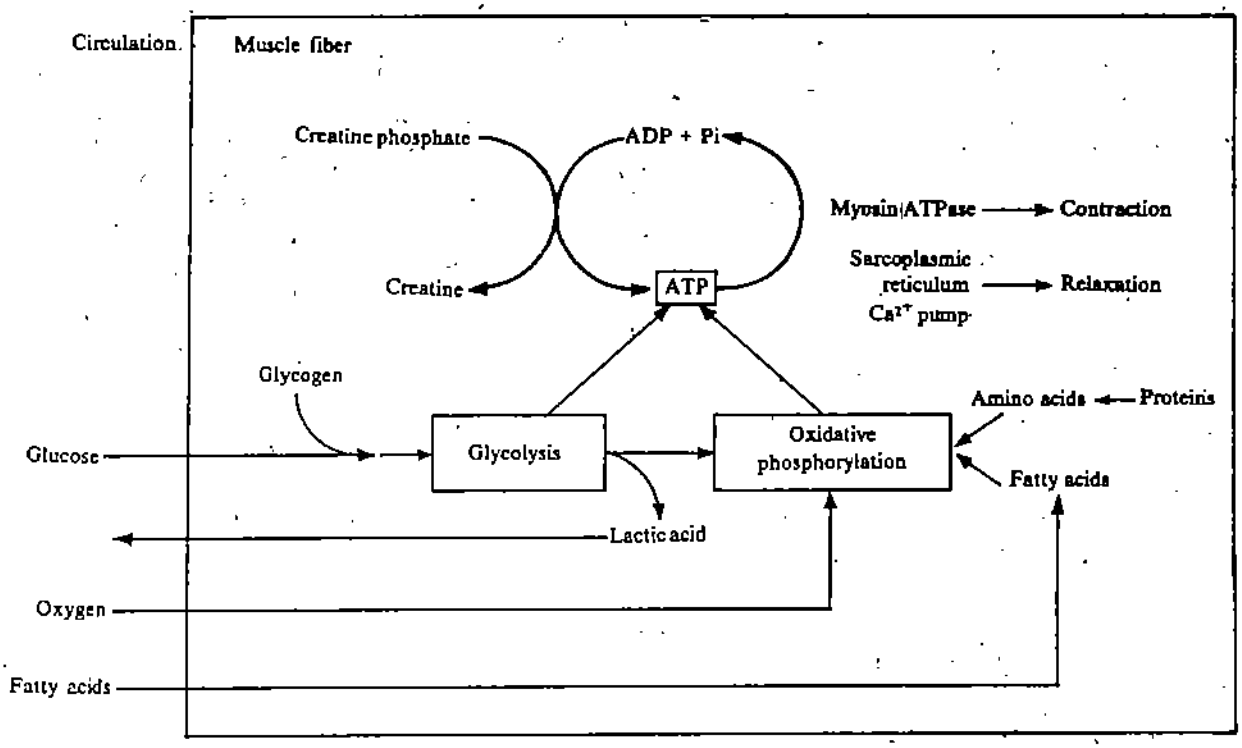



Fig. 6.18 : Biochemical pathways producing ATP utilised during vertebrate muscle contraction.

**SAQ 3**

Fill in the blanks with suitable words and compare your answers with those given at the end of this unit.

a) ..... and ..... are called striated muscles, because under the microscope they exhibit transverse light and dark bands alternate to each other.

b) ..... muscles are under the control of will therefore, are usually called ..... ..... are not under the control of conscious mind, and therefore, called .....

c) H.E. Huxley and Hanson and A.F. Huxley and Niedergerke in 1954 independently proposed ..... model to explain the mechanism of muscle contraction.

d) The junction of the nerve and the muscle is called ..... or .....

e) During muscle contraction ATP is generated immediately from energy rich compounds known as .....

## 6.5 CARDIAC MUSCLES AND SMOOTH MUSCLES

In the earlier section you have read about the structure and function of skeletal muscles. In this section you will read about vertebrate cardiac muscles and smooth muscles.

## Cardiac Muscles

Cardiac muscles exhibit cross-banded appearance under the microscope similar to the skeletal muscles. Therefore, these are also called striated muscles, but the striations are not aligned as found in the skeletal muscles. Therefore, the striated appearance is less distinct in them. These also contain actin and myosin filaments. Cardiac muscle fibres are smaller in size than the skeletal muscle fibres. They are mononucleated but contain abundant mitochondria. Sarcoplasmic reticulum and T- tubules may be well developed or absent (Fig. 6.19)

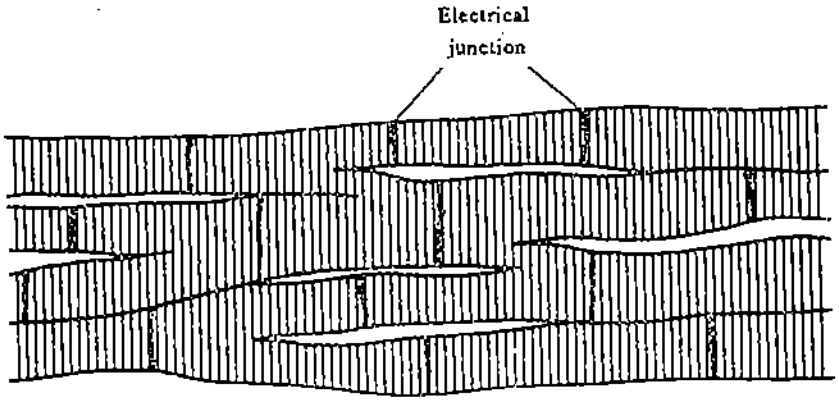



Fig. 6.19 : Cardiac Muscles.

The functional properties of cardiac muscle differ from the skeletal muscle in two important respects. One is that, when a contraction starts in one area of the heart muscle mass, it rapidly spreads throughout the muscle mass. During contraction the cell membrance of the cardiac muscle fibres undergoes electric changes, known as **action potentials**. The second peculiarity of the heart muscle is that, the cell membrane after completion of an action potential, remains in a refractory state for a long enough time to allow the muscle to relax. Because of this refractory period, the cardiac muscle cannot go into a sustained contraction. The refractory period is thus essential for the alternation between contraction and relaxation vis-a-vis normal rhythmic contraction of the heart.

## Smooth Muscles

Smooth muscles do not have transverse striations like those of skeletal and cardiac muscles. You have already studied in Section 6.4 that smooth muscles line the blood vessels, digestive tract, urinary bladder, uterus etc. These are also present in the iris and skin.

Smooth muscles are small and spindle shaped. Each muscle has a single nucleus. The cytoplasm of the smooth muscle cells contains actin and myosin filaments arranged in a random manner. Despite the lack of internal organisation of the filaments, there is cross bridge formation between the actin and myosin filaments. The contraction mechanism in smooth muscles is similar to the sliding filament type of contraction as found in the skeletal muscles (Fig. 6.20).

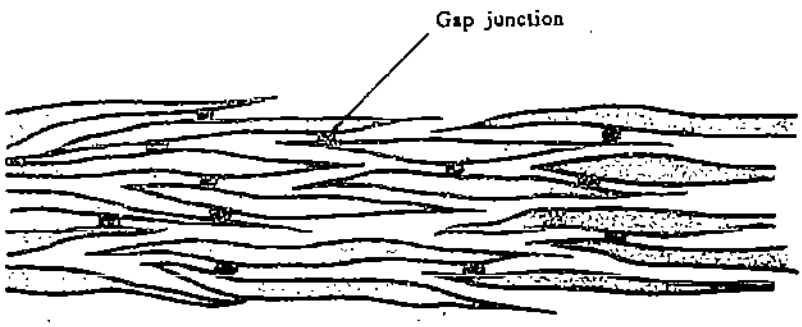



Fig. 6.20 : Smooth Muscles.

In order to contract, smooth muscle need not be stimulated through the nerves. They show spontaneous rhythmic contractions that can vary in both frequency and intensity. This spontaneous activity of smooth muscle, however, can be modified by nerves as well as by hormones such as **epinephrine** and **norepinephrine**. A distinctive feature of vertebrate

smooth muscle is the slowness of response. Another important property of it is that the smooth muscle can maintain contraction for prolonged periods with very little energy expenditure.

## 6.6 SUMMARY

In this unit you have read about the physiology of movement in animals. You have read that :

- movement in amoeba is due to cytoplasmic streaming, change in the cell shape and extension of pseudopodia,
- cilia and flagella have a similar internal structure consisting of microtubules in 9+2 configuration. The filaments of the cilium move past one another to produce movement similar to the sliding filaments of muscle contraction,
- in vertebrates, there are three types of muscles: skeletal muscles, cardiac muscles and smooth muscles. Skeletal muscles and cardiac muscles exhibit transverse light and dark band alternating with each other. Therefore, they are known as striated muscles. Smooth muscles do not exhibit striations,
- contraction of the muscle fibre is brought about by sliding of actin and myosin filaments past each other. This is brought about by formation of cross bridges by the globular heads of the myosin filament with the actin filament which then swivel,
- ATP is the immediate source of energy for muscle contraction,
- calcium, troponin and tropomyosin regulate contraction of the muscle.

## 6.7 TERMINAL QUESTIONS

1) Explain briefly in the space given below the mechanism of movement of cilia.

2) Explain briefly in the space given below the role of myosin in muscle contraction.

3) Explain briefly in the space given below the role of calcium in the regulation of muscle contraction.

4) Illustrate the biochemical pathways that produce ATP during vertebrate muscle contraction.

## 6.8 ANSWERS

### Self-assessment Questions

1) a) Protozoans, slime molds, white blood cells
   b) Pseudopodia
   c) ectoplasm, endoplasm

2) a) kinetosome
   b) 9 + 2
   c) A, outer doublets

3) a) striated muscles, cardiac
   b) skeletal muscles, voluntary, smooth muscle, involuntary
   c) sliding filament model
   d) neuromuscular junction or motor end-plate
   e) phosphogens

### Terminal Questions

1) Electron miscroscopic studies demonstrated that in order to move, the filaments of the cilium do not change shape, but move past one another to produce a curvature, similar to the sliding filaments of muscle contraction. This is done by attatchment of the dynein arms to the neighbouring tubule and walk along it, inducing sliding movement.

2) A myosin molecule contains two heavy chains; part of a heavy chain makes up one head and the other part forms the tail. The heads of the myosin molecules form cross bridges with the actin molecules. These cross bridges cyclically attatch to actin molecules and swivel, acting as oars that pull the actin and myosin filaments past each other affecting sliding movement.

3) The thin filaments of the myofibril consist of actin and two regulatory proteins, troponin and tropomyosin. In a resting state of the muscle, tropomyosin blocks the cross bridges binding sites of the actin molecules. When a muscle is stimulated the calcium ion concentration of the muscle fibre increases. The calcium ions bind to the troponin molecules. Binding of calcium ions to the troponin causes conformational changes in both troponin and tropomyosin, thereby uncovering the cross bridges binding sites of the actin molecules, affecting muscle contraction.

4) Please refer Fig. 6.16.

# UNIT 7 TEMPERATURE RELATIONS IN ANIMALS

## Structure



## 7.1 INTRODUCTION

In the previous unit you have studied about physiology of movement in the animals. In this unit you will learn how the animal's life is related to the temperature of the environment. Basically there are two types of animals, (1) the animals whose body temperature changes with that of the environment, and (2) the animals who maintain their body temperature constant, independent of the environment. In this unit you will learn the effect of temperature on animals, their tolerance to the heat and cold, their behavioural and physiological adaptations to escape the rigors of extreme temperatures and also about regulatory mechanisms that operate in maintaining the body temperature.

### Objectives

After reading this unit you should be able to :

- differentiate poikilothermy and homeothermy
- explain the effect of temperature on animals and explain how animals tolerate the extreme temperatures
- explain the behavioural and physiological adaptations of animals to escape the rigors of extreme temperatures
- elucidate the thermoregulatory mechanisms that influence the behaviour and physiology of the animals.

## 7.2 THERMAL RELATIONS

You are aware that almost all physiological processes are regulated by the action of specific enzymes, and that the rate of an enzyme mediated reaction is related to temperatures.

Therefore, the temperature of an animal's body generally has profound effects on function. The cells, tissues and organs of all animals function within a narrow range of temperatures. However, outside the favourable temperature range, many animals can survive in an inactive or **torpid** state. In fact, some can survive freezing at extremely low temperatures. For instance, in polar regions numerous fish and invertebrates live in water at – 1.8°C. At the other extreme, in hot springs, a few animals can live at about 70°C, and a few thermophilic bacteria thrive above the boiling point of water. Body temperature of most animals, particularly that of all aquatic invertebrates remains the same as that of their surroundings. Thus, the body temperature of these animals changes with the changing ambient temperature. Birds and mammals in contrast, usually maintain their body temperature nearly constant and independent of the environment. All the animals whose body temperature fluctuates with that of the environment have traditionally been called **poikilothermic** (poikilo = changing). To this category belong all the so-called **cold blooded** animals. The birds and mammals which maintain nearly constant body temperatures are classified as **homeothermic** or the so-called **warm blooded** animals. Nevertheless, it should be noted that there is no easy way to classify satisfactorily the various responses to the thermal environment. The above terminology is not very accurate. For instance, the blood of cold blooded animal is not alwlays cold; or a tropical fish or a desert lizard or an insect sitting in the sun may have a higher body temperature than a mammal. Furthermore, a few birds and mammals exhibit **torpor** or **hibernation** during which period their temperature decreases to near the freezing point of water. These examples should serve to explain the inaccuracy of the terminology used. However, the basic difference between the so-called poikilothermic and homeothermic animals is that the homeothermic are able to maintain their body temperature by metabolic heat production whereas, the poikilothermic are unable to do so. Consequently, in recent years, the terms **ectothermic** and **endothermic** are used to denote the animals that depend on external heat sources (mainly solar radiation) and others which are able to maintain a high body temperature by endogenous heat production (endothermic) respectively. These definitions also have their limitations since several invertebrates and vertebrates can at times maintain a substantial difference between their own temperature and that of the surroundings. In the following section you will learn such varied relations and their physiological and ecological implications.

**SAQ 1**

Match the following terms given in column A with their definitions in column B.

| A | | B | |
|---|---|---|---|
| 1) Poikilotherms | [ ] | a) | Animals that depend on external heat sources |
| 2) Homeotherms | [ ] | b) | Animals which are able to maintain a high body temperature by endogenous heat production |
| 3) Ectotherms | [ ] | c) | Animals which maintain their body temperature nearly constant independent of the environment |
| 4) Endotherms | [ ] | d) | Animals whose body temperature fluctuates with that of the environment. |

## 7.3 EFFECTS OF TEMPERATURE

The effects of temperature on individual organisms have profound physiological and ecological significance. The metabolic rate in both homeotherms and poikilotherms is often influenced by the **ambient** temperature. Within limits, a temperature increase accelerates most processes. In general, a rise of 10°C in temperature is known to increase the rate of a reaction to about two to three-fold. The increase in a rate caused by a 10°C increase in temperature is called the $Q_{10}$. If the rate doubles, $Q_{10}$ is 2; if the rate triples, $Q_{10}$ is 3; and so on. Thus, the energetic demands placed on the environment by an organism increase or decrease with thermal circumstances. Temperature is also significant in that the animal must be able to survive various thermal ch... ges imposed on it throughout the year. The

distribution and habitat of species may thus be influenced by thermal effects. When temperature exceeds viable limits at certain times or places it causes a physiological or ecological stress condition. Mammals and birds exposed to heat in deserts, for example, may face critical problems of dehydration if required to expend large amounts of water on **evaporative cooling** to keep their body temperatures from rising beyond the tolerance limits. Fish in summer may have high metabolic rates because their body temperatures are elevated in the warm waters. However, at the same time, they may be forced with relatively low oxygen availability because warm water tends to hold less dissolved oxygen than cold water. The interaction of these factors may prove critical.

Animals differ in the range of temperatures they can tolerate. Some have a very narrow tolerance range, while others exhibit wider range. Furthermore, temperature tolerance may change with time, and a certain degree of adaptation is possible so that continued exposure to a temperature close to the limit of tolerance often extends the limit. Some organisms are more sensitive to extreme temperatures during certain periods of their lives, particularly during the early stages of development.

### 7.3.1 Tolerance to High Temperature

No animal is known to live and carry out its complete life cycle at a temperature over 50°C. However, an animal in a resting stage may be extremely tolerant to high temperatures. For example, a fly larva (*Polypedilum*) from Nigeria and Uganda is known to tolerate dehydration, and in the dehydrated state it can survive a temperature of 102°C for one minute and afterward grow and metamorphose successfully. Likewise, the eggs of a fresh water crustacean (*Triops* from Sudan) survive through winter and early summer in dry mud, where they may be exposed to temperature of up to 80°C. In the laboratory they are known to withstand even higher temperature, close to boiling temperature of water. Apparently, upper temperature limit for life cannot be accurately defined.

When a group of animals of a given species are exposed to a temperature close to the limit of their tolerance, some may die and others survive. The **lethal temperature** is commonly defined as that temperature at which 50% of animals die and 50% survive. It is written as $T_{50}$. Obviously, the lethal temperature value changes with the species, the stage of the life cycle, the previous adaptation of the animal to a given environment and so on. The factors responsible for death due to heat are believed to be the following:

i) Degeneration of proteins which occurs above 45 – 50°C known as **thermal coagulation**

ii) Thermal inactivation of enzymes at the rates that exceed rates of formation

iii) Inadequate oxygen supply

iv) Different temperature effects ($Q_{10}$) on interdependent metabolic reactions

v) Temperature effects on membrane structure

Now let us learn more about these factors.

Denaturisation of proteins due to temperatures above 45 – 50°C is quite common. However, in some animals such as the Antarctic fish of the genus *Trematomus* are very sensitive to heat so that above 6°C the proteins (inclusive of enzymes) denature. It is difficult to explain how such a low temperature (+6°C) can cause denaturation of proteins or inactivation of enzymes in the fish. The third possibility that thermal death occurs due to inadequate oxygen supply is also difficult to explain in certain situations. For example, supplying of insects with pure oxygen instead of air does not enable them to survive at higher temperatures. Likewise, the trout, a cold-water fish, dies in warm water even if the oxygen content of the water is increased several fold by aeration with pure oxygen. The fourth possibility envisages that different temperature sensitivities of the several hundred metabolic enzymes that participate in the intermediary metabolism may lead to a derangement of normal biochemical balance of the organism and eventual death. However, in most cases, heat death is not necessarily always due to enzyme inactivation. In fact, other possibilities mentioned above may be contributory. The last possibility, changes in membrane structure and function (fluidity and permeability), is very important and covers a broad range of subjects. Temperature has profound effects on higher orders of protein structure, protein-lipid interactions, lipid-lipid interactions, and so on. Such disturbance in the integrity of membrane function appears to be the primary factor in heat damage to organisms.

### 7.3.2 Tolerance to Cold and Freezing Temperatures

In the earlier section you have been studying about tolerance of animals to high temperature. Now in this subsection we will study about tolerance to cold and freezing temperatures.

The effects of low temperature are equally perplexing as those of high temperature. Some organisms can tolerate extensive freezing but most animals cannot. Animals that live in temperature and cold regions are often exposed to long periods of winter temperatures that are far below the freezing point of water. Survival of ectothermic animals at such subzero temperature depends upon the physiological and biochemical characteristics that can be described as **cold hardiness**. An animal can develop cold hardiness either by developing capacity for **freeze tolerance** or by avoiding ice formation even if exposed to temperatures as low as –40°C to –50°C. The latter are regarded as **freeze intolerant**. The intertidal marine invertebrates of colder zones are freeze tolerant in the sense that they survive extensive ice formation within their bodies. Many other animals also survive in spite of extensive ice formation. For example, midge *Chironomous* larva from Alaska can be frozen and thawed repeatedly without injury. Several species of insects are known to contain high concentration of **glycerol** in their body fluids. It is well-known that glycerol protects red blood cells and mammalian spermatozoa from injury caused by freezing. Therefore, glycerol is widely used for this purpose and samples of human or bull sperm can be kept frozen and viable for several years using glycerol. Without such treatment, freezing is lethal to sperms. Only a few vertebrates tolerate extensive ice formation. Birds and mammals, however, are not known to tolerate freezing.

**Super cooling** is a phenomenon where body water is allowed to cool far below 0°C without formation of ice. Glycerol is effective in lowering both the freezing point and also the super cooling point . In addition, glycerol improves the tolerance to freezing in animals that tolerate ice formation. In some animals antifreeze compounds are found. For example in the Antarctic fish *Trematomous borchgrevinki* the blood contains a glycoprotein that acts as an antifreeze substance.

### 7.3.3 Acclimation and Acclimatisation

The tolerance limit of a given species is not fixed. Exposure to a near lethal temperature often leads to a certain degree of adaptation so that a previously lethal temperature is tolerated. Frequently, the range of thermal tolerance is different for the same species in summer and in winter. A winter animal exhibits tolerance for temperature so low that it is lethal to a summer animal; conversely, the winter animal is less tolerant to high temperature than a summer animal. Such changes in the temperature tolerance with climatic changes are called **acclimatisation**. Similar effects can be simulated in laboratory experiments by keeping animals for some time at given temperatures. To distinguish the adaptation or adjustment that takes place in laboratory experiments from natural acclimatisation, the response to experimental conditions is often described by the term **acclimation**. Indeed, animals may show long-term physiological adjustments in response to diverse environmental agents, including (in addition to temperature) humidity, salinity, oxygen supply, photoperiod and food supply, to name a few. Furthermore, acclimation or acclimatisation can potentially be exhibited in virtually any physiological property and sometimes in behavioural and morphological properties as well.

SAQ 2

Fill in the blank spaces and compare your answers with that given in Section 8.8.

a) A rise in 10°C in temperature is known to increase the rate of a reaction to about two to three-fold. The increase in a rate caused by 10°C increase in temperature is called the .....

b) Some organisms are more sensitive to extreme temperature specially during .....

c) No animal is known to live and carry out its complete life cycle at a temperature over .....

d) Freeze tolerant species of insects are known to contain high concentration of ..... in their body fluids, which protects their tissues from injury caused by freezing.

e) Changes in the temperature tolerance with climatic changes are called : .....

## 7.4 TEMPERATURE REGULATION IN POIKILOTHERMS

After studying the effects of temperature on animals, you will now learn about regulation of temperature in the poikilotherms.

The term poikilotherm refers to the lack of regulated constancy of the body temperature and it describes the actual physiological status of these organisms. As stated earlier, in recent years these animals have increasingly been termed **ectotherms**, with reference to the fact that their body temperatures are determined primarily by external thermal conditions. The term ectotherm emphasises the mechanism by which body temperature is determined, whereas poikilotherm emphasises variation of the body temperature with environmental conditions.

Although poikilotherms lack physiological mechanisms of controlling their body temperature, they are not necessarily devoid of means of control. They often exert exquisite control behaviourally, by selecting their thermal environment. Animals at all levels of phylogeny have developed mechanisms to meet the normal temperature variations of their surroundings and to withstand the extremes for periods of varying length. Basically, three types of compensatory mechanisms have been recognised:

i) Acclimation or acclimatisation

ii) Body temperature adjustment or regulation

iii) Genetic or evolutionary adaptation

The basic biochemical and cellular events occurring during acclimation and acclimatisation appear to be similar. These processes involve appropriate changes in the activity of various enzymes involved in the metabolism, changes in the lipid composition of the membranes, production of antifreeze substances, super cooling and freeze tolerance. Now we will study how poikilotherms regulate their body temperature.

### 7.4.1 Hibernation and Aestivation

Poikilotherms pass the winter in a resting state, known as **hibernation**. When they enter a resting state in response to heat or drought, the condition is called **aestivation**. In general poikilotherms derive two types of benefit from the resting states that may enter during times of environmental stress. First, by virtue of their special physiological state, the animals may enjoy an enhanced physiological ability to cope with the extreme conditions. (Example, increased freezing resistance etc.) Second, they are often permitted to remain continuously in favourable microhabitats. Their metabolism is depressed to such an extent that the store of nutrients (example, body fat) can augment. The variety of mechanisms exploited by poikilotherms in escaping some of the rigors of extreme temperatures may be divided into behavioural and physiological adaptations.

### 7.4.2 Behavioural Adjustments

When faced with a sudden temperature change, most animals make behavioural responses that enable them to avoid extreme or lethal conditions. Among the invertebrates and aquatic vertebrates this is the only kind of thermal adjustment. The terrestrial environment is more prone than aquatic to show sharp temperature changes. Insects and reptiles which are the most successful among the terrestrial poikilotherms exhibit many complex thermal

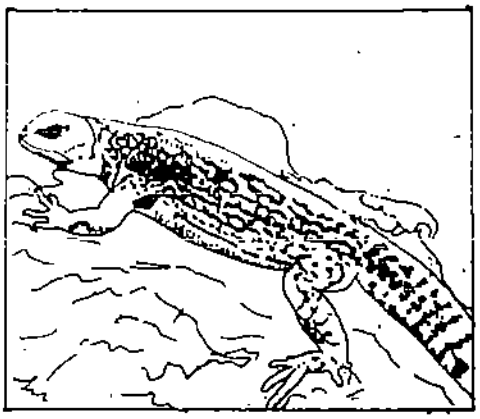


**Fig. 7.1 : A Lizard basking on a rock.**

responses. Capacities for rapid and appropriate responses to temperature changes are based on well developed sensory system. For example, the infrared sense organs located in the facial pit of the rattle snake can detect even very small temperature differences (0.001 to 0.005°C) which helps them in detecting warm blooded or cool (moist) prey as well as in orienting the animals to warm or cool environments.

Many insects and reptiles bask in the sun to warm their bodies and avoid extreme heat by seeking the shade or burrowing (Fig. 7.1).

### 7.4.3 Physiological Adjustments

Three examples of physiological thermoregulation in poikilotherms can be noted. These include:

i) Prompt cardiovascular response to a sharp change in environmental temperature

ii) Cutaneous vasodilation in the heat

iii) Vasoconstriction in cold as frequently noted in lizards and crocodiles.

A measure of temperature control is attained by minimising the flow of blood to the surface during the cold and increasing the flow during the heat. Thus the situation in reptiles, known as **dermovascular responses**, marks an evolutionary step between the vascular respiratory skin of amphibians and the dry but moisture permeable and sweating skin of mammals. Many animals cool their bodies through evaporation of moisture.

Poikilotherms that live in temperate zone experience drastic seasonal changes in the temperature. In these, the adaptations occur slowly and prepare the animals for winter cold or summer heat. Such preparations (example, formation of antifreezes etc.) involve the **neuroendocrine system** and **photoreceptors**. The latter, in fact, serves as a reliable clue to the adjustment of physiology in anticipation of seasonal changes.

Thirdly, some fishes and insects avoid thermal extremes by maintaining a relatively constant body temperature. This capacity of endothermy represents a third level of compensation, involving both morphological and physiological adaptations. In short, it represents the genetic and phylogenetic level of physiological thermoregulation. Several fishes and sharks are known to possess **heat exchangers** especially in the heavy trunk musculature with extensive network of blood vessels. Such heat exchangers are also found in association with the brain, retina and viscera.

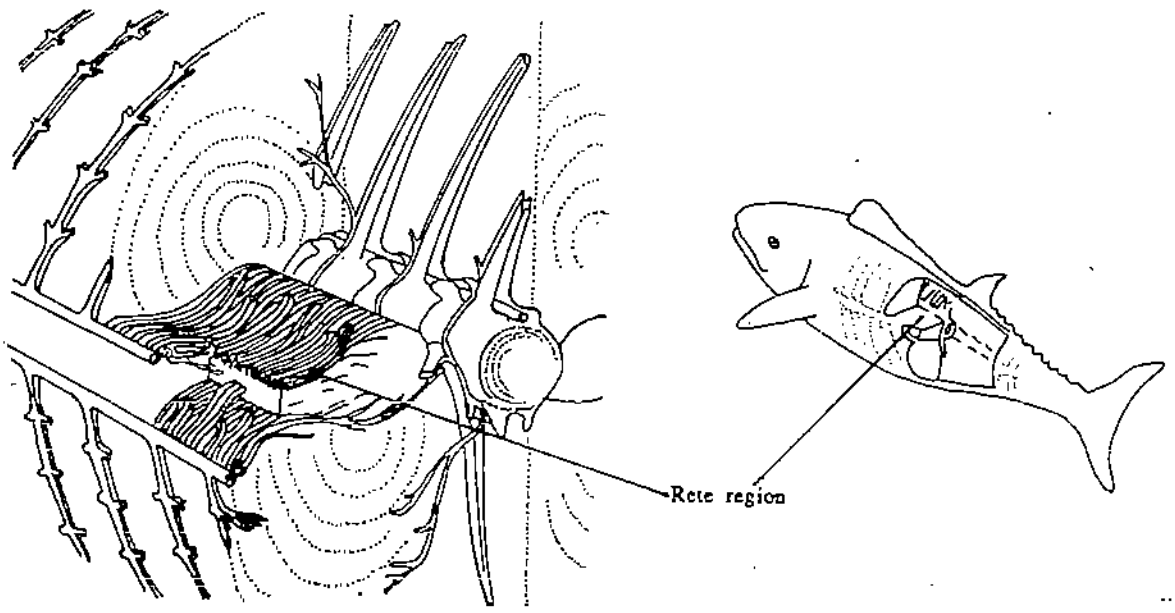



Fig. 7.2 : Rete mirabila in bluefin tuna fish.

Although heat exchangers vary in structure, the physiological principle involved is the same in all of them. They are, in fact, elaborate **retia mirabilia** composed of numerous, relatively long arterioles and venules. These small vessels are intermingled and lie in close contact parallel to one another with arterial and venous blood flowing in opposite directions (counter current). Cold arterial blood from the gills flows through the rete, where it is warmed by the venous blood heated in the actively metabolising muscles or visceral organs. Heat exchange is facilitated by the large number and arrangement of the retial vessels and by the slow passage of blood through them (Fig. 7.2).

Some insects are also able to maintain a warm body while foraging in cool environment. The bumble bee forages for nectar at temperatures as low as 5°C with a thoracic temperature at about 30°C which is essential for flight muscle activity. Bumble bees warm before flying by shivering. The queen bees generate heat and thus raise the temperatures in their hives. Several such examples can be found among the insects.

**SAQ 3**

Fill in the blank spaces and compare your answers with those given in Section 7.8.

a) The infrared sense organs of the rattle snake located in the facial pit can detect temperature differences as small as ..... to ..... C.

b) The ..... response in reptiles mark an evolutionary step between the vascular respiratory skin of amphibians and the dry but moisture permeable and sweating skin of mammals.

## 7.5 TEMPERATURE REGULATION IN HOMEOTHERMS

Homeothermy is regulation of body temperature by physiological means. The stabilisation of body temperature permits a steady high level of activity, both metabolic and locomotory. The advantages are obvious in behavioural, social and cultural evolution, demanding continuous association of individuals. Maintenance of a constant body temperature involves a perfect balance between heat production and heat loss. It demands a sensitive thermostat in the brain, a capacity not only to use heat formed as a byproduct of metabolism but also to increase the output of metabolic energy in accordance with demands. In addition, it requires several anatomical modifications such as appropriate insulation and special heat exchangers. In extreme conditions, the metabolic price of a regulated body temperature may become too high so that some species temporarily suspend temperature control (torpidity and hibernation) or migrate to more favourable climates. In man, there is behavioural evasion of extremes with the development of clothing, air conditioning and other technological devices.

### 7.5.1 Heat Production

In the homeotherms heat production must be elevated if the ambient temperature falls below the critical temperature. Although all metabolic processes result indirectly in the production of heat, birds and mammals have evolved processes that have the specific function of generating heat for thermoregulation. These thermogenic processes are basically meant to convert chemical energy to heat. They are :

i) **Shivering:** The mechanism of thermogenesis with which we are more familiar is shivering and all adult birds and mammals seem to use these mechanisms. Shivering is a high frequency, relatively uncoordinated contraction of skeletal muscles. All muscular contraction liberates heat, and obviously the conversion of chemical energy into thermal energy becomes the primary function of the contraction.

ii) **Nonshivering Thermogenesis:** The nonshivering thermogenesis is a widespread phenomenon in mammals. It refers to the processes that produce heat by means other than shivering. It is not certain whether birds produce heat by nonshivering. One of the well-known sites of nonshivering thermogenesis in mammals is the **brown adipose tissue,** also called **brown fat.** The brown adipose tissue contains great numbers of mitochondria and is richly vascularised. Release of norepinephrine into the tissue by the sympathetic nervous system results in a great increase in oxidation of lipid and release of heat. Brown fat, like nonshivering thermogenesis, is specially prominent in new born individuals, hibernators and cold acclimated adult mammals. The brown fat tends to occur in discrete masses, located in the neck, interscapular region, axillae and abdomen. Among hibernators

brown fat is believed to help in rewarming the body during emergence from hibernation. New born mammals use brown fat in routine thermogenesis (Fig 7.3). The principal mechanism of heat production by brown fat is by uncoupling the oxidative phosphorylation that occurs in the mitochondria. Thus, oxidation of food-stuffs results in the production of heat.

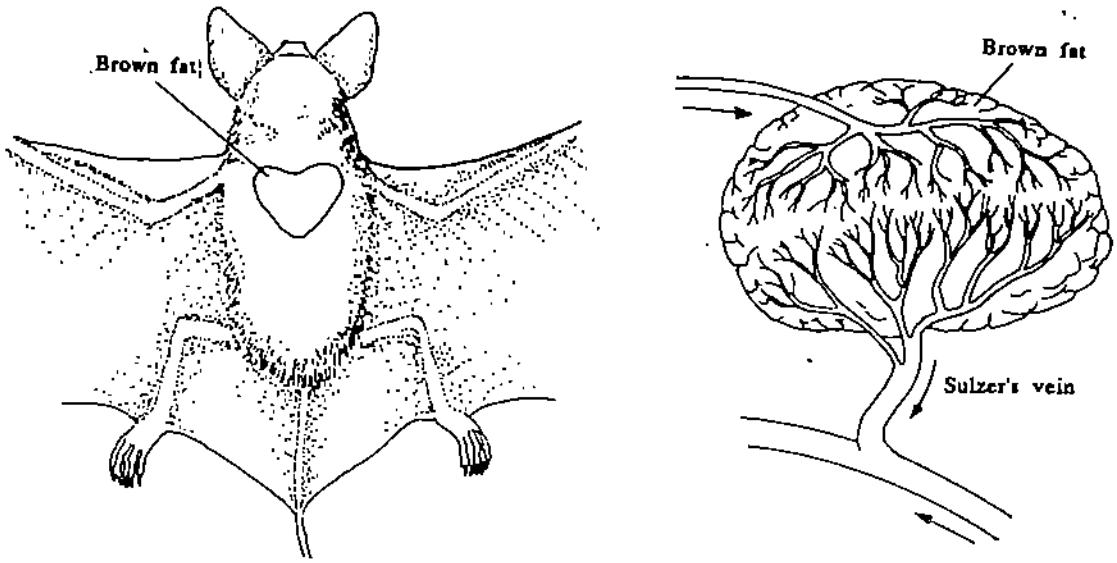



Fig. 7.3 : Brown fat deposition in the bat.

**iii) Exercise:** During physical activity heat production by exercise can to some extent substitute for heat generated by shivering. However, exercise also tends to facilitate heat loss by degrading body insulation. Therefore, relative significance of exercise in thermoregulation of birds and mammals is not very clear. After studying the ways in which homeotherms gain heat, we will now study how they lose the excess heat.

### 7.5.2 Heat Loss

Temperature regulation is extremely uneconomical if it depends only on variations in metabolism. Therefore, mechanisms for losing excess heat have been developed by both birds and mammals. In an aquatic environment, the entire heat transfer between the animal and the media is through **conduction.** However, in terrestrial habitat, only small amounts of heat are exchanged this way. Most of the homeotherms are terrestrial. In man, heat loss due to radiation accounts for about 55% and heat loss due to evaporation is about 40%. The amount of heat loss depends on the ambient temperature and the humidity. Loss of heat by radiation and conduction is usually effective in a cool environment. Whereas, at high temperature, the animals will actually receive heat by these routes. Evaporation however, is always a negative factor and it requires considerable energy expenditure. For instance, to vaporise one gram of water from the moist surfaces of the skin or respiratory epithelia, 0.6 kcal is required. This technique of cooling has been exploited in quite different ways by birds and mammals.

Birds have a dry and insulated skin with no integumentary organs to increase cooling by vaporisation. However, evaporative cooling occurs in birds through buccal and respiratory surfaces. The four major mechanisms of actively enhancing evaporative cooling that are known to be employed by birds and mammals are :

i) **sweating**

ii) **panting**

iii) **gular fluttering**

iv) **saliva spreading**

During **sweating** fluid is secreted by way of the sweat gland ducts through the epidermis onto the skin surface. Vigorous sweating occurs in many mammals including humans during hot environmental conditions. Sweating does not occur in birds. **Panting** is an increase in the breathing in response to heat stress and occurs widely in birds as well as mammals. Panting requires less muscular effort and thus is of advantage in losing heat. By comparison to sweating, panting holds at least two advantages. First, no loss of salts occurs. Second, the breathing activities of panting assure that the air saturated with water vapour is driven forcibly away from the evaporative surfaces. But during sweating the removal of water

laden air is dependent on several other forces such as external winds. However, panting requires more energy than sweating. Many birds augment evaporative cooling by rapidly vibrating their gular area (the floor of their mouth) while holding their mouth open. These **gular flutterings** promote evaporation by increasing the flow of air over the bird's moist and highly vascular oral membranes. The fourth technique, **saliva spreading** is employed by many rodents and marsupials when exposed to heat stress. They spread saliva on their limbs, tails, chest or other body parts for further evaporative cooling. After studying about the ways by which the homeotherms gain heat or lose heat to keep their body temperature constant, we will study about the anatomical features that help the homeotherms in maintaining their body temperature constant.

### 7.5.3 Insulation by Fur and Feathers

There is a strong correlation between the extent of integumentary insulation and the rigors of natural environment. In general, Arctic or Antarctic species are better insulated than tropical species, with well marked seasonal variations in the thickness of fur (example, Polar bear) or feathers (example, penguins). Likewise, the aquatic mammals living in these areas such as seals and whales have thick layers of subcutaneous **blubber** (fatty layer) as a means of major insulation. However, it should be noted that the extent of fat deposition (blubber) is not the same in all parts of the body.

### 7.5.4 Heat Exchangers

Seals and whales have **flippers** and **flukes** that lack blubber and are poorly insulated. These appendages are well supplied with blood vessels and receive rich blood supply. Therefore, these thin structures with their large surface areas can lose substantial amounts of heat and aid in heat dissipation. However, excessive loss of heat is prevented due to the counter-current heat exchangers in which the blood flows in opposite direction. In the whale flipper, each artery is completely surrounded by veins, and as warm arterial blood flows into the flipper it is cooled by the cold venous blood that surrounds it on all sides. The arterial blood therefore, reaches the periphery precooled and hence loses little heat to the water. The heat has been transferred to the venous blood, which thus, is prewarmed before it reenters the body (Fig. 7.4).

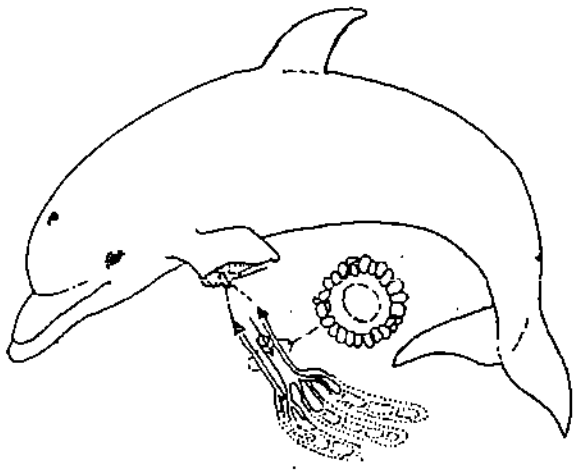


Fig. 7.4 : Countercurrent system for conserving heat in the flippers.

It is interesting to note that such countercurrent heat exchangers are found in a number of other animals. For example, sea cows, which live in tropical and subtropical water, have heat exchangers in their appendages. Even in human limbs some heat exchange takes place between the main arteries and the adjacent larger veins located deep within the tissue. In birds, heat exchange in the legs is very important especially in those that stand or swim in cold water. After studying about the anatomical features that help in maintaining the body temperature, we will now study the regulatory mechanisms that operate to maintain the body temperature.

### 7.5.5 Regulatory Mechanisms

The immediate response to acute temperature change is mediated through the central nervous system. The physiological thermostat has been located in the hypothalamus of the

mammal and the spinal cord of the bird. These are the centres of reflex action and are activated by the temperature receptors of the skin or mucous membranes or directly through the changes in the temperature of the hypothalamus (or the blood circulating through it). The physiological thermostat through the efferent nerve fibres stimulate the muscles for panting and shivering and through the autonomic system it regulates the cutaneous blood vessels, the sweat glands and so on. In some of the amniotes, the pineal and parapineal organs influence thermoregulatory behaviour and physiology. Cardiovascular and cutaneous responses are acute reactions to temperature change. If the exposure is more prolonged, the endocrine system enters the picture and the metabolism is altered particularly by way of the thyroid and adrenal glands.

## Hibernation, Aestivation and Daily Torpor

Although birds and mammals frequently function in the homeothermic mode and maintain relatively high stable temperatures, many mammals and some birds have the ability to relax their homeothermic responses and allow their body temperatures to fall, close to the level of low ambient temperatures. This phenomenon is called **controlled hypothermia.**

Hibernation, aestivation and daily torpor are states in which the animal relaxes its homeothermic processes almost completely within a certain range of ambient temperatures and, like a poikilotherm, allows its body temperature to equal ambient temperature. When body temperature is fixed to approximate ambient temperature for periods of several days or longer during winter, the phenomenon is termed **hibernation.** When this occurs during summer, it is called **aestivation.** If body temperature is freed to approximate ambient temperature for only part of each day, generally on many consecutive days, the phenomenon is called **daily torpor**, regardless of season. These three forms of controlled hypothermia are thus different manifestations of a single fundamental physiological process. During episodes of hypothermia, the heart rate and breathing rate decline along with metabolism. The animal may retain some ability to move and respond behaviourally to its environment at body temperatures well below normal, but there is increasing lethargy as body temperature falls, and at low temperatures the lethargy becomes extreme.

The chief benefit of these hypothermic states is a reduction in the animals' energy demand. This is evidently due to the fact that the animal no longer elevates its rate of metabolism to keep itself warm. Secondly, resultant body temperature itself lowers metabolism ("$Q_{10}$ effect" about which you have studied in Section 7.3). Entry into hypothermic state also reduces an animal's water expenditures. In fact, for individuals suffering water shortage, the savings of water may be of greater significance than those of energy. Respiratory water losses are reduced during hypothermia for two reasons :

i) oxygen requirement as well as the rate of ventilation of lungs is reduced, and

ii) body temperature is lowered, and the exhaled air is cooler than during homeothermy and thus carries less water vapour with it.

Transcutaneous (through skin) water losses are also reduced because the drop in body temperature lowers the vapour pressures of the body fluids. Birds and mammals that are capable of hibernation, aestivation, or daily torpor are often termed **heterotherms**. A heterotherm is an animal that sometimes regulates its body temperature physiologically and some times does not. The heterotherm can enjoy, in a sense, the best of both the homeothermic and poikilothermic worlds. While thermoregulating at high body temperatures, the animal is able to move about with the independence of external thermal conditions and enjoy a prime advantage of homeothermy. When in hypothermia, on the other hand, it enjoys the comparatively low requirements for energy and water characteristic of poikilothermy. Insects that thermoregulate physiologically during flight are also heterotherms and enjoy similar benefits.

During hibernation, aestivation and daily torpor, the body temperature is brought about by a change in the operation of the animal's thermoregulatory control centres. The most striking is the ability of these animals to arouse from the hypothermic conditions. The hypothermic individuals are able to warm themselves back to a high body temperature using their own metabolic heat production. Arousal is accomplished by intense shivering and, in the case of mammals by nonshivering thermogenesis.

Hibernation is known in many mammals, including hamsters, many ground squirrels, dormice, woodchucks, some bats, some monotremes and some marsupials. Such mammals store large quantities of body fat during the months preceding entry to hibernation for use during the winter sleep. Hibernators arouse periodically and at such times they may excrete

urine and faeces and consume food they have stored in their burrow or den. Aestivation has received much less attention than hibernation partly because it is not easy to detect. It has been reported mostly in species of desert ground squirrels.

Daily torpor is found in great many mammals and birds in both warm and cold situations. It occurs in numerous species of bats and rodents and in certain humming birds, swallows, swifts and so on. A characteristic of daily torpor is that the animal is hypothermic for part of each day but maintains an elevated body temperature during the rest of the day. Feeding and other activities are carried out during the later periods. When bats are undergoing daily torpor, they become hypothermic during day light hours and emerge to forage at night; humming birds become torpid at night and feed in day light. The mechanisms that regulate hibernation or daily torpor are complex and vary between the different species. It is believed that the hibernation may be under the control of **biological clock** in certain animals. Daily torpor may be employed as a response to immediate hardship such as storage of food. Effects of day length on hibernation or daily torpor are possibly mediated in at least some species by the **pineal gland** and its hormone **melatonin.**

In ending this unit it is perhaps necessary to offer a counter argument to the common belief that homeothermy is superior to poikilothermy. After all, nature being indifferent, the only criterion of success is perpetuation of the species, involving several subsidiary means for survival and reproduction at the individual level. When such a criteria is applied, poikilothermy can be as successful a mode of life as homeothermy. In fact, many poikilothermic taxa have persisted for hundreds of millions of years. Therefore, poikilothermy and homeothermy may be simply regarded as two extremes in a continuum of thermal relations that have been exploited in the evolution of animals in different habitats. Consequently, the so called advantages and disadvantages of various thermal relations, that we often visualise are in reality only highly relative judgements.

## 7.6 SUMMARY

You have studied in this unit that :

- Temperature of an animal's body has profound effects on the function of the animal.
- Poikilotherms are those animals whose body temperature changes with the changing ambient temperature and homeotherms are the animals who usually maintain their body temperature nearly constant and independent of the environment.
- Animals differ in the range of temperature they can tolerate. Some have a very narrow tolerance range, while others exhibit wider range.
- The factors responsible for death due to heat are : degeneration of proteins, thermal inactivation of enzymes, inadequate oxygen supply, different temperature effects on interdependent metabolic reactions and due to temperature effects on membrane structure.
- Poikilotherms, which lack physiological mechanisms of controlling their body temperature, often exert control behaviourally by hibernation and aestivation. Some of them also exhibit physiological thermoregulation.
- Homeotherms regulate body temperature by physiological means. They produce heat by shivering, by oxidative phosphorylation in the brown fat, and by doing exercise. They lose heat by radiation, evaporation, conduction, sweating, panting, gular fluttering and saliva spreading.
- Homeotherms have fur, feathers, flippers, flukes which help in maintaining the body temperature. They also exhibit hibernation, aestivation and daily torpor.
- The physiological thermostat located at the hypothalamus, pineal, parapinal organ, thyroid and adrenal glands influence thermoregulatory behaviour and physiology of the homeotherms.

## 7.7 TERMINAL QUESTIONS

1) Give two examples of the animals which survive outside the favourable temperature range.

.....

2) What are the factors responsible for death due to heat ?

3) Explain at least two ways by which homeotherms produce heat and lose heat in order to regulate their body temperature.

4) Explain briefly in the space given below the four major mechanisms of actively enhancing evaporative cooling employed by birds and mammals.

5) What is meant by the phenomenon of controlled hypothermia ?

## 7.8 ANSWERS

**Self-assessment Questions**

1) 1 —— d, 2 —— c, 3 —— a, 4 —— b.

2) (a) $Q_{10}$ (b) early stages of development (c) 50°C (d) glycerol (e) acclimatisation

3) (a) 0.001 to 0.005°C (b) dermovascular

4) (a) Shivering (b) neck, interscapular region, new born, hibernators
(c) 50%, 40% (d) 0.6 (e) heat exchangers (f) hypothalamus, spinal cord
(g) daily torpor (h) biological clock

**Terminal Questions**

1) Please refer Subsection 7.3.1

2) Please refer Subsection 7.3.1

3) Please refer Section 7.5

4) Please refer Subsection 7.5.2

# UNIT 8 REPRODUCTION

Structure



## 8.1 INTRODUCTION

The various physiological processes that you studied in the earlier units of this course are concerned with the survival of animals. In this Unit you shall study about physiology of reproduction in animals which is concerned with the survival of the species rather than the individual.

Animals may develop, grow and live a normal life span without reproducing. However, the continuance of the species and all evolutionary changes would come to an end without reproduction. Reproduction is a process by which organisms constantly replace the old with similar but somewhat variable offspring. Successful reproduction in fact is the ultimate objective of all life processes. In this Unit you shall study various reproductive mechanisms, functional morphology of reproductive organs, the process of gametogenesis, hormones of reproduction, breeding cycles and also about regulation of reproduction.

### Objectives

After reading this unit you should be able to :

- explain the need for reproduction
- outline the reproductive mechanisms in animals
- explain the functional morphiology of reproductive organs
- outline the breeding cycles in animals, and
- explain the mechanisms that regulate reproduction.

## 8.2 REPRODUCTIVE MECHANISMS

Organisms reproduce in two different ways, (i) asexually and (ii) sexually. In asexual reproduction there is only one parent and there are no special reproductive organs or cells. Each organism is capable of producing genetically identical copies of itself as soon as it becomes an adult. Sexual reproduction involves two parents, each of which contributes special sex cells, or gametes. These gametes fuse to form a zygote. Since the zygote receives genetic material, the offspring bear the characteristics of the species, but also bear traits that make them different from their parents. In the following sections you shall study about these reproductive mechanisms.

## 8.2.1 Asexual Reproduction

Asexual reproduction is also known as **agamic** reproduction because there is no involvement of gametes in it. Asexual methods of reproduction are (i) Fission (ii) Budding (iii) Fragmentation and (iv) Parthenogenesis. In the following section we shall study about these methods in brief. In the course LSE-09, **Animal Diversity-I**, you shall study these modes of reproduction in detail.

i) **Fission :** In this process the organisms divide mitotically into two or more equal sized parts. Division of the organism into two daughter cells is known as **binary fission,** which is characteristics of protozoans. The daughter organisms produced carry all the cytoplasmic organelles of the parent individual. They also carry the same genetic material as found in the parent organisms. Some organelles like mitochondria divide at the time of division, while others like flagella and contractile vacuoles are formed afresh by the daughter organisms. Protozoans at certain times also exhibit sexual reproduction. Their gametes are produced meiotically and mating takes place. In the following subsection you shall read about sexual reproduction in protozoans.

ii) **Budding :** In this method of reproduction an organism develops an outgrowth which on detachment from the parent becomes a self-supporting individual. Budding is found in protozoans, coelentrates, platyhelminths and several groups of annelida (Fig. 8.1).

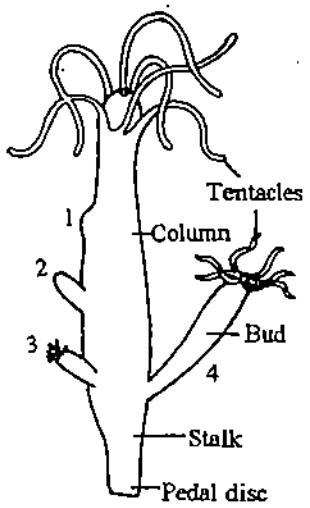



**Fig. 8.1 : Budding in Hydra**

iii) **Fragmentation :** In this type an organism breaks down into two or more pieces, each of which grows into a new individual. This means of reproduction is found in the organisms which have good power of regeneration. Sponges and hydroid coelentrates exhibit this type of reproduction. If a sponge is macerated by pressing it through fine gauge, the separated cells come together in groups and grow into new individuals. Very small fragments of free-living flatworms will regenerate into new individuals if right conditions are provided to them. A nemertine worm, *Lineus*, develops rings of constriction which cut the body into short fragments. These animals possess large number of undifferentiated cells which, when required can proliferate and develop into any kind of tissue.

iv) **Parthenogenesis :** In this type of reproduction, development of a new individual takes place from an egg or a spermatozoon without the participation of a germ cell from the opposite sex. In animals only the maternal cell give rise to a parthenogenetic individual. Certain algae however, give rise to individual from paternal germ cell, therefore, parthenogenesis is considered an asexual reproductive mechanism.

Natural Parthenogenesis is known to occur in rotifers, some nematodes, crustaceans, insects and several species of fish, amphibians and desert lizards.

In many parthenogenetic invertebrates there is a cyclical alternation of asexual with bisexual reproduction. Parthenogenesis may be seasonal and related to temperature or food supply, or it may appear at irregular intervals. In the honeybees and some wasps unfertilised eggs develop into haploid males and fertilised eggs give rise to diploid females.

## 8.2.2 Sexual Reproduction

You have learnt at the beginning of this section that in sexual reproduction there is involvement of two genetically different parents. They combine their genetic material to produce a cell having a new genotype. Asexual reproduction on the other hand does not allow genetic mixing to occur. However, it should not be mistaken that asexual reproduction is a "defective" form of reproduction restricted to the primitive forms of life. If we look into the abundance of these organisms who have persisted on earth for 3.5 million years, and their involvement in some important events of life such as food chain, clearly demonstrates that these organisms are successful and very important. These organisms must have restricted to asexual reproduction because of its simplicity to produce and also no time and energy required to find out a mate.

The union of gametes is known as **syngamy.** Generally the gametes differ from each other in structure, size and behaviour, for which reason these are known as **heterogametes. Ovum** (egg), is produced by the female and the **spermatozoon** (sperm) is produced by the male. Ova are large, non-motile and produced in relatively small numbers. Sperms are small, motile, and produced in enormous numbers. Syngamy takes place by a process known as **fertilization,** in which a sperm penetrates an egg and donates its nucleus to the egg.

Sexual reproduction is found in all the multicellular organisms. Protozoans also reproduce

sexually during certain periods of life. Sexual reproduction in them may or may not involve male and female gametes. Some times two mature sexual parents merely join together to exchange nuclear material or merge their cytoplasm. It is not possible to distinguish sexes in them (you will study details of sexual reproduction in protozoans, in the course LSE-09, Animal Diversity-I). The male-female distinction is more clearly evident in metazoa. The organs that produce germ cells are known as **gonads**. The gonad that produces the sperm is called **testis** and that which forms the egg is known as the **Ovary**.

In sexually reproducing metazoans, there are separate and distinct male and female individuals. Each has its own reproductive system and produces one kind of sex cell, spermatozoon or egg. Nearly all vertebrates and many invertebrates have separate sexes, and such a condition is celled **dioecious** and this type of reproduction is called **biparental** reproduction. However, some animals such as most flatworms, some hydroids, annelids, crustaceans and some fishes have both male and female organs in the same individual. Such a condition is called **hermaphroditism.** In contrast to the dioecious state of separate sexes, hermaphrodites are **monoecious,** having both male and female organs in the same organism. Most of the hermaphrodites avoid self-fertilization by exchanging germ cells with each other. For example, although the earthworm bears both male and female organs, its eggs are fertilised by the copulating male and vice versa. Hermaphrodites also prevent self-fertilisation by developing eggs and sperms at different times.

If we admit that all living things are mortal, that every living organism is endowed with a life span that must eventually end, then reproduction is indispensable for the continuance of species. During evolution the efficiency of reproduction increased where the parents protect and provide nutrition for the young before or after birth. The adaptations that are found in vertebrates are **internal fertilisation, the cleidoic** (enclosed in shell) **egg**, and the **foetal membranes.** Parallel adaptations are also found in lower animals.

For successful fertilisation to occur, animals have adapted internal fertilisation, connected with copulation. As an adaptation to protect the developing embryos reptiles and birds secrete calcarious shell around the fertilised eggs, the cleiodic eggs. The organisms in which the eggs are laid soon after ovulation are called **oviparous** animals. The animals in which egg laying is delayed and the eggs develop within the maternal organism and the young emerge from the cleiodic eggs at about the same time of egg deposition, are called **Ovoviviparous** animals. Mammals are adapted for internal development of the embryos. Here the eggs develop within the maternal organism and the young are born. Such a type of animals are called **viviparous** animals.

You have learnt that genetic mixing is an advantage of sexual reproduction. Sexual reproduction, by combining the parental characters, tend to multiply variations and make possible a richer and most diversified evolution. You have studied earlier that mechanism of interchange of genes between individuals are more limited in organisms with only asexual reproduction. The process of genetic mixing in sexually reproducing organism is brought about by meiosis. You have studied in Unit 17 of the course **LSE-01; Cell Biology** that meiosis is a distinctive type of gamete-producing nuclear division in which the chromosomes split once and the cell divides twice, producing four daughter cells, each bearing haploid number of chromosomes. At fertilisation the two haploid gametes combine to restore the normal (diploid) chromosomal number of the species. The zygote although has equal numbers of chromosomes from each parent, is genetically different from each other because of the genetic recombination. Nevertheless the individual which develops from this zygote bears a random assortment of parental characteristics. It is in this way sexual reproduction introduces new varieties into the population.

After studying the essential features of sexual reproduction, we shall now study the mechanism of sex determination.

### Sex Determination

Sex, whether an individual will be a male or a female is determined at fertilisation, and this directs and controls all the later processes involved in male-female differentiation of the genital system. The genetic determination, however is not final and irrevocable. Many internal and external environmental factors come into operation during the developmental process and modify or completely reverse the sexual constitution of the individual.

Sex which is established at fertilisation, depends upon the "sex" chromosomes contributed by the parents. You are aware that cells of multicellular organisms contain two types of chromosomes; autosomes and sex chromosomes. In human for example, there are twenty

two pairs of autosomes and one pair of sex chromosomes. In mammals, most frogs, some fishes, and dipterus insects there are two types of sex chromosomes, X and Y. Y chromosome is strongly "male determining". The zygote bearing XY chromosomes gives rise to male and it is called **heterozygote**, where as zygote of XX chromosomes develops into a female, and it is **homozygote.** Since a mammalian male is a **heterogamete**, containing XY chromosomes, therefore, half of the spermatozoa bear X chromosomes and the other half Y chromosomes. A mammalian female is a **homogamete** containing XX chromosomes, therefore ova contain only the X chromosomes. The union of the spermatozoon bearing X chromosome with the egg gives rise to a female and union of the spermatozoon bearing Y chromosome with the egg will be the genetic male.

In birds, female is the heterogametic sex. The small chromosome, equalent to the Y in mammals, is designated by the letter W, and the X chromosome is designated in this case as Z. Half of the eggs carry a W chromosome and the other half a Z chromosome. All of the sperms carry a Z chromosome. The homozygous (ZZ) condition produces males, and the heterozygous (ZW) produces females. This is the type of mechanism that operates in birds, most reptiles, salamanders, some fishes and insects. XX-XY type of sex determination is called mammalian type of sex determination and ZZ-ZW type is called avian type of sex determination.

**SAQ 1**

Match the terms given in column A with their definitions given in column B and compare your answers with those given at the end of the unit.

| | A | | | B |
|---|---|---|---|---|
| i) | Agamic reproduction | [ ] | (a) | A type of reproduction in which development of an individual takes place from an egg or a sperm without the participation of a germ cell from the opposite sex. |
| ii) | Parthenogenesis | [ ] | (b) | A type of reproduction in which there is no fusion of gametes. |
| iii) | Binary fission | [ ] | (c) | A phenomenon in which eggs develop within the maternal organism and the young ones are born. |
| iv) | Syngamy | [ ] | (d) | A phenomenon in which eggs are laid soon after ovulation. |
| v) | Dioecious | [ ] | (e) | A condition in which both male and female gonads are present in the same individual. |
| vi) | Heterogametes | [ ] | (f) | Gametes which differ from each other in structure, size and behaviour. |
| vii) | Hermaphroditism | [ ] | (g) | A condition in which the individuals have separate sexes. |
| viii) | Oviparity | [ ] | (h) | Union of gametes |
| ix) | Viviparity | [ ] | (i) | Division of the organism into two daughter cells. |

## 8.3 FUNCTIONAL MORPHOLOGY OF REPRODUCTIVE ORGANS

In the earlier section you have learnt about the essential features of sexual reproduction. In this section we shall learn about the organs of reproduction.

The organs that produce germ cells are known as **gonads.** The gonads that produce the sperm are called **testis** and that which form the eggs is known as the **ovary.** Gonads are the primary sex organs found in all the animals. In some primitive animals the gamete-producing tissues are diffuse, consisting of numerous scattered loci for the proliferation of sex cells. In all the more advanced animals the gonads are localised, and in the bilaterally symmetrical animals they arise as paired structures. Sometimes one of the gonads degenerates secondarily. The birds provide a familiar example with paired testis in the male and a single left ovary in most females.

Apart from the gonads most metazoans have various **accessory reproductive organs** that transfer and receive sex cells such as penis, vagina, oviducts, uterus and vas deferens. In the courses LSE-9; Animal Diversity-I and LSE-10; Animal Diversity- II, you shall study anatomy of these organs in the various groups of animals. Details of the functional morphology of the reproductive organs of various groups of animals is beyond the scope of this unit, therefore we shall restrict our studies to vertebrates.

The gonads of vertebrates can be classified into the following two types (i) Mammalian type and (ii) Non-mammalian type. They differ from each other anatomically, but perform the same functions. In the following subsection we shall learn about the gonads of both mammalian and non-mammalian vertebrates.

## 8.3.1 Ovary

The mammalian ovaries are flattened structures lying on the side of the pelvic cavity. These are attached to the peritoneum by mesovaria and ovarian ligaments. The free surface of the organ is covered by a single layer of **germinal epithelium.** The ovary is roughly divisible into a **cortex** and a **medulla.** In the mature ovary the cortex contains **follicles** and **corpora lutea** in their different stages of differentiation and destruction. Whereas medulla contains large blood vessels. Apart from these structures ovary contains **interstitial cells** which fill all the space not occupied by follicles, corpora lutea and blood vessels (Fig. 8.2).

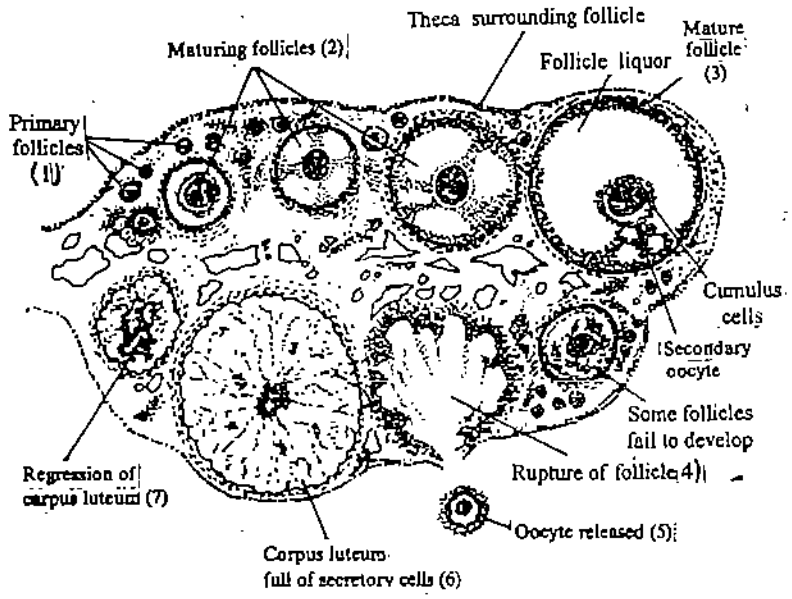



Fig. 8.2 : Diagram of a Mammalian Ovary.

Ovarian follicles are the actual gamete containing structures. These are derived from the cells of germinal epithelium, which are later surrounded by layers of follicular cells. A germ cell surrounded by layers of follicular cells is called a **follicle.** A follicle undergoes stages of differentiation to become a mature follicle known as **antral follicle** or the **Graafian follicle** (see Fig. 8.2). After expulsion of the egg at ovulation, a follicle differentiates into a **corpus luteum.** The ovary also contains some follicles which due to unavailability of hormones fail to develop into a Graafian follicle and undergo degeneration. The degenerating follicles are known as the **atretic follicles.** In an ovary one can find atretic follicles and corpora lutea in different stages of retrogression.

You must have noted in Figure 8.2 that mammalian ovary contains follicles in different stages of development, i.e. there may be primary follicles, growing follicles, preantral follicles and Graafian follicle. Whereas the ovary of non-mammalian vertebrates contains follicles mostly in the same stage of differentiation, present in groups enclosed by a membrane. Such a type of group of follicles is called a **cyst.** In non-mammalian vertebrates

ovarian follicles grow synchronously therefore one can find follicles of the same stage of development. The other major difference between the mammalian ovary and the non-mammalian ovary is the presence of yolk. The mammalian follicles contain negligible quantities of yolk, whereas the follicles of non-mammalian vertebrates are laiden with yolk (Fig. 8.3).

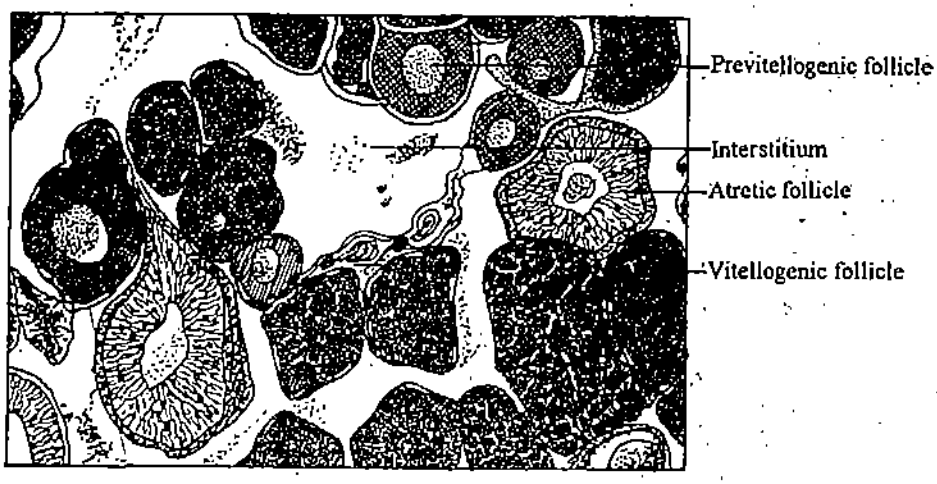



Fig. 8.3 : Diagram of non-mammalian Ovary.

The ovary of vertebrates performs the following functions :

i) production of eggs,

ii) synthesis of hormones needed for the chemical coordination of reproduction,

iii) elaboration of nutrient material (yolk) for the early stages of embryonic development, and

iv) maintenance of pregnancy i.e. housing, nourishment and development of the young in the viviparous animals.

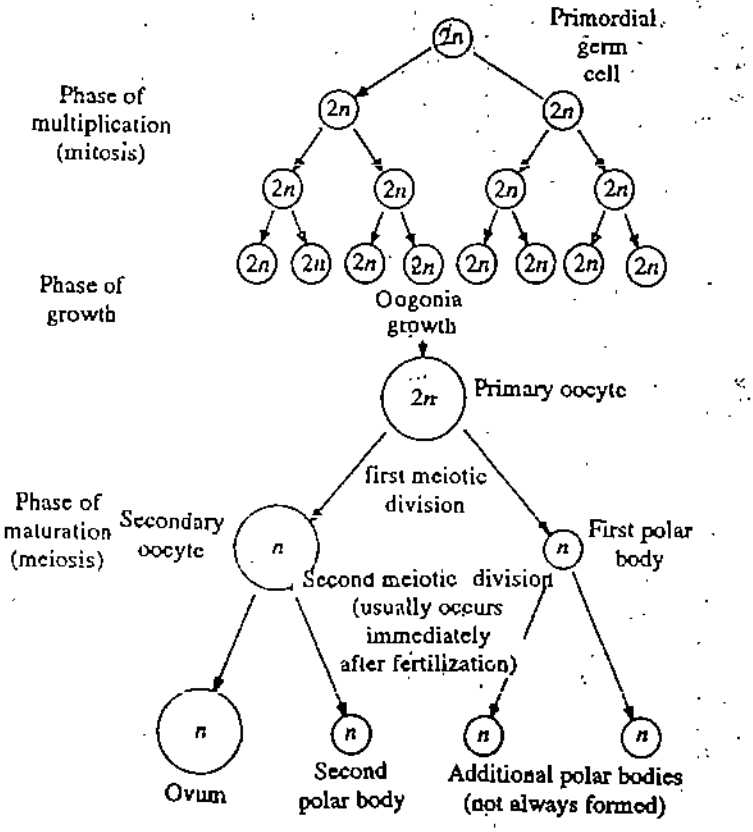



Fig. 8.4 : Steps of Oogenesis.

## Production of Eggs

The origin of female gamete is from the gonocytes of embryonic ovary, which undergo a period of vigorous multiplication and then differentiate successfully into oogonia and oocytes. The oocytes undergo meiotic division to become a haploid egg. The first meiotic division of the oocytes begins just prior to the birth or just after birth depending on the species and is arrested at late prophase stage. In human ovary the oocytes are held at late prophase stage from the time of birth until ovulation occurs after the onset of puberty. The haploid secondary oocytes begin the second meiotic division but remain in metaphase, and do not extrude a second polar body until the oocyte has been penetrated by a sperm. The germ cell becomes a mature ovum after second polar body is released (Fig. 8.4).

During the late foetal life, as well as in the post-natal female, cluster of cells arise from the ovarian epithelium. One cell in the cluster enlarges more rapidly than the others and is called **oogonium,** whereas the remaining cells constitute the early follicles. After the oogonian enlarges and becomes distinguishable from its neighbours, it is called a **primary oocyte.** It is then surrounded by follicular cells. A homogenous membrane, **zona pellucida** appears between the primary oocyte and the follicular cells. The primary oocyte is now called the **primary follicle.** The follicle cells increase rapidly forming layers. They are later differentiated into **theca** and **granulosa.** Under the influence of pituitary gonadotropins fluid-filled spaces appear in the granulosa and the follicle becomes a Graafian follicle, follicle containing mature ovum (see Fig. 8.3).

> Gonadotropins are hormones that stimulate the gonad to synthesise and release sex hormones. You shall study more about hormone action in Unit 10.

## Synthesis of Hormones

Apart from production of mature eggs, the other chief function of the ovary is elaboration of hormones that regulate reproductive tract and secondary sexual characters, condition the mating reaction and exert other metabolic processes.

The ovary elaborates steroid hormones such as **estrogens, progestogens, androgens** and a non-steroid hormone called **relaxin.** Most workers agree that mature follicle is an important source of estrogen. Most of the evidence implicate that it is synthesised either at the membrana granulosa or theca interna. The corpus luteum elaborates both estrogenic and progestational steroids. The cellular source of relaxin and ovarian androgens is not known.

Now let us learn about the structure and function of these hormones.

## The Estrogens

The predominant natural estrogens of the human are estradiol-17β, estrone and estriol. Estrogens contain eighteen carbon atoms (C-18). Steroid hormones are derived from cholesterol. The basic structure of all the steroid hormones is the steroid ring **cyclopentanoperhydrophenantherene,** having 17 carbon atoms (Fig. 8.5).

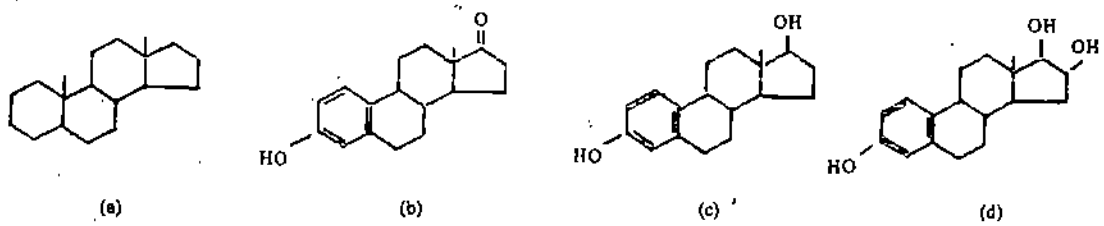



Fig. 8.5 : (a) Cyclopentanoperhydrophenantherene (b) Estrone (c) Estradiol-17β (d) Estriol

The estrogens act directly or in cooperation with other hormones to produce a great variety of effects on specific target organs and on the chemistry of the body as a whole. We shall now discuss some of the actions of estrogens.

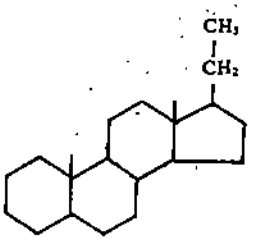
i) The most general effect of the estrogens is to promote tissue growth. It is more pronounced in the accessory sex tissues. Estrogens stimulate cell division in the deeper parts of the skin and causes a more rapid replacement of the outer cornified layers of the skin. There is some evidence that high levels of estrogens (under pathological conditions) may be potentially dangerous as they may encourage the formation of cancer in certain individuals.

ii) Estrogens are required for the maintenance of vaginal lining and uterine growth. It has been observed that in the experimental animals deprived of estrogens, the vaginal lining and the uterine wall become thin, and mitotic divisions seldom occur in these tissues. Administration of estrogens to the estrogen-deprived animals causes rapid growth of the vaginal and the uterine tissue. The metabolic activity of these tissues also increases as evidenced by augmented uptake of water and electrolytes by the tissues and rise in the RNA content of the tissue.

iii) Estrogens are essential for the anatomic preparation of the mammary glands for milk secretion. In some species it affects mammary development in combination with progesterone. But in some species, estrogens alone or progesterone alone produce the effect. As a general rule, the mammary glands require pre-treatment with estrogens before the progestogens are effective.

iv) In mammals **sexual receptivity** or **heat** coincides with a period during which the ovaries are secreting large amounts of estrogens. Full mating behaviour generally depends upon both estrogen and progesterone. These ovarian hormones probably act through the central nervous system (hypothalamus) to condition the psychic manifestations such as increased spontaneous activity, lordosis, sexual receptivity etc.

Biochemical studies revealed that estrogens stimulate synthesis of mRNA, proteins and DNA. It enters the cells and becomes associated with an cytoplasmic receptor protein. The hormone-receptor complex enters the nucleus and initiates transcription and RNA synthesis. You have already studied in detail about the mechanism of action of steroid hormones in Block 2 of LSE-01; Cell Biology.

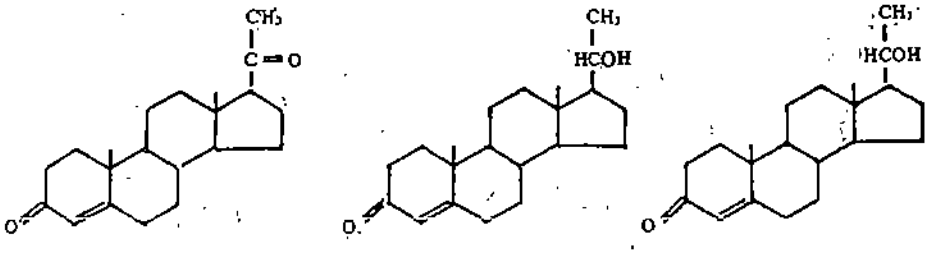



Pregnane

Fig. 8.6 : Pregnane nucleus

### The Progestogens

The progestogens are C-21 steroids having the basic structure of the **pregnane** nucleus (Fig. 8.6). Progesterone, 20 α- hydroxypregn-4-en-3-one and 20β-hydroxypregn-4-en-3-one are the known naturally occurring progestogens in mammals (Fig. 8.7).




Progesterone 20β-Hydroxypregn-4-en-3-one 20α-Hydroxypregn-4-en-3-one

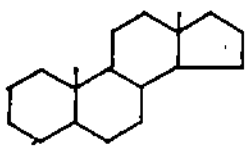
Fig. 8.7 : Naturally occurring Progestogens.

These hormones are present in ovarian follicles, corpora lutea, placenta and blood. The cellular sites of its synthesis are the granulosa lutein cells of the corpus luteum. Placenta also secretes progesterone. Progestogens have varied actions upon female reproductive organs under physiological (normal) conditions. They often act synergistically with estrogens.

However, these hormones are capable of inhibiting the actions of each other. Under these conditions they are considered to act antagonistically.

Progestogens are of special importance in preparing the uterus for the implantation of blastocyst, in maintaining pregnancy and in regulating the accessory reproductive organs during the reproductive cycle.

### Ovarian Androgens



Androstane

Fig. 8.8 : Androstane

Androgens are masculinising compounds that are produced chiefly by the testis under normal conditions. They also arise from the adrenal cortex, ovaries and placenta. These are C-19 compounds derived from the basic structure **androstane** (Fig. 8.8). **Androsterone** and **testosterone** are the principal androgens about which you will learn in Sub-section 8.3.2. Since testosterone is an intermediate in the biosynthesis of estrogens, therefore, this hormone may be present/secreted by the ovaries. The ovarian androgens exhibit the same biological activity as that of testicular androgens. Pathological ovaries may release tremendous amount of androgens, but the amount of androgens secreted by the normal ovaries is not significant.

### Relaxin

This is a water soluble hormone present in the ovaries, placentae and uteri of various mammalian species during pregnancy. Its role in human physiology is not clear. It is apparently a hormone of pregnancy and has not been found in the blood of men or non-pregnant women. Relaxin levels in the blood reach a high peak during the terminal stages of pregnancy and disappear within one day after delivery. It is thought that relaxin helps in the enlargement of birth canal by relaxation of uterine cervix and pelvic ligaments.

### Elaboration of Yolk

Elaboration of nutritive material, **vitellin** or the **yolk**, for the early states of embryonic development is one of the important functions of the non-mammalian ovary. You are aware that mammals except monotremes and marsupials are viviparous. The development of the embryos in the viviparous animals takes place inside the mothers' body. Nutrition and other required things are provided to the embryos through maternal circulation. Most of the non-mammalian vertebrates are oviparous forms in which development of the embryos takes place outside the mothers' body. Therefore they have to be provided with nutrient material enough for the development till they reach a stage at which they manage to procure food by themselves.

The nutritive material in the oviparous animals is stored in the egg cytoplasm in the form of yolk. The constituents of the yolk are proteins, lipids, glycogen, nucleic acids and some minerals like phosphorus. The process of yolk synthesis is called **vitellogenesis.** Vitellogenesis is under the control of gonadotropins. Gonadotropins induce the synthesis and secretion of estrogens in the ovary, which in turn stimulate the synthesis of **vitellogenin,** a yolk protein in the liver secreted into the blood stream. Vitellogenin reaches the ovary through blood and enters the egg cell. In the cytoplasm it is used in the yolk synthesis.

Apart from egg production, synthesis of hormones and yolk, ovaries also play a vital role in the maintenance of pregnancy.

### Regulation of Ovarian Activity

The ovary is not an autonomous organ; its functional capacity is influenced by the wide variety of external stimuli which are funneled into the central nervous system and then "translated" into chemical messengers which act directly upon it. Perhaps all endocrine glands have at least a modulating influence upon the production of gametes and hormones by the gonads.

The ovary is most profoundly regulated by the pituitary gonadotropins, namely **follicle stimulating hormone (FSH)** and **luteinising hormone (LH).** The growth and development of ovarian follicles of mammals depend upon FSH, but LH is required for final maturation. LH acts upon FSH-primed follicles and stimulates estrogen secretion. Corpora lutea secrete progesterone. Its maintenance and secretion is under the influence of **prolactin,** a hormone of the pituitary also known as **luteotropic hormone.** Prolactin is not a luteotropic hormone in the majority of mammals. In some species of mammals it causes regression of the testes and ovaries.

Large amounts of estrogens, given to intact animals, inhibit the gonads by altering the release of pituitary gonadotropins. There is a negative feedback mechanism regulating the ovarian activity. The increased levels of gonadotropins stimulate the synthesis of estrogens by the ovary and the increased levels of estrogens in the blood suppress the gonadotropin secretion, which in turn inhibit the estrogen synthesis and the related activities (Fig. 8.9).

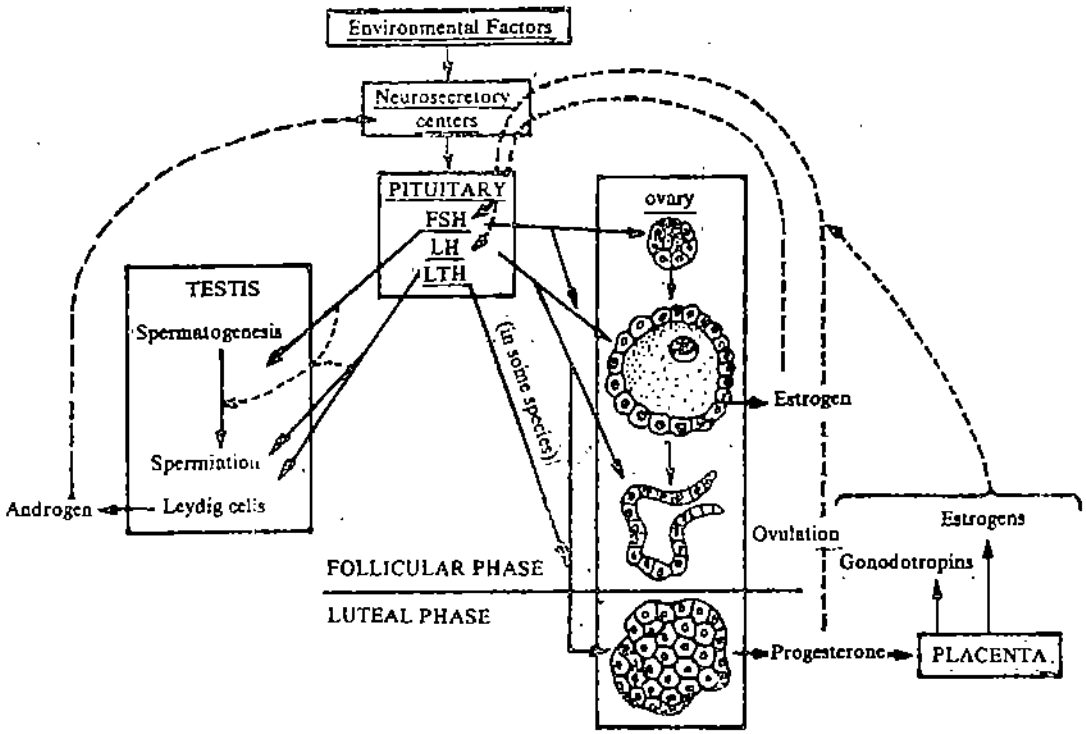



Fig. 8.9: Regulation of Ovarian and testicular activity.

SAQ 2

Fill in the blanks and compare your answers with those given at the end of the unit.

a) The gonads that produce the sperm are called ..... and that which form the eggs is known as .....

b) The gonads are in pairs in the ..... symmetrical animals. In female ..... one of the ovaries is secondarily degenerated.

c) In mammals the mature ovarian follicle is called .....

d) After expulsion of the egg at ovulation, a follicle differentiates into .....

e) The degenerating ovarian follicles are known as ... .....

f) The earliest germ cell of the ovary is known as .....

g) The predominant natural estrogens of the human are (i)..... (ii)..... and (iii) .....

h) Naturally occurring progestogens in the mammals are (i) ..... (ii)..... and (iii) .....

i) The process of yolk synthesis is called .....

## 8.3.2 Testis

You are aware that gonads of the male are called testis. In man and other mammals testes are lodged in an integumentary pouch called the **scrotum.** It is an adaptation for regulating the internal temperature of the testis. Whereas in non-mammalian vertebrates testes are present inside the body cavity, the **peritonium.**

Mammalian testis is made of a series of elongated follicles or **seminiferous tubules.** The lining of the tubules constitutes an epithelium from which the spermatozoa are proliferated. The interspaces between the seminiferous tubules are occupied by blood vessels, connective tissues, and the **interstitial cells of Leydig.** Seminiferous tubule is limited by a thin

basement membrane. In an adult testis often all stages of spermatogenesis are found at any level in cross-section of the tubule (Fig.8.10).

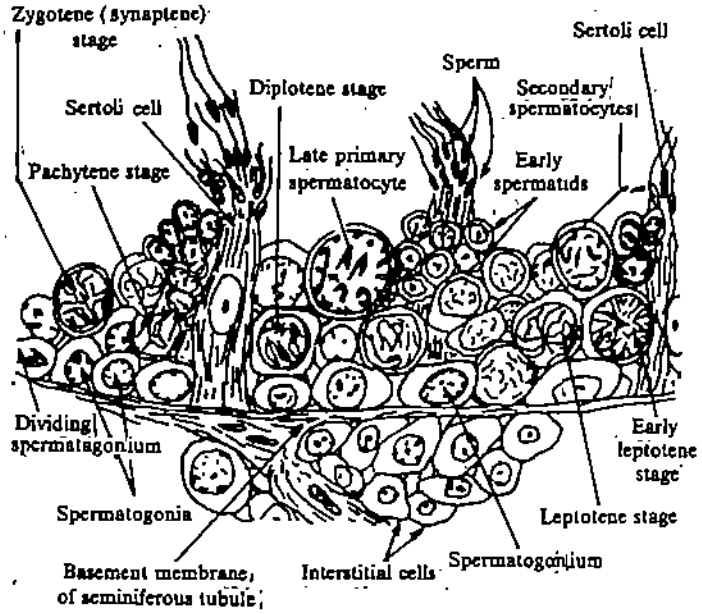



Fig. 8.10 : Diagram of Mammalian testis.

**Spermatogonia** are the youngest germ cells from which spermatozoa proliferate. These lie next to the basement membrane and undergo series of mitotic divisions leading to the formation of **primary spermatocytes.** The primary spermatocytes undergo the first meiotic division and give rise to haploid cells called **secondary spermatocytes.** The secondary spermatocytes begin the second meiotic division and produce smaller cells called **spermatids.** The spermatids later transform into **spermatozoa** by a process known as **spermiogenesis.** The transformation of spermatids into spermatozoa involves principally cytoplasmic loss and the differentiation of the tail piece (Fig. 8.11).

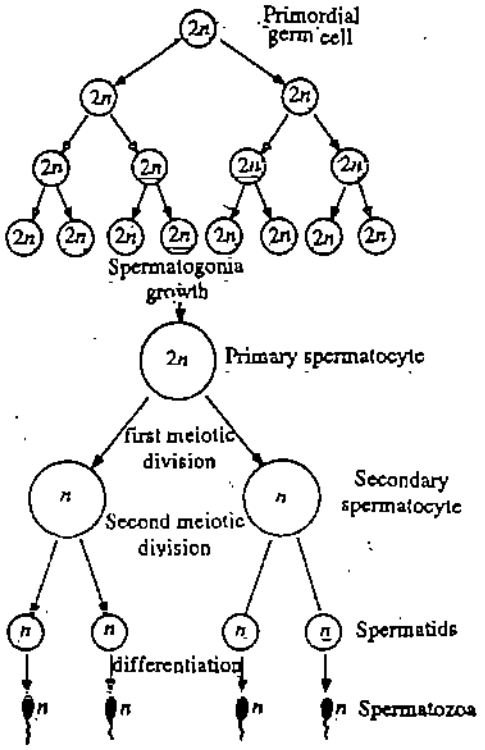



Fig. 8.11 : Steps of Spermatogenesis.

In a cross-section of the seminiferous tubule you will find that spermatogonia are located just inside the basement membrane of the tubules, and the series of maturing stages i.e. from

spermatogonia-spermatozoa, occur towards the lumen where mature sperms are released. Studies have indicated that in man it requires about 74 days for a spermatogonium to transform into functional spermatozoa. The rate of germ cell development is constant for a given species and strains of mammals, and that this rate cannot be accelerated by hormones. Cytological observations suggest that germ cells must move forward during their differentiation; if unfavourable environment make it impossible for them to pursue their differentiation at the normal rate, they degenerate and are eliminated from the system. Although hormones do not accelerate the rate of germ cell differentiation, they must contribute to the creation of favourable environments for their transformation into the spermatozoa.

The seminiferous tubules contain another type of cells called **Sertoli cells.** These are relatively large elements extending from the basement membrane towards the lumen. They are regarded as supporting cells, which probably provide nourishment for the spermatids. In some species, the sperm heads remain embedded in the Sertoli cells for relatively long periods. The release of spermatozoa from the Sertoli cells is termed **spermiation,** a process that is analogous to **ovulation** in the female (Fig. 8.12).

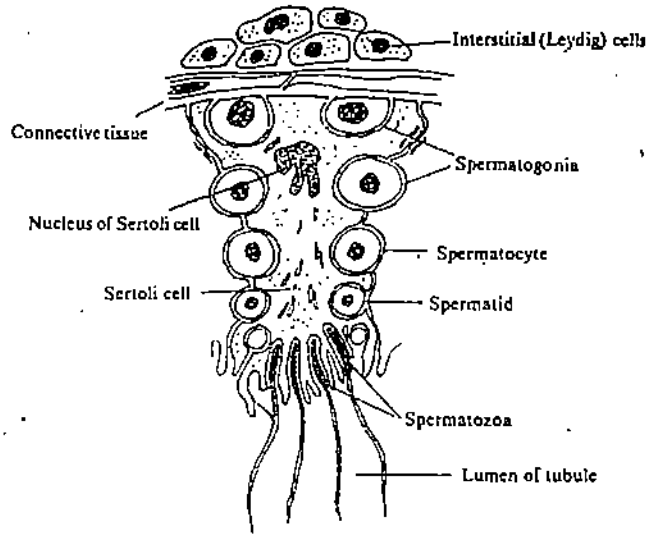



Fig. 8.12 : Diagrammatic section through a mammalian seminiferous tubule showing sertoli cell and a mobile population of differentiating germ cells.

The testis of non-mammalian vertebrates is also made up of tubules. The tubules contain nest of cells which undergo spermatogenesis asynchronously. Each nest of cells is called a **cyst**, which contains germ cells of the same stage of differentiation. For example in a tubule there may be cysts of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. In the breeding season testis lobules contain maximum number of cysts of spermatozoa and also the spermatozoa released into the lumen of the tubule. During the non-breeding season there are more number of cysts of spermatogonia and spermatocytes. You have studied earlier in this section that in mammalian testis the interspaces between the seminiferous tubules is occupied by Leydig cells. In the testis of non-mammalian vertebrates the interspaces between the tubules is occupied by the **lobule boundary cells.** These are homologous to the Leydig cells and apparently have the same endocrine function, which will be dealt with later in this section (Fig. 8.13).

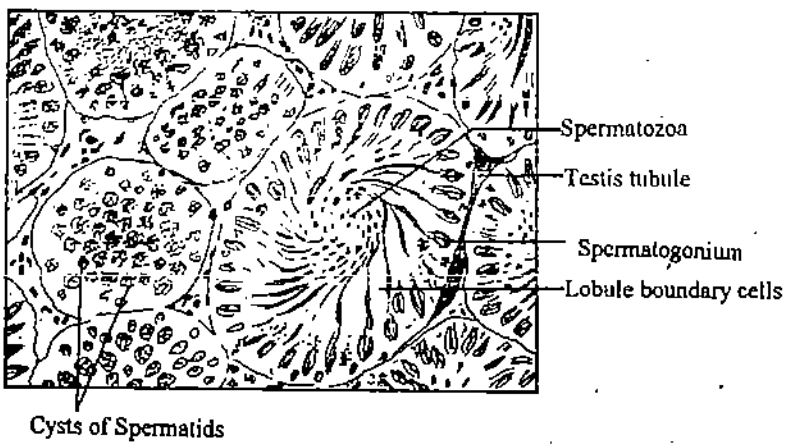



Fig. 8.13 : Diagrammatic section of non-mammalian testis.

The testis performs the following two functions:

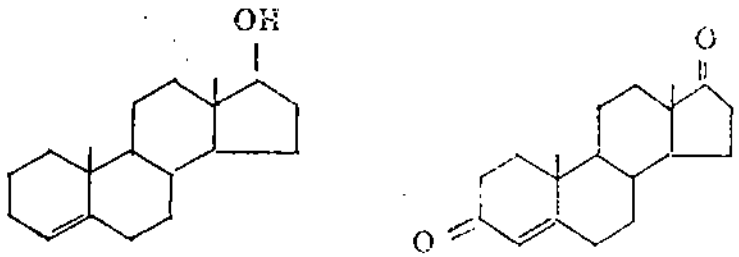
i) proliferation of spermatozoa, and

ii) secretion of steroid hormones.

You have already studied about sperm production by the testis. We shall now learn about the hormones synthesised by the testis.

### Synthesis of Hormones

Leydig cells and the sertoli cells are the steroid hormone synthesising cellular sites of the testis. Leydig cells are the major source of these hormones. Testosterone and **androstenedione** are the main circulating androgens of testicular origin (Fig. 8.14).




(a) Testosterone (b) Androstenedione

Fig. 8.14 : (a) Testosterone (b) Androstenedione

The common metabolites of testosterone are epitestosterone, androstenedione, androstanedione, etiocholanolone, and androsterone. The mechanism of action of androgen is similar to that of the other steroid hormones.

Androgens are essential for the control of secondary sex characters of the male and for the functional competence of the accessory reproductive glands and the ducts. The more pronounced metabolic actions of these steroids is the promotion of protein anabolism. Androgens decrease the urinary loss of nitrogen without increasing non-protein nitrogen of the blood, and produce at least a temporary increase in body weight. Since androgens increase protein matrix of bone, they have been used in the clinical treatment of certain skeletal defects. They also promote muscle growth.

At the human level, androgens are involved in the control of hair patterns, voice changes, skeletal configurations, and regulation of the sebaceous-gland activity. Androgens also exert effects upon the germinal epithelium of the testis tubules and thus influence sperm production. Testosterone, which is synthesised in the cells of Leydig, diffuses into the tubule where it is the principal stimulus for germ cell differentiation.

The accessory system of male ducts and glands about which you will learn in Sub-section 8.3.3, are morphologically and physiologically dependent upon the production of androgens. In the experimental adult animals deprived of androgens these organs involute until that approximate the same structure of juvenile animals. Administration of androgens completely restores all of these organs to the normal conditions.

### Regulation of Testicular Activity

The production of spermatozoa is under the influence of pituitary hormones and of androgens derived from the testis. The testicular tissue involute in the experimental animals deprived of pituitary hormones. FSH is required for the differentiation of spermatogonium into spermatozoa, and LH (also known as **interstitial cells stimulating hormone (ICSH)** in males) to stimulate the Leydig cells for the production of androgens. The increased levels of androgens in the blood suppress the synthesis and secretion of gonadotropins in the pituitary.

You have studied earlier that the time required for the spermatogonia to differentiate into spermatozoa is a biologic constant, varying with the species and strain, which cannot be altered by hormones and other factors. On the other hand, the number of spermatozoa produced is dependent upon pituitary gonadotropins, androgens, nutritional factors, temperature, light etc.

**SAQ 3**

Explain the function of the following in two sentences and compare your answers with those given at the end of the unit.

a) Scrotum : .....

.....

.....

b) Interstitial cells of Leydig : .....

.....

.....

c) Sertoli cells : .....

.....

.....

d) Androgens: .....

.....

.....

## 8.3.3 Accessory Reproductive Organs

The accessory reproductive organs consist of ducts and glands specialised for storage and conveyance of the gametes. The functional status of these organs is conditioned by the respective gonadal hormones. In the course LSE-10; Animal Diversity-II, you shall study comparative account of the anatomy of these organs. In this section we shall study functional anatomy of these organs in both female and male separately.

### The Female Accessory Sex Organs

The female accessories include **oviducts** or **fallopian tubules** as they are generally termed in the human subjects, the **uterus**, the vagina and the **external genitalia.**

In mammals, oviducts provide a passageway between the ovary and uterus. The ovarian end of this tube is expanded into a funnel, having fimbriated margin. Some of the epithelial cells lining the tube possess cilia that beat inwardly. Beating of cilia together with the increased activity of the fimbria at the time of ovulation assist the movement of the ovum towards the uterus. Fertilisation typically occurs in the oviducts. In human beings fertilisation occurs usually within 24 hours after the egg has been released from the ovary.

The primate uterus is a pear-shaped muscular organ. The uterine wall is composed of a thick mass of smooth muscle cells, called **myometrium** and glandular lining known as **endometrium.** The narrow caudal end of the uterus is called cervix. During pregnancy the uterine wall becomes highly modified to facilitate implantation of the blastocyst and its subsequent development.

The human vagina is an unpaired, dilated tube, approximately 4 inches in length extending from the caudal end of the uterus to the vestibule. The lining of the vagina is a stratified squamous epithelium devoid of glands. It has a very thin layer of muscles. The vagina does not provide a satisfactory environment for the survival of spermatozoa. In humans, they die within a few hours, whereas they may remain viable for two or three days in the uterus and fallopian tubes.

The external genitalia of the female consist of the **clitoris,** the **labia majora** and **minora,** and certain glands that open into the vestibule (Fig. 8.15). The clitoris is an erectile organ homologous to the penis.

In reptiles and birds the oviduct supplies water, albuminous coating (egg white), shell membranes and shell to the eggs as they traverse through it. In birds only the left oviduct grows to functional size; the right is lost entirely or persists as a rudiment (Fig. 8.15).

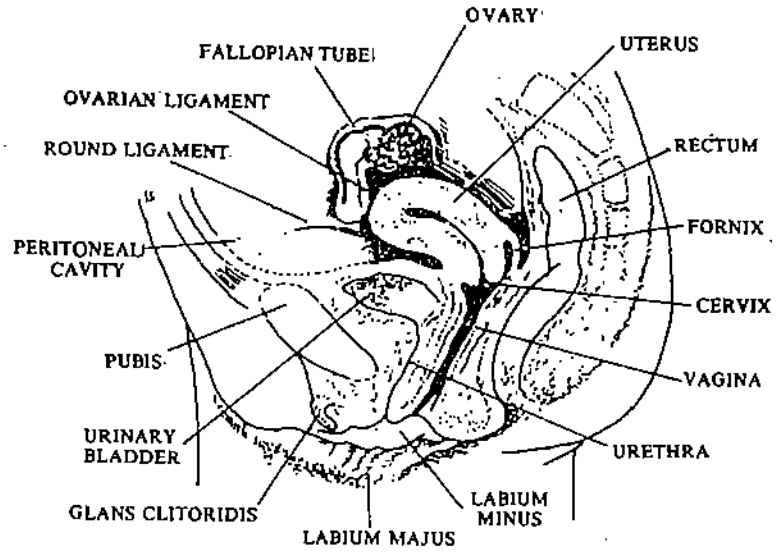



Fig. 8.15 : Diagrammatic section of the female pelvis, showing the genital organs.

## The Male Accessory Sex Organs

The accessory ducts and glands of the male are specialisations for the storage of spermatozoa and their conveyance in an adequate vehicle to the exterior at the proper time. These structures in the human male include multiple **ductuli efferntes**, paired **epididymis**, **vasa deferentia**, seminal vesicles, **ejaculatory ducts**, **cowpers' glands**, a **prostate gland**, the **urethra** and the copulatory organ, **penis**.

The epididymis is an extremely convoluted tubule that measures around 20 feet in length when straightened out. It is a storage place for spermatozoa collected from the testis lumen. Spermatozoa improve the capacity for motility and fertilisation after a period of residence in this organ.

Vas deferens begins from the terminal end of the epididymis. It receives a duct from the seminal vesicles and then becomes known as ejaculatory duct. It also courses through the prostate gland and enters the urethra. The vas deferens contains well developed muscle layers and is largely responsible for the movement of sperm along the tract. Sperm storage also occurs at the proximal end of the vas deferens. The spermatozoa are believed to be nonmotile in the storage structures such as epididymis and the proximal portion of the vas deferens, but become motile when mixed with the accessory gland secretions. The seminal fluid emanating from the accessory glands furnishes a vehicle for the conveyance of sperm and perhaps provides an environment in which they can attain their greatest fertilising capacity. All of the accessory sex organs mentioned above depend on androgens for full functional development and are quite inactive until the advent of puberty (Fig. 8.16).

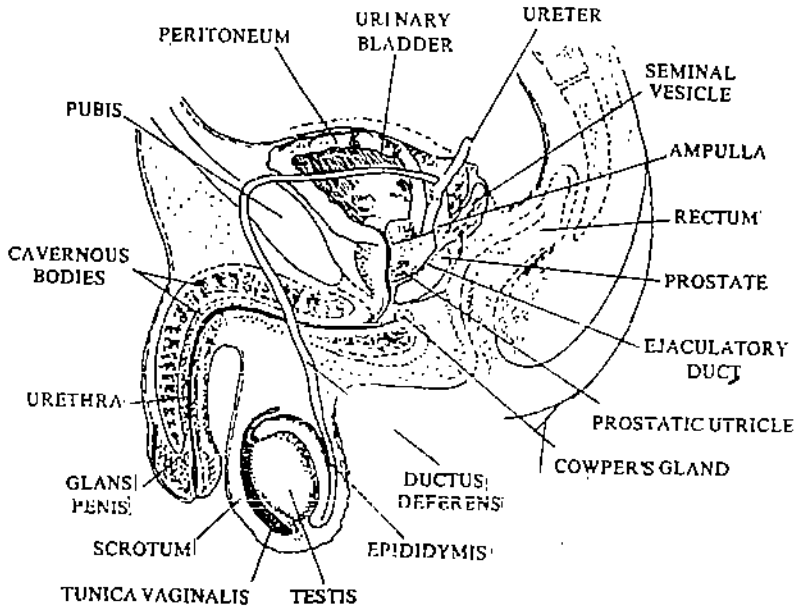



Fig. 8.16 : Diagrammatic section of the male pelvis showing genital organs.

**SAQ 4**

From the list given below identify the organs belonging to the male reproductive system and female reproductive system. Write M for male and F for female.

| | | | | | |
|---|---|---|---|---|---|
| a) | Fallopian tubes | [ ] | b) | Endometrium | [ ] |
| c) | Cervix | [ ] | d) | Labia majoria | [ ] |
| e) | Epididymis | [ ] | f) | Seminal vesicles | [ ] |
| g) | Cowpers' gland | [ ] | h) | Prostate gland | [ ] |
| i) | Vas deferens | [ ] | j) | Urethra | [ ] |

## 8.4 REPRODUCTIVE CYCLES

In the earlier sections you have learnt about structure and function of the reproductive organs. In this section we shall learn about the reproductive activities.

Gamete production is not a continuous phenomenon. It is recurring and cyclical. In most of the animals maturation of the gametes takes place during the seasons which are most favourable for the development and growth of the young.

Based on the frequency of gamete maturation followed by breeding activity, animals are termed as annual breeders, biannial breeders and so on. Most of the non-mammalian vertebrates exhibit annual reproductive cycle. The gonads of such animals mature once in a year and hence they reproduce once in a year. Studies have shown that it is the ovary which exhibits gamete differentiation in phases whereas spermatogenesis is a continuous process.

In non-mammalian vertebrates the **ovarian cycle** consists of the following phase; (i) **prebreeding phase** (ii) **breeding phase** and (iii) **postbreeding phase.**

During prebreeding phase growth and differentiation of the ovarian follicles takes place. The follicles start accumulating yolk and at the end of this phase the follicles are ready for ovulation and fertilisation.

Breeding phase is the period of ovulation, mating and fertilisation. This phase is followed by the postbreeding phase in which ovary contains the spent follicles, or **postovulatory follicles** (the follicles from which the ovum is expelled during ovulation), some follicles which failed to ovulate, and atretic follicles. Degeneration and disintegration of postovulatory and atretic follicles also take place during postbreeding phase. In some of the animals there is a **resting phase** in which no gametoganic activity takes place. All the germ cells are at rest for some time before entering into the follicular or the prebreeding phase. In the male animals testicular activity is a continuous process. Spermatogenesis takes place throughout the year, but **spermiation** (release of sperms) parallels with the ovulatory phase of the females.

The reproductive cycles are regulated by hormones from the pituitary and the gonads. Apart from the endogenous mechanisms, external stimuli also effect the gonadal activity.

In the earlier paragraphs of this section you have learnt about reproductive cycles in non-mammalian vertebrates. We shall now study about mammals.

Mammals exhibit two types of ovarian cycles: i) **Estrous Cycle**, exhibited by non-primates such as rats, cats, dogs, pigs, and ii) **Menstrual Cycle**, found in the primates (Monkeys, Chimpanzees and humans). Let us study estrous cycle in rat and menstrual cycle in humans.

### Estrous cycle

The estrous cycle of the rat is completed in four to five days, although the timing of the cycle may be influenced by external factors such as light, temperature, nutritional status, and social relationships. In species having such short cycles, the ovaries contain follicles in various stages of formation, as well as corpora lutea of several past estrous cycles. The cycle is roughly divisible into four stages.

i) **Estrus :** This is the period of heat, and copulation is permitted only at this time. This condition lasts from 9 to 15 hours and is characterised by a high rate of running activity.

Under the influence of FSH, a dozen or more ovarian follicles grow rapidly; estrus is thus a period of heightened estrogen secretion. Behavioural changes include quivering of the ears and lordosis (arching the back in response to handling or to approaches by the male). The uteri undergo progressive enlargement and become distended owing to the accumulation of luminal fluid. Many mitosis occur in the vaginal epithelium and as new cells accumulate, the superficial layers become squamous and cornified. The latter cells are exfoliated into the vaginal lumen, and their presence in vaginal smears is indicative of estrus (Fig. 8.17). During late estrus, there are cheesy masses of cornified cells with degenerate nuclei present in the vaginal lumen, but few if any leukocytes are found during estrus. Ovulation occurs during estrus and is preceded by histologic changes in the follicle suggestive of early luteinisation. Much of the luminal fluid in the uteri is lost before ovulation.

ii) **Metestrus :** This occurs shortly after ovulation and is intermediate between estrus and diestrus. The period lasts for 10 to 14 hours and mating is usually not permitted. The ovaries contain corpora lutea and small follicles, and the uteri have diminished in vascularity and contractility. Many leukocytes appear in the vaginal lumen along with a few cornified cells (Fig. 8.17).

iii) **Diestrus :** This lasts 60 to 70 hours during which functional regression of the corpora lutea occurs. The uteri are small, anaemic, and only slightly contractile. The vaginal mucosa is thin, and leukocytes migrate through it, giving a vaginal smear consisting almost entirely of these cells (Fig. 8.17).

iv) **Proestrus :** This precedes the next heat and is characterised by functional involution of the corpora lutea and preovulatory swelling of the follicles. Fluid collects in the uteri and they become highly contractile. The vaginal smear is dominated by nucleated epithelial cells, which occur singly or in sheets.

In case pregnancy occurs, the cycles are interrupted for the duration of gestation, which lasts for 20 to 22 days in the rat. The animals come into estrus at the end of pregnancy, but the cycles are again delayed until the termination of lactation (Fig. 8.17).

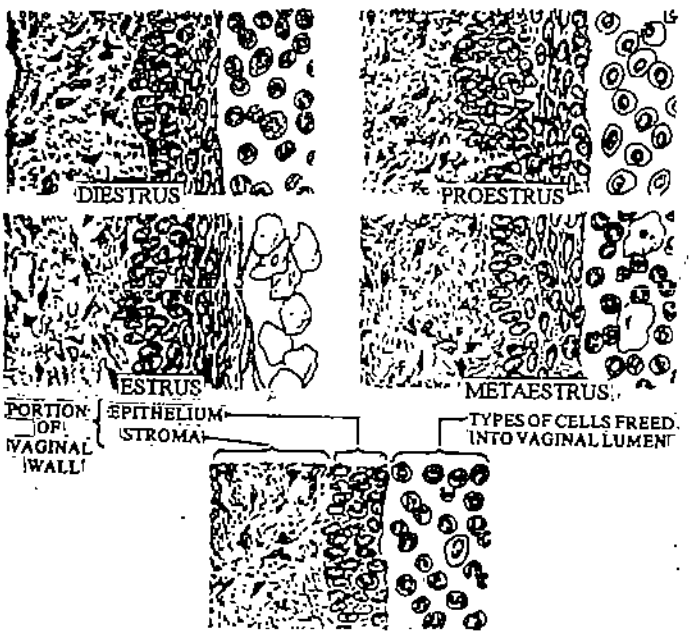



Fig. 8.17 : Section through vaginal wall of the rat during different stages of the estrous cycle.

## Endocrine Regulation of the Cycle

You have learnt above that the reproductive cycles are governed by the interplay of pituitary and gonadal hormones. According to current concepts, a feedback mechanism operates whereby the pituitary release of FSH and LH is controlled by the levels of estrogen and progesterone in the circulation. It is not known what factors are originally responsible for the activation of the pituitary-ovarian axis, but it has been postulated that very low levels of estrogens, coming from the immature follicles or extragonadal sources may stimulate the pituitary to augment its release of FSH and LH. When the level of estrogen in the blood becomes high, indicating that the ovarian follicles are full-grown, it acts to prevent a greater release of FSH by the hypophysis and to promote an augmented release of LH. Under the influence of rising titers of LH, preovulatory swelling ensures and definite lutein changes

occur in the walls of the mature follicles. The preovulatory follicle secretes some progesterone as well as large quantities of estrogens. Ovulation occurs while LH is in ascendancy and there is an immediate fall in the circulating estrogens after ovulation. The ruptured follicle becomes transformed into a corpus luteum, which becomes functional under the influence of **prolactin,** a hormone from pituitary which is also known as **luteotropic hormone.** The discharge of LH from the pituitary seems to be inhibited by rising titers of progesterone.

The corpora lutea remain functional for only a short period unless pregnancy or pseudopregnancy supervenes, but the ovaries of cyclic rats always contain several sets of corpora lutea in different stages of disintegration. Changes in the ovaries must be regarded as resulting from the interaction of the gonadotropins and changes in the sex accessories as consequences of the interaction of the various ovarian hormones.

There is ample evidence that in many mammalian species the secretion of progesterone by the follicle begins before ovulation has occurred during the period of preovulatory swelling. Even in species that ovulate spontaneously it is an interesting fact that sexual receptivity precedes ovulation. In the cow ovulation is spontaneous, but it does not occur until 13 to 15 hours after the end of heat. The secretion of progesterone by the ovarian follicles of the rat, guinea pig, and perhaps other species probably coincides with the onset of sexual receptivity.

The uteri of the rat become quite small and anaemic during diestrus, indicating that while the corpora lutea persist they secrete progesterone only for a brief time in the reproductive cycle. When pregnancy or pseudopregnancy follows a period of estrus, the corpora lutea remain functional much longer, probably owing to the action of prolactin. The progestational changes in rats and mice are much less extensive than those that occur in the uteri of such forms as the rabbit. However, the progestational uteri conditioned by estrogen plus progesterone, are equally sensitive to implanting blastocytes or to endometrial trauma.

### The Menstrual Cycle

Menstrual cycles are characteristic of primates and do not occur in other vertebrate groups. The length of the cycle is highly variable, though 28 days is generally regarded as typical for human female. The cycle in the chimpanzee requires about 35 days. Both estrous and menstrual cycles are regulated by the same interplay of pituitary and ovarian hormones, and the effects of the ovarian hormones on the reproductive tract are comparable in most respects. The chief differences between the two types of cycles are; i) the presence of a **menstrual phase** in primates, and ii) the spreading of sexual receptivity throughout the cycle, rather than the limitation of it to a definite period as found in nonprimates.

During the menstrual phase the superficial layers of the endometrium are sloughed rupturing spiral arteries, resulting in bleeding. This type of bleeding does not occur in nonprimates. Spiral arteries are absent from the uteri of estrous mammals but are present in primates with the exception of the New World monkeys. New World monkeys menstruate, but the loss of blood is greatly reduced.

The menstrual phase lasting four to seven days is regarded as the beginning of the primate cycle. This arrangement is sanctioned because menstruation is the easiest period of the cycle to recognise and because it corresponds with the formation of new follicles in the ovaries (Fig. 8.18). However, if the uterus alone is considered, menstruation represents the terminal event with subsidence of the corpus luteum and a consequent deficiency of ovarian hormones. The endometrium cannot maintain itself and hence regresses and the surface disintegrates.

Four phases of the menstrual cycle are usually distinguished : the **menstrual, proliferative (follicular), ovulatory** and **progestational (luteal).**

The proliferative phase is conditioned by estrogen and extends from the end of menstruation to ovulation. Ovulation occurs near the middle of the cycle. At the end of menstrual disintegration, the endometrium is thin and poorly vascularised and only the basal parts of the endometrial glands remain. The endometrium thickens as the estrogen titers rise and the glandular and vascular patterns are restored.

No conspicuous changes occur in the endometrium during the ovulatory process. Cyclic variations in the body temperature of the human female correlate with menstrual changes. A distinct rise in basal body temperature occurs at ovulation and remains high until the onset of the next menstrual period. The changing titers of hormones during the menstrual cycle apparently account for the temperature fluctuations.

During the progestational phase the uterus is under the influence of both estrogens and progestogens and the endometrium differentiates into a tissue that can fulfil the requirements of an embryo ready to implant. The progestational endometrium, normally requires both estrogens and progesterone. It is the only type of structure in which blastocysts can readily implant and develop normally. If implantation has not occurred the corpus luteum diminishes in function and degenerative changes are observable in the endometrium. With menstruation the outer portion of the endometrium is lost and there is bleeding into the uterine cavity.

### Endocrine Interactions

At the beginning of menstruation the inhibitory influence of the corpus luteum on the pituitary is removed and FSH is secreted in increasing amounts. This stimulates the growth of the young follicles, and they grow, they release increasing quantities of estrogens. The high estrogen content of the blood causes the pituitary to diminish its production of FSH and increase the output of LH. Ovulation occurs when the balance between FSH and LH has swung sufficiently in favour of LH. There is evidence that small amounts of progesterone are produced by the preovulatory follicle, and this hormone may be involved in the ovulatory process perhaps through its action on the brain or the anterior hypophysis. After ovulation, the corpus luteum begins to form in the ruptured follicle under the influence of LH.

Gonadotropins activate the corpus luteum and cause it to secrete progesterone and small amounts of estrogen. If a fertilised egg is not produced, functional degeneration of the corpus luteum begins eight to ten days after ovulation. The onset of menstrual bleeding correlates with the withdrawal of progesterone and, to a lesser extent of estrogen in the breakdown of the endometrial blood vessels with subsequent bleeding remain largely unknown.

If the egg is fertilised, the pituitary continues to release luteinizing hormone, and the corpus luteum increases in size and augments its output of hormones. Secretory competence of the corpus luteum diminishes slowly after the fourth month of pregnancy, although it remains structurally intact until the end of pregnancy. The **placenta,** rather than the ovary, is the principal source of progesterone and estrogen during the latter half of pregnancy. Removal of the ovaries after midpregnancy neither terminates pregnancy nor diminishes the levels of the two types of steroid hormones in the circulation (Fig. 8.18).

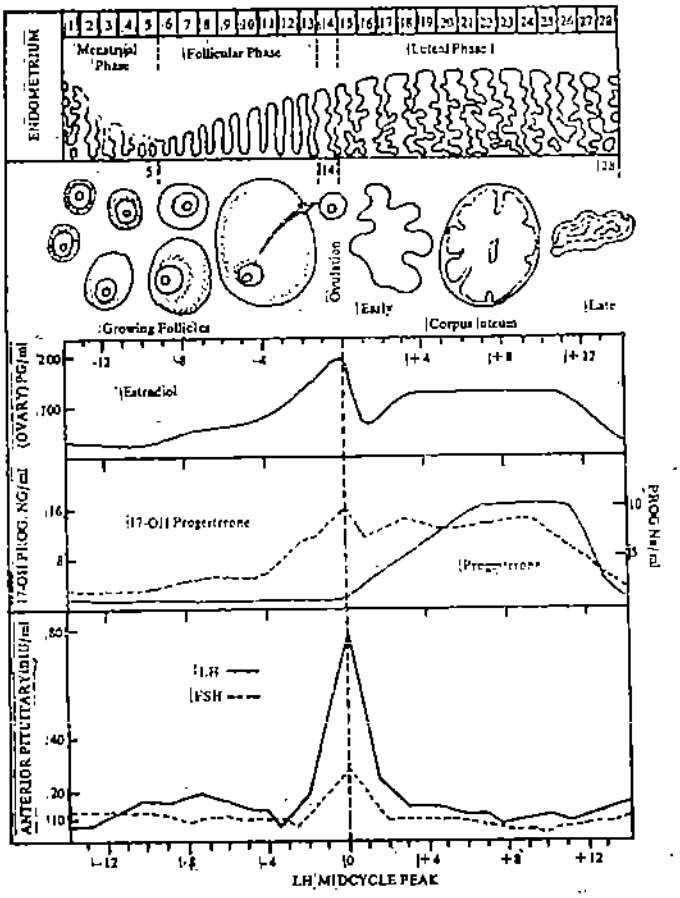



**Fig. 8.18 : Diagram showing changes in the endometrium, the ovaries and the circulating ovarian hormones during the menstrual cycle.**

**Contraceptive Pill**

About sixty million women in the world are currently using oral steroid contraceptives. These contraceptives usually consist of a synthetic estrogen combined with a synthetic progesterone in the form of pills that are taken once each day for three weeks after the last day of menstrual period. This procedure causes an immediate increase in blood levels of ovarian steroids (from the pill), which is maintained for the normal duration of a monthly cycle. As a result of negative feedback inhibition of gonadotropin secretion, ovulation never occurs. The entire cycle is like a false luteal phase, with high levels of progesterone, estrogen and low levels of gonadotropins.

Since the contraceptive pills contain ovarian steroid hormones, the endometrium proliferates and becomes secretory just as it does during a normal cycle. In order to prevent an abnormal growth of the endometrium, women stop taking the pill after three weeks. This causes estrogen and progesterone levels to fall, and permits menstruation to occur. The contraceptive pill is an extremely effective method of birth control, but it does have potentially serious side effects—including an increased incidence of thromboembolism, cardiovascular disorders, and endometrial and breast cancer. It has been pointed out, however, that the mortality risk of contraceptive pills is still much lower than the risk of death from the complications of pregnancy—or from automobile accidents.

**SAQ 5**

What are the two main differences between the estrous cycle and the menstrual cycle? Explain in the space given below.

.....

.....

.....

.....

.....

## 8.5 SUMMARY

You have learnt in this unit that :

- Organisms reproduce asexually and sexually. Fission, budding, fragmentation and parthenogenesis are the asexual methods of reproduction. In sexual reproduction there is involvement of two parents of different sexes; male and female.
- The gonads of the male (testes) produce male gametes; the sperm, and the female gonad (ovary) produces the egg.
- Most of the animals are dioecious, i.e. having separate sexes, but some animals have both the sexes in the same animal, a condition called hermaphroditism.
- During evolution animals have increased the efficiency of reproduction to provide nutrition and protection to the young before or after birth. The adaptations in vertebrates are internal fertilisation, cleidoic eggs and foetal membranes (viviparity).
- Sex of an individual is determined at fertilisation depending upon the genetic constitution. A zygote bearing XX chromosomes differentiates into a genetic female and XY into a genetic male. The genetic determination also gets modified or reversed by some internal and external environmental factors.
- Ovary produces eggs, elaborates hormones and yolk.
- The ovarian activity is regulated by pituitary gonadoropins, and by its own hormones (estrogens) by negative feedback mechanism.
- Testis produces spermatozoa and synthesises and secretes steroid hormones. Its activity is also regulated by pituitary gondotropins and its own hormones (androgens) by negative feedback mechanism.

- The accessory reproductive organs consist of ducts and glands specialised for storage and conveyance of the gametes. The female accessory reproductive organs are the oviducts, the uterus, the vagina and the external genitilia. The male accessory reproductive organs are multiple ductuli efferentes, paired epididymis, vasa deferentia, seminal vesicles, ejaculatory ducts, cowpers' glands, prostate gland, the urethera and the penis.
- Gamete production is not a continuous phenomenon. It is recurring and cyclic, takes place during the seasons which are most favourable for the development and growth of the young.
- Most of the non-mammalian vertebrates exhibit annual reproductive cycle in which maturation of the gametes and breeding activity takes place only once in a year. Gametogenic activity in the males is usually continuous. The mammalian females exhibit two types of ovarian cycles;
  - i) The estrous cycle, exhibited by nonprimate mammals and
  - ii) The menstrual cycle, exhibited by primates. The chief difference between the two cycles are (i) the presence of a menstrual phase (bleeding) in primates and (ii) the spreading of sexual receptivity throughout the cycle in the menstrual cycle, rather than limited to a definite period found in estrous cycle.

## 8.6 TERMINAL QUESTIONS

1) Name the various types of asexual mode of reproduction and briefly describe each of them in the space provided.

.....

.....

.....

.....

2) Draw a section of a mammalian ovary and label the various structures found in it.

3) Briefly write about FSH, LH, estrogens, and progesterone. How do these hormones interact in maintaining reproductive cycles ?

4) Briefly discuss the estrous cycle.

## 8.7 ANSWERS

**SAQs**

1) (i)-(b), (ii)-(a), (iii)-(i), (iv)-(h), (v)-(g), (vi)-(f), (vii)-(e), (viii)-(d), (ix)-(c)

2) a) Testis, ovary
   b) bilaterally, birds
   c) Graafian follicle
   d) corpus luteum

e) atretic follicles

f) oogonium

g) estrone, estradiol, estriol

h) progesterone, 20α-hydroxypregn-4-en-3-one, 20β-hydroxypregn-4-en-3-one

i) vitellogenesis

3) a) It regulates the internal temperature of the testes. During warmer days scrotum keeps the testes away from the body to keep them cool and during cooler days it ascends then touching the body and keeps them warm.

(b) There are the steroid hormone synthesising cells.

(c) These are the supporting cells for spermatogenesis. These cells are also known to synthesise androgens and supply nutrition to the germ cells.

(d) These are masculinising hormones, maintain spermatogenic activity and accessory reproductive organs.

4) (a) F, (b) F, (c) F, (d) F, (e) M, (g) M (h) M, (i) M, (j) M

5) i) Presence of menstrual phase (bleeding) in menstrual cycle which is not found in estrous cycle.

ii) Animals are receptive for sex throughout the menstrual cycle, where as animals exhibiting estrous cycle are receptive only for a very short period of the cycle.

**Terminal Questions**

1) Please refer to Subsection 8.2.1

2) Please refer to Fig. 8.2

3) Please refer to Section 8.4

4) Please refer to Section 8.4

# UNIT 9 COMMUNICATION—I

## Structure



## 9.1 INTRODUCTION

In the previous units of this course you have studied that all multicellular animals possess organ systems of varying degrees of complexity to perform the physiological processes necessary for life. Such complexity requires an internal communication system that coordinates the activities of each system with those of the others and with the environmental conditions. Perception of environmental as well as internal information is necessary for the proper physiological functioning of an organism.

The nervous system is one such anatomically oriented system of communication in nature. You can think of it as a telephone exchange. When you dial a number your message goes coded as an electric current to a specific place or 'target' where it is interpreted by the person who picks up the receiver of the telephone. Similarly in the nervous system messages are sent through cables i.e. nerve cells and nerve to a specific place for an instant reaction.

In this unit you will study the structure and functioning of the nerve cells that make up the complete nervous system. You will learn how messages are generated and transmitted along these nerve cells to specific organs or cells in response to a stimulus. The language in which the message is sent is the same regardless of the stimulus and the interpretation of the message lies in the brain which directs an appropriate response.

You have been introduced to the structure of brain, the organisation of central nervous system and peripheral nervous system in FST-1, Block-6. It would be useful for you to read Units 23-24 again before you start a study of this unit.

In the physiology of communication we have deliberately omitted certain functions of the central nervous system, the so called higher functions of the brain such as consciousness sleep, and memory. These topics can form a full course in their own right! However, you shall study the role of neural circuits in nervous integration briefly with the help of some of the involuntary functions like the simple reflex arc and the sensory filtering system.

In the last unit of this block, we will deal with the complex of chemical meassengers, called hormones that are secreted by endocrine glands.

## Objectives

After studying this unit you should be able to:

- describe the parts of a neuron and their functional significance,
- describe the events that occur during the production of action potential and explain the significance of the all or none law,
- describe the conduction of action potentials along myelinated and non myelinated axons and explain why saltatory conduction improves the speed of conduction,
- list some neurotransmitters and describe the process that takes place at synaptic junction,
- describe a typical reflex arc,
- explain the importance of reticular system.

# 9.2 NERVOUS SYSTEM AND NERVE CELLS

Living matter has the intrinsic capability to respond to changes in environmental (physical, biological and chemical) factors. Single cell organisms such as *Amoeba, Paramecium* also respond to the environment, interact with it and generate appropriate responses. With the advent of multicellularity and increase in the size of animals, arose the need for coordination and rapid conduction of information within the organism. To fulfil this need, some of the cells in the multicellular organisms became more and more specialized, acquired particular ability for receiving environmental information, for conducting it through the organism and finally for generating appropriate responses. And thus a cell type, the nerve cell or **neuron** came into being. The neuron itself underwent further morphological and physiological specialisation resulting in the evolution of a great variety of neurons (nerve cells) differing markedly in size and morphological appearances according to the specialized job they were required to perform. These cells became organised in what is known as the nervous system.

Glial cells are about 5 times more than neurons. Brain tumors that occur in adults are usually composed of glial cells rather than neurons.

In the nervous tissue, besides the nerve cells there is one other cell type — the **neuroglia** which are present in large numbers and occupy practically all the space between the nerve cells. Neuroglia cells themselves are of several types better recognised in vertebrate nervous tissues than in the invertebrate nervous tissues. Some categories of glial cells are listed in Table 9.1.

**Table 9.1 : Some types of glial cells and their functions**

| Neuroglia | Function |
|---|---|
| Schwann Cells | surround axons of all peripheral nerve fibres, form the myelin sheath. |
| Oligodendrocytes | form myelin sheath around central axons producing the white matter of central nervous system. |
| Astrocytes | cover capillaries of brain to form the blood brain barrier and help regulate passage of molecules from blood to brain. |
| Ependyma | line the ventricles or brain cavities and central canal of spinal cord. |
| Microglia | phagocytic amoeboid cells in central nervous system that remove foreign and degenerate material from the brain. |

### Neurons

Although neurons occur in a great variety of sizes and morphological forms, they have a basic plan of structure representing certain common characteristics. You have studied this basic structure in FST-1 Unit 23. In general, the neurons exhibit the following cytological features (Fig. 9.1). The cell body of the neuron is called **perikaryon.** A large number of processes called **dendrites** emerge out of the perikaryon. these are relatively short, frequently branched, irregular in diameter and tapering. Besides these processes there is another structure, the **axon** that emerges from the perikaryon. It is a relatively long process and branches at its tip into terminal processes. The neuron all over is covered by a cell membrane named **neurilemma.**

Axons are of two types ; (i) myelinated and (ii) nonmyelinated. In the case of **myelinated axons** the neurilemma is ensheathed by another covering, **the myelin sheath**, composed of complex lipoproteins. The terminal branches, dendrites, cell body (perikaryon) and the initial region of the axon (the axon hillock) are devoid of myelin sheath. Although the dendrites are relatively short, unsheathed, tapering, irregular in diameter, and branched in a wide array of patterns, the cell body cytoplasm extends into them. The dendrites receive synaptic endings over much of their surface and thus constitute the receptive surface of the neuron. They are like receiving antennae as a nerve cell receives messages along the dendrites but sends messages along the axon.

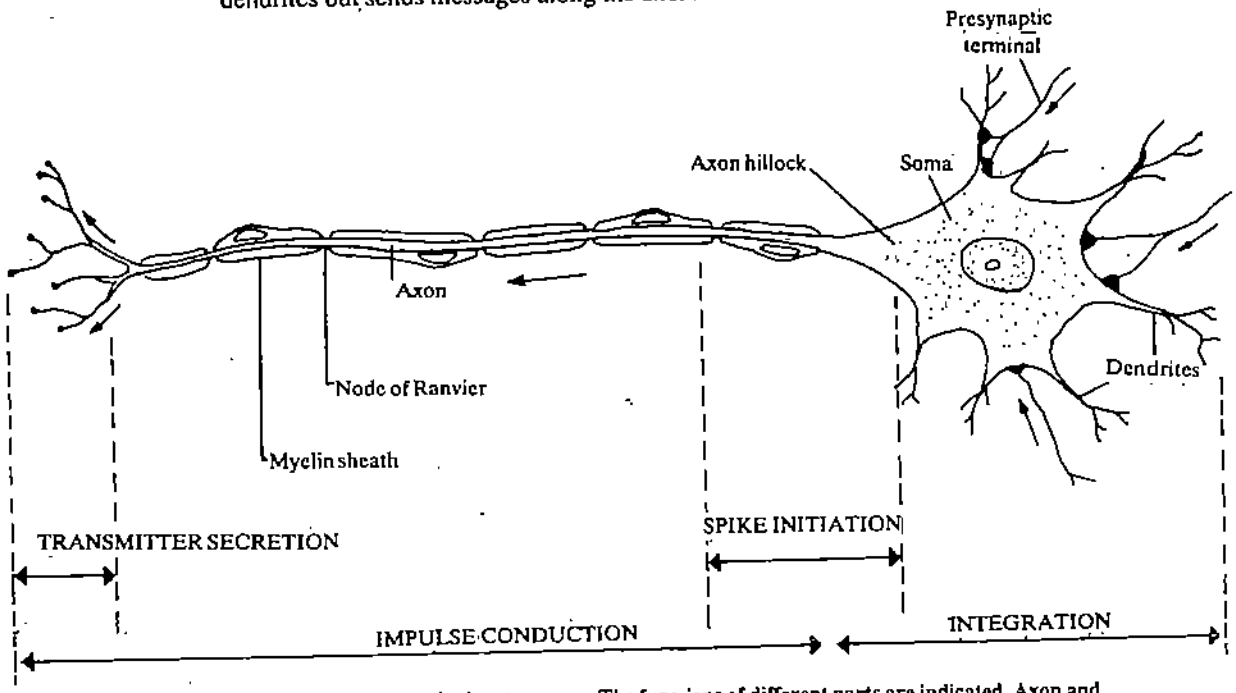



**Fig. 9.1: Vertebrate spinal motoneuron. The functions of different parts are indicated. Axon and surrounding sheath are shown in longitudinal section.**

Another structural feature of unique importance in the nervous tissue is the **synapse**. A synapase is the anatomical site at which axon terminal processes of one nerve cell establish a functional contact with another nerve cell. At this contact site the membranes of the two cells in contact are separated by a physical space. Special mechanisms operate to transmit signal from one i.e. presynaptic to the other i.e. postsynaptic. (Further description will be given when we deal with synaptic transmission.)

The cell body constitutes the trophic apparatus of the neuron in which most biosynthetic activity occurs producing chemicals needed for other parts of the cell, for example, the axon. Rough endoplasmic reticulum is present in the cell body and dendrites but is absent in axon. Since rough endoplasmic reticulum is the principal protein-manufacturing machinery of the cell, the axon gets its proteins from the cell body. Movement of a large variety of substances from the perikaryon to and along the axon constitutes phenomenon of **axonal transport**. Extending throughout the cytoplasm from dendrites to the axon, are present fine fibrilar structures called **neurofilaments and microtubules**.

The length of the axon provides one of the adaptive advantages of having neurons. The neurons that carry messages from the brain of giraffe to its toes are over 3 meters in length. The longest cells in the animal world!

Axon is a relatively long process (also quite often referred to as nerve fibre) and can be considered as functionally specialised for conduction of excitation over considerable distances. In vertebrates the axons of high velocity neurons are covered by insulating sheaths of lipid containing myelin. This myelin is made up of special glial cells—the **Schwann cells** (Fig. 9.2). The myelin covering is not continuous. There are tiny uninsulated gaps between adjacent Schwann cells. These are the **nodes of Ranvier**. The myelin covering and nodes of Ranvier help accelerate the speed of conduction of impulses by a mechanism which we will discuss later.

The terminal part of the axon branches, terminating into small ramifications whose tips form **end knobs** which synaptically contact with other nerve cells. In the end knobs are present characteristic structures called **synaptic vesicles** which contain and store chemical substances called **neurotransmitters.**

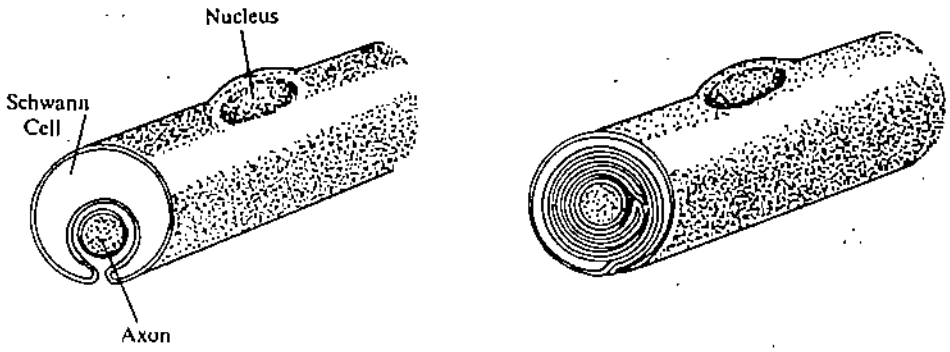



Fig. 9.2 : Myelinated fibre. The myelin sheath develops from Schwann cells that grow around the nerve fibre until it is covered by a multi-layered wrapping of supporting cell membrane.

The diversity in neuron form, however, is so much that there can be many variations from the generalized description given above. For example, the nerve cells called **amacrine cells** found in retina contain processes without a demonstrable axon.

**SAQ 1 Pick the correct answer**

a) In vertebrates myelin sheaths cover the

i) nerve cell body

ii) dendrites

iii) axons

iv) all of the above

b) Which of the following statements describes a synapse best

i) A synapse is a point of functional contact between nerve cells where nerve impulses are transmitted.

ii) A synapse is the functional connection between a neuron and a second cell for transmission of messages.

iii) A synapse is a functional contact between a nerve cell and another cell across which nerve impulses are transmitted via neurotransmitters.

## 9.3 NERVE IMPULSE

Neurons produce and conduct nerve impulses which are basic to all neurologic functions from sensory perception to physiological experiences. It is, therefore, of interest to understand as to how impulses are generated and conducted in the nerve cells before we examine how the nervous system as a whole is organised and functions.

For instance how can pricking your finger with a sewing needle generate an electrical change that tells your brain to experience pain? The source of electricity is certainly not in the needle. It lies in the nerve cell itself. The property responsible for generation of electrical currents is largely in the neuron's plasma membrane which shows regional functional specialisation (see Fig. 9:1).

### 9.3.1 Membrane Potential

Even in their resting state i.e. when they are not conducting any impulses, nerve cells are generating an electric charge. Actually a resting neuron is not realy resting but maintaining its **excitability** i.e. the capacity to conduct an impulse in response to a stimulus.

The nervous ability to accept and relay information results from differences in ion distribution between inside and outside the cell at rest and the changed permeability of the plasma membrane when a stimulus is given. You have learnt in LSE-01 Units 6 and 7 that the interstitial fluid around the cell has relatively higher concentration of sodium ($Na^+$) and chloride ($Cl^-$) ions, but low concentration of potassium ions ($K^+$). Inside the cell the concentration of $K^+$ is more than $Na^+$ and $Cl^-$ (Fig. 9.3). These differences are pronounced

as there is approximately 10 times more $Na^+$ outside the cell than inside and 25 to 30 times $K^+$ inside than outside the cell.

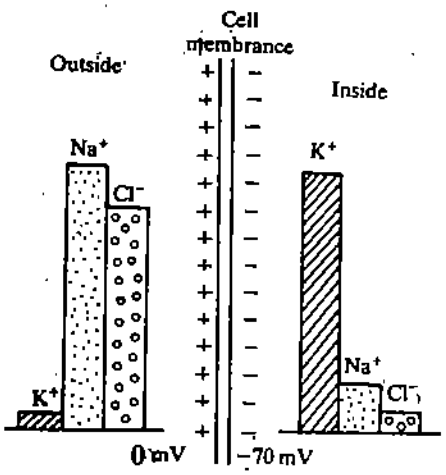



**Fig. 9.3 : Ion distribution inside and outside a neuron. An active sodium pump drives $Na^+$ outside keeping concentration low inside the cell. Even though $K^+$ leaks outside, the concentration is more inside.**

When at rest, the nerve cell is selectively permeable to $K^+$, which can pass through the membrane through passive ion specific channels. The permeability to $Na^+$ and $Cl^-$ at this time is almost zero because these channels are closed and $K^+$ tends to diffuse out down its concentration gradient as there are less $K^+$ outside the cell. Each $K^+$ that leaves the cell without $Cl^-$ following it, gives a positive charge to the outside of the membrane. This positive charge quickly reaches a level that prevents further outflow of $K^+$. The resting membrane attains equilibrium and the positive charge outside exactly balances the concentration gradient that drives the $K^+$ out. This gives the **resting membrane potential** which can be calculated by Nerst's equation. (Refer to Units 6-7 LSE - 01.)

$$E = \frac{RT}{F} \log e \frac{[K]o}{[K]i}$$

In giant squid it is calculated to be –75 mV.

The most dramatic experiment to show that nerve membrane is the essential element in generating a resting potential and not the axoplasm was shown by replacing the entire content of the axon with artificial salt solution. Such an axon behaves amazingly like a normal axon. It can remain excitable for several hours.

Squids and many other active invertebrates have neurons with giant axons of such large diameter that they are easier to study then neurons of vertebrates. The basic functioning of all nerve cells, however, is the same. A.L Hodgkin and A.F. Huxley studied the giant axons of squids and received a Nobel prize for showing how this potential difference contributes to the functioning of the nerve cell.

The membrane potential can be measured by inserting a tiny micro-electrode into the axon and reading the potential relative to the outside. The observed resting potential in the giant squid axon is close to the calculated potential but several millivolts less, about –60 to –70 mV. (The negative sign preceeding the value denotes that the inside of the cell is negative with respect to outside.)

Let us see what causes the concentration difference of ions inside and outside the nerve cell. It is due to:

i) electrical attraction by negatively charged proteins and organic phosphates that are inside the cell and cannot move out

ii) the greater permeability of the cell membrane to $K^+$ than $Na^+$

iii) active transport by $Na^+/K^+$ pumps.

You would recall from LSE–01 Units 6 and 7 that the lipid bilayers of membranes have the property of capacitance and that proteins in the membrane have the property of electrical conductance as they permit physical passage of ions across the membrane. An excitable membrane has ion conductance channels or gates. These are composed of polypeptide chains that can open or close a membrane channel according to specific conditions. When the gates for an ion are open, the membrane becomes very permeable to that particular ion and when the gates are shut the permeability to that ion decreases. $K^+$ has two types of

channels. One, which lacks gates and is always open, the other type has gates that are closed in a resting cell. The resting cell, therefore, is more permeable to $K^+$ than $Na^+$ as channels for $Na^+$ are always gated and these are closed in a resting cell.

If a pair of stimulating electrodes are put in a region of the membrane, (One electrode within the axon and the other outside) a sudden and very rapid change is observed in the membrane potential. The membrane is **depolarised** that is, it causes the $Na^+$ gates to open and $Na^+$ flows in down its concentration gradient. (Fig. 9.4). At this time, $K^+$ gates are closed. The potential difference between the two recording electrodes is reduced. A fraction of a second later the $Na^+$ gates close and the $K^+$ gates open. This makes the membrane more permeable to $K^+$ than it is at rest and $K^+$ diffuses out of the cell along its concentration gradient. The $K^+$ gates then close and membrane potential is restored to what it was at rest or the membrane is **repolarised**. If the situation results in the membrane becoming more negative on the inside of the membrane the needle of the oscilloscope defects down leading to **hyperpolarisation**.

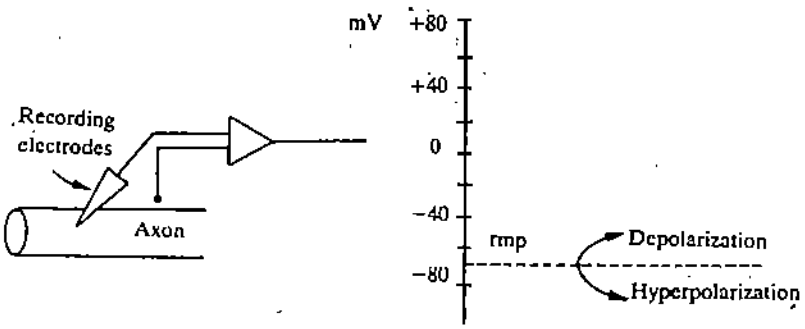



**Fig. 9.4 : The difference in potential between an intracellular and extracellular recording electrode is shown on an oscilloscope screen. The resting membrane potential rmp may be increased (hyperpolarisation) or decreased (depolarisation).**

You would note that the gates for $Na^+$ and $K^+$ are open or closed depending on the membrane potential. The gated channels are closed at resting potential and open when the membrane is depolarised to a certain **threshold level**. These gates are, therefore, said to be **voltage regulated**.

## 9.3.2 Action Potential

Events that underlie the genesis of a nerve impulse can now be outlined. When an appropriate stimulus interacts with the neurolemma, it is the resting membrane potential that is disturbed. When the $Na^+$ gates open to permit $Na^+$ to enter the axon membrane is further depolarised (the inside becomes more positive). This depolarisation allows even more sodium ions to enter the cell and a positive feedback loop is established (Fig. 9.5) which causes the rate of $Na^+$ entry and depolarisation to accelerate. The diffusion of $K^+$ out of the cell makes the inside more negative and original resting potential is restored. This repolarisation is therefore under a negative feedback loop (Fig. 9.5).

Feedback loops are discussed in greater detail in unit-10.

Fig. 9.6 shows the movement of Na and K ions in response to a stimulus. The rapid increase of $Na^+$ movement causes rapid depolarisation to zero millivolts(mV)and then overshoot so that the polarity of the membrane is reversed i.e. it becomes positively charged to almost +40mV.The conductance of $Na^+$ stops suddenly and the conductance of $K^+$ starts, resulting in repolarisation. These changes in $Na^+$ and $K^+$ conductance produce an event known as **action potential or nerve impulse** and the entire sequence takes place in one thousandth of a second. Once an action potential is completed the $Na^+/K^+$ pump starts working and extra $Na^+$ are pumped out and $K^+$ pumped in the cell. The $Na^+/K^+$ pump transports 3 $Na^+$ out of the cell for every 2$K^+$ it brings inside the cell. Thus it actually helps to maintain the potential difference. Small amounts of $Na^+$ and $K^+$, however, actually diffuse out and a cell has, therefore, a relatively constant intercellular concentration of $Na^+$ and $K^+$ and a constant membrane potential of –65mV to –85mV is maintained in absence of stimulation. You would notice by reading the above description that **active transport processes are not directly involved in the production of action potential**. Giant axons which had been poisoned with cynide to disrupt the sodium pump continue to show normal excitability and action potentials for several hours. But the system finally collapses because of $Na^+$ accumulation in

the cell. The $Na^+/K^+$ pumps are, therefore, needed to maintain the concentration gradients for diffusion of $Na^+$ and $K^+$ during the action potentials.

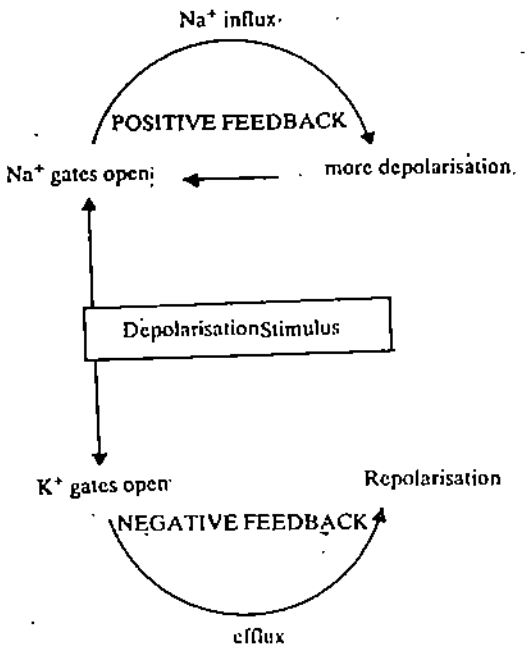



Fig. 9.5: Changes in conductance of sodium and potassium. The increase in sodium conductance is by positive feedback and in potassium conductance is by negative feedback.

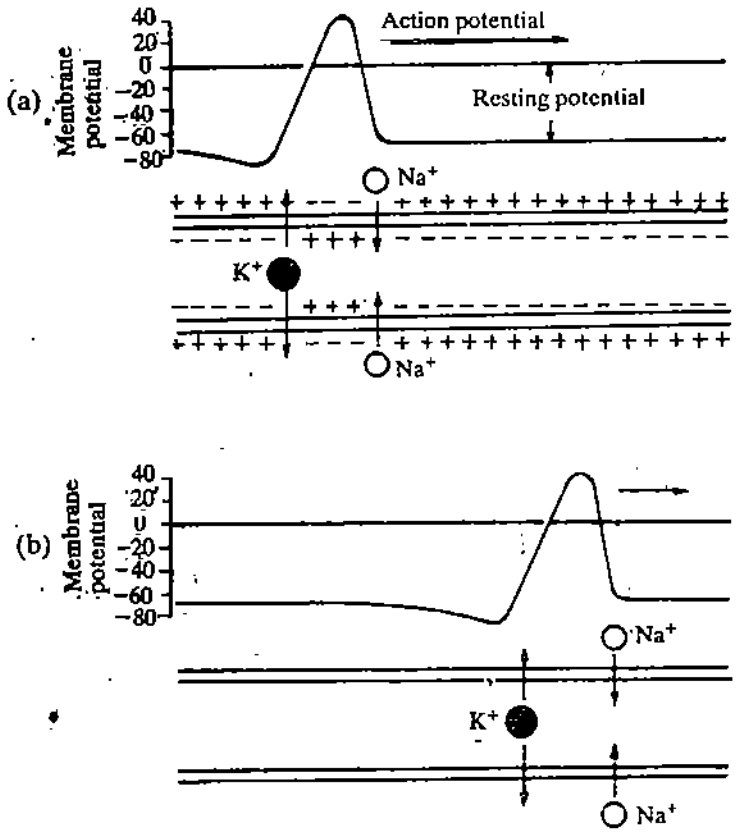



Fig. 9.6 : Recording an action potential in an axon (a) shows the electrical events and changes in ion permeability associated with it. The position of action potential in (b) is about 4 milliseconds after (a).

### 9.3.3 All-or-None Response

A very weak stimulus does not result in an action potential. The stimulus must have a certain strength to evoke an excitation. This is the **threshold value** below which no action potential results. Once generated the size of the action potential is not influenced by the magnitude or amplitude of the stimulus. Even if we double or triple the stimulus, the action potential remains the same. The stimulus causes either a full response or none at all. In physiology, this type of response is called **all-or-none response**. When a greater stimulus strength is applied to a neuron identical action potentials are produced more frequently.

When an entire collection of axons is stimulated as in a nerve, different axons will be stimulated at different frequencies. A low intensity stimulus will activate fibres with low threshold values and as the stimulus intensity is increased more and more fibres will be activated. This process is known as **recruitment**.

Another important property of nerve impulse to be noted is that during the time that a patch of axon membrane is producing an action potential, it is incapable of responding to another stimulus i.e. it is **refractory** to further stimulation. If the second stimulus is applied when the $Na^+$ gates are open the membrane remains in **absolute refractory period** but if the stimulus is applied when the $K^+$ gates are open the membrane is in **relative refractory period** and can be depolarised only if the stimulus is very strong.

### 9.3.4 Conduction

An action potential occurs at one point along the axon. Yet we know that neurological impulses are not fixed, they travel along a neuron. So how can the action potential at one point excite a neuron at another point? The action potential itself is also a stimulus. The $Na^+$ that rush in the axon after depolarisation are conducted by the **cable properties** (the ability of a neuron to transmit charges through the cytoplasm) to the adjacent region that still has a membrane potential of –70mV. The limits of transmitting electric charge through cytoplasm is one to two millimeters. When the stimulus is of threshold value in the adjacent region it too produces an action potential. Therefore, an action potential is a self propagating event.

#### Conduction in Unmyelinated Axons

The region of the membrane at which the action potential spike has been generated has reversed polarity i.e., its outside is negatively charged with respect to inside where it is positively charged. Adjacent to this depolarised region is the normal polarised region having positive charge on the outside. During depolarisation of the membrane the $Na^+$ that enter the cell carry a momentary strong current into the newly excited region of the axon. To complete the circuit the current must flow out across the unexcited portions of the membrane ahead of the region of sodium influx where it again stimulates another action potential and then flows back to the active region (see Fig. 9.7a).

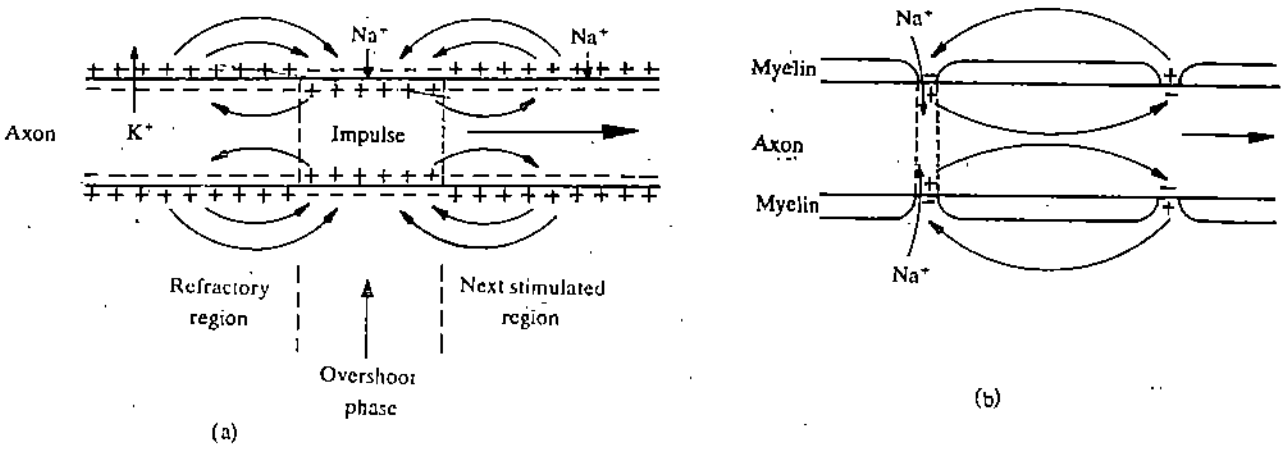



Fig. 9.7: Conduction of action potential in (a) unmyelinated nerve fibre, (b) myelinated nerve fibre.

Some of the current that enters the axon at the excited portion spreads backwards within the axon i.e. in the direction from which the impulse was generated but the membrane is not excited as it is in the refractory state. The potassium channels in that area are still open and carry this current out of the cell as an efflux of $K^+$. The action potentials are, therefore, not really conducted but regenerated along the axons length. The last action potential has the same amplitude as the first. Thus they are said to be **conducted without decrement.**

The biological role of high speed conduction is obvious. It is always related to quick response mechanism in locomotion to avoid predators. One of the fastest responses is that of the cockroach which reacts to a puff of air on the tip of the abdomine within 25 meters.

The spread of conduction in unmyelinated fibres is faster if the axon is thicker because the ability of fibres to conduct by cable properties improves with increasing diameter as can be seen in the giant axons of squids in comparision to ordinary axons from the same animal.

### Conduction in Myelinated Axons

In Section 9.2, we told you that myelin sheath provides insulation for the axon preventing movements of $Na^+$ and $K^+$ through the membrane. Therefore, if this myelin sheath was continuous no action potential would be generated. Fortunately, gaps or nodes of Ranvier occur in the myelin. Since the cable properties of axon can conduct depolarisations over very short distance (1–2 mm), the nodes of Ranvier must be close together (actually they are 1 mm apart). Studies have shown that the $Na^+$ channels are concentrated at the nodes and absent from the regions between two nodes. Action potential therefore occurs only at the nodes of Ranvier and seems to leap from node to node (Fig. 9.7b). This is called **saltatory conduction**. The spread of depolarisation between the nodes is very fast and fewer action potentials are needed. Therefore, the rate of conduction is much faster than in unmyelinated fibres.

Imagine the size of the optic nerve in humans if a high conduction velocity were to be achieved without myelination. The optic nerve has a diameter of 3 µm, if it were to contain the same number of fibres without myelination and conduct at the same speed it would require a diameter of 300 mm.

This peculiar structure and mode of conduction in myelinated fibres is responsible for the fast conduction of impulses in motor nerves of vertebrates even though the nerves are very thin. The greatest advantage of myelinated fibres comes from their smaller size and so a highly complex nervous system with high conduction velocities can occur without occupying too much of space.

**SAQ 2 : Choose the correct answer :**

a) Depolarisation of an axon is produced by:

i) inward diffusion of $Na^+$

ii) inward diffusion of $K^+$

iii) inward active transport of $Na^+$

iv) active extrusion of $K^+$

b) Repolarisation of an axon during an action potential is produced by:

i) inward diffusion of $Na^+$

ii) active transport of $Na^+$ out of the cell

iii) active transport of $K^+$ out of the cell

iv) outward diffusion of $K^+$

c) What would happen if we increase the strength of the stimulus to an axon?

d) Fill in the blanks with suitable words:

i) The leaping of action potential between the nodes of Ranvier is known as .....

ii) Action potentials are conducted without .....

iii) The ..... like spread of depolarisation induced by $Na^+$ influx during an ..... helps to ..... the adjacent regions.

## 9.4 SYNAPTIC TRANSMISSION

Now that you know how a nerve impulse is generated and conducted along the axon we come to the most important aspect of the functioning of the nervous system. The transfer of information from one neuron to another neuron or another cell after it reaches the end of the axon.

We mentioned in the beginning of the unit that the ends of axons split into fine extensions which form synapses with other cells. You also know that a synapse is an area where the membrane of the axon terminal lies very close to the membrane of the other neuron, muscle or gland. A synapse close to a muscle cell is called **neuromuscular junction**.

Information passes across a synapse in two ways, either chemically or electrically. Chemical transmission however, is more common and takes place through chemicals called **neurotransmitters**. The end of the axon terminals are known as **synaptic knobs or**

**boutons** (bouton = button) (Fig. 9.8). The membranes of the synaptic knobs do not touch the membrane of the other cell but are separated by a narrow space, 20 nanometer (nm) apart, known as **synaptic cleft**. The axon knob is referred to as **presynaptic** and the dendrite or other cell with which it communicates is **postsynaptic**.

1 nanometer = $10^{-9}$ meter

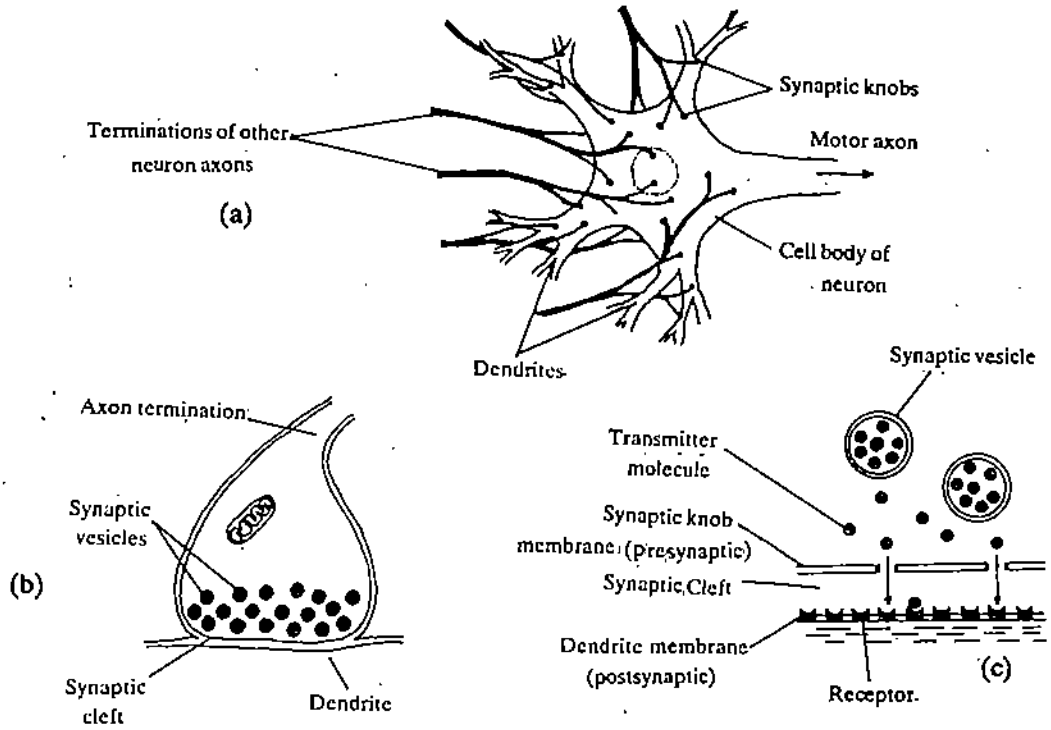



**Fig. 9.8 : Transmission across nerve endings.**

a) **Cell body of a neuron with many terminal synaptic knobs.**

b) **a synaptic knob enlarged.**

c) **a synaptic cleft as it might appear under a high resolution electronmicroscope. Neurotransmitters move across the cleft to bind to receptors in the postsynaptic cell membrane.**

The appearance and width of synaptic cleft is remarkably similar throughout the animal kingdom but all the chemicals released in each cleft are yet not known. The synaptic knob contains a large number of small vesicles that are usually 20-100 nm in diameter. These vesicles enclose the neurotransmitters.

The neurotransmitter molecules are synthesised in the presynaptic terminal itself from the respective precursor molecules. For example, **acetylcholine** is synthesised from choline and acetyl coenzyme A, the reaction being catalyzed by the enzyme **choline acetyl-transferase**. The newly synthesised acetylcholine is stored within the synaptic vesicles. Each vesicle may contain 10,000 to 50,000 molecules of a neurotransmitter. The whole neurotransmitter content of a vesicle represents one quantum of the neurotransmitter.

The postsynaptic membrane has molecular receptors that specifically interact with the released neurotransmitter molecules. Binding of the neurotransmitter molecules with the receptors activates ion channels causing changes in the membrane permeability. Both excitatory and inhibitory receptors can exist on the postysynaptic cell. Activation of the excitatory receptors depolarises the membrane generating an excitatory response while excitation of the inhibitory receptors hyperpolarises the membrane resulting in inhibition of production of signals.

## 9.4.1 Chemical Synaptic Transmission

The arrival of an impulse at the presynaptic terminal depolarises the membrane. The depolarisation opens calcium channels allowing calcium ions to move through these channels into the axon terminal. The elevated $Ca^{2+}$ concentration causes the translocation (propulsion) of the synaptic vesicles toward the presynaptic membrane. The synaptic vesicles become oriented and aligned with the synaptic plasma membrane. A number of vesicles ultimately fuse with presynaptic membrane leading to the release of their neurotransmitter contents by exocytosis into the synaptic cleft. After releasing their neurotransmitter, the synaptic vesicles merge with the membrane. New vesicles are formed

from invaginations of the synaptic membrane, which are filled with the transmitter and can be reused again. Figure 9.9 summarises the process.

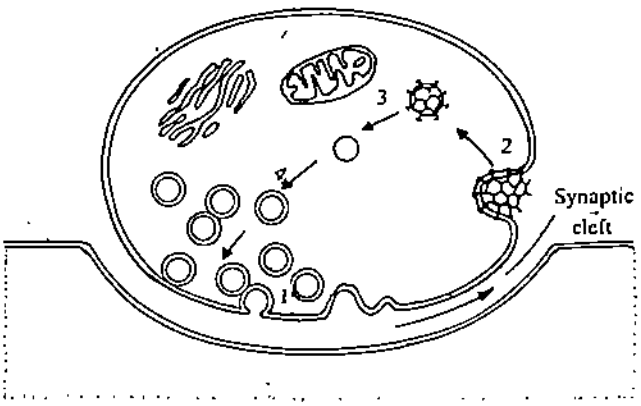



**Fig. 9.9 : Vesicular hypothesis of transmitter release.**

**1) vesicle fuses with presynaptic membrane releasing transmitter substance**

**2) endocytosis of membrane pinches off new vesicles**

**3 and 4) new vesicles fill with transmitter substance.**

The released neurotransmitter diffuses across the cleft and binds with the specific receptor molecules. This process takes a fraction of a millisecond to occur. Binding of the neurotransmitter to a postsynaptic receptor causes the opening of ion channels. These channels are therefore **chemically gated** and not voltage dependent. Flowing of ions across the postsynaptic membrane changes the potential of the postsynaptic membrane thus producing a new signal, the **postsynaptic potential (PSP)**, in the postsynaptic neuron. Our description about the transfer of information from a single synapse seems simple and does not reflect the complexity of the functions of the nervous system. You must recall that most axons are highly branched at their ends and may connect to a large number of other neurons. Similarly a single neuron would also receive branches from a large number of axons that terminate on its cell body or its dendrites (see Fig. 9.8 again).

Some specialised neurons are so densely covered with synaptic knobs that a single such neuron may have about 10,000 synaptic connections. You would appreciate the complexity of the central nervous system when you couple this with the number of neurons present which are more than $10^{11}$. These connections are not random but highly specific and have been studied in great detail by neuroanatomists and neurophysiologists.

## 9.4.2 Postsynaptic Potential

Let us now examine the postsynaptic potential in some detail. It has been found that some of the axons carry inhibiting impulses and some carry exciting impulses to a neuron. But you must remember that there is no difference in the impulse carried in exitatory and inhibitory impulses, the action potential is the same but opposing effects are achieved by the release of different chemical transmitters from the presynaptic endings. The ion gates that open up in the postsynaptic membrane depend upon the type of neurotransmitter involved.

Let us see how the postsynaptic potential (PSP) works by using the example of a well known neurotransmitter **acetylcholine** which has been studied at neuromuscular synapses. The interaction of acetylcholine results in the opening of both $Na^+$ and $K^+$ gates allowing the flow of ions simultaneously. The depolarisation effect predominates and the membrane potential takes a value of –25 mV which is nearer zero and the characteristic overshoot of action potential is not achieved. Because of this, the neurotransmitter cannot produce action potential but only depolarisation which may produce an action potential at a short distance away from the site of synapse. The magnitude of the PSP at the synapse is dependent on the amount of neurotransmitter released (see Box 9.1). Acetylcholine must also be removed quickly or there would be a continuous PSP. An enzyme **acetylcholinesterase** present at the synapse removes the transmitter.

Now if a second impulse arrives before the first one dies off, it adds to the first one evoking a stronger action potential at the axon hillock (see Fig. 9.1 again). This is known as **temporal summation.**

The PSP is a direct expression of the impulse frequency in the axon. The PSP also exhibits **spatial summation** because the potential can spread to adjacent regions of the membrane altering the membrane potential and if a new PSP arrives at a neighbouring synapse it would be added to the existing PSP. These two types of summation—temporal and spatial form the basis for computation in each neuron and so the entire nervous system.

As has been stated above that a neuron ordinarily has both excitatory and inhibitory synapses. An **excitory postsynaptic potential (EPSP)** generally represents a depolarisation caused by neurotransmitter receptor interaction which opens up channels for both $Na^+$ and $K^+$ (as in the case of acetylcholine). If, however, the neurotransmitter-receptor-interaction opens $K^+$ channels only, some $K^+$ will leave the cell increasing the membrane potential i.e. hyperpolarisation. This increase in potential is **inhibitory postsynaptic potential (IPSP).** If the channel opened by the neurotransmitter is for $Cl^-$, then also an IPSP will develop.

Thus, excitatory (EPSP) and inhibitory (IPSP) postsynaptic potentials are constantly developing at many points on the surface of the postsynaptic cell. The two types of postsynaptic potentials will have opposing effects on the net membrane potential. If the postsynaptic potential at the axon hillocks is of sufficient size to reach threshold value, an action potential is initiated and travels along the axon of the receiving neuron. If inhibitory inputs prevail then no action potential is generated. Thus synapses act as relay stations in the line of communication in the nervous system.

**Box 9.1**

The understanding of synaptic transmission became clear when methods were devised to record electric impulses from a single neuron. Extremely fine glass pipettes with tip diameter less than 1 micrometer are made. These are filled with salt solution (concentrated potassium chloride) to make micro electrodes that can be used to make recordings of potential changes between the tip and a common ground. When this tip is moved towards a neuron, at first it shows the same potential as the common ground but as soon as it penetrates the neuron membrane it shows a negative potential in relation to the ground. That is, the outside of the membrane is positively charged in relation to the inside. The membrane seems to seal around the pipette and the neuron gives normal recordings for several hours. By using pipettes with two or three channels it is possible to apply minute amounts of various chemicals at the site where recordings are being taken.

With this technique, events at the synapses can be studied. As an impulse arrives there is a delay of a fraction of millisecond before a change occurs in the potential at the postsynaptic membrane. This is the postsynaptic potential which rises rapidly at first but decays more slowly.

## 9.4.3 Electrical Synaptic Transmission

As described above, the mode of chemical synaptic transmission involves the secretion of a chemical transmitter from the presynaptic terminal to affect the permeability of the postsynaptic membrane causing an action potential to develop in the postsynaptic neuron. The mode of electrical transmission, however, involves the direct spread of ionic current from the presynaptic terminal to the postsynaptic membrane to depolarise the postsynaptic cell to the threshold that would generate an electric potential.

Microstructure of the electrical synapses differs from that of the chemically transmitting synapses. In an electrical synapse, the extracellular space between presynaptic and the postsynaptic membrane is greatly reduced (about 2 nm) making the cell membranes more closely approximated and the resistance of the two terminal membranes is also reduced. Furthermore the cleft width of 2-3 nm is bridged by the large tubular protein molecules which are embedded in the presynaptic and postsynaptic membranes, thus providing channels through which inorganic ions can pass. This is indicative of some cytoplasmic continuity. These structural features make it possible for the current to flow readily from the presynaptic to postsynaptic side and there is essentially no delay between potential changes in the presynaptic and postsynaptic neurons making the two neurons electrically continuous. The electrical synapse can transmit equally well in both directions. In some electrical synapses, however, it has been found that current passes more easily in one direction than in the other. At chemical synapses the distance across the synaptic cleft is too large (20 nm) to allow the presynaptic current to invade the postsynaptic membrane.

### Properties and functions of synapses

There is an interval between depolarisation of the presynaptic terminal and the earliest appearance of a postsynaptic response. This interval is called **synaptic delay.**

The synapse is the prime locus in the integration of neuronal signals. The synapse is also a site where functional properties are changed as a result of previous activity. For example, prolonged activity transmitted across a synaptic junction may result in a reduction of the threshold of firing of the neurons to further afferent inputs. This indicates that synapses are the site of changes in the storage of information (learning and memory). The synapse is thus modifiable i.e., it shows **plasticity.**

A variety of chemical agents (drugs etc.) act at synapses to alter nervous system's behaviour. A postsynaptic neuron is an integrator of information that is received from presynaptic neurons. Synaptic transmission is a one way process except in electric synapses.

Synapses are the site of several pathological disorders viz., Parkinsonism, myasthenia gravis, and psychiatric disorders like schizophrenia.

So far we have discussed the action potential and its effect at the synapse i.e. release of neurotransmitter. Acetylcholine is just one of the examples of a neurotransmitter. There are many more physiologically active chemicals that are produced by nerve cells. These have profound effects as hormones which we will study in Unit 10. In the next section we will learn about neurotransmitters.

**SAQ 3**

a) Why do chemical synapses transmit impulses in only one direction?

.....

.....

.....

b) Mark True or False against the statements given below:

i) The axon of one neuron communicates with another cell through a junction called synapse.

ii) Neurotransmitters are released from the postsynaptic cell on activation by an action potential.

iii) Neurotransmitters are either reabsorbed or broken down by enzymes after they have evoked a response.

iv) Excitatory neurotransmitters cause opening of $Na^+$ gates while inhibitory transmitters cause $K^+$ gates or $Cl^-$ gates to open.

## 9.5 NEUROTRANSMITTERS

We know that transmission of signals from nerves to muscles is affected by acetylcholine a transmitter substance. Similarly neuron-neuron transmission is also achieved by acetylcholine. But there are several other chemicals that function as neurotransmitters. A limited number has already been identified and new ones are being identified rapidly. Some of these are listed in Table 9.2

A chemical can be called a neurotransmitter in a given tissue only if it fulfils certain conditions like:

- When applied to the postsynaptic membrane it should be able to produce the same effect as does presynaptic stimulation
- It should also be released by the presynaptic membrane
- Its action should be blocked by the same agents that block neural transmission.

**Table 9.2 : Some neurotransmitters and their effects (+ denotes excitatory, and – denotes inhibitory effects).**

| Neurotransmitter | Action + or– | Target Cells | Effect |
|---|---|---|---|
| Acetylcholine | + | Voluntary muscle neuromuscular junction | Stimulates muscle contraction. |
| | – | Autonomic nervous system Vertebrate central nervous system Heart muscle | Increases threshold of contraction. |
| Norepinephrine | + | Neurons of central nervous system responsible for arousal, attention, moods; involuntary muscles, glands. | Increases alertness and attention, prepares for muscular activity. |
| Dopamine | – | Neurons that produce acetylcholine. | Prevents overactivity of neurons that activate muscles; deficiency causes uncontrolled muscle contractions of **Parkinson's disease.** |
| Glycine | – | Motor neurons to voluntary muscles. | Prevents uncontrolled muscle contractions. |
| Serotonin | – | Neurons in the brain that maintain wakefulness. | Induces sleep, may modulate moods. |
| GABA | – | Motor neurons to voluntary muscle. | Prevents uncontrolled muscle contraction. |

The neurotransmitters are either derived from the amino acid tyrosine, which include dopamine, norepinephrine, epinephrine and serotonin or from neuropeptides. Peptide neurotransmitters include the **endorphins** and **enkephalins** that reduce pain perception. Therefore, these are called natural opiates (morphine like substances). The neurons that are involved in pain reception have receptor molecules to which internal opiates attach so that pain receptors are inhibited from releasing their own transmitter substance (substance P) on to neurons in the pain perception centres. Thus relay of information to the brain is blocked. The pain killing effectiveness of endorphins is similar to that of morphine.

The hallucinating effects of drugs like morphine, heroin, mescaline etc., are due to their competition with receptor sites of natural opiates. The old method of acupuncture in China may also be related to normal action of endorphins.

Neurons do not always function in the way they should. Things can go wrong in a deadly way if the synaptic cleft is occupied by chemicals that interfere with neurotransmission (see Box 9.2).

**Box 9.2**

**Nerve Poisons**

In general nerve poisons are the most toxic substances known. In minute quantities they can interfere with acetylcholine activity and sabotage the normal functioning at the synapse.

**Curare:**

Curare is a deadly extract of tropical plants of South America. The native hunters of South America dip their darts in this neurotoxin and blow it from their small blowguns. Curare blocks the neurotransmitter sites on the postsynaptic membrane so that the muscles stop contracting even though there is enough acetylcholine in the synaptic clefts. Death occurs quickly from suffocation when the muscles used form breathing stop working.

**Organophosphates and Nerve Gas**

Organophosphates and nerve gas inhibit the enzymatic removal of acetylcholine so that the neurotransmitter remains in the cleft and the victim suffers from spastic paralysis.

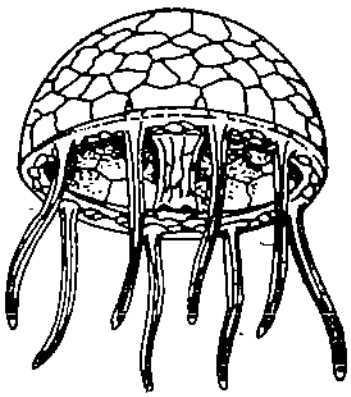
**Tetanus**

Tetanus also causes spastic paralysis and is associated with simple bacterial infection of wounds. The toxin released by the bacteria affects inhibitory synapses by blocking glycine sites on the postsynaptic membrane. The muscles become locked in permanent contraction. All voluntary muscles are eventually affected and the victim looses the ability to breathe and dies.

## 9.6 NEURAL CIRCUITS

The simple all or none activities of a single neuron can hardly provide the adaptibility needed for the constant changes faced by the organism in its internal and external environments. Information about the external environment is integrated with signals arising within the organism and transmitted to effectors to elicit a coordinated response. Thus each neuron forms a unit in a communication circuit.

A survey of the features of the nervous system in the animal groups at various points on the phylogenetic tree shows that a long evolutionary process has produced the outstanding complex structure of the human brain. Protozoans are single-celled organisms and clearly cannot have a nervous system. An examination of the electrical properties of the protozoan cell membrane would, however, show many similarities to those of nerve cells including electrical potential changes and currents associated with activity. Coelenterates are of great interest neurologically since they are the first animals to possess a true nervous system. The coelenterate nervous system consists of a diffuse network of neurons that are distributed throughout the body wall. Such a simple and primitive nervous system is termed a nerve net in which neurons are dispersed mostly at random, (Fig. 9.10). Though primitive, this arrangement serves the need of a radially symmetrical animal whose food and enemies may approach from all directions. The animal's reaction depends on the strength of the stimulus. Only a part of the body reacts to a weak stimulus and a strong stimulus causes the entire animal to respond. From such diffuse primitively organised system of nerve cells, evolution has produced a complex organized nervous system such as that of man. The system of local nerve nets, however, continues to exist even in many advanced invertebrate groups and in the intestines of vertebrates.



Jellyfish

Fig. 9.10 : Nerve net in Jelly fish.

With evolution into higher forms, the nerve net arrangement was progressively replaced by a compact centralised nervous system consisting of an elaborate system of nerves connected with sense organs etc., and a brain and spinal cord.

The vertebrate nervous system can be divided into a **central** and a **peripheral nervous system.** The central nervous system consists of the brain and spinal cord. The vast majority of all neuron cell bodies and in most cases the dendrites and axons lie in the central nervous system. The sensory information is integrated, compared, changed, added up or suppressed—assuring that the response to the stimulus is appropriate. You have learnt in FST-I Unit 23 that in the human nervous system, some of the activities go on automatically without our being aware of them. Others are carried out at the level of consciousness by the higher centres of the nervous system. Lying between the sensory inputs and the motor response of the central nervous system are highly complex **neural networks.** These are responsible for all the reflexive and higher functions of the nervous system. The connection in the neural networks are established during development and can be modified by use during the organism's life time. However, disuse can lead to major loss of function. By and large they appear to be preprogrammed genetically.

Let us now examine the simplest neural network in vertebrates. The reflex arc. The primordial nerve arc must have consisted of a receptor cell directly connected to an effector cell. (Fig. 9.11(a)).

As neural circuitry became complicated centralised nervous system developed to permit compactness and complexity of interneurons. Long sensory and motor axons became useful to connect the receptors and effectors at the perifery of the central nervous system. This gave rise to the **monosynaptic reflex arc.** (Fig. 9.11b).

The most familiar example of this is the stretch reflex of vertebrates. Elongation of a muscle stimulates its stretch receptors, which include the sensory endings of afferent axons. These fibres enter the spinal cord and make direct synaptic connections with the motoneurons that activate contraction of muscle. This reflex contraction counteracts the force that produced the initial elongation of the muscle causing it to shorten to its original length. This mechanism operates without any conscious control and is important in maintaining posture. Pathways with many synapses including **interneurons** connecting the sensory and motor neurons are more common (Fig. 9.11c). You would recall the familiar knee jerk described in FST-1—Unit 23 as an example of this.

It is an interesting fact that the complexity and variety of functions of the nervous system are manifestations of the complexity and variety of neural circuits and not because the signals

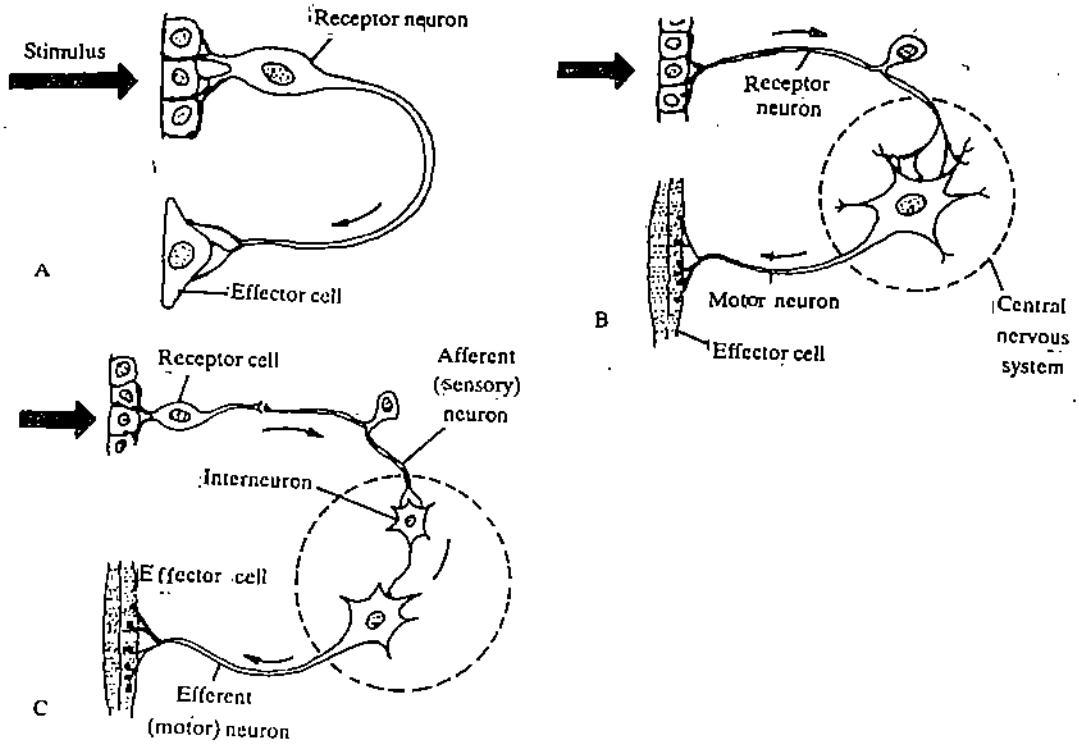



Fig. 9.11 : Examples of simple reflex arcs. (a) hypothetical primordial nerve arc., (b) monosynaptic reflex arc, (c) reflex arc with many relay synapses.

are complex or different. We had said earlier that all nerve impulses have the same fundamental nature. For example, the optic nerve carries the same kinds of nerve impulses as the auditory nerve and the central nervous system recognise what the original stimulus was. If the auditory nerve is stimulated artificially the impulse is interpreted as sound and if the optic nerve is stimulated then the impulse is interpreted as light. Most of us have experienced this kind of interpretation if you put mechanical pressure on your eyeball it is interpreted as light and any sharp blow on the eye makes you see stars though no light is involved.

Neural networks can be grouped into :

1) Sensory filter networks that are organised so as to pass on only certain features of a complex sensory input while ignoring other features. You must have tried to gather your attention while studying this unit even if the surroundings are noisy. Once your attention is caught you hardly perceive the sound as noise and can study undisturbed.

2) Pattern generating networks that are responsible for the production of motor output that regulates stereotyped movements. Examples are networks that govern respiration and locomotion.

The number of combination with which neurons form different neural circuits is astonishing. You know that a single neuron may receive thousands of presynaptic terminals from other neurons. Some of which may be excitatory and some inhibitory. The neuron itself may branch many times and innervate many other neurons. Thus divergence gives it a widespread influence on many other postsynaptic neurons and convergence of inputs allows that unit to integrate signals from numerous presynaptic neurons.

The nerve cell population in the nervous system has consolidated into distinct functional and morphological structures that can generate psychological experiences. It is worthwhile to point out that the nervous system is the site of ones personhood.

**SAQ 4**

Suppose the optic nerve of an animal is cut and connected to the auditary nerve will it still perceive the signals as light ?

.....

.....

.....

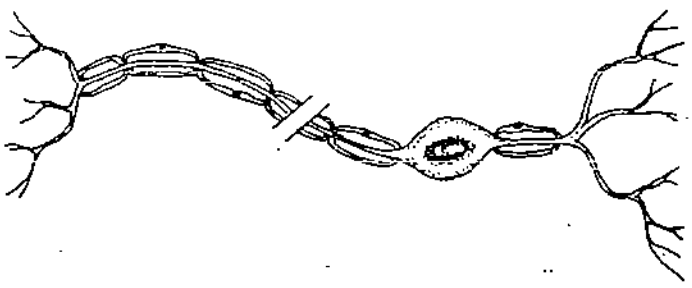
## 9.7 SUMMARY

In this unit : have studied that :

- the functional unit of all nervous systems is the neuron which typically consist of a cell body containing the nucleus, a variable number of dendrites that receive inputs and a single axon that carries information in the form of electrical impulses. These impulses are conveyed to other neurons or cells by release of neurotransmitters from the axon terminal ends or synaptic terminals,
- the nervous system functions as a result of differences in ion distribution across the neuron membrane at rest and after stimulation. Action potential (AP) is generated when the membrane is sufficiently depolarised in response to a stimulus to attain a threshold level. It is an all-or-none phenomenon. The AP is conducted due to the cable property of neurons in unmyelinated fibres and by saltatory conduction in myelinated fibres,
- synaptic transmission of information is mostly through chemical synapses and in some instances through electrical synapses. Chemical synapses involve neurotransmitter substances that are produced in presynaptic terminals and released in the narrow cleft that separates the presynaptic cell from postsynaptic cell. Receptors in the postsynaptic membrane pick up these transmitter molecules. These receptors are linked to chemically gated ion channels and change in membrane permeability generates a postsynaptic potential which may be inhibitory or excitatory. In excitatory postsynaptic potential the action of the transmitter molecules is to shift the membrane potential beyond the threshold for initiation of action potential in the axon hillock. Inhibitory postsynaptic potential changes ion conduction so as to counteract depolarisation of the membrane,
- the neurotransmitter substance in the cleft is either destroyed or picked up by surrounding glial cells or reused by the presynaptic cell. Basically action potentials are the result of voltage regulated ion channels and postsynaptic potentials are due to chemically gated ion channels. Several chemicals are known to be neurotransmitters and they may have excitatory or inhibitary effect depending on the type of synapse being investigated,
- sensory receptors transform environmental stimuli into action potentials by depolarising the membrane. The resulting impulses are coded according to their frequency. The integration of impulses occurs in the postsynaptic membrane which acts as an analyser of inputs,
- lower animals have simple neural networks while higher animals have more organised nervous systems. Lying between sensory inputs and motor outputs of the central nervous system are complex neural networks responsible for all reflex actions and higher functions of the nervous system. The simplest neural networks are monosynaptic reflex arc. The neural networks act as filters of sensory inputs that enhance some incoming stimuli while suppressing some other features.

## 9.8 TERMINAL QUESTIONS

1) Label the figure given below and show the direction of flow of impulses.



2) The membrane potential of an axon is normally –75 mV What would happen to the membrane immediately after each of the following situations :

a) A sudden increase in $Na^+$ permeability

b) A sudden increase in $Cl^-$ permeability

c) An accumulation of $K^+$ on the outside of the membrane

d) An accumulation of $Na^+$ on the outside of the membrane

.....

.....

.....

.....

3) Data regarding velocity of conduction and diameter of nerve fibre of two species A and B is given below. Which species is an invertebrate and why is the velocity of conduction in species B more than species A.

| | diameter/μm | Velocity of conduction $ms^{-1}$ |
|---|---|---|
| Species A | 500 | 33 |
| Species B | 15 | 90 |

.....

.....

.....

.....

4) Make a diagram to summarise the major events between the arrival of action potential in an axonal terminal and the generation of another in the axon hillock of a receiving neuron.

## 9.9 ANSWERS

**Self-assessment Questions**

1) a) iii), b) iii)

2) a) i), b) iv)

c) If the threshold level is already reached and action potential generated then increasing the strength of the stimulus would still produce the same action potential only frequency of action potential may increase.

d) i) saltatory conduction

ii) decrement

iii) cable like, action potential, depolarise

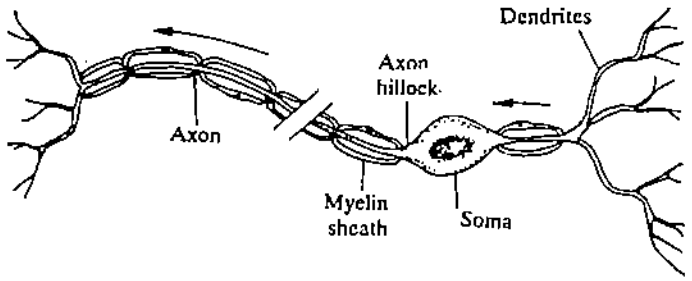
3) a) Because transmission depends on release of transmitter substance that is present only in the presynaptic knob. There is no means for transferring impulses in the opposite direction.

b) i) True ii) False iii) True iv) True

4) No.

**Terminal Questions**

1) Arrow shows direction of impulse from dendrite to axon.




2) a) increase in $Na^+$ permeability would depolarise the membrane

b) increase in permeability to $Cl^-$ would hyperpolarise the membrane

c) A rise in concentration of $K^+$ on the outside would depolarise the membrane

d) An accumulation of $Na^+$ inside the cells would bring the membrane potential to zero.

3) Species A is an invertebrate as only invertebrates have giant fibres (500 µm). Species B has a small diameter of fibre but still high velocity of conduction. This indicates that the fibre is myelinated, therefore insulated.

4) Base your diagram on Fig. 9.8.

# UNIT 10 COMMUNICATION—II

## Structure



## 10.1 INTRODUCTION

You would be aware by now that in any multicellular organism, the work done is divided among many different types of cells. These cells must exchange information and work together if the organism is to survive.

In the previous unit we studied one of the two great communication networks—the nervous system, that sends electrical messages via the neurons and nerves to various parts of the body. These messages are transmitted across the synapses in the form of chemicals. In this unit, we shall study the other communication network of chemical messengers — the hormones. The nervous system and the body's chemical messengers work together in coordinating the activities of the different cells of the body.

Originally, hormones were defined as chemicals produced by ductless endocrine glands that travel through the blood to exert their effect at some distance from where they are produced. Now, we know that animals have many other chemical messengers in addition to the hormones produced by endocrine glands. These include the neurotransmitters we encountered in Unit 9; local chemical messengers such as histamine that participate in inflammatory and immune reactions (LSE–01, Unit 15), growth factors that stimulate growth by particular tissues; prostaglandins that are lipids with a variety of effects and pheromones that are chemical signals emitted into the environment that affect the behaviour of other individuals of the same species.

This unit considers general patterns seen in the chemistry and activities of hormones produced by endocrine glands and nervous tissue. You are not expected to remember the chemical structures of different hormones. These structures are included in the text to illustrate the similarities or relationships among several hormones. We shall see how hormones affect the homeostasis and the animal's response to external stimuli and also learn about some of the roles played by pheromones in influencing the behaviour of different individuals of the same species.

Before you begin a study of this unit we suggest that you read Unit 15 of the Cell Biology

Course (LSE–01) again for, we assume your knowledge of certain concepts of hormone action at cellular level.

## Objectives

After studying this unit, you should be able to:

- define the terms hormones, endocrine glands and identify sources of hormones other than endocrine glands,
- describe the chemical classification of hormones and compare the hormone action in case of proteins and steroid hormones,
- describe the neuroendocrine relationship and the mechanism by which hypothalamus regulates the secretion from pituitary,
- explain how concentration and action of hormones are regulated by negative feedback mechanism,
- explain the action of insect hormones in controlling metamorphosis,
- describe the role of pheromones in communication and compare it to hormonal action.

# 10.2 HORMONAL CONTROL SYSTEMS

Throughout the animal kingdom we see that chemical signals transmitted from cell to cell coordinate body function. The three basic modes of chemical signalling are shown in Fig. 10.1.Each involves specific receptor proteins on the surface of the cells that receive the signals. Local chemical mediators are produced by most cells and vary in type and function but they affect the cells in the immediate vicinity only.

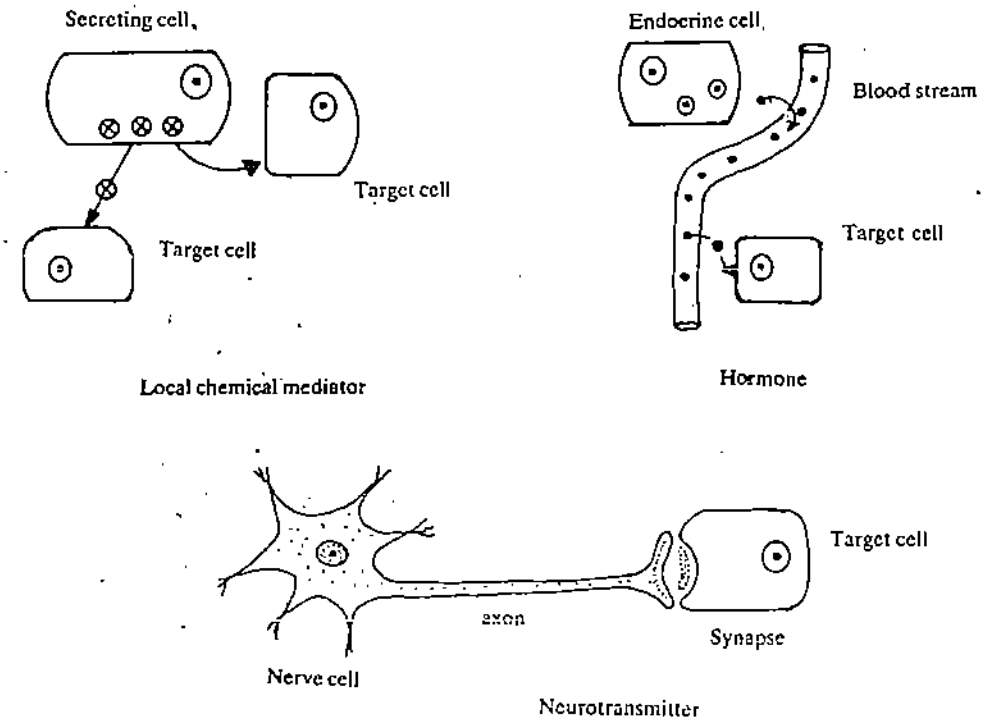



**Fig. 10.1: Chemical signalling system.**

Hormones in contrast are chemical substances produced by a specific group of cells and released into the blood stream. They may affect distant parts of the body. Even though hormones in blood come in contact with every organ they affect only specific target organs or tissues. These target cells are equipped with receptor cells for a particular hormone or a group of hormones.

The major mammalian endocrine glands are shown in Fig. 10.2 and the most important vertebrate hormones and their functions are listed in Table 10.1. Many of these hormones are almost similar throughout the vertebrate classes; others have specific functions that differ from group to group. For example, prolactin has 365 known effects! Prolactin in mammals stimulates milk secretion, in pigeons it stimulates the formation of crop milk and in fish it affects renal functions and osmotic permeability of the gills.

Fish has maximum hormones, as we go up the evolutionary tree the number of hormones have decreased but specialisation increases.

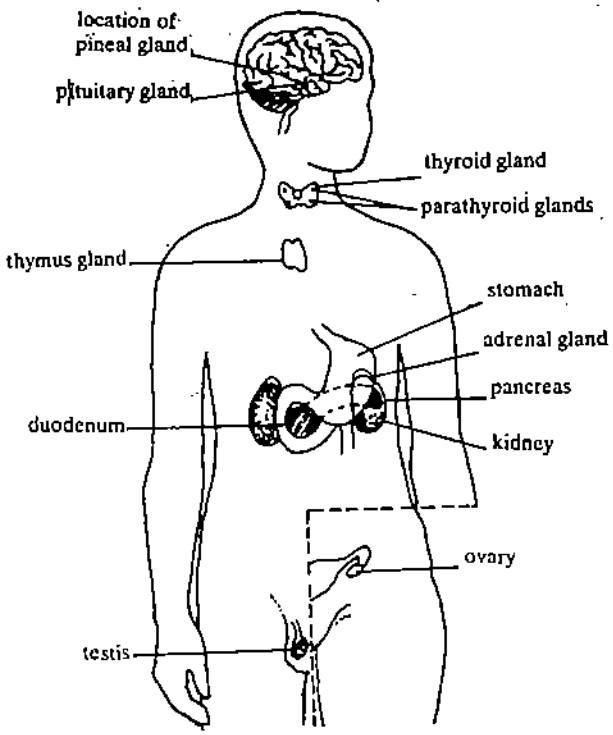



**Fig. 10.2: Location of endocrine glands.**

**Table 10.1 : Some vertebrate hormones grouped according to source and their action (hormones from hypothalamus and pituitary given in Table 10.2 and 10.3)**

| | Hormones and Source | Chemical Nature | Action. |
|---|---|---|---|
| 1) | **Thyroid Gland** | | |
| | Calcitonin | Polypeptide | Decreases blood calcium levels by promoting calcium deposition in bones |
| | Thyroxine ($T_4$) | Iodinated tyrosine derivative | 1. Growth and metabolism<br>2. Metamorphosis in amphibians |
| | Triiodothyronine ($T_3$) | -do- | |
| 2) | **Parathyroids** | | |
| | Parathormone (PTH) | Polypeptide | Increases blood calcium and phosphorus by releasing calcium stored in bones, decreases excretion of calcium from kidneys |
| 3) | **Pancreas** | | |
| | Insulin (from B-cell) | Polypeptide | Decreases blood glucose, alters protein and lipid metabolism |
| | Glucagon (from A cell) | Polypeptide | Increases blood glucose by increasing glycogenolysis |
| | Gastrin (D-cells also found in stomach) | Polypeptide | Secretion of HCl by stomach |
| 4) | **Blood** | | |
| | Erythropoietin | Polypeptide | Increases erythrocyte production in bone marrow |
| | Angiotensin I<br>Angiotensin II | Small peptide | Stimulate aldosterone synthesis in adrenal cortex |
| 5) | **Small Intestine** | | |
| | Enterogasterone | | Inhibits acid secretion in stomach |
| | Cholecystokinin (CCK) | Polypeptide | Stimulates contraction of gall bladder and release of bile salts; digestive enzymes from pancreas |
| | Secretin | Polypeptide | Stimulates secretion of water and inorganic salts from pancreas |

Table 10.1 Continued

| | Hormones and Source | Chemical Nature | Action |
|---|---|---|---|
| 6) | **Adrenal Medulla** | | |
| | Epinephrine or adrenalin | Amine | Neurotransmitter, contraction/relaxation smooth muscles |
| | Norepinephrine or noradrenalin | Amine | Dilation of blood vessels, increase in blood sugar and blood pressure, increases heart rate and cardiac output |
| 7) | **Adrenal Cortex** | | |
| | Glucocorticoids (Corticosterone, Cortisol etc.) | Steroid | Metabolism of carbohydrate, protein and fat in liver; important in fasting or hibernating animals; have anti-inflammatory action; involved in termination of pregnancy |
| | Minerelocorticoids (Aldosterone) | Steroid | Resorption of $K^+$ and $Na^+$ in kidney; sweat, salivary glands, gut, amphibian skin, bladder, fish gills |
| | Small amounts of sex hormones (androgens and progesterone) | Steriods | Promotes secondary sexual characters predominently male. |
| 8) | **Testes** | | |
| | Androgens (testosterone, 5 α-dihydroxy testosterone) | Steroid | Development and maintenance of male characteristics and behaviour |
| 9) | **Ovaries** | | |
| | Estrogens | Steroid | Development and maintenance of female characteristics and behaviour |
| 10) | **Corpus Luteum** | | |
| | Progesterone | Steroid | Maintenance of uterine endometrium, stimulation of mammary duct formation; acts with estrogens to maintain estrous and menstrual cycle |

## 10.2.1 Chemical Nature

All hormones are chemical compounds that can broadly be grouped as:

1) Those that are synthesised from fatty acid precursors.

2) Those that are synthesised from amino acids or closely related compounds.

Hormones based on fatty acids are relatively small in size and have basically similar structure. All steroid hormones are based on 4 ring structures synthesised from cholesterol (Fig. 10.3).

In invertebrates the ecdysone found in insects is included in the steroid group and in vertebrates the important steroid hormones are estrogens, androgens and corticosteroids. Within each of these groups different hormones are synthesised by addition or removal of oxygen and/or hydrogen (Fig. 10.3b).

The **peptide hormones** or those based on amino acids vary in both size and structure. For example, oxytocin and antidiuretic hormone (ADH) are both small peptide hormones that differ by only two amino acids (Fig. 10.4a) but produce entirely different effects.

Oxytocin is present in all vertebrates but different vertebrate classes have slightly different versions of it. Thyroxine ($T_4$) which is involved in control of tissue metabolism is derived from the tyrosine residues while growth hormone is composed of 190 amino acids. Most neurotransmitters are essentially modified amino acids (Fig. 10.4b).

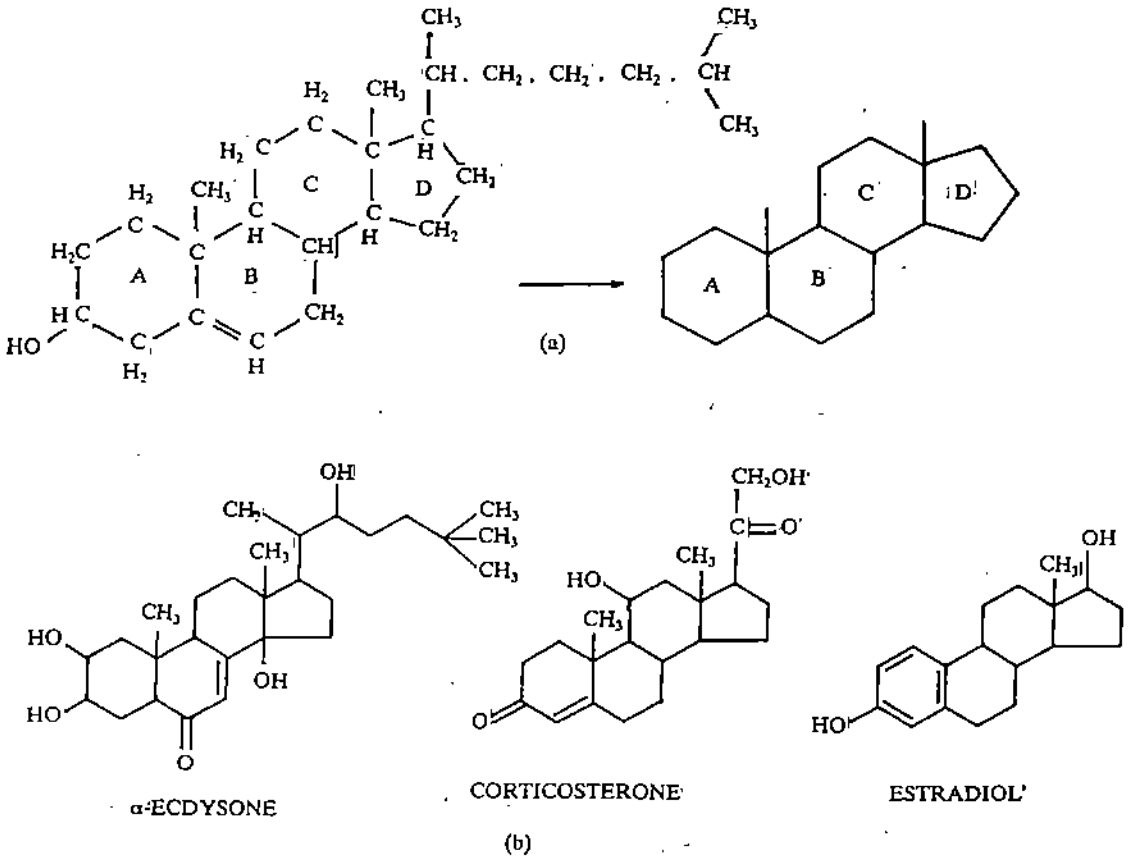



Fig. 10.3 : a) Relationship between cholesterol and basic steroid nucleus. b) All naturally occurring steroids have the basic ring structure of 17 carbon atoms. They differ in the number of carbon, hydrogen and oxygen atoms attached to the basic nucleus

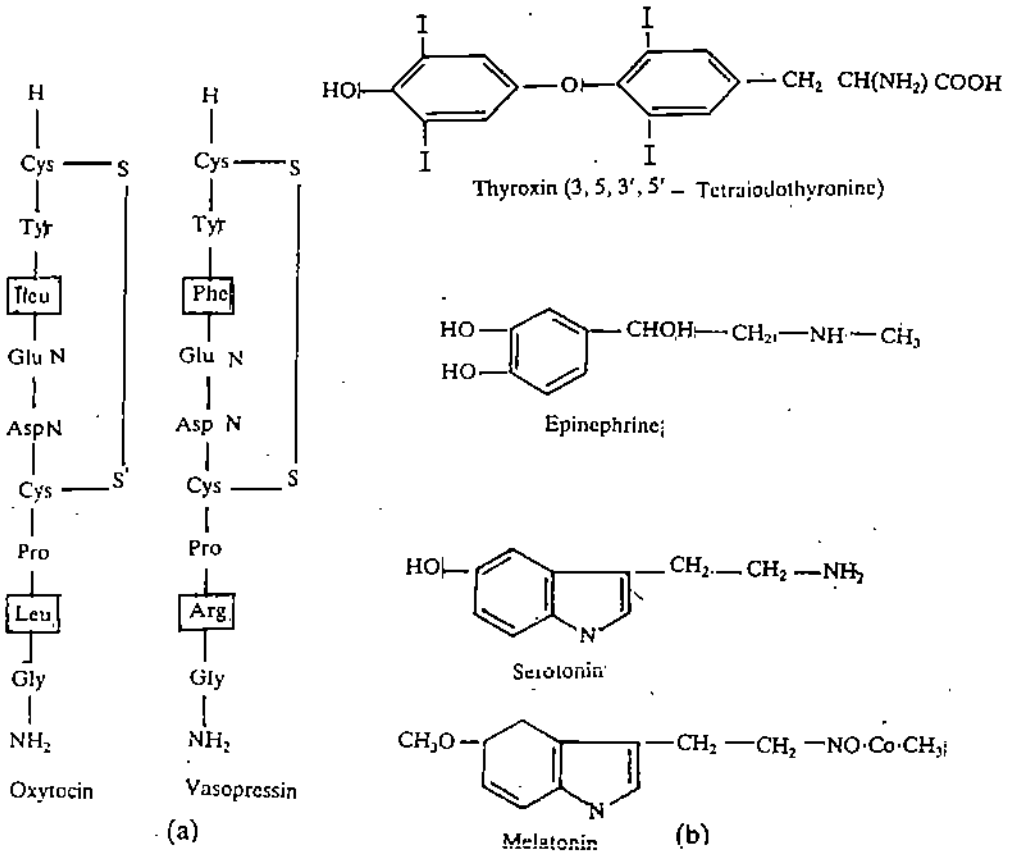



Fig. 10.4 a): Amino acide sequences of oxylocin and vasopressin. b) Amino acid based hormones

### 10.2.2 Synthesis and Storage

The synthesis and storage of hormone depends on the nature of the hormone. Steroid hormones are secreted in diffuse molecular form and usually accumulate in the cell as clear lipid droplets, or may sometimes be bound to the membrane as lipids-protein aggregate. Hormones based on amino acids are packaged in membrane bound vesicles that are later liberated in extracellular space.

The duration of storage of a hormone within a secretory tissue also varies. Steroid hormones appear to diffuse out of the cells across the membrane (being lipid soluble) in a matter of minutes after synthesis. Secretory residues of most endocrine cells, however, are held till they are given the signal to be released. Thyroid hormone is secreted into extracellular spaces of the cells called follicles and can be stored for several months.

### 10.2.3 Secretion of Hormones

The secretion of most hormones (except steroid) is by the process of exocytosis. Fig. 10.5 summarises the formation, transport, release and reconstitution of secretory vesicles.

The release of hormones from the endocrine glands is controlled by nervous, hormonal or metabolic stimuli which also control the rate of release into the bloodstream. As with neurotransmitters, $Ca^{2+}$ seems to play an important role in the release of hormones. The secretion is not random but follows a certain pattern. This may be circadian (i.e. approximately a 24 hour cycle) or seasonal or may be periodical (e.g. the human menstrual cycle). Some hormones like the thyroxine or triiodothyronine are secreted continuously by the thyroid gland).

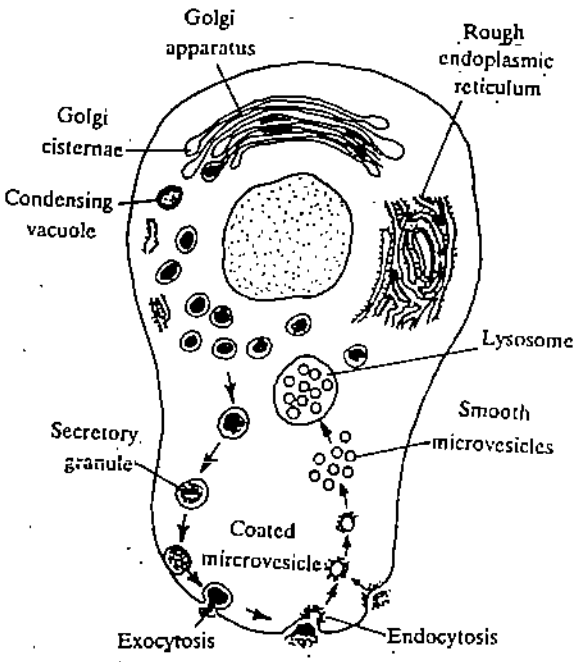



**Fig. 10.5 : Formation and secretion of hormones. After their formation in the golgi apparatus secretory vesicles are transported to the site of release. After the release of hormone by exocytosis the new vesicles may form by process of endocytosis**

The duration of hormone in the bloodstream is indicated by its 'half life' which varies from a few seconds to almost a week depending on the hormone. The hormone is deactivated in the liver and in the target tissue that break down the hormone, or the hormone may be lost through the kidneys. Hormones of molecular mass of 10,000 or less are selectively filtered from the kidneys and excreted in the urine.

Many hormones are bound to the protein molecules in the blood plasma forming aggregates. This prevents a) the hormones from being excreted out of the body via the kidney, b) rapid destruction of hormones by enzyme. Hormone-protein aggregate is a source of slow release of hormones.

The slow release of hormones occurs because the amount of hormone bound to the plasma protein is always in equilibrium with a small amount of free hormone. Receptor molecules have a greater affinity for hormones than the protein molecules in the plasma. So, free hormones tend to bind to the receptors shifting the equilibrium and more hormone dissociates from the plasma proteins. Because free hormone is also broken down continually by action of enzymes, a steady trickle of hormone is maintained.

Hormone concentrations in the blood are related to the volume of blood, physiological state, metabolic rate and hormone binding capacity of plasma proteins. However, it is seen that hormone levels in blood are always very low in comparison to other substances. Let us take the example of antidiuretic hormone (ADH). Within the blood of rat ADH level is approximately $10^{-11}$ mole per litre, but if the rat faces dehydration the level increases to $2.5 \times 10^{-10}$ mole per litre. Hormones like insulin, glucagon that are concerned with maintaining blood glucose levels are in the range of $10^{-9}$ mole per litre. Sex hormones are in the range of $10^{-7}$ mole per litre to $10^{-10}$ mole per litre. Detecting this amount of hormone in blood is equivalent to detecting a teaspoon of sugar in a swimming pool! What does this example illustrate? It shows that receptor molecules must be very sensitive and have a very high affinity for hormone molecules. Therefore we can say that hormones are simply chemicals it is the receptors that makes them hormones.

**SAQ 1**

Which of the following statements are true and which are false. Give reasons.

i) Peptide hormones are related more closely to neurotransmitters than steroid hormones.

ii) All hormones have similar effects on all animals.

iii) All steroid hormones have basically a 4 ring structure.

iv) Hormone bound to proteins in blood is released quickly and secreted by kidneys immediately.

**SAQ 2**

Fill in the blanks to complete the definition.

A hormone may be defined as a ..... produced by one type of cell that has a ....., ..... effect on the activity of another type of cell.

## 10.3 ACTION OF HORMONES

We said earlier that hormones are released into the blood stream or extracellular fluid and therefore, reach most of the cells of the body. However, they are specific and influence only their destined **target cells**. Other cells do not react. For instance, insulin passes throughout the body but only the liver and muscle cells respond to it by taking up glucose. Similarly the target cells respond to the hormones differently at different times. Injections of thyroxine will not make a very young tadpole develop into an adult since the cells cannot respond to the hormones as they will in later life. This suggests that only specific receptor molecules at the target cells recognise and bind to particular hormones, and other cells do not possess these receptor molecules. When the hormone reaches a target cell many changes may occur in the cell.

- The activities of various enzymes may increase or decrease i.e. inactive enzymes are activated.
- permeability of plasma membranes may be altered.
- increase in activity of certain genes may alter the types of messenger RNA and proteins produced by the target cells.

The way in which hormones act upon their target cells is similar to the mechanism involved in the case of neurotransmitters. We must not forget that the 'language' used in both types of communications is chemical. Basically, three steps are involved that have been shown graphically in Fig. 10.6.

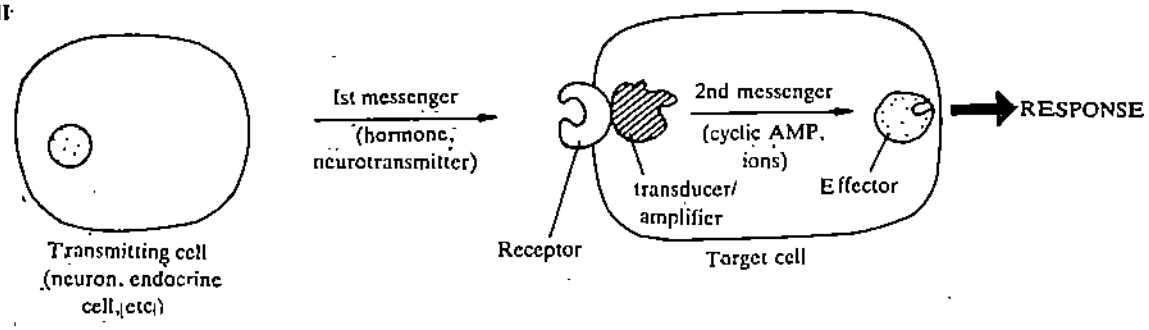



Fig. 10.6: Intracellular communication system involving chemical messengers

The speed with which the hormone acts depends on how it acts. Insulin for example, affects tissues within minutes after it appears in the bloodstream because it crosses the plasma membrane and acts on the cell's metabolism directly. Other hormones do not enter the cell but bind to receptors on plasma membrane and affect the metabolism and transcription. Hormones that induce genetic activity and protein production are the slowest in their effect.

Hormones can be divided into two groups: 1) those like steroids and other lipid soluble hormones penetrate the surface membrane of the target cells and 2) Proteinaceous hormones and catecholamines that cannot or can poorly penetrate the membrane of the target cell. Let us consider the action of steroid and thyroid hormones first.

In general, the steroid hormones induce the transcription activity of the target cells and proteinaceous hormones affect the membrane permeability and enzyme action.

### 10.3.1 Steroid and Thyroid Hormones

Cytoplasmic receptors for steroids are proteins with two subunits that bind to the steroid molecules. When both receptor sites are occupied by the steroid then the receptor-steroid complex migrates to the nucleus where one subunit ensures that the complex binds to the specific site on the chromatin. Then the receptor subunits separate and the other subunit interacts directly with the adjacent region of DNA molecule resulting in the transcription of the DNA segment into mRNA (see Box 10.1 and Fig. 10.7a). Thyroid hormone acts in a similar manner (Fig. 10.7b) except that the receptor is located in the nucleus. The major hormone secreted by thyroid is thyroxine ($T_4$). It travels in blood attached to carrier proteins. Thyroid also secretes a small amount of triiodothyronine ($T_3$). Carrier proteins have higher affinity for $T_4$ and very little $T_4$ is free in the plasma. Only the free $T_4$ and $T_3$ enter the cells. The rest of the bound $T_4$ acts as a reservoir for slow release. The free $T_4$ that enters the cell is also converted to $T_3$ enzymatically. Therefore, $T_3$ is the chemically more potent and active form of thyroid hormone.

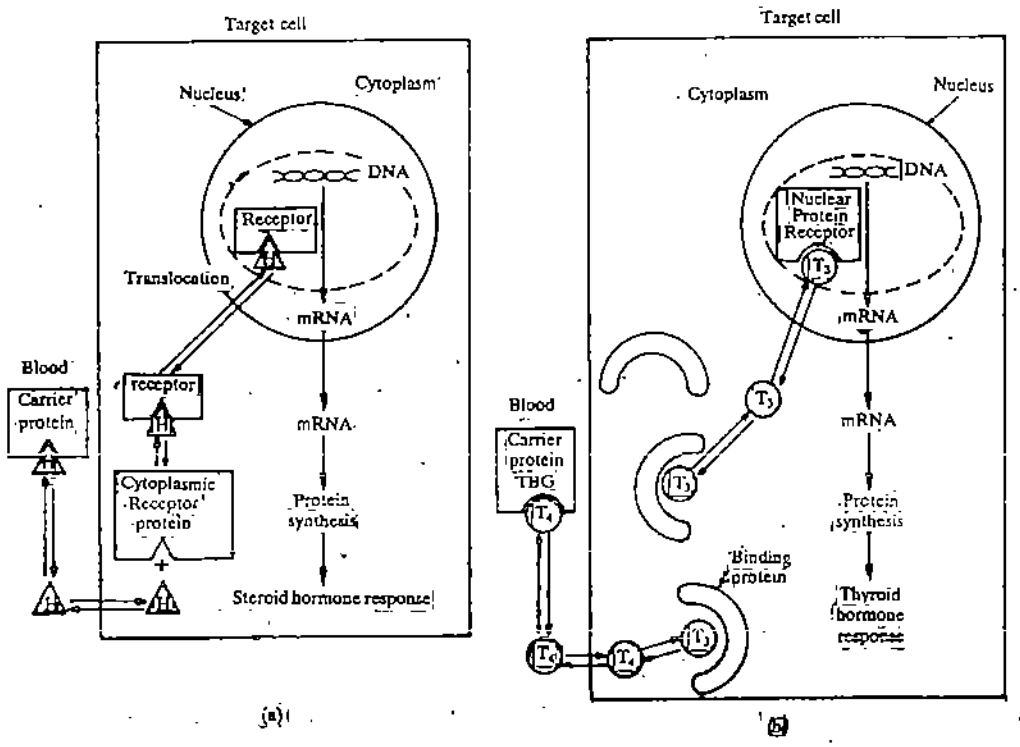



Fig. 10.7 : Molecular action of (a) sterold hormones, (b) thyroid hormone.

The techniques of autoradiography showed that steroid hormones accumulate in the nuclei of target cells but not in the nuclei of other cells. This accumulation occurs very rapidly and remains for some time even after the labelled hormone is removed for circulation. These findings suggested that steroid binding receptors were in the cytoplasm of target cells. By fractionating target tissue (rat uterus) incubated with labelled hormone (estradiol). Roger Gorske (1979) and associates identified the receptor—that was a protein (molecular weight 200,000). This protein binds very strongly to estradiol and was present only in uterine tissue. One significant finding was that substances that have similar hormonal action to that of estradiol are all bound by this protein. This suggests that receptor-steroid complex is an intermediate step in the final action of the hormone. Similar receptor proteins have been identified in target tissues of other steroid hormones.

## 10.3.2 Peptide Hormones

Protein hormones exert their effect in a different manner than steroid hormones. The hormone does not enter the cell itself but binds to a specific protein receptor on the target cell surface and this results in either

i) stimulation of membrane-bound enzymes or

ii) opening of ion channels in the membrane

In the first system the enzyme activated is **adenylate cyclase** which catalyses the production of cyclic AMP from ATP. A single activated adenylate cyclase can produce about 1000 cyclic AMP molecules per minute so that the enzyme amplifies the interaction of the 1st messenger and receptor (Fig. 10.8). Cyclic AMP operates as the second messenger working inside the cell by activating a set of enzymes known as **protein kinases** which in turn phosphorylate proteins. These proteins can be other enzymes, structural proteins or nuclear proteins and their configuration changes. As a result enzymes may be activated or membrane permeability may change depending on the type of cell.

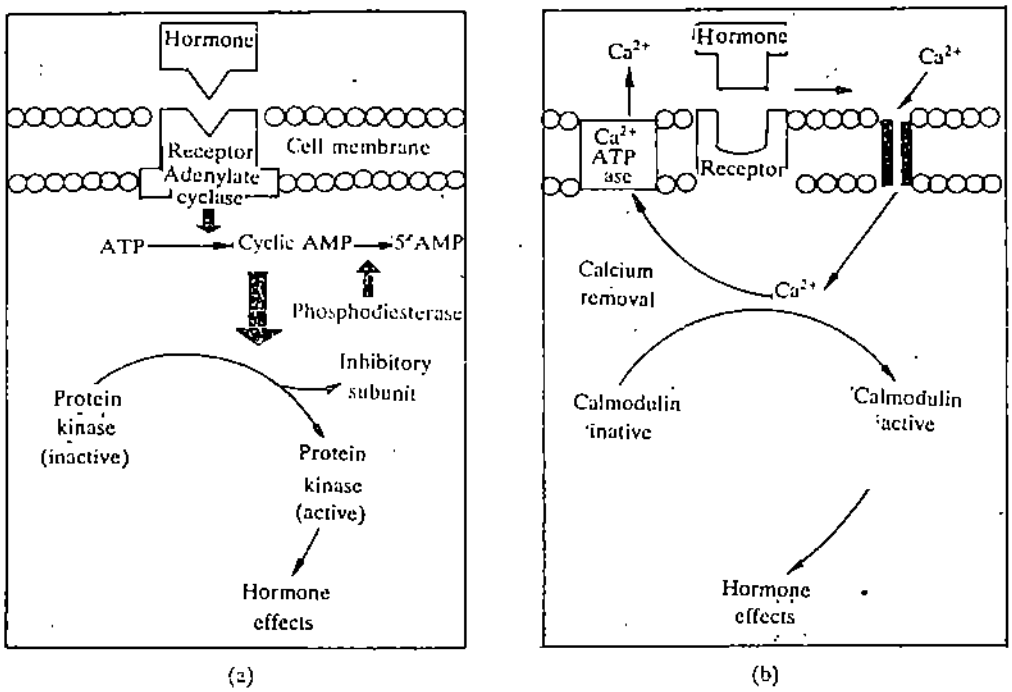



Fig. 10.8: Second messenger concept of hormones
a) By formation of cyclic AMP
b) By Ca-calmodulin system.

In the second system (Fig. 10.8b) the action of receptor and hormone leads to opening of simple ion channels to permit calcium ions ($Ca^{2+}$) in the membrance. $Ca^{2+}$ enter in and the interaction of 1st messenger and receptor is amplified. $Ca^{2+}$ serves as the second messenger. Many cells produce **calmodulins** (regulatory proteins that depend on calcium) that are analogus to protein kinase. These proteins then produce a wide range of effects depending on the target cell type.

The important feature of these two second messenger systems is that the target cells do not differ in terms of second messenger but in **terms of receptors that are capable of activating the second messenger**. Thus the action of many hormones can be mimicked by raising the level of cyclic AMP or calcium ions by means of various drugs.

In some target cells the $Ca^{2+}$ and cyclic AMP act antagonestically in response to different hormones. For example, $Ca^{2+}$ causes smooth muscles to contract and cyclic AMP causes them to relax. In other situations the two second messengers coordinate or act synergestically (Fig. 10.9 A to D). For example, acetylcholine causes calcium ion gates to open (A) in adrenal medulla to release adrenalin. Medulla cells also have receptors linked to adenylate cylase (B) and a rise in cyclic AMP causes more calcium ions to be released from calcium stores within the cell (C). Thus cyclic AMP acts in coordination with $Ca^{2+}$. A further rise in cyclic AMP levels increases the synthesis of adrenalin (D).

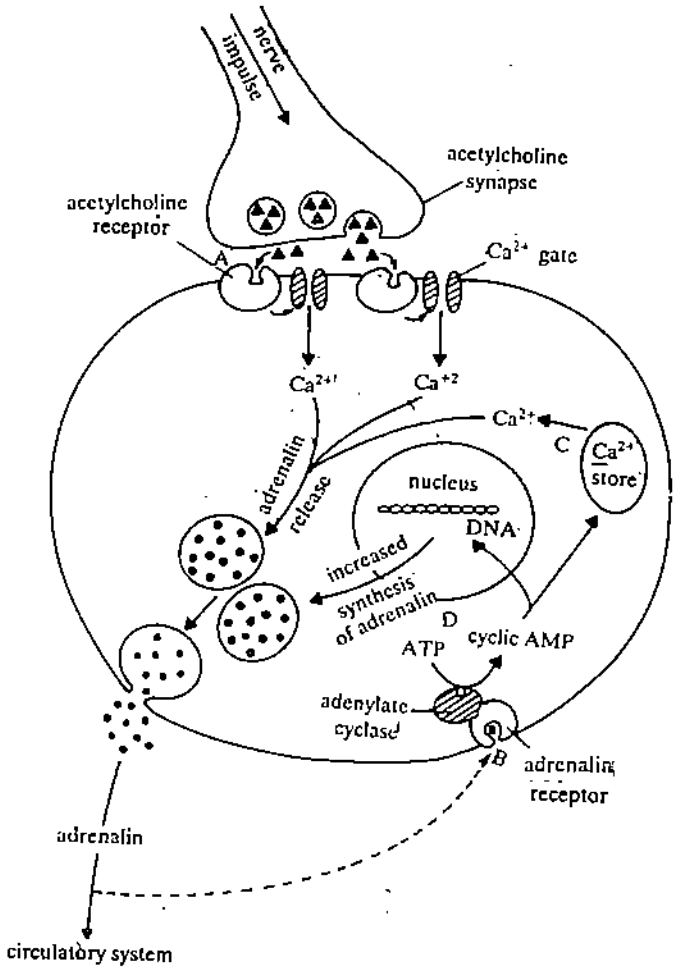



**Fig. 10.9 : Factors that affect the synthesis of adrenalin from adrenal medulla. (A to D)**

**SAQ 3**

Hormones are known to cause all the following changes in target cells except one:

i) changes in membrane permeability

ii) changes in metabolic rate

iii) increase in AMP concentration inside cells

iv) changes in genetic makeup of cells

v) synthesis of different mRNA and protein.

## 10.4 NEUROENDOCRINE CONNECTION

Some endocrine glands are part of the central nervous system. There is a close relationship between the neural and endocrine function which we will analyse towards the end of this section.

## 10.4.1 Hypothalamus and Pituitary

The most obvious neuroendocrine link is between the hypothalamus and pituitary (Box 10.3). The hypothalamus is a part of the brain which is connected to the pituitary gland a small organ situated in the floor of the skull just above the roof of the mouth (Fig. 10.10a)

The pituitary gland was discovered in the 16th century by Vesalius who thought that it was responsible for secretion of pituita (nasal fluid).

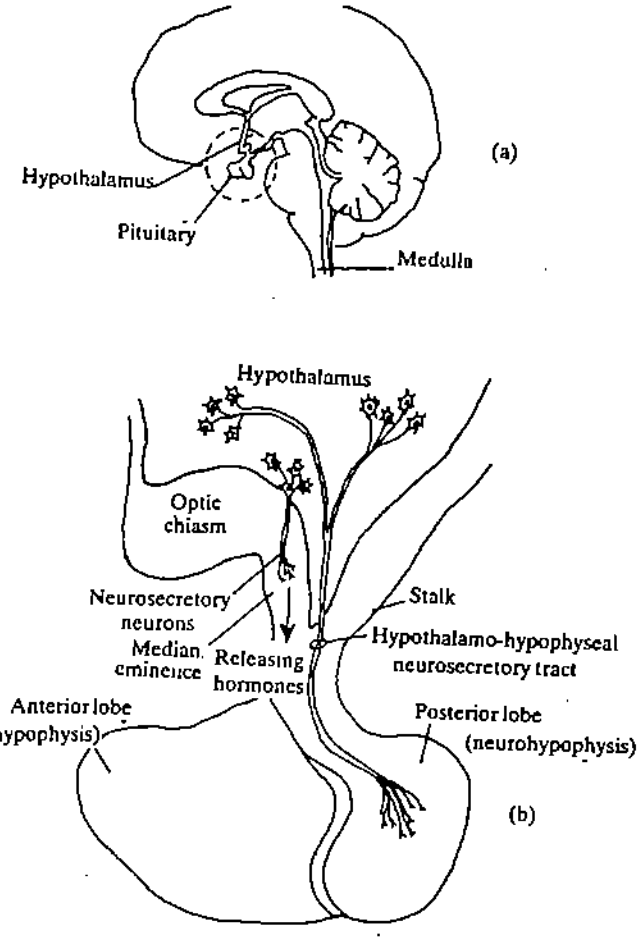



**Fig. 10.10: a) Location of pituitary gland and hypothalamus. b) Major components of pituitary-anterior lobe, posterior lobe and stalk. Note the neurosecretary neurons.**

Box 10.2

In the 1920s a German scientist Ernst Scharrer observed that the neurons of the hypothalamic region of brain in the bird minnow (*Phoxinus phoxinus*) were structurally similar to endocrine cells. The axons of these neurons passed out of the hypothalmus, into the pituitary gland. These axons ended into large bulbous swellings that were closely applied to certain blood capillaries passing through the pituitary gland. Scharrer concluded that atleast some of the hormones secreted by pituitary were actually synthesised in the hypothalamus and that pituitary was more of a storage organ. At that time the idea was rejected outright. However, Scharrer and his wife Berta persisted in their studies looking for similar phenomena in other animals. By early 1950s a considerable amount of evidence was collected to support Scharrer's hypothesis in invertebrates. This phenomenon came to be known as **neurosecretion** and the secretory products were called **neurohormones** to indicate their origin in the nervous tissue. The storage organs involved were called **neurohaemal organs** because of the close connection between the blood capillaries and nerve endings in these organs.

The pituitary is also known as **hypophysis**. It is composed of two embryologically distinct tissue. The **anterior pituitary** or **adenohypophysis** is derived from the roof of the mouth and the **posterior pituitary** or **neurohypophysis** is derived from the hypothalamus. Pituitary is joined to the hypothalamus by a slender **stalk** of nervous tissue.
The neurohypophysis consists mostly of **neurosecretory nerve endings** (Fig. 10.10b). The cell bodies of these neurons originate in the hypothalamus. (These were the neurons investigated by Scharrer and his associates). Neurosecretory cells differ from conventional neurons in position and structure of nerve endings. They have more nerve endings so that

they secrete greater amounts of neurohormones when stimulated. The size of the neurosecretory granules is also larger (200–500 nm) than those found in other nerve synapses (40–100 nm). These hypothalamic nerve cells synthesise two hormones, vasopressin or **antidiuretic hormone** (ADH) and **oxytocin**. These are transported along the axons, stored and ultimately released from nerve endings, in the posterior pituitary lobe.

The anterior pituitary lobe, however, synthesises and releases atleast 7 peptide hormones. Table 10.2 gives a brief account of their structure and their action on the target tissues.

**Table 10.2: Hormones of anterior lobe of pituitary gland**

| Hormone | Structure | Target Tissue | Action |
|---|---|---|---|
| Adrenocorticotropin (ACTH) | Peptide | Adrenal Cortex | Stimulates synthesis and release of corticosteroids |
| Thyroid Stimulating Hormone (TSH) | Glycoprotein | Thyroid gland | Stimulates synthesis and release of thyroxin and triidothyronine |
| Growth Hormone (GH) | Peptide | Liver | Stimulates the liver to produce somatomedins which alter the metabolism of all tissues (liver, muscle, adipose tissue) |
| Follicle Stimulating Hormone (FSH) | Glycoprotein | Testes and Ovary | Controls development and maturation of germ cells |
| Luteinizing Hormone (LH) | Glycoprotein | Testes and Ovary | Controls secretion of steroid hormones responsible for male and female sexual characters, also triggers ovulation |
| Prolactin (PL) | Peptide | Mammary glands | Stimulates milk production |
| | | Corpus luteum | Maintains secretion of estrogens and progesterone |
| | | Liver | Stimulates production of pheromones that control maternal behaviour in some mammals |
| | | Fish gills | Involved in maintenance of salt balance and osmoregulation |
| Melanocyte Stimulating Hormone (MSH) | Peptide | Pigment cells | Promotes melanin synthesis, (Darkens the skin in lower vertebrates;) controls hair colouration in some mammals; affects activity of some neurons |

All except GH and MSH are **tropic hormones** (from Greek for 'to turn' or change) i.e. they stimulate the secretion of other hormones from endocrine glands of the different regions of the body. GH and MSH are direct acting hormones.

The secretory activity of anterior pituitary is regulated by hormones or factors that are secreted by the hypothalamus some are **releasing factors** and some are **releasing-inhibiting factors**. These hormones are all small peptides and named according to their action on the anterior pituitary (Box 10.3). Table 10.3 gives the various factors and there action on anterior pituitary.

**Box 10.3**

Geoffrey Harris of Oxford University put forward the hypothesis in the 1940s that some neurohormones released in the blood from hypothalmic neurosecretory nerves after electical stimulation either inhibit or stimulate the secretion of hormones from the anterior pituitary. This idea was not easily substantiated as it was very difficult to isolate these neurohormones or releasing and release-inhibiting factors.

In 1969, two different groups isolated and chemically identified a releasing factor—the **thyroid-stimulating hormone releasing factor** (TRF) which stimulates the concerned cells of anterior pituitary to release TSH. The two group leaders Roger Guillemin and Andrew Schally were awarded the Nobel Prize for Physiology and Medicine in 1977. According to Guillemin, the cost of isolating the 1st milligram of TRF was stupendous. His group alone extracted 5 million fragments of hypothalmic region from 500 ton of sheep brain over a period of 4 years. The cost was comparable to bringing 1 g of moon rock to earth.

**Table 10.3 : Hypothalmic hormones that release or inhibit hormones from anterior pituitary**

| Hormone | Structure | Action |
|---|---|---|
| Corticotropin Releasing Hormone. (CRH) | Peptide | Stimulates ACTH release |
| TSH Releasing Hormone (TRH) | Peptide | Stimulates release of TSH and prolactin |
| GH Releasing Hormone (GRH) | Peptide | Stimulates GH release |
| FSH and LH Releasing Hormone or Gonadotropin Releasing Hormone (GrRH) | Peptide | Stimulates release of FSH and LH |
| GH Inhibiting Hormone (GIH) (somatostatin) | Peptide | Inhibits GH release and interferes with TSH release |
| Prolactin Release Inhibiting Hormone (PIH) (Tentatives as yet) | Dopamine | Inhibits prolactin release |
| MSH Release Inhibiting Hormone (MIH) | Peptide | Inhibits MSH release |

The releasing factors are carried from the hypothalamus to the anterior lobe via the **porta vessels** (Fig. 10.11 A)

A portal system is a series of blood vessels that carry blood between two sets of capillaries without first returning to the heart.

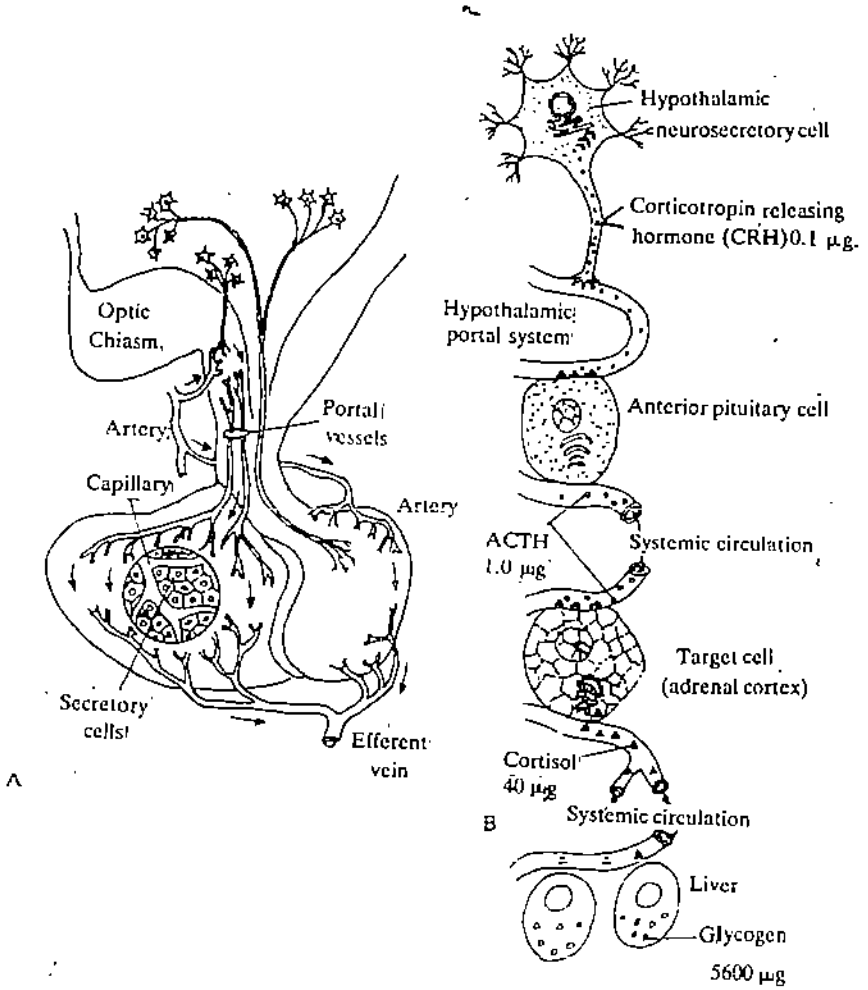



Fig. 10.11 : The hypothalmo-hypophyseal-portal system (A). Amplification in ACTH-endocrine system (B).

By now you would appreciate that the pituitary and hypothalamus are the main centres of physiological control. Unlike the brain, the hypothalamus is not protected by the blood brain barrier hence it detects any changes that take place in the blood stream. Various sensory devices in the hypothalamus also detect any change. Any sensory information from the brain is also routed through the hypothalamus. Thus, it connects the neural and blood borne information and coordinates both sets of inputs to give out and appropriate response. The sequence of hypothalamus → pituitary → endocrine gland enables a weak signal to be amplified many times. Examine Fig 10.11 (B) carefully. It shows stepwise, how 0.1 µg of CRF can stimulate the deposition of 5600 µg of glycogen in the liver.

## 10.4.2 Regulation of Hormone Secretion

The secretion of hormones occurs normally at a basal or 'resting' level. This level is required by the body to regulate the composition of body fluids or the rate of metabolism or other bodily functions. This basal level is raised or lowered by signals acting on the endocrine tissue. The signals may be in the form of neurotransmitters released directly on to the endocine tissue or they may be hormones released from other endocrine tissue.

Endocrine tissues may simply secrete in response to internal or external stimuli without modulation or they may be a part of a **feedback circuit**. You are already familiar with the basic meaning of a feedback circuit (Unit FST-1). The secretion thus is affected by one or more consequences of the secretion.

The secretion from endocrine tissues is generally under **negative feedback control** i.e., the concentration of the hormone itself or a response to that hormone by the target tissue has an inhibitary effect on the further secretion of the hormone. Such negative feedback may be short-looped or long-looped.

Let us take some examples to explain this further. A drop in the calcium level in the blood causes the secretion of parathormone from the parathyroid gland. Parathormone causes the release of calcium from bones, decreases excretion of calcium from kidneys and increases the absorption of calcium from intestines. All these processes increase the calcium concentration in the blood so that it is back to normal within a few hours. The rise in calcium in the blood now inhibits the secretion of parathormone (Fig. 10.12). This is an example of a short-loop negative feedback system. Similar simple feedback loops control hormone secretion from posterior pituitary.

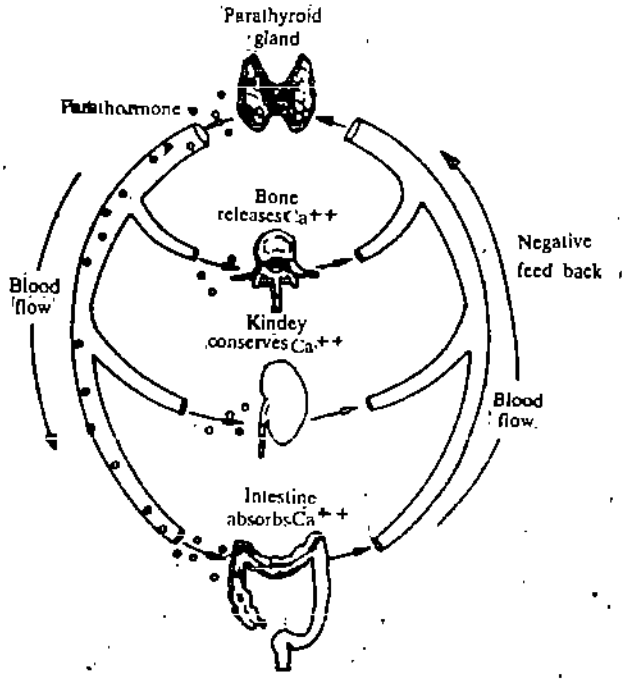



**Fig. 10.12 : Feedback control of parathormone secretion**

Sometimes inhibition may occur at two sites forming a short and a long loop circuit. Hormone secretion from anterior pituitary is an example of a more complex negative feedback loop (Fig. 10.13).

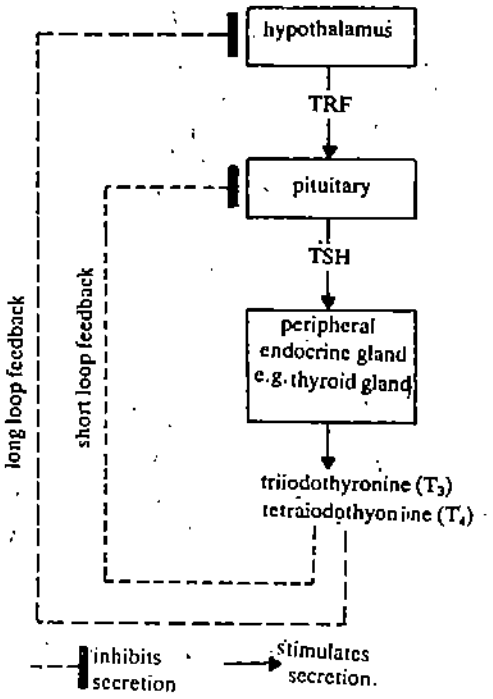



**Fig. 10.13 : Feedback control of thyroid hormones as an example of tropic hormones.**

A high level of thyroxine in the blood inhibits the release of thyroid stimulating hormone (TSH). This inhibition takes place at the anterior pituitary as well as hypothalamus.

Apart from negative feedback there is also a process of control that feeds upon itself—i.e., the secretion of hormone leads to its increased secretion. This is known as **positive feedback** and occurs in some vertebrate reproductive cycles in which strong response must be built up to a peak relatively quickly.

Upto now we have studied the general principles involved in endocrine functions. Most of the examples used to emphasise a concept have been taken from vertebrates, mammals in particular. In the next section, we shall study the invertebrate hormonal system using some insect hormones.

**SAQ 4**

a) Given below are some statements that are not entirely incorrect but need to be modified if they are to be precise. Make corrections wherever necessary.

   i) The secretion of hormones from the anterior pituitary lobe is controlled by releasing factors secreted by the brain.

   ii) The cells of the anterior pituitary are connected to the hypothalamus by neurons.

   iii) The secretion of thyroxine is controlled by pituitary.

b) Fill in the blanks with appropriate words from the text.

   Hormone secretion is regulated by a ..... control mechanism. A control process that leads to increased secretion of hormone in response to initial secretion levels is known as .....

## 10.5 INSECT HORMONES

During the last half century, physiologist have seen that many invertebrates too have endocrine systems that approach the complexity of vertebrate endocrine systems. However, there are few similarities in the hormones of both groups of animals.

The principal source of hormones in invertebrates are the neurosecretory cells and their products are secreted directly into the circulation. The most extensively studied group of invertebrates is Insecta hence we shall limit our discussion to hormones found in insects.

Insects being fairly hardy organisms, proved to be ideal subject for the kind of experiments conducted on them (Box 10.4). It has been found that control of development and moulting in insects depends on several major hormones (listed in Table 10.4).

**Table 10.4 : Developmental hormones in insects.**

| Hormone | Tissue of Origin | Structure | Target Tissue | Primary Action |
|---|---|---|---|---|
| Prothoracicotropin, PTTH (brain hormone) | Neurosecretory cells in brain | Peptide | Prothoracic gland | Stimulates ecdysone release |
| Ecdysone (molting hormone) | Prothoracic glands, ovarian follicle | Steroid | Epidermis, fat body, imaginal disks | Increases synthesis of RNA, protein, mitochondria, endoplasmic reticulum; stimulates secretion of new cuticle |
| Juvenile hormone(JH) | Corpus allatum | Terpene derivative | Epidermis, ovarian follicles, accessory sex glands, fat body | In larvae promotes synthesis of larvae structures; inhibits metamorphosis; in adult stimulates yolk protein synthesis and uptake, activates ovarian follicles and accessory sex glands |
| Bursicon | Neurosecretory cells of CNS | Peptide | Epidermis | Promotes cuticle development; induces tanning of cuticle of newly molted adults |
| Diapause hormone (silk moth, *Bombyx*) | Neurosecretory cells in subesophageal ganglion | Peptide | Ovaries, eggs | Induces diapause of egg |
| Eclosion hormone | Neurosecretory cells of brain | Peptide | Nervous system | Induces emergence of adult from puparium |

Box 10.4

The first experiments to find whether endocrine secretions control insect development were done by S Kopeč between 1917 and 1922. Kopeč ligated the last instar larvae of a moth at different times during the instar. He found that if the ligation was done before a critical period the insect would develop into an adult anteriorly and remain a larva posteriorly. Cutting the nerve cord was of no effect but if the brain was removed the larvae would not become an adult. Reimplantation of brain, however, allowed pupation to occur. It was ultimately found that a substance secreted from neurosecretory cells of brain induced the prothoracic gland to secrete a hormone that induces moulting. The substance was named **prothoracicotropic hormone** (PTTH).

Fig. 10.14 summarises the role of insect endocrine system in moulting and development.

About a ton of silkworm pupae were required to find out the steroid structure of ecdysone.

1) Neurosecretory cells in the brain synthesise PTTH which is stored in their terminal axons that end in **corpus cardiacum**. The hormone is released into the blood.

2) PTTH in blood activates the prothoracic gland to synthesise the moult-inducing hormone **α - ecdysone** (Fig. 10.3(b))|which is a steroid resembling cholesterol. α-ecdysone is now considered to be a prohormone and is converted to active form β-ecdysone in several target tissues.

3) The corpora allatum contains nonneural endocrine tissue that produce and secrete another hormone-**juvenile hormone (JH)** (Fig. 10.15). This promotes the retention of juvenile characters till larval development is complete.

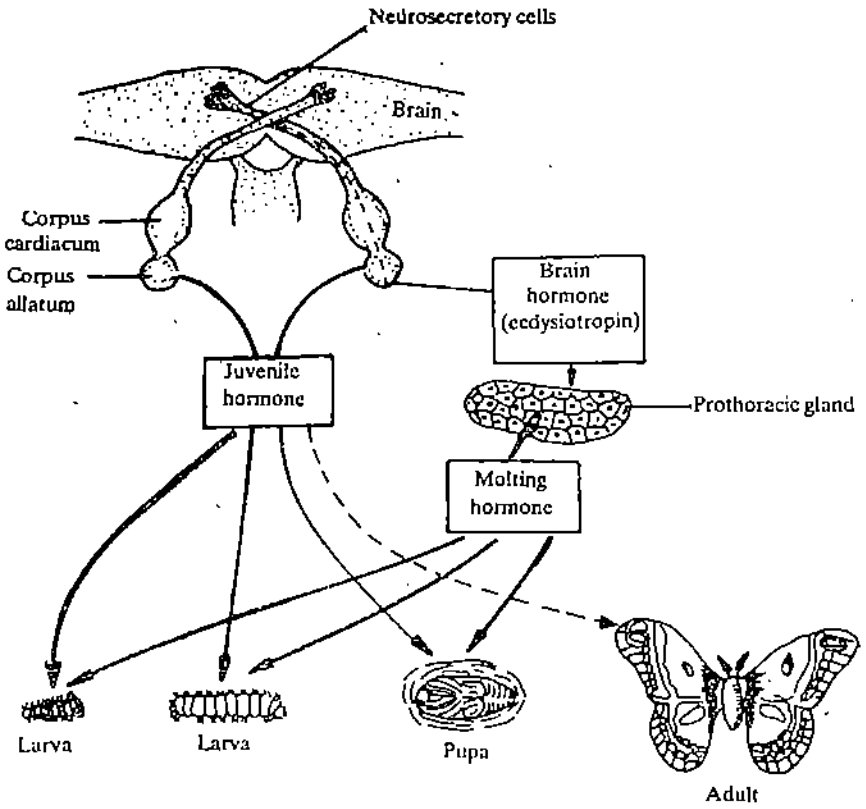



**Fig. 10.14 : Role of insect hormones in moulting and development.**

The circulating JH level is highest in early larval stage and gradually drops till metamorphosis to adult stage occurs when JH disappears from circulation. The concentration rises again in adult reproductive life. JH promotes development of accessory sexual organs in males of certain insect species and induces yolk synthesis and maturation of eggs in many female insects.

Two additional hormones eclosion hormone and bursicon regulate the terminal phase of moulting or shedding of the cuticle.

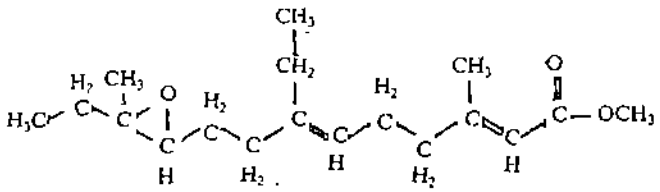


**Fig. 10.15 : Structure of juvenile hormone extracted from moth (*Hyalophora cecropia*).**

The normal development of an insect depends on precise adjustments in concentration of JH at each stage. Synthetic compounds with juvenile hormone like activities hold great promise as future insecticides. These compounds are effective in very small quantities and if they are applied at the appropriate times, they can prevent the formation of adults and thus reproduction. JH analogs would be a more attractive nontoxic prospect than application of DDT and one against which insects would find it difficult to develop resistance.

**SAQ 5**

*Dysdercus cingulatus*, the red cotton bug is a pest on cotton plants. What is the role envisaged for a JH analogue in the control of this pest?

.....

.....

.....

.....

.....

## 10.6 PHEROMONES

The word pheromone is derived from Greek word "pherein" meaning to transfer and hormone meaning to excite.

Earlier in the unit we had mentioned about local chemical mediators like histamines, prostaglandins and growth factors. You have read about their mode of action in Unit 15 of LSE-01. You have also studied that both neurotransmitters and hormones are chemical messengers in the body's communication system. While these are chemicals that carry messages inside the body, certain chemicals known as **pheromones** carry information outside the body to other members of the same species.

A pheromone is, therefore, a chemical substance released by an animal into its surroundings which influences the behaviour or development of the individuals of the same species. In many insect species, females release pheromones which function as sex attractants and induce males to come to females to participate in the reproductive behaviour. In honey bee colony, the queen bee produces a chemical called **queen-bee substance** that inhibits development of other queens. Animals of many kinds including protozoa , mammals, fish, amphibian and insects are known to employ pheromones as a primary means of communication, i.e. transmitting information. The pheromonal communication may be used for a variety of purposes in different species: attracting a mating partner, for causing others to stay away when it is appropriate, for directing others to suitable food or resting sites etc.

Mammals in particular use pheromones in urine or faeces or from special scent gland to mark traits and territories. When a dog urinates on a tree, he is depositing a pheromone that will tell other dogs that the tree is a part of his territory. Other large cats such as lions and tigers do the same in the forest. Pheromones also accelerate reproductive maturity in a number of species and permit members of one sex to distinguish which members of the opposite sex are in breeding condition.

The following example will illustrate how pheromones work. Female gypsy moths *Lymantria dispar* emit into a prevailing breeze plumes of a chemical substance known as **gyplure** to "call" male moths that may be hundreds of meters downwind. The male moths are able to recognise the wind-borne pheromone plume by means of receptors located on their antennae. It should be understood that the pheromone emitted by the female contains molecules in a species-specific proportion. The specificity is remarkable since only the males of the particular species will respond to it, and the male will be able to distinguish his species pheromone from another even when many other species are signalling at the same time. The pheromone acts as an odorous (olfactory) stimulus for males. The odor constitutes a message disclosing the presence of conspecific female. Once the male has reached close to the female, male and female exchange another set of messages mediated by chemicals (pheromones), a mating procedure is initiated resulting in copulation. Thus, close-range sexual behaviour is mediated by another set of pheromones. In a few insect species, male insects also produce sex pheromones which facilitate their mating behaviour and may attract females.

Many male animals are known to perform complex courtship displays when near females. For example, male rhesus monkeys continue to groom their sexual partner for quite some time when they smell her sex pheromone. Many male fish perform complex courtship displays when near females, which are caused by pheromones released by female fish. For example, males of the fish *Bathygobius soporator* make rapid fanning and gaping movements and change body colour as courtship behaviour induced by female pheromone.

The pheromones used to mark territories or attract a mate produce immediate effect on the nervous system, physiology and behaviour of the receiving animal.

### 10.6.1 Neural Basis of Resposes to Sex Pheromones

The ability of insects to detect and discriminate between a variety of pheromones with great sensitivity depends on the presence of specific neural structures in the neuropile of the male antennal lobes. The female antennal lobe lacks these structures. The electrophysiological signals resulting from interaction between pheromonal molecules and antennal olfactory receptors are passed via the olfactory nerve to the specific part of the brain containing specific neural circuitary to decode complex pheromonal signals. The brain then translates pheromonal information into the act of directional locomotion or orientational responses.

There are pheromones that act more slowly and have longer lasting effects. For example, if a newly fertilised female mouse is caged with a strange male, the odor of that male will terminate pregnancy. The pheromone responsible comes from the urine of the male. It is received by the olfactory receptors of the female and triggers activity in the hypothalamus which directs the pituitary to release a hormone that reduces the steroid hormone output of the ovaries. The uterus does not receive adequate hormones for implantation of foetus and the pregnancy aborts.

### 10.6.2 Phermones Serving Functions Other than Reproduction

Besides sexual responses as described above pheromones mediate several other types of behaviour. **Alarm pheromones** have been found in termites and bees. The termite soldiers liberate alarm pheromones attracting (communicating alarm signals) the aggressive workers that participate in attacking the intrusive individuals. **Trait pheromones** secreted by insects help social integrity during migration of colonies by orienting along an invisible chemical trail that has been laid out by one or more conspecific insects. Both aerial and terrestrial trails are possible. Some species of ants and termites deploy trait pheromones in the recruitment of workers to food sources. **Aggregate pheromones** make it possible for both sexes to aggregate. They are produced by members of both sexes. Pheromone-mediated aggregation help to aggregate insects for feeding, protection, reproduction etc. A pheromone, **2-methoxy-5- ethylaphenol** is produced from faeces of the migratory locust, *Locusta migratoria migratorioides*. The pheromone causes the young hoppers to aggregate and is also associated with the induction of morphological and physiological changes that result in the transformation of the hoppers to the migratory phase. In cockroaches also, the pheromones released from various surfaces of the body and from the faeces cause aggregation. In the protozoan *Dictyostelium discoideum* (amoebae, also known as cellular slime molds) the amoebae release pulses of a pheromone, which has been identified as cyclic 3′–5′AMP resulting in the aggregation.

## 10.7 CONCLUSION

Now that you have worked through both Units 9 and 10, you should be able to see clearly the basic principles involved in physiological communication. A stimulus from within or outside the animal triggers the release of chemical messengers that communicate with specific target organs.

In these units we have laid emphasis on the similarities between the action of hormones and nerves not only in their use of chemicals as messengers but also in the way the messengers and receptors interact. But two important differences can be noted:

One related to the speed of action and the other to the size of target. Whenever a quick response is required the nervous system reacts. Nerve impulses move at a speed upto 100 m per second and delay in transmission is hardly more than a few milliseconds. A process regulated by hormones requires that the hormone reach the target organ and this takes place through the blood stream. The minimum response time will be in the magnitude of seconds. Hormones usually control processes that are slow for example the secretion of gastric juices, development of gonads and growth of body.

The second important difference is that action of hormones is diffuse. For instance, hormonal action on liver causes all the cells of the liver to release sugar while neural action permits stimulation of even a single muscle fibre without affecting the others.

However, there is no sharp demarcation between nervous and hormonal control. The nervous system not only regulates the endocrine function it is important in the actual production of hormones. Both systems work hand in hand supervising the animal's complex body functions.

## 10.8 SUMMARY

In this unit you have studied that :

- Hormones can be broadly subdivided into a ) steroids, based on cholesterol structures; and b) polypeptides based on amino acids. The amino acid sequence of a particular polypeptide hormone may vary in different animal species.
- All hormones except steroids are secreted by process of exocytosis; steroids can diffuse through the membrane. Duration of hormone action depends on its longivity and rate of breakdown by liver or excretion from the body. Since receptor cells for hormones are very sensitive and specific, the hormones are required in very minute quantities.
- Steroid hormone receptors lie in the cytoplasm and carry the hormones to the nucleus where it induces transcription activity whereas, receptors for peptide hormones are plasma membrane bound. This results in the formation of a second messenger molecule that interacts with the cellular effectors that may be enzymes, membranes or microfilaments etc. The overall response depends on the particular effector.
- Specialised neurosecretory cells secrete neurohormones into the blood stream which transports them to the target cell. Because of neurosecretory cells, nervous system coordinates the activity of the endocrine system.
- The hypothalamus and pituitary are important centres of physiological regulation. The pituitary gland is divided into two lobes. The anterior lobe which secretes mainly tropic hormones i.e., they regulate the release of hormones from other endocine cells. The hormones of anterior pituitary are in turn regulated by releasing and release-inhibiting factors secreted by neurosecretory cells of hypothalamus. The posterior lobe of pituitary acts as releasing site of hormones produced in hypothalamus.
- The neurosecretory substance → endocrine cell (pituitary) → other endocrine cell arrangement in vertebrates amplifies the original signal. It also forms several feedback loops that finally regulate the whole system.
- Invertebrates also possess endocrine systems. The principal source of hormones are neurosecretory cells. In insects juvenile hormone and ecdysone are the two hormones that control and regulate moulting and development.
- Pheromones are chemical substances that carry information outside the body to other members of the same species and influence their behaviour or development. Pheromones affect the olfactory receptors which transmit the signal to the specific neural circuits to decode the pheromonal signals.
- Communication systems in animals, whether hormonal or nervous basically utilise chemical signals that interact with specific target organs. The two differences, however, are that neural signals are fast acting and can affect even a single cell without affecting others, whereas, hormonal action is slower and diffuse.

## 10.9 TERMINAL QUESTIONS

1) What are the factors that determine

   a) which target tissue responds to a particular hormone and

   b) duration of response?

   .....

   .....

   .....

2) Compare the action of steroid hormones and protein hormones with respect to

a) structure

b) receptors

c) mechanism of action

.....

.....

.....

3) List the hormones secreted by each of the following endocrine glands and give their action briefly.

a) posterior lobe of pituitary

b) anterior lobe of pituitary

c) adrenal medula

d) β-cells of Pancreas

.....

.....

.....

.....

4) How does the hypothalamus regulate endocrine activity?

.....

.....

5) Draw a flow chart to illustrate the hormonal control of development in insects.

6) Pheromones are also part of the chemical signal systems in animals. In what way are they different from hormones.

.....

.....

.....

## 10.10 ANSWERS

### Self-assessment Questions

1) i) True. Both neurotransmiter and peptide hormones are composed of amino acids.

ii) False. Hormones in different animal groups may differ slightly in structure so they have different effects on different animals.

iii) True. They are synthesised from cholesterol molecule.

iv) False. Protein-hormone aggregates prevent them from being excreted by kidney. They are a source of slow release of hormones.

2) Chemical messenger; specific regulatory.

3) iv)

4) a) i) Release as well as release-inhibiting factors are secreted by a specific region of the hypothalamus and not the whole brain.

ii) Not by neurons directly but via a portal system in which the neurohormones are released that affect the anterior pituitary.

iii) The hypothalamus is also involved in the feedback loop.

b) negative feedback; positive feedback.

5) Since juvenile hormone suppresses metamorphosis, any compound showing activity similar to its action would result in the larvae retaining their larval characters and never metamorphosise into adults. They are not able to reproduce and increase their population.

**Terminal Questions**

1) a) Presence of appropriate receptor molecules in the target cells

b) The rate at which the hormone is secreted, the rate at which the hormone is broken down in the liver and the rate at which it is excreted in urine determine the duration of response.

2) Refer to Section 10.3.

3) Refer to Table 10.1 and 10.2.

4) **Hint**: By regulating activity of pituitary gland which in turn stimulates other endocrine glands to secrete hormones. Refer to Section 10.4 for details.

5) a) Neurosecretory cells in brain

↓ PTTH

Corpora cardiacum

↓ PTTH

Prothoracic gland

↓ ecdysone

induces moulting

b) Endocrine tissue in

Corpora allatum

↓ JH

retention of juvenile character.

6) Hormones are chemical signals that carry information inside the body while pheromones are chemicals deposited in air or as liquids outside the body that affect the behaviour of other individuals of the same species. Pheromones act on the olfactory receptors which carry the information to the brain which directs an appropriate response.

## GLOSSARY

**acclimation** : the persisting change in a specific function of the body due to prolonged exposure to an environmental condition such as high or low temperature

**acclimatisation**: the persisting spectrum of changes due to prolonged exposurs to an environmental condition such as high or low temperature

**action potential (nerve impulse, spike)** : an all-or-none electrical event in a neuron or muscle fibre, in which the polarity of the membrane is reversed rapidly, and reestablished

**adrenal cortex**: the outer part of the adrenal gland derived from embryonic mesoderm. It secretes steroid hormones

**adrenal medulla** : the inner part of adrenal gland derived from postganglionic sympathetic neurons. Secretes catecholamine hormones

**amacrine cells**: neurons without axons, found in inner plexiform layer of retina

**ambient temperature**: surrounding or prevailing temperature

**amniotes**: any reptile, bird or mammal. Vertebrates whose embryos are enclosed in inner foetal membranes that contain fluid

**anaminiotes**: vertebrate classes that do not have foetal membranes and fluid surrounding the foetus. Consist of Agntha, fishes and Amphibia

**androgens**: steroids containing eighteen carbons that have masculinising effects

**birth canal**: formed by dilation of cervix due to the action of relaxin to facilitate parturation

**blastocyst**: stage in mammalian development resulting from cleavage. A thin walled hollow sphere containing at one side a knob of cells destined to become embryo proper

**blood-brain-barrier**: the structure and cells that selectively prevent particular molecules in the plasma from entering the central nervous system.

**catecholamines**: a group of molecules including epinephrine, norepinephrine, L-dopa, and related molecules that have effects similar to those produced by activation of sympathetic nervous system

**circadian rhythms** : physiological changes that repeat at about a 24-hour period which are synchronised to changes in external environment such as day-night cycles

**corpora allata**: nonneural insect glands existing as paired organs or group of cells dorsal and posterior to corpus cardiaca. They secrete juvenile hormone

**corpora cardiaca**: major insect neurohaemal organs. Paired structures posterior to brain. They liberate brain hormone

**estrogens**: a family of female sex hormones responsible for producing estrous and female secondary characters

**estrous cycle**: periodic changes in structure and function of ovaries and female reproductive tract in mammalian species. This is accompanied by a period of receptivity

**evaporative cooling**: cooling of body by application of saliva which evaporates and lowers the body temperature

**GABA**: gamma-aminobutyric acid; it is believed to function as inhibitory neurotransmitter in central nervous system

**gel state**: stiff high-viscosity state of cytoplasm

**implantation**: attachment of mammalian embryo to lining of uterus preparatory to forming placenta

**lordosis**: arching of back in female rats in response to handing and when approached by male rats.

**lutinisation**: formation of corpus luteum and secretion from it

**pathological conditions**: diseased conditions

**puberty**: the period of time in an individual's life span when secondary sexual characteristics and fertility develop

**secondary sexual characters**: characters that distinguish between opposite sexes in animals excluding the gonads, the duct and accessory glands

**sol state**: low viscosity state of cytoplasm

**vaginal smear**: scrapings from vaginal wall which is lined by stratified non-glandular epithelium that undergoes cyclic changes

**viseral organs**: organs lying in abdominal cavity

## FURTHER READING

*General and Comparative Physiology*, William S. Hoar (Third Edition) 1991. Prentice Hall of India Pvt. Ltd., New Delhi.

Dear Student,

While studying the units of this block, you may have found certain portion of the text difficult to comprehend. We wish to know your difficulties and suggestions, in order to improve the course. Therefore, we request you to fill and send us the following questionnaire, which pertains to this block. If you find the space provided insufficient kindly use a separate sheet.

## QUESTIONNAIRE

LSE-05
Block-2

Enrolment No.

1) How many hours did you need for studying the units?

| Unit Number | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|
| No. of Hours | | | | | |

2) How many hours (approximately) did you take to do the assignment pertaining to this block?

| Assignment Number | | |
|---|---|---|
| No. of hours | | |

3) In the following we have listed 4 kinds of difficulties that we thought you might have come across. Kindly tick (√ ) the type of difficulty and give the relevant page number in the appropriate columns.

| Page Number and line Number | Type of difficulties | | | |
|---|---|---|---|---|
| | Presentation is not clear | Language is difficult | Diagram is not clear | World/Terms are not explained |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

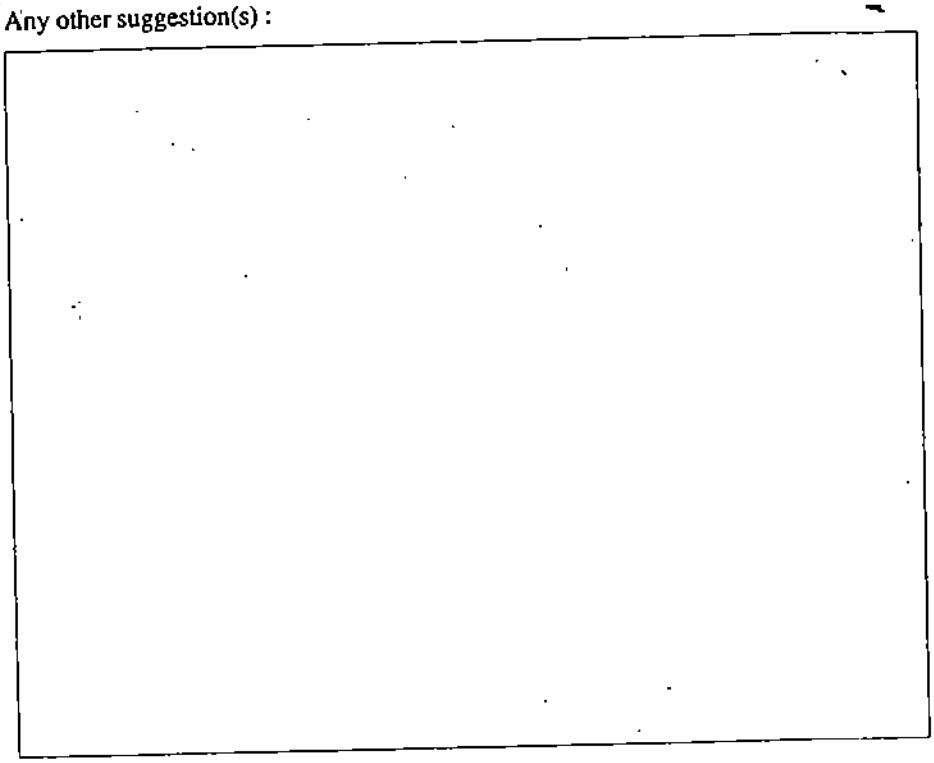
4) It is possible that you could not attempt SAQs and TQs. In the following table are listed the possible difficulties. Kindly tick ( √ ),the type of difficulty and the relevant unit and question numbers in the appropriate columns.

| Unit No. | SAQ No. | TQ No. | Type of Difficulties | | | |
|---|---|---|---|---|---|---|
| | | | Not clearly posed | Cannot answer on basis of information given | Answer given (at end of Unit) not clear | Answer given is not sufficient |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |

5) Where all the difficult terms included in the glossary? If not please list the words in the space given below.

| |
|---|
| |

6) Any other suggestion(s) :



---

---

To

The Course Coordinator (LSE-05; Physiology; Blocks 1 & 2)
School of Sciences
Indira Gandhi National Open University
Maidan Garhi
New Delhi-110068

# NOTES

# NOTES

Uttar Pradesh
Rajarshi Tandon Open University

# UGZY/BY-08
# Physiology

Block

3

## PLANT PHYSIOLOGY — I

# BLOCK 3 PLANT PHYSIOLOGY—I

In the previous two Blocks you have studied Animal Physiology. In this and the following Block we will deal with Plant Physiology. Because of the different modes of life that plants and animals have adopted over millions of years there necessarily are many differences between the two forms of life especially so far as their physiology is concerned. Water absorption and transport, uptake of mineral ions from the soil, photosynthesis and $N_2$-fixation are unique to plants.

In broad sense this Block concerns with nutrition of plants, particularly higher plants. Plants need enormous amounts of water in order to live and grow. They synthesise all their food and other materials from the simplest of chemicals — $H_2O$, mineral nutrients and $CO_2$. The roots absorb water and minerals and leaves take in $CO_2$ from the atmosphere. In Unit 11 of this Block we discuss absorption and loss of water and mechanism of water transport to great heights. Minerals are transported along with water; however, the uptake of minerals into the root cells involves special transport mechanisms which is dealt in Unit 12.

One process which is absolutely unique to plant kingdom is photosynthesis about which you would learn in Unit 13. The food produced in the leaves by photosynthesis is supplied to all parts of the plant by an efficient distribution system which is discussed in the last unit of this Block.

**Objectives**

After studying this block you will be able to:

- explain the water relations of plants with reference to water absorption, water loss and transport,
- describe the role of essential nutrients and the mechanism of their uptake,
- describe the reactions involved in the process of photosynthesis and photorespiration,
- discuss the role of biotechnology in improving the photosynthetic efficiency in crop plants,
- discuss various hypotheses proposed for the transport of food through phloem.

## General Study Guide

To get maximum benefit out of this study material please take note of the following points:

i) Make a notebook with one side ruled and the other side plain, keep a pen and some coloured pens/pencils with you.

ii) Read the material slowly and attentively. Spend enough time on figures and flow charts. Try to draw the figures/flow charts and label them properly. This will help you in better understanding of the text.

iii) While studying the text, underline the important points with a distinct colour (red, green, blue etc.) in the block itself. Write down salient points in the space provided on each page, or in your notebook, if necessary.

v) After finishing a section or subsection; ask yourself — what have I learnt? Try to list the important points in your notebook and compare them with the text and see if you have missed any.

) Attempt all the self-assessment questions (SAQs), wherever they appear. Don't skip any of them as they are designed to assess your understanding of the subject. If you cannot answer, read the text again.

i) The answer to the SAQs and Terminal Questions are given at the end of each unit. **Don't** get tempted to see the answers, before you try them.

ii) If you don't understand any word in the text, consult a dictionary. For scientific and technical words consult the glossary given at the end of each block or a scientific dictionary, if necessary.

iii) In case you get stuck with some fundamental concept, consult NCERT school books. You will find them very useful and easy to understand. For exploring

the topics further we have given a list of suggested readings at the end of each block. Most of these books and NCERT books are available at your Study Centre.

## Study Guide for the Block

The understanding of this block requires knowledge of Cell Biology Course. At various places in the text we have indicated the sections that you may like to revise before reading further. Some of the captions are lengthy because the explanations are avoided in the text; the captions explain the details of the figures. We advise you to spend some time for reading them carefully so as to have a complete understanding of the text as well as figures.

**Acknowledgements**

Dr. V.V. Raghavan, IGNOU, for his useful comments on some units of this Block, and Dr. Anil Grover, Delhi University, for Unit 11 of this Block.

# UNIT 11 PLANT WATER RELATIONS

**Structure**



## 11.1 INTRODUCTION

At one time it was thought that man would be able to colonise the moon and other planets. However, even before man landed on moon his hope was shattered. The reason, neither there was the atmosphere containing gases which sustain us on the earth nor was there any water which is essential for life. Life originated in water of the oceans and water remains a key molecule in maintaining life on earth.

The intimate relationship between water and plant is apparent from the greenery in areas where water is abundant and barren deserts in areas where it is in extreme deficit. Among the environmental factors — light, temperature, water and soil, it is the water that limits plant growth in virtually all environments.

The quantity of water absorbed by plant is enormous and is far greater than taken by animals of the same weight. This is because the loss of water by plants through transpiration is enormous. A major challenge before plant physiologists is to find ways to decrease water losses and increase the efficiency of water use by the plants.

Plants can move water to great heights in trees. Even the tallest tree *Sequoia sempervirens* (113.1m, and still growing) found in California faces no problem in moving water to its top leaves. This was puzzling for the scientists. They tried to find out the forces that move water to such great heights.

We have the following questions before us with regard to water relations of plants:

i) What is the force that drives the flow and direction of water in plants? ii) What are the forces that drive water to great heights in trees? iii) What are the factors that control the opening and closure of stomatal aperture? and iv) Why do plants transpire so much water in an apparently wasteful way?

In this unit we will try to find answer to these questions.

### Objectives

- describe early experiments on water movement through vascular plants,
- explain the cohesion-tension theory of water movement in plants,
- draw and explain radial movement of water from soil to roots and long distance transport from xylem to leaves,
- describe the factors that affect water potential and explain their significance in the transport of water in soil-plant-atmosphere system,
- explain how differences in water potential, $\Delta\psi_w$, affect the direction of water movement,
- calculate $\psi_\pi$, $\psi_p$, $\Delta\psi_w$, water flux and resistance using mathematical expression.

- discuss various resistances that impede water flow in plant and explain their significance to the plant,
- describe the factors that affect water absorption and water loss,
- relate the structure and properties of stomata to their function,
- explain how changes in turgor bring about opening and closure of stomata,
- explain the causes that alter the relative turgor in the guard cells, and
- list factors that control movement of stomatal aperture.

## 11.2 PLANT WATER

### Role of Water in Plants

You know that water is the main constituent of plant cells. It performs the following major functions.

#### i) Water as a Solvent

Water is a very good solvent. It easily dissolves electrolytes and small molecules such as glucose and amino acids. As you know life evolved as a result of chemical reactions that occurred in aqueous medium of the oceans and even today we know that all reactions in a cell occur in aqueous medium.

#### ii) Water as a Chemical Reactant

Water participates in many biochemical reactions. It is involved in photosynthesis, the most important process of life. The photochemical splitting of water evolves oxygen, and the hydrogen atoms are denotated to $CO_2$ for making glucose. During catabolism, carbohydrates, fats and proteins require water for hydrolyses. In the course of your study, you will come across many other biochemical reactions which also involve water.

#### iii) Water Provides Turgidity to the Cells

Plants maintain their shape due to turgidity which is brought about by the hydrostatic pressure of water in the cells. If water moves out, the cells become flaccid (Fig. 11.1). Hydrostatic pressure is also necessary for the enlargement of cells and as a consequence growth results.

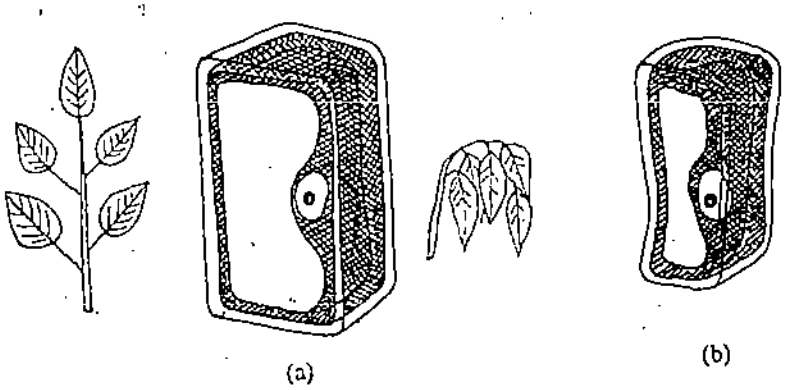



Fig. 11.1 : Cells of a normal (a) and a wilted plant (b). In fully turgid plant the central vacuole of each cell is filled with water and the protoplasm is pushed towards the cell wall, stretching it tight, while in the wilted plant the cell walls may partially deflate after the vacuole shrinks.

### How much Water is Present in Plants?

The amount of water in plants varies among different species depending upon their structure. Aquatic plants such as *Chlamydomonas, Spirogyra, Chara,* red algae and others contain 97-98% of their weight as water. This is true also of *Azolla, Eichhornia* and other fresh water plants. However, among terrestrial plants, whether they are crops such as wheat, rice, maize, or trees such as sheesham, mango, neem and others, the amount of water varies in different plant parts. It would be as high as 95% or more in young roots and as low as 30-40% in wood of a tree trunk. Young leaves often contain 85-90% water whereas mature leaves contain 60-80%. This means that the amount of water changes during the growth of the plant as well as during the growth of an organ such as leaf and seed.

**Relation of Water Content to Functions**

If an aquatic plant appearing turgid is removed from water and left in the open, it quickly wilts. The water content of the leaves may change hardly by 3 to 5 per cent but the plant looks functionless. This is also true for young leaves of wheat which have 90-95% water, they wilt if water content drops by 3-4 per cent. However, an older leaf has only 80-85% water but is fully turgid. Thus, water content cannot be the basis of judging the activity of leaves. Therefore, it is necessary to have an expression for plant water status which could be related to plant function. We will discuss this in one of the sections later.

## 11.3 EARLY HISTORY OF ASCENT OF SAP

It is a familiar fact that water runs downhill. But plants can raise water upwards from soil by roots to great heights of trees. This was one of the puzzles of early plant physiologists. They tried to know what the forces are that drive water upwards. They considered two possibilities : i) water is either pushed up by the driving forces that might develop at the bottom (roots) or ii) it is pulled up by the forces created at the top (leaf) of the plant.

Even 400 years ago, it was recognised that it is the xylem conduits and not the phloem that translocate water in plants. It was Italian anatomist, Marcello Malpighi who in 1679 demonstrated that if a ring of phloem was removed from the stem the path of water was unaffected. Water moves through dead xylem cells was shown by Edward Strasburger in 1883 who sawed an oak tree and placed it in a bucket of water containing picric acid or $CuSO_4$. Though the chemical killed the bark and other living cells the movement of water was uninterrupted. Experiments have also been done using coloured or radioactive water. The water is observed to move into the root system and upward through xylem. Now, we know that xylem vessels form an intricate plumbing network whose supply lines extend to all parts of the plant.

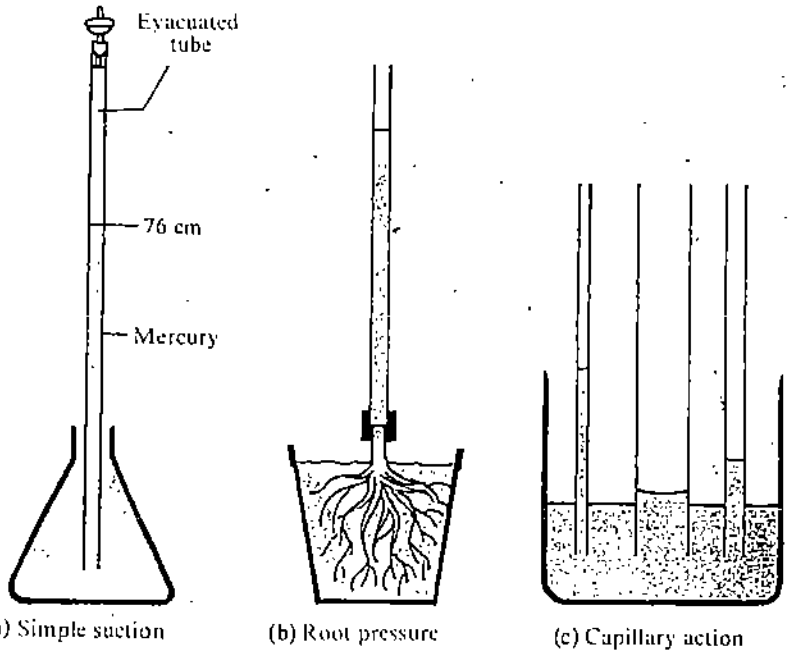



Fig. 11.2 : Experiments on water movement through vascular plants. a) Simple suction — mercury is raised 76 cm up in an evacuated tube by atmospheric pressure. b) Root pressure — a glass tube is fitted on the cut stem of a potted plant. The soil is kept well watered. Root pressure forces water to exude out from the cut stem and up the tube. The water column may rise up to a height of 14 m or more. c) Capillary action — water rises in the capillary tube due to high surface tension of water. The greater the surface tension greater the rise in the capillary.

For investigating which of the forces were responsible for the upward movement of water, initially forces such as atmospheric pressure, capillary action and root pressure were considered (Fig. 11.2 a, b and c). But none of these forces could account for the movement of water beyond a height of 100 cm. It was an Irish Botanist Josef Dohm (1883) who demonstrated by a simple experiment that if water were to be evaporated from a closed system like a porous pot connected to a set up as shown in Fig. 11.3, mercury could be lifted to a height of more than 100 cm, considerably

**Water has a great tensile strength**

Theoretical calculations, from heats of evaporation and surface tension, indicate a tensile strength for water of several thousand atmospheres. Experimental values, however, are somewhat lower, ranging from 25 to 300 atmospheres. In one illustrative experiment the British investigator H.M. Budgett wrung two polished steel plates together with a film of water between them. Tensions of up to 60 kilograms per square centimeter was required to pull the plates apart.

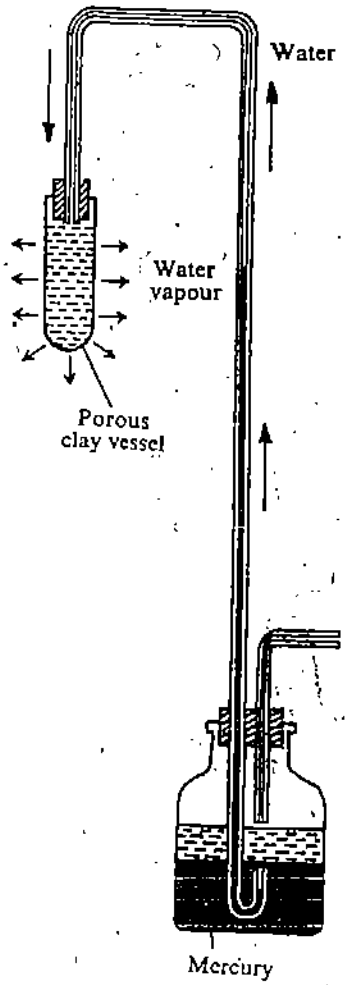



Fig. 11.3 : Demonstration of the principle of water movement up the plant stem by a physical model. Evaporation of water from the porous clay pot exerts more pulling force than simple suction. It can pull mercury behind it to a height of more than 100 cm.

**Guttation**
Under certain environmental conditions root pressure forces water through special water pores around the edge of the leaves or at the tip. This is known as **guttation.**

higher than 76 cm to which it can be pulled by a vacuum. Irish Botanist H.H. Dixon and his collaborator J. Joly repeated the same experiment using a transpiring pine leaf instead of a porous pot and got the same results. A similar experiment shown in Fig. 11.4 illustrates the rise of water in plants. Dixon and Joly (1895) then proposed the now famous **cohesion theory of ascent of sap.**

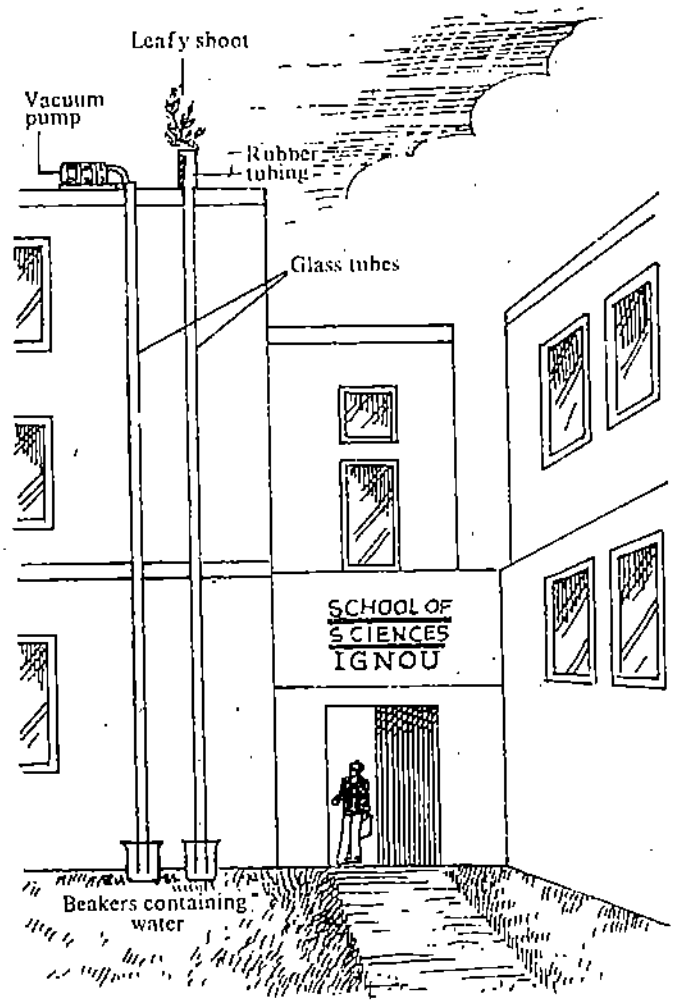



**Fig. 11.4 : Experiment to show the cohesion-tension theory of water movement in the plant. Two long slender tubes dipped in water from below are extended up to the height of the building. A vacuum pump fails to suction the water to this height while a tiny leafy shoot succeeds. A short piece of rubber tubing is used to connect the cut tip of a leafy shoot to a slender glass tubing (14m length and 0.5 mm in diameter) which is dipped in a beaker containing water and dye. The entire system is made continuous water filled air proof system.**

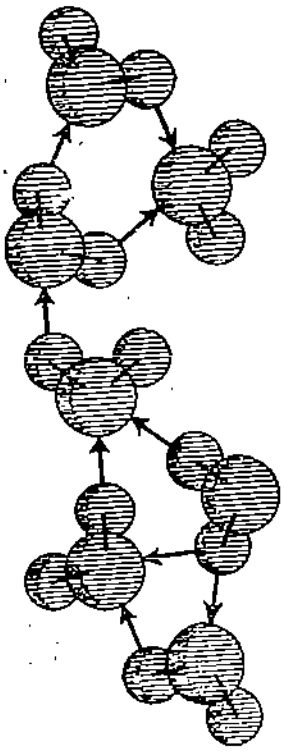


**Fig. 11.5 : Diagrammatic representation of the cohesiveness of water molecules due to hydrogen bonds. Water molecules cling together due to electrostatic attraction between the partial negative charge on the oxygen atom of one water molecule and the partial positive charge on the hydrogen atom of an adjacent water molecule.**

You are aware that plants lose water through stomatal openings by the process of transpiration. Dixon's theory suggests that water can be continuously pulled upward due to the transpiration pull. As the water molecules vacate transpiration sites (the mesophyll cells of leaf lining stomata) due to evaporation, other water molecules are pulled to fill their places. This results in the creation of a negative hydrostatic pressure downward extending from leaves to the xylem vessels and finally to the roots. In other words, a state of water tension is created inside the xylem conduits. The water molecules do not snap away from each other due to tension in the xylem, instead they travel as a continuous column due to their cohesive property. Cohesion is a phenomenon by which water molecules cling tightly to one another by hydrogen bonds (Fig. 11.5) and resist being pulled apart. If water is indeed moving through an upward pull, it would be expected to recede in the xylem, if its continuum is broken by cutting a branch above the ground level. The state of water in xylem is more or less analogous to a stretched rubber band. Suppose if we cut a stretched rubber band at some point, it would immediately recede in both direction from the point where it is cut. A similar receding occurs in xylem vessels and water pulls away from the cut point. The pulling force of water column or tension in the xylem can be measured by a Scholander pressure bomb (Fig. 11.6) by forcing the xylem sap back to the original point by applying pressure.

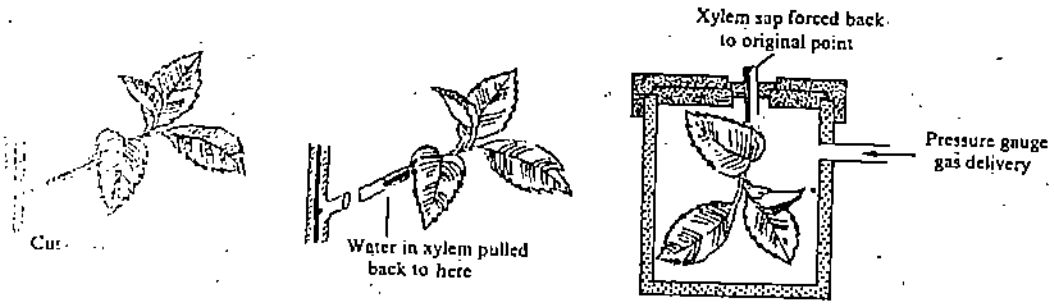



**Fig. 11.6 : Demonstration of tension in xylem sap. When a twig is cut the tension in xylem pulls the sap away from the cut point. The pressure applied in Scholander Pressure Bomb forces xylem sap back to the cut point.**

It is important to point out here that the dead cells of the xylem are greatly strengthened with cellulose fibrils encrusted with lignin. Such strengthening of walls can be compared with reinforced concrete and thus the xylem cells are strong enough not to collapse when water is pulled through them.

Studies on the movement of water at various times of the day also suggest that the "motor" for ascent of sap lies in the leaves of the tree. The velocity of sap flow in the wood was also measured by inserting a heating element into the xylem. After a time interval the distance covered by the heated xylem sap was measured by a thermocouple. The velocities as high as 75-100 cm/hr were recorded (Fig. 11.7).

Although, the cohesion theory of water was proposed about a century ago yet it holds good even today. However, it explains only the long distance transport of water through dead xylem tissue. Many questions — such as what the factors are that affect water absorption from soil, how does water move from cell to cell and in tissues other than xylem and what are the driving forces that determine the direction of water movement — remain to be answered.

In the following sections we explain to you the concept of water potential which can tell us not only the water status, but also the direction and rate of movement of water in a plant.

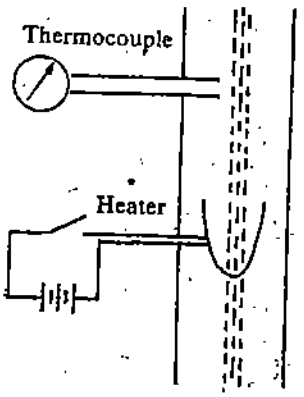



**Fig. 11.7 : Measurement of velocity of xylem sap. A small heating element can be inserted at a point in the trunk upto the xylem. After a time interval the upward movement of the sap can be detected by a thermocouple.**

## 11.4 THE PATHWAY OF TRANSPORT OF WATER

Plants absorb water from the soil by roots, mainly near the root-hair zone in the region of maturation (Fig. 11.8). The radial movement of water is shown in

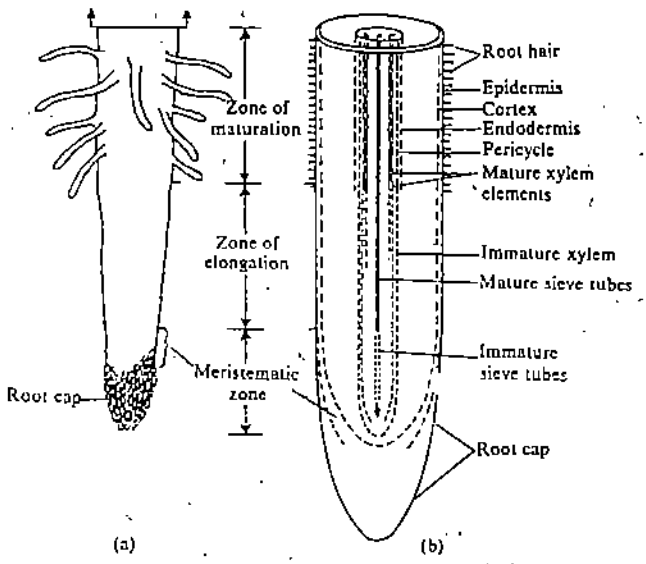
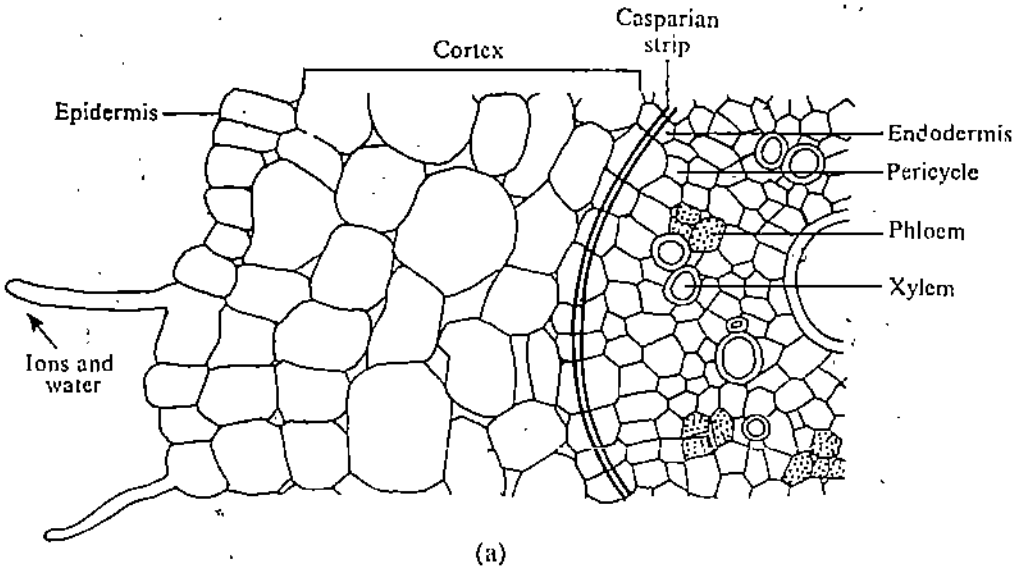



**Fig. 11.8 : a) General morphology of root tip showing root cap, meristematic zone, zone of elongation and zone of maturation with root hair. b) position of various tissues including xylem and phloem.**

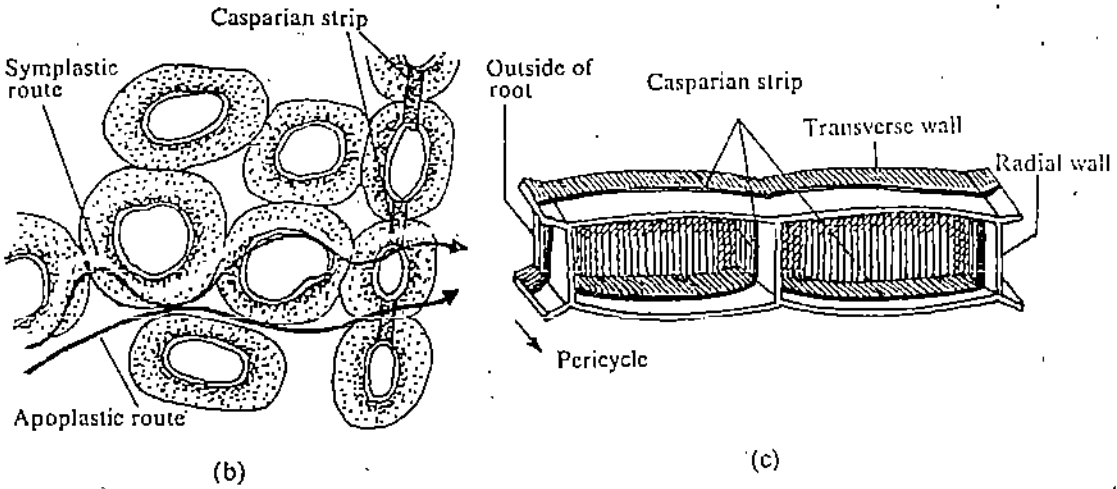
Fig. 11.9a. At most stages of its journey water molecules have the option of moving either through protoplast or through cell walls. Water may first enter the epidermal cells of the root by crossing plasma membrane and then move along through the cytoplasm of the cortical cells, the endodermis, the pericycle and finally into the xylem vessels and/or tracheids. The movement of water through cell to cell occurs probably via plasmodesmata — the protoplasmic bridges between the cells. This transport route is termed **symplastic pathway** (Fig. 11.9b). Since cells are joined via intercellular connections, the entire living portion of the plant forms a continuous single entity and is called **symplasm**.

The other route for the transport of water is through cell walls, intercellular spaces and non-living cells of the xylem (Fig. 11.9b). This is called **apoplastic pathway** and the non-living portion of plants is called **apoplasm**. The cell wall is composed of hydrophilic substances like cellulose, hemicellulose, pectin, lignin and hydrophilic proteins, and carbohydrates, polymers of gums and mucilages. The molecules of these substances retain a lot of water and let it permeate easily without any resistance, when the supply of water is in plenty. When water moves through the cell walls of epidermis and the cortex, its movement is restricted at the highly packed endodermal cells, because the radial and transverse cell walls but not the tangential walls of these cells are lined with suberin a hydrophobic substance in the form of strip called Casparian strip (Fig. 11.9c).

Chemically suberin is like cutin and lignin of cuticle which are impermeable to water. Therefore, water crosses membranes in order to enter endodermal cells and joins the symplastic route. Some water may enter cells at any point prior to endodermis and




(a)




(b) (c)

Fig. 11.9 : a) Cross-section of a root showing radial movement of water from root to xylem. b) symplastic and apoplastic routes. c) Casparian strips.

join the symplastic pathway. Once the water reaches xylem conduits it spontaneously moves upwards through xylem of root, shoot, petiole and finally in the tracheid of leaf vein and mesophyll cells (Fig. 11.10). Most of the water evaporates from the mesophyll cells that line the stomatal cavity and diffuses out to the air through stomatal pore.

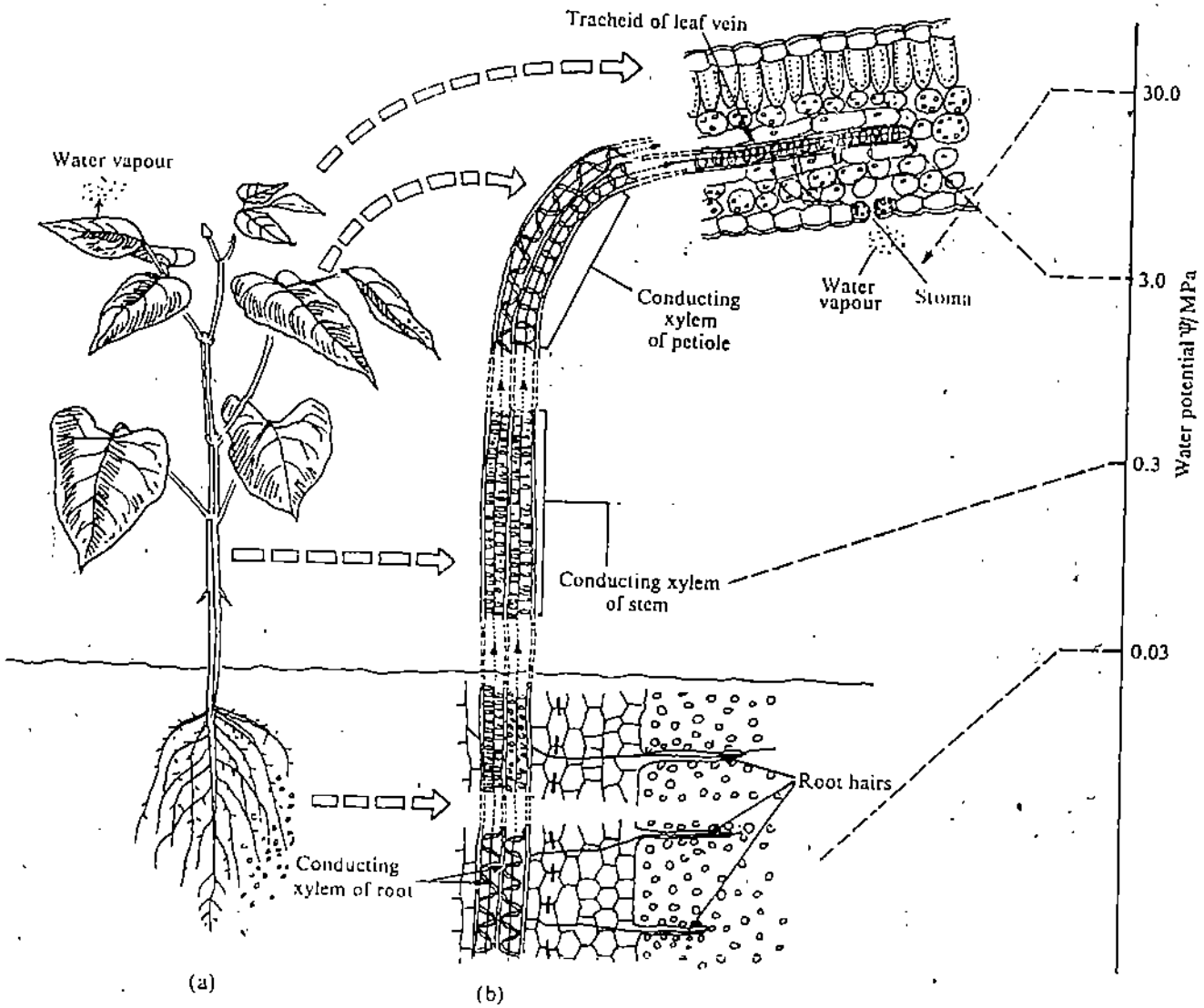



Fig. 11.10 : The pathway of water movement in a plant : a) whole plant. b) movement through xylem. The scale shows water potential at different points in the plant.

**SAQ 1**

a) Which of the following statements are true :

i) A plant (without roots) would not translocate a dye solution if first placed in picric acid solution for about one hour. ☐

ii) If a rapidly transpiring plant is cut off just above the ground, water will ooze out of xylem vessels. ☐

iii) Removing all the leaves from a plant will probably reduce flow of water up the stem. ☐

iv) A soap solution injected in the xylem of a tree-trunk could prevent water from reaching at the top. ☐

**Hint :** Soap solution reduces the surface tension of water.

## 11.5 SOME BASIC PHYSICAL CONCEPTS

The main idea of this section is to explain the concept of chemical potential of water or water potential and the effect of various factors that change its value in plant cell and its immediate environment. The flow of water depends upon the gradient of water potential in soil-plant-atmospheric system. The understanding of this section requires some knowledge of certain basic physical concepts such as diffusion, osmosis, imbibition, and chemical potential. We will explain them briefly. (You need to revise Section 7.3 of Cell Biology Course before reading this section.)

**Diffusion**

Diffusion is a spontaneous process that leads to net movement of a substance from a region of higher concentration to a region of lower concentration. It can also be defined as the net movement of molecules from region of **high free energy** to a region of **low free energy**.

The principle of diffusion is of importance in plants because several of the transport processes such as uptake of water and minerals, intake of $CO_2$ and release of $O_2$ by leaf cells, loss of water due to transpiration depend in part on diffusion.

**Osmosis**

Osmosis is a special case of diffusion. Here, the movement of water (solvent) takes place from a region of higher concentration to a region of lower concentration, if the two are separated by a semi-permeable membrane.

**Osmotic Pressure**

It is the pressure necessary to prevent the flow of water or a pure solvent to a solution (osmosis) when the two are separated by a semi-permeable membrane. Osmotic pressure is given by the following equation

$$\pi = CRT \qquad \ldots (11.1)$$

$\pi$ = osmotic pressure in bars

C = concentration of solution in mol/l

R = gas constant litre bar $mol^{-1}K^{-1}$
Its value is 0.08 litre bar $mol^{-1}K^{-1}$

T = absolute temperature in Kelvin

Vapour Pressure
It is the pressure exerted by vapour over a liquid where it is in equilibrium with itself.

The osmotic pressure of a molar solution (i.e. one mole/litre) is thus

$$= \frac{1 \text{ mol}}{\text{litre}} \times \frac{0.08 \text{ litre bar}}{\text{mol K}} \times 273 \text{ K}$$

$$= 21.84 \text{ bars} \qquad (T = 273 \text{ K})$$

**Imbibition**

It is the process of absorption of water to the nearly dry surface such as seeds and wood. There is liberation of substantial amount of heat during the process.

**Gradient**

It is the difference between concentration or pressure or any other parameter indicative of energy between two specified points. The direction of flow is always from higher energy towards lower energy.

**Chemical Potential**

The chemical potential of a substance in a system is a measure of its free energy, i.e. energy available to do work. Here the system refers to thermodynamic concept wherein studies are of system rather than individuals or bodies. Thus when we study the properties of a solution in the container, we are studying a system wherein each individual component interacts internally. For example, suppose we have pure water in a system be it in a beaker or soil, all the molecules of water can do work, so their free energy is maximum. When a small amount of sugar is added a few molecules of water get associated with each molecule of sugar, the free energy of water decreases. Hence, the free energy of pure water is always maximum. Addition of any solute lowers the free energy or chemical potential of water.

The chemical potential of water in a solution is related to its vapour pressure and is given by the following equation.

$$\mu_w - \mu w^\circ = RT \ln \frac{e}{e^\circ}$$

$$\Delta\mu = RT \ln \frac{e}{e^\circ} \quad \ldots (11.2)$$

$\mu_w$ = chemical potential of water in question (joules/mole)
$\mu w^\circ$ = chemical potential of pure water under STP
$\Delta\mu$ = change in free energy
R = gas constant
T = absolute temperature
e = vapour pressure of water in question
$e^\circ$ = vapour pressure of pure water

Note that

$$\text{Relative humidity} = \frac{e}{e^\circ} \times 100$$

If e is also pure water then $\ln \frac{e}{e^\circ}$ is zero and $\Delta\mu$ becomes zero. So the chemical potential of pure water is set to zero. If e is less than that of pure water then $\ln \frac{e}{e^\circ}$ will be negative number, therefore, $\Delta\mu$ will be less than zero – a negative number.

**Water Potential**

It is the difference between chemical potential of water at any point in a system and that of pure water at STP. By convention the chemical potential of water is referred to as water potential and is denoted by $\psi_w$ ($\psi$—pronounced as psi).

$\psi_w$ is expressed in pressure units. It is the free energy per unit volume of water (joules per $cm^3$).

$$\psi_w = \frac{\Delta\mu}{\bar{\upsilon}}$$

$\bar{\upsilon}$ = partial molal volume

If water potential of the source (the region supplying the water) is higher than the water potential of the sink (the receiving region), then there is a spontaneous transfer of water from source to sink ($\psi_{source} > \psi_{sink}$).

**Partial molal valume (J)** of a solution is the change in volume of a solution when one mole of a substance is added to it.

By convention the water potential of pure water is taken as zero. Therefore, water potentials other than that of pure water will generally be negative. Thus lower potential means a more negative value, and higher potential a less negative value.

Water potential in plants is affected by the solute, hydrostatic pressure and matric forces. In order to predict the movement of water inside or outside a cell we must consider the effect of the above three factors.

**Effect of Solute**

Let us take pure water in two chambers A and B separated by a semi-permeable membrane (Fig. 11.11 a). The water potential of both the chambers is zero. Addition of solute in chamber A (Fig. 11.11b) would reduce the free energy of water and the water potential will fall below zero. Consequently, water will move from B to A (Fig. 11.11c). The effect of dissolved solutes on water potential ($\psi_w$) is called osmotic potential ($\psi_\pi$). It can be estimated numerically if we know the osmotic pressure of the solution. The two are related as

$$\pi = -\pi \quad \ldots (11.3)$$

For example, if $\pi$ of a solution is 5 bars then $\psi_\pi$ would be −5 bar.

Water potential is expressed in pressure units. Energy per unit volume of water is expressed in joules per $cm^3$. This is equivalent to dynes per $cm^2$. $10^6$ dynes = 1 bar. The present unit used for pressure is pascal (Pa). It is a pressure equal to the force of one Newton acting uniformly over one square meter.

1 bar = $10^5$ Pa
$10^3$ Pa = 1 KPa (1 kilo pascal)
$10^3$ KPa = 1 MPa (mega Pascal)

**Effect of Pressure**

Let us now see the effect of pressure on water potential. As Fig. 11.11d illustrates, when pressure is applied the flow of water begins from chamber A to chamber B

through a semi-permeable membrane. This means that pressure increases the free energy of water and thus raises the potential of pure water above zero. The effect of pressure on water potential is called pressure potential and is designated as $\psi_p$. The level of water in B rises due to increase in water potential of A (Fig. 11.11e).

What would happen if pressure is applied on the chamber containing solutes? Now the $\psi_w$ will be affected by both solute and pressure. The solute would lower the water potential and pressure will raise the water potential. So the flow of water from B to A would start decreasing. An equilibrium condition will reach when $\psi_p$ will be equal but opposite in magnitude to $\psi_\pi$ and there will no net flow of water in the two chambers (Fig. 11.11e). This can be represented by the following equation:

$$\psi_{w(B)} = (\psi_\pi + \psi_p)_B \quad \ldots (11.4)$$

Let us suppose if $\psi_p$ is equal and opposite to $\psi_\pi$. Then

$$\psi_{wA} = (\psi_{\pi A} + \psi_{\pi B})$$
$$= 0$$

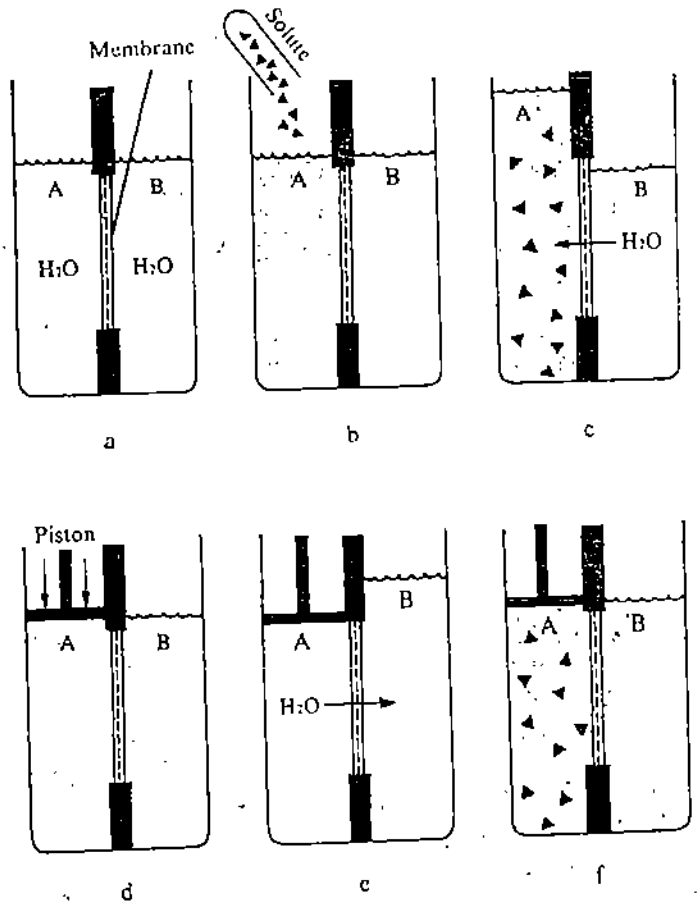



Fig. 11.11 : Experiment to show the effect of solute and pressure on water potential, see text for details.

### Effect of Matric Pressure

Water can get absorbed to the wettable surface of solids such as soil, wood, seeds and cellular constituents. A force operates between solid-liquid interface and is called matric suction or matric pressure. The absorption process is accompanied by heat loss and results in decrease in free energy of water. In other words, the effect of matric forces on water potential is called matric potential ($\psi_m$). Its value will be negative.

In a well watered soil $\psi_m$ is not very significant, however, when the soil is near drying $\psi_m$ determines water potential of the soil.

So we find that the $\psi_m$ is composed of the following main component forces:

$$\psi_w = \psi_\pi + \psi_p + \psi_m + \psi_{...} \quad \ldots (11.5)$$

$\psi_{...}$ = any other force that may influence $\psi_w$.

## 11.6 RESISTANCES TO WATER MOVEMENT AND WATER FLUX

We have explained earlier that if water potential drops from source to sink ($\psi_{source} > \psi_{sink}$) than there will be spontaneous flow of water. But we do not know the rate of this transfer i.e. flux.

Recall from Section 7.2 of the Unit 7, Cell Biology Course, the rate of diffusion $dc/dt$ is flux. The flux for water flow is denoted by $J_w$ which is volume of water flow through unit surface area per unit time. Water in plants flows from cell to cell and also through cell wall.

When water moves from cell to cell the flow is the function of water permeability of the membrane. The flux of water is given by

$$J_w = L_p \Delta\psi_w \quad \ldots (11.6)$$

$L_p$ = permeability coefficient of limiting membranes

$\Delta\psi_w$ = difference in water potential at two points

From equation (11.4) we know that

$$\psi_w = (\psi_\pi + \psi_p)$$

$$\Delta\psi_w = (\Delta\psi_\pi + \Delta\psi_p)$$

substituting the value of $\Delta\psi_w$ in above equation

$$J_w = L_p (\Delta\psi_\pi + \Delta\psi_p) \quad \ldots (11.7)$$

Thus, the inward or outward rate of flow or water from cell to cell and tissues can be calculated from the above expression.

Let us now consider the flow of water in the plant through intercellular spaces (apoplasm) where the limiting membranes are absent. Then the flux is given by

$$J_w = H_c \Delta\psi_w$$

$H_c$ = hydraulic conductance

Because $H_c = \frac{1}{R}$

R = Hydraulic resistance to flow of water

$$J_w = \frac{\Delta\psi_w}{R} \quad \ldots (11.8)$$

The water flux is directly proportional to $\Delta\psi$ and inversely proportional to hydraulic resistance (R). In other words, higher the $\Delta\psi_w$ more will be flux but high R will decrease the flux.

In plants water will move through the pathway which offers least resistance. Between the two routes — cell walls and cell to cell, the membranes of the cells exert more resistance (because of low permeability) than the cell walls. Therefore, water can flow relatively easily through cell walls. Water will not experience the resistance of plasma membrane when it moves from cell to cell via plasmodesmata. The xylem conduits which are not obstructed by cell membranes have least resistance and the rate of flow of water is very high. The ratio of R in xylem, cell walls and cell membranes is in the order of 0·3 : 1 : 50. This explains why xylem is the pathway for long distance transport as has been observed experimentally. The resistance in xylem varies inversely with the diameter of xylem elements. The smaller the diameter of xylem greater will be the resistance.

**Hydrostatic pressure** is the pressure exerted by or on a liquid above or below atmospheric pressure.

In soil, pressure potential is insignificant and osmotic potential is zero because there are no membranes (solute and water move together). Hence, the driving force $\Delta\psi_w$ in soil is determined by the matric pressure.

$$\Delta\psi_{w(soil)} = -\Delta\psi_{m(soil)}$$

The hydraulic resistance varies from soil to soil. The fine soil particles with small space between them offer more resistance than coarse particles with large spaces. When $\psi_w$ of soil falls R increases and then the plant take up less water.

Also, in the cell walls the driving force is determined by $\Delta\psi_m$. In those leaf cells which are losing water rapidly, matric forces become significant and consequently $\psi_w$ of leaf decreases. So water moves from the wetter cells. Thus a continuous gradient develops which operates along the cell walls throughout the plants. Of course, it will break at points where cell walls are impregnated with hydrophobic substance for example — Casparian strip.

SAQ 2

a) List the three factors that determine the value of $\psi_w$ in plant.

.....

.....

.....

b) The water potential in a cup ($\psi_c$) containing salt solution will be

$\psi_c > \psi_w$

$\psi_c \geq \psi_w$

$\psi_c = \psi_w$

.....

.....

.....

c) What will be the water potential of a plasmolysed cell if its osmotic pressure is 7.9 MPa.

.....

.....

d) Fill in the blank spaces in the following statements with appropriate words:

i) At full turgor $\psi_w$ of a cell will be .....

ii) The net flow of water movement in a system will stop when $\psi_p$ will be equal and ..... to $\psi_\pi$.

iii) Greater matric suction will ..... the water potential in a system.

## 11.7 GRADIENTS OF WATER POTENTIAL

The absorption of water by a plant involves the water relations of an individual cell, a group of cells and finally of the whole plant. Therefore, we will consider water relations at different levels of organisation. We have already discussed the long distance transport of water.

### Movement of Water in a Single Cell

Isolated cells, single celled organisms and root hairs absorb water directly from their surrounding media. Let us consider an ideal parenchymatous cell. The vacuole occupies 90% of the cell and contains cell sap which is a dilute solution of salt and other small molecules. Due to osmotic potential the cell sap has a lower water potential than pure water. When such a cell is placed in pure water a gradient develops due to the difference in water potential. This results in the movement of water inside the cell (Fig. 11.12). However, in no time the concentration of sap also decreases which lowers the osmotic potential of the sap. Thus, the difference between the potential of pure water and cell sap gets reduced. This lowers the force by which water enters the cell. We can represent the relationship with the following equation:

$$\psi_w \text{ (outside the cell)} = (\psi_\pi + \psi_P)_r \text{ inside the cell}$$

where, $\psi_w$ is total water potential of the system, $\psi_\pi$ is the osmotic or solute potential and $\psi_p$ is pressure potential due to cell wall pressure or turgor pressure. At full turgor pressure the sum of $\psi_p$ and $\psi_\pi$ is zero. Hence $\psi_w$ is zero.

The driving force F that causes water to move can be represented by the following equation:

$$F = \text{gradient } (\psi_c - \psi_c)$$

where $\psi_c$ is the total water potential of the cell including that of the cell sap and $\psi_e$ is the total water potential of the external medium. If the latter is pure water then its value will be zero. In that case the driving force will be equal to $\psi_c$. However, as the water will move into the cell $\psi_c$ will be regulated by $\psi_\pi$ and $\psi_p$. In this relationship the elasticity of the cell wall would also play an important role. The volume of the cell would increase upto a certain limit with the dilution of the cell sap and this will increase the total osmotic potential. This in turn would influence the force developed due to the gradient between the cell and the medium.

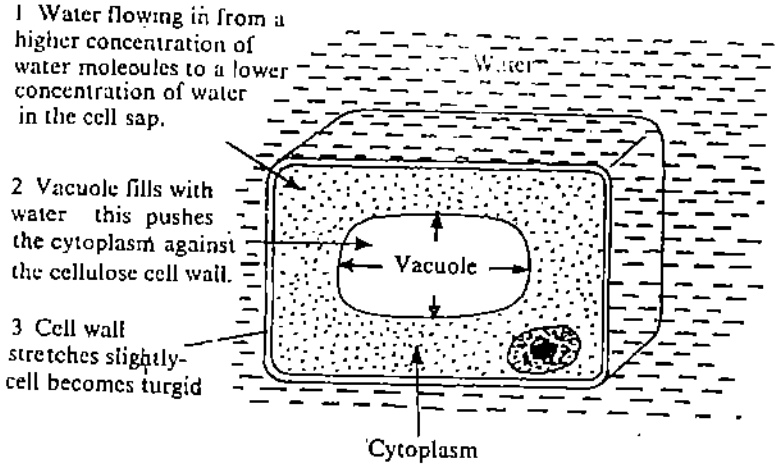



**Fig. 11.12 : A single cell surrounded by water.**

In the cells of root, leaf and other parts of plant, the external medium is the water in the cell walls and intercellular spaces (apoplast) which is under atmospheric pressure and has very low osmotic potential. But the matric forces exerted by the cell walls are higher, therefore, the water potential in apoplasm is determined by matric forces exerted in cell wall.

### Water Relations of a Tissue

In higher plants no cell exists in isolation from others. Even a root hair which is projected outside into the surrounding medium is attached with other cells on all the remaining sides. In a transverse section, it would appear to be surrounded on the three sides by other cells. Thus, the water relations of a root hair are governed on one side by the surrounding medium and on the other by other cells. Let us just consider two cells A and B joined with each other through a common cell wall. If these two cells individually have the same total water potential, then there will not be any net exchange of water between them. This can be shown as follows:

The total water potential of cell A ($\psi_{wA}$) will be equal to the sum of its osmotic potential and pressure potential ($\psi_{\pi A} + \psi_{pA}$). Let us say that for the cells A and B these values are

$$\psi_{wA} = (\psi_{\pi A} + \psi_{pA})$$

$$\psi_{wB} = (\psi_{\pi B} + \psi_{pB})$$

In a cell the matric forces are much less. Here the $\psi_w$ is lowered because of $\psi_\pi$. If the $\psi_w$ in the vacuole is lower than $\psi_w$ in apoplast, then water flows inwards.

Now, the driving force (F) will be the difference in the water potential of two cells.

$$F = (\psi_{wA} - \psi_{wB})$$

If $\psi_{wA}$ is equal to $\psi_{wB}$ the gradient will be zero and so will be the F. Therefore, no net exchange of water will take place between the cells A and B. However, we must realise that the same value of $\psi_{wA}$ and $\psi_{wB}$ does not necessarily mean that the two cells should have the same osmotic potential and the same turgor pressure or wall pressure.

On the other hand, if the total water potential of cell B is lower than that of cell A, a driving force will develop which would cause influx of water into cell B till the two cells attain the same water potential. Now, this example can be extended to a larger number of cells which are connected with each other in tissue. If there are 20 cells beginning from 1 to 20 they will attain an equilibrium amongst themselves, depending on their total water potential in the same way as discussed for the two cells attached to each other. Under such conditions a situation may come when water absorption and movement will come to a standstill.

**Water Relations of a Whole Plant**

Let us now consider the water relations of a plant considering leaves, stem and roots, that provide a continuum for water in soil-plant-atmosphere system. The total water potential in the atmosphere could be very low, depending upon the temperature and humidity. Table 11.1 shows an approximate magnitude of water potential in the soil-plant-atmosphere-system.

**Table 11.1 : Approximate magnitudes of water potential in the soil-plant-atmosphere system**

| Component | Water Potential (Bar) |
|---|---|
| Soil | −0.1 to −20.0 |
| Leaf | −5.0 to −50.0 |
| Atmosphere | −100 to −2000 |

It is clear from the data in Table 11.1 that the difference in total water potential in the soil-plant-atmosphere system would generate a driving force for water movement from the soil through the plant to atmosphere. If this continuum is broken, the driving force would automatically disappear.

SAQ 3

a) In a plant water moves from an organ A to an organ B bacause

   i) $\psi_{wA} < \psi_{wB}$

   ii) $\psi_{wA} > \psi_{wB}$

   iii) $\psi_{wA} = \psi_{wB}$

b) Calculate the value of $\Delta\psi_w$ if water potential of xylem is −0.5 MPa at the base of the tree trunk and −1.5 MPa at the top.

.....

.....

.....

.....

c) Water potential in a tree in three tissues A, B, and C was found to be −0.4, −3.1 and −0.09 MPa. What would be the direction of movement of water.

.....

.....

d) A raisin swells in water because

   i) $\psi_{w\ raisin} > \psi_w$ or

   ii) $\psi_w > \psi_{w\ raisin}$

## 11.8 WATER ABSORPTION

Water status of a plant is determined by two major factors: i) water absorption and ii) water loss. We deal with water absorption in this section and water loss in the following section.

Water absorption is regulated by soil factors, rate of transpiration and size and distribution of roots. The soil factors that regulate water absorption are: i) soil water content, ii) difference in water potential between soil and root, iii) concentration of soil solution, iv) soil temperature and v) aeration of the soil.

**Soil Characteristics**

The physical properties of soil govern water-holding capacity and the water availability to the plant. The fine soil particles of clay have much more water-holding

capacity than silt and sand. Addition of humus increases the water-holding capacity of soil. Water moves through pores present in soil. The pores form because individual soil particles aggregate to form large particles of varying sizes called micelles. The pores are spaces left between micelles. The size of pores can be small (micropores) or large (macropores) depending upon the soil type. The pores get filled with water.

A soil freshly wet with irrigation or rain water cannot retain all the water. Much of the water percolates through macropores due to gravity. This water has been termed as **gravitational water**. The remaining water that is held tightly by hydrogen bonds to the soil particles against gravitational forces is called **capillary water**. This water is available to the roots. Part of the capillary water that is held very tightly and is not available to the root is termed **hygroscopic water**.

The water content of a soil is expressed as

$$\% \text{ of soil water} = \frac{FW - DW}{DW} \times 100$$

FW = Field weight of wet soil

DW = Dry weight (obtained by heating soil to 60°C)

Two terms field capacity (FC) and permanent wilting percentage (PWP) are often used to describe the water status (capillary water) of the soil.

**Field Capacity (FC)**

It is the capacity of a field to hold the amount of water against gravitational forces. It is expressed as the percentage of the dry weight of the soil. Field capacity represents the upper limit of water availability and its value differs from soil to soil. The capacity of clay soil on an average is about 40-45%, silt 20% and sand 5-10%.

**Permanent Wilting Percentage (PWP)**

It is the percentage of moisture in the soil at which a plant wilts and does not recover unless water is added to the soil. PWP for clay is about 26%, for silt 10% and for sand 3-5%. It must be noted that PWP is used to express property of a soil not any feature of the plant.

However, the moisture content at which any plant shows permanent wilting need not be the same for all plants even in the same soil. This happens because some plants are prone to wilting at fairly reasonable water content of soil while others wilt only when the moisture content is very much reduced.

The water status of a plant in terms of FC and PWP is significant only if the soil properties are known. This is because the soil determines the availability of water. However, it is desirable as well as easier to express soil water status in terms of water potential so that soils become uniform with respect to water.

**Soil Temperature**

Soil temperature is known to influence water absorption and ultimately transpiration to a considerable extent. In many plants water absorption is reduced sharply below 10°C. Water absorption also slows down above 25°C. In most instances, temperatures above 40°C in the rhizosphere does not support water absorption and plant may show signs of wilting. The following are the reasons suggested for the reduced absorption of water at low temperature: i) decreased root growth, ii) increased viscosity of water, iii) increased resistance of water into roots due to decreased permeability (increased hydraulic resistance) of cell membranes and iv) decreased metabolic activity of root cells.

**Soil Aeration and Flooding**

It is not unusual to observe some plants wilting while they stand in water. The following are the possible reasons of flood injury.

i) Poor availability of $O_2$ and accumulation of high concentration of $CO_2$ around roots.

ii) Changes in the pattern of ion uptake resulting in the accumulation of some ions to toxic levels.

iii) Accumulation of toxic substances in the root and/or around them.

Poor availability of oxygen affects respiratory activity of roots, thus lowering the ATP supply. In the following units you will learn that ATP is required for the active uptake of ions. Low uptake lowers the osmotic potential of roots cells and hence water cannot enter because $\Delta\psi_w$ between root cells and soil is not sustained. Increased concentration of $CO_2$ affects the permeability of membranes and thus affects water uptake adversely.

Among toxic substances are the metabolites produced as a result of anaerobic respiration. These could be alcohol or aldehyde and even ethylene. Plants usually become chlorotic under waterlogged conditions. This is apparently due to reduction in translocation of iron from roots to the top of the plant.

**Root**

Root system is directly related to the absorption of water and its growth under water-logged conditions is very much influenced by soil. In dryland agriculture, particularly root structure has apparently great significance. The rates of water absorption in roots of different plants differ in their stage of growth. Highest rates of water entry are associated with root hair and unsuberised roots and lowest with suberised woody root.

**Water Absorption and Transpiration**

The rate of water absorption is controlled by the rate of transpiration. A high water potential in the atmosphere would reduce water loss from the plant and consequently slow down water absorption. But it does not follow from this that water will be continuously absorbed if the water potential of the atmosphere is very low and soil is near drying. When the equilibrium in the soil-plant-atmosphere system is disturbed either due to soil or atmosphere, the plant can respond appropriately and can control water absorption or water loses. We discuss below some of these aspects.

Let us first see the relationship of water absorption and transpiration in a well watered plant (Fig. 11.13). By and large all plants show a diurnal behaviour in their rates of transpiration. There is a rapid increase in transpiration during the morning hours and a peak is reached in the early afternoon. Then a decline begins and a minimum is reached during the night.

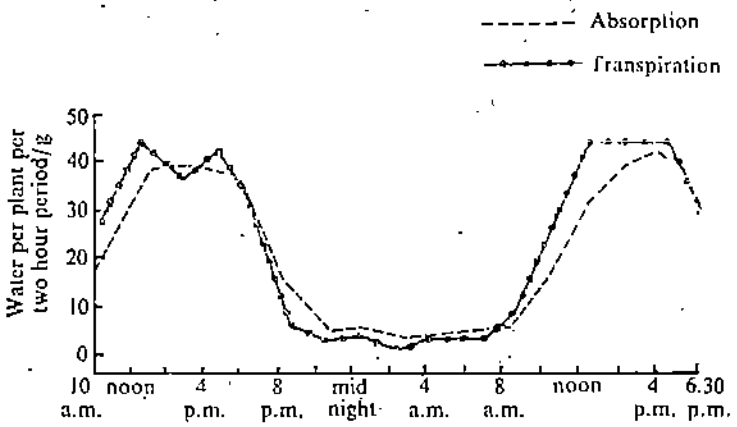



Fig. 11.13 : Diurnal variations in rates of transpiration and absorption of water by a plant.

You may note in the graph that water absorption keeps pace with losses due to transpiration except around noon when it lags behind. Why is it so? You have learnt that the resistance to the movement of water across the roots is generally higher than in the leaves because in roots water moves through sympiasm. In roots it faces high resistance of the membranes of cortical cell and that is why there is an absorption lag. This idea is supported by an experiment where water absorption was recorded after removing the roots of a plant. As shown in the graph (Fig. 11.14) the absorption lag is reduced greatly.

Let us now see what happens to water loss if soil is not moist. Ralph O. Slater (1967), a plant physiologist at Australian National University, Canberra, studied the

relationship between water potential of soil ($\psi_{w\ soil}$), root ($\psi_{w\ root}$) and leaf ($\psi_{w\ leaf}$) of a plant where water supply was withheld for five days. The graph (Fig. 11.15) shows diurnal changes in leaf potential, water stress around the noon time and recovery of lag in absorption at night. The water potential of the soil decreased almost linearly

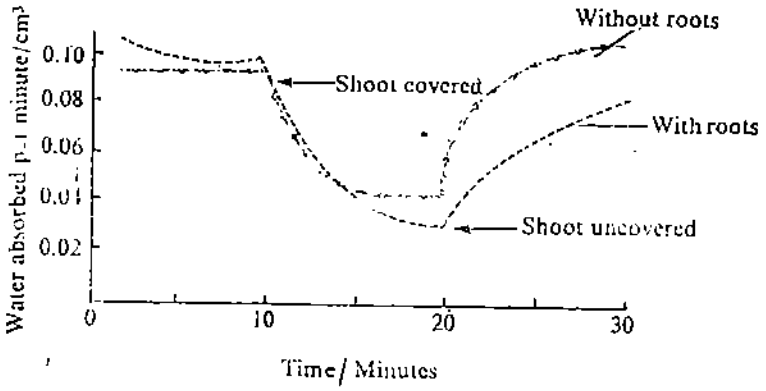



**Fig. 11.14 : Comparison of rate of water absorption in rooted and rootless plant. When transpiration is high (shoot uncovered), the rate of water absorption in a rooted plant is less than rootless plant.**

after second day. It must be pointed out that when plants are under water stress, water tends to move out of the cells that are not situated in the main pathway of transport of water. This causes reduction in turgor, i.e. cell volume. In fact, it was demonstrated a century ago by Josef Friedrich that there are daily fluctuations in the diameter of tree trunk, shrinkage in the morning and recovery in the diameter by night.

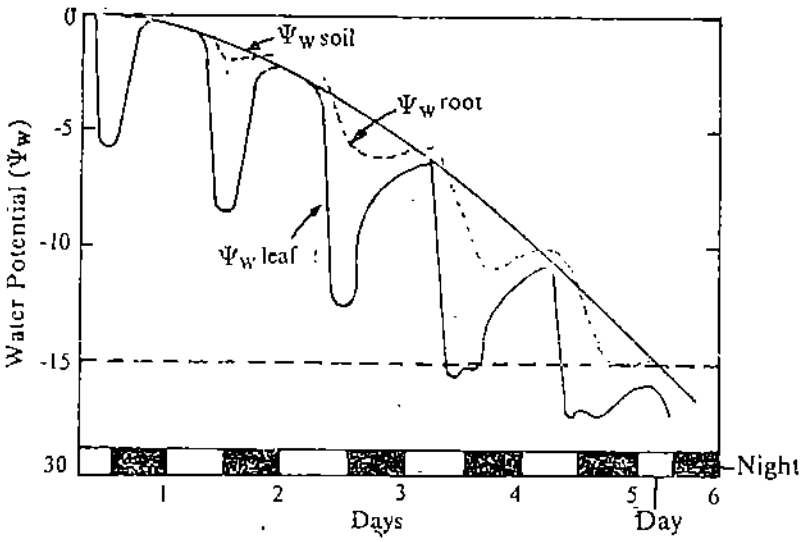



**Fig. 11.15 : Diagram showing probable changes in leaf water potential ($\psi_{w\ leaf}$) and root water potential ($\psi_{w\ root}$) of a transpiring plant rooted in soil allowed to dry from a water potential near zero to a water potential ($\psi_{w\ soil}$) at which wilting occurs.**

Which among the following suggestions are desirable for growing healthy plants. Put tick mark for the correct ones :

i) Excess use of fertilisers. ☐

ii) Saturation of plants, particularly growing in pot, with water. ☐

iii) High humidity area for water-sensitive plant. ☐

iv) Use of very cold water in warm season. ☐

v) Use of lukewarm water in cold season. ☐

## 11.9 WATER LOSS

Plants lose about 98% of water to the atmosphere by transpiration. Often water loss by transpiration exceeds gain by absorption and results in negative water balance within the plant. Small and moderate deficit that occur due to high temperature during the day are compensated during the night but prolonged deficit causes irreversible damages and threatens the plant's survival.

Transpiration is essentially evaporation of water from the aerial portion of the plant. However, evaporation of water from open surface meets less resistance while evaporation of water from leaves faces considerable resistance. Transpiration occurs mainly through stomata of the leaves. This is called **stomatal transpiration**. About 5% of the water is lost from the leaf through the cuticle. This is called **cuticular transpiration**. In woody plants there are lenticels opening within the bark that function in gas exchange. The water loss through these cells is called **lenticular transpiration**.

### 11.9.1 Stomata

The cross-section of a leaf shown in Fig. 11.16a shows the position of a typical stoma (plural stomata) which however, differs from species to species, with respect to the size of the pore, structure and size of the guard cells and depth and size of the stomatal cavity. As indicated in the diagram b, water evaporates from wet mesophyll cell walls that border intercellular spaces, the vapours then diffuse out through sub-stomatal cavity and stomatal pores to the air outside the leaf. The water potential gradient develops in the sub-stomatal cavity, stomatal pore, boundary layer and the atmosphere. During transpiration the sub-stomatal cavity has relatively much higher water potential as compared to the atmosphere, therefore, the water vapours move out. This is turn lowers the water potential of the sub-stomatal cavity. Consequently, the cells surrounding the sub-stomatal cavity evaporate water through their cell walls. Depending upon the water potential of the environment, the water potential of the sub-stomatal cavity and the surrounding cells is lowered. This gradient eventually acts as a 'pull' on the water column which maintains continuity through the vascular bundles of the leaf. The intercellular spaces also play an important role in this respect because they are in continuity with the sub-stomatal cavity and cause a gradient quickly.

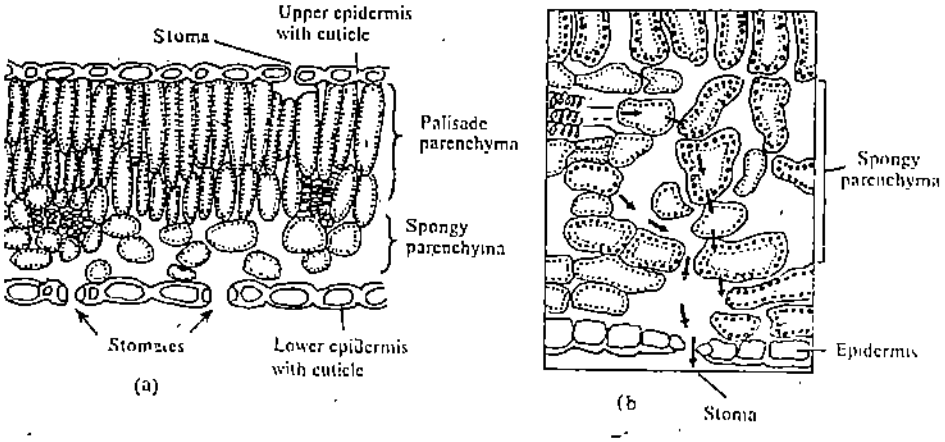



**Fig. 11.16 : a) Cross-section of a leaf showing the relationship of stoma with other leaf tissues. b) Stoma enlarged to show the path of water vapours from leaf to atmosphere.**

When water evaporates through leaves it experiences considerable amount of resistance (Fig. 11.17) which can be grouped into the following two categories: a) leaf resistance (internal resistance), and b) air boundary resistance (external resistance).

The components of leaf resistance are cuticle, mesophyll cells, intercellular spaces of the leaf and stomata. The cuticular resistance is maximum followed by stomatal resistance and air boundary layer resistance. The pathway of movement of water vapours is analogous to the electric current moving in circuit, the greater the resistance, the smaller the flow of water and vice versa.

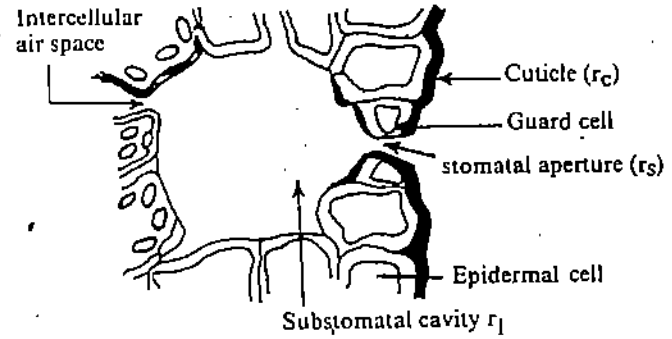



**Fig. 11.17 : Schematic cross-section of a leaf showing different resistances.**

The cuticle forms outermost surface of the leaf and offers resistance to the evaporation of water vapour and entry of carbon dioxide necessary for photosynthesis. Stomata perform the following main functions:

i) They allow entry of $CO_2$ necessary for photosynthesis,

ii) They control water loss through transpiration and thus protect the plant from desiccation,

iii) At higher temperature (above 35°C) they promote transpiration which serves to cool the leaves.

Stomatal resistance is most important because gas exchange between leaves and external atmosphere takes place entirely through stomatal pores. Stomatal resistance depends mainly on the size and shape of the stomatal cavity and size of stomatal aperture. Can you tell which pore size, small or large would exert greater resistance?. Of course, smaller the pore greater would be the resistance for the outward movement of water vapour. On an average a standard pore measures about 20 μm in length and about 11 μm wide at its widest point when fully open.

Water loss from the leaf also depends on the number of stomata per leaf. One of the aims of agricultural scientists is to find ways of minimising the water loss from the plants so as to increase the efficiency of water use. One way is through a study of the number of stomata per leaf. Some investigations have been carried out keeping the following questions in view:

i) What is the distribution of stomata and how is it related to water loss and carbon dioxide intake?

ii) How can water loss be reduced without seriously impairing carbon dioxide intake?

iii) Is it possible to evolve varieties which possess characteristics associated with low evaporation of water?

There are two parameters normally used for expressing the distribution of stomata.

a) **Stomatal frequency :** The number of stomata per unit area.

b) **Stomatal index :** The ratio of number of stomata to the total number of cells per unit area.

Plants differ in their stomatal frequency. In monocots the upper and lower surfaces usually have the same frequency but in dicots the lower surface usually has a higher frequency than the upper surface. However, the frequency of stomata can change with the position of leaves on the plant.

Studies have been carried out to see if any relationship exists between i) stomatal frequency or stomatal index and transpiration and ii) stomatal opening or stomatal index and photosynthesis. Using two barley lines which were high and low in stomatal frequency Misken and coworkers (1972) found that stomatal resistance and transpiration rates differed significantly between lines but photosynthetic rates were the same. The lines having low stomatal frequency had higher stomatal resistance, transpired less water than lines having more stomata. A decrease of 25% in stomatal frequency reduced transpiration rates by about 25%. However, the photosynthetic rate was unaffected by decrease in stomatal frequency. These studies suggested that it should be possible to alter transpiration without altering photosynthesis by selecting varieties with fewer stomata in barley or other crops.

Let us first have a close look at the stomatal aperture. Fig. 11.18 illustrates an open (a) and a closed (b) stoma in surface view. As you may know each stomatal pore is surrounded by a pair of guard cells. In closed stoma guard cells appear like two joined kidney beans. In monocots guard cells are dumbell shaped and are arranged in pairs in contact at the bulbous ends (Fig. 11.18c). In some species the epidermal cell adjoining the guard cells are specialised and are called subsidiary cells (Fig. 11.18d). It is important to mention here that guard cells contain chloroplasts and mitochondria and can synthesise starch while no other epidermal cells contain chloroplasts.

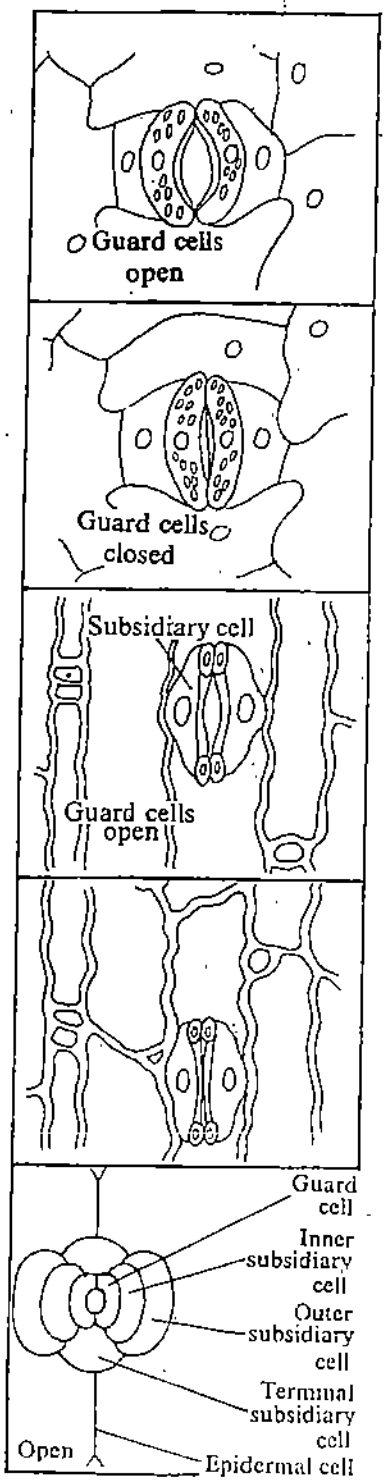



Fig. 11.18 : Surface view of an open a) and a closed b) stomatal aperture in a leaf of a dicotyledon c) and d) of monocotyledon e) guard cells surrounded by subsidiary cells.

## 11.9.2 The Mechanism of Stomatal Opening

It has been known for over a century that stomata open because of reversible turgor changes in the guard cells. Stomata open when turgor in the guard cells is high and close when it is low. It is clear that the turgor would increase only if the solute content of guard cells would be higher than the neighbouring epidermal cells. Falling of solute content would decrease turgor.

How do the two guard cells on swelling form an aperture? The cell wall of guard cells is peculiar because the cellulose microfibrils which constitute the major structural feature of plant cell are arranged radially extending from the centre towards the periphery (Fig. 11.19).

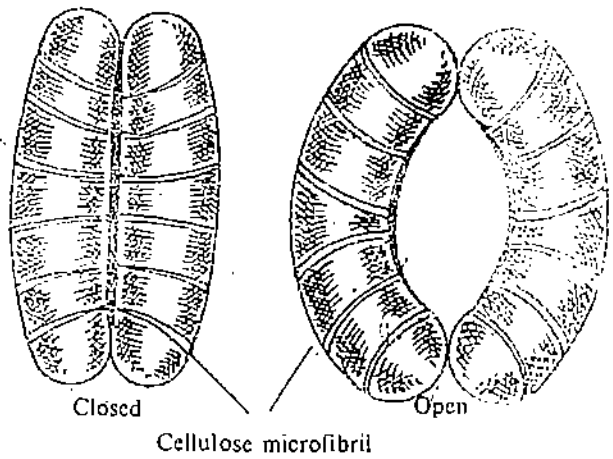



Fig. 11.19 : Diagrammatic representation of arrangement of cellulose microfibrils (lines) in guard cells.

This arrangement restricts the expansion of cell wall in transverse direction. Since the inner wall (towards the pore) is thickened and is less elastic than the outer wall, the uneven longitudinal expansion causes the cells to arch away from each other forming a pore in the centre.

Before we discuss the mechanism of control of stomata, it is important to bear in mind the following observations made in this regard.

i) Normally, stomata are open in the day and are closed at night. However, a drop in supply of water leads to the day time closure of stomata.

ii) Stomata open when the internal concentration of $CO_2$ drops and close when the internal concentration of $CO_2$ is maximum.

iii) Dark $CO_2$ fixation (CAM plants refer to Unit 13) occurs in the guard cells.

iv) In guard cells there is a change of pH in light (day) and dark (night) which is associated with the interconversion of starch and sugar.

v) Opening and closing of stomata are related to the osmotic potential of the guard cells and the permeability of membranes.

vi) At the time of opening of stomata, there is an inflow of potassium ions into the guard cells but when the stomata close, the guard cells lose their acquired potassium to the surrounding cells.

vii) Inhibitors of cyclic phosphorylation can also close stomata.

viii) Blue light also brings about changes in stomatal movement — serving to open stomata.

ix) Abscisic acid — a plant hormone, at a very low concentration can lead to the closure of stomata.

There are two ways in which the relative turgor of guard cells may be altered. i) a decrease in osmotic potential or ii) a decrease in pressure potential. In both instances the water potential of guard cells will fall and hence water from neighbouring cells would move in. Alternatively, guard cells may face mechanical pressure from the subsidiary cells if they face a sudden change in their turgor.

There is overwhelming evidence that the osmotic potential of the guard cells decreases due to the migration of $K^+$ ions from the surrounding cells into the guard cells (Fig. 11.20). It is also observed that the uptake of $K^+$ requires ATP which may be generated by degradation of starch through respiratory metabolism and photophosphorylation. ATP is utilised to pump out protons out of the guard cells by a membrane bound ATPase (refer to section 8.4 LSE-01). The supply of protons is maintained by malic acid which is synthesised from stored starch. Due to the active pumping of protons, an electrochemical gradient develops across guard cell membrane. Now the ions can move passively into the guard cells. In some species $Cl^-$ ions or other anions move along with $K^+$ in and out of the guard cells. Thus, there is a decrease in osmotic potential due to accumulation of $K^+$, $Cl^-$ and/or potassium salt of organic acid mostly malate. Consequently, water enters guard cells and builds up turgor. Reverse events would bring about closure of stomata.

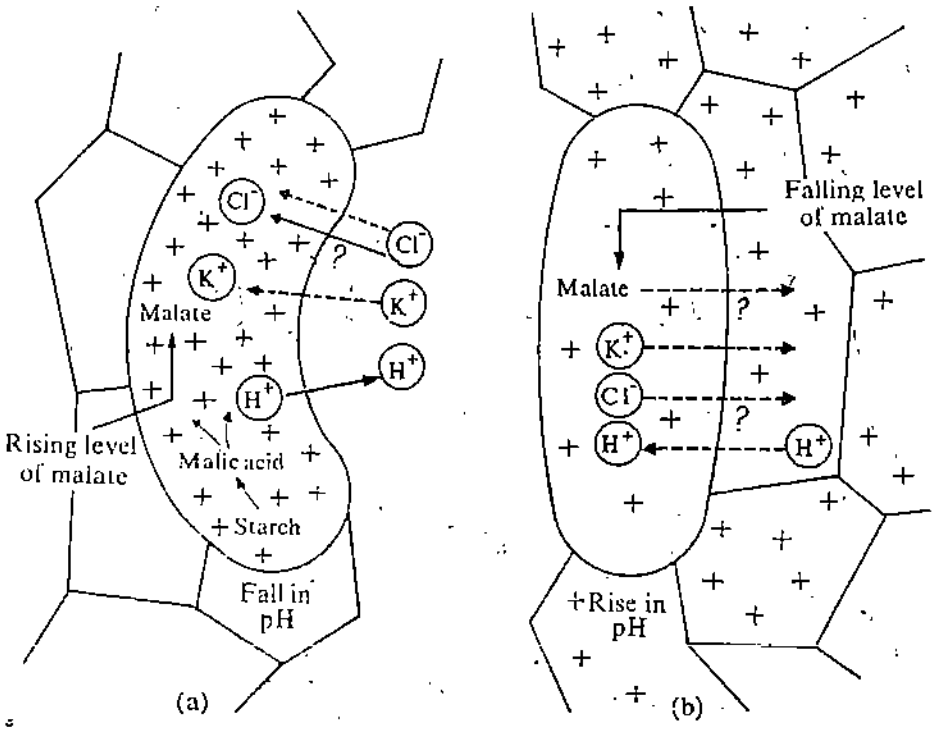



Fig. 11.20 : Changes in the turgor of guard cells by the movement of $K^+$ and other ions. Solid lines indicate active transport, broken line passive fluxes and ? mark uncertainty. Upward arrow indicates rising level of malate (a) and downward arrow falling level (b).

## 11.10 FACTORS CONTROLLING STOMATAL APERTURE

'Khul Ja Sim Sim' the two rocks slide and the gates to a great treasure open. With these magic words "Ali Baba and Chalis Chor" could open the door. Let us now find out what is 'Khul Ja Sim Sim' for moving the guard cells of the stomata, so that $CO_2$ can enter the stomata and make food for organisms of the earth. You have learnt that more than one factor can trigger the change in the turgor of the guard cells and bring about stomatal movements and control the size of stomatal aperture. The main controlling factors are:

i) light,
ii) the level of $CO_2$, and
iii) high temperature.

To understand better you can imagine the factors as hands that can open and close stomata by moving the turgor operated valve — the guard cells (Fig. 11.21). The stomata open when the level of $CO_2$ falls due to its rapid utilisation in photosynthesis during the day. However, it is not known how guard cells sense the low level of $CO_2$. The highest rates of photosynthesis are obtained when the conditions for light and temperature are optimum and plants are well watered. But if the water potential in the leaf falls under low level of $CO_2$, then conserving water becomes more urgent than food production for the plant and the stomata close. In other words, the water status of plant overrides the control by $CO_2$.

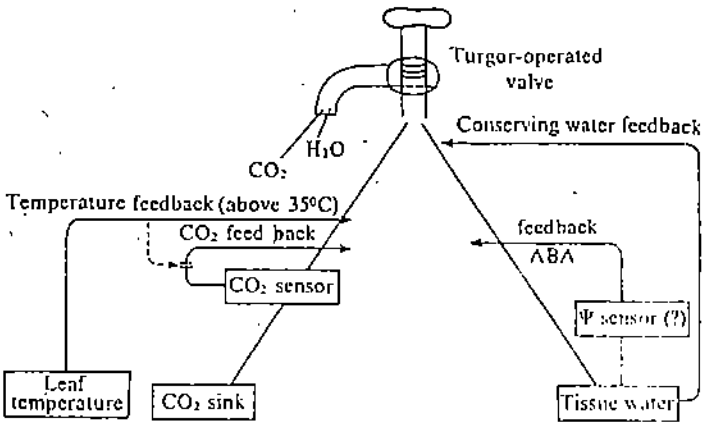



Fig. 11.21 : A simplified model showing the effect of various factors on stomatal aperture.

The rate of photosynthesis increases with increase of temperature but above 35°C cooling of the plant becomes necessary. Therefore, irrespective of $CO_2$ concentration stomata open widely above 35°C provided that there is no shortage of water. Here again the temperature overrides the control by $CO_2$ concentration. This makes sense because high temperatures are deleterious to the health of the leaves, and evaporation of water through transpiration can lower the temperature of leaves.

Before we close this unit, it is important to point out that the water loss by a plant due to transpiration is very high usually in the range of 0.1 to 2.5 g $dm^{-2} h^{-1}$ during day time. However, it plays no major role in growth and development of plant so far is known. We have mentioned above that it cools the plant at high temperatures. Perhaps, the transpiration stream facilitates the availability of mineral ions to all parts of the plant. Soil contains minerals in very dilute concentration but in cells they get accumulated in much higher concentration due to active transport aided by their rapid availability in the transpiration stream.

You have learnt that the external application of plant hormone abscisic acid (ABA) to leaves causes stomata to close. During water stress, increase in the level of ABA of the leaves has also been observed. Therefore, it is quite likely that ABA is an internal control for the regulation of water content. Phenyl mercuric acetate — a fungicide has been used as foliar spray in low concentration ($10^{-4}$M). It brings about partial closure of stomata for about two weeks without visible damaging effects to

the plant. Other chemicals such as colourless plastics, silicon oils and low-viscosity waxes are also used as foliar spray. These chemicals form on leaves a film permeable to $CO_2$ and $O_2$ but not to $H_2O$. Water-saving strategy by reducing transpiration though seems quite promising, yet it is a big challenge for plant physiologists.

SAQ 5

a) List the features of guard cells essential for opening the stomata.

.....

.....

.....

.....

.....

## 11.11 SUMMARY

- Water is the key molecule for the maintenance of life on the earth. About 85 to 90% of a plant is made up of water. The quantities of water used by plants are enormous.
- Water is a good solvent and reactant in the cell. For plants water is also crucial because the hydrostatic pressure of water provides turgidity to the cells, so that the soft stem parts are able to stand erect.
- Water moves up into the trees due to negative hydrostatic pressure created in xylem by the transpiring leaves. The continuity of water column in the xylem is maintained because of cohesive property of water.
- In plant tissue water molecules move through two routes — apoplasm and symplasm. Apoplast transport is through non-living portion of the plant and symplast is through cell to cell via plasmodesmata.
- Water status in a plant is expressed by water potential ($\psi_w$). The rate of flow and the direction of movement depend upon the gradient of water potential between the two points. Water tends to move from a region of high water potential to a region of low water potential.
- Water potential is affected by solute concentration, pressure and absorptive or matric forces. Addition of solute lowers the water potential while increased pressure on water causes a rise in water potential. Absorptive forces between solid-liquid interfaces lower the water potential. Water potential is computed as the algebraic sum of component forces: osmotic potential (due to solute), pressure potential (due to hydrostatic pressure, matric or suction potential (due to adhesive forces).
- In a cell both solute concentration and turgor pressure can have offsetting effect on water potential. The combined effect of the two in cells can build up the gradient and determine the rate of flow and direction of water movement.
- The gradient of water potential — the driving force for water movement in soil is due to differences in matric potential. This gradient also occurs along the cell walls.
- Water movement within the dead xylem cells occurs along the gradient of hydrostatic pressure.
- There is resistance to water flow in the soil and in the plant. Water takes up the path of least resistance. Membranes of cells exert greatest resistance while xylem offers least resistance.
- Water status of a plant depends upon water absorption and water loss due to transpiration. Soil characteristics, soil temperature, aeration, flooding and structure of roots are the factors which affect water absorption.
- The rate of water movement in plant is determined by the rate of transpiration in a well watered plant. Temperature, wind velocity, humidity of the air, light intensity and degree of stomatal opening affect the rate of transpiration. However, ultimate limit to transpiration is the supply of water from soil.

- Among stomatal, cuticular and lenticular transpiration, stomatal transpiration accounts for major water loss. Evaporation of water faces considerable resistance from the leaf — cuticle, mesophyll cell, intercellular spaces, stomata and air boundary layer of the leaf.
- Stomata control water loss through transpiration, allow gas exchange and control temperature of the leaf. Some efforts have been made to reduce water loss through stomata without affecting $CO_2$ intake.
- Stomatal aperture is controlled by the changes in the turgor of guard cells. The turgor in turn is affected by the metabolism and the movement of $K^+$ and other ions into and out of the guard cells. A proton pump powered by ATPase operates in the plasma membrane of the guard cell and the $K^+$ uptake is a passive uptake.
- Stomatal aperture is controlled by the level of $CO_2$, light and high temperature. However, if conserving water or saving plants from deleterious effects of high temperature becomes more urgent than food production then these factors override the system.

## 11.12 TERMINAL QUESTIONS

1) Why do animals need less water than plants?

.....

.....

.....

.....

2) What would happen if a twig of a potted plant is cut into two pieces and joined with tygon tubing in such a way that it leaves an air gap between the cut ends? Will water continue to move?

.....

.....

.....

.....

.....

3) A plant cell with an osmotic potential of −7.6 bars and pressure potential of 3 bars is placed in a slide with a drop of pure water, in which direction the water will flow? What is the $\psi_\pi$, $\psi_p$ and $\psi_w$ of the cell and water initially and after equilibrium?

.....

.....

4) Draw the structure of stomata and explain how stomatal aperture opens by the increase in the turgor of the guard cells.

.....

.....

.....

.....

## 11.13 ANSWERS

**Self-assessment Questions**

1) i) F, ii) F, iii) T, iv) T

2) a) Osmotic potential ($\psi_\pi$), pressure potential ($\psi_p$) and matric potential ($\psi_m$)

b) $\psi_c = \psi_w$. The $\psi_c$ of salt solution in a cup will be zero because there are no membranes present. Therefore, its water potential is equal to pure water.

c) −7.9 MPa.
We know that $\pi = -\psi_\pi$. Therefore, the osmotic potential will be −7.9 MPa.
Since $\psi_p = 0$, therefore, $\psi_{wc} = \psi_\pi$

d) i) zero ii) opposite iii) lower

3) a) ii

b) $\Delta\psi_w = \psi_{w\,Base} - \psi_{w\,top}$
$= -0.5 - (-15)$
$= -0.5 + 1.5$
$= 1$ MPa

c) A = −0.4 MPa, B = −3.1 MPa, C = 0.09 MPa
$\psi_w$ = $0.09 > -0.4 > -3.1$
the flow of water will be from C → A → B

d) ii)

4) iii) and iv) are the right suggestions

5) a) i) A thickened cell wall, facing the pore,
ii) presence of chloroplasts,
iii) supply of ATP,
iv) a proton pump in the membrane,
v) the capacity to generate higher turgor pressure than adjacent cells.

b) i) F, ii) T, iii) T, iv) F.

**Terminal Questions**

1) In animals a great deal of water is recycled through the body in the form of blood plasma and other fluids in vertebrates. In plants more than 90% of the water absorbed by leaves is lost into the air as water vapour through the process of transpiration.

2) The plant will absorb water but the upper part of the plant will wilt. This is because the air column between the two stem pieces does not provide a continuum. In plants the movement of water takes place only in liquid phase which can stand enormous pressure without breaking the liquid column.

3) Water will flow into the cell until the water potential of cell and water drop is equal.

The following are initial and final (equilibrium potentials

| | Initial | | Equilibrium | |
|---|---|---|---|---|
| | water drop | cell | water drop | cell |
| $\psi_\pi$ | 0 | −7.9 | 0 | −7.9 |
| $\psi_p$ | 0 | +3 | 0 | +7.9 |
| $\psi_w$ | 0 | −4.9 | 0 | 0 |

4) Please see text for the answer.

# UNIT 12 MINERAL NUTRITION

## Structure



## 12.1 INTRODUCTION

Three basic needs of human beings are food, clothing and shelter. For all these, we directly or indirectly depend upon plants. Late (Prof.) P. Maheswari once said, "We are all guests of plants on this earth." What an apt remark on our dependence on plants! Modern living, in addition requires many industrial products, the raw materials for many of these again come from plants. Therefore, successful cultivation of plants, on which we depend so much, is of prime importance.

Like man and animals, plants too need wholesome nutrition for healthy growth and development. While man and animals are mobile and can gather their food from wherever it is available, plants are stationary and manufacture their own food from simple inorganic nutrients, relying mostly on what they get from their immediate environment. For successful production of a healthy crop, an in-depth knowledge of their nutritional requirements is a must. Our attempt in this unit will be to find out what these requirements are, how plants obtain them from the environment and how they are transported to all parts of the plant body.

### Objectives

After studying this unit you should be able to :

- list the essential macro and micronutrients that a plant must absorb in order to live and grow,
- describe the role of the essential elements in plants,
- list the factors that influence the uptake of ions by the root from soil,
- explain how these elements are taken in selectively by root cells from the soil and transported to different parts of the plant body,
- list a few deficiency symptoms of specific nutrients in a plant and name the chemicals that are applied to correct the deficiency.

### Study Guide

The understanding of Section 12.4 of this unit requires knowledge of membrane transport processes. You may revise Sections 7.4, 7.5, 8.2, 8.3 and 8.4 of LSE-01 Block II. Section 12.5 on the role of essential nutrients is lengthy but it makes easy reading. To save time jot down the important information in the first reading itself.

## 12.2 NUTRIENT ELEMENTS OF PLANTS

Let us first find out the chemical composition of plants and see which [illegible] nature has selected to support their healthy growth. Only then we will be able to know the nutritional requirement of plants.

As you know a major part of plant tissue is comprised of water. This we can demonstrate by taking a known amount of plant tissue and drying it for a few hours in an oven at a temperature of 65-80°C. If we condense and analyse the vapours coming out from the plant tissue we will find that it is nothing but water. In fact, about 85-90 per cent of the tissue is composed of water. The part of the tissue which is left behind is called the dry matter and typically it is about 10-15 per cent of the original weight. The dry matter consists mainly of organic compounds. About 90% of the dry matter consists of plant cell walls, primarily cellulose and related carbohydrates. This can be eliminated in the form of gases on combustion at 600°C. The residue now left is the ash which varies in different plant tissue from about 1 per cent to 0.15 per cent of the dry weight. Interestingly, a careful analysis of the ash shows that it contains almost all of the chemical elements present in the soil surrounding the plant.

Now, the question is whether all the elements found in the ash are essential for the plant in order to lead a healthy life? How do we distinguish the essential elements from the non-essential ones?

### 12.2.1 Criteria of Essentiality

Arnon and Stout (University of California, USA), as early as 1939, suggested certain criteria that an element must fulfil in order to be classified as essential. These criteria are listed below.

1) An element is essential if in its absence the plant cannot complete its life cycle and form viable seeds.
2) An element is essential if it forms a part of any molecule or constituent of the plant that in itself is essential for the plant. For example, nitrogen in protein, magnesium in chlorophyll and iron in cytochromes.
3) The element must act directly inside the plant, and not enhance or su[illegible] availability of some other element.

Although the first two criteria mentioned above are sufficient to judge if an element is essential, the third criterion can eliminate doubtful candidates from the list. For example, the plant *Astragalus* is a selenium accumulator. When grown in seleniferous soils the element shows growth promoting effect. However experiments have shown that this property is due to the ability of selenate ion to inhibit the absorption of phosphate which otherwise is absorbed by the plant in toxic amounts. From a practical point of view, an element is considered essential if plants show deficiency symptoms when they are raised without that element in the medium even if the plants are able to form viable seeds. You may note that rigorous exclusion of elements, specially trace elements is very difficult as they can come from seeds themselves, from dust in air, or as contaminants of major salts.

Table 12.1 shows the list of sixteen elements that fulfil the criteria of essentiality stated above, their approximate adequate concentration and approximate number of atoms required with molybdenum (Mo) serving as the reference point. Look at the difference! The requirement of hydrogen atoms is about 60 million times the number of Mo atoms. It is because H is an essential part of thousands of chemical compounds with which the plant is made of, whereas Mo is required only in one or two enzyme mediated reactions.

Scientists have added a few more elements to the list of sixteen though they have found them essential only for certain group of plants. These are, sodium (Na), cobalt (Co), silicon (Si), nickel (Ni) and even chromium (Cr), tin (Sn) and fluorine (F). Some specific organisms may require other elements. For example, certain algae apparently also require the elements vanadium (Va), silicon (Si) or iodine (I), while some ferns utilise aluminium (Al) and some local weeds absorb and accumulate selenium (Se) in high amounts. Fig. 12.1 shows the effect of nutrient deficiency on the growth of barley seedlings.

The following couplet will help you to remember the mineral requirement of plants.

See Hopkins Cafe managed By Mine Cousin Mo Very Clean Naturally Cool.

(C H O P K I N S Ca Fe Mg B Mn Cu Zn Mo Va Cl Na Co)

You will notice that the couplet includes iodine. Actually plants do not need iodine, while animals do, as you know the lack of which causes goiter.

It has been shown that $Na^+$ as micronutrient is required by certain desert species, such as *Atriplex vesicaria* and some species that assimilate $CO_2$ in photosynthesis by $C_4$ pathway or crassulacean acid metabolism. Deficiency of $Na^+$ is manifested by severe chlorosis in leaves and necrosis in leaf margin and tips.

Table 12.1 : The essential elements required by higher plants**

| Element | Chemical Symbol | Form available to plants | Concentration in dry matter (%) | Relative no. of atoms compared of Molybdenum |
|---|---|---|---|---|
| 1. Hydrogen | H | $H_2O$ | 6.0 | 60,000,000 |
| 2. Carbon | C | $CO_2$ | 45 | 35,000,000 |
| 3. Oxygen | O | $O_2$, $H_2O$, $CO_2$ | 45 | 30,000,000 |
| 4. Nitrogen | N | *$NO_3^-$, $NH_4^+$ | 1.5 | 1,000,000 |
| 5. Potassium | K | $K^+$ | 1.0 | 250,000 |
| 6. Calcium | Ca | $Ca^{2+}$ | 0.5 | 125,000 |
| 7. Magnesium | Mg | $Mg^{2+}$ | 0.2 | 80,000 |
| 8. Phosphorus | P | *$H_2PO_4^-$, $HPO_4^{2-}$ | 0.2 | 60,000 |
| 9. Sulphur | S | $SO_4^{2-}$ | 0.1 | 30,000 |
| 10. Chlorine | Cl | $Cl^-$ | 0.010 | 3,000 |
| 11. Iron | Fe | $Fe^{3+}$, *$Fe^{2+}$ | 0.010 | 2,000 |
| 12. Boron | B | $H_3BO_3$ | 0.002 | 2,000 |
| 13. Manganese | Mn | $Mn^{2+}$ | 0.005 | 1,000 |
| 14. Zinc | Zn | $Zn^{2+}$ | 0.002 | 300 |
| 15. Copper | Cu | $Cu^+$, *$Cu^{2+}$ | 0.0006 | 100 |
| 16. Molybdenum | Mo | $MoO_4^{2-}$ | 0.00001 | 1 |

* More common form.

** Modified after P.R. Stout (1961), Proceedings of the Ninth Annual California Fertiliser Conference.

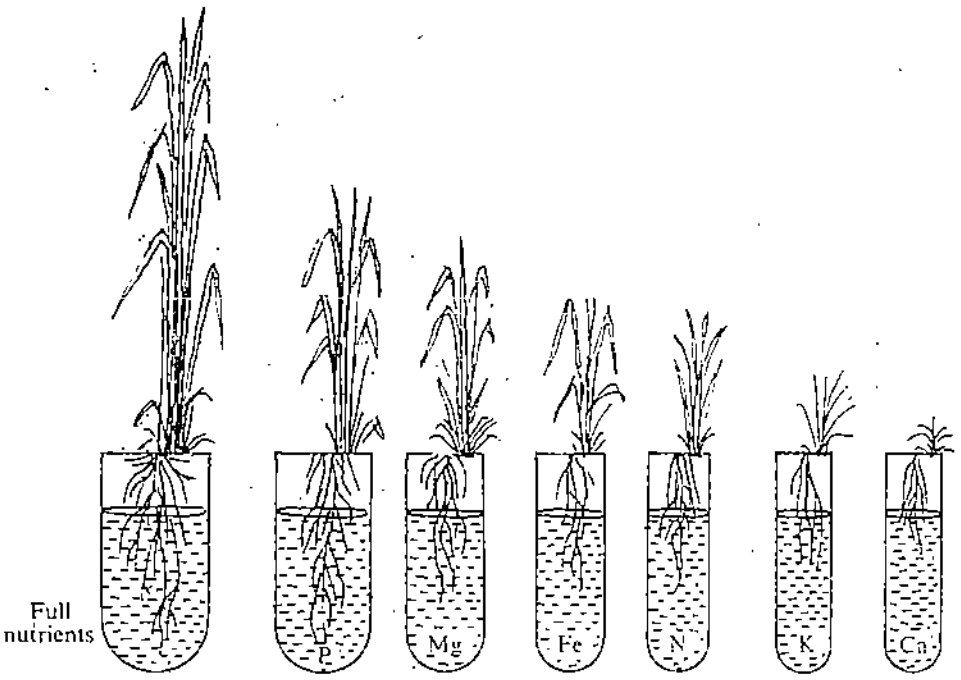



Fig. 12.1 : Effect of nutrient deficiency on growth of barley plants in water culture.

You will have opportunity to learn more about the essential elements towards the end of the unit.

## 12.2.2 Classification of Elements

The conventional system of classification is based on the concentration of element $g^{-1}$ dry matter found in the plant. Those which are found in concentration of 1000 μg or more are called **macronutrients** and those found in less than 100 μg $g^{-1}$ dry matter are designated as **micronutrients**. Thus, the first nine elements listed in Table 12.1 are macronutrients and the rest seven are micronutrients.

This classification though useful in some respects, is arbitrary and in many cases the difference between the contents of the two groups is not much. To give an example, the Fe and Mn contents of plant tissues very often are as high as the content of S or Mg. Hence, scientists are trying to evolve a more satisfactory classification based on physiological and biochemical parameters.

### 12.2.3 Functions of Essential Elements

Essential elements in plants serve the following three functions:

1) **Osmotic Function :** Plant cells generally contain mineral ions 10 to 1000 times higher in concentration than the surrounding soil. That is why water enters the cells by osmosis and builds up turgor. You know that turgor maintains the shape and size of non-rigid plant parts such as leaves. Potassium is a key ion in this respect. Changes in its concentration in guard cells affect turgor and thus result in opening and closing of stomata. Turgor is also essential for the growth of plant cells.

2) **Structural Function** : The elements nitrogen, phosphorus and sulphur absorbed from the soil are essential components of amino acids and nucleotides. The other two elements that are constituents of important compounds are $Mg^{2+}$ and $Ca^{2+}$. While $Mg^{2+}$ is part of chlorophyll molecule and $Ca^{2+}$ is an integral part of the middle lamella and is thought to be essential for maintaining structural characteristic of cell walls. It also maintains the permeability of plasma membrane. In its absence cells begin to leak out. In recent years a new regulatory role that $Ca^{2+}$ plays in the cell is beginning to be appreciated. It plays the role of a second messenger.

3) **Biochemical Function** : Elements Mg, Mn, K, Ca and Fe are cofactors for many enzymatic reactions. Fe is carrier of electrons in electron transfer chain. Phosphorus plays a great role in cell chemistry. Phosphorylated sugars are essential for photosynthesis and respiratory metabolism, and phosphorus in the form of phosphate is essential for the formation of the high-energy bonds of ATP.

**SAQ 1**

a) Which of the following statements are true? Write T for true and F for false in the given boxes.

i) The matter which is left after drying the plant tissue is called ash. ☐

ii) Plants contain more calcium than phosphorus. ☐

iii) The elements that are found in concentration of 1000 μg are called micronutrients. ☐

iv) The element sodium plays a key role in maintaining the turgor of plant cells. ☐

b) Fill in the blanks with appropriate words.

i) The key element that performs osmotic function in plants is .....

ii) ..... maintains the permeability of plasma membrane.

iii) $Mg^{2+}$ is part of ..... molecule.

iv) ..... is carrier of electrons in electron transfer chain.

## 12.3 NUTRIENT ABSORPTION

Except for carbon, oxygen and hydrogen which are provided by $CO_2$ and water, all the other elements essential for plants are provided by the soil. Hence, hereafter, in this unit on 'Mineral Nutrition' we will confine our discussion to those elements which are acquired from the soil by the plants.

**Hydroponics**
Plants can be grown without soil in a solution of mineral salts of required composition. This technique of growing plants is called hydroponics, soil-less culture or solution culture.

### 12.3.1 Nutrients and the Soil

Early experiments on mineral uptake were performed by Hoagland, Stout and Arnon in 1923. They showed that minerals were taken up from the soil primarily in ionic form. The rate of uptake of different ions by roots varied and one ion influenced the

uptake of other ions. As soil is the medium for the storage and exchange of mineral ions, its properties, ion exchange capacity, pH and the presence of different cations and anions affect the availability of ions to the plant. In other words, the presence of a certain mineral ions in abundance in the soil cannot ensure its availability to the plant because ions may adhere to clay or precipitate out of the solution as insoluble salts. The soil with high water holding capacity generally has high mineral holding capacity as well. The fine particles of clay and humus possess a relatively large surface to volume ratio and are negatively charged. Hence, they have higher ion-binding capacity than the soil composed of coarse particles. Fig. 12.2 shows the colloidal clay crystals (micelles) with innumerable negative surface charges. The cations are loosely bound to negative charges by ionic bond and are capable of exchanging rapidly and reversibly with those in the soil solution. $H^+$ ions have greater affinity for charged soil particles than $Ca^{2+}$, $Mg^{2+}$ or $K^+$ ions. Therefore, these cations are released in soil water by $H^+$ ions and made available for uptake by roots. The acidity of soil also increases due to respiration because $CO_2$ released reacts with soil water to form carbonic acid.

$$\text{Micelle-Mg}^{2+} + 2\,H^+ \rightarrow \text{Micelle-}H^+ + Mg^{2+}$$
(In soil solution)

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$
$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$
(From respiration)

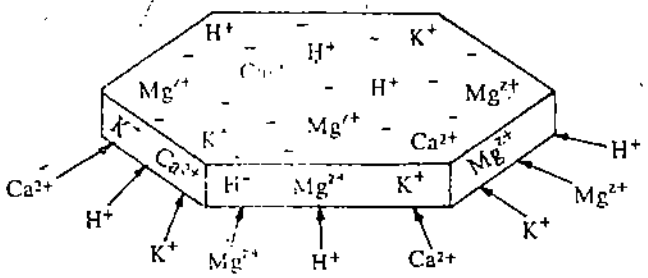



**Fig. 12.2 : Diagrammatic representation of the outside surfaces of a clay micelle. Note many negative surface charges and the various cations surrounding them.**

The ion exchange capacity of mineral ions is affected by the pH of soil which in turn affects the availability of different ions to the plant as shown in the graph below (Fig. 12.3).

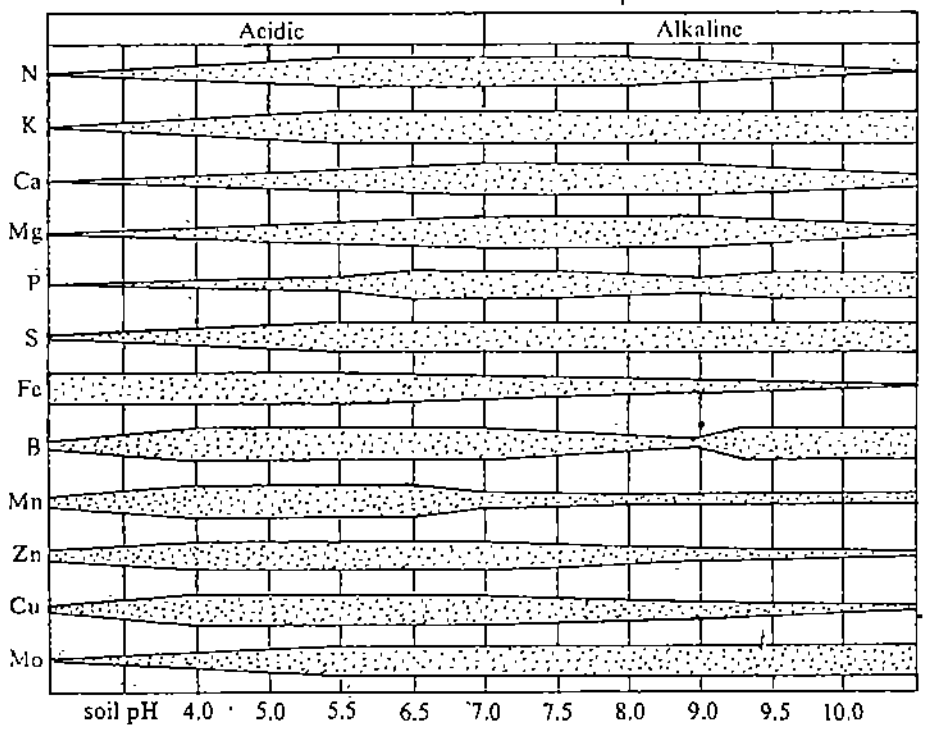



**Fig. 12.3 : The effect of soil pH on the availability of different ions to the plant. The wider the bar greater the availability.**

## 12.3.2 Uptake of Mineral Ions

Let us now see, what happens to the solute concentration in the root cells when plants are raised for a few days in a nutrient solution of known concentration. Data from a typical experiment are given in Table 12.2. Two plants, maize and beans are selected for comparison.

Table 12.2: Changes in the ion concentration of the external nutrient solution and in the root sap of maize and bean expressed in mM. The data are recorded after 4 days

| Ion | After 4 days | | | | |
|---|---|---|---|---|---|
| | External Concentration | | | Concentration in the root sap | |
| | Initial Concentration | Maize | Bean | Maize | Bean |
| Potassium | 2.00 | 0.14 | 0.67 | [illegible] | [illegible] |
| Nitrate | 2.00 | 0.13 | 0.07 | [illegible] | 35 |
| Calcium | 1.00 | 0.94 | 0.59 | 3 | 10 |
| Sulphate | 0.67 | 0.61 | 0.81 | 14 | 6 |
| Sodium | 0.32 | 0.51 | 0.58 | 0.6 | 6 |
| Phosphate | 0.25 | 0.06 | 0.09 | 6 | 12 |

A careful study of the above table shows the following:

i) The concentration of potassium, phosphate and nitrate declined significantly in the bathing medium within four days.

ii) The concentration of sodium and sulphate, however, increased indicating that water was absorbed faster than either of the two ions, or possibly they leach out.

iii) The rate of uptake especially of potassium and calcium, differed between the two plant species, maize and bean.

iv) The ion concentration (particularly of $K^+$, $NO_3^-$ and $SO_4^{2-}$) in the root was considerably higher than in the nutrient solution used for the experiment.

These results show certain characteristics of nutrient uptake.

1) **Selectivity** : Certain mineral elements are taken up preferentially while others are discriminated against or nearly excluded.

2) **Accumulation** : The concentration of mineral elements can be much higher in the plant sap than in external solution. This means the uptake is against concentration gradient.

3) **Genotype** : Plant species differ genetically in nutrient uptake characteristics.

You know that chemically $Na^+$ resembles $K^+$ very closely but the rate of absorption of $K^+$ is not influenced by similar concentration of $Na^+$ ions in the medium. The process of $K^+$ absorption is, therefore, selective and uninfluenced by a related ion. Similarly, several other monovalent and less related divalent ions also have no effect on $K^+$ uptake. Likewise, absorption of $Cl^-$ is unaffected by related halides, fluoride and iodide, as well as other anions like $NO_3^-$, $H_2PO_4^-$ or $SO_4^{2-}$. However, interestingly, $Ca^{2+}$ is an absolute requirement for this selectivity. For example, in the absence of $Ca^{2+}$, $K^+$ absorption is inhibited by $Na^+$

Even though the ion uptake mechanism is highly specific, yet it can often by 'fooled' by similar ions. For example, it has been seen that absorption of $K^+$ can be competitively inhibited by rubidium ($Rb^+$). Similarly, competitive inhibition of $Cl^-$ by $Br^-$, of $Ca^{2+}$, $Sr^{2+}$ by $Mg^{2+}$, and sulphate by selenate ($SeO_4^{2-}$) has also been observed.

The selectivity and the rate of uptake of the nutrients and metabolites are influenced by temperature, $O_2$, poisons, carbohydrate content of the tissues and light. Such effects are similar to enzyme-mediated reactions and indicate that proteins are involved in solute uptake. You will learn about transport proteins and mechanism of ion uptake in a later section.

### 12.3.3 Movement of Nutrients into the Roots

In the previous unit we told you about the two main routes,— apoplast and symplast — by which water and dissolved solutes are conducted across the root interior into xylem vessels and tracheids. The cell wall spaces and intercellular spaces in the root's epidermis and cortex are virtually continuous with the external soil solution.

As shown in the Fig. 11.9 of the previous unit ions can move up into the root hair as well as epidermal cells. An io[illegible]hat is absorbed by an epidermal cell and moving

Many fungi grow in soil in close association or even into the roots in symbiosis called mycorrhizae. The fungal hyphae have superior mineral absorptive ability and supply plant with more nitrogen, phosphorus and potassium. Plants in return provide food to the fungi.

towards the xylem in the symplast must cross the epidermis, several cortical cells, the endodermis and the pericycle. The movement would involve transport directly through each of the two primary walls, middle lamella and plasma membranes between the cytosol of adjacent cells. Alternatively, the solute could move through plasmodesmata without crossing the plasma membrane or diffusing through cell walls.

The nutrient movement along the apoplasm is prevented at root endodermal cells because these cells are lined with Casparian strips. Therefore, the water and dissolved substances must enter the cell and pass through them via the symplastic route. Thus all minerals must pass through the cytoplasm in order to reach the xylem.

With this background we are now ready to trace the path of a solute (or nutrient) entering the root from the surrounding soil solution.

### Free Space

You have learnt that the primary cell wall of the plant cell consists of mainly cellulose microfibrils which are embedded in an amorphous matrix of two polysaccharides, hemicelluloses and pectic substances. Hemicelluloses are made of sugars other than glucose (e.g. xyloglucans) while pectic substances are partly made from polygalacturonic acids. These acids have weak carboxylic acid groups ($-COOH$) that ionize and give rise to negative charges ($-COO^-$) on which hydrogen ions are loosely held. When positively charged ions such as $K^+$, $Mg^{2+}$, $Ca^{2+}$ pass through the plant cell wall they displace hydrogen ions of the carboxyl groups and are held there by the weak inter-ionic attractive forces. The negative charges of the $COO^-$ group cell wall are termed cation adsorption sites or cation exchange sites or Donnan Free Space. A cation such as $Ca^{2+}$ with a relatively high adsorptive capacity will displace ions with a lesser adsorptive affinity (e.g. $K^+$).

**Table 12.3 : Comparative Dimensions in (nm)**

| | |
|---|---|
| Cortical cell wall of maize | 100-200 |
| Pores of cell wall | >5.0 |
| Sucrose | 1.0 |
| Hydrated ions | |
| $K^+$ | 0.66 |
| $Ca^{2+}$ | 0.82 |

The cellulose microfibrils are not very tightly packed; as a result they leave small pores between them. The pores are large enough to allow free movement of water and dissolved substances. The diameter of these pores is in the range of 5.0 nm whereas, the dimensions of hydrated ions such as $K^+$ and $Ca^{2+}$ are smaller (Table 12.3). So, the pores do not restrict the movement of ions. Water and dissolved nutrient molecules, ions and metabolites of the size of glucose, sucrose, and amino acids diffuse readily across primary cell walls.

The intercellular spaces, the negatively charged regions (Donnan Free Space) in the amorphous matrix and the pores in the cellulose microfibrils are readily accessible to water and dissolved ions. The fraction of the volume of the plant tissue readily accessible to diffusion of an external solute dissolved in water is termed 'Free space'. The free space in the root is bound by the plasma membrane of the epidermal and cortical cells and the Casparian strip of the endodermis.

Any substance which can easily pass through the free space then reaches the external surface of the protoplasm. Here, it encounters the plasma membrane which is an effective barrier to its movement further inward. Nevertheless, the plasma membrane does not act like a passive barrier, it has the ability to allow the passage of some substances into the cell interior while selectively inhibiting the passage of certain others. In the following section, we will discuss the transport of nutrients across the plasma membrane. You can check your progress by trying the SAQ given below.

SAQ 2

a) Tick mark the correct alternative from the words given in each parenthesis.

i) The clay micelles have (negative/positive) charge.

ii) Cytoplasmic strands connecting the adjacent cells are (plasmodesmata/ Casparian strip).

iii) The iron-exchange capacity is affected by (temperature/pH) of the soil.

iv) The apoplastic pathway is broken at the (Casparian strip/plasmodesmata) of the endodermal cells.

v) The fixed negative charges formed by the weakly acidic carboxyl groups of polygalacturonic acids form (cation exchange sites/anion exchange sites)

vi) $K^+$ absorption can be competitively inhibited by (Sodium/Rubidium)

## 12.4 TRANSPORT OF IONS

In this section, we will study transport of solutes particularly inorganic ions across plasma membrane, their entry into xylem elements and long distance transport from root to shoot. In Units 7 and 8 on membrane transport processes (Cell Biology course), you had learnt that the transport of solutes across the membrane can take place by simple diffusion, facilitated diffusion and active transport (Fig. 12.4).

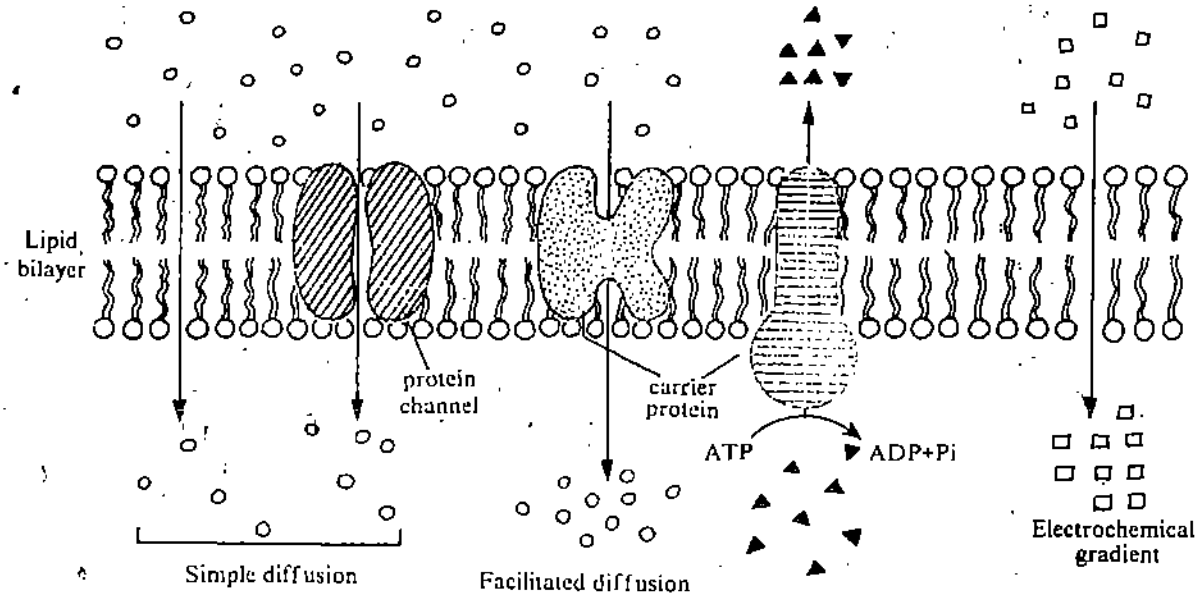



**Fig. 12.4 : Passive and active transport mechanisms of mineral ions and other molecules across the plasma-membrane.**

### 12.4.1 Ion Transport Across the Plasma Membrane

As we have mentioned earlier ions must cross plasma membrane either before Casparian strip or at the Casparian strip in order to move further. The transport can take place by passive as well as active processes.

**Diffusion**

Simple diffusion can be a mechanism of transport of ions across plant cell membrane. Let us imagine a lipid bilayer without the various proteins in it (Fig. 12.5). Such a

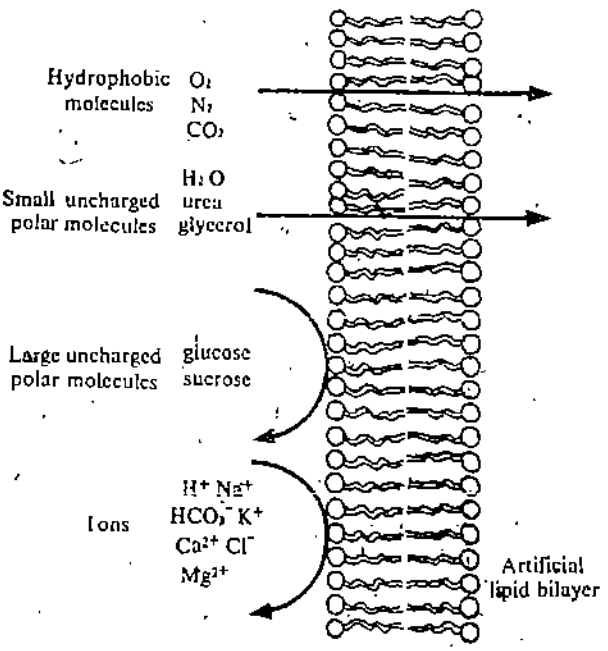



**Fig. 12.5 : The relative permeability of an artificial lipid bilayer to different class of molecules.**

membrane can be made in the laboratory. Given enough time, molecules can diffuse across the lipid bilayer down its concentration gradient by virtue of their own kinetic energy. However, the rate at which a molecule diffuses across such a lipid bilayer depends on the size of the molecule and its relative solubility in the lipid. Non-ionic hydrophilic substances are generally taken up as inverse function of their size, whereas hydrophobic substances are transported as a function of their lipid solubility. Small non-polar and hydrophobic molecules like $O_2$ and $N_2$ readily dissolve in lipid and therefore, rapidly diffuse across the bilayer. Ethanol (46 dalton) carbon dioxide (44 dalton) and urea (60 dalton) cross the bilayer rapidly while bigger molecules like glycerol (92 dalton) less rapidly and glucose (180 dalton) hardly at all. Water molecule (18 dalton) even though is relatively insoluble in lipids diffuses very rapidly across a lipid bilayer. On the contrary, lipid bilayers are impermeable to charged molecules (ions), even if they are very small. Because the charge and large hydration shell around prevent them from entering the hydrocarbon phase of the bilayer. As a result, lipid bilayers are $10^9$ times more permeable to water than to even such small ions as $Na^+$ or $K^+$ (Fig. 12.6).

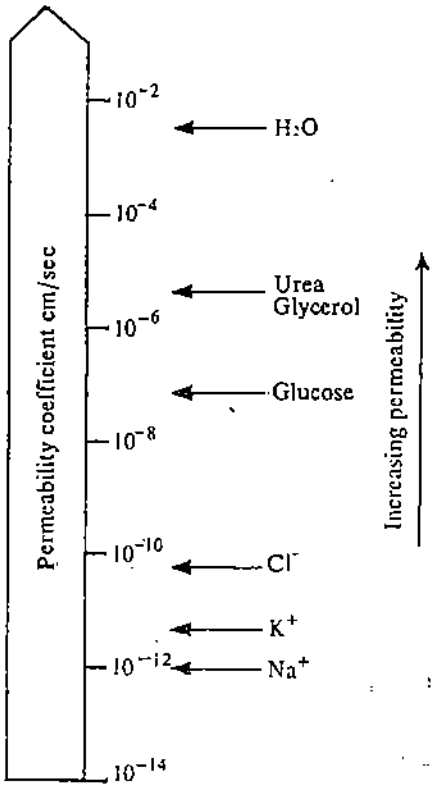



**Fig. 12.6 : Permeability coefficients cm $sec^{-1}$ for the passage of various molecules through artificial lipid bilayers.**

$K^+$ is more permeable than most ions. Its permeability coefficient is arbitrarily set at 1.0 and is taken as standard. The permeability of other ions is compared with $K^+$

The ability of the lipid bilayer to discriminate between low and high molecular weight hydrophilic materials, permitting the former to diffuse across but not the latter is due to the presence of pores in the bilayer. These pores are formed randomly as a result of thermal movement of acyl phospholipid chains. They are called 'statistical pores'. Since these pores are only transient, they cannot be viewed even under the electron microscope.

The driving force for diffusion is a concentration gradient and obeys Fick's law; which states that the rate of movement of a substance is directly proportional to the concentration gradient. Simple diffusion thus shows a linear relationship between the concentration of solute and its rate of transport across the membrane.

## 12.4.2 Transport with the Help of Membrane Protein

Even though, the lipid bilayers do not permit the entry of polar molecules such as ions, sugars, amino acids, nucleotides and cell metabolities, these molecules enter the cell through i) aqueous protein channels (pores), ii) carrier proteins that rotate and move across the membrane and iii) transmembrane proteins that transport solute by undergoing changes in shape, (Fig. 12.7).

Studies on plants cell membrane suggest that inorganic ions can permeate through aqueous protein channels called permeases. The permeases are ion specific because the permeability of different ions varies. Most membranes are more permeable to $K^+$ than to other ions.

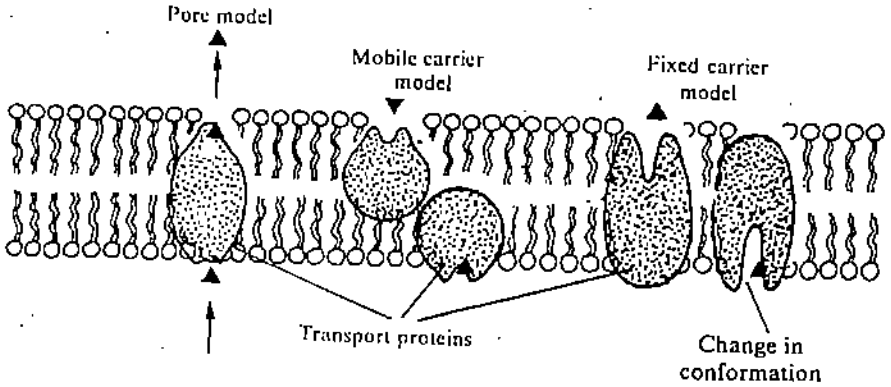



**Fig. 12.7 : Three different possible modes of transport of ions and other molecules across the plasma membrane.**

We had mentioned in Section 12.3.1 that ion uptake though specific can be fooled by similar ions. It seems probable that certain proteins transport more than one ion at the same site and so the ions compete with each other. For example, $K^+$, $Cs^+$, $Rb^+$ are apparently transported at the same site and $Na^+$ and $Li^+$ both are transported at the other site. But ions transported at different sites do not compete with each other; for instance $K^+$ does not compete with $Na^+$.

Ion transport in plants also takes place through ionophores. As you know, ionophores are small polypeptides and proteins that shield the charge of ions from hydrophobic environment of the membrane. Ionophores have been isolated from bacteria and fungi. When they are added to the artificial lipid bilayer, they increase the rate of diffusion of specific ions by as much as one million fold!

There is much indirect evidence for mobile carrier proteins in plant membranes. So far only sucrose carrier is identified.

**Driving Force**

Let us now find out what is the driving force involved in protein mediated transport. Many membrane transport proteins allow specific solutes to move across the lipid bilayer. If the transported molecule is uncharged, then the difference in its concentration on the two sides of the membrane, that is its concentration gradient — determines the direction of transport. However, if the solute to be transported carries a net charge, then both its concentration gradient and the total electrical gradient across the membrane influence its transport. For instance, an ion will move across a membrane if there is sufficient electrical gradient across the membrane even if the concentration gradient does not favour such a movement. In other words, the direction of movement is decided by which of the two forces is steepest. The two gradients together constitute the electrochemical gradient. The gradient can develop in part due to the selective permeability of the membrane. So the related diffusion of cations may be more than anions or vice versa. For example, $K^+$ diffuses out more rapidly due to differences in electrical gradient than $Cl^-$ in the immediate exterior and hence excess of $Cl^-$ in the cell gives it a negative charge.

In fact, all plasma membranes have electric potentials (transmembrane potential) across them with inside of the cell more negative compared to the outside. This is due to active transport of ions particularly $H^+$ ions out of the cell. This potential difference allows the entry of positively charged ions into the cell but opposes the entry of negatively charged ions.

The relationship of electrical potential inside of a cell to the distribution of charged ions inside and outside of a cell is given by Nernst equation. You have already learnt this equation in Cell Biology course (Unit 7, Section 7.4). It is possible to measure transmembrane potential by a very fine glass microelectrode (μm in diameter). In plants the difference in potential of cell vacuole and cellular exterior is measured by inserting a narrow tipped fine ions-selective micro electrode through the cell wall and

When different concentrations of freely diffusing ions are separated by a membrane, a voltage gradient develops across the membrane. This is called transmembrane potential.

plasmalemma into the vacuole (Fig. 12.8). The other larger reference electrode is placed in the solution bathing the tissue. Such measurements show potential difference ranging between 50 to 200 millivolts (mv), the interior of cell being more negative.

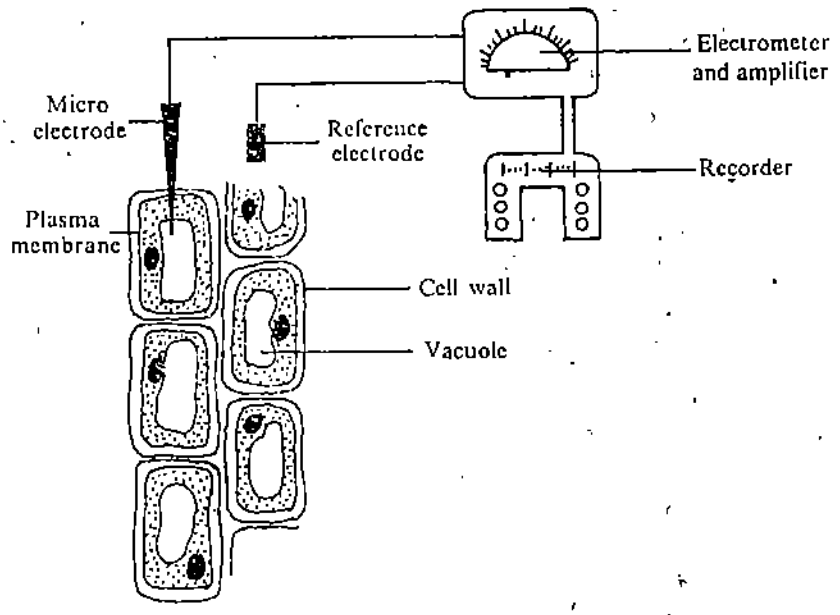



**Fig. 12.8 : Equipment used to measure the transmembrane potential of plant cells (see text for details).**

### Active Transport

We have mentioned above that transmembrane potential develops due to active transport of ions ($H^+$) outside the cell. Since this transport takes place against a concentration gradient it utilises energy of hydrolysis of ATP. The proton motive force generated by proton pumping provides the driving force for the transport of solutes including cations, anions, amino acids and sugars. Electrical potential and pH measurements of intact plant cells have suggested that proton pumps are localised on the plasma membrane.

A dalton is a unit of mass equivalent of 1/12 of the mass of $^{12}C$. Thus the mass of $^{12}C$ is 12 dalton. Dalton can be converted to grams by multiplying with $1.66 \times 10^{-24}$ grams.

The plant plasma membrane ATPase is a transmembrane protein composed of a single polypeptide chain of 100 KD. The most possible coupling mechanism between ATP hydrolysis and proton transport is shown in Fig. 12.9. The enzyme exists in two conformations differing in catalytic and transport properties. In conformation I, the transport site faces the cytoplasm and has high affinity for protons. In conformation II, the transport site is externally oriented and has low affinity for protons. The enzyme is forced to alternate between these two conformations and to bind and release the transported proton because neither conformation can affect the complete catalytic cycle. In conformation I, the enzyme acts as a kinase; after binding a proton it catalyses the formation of phosphorylated intermediate. In the new state (conformation II) it acts as a phosphatase and after releasing the proton it returns to its original state, conformation I. Thus, when steady state is reached, a proton

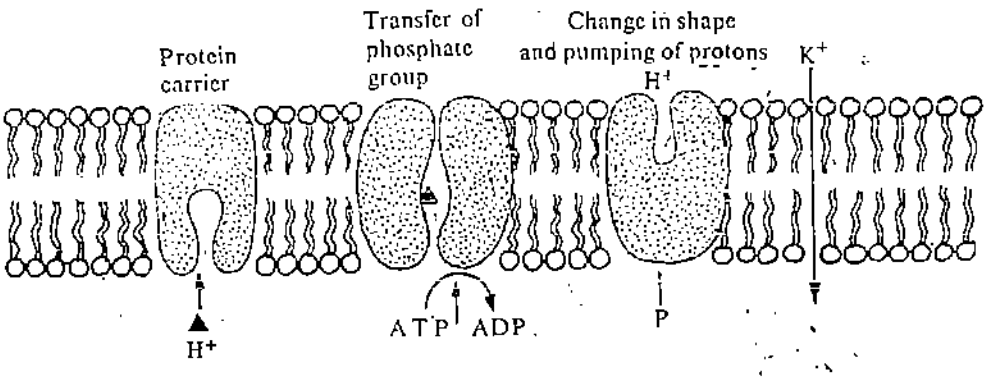



**Fig. 12.9 : Simplified picture of active transport. Transport of proton across the membrane is coupled with transport of other cations in opposite direction. The transport protein receives an energy boost from ATPase and thereby undergoes changes in its shape that are necessary to the transport process.**

gradient is developed, the concentration of protons being more on the outside of the membrane (i.e. around the cell wall region) and less in the cytoplasm. This leads to the generation of **proton motive force.** The net result of the proton pump is that the pH of the medium outside the plasma membrane becomes more acidic and the cytoplasm more alkaline.

An enzyme that promotes hydrolysis of ATP in the presence of $K^+$ has also been isolated from plants. It is suggested that enzyme binds $K^+$ ion at specific site and changes conformation on binding ATP. After hydrolysis of ATP it returns to its original conformation and the ion is released to the other side of the membrane. A working model depicting the active transport in plant cell with $H^+$ translocating ATPase located in plasma membrane and tonoplast is shown in Fig. 12.9.

A study on the rate of uptake of $K^+$ using radioactive isotope shows that uptake resembles an enzyme-substrate reaction curve and carriers involved behave like specialised membrane-bound enzymes. At higher concentration the carrier site for ions gets saturated, hence the uptake does not increase further with increase in concentration of ions in the solution. The binding sites of the carrier can also be inhibited by competitive inhibitors.

Transmembrane potential thus generated by proton pumping provides the driving force for the subsequent movement of cations, anions, amino acids and sugars by specific carrier or ion channels. Movement of sucrose into and out of phloem cells occurs along with $H^+$ (symport) through a permease. In roots hydrogen is frequently exchanged for $K^+$, (Fig 12.10). Various ions and metabolites also accumulate in plant cell vacuole via ion channels and proton antiports present in tonoplast Fig. 12.10.

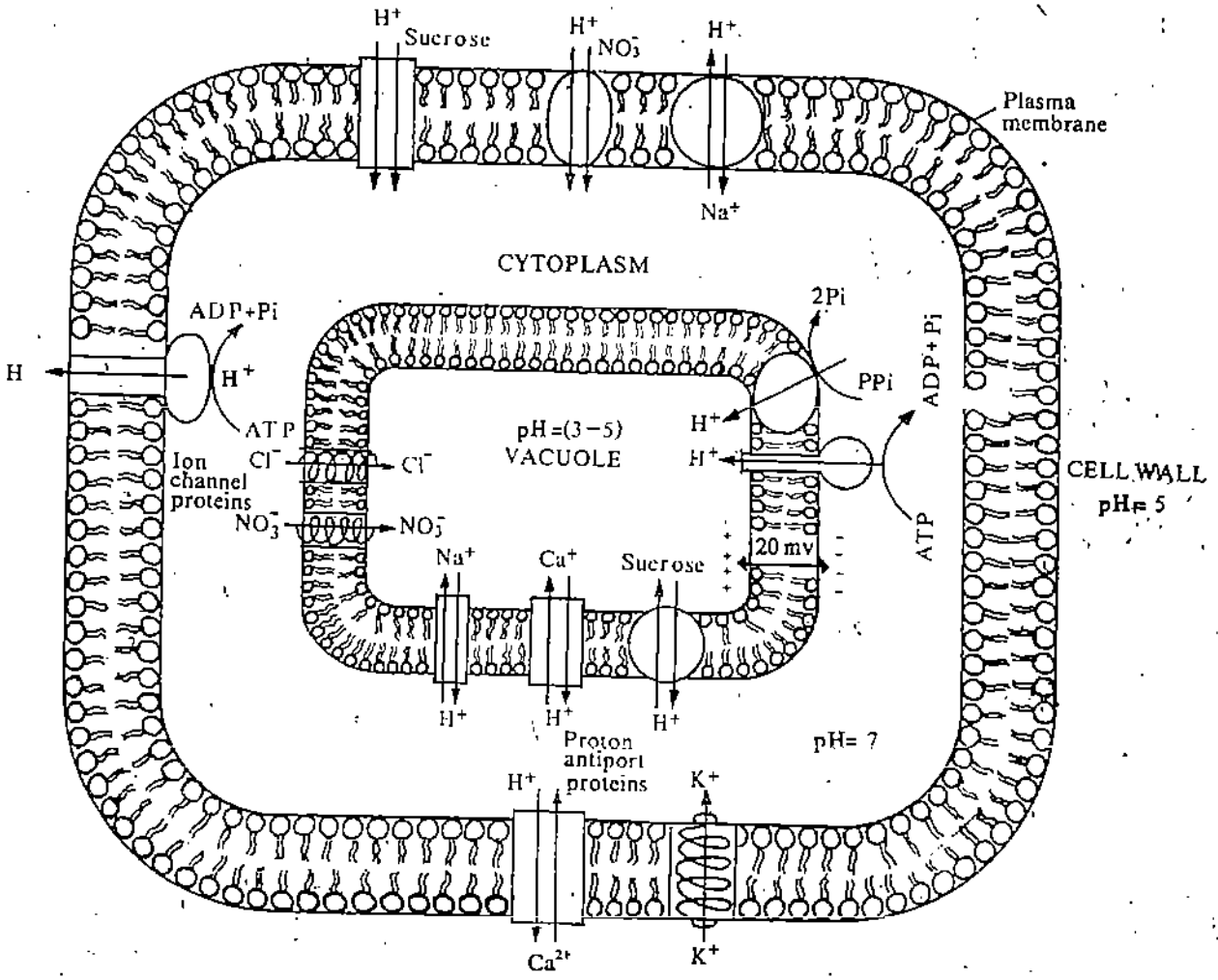



**Fig. 12.10 : The transport of ions across cell and vacuole membranes. Note the two types of proton pumps — ATPase and unique pyrophosphate-hydrolysing proton pump. The pH of vacuole falls because of transport of protons inside the vacuole.**

The proton pumping activity of the enzyme directly controls intracellular pH, nutrient uptake, turgor, cell growth, loading of nutrients into root xylem, loading of leaf phloem with organic nutrients, turgor changes responsible for stomata and pulvini movements, regulation of cell elongation and cell wall synthesis, early response to hormones especially IAA and many other functions. Thus, proton pumps have a central role in plant physiology and are considered as master enzymes. The proton pump has now been isolated and characterised biochemically.

### 12.4.3 Radial Movement of Ions into the Roots

Fig. 12.11 shows schematic drawing of the radial movement of ions by symplastic and apoplastic routes. As nutrients move along the cell wall and intercellular spaces of the epidermal and cortical cells, some nutrients are absorbed by these cells and enter

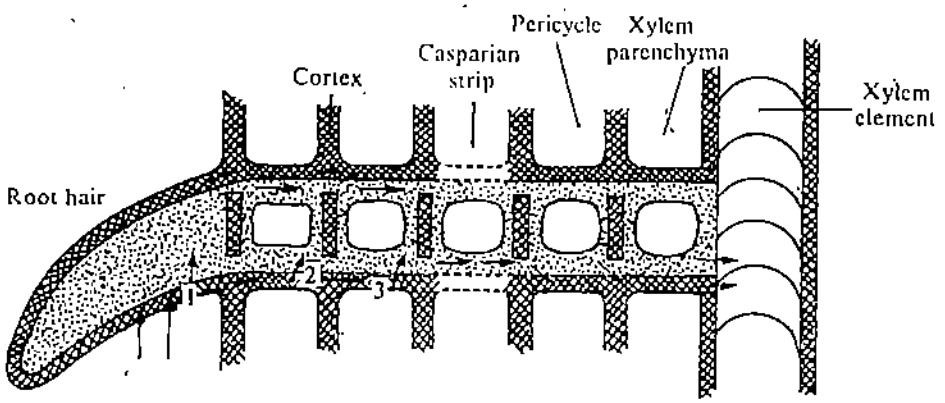



**Fig. 12.11 : Schematic drawing showing the apoplast and symplast in cross-section of a root. The heavily stippled dark regions constitute the apoplast while the lightly stippled regions constitute the symplast. The vacuole is not part of either system. The Casparian strip creates a discontinuity in the apoplast. Therefore, all ions absorbed by root hairs must cross the plasma membrane of a cell (1, 2 or 3) exterior to the Casparian strip, thereby entering the symplast.**

symplastic pathway in cytoplasm through plasmodesmata. Rest of the ions are screened by the membranes of endodermal cells which control the rates of absorption and type of solute absorbed. Some of these solutes are transported into the vacuole, where they contribute greatly to the negative osmotic potential of root facilitating water uptake, turgor pressure and growth of roots through soil.

The preferential use of apoplastic or symplastic pathway by different ions is not clearly known. Experiments with radioisotopes of $^{86}Rb$ and $^{36}Cl$ on the water plant *Vallisneria* show that these ions take only symplastic pathway probably through plasmodesmata. So far, it has been technically difficult to ascertain the role of plasmodesmata, however, their presence in regions where transport is active indirectly indicates that ions move through them in symplastic routes.

Studies using radioisotopes of $^{45}Ca$ in barley seedlings show that it is preferentially transported along apoplastic pathway and its concentration in the cytoplasm remains minimum because it can precipitate both organic and inorganic phosphates within the cells. $Mg^{2+}$ also moves slowly through apoplastic pathway.

Regardless of the pathway, the radial movement through symplasm brings the mineral elements and other solutes to the stele where they are released into the xylem. This transfer is an energy requiring process in which xylem parenchyma plays an important role. The mechanism is more or less similar to that described above for loading of nutrients into the cells and involves carrier proteins.

### 12.4.4 Long Distance Transport

Once in the xylem vessels, the transport of the mineral elements from the root to the shoot is driven by the gradient of hydrostatic pressure (root pressure) and by the gradient of water potential. The gradient of water potential between roots and shoots is usually quite steep during the day when the stomata are open. It follows the pattern: atmosphere << leaf cells << xylem sap < root cells < external solution (soil). Transport in xylem vessels is mainly unidirectional. An increase in the transpiration rate enhances both the uptake and the translocation of mineral elements in the xylem.

The lateral transport of ions from root xylem to leaves probably takes place via xylem transfer cells (Fig. 12.12) which have two special features:

i) the cell wall of these cells facing xylem is elaborately corrugated for providing large surface area for absorption; and

ii) the cells contain many mitochondria that are located close to the corrugated wall in order to supply ATP for the active transport that takes place across these walls.

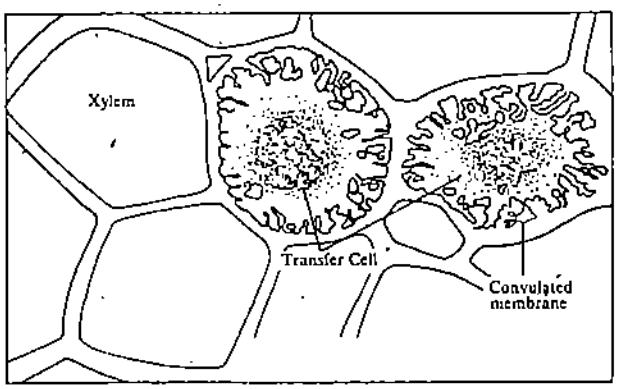



**Fig. 12.12 : Xylem transfer cells.**

The transfer cells are also present at places where large quantities of ions or organic solutes are moved into and out of conducting cells or storage tissue. You will learn more about transfer cells in Unit 14 on phloem transport.

Mineral elements which are phloem mobile can be retranslocated from the shoot to the root via phloem, though the main transported compound in the phloem is sucrose and other organic compounds. The transport in phloem is bidirectional. The direction of transport is determined b the nutritional requirements of various plant organs or tissues.

**SAQ 3**

a) In the following statements fill in the blank spaces with appropriate words.

i) The driving force for diffusion is ..... .

ii) The proton pump in plant is an ATP hydrolysing enzyme called .....

iii) When proton pump works, protons are transported from ..... to ..... of the cell.

b) In the following statements tick mark the correct word given in the parenthesis.

i) Most membranes are permeable to ($K^+$/$Na^+$).

ii) When ionophores are added to the artificial lipid bilayer the permeability of the membrane (decreases/increases).

iii) Transport of the proton across the membrane develops an electric potential gradient which is (negative/positive) outside of the cell.

iv) The net result of the operation of the proton pump is that the pH of the medium outside the plasma membrane becomes (acidic/alkaline).

v) An element which is preferentially translocated by the apoplastic pathway is (potassium/calcium).

## 12.5 ROLE OF ESSENTIAL ELEMENTS

### 11.5.1 Macronutrients

**Nitrogen (N)**

In the atmosphere nitrogen (N) is present as gas ($N_2$) to the extent of 79% by volume. However, plants with a few exceptions cannot use it. From the soil only a very small portion of nitrogen is available to plants. The available forms in soil are $NO_3^-$ and $NH_4^+$ ions. Because of the numerous factors which affect nitrogen turnover in the

soil, the concentration of N dissolved in the soil solution can change considerably over short periods. This is particularly true of $NO_3^-$. Usually the $NO_3^-$ content in the soil solution is of major importance in plant nitrogen nutrition.

Nitrogen is an indispensable elementary constituent of important organic compounds like amino acids, proteins and nucleic acids. Dry plant material contain about 2 to 4% N. In green plant parts, protein nitrogen is by far the largest N fraction and accounts for 80 to 85% of the total nitrogen. In vegetative parts, the proteins are mainly enzyme proteins, whereas in seeds and grains, special storage protein make up the major protein fraction. Nitrogen is also an essential constituent of various coenzymes.

Although nitrate ion is preferred, plants can absorb $NH_4^+$ as well. Crops mainly take up $NO_3^-$ even when $NH_4^+$ fertilisers are applied because of the rapid microbial oxidation of $NH_4^+$ to $NO_3^-$ in the soil. The rate of uptake of $NO_3^-$ is generally very high as plants require large amounts of Nitrogen. An important difference between uptake of $NO_3^-$ and $NH_4^+$ ion is in their sensitivity to pH. $NH_4^+$ uptake takes place best in a neutral medium and it is depressed as the pH falls. The converse is true for $NO_3^-$ absorption; a more rapid uptake occurs at low pH values. Gaseous ammonia may also be absorbed by the upper plant parts via the stomata.

Within the plant, nitrate is to be reduced to ammonia before it is incorporated into amino acids and proteins. In Unit 15, you will learn about metabolism of nitrogen.

The form in which translocation occurs depends on the nitrogen source and metabolism in the root. Nearly all the $NH_4^+$ ions absorbed are assimilated in the root tissue and translocated as amino acids. Nitrate can be translocated as such to shoots and leaves but this depends on the nitrate reduction potential of the roots.

When the supply of nitrogen from the root is inadequate, nitrogen from older leaves is mobilised to feed the younger plant organs. For this reason plants suffering from N-deficiency first show deficiency symptoms in older leaves.

Nitrogen deficiency is characterised by a poor growth rate. The leaves are small and the older ones often fall off prematurely. Shoot growth is affected and in particular branching is restricted. Leaves deficient in nitrogen show chlorosis which is generally rather evenly distributed over the whole leaf. Necrosis of leaves occurs when deficiency is severe. Deficiency symptoms of Fe, Ca, S are also characterised by yellowish and pale leaves similar to nitrogen deficiency. In these deficiencies, however, the symptoms occur first in the younger leaves. Nitrogen deficiency in cereals is characterised by poor tillering, the reduction in the number of ears per unit area and also the number of grains per ear.

Of all the nutrient amendments made to soils, nitrogen fertiliser application by far has been the most effective in increasing crop production. High yielding crop cultivars in particular respond to N-fertilisers. High physiological efficiency of nitrogen usage in cereal crop is achieved when a large proportion of the nitrogen taken up is used in grain formation.

The most common N-fertilisers are given in the Table 12.4.

Table 12.4 : Nitrogen Fertilisers

| N-Fertiliser | Formula | % N |
|---|---|---|
| Ammonium sulphate | $(NH_4)_2SO_4$ | 21 |
| Ammonium chloride | $NH_4Cl$ | 26 |
| Ammonium nitrate | $NH_4NO_3$ | 35 |
| Potassium nitrate | $KNO_3$ | 14 |
| Urea | $CO(NH_2)_2$ | 46 |
| Calcium cynamide | $CaCN_2$ | 21 |
| Anhydrous ammonia | $NH_3$ | 82 |

Nitrogen fertilisers supply $NO_3^-$ and $NH_4^+$ to the soil. Between the two, $NH_4^+$ is partially adsorbed on soil colloids and its uptake rate is usually, therefore, lower than that of $NO_3^-$ under field conditions. For this reason most crops do not respond as quickly to $NH_4^+$ fertilisers as to $NO_3^-$ application.

In paddy soils, nitrogen is lost as a result of denitrification. These soils should not, therefore, receive $NO_3^-$ containing fertilisers. Hence urea and $NH_4^+$ fertilisers are recommended. The main drawback of anhydrous ammonia is the special equipment required for its transport and application.

**Phosphorus (P)**

In soil, phosphorus occurs almost exclusively in the form of orthophosphate. Substantial amount of P is associated with the soil organic matter. The major P-containing ions in soil solutions are $HPO_4^{2-}$ and $H_2PO_4^-$.

Roots are capable of absorbing phosphate from solution low in phosphate content. The phosphate content of roots and xylem sap is about 100-1000 fold higher than that of the soil solution. This shows that phosphate is absorbed by plants against a very steep concentration gradient. Phosphate is highly mobile in the plant and can be translocated in upward or downward direction.

The inorganic form of phosphate found in plants are orthophosphate and to a minor extent, pyrophosphate. The organic form of phosphates are compounds in which the orthophosphate is esterified with hydroxyl groups of sugars and alcohols or bound through a pyrophosphate bond to another phosphate group. You know that phosphorylated sugars and alcohols are the major intermediary compounds of metabolism. Phosphate is also present in phospholipids. The nucleotide phosphates — ATP, UTP, GTP, CTP supply energy to various endergonic processes including active ion uptake and the synthesis of various organic compounds.

Another important phosphorus containing compound is phytin which is mainly found in seeds. It is Ca or Mg salt of phytic acid and is formed during seed formation. Phytic acid is the hexaphosphoric ester of inositol. Immediately after pollination there is an increase in P transport towards the developing seeds. Phosphorus in the phytin of seeds is regarded as a P reserve. During seed germination phytin is mobilised and converted into other phosphate forms that are needed in the metabolism of young plants.

Inositol is a sugar (hexose) alcohol.

Plants suffering from P deficiency are retarded in growth. In cereals tillering is reduced. Generally, the symptoms of P deficiency appear in the older leaves which become darkish green in colour. The stems of many annual plant species suffering from P deficiency develop reddish colour due to enhanced formation of anthocyanin pigments.

**Potassium (K)**

The main source of $K^+$ for plants comes from withering of K containing minerals. Potassium released by withering dissolves in the soil solution. It can then be taken up by plants or adsorbed onto soil colloids.

In plants, $K^+$ is an important cation. It is taken up by the plant at high rates through $K^+$ channels present in the membranes.

The concentration of $K^+$ in the cytoplasm is about 100 mM which is 5-10 times higher than $K^+$ concentration in the vacuole. The phloem sap is rich in $K^+$. It is the most abundant cation present in phloem with the concentration approaching that in the cytoplasm. As the solutes of the phloem sap can be translocated both upwards and downwards in the plant, $K^+$ movement is bidirectional.

Potassium is necessary for meristematic growth. It is involved in controlling water status of plants and maintains cell turgor. There is less water loss from plants supplied with $K^+$ due to a reduction in transpiration rate. As you know $K^+$ plays an important role in opening and closing of stomata. Plants inadequately supplied with $K^+$ have impaired stomatal activity. It is also involved in the translocation of photosynthates. The main biochemical function of $K^+$ is the activation of various enzymes.

Potassium deficiency does not immediately result in visible symptoms. At first there is only a reduction in growth rate. Chlorosis and necrosis appear later. These symptoms generally apppear on the margins and tips of older leaves. Plants suffering from $K^+$ deficiency show a decrease in turgor. They easily become flaccid under water stress. Resistance to drought is, therefore, poor. Inadequate soil $K^+$ levels can be corrected by the use of $K^+$ fertilisers. The most widely used and cheapest potash fertilisers is potassium chloride (KCl) which is known commercially as muriate of potash.

## Sulphur (S)

Sulphur is present in the soil in inorganic and organic forms. In most soils organically bound S is the major S reservoir. The inorganic forms of S in soil consists mainly of $SO_4^{2-}$. In arid regions, soil may accumulate high amounts of sulphur salts such as $CaSO_4$, $MgSO_4$ and $Na_2SO_4$. Under humid conditions however, $SO_4^{2-}$ is present either in soil solution or is adsorbed on soil colloids.

The organic S of the soil is made available to plants by microbial activity. In this process of mineralisation $H_2S$ is formed which under aerobic conditions readily undergoes autooxidation and forms $SO_4^{2-}$. In anaerobic media, however, $H_2S$ is oxidised to elemental S by chemotrophic sulphur bacteria such as *Beggiatoa*, and *Thiothrix*. Further oxidation of S results in the formation of $H_2SO_4$. As a result increase in soil acidity can occur.

Plants mainly absorb S in the form of $SO_4^{2-}$. It is mainly translocated in an upward (acropetal) direction. Downward (basipetal) movement of S is relatively poor. There is now a considerable evidence to show that plants can utilise sulphur dioxide also.

Many plant species contain a small amount of volatile S compounds. These are mainly di- or polysulphides. In onions these compounds are responsible for the lachrymatory (tear producing) effect. The main component of garlic oil is diallyl-disulphide.

The most important sulphur containing compounds are cysteine, methionine, lipoic acid, coenzyme A, biotin, thiamin and ferredoxin (an electron carrier, a type of non-heme iron-sulphur protein). Sulphur forms disulphide bridges in polypeptides.

In field crops sulphur deficiency and nitrogen deficiency are sometimes difficult to distinguish. In plants suffering from S deficiency the rate of plant growth is reduced. Generally, the growth of the shoots is more affected than root. In contrast to N deficiency, chlorotic symptoms occur first in the younger, the most recently formed leaves.

Although the content of S in crops is similar to P content, S application does not play an important role as P fertilisation. This is because $SO_4^{2-}$ is not strongly bound to soil particles as phosphate and is thus more available to plants. In addition, substantial amounts of S can come from the atmosphere or from fertilisers which contain S along with other major nutrients being applied e.g. ammonium sulphate or potassium sulphate. The most important sulphur containing fertilisers are gypsum, super-phosphate, ammonium sulphate and potassium sulphate.

## Calcium (Ca)

Soils differ very widely in their Ca content. Plant species may be classified into calcicoles and calcifuges. The calcicoles are those growing on calcareous soils where as the calcifuge species grow on acidic soils poor in Ca.

Generally, $Ca^{2+}$ concentration of the soil solution is about 10 times higher than that of $K^+$ but the uptake rate of $Ca^{2+}$ is usually lower than that of $K^+$. This low $Ca^{2+}$ uptake is because $Ca^{2+}$ can be absorbed only by young root tips in which the cell walls of the endodermis are still not suberised. The uptake of $Ca^{2+}$ can also be competitively depressed by $K^+$ and $NH_4^+$ which are rapidly taken up by roots. Calcium is translocated in an upward direction in the xylem with the transpiration stream. It is translocated only in very small concentrations in the phloem. Once Ca is deposited in older leaves it cannot be mobilised to the growing tips.

In the absence of $Ca^{2+}$, growth rate is reduced and after a few days the root tips become brown and gradually die. $Ca^{2+}$ is required for cell elongation and cell division. It is essential for the stabilisation of newly synthesised membranes. In the absence of $Ca^{2+}$, membrane permeability increases to such an extent that inorganic and organic constituents can diffuse out of the cell causing considerable damage to the cells. In whole plants, this disorder occurs first in the meristematic tissue such as root tips, growing points of the upper plant parts and storage organs.

Most of the $Ca^{2+}$ present in plant tissues is located in the apoplast and in the vacuoles. The $Ca^{2+}$ concentration of the cytoplasm is low. The maintenance of low cytoplasmic $Ca^{2+}$ is of vital importance for the plant cell because the evidence shows that $Ca^{2+}$ may inhibit various cytoplasmic enzymes, and also precipitate as Ca-phosphate. The maintenance of low $Ca^{2+}$ is achieved by pumping $Ca^{2+}$ out of the cytoplasm into the apoplast or into the vacuole.

In the cytoplasm, the function of $Ca^{2+}$ is related to calmodulin, which is involved in the activation of many enzymes by allosteric induction. Calcium is present in plant tissues as free $Ca^{2+}$ adsorbed to indiffusible ions such as carboxylic, phosphoric and

phenolic hydroxyl groups. It is also present as Ca oxalates, carbonates and phosphates. These compounds often occur as deposits in cell vacuoles. Calcium in the cell wall is associated with the free carboxylic groups of pectins.

Calcium deficiency is characterised by a reduction in growth of meristematic tissues. The deficiency can be first observed in the growing tips and young leaves. They become deformed and chlorotic. At a more advanced stage necrosis occurs at the leaf margins. The affected tissue becomes soft due to a dissolution of the cell walls. Brown coloured substances accumulate in intracellular spaces and also in the vascular tissue where they can affect the transport mechanism. In apple the disease is called 'bitter pit' because the surface of the apple is pitted with small brown necrotic spots. In tomato the disease is known as 'blossom end rot' and is characterised by a cellular breakdown at the distal end of fruit.

**Magnesium (Mg)**

Magnesium like $Ca^{2+}$ is present in fairly high concentrations in the soil solution. Generally the concentration in soil solution is higher than that of $K^+$ but the rate of uptake of $Mg^{2+}$ is much lower than the uptake rate of $K^+$. $Mg^{2+}$ is very mobile in the phloem and can be translocated from older to younger leaves or to the apex.

Mg is constituent of chlorophyll molecule. It is a cofactor in almost all phosphorylation reactions. $Mg^{2+}$ forms a bridge between the pyrophosphate structure of ATP or ADP and the enzyme molecule. The activation of ATPase by $Mg^{2+}$ is brought about by this bridge formation. Another key function of $Mg^{2+}$ is in the activation of ribulose bisphosphate carboxylase. Light triggers the import of $Mg^{2+}$ into the stroma of the chloroplast in exchange of $H^+$ thus providing optimum conditions for the carboxylase reaction. Mg deficiency inhibits protein synthesis.

Mg is mobile in the plant and deficiency always begins in the older and then moves to the younger leaves. Interveinal yellowing or chlorosis occurs and in extreme cases the areas become necrotic.

Mg application is recommended for all crops growing on soils with less than 25 ppm exchangeable Mg. The major Mg fertilisers are, magnesium limestone ($MgCO_3$ — 5 to 20% MgO) ground burnt magnesium lime (Mg oxide — 10 to 33% MgO), kiesernite ($MgSO_4.H_2O$ — 27% MgO), Epsom salt ($MgSO_4.7H_2O$ — 16% MgO) and Magnesite ($MgCO_3$ — 45% MgO).

**SAQ 4**

a) In the following sentences choose the right alternate word given in the parenthesis.

i) The form of nitrogen preferred by plant is ($NH_4^+/NO_3^-$).

ii) Largest fraction of N present in plant is in the form of (proteins/nucleic acids).

iii) In soil ($NO_3^-/NH_4^+$) is converted by microbial action into ($NO_3^-/NH_4^+$).

iv) Uptake of $NH_4^+$ is best in neutral medium. It (decreases/increases) as pH falls.

v) In plants ($NH_4^+/NO_3^-$) is converted into ($NH_4^+/NO_3^-$) before it is incorporated into proteins.

vi) The symptoms of nitrogen deficiency first appear in (older/younger) leaves

vii) Generally the concentration of ($Ca^{2+}/K^+$) is higher in soil but uptake rate of ($Ca^{2+}/K^+$) is higher.

viii) Calcium is mainly translocated through (xylem/phloem). It (can/cannot) be mobilised from older leaves.

ix) The rate of uptake of ($Mg^{2+}/K^+$) is lower than ($Mg^2/K^+$).

x) Deficiency of phosphorus would affect (carbohydrate/protein) metabolism

xi) The colour of stem of some annuals becomes red due to the formation of anthocyanin pigment. This is due to the deficiency of (phosphorus/sulphur)

xii) Phosphorus is highly (mobile/immobile) in plants.

xiii) The most mobile nutrient in plants is (K/P).

xiv) K deficiency results in loss of (membrane permeability/turgor),

b) List four important compounds of plant containing sulphur.

.....

.....

.....

c) Match the content of column I corresponding with those of column 2.

| Column I | Column 2 |
|---|---|
| i) Magnesium | a) deficiency affects cell division and cell elongation. |
| ii) Calcium | b) cofactor in all phosphorylation reactions. |
| iii) Sulphur | c) component of coenzyme A |

## 12.5.2 Micronutrients

### Iron (Fe)

Iron is present in all soils. Soluble Fe in soil is extremely low in comparison with its total content. Soluble inorganic forms are $Fe^{3+}$, $Fe(OH)^{2+}$ (hydroxoferric ion), $Fe(OH)_2^+$ (dihydroxoferric ion) and $Fe^{2+}$.

When soils are waterlogged, a reduction of $Fe^{3+}$ to $Fe^{2+}$ occurs which is accompanied by an increase in solubility. Anaerobic bacteria which use Fe oxides as electron acceptors in respiration, bring about this reduction. This process is of particular interest in paddy soils where it can lead to high $Fe^{2+}$ concentrations. Such high concentrations often produce toxic effects in rice plants.

Fe must be reduced before it can be taken up by the roots. Iron in the free spaces of roots may be present in the ionic form or as chelate. The reduction of $Fe^{3+}$ chelate destabilises the complex and the resulting $Fe^{2+}$ can be absorbed. The rate of Fe reduction is pH dependent and is higher at low pH.

The uptake of iron is competitively inhibited by other cations, like $Mn^{2+}$, $Cu^{2+}$, $Ca^{2+}$, $Mg^{2+}$, $K^+$ and $Zn^{2+}$. Iron is not readily mobile between the various plant parts. Green plants lacking Fe become chlorotic in the younger plant parts, whilst older parts remain green. Younger parts are, therefore, dependent on a continuous supply through the xylem. The major form in which Fe in translocated in the xylem is considered to be as ferric citrate.

The well-known function of Fe is in enzyme systems in which heme forms the prosthetic group. Here, Fe plays a somewhat similar role to Mg in the chlorophyll molecule. The heme enzyme system include catalase, peroxidase, cytochrome oxidase and the various cytochromes. Another important compound containing Fe is the non-heme iron protein 'ferredoxin' which is an iron sulfur protein. It is an electron carrier, in photosynthetic light reactions.

The deficiency of Fe and Mg are more or less similar as both are characterised by a failure of chlorophyll production. Iron deficiency, however, unlike Mg deficiency, always begins in the younger leaves, the darker green veins contrasting markedly against a lighter green or yellow background. The youngest leaves may often be completely white and totally devoid of chlorophyll. In the leaves of cereals the deficiency is shown by alternate yellow and green stripes along the length of the leaf.

Iron toxicity is particularly a problem in flooded rice soils, since within a few weeks of flooding it may increase 500-1000 folds. Iron toxicity in rice is known as 'Bronzing'. In this disorder the leaves are first covered by tiny brown spots which develop into a uniform brown colour. This frequently occurs in rice leaves containing excessively high Fe concentrations.

In the treatment of Fe chlorosis, the addition of inorganic Fe salts to the soil is mostly without effect for the Fe is rapidly made insoluble as oxides. Foliar treatment with ferrous salts is also not satisfactory. Iron chelates are more effective and can be used as fertilisers applied to the soil or as a foliar spary.

### Manganese (Mn)

$Mn^{2+}$ and Mn oxides in which Mn is present in trivalent or tetravalent forms are the important soil fractions. In soil solutions, divalent Mn is the most important form.

$Mn^{2+}$ resembles $Mg^{2+}$ in its biochemical functions. Both ions bridge ATP with the enzyme complex. Decarboxylases and dehydrogenases of the TCA cycle are also

activated by $Mn^{2+}$. Nevertheless, $Mn^{2+}$ is not specific for these enzymes and can be substituted by $Mg^{2+}$. The most well documented role of Mn in green plants is in the Hill reaction of photosynthesis where a manganese protein catalyses water splitting and $O_2$ evolution.

Mn deficiency causes disorganisation of the lamellar system of chloroplasts. Mn deficiency symptoms resemble Mg deficiency, as in both cases interveinal chlorosis occurs in the leaves. In contrast to Mg deficiency, however, Mn deficiency symptoms are first visible in the younger leaves whereas in Mg deficiency older leaves are first affected. Organic soils high in pH are particularly low in available Mn and it is in crops growing on these soils Mn deficiency often occurs. Application of Mn salts to the soil, e.g. $MnSO_4$, usually does not alleviate the deficiency because the applied $Mn^{2+}$ is rapidly oxidised. Spraying 1 to 5 kg $MnSO_4$/ha is the best way of removing the deficiency in most crops. Of the organic Mn carriers, Mn-EDTA gives the best response.

Zinc (Zn)

In its function in some enzyme systems, $Zn^{2+}$ resembles $Mn^{2+}$ in that it brings about substrate binding and conformational changes in enzymes. A number of enzymes are thus activated in more or less the same way by $Mn^{2+}$, $Mg^{2+}$, or $Zn^{2+}$.

Zn is actively involved in the N metabolism of the plant. In Zn deficient plants, protein synthesis and protein levels are markedly lowered, and amino acids and amides accumulate. Zn deficiency affects protein metabolism through the inactivation of RNA polymerase. It affects structural integrity of ribosomes, and enhances RNA degradation by increasing RNase activity. Zn is also required for the synthesis of indole acetic acid from tryptophan.

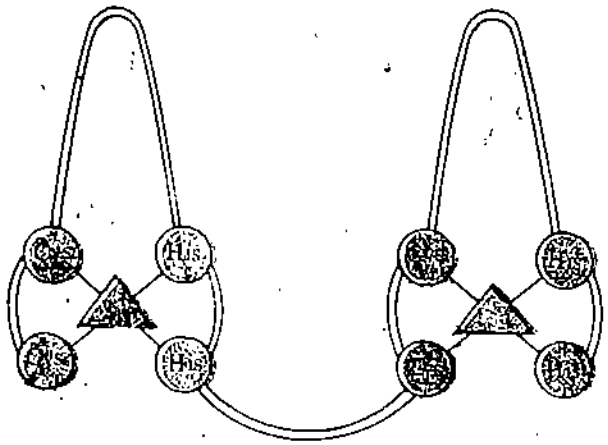



**Fig. 12.13: The zinc in each finger is bound to four amino acids: two cysteines and two histidines, holding the finger in the proper shape for DNA binding. The four key amino acids are found in the same places in each finger.**

Plants suffering from Zn-deficiency often show chlorosis in the interveinal areas of the leaves. They become pale green, yellow or even white. In fruit trees leaf development is adversely affected. Unevenly distributed clusters or rosettes of small stiff leaves are formed at the ends of the young shoots. Frequently, the shoots die off and the leaves fall prematurely. In apple trees the disease is known as 'rosette' or 'little leaf'.

Zn toxicity may occur in areas of Zn ore deposits. plant species are, however, Zn tolerant and are able to withstand high soil-Zn l. cls.

Zinc deficiency is one of the commonest micronutrient deficiencies and it is becoming increasingly important in areas of high yield agriculture. Plant species and even cultivars vary considerably in the susceptibility to Zn deficiency.

Zn-deficiency can be alleviated either by spraying or by soil application of Zn fertilisers. $ZnSO_4$ is the most commonly used fertiliser because of its high solubility. On acid sandy soils it is preferable to spray the crop because $ZnSO_4$ is very easily leached from soil.

Recently a very special role that Zinc plays in gene activation has been discovered. There are proteins which act as transcription factors. These are folded in the form of fingers with zinc liganded at the bottom of each finger. Interestingly, as soon as the fingers touch the DNA the gene is turned on.

Copper (Cu)

Copper occurs in the soil almost exclusively in its divalent form. Copper is absorbed by the plant only in very small quantities [illegible] inhibits the uptake of Cu and vice versa. It is not definitely known whether Cu is taken up as $Cu^{2+}$ or as copper chelate. Copper is not readily mobile in the plant although it can be translocated from older to younger leaves.

Copper has a number of attributes which control its biochemical behaviour. Cu bound to protein participates in redox reactions which are mostly dependent on the valency change. ($Cu^{2+} + e^- \longrightarrow Cu^+$).

The most important copper containing proteins are plastocyanin, superoxide dismutase, amine oxidases, ascorbic acid oxidase and lactase. Cytochrome oxidase, the terminal oxidase in the mitochondrial transport chain is one of the most well studied of the Cu containing enzymes. As in the case of Fe, high concentration of Cu is found in chloroplasts. There is also a specific requirement of Cu in symbiotic $N_2$-fixation. In the absence of Cu, nodule development and $N_2$-fixation are depressed.

Cu deficiency symptoms appear first in the younger leaves. In cereals, the deficiency appears first on the leaf tips. The plants develop a bushy habit with white twisted tips accompanied by a reduction in panicle formation. Copper chelates can be used either as foliar spray or as soil dressing to overcome the deficiency.

Molybdenum (Mo)

Mo is absorbed as molybdate ($MoO_4^{2-}$) ion by plants. Its uptake can be competitively reduced by $SO_4^{2-}$. The requirement of plants for Mo is very low. Mo is an essential component of two major enzymes, nitrogenase and nitrate reductase. The functional mechanism of both enzymes probably depends on valency changes of Mo.

The most important function of Mo in plant metabolism is in N assimilation. Mo deficiency resembles N-deficiency. Older leaves becoming chlorotic first, but in contrast to N-deficiency, necrotic symptoms rapidly appear at the leaf margins because of nitrate accumulation. In Cruciferae, in extreme deficiency of Mo, leaf laminae are not formed and only the mid rib is formed. The leaf thus appears like a whip and for this reason the deficiency is called 'Whip tail'.

**Boron (B)**

It is absorbed by the plants as the undissociated boric acid. Though it has convincingly been shown that B is an essential element for higher plants, its functional role is not well understood. Unlike other plant nutrients B is not known to be a component of any enzyme.

Abnormal or retarded growth of the apical growing points is the first deficiency symptom. The youngest leaves are malformed, wrinkled or are often thicker and of darkish blue-green in colour. The leaves and the stem become brittle indicating a disturbance in transpiration. As the deficiency progresses the terminal growing point dies and flower and fruit formation is restricted or inhibited. Boron is known to have a role in germination of pollen and formation of pollen tubes. Thus plants growing on boron deficient soils show a disturbance in pollen germination and impairment of in fruit formation. In some plant species, the affected growth of pollen leads to parthenogenesis. The most well-known B deficiency symptoms are 'crown' and 'heart rot' in sugarbeet.

Boron deficiency is more pronounced in a wide range of crops under wide climatic conditions than deficiencies of any other micronutrient. The most well-known B fertiliser is borax ($Na_2B_4O_4.10H_2O$). Borated super phosphates are also available. Boric acid ($H_3BO_3$) is frequently applied as leaf spray particularly when the soil is potentially capable of fixing high amounts of boron.

**Chlorine (Cl)**

The role of $Cl^-$ in plant is not clearly understood. $Cl^-$ is required in Hill reaction the water splitting reaction of photosystem II about which you will learn in Unit 13. In the presence of $Cl^-$, both the evolution of $O_2$ and photophosphorylation are enhanced. $Cl^-$ may also influence photosynthesis indirectly via its effect on stomatal regulation of the guard cells.

Wilting of leaves at the margin is a deficiency symptom of chloride. $Cl^-$ deficiency is noticed only rarely. The presence of $Cl^-$ in the atmosphere and in the rain water is more than enough to meet the demand of the crops. In fact, its presence in excess in plants is a more serious problem. Crops growing on salt affected soils often show symptoms of $Cl^-$ toxicity. These are burning of leaf tips or margins, bronzing, premature yellowing and abscission of leaves.

**Silicon (Si)**

Plant species may be divided into Si accumulators and non-accumulators. The accumulators include paddy rice (*Oryza sativa*), horse tails (*Equisetum arvense*) and members of the Pineceae, all of which contain 10-15% $SiO_2$ in the dry matter. Other cereals, sugarcane and a number of dicots with 1 to 3% $SiO_2$ are also included in this category. The non-accumulators are most of the dicots including the legumes with less than 0.5% $SiO_2$.

Necrosis of older leaves and wilting associated with higher rates of transpiration are the typical deficiency symptoms. There is little biochemical evidence to justify Si as an essential element for higher plants, however, it shows a number of well established beneficial effects on plant growth. In plants well supplied with Si, cuticular water loss is lowered because of the epidermal accumulation of silica. In cereals, the presence of silicon is important for keeping the leaves erect and decreasing susceptibility to logging. In rice, a significant relationship is observed between the Si content of the straw and the yield of rice. Silicon especially promotes the formation of reproductive organ in rice. The important silica fertilisers are soluble silicates, sinter phosphates and Ca silicate slags.

**Cobalt (Co)**

The Co concentration in the dry matter of plants grown in soil normally lies around 0.02 to 0.5 ppm. In soils the content varies from 1 to 40 ppm. Cobalt is not readily mobile in the plant.

Co is essential for symbiotic $N_2$-fixation. Increasing the supply of Co increases rhizobial growth, $N_2$-fixation and the formation of leghaemoglobin in nodules. Co is essential component of vitamin cyanocobalamin.

**SAQ 5**

a) List three molecules that contain heme iron in their prosthetic group.

.....

.....

b) i) Protein containing Mn ion catalyse ..... during photosynthesis.

ii) Zinc deficiency enhances the degradation of ..... and inactivates enzyme .....

iii) Zinc is required for the synthesis of hormone .....

iv) In cell, copper ions play a role in ..... reaction by undergoing valancy change.

v) Enzyme nitrogenase and nitrate reductase contain .....

vi) ..... is an essential element but it is not known to be a component of any plant chemical.

vii) In some plants boron deficiency leads to fruit formation by .....

viii) Chlorine is required in ..... reaction of photosynthesis.

ix) Excess of chlorine result in ..... leaf tips or margins, ..... and premature yellowing and abscission of leaves.

x) ..... and ..... are essential for symbiotic nitrogen fixation.

xi) Iron deficiency is corrected by fertiliser application foliar spray of .....

c) List three important compounds containing copper.

.....

.....

## 12.6 SUMMARY

In this unit you have learnt that :

- On an average mineral elements account for about 1.5 per cent of fresh weight of plant. Not all the elements detected may be essential for a plant. Essential elements are necessary for the completion of the plant's life-cycle.
- Besides, C, H, and O that make the backbone of organic molecules, N, P, K, Ca, S and Mg are required by plants in a relatively large amounts and are referred to as macronutrients, whereas Fe, Cu, Mn, Zn, Cl, B and Mo are required in lesser amounts and are referred to as micronutrients.
- Mineral elements perform structural, osmotic and biochemical functions in plants.
- Plants obtain carbon, hydrogen and oxygen from $CO_2$ and water Other elements are absorbed by roots from the soil in ionic form.
- The uptake of ions by roots from the soil is influenced by ion exchange capacity of the soil, pH and the presence of different cations and anions.
- Minerals move rapidly towards root interior either through intercellular spaces in root epidermis and cortex or across the selectively permeable membrane of the epidermis or cortex and join symplasm. All minerals must cross cell membrane and pass through cytoplasm, if they are to reach the xylem vessels which carry them upward alongwith water and transports them throughout the plant body.
- Ions are transported across plasma membrane with the help of proteins in the lipid bilayer. Some of the proteins such as permeases form ion channels and others function as carriers. The driving force involved in the transport is a concentration gradient or electrochemical gradient. The movement of ions against electrochemical gradient is an active transport process and requires energy in the form of ATP. Electrochemical gradient is also maintained by proton pumps which operate constantly and generate transmembrane potential across the membrane and facilitate transport of cations, anions, amino acids and sugars.
- Long distance transport of ions from root to shoot is driven by the gradient of hydrostatic pressure and water potential.
- Nitrate and ammonia are two nitrogen sources of plants. Between the two, $NO_3^-$ is the preferred source and is absorbed at low pH. Nitrate may be translocated as such or many get reduced to nitrite in the roots before translocation. The deficiency symptom — chlorosis appears first in older leaves and plants show poor growth rates.
- Phosphorus is absorbed by plants against steep concentration gradient. It is highly mobile. Its deficiency manifests by the formation of anthocyanin pigments and by the older leaves becoming darkish green.
- Potassium is required in large amounts because it maintains turgidity of cells. In its deficiency plants resistance to drought is poor.
- Sulphur is an important constituent of several compounds in plants. Its deficiency results in poor growth rates.
- Calcium is required for cell elongation and cell division. In its deficiency the permeability of membranes increases and thus causes damage to cells.
- Mg and Mn are cofactors in phosphorylation reaction. Mg is part of chlorophyll. Mn and Cl are necessary for an important reaction in photosynthesis.
- Iron, copper and Mo are constituents of proteins that take part in redox reactions. Iron and Mg both are necessary for chlorophyll synthesis, zinc for protein synthesis and Cobalt for $N_2$-fixation.

## 12.7 TERMINAL QUESTIONS

1) In what form the following elements are available to the plant from soil.
   i) Nitrogen
   ii) Phosphorus

iii) Boron
iv) Molybdenum

.....

.....

.....

2) a) The uptake of minerals is very specific. Explain the mechanism that ensures this specificity.

.....

.....

.....

.....

b) How is the uptake of ions in some cases fooled by similar ones?

.....

.....

3) List the three factors that influence the uptake of ions by the roots from soil.

.....

.....

.....

4) Why can't potassium and sodium ions cross the artificial lipid bilayer?

.....

.....

.....

5) While studying uptake of $K^+$ by plant membrane it was observed that uptake was affected by chemicals that inhibit respiration. What inference can be drawn from this observation.

.....

.....

.....

.....

## 12.8 ANSWERS

**Self-assessment Questions**

1) a) i) F, ii) T, iii) F, iv) F

b) i) Potassium
ii) Calcium
iii) Chlorophyll
iv) Iron

2) i) Negative
ii) Plasmodesmata
iii) pH
iv) Casparian strip
v) Cation exchange sites
vi) Rubidium

3) a) i) Concentration gradient
ii) ATPase
iii) Cytoplasm, Outside

b) i) $K^+$
ii) Increases
iii) Positive
iv) Acidic
v) Calcium

4) a) i) $NO_3^-$
ii) protein
iii) $NH_4^+$, $NO_3^-$
iv) decreases
v) $NO_3^-$, $NH_4^+$
vi) older
vii) $Ca^{2+}$, $K^+$
viii) xylem, cannot
ix) Mg, $K^+$
x) carbohydrate
xi) phosphorus
xii) mobile
xiii) $K^+$
xiv) turgor

b) Ferredoxin, cysteine, methionine, coenzyme A, biotin, thiamine

c) i) b, ii) a, iii) c,

5) Any three of the following

a) cytochrome oxidase, catalase, peroxidase, cytochrome f and cytochrome c.

b) i) water splitting
ii) RNA, RNA polymerase
iii) indole acetic acid
iv) oxidation — reduction reaction.
v) molybdenum
vi) boron
vii) parthenogenesis
viii) water splitting
ix) burning of, bronzing
x) Mo and Co
xi) iron chelates

c) plastocyanin, cytochrome oxidase, lactase, ascorbic acid oxidase, superoxide dismutase.

**Terminal Questions**

1) i) $NO_3^-$, $NH_4^+$, ii) $H_2PO_4^-$, $HPO_4^{2-}$, iii) $H_3BO_3$, iv) $SO_4^{2-}$

2) a) The specificity of transport of certain ions is due to carrier proteins which behave more like enzymes and the transport is similar to enzyme mediated reactions. The carrier protein has a specific site for binding an ion that is to be transported.

b) Uptake is fooled when two or more than two ions can bind at the same site of carrier protein.

3) i) Ion exchange capacity of soil
ii) pH
iii) Presence of different cations and anions.

4) Although both $K^+$ and $Na^+$ ions are small in size but the charge and hydration shell around them prevents them from entering the hydrophobic hydrocarbon phase of the lipid bilayer.

5) Inhibition of $K^+$ uptake by respiratory inhibitors shows that $K^+$ transport is linked to, and dependent on ATP produced from respiration.

# UNIT 13 PHOTOSYNTHESIS

**Structure**



## 13.1 INTRODUCTION

Our concern in this Unit will be with photosynthesis — the process by which plants utilise energy from sunlight to convert carbon dioxide and water for the synthesis of sugar. This sugar can then be converted to other carbohydrates or other food materials like fats and proteins. The general importance of the process was recognised as long ago as 2000 years. The biblical saint, Isaiah, who lived between 700-600 B.C., said *"All flesh is grass"* recognising that all food chains are finally traced to plants. Plants are also responsible for the fossil fuels such as petroleum, oil and coal, which represent products of photosynthesis carried out millions of years ago in the carboniferous era. It is through this process that plants continuously purify air during daytime and thus allow animals to breathe.

The overall importance of this process is best expressed in the words of Eugene Rabinowitch one of the great authors and researchers of photosynthesis, who said *"Physiologically speaking, all the animals on land and in the sea, including man, are but a small brood of parasites living off the great body of the plant kingdom"*, and *"if plants could express themselves, they would probably have the same low opinion of animals as we have of fleas and tapeworms — organisms that must lazily depend on others for survival."*

The photosynthetic products are utilised by humans and other animals to provide energy. He proceeded to state that *"without them no heart could beat, no amoeba could swim, no sensation could speed along a nerve, no thought could flash in the human brain"*. Clearly, for all these activities we are dependent on plants.

In sheer magnitude, too, the process dwarfs any other chemical activity on earth. It has been estimated that photosynthesis gives $200 \times 10^9$ tonnes of solid plant material per year which comes to about 70 to 80 tonnes of sugar equivalent per person! Clearly, photosynthesis represents the greatest chemical factory on earth. Unravelling the mechanism of the process has, therefore, been one of the most important tasks of plant biology.

The subject matter in this unit you may find quite unique and interesting. Here, we give in a story form an historical account of major experiments that led to the detailed knowledge of photosynthesis such as we have today. We have particularly emphasised how the various key concepts in photosynthesis were formulated.

The various sections and subsections are arranged in a chronological order. Beginning with experiments that led to the formulation of basic equation, we tell you about the light and dark reactions of photosynthesis, that is, photolysis of water and synthesis of ATP by photophosphorylation, the role of pigments in these processes and then to fixation of carbon dioxide by the $C_3$ and $C_4$ pathways. The intricate details of photosynthetic machinery which have been unravelled partly in the last two decades are discussed later.

A section on photorespiration, and the relevance of photosynthesis to agriculture and human welfare is also included. Finally, we briefly discuss the evolutionary aspects of the origin of chloroplast.

### Objectives

After studying this unit you should be able to :

- outline the scientific developments that led to recognition of the necessary raw materials of photosynthesis and the important end products,
- list the main photosynthetic pigments and describe their functions,
- list the evidences that led first to the discovery of light and dark reactions and later to the two photochemical reactions,
- outline the path of electrons in electron transport chain from water to the final electron acceptor,
- outline the $C_3$ or the Calvin cycle and illustrate its connection with the energy capturing reactions in the thylakoid membrane,
- draw and label the structure of a chloroplast and its membranes and show the sites where PS I and II and the components of electron transport chain are located and various processes such as photolysis of water, photoreduction of $NADP^+$, photophosphorylation, and carbon dioxide fixation go on,
- outline the reactions that result in the loss of carbon dioxide during photorespiration,
- compare the fixation of $CO_2$ in $C_3$, $C_4$ and CAM plants and explain why $C_4$ plants are photosynthetically more efficient than $C_3$ plants,
- gain an idea of the future prospects of increasing photosynthetic efficiency through biotechnology,
- give reasons for considering the present day chloroplast as one time free-living prokaryote which became an endosymbiont during the course of evolution.

**Study Guide**

Since this is a double unit, you will find it very lengthy. It is important that you spend more time in learning it.

## 13.2 FORMULATION OF BASIC CONCEPTS

### 13.2.1 The Beginnings

We can trace the beginnings of research on photosynthesis to about 300 years ago. The idea that water is an important reactant came from experiments of a Dutch alchemist, Van Helmont, performed in 1648 but published posthumously in 1740, under the title "*By Experiments, that All Vegetable Matter is Totally and Materially of Water Alone*". He grew a 5 lb sapling of the willow tree (*Salix*) in an earthenware pot containing soil which he carefully dried and weighed — it was 200 lbs. He watered the sapling regularly with distilled water, if rain failed, and at the end of five years decided to take stock of the experiment. He found that the weight of the tree increased to about 169 lbs, but the weight of the soil was nearly the same, decreasing by only 2 ounces (Fig. 13.1). He concluded, therefore, 164 lbs of wood, bark and roots were formed from water **alone**. Of course, we know today that Van Helmont was only partially right and was totally unaware of the contribution of carbon dioxide.

Fig. 13.1 : Van Helmont's experiment in which it was concluded that a plant grows from water alone (see text for further details).

The knowledge that gases also participate in the process of photosynthesis came from studies of an English clergyman Joseph Priestley, who was intensely interested in the process by which bad air could be purified. In a contribution entitled *"Observations on Different Kinds of Air"* he wrote that *"I have been so happy as by accident to hit upon a method of restoring air which has been injured by the burning of candles, and that to have discovered at least one of the restoratives which nature employs for this purpose. It is vegetation"*. He had found that animals such as mice, vitiated the common air and a candle no longer burnt in it (Fig. 13.2). He conducted a series of experiments in 1771 and showed that plants had the remarkable ability of turning impure air into pure air. In his own words. *"Accordingly, on the 17th of August, 1771, I put a sprig of mint into a quantity of air, in which a wax candle had burned out, and found that, on the 27th of the same month, another candle burned perfectly well in it"*. He added *"I generally found that five or six days were sufficient to restore this air, when the plant was in its vigour"*. At that time, chemists were obsessed with the idea of phlogiston, then considered a principle of flammability. According to Priestley, plants dephlogisticated the foul air. Further, the pure air had properties similar to the gas which he had discovered and was released by focusing sunrays on the red oxide of mercury with the help of a huge lens — he had procured one almost a foot in diameter.

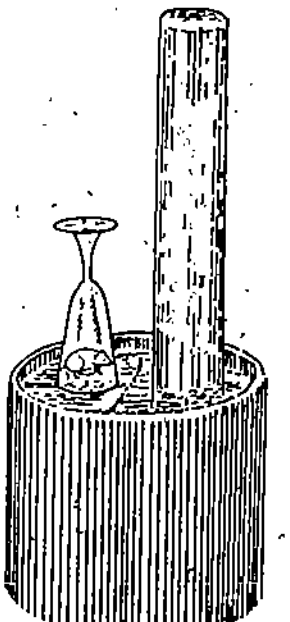


Fig. 13.2 : Priestley grew small twigs of mint in an inverted tube and piped air to a jar containing mouse. By such experiments, he proved that plants have the capacity of purifying air.

Priestley's experiments excited the interest of Jan Ingen-Housz in Vienna who was a court physician to Empress Maria Theresa of Austria. In 1778, on a visit to England for a three-month vacation he rented a villa and conducted some 500 experiments. He confirmed that not only mint but even other plants purified air; but, more importantly, he found that the process will proceed only in the presence of sunlight and plants could purify air significantly even in a few hours. To quote from a book *"Experiments on Vegetables Discovering Their Great Power of Purifying the Common Air in Sunshine, and of Injuring at Night"* he said *"I was not long engaged in this enquiry before I saw a most important scene opened to my view: I observed, that plants not only have a faculty to correct bad air in six or ten days, by growing in it; as the experiments of Dr. Priestley indicate, but that they perform this important office in a compleat (sic) manner in a few hours; that this wonderful operation is by no means owing to the vegetation of the plant, but to the influence of the light of the sun upon the plant"*. He also found that only the green parts of the plant purified air and not the non-green parts and that so long as the plants were green, the *"acrid, ill-scented, and even the most poisonous plants perform this office in common with the mildness and the most salutary"*.

Phlogiston : The alchemists thought that when metals rust or a candle burns something was lost. This something they called phlogiston. Today, we know that actually nothing is lost and, in fact, a burning substance unites with oxygen. Similarly, rusted metal combines with oxygen and actually weighs more than the pure metals.

Neither Priestley nor Ingen-Housz knew the true chemical nature either of impure or of pure air and it was the brilliant French chemist Antoine Lavoisier who discovered the principle of combustion and identified the "pure" component of air as oxygen ($O_2$) and the "impure" air as carbon dioxide ($CO_2$). (Unfortunately, Lavoisier was guillotined by terrorists during the French revolution but that is the way fate overcomes occasionally the best of men). Another important advance was made by a Genevan, Jean Senebier, who found that the quantity of pure air ($O_2$) generated depended on the presence of noxious or vitiated air ($CO_2$) at the start of an experiment.

**SAQ 1**

a) Match the experimental findings related to photosynthesis (given in column 1) with the names of scientists (given in column 2) who were responsible for the findings.

| | Column 1 | | Column 2 |
|---|---|---|---|
| i) | A sprig of mint can purify air injured by breathing of animals | a) | Antoine Lavoisier |
| ii) | Plants are made of water alone | b) | Jan Ingen-Housz |
| iii) | All kinds of plant purify bad air, but light is necessary for such purification | c) | Van Helmont |
| iv) | Identified pure air as $O_2$ and impure air $CO_2$ | d) | Joseph Priestley |

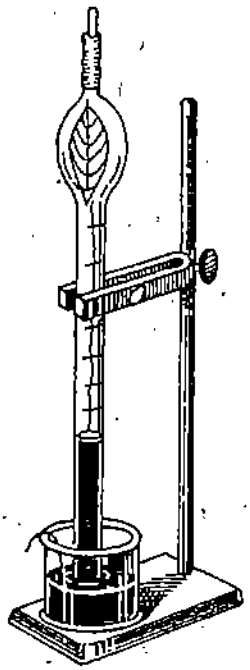


Fig. 13.3: An apparatus for determining gas exchange during the process of photosynthesis. This type of set-up was employed by Nicolas Theodore de Saussure. A leaf or other plant parts can be put in the area of the bulb with a suitable support. The composition of the air can be determined by use of an alkali water and pyrogallol at the end of the experiment.

## 13.2.2 Formulation of the Equation of Photosynthesis

By 1804, methods of quantitative measurements of gases were well established. By the use of an eudiometer (Fig. 13.3) followed by simple methods of gas analysis Nicolas Theodore de Saussure, also a Genevan, confirmed the equivalence of release of $O_2$ to consumption of $CO_2$ in the process of photosynthesis. But much more important, he once again drew attention to the role of water, an ingredient whose participation in photosynthesis had been totally ignored after Van Helmont. In 1837 chloroplasts were also described. By the end of the last century, the stage was thus set for formulating the following equation of photosynthesis:

$$6CO_2 + 6H_2O \xrightarrow[\text{green colouring matter (chlorophyll pigments)}]{\text{sunlight}} C_6H_{12}O_6 + 6O_2$$

Another very important development which took place at the end of the last century was the determination of the action spectrum of photosynthesis. Earlier, the use of spectroscopy easily established that chlorophyll absorbed strongly in the blue and red regions of the spectrum (it had hardly any absorption in the green region, explaining why plants appear green). However, there was one uncertainty, although researchers of that time associated, "the green colouring matter" with the process of photosynthesis, there was no conclusive evidence that chloroplasts were the site of photosynthesis and the pigments in them participated in this important reaction. The German botanist Theodore Engelmann (1882) determined the action spectrum of photosynthesis and showed that it indeed closely matched the absorption spectrum of the chlorophyll pigments.

Absorption spectrum : A graph depicting absorption as a function of wavelength is called absorption spectrum. The absorption spectra of chl *a* and chl *b* indicate that very little of green and yellow green light between 500 to 600 nm are absorbed but violet, blue, orange and red wavelengths are absorbed strongly.

By way of explanation, it can be said that the **absorption spectrum** is an optical property of a solution. With the help of a spectroscope one learns about the wavelengths absorbed by a plant extract or a solution such as of chloroplast pigments in acetone. Now with a modern spectrophotometer one can even know the degree to which they are absorbed. An **action spectrum**, on the other hand, tells us about the **relative activity** of a physiological process in different parts of the spectrum (to obtain an action spectrum one must illuminate the living cell, tissue or the organism with monochromatic light in different regions of the spectrum). Obviously, to associate a photoreceptor convincingly — to a certain process or action, the two spectra must match (Fig. 13.4).

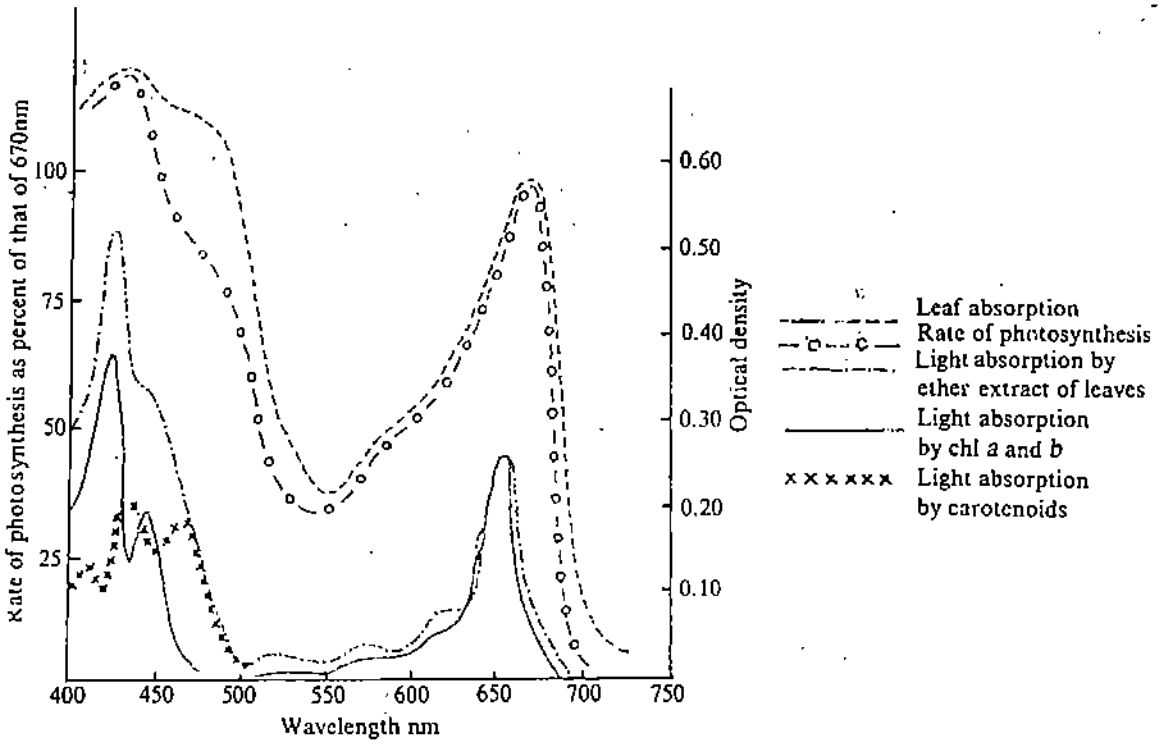



Fig. 13.4 : Comparison of action spectrum for the rate of photosynthesis by *Elodea densa* leaf with absorption spectra of the intact leaf and with extracted pigments. (Adapted from G. Ray Noggle and George V. Fritz, Plant physiology, 1989, Prentice-Hall of India)

Engelmann's experiments — which are among the most ingenious ever devised — were carried out with filamentous algae such as *Cladophora*. The algal filament was laid on a slide on the stage of a microscope. In the optical path of the microscope a prism was inserted such that a small spectrum illuminated the algal filament and the spectrum could be seen readily through the eyepiece by the viewer. To determine which wavelengths of light were effective in evolving oxygen, Engelmann made use of a species of highly aerobic motile bacteria. A drop of the bacterial suspension was introduced over the algal filament, whereupon the bacteria clustered around those regions of the algal filament which received the red and blue light (see Fig. 13.5).

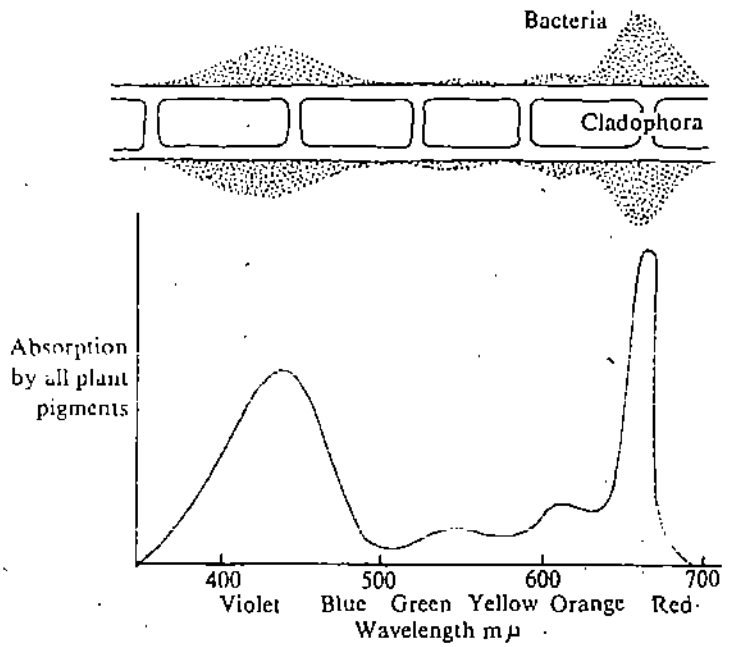



Fig. 13.5 : Engelmann's experiment to determine the wavelengths of light that are most effective for photosynthesis (see text for further details). On the lower side is given an absorption spectrum of all plant pigments extracted in a solvent. (Adapted from John W. Kimball, Biology, Addison-Wesley, 1965)

Thus, the density of distribution of bacteria provided an idea of the relative effectiveness of various spectral regions of visible light, depicting the action spectrum.

To summarise, by the end of the 19th century it was clear that water and carbon dioxide were converted to sugar and oxygen with the help of chlorophyll pigments and light. Meanwhile, in 1842, Julius Mayer, a surgeon in Germany, also formulated the "Law of Conservation of Energy" and expounded the theme that photosynthesis, in the main, represented a process in which physical energy was conserved as chemical energy. He wrote in 1845, *"Nature has put itself the problem how to catch in flight light streaming to the earth and to store the most elusive of all powers in rigid form. To achieve this aim, it has covered the crust of earth with organisms which in their life processes absorb the light of the sun and use this power to produce a continuously accumulating chemical difference .... . These organisms are the plants; the plant kingdom forms a reservoir in which the fleeting sun rays are fixed and skillfully stored for future use; an economic provision to which the physical existence of mankind is inexorably bound. The plants take in one form of power, light; and produce another power : chemical difference".*

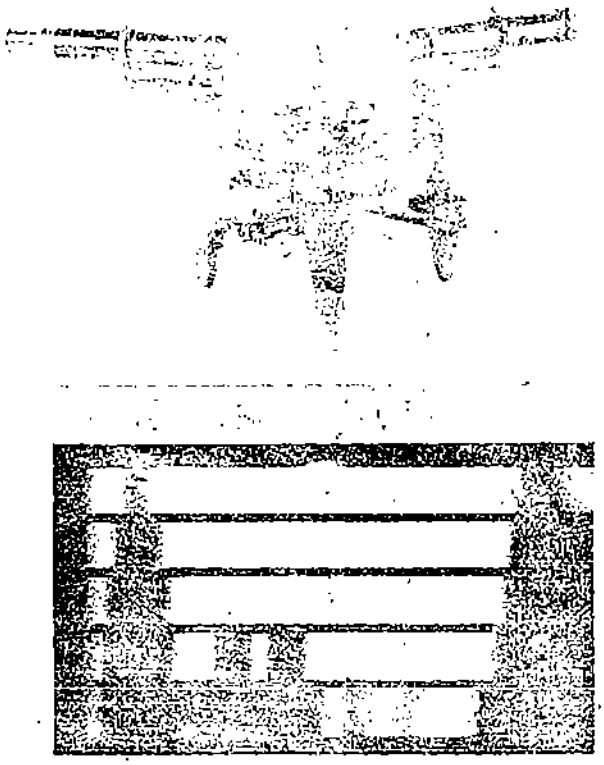


Fig : a): A spectroscope. b): Absorption spectra of five different concentrations of chlorophyll *a* The wavelengths in $m\mu$ are indicated above.

### Spectroscope

The figure above (a) illustrates a spectroscope. Light is passed through a slit at one end and a spectrum emerges at the other end. If a solution containing photosynthetic pigments is interposed between the viewer and the light, dark bands are seen — in the blue and red regions of the spectrum in case of chlorophyll pigments and the blue and violet regions in case of carotene and xanthophyll. If the concentration of chlorophyll pigment is high some absorption can also be seen in the other regions. If photographs are taken of the absorption spectra at various dilutions and placed one below the other, one can determine absorption peaks more exactly. Absorption spectra of this type were obtained by the German chemist, Willstätter as seen in Fig. b. They are similar to the absorption spectra which are obtained by a modern spectrophotometer (which essentially represents a spectroscope fitted with accessories such that the emerging light can be measured electrically by having it fall on a photocell or a photomultiplier). For obtaining an absorption spectrum, the prism has to be rotated to allow scanning of absorption values from the blue end of the spectrum to the red end. The slow rotation can be accomplished by a motor.

## Measurement of Photosynthesis

The rate of photosynthesis can be measured in many different ways and, with time, more and more sophisticated techniques have been developed so that results can be had almost instantly or at the most within a few seconds.

Fig. a represents the simplest method of measuring photosynthesis which can be employed for aquatic plants. Oxygen bubbles, escaping from cut stems or

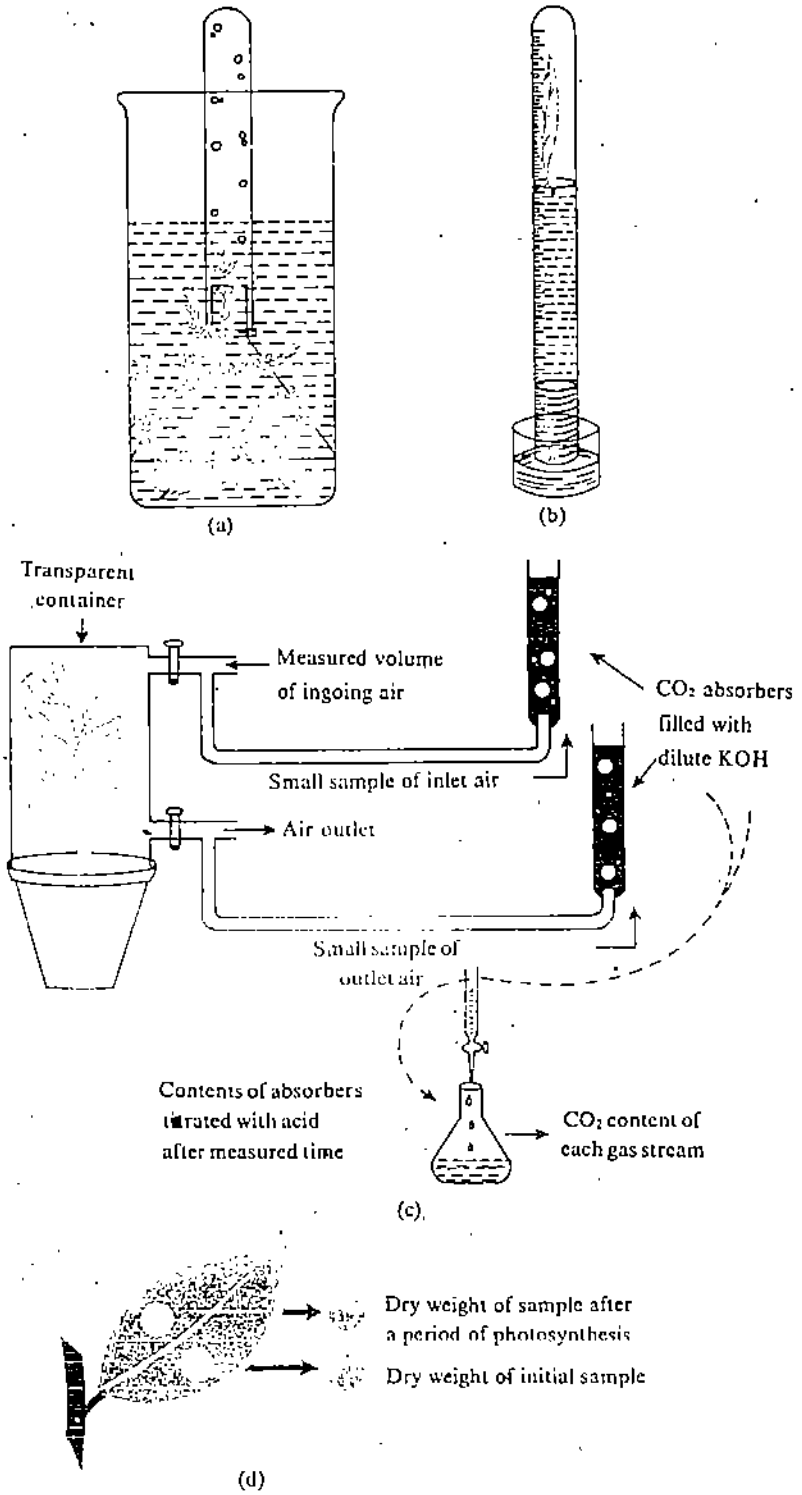
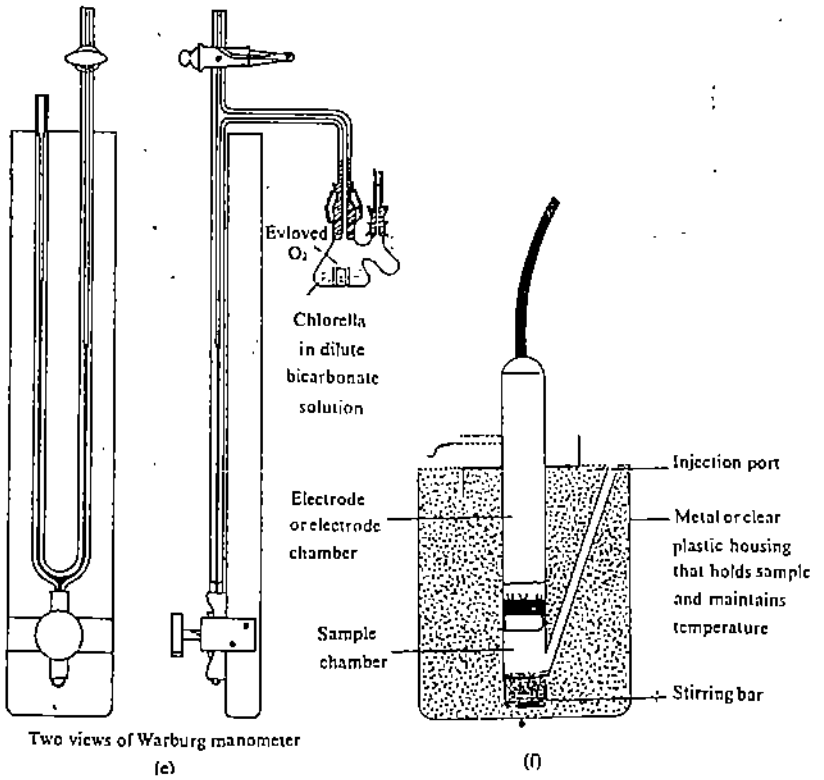



Fig a): Collection of oxygen from water plants in light.
b): Leaf in position in a measuring tube, for demonstration of absorption of carbon dioxide and elimination of oxygen during photosynthesis.
c): Determinations of photosynthetic rate, based on measurement of $CO_2$ uptake from a moving gas stream, depend on analyses of the $CO_2$ content of small samples of the inlet and outlet air.
d): The net photosynthetic rate may be roughly determined from the dry weight increase of a measured area of leaf surface during a period of $CO_2$ assimilation.

other parts of a plant can be collected in an inverted test tube. For more precision, the composition of the gas can be checked by chemical methods of analysis such as through use of pyrogallol or KOH. Fig. b represents another technique adopted by early workers for determining the rate of photosynthesis in leaves or other similar organs of land plants which can be enclosed in a graduated tube. The tube is inverted over mercury and gas exchange after a period of time determined by chemical methods of analysis. Fig. c depicts the gas train method. This method came into wide use in the early part of the century (this was employed by Blackman for determining the $Q_{10}$ of the photosynthesis reaction). The air is led into a chamber containing the plant material and the amount of carbon dioxide assimilated from the air can be measured by titrating the carbon dioxide absorbing solution i.e. the alkali (e.g. barium hydroxide) with a standard solution of acid after a measured time. A blank run without plant material gives the quantity of carbon dioxide in air sample; but when there is an experimental run, less $BaCO_3$ is precipitated and the difference in titration values in the control and experimental runs gives us a measure of photosynthesis.

Another method popular in the early days was to take samples of leaf material as shown in Fig. d and determine their dry weights — at start and after a period of photosynthesis. The difference in weights gives us a measure of photosynthesis. It is important to realise that all these methods suffer from the drawback that what one measures is net photosynthesis, since respiration also takes place simultaneously and losses of carbon fixed do occur. In order to estimate the real rate of photosynthesis, the rate of respiration should also be measured in darkness and appropriate corrections made.




e): The Warburg manometer and reaction vessel. f) Features of a typical oxygen electrode system.

In the manometric technique, developed by Warburg in 1920s and which remained extremely popular till the sixties, the volume of oxygen evolved is measured directly by reading against a graduated scale attached to the manometer (Fig. e). The technique is specially suited for algae or other small samples of green tissue which are suspended in a dilute solution of sodium bicarbonate (which gives $HCO_3^-$ or $CO_2$). Lowering the level of the manometric fluid in the closed arm is due to oxygen evolution and consequent rise in pressure. A high degree of sensitivity in estimation of oxygen output can be obtained by employing a thin-diameter glass tubing for fabrication of the manometer. The reaction flask is always kept submerged in constant temperature water bath to maintain constant conditions and up to 14

manometers can be used simultaneously in a single apparatus. However, one of the manometers is left without any plant material so that it can serve as a barometer and appropriate corrections can be made for the estimation of the true volume of gas exchange.

The most recent method, however which has come into wide use is the oxygen electrode technique (Fig. f). The oxygen electrode is sensitive to concentration of oxygen and the output of oxygen can be measured electrically.

**SAQ 2**

a) Describe the experiment which led to the discovery of:

i) the role of chlorophyll in photosynthesis.

.....

.....

.....

.....

ii) the wavelengths of visible spectrum that are effective in photosynthesis.

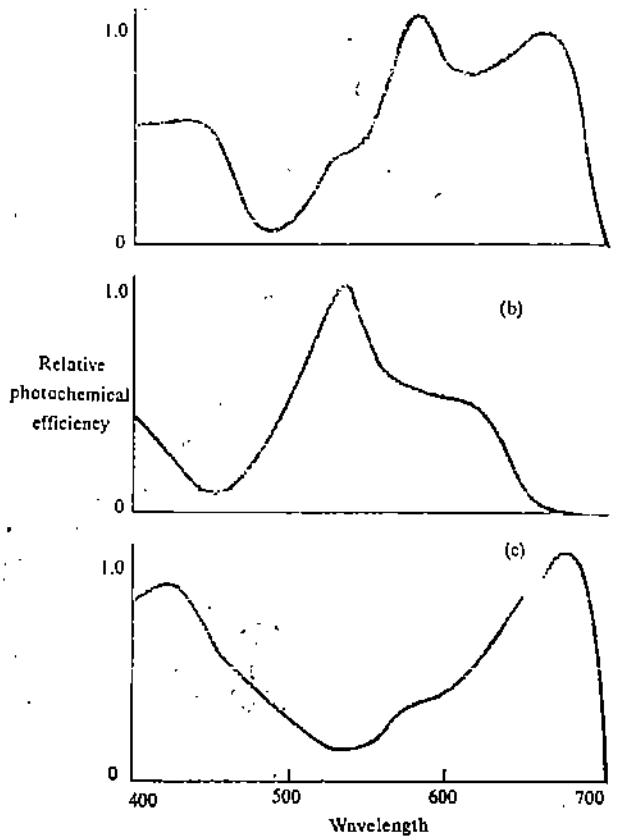
.....

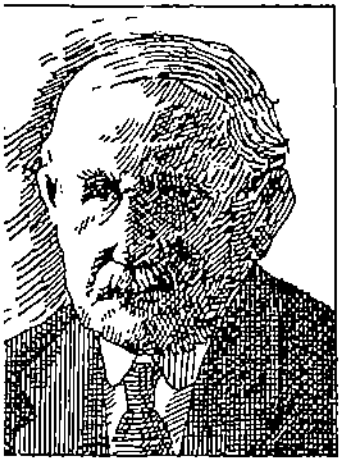
.....

.....

.....

b) The following three action spectra relate to photosynthesis in blue green algae, purple sulphur bacteria and barley leaves. Identify the spectrum that most likely corresponds to a particular organism.





F.F. Blackman

## 13.3 UNDERSTANDING THE MECHANISM OF PHOTOSYNTHESIS

### 13.3.1 Evidence for the Existence of Light and Dark Reactions

As discussed above, the process of photosynthesis was known in its bare outline already at the beginning of this century. But the phenomenon was still much like a mysterious black box and scientists had little idea of the events that proceeded inside the box. Unfortunately, the box could also not be opened then since photosynthesis ceased immediately if one broke up the cell or tissue where photosynthesis was going on. For a long time there was no clue, till at the turn of the century, an English plant physiologist, F.F. Blackman, working in Cambridge, started his experiments. He was the first to give the idea that photosynthesis consists of at least two kinds of reactions — the light and dark reaction(s) (Fig. 13.6). He estimated $Q_{10}$ of the photosynthetic reaction and found it about 2.5, provided that photosynthesis was studied under optimal conditions, specially of adequate light and supply of carbon dioxide. That

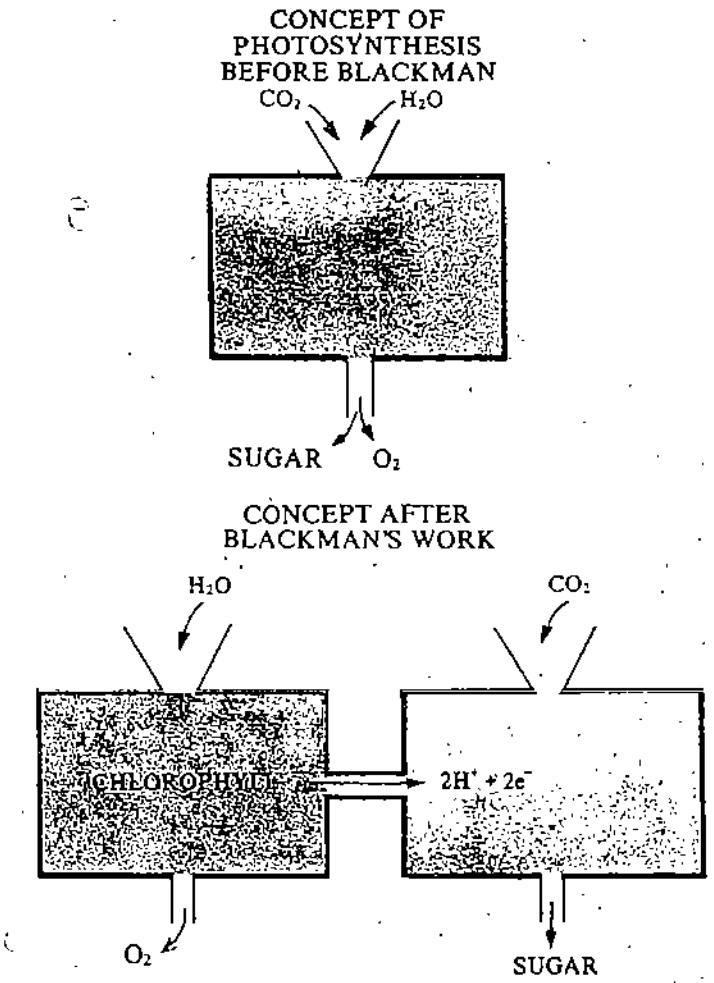



Fig. 13.6 : Diagram to show evolution of concepts concerning the mechanism of photosynthesis. The box above represents the concept at the end of the last century. After Blackman conducted the experiments to determine the $Q_{10}$ of the photosynthetic reaction (see text for further details), it was concluded that the reactions in the "black magic box" consisted at least of two sub-sets of reactions, the light and the dark reactions. The actual roles, that in the light box there is photolysis of water and in the dark box there is reduction of carbon dioxide was established much later.

photosynthesis involved some photochemical reaction(s) was of course obvious because of the necessity of light and chlorophyll pigments. However, the derivation of $Q_{10}$ value gave a unique insight into the complexity of the reactions. The $Q_{10}$ of photochemical reaction is 1, whereas for chemical reactions it is generally 2-3. By a simple study of the effect of temperature and analysis of results indicated that photosynthesis consists also of chemical reaction(s) often called 'dark' reactions since they do not need light. To put in other words, one learnt immediately that instead

**$Q_{10}$**

For chemical and biochemical reactions it is commonly observed that increase of temperature by 10°C, increases the rate of thermal reaction by twofold. The minimum energy which the reactants must possess for a reaction to proceed is called activation energy. The increase in temperature increases molecular collisions and so the proportion of molecules possessing activation energy for reaction to occur increases. Hence, the reaction rate increases.

The ratio of the rates of reactions at t°C and (t + 10°C) is denoted by $Q_{10}$.

$$Q_{10} = \frac{\text{Reaction rate at } (t + 10)°C}{\text{Reaction rate at } t°C}$$

$$= 2$$

(Q denotes Quotient and 10 stands for 10°C rise in temperature.)

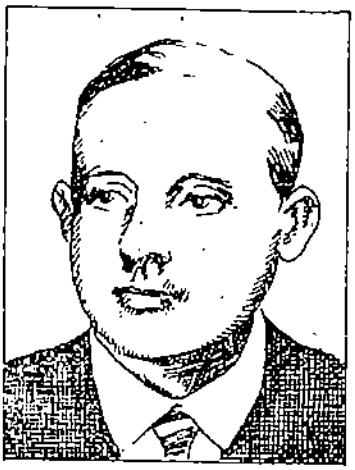
For physical processes, the $Q_{10}$ is in the range of 1.2 to 1.4. Enzymatic and physiological processes have $Q_{10}$, 2 to 3.

The rate of photochemical reaction is not affected by temperature increase because the activation energy to substrate is supplied by photons of required energy and the energy for bond breaking process is also given by photons. Photochemical reactions have a $Q_{10}$ of 1.

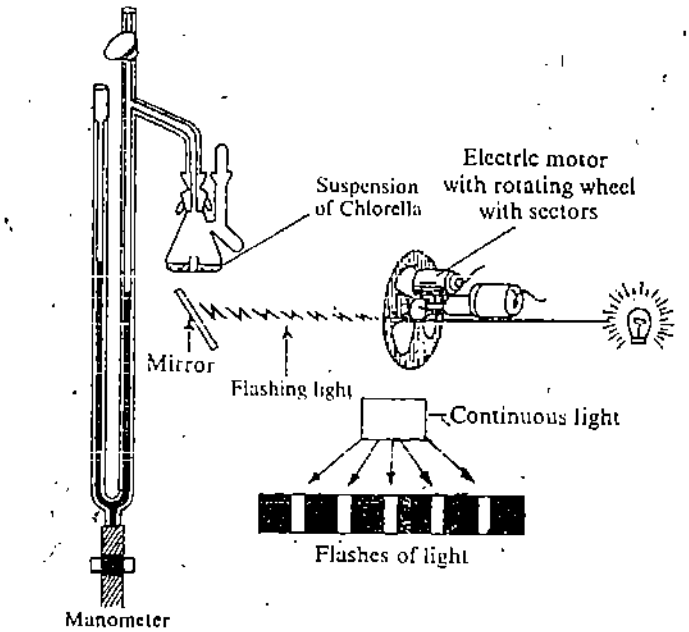
of the single magic box (alluded to earlier and representing the photosynthetic process), there were really two boxes (Fig. 13.6):

1) One box contained the chlorophyll pigments on which light shone and which represented the light reactions, and
2) the other was a "dark" box which represented the dark reactions.

The existence of dark and light reactions was further confirmed when the German Nobel laureate, Otto Warburg, and later his American pupils, Robert Emerson and William Arnold — all of them with a deep understanding of biochemistry, biophysics and cell biology — carried out experiments with alternating flashes of light and darkness given to the unicellular green alga, *Chlorella*. A mechanical device, consisting of an electric motor to which was attached a rotating disc with sectors cut in different sizes (see Fig. 13.7) was set in the light path. With this they could obtain intermittent light and darkness. In later experiments the mechanical flashing device was replaced by electronic flash discharges. It was shown that, if light and dark periods were separated by appropriate intervals, the oxygen yield per unit light shone increased by as much as 400% over the control where the same amount of light was given continuously. Thus, it was confirmed that photosynthesis consisted of *both* light



Otto Warburg




Robert Emerson

Fig. 13.7 : The experimental set-up employed for the flashing light experiments (see text for further details).

Cornelius B. Van Niel

and dark reactions. By experiments with different durations of light and darkness, it was found further that dark reactions were much slower than light reactions and that was the reason why light given continuously was utilised less efficiently.

## 13.3.2 The Role of Light Reaction

**Photolysis of Water**

The identities and roles of light and dark reactions were not clear until this time. In 1930s a dutch microbiologist, Cornelius B. Van Niel, began experiments on the photosynthesis of green sulphur bacteria. These contain bacteriochlorophyll (a slightly different form of chlorophyll) and survive on hydrogen sulphide ($H_2S$) but release elemental sulphur (in nature wherever these bacteria grow there are picturesque deposits of sulphur). He found that photosynthesis in these organisms followed the reaction as per the equation given below:

$$6CO_2 + 12H_2S \xrightarrow[\text{bacteriochlorophyll}]{\text{light}} C_6H_{12}O_6 + 6H_2O + 12S$$

The equation indicated that the primary role of $H_2S$ was that of a reductant to supply electrons. By simple analogy, he reasoned that $H_2O$, too, in case of higher plants, must serve as reductant and the $O_2$ evolved in photosynthesis must come entirely from $H_2O$ rather than from both $H_2O$ and $CO_2$ or $CO_2$ alone, thus clearly contradicting the ideas that existed before. In fact, he predicted that the equation for photosynthesis of higher plants would be:

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

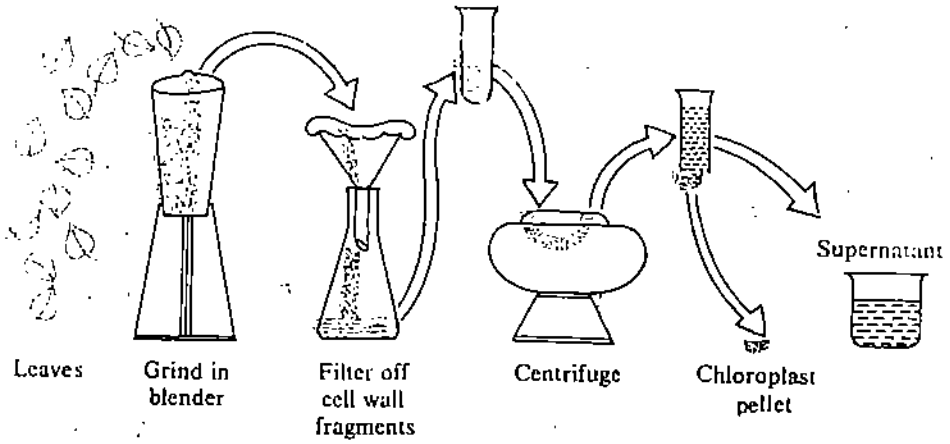
Equally important, he also proposed on simple, purely thermodynamic consideration that the role of the light reaction would be to split $H_2O$ (a process for which he coined the term "photolysis") since this was the step that would require the maximum input of energy. The role of $H_2O$ or $H_2S$, thus could be depicted by the following equation:

$$H_2S \longrightarrow 2H^+ + 2e^- + S$$
$$H_2O \longrightarrow 2H^+ + 2e^- + \tfrac{1}{2}O_2$$

The proof that $O_2$ is indeed evolved from $H_2O$ came soon after from experiments of two American chemists, Samuel Ruben and Martin D. Kamen, who worked for the U.S. Atomic Energy Commission. These workers used water labelled with $O^{18}$, the heavy isotope of oxygen. Employing a mass spectrometer for analysis they showed that — if $H_2O^{18}$ was used — the oxygen evolved was largely $O^{18}$ rather than $O^{16}$, confirming thereby that the new equation for photosynthesis formulated by Van Niel was the correct one. Photosynthesis had now to be viewed as a chemical process in which the central reaction was a "redox" reaction (i.e. consisting of a reduction and another an oxidation event). It involves transfer of hydrogen or electrons from a suitable raw material such as $H_2S$ or $H_2O$ to $CO_2$. Overall, a reductant such as $H_2O$ or $H_2S$ was oxidised and an acceptor such as $CO_2$ was reduced.

**Photoreduction — Production of Reducing Power — NADPH**

The work with the heavy isotope of oxygen ($O^{18}$) was followed by another significant discovery. The English biochemist Robin Hill working in Cambridge, U.K., found that even isolated cell organelles could evolve $O_2$ from $H_2O$, if they were provided with light and a suitable electron acceptor such as ferroxalate or ferricyanide (Fig. 13.8). Later workers have shown that many other electron donors such as benzoquinone and 2,6-dichlorophenol indophenol can also be used (see reaction below the Figure). The general reaction is now called Hill reaction in honour of its discoverer. Since $O_2$ evolved by isolated chloroplast was rather in small quantities, Hill adopted a clever strategy : he put some reduced haemoglobin in the chloroplast suspension which was readily oxidised by the $O_2$ released and which he monitored by a spectroscope. Clearly, by the end of the first half of this century, the basic role of at least one of the two "boxes", the box in which electrons are produced from $H_2O$ for reducing $CO_2$, was established beyond doubt




$$2H_2O + 2O{=}\langle\bigcirc\rangle{=}O \xrightarrow[\text{Isolated chloroplasts}]{\text{Light}} 2HO{-}\langle\bigcirc\rangle{-}OH + O_2$$

Benzoquinone — Hydroquinone

**Fig. 13.8 : A common procedure for the isolation of chloroplasts from leaves for demonstrating Hill's reaction given in the lower part of the figure.**

The role of the other box was clarified by the American biochemists Wolf Vishniac and Severo Ochoa. It was shown that photolysis of $H_2O$ by illuminated chloroplasts led to the reduction of TPN to $TPNH_2$ (now referred to as $NADPH_2$) which could be coupled directly to fixation of $CO_2$ by reactions such as carboxylation of pyruvic acid to malic acid—as shown per equations below. Earlier in the thirties and forties, parallel work on respiration of animal cells and yeast had firmly established the role in living cells of $NAD^+$ and $NADP^+$ as electron acceptors and NADH and NADPH as donors. The moral of the new experiment was that fixation of $CO_2$, by itself, required no light: indeed, parallel work in various laboratories showed that if reduced $NADP^+$ was provided, fixation of carbon into organic acids could be carried out even by extracts of animal cells.

(i) $$\left.\begin{aligned} H_2O &\longrightarrow 2H^+ + 2e^- + \tfrac{1}{2}O_2 \\ NADP^+ + 2H^+ &\longrightarrow NADPH + H^+ \end{aligned}\right\}$$

(ii) $$\begin{array}{l} \text{COOH} \\ \text{C=O} \\ \text{CH}_3 \\ \text{Pyruvic acid} \end{array} + CO_2 + NADPH + H^+ \longrightarrow \begin{array}{l} \text{COOH} \\ \text{CH}_2 \\ \text{CHOH} \\ \text{COOH} \\ \text{malic acid} \end{array} + NADP^+$$

Dark reactions

or to put more generally

$$CO_2 + NADPH + H^+ \longrightarrow [CH_2O] + NADP^+$$

The actual acceptor of $CO_2$, as you may know is a phosphorylated pentose sugar, ribulose bisphosphate (RuBP), but this matter will be discussed in more detail later.

**Photophosphorylation — Production of ATP**

We have just learnt that one major role of light in photosynthesis is to produce reducing power in the form of NADPH. However, the photosynthetic process also requires considerable energy in the form of ATP molecules

Daniel I. Arnon, working at the University of California, Berkeley made an outstanding discovery in the fifties. By using radioactive phosphate, labelled with $P^{32}$ he showed that isolated chloroplasts when exposed to light could synthesise ATP even without oxygen.

$$ADP + Pi \xrightarrow[\text{chloroplasts}]{\text{light}} ATP$$

The process is called photosynthetic phosphorylation or **photophosphorylation.** The discovery made good sense, since photosynthesis is generally 10-20 times faster than respiration. Obviously, the requirement for ATP in dark reactions of photosynthesis (for example in phosphorylating ribulose to RuBP) could not be met by the ATP supplied by respiration via the ordinary pathway of oxidative phosphorylation. By this time a general scheme for primary and secondary processes as shown in Fig. 13.9 was formulated.

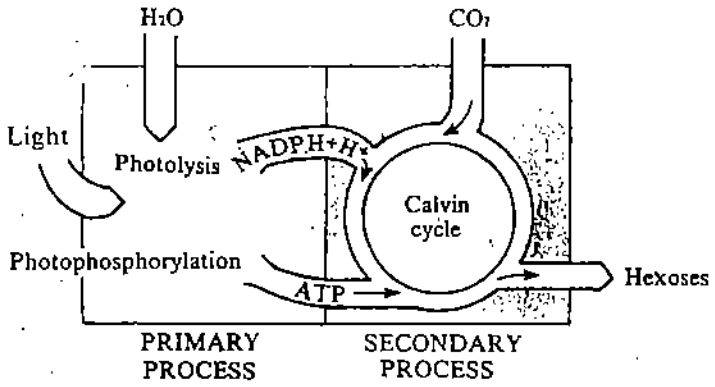



**Fig. 13.9 : A scheme of primary and secondary processes of photosynthesis. Through light reactions, not only electrons are transferred from water to $NADP^+$ to make NADPH, but ATP is also synthesised. Both NADPH and ATP are then used for driving the Calvin cycle through which $CO_2$ is fixed.**

SAQ 3

a) i) Which of the experimental findings in photosynthesis gave the clue that the process consists of both light and dark reactions?

.....

ii) What exactly is the role of the photochemical reaction?

.....

.....

b) Fill in the blanks in the following statements with appropriate words

i) In photosynthesis the $O_2$ is released because of ..... This was reasoned by ..... who observed analogous splitting of $H_2S$ in photosynthetic ..... bacteria.

ii) Light splits ..... water molecules by using an isotope of $H_2O$ containing .....

iii) The action of light leads finally to reduction of ..... to ..... as also synthesis of ..... from ..... and .....

iv) Reduction of an electron acceptor and evolution of $O_2$ by isolated chloroplast on illumination is called .....

## 13.4 CHEMISTRY OF CHLOROPLAST PIGMENTS

Before we proceed to the more modern ideas of the mechanism of photosynthesis it is necessary for us to understand some other fundamentals, for example, the chemistry and role of photosynthetic pigments. Anyone could infer from yellowing leaves that they have more than one pigment. Mikhail Tswett, a Russian botanist, at the beginning of the century, separated the chloroplast pigments by chromatography — a technique he developed — and showed that leaves have four types of photosynthetic pigments :

| Pigment | Colour | Range of Maximal Absorption | |
|---|---|---|---|
| | | Blue Region | Red Region |
| 1) Chloophyll *a* | Blue green | (400-500 nm) Peak at 430 nm | (600-700 nm) Peak at 670 nm |
| 2) Chlorophyll *b* | Green | (400-500 nm) Peak at 470 nm | (600-700 nm) Peak at 650 nm |
| 3) Carotenoids | Yellow or Orange | (400-500 nm) Peak at 450 nm | |

Note : Absorption peak changes with the solvent used.

The carotenes and xanthophylls are basically hydrocarbons with two aromatic rings at the ends joined together with a long polyene aliphatic chain — because of their structure they are highly non-polar or hydrophobic (Fig. 13.10a). On the other hand, the chlorophylls are comparatively less hydrophobic and more polar on account of the existence of a porphyrin "head" and charged N atoms. The "head" or the "flag" is borne on a long hydrocarbon "tail" or "pole" of an aliphatic alcohol (Fig. 13.10b). The difference in chl *a* and chl *b* is minor in nature — in chl *b*, a methyl group in the porphyrin "head" is replaced by $-CHO$, an aldehyde group. In the centre of the porphyrin ring there is a Mg ion. The haem of haemoglobin has similar structure, but there the metal ion in the porphyrin ring is $Fe^{3+}/Fe^{2+}$ rather than $Mg^{2+}$.

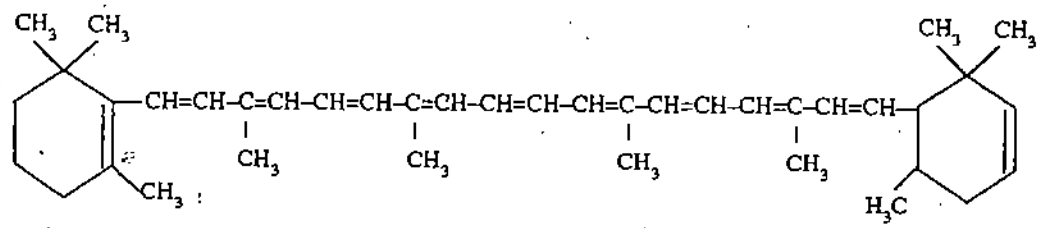


Fig. 13.10 b) : Structure of carotene.

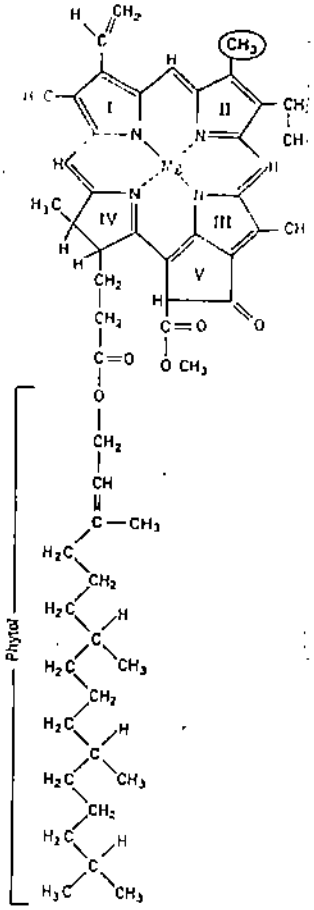


Fig. 13.10 a) : Structure of chlorophyll molecule. In chl *a*, the circled methyl group is replaced by an aldehyde group (CHO) in chlorophyll *b* as shown here.

A unique feature of both carotenoids and chlorophylls is the presence of a system of alternating double bonds with resonating electrons which are rather easily excited by photons of the visible light, specially at the blue and red ends (Fig. 13.4). In fact, the chloroplast pigments are among the most intensely light absorbing molecules in nature.

We should now have a look at the chemical mechanism by which the pigments help in transferring electrons from $H_2O$ to $CO_2$ via $NADP^+$. The most critical role is played by a special pair of chl *a* molecules ($P_{680}$ and $P_{700}$) which may be called "daddy" molecules. By virtue of the fact that chl *a* is the longest wavelength absorbing pigment, it can receive energy from an excited chl *b* molecule, provided it is sufficiently close. The chl *b* molecule can, in turn, absorb energy from excited molecules of carotenes and xanthophylls. The direction of transfer of electrons is always from a pigment which absorbs photons of higher energy to one that can be excited with those of lower energy, as given below:

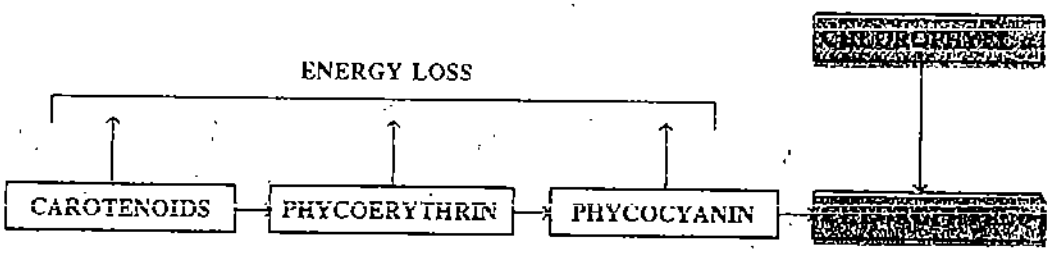



Fig. 13.11 : Scheme of energy transfer between various pigments.

The chemistry of chlorophyll *a* and *b* as also carotene and xanthophyll was worked out by German chemist Richard Willstätter and his associates. Willstätter was given a Nobel Prize.

Since the energy of light varies inversely with wavelength of a photon this means that its transfer is always from a lower wavelength (but higher energy photon) absorbing

molecule (like carotenoids), to a higher wavelength (low energy photon) absorbing molecule (for example chl *a*). Of course, the photon may marginally lose some energy in the process of transfer and that is why energy can never be transferred in the reverse direction.

Light is absorbed in discrete packets referred to as photon or quanta (sing. quantum) of light, the energy of a photon is given by

$$E = h \frac{c}{\lambda}$$

where $h$ = Planck's constant ($6.626 \times 10^{-34}$ J*-s)

$c$ = velocity of light ($3.0 \times 10^{8}$ m $s^{-1}$),

$\lambda$ = wavelength of light (in m)

$$E = \frac{6.626 \times 10^{-34} \times 3.0 \times 10^{8}}{\lambda} \text{ J photon}^{-1}$$

$$= \frac{1.988 \times 10^{-25}}{\lambda} \text{ J photon}^{-1} \quad \ldots (1)$$

For example $E$ for blue light ($\lambda$ = 450 nm) is

$$E = \frac{1.988 \times 10^{-25}}{450 \times 10^{-9}} \text{ J photon}^{-1}$$

$$= 4.42 \times 10^{-19} \text{ J photon}^{-1}$$

The energy for 1 mole of photon (i.e. for 1 einstein) would be

$$E = N \times h \frac{c}{\lambda}$$

N = Avogadro's number ($6.022 \times 10^{23}$ photons $mol^{-1}$ or 1 einstein)

From equation (1) $E = \frac{6.022 \times 10^{23} \times 1.988 \times 10^{-25}}{\lambda}$

$$= \frac{0.1197}{\lambda} \text{ J einstein}^{-1}$$

So, we can calculate energy of one einstein of photons by dividing 0.1197 by wavelength of light.

*J = joule

4.184 J = 1 calorie

Now let us enquire into the precise mode by which chlorophyll molecules help transfer electrons from $H_2O$ to $NADP^+$. According to the laws of physics, an electron electron hit by a photon — of a certain minimal energy — can pass from a lower atomic orbital to the next higher atomic orbital. In fact, with a higher energy photon — as in case of blue light — the electron can jump even to the next orbital. Nonetheless, since the excited states are short-lived, the electron tends to readily pass its energy to a neighbouring molecule and especially so to molecules that absorb longer wavelengths. The exact mechanism of transfer is still a matter of debate among physicists, but a particle called "exciton" may mediate this process. Ultimately, a special chlorophyll molecule in the reaction centre of a photosynthetic unit acts as the final trapping centre for the energy of the photon.

Although the evidence for the existence of the organisation of the photosynthetic machinery in the form of photosynthetic units of two kinds of photosystems (PS I and PS II embedded in the thylakoid membrane) will be discussed in the following section, we can leave for a while the matter of the history of evolution of the concept and consider a bit more closely the arrangement of the chlorophyll molecules in them. Each of the photosynthetic unit contains some 200-300 chlorophyll molecules. The photosynthetic units in fact consist also of several proteins (about 2 dozen in each photosynthetic unit). The peripheral components of each unit are the so called Light Harvesting Complexes (LHC), where the large majority of chlorophyll molecules are bound. The Light Harvesting Complex surrounds an inner core and at the heart of each Core Complex is the Reaction Centre. The light harvesting complexes also

contain the accessory pigments and absorb light throughout the range of photosynthetically active radiation (PAR). They are popularly referred to as the antenna since they function much like the ones we have on roof-tops connected to the TV sets inside our homes. The energy in photons captured by the antennae, is passed on to the special (daddy) chlorophyll *a* molecules which constitute the heart of the photosynthetic machinery.

The difference in chl *a* at the reaction centre and other chl *a* molecules—for example in the antenna—is that when it is excited, the electron is lost altogether from the orbit causing **photoionisation**. This electron finally passes to $NADP^+$. The reaction centre chl *a* — now positively charged — regains an electron from water, and setting free in this process protons as well as oxygen (we shall discuss the fate of the protons later). To put it very simply, chlorophyll acts as a pump for transferring electrons and light provides the energy for this process. And as someone has observed the jump of the electron from chl *a* is more significant for life on earth than the highest "jump" of any object that humans can effect!

$$\text{Chl} \xrightarrow{\text{light}} \text{Chl}^* + e^-$$

$$NADP^+ + 2H^+ + 2e^- \longrightarrow NADPH + H^+$$

## 13.5 DISCOVERY OF TWO LIGHT REACTIONS

After our short diversion in matters of pure chemistry (essential, nonetheless, to gain proper understanding), let us now enquire into detailed mechanism of both the light as well as the dark reactions. We shall start this section with the light "box" — and discuss one of the great conceptual advances in photosynthesis i.e. the discovery that there are two light reactions (or boxes), and not one as was originally believed.

### 13.5.1 Quantum Requirement of Photosynthesis

In the earlier section on Understanding the Mechanism of Photosynthesis, while we were discussing flashing light experiments (page 65), the emphasis was laid on confirmation of the idea of the existence of light and dark reactions. But a second objective of doing these experiments was to determine the quantum requirement for photosynthesis to obtain an overall idea of the efficiency of the process and its mechanism. Since oxygen evolution is a measure of the photosynthetic process, the question that needed to be resolved experimentally was "How many quanta of light are necessary for evolving one molecule of oxygen"?

With the development of the Atomic theory in the early part of this century as also the new equation of photosynthesis (that a minimum of 4 electrons are needed to evolve a molecule of oxygen) and Einstein's Law of Photochemical Equivalence, the studies of quantum requirement of photosynthesis took an entirely new turn. According to the concept advanced by Einstein, in a photoelectric effect one hit by a photon leads to expulsion of only one electron at a time from a molecule. Thus, the overall quantum requirement (number of quanta required for evolution of one oxygen molecule) could serve as an indicator of the number of hits needed for an electron to be pulled up via chlorophyll from water to a higher energy state as in NADPH.

As per the new equation of photosynthesis, four electrons need to be extracted from a $H_2O$ molecule for evolution of one oxygen molecule. Thus, if the quantum requirement is 4, this may mean that to drive a single electron only one photochemical reaction is involved in photosynthesis. However, if the quantum requirement is 8, it would mean that for each electron to move up two steps may be required and so on (it should be understood that quantum requirement has to be in simple multiples and so one has to choose a figure from amongst 4, 8, 12 and so on Fig. 13.12). Although Warburg—who initiated these studies—found a quantum requirement of 4 (giving a rather misleading idea of the extraordinary efficiency of the photosynthetic process), Emerson and Arnold and later many other workers found that the quantum requirement was about 8, meaning thereby that the overall photochemical process involved a two-step reaction.

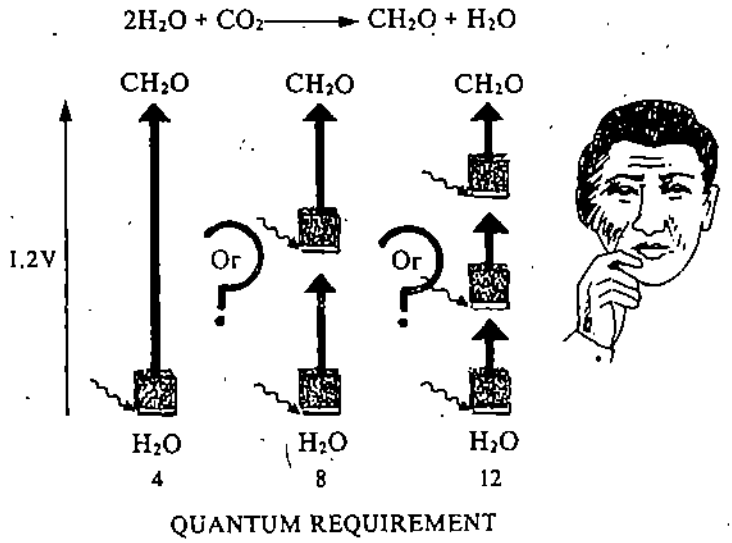



Fig. 13.12 : a) The diagram illustrates the significance of studies on quantum requirement to evolve oxygen during photosynthesis. As shown in the equation above, four electrons have to be released from water by photons hitting chlorophyll molecules. Square boxes represent chlorophyll molecules at the reaction centres. A quantum requirement of 8 indicates that at least two photochemical reactions may be involved in the transfer of each electron in photosynthesis.

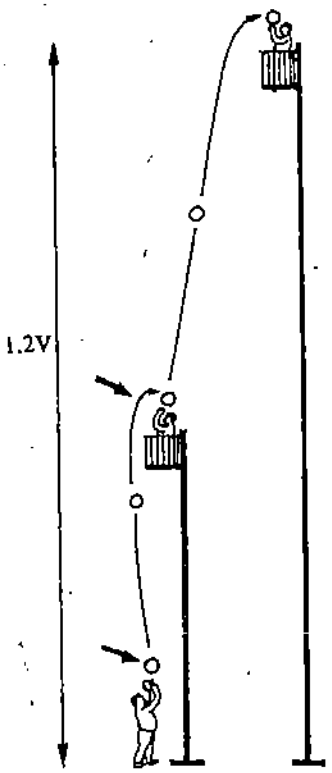



Fig. 13.12 : b) Cartoon

## 13.5.2 Red Drop

Another hint that there are two photochemical reactions — and which proceed in two distinct photosystems came from studies of quantum requirement as a function of wavelength of light. Since quantum requirement is calculated in terms of the amount of oxygen evolved per unit of light energy **actually absorbed**, it was expected that the curve for quantum requirement should stay more or less constant from 400 to 700 nm, i.e. covering both ends of the spectrum (until some absorption of light by one or other photosynthetic pigment still occurred). Strangely, the quantum yield (this is simply the number of oxygen molecules evolved per quantum of light absorbed) dropped precipitously when $O_2$ yield was determined employing red light for photosynthesis (and even when chl a still absorbed). The "red drop" specially clear in experiments with red algae (Fig. 13.13) was highly perplexing since chl *a* has been considered as the most important of all photosynthetic pigment molecules and to which in fact all energy is ultimately transferred from the other pigments.

## 13.5.3 Emerson Enhancement Effect

When Emerson continued his experiments to unravel the mystery of red drop, he found that if simultaneously a weak beam i.e. of low intensity light of any wavelength in the visible spectrum was shone, the "red drop" was abolished. Indeed, *the oxygen yield with two light beams shining together was more than the sum of yields obtained from two lights given individually*. By this experiment we now know that there is not one but two photochemical reactions in photosynthesis which need to be driven simultaneously.

Ultimately, finer work led to the realisation that chl *a* itself existed in at least two forms — one absorbing at longer and the other at shorter wavelengths. Thus it was established that there are two energy-capturing reactions, each using a different cluster of pigments and a reaction centre chlorophyll. They are now termed photosystems (PS) I and II and Robin Hill proposed the 'Z' scheme of photosynthesis, now universally accepted by all (Fig. 13.14). Ingenious experiments by L.N.M. Duysens, a dutch biophysicist, showed in the sixties that the photosystem II is driven by the shorter wavelength light and accepts electrons from $H_2O$ whereas photosystem I by longer wavelength light provides electrons to $NADP^+$. We cannot go in a description of the experiment and you may like to consult more advanced texts. Let us, however now learn a little more of PS I and PS II.

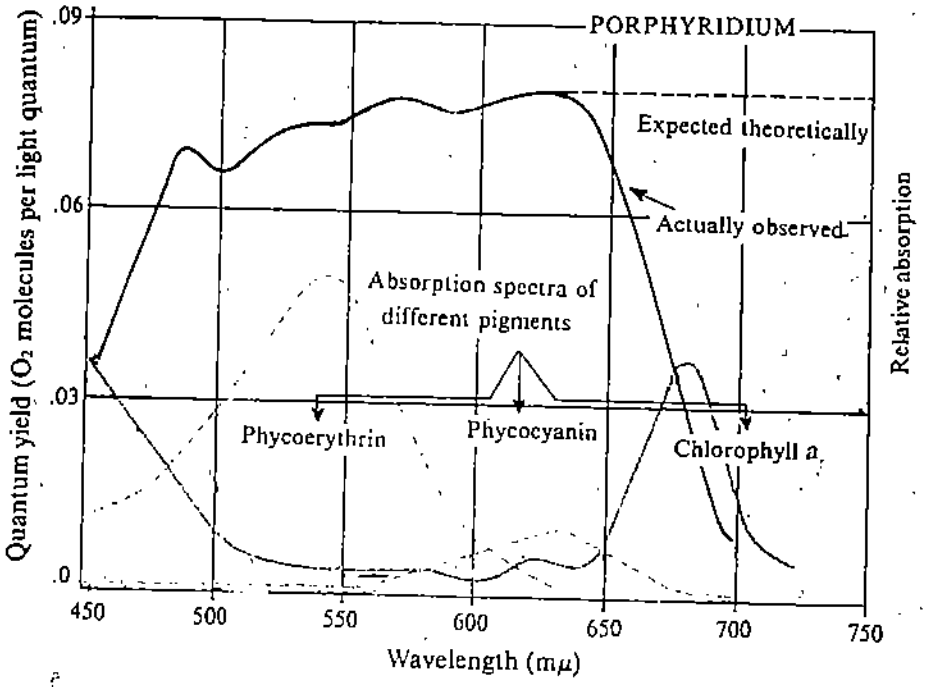



Fig. 13.13 : An action spectrum of the quantum yield for photosynthesis, i.e. the yield of oxygen evolution ***per quantum absorbed*** as a function of wavelength. The straight dotted line on top represents the quantum yield as expected theoretically, through different regions of the spectrum. The solid line shows that actually there is a fall in the red region even though chl *a* is still absorbing. There is, however, restoration of the quantum yield if weak light of a lower wavelength is shone simultaneously.

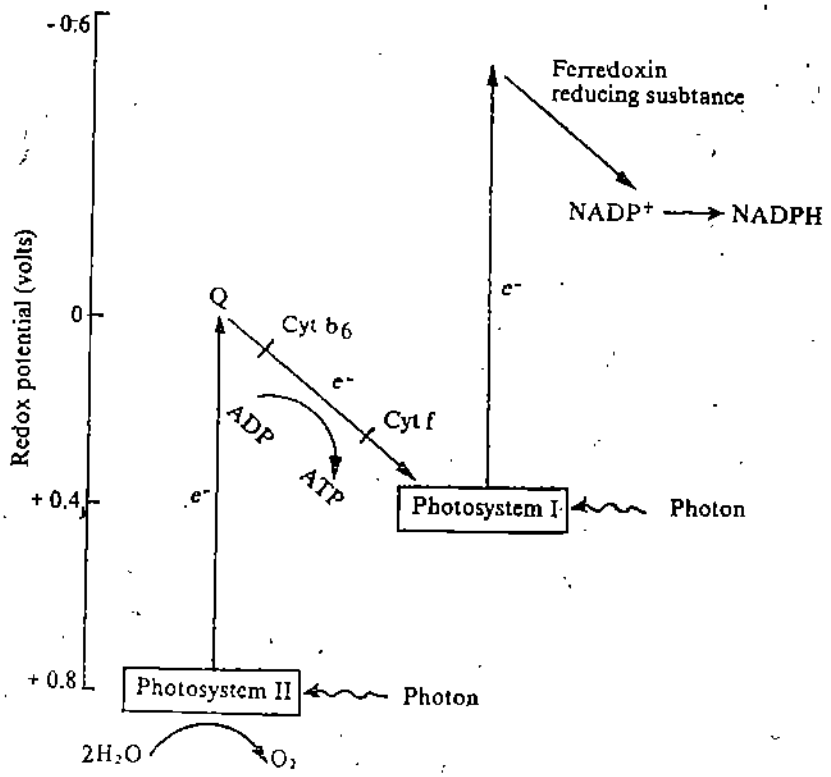



Fig. 13.14 : The simplified 'Z' scheme of photosynthesis and the pathway of electron transfer from $H_2O$ to $NADP^+$ via PS I and PS II essentially in the form first proposed by Hill. Q is an unidentified electron acceptor of PS II.

### 13.5.4 Photosystems I and II

Since the seventies, biochemical methods have been developed which enable isolation of the two photosystems by density gradient centrifugation. Many studies have naturally been done on the properties of the two photosystems. By isolating each system and carrying out appropriate experiments, it is clear that photosystem II (heavier) is responsible for the evolution of $O_2$ from $H_2O$ — it has a shorter wavelength absorbing form of chl *a* ($P_{680}$). In contrast, photosystem I is responsible for the reduction of $NADP^+$ to NADPH and has a longer wavelength absorbing form of chl *a* ($P_{700}$). The ratio of chl *a* to *b* is also different in the two photosystems. In a plant, as a whole, there are about 3 molecules of chl *a* for 1 of chl *b*. However, chl *a* (the longer wavelength absorbing pigment) may be 5 times as much in photosystem I and only 2.0-2.5 times the concentration of chl *b* in photosystem II. This explains why a mixture of both longer and shorter wavelength lights drives photosynthesis better than if only one kind of light is employed. Our current understanding of the two photosystems is shown in Fig. 13.15, which is a modern version of the 'Z' scheme in which all the reactions are arranged against the redox scale to illustrate as to how an electron from a lower energy level (as in water) is being raised to a higher energy state (as in NADPH).

A principal feature of the electron transport pathway is (see Fig. 13.15) that there several electron carriers involved in electron transfer from $H_2O$ to $NADP^+$. Two of them, cyt $b_6$ and cyt f, bear some similarity to cytochromes involved in respiration (however, the cytochromes in the green leaf are unique). In addition, there are others such as plastoquinone, plastocyanin and ferredoxin, which too have similarity with respiratory electron transfer carriers (refer to Unit 11 of Cell Biology). It can also be seen that the two photosystems are connected by a "dark bridge" in which, for a short distance, electrons move from one carrier to another without any need of light. This is made possible by the fact that in each photochemical act, the electron is

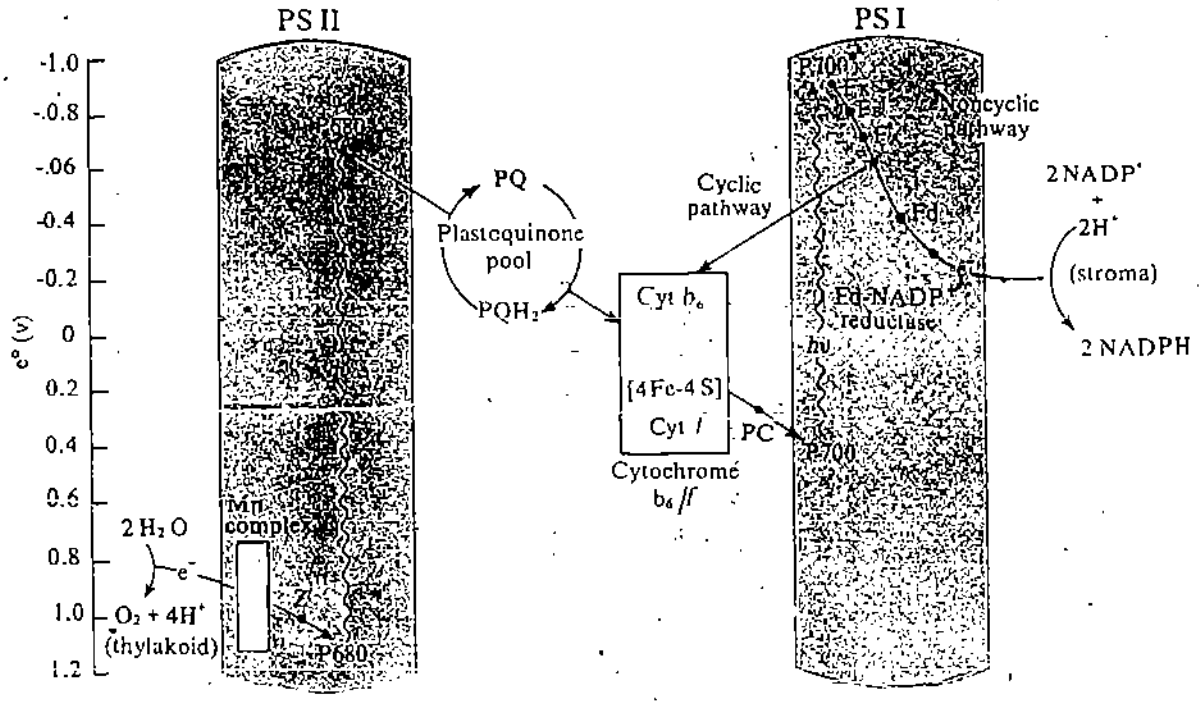



Fig. 13.15 : A modern version of the 'Z' scheme. Since Hill first proposed the 'Z' scheme of photosynthesis, a number of electron carriers and intermediates in the electron transfer chain have been discovered. It has been established that Mn-centre plays an important role in the transfer of electrons from $H_2O$ to $P_{680}$, but there is some evidence that an unknown electron carrier —Z may exist between the Mn-centre and $P_{680}$. An excited molecule of $P_{680}$ transfers electron to phaeophytin (Ph) which is a modified form of chl a. Phaeophytin, in turn, transfers electrons to a plastoquinone (PQ) which is reduced to $PQH_2$ through a step generating a semiquinone and requiring protons. From plastoquinone, which is a mobile carrier the electron goes to the cyt $b_6/f$ complex which, then, transfers it to plastocyanin (PC), again a mobile carrier. From PC the electron moves to $P_{700}$. In the excited state, the electron is transferred to a Fe-S complex, ferredoxin (Fd), and finally to $NADP^+$ via the enzyme Fd-$NADP^+$ reductase. It is believed that there may be three other forms of Fe-S clusters ($F_X$, $F_B$ and $F_A$) between $P_{700}$ and Fd complex yet to be completely characterised.

excited to a level slightly more than that required for the next photosystem to be excited. It is believed that the "downhill" journey of electrons provides a mechanism of synthesis of extra ATP molecules.

**Redox Reactions**

Reactions that involve transfer of electrons from one molecule to another are called oxidation-reduction reactions or redox reactions. The molecule that loses electron is oxidised and the molecule that gains electron is reduced. In a complete redox reaction the two would occur simultaneously.

$$y_{(red)} \longrightarrow y_{(ox)} + e^-$$

$$x_{(ox)} + e^- \longrightarrow x_{(red)}$$

$$x_{(ox)} + y_{(red)} \longrightarrow x_{(red)} + y_{(ox)}$$

The oxidised and reduced forms of x, are called a **redox couple** written as $x_{(ox)}/x_{(red)}$. The energy associated with the transfer of electrons (under standard biological conditions) is expressed as standard reduction potential, $E_0'$, in units of volts. Since the energy associated with electrons of different redox couple varies, it can be arranged on a scale taking an arbitrary standard, the hydrogen half-reaction $2H^+/H_2$ (under standard conditions, pH = 0, $E_0'$ = 0.00 volt, at pH =.7 its value is $E_0'$ −0.42 volts). The $E_0'$ values for redox couple involving electrons with higher energy than $2H^+/H_2$ are assigned a negative sign and those involving electrons with lower energy than $2H^+/H_2$ are assigned a positive sign. The redox couples with highest energy electrons (most negative) are arranged at the top and the one with least energy (most positive) at the bottom of redox scale. The spontaneous transfer of electrons can occur only in downward direction. Some important redox couples of biological systems are given in Table 13.1.

**Table 13.1 : Some Standard Redox Couple Potentials of Biological Interest**

| Redox Couple | Number of electrons transferred | $E_0'$ (volts) |
|---|---|---|
| acetate + $CO_2$ + $2H^+$/pyruvate + $H_2O$ | 2 | −0.70 |
| chlorophyll : $P_I^*/P^*$ | 1 | −0.6 |
| ferredoxin ox/red | 1 | −0.43 |
| $2H^+/H_2$ | | −0.42 |
| S + $2H^+/H_2S$ | 2 | −0.23 |
| chlorophyll : $P_{II}^*/P^*_{II}$ | 1 | −0.2 |
| FAD (flavin adenine dinucleotide) $2H^+/FADH_2$ (Free) | 2 | −0.18 |
| Standard hydrogen half cell ($2H^+/H_2$) | 2 | $E_0'$ = 0.00 |
| cytochrome b ($Fe^{3+}/Fe^{2+}$) | 1 | 0.06 |
| ubiquinone ox/red | 2 | 0.10 |
| haemoglobin ($Fe^{3+}/Fe^{2+}$) | 1 | 0.17 |
| cytochrome c ($Fe^{3+}/Fe^{2+}$) | 1 | 0.22 |
| cytochrome a ($Fe^{3+}/Fe^{2+}$) | 1 | 0.29 |
| $2I^- + O_2/H_2O_2$ | 2 | 0.30 |
| chlorophyll : $P_I^*/P_I^o$ | 1 | 0.4 |
| $Fe^{3+}/Fe^{2+}$ | 1 | 0.77 |
| $2H^+ + 1/2\ O_2/H_2O$ | 2 | 0.82 |
| chlorophyll : $P_{II}^*/P_{II}^o$ | 1 | 0.9 |

$P_I^*$, $P_I^*$ and $P^o$ represent the excited, the electron-deficient, and the ground state of chlorophyll at photoreactive centres, PS I and PS II.

**SAQ 4**

a) List the evidences for the involvement of two light reactions instead of one in photosynthesis.

b) In the following statements fill in the blanks with appropriate words:

i) The photosensitive pigments transfer ..... electron at a time, while a molecule of $NADP^+$ requires ..... electrons for its reduction.

ii) Light energy captured by chlorophyll is used to produce a strong ..... ..... from a weak one.

iii) Light energy absorbed by the ..... ..... pigments is funnelled to a single molecule of ..... ..... .

iv) The expelled electron from chl P..... travels through the electron transfer chain and reaches chl P..... The final acceptor of electron is ..... .

c) In the following statements choose the correct word from the alternate given in parenthesis:

i) The relative quantum efficiency of photosynthesis (drops/increases) sharply in the red region of the spectrum.

ii) When a beam of blue light is given along with red light to a photosynthesizing cell, there is (enhancement/red drop) in the quantum yield.

iii) The reaction centre chl *a* molecule at PS I absorbs a (higher/lower) energy photon than the one at PS II.

d) Which among the following statements are true. Write T for true and F for false in given boxes.

i) Photoreduction of $NADP^+$ requires $CO_2$. [ ]

ii) Electron can move from a redox couple with positive redox potential to another couple with negative redox potential. [ ]

iii) ATP is formed when high energy electrons move down from negative redox potential to a positive one in an electron transfer chain. [ ]

iv) The reaction centre chl *a* in PS I absorbs photon of a higher energy than the reaction centre chl *a* in PS II. [ ]

v) The pigment molecules absorbing at yellow region can transfer their energy to the ones absorbing in orange region of the visible spectrum. [ ]

vi) Evolution of a molecule of $O_2$ requires 8 quanta of light. It means a total of 8 electrons are expelled from the two chl *a* molecules in the reaction centres of PS I and II. [ ]

Melvin Calvim

The introduction of *Chlorella* as a material of choice for photosynthetic studies and the development of the manometric technique for the estimation of oxygen evolution (for which the alga was eminently suited) greatly advanced the studies.

## 13.6 THE DARK REACTIONS

### 13.6.1 The Calvin Cycle

It is time now to consider the dark reactions of photosynthesis. The process of fixation of $CO_2$ was elucidated by Melvin Calvin, who was a Professor of Chemistry in the University of California at Berkeley. By the use of the radioactive isotope of carbon, $C^{14}$, he discovered what is now called the Calvin cycle in his honour and the unravelling of which merited a Nobel Prize.

A suspension of *Chlorella* cells was fed radioactive sodium bicarbonate ($NaHC^{14}O_3$), which results in the production of $C^{14}O_2$). The suspension was placed in a glass container that looked like a "Lollipop" (Fig. 13.16) and then the contents were extracted in hot alcohol after brief period of illumination and concentrated by evaporation. Its constituents were then separated by chromatography, and those that were radioactive identified by the technique of radioautography. Once a certain spot was identified on the autoradiogram, one could go back to the original chromatogram, cut it out, elute the compound and determine its structure or investigate other properties.

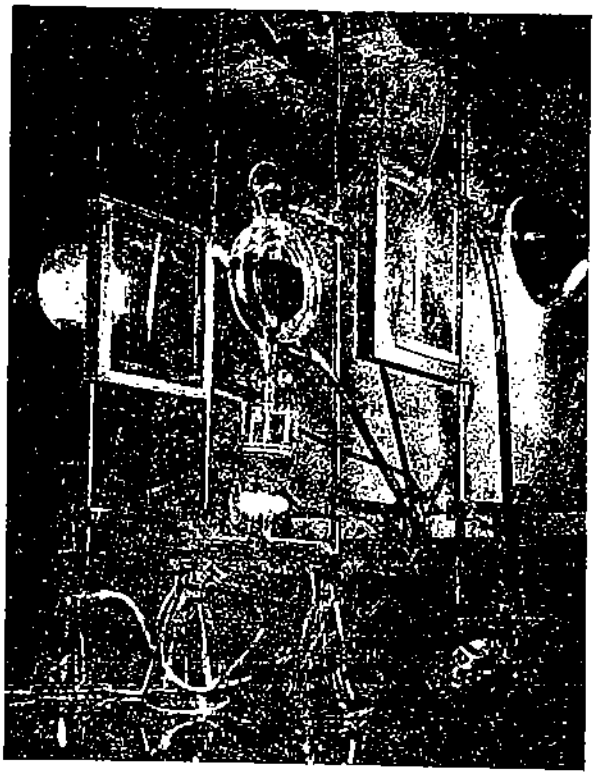


Fig. 13.16 : The 'lollipop' apparatus employed by Calvin for experiments to elucidate the pathway of $CO_2$ fixation.

Being a chemist, Calvin went a step further and employed a technique of "molecular dissection", whereby the terminal carbon atom in a radioactive intermediate was oxidised to carbon dioxide which was trapped as $BaCO_3$ by passing it through a barium hydroxide solution. The radioactivity in the carbon atoms was then determined by a Geiger-Müller counter. He could thus not only tell whether a particular carbon atom was radioactive, but also to what extent in a given sample. It was found that the first compound to become radioactive, already within a few seconds of photosynthesis, was the three-carbon compound, 3-phosphoglyceric acid (3-PGA see Fig. 13.17). Since only the terminal carbon atom was radioactive, it was clear that $CO_2$ was added to a pre-existing acceptor molecule. Initially, thought to be a 2-C molecule, later the acceptor was found to be a 5-C sugar, ribulose bisphosphate (RuBP) and the corresponding enzyme ribulose-

bisphosphate (RuBP) and the corresponding enzyme ribulose-bisphosphate carboxylase (called Rubisco) that fixes $CO_2$ was also discovered. Since no 6-carbon intermediate has ever been detected, the intermediate obviously fragments immediately into two 3-carbon compounds: phosphoglyceric acid and dihydroxy acetone phosphate, and both of which are well-known as intermediates arising also via the glycolytic pathway in respiration.

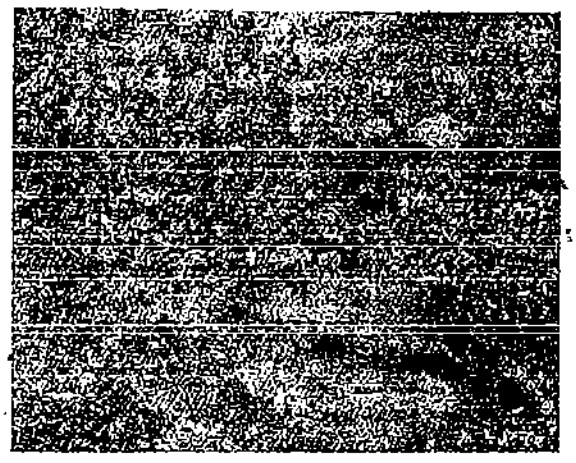


Fig. 13.17 : Radioautograph showing products of $CO_2$ fixation (see text for the details of experiment). It can be seen here that most of the radioactivity is localised in PGA.

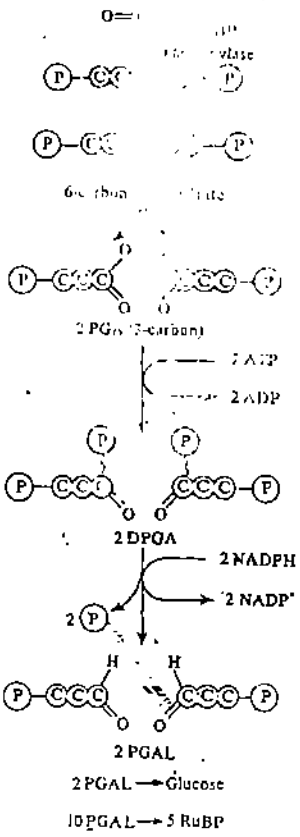



Fig. 13.18 : Carbon-fixation via Calvin cycle. Carbon dioxide combine with ribulose bisphosphate and forms an unstable 6-carbon intermediate which splits into two phosphoglycerates (PGA). Phosphorylation of PGA by ATP forms diphosphoglycerate (DPGA), which is reduced by NADPH to phosphoglyceraldehyde (PGAL) . Out of 12 PGALS produced by fixation of 6 $CO_2$ molecules, 2 combine to produce a molecule of glucose, whereas the remaining 10 PGAL molecules recombine to regenerate the 6 molecules of RuBP that are needed to start the Calvin cycle. Each PGA requires 1 ATP and 1 NADH to form PGAL. A third ATP is required for the regeneration of RuBP. In all, a total of 12 NADPH and 18 ATP molecules are needed for producing a molecule of glucose.

Obviously, in order for photosynthesis to proceed to some significant level, an acceptor has to be continuously generated. The Calvin cycle illustrated in Fig. 13.18 shows in brief the mechanism by which RuBP, the acceptor molecule is regenerated. If we start with 6 RuBP, then out of 12 PGA molecules that are formed, in reality only two are available to go into the pool for the synthesis of a molecule of glucose.

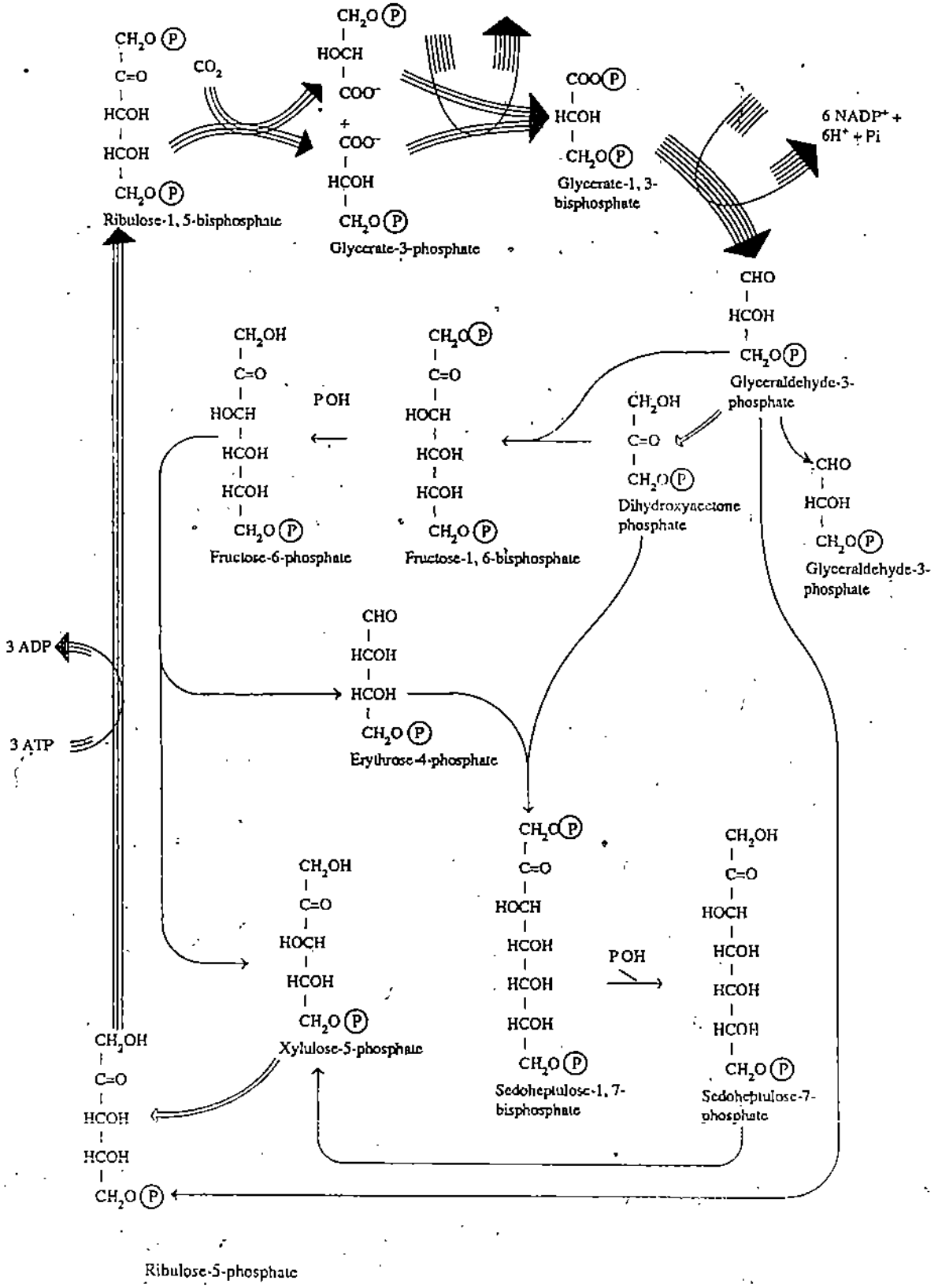



Fig. 13.19 : Detailed reactions of calvin cycle. The number of arrows drawn at each step in the diagramme indicates the number of molecules proceeding through that step for every three molecules of $CO_2$ that enter the cycle. The entry of three molecules of $CO_2$ results in the formation of one molecule of glyceraldehyde-3-phosphate (box on right), and requires the oxidation of six molecules of NADPH to $NAPD^+$ and the breakdown of nine molecules of ATP to ADP.

and from it to other substances such as starch, cellulose and so on. The remaining 10 molecules recycle through a series of reactions involving 4–, 5– and 7-C intermediates to regenerate the five molecules of RuBP from 10 PGA. Confirmation of the existence of the cycle has come not only by an analysis of distribution of radioactivity in the C atom in different sugars, but also by isolation of various enzymes involved in the process (other than Rubisco already mentioned). Figure 13.19 also illustrates that ATP and NADPH molecules are also required to run the cycle. The overall reaction of carbon fixation is given below:

$$6\,RuBP + 6\,CO_2 + 12\,NADPH + 12\,H^+ + 18\,ATP \longrightarrow 6\,RuBP + C_6H_{12}O_6 + 12\,NADP^+ + 18\,ADP + 18\,Pi$$

**SAQ 5**

a) Melvin Calvin elucidated the path of carbon in fixation of $CO_2$. The following statements highlight his approach. Fill in the missing words in the statements:

i) ..... $NaHCO_3$ was used for the production of $CO_2$.

ii) ..... was the technique used in tracing the pathway of radioactive carbon.

iii) The technique of ..... converted the terminal carbon atom to $CO_2$.

iv) The radioactivity in carbon atoms was detected by ..... counter.

b) Give one word answer:

i) The first compound in which the radioactivity appeared was .....

ii) The $CO_2$ acceptor molecule in Calvin Cycle is .....

iii) The final product of $CO_2$ fixation is ..... .

iv) The number of ATP molecules required for the fixation of six molecules of $CO_2$ is ..... .

v) The number of ATP molecules used for the production of a molecule of glucose is ..... .

vi) The number of NADPH used for fixation of 6 $CO_2$ is ..... .

vii) The number of molecules of ATP required for the regeneration of one RuDP is ..... .

## 13.7 PHOTORESPIRATION AND THE C4 PLANTS

### 13.7.1 Photorespiration

Since photosynthesis has been evolving for millenia, it might be expected that the development of the machinery of carbon dioxide assimilation may have reached a stage of perfection. Yet, one of the great problems in photosynthesis lies with ribulose bisphosphate carboxylase itself, the key enzyme concerned with $CO_2$ fixation. Its catalytic site is such that the enzyme cannot make an absolute distinction between $CO_2$ and $O_2$. Thus, $O_2$ also competes with molecules of $CO_2$ for binding at the catalytic site, and often fragments ribulose bisphosphate into phosphoglycolic acid (a 2-carbon compound) and phosphoglycerie acid (Fig. 13.20), instead of two 3-carbon fragments of PGA which should be normally produced. After the discovery of this reaction, the enzyme is now commonly called ribulose bisphosphate carboxylase/

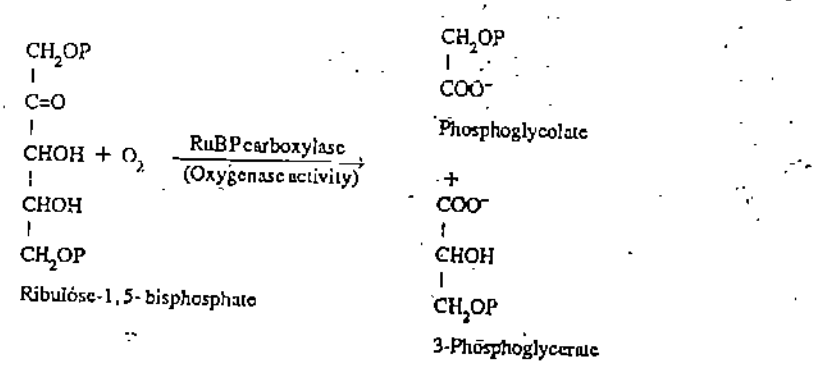



Fig. 13.20 : Reaction showing the oxygenase activity of the enzyme RuBP carboxylase.

oxygenase. The oxygenase activity is very significant because in nature molecules of $O_2$ are far in excess to those of $CO_2$. Locally, i.e. in the interior of leaf, photosynthesis can lead to accumulation of even higher concentration of $O_2$ than is present in the atmosphere.

Production of glycolic acid is a disadvantage since, apart from loss of a potential carbon atom that could have been fixed, the state of oxidation becomes higher than had been existing before as a result of the oxygenation reaction. Eventually, one of the two carbon atoms constituting glycolate is respired away as $CO_2$. Thus, instead of fixation of $CO_2$, plants under conditions of high intensity light actually evolve $CO_2$, a process which is called "photorespiration" and yet in this process no energy is released. It is estimated that if oxygenase activity and photorespiration could be avoided, plants would have fixed 30% more carbon.

Long ago, Otto Warburg himself had noted that with increasing $O_2$ concentration, photosynthesis in *Chlorella* was inhibited. Later, in the sixties, botanists started experiments with higher plants. They observed that if $CO_2$ output was monitored continuously in light and then upon transfer to darkness, immediately there was a transient "burst" in the net $CO_2$ output in certain plants. Curiously, the "burst" became more intense if $O_2$ concentration in atmosphere was higher. Thus, the existence of photorespiration has been suspected for long. However, definitive evidence came only by the use of $O^{18}$ which allowed measurement of oxygen uptake even upon exposure of a plant to constant illumination. When the consumption of $O^{18}$ was monitored by mass spectrometry in the surrounding atmosphere, it was discovered that the rate of uptake of $O^{18}$ became significantly higher after illumination.

The detailed mechanism whereby $CO_2$ is evolved has been investigated by American biochemists and is illustrated in Fig. 13.21. According to this scheme, phosphoglycolic

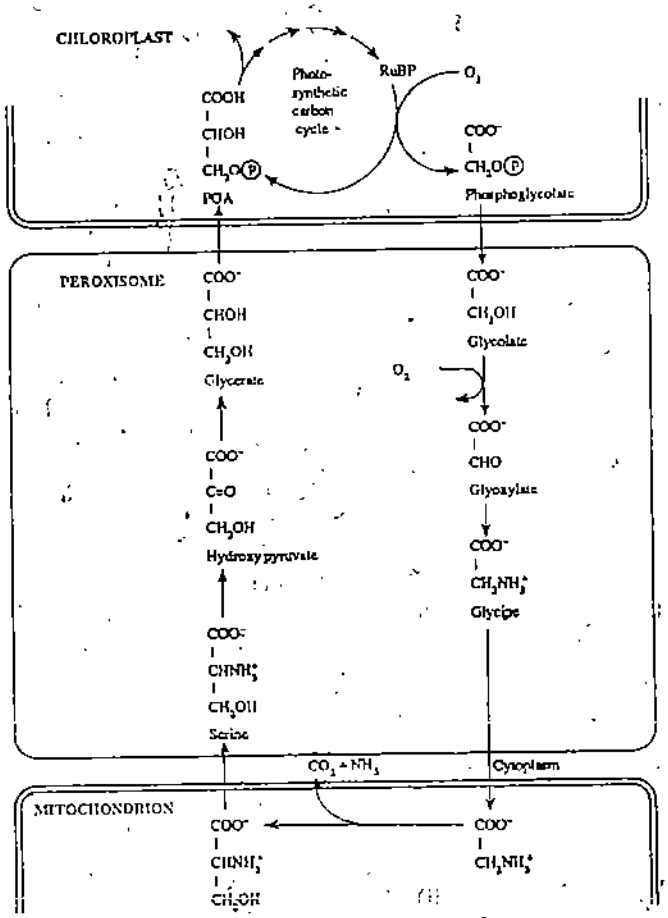



**Fig. 13.21 : A simplified scheme indicating the reactions involved in the glycolate metabolism. Only the oxygenase activity of the enzyme RuBP carboxylase is shown, which results in the formation of glycolic acid. These molecules move out through the cytoplasm, to organelles called peroxisomes and glyoxysomes which convert glycolate to glyoxylate and then to glycine, which then moves into the mitochondria. Although *photorespiratory* $CO_2$ is ultimately believed to be liberated from mitochondria, as can be readily seen, this mechanism of respiration is quite different from that which normally occurs in mitochondria.**

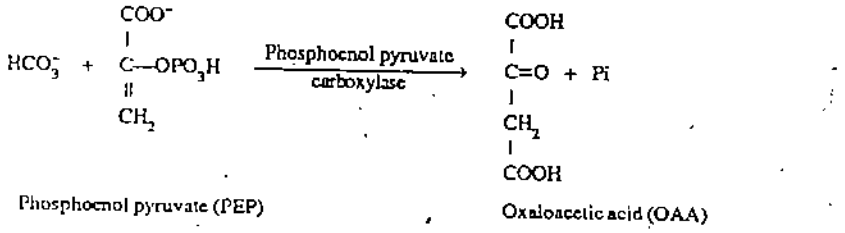
acid enters peroxisomes where it is converted to glycolate and gets further oxidised to glyoxylate. The transamination of glyoxylate, yields glycine (a 2-C amino acid). Glycine then enters the mitochondrion, where one molecule of serine is formed from two molecules of glycine with the release of a molecule each of $CO_2$ and $NH_3$. The amino acid serine (a 3-carbon compound) then reenters peroxisomes and gets deaminated to form glyceric acid, which is again converted to phosphoglyceric acid in chloroplasts. The pathway obviously requires close cooperation of biochemical activities among three organelles — the chloroplasts, the peroxisomes and the mitochondria. Remarkably, electron micrographs do show these three organelles very closely appressed to each other indicating that there is indeed some important functional relationship among them.

As would be clear, the pathway serves to *recycle three carbon atoms (ending up as PGA) out of the 4 carbon atoms i.e. 2 molecules of glycolate.* There is loss of one of them as $CO_2$. Also, of the two $-NH_2$ groups donated in the transamination reaction, one is given up as $NH_3$.

At first sight, the loss of $CO_2$ and $NH_3$ does appear as a wasteful process. However, there is another way of looking at the cycle. At least, by loss of one atom of carbon (and another of nitrogen), the three other carbon atoms are conserved, and one molecule of phosphoglycerate is formed, ready to be reduced to phosphoglyceraldehyde and enter the Calvin cycle, or be converted to sugar directly. Viewed this way, photorespiration is of benefit to plants — one should then look upon it as a "necessary evil".

## 13.7.2 The $C_4$ Plants

Another interesting mechanism plants have developed to capture $CO_2$ (over and above what is possible through $C_3$ cycle) is via another enzyme, phosphoenol pyruvate carboxylase (PEP carboxylase), according to the following reaction:




Phosphoenol pyruvate (PEP) Oxaloacetic acid (OAA)

Carbon dioxide is accepted by a 3-carbon compound, phosphoenol pyruvic acid (PEP), and fixed in the form of a 4-carbon acid, oxaloacetic acid (OAA), which is readily reduced with the help of NADH to malic acid (an amination reaction also leads to production of aspartic acid). Since in this pathway a 4-C acid is the first product of $CO_2$ fixation, it is called the $C_4$ cycle to distinguish it from Calvin cycle where fixation of $CO_2$ takes place in the form of a 3-C acid. The discovery of the new cycle took place rather accidentally, when Kortshak and co-workers began work in 1965 on mode of fixation of $CO_2$ in sugarcane at the Sugarcane Experimental Station in Hawaii (where they were employed). They were puzzled to find that in sugarcane the first stable compound of photosynthesis was not PGA. Instead, the radioactivity appeared in acids such as oxaloacetate, malate and aspartate, following a pattern quite different from that discovered by Calvin in *Chlorella* and later confirmed in many higher plants like spinach. Further work by M.D. Hatch and C.R. Slack in Australia showed that this represented an additional pathway of $CO_2$ assimilation.

Despite the novelty of the $C_4$ cycle, a point to note about this pathway is that it is not totally independent of the Calvin cycle. By a mechanism, yet to be explained satisfactorily, the 4-carbon acid, i.e. oxaloacetate, immediately gives up the $CO_2$ to be refixed by ribulose bisphosphate carboxylase via the Calvin cycle. In the process, the phosphoenol pyruvate, which is the acceptor for $C_4$ cycle, regenerates. The $C_4$ cycle has, therefore, to be viewed not as an independent cycle, but as one adjunct to the Calvin cycle. Nonetheless, plants do not fix $CO_2$ in photosynthetic cells by both $C_3$ and $C_4$ pathways in any one cell. Indeed, in plants, like the grasses, which have the $C_4$ cycle, there is a spatial separation. As is well-known, these plants have the so-called Kranz (German, wreath-like) anatomy (Fig. 13.22) — there being a central

Some common examples of photorespiring ($C_3$) and non-photorespiring ($C_4$) plants

| Photo respiring $C_3$ | Non-photo respiring $C_4$ |
|---|---|
| Wheat | Corn |
| Rice | Sugarcane |
| Legumes | Sorghum |
| Potato | Sugar-beet |
| Tomato | Bajra |

bundle sheath region consisting of a ring of large chlorophyll containing cells, surrounded by more loosely arranged spongy mesophyll cells. The enzyme PEP carboxylase abounds in the outer mesophyll cells whereas ribulose bisphosphate carboxylase is restricted to the bundle sheath cells.

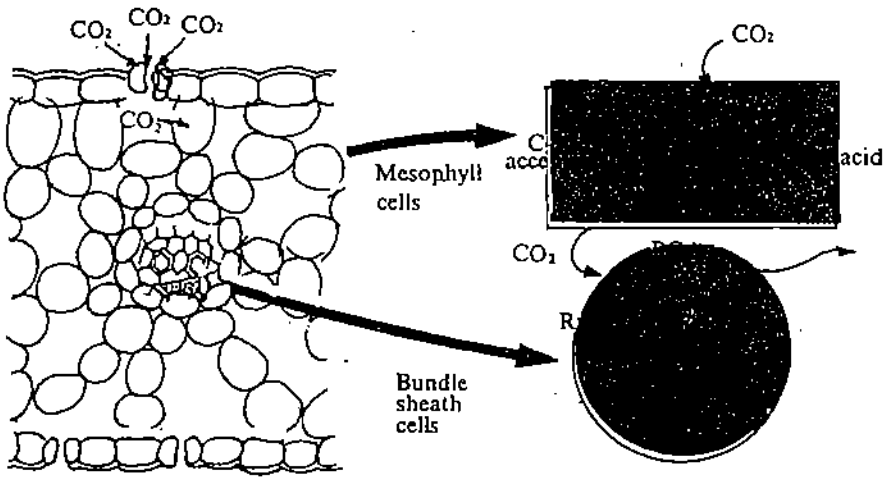



**Fig. 13.22: The diagram illustrates the anatomy of plants which apparently do not photorespire and the C-4 pathway of carbon dioxide fixation with which they are endowed. Here, $CO_2$ is captured initially, that is, from the atmosphere by the enzyme PEP carboxylase, instead of by RuBP carboxylase as in the Calvin cycle plants, which are now commonly referred to as the $C_3$ plants since the first product of $CO_2$ fixation in this cycle is the 3-carbon containing molecule of phosphoglyceric acid rather than oxaloacetic acid. If some photorespiration does occur in the $C_4$ plants in the bundle sheath cells, the $CO_2$ so released would probably be captured and cycled back.**

Another interesting point is that PEP carboxylase has a much higher affinity for $CO_2$ than RuBP carboxylase has. It would seem, therefore, that PEP carboxylase is ideally suited to fix $CO_2$ from the atmosphere. As we have learnt above, the $CO_2$, so fixed, has to enter the Calvin cycle. Electron micrographs show that the bundle sheath cells on their outer surface have a peculiar network of membranes — this may provide the extra absorptive surface for 4-C acids, like malate, to be transported into the bundle sheath cells to give off their carbon to the Calvin cycle. The more successful plants, then, have a rather unique and interesting mechanism to capture $CO_2$ from the atmosphere by a more efficient enzyme.

One cannot fail to appreciate also another advantage to the plants by virtue of the Kranz anatomy. Oxygen tension is likely to be high in the outer areas and low in the innermost parts of the leaf, thus, diminishing oxygenase activity of Rubisco. However, if there is some unavoidable photorespiration in the inner bundle sheath cells, the $CO_2$ so formed can be trapped again by PEP carboxylase, as it tries to escape. No wonder, then, the $C_4$ plants are highly efficient photosynthesisers. Grasses are widely considered to be one of the most photosynthetically efficient species colonising the earth and this may be due to the $C_4$ pathway they possess. Many of our important crops like corn, besides sugarcane mentioned above, are also $C_4$ plants. On the other hand, the dicots are by and large $C_3$ plants. However, there are some notable exceptions among dicots, for example, sugarbeet and members belonging to the family Chenopodiaceae and Portulacaceae. It is of interest that these plants also have the "Kranz" anatomy — much like monocots — instead of a palisade layer and spongy parenchyma typical of dicotyledonous leaves. Overall, $C_4$ pathway is an adaptation to enable plants to survive better under conditions of higher temperatures and poor water supply — it is of interest that if one studies the distribution of $C_3$ and $C_4$ species geographically, the former dominate in the temperate regions, but as one approaches the equator, the $C_4$ species dominate.

### 13.7.3 The CAM Plants

Exceptional among dicots from the viewpoint of $CO_2$ fixation are also *Bryophyllum*, *Kalanchoe*, the cacti, and some members belonging to the Euphorbiaceae that grow in the desert. These members have basically the $C_4$ pathway of C-fixation, but they

are called CAM plants (standing for Crassulacean Acid Metabolism) since they have some additional unique features.

Botanists have known for long that many plants with fleshy leaves have a rather sour taste on account of large quantities of organic acids such as malic and oxalic. However, it is only now that the role of these acids has been appreciated. Many of these plants fix their carbon largely in the form of 4-C organic acids which represents a unique and truly clever adaption for survival in their desert surroundings (where the day temperatures are high and water must be conserved). The strategy these plants have adopted, therefore, is to fix $CO_2$ at night and reduce the organic acids *via* the Calvin cycle pathway, using NADPH formed during the day (Fig. 13.23 and 13.24). The plants, thus, close their stomates during the day (to avoid transpiration) but open them at night (to permit inflow of $CO_2$)! The CAM plants have, therefore, separated the function of $CO_2$ fixation and electron transfer for reduction of organic acids (to aldehyde and alcoholic groups as in sugar) in a temporal sense i.e. one process occurring during the day and the other at night.

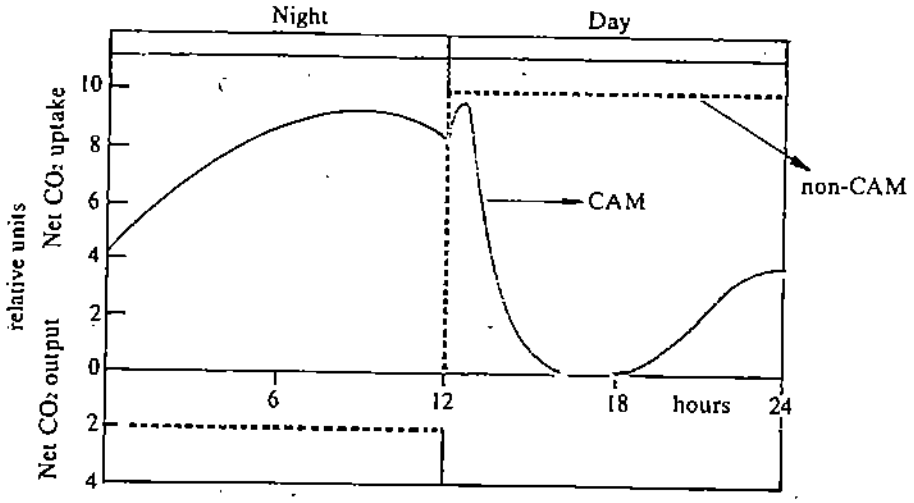



**Fig. 13.23 : Typical pattern of gas exchange in a CAM plant. Uptake of $CO_2$ occurs largely at night. During the day, there is decline in $CO_2$ as it is reabsorbed for production of organic acids. Dashed line shows the $CO_2$ exchange curve for a non-CAM plant.**

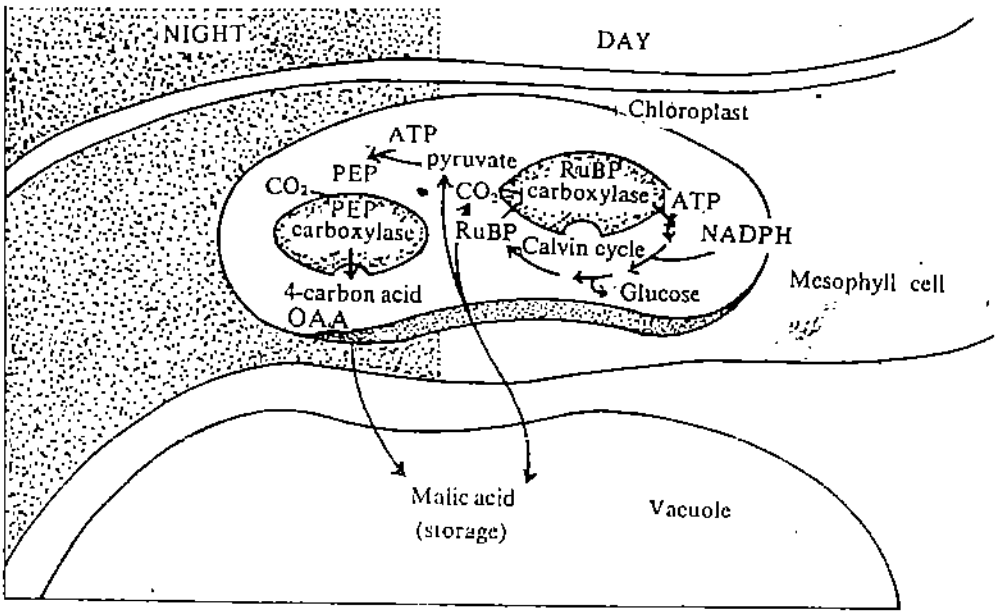



**Fig. 13.24 : CAM synthesis. The reactions are identical to $C_4$ synthesis reactions except that they take place at different times within the same mesophyll cell. At night, stomate pores open to take up $CO_2$. The $CO_2$ is fixed to PEP by PEP carboxylase producing oxaloacetate. The oxaloacetate is then converted to malic acid, which is then stored in the cell's vacuole. During the daytime, CAM plants close their stomates, conserving water. The stores of malic acid are gradually moved back to the chloroplasts and split to release $CO_2$ and pyruvate. The released $CO_2$ is then fixed to RuBP by RuBP carboxylase and is introduced into the Calvin cycle, while the pyruvate is converted into PEP with energy from ATP.**

**SAQ 6**

a) List the conditions required for photorespiration:

.....

.....

b) Match the reactions of photorespiration in column 1 with the site of their occurrence in column 2.

| Column 1 | Column 2 |
|---|---|
| i) Evolution of $CO_2$ from two molecules of glycine | a) Chloroplast |
| ii) Oxidation of RuBP | b) Peroxisomes |
| iii) Formation of serine | c) Mitochondria |
| iv) Formation of glyoxylate | |
| v) Regeneration of PGA | |

c) Complete the following statements about $C_4$ plants and the $C_4$ pathway.

i) The acceptor of $CO_2$ is a three-carbon compound .....

ii) The enzyme for fixation of $CO_2$ is .....

iii) A molecule of ..... is used per molecule of $CO_2$ fixed.

iv) The pathway occurs in the..... of mesophyll cells.

v) Plants that use $C_4$ pathway also use ..... ..... which operates in ..... ..... cells.

vi) PEP carboxylase has higher affinity for $CO_2$ than .....

vii) Bundle sheath cells of $C_4$ plants have peculiar .....of..... for $CO_2$ absorption.

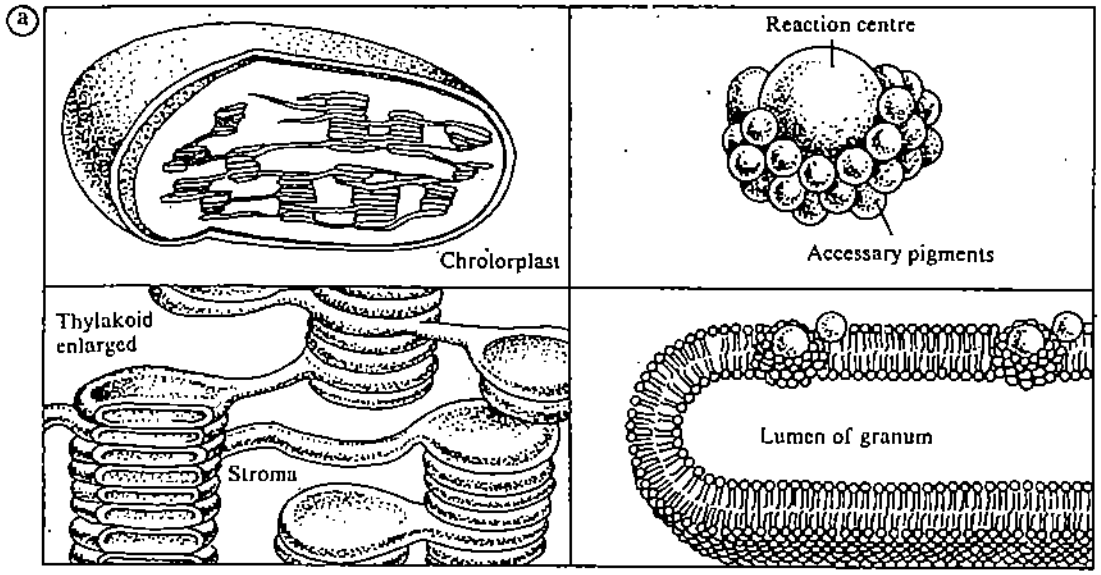
viii) $C_4$ plants are ..... ..... .....than $C_3$ plants.

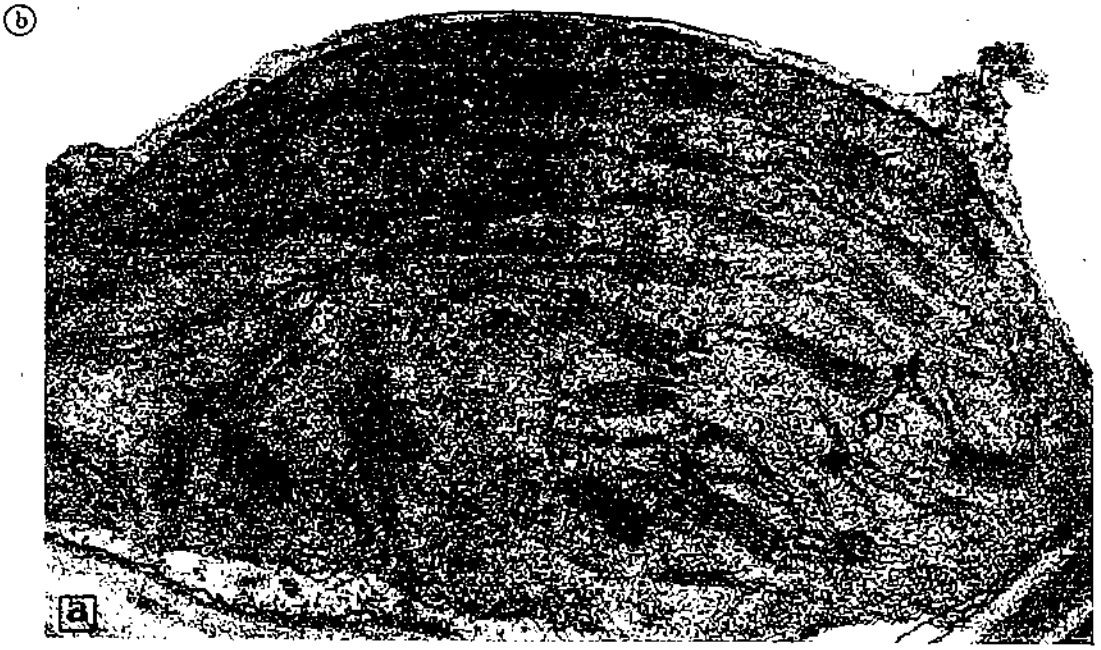
## 13.8 THE CHLOROPLASTS — ULTRASTRUCTURE AND ORGANISATION OF PHOTOSYNTHETIC MACHINERY

We can now have a close look at the structure of chloroplast and the photosynthetic machinery inside (Fig. 13.25). Since the chloroplast was first described by the German botanist von Mohl in 1837, and Engelmann in 1882 could definitively correlate photosynthesis with this organelle, we have come a long way towards the understanding of the chloroplast structure. The major breakthrough came in the fifties when the techniques of preparing ultrathin sections and electron microscopy came into existence. The chloroplast was found to have an extensive internal membrane system comprising the thylakoids (Thyla, Greek = sacs; thylakoid = sac-like) connected to each other through tubular connections and all embedded within the stroma which contains ribosomes and other soluble components in addition to DNA. Thylakoids pile upon one another to produce grana which are discoid in shape. Grana are much like a pile of coins. The techniques of disruption of cells and organelles by lysis or sonication and of high speed centrifugation allow grana to be separated—and from experiments with purified grana it is clear that it is here that the real photosynthetic machinery lies.

More sophisticated specimen preparation techniques for electron microscopy developed in the sixties and seventies, i.e. use of freeze-fracturing and shadowing methods (refer LSE-01, Section 2.3.3), have revealed also the presence of smaller granular particles which may represent the ultimate units of the photosynthetic machinery. This is discussed below further.

The idea that the photosynthetic machinery is not distributed randomly in the chloroplast, but organised in small units first came from the experiments of Emerson,




Chloroplast envelop (CE),
Stroma (S)
Stroma lamellae (SL)
Grana (G)

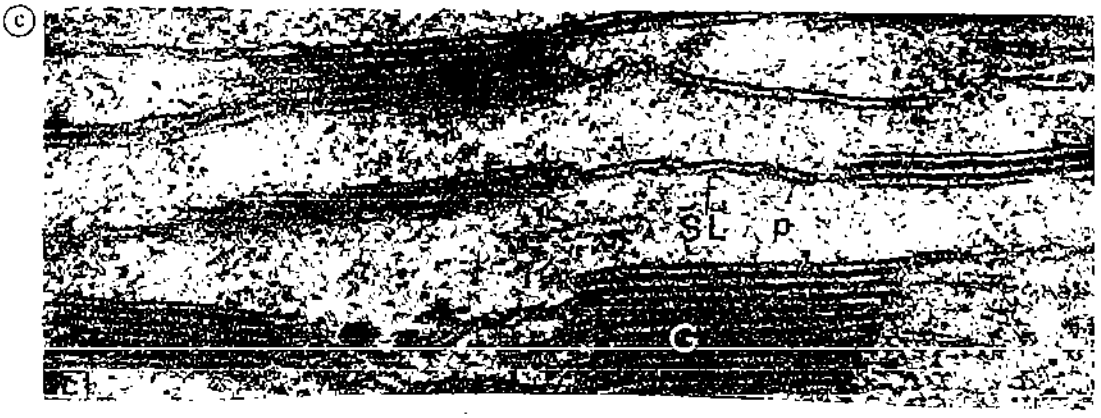



Fig. 13.25 : Organisation for photosynthesis. Photosynthesis takes place in the chloroplasts of plant cells. The thylakoid system of the chloroplast is the site of the light reactions, whereas the stroma is the site of the synthesis reactions. Photosynthetic pigments are precisely arranged in the thylakoid membrane to form light-harvesting photosystems. The reaction centre of each photosystem receives energy from surrounding antenna pigments. (b) E M photograph of chloroplast (c) Thylakoids enlarged (by Swadesh Taneja, 1973)

some fifty years ago, when he carried out the flashing light experiments and measured $O_2$ output per flash. Emerson became interested in discovering as to how many chlorophyll molecules are required to evolve one oxygen molecule. He found that even when experiments were conducted under the best conditions the ratio of number

of chlorophyll molecules to a molecule of $O_2$ evolved was always between 2,000 and 2,500 corresponding to a value of about 250-300 per quantum of light absorbed (this calculation assumes that 8 quanta are required for one $O_2$ molecule to evolve). Clearly, chlorophyll molecules are in great excess and if the photosynthetic unit was made, let us say of 250 chlorophylls, for one chlorophyll molecule (of the special pair) actively participating in electron transfer, 249 molecules were "standby" molecules. This immediately led to the concept of existence of photosynthetic units comprising one chlorophyll molecule as part of a "reaction centre" and the others forming an antenna (Fig. 13.26).

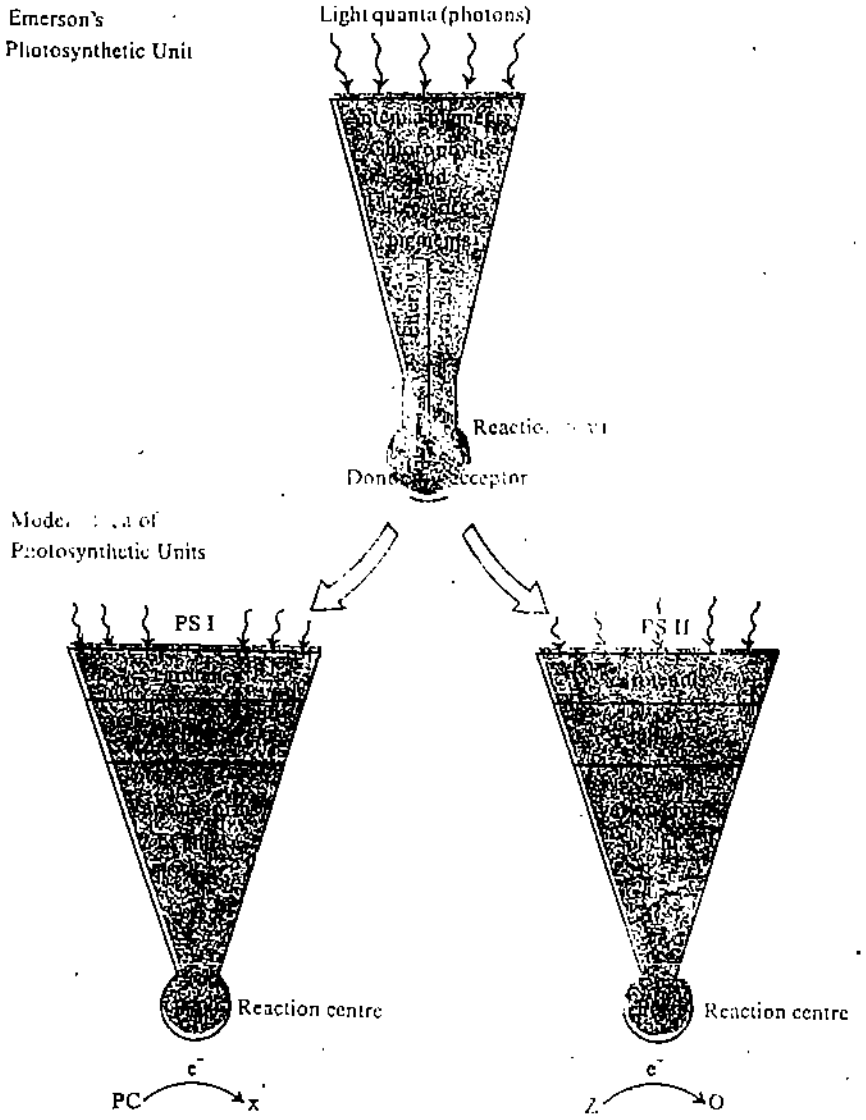



**Fig. 13.26 : The photosynthetic unit. At the top is a simplified view of the photosynthetic unit as conceived by Emerson and coworkers and representing the antenna pigments and the reaction centre. Below is a modern version of the photosynthetic unit. The diagrams depict only the contrast and distribution of the photosynthetic pigments. It is well established now that there are two photosynthetic units, each containing carotenes, chl *b* and various forms of chl *a*. The chlorophylls at reaction centres are called $P_{700}$ and $P_{680}$.**

The new electron microscopic observations employing the freeze-fracturing technique show that particles of approximately the predicted size of photosynthetic units do exist in the membranes. The first such observations with clear images of particles came in the late sixties. The particles were called "quantasomes" as it was thought that each can catch one quantum of light energy (Fig. 13.27). Since it is now recognised that there are two photosystems, the term quantasome is no longer in much use. Instead, now one refers to them as "photosystems" and further electron microscopy studies show that the particles are not homogenous but are at least of two types, corresponding to the currently accepted concept of two photochemical reactions. Photosystem II is the larger and shows rather regular crystalline structure when viewed from the luminal side. On the other hand, the smaller particles are

thought to represent photosystem I. Other particles may represent the cytochrome $b_6f$ complex as also the ATP synthetase complex which carries out photophosphorylation (Fig. 13.28).

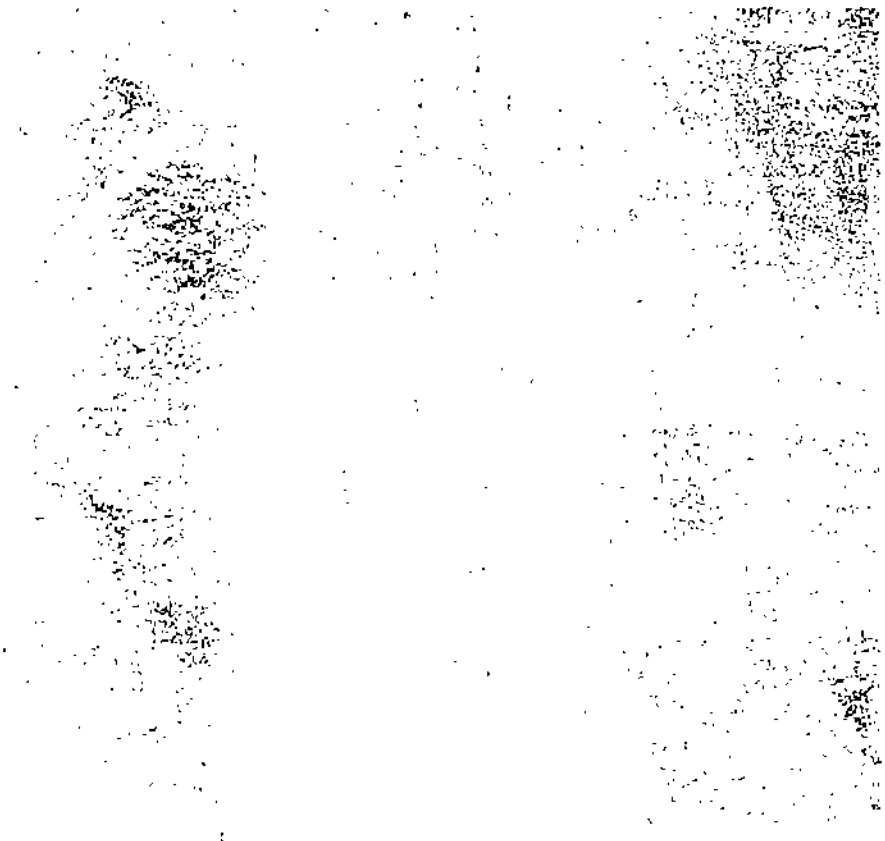


**Fig. 13.27 : Electron micrographs made by employing the freeze-fracture and freeze-etching methods to show the photosynthetic units. The photograph on top was made by Roderic Park and Biggins and they called the units "quantasomes".**

Unfortunately, at present, even the best electron microscopic techniques do not have sufficient power to resolve the inner sub-structure of these particles. Therefore, biochemical techniques are being relied upon to elucidate the structure. The arrangement illustrated in Fig. 13.29 is due largely to the work of biochemists and molecular biologists. The basic feature of the structure of photosystems I and II is that there is an inner core complex (called the reaction centre) which contains the "daddy" chlorophyll molecules and which participates in all electron transfer reactions (the daddy chlorophyll molecules here undergo photoionisation). The core is, however, surrounded by the antenna or the light harvesting complex, where extra pigment molecules are located. A photon is more likely to be absorbed by an outer chlorophyll molecule, but according to the principles of physics we have discussed earlier, the energy is ultimately trapped by the reaction centre chlorophyll molecules. The movement of electron across the thylakoid membrane is shown in Fig. 13.30

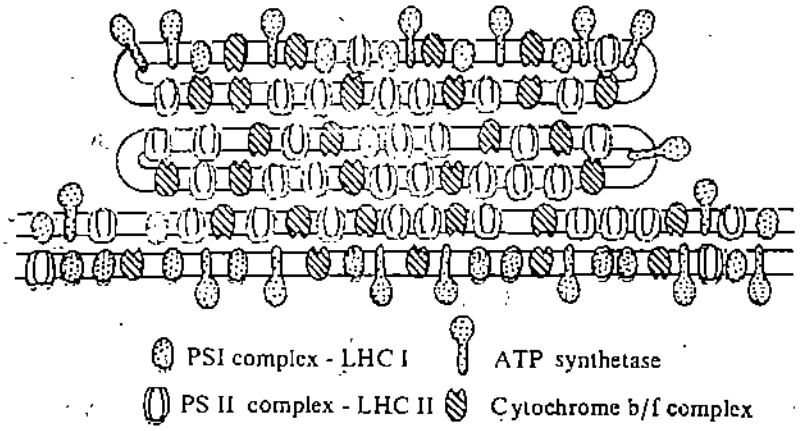



**Fig. 13.28 : Schematic diagram to show the location of PS I, PS II systems in the thlakoid membrane. Shown also are cyt $b_6/f$ and ATP synthetase complexes. Note that PS II is located mostly in appressed membranes.**

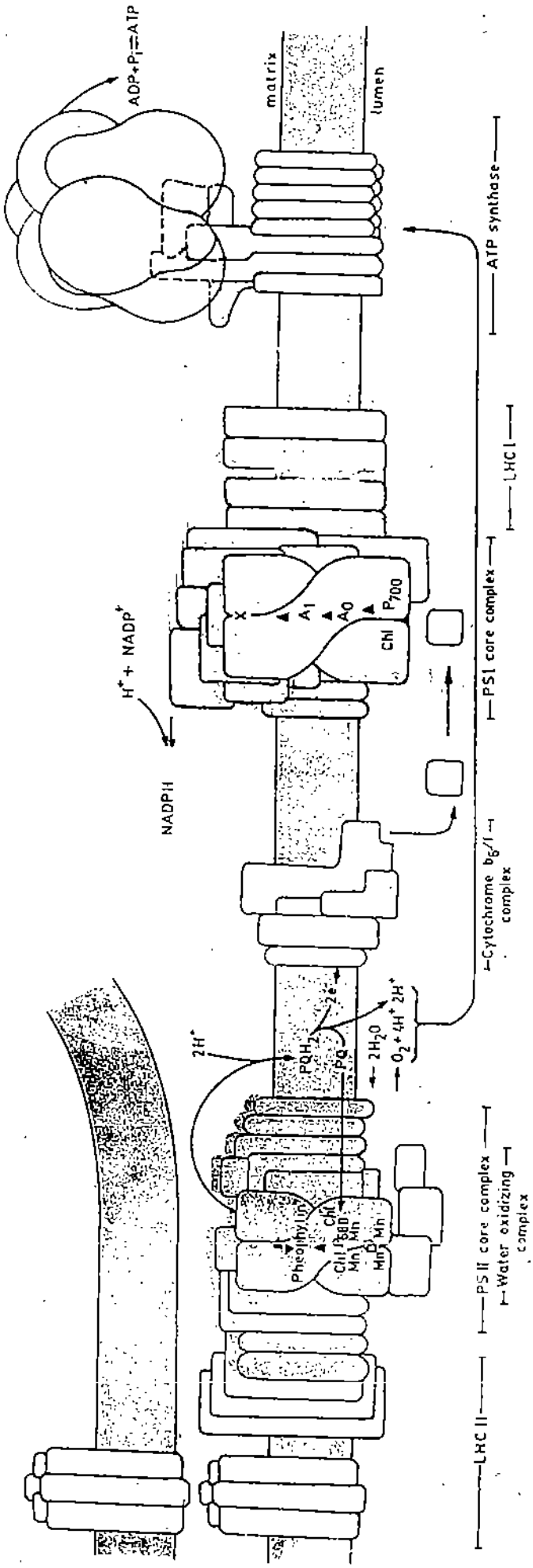



Fig. 13.29 : A more detailed representation of the structure of the photosynthetic machinery. The complexes from left to right are photosystem II, Cyt $b_6/f$ complex, photosystem I and ATPase complex Each complex consists of various polypeptides, the precise orientation of which still remains to be determined. Electrons are transferred from photosystem I to Cyt $b_6/f$ complex via a pool of plastoquinone (not shown) and from the Cyt $b_6/f$ complex to photosystem I via plastocyanin which is thought to be mobile.

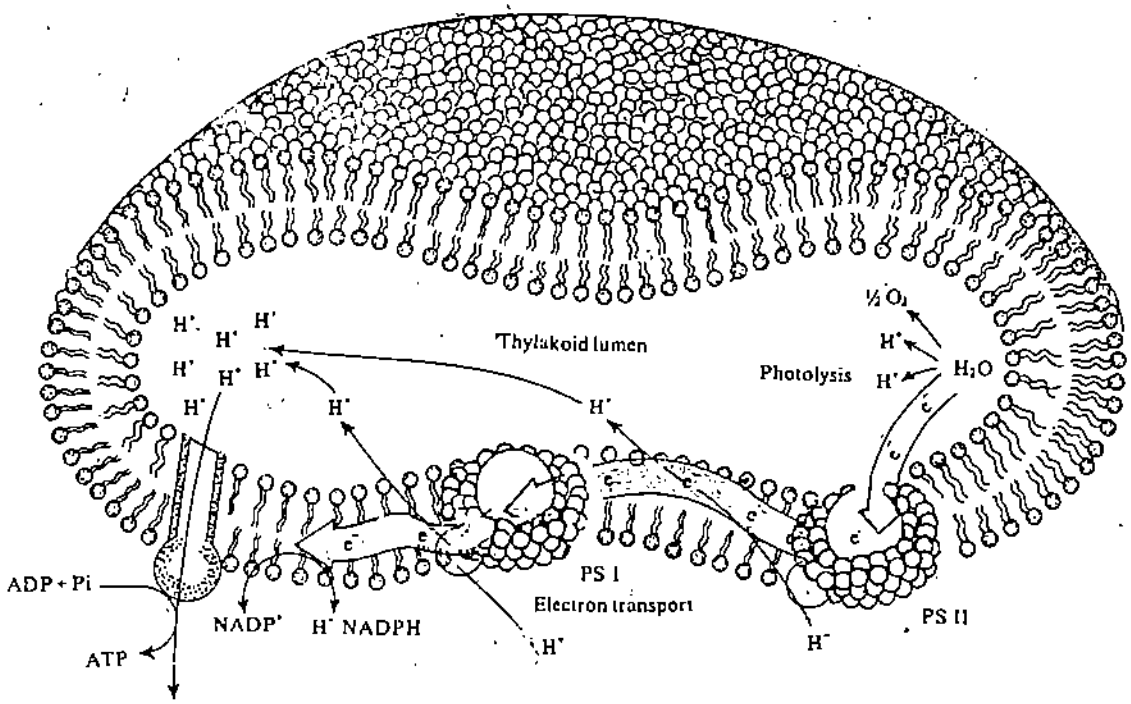



**Fig. 13.30 : Illustrates how electrons are transferred from water to $NADP^+$ with the help of light energy captured in photosystems I and II. The diagram also illustrates how ATP is synthesized as a result of a concentration gradient of protons which develops due to splitting of water. As the protons escape through the proton tunnel of ATPase, ATP is synthesized.**

While the basic design is similar for both photosystems, the detailed structures are naturally different. Indeed, the structure of each photosystem is unique and rather complex. By electrophoresis of purified photosystems, but pretreated in such a way as to disassemble all components, many unique polypeptides have been resolved in each photosystem. For example, in photosystem II a few polypeptides are concerned with $H_2O$ splitting complex which are absent in photosystem I. Similarly, certain polypeptides in photosystem I have no corresponding components in photosystem II. However, it must be emphasised that the evidence for detailed structure is mostly indirect and highly speculative. The technique most extensively employed by biochemists for unravelling detailed structure of macromolecules or their complexes is X-ray crystallography. However, researchers of photosynthesis have faced considerable difficulty in crystallising the photosystems, essential for such work. Nevertheless, three German scientists, Hartmut Michel, Johann Deisenhofer and Robert Huber have, recently provided a detailed structure by X-ray crystallography of the bacterial photosystem, fetching them a Nobel prize. Similar work has yet to be done in higher plants.

**SAQ 7.**

In column 1 we have listed processes or components related to photosynthesis, match them with their locations given in column 2.

| Column 1 | | Column 2 |
|---|---|---|
| i) | The "heart" of photosynthetic machinery | a) stroma |
| ii) | $C_4$ cycle | b) grana |
| iii) | P700 | c) thylakoid membrane |
| iv) | P680 | d) in the thylakoid membrane towards the lumen |
| v) | Electron carriers | e) from stroma to the lumen of thylakoid |
| vi) | Water splitting apparatus | f) transmembrane protein more towards stroma |
| vii) | Light harvesting complexes | |
| viii) | Site of release of NADPH | |
| ix) | Site of release of ATP | |
| x) | ATPase | |

## 13.9 PHOTOSYNTHESIS, AGRICULTURE AND HUMAN WELFARE

Finally, let us have a look at the relationship of studies on the mechanism of photosynthesis to agriculture and productivity. We shall first take up the question: What is the theoretical, maximal efficiency of photosynthesis. We shall then look into the actual efficiency prevailing in the field and in nature. And, if it is low, we shall enquire into the reasons and examine if anything can be done about it.

### 13.9.1 Efficiency of Photosynthesis

Many investigators have looked for an answer to the question of theoretical efficiency, from the time Warburg first undertook an enquiry into it in the twenties of this century. Warburg found a quantum requirement of 4 for every $CO_2$ molecule reduced (or $O_2$ evolved). From a purely theoretical angle, $4 \times 6 = 24$ quanta should be more than sufficient for reduction of 6 molecules of $CO_2$ since one mole of glucose contains 686 Kcal of energy, whereas 24 mole quanta of red light will have $24 \times 41 = 984$ Kcal, well above the maximal energy requirement. But if we consider the true quantum requirement for photosynthesis as 8, as established by later workers, the efficiency should be about 35%.

Nonetheless, in stark contrast to the above figure of even 35% in natural conditions, the efficiency of photosynthesis is rarely more than 1 to 2% of the total solar receipt of energy, which speaks poorly of man's ability to tap free energy from sunlight. There are many reasons for this. Firstly, of the total light energy incident upon a leaf, more than 50% consists of radiation which is not available for photosynthesis — for example UV, infrared and other such rays etc. Even of the photosynthetically active band of radiation (PAR) from 400 to 800 nm — for example, green light — is reflected from leaf surface (only certain algae have the ability to use this light). Some is simply transmitted through the leaf and a great deal is just converted to heat — in point of fact, nearly 20% of even the PAR is wasted Fig. 13.31. If we consider total

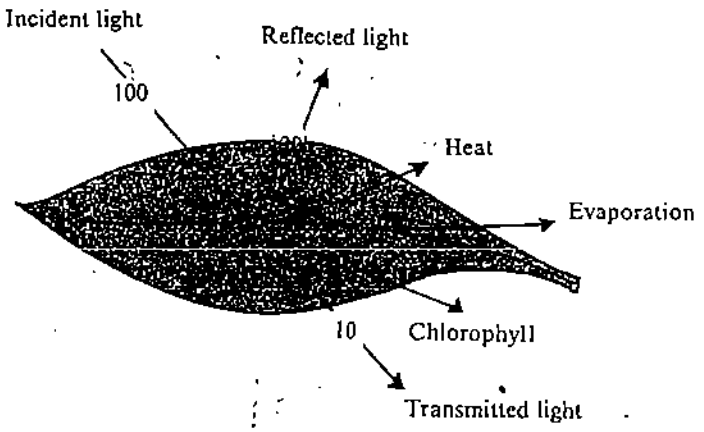



Fig. 13.31 : A diagram to show that distribution of light energy consumed or wasted at various steps shown as the ratio of incident light falling on a typical leaf directly.

plant productivity for example of a crop through a whole year, many other wastages are immediately apparent. Thus, there are times when land is lying barren without any plant cover and even if the land is fertile, most crops are seasonal and it is rather a short span of time when a plant has a fully developed leaf surface. Further, in a crop plant, not every part is edible or useful. If we consider only the usable parts, the efficiency becomes still lower. But then all tracts are not fertile and if we consider the drought affected areas and the deserts — on very large tracts of the surface of the earth there is no plant cover to intercept sunlight.

Of the various types of plants, the maximum efficiency is attained by trees (5%), because they provide a permanent cover in terms of leaf surface. For crops, the efficiency calculated for a season or on an yearly basis is much lower, of the order of only 1-2% or so. However, under the best conditions of irrigation, optimum use of fertilisers, and if photosynthesis is measured over a short period of time, the efficiency may reach up to 12% which is about one-third of the maximal theoretical

efficiency. Such levels of efficiency have, however, been possible in countries like Switzerland and Denmark, with advanced crop husbandry techniques. But the world average is much lower.

Many estimates have been made of photosynthetic productivity considered in the world as a whole. The total photosynthetically active radiation available is around 0.17%, equivalent to energy capable of fixing $0.4 \times 10^{11}$ tons of carbon per annum. One important point to know is that perhaps more than about one-third of the total photosynthesis is carried out in oceans. The average efficiency of photosynthesis in oceans is lower (almost half as much as on land) on a per square meter basis. However, since oceans occupy nearly two-third of the surface of the earth, their total contribution is quite significant.

### 13.9.2 Environment and Photosynthesis

We turn now to the question as to how man can have increased productivity via photosynthesis, in view of the fact that the world productivity is much lower than attainable theoretically. One of the pressing needs of agriculture is improvement of the microenvironment.

Many studies have been made of the role of environmental factors affecting photosynthesis. One of the most critical factors is $CO_2$ concentration which is only 0.03% in natural atmosphere. Blackman's experiments showed that if $CO_2$ concentration is increased, the photosynthetic rate increases, and even 10-20 fold higher concentration of $CO_2$ is by no means injurious to plants, at least in the short-term. Unfortunately, however, there is not much that can be done to increase the $CO_2$ concentration in large tracts of agricultural land or of forest areas, though $CO_2$ concentration is rising in the atmosphere because of use of fossil fuels and industrial activity. Very low or high temperatures, too, limit photosynthesis : whereas high temperature results in photobleaching and destruction of chlorophyll, low temperatures, although not destructive slow down the thermochemical dark reactions. Similarly, although water too seriously limits photosynthesis in many regions of the world (and stomates often close during the day preventing $CO_2$ from entering the leaf cell), again not much can be done. Irrigation can certainly help and schemes like Indira Gandhi Canal in the Western Rajasthan desert is fast changing the scenario converting barren lands into rich fields of bumper crops. Other better husbandry practices and better way of nutrition especially of nitrogen and phosphorus which are limiting in the environment, if corrected, can markedly improve overall photosynthesis. Another important research areas could concern with improvement of plant architecture, structure of canopy so that the light interception is more efficient. The erect leaves are also better than those that stretch out horizontally. One could also have dwarf varieties as in wheat since such varieties tend to have higher chlorophyll in the ears as compared to stems and stalks, other than the obvious advantage the dwarfs have. The second area of improvement is development of early maturing varieties so that over the years there is higher proportion of plant cover on the earth.

### 13.9.3 Agricultural Biotechnology

It would seem from the foregoing discussion that there is little scope for increasing efficiency of photosynthesis under existing conditions, unless the environment itself is improved in terms of increasing water supply or providing fertilisers etc. A question that we ask now is can we do anything to adapt a plant better to existing conditions. There is much hope that, with the rise of modern molecular biology and biotechnology, plants can indeed be modified such that the efficiency of photosynthesis or the yield can be increased in a permanent way.

At this point some brief remarks are appropriate on the molecular biology of chloroplast, also from the viewpoint of fundamental knowledge. Work in the sixties and seventies has shown that the chloroplasts, as also the mitochondria, have not only their own DNA, but they also have RNA and protein synthesising machinery. However, the size of chloroplast DNA is relatively small and is just enough to code for about 100 genes. The actual number of proteins that exist in chloroplasts is much larger, in thousands — and a great majority of the proteins therefore are encoded by the nucleus. There are, in fact, many interesting cases, for example in case of multi-subunit proteins or enzymes (such as Rubisco), where some of the polypeptides are encoded by the nucleus and others by the chloroplast, which demands a very high degree of synchronisation between the two cellular constituents. The unique origin

of such proteins provides an excellent opportunity for studying the biochemistry of such interaction and general regulation of chloroplast development. The various genes are gradually being identified both in the chloroplast and the nuclear genome. Since the chloroplast genome is smaller, it has been the focus of much work. In fact, it has been recently completely sequenced by Sugiura and co-workers in Japan in certain plants, namely *Marchantia*, tobacco and rice and most of the genes already identified. The work on nuclear genes encoding chloroplast proteins is now just beginning. When the important genes have been identified, one can expect research to modify them. How to make them more efficient, however, remains a task for the future.

From an agricultural viewpoint, one could give a few exmaples of future directions of research. One can possibly make Rubisco more efficient such that it does not accept oxygen and thus the process of photorespiration is avoided altogether. One could even attempt to transfer characteristics of $C_4$ plants into $C_3$ such as an active PEP carboxylase. Thinking of still other ways of enhancing photosynthesis perhaps one could engineer chloroplasts to live longer or divide more rapidly. Perhaps one could change the metabolic pathways so that one can enhance the synthesis of amino acids rather than largely manufacture carbohydrates. This modification can be of far-reaching consequence in management of global nutrition. Also, one could engineer plants resistant to herbicides by altering proteins in chloroplasts (which are often the target sites for their action), and without impairing electron transfer functions. Some genetically engineered herbicide resistant crops have in fact already been developed in the West, and it is likely to be big business.

The techniques for transferring foreign genes into nuclei are already will-established and, when nuclear-encoded genes are involved, their modification should not be too difficult. However, the stable transformation of the chloroplast genome poses a more serious challenge since the usual vectors — such as the Ti plasmid — do not stably integrate into chloroplast genome. Another difficulty is that the chloroplast genome is very compactly organised — often two genes reside in the same region of DNA and transcribe in opposite directions. It may therefore, be difficult to insert genes in the chloroplast without inactivating some already existing useful gene, even though some success in transferring a gene — purely as a test case, for example to correct a gene deliberately mutated — directly in chloroplasts by the particle gun technique has been reported. Perhaps a more useful strategy will be to transfer a required gene in the nucleus in such a way that the protein product ultimately finds its way into the chloroplast. For this, one can attach a region of DNA coding for signal polypeptides, which have the ability to lead a polypeptide chain smoothly into the chloroplast. In fact, most of the nuclear-encoded chloroplast polypeptides have some kind of a signal polypeptide chain attached to them, which makes it possible for them to enter the chloroplast. Man could adopt this strategy for *directed* genetic changes.

## 13.10 EVOLUTIONARY ASPECTS OF THE CHLOROPLAST

Finally, one can touch briefly on the evolutionary aspect of the photosynthetic process and of chloroplast itself. It is believed that photosynthesis preceded aerobic respiration. According to current evidence, in the primitive world there was not much of free oxygen. Organisms survived by virtue of anaerobic respiration, and gradually thus there was accumulation of carbon dioxide in the atmosphere. It is only then that photosynthesis started. Finally, when $O_2$ started accumulating in the atmosphere, aerobic respiration evolved.

Another fact of great interest is that the modern-day chloroplast probably represents a prokaryotic unicellular alga which invaded ordinary non-photosynthetic eukaryotic cells. There are indeed many pieces of evidence pointing towards a prokaryotic origin of chloroplasts e.g. the circularity of the chloroplast genome, existence of smaller ribosomes such as in bacteria, or of an extra set of tRNAs unique to this organelle. When the unicellular alga became an endo-symbiont, most of the genes (not essential any more for corporate existence) were gradually lost; and others — still essential — gradually moved to the nucleus. Some evidence that genes are still in the process of movement comes from a study of some lower plants. Here certain genes have been found in chloroplasts which have moved to the nucleus in the higher land plants. It appears that it is basically the genes for the photochemical machinery which continue

to reside in the chloroplast. Perhaps this is because proteins are highly hydrophobic and it is thus easier to synthesise and assemble them in the thylakoid machinery rather than move them through the aqueous environment of the cytoplasm.

## 13.11 CONCLUSIONS

To conclude, photosynthesis is the most basic of all phenomena in the living world. Photosynthesis is a subject which has attracted the attention of the scientists from all disciplines such as from physics, chemistry, biochemistry, molecular biology, genetics, botany, zoology, and evolution. A great deal has been learnt and the outline of the process is now sufficiently clear. Despite so much work, however, the real structure of the photosystems, and the placement of chlorophyll and other constituents, remains uncertain. This remains a task for the future.

Biotechnology and genetic engineering have opened up a new approach towards increasing yield. However, increasing efficiency of the plants by these techniques remains, also, a task for the future since we need to know a lot more about the molecular biology of chloroplasts, before we can intelligently modify the photosynthetic machinery to our advantage. What is certain is that because of the rich interaction of physics, chemistry, biochemistry and molecular biology, photosynthesis will continue to remain a subject to great interest of scientists for decades to come.

## 13.12 SUMMARY

- Photosynthesis is the process by which carbon dioxide and water are assimilated into carbohydrates with the help of energy of sunlight. The reaction is carried out by all green plants including the algae and is of the greatest significance — since not only animals derive their food through this process, but also our energy requirements are largely met by fossil fuels like coal and petroleum.
- The photosynthetic process is known to consist of two types of reactions — the light and the dark reactions. In the former, the key role is played by four pigments — chl *a*, chl *b*, xanthophylls and carotenoids — all located in thylakoid membranes of chloroplast. Carotenoids and xanthophylls absorb the blue region of the spectrum whereas chlorophylls absorb both blue and red regions. Since chl *a* absorbs light of the longest wavelength, it can collect the energy captured by all other pigments. From chl *a* the energy is transferred finally to a special form of chl *a*.
- The capture of photons by chlorophyll pigments ultimately leads to the photolysis of the water molecule resulting in the release of electrons, protons and oxygen. Splitting of a water molecule follows ionization of the special chl *a* molecule, since on release of the electron chl *a* becomes positively charged (chl $a^+$). An electron is regained from water; the chl *a* molecule, therefore, acts as a pump that drives electrons from $H_2O$ to reduce $CO_2$ and synthesise carbohydrates. The energy for driving the pump comes from sunlight. The common electron carrier $NADP^+$ serves as the final acceptor of electrons (and it is through NADPH that reduction of $CO_2$ occurs).
- In reality, the transfer of electrons from $H_2O$ to NADPH is mediated by two separate photochemical reactions through two sets of pigment molecules organised in the form of photosynthetic units, representing the PS I and PS II. Two special kinds of chl *a* molecules exist in these photosystems. The special chl *a* molecule in PS II maximally absorbs at 680 nm and is called $P_{680}$ whereas the special chl *a* molecule in PS I absorbs at 700 nm and is called $P_{700}$. Transfer of light energy to $P_{680}$ and $P_{700}$ is greatly facilitated by the existence of antennae attached to the photosystems and each of which comprises about 250-300 chlorophyll molecules bound in special pigment-protein complexes. Photolysis of water takes place in PS II and the electron is transferred via a cytochrome $b_6/f$ complex, to complex, to PS I. Here, an electron can gain further energy by absorbing energy from an additional photon and then reach $NADP^+$ to reduce it to NADPH.
- To drive the dark reactions of photosynthesis, apart from NADPH, ATP is also necessary. Since photosynthesis proceeds at a much faster rate than respiration, green plants are able to synthesise ATP by a process known as photophos-

phorylation, independent of oxidative phosphorylation carried on in mitochondria. This is made possible by the release of protons during splitting of $H_2O$, and which results in the generation of a proton gradient (with a high concentration of $H^+$ in the luminal space of thylakoids). As the gradient dissipates — by the movement of protons through the channel of an ATPase complex outside the stroma — ATP is synthesised by the "chemiosmotic" mechanism.

- The fixation of carbon dioxide occurs in most plants via the Calvin cycle through ribulose bisphosphate carboxylase. Ribulose bisphosphate (RuBP, a 5-carbon sugar) serves as the primary acceptor. Since the intermediate is unstable, the first stable product is a 3-carbon compound, phosphoglyceric acid, which is reduced to phosphoglyceraldehyde with the help of NADPH generated in the photochemical reactions. Two molecules of phosphoglyceraldehyde combine to form a molecule of glucose by reversal of reactions in glycolysis. Some of the phosphoglyceraldehyde molecules, however, serve to regenerate the acceptor, RuBP, through reactions of the Calvin cycle.
- The $C_4$ plants are a special group of plants (mainly represented by the grasses) where the primary acceptor of carbon dioxide is a 3-carbon acid, phosphoenol pyruvic acid, and the first product is a 4-carbon compound, oxaloacetic acid, which is reduced to malic acid. The enzyme which fixes $CO_2$ in these plants is called PEP-carboxylase. Since this enzyme has higher affinity of $CO_2$ (than RuBP carboxylase), the $C_4$ plants carry out photosynthesis more efficiently. The 4-carbon acids cannot, however, be converted to carbohydrates directly. Instead, the fixed $CO_2$ reenters the Calvin cycle by a reaction which is still not understood properly. But, it is well established that in a typical monocot leaf, the outer cells have PEP carboxylase; on the other hand, the bundle sheath cells — more centrally located — have RuBP carboxylase. The organic acids are believed to be transported to bundle sheath cells, when the $CO_2$ is released to be refixed by the $C_3$ cycle. The CAM plants (a group to which cacti and many other succulents belong) essentially constitute a variant group of $C_4$ plants in which stomates open at night to allow $CO_2$ fixation by PEP-carboxylase whereas the process of reentry of $CO_2$ into Calvin cycle and reduction of PGA to phosphoglyceraldehyde by NADPH takes place during the day.
- The enzyme RuBP carboxylase, is unable to discriminate totally $CO_2$ from $O_2$, leading to an oxygenase activity and photorespiration. Often, therefore, instead of two molecules of PGA only one molecule of PGA is formed — the other product being a molecule of 2-carbon glycolic acid. Two molecules of glycolic acid can be recycled to yield another molecule of phosphoglyceric acid with the loss of molecule of $CO_2$. However, because of the more efficient process of $CO_2$ fixation in $C_4$ plants, any $CO_2$ released in light is wholly recaptured by PEP-carboxylase. Hence, they do not show photorespiration. One of the goals researchers have is to convert $C_3$ plants into $C_4$ plants and eliminate photorespiration so as to conserve fixed carbon lost during the process.
- In recent years, the techniques of electron microscopy and X-ray diffraction crystallography have allowed us to understand the structure of the photosynthetic machinery in surprising detail. The photosynthetic units — originally proposed on purely theoretical grounds — can be seen in electron micrographs. In bacteria even a detailed model of the reaction centre of the photosynthetic machinery has become recently available. It is expected that in the near future, a detailed model will become available also of the organisation of the photosynthetic machinery in higher plants.
- The area of molecular biology of chloroplast is also developing at a rapid rate and not only proteins, but each gene related to photosynthesis is being identified. Simultaneously, genetic engineering techniques to transform the photosynthetic machinery in chloroplasts are also being developed and it is possible that photosynthesis can be made more efficient in future in selected economically useful plants.
- Finally, the origin of the chloroplast is very intriguing subject from the viewpoint of evolution and taxonomy. It is widely believed that chloroplasts represent primitive prokaryotic cells which were capable of carrying out photosynthesis and which got entrapped in the eukaryotic cell giving rise to higher plants such as we know today.

## 13.13 TERMINAL QUESTIONS

1. The photochemical reactions in grana capture light energy and convert it into chemical energy as ATP and NADPH. What is the necessity of carbon fixation?

.....

.....

.....

2. Give the raw materials and end-products of the following reactions of photosynthesis.

| Photosynthesis Reactions | Raw Material | End-Product |
|---|---|---|
| i) PS I | | |
| ii) PS I followed by electron transport | | |
| iii) PS II | | |
| iv) PS II followed by electron transport | | |
| v) Cyclic phosphorylation | | |
| vi) Carbon fixation | | |

3. Compare photorespiration and mitochondrial respiration.
(**Hint** : Compare with respect to substrate, enzyme, waste products, gain of energy and loss of carbon.)

.....

.....

.....

.....

.....

.....

## 13.14 ANSWERS

**Self-assessment Questions**

1) a) i) d, ii) c, iii) b, iv) a

2) a) i) Determination of relative photochemical efficiency of different wavelength of visible spectrum. It closely matched with the absorption spectrum of chlorophyll.

ii) Exposure of portions of algal filament to different wavelengths and measurement of photochemical efficiency ($O_2$ evolution was measured by differential accumulation of aerobic motile bacteria on the filament).

b) The colour of an object is due to the light it reflects. The three organisms — purple sulphur bacteria, blue green algae and leaves of barley reflect purple, blue-green and green light respectively and hence they appear purple, blue-green and green. They absorb the colours of visible spectrum other than the colours they reflect. Since the action spectrum and absorption spectrum generally overlap, therefore, the spectra represent a) leaves, b) blue green algae, and c) purple sulphur bacteria.

3) a) i) The value of $Q_{10}$ was more than 1.

ii) Splitting of water, formation of ATP and reducing power.

b) i) Photolysis, $H_2O$, Van Niel, sulphur ii) $H_2O$, $O^{18}$

iii) $NADP^+$, NADPH, ATP, ADP, Pi. iv) Hill reaction

4) a) 1) Quantum requirement (number of light quanta required for each molecule of $O_2$ produced was 8 instead of 4.

2) The quantum yield drops at higher wavelengths (red region) but it is abolished/when a beam of shorter wavelength was simultaneously given.

b) i) one, two
ii) electron donor
iii) antenna complex, reaction centre chl *a*
iv) 680, 700, $NADP^+$

c) i) drops, ii) enhancement, iii) lower

d) i) F, ii) F, iii) T, iv) F, v) T, vi) F

5) a) i) Radioactive, ii) Radioautography, iii) molecular dissection, iv) Geiger-Müller

b) i) PGA, ii) RuBP, iii) Glucose, iv) 18, v) 12, vi) 12, vii) one

6) a) i) Light, ii) RuBP, iii) higher concentration of $O_2$ in leaves, iv) high intensity of light, v) $C_3$ plants, vi) peroxisomes

b) i) c, ii) a, iii) c, iv) b, v) a

c) i) phosphoenol pyruvate ii) pyruvate carboxylase
iii) ATP iv) chloroplasts
v) $C_3$ pathway, bundle sheath cells vi) RuBP carboxylase
vii) network, membranes viii) photosynthetically more efficien

7) i) b, ii) a, iii) c, iv) c, v) c, vi) d, vii) c, viii) a, ix) a, x) f,

**Terminal Questions**

1) Firstly, the storage of energy in the form of carbohydrates is much more convenient and lot more energy can be stored in this form. Secondly, carbon skeleton of carbohydrates is needed for various biosynthesis.

2)

| | Photochemical Reaction | Raw Material | End Product |
|---|---|---|---|
| i) | Excitation of PS I | hν + light harvesting pigment complexes + $P_{700}$ | excited electrons |
| ii) | PS I followed by electron transport | hν + (light harvesting pigment complexes + $P_{700}$) + $NADP^+$ | NADPH |
| iii) | Excitation of PS II | hν + light harvesting pigment complexes, $P_{680}$ | electrons |
| iv) | Excitation of PS II followed by electron transport | hν + light harvesting pigment complexes + $P_{680}$ + ADP + Pi | ATP + electrons |
| v) | Cyclic photo-phosphorylation | $H^+$ reservoir, ADP + Pi | ATP |
| vi) | Carbon fixation | RuBP + $CO_2$ + ATP + NADPH | Sugars + ADP + Pi + $NADP^+$ |

3)

| Item | Respiration | Photorespiration |
|---|---|---|
| Substrate | Carbohydrates, fats, proteins or their monomeric units + $O_2$ | RuBP |
| Enzymes | Various enzymes of glycolysis, TCA cycle, electron transfer chain | RuBP carboxylase/ oxygenase |
| Loss | Loss of carbon | Loss of carbon |
| Gain | Energy 36 ATP/glucose | No ATP |
| Waste products | $CO_2$, $H_2O$ | $CO_2$, $NH_3$, |

# UNIT 14 TRANSPORT IN THE PHLOEM

**Structure**



## 14.1 INTRODUCTION

So far you have learnt that one of the basic necessities of plants, water, is taken up by the roots. Another purpose served by the roots is to absorb water soluble mineral nutrients from the soil. Mineral nutrients move together with water in long distance pathways provided by the vascular system to reach the entire plant. Roots cannot feed themselves in the darkness of soil. Several other tissues in the plant body also lack photosynthetic apparatus completely or possess it partly in the sense that they cannot manufacture enough food required to support their life processes. Leaves on the other hand rely on roots for water and minerals but manufacture more food than they actually need. Hence, leaves serve as the source of food for other tissues which may store excess of it so that it can be used by plant for the perpetuation and spread of species. Now, how is such a complementary division of labour in tissues particularly between roots and leaves made possible? And how are the organic molecules made by photosynthesis and other biosynthesis are distributed in various parts of plants?

In this unit you will learn about the delivery of food from leaves to various parts of plants such as seeds, fruits and storage tissues. What sort of questions come to your mind when you think about transport of food? Perhaps you would like to know the following: i) Where does the translocation occur in plants? ii) What sort of materials are transported? iii) What is the mechanism of translocation? iv) What are the factors, internal or external, influencing the transport? For instance, how do water melons or grapes manage to be so juicy while the leaves just below are papery and non-tasty?

In this unit we will try to find answers to the above questions.

### Objectives

- describe transport network for the translocation of food material,
- explain the concept of source and sink with respect to translocation,
- sketch and describe the structural and functional organisation of phloem particularly near the region of loading and unloading in the sieve tubes,
- list substances transported by phloem conduits,
- describe various experiments performed for the study of phloem transport,
- explain and compare various models proposed for the mechanism of translocation through phloem.

## Study Guide

For the study of this unit you should have at least a rudimentary knowledge of hydrodynamics: flow of viscous fluids in capillaries, concept of turgor pressure, osmotic pressure and water potential. Some of these have already been covered in the previous units. You should also know the anatomy of root, stem and leaves and the various types of cells that constitute them; so keep Block 4 of Cell Biology course handy for ready reference.

## 14.2 WHY IS TRANSPORT NECESSARY?

As you know leaves produce photoassimilates and support various tissues including roots. The excess photoassimilates and metabolites stored in specialised tissues produce fruits and seeds which germinate and regenerate the plant in the appropriate season. The leaves at the canopy of a tree are at a distance from roots. This can vary from a few millimeters to more than a 100 meters. All in all we need an extensive transport system to carry the products of photosynthesis and nitrogen metabolism over medium and long distances. Diffusion is too slow a process to achieve this task over such large distances; what is required is a convective flow in the specialised vascular system so that the photosynthates reach all the needy tissues. The transport system has to be as extensive and ramified as the arterial and venous network in an animal body. However, plants are devoid of a specialised pump such as heart meant for blood circulation. Though we have not yet fully understood how plants achieve this goal, they do carry out this exceedingly difficult task without a heart. Leaves manage to feed the tissues over vast expanses of the plant body with photosynthates necessary to fulfil their requirement.

## 14.3 THE TRANSPORT NETWORK

You may know that plants have elaborate plumbing network which serves to link their various parts. Phloem forms such a network for the transport of food in higher

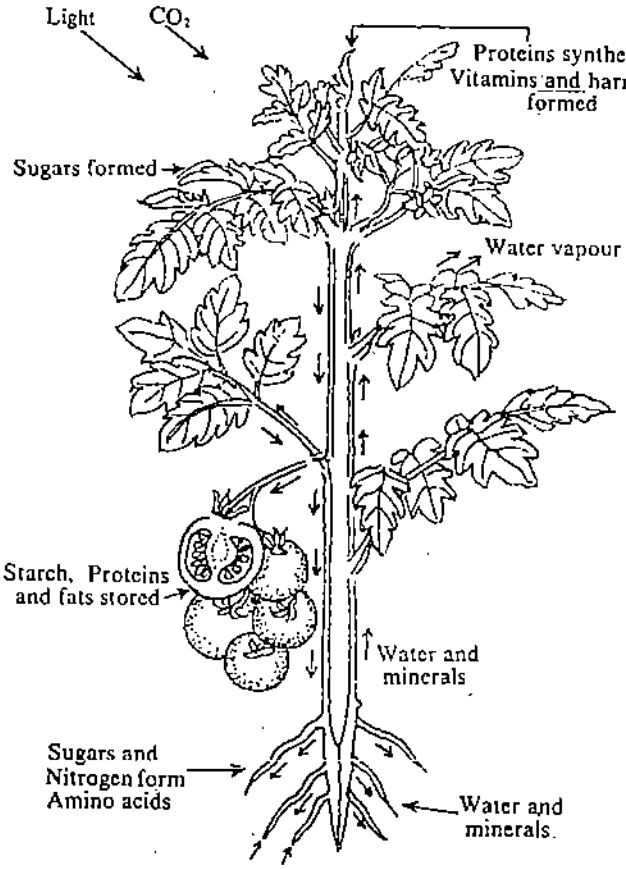



**Fig. 14.1 : Plants are devoid of a heart but carry out massive transport processes within their body. Transpiration stream (→) transports water and inorganic nutrients from their subsoil roots to the shoots, sometimes more than a 100 meter high. A viscous solution of metabolites generated in the leaves from the assimilation of carbon from $CO_2$ by the capture of solar energy in the process of photosynthesis, is distributed to sink tissues by translocation (—→).**

plants. It runs parallel to other major transport system — the xylem (Fig. 14.1) which takes up water and mineral nutrients from roots and distributes them throughout the plant. The sieve tubes in phloem are joined into long interconnecting pipelines. Unlike water conducting xylem cells, sieve tube members are alive at maturity. The two long distance transport systems and their radial branching establish supply lines to every small region of the plant body.

The direction of transport in phloem varies during the developing stages of the plant. For example, a young seedling moves food upwards from seed to juvenile leaves until they begin to synthesise food. While during fruit formation or for storage in roots or stem the food moves in downward direction. So food moves from the tissues where it is in excess called source to those where it is needed — called sink (Fig. 14.2).

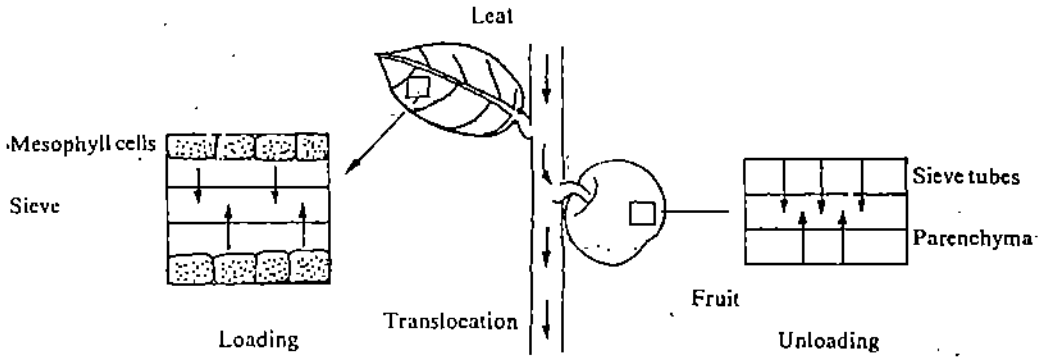



Fig. 14.2 : Source and sink, a schematic diagram.

Essentially, the phloem transport can be subdivided into the following:

i) The "loading" of the organic nutrients from the mesophyll cell into the phloem of the leaves,

ii) Its translocation over long distances to the tissues awaiting supply,

iii) The "unloading" of the nutrients from the phloem into the cells of the sink.

All these three processes are interlinked. The rate of transport is not constant but depends on the metabolic needs of the sinks on one hand and on the rate of photosynthesis in the leaves — the sources on the other. Phloem transport is a very complex phenomenon and we may indicate, at the beginning itself, that in spite of intensive research, it is not fully understood even today. As such this field offers a very challenging subject for future research in plant anatomy and physiology.

## 14.4 ORIGIN AND DELIVERY — THE SOURCE AND THE SINK

We have stated above that transport of photosynthates starts from leaves and ends up in one or the other sink tissue. Though this is true, however, it is necessary to define the concept of sinks and sources at any given point of time. For this consider an experiment on the plant *Saxifraga* (Fig. 14.3). This plant spreads by giving out long offshoots with a bunch of leaves and a potential root system at the end. If the latter comes in contact with moist soil, it develops into a self-sufficient shoot-root system. However, so long this has not happened, the long link provides water and minerals taken up by the root of the parent plant to the juvenile bunch of leaves. The phloem transport system initially provides the buds of the distant shoot with the necessary nutrition. When fully grown, the new cluster of leaves becomes excess producers of photosynthates and contributes their output to the parent plant. If we shut off the light falling on the parent plant or the off-shoot (Fig. 14.3b and c) over a substantial period of time, so that either of them is incapable of photosynthesis, which way will the phloem translocate the food? We are sure that you have already arrived at the correct answer. It is observed that translocation is from the system in which photosynthesis takes place to that in which it is prevented. Thus, the two systems of leaves can be sink at one time and source at another. The direction of phloem transport in the above case is, therefore, dependent on the relative production of photosynthates in the two systems of leaves.

Similar situations among typical sinks also exist. In tropics when the new leaves in deciduous plants emerge in the spring on a denuded tree, they need the supply of nutrients. What could be the possible source of nutrients in the absence of pre-existing mature leaves? Naturally from the stored stock of metabolites in sink tissues. In spring, both the xylem and the phloem receive metabolites from all over the plant body and deliver them to the buds.

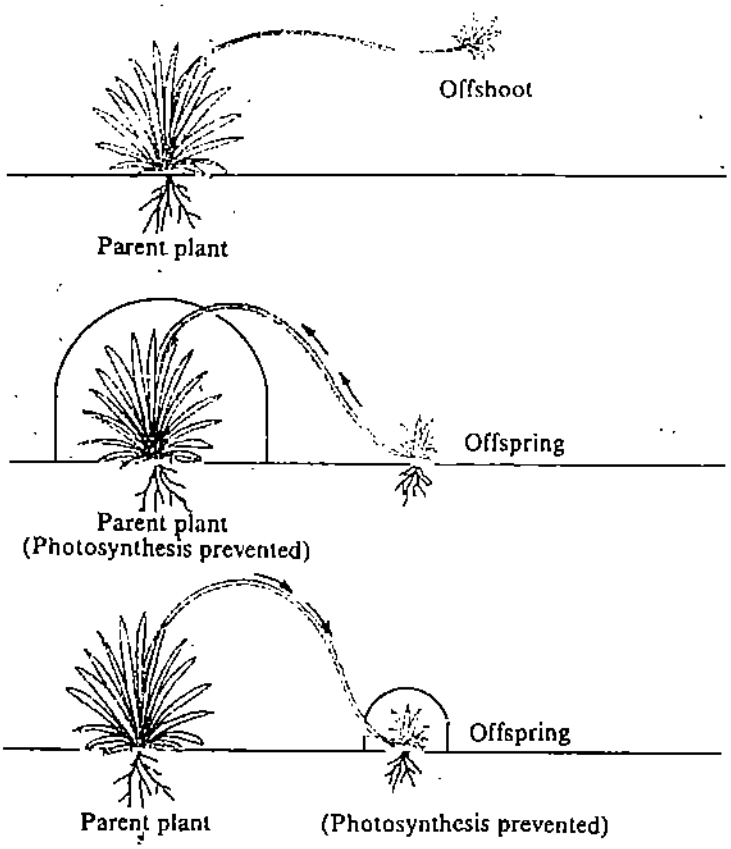



**Fig. 14.3 : a) *Saxifraga* plant with an offshoot, b) Supply of food by the offspring, c) Supply of food by the parent plant.**

SAQ 1

i) Plants need extensive ... and ... metabolites to their various parts.

ii) The two elaborate transport networks ... and ... run parallel to each other in plants.

iii) ..... are arranged in a linear array running vertically through the length of phloem.

iv) Phloem translocates food from ... to ...

v) Loading in sieve tube takes place at the ..... and unloading at the .....

## 14.5 PHLOEM — STRUCTURAL AND FUNCTIONAL RELATIONSHIP

In this section we will explain the structural and functional relationship of phloem and compare it with xylem. The location of phloem with respect to other tissues can be examined by a single transverse section, but to get an idea of their longitudinal route from stem to various branches requires a painstaking study of innumerable serial transverse sections along the height of the plant. Fig. 14.4 shows a schematic ...ing envisaged from serial-sections. Note the branching, criss-crossing and ...ification of vascular bundles. The longitudinal branching and fusion of phloem, ...rticularly at the node show that phloem of one side of a stem can effectively cross

Callose : It is a special carbohydrate polymer which is deposited at the sieve plate usually around plasmodesmata and forms artefacts in the pores.

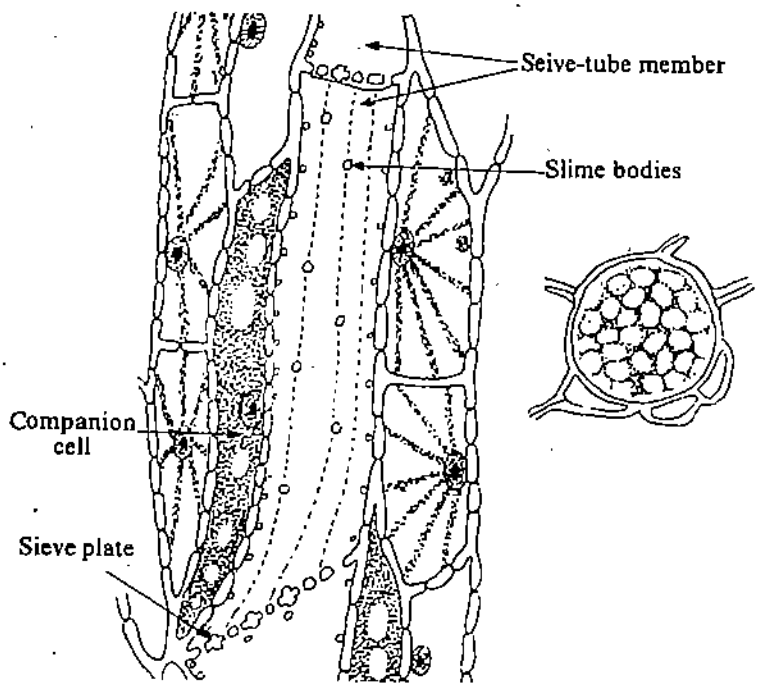



**Fig. 14.5 : Fine-structure of phloem showing a full sieve tube element, its companion cell and other cells of phloem. The sieve plate pores are circular openings in the cell walls of adjacent sieve element (inset).**

over to the opposite side on its longitudinal path. We know that phloem consists of 4 types of cells: i) sieve elements, ii) companion cells, iii) phloem parenchyma and sometimes iv) phloem fibres. The metabolites flow only in sieve elements which resemble hollow pipelines referred to as cellular channels (Fig. 14.5). The sieve tube elements are devoid of nucleus but plastids and mitochondria are present. The cell lumen of the sieve element appears open except for transcellular strands that merge with neighbouring sieve cells at the sieve pore plate via plasmodesmata (Fig. 14.6). The continuity of plasmalemma and cytoplasm makes the sieve tubes a longitudinally extending symplast. Do you remember as to what the xylem vessels form; symplast or apoplast?

Phloem carries a viscous solution of metabolites mainly sucrose. The sieve tube elements are of a much smaller radius than xylary vessels and tracheids. The free flow of the viscous phloem sap is apparently not affected by the narrow sieve plate pores.

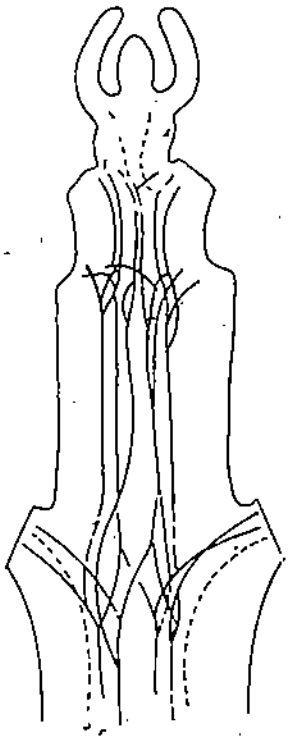


**Fig. 14.4 : Branching, criss-crossing and remification of vascular bundles in a stem of *clematis vitalba* (schematic).**

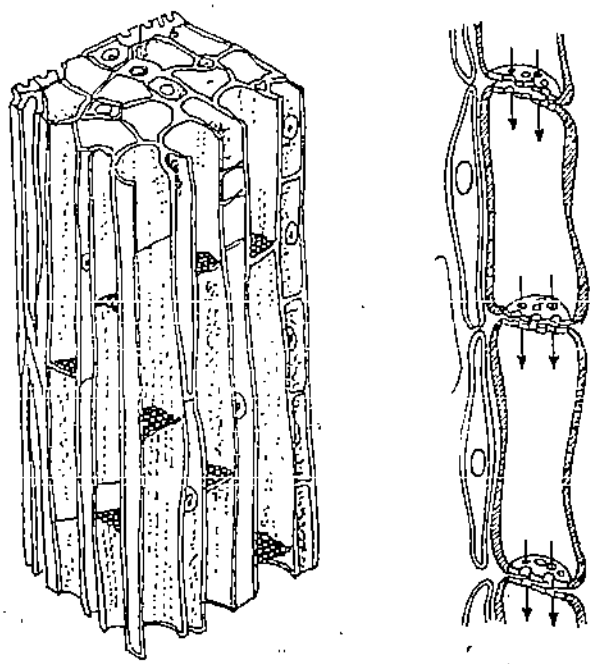


**Fig. 14.6 : Detailed 3-dimensional schematic representation of phloem.**

EM pictures reveal that sieve plate pores are callose-lined and are often occluded with endoplasmic reticulum and phloem protein (P-Protein). Earlier it was not conclusively known whether the sieve plate pores are normally occluded or the occlusions were possible artefacts introduced while preparing specimens for electron microscopic studies. Later, using special techniques (that avoid artefacts) it has been suggested that sieve pore plates are open in normal growing plants and flow of solutes occurs through unplugged sieve plate pores. When plants are cut or injured, P-proteins flow to the sieve plate thereby blocking the pores. In their absence, metabolites would continue to flow out of the wounded part and plant will eventually die. The P-protein apparently play a role similar to blood protein fibrin. In the absence of fibrin the person would bleed to death.

Each sieve element is compulsorily associated with a neighbouring companion cell. Both are usually connected with many plasmodesmata. The two arise from a common cell. The function of companion cells is not known but they live as long as the sieve cells live. In this sense, they are true companions. They contain the same concentration of sucrose and have the same osmotic potential as the sieve tube elements. The companion cells have dense cytoplasm and numerous cell organelles, specially mitochondria which indicate that they have a high metabolic activity. It is very likely that companion cells support translocation. Furthermore, the numerous plasmodesmatal connections between the two cells support the idea that companion cells supply large quantities of food materials to the sieve tube elements and provide the latter with proteins and enzymes which they cannot synthesise.

In some species at the phloem loading (near the sieve tubes of leaves) and unloading sites (near the sieve tubes into any sink) there are special kinds of cells called **transfer cells**. As the name suggests they play a major functional role in the transfer of metabolites from the source to the sink. These cells are modified companion cells or cells of phloem parenchyma. Their location in xylem or in phloem varies depending on the part of the plant but in general they are located at the critical sites of heavy transport of inorganic solutes or metabolites. The plasma membrane of transfer cells is highly convoluted which serves to increase the surface area for solute exchange across the cell wall (apoplast) as well as through numerous plasmodesmata (symplast). Moreover, the cells have dense cytoplasm and numerous mitochondria which indicate a high level of metabolic activity.

## 14.6 LOADING AND UNLOADING OF SIEVE TUBES

In order to understand the loading of food from manufacturing leaf cells to sieve tubes we must examine the anatomy of a minor vein shown in Fig. 14.7. We can see

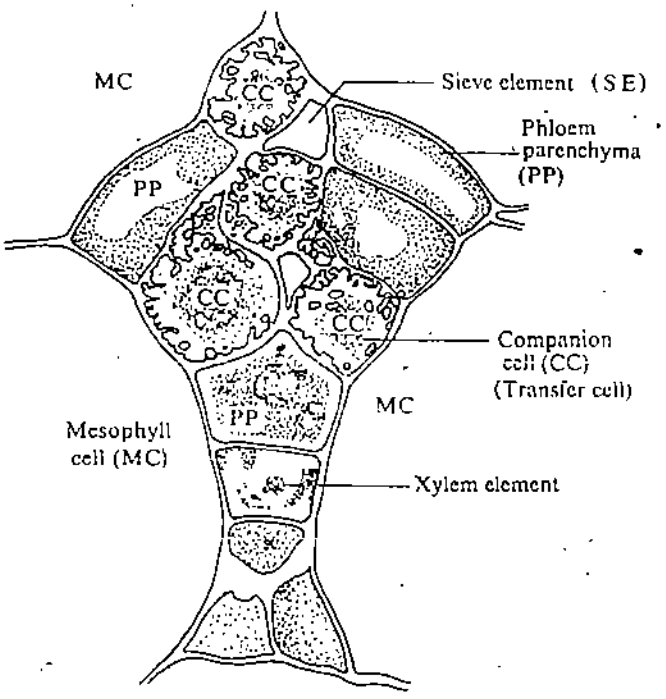



Fig. 14.7 : A cross-section of a minor leaf vein. (MC—Mesophyll Cell, XE—Xylem Elements, SE—Sieve Element, TC—Transfer Cells, PP—Phloem Parenchyma cell.

a xylem vessel (X), two sieve elements (SE) which are relatively smaller than other cells and without cytoplasm, the modified companion cells or transfer cells (TC) with dense cytoplasm and phloem parenchyma (PP) which is less dense and vacuolated. The transfer cells are associated with bundle sheath and mesophyll cells. The ingrowths of cell walls in the companion cells of leaves occur as the leaf matures, perhaps to increase its efficiency to load the sieve tube elements. The metabolites of all the mesophyll cells around the sieve elements join in a common pool to load via the surrounding transfer cells. The path of metabolites and other solutes is shown in Fig. 14.8. Presumably the metabolites are poured in the sieve tubes by the following three types of transport processes:

i) Symplastic transport via the plasmodesmatal connections,
ii) apoplastic transport through the cell wall (passive transport), and
iii) active transport (symport and antiport).

The latter two occur across the plasma membrane of the transfer cells.

All the non-photosynthetic cells and young buds which require more energy than they produce by photosynthesis are the sites of delivery (unloading points) of phloem sap. Grossly speaking, roots are the major importers of metabolites. Buds, phloem and xylem-parenchyma get their supply along the whole length of the vascular system. Seeds, fruits, tubers etc. become the major importers during their growth and development. The morphology of phloem at the sites of transfer of metabolites to seeds, buds, fruits etc. becomes specialised because of the layer of transfer cells which are arranged similar to the placenta of an animal embryo. The process of unloading is similar to loading except that the events occur in reverse direction.

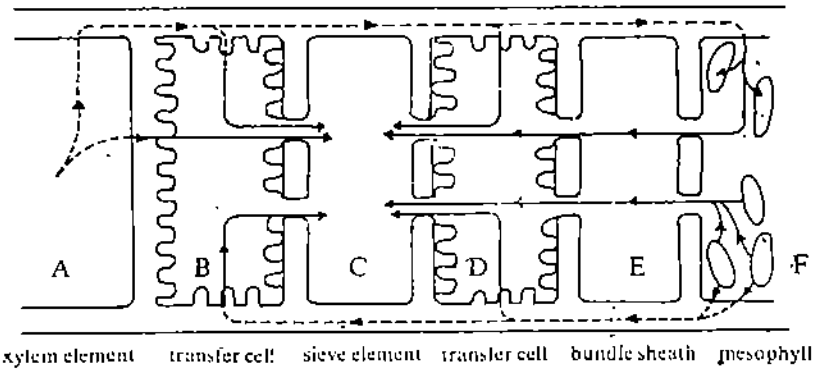



**Fig. 14.8 : The role of transfer cells in a minor vein of a leaf is to mediate solute fluxes from leaf mesophyll cells via leaf apoplasm, into the sieve elements ("loading"). Water and mineral nutrients reach mesophyll cells through the cell wall phase (apoplasm). Membrane permeable metabolites and $K^+$ released into the apoplasm by the mesophyll cells enter sieve elements via the transfer cells.**

## 14.7 THE NATURE OF METABOLITES IN SIEVE TUBES

The phloem sap contains three major classes of organic compounds — organic acids, amino acids and sucrose besides some cations, anions and hormones. Their concentrations are represented in the histogram shown below (Fig. 14.9). Sucrose — the major energy source in plants is the major metabolite transported from leaves to sinks. Rarely unusual sugars such as raffinose or mannitol are transported in some species. Of the total solute content in phloem, sucrose content ranges from 50 to 90%. The reason why sucrose is the major transporter of energy is not known. It is loaded by active transport because of its high concentration in sieve tubes. Amino acids and organic acids, mainly malate, are also substantial components of phloem sap. Among cations, the concentration of $K^+$ is much higher. In the xylem sap the concentrations of $NO_3^-$ and $K^+$ are about the same, but in phloem concentration of $NO_3^-$ is very small.

Can you think where the $NO_3^-$ could disappear? Nitrate is reduced in the leaves and used for the synthesis of amino acids and several other nitrogen containing

compounds. Now, can you guess why there is so much $K^+$ in the phloem sap? Unlike $NO_3^-$, $K^+$ is not incorporated into organic molecules. Due to transpiration there is a persistent influx of $K^+$ in the leaves. Because water evaporates leaving $K^+$ behind, its enhanced levels in the shoot require recycling in the xylem.

The presence of excess of ions in root tissues in turn affects the uptake of these mineral nutrients from soil by influencing the ionic relationships of the cells of the root. Here, we would like to emphasise that ions that accumulate in excess in the leaf apoplasm are passed on to the sieve elements via the transfer cells. The presence of $K^+$ is important as a counter ion to organic acids and amino acids within the cytoplasm of all cells and it is gradually assimilated in the cells of growing plant tissues.

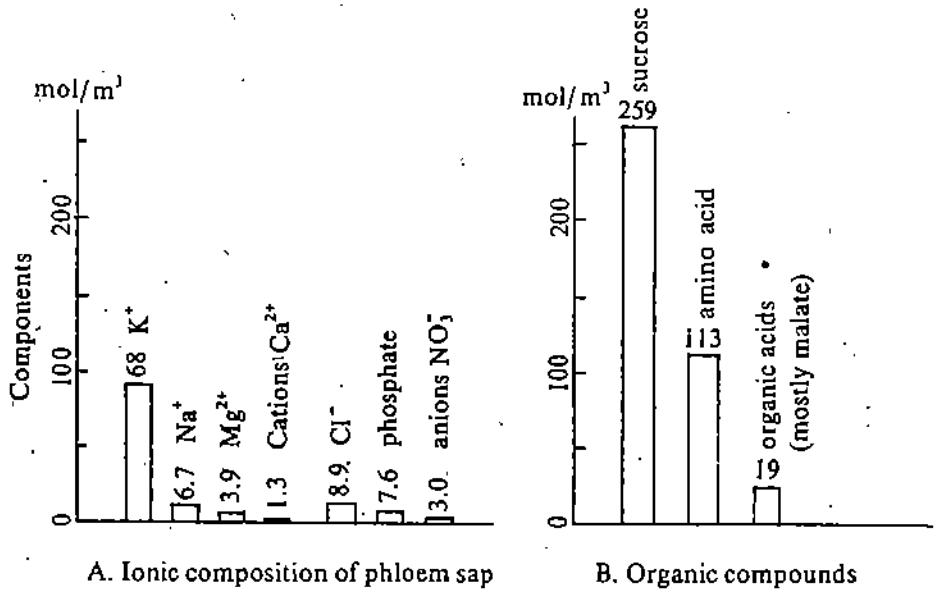



**Fig. 14.9 : Ionic and organic compounds of phloem sap of *Ricinus*. In general phloem sap is alkaline (pH 7.2-8.5).**

SAQ 2

a) Match the items given in column 1 with those listed in column 2.

| Column 1 | Column 2 |
|---|---|
| i) Sieve tubes | a) prevent the flow of phloem sap from injured cells |
| ii) Xylem vessels | b) cellular channels running throughout the plant |
| iii) P-proteins | c) alive at maturity |
| iv) Sieve tube element | d) cellulose pipelines running throughout the plant |

b) Which of the following statements are true? Write T for true and F for false in the given boxes.

i) Sieve tube elements are smaller in radius than xylem vessels and tracheids. ☐

ii) Sieve tube elements can carry out protein synthesis. ☐

iii) Companion cells have numerous mitochondria to carry high metabolic activity. ☐

iv) Transfer cells are located at the site of heavy transport of organic solutes and metabolites. ☐

v) Transfer cells transfer metabolites to mesophyll cells and load them into the sieve tubes. ☐

vi) Sugars are transported via both symplastic and apoplastic route. ☐

## 14.8 EXPERIMENTS ON PHLOEM TRANSPORT

To begin with, it was necessary to establish the basic fact that the metabolites flow from the source to the sink through phloem. One of the earliest experiments was to cut the bark of a stem (to remove phloem) in the form of a ring leaving the xylem intact. After a few weeks, the bark on the upper side showed swelling while the lower side retained the initial diameter (Fig. 14.10), thereby suggesting that the food materials moved via the phloem and accumulated in the upperpart.

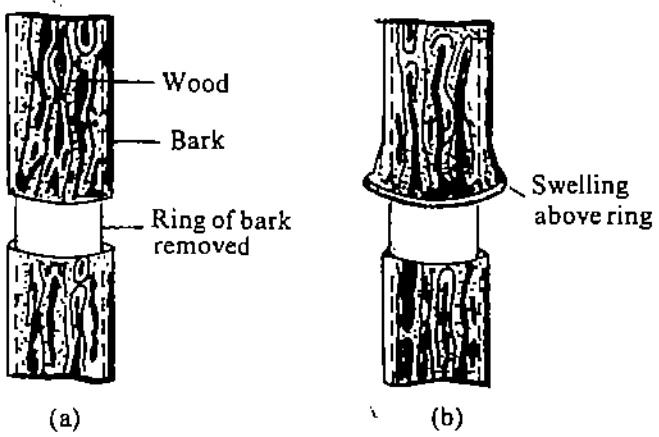



**Fig. 14.10 : Ringing (girdling) a tree. a) A ring of bark is removed from the tree, stripping the bark away, down to the xylem. b) Swelling in the bark above the ring is thought to occur from accumulation of downwardly transported material and the continued rapid development of cells just above the ring as compared with those below the ring. The tree eventually dies as the lower part does not receive food material synthesised in the leaves.**

Early in this century Münch cut away two such rings in an apple tree as shown in Fig. 14.11. The apple B with girdles both below and above it stopped growing, while the other two apples A and C grew perfectly well because they could draw their metabolites from above and below respectively.

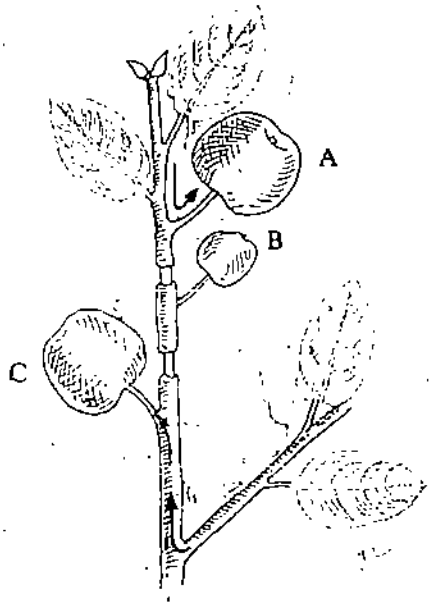



**Fig. 14.11 : Girdling (or ringing at two positions), above and below the apple B deprived the apple with organic nutrition and stopped its growth. A and C could obtain photosynthates from the leaves above and below them, respectively.**

At the end of World War II when radioisotopes were made available as markers, experiments were conducted to trace the pathway of photosynthates using radioactive $^{14}CO_2$. A single leaf was given $^{14}CO_2$ and light so that is would be the only leaf to fix $^{14}CO_2$ by photosynthesis (Fig. 14.12). After a lapse of few hours the stem was cut into a number of segments in order to see the distribution of photosynthate made from $^{14}CO_2$. From the radioactive count of $^{14}C$, in the various segments of the stem, it was found that only those parts of plant contained radioactive photoassimilate that were in communication with the leaf receiving $^{14}CO_2$.

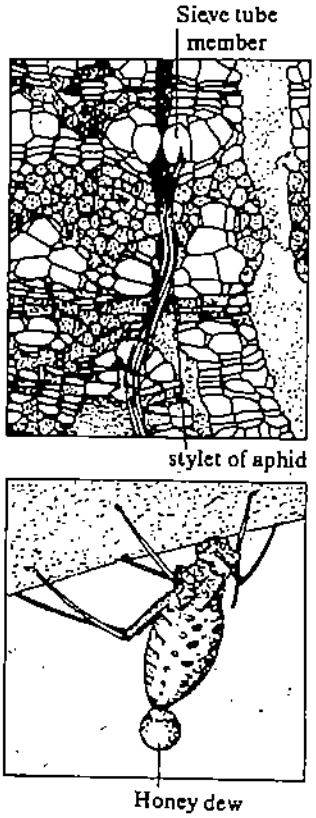



Fig. 14.13 : Naturally occurring research tool. a) An aphid penetrating the bark. A honey-dew droplet can be seen at the anal end of aphid. b) The stylets of aphid penetrating into the sieve tube element.

Now there are many evidences which prove that photoassimilates and metabolites flow in the sieve tube elements which are the actual channels. Translocation of sugar from leaf to other parts can be followed by placing Geiger tube which detects radioactivity (Fig. 14.12). The velocity of the phloem sap can also be determined by positioning two Geiger tubes against the stem and noting the time required for the movement of radioactivity from tube 1 to tube 2. The values of velocity so obtained usually range from 20 to 100 cm $hr^{-1}$ but occasionally could be as high as three meters $hr^{-1}$.

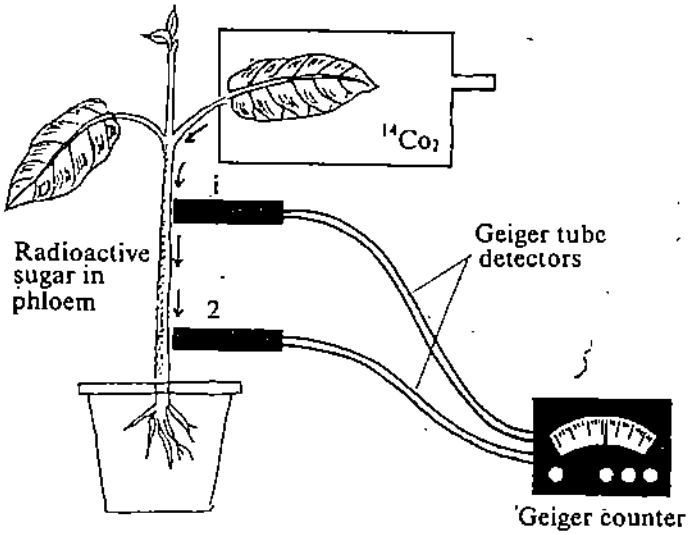



**Fig. 14.12 : Experimental design for measuring the velocity of phloem sap movement. A leaf is sealed in a closed chamber containing radioactive carbon dioxide ($^{14}CO_2$). The leaf is illuminated and the radioactive carbon dioxide is incorporated into sugar via photosynthesis. The translocation of the radioactive sugar can be detected by a Geiger tube positioned against the stem. The time required for sugar to move a known distance from Geiger tube 1 to Geiger tube 2 can be used to calculate the velocity of phloem sap movement.**

The velocity of phloem sap can also be determined by slightly heating small sections of the phloem sap. The sensitive thermocouple is applied further down the stem to detect the arrival of the warmed sap. You may recall that velocities of xylem sap flow is also determined by this method.

The mechanism of transport in phloem is difficult to study because when the cells of phloem are cut, the sieve tube plates get instantly plugged and transport stops altogether. Further, the cellular structure is altered or destroyed.

A simple, neat technique for the study of phloem transport is provided by nature. Aphids, phloem sap sucking insects draw their nutrition from phloem by inserting their stylets right into a sieve tube element (Fig. 14.13). The turgor pressure of the sieve tube is sufficiently high so the sap simply flows into the aphid's alimentary canal. A honey-dew can be seen at the anal end of a well fed aphid. If the insect is cut off from the plant just before the point of entry of stylets, with the stylets still inserted into the bark, phloem sap keeps exuding from the cut end. The exudate called honey dew provides information on the content of the phloem sap. The location of the sharp tip of the stylets is determined by microscopic observations which show that stylets penetrate single sieve element. This simple technique provides valuable information on the transported material and the rate of transport under different conditions (temperature, soil, water content etc.) in a fully functioning intact plant.

**SAQ 3**

Tick mark the correct alternate word(s) given in parenthesis for the statements listed below:

i) Phloem sap is rich in (amino acid/nucleic acid).

ii) Due to transpiration there is persistent influx of ($Ca^{2+}$/$K^{+}$) in leaves.

iii) (Nitrate/Sulphate) is reduced for the synthesis of amino acids.

iv) The technique of (photography/autoradiography) is used to see the location of radioactive carbon in the cell organelles.

## 14.9 MECHANISM OF PHLOEM TRANSPORT

The efficiency and magnitude of translocation of food material are evident from the annual yields of various crops and fruits. Now, the question is, what is the mechanism of translocation? Diffusion is too slow a process to account for the known velocities of translocation. Moreover, often the direction of movement is from lesser concentration to greater concentration. A few theories proposed for the mechanism of translocation in phloem are discussed below.

### 14.9.1 Münch Pressure Flow Model

Münch, a German plant physiologist, proposed in 1930, a simple physical model which can be tested in the laboratory for the mechanism of phloem transport. As shown in Fig. 14.14, two osmometers, one containing solute at higher concentration (A) than the other (B), are connected by a tube (C) and dipped in water. Due to high solute concentration in A water flows in to it by the process of osmosis. Consequently, the pressure develops which forces the solution to rise in the connecting tube and the content of A flow into B. This pressure forces water to flow out of B through its membrane in the medium.

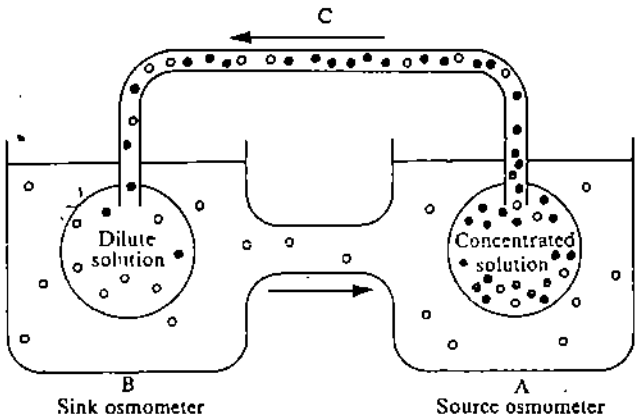



Fig. 14.14 : Model system illustrating Münch pressure flow hypothesis. (See text for details).

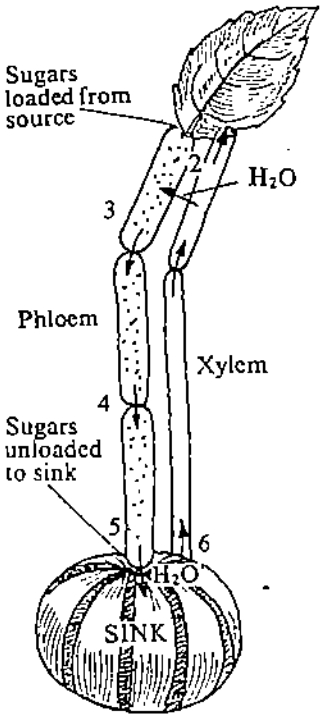



Fig. 14.15 : Münch Pressure Flow Model as applied to the plant. (1) Sugar is actively loaded from the leaf into the uppermost sieve tube member. (2) The higher sugar concentration causes water to move in osmotically from the xylem, building up a high water pressure. (3), (4) The high water pressure causes sieve tube sap to flow into the remaining sieve tube members towards the fruit. (5) Sugar is actively unloaded from the lowermost sieve tube member into the fruit. (6) The excess water delivered to the sink enters the xylem and moves back up the plant.

In plants the state of source and sink is analogous to the two osmometers. The source regions have higher solute concentration than the sink region. The phloem conduits in the source region would imbibe water by the process of osmosis and generate a high turgor zone. On the other hand, the phloem in the sink region will be at low turgor. Thus, a pressure gradient exists along the length of the phloem which would cause a mass flow of solvent and solutes with equal velocity.

There are several difficulties in accepting this, otherwise admirably simple and appealing hypothesis. Firstly, the generation of pressure gradient is possible in the above given model only if the water potential is about the same in both regions. However, in plants, water potential is lower in the leaves and higher in the roots. Thus, the tendency of water to enter the phloem in the leaf region is reduced. Whereas in the root region, water can be readily imbibed by sieve elements even at lesser solute concentration of the phloem sap, from neighbouring cells and/or apoplasm. Therefore, it cannot be assumed that the gradient in solute concentration

would generate a pressure gradient from sources to sinks. Moreover, the observed differential, mobilities of solute molecules, highest for $K^+$ and lowest for $Ca^{2+}$, speak against the pressure flow hypothesis. The original model has been modified, as shown in Fig. 14.15 to remove this problem.

Pressure gradients sufficient for causing mass flow have not been recorded in any plant. Much depends, on the radius of the sieve plate pores, the presence of P-proteins and the degree of callose formation. Hence the debate on the Münch's models is still continuing, primarily because there is still no other alternative model to explain precisely the mechanism of Phloem transport. You may note that at the sites of loading and unloading there is active transport operating which may account for the differential mobilities of ions and accumulation of molecules such as sugars in large amount against concentration gradient.

### 14.9.2 Fensom and Spanner Electroosmotic Flow Hypothesis

In electroosmosis the ions flow across a membrane in response to electrical gradient. Ions pull along water and other contents because of solvent drag. In this hypothesis it is visualised that sap flows in the lumen of sieve element and electroosmosis occurs across the sieve plate. The basic idea of this model is represented in Fig. 14.16. The pores of sieve are negatively charged and many positive ions are associated with them. The companion cells of consecutive sieve elements are shown to be engaged in $K^+$ uptake and release. This generates a ($K^+$) gradient in the direction of phloem sap flux; the fluxes of other solutes are coupled to $K^+$ flux and move alongwith the electroosmotic flow from one sieve tube to the next.

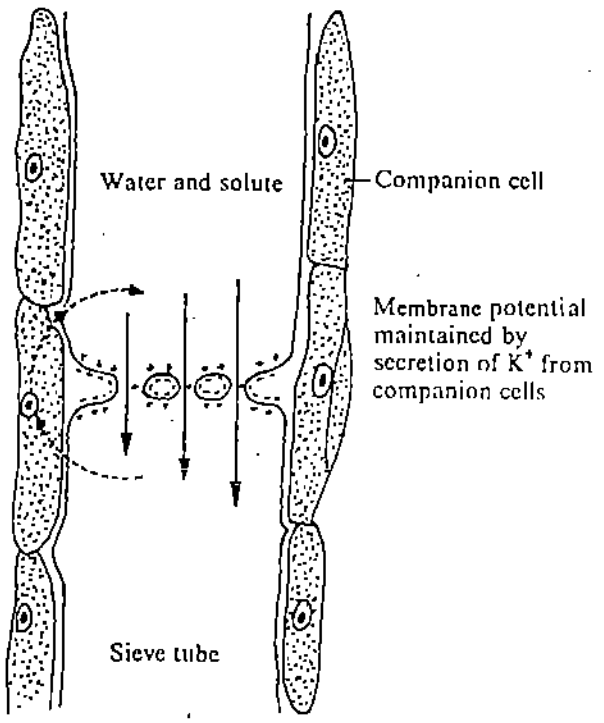



**Fig. 14.16 : Active uptake of $K^+$ by the companion cell from its associated sieve tube on the left and secretion of $K^+$ into the sieve tube at the right side by its companion cell generate both a potential difference and a $K^+$ concentration gradient. This causes a flow of $K^+$ ions and other solutes from right hand side to left.**

The model has several advantages over Münch's model. The presence of P-proteins, and the occlusion of sieve plate pores by filaments bearing negative fixed charges are in favour of this model. While these filaments would greatly impede the flow of phloem sap under a pressure gradient, they would make electroosmotic flow more efficient. The negative aspects of this model are:

a) the apparent irrelevance of the source-sink long distance relationship.

b) prohibitively large expenditure of energy (ATP) for $K^+$ uptake and release by the companion cells and

c) contradiction of the model in respect of anionic fluxes which would be excluded by electroosmosis across channels bearing negative fixed charges.

So long the basic premises of this model are not established by experimental evidences, the model remains an interesting candidate for the mechanism of translocation in phloem.

### 14.9.3 Protoplasmic Streaming and Tubular Peristaltic Flow Model

The first of the above two models involves the well known phenomenon of cytoplasmic streaming in giant algal cells. Coupling the regular cyclic movement of cytoplasm with active transport across sieve plate pores is thought to lead to a net flux of phloem sap in the direction of sinks. But even the highest estimates of movement by cyclosis are inadequate to explain the observed rates of transport through phloem. The second model is based on the hypothesis of trans-sieve element tubular structures which undergo peristaltic movements similar to the action of our alimentary canal. Both models need experimental support.

### 14.9.4 Protoosmotic Model

This model as proposed by M.M. Amin (1982), is based on the fact that there exists a metabolically generated pH imbalance between the sinks and the sources. As a rule all cells which use exogenous metabolites and whose metabolism is based on energy derived from respiration generate excess $H^+$, whereas cells in which photosynthesis and nitrate reduction processes exceed their respiratory activity require uptake of $H^+$ to maintain the cytoplasm at neutral pH. Thus $H^+$ flux from sinks (roots) to sources (leaves), is needed (Fig. 14.17). Being a downhill process it provides a source of energy which can be used for phloem transport. The model conceives a long distance translocation of $H^+/K^+$ antiport process, well known for plant cell membranes. This flux of $H^+$ from sinks to sources is charge compensated by $K^+$ Flux (Fig. 14.17). The $K^+$ flux is electroosmotic in nature which carries other solutes with it (hence named protoosmosis). As against the case of electroosmotic model discussed above, sink-source relationship in exchanging materials as per their metabolic activities is fully incorporated in this model. No experimental evidence has been advanced as yet either to substantiate or refute this model.

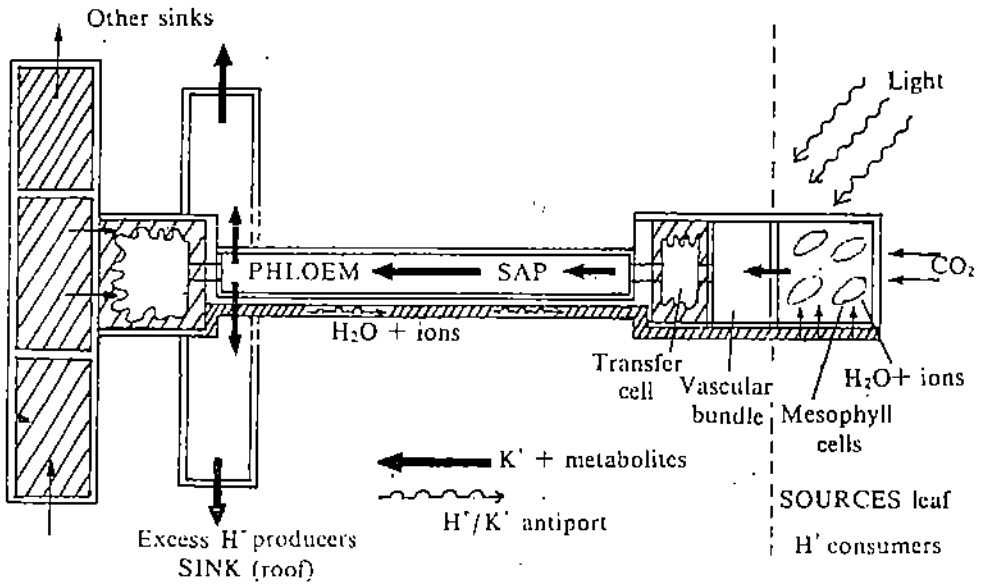



Fig. 14.17 : Proton flux down its gradient from sinks to sources causes $K^+$ — counter flux in the phloem which is thought to drive metabolites to sinks. The energy for phloem transport is provided by the imbalance in the pH of sources (alkaline) and sinks (acidic), tendencies that arise from their photosynthetic/N-fixing and respiring activities. Potassium ions are recycled in the two vascular systems.

M = Mesophyll, T = Transfer Cell, B = Bundle sheath

Interestingly this model is applicable to radial transport between phloem and xylem. Xylary sap is always acidic while phloem sap is invariably alkaline and rich in $K^+$. Flux of $H^+$ from xylem to phloem would in this case bring $K^+$ and water into xylem by protoosmosis. This would enrich phloem sap (i.e. enhance the concentration of

sugars) as the sap moves towards a sink, e.g. fruit. Indeed tracer experiments have shown this movement of water (tritium) and $K^+$ from phloem to xylem in a cyclic form : phloem of stem → xylem → leaf → phloem.

SAQ 4

Give one word for each of the statements listed below:

i) Uptake of organic solutes by sieve elements from adjacent parenchyma cells, companion cells or transfer cells.

ii) The movement of ions across a membrane in response to electrical gradient that pulls along water and other contents because of solvent drag.

iii) The flow of solute from source to sink due to pressure gradient.

iv) The flux of proton down its gradient from sink to source and counter flux of $K^+$ from source to sink to drive metabolites to sink.

## 14.10 SUMMARY

In this unit you have learnt that :

- Plants need extensive plumbing network to transport organic material, primarily the products of photosynthesis from the sites where they are synthesised to the sites where they are consumed or stored.
- This transport is carried out through a network of phloem tissue that extends from roots through the stem to the very tip of each and every leaf.
- The sites or the parts of plant from which the organic materials are transported are called the sources and the sites or parts of the plant its receiving the material from the source are called the sinks. A one-time source can also become sink depending on the need of the plant.
- The sieve tube elements are joined together by cytoplasmic connections through holes in the sieve plate, thus forming continuous cytoplasmic channels called sieve tubes.
- Experimental studies show that organic materials move through sieve-tubes.
- The location and special structure of companion cells suggest that they support translocation.
- Transfer cells are modified companion cells. Their convoluted plasma membrane and numerous plasmodesmata are perhaps for increasing the area of loading material in the sieve tubes.
- Phloem sap contains mainly sucrose, amino acids and potassium ions. Some other ions and organic acids are also present.
- The mechanism of phloem transport is poorly understood. According to Münch pressure-flow hypothesis water enters in sieve tube elements by osmosis and creates hydrostatic pressure which pushes the phloem contents from one cell to the next.
- Electroosmotic model visualises the flow of material due to electrical gradient created by uptake and release of $K^+$ ions by consecutive sieve elements.
- Protoosmotic model proposes a continuous flux of $H^+$ ions from sink to source compensated by $K^+$ flux from source to sink. The counter flux of $K^+$ is thought to drive metabolites to sink.

## 14.11 TERMINAL QUESTIONS

1) Compare vessels and tracheids of xylem with sieve tube members of phloem.

.....

.....

.....

.....

.....

2) Why is the phenomenon of translocation of food in sieve element a difficult process to study?

.....

.....

.....

.....

3) Phloem sap can be collected for the analysis by making incision in the bark. However, the collection of sap by Aphid method is considered to be superior. Why?

.....

.....

.....

.....

4) Why is cyclosis or diffusion cannot account for the transport of organic solutes in a plant?

.....

.....

## 14.12 ANSWERS

**Self-assessment Questions**

1) i) photosynthesis, nitrogen ii) xylem, phloem iii) Sieve tube elements
iv) source, sink v) source, sink

2) a) i) b, ii) d, iii) a, iv) c
b) i) T, ii) F, iii) T, iv) T, v) F, vi) F

3) i) amino acid ii) $K^+$ iii) Nitrate iv) autoradiography

4) i) loading ii) electroosmosis iii) Munch pressure flow hypothesis
iv) protoosmotic hypothesis

**Terminal Questions**

1) Xylem vessels are dead cellulose pipes running through the plant. They form part of apoplastic route whereas the sieve-tubes of phloem are living cytoplasmic channels forming symplasm. Water and mineral ions are tansported through xylem vessels and trachieds, whereas photoassimilates, organic solutes including hormones are translocated through sieve-tubes of phloem.

2) Transport in phloem is difficult to study because the cells involved are very delicate and get easily damaged. When phloem cells are injured, beaded chains of P-protein filaments are formed. Besides a slimy plug of callose develops in each plate pore.

3) Collection of phloem sap does not provide selective sampling of the sieve tube contents, since the phloem contains numerous other cells. Whereas, aphid method provides only sieve tube sap because the stylets are inserted in a single sieve tube element.

4) The observed rates of phloem transport are much higher than the rates that can possibly be achieved by cyclosis or diffusion.

# GLOSSARY

**Adhesion** : The tendency for one substance to cling to another substance due to intermolecular forces.

**Bacteriochlorophyll** : A type of chlorophyll, occurring in forms a and b, found in photosynthetic bacteria.

**Bark** : All tissues outside the vascular cambium; in old trees, divided into inner living bark (functional secondary phloem) and dead outer bark.

**Bundle sheath** : One or more layers of cells surrounding a vascular bundle.

**Callose** : A complex carbohydrate (1, 3-linked glucan) associated with the pores of sieve tube members, pollen grains, pollen tubes, and primary walls of many living cells.

**Capillary water** : That portion of soil water that is held in pores of the soil by capillarity.

**Carotenoid** : Any of a group of fat-soluble, yellow, orange, red, or purple pigments widely distributed among plants. They are subclassified into two groups, carotenes and xanthophylls.

**Cell wall** : The non-living layer that encloses the plant protoplast, is composed of cellulose, hemicellulose, pectin, lignin, and other substances and consists of a primary cell wall (the outer wall deposited during cell expansion) and a secondary wall (the inner wall layer(s) deposited after expansion has ceased).

**Chlorosis** : Decreased chlorophyll content due to its loss or reduced production.

**Cohesion** : The tendency of molecules of the same substance to cling to one another due to intermolecular forces.

**Cutin** : The fatty layer of cutin on the outer wall of epidermal cells.

**Cyclic phosphorylation** : ATP synthesis associated with electron transport in a cyclic fashion in photosystem I of chloroplasts.

**Cyclosis** : The movement of cytoplasm within cells. Also called cytoplasmic streaming.

**Dry land farming** : Type of agricultural operation involving deep cultivation of the soil to form a sufficient reservoir for the moisture as it falls, surface cultivation to prevent or reduce evaporation.

**Electrophoresis** : A procedure by which mixture of charged compounds (such as proteins) can be separated in an electrical field on a matrix such as starch gel.

**Emerson effect** : The experimental observation made by Robert Emerson in 1957 that photosynthetic efficiency is enhanced in the presence of long wavelength red light when that light is supplemented with shorter wavelength than red light.

**Epinasty** : Curling and overgrowth of leaves on their upper sides as a result of auxin and ethylene applications.

**Field capacity** : A measure of the water-holding capacity of the soil; soil water content (per cent by weight) after saturating soil with water and allowing gravitational runoff.

**Fixation** : The incorporation of components of free gas into organic materials. In photosynthesis, the carbon of carbon dioxide is fixed into carbohydrate. In nitrogen fixation, gaseous nitrogen is fixed into ammonia and eventually carbon compounds.

**Freeze-fracture** : A technique for preparing material for viewing under the electron microscope in which the specimen is frozen and then fractured with a cutting blade often causing membranes to split down the middle revealing internal components.

**Gravitational water** : That component of water added to the soil which is lost by runoff under the force of gravity.

**Guttation** : The secretion of liquid water from hydathodes, along the edge and tip of a leaf.

**Humus** : A complex mixture of colloidal matter in the soil composed of those fractions of the organic matter of plants, animals, and microoganisms that are most resistant to degradation.

**Hygroscopic water** : The component of soil water that is held by adsorption to the surface of soil particles and is not available to plants.

**Lignin** : A complex polymer, made up of coniferyl, sinapyl, or p-coumaryl alcohols, which becomes associated with cellulose in primary and secondary cell walls, especially in secondary xylem, and gives strength to the cell wall.

**Mass spectrometer** : An apparatus for obtaining the mass spectrum of a beam of ions by means of suitably disposed magnetic and electric fields.

**Middle lamella** : The layer of cementing substance between the primary walls of adjacent cells.

**Monochromatic** : Having one colour or wavelength only.

**Necrosis** : Death of a cell or group of cells as a result of injury, disease, or nutrient deficiency.

**Net photosynthesis** : Photosynthetic carbon fixation minus the carbon released as carbon dioxide by processes such as respiration.

**$P_{680}$** : A special molecule of chlorophyll *a* that accepts energy from the light-harvesting pigments of photosystem II and transfers it by loss of a high-energy electron to an electron acceptor.

**$P_{700}$** : A special molecule of chlorophyll *a* that accepts energy from the light-harvesting pigments of photosystem I and transfers it by loss of a high-energy electron to an electron acceptor.

**Parthenocarpy** : The development of a fruit in the absence of fertilization.

**Parthenogenesis** : The development of an egg without fertilization. The production of an organism from an unfertilized egg.

**Pectin** : A cell wall polymer made of β-1, 4-linked galacturonic acid residues with the carboxyl groups esterified with methanol, rhamnogalacturonan (rhamnose and galactose), and arabinogalactan (arabinose and galactose).

**Photoreceptor** : Light sensitive spot; eye of vertebrate.

**Photosystem** : One of two interacting energy-collecting and energy-transferring systems that operate in chloroplasts.

**Phycocyanin** : Any of several blue, water-soluble protein pigments present in most blue-green algae.

**Phycoerythrin** : Any of several water-insoluble red protein pigments present in most blue-green algae and all red algae.

**Quantum** : A discrete unit of electromagnetic energy. An entity having particle like properties. With reference to light, the amount of energy associated with one particle like unit or photon.

**Sieve plate** : A region of the cell wall of a sieve tube member where pores are concentrated.

**Sieve tube:** A column of sieve tube members that functions in the transport of organic solutes in the phloem of angiosperms.

**Sieve tube member** : An elongated cell with pores on its end walls.

**Stomatal apparatus** : A pair of guard cells and associated subsidiary cells involved in the opening and closing of the pore between the guard cells.

**Stroma** : The fluid substance within an organelle, such as a plastid. In fungi, a large mass of somatic (vegetative) hyphal tissue.

**Thylakoid** : A photosynthetic membrane in chloroplasts of eukaryotic cells. A stack of thylakoids in a chloroplast is called a granum.

**Tracheid** : An elongated, empty cell of the xylem without perforated walls that is active in longitudinal transport of water and mineral nutrients in vascular plants.

**Transfer cell** : A parenchyma cell modified with internal extensions of the cell wall that greatly increase the surface of the plasma membrane.

**Turgor** : The positive hydrostatic pressure that develops within plant cells as a result of osmotic water entry. Also, the distension that results from the pressure.

**Ureides** : The major derivatives of urea which have been found in nodulated plants are allantoin, allantoic acid and citrulline.

**Vessel** : A long, hollow series of vessel members connected to each other end-to-end in the xylem that functions in longitudinal transport of water and mineral nutrients in angiosperms and some ferns.

**Water potential** : A measure of the potential energy of osmotic potential and the pressure potential. A measure of the tendency of water to move away from a given location.

**Water use efficiency** : The ratio of organic material produced by a plant to water utilized (including water taken up from the soil and lost by transpiration).

**Wilting** : Drooping of leaves or other plant parts due to decreased turgor within the cells as a result of excess water loss by transpiration.

## FURTHER READING

1) Noggle, G. Ray and Fritz, George J. *Plant Physiology*, 2nd edition, 1989, Prentice-Hall of India Pvt., New Delhi.

2) Devlin, Robert M. and Witham Francis H. *Plant Physiology*, 4th edition, 1986, CBS Publishers, Delhi.

3) Salisbury, Frank B. and Ross, Cleon *W. Plant Physiology*, 4th edition, 1989, CBS Publishers, Delhi.

Dear Student,

While studying these units you may have found certain portions of the text difficult to comprehend. We wish to know your difficulties and suggestions, in order to improve the course. Therefore, we request you to fill and send us the following questionnaire, which pertains to this block.

## QUESTIONNAIRE

LSE-05
Block-3

Enrolment No. | | | | | | | | | | |

1) How may hours did you need for studying the units?

| Unit Number | | | | | |
|---|---|---|---|---|---|
| No. of Hours | | | | | |

2) How many hours (approximately) did you take to do the assignments pertaining to this block?

| Assignment Number | | |
|---|---|---|
| No. of Hours | | |

3) In the following table we have listed 4 kinds of difficulties that we thought you might have come across. Kindly tick (√) the type of difficulty and give the relevant page number in the appropriate columns.

| Page Number | Types of difficulties | | | |
|---|---|---|---|---|
| | Presentation is not clear | Language is difficult | Diagram is not clear | Terms are not explained |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

4) It is possible that you could not attempt some SAQs and TQs.
In the following table are listed the possible difficulties. Kindly tick (√) the type of difficulty and the relevant unit and question numbers in the appropriate columns.

| Unit No. | SAQ No. | TQ No. | Type of difficulty | | | |
|---|---|---|---|---|---|---|
| | | | Not clearly posed | Cannot answer on basis of information given | Answer given (at end of Unit) not clear | Answer given is not sufficient |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |

5) Were all the difficult terms included in the glossary? If not, please list in the space given below.

| |
|---|
| |

6) Any other suggestion(s):

To,

The Course Coordinator (LSE-05, Physiology),
School of Sciences
Indira Gandhi National Open University
Maidan Garhi
New Delhi-110068

Uttar Pradesh
Rajarshi Tandon Open University

# UGZY/BY-08
# Physiology

Block

4

## PLANT PHYSIOLOGY–II

# BLOCK 4 PLANT PHYSIOLOGY—II

In the previous block on plant physiology we have dealt with nutrition of the plants. The first Unit of this block on inorganic nitrogen and sulphur metabolism is again concerned with nutrition. Plants require nitrogen, in relatively large amounts as it is the constituent of amino acids and nucleic acids. Unfortunately, unlike carbon dioxide, atmospheric hitrogen cannot be fixed by all plants. Only a few gifted prokaryotes, free living (e.g. *Azotobacter, Nostoc)* or in symbiotic association with eukaryotes (e.g. *Rhizobium*–legume) have remarkable capacity of fixing atmospheric nitrogen. We will explain in detail the biochemistry and mechanism of nitrogen fixation and metabolism of nitrate and ammonia. A small section on the metabolism of sulphur is also included.

Units 16 and 17 deal with regulation of growth, differentiation and development. A plant grows and develops from a zygote by a series of steps controlled at three levels—genetic, hormonal and environmental. There is a complicated interaction among the three levels of control. In Unit 16 we discuss the discovery and role of five groups of hormones and their application to agriculture. Plant hormones have multiple effects on growth, and particularly developmental processes are regulated by more than one hormone.

In Unit 17 you will study the environmental factors that control seed germination, vegetative growth, flowering, fruit set, abscission and senescence. There are chemical receptors in plants that receive signals which indicate that environment is favourable and the time is appropriate to switch on genes responsible for a particular developmental event. A small section on tissue culture and biological clock is also dealt with in this unit.

In the last unit of this course we study the various types of stress conditions that plants have to face, and their varied responses to cope up with them. The possibility of manipulating the plants by genetic engineering to survive under stress situations is also explored.

## Objectives

After studying this block, you should be able to :

- describe nitrogen fixation and nitrogen metabolism,
- describe metabolism of sulphur,
- explain the regulation of plant growth, development and differentiation by internal and external factors,
- describe the varied responses of plants under different stress conditions and the ways to adapt to them.

**Acknowledgement**

Dr. Anil Grover, University of Delhi, for the comments on Unit 18 of this course.

# UNIT 15 INORGANIC NITROGEN AND SULPHUR METABOLISM

## Structure



## 15.1 INTRODUCTION

Productivity in agriculture, forestry and other ecosystems is basically limited by the availability of nitrogen nutrients like nitrate or ammonia. The success story of modern agriculture is based on abundant availability of industrially fixed nitrogen fertilisers. However, it is a matter of grave concern that such technology may not last long in view of the diminishing reserves of fossil fuel. Therefore, it is important to intensify efforts to exploit the natural or biological nitrogen fixation such that it could function as the main source of nitrogen in agriculture. In fact, in importance nitrogen nutrition is next only to water in the life of an organism.

In nature there are two major processes that control the balance of nitrogen available for plant. The process of denitrification is the major cause of the loss of combined nitrogen to the atmosphere. While biological, abiological (lightening and combustion) and industrial process are involved in transforming atmospheric nitrogen into combined forms like ammonia and oxides of nitrogen which are readily taken by the organisms.

In Unit 13, you have learnt how carbon is assimilated into glucose during photosynthesis. In this unit, you are introduced to the basics of nitrogen fixation, nitrate assimilation, ammonia assimilation, interaction of nitrogen and carbon assimilation and controls that regulate the form of inorganic nitrogen nutrition. We will also describe briefly assimilation of sulphur which like nitrogen is essential for plants. Its predominant natural form is sulphate and like nitrate it is also reductively assimilated.

### Objectives

After studying this unit, you should be able to:

- name and classify the nitrogen fixing organisms on the basis of mode of living,
- write the conditions necessary for nitrogen fixation and the essential requirements of the process,

- describe the metabolic modifications occurring during bacteroid formation in nodules,
- explain the basis of specificity of *Rhizobium*-legume symbiosis,
- name the components of nitrogenase enzyme, describe the reactions carried out by each component, and explain the significance of $H_2$ evolution during the process,
- describe the steps involved in assimilatory reduction of nitrate and the requirement of the process,
- explain the significance of organelle specific or tissue specific distribution of nitrate reductase and nitrite reductase,
- explain the significance of induction, repression and control of activity of nitrate reductase,
- write the requirements of assimilation of ammonia into organic form,
- write the pathway of sulphate assimilation and its difference from nitrate assimilation,
- discuss the metabolic interrelation of nitrogen, carbon and sulphur.

## 15.2 BIOLOGICAL NITROGEN-FIXATION

The process by which molecular nitrogen ($N_2$) is reduced to ammonia ($NH_3$) is called nitrogen-fixation ($N_2$-fixation).

This is the most important section of this unit and is fairly long. Here we first introduce you to the species capable of $N_2$-fixation and describe the formation of nodules during symbiotic association. Then we go through the reactions and enzymes involved in the conversion of molecular nitrogen to $NH_3$. A brief section on the genetics of $N_2$-fixation will introduce you to the possibility of transferring, $N_2$-fixing capability to non $N_2$-fixers.

### 15.2.1 The Gifted Species

Assimilation of nitrogen ($N_2$) as nitrogen source represents a mode of nutrition called **diazotrophy** and organisms with this mode of nutrition are called **diazotrophs.**

Biological nitrogen fixation remains mainly confined to a few distinct nutritional types of prokaryotes, some of which are free-living while others live in symbiotic association with eukaryotic partners. This process contributes annually about 60% to the earth's newly fixed nitrogen budget. Table 15.1 shows the ranges and types of biological nitrogen fixers. They have been variously classified as free living (*Azotobacter, Nostoc, Anabaena,* Fig. 15.1) or symbiotic (*Rhizobium*-legume association). Free-living can further be classified into phototrophic *(Nostoc),* chemotrophic *(Klebsiella),* aerobic *(Azotobacter)* or anaerobic *(Chromatium)* depending upon the state of existence and mode of nutrition. Agronomically the most important nitrogen fixing systems are *Rhizobium*-legume association (Fig 15.1), *Frankia* (actinorhizal)-woody plant association, *Anabaena-Azolla* and *Cyanobacteria*-rice system. *Rhizobium*-legume association as you will know invariably occurs in the form of root nodules. This group of nitrogen fixers has been divided into three categories: i) *Rhizobium* which includes fast growing species, ii) *Bradyrhizobium* which include slow growing strains and iii) *Azorhizobium* which is a combination of traits from *Rhizobium* and *Bradyrhizobium. Rhizobium* is highly specific with respect to its host and that is why the nomenclature is based on the specific host which it infects. For example the *Rhizobium* infecting clovers and peas are called *trifolii* and *leguminosarum* respectively. The genus *Bradyrhizobium* is not so rigid with regard to the host partners. All the strains of *Rhizobium* and *Bradyrhizobium* produce nodules on the host, but *Azorhizobium* produces nodules on the stem of *Sesbania rostrata* and *Aeschynomene afraspera. Parasponia* is the only non-legume genus where the bacterial partner is *Rhizobium.* This observation has raised the hope of creating nitrogen fixing associations between *Rhizobium* and important crops like cereals. Nitrogen fixing nodules on the roots of woody plants like *Alnus, Casuarina, Myrica* are formed by symbiotic association with *Frankia* a member of actinomycetes. *Anabaena-Azolla* association is experimentally shown to be a strong source of nitrogen fertiliser in wetland rice agriculture. Free-living heterocysts cyanobacteria are also known to contribute significant amount of nitrogen to the wetland rice ecosystem. Lichens are associations between fungi and $N_2$-fixing cyanobacteria. They

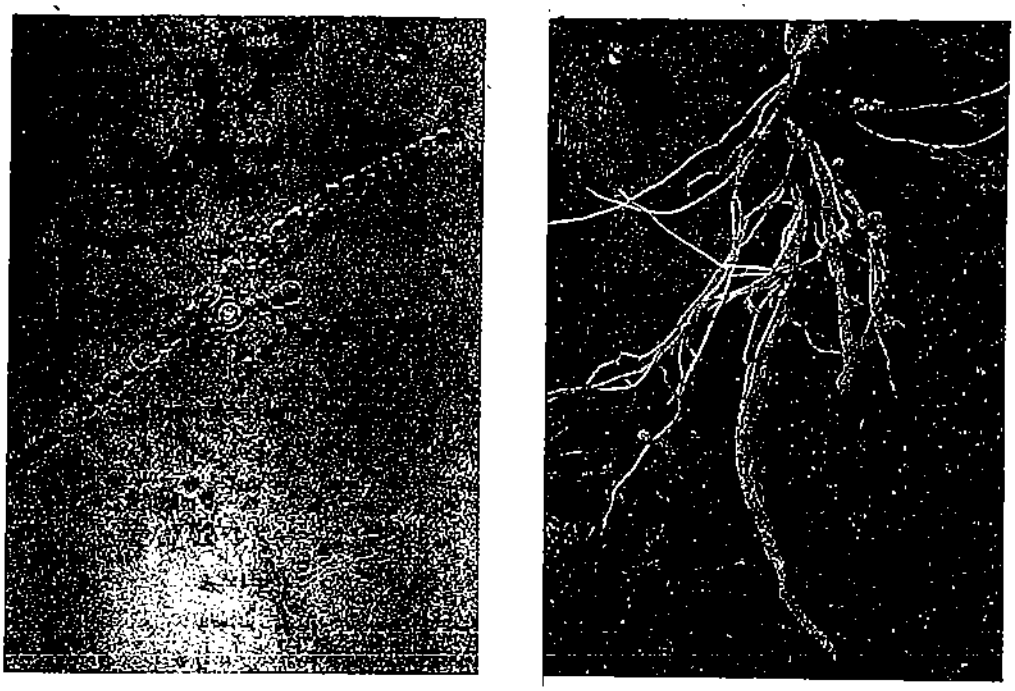


Fig. 15.1: The gifted $N_2$-fixers (photographs). a) *Anabaena* (arrow-heteroyst) b) *Rhizobium*-legume

are very important source of $N_2$ in barren harsh habitats. $N_2$-fixing root nodules are also known to occur in tropical plants like *Psychotria* with *Klebsiella* as the $N_2$-fixing partner.

Table 15.1: Some examples of certain gifted $N_2$-fixing species.

| Type | Examples |
|---|---|
| 1) **Free-living nitrogen-fixing organisms** | |
| i) Anaerobes | *Clostridium* |
| ii) Facultative anaerobes | *Klebsiella, Enterobacter* |
| iii) Microaerobes | *Azospirillum, Aquaspirillum Arthrobacter* |
| iv) Aerobes | *Azotobacter, Derxia* |
| v) Photosynthetic bacteria | *Rhodopseudomonas, Rhodospirillum,* Chromatium |
| vi) Cyanobacteria | *Anabaena, Nostoc* |
| 2) Symbiotic systems | |
| i) *Rhizobium*-legume association | |
| a) Fast growers | *Pisum, Trifolium, Vicia* |
| b) Slow growers | *Arachis, Glycine, Vigna* |
| ii) *Rhizobium*-non-legume associations | |
| Slow growers | *Parasponia* |
| iii) Associated symbionts | *Azospirillum-brasiliense-sorghum* |
| iv) *Frankia*-(actinorhizal) associations | *Alnus, Casuarina, Myrica* |
| v) Cyanobacterial associations | |
| a) angiosperms | *Gunnera* |
| b) gymnosperms | *Agathis, Cycas, Macrozamia* |
| c) pteridophytes | *Azolla* |
| d) bryophytes | *Anthoceros, Blasia, Cavicula* |
| e) lichens | *Collema, Lichina, Peltigera* |

## 15.2.2 Requirements of Nitrogen-Fixation

The following are the essential requirements for $N_2$-fixation.

i) The enzyme catalysing the conversion of molecular $N_2$ to $NH_3$ is called nitrogenase. It is produced by a group of genes called the *nif* genes. Expression

of the nif genes leading to the formation of functional nitrogenase is thus one of the essential requirement.

ii) The activity of nitrogenase is extremely sensitive to oxygen. $N_2$-fixing organisms, therefore, must have a cellular mechanism to protect nitrogenase activity from oxygen inhibition.

iii) The reduction of $N_2$ to $NH_3$ requires electrons and protons. The natural physiological electron donors to the nitrogenase enzyme are reduced ferredoxin ($Fd_{red}$) or reduced flavodoxins. The organism must have provision for such electron donors.

iv) Reduction of $N_2$ to $NH_3$ is a high-energy requiring process consuming 16 to 24 ATP molecules per $N_2$ reduced. Therefore, the organism must have provision for an abundant supply of ATP.

v) Mo and Fe are integral constituent of nitrogenase and therefore, they must be available to the plant in order to ensure the formation of nitrogenase.

vi) Nitrogenase enzyme is not produced in the cells that have access to fixed nitrogen source like nitrate or ammonia. Hence, the process of $N_2$-fixation occurs in situations devoid of such fixed nitrogen sources.

### 15.2.3 Development and Formation of Nodules in Legumes

There are three main reasons why legumes are important in agriculture.

i) They fix nitrogen and thus reduce the consumption of nitrogen fertilisers.

ii) Grains and forage legumes rich in protein content are nutritionally indispensable for human and livestock.

iii) They provide nitrogen inflow to natural habitats such as tropical forest.

$N_2$-fixing nodules of legume include the following distinct stages in its development and formation. They are: i) recognition ii) infection iii) nodule formation iv) nodule physiology.

The specificity of symbiotic interaction between *Rhizobium* and its host occurs at the very first stage and is called **recognition.** There are reciprocal signals between the host and symbiont for determination of host specificity for root nodule development. It is known that this recognition is a function of complementary molecules present on the surface of the two symbiotic partners. There are two types of molecules comprising this complementary group, one is the polysaccharide and the other lectins. The polysaccharide component is characteristic of both partners while the lectin molecules are produced only by the host (legume) concerned. Lectins have many sites specific for binding to a variety of surface polysaccharides of the interacting partners. For example as shown in Fig. 15.2 clover root hair produces a lectin called trifollin with two specific sites, one for binding to polysaccharides at the surface of bacterium *Rhizobium trifolii* and other for binding to polysaccharide on the wall of root hair. Thus lectin functions as an anchor to fix *Rhizobium* on the root surface.

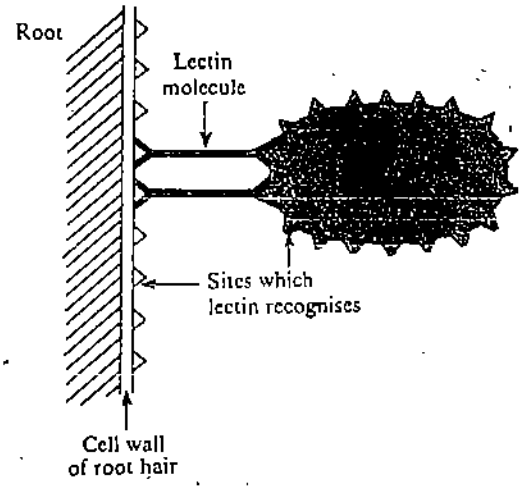



Fig. 15.2: Role of lectins in recognition of host and symbionts. The specific rhizobia attach to the root hair of the correct species because the bifunctional lectin molecules recognise antigenic sites of both partners.

A question will naturally arise in your mind whether there is any specific chemical signal from the host to its compatible symbiotic partner and from the later, in response to the former. Recently, root exudates of alfalfa were found to contain a substance called luteolin (a flavonoid) which is found to induce reactions in *Rhizobium meliloti* leading to the synthesis and excretion of symbiotic signal called sulphate N-acetyl glucosamine.

Next to recognition is the stage of **infection.** The first obvious change in infection involves curling of root hair (see Fig. 15.3). The substance involved in root hair curling is of bacterial origin and is sulphated acylated tetra-N-acetyl glucosamine (symbiotic signal). The symbiotic signal is host specific initiating host recognition and development of infection thread and nodule . It is essential for you to know that root hair curling is not an essential requirement for infection. It is thought merely to facilitate infection by providing a close environment to the bacterium, where it can enter the root hair cell by causing its localised enzymatic dissolution.

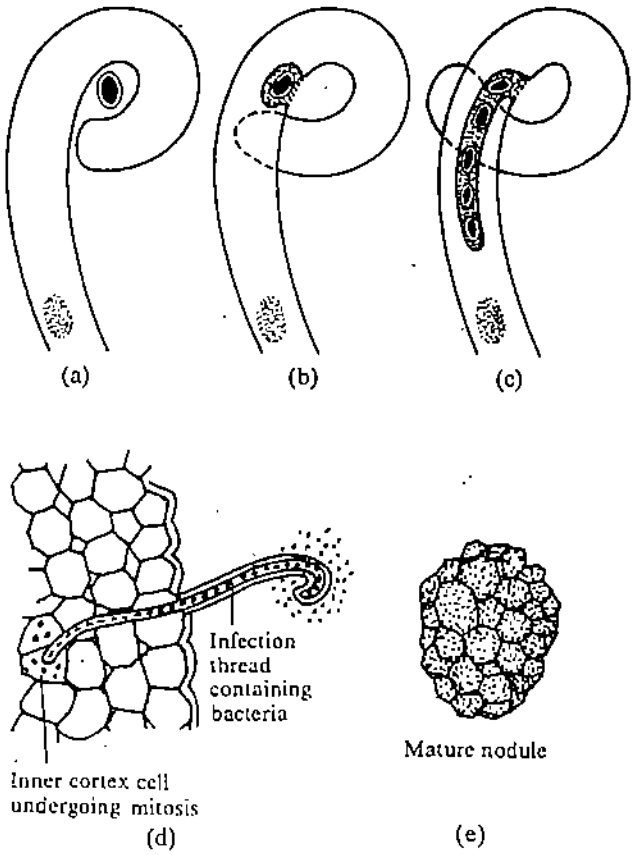



Fig. 15.3: The invasion of legume root hair by *Rhizobium*: a) enclosure of bacterium into the root hair; b) penetration of bacterium into the root hair; c) growth of infection thread; d) infection thread in dividing cortical cells, e) mature nodule.

Following the entry of the bacterium, the root hair wall and plasma membrane invaginate and grow into a tube like structure called infection thread (Fig. 15.3 d). The bacterium present in the infection thread multiplies repeatedly to produce more bacteria each enveloped by an extra cellular polysaccharide. Such infection threads penetrate into the cortex following which the cells of the inner cortical region are stimulated to divide and become tetraploid. One bacterium at a time is released into a cortical cell by pinching of the infection thread at a point devoid of root hair wall. The bacterium may be released from infection thread into a dividing cell in which case all the daughter cells will contain bacteria. Alternatively, the cells in the cortical region may have already undergone many divisions, in which case the entry of bacteria from the infection thread into the daughter cells is a host dependent phenomena. The release of each bacterium into the cortical cell is accompanied by the formation of external membrane envelop of plant origin around the bacterium called peribacteroid membrane.

During the formation of nodules and its maturation, their bacterial cells are transformed into bacteroids (Fig. 15.4). These have functional $N_2$-fixing apparatus

and cytochrome component located in new bacteroid membrane that facilitate respiration at low $O_2$ concentration. During the same time a special protein, leghaemoglobin, is synthesised. It is also localised within peribacteroid membrane. A number of new proteins of enzymatic and transport nature are also produced exclusively by the host. Since these host produced proteins are nodule specific they have been given the name **nodulins**. Nodules are supplied with root vascular tissues.

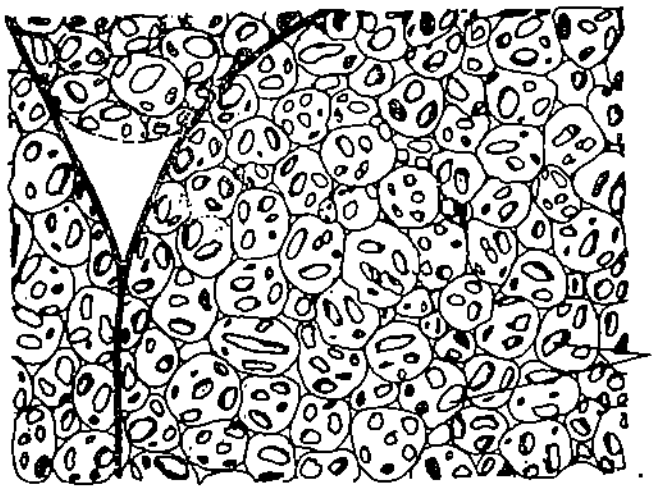



**Fig. 15.4: Diagrammatic representation of bacteroids within root nodules. Numerous bacteroids in groups of four to six. Each group is surrounded by a peribacteroid membrane.**

Mature bacteroids have enzymes for TCA cycle and oxidative phosphorylation. The bacteroids are active in ATP production and in supply of reductant for nitrogenase activity. The carbon requirement for the production of ATP and reductant is met ultimately from photosynthetically produced carbohydrates translocated through phloem. The differentiation of bacterium into bacteroid is accompanied by inhibition of *glutamine synthetase* activity, so that the bacteroid cannot assimilate ammonia. In other words, the bacteroids functions as ammonia producing factories for the host. Consequently, ammonia is released into the cytoplasm of the host cell and here it is assimilated into glutamine and asparagine in the case of pea and clover or it is assimilated into ureides as happens in soya bean. The reaction of conversion of $NH_3$ to glutamine will be discussed in a later section.

**SAQ 1**

a) Given below in Column 1 are some $N_2$-fixing species. Match them with their symbiotic partner in Column 2.

| Column 1 | Column 2 |
|---|---|
| i) *Anabaena* | a) Certain leguminous plants |
| ii) *Rhizobium* | b) *Anthoceros* |
| iii) *Nostoc* | c) *Azolla* (fern) |
| iv) *Frankia-actinorhizal* | d) *Sorghum* |
| v) *Azospirillum* | e) *Casuarina* |

b) Match the asymbiotic $N_2$-fixers in Column 1 with the characteristics given in Column 2.

| Column 1 | Column 2 |
|---|---|
| i) *Nostoc* | a) Anaerobic |
| ii) *Azotobacter* | b) Photosynthetic anaerobe |
| iii) *Clostridium* | c) Heterocyst |
| iv) *Chromatium* | d) Aerobic |

c) Which among the following statements are true? Write T for true and F for false.

i) Only prokaryotes have the ability to fix nitrogen. ☐

ii) Leguminous plants themselves cannot grow without *Rhizobium*. ☐

iii) Enzymes necessary for the fixation of nitrogen are present in the bacteroids. ☐

iv) Enzymes of TCA cycle and electron transport chain are present in bacteroids. ☐

v) Bacteroid secretes asparagine into cortical cells. ☐

vi) Infection thread consists of an infolded and extended plasma membrane of the bacteria. ☐

d) Fill in the blank space with appropriate words in the statements given below:

i) Molecules that function to recognise both symbiotic partners in $N_2$-fixation are ..... and .....

ii) The molecules of ..... anchor by binding to the ..... present on the surface of bacteroid and legume.

iii) Bacteroids release ..... into the cytoplasm of host cell because they lack ..... enzyme necessary for $NH_3$ assimilation.

iv) The peribacteroid membrane is of ..... origin.

### 15.2.4 Biochemistry of Nitrogen-Fixation

Nitrogenase enzyme is made of two subunits. One of these contains non-heme iron and is called Fe-protein or component II or **dinitrogenase reductase,** while the other, contains Fe and Mo and is therefore, called Fe-Mo protein or component I or **dinitrogenase**. Both the components are essential for the reduction of $N_2$ to $NH_3$. For activation dinitrogenase reductase requires ATP which gets hydrolysed to ADP and inorganic phosphate. Only such activated dinitrogenase reductase can mediate the transfer of one electron at a time from reduced ferredoxin or flavodoxin to dinitrogenase which contains the sites for its substrate (Fig. 15.5). Such repeated one electron transfer process results in building up of a pool of electrons in dinitrogenase which are finally used to reduce nitrogenase substrates. The following equation sums the process of $N_2$ fixation to $NH_3$ and molecular $H_2$:

$$N_2 + 8\,\text{electrons} + 16\,\text{MgATP} + 16\,H_2O \longrightarrow 2\,NH_3 + H_2 + 16\,\text{MgADP} + 16\,Pi + 8\,H^+.$$

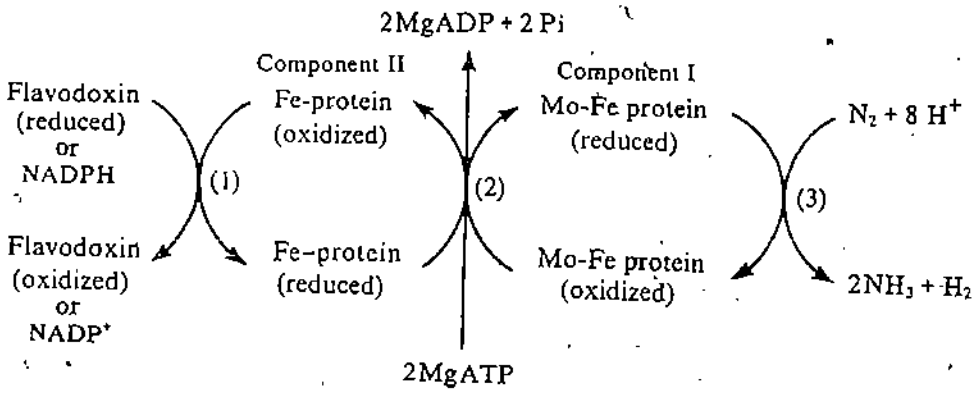



**Fig. 15.5: Details of electron transport from NADPH or flavodoxin to nitrogen via component I and component II of Nitrogenase.**

Fixation of $N_2$ to $NH_3$ and of proton into molecular hydrogen occurs concurrently. It is estimated that two to three molecules of ATP are consumed for the transfer of one electron in the nitrogenase reaction. Since $N_2$-fixation and $H_2$ production, as shown in the equation is an 8 electron requiring process, a total of 16 to 18 molecules of ATP would be the basic need for the two reactions in the process. So, you can see that $N_2$-fixation is indeed a very costly reaction energy-wise. It is believed that this requirement alone might have been the reason for restricting $N_2$-fixation process to organisms efficient in ATP production. You can see hydrogen production during $N_2$-fixation to be a wasteful reaction energy-wise. It also affects nitrogenase activity because molecular $H_2$ is an inhibitor of $N_2$-fixation. Attention, therefore, is being given to eliminate hydrogen generating reaction of nitrogenase during $N_2$-fixation.

As shown in Fig. 15.6, nitrogenase catalyses reduction of a variety of substrates with concomitant hydrolysis of ATP. If hydrogen cyanide is the substrate, it is reduced to

methane and ammonia. If azide is the substrate it is reduced to ammonia and molecular nitrogen. Similarly, if acetylene and protons are the substrate they are respectively reduced to ethylene and molecular $H_2$. Acetylene reduction to ethylene by nitrogenase had led to the development of a method called acetylene reduction method for assaying nitrogenase activity *in vivo* conveniently and inexpensively. The technique of estimating nitrogenase activity would be discussed briefly in a later section.

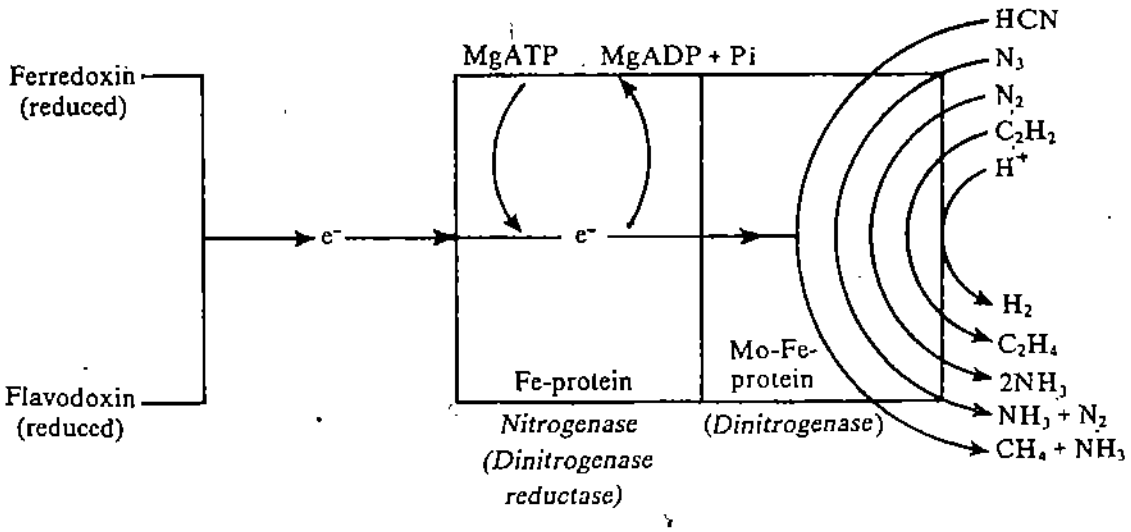



**Fig. 15.6: Nitrogenase enzyme catalysing ATP hydrolysis dependent reduction of hydrogen cyanide (HCN) to methane ($CH_4$) and $NH_3$, of azide ($N_3$) to $NH_3$+$N_2$, of $N_2$ to $NH_3$, of acetylene ($C_2H_2$) to ethylene and protons to molecular $H_2$.**

Recently it has been shown that Azotobacter has vanadium containing nitrogenase in addition to Mo-nitrogenase.

### 15.2.5 Factors Influencing Functions of Nitrogenase

Following are the factors that show considerable influence on the activity of nitrogenase enzyme.

**i) Molybdenum (Mo) and Vanadium (Va)**

Molybdenum must be available in nature in order to meet the demand of Mo for the formation of nitrogenase necessary for $N_2$-fixation. Now, it is known that Mo inhibits formation of Va containing nitrogenase. As you may know the relative distribution of Mo and Va in nature is in favour of Va. Therefore it is quite likely that some ecological habitats could be deficient in Mo not in Va and $N_2$-fixers of such habitat are expected to produce Va containing nitrogenase for $N_2$-fixation. Accordingly, there could be two nitrogen cycles functioning in nature one mediated by Va system and the other mediated by Mo system.

**ii) Molecular Hydrogen**

$N_2$-fixing organisms also produce a membrane bound enzyme called **uptake-hydrogenase** under $N_2$-fixing condition. The physiological significance of the presence of uptake hydrogenase lies in its ability to consume hydrogen produced during nitrogenase activity through aerobic electron transport chain generating ATP in the process. Such nitrogenase uptake-hydrogenase relationship in nitrogen fixing cells provides the following advantages:

i) By aerobic respiration it restores a major part of energy in the form of ATP.

ii) It offers protection of nitrogenase from oxygen.

iii) It prevents inhibitory effect of $H_2$ on nitrogenase activity.

It has been observed that the nitrogenase enzyme catalyses exclusive production of hydrogen provided the $N_2$-fixer is in an atmosphere devoid of nitrogen. Efforts are being made to exploit this special property of the enzyme for photoproduction of $H_2$ by heterocysts of cyanobacteria on a commercial scale. This would require construction of special strains of cyanobacteria that would be genetically deficient in uptake hydrogenase enzyme. It is expected that these strains would continuously generate hydrogen in photosynthetic and $N_2$-fixing condition.

**iii) Oxygen**

Oxygen is a strong inhibitor of $N_2$-fixation because it blocks both the synthesis as well as the activity of nitrogenase. As you have seen in Table 15.1, there are $N_2$-fixers which are anaerobes and there are those which are aerobes. The problem of oxygen in anaerobes is taken care of by their very anaerobic mode of nutrition. The problem is really with aerobes like Azotobacter which require oxygen to fix nitrogen and grow at the expense of nitrogen. Azotobacter is known to possess the following two mechanisms to protect its nitrogenase from oxygen inhibition.

i) respiratory protection and

ii) conformational protection.

In respiratory protection $N_2$-fixing cells adjust the rate of aerobic respiration according to prevailing oxygen tension i.e. rising and falling according to external oxygen concentration. Consequently, anaerobic situation within the cell where $N_2$-fixation occurs is created.

In conformational protection an Fe-S redox protein provides protection to the enzyme. The protein gets oxidised in the presence of oxygen and $Mg^{2+}$ and it forms a reversible complex with nitrogenase enzyme. So the enzyme becomes inaccessible to oxygen and $N_2$. In the absence of oxygen reduction of Fe-S redox protein results in the dissociation of the complex leading to recovery of active nitrogenase.

The biochemistry, physiology and genetics of $N_2$–fixation in *Klebsiella pneumoniae* is fairly well understood than in other $N_2$-fixers . It overcomes the problem of oxygen by repressing or inhibiting the production of nitrogenase enzyme in the presence of oxygen. Whereas, cyanobacteria like *Nostoc* and *Anabaena* have heterocysts as exclusive sites of nitrogen fixation under aerobic growth condition. Heterocysts are the site of both synthesis and activity of nitrogenase enzyme. In such forms heterocyst plays the role of an oxygen protection device for nitrogenase. The mechanism by which heterocysts perform this role is as follows: Heterocysts are specialised cells which are formed from ordinary vegetative cells. During the differentiation process photosystem II activity gets eliminated resulting in the loss of oxygen production during photosynthesis. Further, a glycolipid layer is newly laid in the wall of the heterocyst during its differentiation. This structural modification constitutes a barrier to oxygen entry from the external environment. Thus, loss of PS II activity and formation of new glycolipid layer are the apparent mechanisms by which the heterocyst protects its nitrogenase from inhibition by oxygen.

**iv) Leghaemoglobin**

Leghaemoglobin is a joint product of *Rhizobium* and the host. It is produced during the maturation of nodule. It is commonly observed that nodules containing leghaemoglobin look pink in colour and those deficient in it look colourless. Pink coloured nodules are always effective in $N_2$-fixation while the colourless ones are unable to fix $N_2$. The inability to synthesise leghaemoglobin is associated with lack of $N_2$-fixing ability. Leghaemoglobin serves a dual function. It functions as a reservoir of oxygen and thus provides enough $O_2$ to the bacteroid for the production of ATP through aerobic respiration. Secondly, it has the ability to bind diffusing oxygen within the nodule, so it keeps oxygen away from nitrogenase.

## 15.2.6 Genetics of Nitrogen-fixation

The genetics of nitrogen-fixation is known in detail in *Klebsiella pneumoniae*. There are twenty genes required in organising the complete $N_2$-fixing apparatus. Interestingly, it has been shown that if all the twenty genes of *Klebsiella* are transferred to E coli, a non-$N_2$-fixer, the latter becomes a $N_2$-fixer. Some nif genes code for the structural components of nitrogenase while two genes (nif L and nif A) are regulatory.

The fast growing Rhizobia like *R. leguminosarum* are known to contain three kinds of genes for $N_2$-fixation. They are i) **nif-genes** required for the formation of functional nitrogenase as in *Klebsiella*, ii) **nod-genes** required for the formation of nodules in root and iii) **fix-genes** required for the maintenance of $N_2$-fixation by the nodule. They control oxygen protection of nitrogenase as well as ATP and reductant supply. There are about 18 nod-genes known which are activated by the inducer

flavonoid (leutolin) present in the exudate of roots. They are supposed to be involved in controlling various stages of *Rhizobium* infection leading to the formation of nodule. The nif, the nod and the fix genes are located on a plasmid in the fast growing rhizobia and on the chromosome in slow growing rhizobia.

Nod-genes are known to control the host-range of the bacterium and it should be genetically possible to increase its host range, thus enabling it to infect cereals and produce root nodules on them.

Recently cultured tissues of rice and wheat have been successfully infected with *Rhizobium* although the association does not have the capability of fixing nitrogen. This achievement, however, is a step closer to *Rhizobium*-wheat or *Rhizobium*-rice $N_2$-fixing system.

### 15.2.7 Measurement of Nitrogenase Activity

There are various methods to find out whether an organism is a $N_2$-fixer or not. If an organism can grow in normal atmosphere without any provision of a nitrogen source like nitrate or ammonia, one infers the organism to be a $N_2$-fixer. It is important to mention here that $N_2$-fixer heterocysts cyanobacteria or *Rhizobium*-legume system are known to grow without any supply of fixed nitrogen.

The other more reliable method is the incorporation of $^{15}N$ isotope into cellular nitrogen containing compound which can be estimated in a mass spectrometer. This method is very costly and is, therefore, not easily accessible for day-to-day measurement of nitrogenase activity and $N_2$-fixation.

The most extensively used method for measuring nitrogenase activity is the acetylene reduction method. The method consists of incubating the $N_2$-fixing system with about 10% acetylene for a period of 10 to 30 min during which nitrogenase converts acetylene into ethylene depending on its efficiency. Gas samples are then taken out by syringe from the reaction sample and the acetylene reduced is assayed by injecting the gas sample into a gas chromatograph which separates quantitatively acetylene and ethylene and thus provides easy quantitative assay of ethylene. Nitrogenase catalysed reduction of acetylene to ethylene is not accompanied by reduction of proton to molecular hydrogen as happens in the reduction of $N_2$ to $NH_3$.

The reduction of acetylene to ethylene involves two electrons and if one wants to equate the amount of ethylene produced with the amount of $N_2$ fixed then the amount of ethylene has to be divided by a factor of 4 to find out the optimum level of $N_2$-fixing activity of nitrogenase.

**SAQ 2**

a) Match the characteristics given in Column 2 with enzymes given in Column 1.

| Column 1 | Column 2 |
|---|---|
| i) Dinitrogenase reductase<br>ii) Dinitrogenase | a) Fe-Mo protein<br>b) requires ATP for activation<br>c) transfers one electron at a time<br>d) transfers electrons to $N_2$<br>e) Fe-protein |

b) Fill in the blank spaces with appropriate words in the following statements.

i) In cyanobacteria nitrogenase is present in.....

ii) Uptake-hydrogenase utilises hydrogen by passing it to ..... under aerobic condition and generates .....

iii) In an atmosphere devoid of $N_2$ gas nitrogenase enzyme catalyses the production of ..... only.

iv) A cyanobacterial strain deficient in uptake hydrogenase will produce ..... continuously in $N_2$ free atmosphere.

v) ..... protects nitrogenase against oxygen and it also functions as a ..... of oxygen.

c) Match the genes of $N_2$-fixation given in Column 1 with the functions given in Column 2:

| Column 1 | Column 2 |
|---|---|
| i) nif genes | a) Maintenance of $N_2$-fixation |
| ii) nod genes | b) Functional nitrogenase |
| iii) fix genes | c) Formation of nodules in roots |

## 15.3 NITRATE ASSIMILATION

### 15.3.1 Biochemical Reactions

Nitrate is the most readily available and preferred source of nitrogen for growth. Assimilatory reduction of $NO_3^-$ to $NH_3$ is known to occur in two steps as shown below:

i) $NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$
Nitrate Nitrite

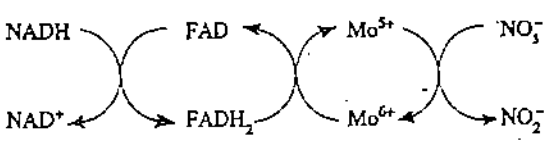
ii) $NO_2^- + 7H^+ + 6e^- \longrightarrow NH_3 + H_2O$
Nitrite Ammonia

The first step is catalysed by **nitrate reductase (NR)** which reduces $NO_3^-$ to $NO_2^-$ at the expense of two electrons. The second step is catalysed by **nitrite reductase** which converts $NO_2^-$ into $NH_3$ at the expense of six electrons. Nitrate reductase is Mo-enzyme like dinitrogenase and nitrite reductase is Fe-protein. The physiological source of reductant for the two reductive processes could be reduced ferredoxin – $Fd_{(red)}$ or reduced pyridine nucleotides (NADH or NADPH) depending upon the system. It is important to point out here that $NO_3^-$ is known to undergo dissimilation in which it is reduced to $N_2$ gas. Such a process of nitrate metabolism is called **nitrate respiration or denitrification** and occurs exclusively in certain bacterial forms under anaerobic condition. The enzymes of denitrification of nitrate are called **dissimilatory nitrate reductases.**

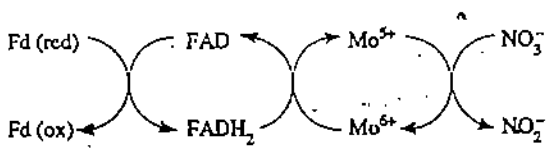
### 15.3.2 Assimilatory Nitrate Reductase and Nitrite Reductase

There are two kinds of nitrate reductases depending upon their specificity to reductant. Nitrate reductase of cyanobacterial system requires reduced ferredoxin (Fd-dependent) to catalyse the reaction. While nitrate reductase in plants and fungi requires reduced pyridine nucleotide (NADH or NADPH – dependent) to carry out the reaction. In general most pyridine nucleotide nitrate reductases are capable of using both NADH and NADPH as source of reductant. The enzyme from photosynthetic organisms like higher plants and algae show preference for NADH but that from fungi show preference for NADPH. This suggests inherent difference between cyanobacterial nitrate reductase and eukaryotic nitrate reductase in respect of reductant requirement. The two types of reductant dependent nitrate reduction reactions are shown below :

**Eukaryotic**




**Cyanobacteria**




In comparison to the Fd-dependent enzyme which contains only molybdenum as prosthetic group, the NADPH-dependent enzyme in addition to molybdenum also contains flavin adenine dinucleotide (FAD) and cytochrome $b_{557}$ as prosthetic

groups. Functionally, while the Fd-dependent enzyme catalyses only reduction of nitrate to nitrite, the NAD(P)H-dependent enzyme catalyses two independent activities, one called **diaphorase** activity in which NAD(P)H is oxidised and one electron acceptor like cytochrome C or flavin mono-nucleotide (FMN) is reduced, and the other called **terminal nitrate reductase** activity which is NAD(P)H independent and in which nitrate is reduced at the expense of reduced flavins ($FMNH_2$) or viologens. *In vivo* both activities participate jointly and sequentially in the transfer of electrons from reduced pyridine nucleotide to nitrate as shown in reaction (Fig. 15.7). Nitrate reductase in cyanobacteria lacks diaphorase activity and contains only Mo as prosthetic group.

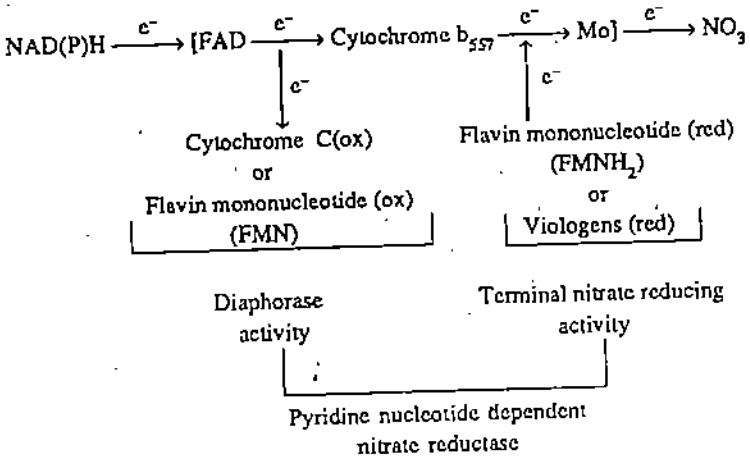
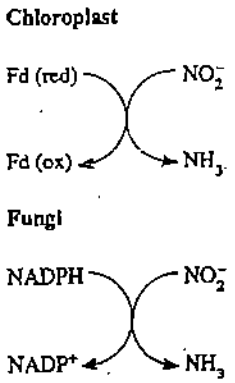



**Fig. 15.7: Activities of $NAD^+(P^+)$-dependent nitrate reductase i) diaphorase activity ii) terminal nitrate reductase activity.**

Reduction of nitrite to ammonia occurs in chloroplasts. The enzyme nitrite reductase in cyanobacteria or chloroplasts requires reduced ferredoxin for reduction. Nitrite reductase of fungi requires NADPH to carry out the reductive function. The two reactions are shown below:




### 15.3.3 Nitrate Uptake

Nitrate must enter the cells before undergoing assimilatory reduction by the joint action of nitrate reductase and nitrite reductase. Cells accumulate $NO_3^-$ against concentration gradient and this accumulation is a result of the presence of active $NO_3^-$ transport system in the cell membrane. Nitrate uptake and nitrate reduction are independent processes because organisms genetically deficient in nitrate reductase activity contain normal transport activity.

### 15.3.4 Distribution of Nitrate Reductase and Nitrite Reductase

Let us see whether nitrate assimilation depends upon the reductants produced in photosynthesis or in oxidative metabolism. It is now known that nitrate reductase and nitrite reductase are located in the photosynthetic membranes of cyanobacteria.

In higher plants ($C_3$) nitrate reductase has been shown to be a cytoplasmic enzyme and nitrite reductase a chloroplastic enzyme.

In $C_4$ plants nitrate assimilation and carbon dioxide assimilation are compartmentalised, the former located in mesophyll cells and the latter is present in bundle sheath cells. It is observed that $C_4$ plants have greater efficiency of nitrate assimilation, in comparison to $C_3$ plants. This is due to the special separation of nitrate and nitrite reduction from the site of $CO_2$ reduction. Cytoplasmic nitrate reductase and chloroplastic nitrite reductase are characteristic of mesophyll cells and not of bundle sheath cells. In non-green tissues, like roots of higher plants nitrate and nitrite reductase also occur in active form. Accordingly, there are the following three basic modes of $NO_3^-$ reduction.

i) Directly dependent upon photosynthesis as in cyanobacteria,

ii) Partly non-photosynthetic and partly photosynthetic as in mesophyll cells, and

iii) Completely non-photosynthetic as in roots.

## 15.3.5 Regulation of Nitrate Assimilation

There are two levels of regulation of nitrate assimilation. One is long-term and another is short-term. The long-term regulation operates at the level of enzyme synthesis and the short-term regulation operates at the level of enzyme activity.

### Enzyme Synthesis

Nitrate assimilating system in general are known to show increase in nitrate uptake system and nitrate reductase system in the presence of nitrate. In other words, nitrate assimilatory system is induced by the presence of nitrate. Similarly, cells or organisms assimilating $NH_3$ as nitrogen source show lack of nitrate assimilatory system. Such control of nitrate is called repression control. Thus nitrate is an inducer while ammonia is repressor of nitrate assimilatory system.

**Red light** is known to enhance synthesis of nitrate assimilatory system and this light effect is mediated by well-known photomorphogenic pigment called phytochrome about which you will learn in detail in Unit 17 of this course.

### Enzyme-activity Control

Availability of the substrate, NADH and nitrate would be an important determinant of the rate of nitrate assimilation. In addition, there are number of substances which are known to cause reversible inactivation of nitrate reductase under reducing condition (Fig. 15.8).

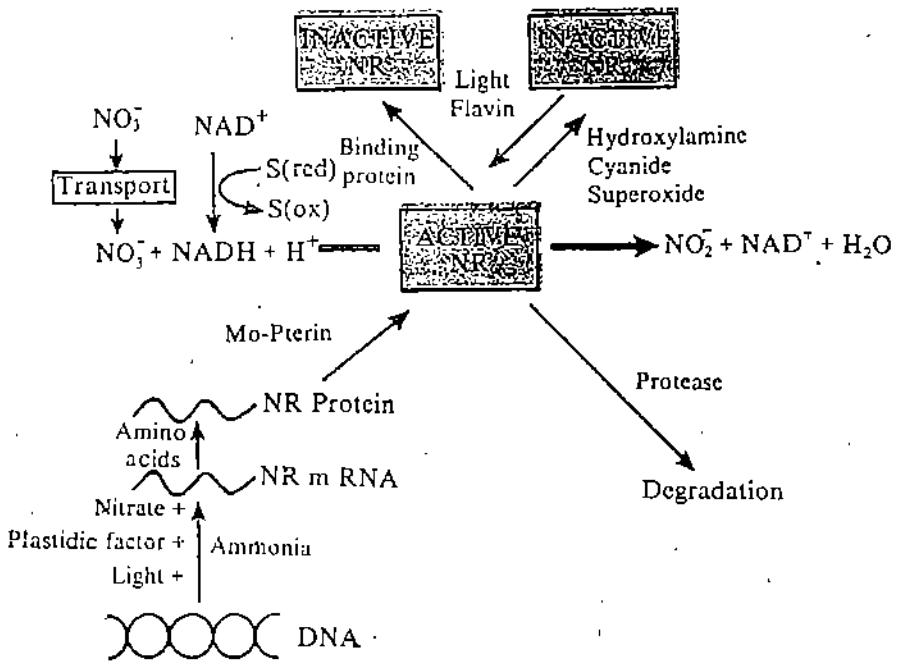



Fig. 15.8: Regulation of assimilatory nitrate reductase (NR). Reversible inactivation of NR by combination with hydroxylamine, cyanide, or superoxide and reversal of this inactivation by blue light in the presence of flavin; inactivation by combination with specific binding proteins or by limited proteolysis. Enzyme synthesis is regulated by positive effector-nitrate, plastidic factor, light and negative effectors derived from ammonia.

**Cyanide :** Plants generate cyanide from cyanogenic glycides and histidines. Ethylene biosynthesis is also accompanied by cyanide production. Nitrate reductase can occur

in reduced form following its interaction with NADH in the absence of nitrate. The reduced form of the enzyme has the ability to combine with cyanide forming enzyme-CN complex which is enzymatically inactive. Nitrate, blue light or oxygen oxidise the enzyme–CN complex releasing cyanide. The cyanide free enzyme regains its activity.

Hydroxyl amine or superoxide inactivate the enzyme which on exposure to blue light gets converted to active form. It is observed that plants grown in blue light are higher in protein contents. Blue light regulation of nitrate reductase activity may be one of the primary factors responsible for higher protein synthesis.

**Inhibitor proteins:** One kind of inhibitor protein found in higher plants is an endopeptidase which degrades nitrate reductase thus causing irreversible loss of the enzyme. The other kind includes binding proteins that specifically bind to nitrate reductase leading to permanent inactivation of enzyme. Such inactivator proteins have been isolated from rice seedings and spinach leaves. These reactions are explained in Fig. 15.8. You will see that the transcription of enzyme is mediated by light. It is unique enzyme in this respect.

## 15.4 INTERACTION OF NITROGEN AND CARBON ASSIMILATION

The requirement of $N_2$-fertilisers in agriculture is because of the low-level of nitrogen in the soil. Application of nitrogenous fertilizers bring about dramatic effects on the growth and performance of the plant. One of the most important consequences is the effect on utilisation of carbohydrates in plants. It is known that the level of carbohydrates in the plants goes down with the level of $N_2$-fertiliser supplied to them. The form in which the fertilisers are supplied are $NO_3^-$, $NH_3$ and $NH_4^+$. In the plant $NO_3^-$ is reduced to $NH_3$ and assimilation of $NH_3$ requires carbon-skeleton like $\alpha$-ketoglutaric acid, an intermediate of TCA cycle for its incorporation into organic form. Naturally, the rate of $NH_3$ assimilation into organic form would depend on the rate at which TCA cycle supplies the carbon skeleton. The C-skeleton removed during the assimilation of $NH_3$ must be replenished through the catabolism of carbohydrates. Consequently, the carbohydrate content of the plant decreases in proportion to the amount of organic $N_2$ produced.

The other place of nitrate assimilation in the plant as you have already seen is photosynthetic tissue and the reductants are reduced ferredoxin and pyridine nucleotide. Since such reductants are also required for carbon assimilation, the production of carbohydrates in photosynthesis is bound to go down in proportion to the increase in rate of reductive assimilation of $NO_3^-$. In other words, the level of $NO_3^-$ utilisation is inversely related to the level of carbohydrate in the plant.

Following are the practical applications of this knowledge in raising crops with desired protein and carbohydrate content. Take the example of celery eaten as salad, the stalks of this plant are most edible when soft and this softness is the result of lower availability of carbohydrates. Now, to raise a celery crop with soft stalks one uses $N_2$-fertilisers which divert a major part of carbohydrate in the synthesis of amino acids and proteins. Another example is sugarcane, a crop of tropical region. It requires a period of six months from the time of planting to the period of harvest. Here, the growers apply nitrogen fertilisers in the beginning and not near the time of harvest. Similar practices have resulted in production of beet roots highly rich in sugar concentration. The reason for rise in sugar in the beet root is again due to withholding of $N_2$-fertiliser near the harvest time, so that the C-skeleton is not diverted for producing amino acids and proteins.

SAQ 3

a) Match the characteristics listed below of Column 2 with enzymes given in Column 1.

| Column 1 | Column 2 |
|---|---|
| i) Nitrate reductase | a) Non-heme Fe-protein |
| ii) Nitrite reductase | b) Present in chloroplast |
| | c) Present in cytoplasm |
| | d) Molybdo-flavo protein |
| | e) Diaphorase activity |

Choose the alternate correct word given in parenthesis in the following statements:

i) Nitrate assimilation in most higher plants occurs mainly in (roots/leaves).

ii) Number of electrons used when $NO_3^-$ is reduced to $NO_2^-$ (1/2).

iii) Number of electrons used when $NO_2^-$ is reduced to ammonia (2/6).

iv) In cyanobacteria nitrate reductase is located in the (photosynthetic membranes/plasma membrane).

v) In higher plants nitrate reductase is in (cytoplasm/chloroplast) and nitrite reductase is in (cytoplasm/chloroplast).

vi) In $C_3$ plants nitrate assimilation occurs in (bundle sheath/mesophyll cells) and $CO_2$ assimilation occurs in (bundle sheath/mesophyll cells).

vii) Nitrate reductase activity is induced in the presence of ($NO_3^-$/$NH_4^+$).

## 15.5 AMMONIA ASSIMILATION

Nitrogen ($N_2$) gas and $NO_3$ are the most common available forms of inorganic nitrogen. Both are enzymatically reduced to ammonia because it is only ammonia that is incorporated into organic form. Since amino acids either free, or part of proteins are the predominant form of organic nitrogen, the major product of ammonia assimilation is usually considered to be amino nitrogen.

### 15.5.1 Biochemical Reactions

Ammonia resulting from $N_2$-fixation or nitrate reduction or supplied exogenously is assimilated in plants by the following two primary pathways, i) glutamate dehydrogenase (GDH) pathway and ii) glutamine synthetase (GS)-glutamate synthase pathway.

i) **High cellular ammonia pathway:**

$$NH_3 + \alpha\text{-Ketoglutarate} + NADPH + H^+ \xrightarrow{GDH} \text{Glutamate} + NADP^+ + H_2O$$

ii) **Low cellular ammonia pathway:**

$$\text{Glutamate} + NH_3 + ATP \xrightarrow{\text{Glutamine Synthetase}} \text{Glutamine} + ADP + Pi$$

$$\text{Glutamine} + \alpha\text{-Ketoglutarate} + NADPH + H^+ \text{ (or } Fd_{(red)}) \xrightarrow[\text{synthase}]{\text{Glutamate}}$$

$$\text{Glutamate} + NADP^+ + H_2O \text{ (or } Fd_{(ox)})$$

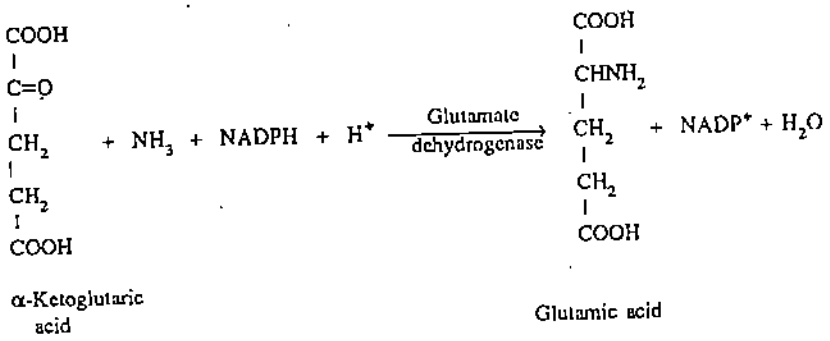
As you may note, both the pathways generate the same amino acid, glutamate as end product of the reaction. The two pathways may operate simultaneously as in higher plants and eukaryotic algae or alternatively as in certain heterotrophic enterobacteria. In cyanobacteria glutamine synthetase—glutamate synthetase (GS) is the main primary pathway of ammonia assimilation.

The two enzymes involved in ammonia assimilation differ significantly in their affinity for ammonia which is very high for glutamine synthetase and significantly low for glutamate dehydrogenase. In other words, glutamate dehydrogenase mediated pathway functions under conditions of high cellular ammonia and glutamine synthetase-mediated pathway under conditions of low cellular ammonia (Fig. 15.9).

The GS-glutamate synthase pathway is characteristically cyclic in nature in which glutamate acts as both the acceptor and product of ammonia assimilation. This pathway of ammonia assimilation is called glutamate synthase cycle. The pathway

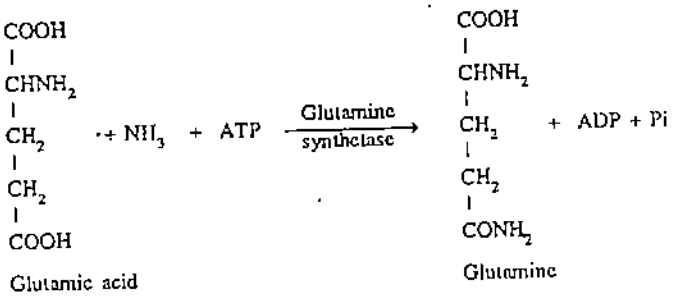
requires ammonia, ATP, reductant and α-ketoglutarate for the net production of a molecule of glutamate. So we find that in comparison to GDH pathway, this pathway of ammonia assimilation is more expensive because of energy cost and material need than that of the GDH pathway.

Glutamate dehydrogenase pathway in high cellular ammonia

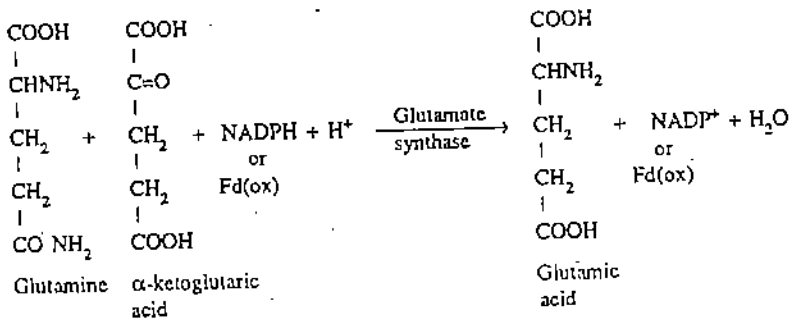



α-Ketoglutaric acid — Glutamic acid

Glutamine synthetase (GS)—glutamate synthase pathway




Glutamic acid — Glutamine




Glutamine — α-ketoglutaric acid — Glutamic acid

Fig. 15.9: a) Glutamate dehydrogenase pathway, b) The Glutamine synthetase – Glutamate synthase cycle, glutamine synthetase, glutamate synthase.

## 15.5.2 Uptake of Ammonia

Ammonia ($NH_3$) diffuses freely across biological membranes according to its concentration gradient. However, ammonium ($NH_4^+$) ion requires a specific transport system to cross the biological membrane. In recent years, activities and regulation of ammonium transport system have been studied in a number of $N_2$-fixing systems. The following are the characteristics of ammonium transport system:

i) ammonium transport system is ammonia repressible as it is absent in cultures grown with ammonia as nitrogen source;

ii) nitrogen limitation for growth appears to be the signal for the induction of the ammonium transport system;

iii) ammonium transport system like other transport processes is an energy requiring active process.

Since higher levels of ammonia in lower plant cells like cyanobacteria exert toxic effects, there must occur a closer interrelationship between uptake and assimilation of ammonia in such systems. Further studies are required to clearly understand the interdependence of the two processes. At this point it is important to point out that the characteristics of ammonium transport process has not been studied in higher plants.

### 15.5.3 Regulation of Ammonia Assimilation

Heterotrophic bacteria like *Escherichia coli* and *Klebsiella aerogenes* induce the operation of GS-glutamate synthase pathway of ammonia assimilation under conditions of low ammonia and of GDH-pathway under conditions of high ammonia. Thus the two pathways are mutually exclusive and the level of cellular ammonia determines which pathway of ammonia assimilation is likely to operate. Cyanobacteria like systems have only GS-glutamate synthase pathway functioning under low or high cellular ammonia. Information about the role of ammonia in metabolic regulation of its two assimilatory pathways in higher plants is virtually unknown.

Animals get rid of excess ammonia through the urea cycle in order to escape ammonia toxicity. Plants have no similar mechanism of escaping ammonia toxicity. This might be because land plants evolved under conditions of nitrogen limitation. However plants seem to have developed controls of nitrogen metabolism which ensure that ammonia is reassimilated.

## 15.6 NITROGEN CONTROL OF NITROGEN ASSIMILATION

$N_2$-fixer like *Klebsiella pneumoniae* and *Nostoc* can grow with $N_2$, $NO_3^-$ or $NH_4^+$ as nitrogen source. You would like to know how these organisms manage to assimilate one of the three forms of $N_2$ when given all the three simultaneously. It is known that $NH_4^+$ is preferred over $NO_3^-$ or $N_2$ as nitrogen source. Now the question is how this preference is realised by such $N_2$-fixers? Ammonia can readily enter the cells by diffusion and the cells assimilate ammonia thus available into glutamine and glutamate. Under such conditions the ratio of glutamine to $\alpha$-ketoglutarate rises which is a signal for sufficient nitrogen and causes repression of both $NO_3^-$ assimilation and $N_2$-fixation system. This is analogous to ATP/ADP ratio which signals the energy-state of a cell. A high ATP/ADP ratio indicates that the cell has sufficient energy to perform its metabolic functions. That is why legumes grown in the medium containing $NH_4^+$ do not form nodules with *Rhizobium*. In this connection it is important to point out that recognition mechanics as explained earlier of *Rhizobium*-legume symbiosis is not seen in root hairs of legume supplied with $NH_4^+$. Similarly, when a $N_2$-fixer is exposed to $NO_3^-$ and $N_2$, it preferentially assimilates $NO_3^-$ and such $NO_3^-$ assimilating organisms do not produce $N_2$-fixing apparatus. The mechanism of $NO_3^-$ inhibition of $N_2$-fixation is the same as that described for $NH_4^+$ inhibition of $N_2$-fixation. The mechanism of ammonium repression of $NO_3^-$ assimilation occurs through the same mechanism as ammonium repression of $N_2$-fixation. This explains why $N_2$-fixation, nodule formation and heterocyst formation occur under conditions of limited nitrogen and not under conditions when nitrogen is available to the plant.

## 15.7 SULPHATE ASSIMILATION

You know that plants take sulphur mainly in the form of sulphate ($SO_4^{2-}$). It is taken up actively against concentration gradient. Sulphur is part of amino acids—cysteine, cystine and methionine. Plants also contain two activated sulphur compounds — adenosine 5′ phosphosulphate (APS) and 3′ phosphoadenosine 5′ phosphosulphate (PAPS) important in sulphate assimilation (Fig. 15.10). These are analogous to ATP. There are also sulphate esters in plants such as polysaccharide sulphate and mustard oil glycosides.

Certain anaerobic bacteria like *Desulfovibrio* use sulphate as a terminal acceptor of electrons during respiration to generate ATP. This is called **sulphate respiration.** Such a reduction of sulphate is called dissimilatory reduction. The other mode of sulphate reduction carried out by plants is assimilatory reduction in which sulphate is reduced to thiol group found in many coenzymes and in the amino acids—cysteine and

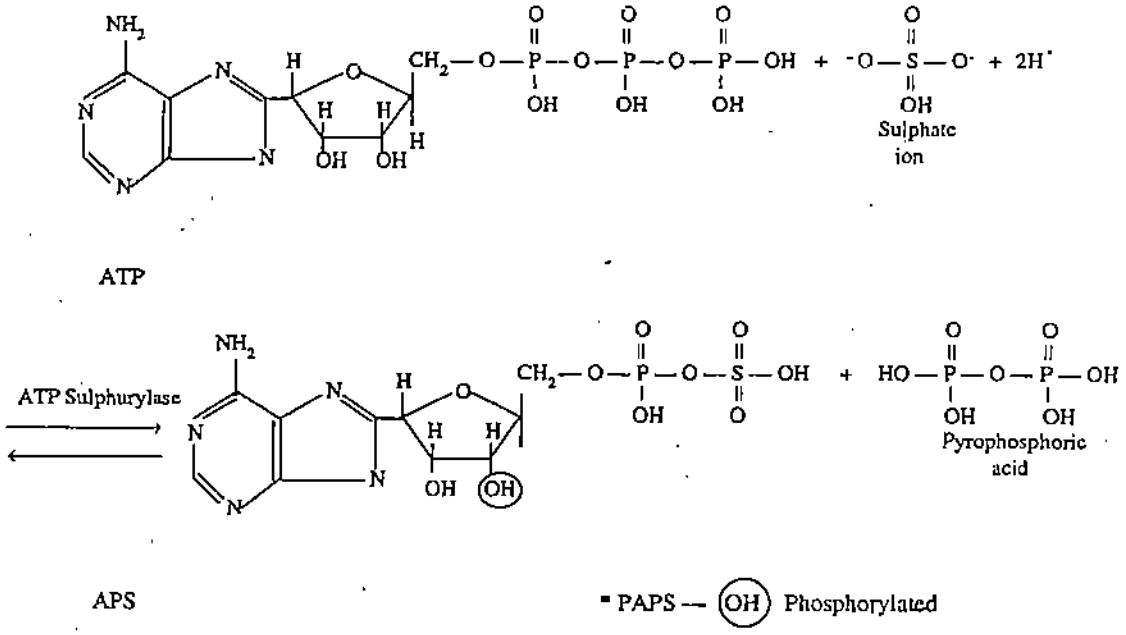



**Fig. 15.10: Structure of ATP and adenosine 5' phosphosulphate (APS). In PAPS 3'-hydroxy group of pentose is also phosphorylated (encircled)**

methionine. As you have learnt hydrogen sulphide and other reduced inorganic sulphur compounds are used as photosynthetic electron donors in bacterial photosynthesis.

Assimilation of $SO_4^{2-}$ into organic compounds occurs through the following two reductive steps.

$$\underset{\text{Sulphate}}{SO_4^{2-}} \longrightarrow \underset{\text{Sulphite}}{SO_3^{2-}} \longrightarrow \underset{\text{Sulphide}}{S^{2-}}$$

The sulphate that has entered the cell is converted into activated PAPS according to the following equation:

$$SO_4^{2-} + ATP \xrightarrow{\text{ATP Sulfurylase}} AMP{-}SO_4^{2-}(APS) + PPi$$

$$APS + ATP \xrightarrow{\text{APS Kinase}} PAPS + ADP$$

$$PPi \xrightarrow[\text{Pyrophosphatase}]{\text{Inorganic}} 2\ Pi$$

APS can enter directly into the assimilatory pathway or it can be acted upon by another molecule of ATP (see equation above) in the presence of enzyme APS kinase to produce PAPS which represents another form of activated sulphate. You have seen that such ATP-requiring activation reactions are not needed in the assimilatory reduction of nitrate or carbon dioxide.

PAPS is used in cells for all esterification reactions of sulphate with organic compounds. APS pathway of sulphate reduction occurs in higher plants, algae and cyanobacteria; PAPS pathway operates in organisms like yeast and *Escherichia coli*.

Sulphite can also serve as a source of sulphur in photosynthetic systems but this pathway of assimilatory sulphite reduction follows a course separate from that of sulphate assimilation and is carried out by a single enzyme called sulphite reductase. The enzyme like nitrite reductase catalyses a 6 electron reaction step for the conversion of sulphite to sulphide. In the PAPS pathway sulphite is an intermediate in the reduction of sulphate to sulphide and sulphate reduction here is catalysed by sulphite reductase leading to the formation of sulphide.

$$SO_3^{2-} + 6\,e^- + 6\,H^+ \xrightarrow[\text{reductase}]{\text{Sulphite}} S^{2-} + 3\,H_2O$$

Most likely the activated forms of $SO_4^{2-}$, APS and PAPS are directly involved in the reduction of sulphate to sulphite. There is evidence that the reduction of sulphite to sulphide in photosynthetic tissues involves reduced ferredoxin.

The enzyme sulhydralase catalyses the incorporation of sulphide into serine to form cysteine. The two other amino acids cystine and methionine are synthesised from cysteine.

$$\text{Serine} + S^{2-} \longrightarrow \text{cysteine}$$

The reactions of PAPS with alcohols results in the formation of sulphate esters as shown below:

$$ROH + PAPS \longrightarrow ROSO_3^- + PAP$$

The regulatory aspects of inorganic sulphur metabolism are not as well understood as those of inorganic nitrogen. Scientists are turning their attention to them now.

## 15.8 METABOLIC INTERRELATION OF NITROGEN, CARBON AND SULPHUR

Plants grow and develop into characteristic individuals as a result of a series of well programmed and regulated biochemical and morphological events. The main elements that enter into the composition of an individual plant are carbon, hydrogen, oxygen, nitrogen and sulphur. The three elements are taken by plants in the oxidised form $CO_2$, $NO_3^-$, $SO_4^{2-}$ and thus need to be reduced for assimilation. Assimilation of nitrate and sulphate, like carbon dioxide, requires ATP and reductant. It seems that plants must have evolved mechanisms to integrate the three assimilatory reductions with light harvesting photosynthetic reactions. Eukaryotic algae and higher plants have achieved this integration by localising most of these assimilatory reactions within chloroplast.

Photosynthetically produced ATP and reductant (ferredoxin or NADPH) together constitute what is called assimilatory power of the plants. Light reactions of photosynthesis produce ATP and reductant and dark reactions of photosynthesis are related to the reductive assimilation of carbon dioxide, nitrate and sulphate.

In plants, since carbon, nitrogen and sulphur also occur together in sulphur amino acids there must be regulatory mechanisms controlling integrated assimilation of carbon dioxide, nitrate and sulphate into organic forms. The results of scientific studies also support this view. Inhibition of photosynthetic carbon dioxide assimilation is known to simultaneously inhibit nitrate assimilation. This is because the carbon skeleton for compounds like α-ketoglutarate is ultimately produced from photosynthetically generated organic carbon from carbon dioxide. Assimilation of $N_2$ by nodulated legumes also depends upon the extent and amount of photosynthate like organic carbon reaching them. There is evidence which suggests that sulphur like carbon also controls nitrogen metabolism. Protein synthesis is known to decline under sulphate limitation and comes to a halt after sulphate exhaustion. During sulphur starvation, nitrate or ammonia is assimilated mainly into free amino acids and not in proteins. Because, in the absence of sulphur cysteine and methionine, (sulphur containing amino acids), cannot be synthesised and the disulphide bridges cannot be formed in proteins. Some of the amino acids preferentially synthesised under these conditions are arginine, glutamine and alanine.

The major regulatory mechanisms controlling interdependence of inorganic carbon, nitrogen and sulphur metabolism are to be studied and understood in detail as they hold the key to the desired production of major plant products at commercial scale.

SAQ 4

a) i) Name the two pathways of ammonia assimilation in plants.

.....

.....

ii) Which of the two pathways operates under high concentration of cellular ammonia?

.....

iii) Which of the two pathways is costly in respect of energy and material needs?

.....

.....

.....

b) i) Which of the source among $NH_4^+$, $NO_3^-$ and $N_2$ is preferred by the plants?

.....

ii) What signal in plant causes repression of both $NO_3^-$ assimilation or $N_2$-fixation?

.....

.....

c) i) Name the two activated sulphur compounds in plants. What is their role in sulphur assimilation?

.....

.....

.....

## 15.9. SUMMARY

- Biological $N_2$-fixation is characteristic of prokaryotes and the process occurs in free-living and symbiotic state with various kinds of eukaryote partners.
- Nitrogenase enzyme is made of two functional components dinitrogenase reductase (Fe-protein) and dinitrogenase (Fe-Mo protein). Both of these are essential for $N_2$-fixation, acetylene reduction, proton reduction and HCN reduction.
- Nitrogenase reaction occurs at the expense of 2 to 3 molecules of ATP consumed per electron transferred.
- Oxygen is inhibitor of both nitrogenase activity and synthesis. The $N_2$-fixers have evolved a strategy to overcome the $O_2$ problem by having respiratory and conformational protection in *Azotobacter*, leghaemoglobin in nodules of legumes and heterocysts in *Nostoc*.
- Nitrate and ammonia are strong inhibitors of $N_2$-fixation. In their presence $N_2$-fixing system is not made at all. Uptake hydrogenase activity is a mechanism of removing hydrogen from the site of nitrogen activity which concurrently carries out reactions of nitrogen fixation and hydrogen production.
- In *Rhizobium*-legume symbiotic $N_2$-fixing system, there are three classes of genes controlling $N_2$-fixation *in situ*. They are nod genes, nif genes and fix-genes.
- Acetylene reduction technique is most common method of measuring nitrogenase activity.
- Nitrate assimilation occurs in two enzymatic steps. Nitrate reduction to nitrite is catalysed by nitrate reductase and nitrite reduction to $NH_3$ is catalysed by nitrite reductase. NADH is the reductant for nitrate reductase in plants, NADPH is the reductant in fungi and reduced ferredoxin in cyanobacteria. Reductant for nitrite reductase in fungi is NADPH and in plants and cyanobacteria is reduced ferredoxin.
- Nitrate reductase is predominantly localised in cytoplasm and nitrite reductase is localised exclusively in chloroplast.
- Nitrate assimilation is regulated by inducer and repressor control at synthetic level and activity control involving reversible inhibition of enzyme activity by cyanide, hydroxyl amine, superoxide radical and reactivation by blue light.
- In $C_4$ plants nitrate assimilatory pathway remains localised within mesophyll cell and $CO_2$ assimilation in bundle sheath cells.
- Plants prefer ammonia as a source of nitrogen over $NO_3^-$ or $N_2$ this is achieved by repression control of nitrate assimilation and $N_2$ fixation.

- Glutamate dehydrogenase and glutamine synthatase are the two primary enzymes of ammonia assimilation.
- Sulphate like $NO_3^-$ is also assimilated reductively. Unlike $NO_3^-$, it is required to undergo ATP dependent activation before entering into pathway of assimilatory reduction.
- A clear understanding of metabolic interrelationship of N, C and S nutrition are a must from agricultural point of view.

## 15.10 TERMINAL QUESTIONS

1. How will you experimentally separate $N_2$-fixers from non-fixers?

2. Why is heterocyst essential for $N_2$-fixation in *Nostoc* and *Anabaena*?

3. What is the role of leghaemoglobin in nodules?

4. Why is Mo an essential nutrient for $N_2$-fixation and nitrate assimilation?

5. What do you understand by nitrate respiration and sulphur respiration?

## 15.11 ANSWERS

**Self-assessment Questions**

1) a) i) c, ii) a, iii) b, iv) c, v) d
   b) i) c, ii) d, iii) a, iv) b
   c) i) T, ii) F, iii) T, iv) T, v) F, vi) F
   d) i) polysaccharide, lectin, ii) lectin, polysaccharide, iii) ammonia, glutamine synthetase, iv) plant

2) a) i) b, c and e, ii) a and d
   b) i) heterocyst, ii) electron transport chain, ATP, iii) hydrogen, iv) hydrogen, v) Leghaemoglobin, reservoir.
   c) i) b, ii) c, iii) a.

3) a) i) c, d, e, ii) a, b
   b) i) leaves, ii) 2, iii) 6, iv) photosynthetic membranes, v) cytoplasm, chloroplast vi) mesophyll cells, bundle sheath cells, vii) $NO_3^-$.

4) a) i) Glutamate dehydrogenase pathway and Glutamine synthetase-glutamate synthase pathway
      ii) Glutamate dehydrogenase pathway
      iii) GS-glutamate synthase
   b) i) $NH_4^+$
      ii) high ratio of glutamine to $\alpha$-ketoglutarate.
   c) i) adenosine 5'-phosphosulphate (APS) and 3'-phosphoadenosine 5'-phosphosulphate (PAPS).
      ii) They are used in the cell for esterification reactions of sulphate with organic substances.

**Terminal Questions**

1. The nitrogen fixers can be separated from non-nitrogen fixer by performing the following experiment. Take cyanobacteria like *Nostoc* and *oscillatoria* and grow them separately in mineral growth medium which lacks $NO_3^-$ or $NH_4^+$. Incubate these two cultures for growth under photosynthetic condition. The culture which will keep on growing without external nitrogen source would be $N_2$-fixer and the other which will not grow would be non-$N_2$-fixer.

2. For $N_2$-fixation it is necessary to have $O_2$-protection mechanism for nitrogenase enzyme. In *Nostoc* and *Anabaena* this is provided by heterocyst which is a specialised structure that has lost PS II activity during differentiation. Hence in heterocyst nitrogenase is protected because of reduced oxygen tension. It would not be possible for these algae to fix $N_2$ without heterocyst because the green parts of algae evolve oxygen which is inhibitor of $N_2$-fixing enzyme.

3. Oxygen is inhibitor of $N_2$-fixing enzyme nitrogenase. The primary role of leghaemoglobin is to bind oxygen in the vicinity of the nitrogenase enzyme. It also serves as a reservoir of oxygen and supplies oxygen to meet the high energy demand during aerobic respiration.

4. Mo is part of component I of enzyme nitrogenase and nitrate reductase and is involved in the reductive process of $N_2$-fixation. Like Fe, Mo ion can also reversibly change from oxidised to reduced state and help in the transfer of electrons from reductants like ferredoxin, flavodoxin, NAD (P) H to $N_2$ or $NO_3^-$.

5. Nitrate or sulphate are also used as terminal acceptor of electron instead of $O_2$ to generate ATP in respiration. This type of respiration is termed accordingly nitrate and sulphate respiration.

# UNIT 16 PLANT HORMONES

**Structure**



## 16.1 INTRODUCTION

In the previous Units 11 to 15 you have learnt about nutrition in plants. In this unit and the one that follows we will deal with control of plant growth and development.

You may have often wondered why in a germinating seedling, roots grow in downward direction whereas shoots grow upwards. Why some flowers bloom during the day, but close at night, as if to sleep. Why one rotten apple in a basket leads to rotting of others or what causes leaf fall? How are the processes of cell division and cell elongation controlled? These are some of the questions, amongst many others, which do not have any simple answers, for most of these phenomena are controlled by complicated interactions among three levels of controls—genetic, hormonal and environmental. The various genes in a species are turned on at precise times to control cell activity and various characteristics of organisms. One class of potent chemicals that coordinate growth and development in plants and animals are hormones. They trigger cellular reactions in target cells and also determine the genes that are to be expressed at a particular stage of development. Environmental factors such as light and temperature also affect and control growth and development. These will be dealt with in the following unit.

In this unit we will tell you about different groups of plant hormones, how they were discovered and the various roles they play in growth and development of plants. Finally we will also discuss the application of plant hormones in agriculture.

**Objectives**

After studying this unit you should be able to:

- explain the experiments that led to the discovery of auxins,
- describe the discovery of gibberellins,
- describe the experimental technique of Went used for the bioassay of auxins,
- list the five groups of plant hormones, their site of production and their role in plant growth and development,
- discuss the possible mechanism of action of plant hormones,
- describe commercial applications of plant hormones.

## 16.2 DISCOVERY AND CHARACTERISTICS OF PLANT HORMONES

To date, five major classes of plant hormones have been discovered namely auxins, gibberellins, cytokinins, abscisic acid and ethylene. It is possible that many other growth regulators, present in plants, may be classified as plant hormone in the future. Not all plant hormones fit the general definition of a hormone which is a chemical synthesised in one part of an organism that stimulates or inhibits a specific response in a target tissue elsewhere in the organism.

The five groups of natural hormones—auxins, gibberellins, cytokinins, ethylene and abscisic acid fit the classical definition of hormones. Synthetic chemicals used as hormones are referred to as **plant growth regulators**.

Most animal hormones trigger highly specific responses in the target tissue. But the responses of plant hormones are quite different compared to that of animal hormones. The following are the important features of the role of plant hormones in growth and development.

i) The hormone may initiate one response in one part of the plant and an entirely different response in another part.
ii) Quite often interaction of various hormones may elicit an expected or different response from what each hormone will individually produce. They may work synergistically or antagonistically.
iii) The effect of a hormone may even vary with concentration, during different times of the year or the developmental stages of the plant.

These variables make it difficult to answer specific questions on the role of plant hormones in growth and development. Thus this area is one of the most challenging areas of physiology.

### 16.2.1 Auxins

The history of discovery of auxin is a fascinating chapter in plant physiology. The hormone auxin was discovered first, through some elegant experiments by Charles Darwin and his son Francis around 1880. They found that if a coleoptile of a grass (*Phalaris*) was illuminated from one side it bends towards light. However, if the tip region was covered, no bending occurred (Fig. 16.1). In his book "The Power of Movement in Plants" published in 1880, Darwin wrote that some matter in the upper part; which is acted upon by light, transmits its effect to the lower part causing the latter to bend.

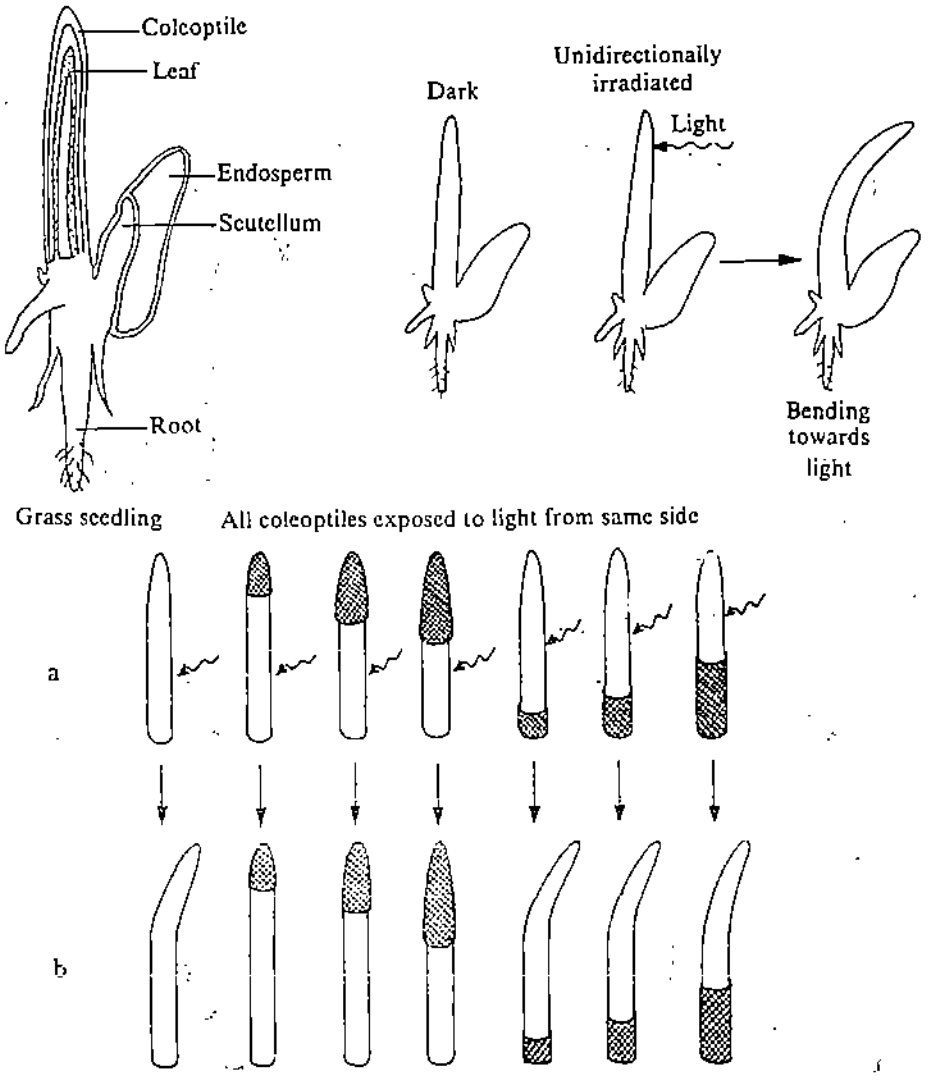



Fig. 16.1: Darwin's experiment on canary grass coleoptiles: a) Various parts were covered with metal caps for the determination of photosensitive region: b) Results observed in each case.

Peter Boysen-Jensen, a Dane and A. Paal a Hungarian did further experiments (Fig. 16.2) on Darwin's observation in order to understand the movement of the stimulus. They decapitated a coleoptile of oat seedlings and in one experiment (a) introduced a tiny gelatin block between the stump and cut tip and in other (b) a thin sheet of mica. The curvature in coleoptile occurred in a but not in b because the stimulus could pass down through gelatin but not through mica. However, when the mica was inserted half way either on lighted side or on darkside the curvature occurred only when the mica was on the lighted side. These observations suggested that the stimulus promotes elongation on dark side. A. Paal observed that in the dark whichever side the cut tip was placed bending occurred in the opposite direction, suggesting that the stimulus in the tip affects cells directly below. This stimulus was later found to be a diffusible substance by a Dutch scientist F.W. Went in 1926. He collected this substance in large amounts by placing decapitated coleoptile tips on agar blocks. He

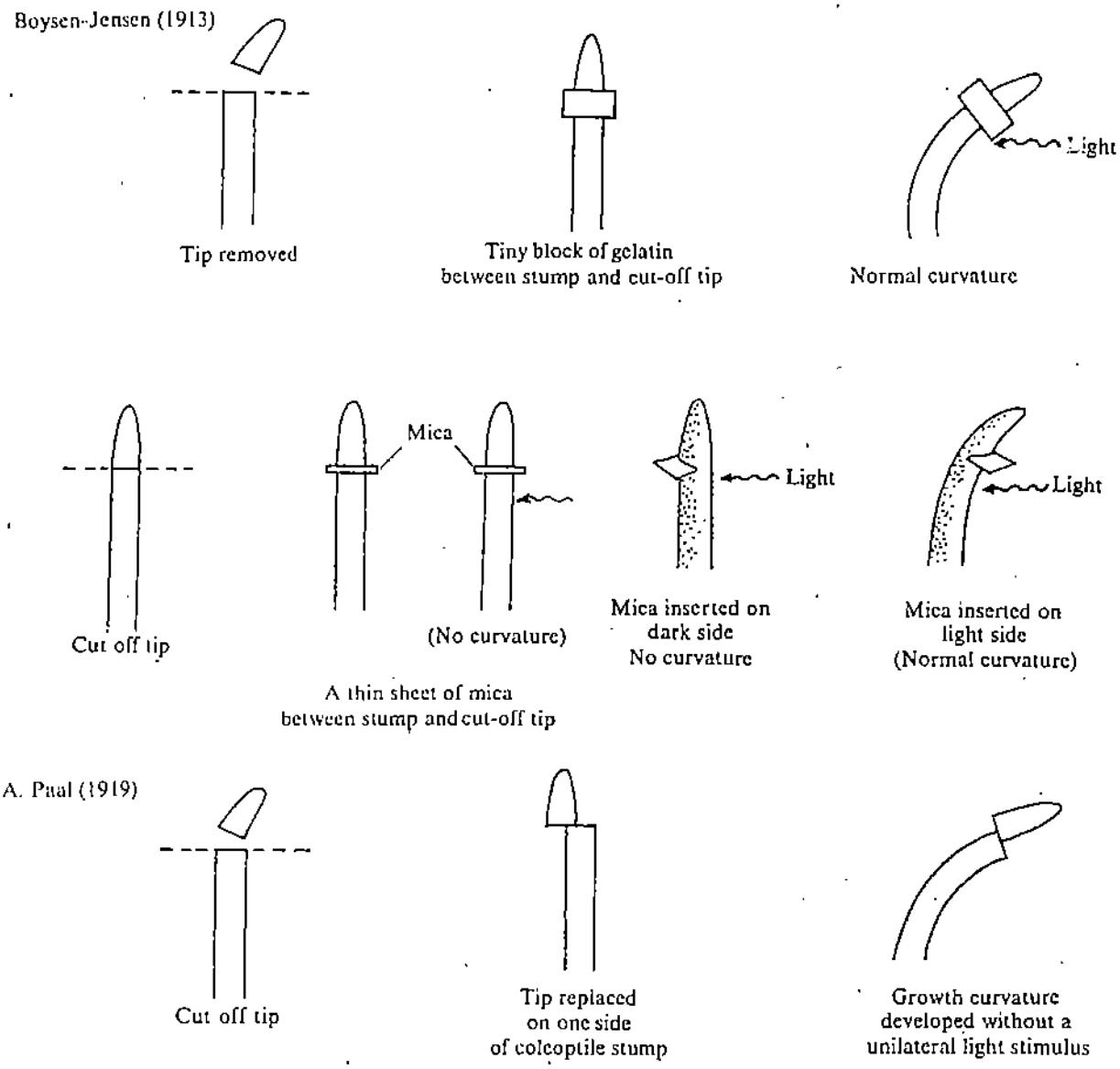



Fig. 16.2: Inserted between stump and the cut-off tip: a) a tiny block of gelatin, b) a thin sheet of mica. Note that on illumination the curvature of the tip of coleoptile was seen in a but not in b: c) displaced tips cause bending in the opposite direction.

then took tiny squares of the agar and placed them eccentrically on the cut end of the decapitated seedlings. Typically bending occurred within an hour after the blocks were applied (Fig. 16.3). He concluded that the substance, present in agar block when placed eccentrically on the coleoptile stump diffused in the coleoptile causing elongation of cells in that side resulting in curvature. Went named it auxin (from the Greek word auxein, "to increase"). Later auxin was chemically characterised as indole-3-acetic acid or IAA (Fig. 16.4).

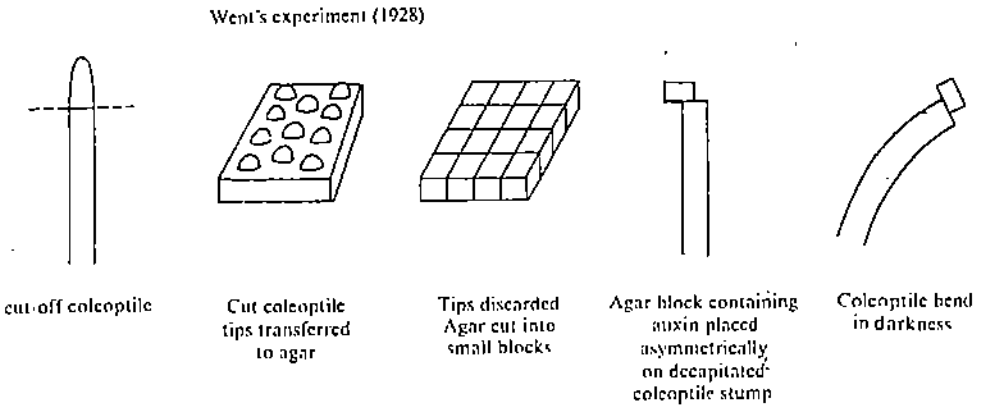



**Fig. 16.3: Went's experiment on auxins: a) Collection of difusible substance from the tips of oat coleoptiles on agar blocks. b) The agar blocks placed eccentrically on the colpoptile's cut edge alongside the growing shoot. The curvature is seen on the opposite side.**

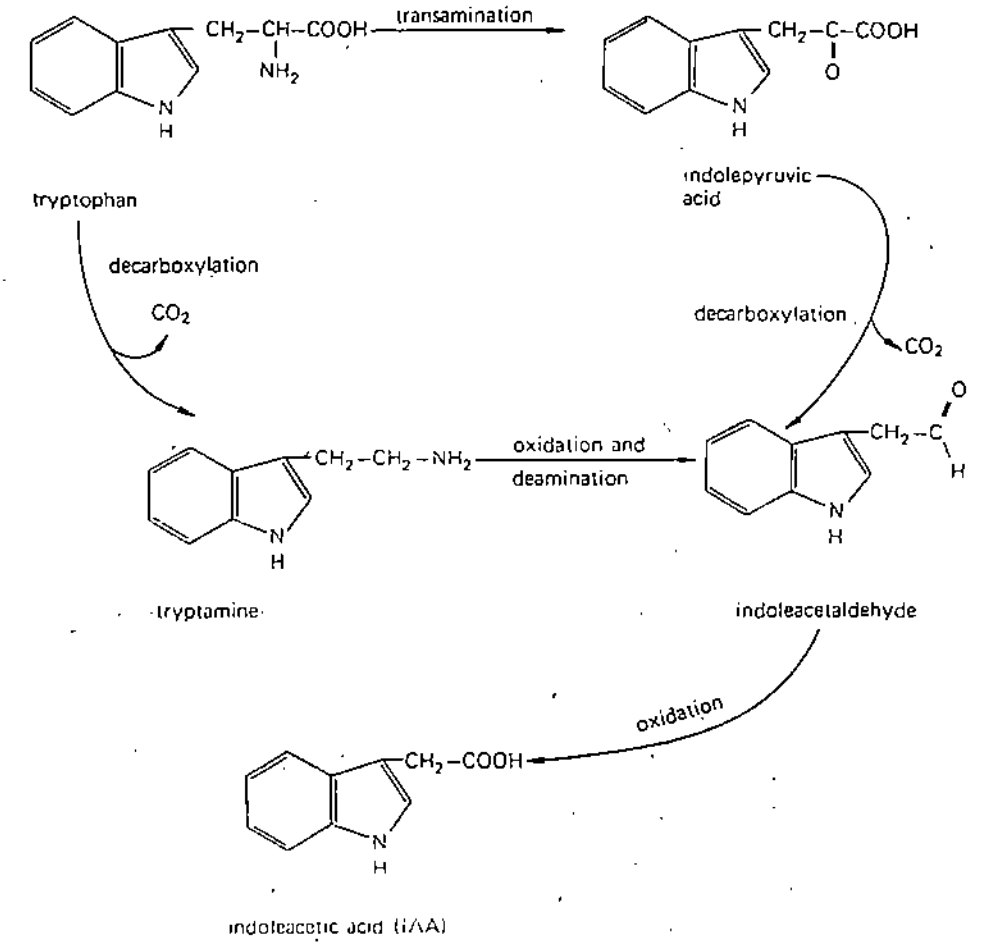



**Fig. 16.4: The structure of auxin-indoleacetic acid (IAA) and the probable scheme of its biosynthesis from tryptophan.**

Auxins are produced in the growing regions of stems and young leaves. Little did people realise when it was discovered about the tremendous impact it will have in agriculture and horticulture. The presence of auxin can be demonstrated and in fact quantified by a sensitive assay system, known as bioassay, based on Went's basic method as shown in the Fig. 16.5. Hormone concentrations are calculated by the degree of bending seen in the oat coleoptile. The greater the angle, the more the hormone present.

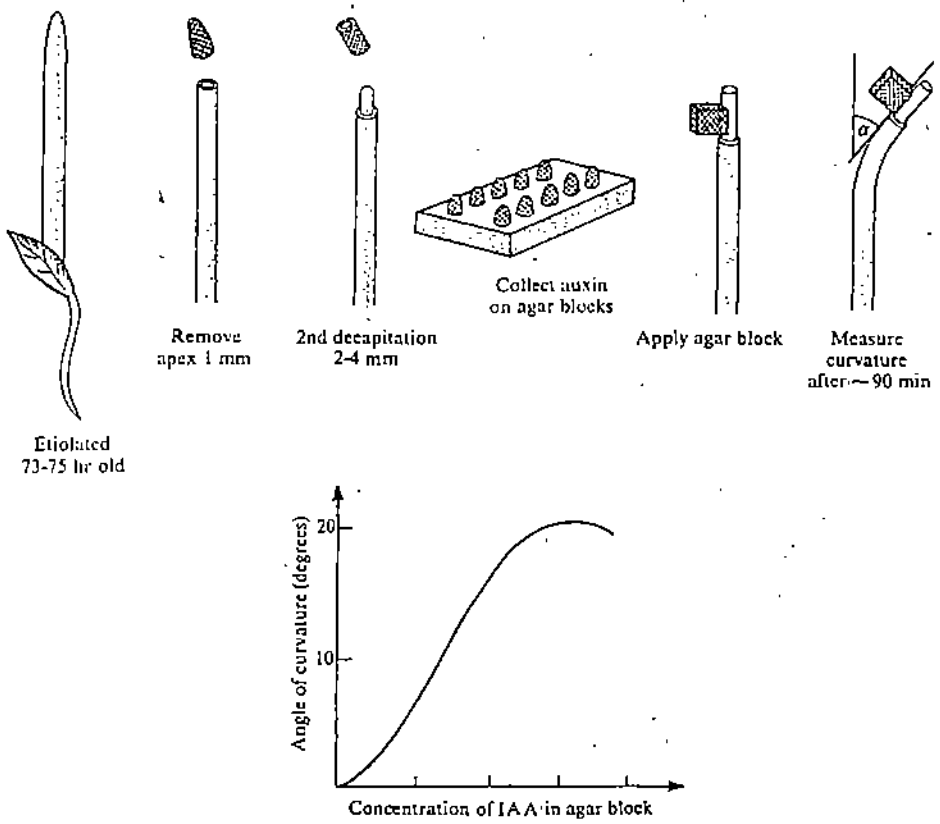



**Fig. 16.5: Bioassay of auxin. The extent of bending as measured by the angle α, is a function of the amount of auxin or IAA present in the agar block. (Source, Leopold 1955).**

The effect of auxin on growth of roots, stems and buds varies. Roots are the most sensitive, followed by stems and buds which can tolerate a much higher dose of auxin. Look at Fig. 16.6, the concentration of auxin that promotes growth in buds or stems actually inhibits growth of roots. If the concentration of auxin is more than the requirement (optimum concentration), it will inhibit growth. Auxin does not cause growth by division but rather promotes cell enlargement through elongation (Fig. 16.7)

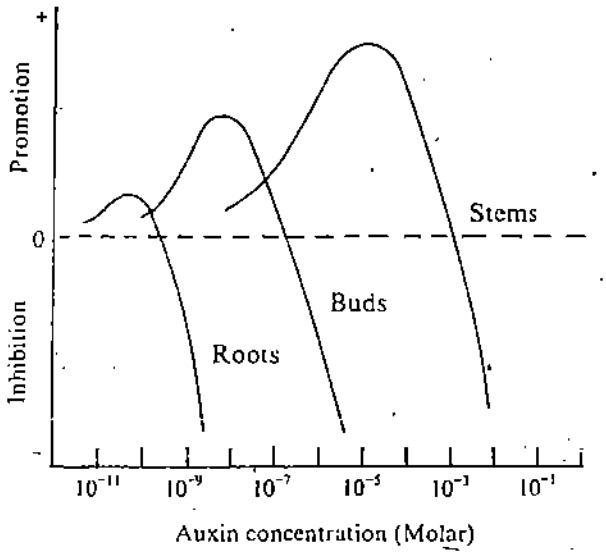



**Fig. 16.6: Effect of various doses of auxin on roots, buds and stems. A biphasic dose-response curve is obtained for different plant organs. (Source: Leopold).**

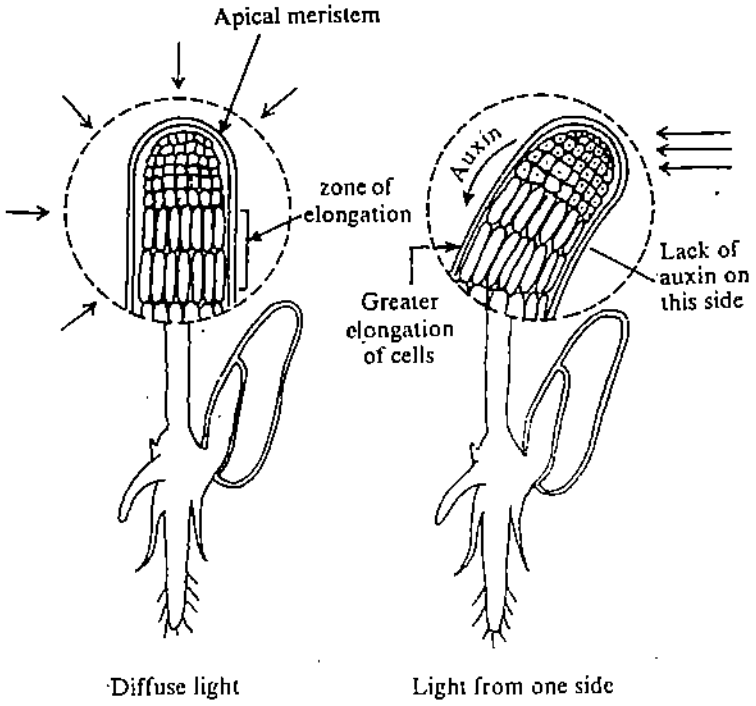



Fig. 16.7: Action of auxins: Cell elongation is promoted by auxin (a) multidirectional light (b) light from one side.

Use of radioactive label technique has shown that IAA is synthesised from tryptophan by the scheme shown in Fig. 16.4. Auxins are synthesised mainly in the shoot apex, young leaves and buds and are transported downward in stems. They promote root initiation and the formation of lateral and adventitious roots, formation and differentiation of secondary vascular tissues, fruits and flower development. Another interesting effect of auxin is apical dominance, where the apical bud exerts an inhibitory effect on the development of the lateral buds. The IAA produced in the apical meristems moves down the stem and inhibits auxillary buds from growing into new leafy stem. If the apical bud is excised then lateral buds develop into sprawling leafy branches. (The same effect is observed if IAA is applied to the cut tips of growing apical buds.) This knowledge is employed by gardeners to make a neat and trim hedge or let a plant acquire a stately appearance. Synthetic auxins are now manufactured commercially. Although chemically different, they show the same effect as the natural auxins produced by the plant. Synthetic auxins are commonly used for mimicking IAA effects. The two commonly employed in laboratories are:

i) naphthalene acetic acid (NAA) and
ii) 2,4-dichlorophenoxy-acetic acid (2,4-D) (Fig. 16.8).

Nearly 9000 kg of oat meristems (a centre for IAA production) were required for the isolation of 1 g of IAA

Synthetic auxins are preferred for experimental work in laboratories because the isolation of natural auxins is tedious and therefore are more expensive. Moreover, unlike IAA, they are destroyed very slowly by plant tissues, and therefore are more stable.


Fig. 16.8: The structure of naphthalene acetic acid (NAA) and 2,4-dichlorophenoxy-acetic acid (2,4-D).

## 16.2.2 Gibberellins

The gibberellin story actually began in the last decade of the nineteenth century. In 1889, Konishi, a semiliterate Japanese farmer described a disease in rice, known as 'bakanae' (foolish seedling). The characteristic symptom is the appearance of spindly

plants which grow so tall that they kneel over and die. Later the causal fungus for the disease was identified as *Gibberella fujikuroi* in 1926. The name gibberellin was assigned to the active factor in *G. fujikuroi* culture filterates in 1935. In 1938 two crystalline biologically active substances named gibberellin A and B were isolated. Today some 72 forms (designated as GA to $GA_{72}$ rather than individually named) have been identified. However, not all of them are active. All gibberellins are diterpenoid acids which have the same basic *ent* gibberellane ring structure (Fig. 16.9) but, vary slightly in their structures depending upon the source from which they are isolated. Some type of gibberellins have been identified in *Gibberella*, and other in higher plants and in many plants both are present. Like *Gibberella* individual angiosperms may contain many different GAs, for instance at least 20 GAs in seeds of the cucurbit and 16 known GAs in seeds of *Phaseolus* species have been identified.

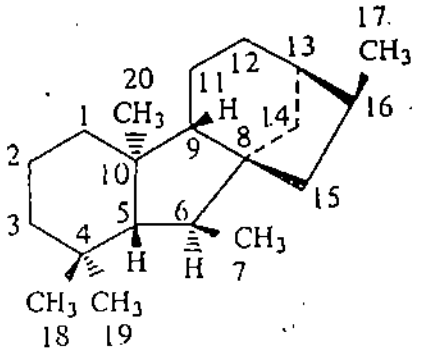



Fig. 16.9: Structural formula of *ent-gibberellane*.

Gibberellins are produced in young leaves around the growing tips, and possibly in roots of some plants. Although their role in root activity is not known.

The effect of GA is dramatically illustrated in genetic dwarf pea or maize where its application to young seedlings, induces them to grow to normal height (Fig. 16.10).

2-Chloro ethyl trimethyl-ammonium chloride (CCC) blocks the synthesis of gibberellic acid in otherwise normal variety of crop

Fig. 16.10: The effect of gibberellic acid ($GA_3$) on normal and dwarf corn.

This illustrates that the dwarf characteristic is due to a block in the synthesis of gibberellins. GA also stimulates the production of numerous enzymes, notably $\alpha$-amylase in germinating cereal grains. As these grains germinate the embryo

secretes gibberellins which traverse to the aleurone layer that surrounds the starchy endosperm (Fig. 16.11), and stimulate the production of $\alpha$-amylase which in turn breaks down starch to sugar and makes it available to the growing embryo.

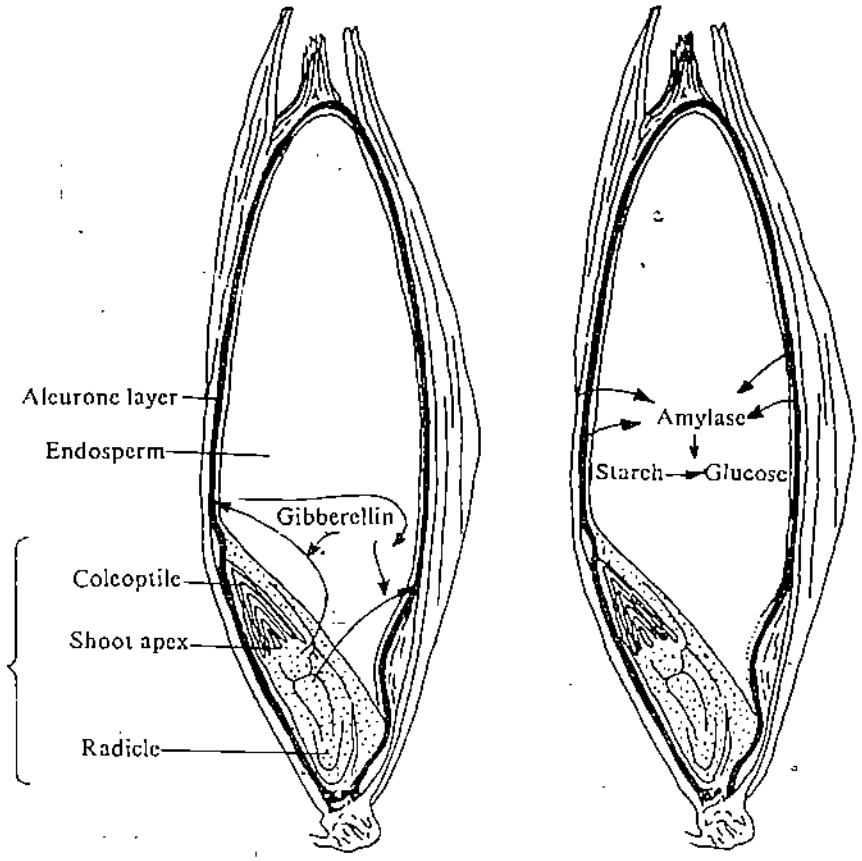



**Fig. 16.11 : Gibberellins secreted by embryo moves to the aleurone layer and stimulate the production of α-amylayse.**

There are many other interesting effects of gibberellins. For instance, they can induce bolting in long day plants, cause stem elongation (it is however, unable to induce curvature in *Avena* coleoptile if applied asymmetrically on cut stump of the coleoptile) and induce germination in seeds that normally require cold or light treatment to germinate.

### 16.2.3 Cytokinins

Both auxins and gibberellins mostly affect growth by stimulating elongation of cells. Therefore, there was a frantic search for specific substance which would induce division of cells. Ultimately cytokinin was discovered during the course of investigations dealing with growth of tissues *in vitro* in the 1950s. A substance called kinetin (6-furfurylamino purine) from an autoclaved sample of herring sperm DNA was demonstrated to be very active in promoting mitosis and cell division in excised pith tissue of tobacco grown on a synthetic medium along with auxin. It stimulated shoot formation if the concentration of kinetin employed was higher than that of auxin and root formation when auxin concentration was higher than kinetin (Fig. 16.12). Thus for the first time a clear picture emerged of the role of interaction of hormones on organogenesis and gave a serious blow to the earlier theory that there are different kinds of hormones present in plants which controlled formation of root, stem and leaf.

Unlike auxins and gibberellins, kinetin the first cytokinin (a name proposed to designate all compounds which promote cell division), was never found in plant tissues, although other natural cytokinins were subsequently extracted from plant tissues. In 1964, the first natural cytokinin, zeatin (Fig. 16.12), was described from maize seeds. Since then three others have been identified. Cytokinins are formed in root tips. In addition to root sites, described above, it has recently been demonstrated that biosynthetic sites of cytokinin are also located in the shoot. Studies on seeds of *zeamays* and fruits of tomato, pea and bean indicate that cytokinins are also produced in them.

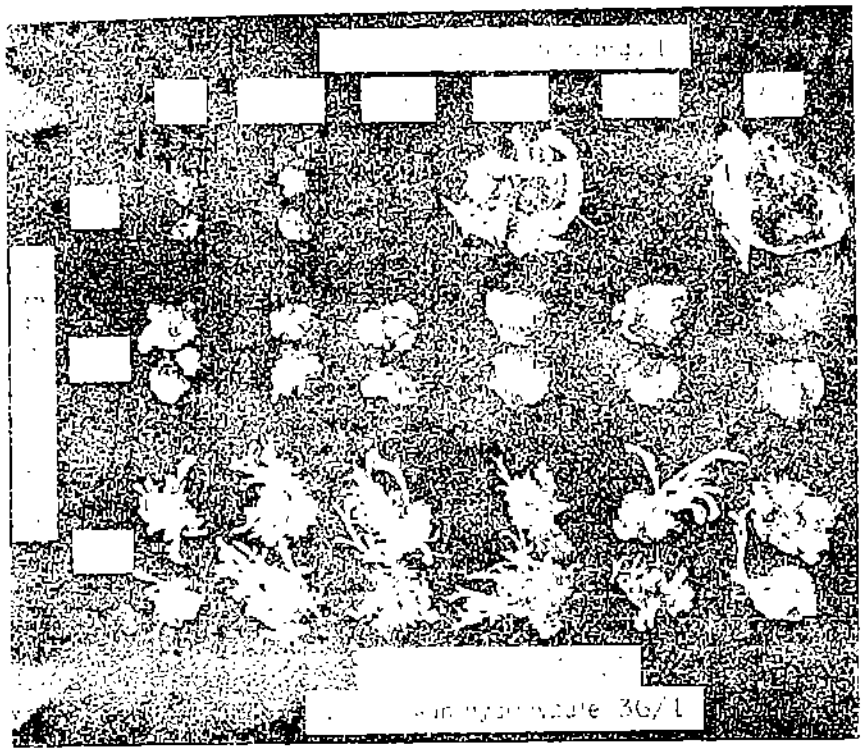


Fig. 16.12: Interaction of hormones in organogenesis. Pith tissue taken from tobacco stem is treated with varying concentration of auxin and cytokinin. (From Skoog and Miller 1957).

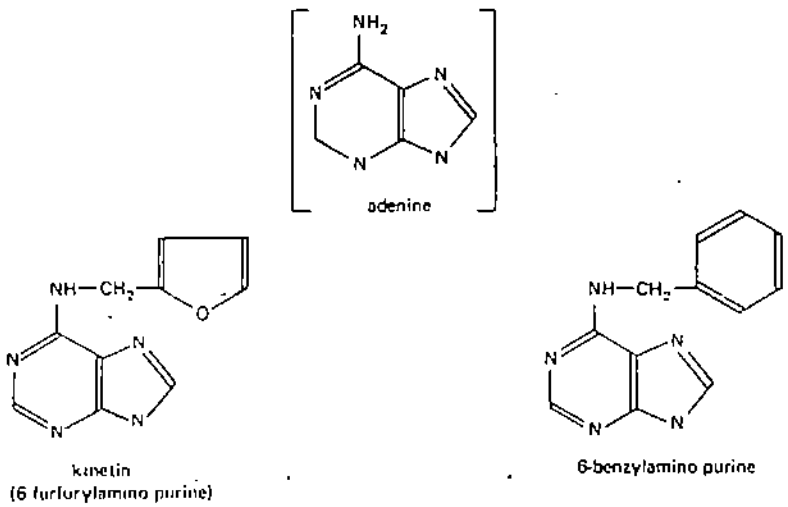



Fig. 16.13: Structure of kinetin and zeatin.

Besides induction of shoots in tissue culture, another effect of cytokinin is associated with senescence or aging in plants. If leaves are treated with cytokinins, aging is retarded, the chlorophyll does not disintegrate and the leaves stay green. Synthetic cytokinins have been applied to harvested vegetable crops such as celery, broccoli and other leafy vegetables to extend their shelf life.

### 16.2.4 Ethylene

Amongst hormones in both plant and animal kingdoms, ethylene, a gaseous hydrocarbon, is unique. Despite its chemical simplicity, it is a potent growth regulator. Even twenty years ago, there was a dispute whether this gas, which had been shown to have a range of multiple effects on plant tissues could be properly called a hormone. Ethylene can be smelt in ripening fruits, as it is involved in the ripening process. Ethylene gas hastens fruit ripening, and as fruit ripens, it produces even more ethylene gas. It is production of ethylene gas by overripe fruits that explains why one rotten apple can spoil the whole basket. Stimulation of fruit ripening by ethylene has been commercially exploited by shipping tomatoes, bananas, oranges, mangoes and many other fruits when green, in ventilated crates (to prevent accumulation of ethylene) and then gased with ethylene before distributing to consumer.

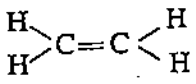


Fig. 16.14: Ethylene

Ethylene may also help in determining sex in certain flowers. Along with gibberellin ethylene controls the ratio of male to female flowers. Ethylene treatment ensures higher ratio of female flowers in some monoecious plants such as cucumber. Therefore, ethylene is widely used in green houses to increase the yield of cucumber by inducing the production of female flowers. Silver nitrate is an antagonist of ethylene. It induces the formation of male flowers.

### 16.2.5 Abscisic Acid

Abscisic acid (ABA) as a naturally occurring growth inhibitor was discovered through independent investigations of different physiological phenomena in two different laboratories. F.T. Addicott and collaborators (University of California) had been investigating natural substances which accelerate leaf abscission, whereas P.F. Wareing et al (University College of Wales at Aberystwyth) had been investigating natural inhibitors which appeared to be related to bud dormancy in woody plants. By 1965, these two paths of research converged upon the discovery that a single hormone was involved in both. The term abscisic acid (ABA), was later proposed to denote this compound, which is a sesquiterpenoid (15-C) compound (Fig. 16.15).

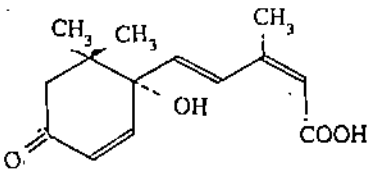



Fig. 16.15: Structure of Abscisic Acid.

Abscisic acid (ABA) is a particularly interesting hormone with regard to the regulation of its own levels. Its levels rise and fall dramatically in several kinds of tissues in response to environmental and developmental changes. Following are the roles of abscisic acid:

i) When leaves of mesophytic plants are water stressed (i.e. under water shortage condition) ABA levels can rise from 10 to 50-fold within 4-8 hours. When the plants are rewatered, the ABA levels drop dramatically within 4-8 hours.

ii) It is generally believed that abscisic acid induces dormancy in seeds to tide over adverse environmental conditions such as freezing temperature or stresses of hot dry periods causing water shortage. Dormancy is induced also in deciduous plants.

iii) You have already learnt that when abscisic acid accumulates in guard cells during periods of water shortage, it causes stomates to close thus enabling the plants to recover water balance.

iv) The name abscisic acid was originally proposed as early investigators believed that it caused flowers, fruits and leaves to abscise (fall). However, now there is dispute over whether ABA is involved at all in the abscission process.

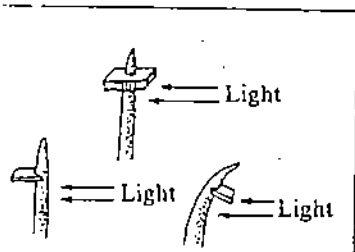
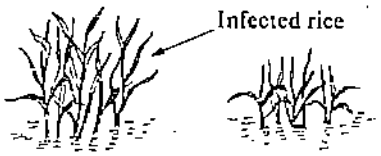
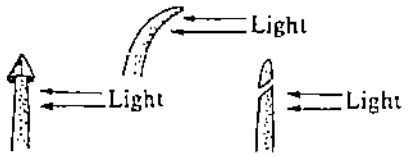
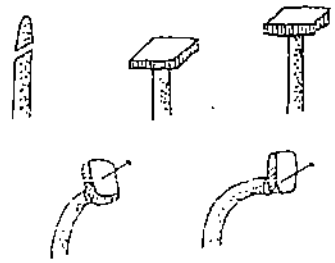
## 16.3 OTHER GROWTH REGULATORS

Besides the major five hormones, polyamines can also exert regulatory control over growth and development at micromolar concentrations. They are widespread and probably occur in all cells. However, there is some controversy as to whether polyamines should be classified as hormones.

Polyamines have a wide range of effects on plant development. It appears polyamines are present in all cells and are not confined to the specific site where they are synthesised. Whether polyamines are classified as plant hormones or not is immaterial as they definitely control plant growth and development to a great extent and are therefore, major plant growth regulators.

SAQ 1

a) Match the scientists given in Column 1 with their experiments illustrated diagrammatically in Column 2.

| Column 1 | Column 2 |
|---|---|
| i) Darwin 1880 |   |
| ii) Boysen-Jensen 1910 |   |
| iii) F. Went 1926 |   |
| iv) E. Kurosawa 1926 |  |

b) In the following statements choose the appropriate word given in parenthesis so as to make the statement correct.

i) (Gibberellins/Abscisic acid) inhibit cell elongation and cell division.

ii) (Cytokinins/Auxins) promote cell division.

iii) (Auxins/Gibberellins) promote root formation, and (cytokinins/auxins) remove apical dominance.

iv) (Gibberellins/Cytokinins) restore normal growth in dwarfs.

v) (Abscisic acid/Cytokinins) retard senescence or aging while (abscisic acid/ cytokinins) may cause leaf abscission.

c) Match the hormones listed in Column I with its functions listed in Column 2.

| Hormone | Function(s) |
|---|---|
| i) Auxins (IAA) | a) Delays senescence |
| ii) Gibberellins | b) Promotes dormancy and stomate closure |
| iii) Cytokinins | c) Root development |
| iv) Ethylene gas | d) Stimulates aleurone layer to produce starch-digesting enzyme |
| v) Abscisic acid | e) Promotes fruit ripening |

d) Draw a small plant and indicate in which of the parts auxins, cytokinins, and gibberellins are mainly synthesised.

## 16.4 HOW DO HORMONES ACT?

All plant hormones show extraordinary varied complex effects in controlling plant growth and development. Extrapolation from how an animal hormone works, a common framework for different plant hormones may explain their varied effects. In animals, the wide variety of effects shown by different hormones are understood by the mechanism of action at the cell level. You may recall that the target cells have appropriate receptors for hormones either on the plasma membrane or are located generally in the interior of the cell. Similar attempts have been made to explain the mechanism of action of plant hormones employing the receptor concept. Both natural and synthetic hormones behave in a similar way as it is assumed that they bind to specific receptors to form a hormone receptor complex to trigger an effect.

Though search for a receptor protein has been generally a frustrating one, recently, such proteins have been demonstrated in pea which bind with auxins before eliciting a response such as embryoid differentiation in tissue culture.

Cell elongation, the most well-known response of auxin, requires that the longitudinal wall stretches, which will involve basic changes in cell wall. In order to stretch, cell wall has to become more plastic, just like a balloon, in which the driving force to increase the volume is proportional to the resistance offered by the balloon wall. Increased plasticity of the cell wall by auxin is considered to be due to breaking of some of the bonds between the polysaccharide components of the cell wall. As it becomes plastic the cell is amenable to stretching.

The structure of gibberellin resembles certain animal steroid neurotransmitters. The search for gibberellin receptor in cytoplasm rather than in the membrane has not been fruitful unlike animal neurotransmitters which bind to cytoplasmic receptors.

Though the biochemical mode of action of plant hormones is poorly understood, still the general assumption in current research work is that plant cells have specific receptors which when bound to hormones activate the signal transduction pathway for various activities. However, though many proteins have been found by various workers to bind with hormones, these may be inactive complexes. Currently search is on to identify the nature of the receptor and decipher its mode of action.

## 16.5 APPLICATION OF PLANT HORMONES

Unknown to the farmer plant hormones were already playing an important role in agriculture and horticulture even prior to their identification. For example, in order to synchronise flowering in mango or pineapple, fires were lit adjacent to fields in which these crops were grown, although the reason was not known at that time. The ethylene generated as a result of incomplete combustion stimulated flowering. The same treatment was followed to stimulate ripening in lemons. Use of ethylene for effective transport of fruits has already been described. Green fruits are preferred during transport which are ripened by treating with ethylene whenever desired.

One major application of synthetic auxins has been in controlling weeds. One such compound is 2, 4-D, which promotes growth at very low concentration whereas it kills plants at high concentrations. Broad leaved plants are more sensitive to this herbicide than narrow leaved ones. This is the basis of killing weeds (generally dicots) in wheat, rice or oat fields.

Weed control agents (herbicides or weedicides) can be very dangerous too. For example, there has been serious questions about the biological effects of 2, 4, 5.T (2, 4, 5, trichloroacetic acid) which was used as weedicide during the Vietnam war. In laboratories, it was found to cause birth defects in mammals, (mice and rats) when treated during early pregnancy. This report along with reports of increased occurrence of human birth defects among South Vietnamese population in 1969 prompted strong protests against its use. However, later the harmful effects were attributed to dioxin, the infamous environmental pollutant, which contaminated 2, 4, 5.T during its production.

Plant hormones have also found practical application in rooting of cuttings, parthenocarpic fruit set, thinning of fruit trees, inhibiting sprouting in cereal grains and potatoes, inhibiting bud growth, inducing flowering, defoliation, and preventing preharvest fruit drop. All of these uses involve auxins, except the induction of flowering in pineapple which can be done with acetylene.

There is a world wide attempt to develop commercial plant growth regulators which will be able to increase basic productivity or yield of a crop such as wheat, rice or peas. However, this has not yet been achieved as the task seems to be too complex to be accomplished. One has to first identify the right crop, the hormone to be applied at the proper concentration at the right stage of development, and then develop a criterion to measure the increase in yield. Success in promoting yields has always occurred when a specific goal is recognised such as increase in sugar content of sugar cane (by gibberellic acid which causes increase in stalk elongation when flowering prevented. This resulted in the increase of sugar content), promote flow of latex in *Hevea* and prevent lodging of small grains.

Finally it is a fact that applications of plant hormones have found use mainly in horticultural crops, as a result of repeated empirical tests by various workers. The range of application can be extended only with clear understanding of regulation of plant growth and development by natural plant hormones and synthetic growth regulators. Time is not yet ripe to put this knowledge to practical use in the case of agricultural crops.

S.Q 2

Which of the growth regulators you will apply for the commercial purposes listed in column 1

| Commercial Application | Growth Regulator |
|---|---|
| i) Control of weeds | |
| ii) Parthenocarpic fruit set | |
| iii) Determining sex in flower | |
| iv) For increasing sugar content in sugarcane | |
| v) Stimulation of synchronised flowering | |
| vi) Inhibition of bud growth | |

## 16.6 SUMMARY

In this unit you have learnt that:

- Growth, development and differentiation are regulated by hormones.
- There are five groups of plant hormones—auxins, gibberellins, cytokinins, ethylene and abscisic acid. Except ABA which inhibits growth all hormones promote growth. Polyamines also exert regulatory control over growth and development, however, they have not been classified as plant hormones so far.
- Auxins promote cell elongation by breaking some of the bonds between the polysaccharides of the cell wall. They also promote root initiation, fruit and flower development and are involved in the development and differentiation of vascular tissues.
- The mechanism of action of plant hormones is under investigation. It is believed that most likely it is similar to that of animal hormones.
- Plant hormones have wider applications in agriculture. Auxins are used for weed control, initiation of rooting in cuttings, in tissue culture, parthenocarpic fruit set, thinning of fruit trees to obtain larger fruit size and inducing flowering. They are also used for inhibiting sprouting and bud growth. Ethylene is used for fruit ripening and synchronising flower formation. Gibberellins are used for stalk elongation of sugarcane to increase sugar content.
- Gibberellins promote growth by cell elongation and cell divisions. They restore normal growth of dwarf corn plants. During seed germination they stimulate aleurone layer to produce starch-digesting enzyme so that the embryo can get food for its development.
- Cytokinins promote cell division, remove apical dominance, delay senescence and aging, help in flower and fruit development. Together with auxin it controls shoot and root development in callus tissue.
- Ethylene is involved in fruit ripening.
- Abscisic acid is a growth inhibitor. Its levels are affected by environmental conditions. During water stress, its level increases 10 to 50 folds. It is involved in seed dormancy, stomate closure and probably in fruit, flower or leaf fall.

## 16.7 TERMINAL QUESTIONS

1. List the various factors that control growth and development.

   .....

   .....

   .....

   .....

   .....

2. It is known that grain yield is unaffected if the stem length of cereal crops is reduced by dwarfing genes or by interfering with plant hormones. This is economical. Can you tell which hormone needs to be interfered with?

   .....

   .....

   .....

   .....

   .....

3. What is the difference between animal and plant hormone?

   .....

   .....

.....

.....

.....

4. Which among the following statements is true? Write T for true and F for false in the given boxes.

   i) NAA and 2, 4-D have same effect as natural auxins produced by plants. ☐

   ii) There is controversy whether polyamines control growth and development. ☐

   iii) To initiate root formation in cuttings, it would be advisable to add little NAA in water. ☐

   iv) The present evidence indicates that the mechanism of action of plant hormones is different from that of animal hormones. ☐

## 16.8 ANSWERS

**Self-assessment Questions**

1. a) i) c, ii) a, iii) d, iv) b.

   b) i) Abscisic acid, ii) Cytokinins, iii) Auxins, cytokinins, iv) Gibberellins, v) Cytokinins, abscisic acid.

   c) i) c, ii) d, iii) a, iv) e, v) b.

   d) Auxins—shoot apex, young leaves and buds. Gibberellins—growing tips. Cytokinins—Root tips, developing seeds and in some fruits.

2. i) 2, 4-D, ii) auxins, iii) ethylene, iv) gibberellins, v) ethylene and auxins, vi) auxins.

**Terminal Questions**

1. These are:

   i) genetic controls,

   ii) hormonal controls and

   iii) environmental control—light, temperature, soil pH, acid rain, humidity and rain fall.

2. Gibberellic acid. Its synthesis can be blocked by the chemical 2-chloroethyl-trimethylammonium chloride (CCC).

3. Animal hormones trigger highly specific response in specific target tissues. Plant hormones work in more general, and probably in more complex way.

4. i) T, ii) F, iii) T and iv) T.

# UNIT 17 DEVELOPMENT AND DIFFERENTIATION

**Structure**



## 17.1 INTRODUCTION

The developmental phases of a flowering plant involve seed germination, vegetative growth, flowering, fruiting and senescence. These phases are precisely timed and are under strict internal and external controls. You must have noticed that a seed remains dormant all winter long buried in the sand, suddenly germinates and the plant flowers in the spring. A short rainfall in desert brings life to many plants and the dormant winter buds become active in the spring. What external or internal factors initiate germination which involves resumption of all the metabolic activities? How does a seed sense that all the environmental conditions are favourable for its germination and growth and how do buds know that the winter is over?

You also know that most fruits, vegetables and flowers are seasonal. *Chrysanthemums* bloom in winters while Gulmohar and Amaltas bloom in the summer. Why do some plants bloom in the spring and others wait for summer, fall or winter? Many flowers open and close on a fixed schedule as if a clock is present in their cells. Now it would be interesting to find out whether it is possible to grow chrysanthemums throughout the year or make Morning glories bloom at dusk? You can ask many such questions with regard to development of a plant.

In the previous unit you have learnt that hormones coordinate growth and development in different parts of an organism by triggering cellular reactions in the target cells. In this unit we will discuss the phases of plant development that are under the control of specific environmental signals. You will see that plant cells receive environmental signals which govern various developmental events. The signals are received by some special molecules and the genes are turned on at a precise time to regulate specific activity and characteristics.

## Objectives

After studying this unit you should be able to:

- describe seed formation and seed germination and the factors that control these processes,
- list the factors that are responsible for flower induction and discuss the nature of the receptor that perceives external stimuli,
- explain the role of phytochrome in controlling the period of light and dark cycles in flowering,
- discuss the role of phytochrome in regulating plant development,
- list the various factors that affect onset of senescence,
- describe the principle and uses of tissue culture technology,
- give examples of circadian rhythms regulated by biological clocks in plants.

# 17.2 VEGETATIVE DEVELOPMENT

Before we move on to the contents of this unit, let us clear our understanding of the terms, growth, differentiation and development.

## 17.2.1 Definitions

**i) Growth:** It is quantitative and irreversible change. It reflects an increase in size and volume of an organism. For example, the change in the size of a leaf or increase in the length or breadth of stem can be referred to as growth. It is usually accomplished by changes in form and shape. There can be growth of the whole plant or only of an organ. At cellular level there is synthesis of various molecules, specially macromolecules such as nucleic acids, proteins, lipid at the expense of energy derived from catabolism in growing structures. The macromolecules organise into membranes and organelles—chloroplast, mitochondria and others. There is also active division of cells.

**ii) Differentiation:** It is referred to as qualitative changes that lead to increased specialisation. For example, the formation of cells and tissues of root, shoot and leaf or changes that transform a vegetative bud to a flowering bud. In simple terms, the process by which cells become specialised is called differentiation.

**iii) Morphogenesis:** This term is coined for the process leading to and determining the form and structure of organs. It is used mainly by experimental morphologists. For our purposes differentiation and morphogenesis would mean the same thing and may be used interchangeably.

In Greek morpho means "form" and genesis means "origin" or beginning

**iv) Development:** The process of growth and differentiation of cells into organs and organisms is often termed development. For example, the development of a higher plant starts from the formation of seed and then its germination results in the formation of root and shoot. With further growth more branches and leaves are produced till a certain time when vegetative buds get converted to flowering buds. After the formation of flowers, fertilisation takes place resulting in the development of fruits and seeds.

## 17.2.2 Seed Formation

A mature seed has seed coat, an embryo and reserve food material in the endosperm in monocots and in cotyledons in the dicots. Fig. 17.1 shows structure of dicots and monocot seeds. The embryo as shown is already well-developed and is differentiated into embryonic root (radicle) and shoot (plumule).

You know that the process of seed formation begins with the fusion of a male gamete with an egg cell. The 'zygote' thus formed undergoes divisions resulting in the development of an embryo. At this stage the development is stopped and there is cessation of metabolic activity which resumes only after the seeds get dispersed from the parent plant and start germination. The process of seed formation can be divided into two phases:

i) The division phase, in which fertilised egg divides mitotically and compl es embryonic development.

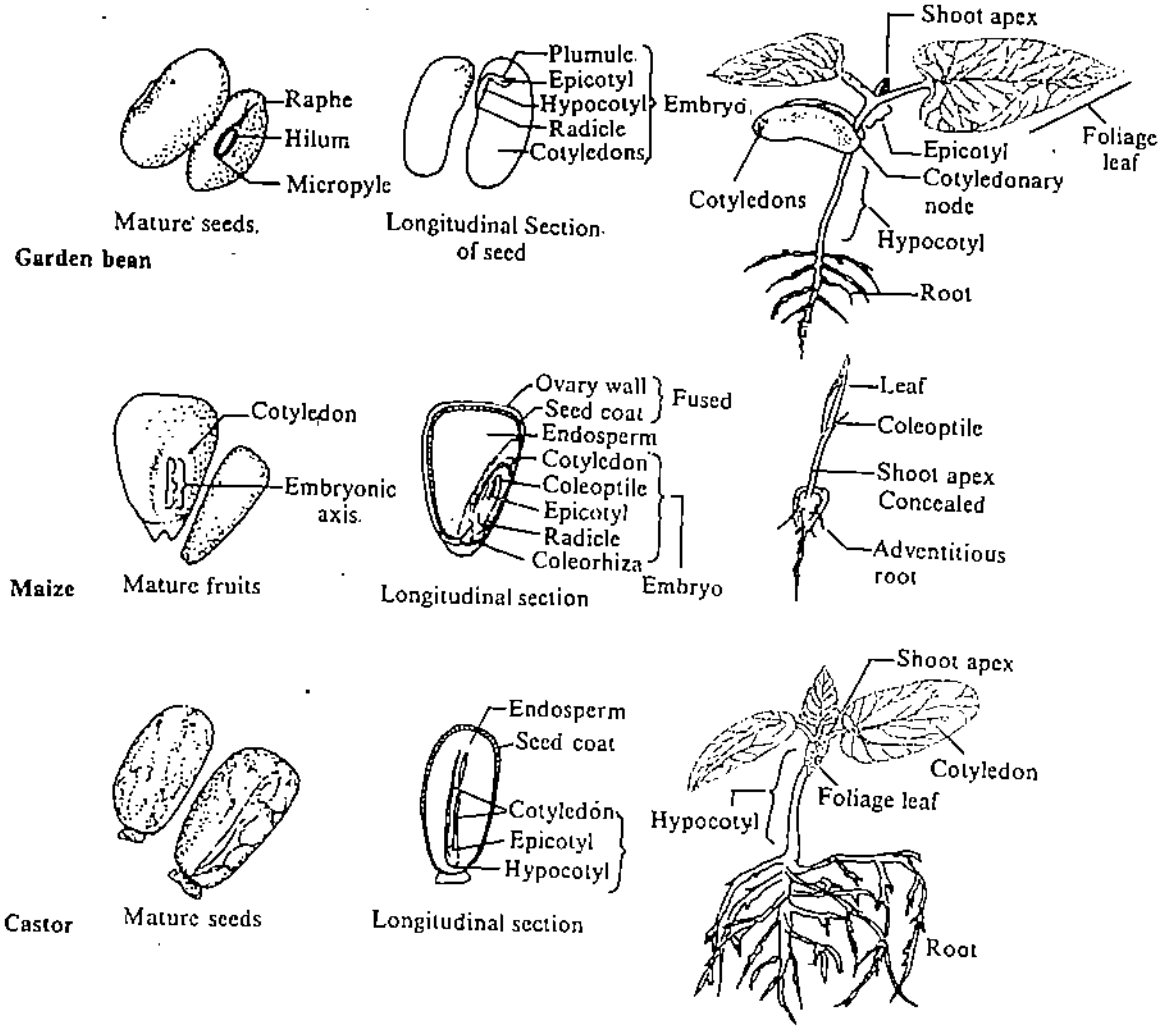



Fig. 17.1: Seed structure and seedlings of 3 different angiosperms. The food is stored in cotyledons (bean, a dicot), endosperm (maize, a monocot, castor bean, a dicot).

ii) The desiccation phase—In this phase the embryo usually enters a period of inactivity or dormancy. Further divisions do not occur and the average water content of the seed, falls below 15%, the respiration slows down, mitochondria start loosing their structure as was seen by using electron microscopy and protein synthesis is also slowly arrested.

Interestingly, it was found that though protein synthesis did not occur, the system still contained enough mRNA which were produced in the division phase. This means that due to some reason although the protein synthesis was blocked but the existing mRNA remained stored in the embryos. Later, these mRNAs were utilised by seeds during its germination for producing some specific proteins. Now, the question arises how does protein synthesis get blocked? It was found that during desiccation phase, abscisic acid, is produced which at higher concentrations blocks the translation of mRNAs.

The process of inhibition of protein synthesis by ABA has great significance for plant development. If this process would not occur the seeds would start germinating on the parent plants itself in the fruit before their dispersal. This would affect the plant survival. The duration of the dormant phase varies from species to species. In cereals, it is very brief. In some plants sometimes seeds do germinate on the parent plant itself as is seen in oranges and papaya sometimes. This phenomenon is called vivipary, which is characteristic of mangrove plants.

### 17.2.3 Seed Germination

The environmental factors that influence the germination of seeds are : i) water availability, ii) optimal light, iii) aeration, iv) temperature and age of the seed. When water is imbibed by the seeds, they swell. In fact, seeds swell because the hydrophilic groups like $NH_2$, $OH^-$, $COO^-$, present in carbohydrates and proteins attract dipolar

water molecules. If oxygen is also available the energy (ATP) for germination is obtained by oxidative phosphorylation.

At optimal temperature many biochemical reactions occur. All these changes lead to germination which finally results in the emergence of radicle and the plumule. The root and the shoot grow (see Fig 17.1), and for their growth, food stored in the seed is available for a short period. After the leaves are developed the plant becomes autotrophic and synthesises its own food material in the presence of light.

Experiments have revealed that certain seeds need light for the germination. The effect of light on seed germination is controlled by the pigment **phytochrome** about which you will learn in Section 17.4. Many seeds of temperate region need a minimum period of pre-chilling (cold-treatment). For example, seeds of lettuce (of a variety called Grand Rapids) exhibit dormancy because they are unable to overcome the physical restraint imposed by seed coat. The seed coat is hard like that of a shell of a nut. The seeds require light for germination. They also germinate in dark if pre-chilled at 2°C. These observations suggest that there is a single on and off switch that can turn to the 'on' position by light or by chilling.

Let us now examine the changes that occur in the seeds after they imbibe water. In general, the most important metabolic events are:

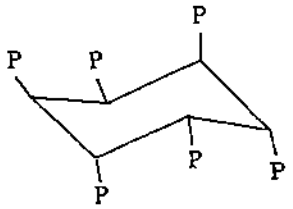
i) degradation of stored food material through the activity of various enzymes,
ii) changes in hormonal levels, and
iii) synthesis of new proteins and enzymes.

Seeds store food material in the form of fats, proteins and starch. These cannot be used directly by the embryo. In order to make them usable, they need to be degraded. Fats are broken down by the enzyme lipase into fatty acids and glycerol. You know that fatty acids are converted to acetyl CoA by $\beta$-oxidation. You have also learnt (Unit 12 of Cell Biology Course) that acetyl CoA through glyoxylate cycle produces succinate which forms glucose via gluconeogenesis. Starch is hydrolysed by $\alpha$-amylase to maltose units which are converted to glucose.

In seeds, proteins are stored as protein bodies. Different plants have different type of proteins. For example, the proteins in maize seeds are called **zein** and in barley **hordeins.** In seeds of soya bean different sizes of protein bodies are present. Plants cannot use these proteins for the growth of seeds. These are hydrolysed by proteases into amino acids which are used for the synthesis of proteins of functional importance to the embryo. Similarly, in some cases nucleic acids are also degraded by nucleases to form nucleotides which are reutilised as building blocks for the synthesis of DNA.

**Zein** — In seeds prolamine and glutelins are storage proteins. Prolamines are deficient in amino acids, lysine and tryptophan which are essential amino acids for nutrition. The seed prolamine are commonly named after the genus of the plant in which they occur. A great deal of research nowadays is being done to analyse the structure and composition to correct any nutritional imbalance.

From the above discussion, you must have realised that one of the initial events during seed germination is to produce large number of hydrolytic enzymes to degrade stored food material into small molecules that can be utilised by the embryo for growth. The degradation of food material takes place in the endosperm or storage tissue (cotyledons) and the energy and raw material formed are mobilised to the developing embryo for its 'nourishment'. Sugars are catabolised via respiration to yield ATP and NADH. It has been found in some cases that initially ATP is produced on breakdown of phosphorus rich compound inositol 6-phosphate or phytin stored in the seeds by an enzyme called phytase.




The phytin structure:
Phytin broken down by phytase releases inorganic phosphate which gets coupled to ADP to form ATP.

During seed germination many changes also occur in the hormonal levels. As mentioned in the previous unit that the embryo of barley seed releases gibberellic acid which acts on the aleurone layers and induces the synthesis of the enzyme $\alpha$-amylase. As you know $\alpha$-amylase degrades starch. Clearly the enzyme was not present in the seeds but is synthesised *de novo*. Experiments show that gibberellin specifically affects synthesis of mRNA of $\alpha$-amylase (i.e. transcription of $\alpha$-amylase gene). As we have mentioned before there are some enzymes which are already present in the seed while others are produced in response to hormones. Some of the changes that occur in a barley seed are shown in Fig 17.2.

A number of other cellular changes like changes in membrane organisation e.g. endoplasmic reticulum, development of mitochondria are also triggered in the seed during germination. The net result of all these changes is to provide nutrition to the embryo in order to make it divide and grow. The visible outcome of these events is the emergence of radicle (the root) followed by the plumule (the shoot). The shoot grows and develops primary leaves and with chloroplasts the plant becomes autotrophic and begins its life by establishing itself firmly by its roots on the ground.

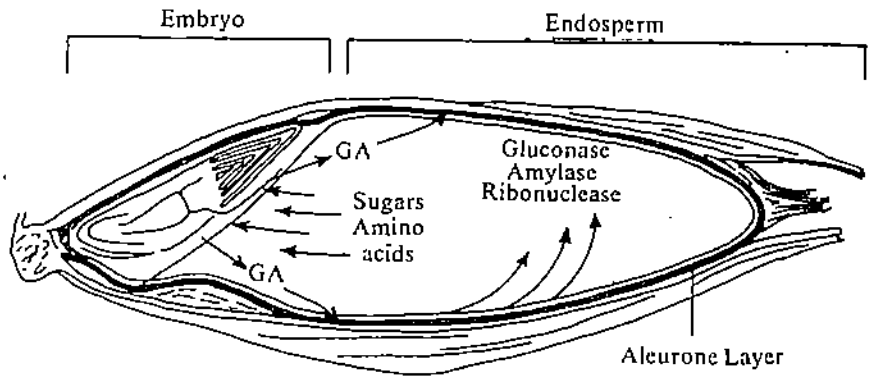



**Fig. 17.2: The working model of initial metabolic changes occurring in barley seed. Gibberellic acid (GA) moves out of the embryo and acts on the aleurone layer to synthesise new enzymes by triggering the synthesis of their mRNAs. These hydrolytic enzymes act on their substrates available in the endosperm and thus provide sugars and amino acids to the developing embryo for its growth.**

### 17.2.4 Dormant Vegetative Structures

You have learnt that light, a period of chilling or application of gibberellin often breaks seed dormancy and stimulates seed germination. Vegetative organs such as tubers, corms, bulb and winter buds can also enter a period of dormancy. What kind of stimulus is required for these dormant structures to resume growth? It is observed that light can stimulate bud break in certain trees and a period of chilling is necessary for triggering the development of dormant buds, and other organs in plants of temperate region. The period of chilling required for the bud break is usually a week or more. Similarly, lengthening of days is necessary for breaking dormancy of winter buds exposed to light. Dormant buds can also be stimulated by the application of gibberellins. As you know gibberellins promote growth and abscisic acid inhibits the growth. Accordingly, on breaking of bud dormancy increase in the level of GA and fall in the level of abscisic acid has been noted. It needs to emphasised here that though all the buds of a plant receive the same dormancy breaking stimulus, however, it is only the apical bud that grows and the growth of other buds is inhibited by it because of **apical dominance** (refer Unit 16).

Plants reproduce vegetatively through rhizomes, runners or stolons. Tuber formation in potatoes can be induced by exposing leaves to daylength less than the critical value. Artificial application of hormones show that in tuber formation gibberellic acid decreases while abscisic acid increases. The results show that the level of a particular hormone can increase growth in some organ and decrease in other.

**SAQ 1**

In the following statements, fill in the blank spaces with the appropriate words.

i) In seed formation once an embryo is formed from a zygote it usually enters a period of ..... .

ii) In desiccation phase of seed formation ..... ..... synthesis ..... ... is blocked but seed contains ..... formed during division phase.

iii) ..... is the phenomenon of seed germination on parent plant itself.

iv) During desiccation phase protein synthesis is blocked by ..... .. ..... .. ..

v) Gibberellic acid released from the embryo diffuses in the ..... ..... .. and produces mRNA for the synthesis of $\alpha$-amylase.

vi) During germination food molecules— carbohydrates, proteins and fats are degraded by ..... ..... enzymes into small molecules to provide nourishment to the embryo.

vii) In certain species the energy for the germination of seed is provided by energy-rich compound ..... stored in the seeds.

## 17.3 FLOWERING

One of the major changes that occur during the life cycle of a plant is the transition from vegetative stage to the flowering stage. In this transition the vegetative meristems change into flowering meristems which form sepals, petals, carpels and anthers instead of branches and leaves. It is a major morphogenetic change. In some plants this change occurs once in life-time and after flowering, fruit and seed set, the plant dies. Whereas, in others, the flowers are produced every year, as in trees. Now the question arises, is it due to an internal annual rhythm in the plants or a requirement for particular environmental condition? In this section we will tell you what controls flowering and how this transition occurs.

One of the major factors that affects flowering, as we know today is related to the effects of the environment and in particular to the duration of light/dark 24 hour cycle or temperature. However, it took a number of years for scientists to realise this fact. We will describe two experimental observations which suggested that the length of the day determines the behaviour of plants with respect to flowering.

The phenomenon of photoperiodism was discovered by two American scientists, Garner and Allard in 1920, working at the U.S. Deptt. of Agriculture at Beltsville, Maryland.

The seeds of soya bean *(Glycine max)* were planted at different times in the month of May, June, July and August. Even though planted at different times, all of them flowered in the month of September. The first planted seeds took 125 days to flower whereas the last set flowered in only 58 days. These observations show that at a particular time of the year the day length was suitable for plants to flower. The second observation was made on a mutant of tobacco called Maryland mammoth, because of the large size of its leaves. In fields it does not flower in winter, however, in glass houses when light duration was provided like that of summer, it was possible to induce flowering even in winter in these plants.

In temperate region the daylength is more during summer.

### 17.3.1 Plant Responses to Light-Dark Cycles

Based on their requirement for day length (number of hours of light) for flowering plants have been grouped under three major categories, 'short-day', 'long-day' and 'day-neutral' plants (Fig. 17.3).

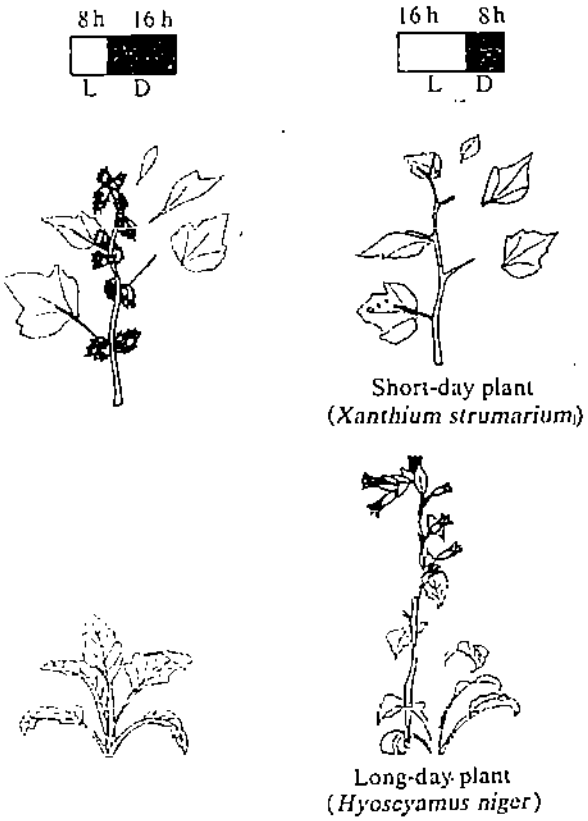



Fig. 17.3: A diagrammatic representation to show the difference between a short-day plant (SDP) and a long-day plant (LDP). A SDP requires more than a critical period of darkness. In this case SDP needed more than 8 hours of darkness or less than 16 hours of light to flower. A LDP requires less than a critical hour of darkness or more than a certain hours of light. In this case, LDP required more than 10 hours of light to flower.

**Short-Day Plants (SDP) :** These plants only flower or flower more profusely and rapidly when given less than a certain (critical) number of hours of light in a day (24 hours cycle). The critical period, let it be clear, is not 12 hours. It can be for example 10 hours. In that case a plant given less than 10 hours of light (say 8 hours light + 16 hours darkness) will flower. And this critical period is determined experimentally and varies from plant to plant. Short-day plants are found in tropical regions where day length varies comparatively little during the year.

**Long-Day Plants (LDP) :** The definition of this is exactly opposite to short-day plant. That is those plants which flower when given more than a certain (critical) number of hours of light (day length). These plants are found in temperate region where they flower during summer.

**Day-Neutral Plants :** Besides SDP and LDP, those plants that flower irrespective of the length of light are called day-neutral plants. For these there is no specific requirement of light duration.

A few examples of these three categories of plants are given in Table 17.1. The day length requirements vary from species to species (Fig. 17.4). Even within a single species, different varieties of either long-day or short-day plant may show a range of critical day length. It is reported that rice plants are able to detect changes in day length of only 15 minutes. Therefore, it seems that plants must have an extremely accurate time-measuring mechanism within the tissues of the plant. Flower induction in some plants can be stimulated by particular day length only if the plant has been previously chilled, for example daffodil and henbane. While some plants, for example, primrose flower solely in response to pre-chilling, otherwise they are day neutral.

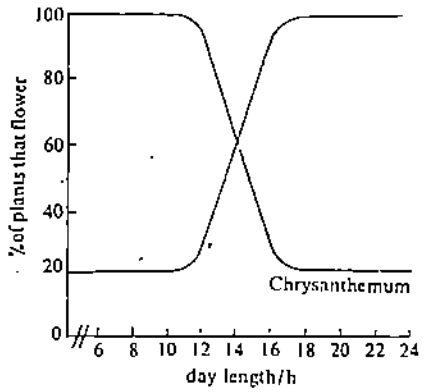



Fig. 17.4: Day length and percentage of flowering in chrysanthemum and in spinach.

Table 17.1: Some examples of short, long and day-neutral plants

| Type | Behaviour | Examples |
|---|---|---|
| Short-Day plant | Flower only in short days | *Chenopodium rubrum, Lemna paucicostata, Oryza sativa. Glycine max, Kalanchoe blossfeldiana, Xanthium strumarium.* |
| Long-Day plant | Flower sooner in long days | *Arabidopsis thaliana, Hyoscyamus niger, Lactuca sativa, Hordeum vulgare, Nicotiana sylvestris, Beta vulgaris.* |
| Day-Neutral plants | Flowering little affected by length of day or night | *Oryza sativa, Cucumis sativa Lycopersicon esculentum, Gossypium hirsutum.* |
| Chill treatment only | Flower when plant returned to normal temperature after chilling | Primrose *(Primula vulgaris)* |
| Chill requiring long-day | Flower in long days after chilling | Henbane *(Hyoscyamus niger)* |
| Chill requiring short-day | Flower in short days after chilling | *Chrysanthemum* hybrids |

## 17.3.2 Importance of Dark Period

For quite sometime the role of light period (photoperiod) was emphasised in flowering. However, based on certain experiments it was realised that it is the dark period that is more important than the light period for inducing a plant to flower. In fact, as early as 1912, from his experiments on flowering, Julien Tournois concluded that flowering occurred "not so much by shortening of day as by lengthening of nights". We describe below an experiment, which was done much later by Hamner and Bonner (1936), that proved this point.

A short-day plant was taken that required 16 hours of darkness and 8 hours of light to flower. If the dark period was reduced, plants did not flower. Interestingly, if the dark period of 16 hours was interrupted by light, again there was no flowering. However, if the light period was interrupted by dark period, or if plants were kept less than 8 hours in light there was no effect on flowering. It was clear that altering the light period had no effect but if the dark period was less than 16 hours, the plants would not flower. A look at Fig 17.5 will further clarify this experiment and confirm that dark period is more important for inducing plants to flower. This concept has now been well established. The role of dark period is to bring about some changes which trigger the development from the vegetative to the flowering state. The role of light period is to realise this change and help in bringing about maximum flowering.

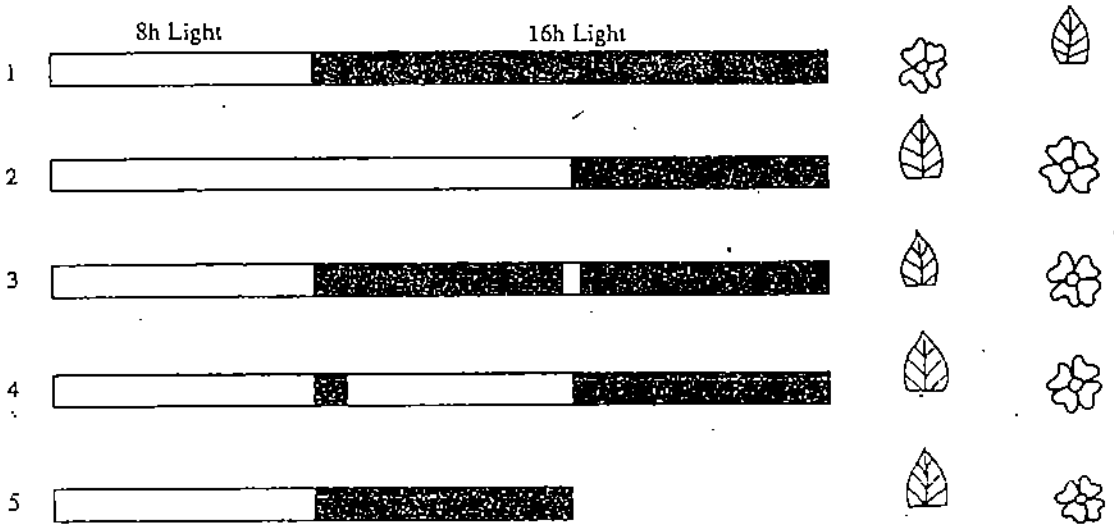



Fig. 17.5: Experiments to show that dark period is important for flowering. If dark period is interrupted (3) SDP do not flower but if light period is interrupted (4) or shortened (5) there is no effect on flowering in LDP.

## 17.3.3 Flowering Hormone

It was in 1880, that Julius Sachs suggested that there is a chemical basis for flowering. Later experiment indicated that some hormone was produced which controlled flowering. The hormone was named florigen. Before we find out what the florigen is, let us first see the experiments which suggested that a chemical is involved in flowering and that it is produced in the leaves.

The concept of florigen—a flowering hormone was given by a Russian Botanist named Chailakhyan in 1930s.

In an experiment only the leaves of **Kalanchoe**, a short-day plant, were given the inductive conditions and not the whole plant. Even then the plant flowered (Fig. 17.6). More such experiments were done where treatment of light-dark cycle was given only to the leaves and the plants were induced to flower. Some scientists performed experiment with a twig, a single leaf of which had been given the inductive condition. It was grafted onto a plant which was not given any inductive condition. Interestingly, it was found that the grafted plant also started flowering (Fig. 17.6). In some cases grafts were taken from plants which were induced by short-days, for example from *Xanthium canadense* and were put on plants which required long-day conditions e.g. *Rudbeckia bicolour*. Even in such graftings the recipient plants, in the above case *Rudbeckia*, started flowering.

The light dark conditions which induce plants to flower are called **inductive conditions.**

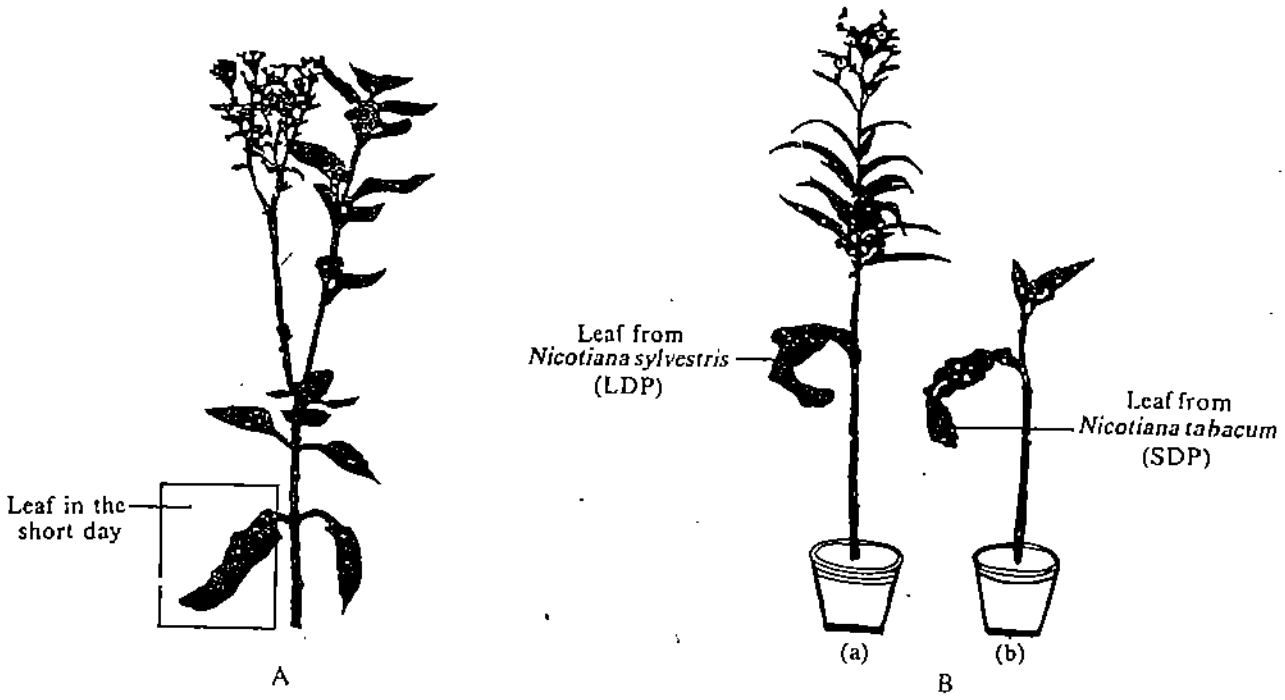



Fig. 17.6: Experiments to demonstrate that photoperiodic stimulus is perceived by the leaf. In (A) *Kalanchoe blossfeldiana* plants begin to flower even if one single leaf is maintained under shortday conditions. In expt. (B) a leaf from LDP, *Nicotiana sylvestris* was grafted on SDP, *N. tabacum*. The plant started flowering even under long-day conditions, (a). However, if a leaf from *N. tabacum* was grafted on *N. tabacum* itself (a control expt b) flowering was not induced.

If you have understood the above experiments perfectly well, you will come to the following conclusion.

i) the leaves perceive the light stimulus, and

ii) some chemical moves out of the leaves (as must be occurring in grafts) and goes to the meristems and changes them from vegetative to flowering. What is this chemical?

This chemical was termed florigen. However, we must point out that nobody has isolated florigen or any other chemical till today which can induce flowering in any plant. In all probability florigen is a plant hormone which at certain concentrations induces flowering. In the Unit on hormones you have already learnt that when supplied exogenously different hormones can induce flowering in different plants.

However, some observations are contrary to this view. It has been shown that florigen can move readily between tissues but it cannot pass between two tissues if separated by a strip of agar while a hormone can travel easily through agar. Further, experiments have suggested that the flowering stimulus moves through the phloem. This suggests that some chemicals, other than hormones may also be involved in flower induction *in vivo*. We can hope that in the near future it would be possible to isolate and identify florigen by analysing and comparing the content of phloem sap before and after flower induction.

### 17.3.4 Chilling and Flower Induction

Some plants flower only after passing a winter season. For example, winter wheat is sown in the autumn for harvest in the following summer. It needs exposure to cold. If winter is mild, plants do not flower and the crop fails. It has been shown that winter wheat and many other plants require a period of chilling 0°–2°C for about a week for flower formation. The cold treatment given for flower induction is called **vernalisation.** The technique of vernalisation was developed in Russia where winter crop required chilling for successful cultivation. The seeds are soaked for small period to initiate germination and then they are buried in the snow. Later, they are planted in the spring when severely cold conditions are over. Thus chilling is also a stimulus for flower induction.

Vernalisation is Latin term, it means "to make springlike"

Which part of the plant requires chilling stimulus for floral induction? Experiments show that only the shoot apex receives the stimulus (needs vernalisation) which then

is passed on to the other parts of the plant. When the shoot apex that has received the stimulus is pinched off, the lateral shoots flower and if their apices are also pinched off, the side shoots develop and flower. Moreover, when extracts of a vernalised plant are applied to a long-day plant growing under short-days the recipient plant flowers. Like light induction, the stimulus can also pass through a graft to a non-vernalised plant. These results show that some kind of flowering stimulus is transmitted from shoot apex to other tissues. The chilling stimulus was named **vernalin.** The nature of this compound has yet not been identified.

### 17.3.5 Biochemical Changes

Many workers have tried to follow the biochemical changes that precede flowering and result in meristems which give rise to flowers instead of vegetative structures. In *Pharbitis,* which is a short-day plant and requires only one dark period for flowering it was found that soon after the dark period, the flowering stimulus begins to move out of the leaves. In this experiment, the plant was given inductive conditions and after specific time intervals, biochemical changes were measured in meristems. An increase in metabolic activity around 40th hour at the floral apex was manifested by an increase in the level of RNA, proteins and ribosomes. Electron microscopic observations also revealed extensive formation of endoplasmic reticulum. These activities were followed by an increase in DNA synthesis and mitotic activity. At about 88th hour after floral induction, the rate of cell division increased at apical and axillary meristems and the increase in cell division was noticed particularly in the central zone and peripheral zones of apical meristems.

Such experiments have also been done in other plants. However, it has not been possible yet to identify which of the RNA or proteins are responsible for the onset of flowering. With the application of newer techniques it has been possible to suggest that there are some specific flowering genes which get **switched on** after receiving specific light-dark cycle. Although we do not know the products of all these genes, some of them have been shown to code for proteins which regulate transcription.

**SAQ 2**

a) Fill in the blank spaces in the following statements.

i) The major morphogenetic change in flowering is the change of.....meristems into.....meristems.

ii) Photoperiodic control of flowering depends on the length of uninterrupted ..... .....given.

iii) .....plant is likely to flower at the same time of the year whether grown in the house, field or green house.

iv) The chemical suspected to induce flowering is given the name..... and is produced in the. ..... .

v) A short-day plant requires more than a critical period of..... .

b) List the biochemical changes that precede flowering.

.....

.....

## 17.4 PHYTOCHROME

You know that plants capture light energy during photosynthesis, now you are familiarised with another important and interesting role of light in developmental phenomena. Just as for photosynthesis light is absorbed by pigments chlorophylls and carotenoids, similarly to bring about developmental response light has to be absorbed by some pigments. One of the pigments that detects the quality of light in the range of 600-800 nm region is phytochrome. It may also be mentioned that some developmental changes do exist even in total darkness. These processes would be independent of active form of phytochrome. Such changes are called skotomorphogenetic as against photomorphogenetic change that occur in light. There also exists an unidentified blue light absorbing pigment which brings about phototropism and nastic movements.

The experiments on reversible effect of red and far-red light to study developmental responses were done by Borthwick and Hendricks at the same place where Garner and Allard had discovered the phenomenon of photoperiodism.

## 17.4.1 Discovery of Phytochrome

In Sub-section 17.3.2 we described the experiment that proved the importance of the dark period in flowering. In that experiment if dark period was interrupted with light, the flowering was not induced in a short-day plant. In further experiments instead of white light, dark period was interrupted by lights of various wavelengths like red, blue, yellow and far-red light. These experiments showed that red light alone was sufficient to inhibit flowering. However, the effect of red light was not always inhibitory. For different developmental response its effect was different. For example, an experiment done to check effect of red light on lettuce seed germination, it was found to have stimulatory effect. Another interesting observation was that any response to red light was nullified by far-red light (Fig. 17.7).

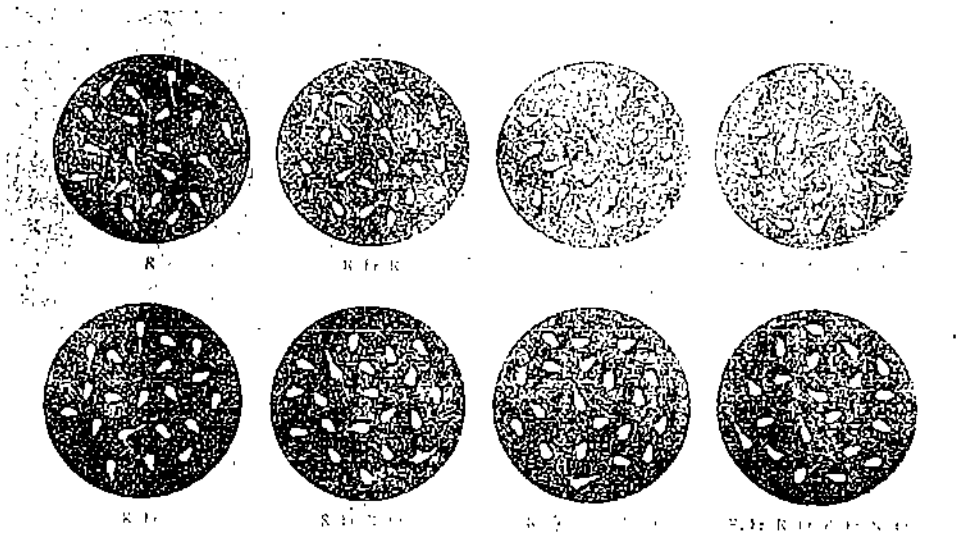


**Fig. 17.7: Reversal of seed germination with red (R) and far-red light (FR). When irradiated with red light lettuce seeds germinate; however, if red light is followed by far-red light, the seeds do not germinate. It was noticed that even after successive irradiations, if the seeds receive red light as last irradiation then they germinate but if far-red light was given at the end (as shown in lower panel) the seeds remain dormant.**

From a number of such experiments it was concluded that some developmental response brought about by red light could be reversed if followed by far-red light and vice versa. On the basis of these physiological experiments it was proposed that both red and far-red lights are absorbed by the same pigment. This pigment was named phytochrome.

Phytochrome, therefore, exists in two forms: a red light absorbing form ($P_r$), which after absorbing red light gets converted to far-red light absorbing form ($P_{fr}$). When $P_{fr}$ form absorbs far-red light it gets converted back to $P_r$ form as shown in Fig. 17.8. So the two forms are interconvertible.

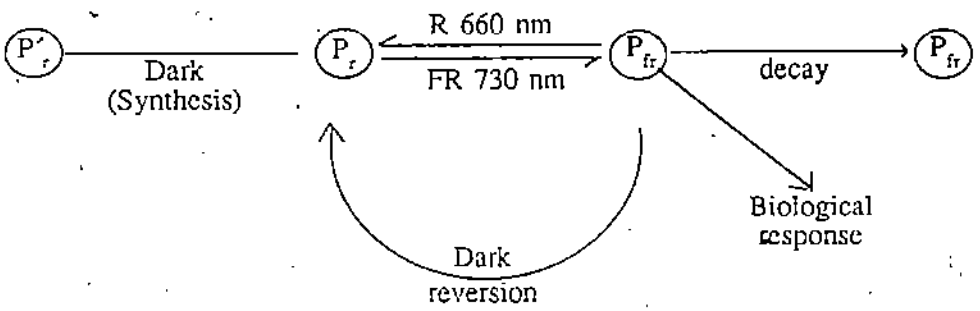



**Fig. 17.8: Schematic representation of photoconversion of phytochrome. Phytochrome is synthesised in dark. On receiving light in red range (R) it is converted from $P_r$ (red light absorbing form) to $P_{fr}$ (far-red light absorbing form). The $P_{fr}$ form is biologically active and induces a response. It also undergoes decay ($P_{fr}$) i.e. it degrades with the passage of time. When plants are left in dark for long, $P_{fr}$ form in some cases can also return to $P_r$ state. It also can be brought back to $P_r$ state by irradiation with far-red light.**

## 17.4.2 Properties of Phytochrome

Phytochrome is a chromoprotein: this means it is composed of a protein and a chromophore. The chromophore is actually attached to the protein and is responsible for its colour and light absorption. It is possible to isolate phytochrome in absolutely pure form by following the procedures of protein purification. The phytochrome in test-tubes also shows similar absorption characteristics. It absorbs red light (maximum absorption occurs at 660 nm wavelength) and on conversion, far-red light (maximum absorption occurs in far-red region at 730 nm). A typical absorption spectrum of phytochrome is shown in Fig 17.9. It is a soluble protein and is present in the cytoplasm. It is believed that $P_{fr}$ form may be associated with the membranes. The intact phytochrome protein has a molecular weight of 124,000 daltons. The total amino acid sequence of the protein is also known and, using recombinant DNA technology, the gene coding for phytochrome has been isolated.

Proteins cannot absorb visible light. They absorb only UV-light. So chromophore part is essential for the absorption of light in the visible range.

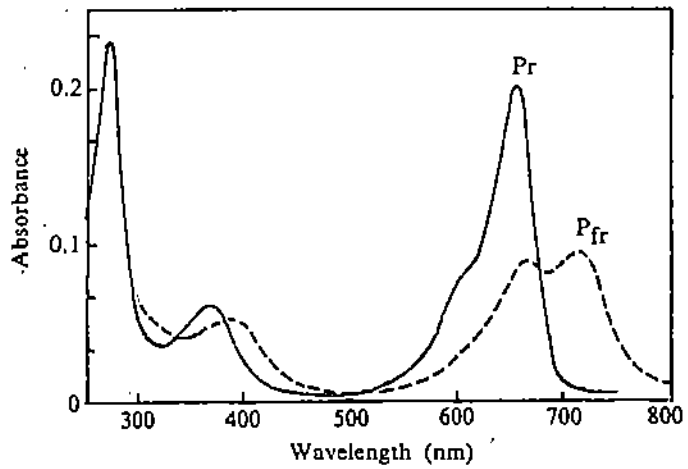



Fig. 17.9: Absorption spectrum of phytochrome *in vitro*. Phytochrome was purified and its absorption spectrum was measured by a spectrophotometer. When $P_r$ was irradiated with red light, the absorption spectrum (dotted line) was obtained. This shows the conversion of $P_r$ to $P_{fr}$. Note that not all $P_r$ can be converted to $P_{fr}$ form and one sees two peaks.

The chromophore is a tetrapyrrole molecule (Fig 17.10) like chlorophyll, but unlike chlorophyll it is an open tetrapyrrole and contains no metal ion. It is covalently

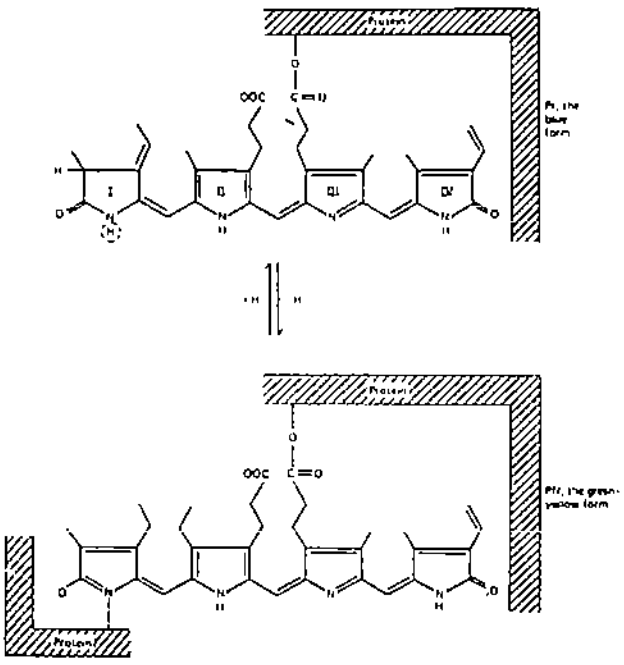


Fig. 17.10: The structure of chromophore and its attachment to protein. Phytochrome chromophore is an open tetrapyrrole. It is attached to the protein at a specific site and undergoes proton shift on conversion from $P_r$ to $P_{fr}$.

attached to the protein. It has been shown that it is the $P_{fr}$ form of phytochrome that is biologically active. Changes in the structure occur during conversion of $P_r$ to $P_{fr}$ (Fig. 17.10) which probably make the $P_{fr}$ form active.

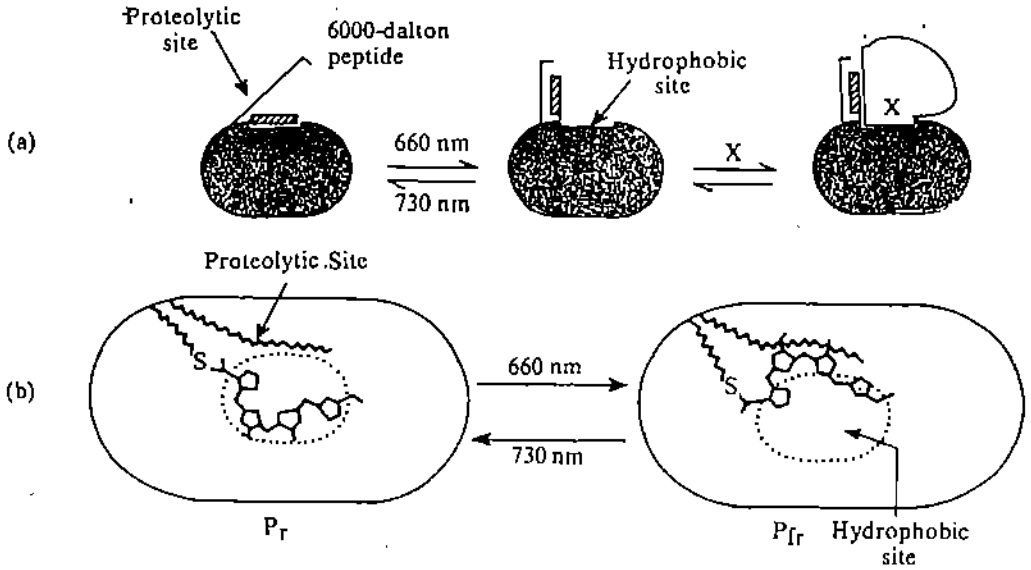



**Fig. 17.11: Model for inter-conversion of $P_r$ to $P_{fr}$ form: a) The tetrapyrrole moves away from the protein when converted from red to far-red form and exposes some hydrophobic sites of the protein in $P_{fr}$ form. This conformational change makes $P_{fr}$ an active species. Some proteins that are essential for biological action (x) may bind to the hydrophobic sites and get activated. b) The movement of tetrapyrrole with respect to protein in $P_r$ and $P_{fr}$ state.**

### A method to estimate phytochrome

Since there is no chemical test, the only method available to test and estimate phytochrome is the spectrophotometric method. Firstly, the difference in the absorbance between 660 and 730 nm is measured after red light irradiation (ΔOD red, No. 1). Next, the sample is irradiated with far-red light and again the difference in ΔOD is measured (ΔOD far-red, No. 2). The content of total phytochrome is measured by subtracting the value obtained after red light irradiation from far-red light irradiated value.

Phytochrome total = Δ (ΔOD) = [ΔOD far-red − ΔOD red]

1) ΔOD red = $[OD_{660} - OD_{730}]$ after red irradiation

2) ΔOD far-red = $[OD_{660} - OD_{730}]$ after far-red irradiation

Nowadays special spectrophotometers are also available which can measure the absorption difference between two wavelengths. These are called dual wavelength spectrophotometers.

## 17.4.3 Biological Responses Controlled by Phytochrome

Phytochrome responses are those which are controlled reversibly by red and far-red light. These can be broadly categorised as:

i) **Fast responses** those which occur within a time span of seconds to minutes and

ii) **Slow responses** those that take hours to days to manifest themselves.

Some of the fast responses are discussed below:

i) It was found that when mung bean root tips were kept in a specific solution (containing ATP, IAA, ascorbic acid, $MnCl_2$, KCl) the root tips adhered to a glass beaker when irradiated with red light. This effect was reversed by far-red light within 30 seconds. It is suggested that the quick response is probably due to change in electric charges on the root tips in response to red light, so the root

tips adhere on to the negatively charged glass surface. Such a response can be classified as a fast response.

ii) Similarly, it was shown that if alga, *Mougeotia* was irradiated, its single chloroplast turned in response to red light but far-red light alone had no effect (Fig 17.12). The red light effect was reversed by far-red light. This movement was also noticed within minutes of irradiation. In fact, in these experiments, the light was irradiated on the cytoplasm or membranes barrier and not onto the chloroplast directly.

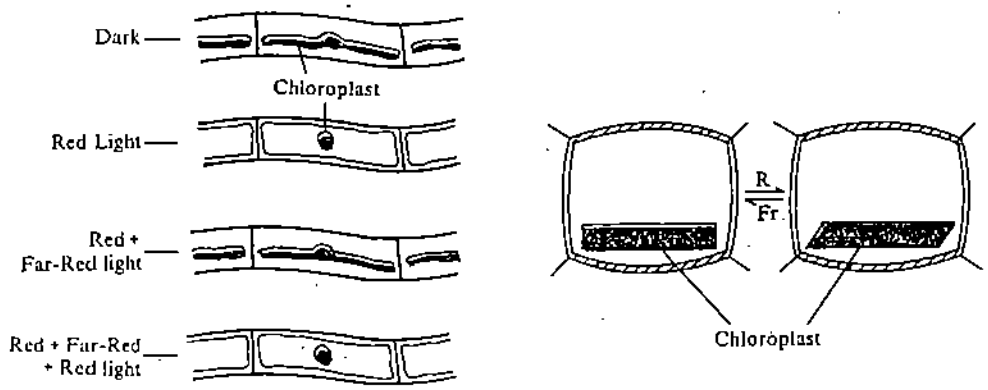



Fig. 17.12: An experiment with *Mougeotia* to demonstrate a fast response. Filaments of *Mougeotia* are made up of long cells each of which contains a single flat plate like chloroplast which can turn inside the cell in response to light. The light induced chloroplast movement was shown to be a red/far-red light reversible response as shown above. The movement starts with a lag phase of only a few minutes.

iii) In many leguminous plants it was found that leaves closed if they were transferred from light to darkness. It has been shown that this response is under phytochrome control (Fig. 17.13).

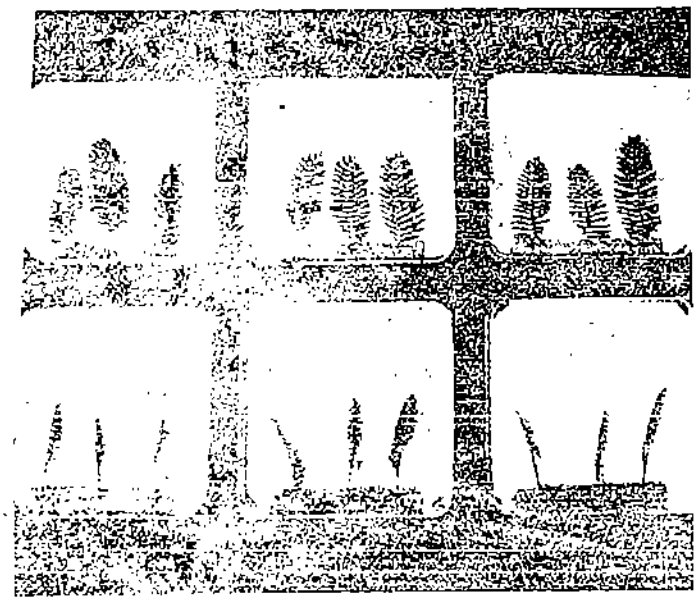


Fig. 17.13: Effect of phytochrome on the opening and closure of pinnae of *Mimosa pudica* (touch me not plant). When far-red light was given alone or after red light, the pinnae remained open (top row) however, if red light was given after far-red even after repeated exposure, the pinnae remained closed (bottom row). The plants were irradiated for 2 min. of each light and then kept in darkness. The response was seen within 30 minutes.

A number of other responses, which could be classified as slow responses like seed germination, hypocotyl elongation, leaf expansion, abscission, flowering, fruiting have been shown to be under phytochrome control. A few examples of effects of red and far-red light on such responses are shown in Fig. 17.14.

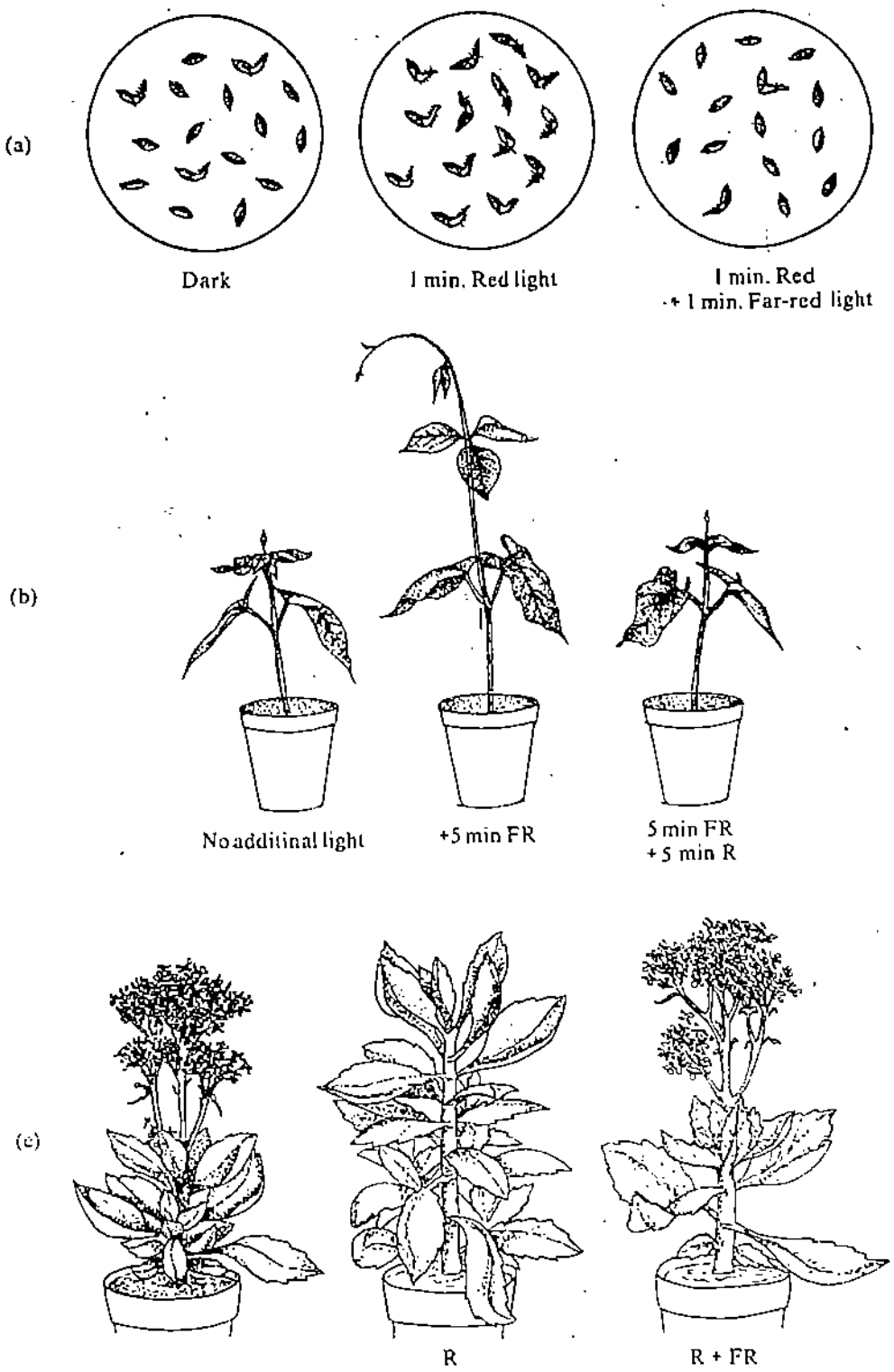



Fig. 17.14: Some of the slow responses controlled by phytochrome
a) reversal of red light stimulated seed germination by far-red light
b) demonstration of control of internodal lengthening by phytochrome, the effects were seen after 8 days. The plants were kept in light-dark cycle received either R or FR light.
c) Flowering in *Kalanchoe* (SDP) is controlled by phytochrome. The plant in the centre received some SD treatment but received red light in the middle of short-day.

### 17.4.4 Mechanism of Action

You have learnt that biochemical and molecular changes are brought about by phytochrome. However, what is not yet clear is the relationship of these changes to a specific developmental event. Since phytochrome controls a large number of processes even in one plant (Table 17.2). This makes the analysis of molecular action a difficult task.

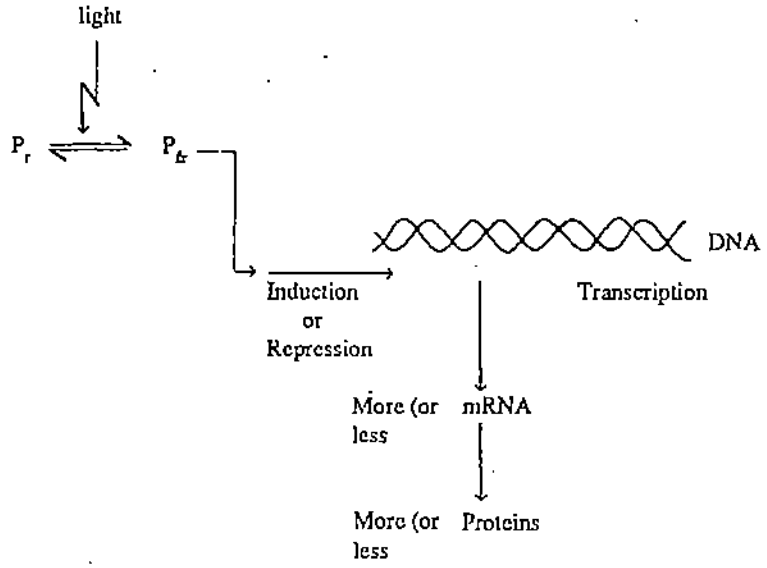



**Fig. 17.15: Hypothesis for differential regulation of gene expression. The biologically active form Pfr can affect the transcription process by either inducing the synthesis of new mRNA or by repressing the synthesis of mRNA already being synthesised in dark. By altering the level and quality of enzymes and proteins, developmental responses can be controlled by phytochrome.**

**Table 17.2: Multiple effects of light on mustard seedlings grown in dark**

| |
|---|
| Inhibition of hypocotyl lengthening |
| Enlargement of cotyledons |
| Opening of the hypocotylar ("Plumular") hook |
| Development of primary leaves |
| Synthesis of anthocyanin |
| Increase of the rate of chlorophyll accumulation (in white light) |
| Changes in the rate of cell respiration |
| Changes in the rate of degradation of storage protein |
| Increase of negative geotropic reactivity of the hypocotyl |
| Increase of protein synthesis in the cotyledons |
| Decrease of RNA contents in the hypocotyl |
| Increase of RNA-contents in the cotyledons |
| Differentiation of stomata in the epidermis of the cotyledons |
| Increase in the rate of carotenoid synthesis |
| Increase in the level of mRNA increase in nitrate reductase, increase in RuBP carboxylase |

Of the various changes brought about by phytochrome many of them occur within a few minutes and some others have a time-lag of 1-2 hour or more. It has been suggested that responses which occur rapidly, say within 0-15 minutes, may be considered as '**membrane associated events**', and the responses with a lag period of few hours may involve changes in amounts or activities of various proteins and enzymes and may be considered as '**gene expression events**' (Fig 17.15). The responses which are measurable as changes in growth may be considered final '**developmental responses**'. It has been found in a number of systems that phytochrome does affect membrane related phenomena. First, it has been reported in a few cases that phytochrome may be associated with membranes. For example, in *Mougeotia*, if the membranes were irradiated with light, chloroplast movement was detectable. Also, experiments suggest that phytochrome binds to the membranes but the results need to be confirmed. However, it has been clearly established for example in *Albizzia* that phytochrome controls transport of $K^+$ ions across the plasma membrane. Similarly, it has been shown that phytochrome also affects the movement of calcium ions. The exact mechanism by which phytochrome affects membrane transport proteins is not yet known

More work has been done on gene expression events. It has been shown that phytochrome does affect the level and activity of enzymes. In some cases, however, the pigment increases the enzyme level as for example, in phenyl alanine ammonia lyase, nitrate reductase, RuBP carboxylase, light harvesting chlorophyll a/b protein, whereas in others the phytochrome decreases the enzyme level, e.g., NADH protochlorophyllide oxidoreductase. An example of phytochrome regulation of nitrate reductase enzyme is shown in Fig. 17.16.

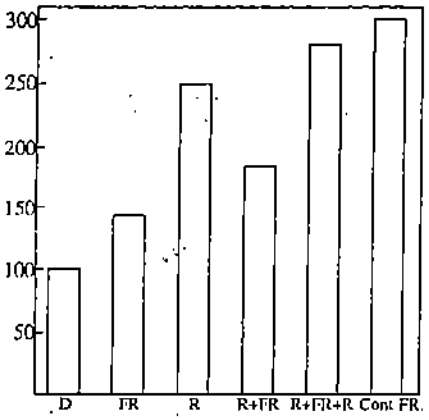



**Fig. 17.16: Photoreversible effect of R and FR light on the induction of nitrate reductase. R and FR lights were given for only 5 minutes to etiolated (plants grown in the absence of light) maize leaves.**

Recently, it has been shown that phytochrome affects the level of enzymes by affecting the process of transcription. An increase in the level of mRNA of a number of proteins in response to light irradiation has been shown.

A major question that remains to be answered is how does phytochrome which is present in the cytoplasm affect gene expression in the nucleus? It is suggested that probably some signals or second messengers are generated by phytochrome which affect at the level of transcription as shown in Fig. 17.17. Lately the role of calcium ions as messenger of light mediated responses has been emphasised. This is based on the findings that in many instances the light affect could be mimicked by calcium ions.

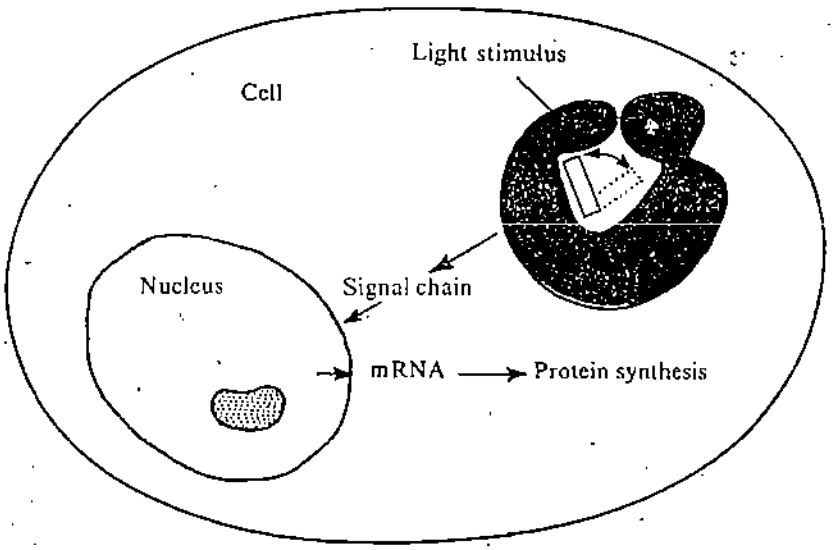



**Fig. 17.17: A general scheme to show that after light absorption and the formation of $P_{fr}$, a biochemical signal has to be sent to the nucleus to make new mRNA for the synthesis of new protein. The nature of the signal is not yet known but some evidences suggest that this could be a simple molecule like calcium ion.**

SAQ 3

a) In the following sentences fill in the spaces with appropriate words.

i) $P_r$ absorbs at ..... nm wavelength of light and $P_{fr}$ absorbs at ..... nm.

ii) The effect of red light could be completely reversed by ..... light.

iii) Phytochrome is a ..... protein. It is present in the ..... of cell.

iv) The biochemical signal sent to the nucleus by phytochrome for making new mRNA is suggested to be ..... .....

v) Biological active form of phytochrome ..... can affect ..... process by induction or repression.

## 17.5 SENESCENCE

Plants begin their development after seed germination. They grow, flower and finally senesce and die. The period from the start to death is called the longevity or age or life span and this period varies from species to species. For example, some plants, like annuals, complete their life cycles within a few months whereas others live for a few centuries. For example, the life of *Juniperus scophularium* is around 3,000 years. The period just before death is called the senescent period. This may be compared to old age in animals. In this period deterioration occurs because there is a consistent decrease in viability and increase in vulnerability. This phase can be prolonged but cannot be reversed.

Senescence may occur very quickly or may be a very slow process. In a plant sometimes the individual organs senesce while the whole plant may remain healthy. In annuals, the whole plant dies; in biennials, the plant dies only after two years, whereas in perennials, year after year the leaves and fruits are shed but the main plant survives. Broadly, therefore, the senescence can be of the following types.

**Overall senescence:** Only the parts above the ground level i.e. the aerial parts die whereas the underground parts survive. For example, potato.

**Deciduous senescence:** Only the leaves senesce as in many trees. The senescence of leaves, or abscission occurs when at the base of a leaf a layer of cells is laid which is called abscission layer (Fig. 17.18). The cells of this layer have high activity of cellulase, an enzyme which degrades cellulose of cell wall. Another enzyme that increases during abscission is polygalacturonase which hydrolyses pectin, a major component of the middle lamella region of the wall. So the cells get separated and the leaves fall. Below this layer, the plant makes a protective layer which has lot of lignified cells. When leaf falls, this layer protects the tissue from desiccation.

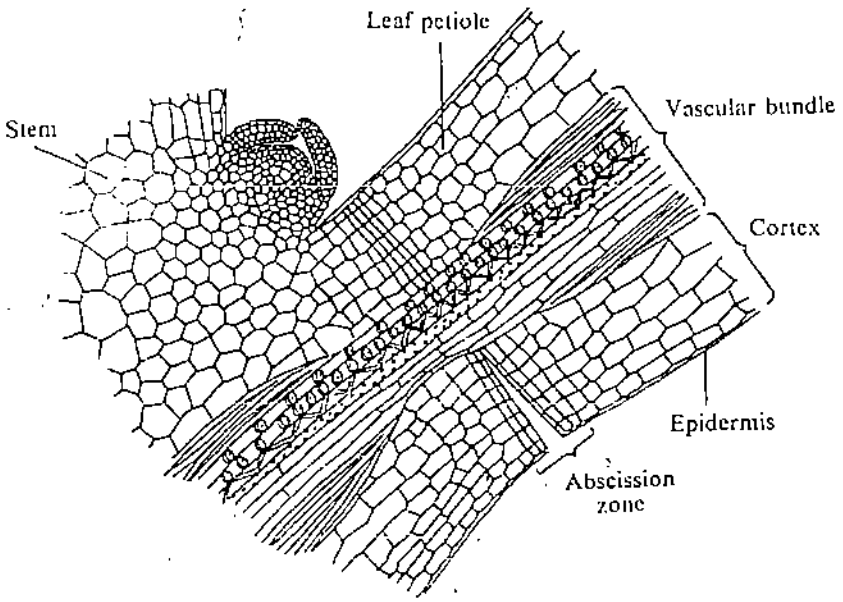



Fig. 17.18: Abscission zone near the base of the stalk of a mature leaf.

**Progressive senescence:** Here also the leaves are shed but it is gradual senescence of leaves up the stem. For example, the palm trees.

### 17.5.1 Regulation of Senescence

Senescence is a part of a developmental sequence of events and has to be a controlled process. You must have seen that if you cut twigs having leaves and flowers and put them in flower vases, these twigs senesce faster than if the twigs were still on the parent plant. It has been found that senescence is controlled by both external and

internal factors. Of the external factors, plant hormones, length of the day, temperature and nutrient supply play an important role. Of the internal factors, size and age of the plant, degree of flowering and time of ripeness of fruits determine the onset of senescence. In one experiment where the normal time of senescence was 120 days, when mature fruits were removed from plants the time of senescence was delayed. It occurred after 140 days. And when young fruits and flowers were removed from plants as soon as they were formed, the senescence was delayed to 160 to 180 days. This means that the senescence is initiated as soon as the process of reproduction is set in. This is probably because a plant needs a lot of nutrition for the growth of flowers and fruits. So to increase the supply to fruits, the stored material from leaves and other parts is translocated to the growing fruits. The demand is so high that the fresh supply of nutrient and photosynthates cannot be replenished. It is a simple case of more demand than supply. Naturally, the system collapses. However, in the process like majority of other organisms the plant tries to ensure that the fruits mature and seeds are set so that it can continue with its progeny. It is a case of self-sacrifice by the parent plant in order to see its seeds develop properly and grow.

Besides environmental and endogenous factors, biotic factors also play a role in inducing senescence. For example, due to an attack of mites, insects or even parasitic fungi, the process is hastened. Also, without realising when you walk in a garden and pluck leaves or break branches and twigs, you are also contributing to initiating senescence in those plants. Fig. 17.19 gives a general view of the factors that affect senescence of various plants.

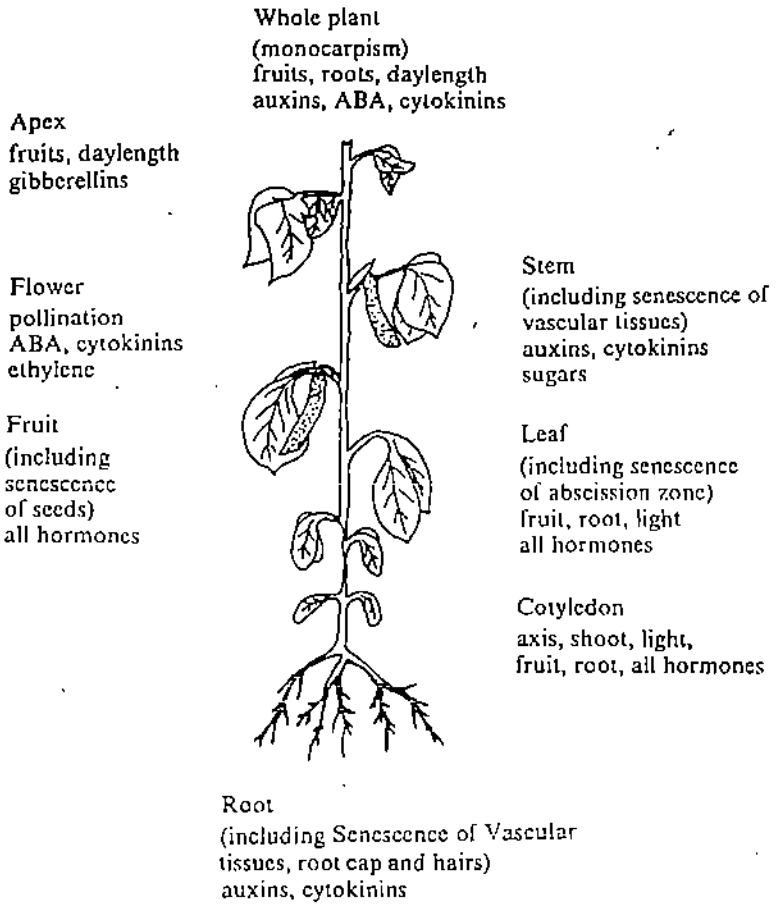



Fig. 17.19: The role of various physical and chemical factors on the senescence of different part of a plant.

## 17.5.2 Biochemical Changes Associated with Senescence

When senescence begins many physiological and biochemical changes take place. For example, one of the important changes observed is in chlorophyll content. Its level starts declining. As a result, the capacity of $CO_2$ fixation also declines and the overall structure of chloroplast in terms of organisation of thylakoids gets disturbed. For quite sometime the rate of respiration remains constant but later it also drops and the supply of ATP is reduced.

It is also shown that a number of degradative enzymes like proteases (hydrolyse proteins), ribonucleases (hydrolyse nucleic acids), β-glucan hydrolases (which loosens cell wall), chlorophyllase (which degrades chlorophyll) are produced which result in catabolism and finally over a period of time, the plant succumbs and dies.

**SAQ 4**

a) What are the associated changes observed in leaves during senescence?

.....

.....

.....

b) List the various factors that induce senescence in plants.

.....

.....

.....

.....

.....

c) Name two enzymes involved in abscission and write their function also.

.....

.....

.....

.....

.....

## 17.6 TISSUE CULTURE

You may be familiar with the technique of growing plants in test-tubes by tissue culture technology. Scientists hope that the technique would possibly help to understand the functioning of plant system, regulation of differentiation and growth in a better way. In this section we will describe the technique of tissue culture and its prospects.

### 17.6.1 Historical Perspective and Development of Techniques

You know that during fertilisation, the egg is fused with a male gamete resulting in the formation of a zygote which on division gives rise to an embryo. After germination, the embryo grows and forms a plant. Since the whole plant arises basically from a single cell, the fertilised egg, as well as the cells of a plant will have the same basic genetic make-up. If so, why does the zygote only form an embryo and develop into a plant; why cannot any other cell of a plant reproduce to form a new plant?

It was German Botanist Gottlieb Haberlandt who got the idea of culturing plant cells.

This curiosity led people to grow different parts and tissues of a plant by controlling the growth conditions. And it was found that if right nutritional and environmental conditions are provided to a cell taken from any part of a plant, it behaves like a zygote and gives rise to an embryo, which grows to form a new plant. This property of the plant cell is called 'totipotency' i.e. any cell of a plant can theoretically give rise to a new plant (Fig. 17.20).

It was in fact in the beginning of this century that culturing of plant cells started. The technique of growing plant cells, tissues, or organs under artificial laboratory conditions is called culturing. By 1936, culture conditions were partially defined. Later, as hormones and growth regulators were discovered, and their role in plant development was understood, scientists used them extensively to culture plants cells.

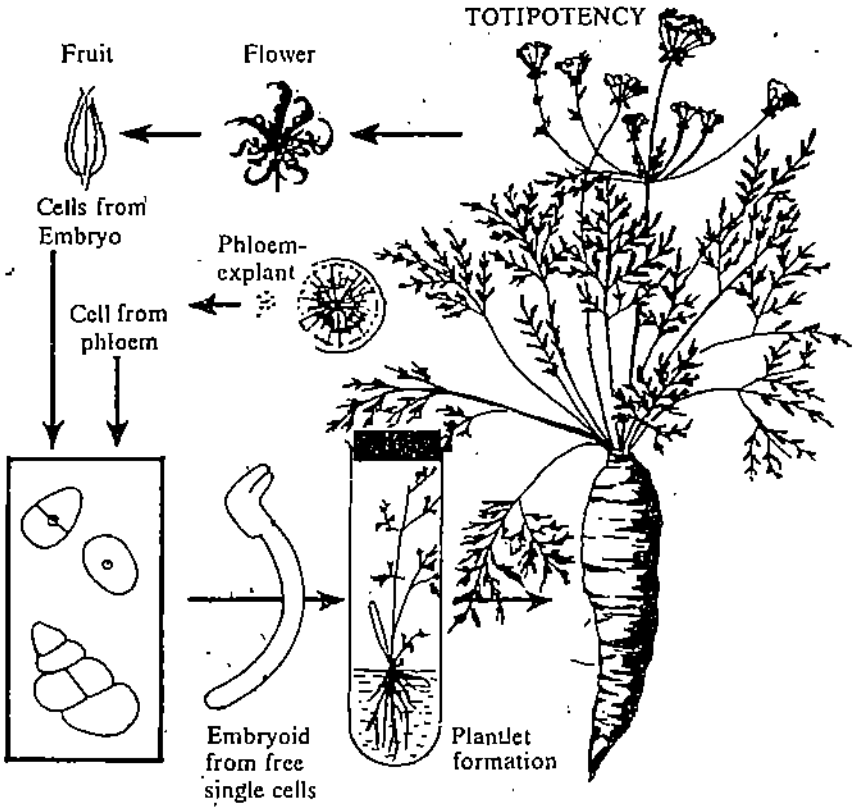



**Fig. 17.20: Demonstration of the phenomenon of totipotency in plants. Cells were taken from phloem explant or from embryos and cultured in medium. On supplying appropriate conditions single cells developed to form embryoids which developed further to form small plantlets. Such plantlets could be grown to complete carrot plants.**

Autoclave is like a big pressure cooker. When its temperature is raised to 120°C, the pressure of steam builds upto 15 lbs/sq inch. Microorganisms get killed under these conditions and sterilisation is achieved.

Before we proceed further let us first understand the method of tissue culture. This will help you to understand the next section easily. One can take any part of the plant. The part is called an explant (since it is excised from the plant) is sterilised and then placed on a medium which is also sterilised by autoclaving. Any medium basically contains essential micro and macro nutrients required for growth, and some vitamins and iron. Since the explant is not autotrophic, sucrose is added as carbon source to provide energy. In the previous unit you have read about the role of hormones in growth and development, they are also added. The medium is either used as such or solidified by addition of agar, sterilised and kept in test-tubes or in petriplates. The explants are then grown on this medium.

## 17.6.2 Organ, Tissue and Protoplast Culture

It has now been possible to culture any part of the plant in aseptic conditions. The nutrient requirements for organ culture varies from species to species. Culture of roots, shoot apices, leaves, embryos, anthers and endosperm has been achieved in a number of plant species, both from dicot and monocot families, and regeneration obtained. Growing roots can be maintained in cultures for a long time in flasks. In fact, in National Botanical Research Institute in Lucknow a technique for keeping root cultures for some years has been developed. And whenever required the roots can be regenerated into plants.

The first major success in tissue culture was achieved by Roger Gautheret in 1939 who established continuous growing cells of carrot. In 1950's Steward and Skoog in USA and Nitsch in France cultured carrot and tobacco cells and showed that they can form embryos and can be grown into complete plants. In India, tissue culture technology was developed by Prof. P. Maheshwari and his group at the Department of Botany, University of Delhi.

Similarly, shoot apices, leaf primodia and leaf segments can be cultured under appropriate conditions to develop callus and regenerate plants from them. At the University of Delhi, an interesting finding was made on the culture of anthers. Whereas in many cases, anther wall developed callus and formed plants, in *Datura innoxia,* it was found by Prof. S.C. Maheshwari and Dr. Sipra Mukherjee* (1964), that pollen grains present inside the anthers started dividing under certain conditions and developed into plants. Since pollen grains are haploid i.e. they have only half the number of chromosomes, the plants which were formed by the division of pollen

* Now, Professor at School of Life Sciences, JNU.)

grains were also haploid. This phenomenon has been shown to occur now in many species. Haploid plants are valuable in agriculture as the time-frame for obtaining homozygous pure lines required for breeding purposes is reduced from about 20 years to a few weeks!

From somatic cells, we can regenerate diploid (2 N) plants, from pollen grains, haploid (1 N) plants, and if we culture endosperms, we should get triploid (3 N) plants. This was also made possible through the work done at the University of Delhi. There are two ways by which the tissue in culture regenerates plants. Either the cell behaves as a fertilised egg and undergoes embryogenesis and the embryos so formed are made to develop further into plantlet. Or, the cells divide and form callus. A few cells of the callus behave like shoot or root primordia and develop into complete plant.

Endosperm is triploid because it is formed by the fusion of a male gamete (N) with 2 central cell (2N) during double fertilisation.

The regeneration of plants is dependent on the growth conditions and on the hormones supplied in the medium. You have read in the earlier chapter that hormones interact with each other to bring about a physiological or a developmental response. This was shown elegantly by Skoog and his coworkers who found that the variations in auxin cytokinin ratio could control the production of shoots or roots in culture.

An entirely different approach to study and manipulate plant cells is via culture of protoplasts. These are cells from which the cell wall has been removed. These 'naked cells', can be grown to form complete plants by modifying the composition of the medium (Fig. 17.21). At present, this technique of growing cells and protoplasts has been standardised for a large number of plants. Protoplast culture is aimed to introduce agriculturally important genes in plants and for getting somatic hybrids for sexually incompatible species.

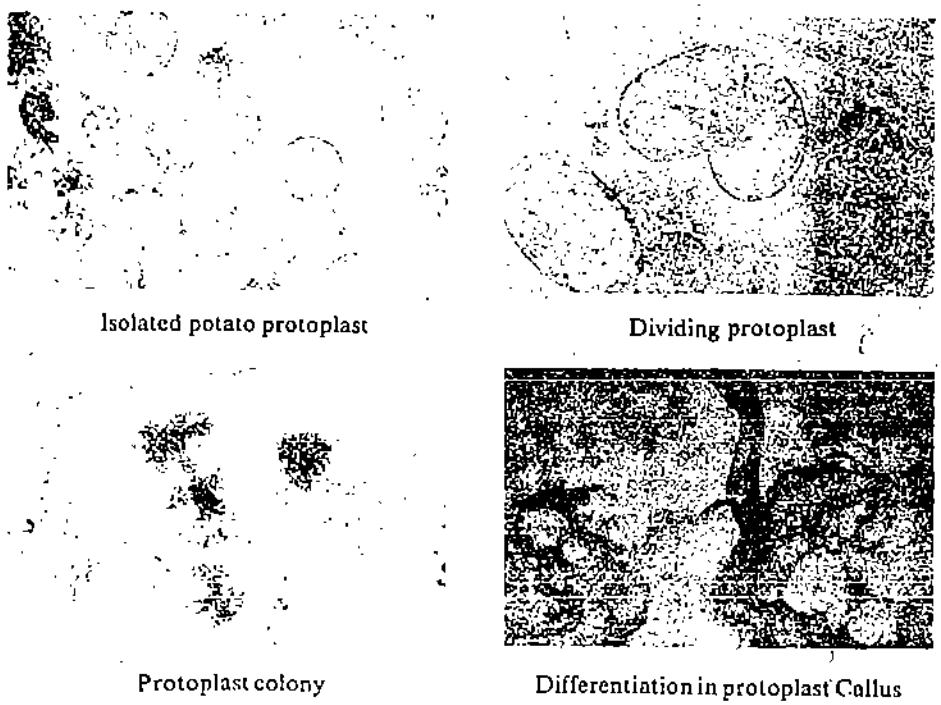



Fig. 17.21: Development of a single protoplast to complete plant. Protoplasts divide to form callus which on culturing on appropriate medium differentiated into plantlets. This particular experiment was done with potato plants.

The tissue culture technique has now become an important tool to study the role of various hormones, chemicals and light, in plant development as the effect can be studied under controlled conditions. Since regeneration can be achieved, tissue culture provides a unique opportunity to study the role of various exogenous factors on morphogenesis of plants.

**SAQ 5**

a) Which among the following statements are true. Write T for true and F for false.

i) Haploid plants can be obtained by anther culture □

ii) Only certain cells can behave like a zygote when provided with appropriate essential nutrients, hormones, vitamins and growth condition. ☐

iii) The regeneration of plant from organ culture is independent of growth hormones supplied in the medium. ☐

iv) 'Naked plant cells' i.e. cells without cell wall are called protoplasts. ☐

b) How does tissue culture technique help in understanding the regulation of differentiation and growth?

.....

.....

.....

.....

.....

## 17.7 BIOLOGICAL CLOCKS

Lastly we are going to learn about a phenomenon which is still not very well understood by scientists. Like animals, which show rhythmicity in their behaviour, it has been found that some developmental events in plants also occur with regular periodicity in time. These processes are rhythmic and if these occur in the absence of external disturbing factor, these are called **endogenous rhythms.** This would be clear if you have ever observed sleep movements of leaves in plants like *Phaseolus*, *Mimosa*, *Albizzia* and *Samanea*. In these plants during the day time the leaves are open and during the night they droop down. In fact, these movements can be recorded. This was done by tying a thread to the leaf that shows periodicity. The thread is rolled over a pulley and then attached to a stylus which is touching a rotating drum as shown in Fig. 17.22.

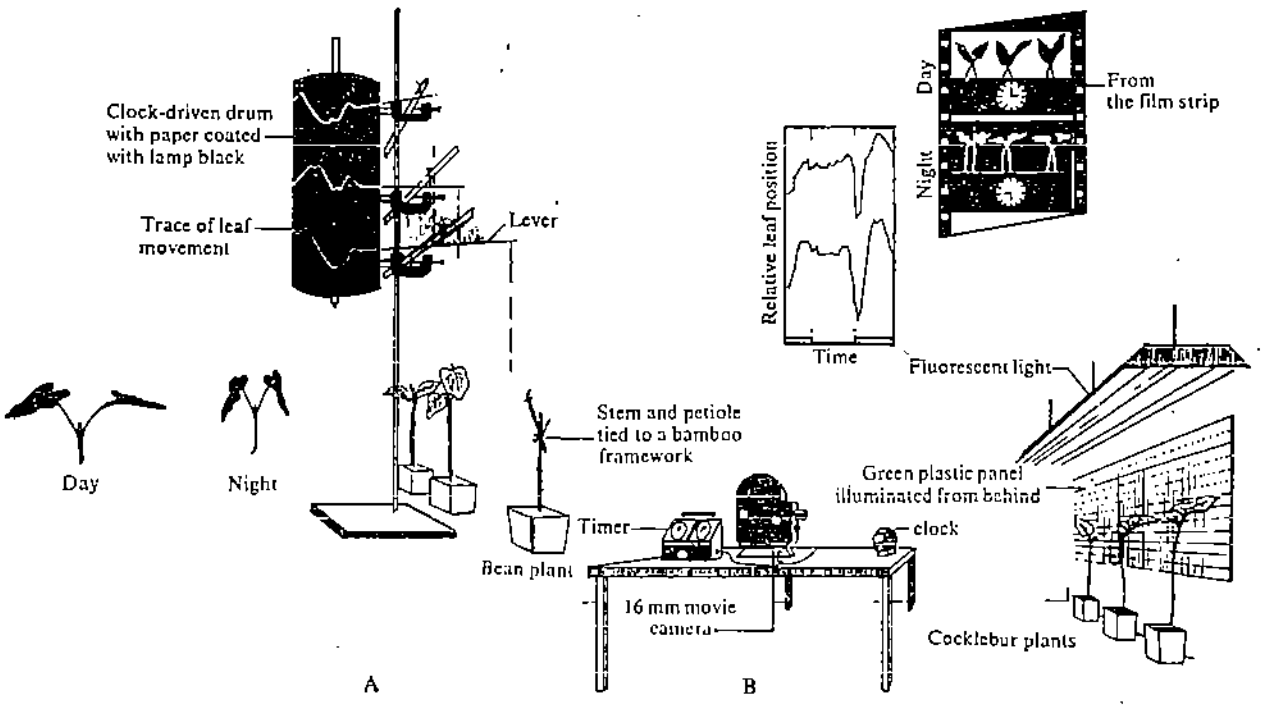



**Fig. 17.22: Methods for recording leaf movements. The leaf of the plant is tied with a thread to a lever that touches the marker running on a clock driven drum (A). Since the drum is moving the trace of leaf movement can be recorded over a period of day and night. A time lapse photography camera is fitted which takes pictures of the plant after short time intervals (B). From the developed film as shown on above right side, the leaf position with respect to time can be recorded.**

How do we know that these movements are independent of external factors? An experiment was done where after the rhythm was induced by light exposure, the plants were kept in total darkness for days. It was found that the leaves would open and close as they would have done under normal conditions. These oscillations were about 23 h apart. Such rhythms with oscillations of 20-23 h are called **circadian rhythms** and the cellular machinery which helps the plant remember and keep these rhythms in time is called the **'Biological clock'**. Interestingly, when plants were put on satellites, the rhythms still persisted.

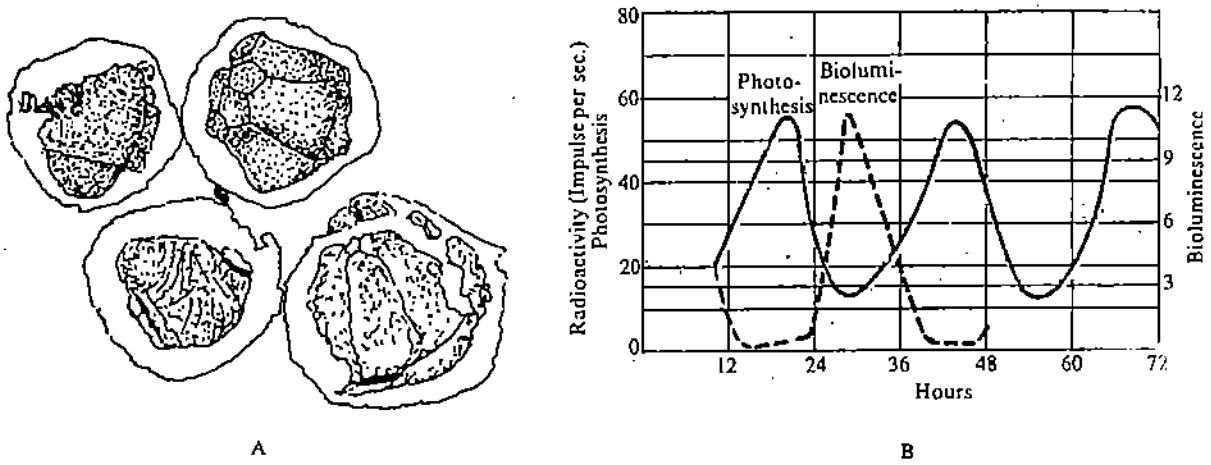



Fig. 17.23: Rhythm in *Gonyaulax polyedra (A).* This organism a dinoflagellate, has been extensively used in the study of biological rhythms. It shows three processes which follow different rhythms. Two processes are shown in B. Photosynthesis increases in light and decreases in darkness, whereas the reverse occurs in bioluminiscence. The third process, cell division, not shown here also follows a rhythmic pattern.

There are some good examples of plants where circadian rhythms have been noticed and studied. These are, sporulation in *Oedogonium cardiacum;* spore discharge in *Pilobolus sphaeroporus:* leaf movement in *Phaseolus multiflorus* and $CO_2$ fixation in darkness in *Bryophyllum fedtschenkoi.*

Very pretty floral clocks have been assembled in tourist places in various parts of the world. One in Canada near the famous Niagara falls tells the time from 6.00 a.m. to 6.00 p.m., beautiful flowers open at sharp 6.00 a.m., 7.00 a.m., 8.00 a.m. and so on, a glorious sight worthseeing. Fig. 17.24 depicts a floral clock found in a garden in Europe.

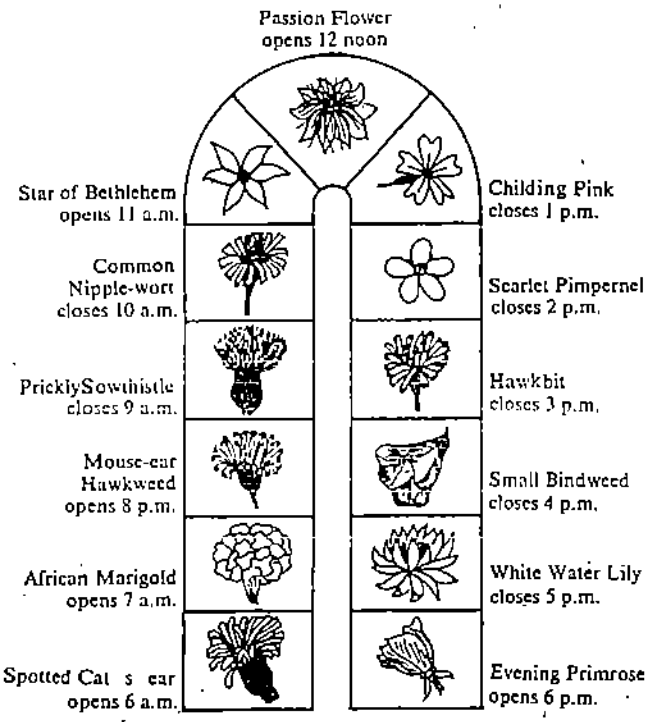



Fig. 17.24: The flower clock.

### 17.7.1 Factors Affecting Rhythms

Two factors, more important than others which regulate or affect the time period and the intensity of a rhythm are temperature and light. It has been found that once the rhythm is set, many circadian processes are independent of temperature over a wide range. The effect of light in many cases has been shown to be regulated by phytochrome, the pigment-protein complex about which you have learnt in Section 17.4. The amount of $P_{fr}$ form changes during the day, i.e. from sunrise to sunset. At sunset, when red light is less, its level would decrease which would further go down in the following darkness. In the morning when red light is more, the $P_{fr}$ level would rise again. This daily change could be one of the factors to set the biological clock.

One of the founders of biological clock research in plants, Erwin Bunning of Botanical Institute in Tübingen, Germany, worked on leaves of different varieties of soya bean and found some correlation between their photoperiodic behaviour and sleep movements. He further found that leaf movement was obvious in short-day varieties whereas in day neutral plants the leaf movement was less pronounced. These observations do suggest that light does have an effect on rhythmic phenomenon in plants.

At present it is still not clear what is the physical basis of the biological clock. Certainly, it is not any specialised timing organ. Some processes affected at the nuclear, protein and membrane level seem to be involved. However, the exact nature is not known with certainty. Probably some chemical inside each cell works as a clock. Although recent work seems to suggest the involvement of membrane bound glycoproteins which intercept environmental signals and set the clock.

## 17.8 SUMMARY

- The development of the plant starts with the formation of seeds. A seed has stored food material and mRNA which it uses during the process of germination. By utilising proteins that are already existing and newly synthesised on stored mRNA or whose transcription is induced, the stored food reserves like lipids, starch and seed storage proteins are degraded to yield compounds that can be utilised by the growing embryo for its growth and development.
- After a certain span of vegetative growth, plants undergo a reproductive development. During this process, flowers are produced. The flowering process is regulated by the duration of light/dark cycles. The dark period seems to be more critical to the flowering process: It is the leaf which perceives the photoperiodic stimulus. After perception the signal is sent to the vegetative meristems which then are converted into flowering meristems.
- The photomorphogenetic events in plants are regulated by a red light and far-red light absorbing pigment called phytochrome, which exists in two states. The far-red light absorbing state—$P_{fr}$ is biologically active and induces a large number of fast and slow responses in plants. The fast responses could be mediated by bringing changes in membrane properties. The slow responses may involve formation of new proteins which would control the transition from one developmental state to another.
- After the plant flowers, it completes its life cycle. The plants then undergo a phase of senescence. In many plants like trees, senescence is a perennial phenomenon leading to leaf fall. In such cases only parts of the plants undergo senescence and death.
- Since plants are complex organisms, many developmental responses are studied using *in vitro* culture techniques. By this technique any of the organs, tissues or cells of the plants can be cultured in test-tubes and made to differentiate and form complete plants. This manipulation is now possible on a large number of plants.
- Although most of the physiological and developmental processes are under the control of the external factors, in some cases they were self-regulatory and show rhythms of endogenous nature. The exact mechanism of these diurnal rhythms is not understood but it forms an essential study of plant development.

## 17.9 TERMINAL QUESTIONS

1. Why do farmers fear rain around harvest time?

.....

.....

.....

.....

.....

2. List the important enzymes and hormones involved in seed germination. Briefly explain their function also.

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

3. Match the following enzymes given in Column 1 with their functions in Column 2.

| Column 1 | Column 2 |
|---|---|
| i) Cellulose | a) degrades chlorophyll |
| ii) Protease | b) hydrolyses pectins in the cell walls |
| iii) Polygalacturonase | c) hydrolyses proteins into amino acids |
| iv) Chlorophyllase | d) hydrolyses cellulose |
| v) Ribonuclease | e) loosens cell wall |
| vi) β-glucan hydrolase | f) degrades RNA |

4. What are the advantages of tissue culture technique?

.....

.....

.....

.....

## 17.10 ANSWERS

**Self-assessment Questions**

1) i) dormancy ii) protein, mRNA
iii) vivipary iv) abscisic acid
v) aleurone layer vi) hydrolytic
vii) phytin

2) a) i) vegetative, floral, ii) dark period
iii) Day-neutral iv) florigen, leaves
v) darkness

b) Increase in the synthesis of following:
i) DNA, ii) RNA, iii) ribosomes, iv) protein.

3) i) 660 nm, 730 nm, ii) far-red, iii) soluble protein, cytoplasm, iv) calcium ion, v) $P_{fr}$, transcription

4) a) i) A decline in the level of chlorophyll.
ii) Consequently, a decline in $CO_2$ fixation.
iii) Disruption in overall organisation of thylakoids of chloroplasts.

b) F) Internal — Age of the plant, degree of flowering and time of ripeness of fruits.
2) External — length of the day, temperature and nutrients.
3) Biotic — attack of mites, insects and other parasites and plucking of leaves.

c) i) Cellulase — hydrolyses cell wall of the cells in abscission zone.
ii) Polygalacturonase — hydrolyses pectin which is a major component of middle lamella region of the wall shared by adjacent cells.

5) a) i) T, ii) F, iii) F, iv) T

b) By tissue culture one can study the stimuli needed for cell differentiation in isolation from whole plant. It gives insight into how relatively unspecialised undifferentiated structures differentiate into special meristems in plants. One can also exploit artificial induction of differentiation in protoplasts, tissues or organs under appropriate growth conditions.

**Terminal Questions**

1. Once the embryo is formed in the seed it enters a period of dormancy so that it does not germinate on the parent plant. In cereals the dormant phase is brief. If it rains around harvest time there is a danger that wheat and barely grains will germinate within the ear, making them useless for human consumption.

2. i) **Hydrolytic enzymes:** The following three hydrolytic enzymes degrade fats, proteins and carbohydrates into simpler units and provide nourishment to the embryo.

Proteinase — hydrolyse proteins into constituent amino acids.

$\alpha$-amylase — hydrolyses starch into maltose units.

Lipase — degrades lipids into fatty acids and glycerol.

ii) **Nucleases** — Degrade nucleic acids into nucleotides which are used as building blocks for the synthesis of new DNA and RNA.

iii) **Enzymes of $\beta$-oxidation and glyoxylate cycle**—Fatty acids are converted into acetyl CoA which produces succinate, a precursor for the production of glucose via gluconeogenesis.

iv) **Hormone**—The embryo releases gibberellic acid which acts on aleurone layer and induces the synthesis of $\alpha$-amylase.

3. i) d, ii) c, iii) b, iv) a, v) f, vi) e.

4. In this technique one can exploit artificial induction of differentiation in protoplasts, tissues or organs under appropriate condition. The technique is applied particularly in the production of large number of desired varieties. It is possible to isolate agriculturally useful mutants rapidly from millions of cultured cells than from millions of plants.

# UNIT 18 RESPONSES OF PLANTS TO STRESS

**Structure**



## 18.1 INTRODUCTION

In the preceding two units you have learnt that growth and development of a plant is governed by hormones—the internal chemical signals and environmental factors — the external signals which are perceived by special molecules present in the cell. In this last unit of the course, you will study the responses of plants to environmental stress.

You know that certain plant species can grow in severe environmental extremes. For example, plants grow below 0°C in the Himalayas and above 45°C in deserts of Rajasthan. The optimal requirements of water, temperature, light intensity, nutrients and soil vary from species to species. Have you ever wondered why grasses flourish in highlight intensities while growth of many plants comes to a halt or is even inhibited? How do rice plants grow under waterlogged condition while maize and wheat cannot? How do thermophiles remain functional above 70°C, though their cells are also composed of molecules that are broadly similar structurally and functionally to those present in cells of other organisms? Again, why are metabolic reactions of theromophiles not disrupted even at 70°C or is there anything special about the enzymes that allow certain species to function optimally at very high temperature and certain others at extremely low temperature. In this unit we will try to find answers to such questions. We will see what are the molecular responses of certain plant tissues that help them to cope environmental extremes — high temperature and light intensity.

Extreme pH of soil, salinity and mineral deficiency drastically limit the total arable land available for the growth of crops, fruits, vegetables and other useful plants in our country and elsewhere in the world. It is estimated that over 50% of the potential crop is lost because of stress of various kinds. Scientists are now studying the responses of plants to various stresses in the laboratory and field conditions. The aim is to get maximal yields despite all deviation from necessary optimal conditions for plant growth in the natural environment. In this unit you will find that results of preliminary studies on plant responses to various kinds of stresses look quite promising and we can hope that it should be possible to manipulate plants by genetic engineering so their performance is improved even under stress conditions.

**Objectives**

After studying this unit you should be able to:

- define stress, photoinhibition, cold hardiness, cold acclimation, an elicitor and osmoregulation,

- describe the various kinds of environmental stresses that plants may have to face,
- discuss the ways adopted by plants to cope with stress conditions,
- explain with examples the different types of responses of plants to cope with various stress conditions,
- discuss the prospects of manipulating and breeding plants in areas that are under environmental extremities in our country.

## 18.2 WHAT IS STRESS?

Sunflecks — small zones of light lasting for much shorter duration on a fixed spot.

Let us recall what happens within the natural communities occupying the same habitat. The relative location of two plants may place them under differing conditions with respect to a given environmental factor such as light. The top cover of a rainforest, for example, consists of relatively tall trees and receives maximal irradiance while the floor dwellers manage with sunflecks. What will happen if we artificially shadow the outer cover plants and illuminate the forest floor? Being a deviation from the natural situation, this is likely to have adverse effects on growth of these plants. Likewise, any deviation from optimal environmental conditions usually with adverse effects on plant growth can be considered as stress. This in turn affects the yield of useful agricultural products. Fig. 18.1 shows the yield gap due to biological and environmental constraints.

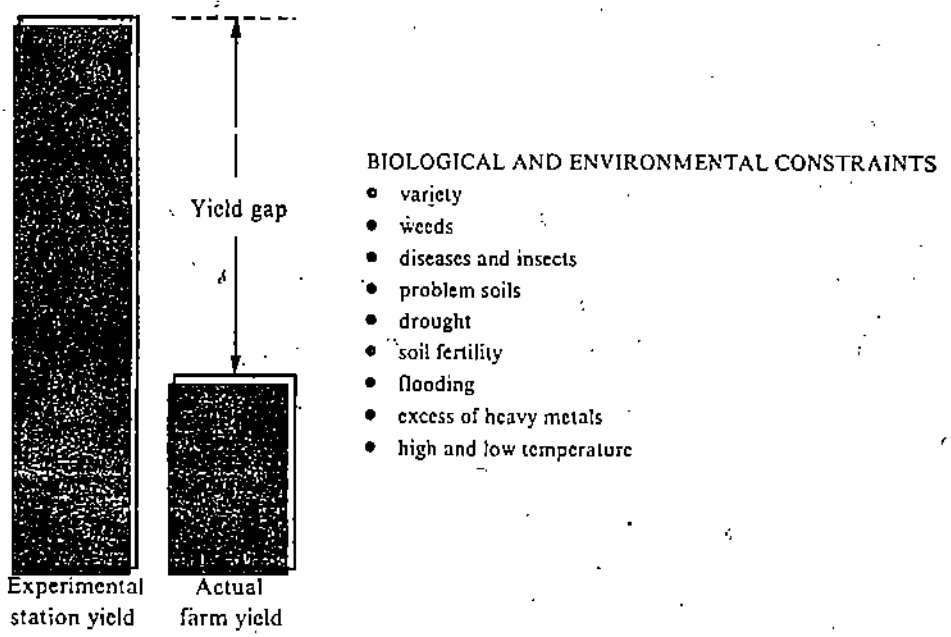



Fig. 18.1 : An illustration of yield gap.

We must, however, realise that in no habitat, all conditions — temperature, light, availability of water, nutrient supply and soil characteristics can be controlled and fixed to optimal level for any species. But since the species must survive, it must adapt itself to the deviations in the environmental condition(s). This could be achieved either by breeding crop varieties tolerant to stress or by offering conditions that help the plants to withstand the stress. For example, if there is a deficit of some nutrient one could try to supplement the same. Plants capable of adapting themselves to changes in environmental conditions perform the best under stressful conditions.

## 18.3 THE NATURE OF STRESS

As mentioned in the preceding section, stress can be considered as any deviation in environmental condition from optimal for the overall performance of the plant species under reference. If you recall the environmental factors, you can tell the various kinds of stress that plants may be subjected to. A broad outline on the nature of possible environmental stress is illustrated in Fig. 18.2.

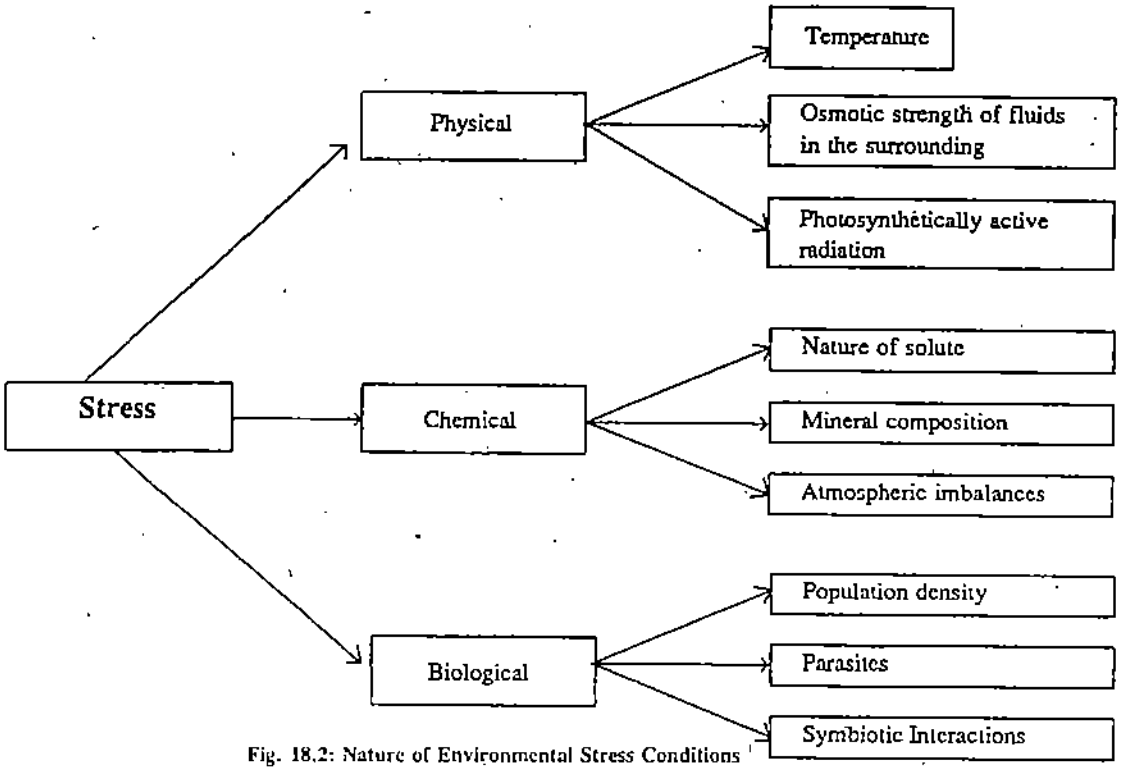



Fig. 18.2: Nature of Environmental Stress Conditions

### 18.3.1 Physical Stress

Physical stress originates from such environmental conditions as:

i) **Temperature** : We are most familiar with the plants and other organisms that live at temperatures close to the temperature range in which we are adapted to live i.e., 15° to 45°C. However, we know that there is life below 0°C in the arctic and above 90°C in the sulphur springs. In the subtropical zones, plants face stress when they get exposed to freezing temperatures during the winters while in the deserts of the tropics the native plants withstand over 55°C during summer. High temperature can be inhibitory for photosynthesis (Fig. 18.3).

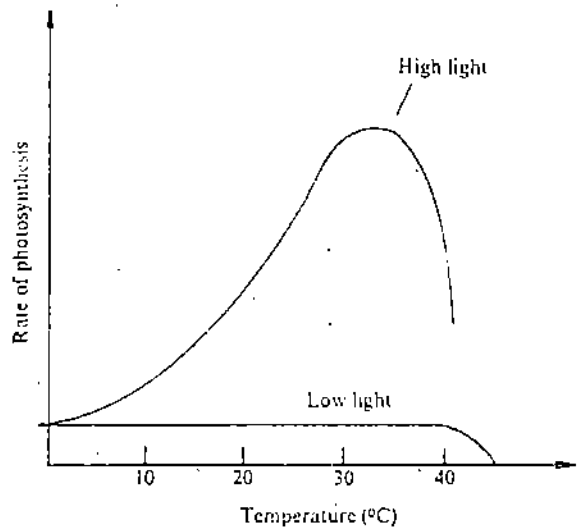



Fig. 18.3 : Effect of light intensity and temperature on the rate of photosynthesis.

ii) **Osmotic strength of the fluids in immediate surrounding:** The availability of soluble mineral salts varies widely from habitat to habitat. In fact, a big chunk of land in our country has been classified as 'wasteland' because of high salinity of the soil cover or the water leachates (Fig. 18.4). Common crop cultivars cannot be grown in such environments.

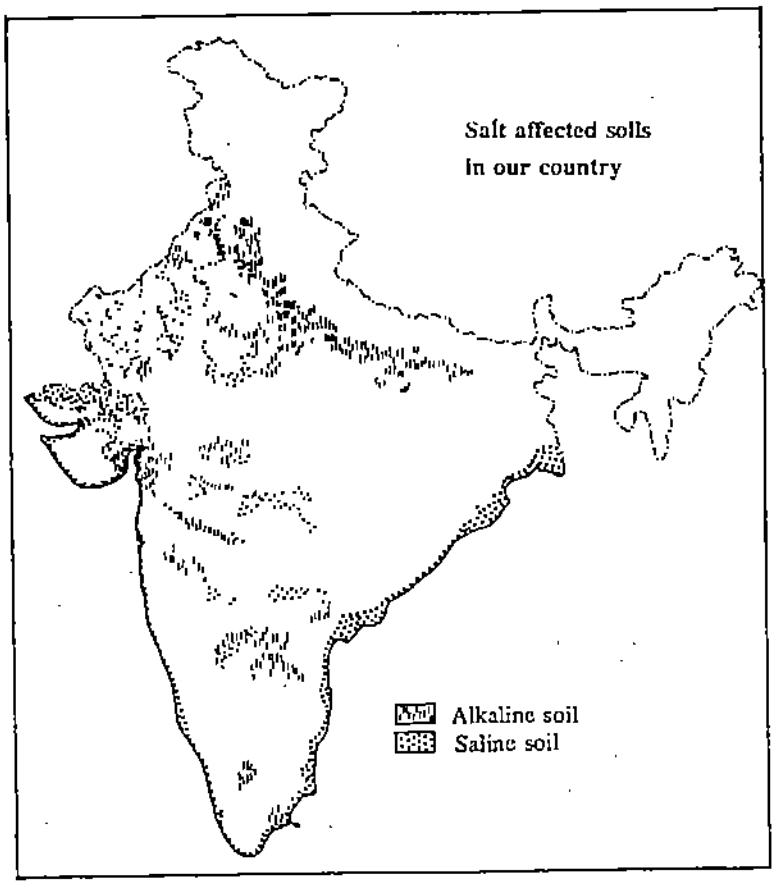



Fig. 18.4: Salt affected soils in India.

**Photosynthetically active radiation** or PAR refers to that part of the light spectrum which is directly absorbed by photosynthetic pigments of a plant. While the light absorption properties of the reaction centres are quite conserved among plants, the nature of light-harvesting pigments may vary. Thus PAR may mean different spectral composition for different species.

iii) **Photosynthetically active radiation :** This is usually in direct proportion with the incident solar radiation. You know that tropics are probably the best illuminated part of the globe. Also, variation in daylength is minimal in this zone. No wonder, light-dependent life has attained maximal density in the tropics. Within a dense population of plants, different organisms may have different degrees of exposure. However, if a plant is exposed to increasing light intensity it shows a corresponding increase in the rate of photosynthesis upto a certain point, beyond which further increase in light causes either no change in the rate or causes an inhibition (Fig. 18.5). Thus, each plant species shows a characteristic photosaturation and/or photoinhibition of photosynthesis. In other words, each species has its own optimum with respect to light intensity. It is under stress if exposed to intensity above or below this optimum.

Quantum efficiency : moles of $CO_2$ fixed per mole of light quanta absorbed.

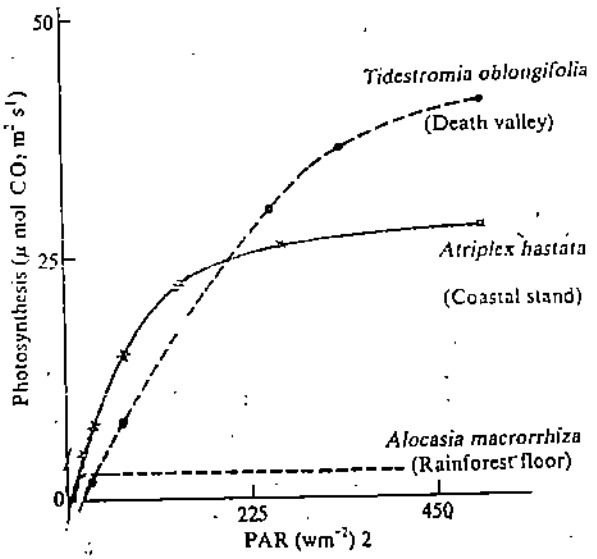



Fig. 18.5: Effect of light intensity on the rate of photosynthesis.

### 18.3.2 Chemical Stress

Survival of cells is dependent on carrying out of a set of chemical reactions (metabolic reactions) in a particular order. This results in a net gain in mass as well as energy in a growing cell. Even for dormant cells as in dormant buds and seeds, where there may not be any net gain in mass, a certain amount of energy must be spent to maintain them in a viable state. Various catabolic and anabolic pathways operate according to the availability of chemical constituents present in the cytoplasm. This may in turn be influenced by the composition of the extracellular environment with respect to the following:

i) **Nature of the solutes :** The acidic or basic reaction of soil and water of a particular habitat reflects its geochemical history beginning with its formation and subsequent interactions with other constituents of the earth up to its current chemical activities. Mineral deposits in their oxide form ('Bhashma') are usually basic in their reaction. The reaction of chloride, sulphate and nitrate is acidic or neutral depending upon the nature of the conjugate ions. You can visualise the reaction of a salt through its acidic and basic radicals. On a natural course, one would expect the neutralisation reactions and consequently change in the character of the habitat towards neutral. However, there are soils which are very high and others very low in pH and certain plant species survive in such soils.

The biosphere has generally developed near the conjunctive zone of the solid, liquid and gaseous components of earth. Beginning with an extremely hot mass of matter, as this planet cooled down, it led to the formation of the rocks, the water bodies and the atmosphere. The long time that has passed between the origin and the present state of earth, must have witnessed physical and chemical changes like melting, cooling and solidification, chemical interactions, erosion, transport, release and absorption of gases and water etc. All these processes put up on a time scale form the geo-chemical and geophysical history of a particular part of earth.

ii) **Mineral composition :** The living systems make use of several mineral ions that they might have encountered at the very origin or during evolution, particularly for transformation of matter involving proteins and nucleic acids. These elements continue to remain essential requirements for life. You have already learnt in Unit 12 that nitrogen, phosphorus, calcium, potassium and magnesium are familiar major requirements for plant growth besides carbon, hydrogen and oxygen. Apart from these, many other micronutrients such as manganese, iron, zinc, cobalt and molybdenum are required for healthy plant growth in much smaller quantities. Availability of these elements in the environment in quantities smaller than required causes deficiency symptoms while a surplus may cause toxicity leading to stunted growth, necrosis, abnormal development of vegetative and reproductive parts.

iii) **Atmospheric imbalances :** At least two components of the environment, oxygen and carbon dioxide, that plants require are predominantly in the gaseous form. As you have learnt, atmospheric nitrogen can be used by nitrogen-fixing bacteria, some prokaryotic algae and plant-bacterial symbiont. The biological cycling of these gases keeps their overall availability buffered within a reasonably stable range. Yet, intensive industrial activities that involve emission of one of these gases or high levels of the oxides of carbon, nitrogen and sulphur has visible effects on the performance of most plant species.

### 18.3.3 Biological Stress

Since in nature, the various organisms do not live in complete isolation from others, stress to a plant species might also be caused by what other organisms in the community require and consume. The following situations exemplify biological stress.

i) **Population density :** You are aware of what might follow an uncontrolled growth of human population. There will be competition for common consumables and for space. But this is not merely a human problem. Too thick population densities of plants can result in the competition for common nutrients, water and photosynthetically active radiation. The effect can be a self inhibition or inhibition of other plant species growing in the same environment. That is one of the reasons why weeds are removed from croplands.

ii) **Parasites :** Many insects and microorganisms can feed upon tissues and saps of living plants. Hence plants must be protected against such parasites either by expressing evasive devices such as inhibitors and toxins against the enzymes of parasites or by developing preventive morphological and biochemical alterations that keep the parasite away from aggregating near the plant.

iii) **Symbiotic interaction :** Presence of symbiotic microorganism can result in differential growth stimulation of those plants which can recognise the beneficial symbionts and utilise their presence in the environment. This interaction is a mutual one. For example in *Rhizobium*-legume symbiosis the rhizobium causes

a drain on the carbon fixed by the plant (refer Unit 15 of this Block, Page 18) while the plant gains access to the nitrogen fixed by the bacteria.

**SAQ 1**

What is the nature of stress faced by the following species if the change mentioned below is brought about in their environment?

i) A plant species growing in Shimla is brought to Jaisalmer during summer.

.....

ii) A sun-loving plant gets shaded by other plants.

.....

iii) A crop gets flooded due to heavy rains for several days.

.....

iv) Trees growing near the pavement are subjected to heavy traffic on the road.

.....

v) Plants acclimatised to grow near Ganga river are grown in coastal areas near Calcutta.

.....

## 18.4 WAYS TO ADAPT TO STRESS

Now that you know the nature of stress, let us see what type of strategies plants have acquired to adapt to stress. You know that biological functions are performed with the help of various kinds of molecules produced in particular tissues and organs. Therefore, it should be possible for an organism to adapt to a change in environmental conditions by correspondingly changing the molecules and/or tissues and organs recruited to perform the affected function. At their extremes, many different types of stresses would elicit similar types of response. This may involve repression/derepression of the synthesis of sets of proteins. Many of these proteins regulate the expression of other proteins (i.e., they are regulatory proteins, refer Cell Biology Course, Unit 14, page 94). Thus, adaptation to stress may be achieved by altering the molecules at work and/or cells and tissues serving a particular purpose. Whatever be the nature of stress, an organisms's response has to be in terms of processes and pathways operative in it. Often, visible changes in the morphology and behaviour of plants are also apparent in response to stress. Let us first consider what sort of changes have been observed in molecules of the cell.

### 18.4.1 Altering the Molecules at Work

Both qualitative and quantitative changes in the biomolecule can occur to help a plant adapt to stress.

**1) Qualitative changes**

**Changes in the conformation of molecules:** You may recall that linear chain of amino acids of a protein folds into a characteristic structure. Acidic residues, glutamic and aspartic acids interact with basic residues, arginine and histidine and form ionic bonds. The thiols of cysteines form inter chain and intrachain disulphide (S-S) bridges which are very important for keeping the protein in desired conformation. The extent of these interactions is influenced by the nature of the environment of protein. If most of the cysteines remain in the reduced state (-SH), there would be minimum folding. The unfolded state of protein molecule may not be as soluble and active as the folded conformation. The qualitative changes in the structure of proteins in response to stress can lead to the following:

i) **Resistance against denaturation of protein:** As mentioned above this is possible by alteration in acidic and basic amino acid side chains and in the thiol group (SH) involved in conferring a safe structure to the protein.

ii) **Change in kinetic property of the molecule :** Conformational changes affect the affinity and Km of protein and change it to a sub-optimal value. For example, receptor molecules involved in the uptake of specific nutrients by plants may change during stress.

**2) Quantitative changes**

The quantitative changes in the availability of the active biomolecules occur in order to compensate for the loss of efficiency of the system caused by the stress. This is illustrated by the following examples.

i) There is an increase in the amount of ribulose bisphosphate carboxylase in plants exposed to bright light than those in shade. As you can correlate, there should be an increase in the level of this enzyme to make use of the extra reducing power (NADPH) generated due to high light intensity (refer Unit 13; Fig. 13.15).

ii) During infection, enhanced synthesis of hydrolytic enzymes for destroying the invader, and synthesis of inhibitors by host that work against the damaging enzymes produced by the invaders has been observed.

iii) In halotolerant species there is an increase in the synthesis of certain amino acids in order to keep the osmotic balance.

iv) To protect against injury due to toxic metal, there is enhanced synthesis of chelating molecules to avoid toxic ions.

## 18.4.2 Changes in the Morphology and Behaviour of Plants

**1) Morphological changes**

Aquatic plants produce different kinds of leaves and produce aerenchyma (tissues with air gaps) where there be a need to do so. Also, a thick cuticle on leaves of many hydrophytes usually prevents direct injury by insects thereby avoiding opportunistic infection by other microbes.

**2) Changes in behaviour and course of development**

Such responses can be placed under the following categories:

i) **Quick transient responses:** Such responses are stimulated by factors showing significant variations over relatively small time period e.g., those showing wide diurnal variations. Opening and closure of stomata in response to light, humidity and temperature conditions is a well known plant response.

ii) **Long-term responses:** Plants alter their course of development to suit the prevailing environmental conditions. For example, leaf maturation and fall, onset of flowering and dormancy of seeds display some of the plant characteristics set in this way. Stress conditions may prepone or postpone the developmental processes. Abscisic acid levels in plant tissues increase under stress caused by nutrient deficiency or toxicity, salinity, chilling and water-logging resulting in reduced growth and metabolism. This can prepone or postpone flowering and seed set.

## 18.4.3 Use of Alternate Metabolic Pathways

Plants surviving under water-logged conditions offer the simplest example of such a response. The submerged parts of such plants use the anaerobic pathways for energy generation under water-logging.

From the above discussion, we can see that plants seem to have ways to escape, avoid or tolerate the stress conditions of varied nature. In the following section we will tell you certain specific responses of plants under stress conditions. Let us now try the following SAQ.

SAQ 2

In the following statements fill in the blank spaces with appropriate words.

i) In plants changes in the conformation of.....make it resistant to.....during stress condition.

ii) During high light intensity the quantity of enzyme..... .....increases many folds.

iii) Plants may change the course of development such as..... .....and seed .....to avoid stress situation.

iv) The hormone..... .....increases during salinity and water-logged condition.

## 18.5 PLANT RESPONSES TO SPECIFIC STRESS CONDITIONS

By now you must have developed a feeling that plants are organised to function optimally under a set of environmental conditions. This is to say that each plant can perform well only within a limited range of variations with respect to a certain environmental factor. However, what is optimal for one species may not be optimal for another. Deviations from any one of these conditions substantially away from optimal, causes stress and initiate an adaptive response of the organism. Obviously, the nature of response would correspond to the particular stress condition deviating from optimal. A considerable amount of research effort has been put into understanding the plant responses to specific stress conditions. This promises to suggest how man could probably overcome the losses caused by stress. Let us review certain studied cases of such plant responses.

**1) Water Stress**

Water stress in a plant can result from drought, excess salinity in the soil and very low or high temperatures. It has been found that during water stress certain organic compounds such as sucrose and amino acids particularly proline accumulate and thus lower the osmotic potential in the cells. But the metabolic activity of the cell is not much affected. However, the activity of some enzymes such as nitrate reductase and phenylalanine ammonia lyase decreases while activity of degrading enzymes like α-amylase and ribonucleases increases. It is proposed that the enzymes break down starch and other molecules into their monomeric units which make osmotic potential of the cells more negative.

Photosynthesis is also inhibited due to low water potential in the leaves. You may recall from the Unit 11 on Plant Water Relations that leaf water potential is the sum of turgor pressure, osmotic potential and matric potential. In very simple words, it represents the degree of saturation of the tissue with water. At low leaf water potentials, photosynthesis is inhibited by:

i) stomatal closure (limiting the gas exchange), and

ii) decrease in photosynthesis.

During dehydration both quantum efficiency (i.e., moles of $CO_2$ fixed per mole of light quanta absorbed) and the rate of photosynthesis are inhibited in leaves exposed to either high or very low light intensity. This phenomenon is called **photoinhibition of photosynthesis**. Photoinhibition is probably caused by accumulation of excessive excitation energy at photosystem II reaction centre complex (see Unit 13, Photosynthesis, Fig. 13.15). In this way exposure to light adds to the water stress when the plant's response already would be to decrease photosynthesis.

We had discussed in Unit 11 that the level of ABA increases in response to drought in the guard cells and results in stomatal closure. In general, increase in levels of ABA about 40 fold has been observed in leaves. In some case it is observed that the application of ABA to drought sensitive cultivar converts them to phenotypically drought resistant variety.

**2) Light Stress in Chloroplasts**

As mentioned in the last section, very high light intensity can inhibit photosynthesis due to accumulation of excess of excitation energy. A portion of this results in the accumulation of reduced plastoquinone in the pool of electron carriers. Low light can exert similar effects if the terminal electron acceptor viz. $CO_2$ is limiting as happens during dehydration followed by closure of stomata.

**3) Response to Osmotic Stress**

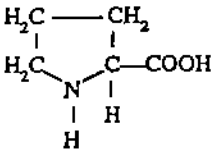
**i) Osmotic adjustment (osmoregulation)**

If concentration of soluble matter (in the form of minerals and small organic molecules) builds up in the external medium, it will create a gradient demanding outward movement of water from the plant cells, thereby causing dehydration. Alternatively, it will create a pressure on the cells to take in the solutes and maintain osmotic equilibrium with its immediate environment. You will appreciate that no organism can freely take these options indiscriminately. Hence, many of them

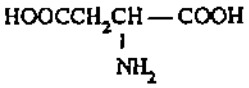
accumulate certain **compatible solutes** within the cells to attain osmotic equilibrium with their immediate surrounding (Table 18.1). In general proline and glycinebetaine are important organic molecules that help bacterial and plant cells to alleviate such osmotic stress. The build up of carbohydrates, amino acids, polyhydric alcohol and other molecules (Fig 18.6) due to water stress or high salt concentration is called **osmotic adjustment** or **osmoregulation.**

Compatible solute — Certain solutes that are accumulated in excess during water stress for providing osmotic adjustment with the surrounding. These can be tolerated because they do not damage the enzymes of the cells.

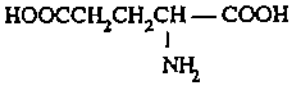
**Amino acids**



Proline

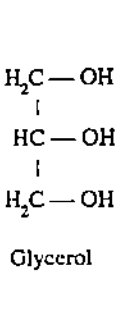


Aspartic acid

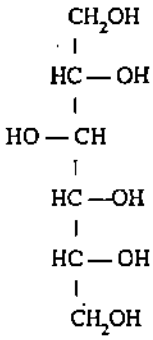


Glutamic acid

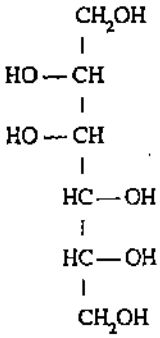
**Polyols**



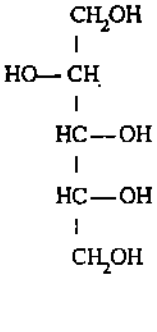
Glycerol



Sorbitol

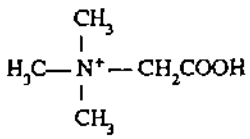


Mannitol

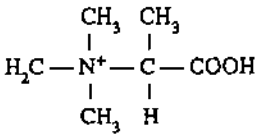


Arabitol

**Methylated quaternary ammonium compounds**



Betaine
(Glycinebetaine)



Alaninebetaine

Fig. 18.6: Molecular structure of compatible solutes found in stressed plants.

Table 18.1 : The compatible solutes that increase in the cells of some organism during stress for osmoregulation.

| Organisms | Compatible Solutes |
|---|---|
| ANGIOSPERMS | |
| glycophytes : *Chloris gayana, Hordeum vulgare* (barley) | betaine and proline |
| halophytes : *Aster tripolium, Salicornia fruticosa, Triglochin maritima* | proline |
| halophytes : *Atriplex spongiosa, Spartina townsendii, Suaeda monoica* | betaine |
| MICROALGAE | |
| *Chlorella pyrenoidosa* (freshwater) | sucrose |
| *Dunaliella* spp. (marine and halophilic) | amino acids, glycerol |
| *Scenedesmus obliquus* (freshwater) | carbohydrate (sucrose + raffinose, glucose, fructose) |
| FUNGI | |
| *Chaetomium globosum* (a terrestrial form) | polyhydric alcohols (mannitol, arabitol, glycerol) |
| *Saccharomyces rouxii* (an osmophilic form) | arabitol |
| BACTERIA | |
| Various halophiles and nonhalophiles (e.g., *Klebsiella, Salmonella, Streptococcus)* | amino acids (glutamate, proline etc.) |
| *Halobacterium salinarium* | NaCl |

**Source :** Flowers et al. 1977 and Yancey et al. 1982.

The betaines accumulated in the stressed cells may function as osmoticum to maintain osmotic balance in the cytoplasm.

ii) **Synthesis of Polyamine**

As the name of this class of compounds suggests, polyamines have several amino groups replacing hydrogen usually in alkyl chain e.g., putrescine is 1,4-diamino butane (Fig. 18.7). Polyamines are known to have significant effect on the stability of various conformational states of RNA and DNA and are often associated with important phases in cell division cycle. They occur in microbes, plants and animals. In plants, they have been implicated to help in developing stress tolerance. Cereal leaf segments exposed to osmotic stress or low pH show up to 60 fold rise in putrescine level.

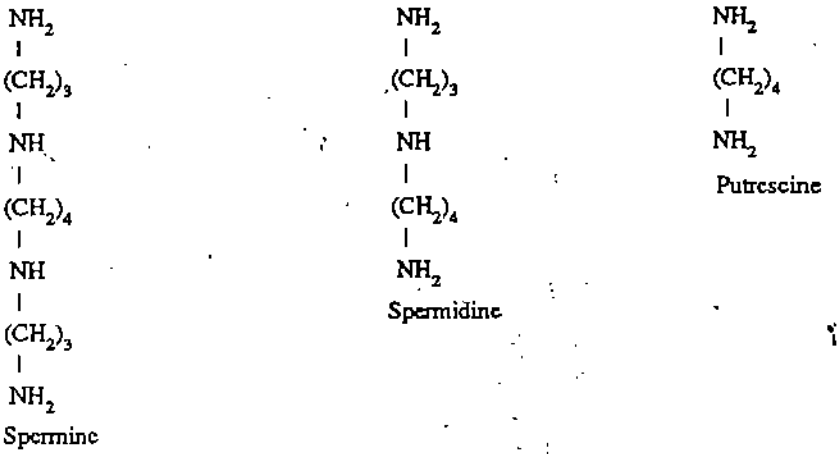



**Fig. 18.7: Molecular structure of common polyamines.**

**4) Response to Heavy Metal Stress**

Several heavy metals emanating from industrial mining and sewage disposal operations contaminate the environment. Cadmium is a common contaminant of this class. It affects human health adversely. It usually retards plant metabolic rates. Yet plants can be selected for their ability to grow on normally toxic concentrations of this metal ion. Low molecular weight proteins capable of binding with metal ions have been found in several plant species. Synthesis of these proteins is induced in response to heavy metal-ions present in the environment. These proteins probably help the plants in accumulating the metals and keeping them away from the sensitive cellular sites.

**5) The Heat-shock Response**

When growing plantlets or tissues of plants are shifted to 42°C and above, the synthesis of normal proteins rapidly declines and instead a set of new proteins appears. Since these proteins are known to be induced by hike in temperature, they are often called **heat-shock proteins (hsp).** These proteins are known to be self-regulatory in that their synthesis is **switched off** after 6 to 8 hours at the elevated temperature while synthesis of the normal proteins resumes. The heat-shock proteins span over a wide range of molecular weight (15 to 102 kd). Several hsps are known to be induced also by heavy metals and arsenites. The hsps are now known to occur in representatives of all the major groups of organisms. Curiously, a pre-treatment at elevated temperature (e.g., 2 hours at 45°C) eliminates the heat-shock response on subsequent exposure to similar conditions. It is believed that heat shock proteins protect essential enzymes and nucleic acids from denaturation.

Induction of synthesis of heat-shock proteins has also been observed under field conditions. In dry fields during summer when the leaf temperature reaches or exceeds the ambient temperature (>40°C), hsp's are synthesised as under experimental conditions. Heat-shock response involves changes in transcriptional as well as translational control. The pre-existing transcripts for normal proteins remain intact for sometime while protein synthesis ceases.

How do hsp's help in heat-shock avoidance? They probably help important cellular proteins to acquire conformations that would be safe and functional under high temperature and the protein will remain in soluble state in the cytoplasm.

### 6) Response to Cold

Let us first see what happen to a plant when it is exposed to a cold climate with temperatures below 0°C. If the temperature is rapidly brought down e.g., >4°C per minute, the extracellular as well as intracellular fluids may freeze. However, if cooling is gradual (<1°C per min) at one stage the extracellular fluids will freeze and insulate the cell's interior. You can guess that intracellular freezing would cause more severe injury to the cell than freezing of the extracellular fluids. However, cooling beyond certain limit can cause fatal injury to the cells. When extracellular water freezes, the intracellular water of hydration moves out and because of the larger space occupied by ice, it squeezes the cell from all around. Thus, freezing can cause the following kinds of stresses.

Supercooling — It is cooling of a liquid below its freezing point without separation of the solid matter. It is possible in those liquids in which the forces of attraction between molecules or atoms are so strong that cooling does not arrange them into regular pattern needed for crystallisation.

i) **Lowering of temperature** to suboptimum thus reducing the activity of most of the common cellular proteins.

ii) **Dehydration** caused by movement of intracellular water to intercellular space,

iii) **Osmotic imbalance** resulting from such movement of water, and

iv) **Physical stress** caused by ice growth and cell contraction.

In connection with cold response we would like to introduce two terms—cold hardiness and cold acclimation.

i) **Cold-hardiness** refers to the ability of a plant tissue to survive ice formation.

ii) **Cold-acclimation** refers to the gradual changes in a plant from a freezing-sensitive to a freezing-resistant state upon exposure to cold conditions.

Remarkably, the cellular fluid rarely freezes at 0°C. It can be supercooled to varying degrees below 0°C without ice formation. Certain compounds are capable of inducing ice crystal formation in water at or below 0°C. Certain bacteria inhabiting the leaf surface of plants in cold climates produce proteins called ice nucleation proteins capable of initiating ice formation. Presence of these proteins on plant surface leads to freezing of the external water before the intracellular freezing. This may partially protect the plant cells by insulating them from more severe injury.

There is a significant correlation between cold-hardiness of plants and percentage of unfrozen water at lethal temperatures. Cold-hardiness in potato is a tolerance against extracellular freezing. Probably cold-hardy plants have proteins that are more stable to freezing than others which are not as capable of withstanding freezing.

Adaptation to cold conditions (cold acclimation) is also influenced by photoperiod, light intensity and quality, water-availability, and nutritional status of the environment. Potatoes can acclimate to cold conditions by exposure to constant day/night low temperature or by stepwise lowering of temperature. Cold-acclimation involves alteration in the plasma membrane, an increase in soluble proteins, and a transient increase in free abscisic acid content. There is also synthesis of **Cold-Acclimation Proteins** (CAPs). These proteins protect chloroplast membranes from freezing damages.

### 7) Response to Flooding

Prolonged flooding or water-logging creates anaerobic conditions for the sub-surface parts of the plant and synthesis of alcohol dehydrogenase (ADH) helps the plant to generate requisite energy anaerobically (Refer Unit 12, Cell Biology Course).

Anaerobic environment induces synthesis of a set of new proteins analogous to the heat-shock proteins. These proteins are, however, different from hsp's. As with the heat-shock system, anaerobic response represses the synthesis of pre-existing types of proteins and induces the synthesis of the **Anaerobic Response Proteins** (ANP's). Five of the ANP's are enzymes of the glycolytic pathway.

Repression — lowering of synthesis of a certain protein by some specific factor.

### 8) Responses to Infection

#### i) Induction of hydrolases

Plants do respond to attack by pathogens. To restrict their spread the cells synthesise and secrete enzymes to hydrolyse the cell wall material of the pathogen. Chitin and $\beta$-1,3 glucans are important constituents of cell walls of many pathogenic fungi. Therefore, it is pertinent that plants have enzymes for degrading them. On microbial

infection the enzymes chitinase and $\beta$-1,3 glucanase are induced for degrading the polymers. Lysozymes are also synthesised in response to infection.

**ii) Ethylene production**

Ethylene is also induced in response to infection. It is also known to be produced under other stress conditions like wounding and application of herbicides.

**iii) Induction of antimicrobial substances**

Phytoalexins are antimicrobial substances produced by plants in response to pathogen. They accumulate at the site of infection. For example, when soya bean (*Glycine max*) is infected by the fungus *Phytophthora megasperma* the chemical phytoalexin glyceollin is accumulated in the plant. Glucan extracted from fungal cell wall can also induce the synthesis of glyceollin. Such a substance which can cause a response in target cells similar to that caused by pathogen is called an **elicitor.** If phytoalexin can be produced in plants by artificial gene transfer, it would probably be possible to have disease resistant crops.

**iv) Production of substances interfering with virus proliferation**

In some cases plants are known to synthesise chemicals that inhibit crucial functions of pathogens. This makes plants immune to infection by respective pathogens. Let us consider what happens when cowpea mosaic virus infects a plant. Certain cultivars of cowpea produce a strong inhibitor of a viral proteinase activity. This proteinase is required to complete the life cycle of the virus as seen in the extracts of the protoplasts of these plants. These cultivars behave as immune against cowpea mosaic virus.

Resistance of many plants to viruses is a passive preformed type e.g., mechanical barriers, lack of compatible infection sites or metabolites required for virus replication. Yet a definite part of resistance is due to active plant response. This includes new synthesis of barriers against infection and spread of virus, and also of new substances interfering with virus replication.

**v) Induction of disease resistance in plants by heat-shock**

The fungus *Cladosporium cucumerinum* infects cucumber and causes disease in the plant. However, if seedling of cucumber are immersed in water at 50° C for 40 seconds prior to experimental inoculation with the fungus, the disease symptoms are reduced by 60%. Resistance develops in 24 to 30 hours after the hot water treatment and remains effective for at least 2 days. One of the earliest observable effects of this treatment is a rise in ethylene production. Soluble peroxidase enzyme associated with the cucumber cell wall also increases in close correlation with the increase in resistance.

**9) Response to Wounding**

**Induction of Synthesis of Inhibitors of Hydrolytic Enzymes**

Wounding of tomato plants by mechanical injury or chewing by insects releases a factor that triggers accumulation of two proteinase inhibitors throughout the plant body. These are inhibitors of serine endopeptidase. Pectic oligomeric fragments derived from cell wall can also induce proteinase inhibitor i.e., they act as elicitors. In fact, cell wall fragments are thought to be generalised elicitors of plant defense responses including phytoalexin synthesis in different plants. The pattern of induction of proteinase inhibitor in these fragments in excised plant tissue is quite like that of mechanical injury.

**10) Protection of Plants against Toxic Substances**

Plants produce several toxic substances themselves and may also encounter the ones produced by other plants or present in the environment. Toxic effects of these substances is prevented by confining them in some part of the plant's organs (e.g., seeds, roots and leaves), tissues (e.g., epidermal layer and the spongy mesophyll), organelles (e.g., vacuoles and chloroplasts) or extracytoplasmic space (cell wall etc.). The enzymes involved in degradation of these substances are also concentrated in these parts. Usually autogenous (self-produced) toxins are stored as their non-toxic derivative. The enzymes that modify self-toxins can also detoxify toxins of foreign origin. Plants have evolved a huge arsenal of enzymes catalysing chemical modification of such toxic compounds. In some cases, a small number of enzymes with each one targeted towards a broad group of chemicals is produced. Both these kinds of enzymes have been found in plants.

**Biochemical warfare : Plant chemicals that mimic animal hormone** — Insects and herbivores have a very big appetite — they eat leaves and other parts of the plants. Certain plants have ways to protect themselves. Some produce toxic chemicals that kill hungry insects, while others can tamper with growth hormones of certain insects and disrupt growth and stages of development by interfering with metamorphosis. Examples of such plants are firs, spruces and yews. Since the insects fail to become adults, they do not reproduce and thus their population falls. Scientists are investigating whether these "lookalike" hormones can be used as insecticides for controlling agricultural pests.

Proteinase inhibitor — certain factors produced in plants during stress for inhibiting the hydrolysis of certain proteins.

An endopeptidase — makes a cleavage inside the protein chain instead of cleavage from one end.

Oligomeric fragments — small fragments of a polymer with up to 5 monomer units.

**Plant chemicals that interfere with insect metamorphosis** — one group of chemicals known as precocenes cause the abnormal (precocious) metamorphosis of insects. Precocenes interfere with the secretion of juvenile hormone — a hormone which regulates the metamorphosis in insects. Such an interference produces adultoids which do not reproduce. This once again causes decrease in insect population.

**11) Induction of Defensive Barriers in Plants**

Several polymers deposited near surface of plants form an effective barrier against invasion or stress. Cuticle (which consists of a structural polymer cutin) is the major barrier between plants and their surroundings. It is often embedded in a complex mixture of soluble lipids collectively called wax. Cutin is built by esterification of hydroxy-fatty acids and hydroxy-epoxy fatty acids. Another polymer similarly embedded in wax and forming a barrier is called suberin.

Wounding triggers suberisation of plant organs even if the natural cover of the injured organ is cutinous. Plants exposed to cold conditions also show an increased deposition of aliphatic polymers. Response of plants to mineral deficiency includes change in the level of suberisation and cutinisation of various plant organs.

In Fig. 18.8 below, we have summarised the responses of plants to various types of stress conditions, so that you can comprehend the detailed text matter.

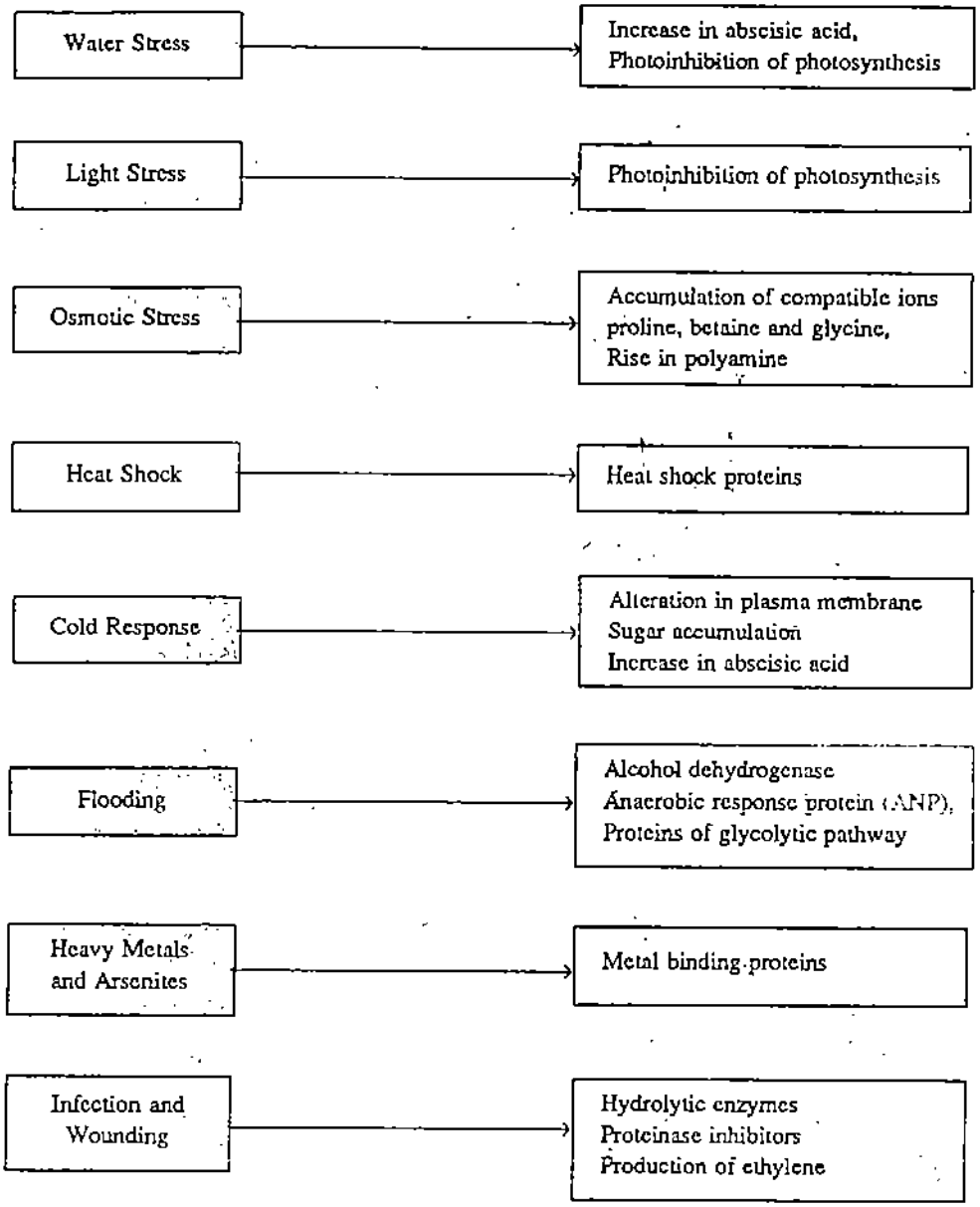



Fig. 18.8: Plants responses to various kinds of stresses

SAQ 3

a) Given below are some events when a tissue is cooled gradually. Arrange the sequence in order in which it occurs.

i) Intracellular fluids move out into intercellular space.
ii) Extracellular fluid freezes.
iii) Extracellular frozen fluid insulates the cell interior.
iv) Physical stress due to the presence of extracellular frozen fluid on the cell all around causing cell contraction.

b) List the changes associated with cold acclimation.

.....

.....

.....

.....

c) Write the type of stress against each of the following responses.

| Plants responses | Type of stresses |
|---|---|
| i) Accumulation of betaine<br>ii) ADH<br>iii) Photoinhibition<br>iv) Chitinase<br>v) Proteinase inhibitor | |

## 18.6 FUTURE PROSPECTS

Plants have evolved a variety of mechanisms to withstand stress conditions. In many cases we can precisely define the way plants enable themselves to survive and perform well under stress conditions. Based on such knowledge, it should be possible to construct and breed plants tolerant to many environmental extremes met in our country and elsewhere on earth while trying to cultivate plants. Fig. 18.4 shows salt affected areas in our country. Such large area can be put to use if salt tolerant varieties can be planted.

One of the ways to manipulate stress tolerant varieties is through genetic engineering technique. With this technique it is possible to isolate a gene and introduce it in a desired organism. This results in the expression of transferred gene in the new organism which then starts behaving like the organism from which this gene was originally isolated. This suggests formation of the gene products related to a particular phenomenon. If this phenomenon consists of one or two proteins it is easy to achieve this objective through genetic engineering technique. Thus, for instance, if genes for betaine synthesis can be transferred to a plant sensitive to osmotic stress, it might confer tolerance against such stress or an elicitor like phytoalexin can be produced by plants by artificial gene transfer it would be possible to have disease resistant plant.

These programmes can be directed towards incorporating the following traits among common crop plants.

1) **Non-photoinhibition** of photosynthesis particularly in the tropics would mean several fold increase in biomass produced over the same priod of time provided water and carbon dioxide supply are non-limiting.

2) **Resistance to high temperature** beyond mesophilic ranges can allow agriculture in several areas that are left uncultivated because of prohibitive temperatures. To a large extent any strategy to achieve this would depend on the water status of the environment.

3) **Cold-hardiness** in cultivars can help curb the losses often incurred because of extremely low temperatures reached during winters in some parts of the world.

4) **Drought resistance** in crop plants will be particularly helpful to our primarily rain-fed agriculture.

5) **Salt tolerance** in plants will bring a lot more of territory under green cover.

6) **Disease resistance** has always been a trait sought after in plants adopted for cultivation. This can improve the present yield by 20 to 50% (depending on the plant under question and the climatic zone).

7) **Pest tolerance** in the crop plants can be achieved by producing proteinase inhibitors or by producing bacterial gene coding for pesticidal protein. This limits their nutritional value but in cases like cotton where our prime interest is fibre instead of food, this can still be a way to achieve upto 50% improvement in yield.

## 18.7 SUMMARY

In this unit, you have learnt that :

- Any deviation in required optimal environmental conditions — light, temperature, water or soil, that would cause detrimental effect(s) on plant growth and development is called stress.
- Stress could be due to variation in environmental conditions viz. i) physical — light, temperature, osmotic strength of the surrounding fluids, ii) chemical — nature of solutes, minerals and pollutant gases and/or iii) biological — pathogens and symbionts.
- Plants adapt to stress by changing the quantity and quality of molecules of the cells. They may adapt alternate suitable metabolic pathway or change their morphology and course of development in order to cope with stress.
- The responses of plants vary according to the nature of stress. They may increase the concentration of certain molecules to keep osmotic balance or synthesise new proteins or enzymes that help in tolerating stress of temperature, light, water-logging, infection etc.
- Studies on plant responses to stress provide useful information. In future, it would be possible to take measures by genetic engineering and other means to prevent losses in yields of crops, fruits, vegetables and other useful products due to stressful environmental conditions. It would also be possible to bring into use chunks of land that at present cannot be used for cultivation.

## 18.8 TERMINAL QUESTIONS

1. The following substances are produced in plants in response to stress conditions. Write the type of stress(es) that induces their production and what functions do these substances perform to cope with the stress.

   i) polyamines, ii) metal-binding proteins, iii) HSPs, iv) ANP's, v) ADH

   .....

   .....

   .....

   .....

   .....

2. Name two elicitors in plants and write the kind of responses they induce.

   .....

   .....

   .....

   .....

3. How do plants respond to toxic substances?

   .....

   .....

4. Why there is photoinhibition of photosynthesis during both water and light stress?

   .....

   .....

   .....

   .....

## 18.9 ANSWERS

**Self-assessment Questions**

1) i) temperature, ii) light, iii) aeration, iv) atmospheric imbalance, v) osmotic

2) i) protein, denaturation, ii) ribulose bisphosphate carboxylase, iii) onset of flowering, iv) abscisic acid

3) a) 3) i, 4) ii, 1) iii), 2) iv

   b) i) Increase in soluble proteins
   ii) Changes in plasma membrane
   iii) Transient increase in abscisic acid
   iv) Sugar accumulation

   c) i) Osmotic stress
   ii) Flooding
   iii) Water stress or light stress
   iv) Infection by fungi
   v) Wounding

**Terminal Questions**

1. i) Polyamines—Rise many folds during osmotic and other kinds of stress. Provide stability to various states of nucleic acid.
   ii) Metal-binding proteins — Bind metal to keep it away from sensitive cellular sites.
   iii) Heat-shock proteins — Help the important cellular proteins to maintain their conformation so that they remain soluble and functional.
   iv) ANPs — Help plant to survive under anaerobic condition.
   v) ADH — Metabolise pyruvate to generate ATP anaerobically.

2. i) Glucan — Induction of synthesis and accumulation of antimicrobial substances phytoalexin glyceollin.
   ii) Pectin fragment — Induction of proteinase inhibitor.

3. i) Store toxins in some part of the plant
   ii) Modify chemically into non-toxic derivatives

4. **Hint :** In both conditions the stomata close.

## GLOSSARY

**Antagonism :** when two compounds produce responses which are opposite to one another.

**Bolt :** to undergo rapid stem elongation, usually resulting in flowering.

**Chelating molecule :** organic molecules that bind metal-ions in ring form, preventing the metal from precipitating as an insoluble salt.

**Circadian :** of or pertaining to events such as biological activities (for example, leaf movement) that repeat themselves approximately every 24 hours.

**Derepression :** removal of a repressor from the gene facilitating transcription.

**Glycophytes :** plants sensitive to relatively high salt concentration.

**Halophytes :** plants which are able to grow in the presence of high salt concentration.

**Phenylalanine ammonia lyase :** the enzyme that catalyses splitting of ammonia from phenylalanine to form cinnamic acid.

**Polyols :** compounds containing many hydroxy groups.

**Repression :** binding of some factor (generally a protein) to a regulatory sequence adjacent to a gene and blocking of the transcription process.

**Synergism :** (means working together) the combined effect of two chemicals is greater than the sum of the effects of two components taken individually.

**Synergism :** (means working together) the combined effect of two chemicals is greater than the sum of the effects of two components taken individually.

**Terpenoids :** a diverse group of compounds with a common structural five carbon unit (isoprene unit – head – $CH_2 - C(CH_3) = CH - CH_2$ – tail). Diterpenoids means compounds with two units and sesquiterpenoids one and a half unit.

**Thermophile :** organisms that survive at very high temperature (80°C). For example bacteria living in sulphur spring.

## FURTHER READING


1) Noggle, G. Ray and Fritz, George J. *Plant Physiology,* 2nd edition, 1989 Prentice-Hall of India Pvt. Ltd., New Delhi.

2) Devlin, Robert M. and Witham Francis H. *Plant Physiology,* 4th edition, 1986, CBS Publishers, Delhi.

3) Salisbury, Frank B. and Ross, Cleon W. *Plant Physiology,* 4th edition, 1989, CBS Publishers, Delhi.

Dear Student,

While studying these units you may have found certain portions of the text difficult to comprehend. We wish to know your difficulties and suggestions, in order to improve the course. Therefore, we request you to fill and send us the following questionnaire, which pertains to this block.

## QUESTIONNAIRE

LSE-05
Block-4

Enrolment No.

1) How may hours did you need for studying the units?

| Unit Number | | | | | |
|---|---|---|---|---|---|
| No. of Hours | | | | | |

2) How many hours (approximately) did you take to do the assignments pertaining to this block?

| Assignment Number | | |
|---|---|---|
| No. of Hours | | |

3) In the following table we have listed 4 kinds of difficulties that we thought you might have come across. Kindly tick (√) the type of difficulty and give the relevant page number in the appropriate columns.

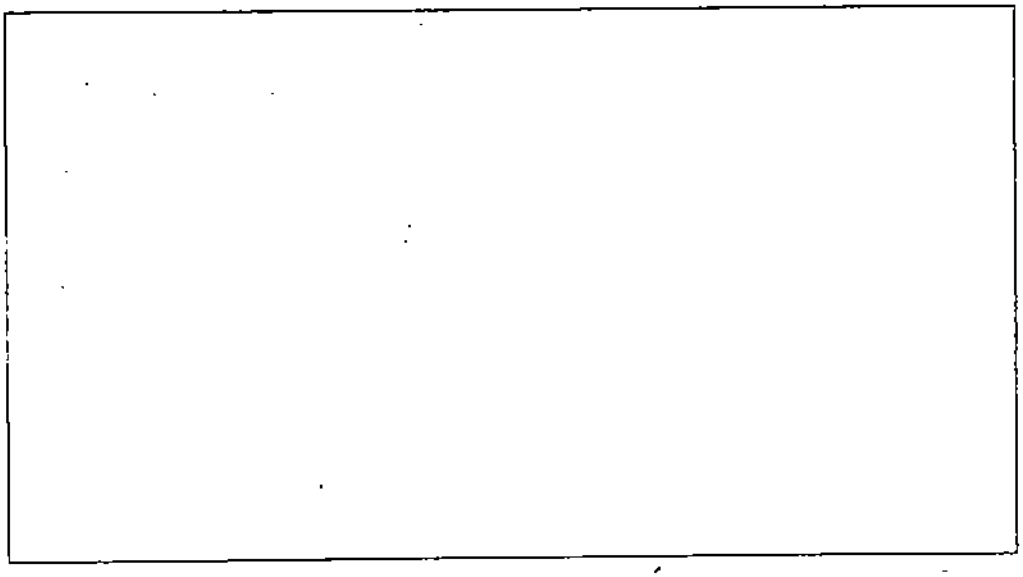
| Page Number | Types of difficulties | | | |
|---|---|---|---|---|
| | Presentation is not clear | Language is difficult | Diagram is not clear | Terms are not explained |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

4) It is possible that you could not attempt some SAQs and TQs.
In the following table are listed the possible difficulties. Kindly tick (√) the type of difficulty and the relevant unit and question numbers in the appropriate columns.

| Unit No. | SAQ No. | TQ No. | Type of difficulty | | | |
|---|---|---|---|---|---|---|
| | | | Not clearly posed | Cannot answer on basis of information given | Answer given (at end of Unit) not clear | Answer given is not sufficient |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |

5) Were all the difficult terms included in the glossary? If not, please list in the space given below.

6) Any other suggestion(s):



To,

The Course Coordinator (LSE-05, Physiology)
School of Sciences
Indira Gandhi National Open University
Maidan Garhi
New Delhi-110068