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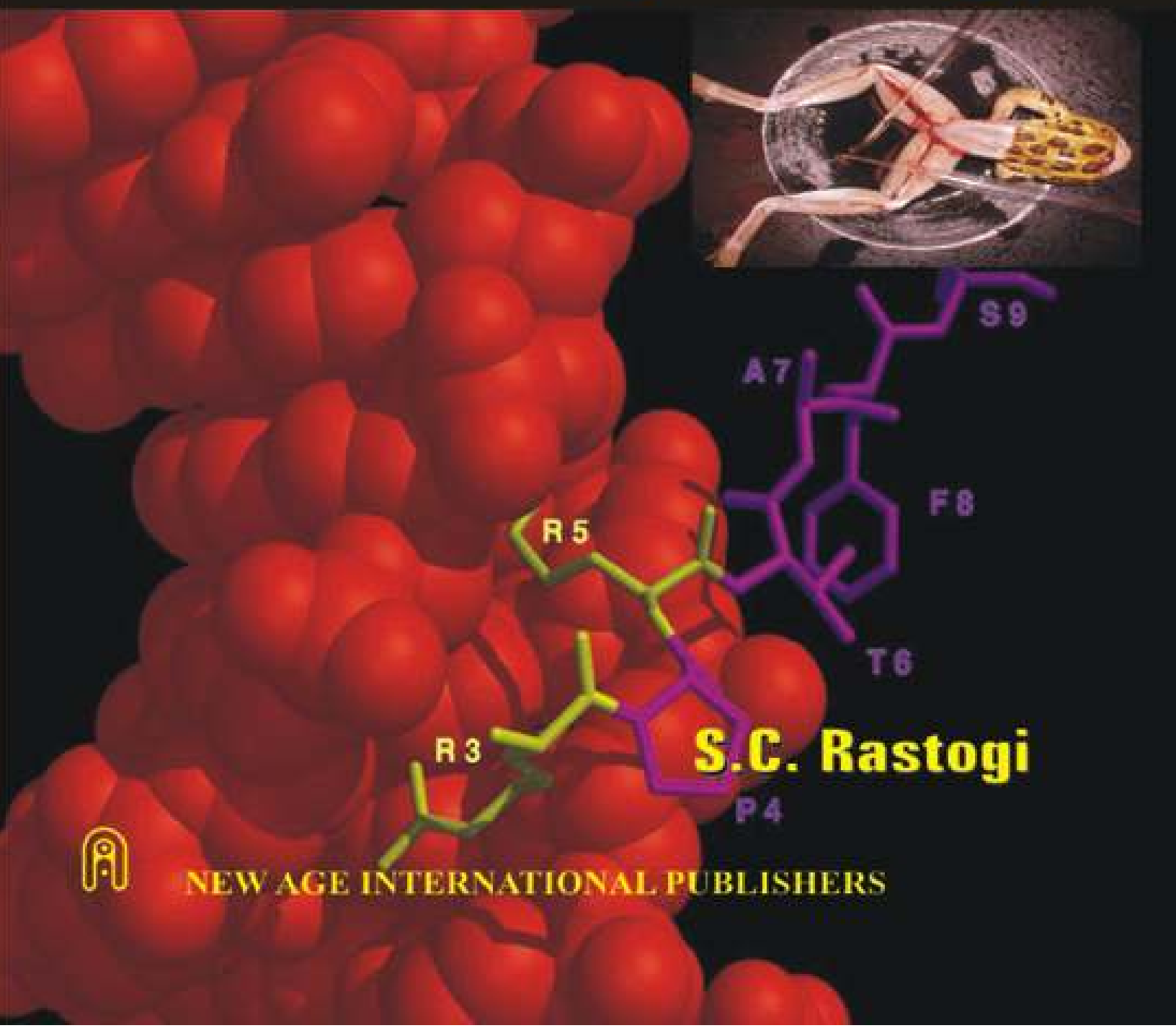
Essentials of Animal Physiology



S.C. Rastogi

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Animal Physiology

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Essentials of
Animal Physiology

(Fourth Edition)

S.C. Rastogi
Formerly Professor of Biological Sciences
B.I.T.S., Pilani

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Preface to the Fourth Edition

The *Essentials of Animal Physiology* has established itself with the academia and served (a) as a text for courses in animal physiology for B.Sc. (Hons.) and B.Sc.(Pass) courses, and (b) as a sound basis for laboratory investigations to analyse animal functions.

Physiology is a synthetic and experimental science which applies physical and chemical methods in biology. It requires a combination of field and laboratory observations of organisms, since their life is influenced by a variety of environmental factors. This fourth edition, wholly reset in its new format, has provided an opportunity for detailed scrutiny and extensive revision. However, the principles of physiology stated in the earlier editions remain sound.

The revision has been impacted by two considerations: these are updating the existing text and adding exciting developments in the field to enhance the utility of the book for an enlarged readership. Consequently, this edition contains new chapters on animal calorimetry, membrane physiology and physiological disturbances emanating from organellar malfunctions and genetic disorders. Besides, certain sections of metabolism and physiology of digestion have been revised to provide new insights. It must be appreciated that physiology offers rational basis for much of medicine, home science and animal husbandry.

It must be emphasised that an effective way of administering a physiology course is to simultaneously plan laboratory exercises to unravel the exciting physiological phenomena. For this the reader is advised to refer "Experimental Physiology" (New Age Publishers), by the same author.

This edition has been reinforced by providing more multiple choice questions for self-assessment. I hope the book will be more appealing to students and instructors in terms of contents and presentation.

S.C. Rastogi

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Preface to the First Edition

Every year, during one semester, I am engaged in the teaching of physiology to senior students. I have often felt the difficulty to cover all areas of physiology owing to deficiency in the background knowledge of students. With the result certain fundamental topics are left uncovered or inadequately treated. In addition, the subject of physiology has recently grown so rapidly that it is impossible for the average student to tread the vast field. Therefore, I felt the necessity of writing this book with the hope that it would cater to the needs of both the categories of students—those who want to study physiology in its essentials, and also those who wish to acquaint themselves with the major areas and latest developments in the field.

While writing the book, I realized that with the development of the core curricula of different universities at various levels of instruction, the presentation of the subject should provide all essential aspects related to it. Still, limits had to be imposed on its treatment since the purpose was not to write a comprehensive treatise. In fact, the objective was to initiate the student in the study of the subject and at the same time to prepare a book that would meet the requirements of various syllabi. Human physiology has been surveyed at appropriate places without exhaustive treatment.

The book is divided into 18 chapters which are arranged in a fashion that the reader can develop his ideas step by step. The subject matter gives a comprehensive coverage to such essential areas as the structure of cells and their function, foodstuffs, digestion and absorption, biological oxidations, metabolism, water relations and ionic regulations, temperature regulation, body fluids and their role, circulation of blood, respiration, excretion, nerve physiology, sensory mechanisms, nerve coordination, effector organs, hormonal regulation, reproduction, and physiological genetics. The discussion of each area is intended to provide an understanding of important facts drawn from relatively new and up-to-date sources that will stimulate students' interest. The book can be profitably used by them whether they are specializing in areas of zoology, veterinary or human medicine, or nutrition.

Perhaps it is not customary to begin a book on animal physiology with a chapter on cell structure and function as has been done in the present case. The cell forms the basic unit of life and all physico-

chemical and vital life functions were first discovered at the cell level and later extended to the organismic level. I consider it difficult, if not impossible, to understand the functioning of the whole organism without a good knowledge of the fundamental processes at the cellular level. One way of trying to understand a complex system is to formulate a model that exhibits the same properties as are found in the entire organism—that model being the cell. Keeping this in mind I have decided to include this chapter which, I believe, will enchance the character of the book in its broad-based bias. The chapter on foodstuffs is comprehensive and highlights chemical details to emphasize the important point, viz. the various types of food eaten by animals are used as fuels for the generation of energy explainable in chemical terms. Chemical details are necessary to explain their functional significance. The types of food and their chemical composition should be an important piece of information to the students to enable them to know as to how animals obtain their energy requirements from the complex foodstuffs. A chapter on biological oxidations has been included. Biology students have a tendency to ignore this area which is very much a part of physiology essential to the strengthening of the basic concepts. It was thought that the initial approach to physiology must be to analyse physiological processes in terms of chemical reactions from the point of view of energetics.

Relevant biochemical details are given to the extent they are necessary. The aim was to explain rather than to describe principles of animal physiology, and therefore, in some parts I have leaned on biochemistry to achieve this end. The pertinence of many areas will be quite obvious. At appropriate places, experimental details have been given in support of the factual statements and hypotheses. The bibliography will be helpful to an alert student interested in more details about the subject.

In a work like this, it is impossible to accomplish the task without the encouragement and invaluable help of many. I, therefore, wish to thank Dr. C.R. Mitra, Director of the Institute; Professor V. Krishnamurty and T.S.K.V. Iyer for the much needed encouragement through the preparation of the book. A text of this type would not be possible without the aid of specialists in the field. Accordingly I wish to thank Professors H.S. Chaudhury (Gorakhpur University), R. Nagbhushanam (Marathwada University) and V.P. Agarwal (D.A.V. College, Muzaffarnagar, Meerut University) who served as members of the University Grants Commission editorial committee, and the reviewer of the National Book Trust. They have read the entire manuscript with meticulous care and offered valuable comments and excellent suggestions about the subject matter. On the basis of the reviewers' comments, substantial additions have been made in the textual matter resulting in rewriting a large part of the manuscript. With the result, the first draft has been thoroughly revised and enlarged. Although I have gratefully adopted many of their suggestions, yet I have sometimes preferred my own viewpoint as well.

I appreciate Dr. H.L. Kundu's sincere cooperation which I have always enjoyed in abundance. I also acknowledge the assistance extended by Dr. M. Ramakrishna, who was associated with the project for some time, in writing Chapters 1 to 3.

Most particularly, I am grateful to the University Grants Commission for financial assistance under its Book Writing Project sanctioned to me for the duration January 1973 to August 1975, without which this book could not have taken shape. However, Chapters 16 to 18 were written after the termination of the project. My sincere thanks are also due to Dr. V.N. Sharma for his valuable help

in the preparation of bibliography, index and some diagrams included in the text. I am grateful to him for his constructive criticism of Chapters 17 to 18. My special thanks are due to Professor S.C. Shukla for his advice on specific points while checking some sections of the manuscript.

Pilani **S.C. Rastogi**
December 1, 1976

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Contents

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CHAPTER

1 Cell Structure and Function

Every multicellular organism is composed of cells, which are the basic units of life. The cell can be likened to a factory. A factory has several machines which are linked to one another in specific order and each one makes a particular component. By sequential operations, the components from these machines are, assembled to produce the desired products. Similarly the cellular constituents, each with their specific function, have a definite arrangement. They produce the components, in this case molecules, which are assembled to synthesize the required products (macromolecules). The function of the organism as a whole is the result of the combination of activities and interactions of the cell units in its body. Hence to understand the essential physiology of animals we need to know the physiological functions of the cell—the cell which is a fundamental unit of life.

The living cell performs all the functions of life such as intake of nutrients, metabolism, growth, reproduction, etc. To perform these life activities the cell has in it various cellular constituents or organelles.

Our knowledge of the structure and function of the cellular constituents has greatly increased with the development of electron microscope by Knoll and Ruska in 1933 and the centrifuge by Swedberg in 1924.

Electron microscope became available in 1940. It paved the way for a more specific knowledge of the cell structure and the structure of organelles within the cell. It permits magnifications of 1,000,000 times or more, i.e. down to the molecular dimensions. It has revealed the strict and orderly patterns of arrangement of macromolecules constituting the organelles of the cell. Hence with the electron microscope it is possible to observe the structural pattern of organelles, but the function of organelles and of their constituent chemical components could be observed by other instruments and techniques. The Swedberg centrifuge which gives quantitative data on sedimentation rates has, to some extent, helped their observation in the above mentioned aspect. The ultra-centrifuges which are now available whirl at 65,000 rpm and produce a centrifugal force which is 425,000 times that of gravity. With the help of ultracentrifuge the constituent parts of the cells from macerated tissue can be

separated into layers depending upon their weights. Under the microscope, these layers can then be identified with the constituent parts and studied for their activities.

The electron microscope and the ultracentrifuge have helped in the merger of cytology and biochemistry, and solved many physiological intricacies of the cell.

1.1 GENERAL STRUCTURE OF CELL

The various cellular organelles as seen in electron microscope are incorporated in Fig. 1.1 to give a comprehensive view of their arrangement in the cell. The detailed structure and function of each organelle is dealt with under separate headings.

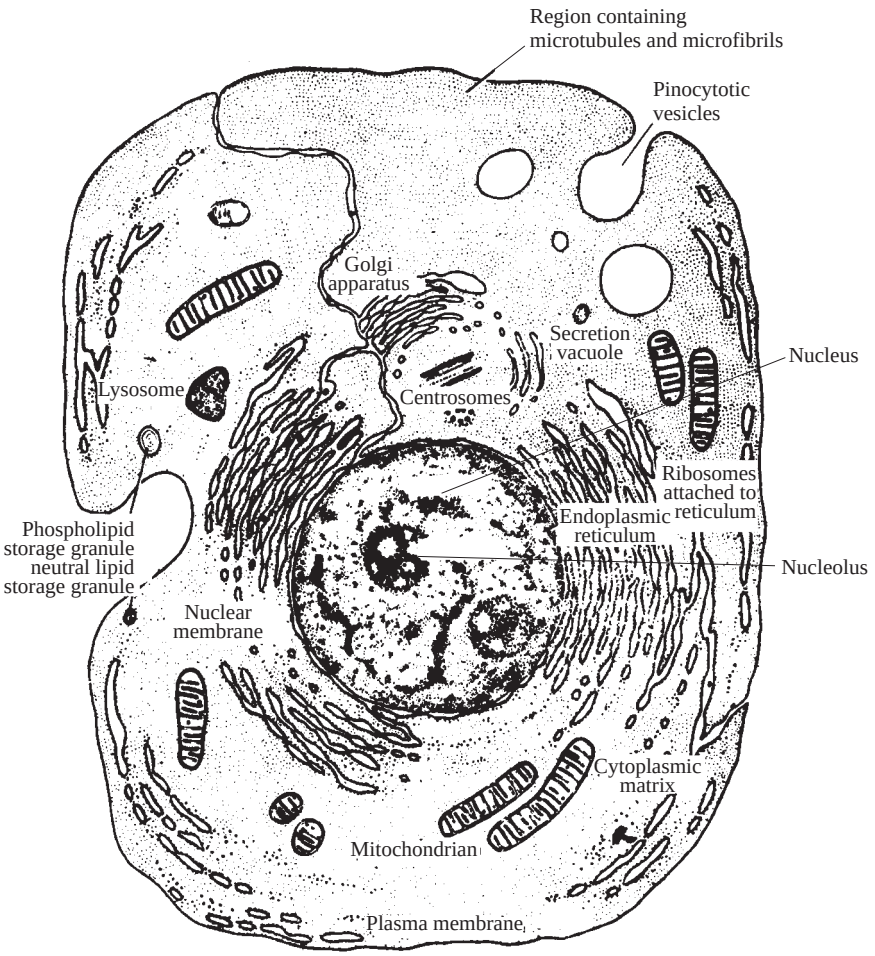


Fig. 1.1 Generalized structure of a cell (adapted from J. Brachet. Sci. Amer. 205:3 (1961)).

The cell contains cytoplasm which is an active fluid medium that helps carry out its life activities. The cytoplasm is a colloidal solution mostly containing water. About 30 per cent of the total mass of this solution consists of various substances. Of these substances, about 60 per cent are proteins, and the remainder consists of carbohydrates, lipids, other organic substances, and inorganic materials. The cytoplasm is enveloped by a membrane known as plasma membrane. The plasma membrane is often termed as cytoplasmic membrane.

Cytoplasmic matrix is a ground substance and usually it is polyphasic in nature. Some authors refer to this matrix as groundplasm. It is the internal environment of the cell. Suspended in the cytoplasm, i.e. matrix, are the various organelles and inclusions. The organelles are the living materials and the inclusions are lifeless and often temporary materials. The latter comprise pigment granules, secretory granules, and nutrients, while the former are the endoplasmic reticulum, the mitochondria, the Golgi complex or apparatus, the ribosomes, the lysosomes, the centrioles and the nucleus.

Three decades ago only the cytoplasm and the nucleus were known to be enveloped by membranes. With the advent of electron microscope it was found that the various organelles and inclusions floating in the cytoplasm are also enveloped by membranes and separated from the cytoplasm.

The commonly represented organelles as well as inclusions which are covered by membranes are: the plasma-membrane, rough and smooth endoplasmic reticulum, Golgi apparatus, lysosomes, mitochondria, nuclear envelope, centrioles, phagosomes, pinocytic vesicles, etc. There does not exist a typical cell in any tissue that is represented by a set of all these organelles. Based on the functional requirements, the cells in various tissues have one or the other of these organelles, i.e. the endoplasmic reticulum is dense in cells of pancreas; the lysosomes are well developed in macrophages; pinocytic vesicles are common in liver cells; Golgi vesicles are conspicuous in storage and secretory tissues; and mitochondria are numerous in the cells of all tissues which expend high energy.

While many cellular organelles consists of a single unit membrane (vide page 6) certain organelles, such as mitochondria and nuclear envelope, have two such membranes, one enveloped by the other. The cellular membranes of the cell help regulate the passage of substances through them and such a passage may be by passive diffusion, or by active transport involving the aid of enzymes (see Chapters 3 and 7) which are located in the membranes. Another important function of the membrane is to provide a surface for harbouring the enzymes.

Nucleus is the most conspicuous structure in a cell. Usually each cell has one nucleus, but cells, such as liver cells, and skeletal muscle cells contain more than one. The fluid matrix of the nucleus is known as karyoplasm or nucleoplasm. It is enveloped by a double layered nuclear membrane. The karyoplasm has densely staining particle called the nucleolus. It is large in growing cells and disappears during cell division. Sometimes the nucleus may have more than one nucleolus. The karyoplasm is not greatly different from the physical and chemical properties of cytoplasm. The most important content of karyoplasm is the chromatin, which is a combination of protein and deoxyribonucleic acid (DNA). It is granular in nature but during cell division it is transformed to long strands called *chromosomes*.

1.2 PLASMA MEMBRANE

There always exists a state of imbalance in the concentration of ions and molecules between the cell and its environment. This difference is maintained by the plasma membrane which is the limiting layer of the cell. In order to maintain this dynamic relationship, nutrients must flow in and reaction products from the cell must flow out through the plasma membrane constantly but in a controlled manner. Once inside the cell the nutrients, i.e. carbohydrates, proteins, lipids, minerals, and vitamins can participate in the metabolic processes. How is this dynamic relation maintained? To answer this we need to know the molecular architecture of the membrane. Such an architecture was conjectured long before the availability of electron microscope. However, it is clear that the performance of plasma membrane is influenced by three factors; one of them is its own capability, second is the supporting cellular activity, third is degree of stress by the environment upon the membrane. The first point, i.e. plasma membrane's own capability in transporting substances, can best be understood by studying its structure.

Structure

Plasma membrane is essential for the life of the cell. It is a bio-membrane that lies close to the cytoplasm. The structure of all biological membranes was deduced from the knowledge of their functional role. All biological membranes have many properties in common. This led to the assumption that they all have the same basic molecular structure. Since lipophilic substances preferably permeate through the membrane, it was conceived that the cell has a lipid covering. But how are the lipid molecules arranged in the membrane? For this an understanding of the behaviour of fatty acid molecules with water medium is required because the lipid molecules in the bio-membrane behave much in the same way. Each fatty acid molecule contains a hydrophilic carboxyl group known as polar head, and a hydrophobic hydrocarbon chain called nonpolar tail. The carboxylic group of the fatty acid is the charged end. This group dissociates forming hydrogen bonds when it comes in contact with water. Thus at the water face several fatty acid molecules arrange themselves in a single layer with their hydrophilic polar heads in contact with water and the hydrophobic hydrocarbon chains away from water surface (Fig. 1.2). Fatty acid molecules would be arranged as double layers in apertures separating two water compartments. In this case the hydrocarbon, chains of the two molecular layers being hydrophobic, extend inwards forming a hydrocarbon phase, whereas the polar heads being hydrophilic lie in contact with aqueous medium (Fig. 1.2).

The cell has aqueous medium inside as well as outside. Hence the lipids in the plasma membrane are arranged in two layers, each layer being one molecule thick. The inner layer with polar heads facing the cell, the outer layer with polar heads facing away from the cell, and the hydrocarbon chains of both the layers facing each other in the same way as in Fig. 1.2. Thus the polar heads of inner and outer layers are in contact with intra-cellular and extra-cellular aqueous media respectively. Further proof as to the bimolecular nature of lipids was provided by the measurements of the amount of lipid present in the cell membranes of red blood cells. The measurements suggested that the quantity of the lipid present was just sufficient to cover the surface of the cell with a bimolecular layer. It has been found that the lipid portions of the membranes are either phospholipids, cerebrosides, or cholesterol. When lipids are phospholipids, the polar heads have charged phosphates. Measurements of the

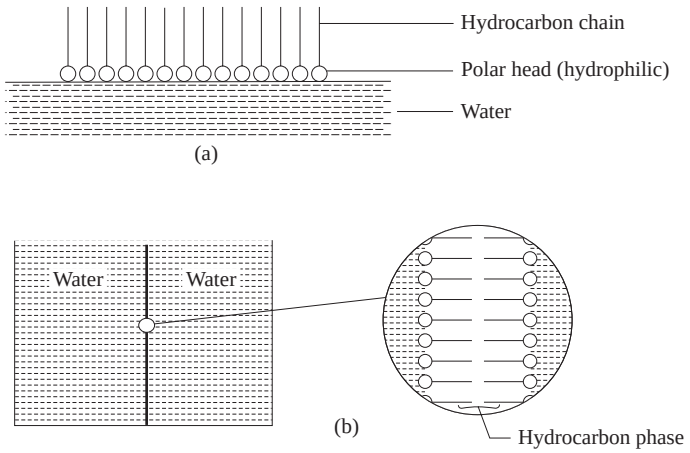


Fig. 1.2 Behaviour of the fatty acid molecules at the water surface.

surface tension of membranes have indicated that it is lower than that of the lipid surface. Such a low surface tension is interpreted to be due to the existence of a protein coating on either side of the lipid bilayer of the cell membrane. Based on the physical properties of the cell membranes, such as preferential permeability to lipid soluble substances, occurrence of low surface tension, and high electrical resistance, Danielli and Davson (1935) deduced the structure of the membranes. They suggested the existence of a continuous layer of lipid molecules with their polar groups directed towards the exterior and interior of the cell; and a coating of a single layer of protein molecules on the polar surfaces; the protein layer consisting of polypeptide chains or meshworks of such chains (Fig. 1.3).

Robertson (1959) suggested the structure of a membrane which nearly corresponds to the one proposed by Danielli and Davson. He called it a unit membrane. However, the structure of this unit membrane was evolved by the studies based on electron microscopy, X-ray diffraction and chemical techniques. According to him the unit membrane has a central core of bimolecular leaflet of lipid on either side by a single layered fully spreadout hydrophilic protein or nonlipid material.

After examining the cell membranes of a variety of tissues from plants and animals, Robertson (1960) postulated the probability of its universal occurrence in animals and plants. The unit membrane may act as a barrier between the cell and its environment, and between the cell-organelles and the cell-matrix. In later studies Robertson observed that the outer and inner protein layers of plasma membranes differ in chemical reactions. This led Robertson to amend the concept of the universality of unit membrane. In such asymetrical plasma membranes he suggested that the layers on one side of the lipid core is made up of protein and the other is made up of carbohydrate perhaps in the form of mucopolysaccharide (Fig. 1.3).

It is well known that the membranes have diverse physiological functions. In accordance to the requirements of organelles, cell and tissues, the membranes select and allow the admission of nutrients. Such diversities in the membranes may be due to: (*a*) the assortment of lipid constituents in

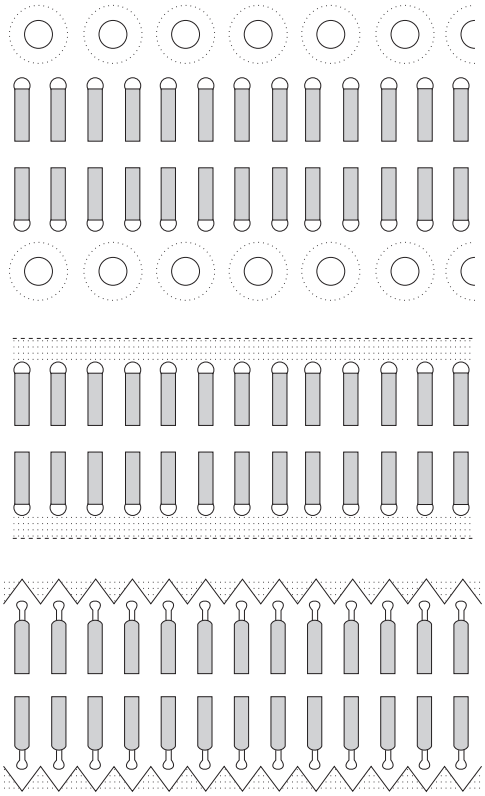


Fig. 1.3 Membrane models proposed by (a) Danielli and Davson (1935); (b) Davson and Danielli (1943); (c) Robertson (1965).

the central core; (*b*) the character of non-lipid monolayer on either side of the lipid bilayer; (*c*) the chemical specificity of certain areas of a continuous membrane.

To explain certain aspects of membrane permeability, Danielli suggested the existence of polar pores lined by protein molecules. According to Solomon (1960) these are not the fixed pores but act as and when required by the intra-and extra-cellular conditions. These conditions cause some pores to open and the rest to close. He supposed that a large part of traffic flows through these pores in the membrane.

The membrane is a barrier to the intra-cellular protein anions whereas it allows water, sodium, potassium, and chloride. Thus the membrane is semipermeable in its nature. As a result of semipermeability of the membrane, chemical and electrical gradients are created (see also Chapter 7).

Fluid-mosaic Model of Membrane

Recently Singer and Nicolson (1972) have proposed a working model which has been widely accepted. Robertson's model envisages a uniform structure of the plasma membrane but according to

the proposed model, in most of the membranes, the lipids are in the form of a fluid bilayer and the proteins do not form a sandwich covering of hydrophilic bilayer lipid covering. The membrane proteins are found to be embedded in the bilayer (Fig. 1.4). The lipids, which are mostly phospholipids and glycolipids in nature, when suspended in water give rise to aggregates of many forms and shapes by forming micelles. These aggregates still preserve the hydrophilic and hydrophobic characteristics of the phospholipids, but the hydrophobic regions are internally arranged in such a way so that water is expelled out of them, while the hydrophilic regions remain in contact with the outer aquatic phase.

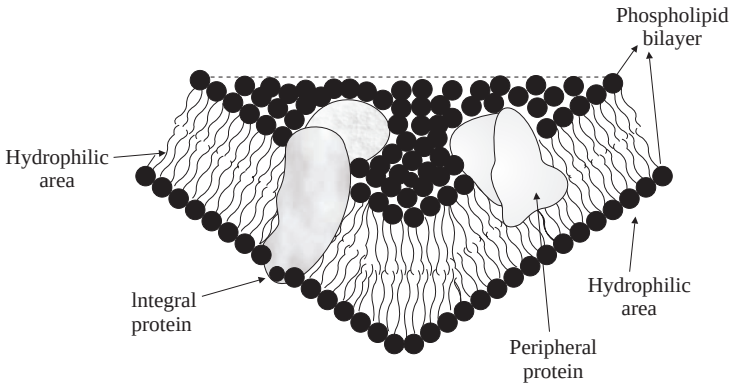


Fig. 1.4 Fluid mosaic model of the plasma membrane as proposed by Singer.

The membrane proteins play a very active role in the structure and functions of the membrane. They are of two types: peripheral (extrinsic) and integral (intrinsic). The peripheral proteins are superficially located and many of these are enzyme proteins. The integral proteins associated with the bilayer of phospholipids penetrate into the interior of the membrane along with the fatty acid side chains. They are tightly bound to the lipids and constitute the functional proteins not easily separable. All membrane bound enzymes, carriers etc. are included in this category. Peripheral proteins have a loose affinity and can be easily displaced. Such a membrane is dynamically more stable and can explain the intricate transport phenomena across the membrane.

Chemical Gradient

The cell has in it, higher concentrations of potassium, protein and related anions whereas outside it has sodium and chloride in higher concentrations. Hence a chemical gradient exists between a region of high concentration and a region of low concentration. Solutes from higher concentration tend to diffuse through the plasma membrane towards low concentration. The movement of potassium ions from the cell to the exterior is said to be passive and down the concentration gradient. The movement of sodium and chloride into the cell is said to be down in the gradient. To maintain the gradients the substances moving passively along the gradients must be counter-balanced by active transport, which restores the extruding potassium ions to the cell and the intruding sodium ions to the environment.

Electrical Gradient

The membranes of all living cells exhibit a difference of electrical potential. The potential difference of most membranes is found to be of the order of 100 mV. Such a potential difference strongly influences the movement of charged materials, particularly inorganic ions, across the membranes.

Usually the interior of the cell is electrically negative. The chloride, which is negatively charged, is known to exist in high concentration outside the cell. If it diffuses down the concentration gradient into the cell, it would promptly be forced back down the potential gradient.

1.3 ENDOPLASMIC RETICULUM

Endoplasmic reticulum is a membranous system of canals extending from plasma membrane to the nuclear membrane. These canals have the same environment that exists around the cell because they are in direct connection with extracellular medium. In other words, the network of canals provide extracellular environment deep inside the cell and surrounding the nucleus. The advantage of such an environment within the cell is that it provides opportunity for a rapid transfer of substances between extracellular and intracellular environments. The endoplasmic reticulum supplies nutrients to the organelles in the cytoplasmic matric and removes from them the products of synthesis and degradation. In a three dimensional view (Fig. 1.5) the endoplasmic reticulum exhibits cavities of varying sizes and shapes. These appear as vesicles and tubules or as flattened sacs. For laboratory studies, fragmentation of the endoplasmic reticulum is brought about by ultracentrifugation.

Fig. 1.5 The three-dimensional view of the endoplasmic reticulum.

The endoplasmic reticular membrane, like plasma membrane is a unit membrane of the type described by Robertson. The surface layers of two membranes are connected by protein septa. The endoplasmic reticulum exists in all cells of higher animals except in mature erythrocytes. The complexity of reticulum increases with an increase in the degree of protein synthesis activity within the cell. Accordingly, in secretory cells the reticulum is well developed. The absence of both the nucleus and the endoplasmic reticulum is explained to be the reason for the absence of enzymatic synthesis in mature erythrocytes.

The endoplasmic reticulum is subdivided into areas with specialized functions. These areas include the granular or rough endoplasmic reticulum, the agranular or smooth endoplasmic reticulum, the nuclear envelop, and the Golgi apparatus. The functional significance of these various specialized areas is discussed under the title Golgi apparatus.

Granular or Rough Endoplasmic Reticulum

Growing cells as well as those engaged in protein synthesis are rich in granular or rough endosplasmic reticulum. The membrane of this reticulum, all along its outer surface facing the cytoplasmic matrix, is studded with uniform size of particles called ribosomes. High density of ribosomes would mean greater protein synthetic activity.

Agranular or Smooth Endoplasmic Reticulum

The outer membrane of this reticulum is devoid of the ribosomes and hence it is termed agranular or smooth endoplasmic reticulum. It is continuous with rough endoplasmic reticulum and with Golgi apparatus. It is present in cells synthesizing steroids, in voluntary muscle cells and in liver cells.

The Nuclear Envelope

The nuclear membrance is covered over by a large cisternal unit of granular endoplasmic reticulum. At intervals the nuclear and recular membranes join forming pores. These pores are continuous with the cytoplasmic matrix of the endoplasmic reticulum. These pores allow the molecules from the nucleus to the cytoplasmic matrix (Moses, 1964). Some investigators suggest that the pores are covered and open only when traffic is warranted. In protein synthesis, the mRNA, tRNA, and rRNA (as ribosomes) travel from the nucleus to the cytoplasmic matrix. The direct route for such a traffic would be through the nuclear pore. Through these pores the nucleus receives the nutrients from the intracellular environment. The channels of the endoplasmic reticulum act as extracellular environment and extend from the plasma membrane to the nuclear envelope. In other words the nucleus is surrounded by the extracellular medium. Thus the nucleus also receives nutrients direct from the extracellular environment.

Functions

Endoplasmic reticulum carries out specialized functions. These functions are localized in various substructures:

(i) One of the important functions, viz. the transport, is carried out by the channels.

(ii) Protein synthesis is associated with the ribosomes of the granular endoplasmic reticulum.

(iii) Concentrating and packaging of enzymes is localized in the Golgi apparatus.
(iv) Steroid synthesis takes place in the smooth reticulum.
(v) The intracellular stability, movement and the activation of amino acids for protein synthesis, and finally the glycolysis, are localized in the cytoplasmic matrix.

1.4 GOLGI APPARATUS

The Golgi apparatus, endoplasmic reticulum, membrane bound vesicles and lysosomes constitute a part of the membrane system present in the cytoplasm of the cell. The constituents, though always present, exist in a state of constant change—formation, transformation, breaking down, and reformation. They also move within the cytoplasm. The cellular organelles such as, nuclear envelope, the rough and smooth endoplasmic reticulum, the Golgi apparatus, the lysosomes, the pinocytic vesicles, all have membranous covering. These organelles or membrane bound spaces have been connected either by functional continuity or by morphological connection and consequently facilitating transport of substances not only within the cell but also to the exterior, and in some cells from the exterior into vesicles and lysosomes.

In this membrane bound transport system, the Golgi apparatus occupies a position where the nucleus and endoplasmic reticulum are at one end, and the vesicles, lysosomes and plasma membrane at the other end. In this system, proteins, polysaccharides, glycoproteins, and probably lipids and lipoproteins are formed and transported. Nucleus acts as a central control site for transport of substances.

Form of Golgi Apparatus

The form of Golgi apparatus varies from a compact discrete granule or mass to a well dispersed filamentous reticulum. It is pleomorphic and a variation in shape can be observed with the metabolic and developmental state of the cell. It occurs in almost all cells of animals and plants. It is easily recognizable and consists of 3 to 12 disc-shaped cisternae or saccules arranged compactly one above the other like a stack of neatly arranged saucers. The cisternae are slightly curved and for this reason the entire Golgi apparatus appears concave at one surface and convex at the other (Fig. 1.6). The material between the cisternae is known as intercisternal structure. A network of tubules arises from the edge of each cisternae and swell to form various types of vesicles.

Formation of Golgi Apparatus

Golgi apparatus is formed by conversion of the membrane. The development and formation of Golgi apparatus in the cell takes place in a series of processes. These processes are: (1) the synthesis of a pile of cisternae in the absence of pre-existing Golgi apparatus; (2) the alteration in the type and number of vesicles; and (3) the increase in number of piles or stacks, in the number and size of cisternae, and in the number of tubular and vesicular regions of the stack. Another way of formation of individual stacks of cisternae is by fragmentation of the preexisting stacks. Cytological, biochemical and chemical evidences indicate a flow of membrane material from the rough endoplasmic reticulum via the Golgi apparatus to the plasma membrane. This suggests that for the

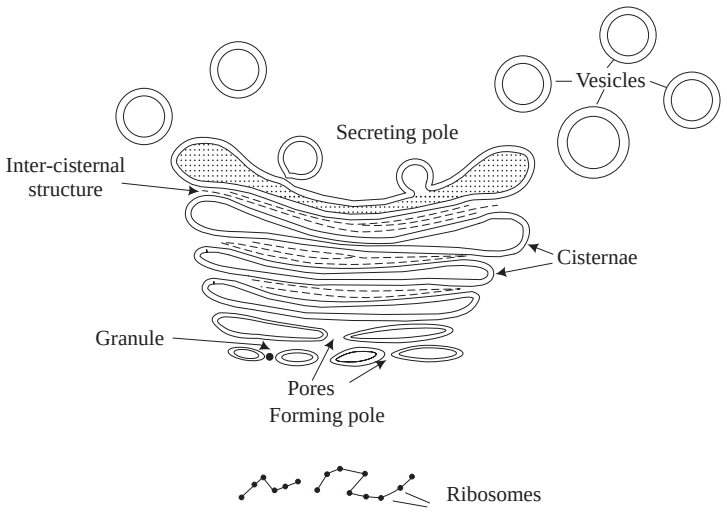


Fig. 1.6 Structure of the Golgi apparatus.

formation of the cisternal membrane, the necessary membrane material come from the rough endoplasmic reticulum. To achieve the formation of cisternal membranes of the Golgi apparatus, it is believed that first the rough endoplasmic reticulum changes to smooth endoplasmic reticulum. Smooth endoplasmic reticulum then becomes the Golgi cisternae and these cisternae break down to form vesicles. The vesicles can fuse with the plasma membrane in order to extend it (Fig. 1.7).

In the absence of nucleus or in the presence of actinomycin D, the Golgi apparatus gradually decreases in size and finally disappears. The renucleation of enucleated amoebae restores the smooth cisternae within half an hour to one hour, and within 6-24 hours the Golgi complexes increase in size and number.

During this time dense material can be observed both in the lumen of endoplasmic reticulum and in the lumen of cisternae which participates in the membrane production.

Autoradiographic studies by G.E. Palade and his co-workers (1964-1967) showed that dense material from the rough endoplasmic reticulum was transferred to proximal cisternae of the Golgi apparatus with the aid of small vesicles. In other words the endoplasmic reticulum is in continuity with Golgi apparatus.

While the vesicles derived from the reticulum fuse constantly forming the proximal cisternae, the distal cisternae of the stack give off vesicles, i.e., Golgi apparatus is conceived as having a newly forming face at one surface and mature secreting face at the other surface.

The membranes of Golgi apparatus have a chemical composition intermediate to that of endoplasmic reticulum and plasma membrane (Keenan and Morre, 1970). The Golgi cisternae occupy an intermediate position (central position) with precise unit membranous structures such as the plasma membrane and the vesicles at the distal or mature face, and with the nuclear envelope and the endoplasmic reticulum membranes at the other extreme. Being thin (25— 40 Å) the latter group of

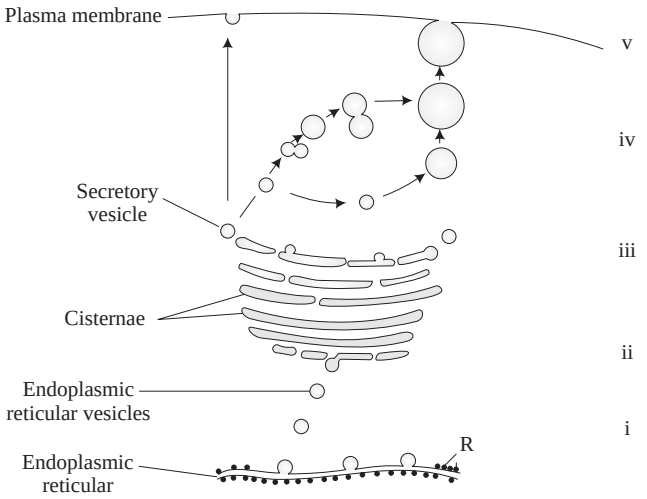


Fig. 1.7 Formation of the Golgi Apparatus

I. Material is transferred from endoplasmic reticulum to the Golgi apparatus. The endoplasmic reticular vesicles fuse to form cisternae at the proximal end of the Golgi apparatus.
II. Cisternal contents and membranes are transferred as the cisterna is displaced distally.
III. Cisternae at the distal pole give rise to secretory vesicles.
IV. Secretory vesicles migrate to the plasma membrane at the apex. Some increase in size while others fuse with other vesicles.
V. Vesicles fuse with the plasma membrane of the apex liberating their contents on to the surface of the cell.

membranes are faintly stained, whereas the former group being thick (75 Å) are brightly stained (Grove, Bracker, and Morre, 1968). The membrane system within the Golgi apparatus exhibits difference, i.e. the cisternal membranes at the forming face are similar to the membranes of endoplasmic reticulum and the nucleus; the membranes at the mature face are similar to plasma membrane; and the membranes between these two faces are intermediate in nature. The membranes in the Golgi apparatus are thus modified. The function of Golgi apparatus is to alter the membranes of endoplasmic reticulum for the formation of plasma membrane.

The following scheme illustrates the relationship of the Golgi apparatus with the rest of the membrane system:

Functions

Golgi apparatus serves as part of an internal transport system of the cell. It carries out secretory and digestive processes. It synthesizes the lipoprotein membranes. The Golgi apparatus is concerned with the formation and packaging of material for export across the plasma membrane. The process of exporting is reverse of pinocytosis. The cell structures such as the acrosome of the maturing spermatids (Fawcett, 1966) and the tubular inclusions of endothelial cells (Sengel and Stoebner, 1970) are few among several examples of secretions derived from the Golgi apparatus. It is now known that there are several materials which are packaged and passed through the Golgi apparatus and its

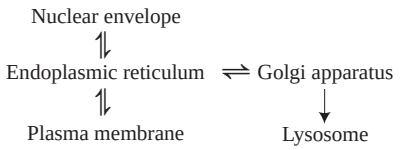


Fig. 1.8 The relationship of Golgi apparatus with other organelles.

associated vesicles. Such materials are mostly polysaccharides or proteins or lipids in the form of glycoproteins or glycolipoproteins, and such a conjugation with carbohydrates is a prerequisite for subsequent transport across the plasma membrane.

Palade and coworkers (1964 and 1967) have shown that the digestive enzymes (e.g. α—amylase) or their precursors (e.g. α—chymotrypsinogen) synthesized on ribosomes at the outer surface of the rough endoplasmic reticulum are passed into the lumen across the reticulum membrane. They are then transferred to the smooth endoplasmic reticulum. Here they are packed into small granules and sent into the forming surface of the Golgi apparatus. Then they are concentrated as zymogen granules into the vesicles which are detached from the cisternae. The granule containing vesicles then migrate to the cell apex, where the material is passed out of the cell by reverse pinocytosis. In this process the membrane of the vesicles fuses with the cell membrane and becomes a part of it.

In case of pancreatic cells, the material thus ejected out of the cell finds its way into the pancreatic duct and eventually into the intestine. Similar events have been observed for the mucous production in the goblet cells of the colon of rats.

1.5 THE LYSOSOME SYSTEM

Present in the cytoplasm are small membrane-bound vesicles containing a group of hydrolytic enzymes. Biochemical studies reveal that all the enzymes in these vesicles show an acid pH optimum. These enzymes serve to destroy certain parts not required by the cell. For this reason de Duve (1955) named the vesicles containing these enzymes as lysosomes. The lysosomes also digest the macromolecules taken in across the plasma membrane. Hence the lysosomes can be described as the digestive system of the cell.

Large numbers of lysosomes are present in white blood cells, macrophages, and in the cells of liver, kidney, thymus and spleen. They were biochemically separated by Novikoff et al. (1956). They observed these as surrounded by a unit membrane containing acid phosphatase. This is a characteristic enzyme of lysosome.

The lysosome is the seat for a minimum of twelve hydrolytic enzymes. All these act in acid pH and split the biological substances such as proteins, nucleic acids, and polysaccharides. The lipoprotein of the unit membrane prevents these enzymes from being harmful to the cell. Inspite of this, the substances can be digested by the action of enzymes if they come to a reasonable distance from the lysosomal membrane. The lysosome may be described as a packet containing enzymes inaccessible to the rest of the cell. However, the enzymes are released within the cell, if the membrane is injured. In such a case the cell would be digested.

Structure

The lysosome is small in size, lacks uniform appearance, and has no characteristic fine structure. It differs not only among the cell types but also within the cell types. Some of the differences have already been known while much awaits investigation. According to de Duve, the lysosome exists in four functional forms, viz. storage granule or protolysosome, phagolysosome or digestive vacuole, autophagic vacuole, and residual body (Fig. 1.9). The following paragraph deals with the formation of these lysosome types and their inter-relationships.

Some of the substances required to nourish the cell may be in the form of large molecules, bacteria, or other cells. Such particulate materials cannot be absorbed through the cell membrane.

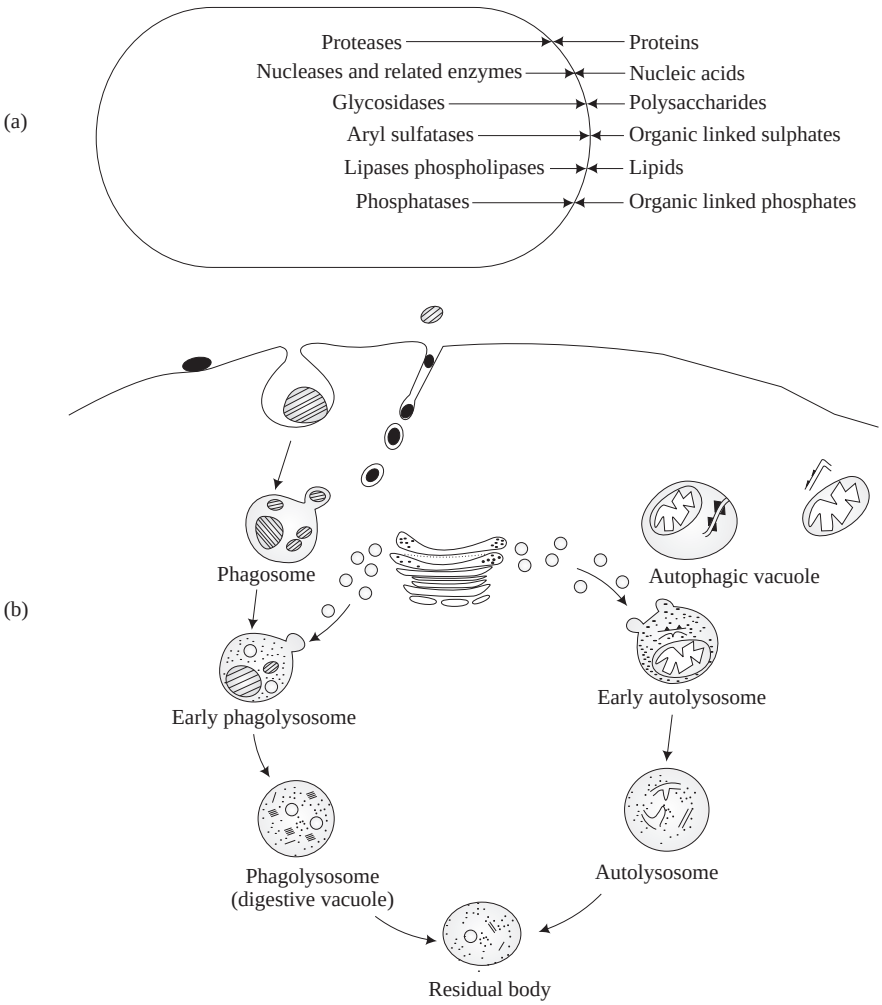


Fig. 1.9 Lysosome: (a) biochemical nature; (b) formation of the lysosomal types.

Therefore, these particles are engulfed by the cell through a process known as endocytosis. In this process first, the required susbtance or particulate material attaches itself to a portion of the cell membrane (Fig. 1.9). Then at the region of attachment, the particle along with the membrane is drawn inward to form a small internal pocket. This pocket is then pinched free from the cell membrane and taken inside the cell. The structure thus formed consists of particulate material surrounded by cell membrane, and this structure is called the phagosome or food vacuole. The autophagic vacuole is formed from intracellular material. The enzymes required for the formation of protolysosomes are synthesized by the ribosomes. These enzymes then find their way into the Golgi apparatus along with the endoplasmic reticular vesicles (Fig. 1.7). The Golgi apparatus releases them packed in the form of protolysosomes. The phagosome and the autophagic vacuole merge with the protolysosomes to form phagolysosome or digestive vacuole and autolysosome respectively. The fourth type, i.e. the residual body contains substances undigested by the lysosome enzymes.

The only one and important characteristic feature of lysosome is the presence of a single unit membrane (Novikoff, 1963). The unit membrane of lysosome serves as the semipermeable membrane. Neither the inner structure nor the lipoprotein membrane of lysosome binds the hydrolases within them. Consequently, they are free to move and escape if lysosomal membrane is injured.

The membrane is unaffected by the highly active hydrolytic enzymes, for the simple reason that none of them is lipase or phospholipase. The lysosome, however, contains enzyme acting on proteins, glycogen, nucleic acids, and mucopolysaccharides. Inspite of the presence of these enzymes, destruction of the cellular organelles does not take place. Only when a substrate comes within the boundaries of the lysosome, the digestive action is initiated.

The membrane, according to de Duve (1963), is responsible for the inactivity of the enzymes. Koeing (1962) suggested that the enzymes are ionically bound to the acidic glycolipids, which makes them inactive. The existence of a fine structure inside the lysosome is yet to be revealed, and when disclosed it would be possible, perhaps, to pinpoint its role in latency.

The stability of the lysosomal membrane is important to carry out its normal physiological activity. There are two agents influencing the stability and disruptivity of the lysosomal membrane. Those favouring membrane stability are termed stabilizers; and those disrupting it are termed labilizers. The agents which disturb cellular pH, osmotic relations, chemical stability, and electrical charge of the membrane are the labilizers and they would interrupt the orderly course and liberate the enzymes into the cell. A balanced proportion of stabilizers and labilizers is maintained to keep the membrane intact. Since the organelles depend on nutrients available in the intracellular environment, it is believed that nutrients have stabilizing and labilizing effect on the lysosomal membrane.

Vitamin A is a labilizer of the lysosomal membrane. It is suggested that the vitamin A penetrates the membrane and causes the expansion of the lipoprotein constituent. Thus expanded membrane is weak to retain the enzymes and hence releases them into the cell. Ultraviolet light and ionizing radiations are the other labilizers of the membrane. The water in the cell absorbs the ionizing radiations and decomposes to give rise to free radicals such as hydroxyl, perhydroxyl radicals. Along with these radicals oxidizing compounds such as hydrogen peroxide and organic peroxides also are formed. The membrane is sensitive to the damage by these free radicals. As a result, the enzymes escape through the damaged membrane. However, the toxic effects by peroxides are counteracted by

the iron containing enzyme catalase which decomposes hydrogen peroxide to water and oxygen. But if the production of free radicals exceeds the capacity of the catalase reaction, the toxicity is inevitable.

Vitamin E is a membrane stabilizer in that it prevents the formation of free radicals due to lipid peroxidation. Deficiency of vitamin E would allow the formation of free radicals which disrupt the lysosomal membrane. Rabbits and chicks deficient in vitamin E are prone to muscular distrophy.

Functions

The enzyme containing lysosome and the substrate containing phagosome merge to perform the digestive process within the enclosed membrane. The worn out, damaged or other unwanted accessory structures in the cell may be disposed by means of an autophagic vacuole. Even the outlived whole organs, such as tadpole tail and Mullerian ducts in male chick embryo, are disposed in this way. During starvation, the digestion of cellular contents by lysosome provides essential nutrients to carry out important functions in the cells.

1.6 MITOCHONDRIA

In order to carry out life processes the organisms are equipped with dependable machinery to perform two basic functions; to reproduce themselves by deoxyribonucleic acid (DNA) and to generate energy by adenosine triphosphate (ATP). DNA is the genetic material responsible for the replication of key substances of life—proteins and nucleic acids. ATP molecule is as important as the DNA and acts as a kind of storage battery. It supplies energy for all processes of life including replication. The enzymes which catalyze the process of glycolysis, and the subcellular particles, namely the chloroplasts (a plant cell organelle) and the mitochondrion are the three systems responsible for generating energy in the form of ATP. Of these, the process of glycolysis which is the breakdown of sugars by enzymes in the absence of free oxygen is the most primitive. In this process only one molecule of ATP is produced for each pair of electrons released. The chloroplast in the green plants, and the mitochondrion in the animal kingdom produce three molecules of ATP for each pair of electrons released. The ATP is the universal intracellular carrier of chemical energy. From the single cell of the protist to each of the myriad cells of the complex organisms, mitochondrion is the site for a series of integrated enzymatically controlled reactions which end in the formation of ATP.

There are other functions which the mitochondrion performs, besides generating energy. One of them is the metabolism. There are two distinct forms of metabolism in the mitochondrion, one form of metabolism involves the oxidation of metabolites for the purpose of energy gain to the cell and the synthetic reactions. The other form of metabolism is involved in the biogenesis of mitochondrion itself. Krebs cycle, fatty acid oxidation sequence, and enzymes responsible for ketone body oxidation and formation are some of the metabolic pathways present within the mitochondrion. Through these cycles, the mitochondrion yields large number of reduced enzymes and hydrogens. The hydrogens are then taken into the electron transport chain.

The mitochondrion has the ability to synthesize and catabolize proteins with the mediation of ribosomal particles. They also participate in ionic regulation in the cell and change the permeability of

its membranes to make new substrate available. Another property of mitochondrion, as observed in some cells, is the production of mitochondria from pre-existing mitochondria by growth and division.

Shape

There is variation in the shape of mitochondria. They may be minute globules, vesicles, straight or curved rods, straight or branched threads, chain of granules, nets and other irregular bodies. However, the major variation is among cell types but not among cells of the same type. Mostly they are sausage shaped, but with altered conditions the mitochondria are observed changing their form, hence pleomorphic.

The number of mitochondria per cell varies depending on the cell type but within a kind of cell the number remains fairly constant. In numbers they vary from 500 in case of some liver cells, to more than 100,000 in case of some single celled organisms.

Location

Mitochondria are situated close to the source from where metabolities enter the cell. They are generally found oriented with their long axes indicating the direction of flow of materials in the cells performing active transport. Mitochondria are found in those areas of the cell which depend on the ATP they contain. In neurons they accumulate at the internodes where the passage of nervous impulse is supposed to take place. In secretory cells they accumulate at the basal regions, and in retina at one end of the rod cells. The mitochondria of some cells can move about freely and passively, while in other cells they are stationary.

Structure

A striking similarity exists in the fundamental structure of mitochondria in all forms from protozoa to primates. In most cases it is a sausage shaped object measuring 15,000 Å units in length and 5,000 Å units in diameter. The complex ultra-structure of the mitochondria as revealed by electron microscope was first described by Palade (1952). It has a two layered wrapping constituting an outer and an inner membrane and in this respect it resembles a thermos bottle (Fig. 1.10). The outer membrane is elastic and at times it may be stretched to 200 times its original dimensions. All over the surface it is covered by stalkless particles. The outer membrane is separated from the inner membrane by a fluid matrix which is structureless. This matrix provides communication between the two membranes and supplies

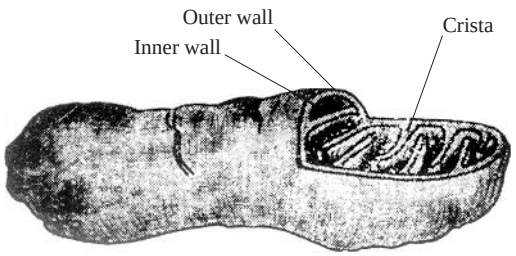


Fig. 1.10 Structure of the mitochondrion.

coenzymes to the enzymes present in the membrane. The inner membrane encloses another matrix which is not in contact with the fluid between the two membranes. The membrane contains some protein and lipid material and as a result may be semirigid. The surface of the inner membrane is much expanded and thrown into numerous transverse folds or cristae which offer an increased surface area to this membrane. While this is the generalized structure, there are mitochondria which do not bear cristae all along their length but are limited to a small part of it. In some they are tubular. The outside surface of the outer membrane and the inside surface of the inner membrane are sprinkled with thousands of smaller particles. These particles are the elementary units which carry out the chemical activities of the mitochondrion. The two membranes are the structural back bones of the mitochondrion and have three properties—good tensile strength, stability, and flexibility. In other words, the mitochondrial membranes are strong enough to hold a structural shape, stable enough to allow membrane phenomena to occur and flexible enough to allow movement.

The fine structure of the mitochondrion is better understood than before, as a result of special staining methods and very highly magnified electron-micrographs. The permanganate fixed mitochondrion revealed the two membranes as separated from each other by a width of about 100 Å. Each mitochondrial membrane closely resembles the membrane of the cell itself. It is about 75 Å thick. It is made up of two materials apart from the particles attached to it. Under the electron microscope each membrane can be seen as a three layered unit. Each of the two protein layers of the membrane is about 20 Å thick. The inner layer is sandwiched by two protein or other nonlipid outer layers (darker layers). The inner layer (lighter layer) is made up of two rows of lipid molecules with their nonpolar ends facing each other (Robertson, 1959) and is 35 Å thick. Thus the two membranes together measure about 250 Å thick.

The principle material is the structural protein which is insoluble in water and accounts for four fifths of the weight of the membrane. The remainder of the material is lipoid, primarily phospholipid.

The protein of the membrane is of two types. Strucutral and the mitochondrial actomyosin. The structural protein is a polymer, composed of basic subunits of a small protein. Half of the amino acid content in these molecules have hydrocarbon side chains which are insoluble in water and form hydrophobic bonds. The mitochondrial actomyocin, in reaction with ATP, is presumed to transform the chemical energy into mechanical energy (Lehninger, 1962).

The belief that the outer surface of the membrane has loosely attached to it thousands of globular particles, is not universal. Logistics suggest, however, that the enzymes responsible for providing "energetic" electrons by carrying out the oxidation reactions such as those of the Krebs and the fatty acid cycles are located in the outer membrane.

The ultrastructure of the inner membrane and its extensions are of great interest. The inner surface of the inner membrane is lined by thousands of particles (Fig. 1.11). These are elementary particles as termed by Fernandez Moran (1962). Each particle has a terminal globular head of about 80 Å in diameter and a stalk of about 50 Å long and 30 Å width. The base with which it is connected to the membrane has the same diameter as does the head piece. These elementary particles are the respiratory assemblies. They are multi-enzyme aggregates and serve in transporting the electrons over a chain of complexes that synthesize ATP. Hence they are conveniently known as electron transport particles. They are composed of a protein cortex with a phospholipid core and thus regarded as enzyme (protein) lipid complexes.

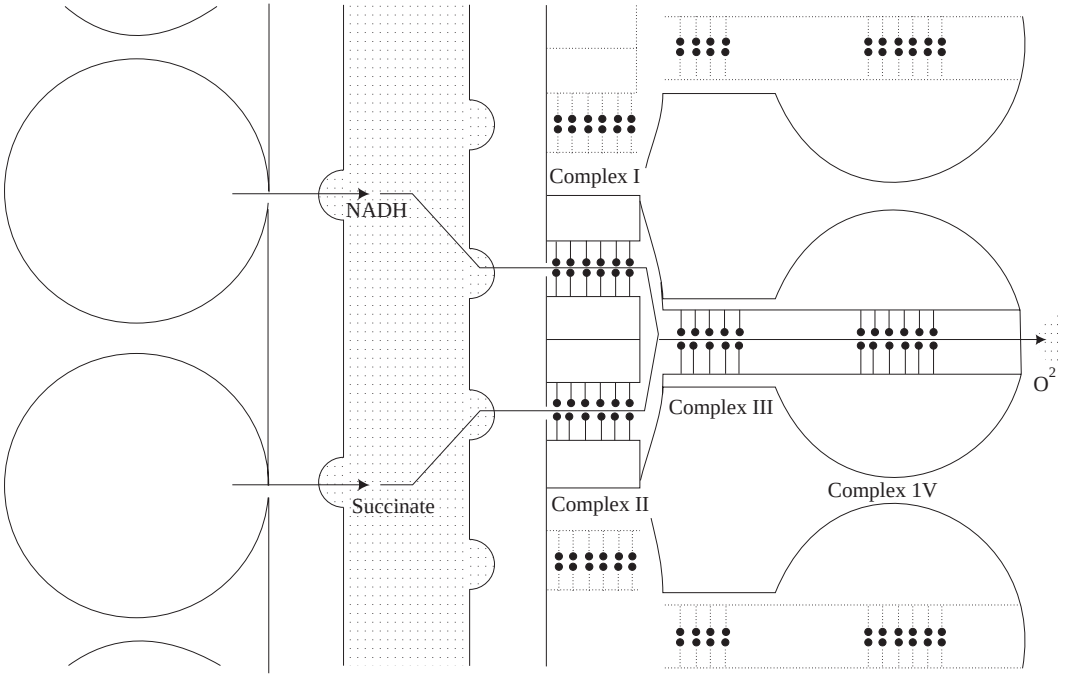


Fig. 1.11 Ultrastructure of the membrane of mitochondrion.

Functions

The carbohydrates, fats, and proteins are oxidised, via tricarboxylic acid (TCA) or Krebs citric acid cycle which is the common pathway in the conversion of the energy from foodstuffs to a form that the cells can use. This usable form of energy, i.e. the currency of the cell, is Adenosine triphosphate (ATP). The energy or ATP producing system is the electron transport system or respiratory chain and it is present in the electron transport particles. The electron transport system transforms the energy of oxidation of TCA cycle substrates to form phosphate bond energy that is needed for the cellular processes.

1.7 CENTRIOLE

Centriole is a minor cell organelle and in light microscopy it appears as a single dot enclosed in a tiny vesicle. It is situated in the region of the nucleus and Golgi apparatus. Electron microscopic studies revealed that it consists of a pair of bundles and each bundle consisting of nine filaments. At mitosis the filaments in the bundle subdivide first, and then the bundles. As a result two pairs of bundles, each pair with nine filaments are formed. Subsequently each pair moves to either side of the nucleus to mark the two poles of the spindle.

1.8 NUCLEUS

The nucleus contains karyoplasm which is covered by a double layered membrane. It has in it a densely stained area called nucleolus, which consists of a rich concentration of RNA and certain proteins. Nucleus also contains chromatin which is a combination of nuclear protein (either protamines or histones) with deoxyribonucleic acid (DNA). During cell division the chromatin takes the form of long strands which are called chromosomes.

Among minerals, the calcium, magnesium, sodium and the phosphates are found in the nucleus. Calcium is bound to the protein, and magnesium to DNA. Sodium ions are required for the active transport of amino acids through the nuclear membrane for which energy is supplied by ATP.

The nucleus is covered by a double membrane. The space between the outer and inner membrane is in continuation with the endoplasmic reticulum thereby forming a continuous channel. Through this channel, materials which are refused transport through by the plasma membrane, reach the nuclear envelope from the extracellular medium and get selectively absorbed through the nuclear membrane. The two nuclear membranes, at intervals, join to form pores which facilitate communication between karyoplasm and cytoplasm. These pores allow the escape of three forms of ribonucleic acid (RNA) to the cytoplasm.

Chromosomes

The nucleus of the resting cell contains long chromatin reticulum which is in combination of DNA and proteins. During the process of mitosis, the chromatin reticulum condenses into compact bodies called chromosomes. The typical chromosome has bands which are known as euchromatic, and heterochromatic bands. The former are composed of DNA associated with histones. The latter, i.e. heterochromatic bands are formed of both DNA and RNA.

During mitosis the chromosome is longitudinally sub-divided into two chromatids. Each of the two chromatids is composed of paired filaments called chromonema.

The chromosome has a region of constriction called kinetochore. Based on its position the chromosomes can be identified as acrocentric, metacentric and submetacentric types. During cell division the kinetochores are subjected to the pulling influence of the spindle fibres.

There are several hypotheses over the fine structure of the chromosome. Some believe the chromosome as containing a single strand of double helical DNA supported by a backbone of protein. According to another hypothesis, the chromosome consists of a continuous non-DNA backbone to which the DNA molecules are attached at regular intervals. Yet another hypothesis is that several DNA molecules get attached end to end to form a continuous strand.

The arrangement of the proteins in the chromosome is unknown. Although the histones seem to bound closely to DNA, some histone molecules merely run along the double helix.

A discussion of the chromosome is incomplete unless the concept of gene is considered. The generally accepted view is that the genes are fragments of DNA molecule. Each gene is able to exert its activity which is different from that of other gene. The reason for the difference in the activities of the genes is now believed to be due to the sequence of the nucleotides in the DNA or in the spatial relationship of the nucleotides to each other. Such genes are located in a linear sequence on the

chromosomes and when the chromosomes divide during meiotic division the hereditary information of the organism is carried into the germ cells.

Nucleic Acids

Nucleic acids are complex molecules and consist of a number of nucleotides joined chainwise. Each nucleotide is a monomer and is made up of three parts, a purine or pyrimidine, a pentose sugar and a phosphate group. The purines are adenine and guanine, while pyrimidines are thymine, cytosine and uracil (Fig. 1.12). Both the purines and pyrimidines are bases.

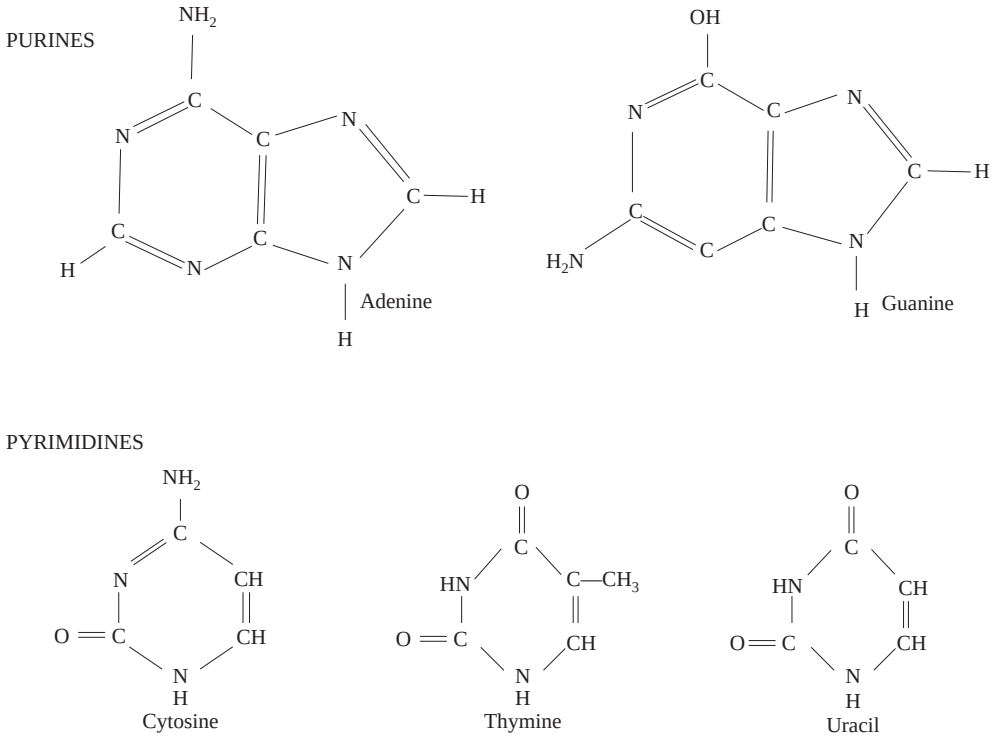


Fig. 1.12 Chemical structure of purines and pyrimidines.

The pentose sugar molecules form a part of the nucleic acids and they exist in the form of a ring. The sugar gets attached either to a purine, or to a pyrimidine base forming a nucleoside. The base attaches to the carbon of the pentose sugar.

The third part, i.e. the phosphate group gets attached with one arm to the fifth position of the pentose sugar to form a nucleotide. For the formation of a nucleic acid chain several nucleotides join, each by its phosphate group binding to the third position of the pentose sugar belonging to the other. Thus the phosphate group in the nucleic acids is known to bind to the fifth position of the pentose sugar of a nucleotide with one arm, and with another arm to the third position of the pentose sugar of

the adjacent nucleotide belonging to the same chain. The sugar and phosphate form the backbone of the nucleic acid and the bases (purines or pyrimidines) are attached perpendicular to it.

The nucleic acids are of two kinds—the ribonucleic acid (RNA) and the deoxyribonucleic acid (DNA). The RNA has a characteristic pyrimidine, the uracil, in its structure instead of thymine which is present in DNA. In addition, the RNA has a five carbon ribose sugar while the DNA has the deoxyribose sugar in its structure.

DNA: The deoxyribonucleic acid consists of adenine, thymine, cytosine, and guanine as bases. In an intact nucleic acid molecule these bases are attached to a five carbon sugar deoxyribose, thus forming a deoxynucleoside. As described above, the phosphate group helps linking the deoxyribose of the deoxynucleoside to form a strand of polynucleotide chain. A DNA molecule has two such polynucleotide chains arranged in a helical structure and held together by hydrogen bonding between bases. Bonding occurs between the purine base of one strand and the pyrimidine base of the partner strand. Chemical data reveals only two base pair combinations, i.e. adenine-thymine, and cytosine-guanine (Fig. 1.13). Pairing occurs by the sharing of two hydrogen bonds between adenine-thymine, and by sharing three hydrogen bonds between cytosine-guanine. Since pairing occurs always between

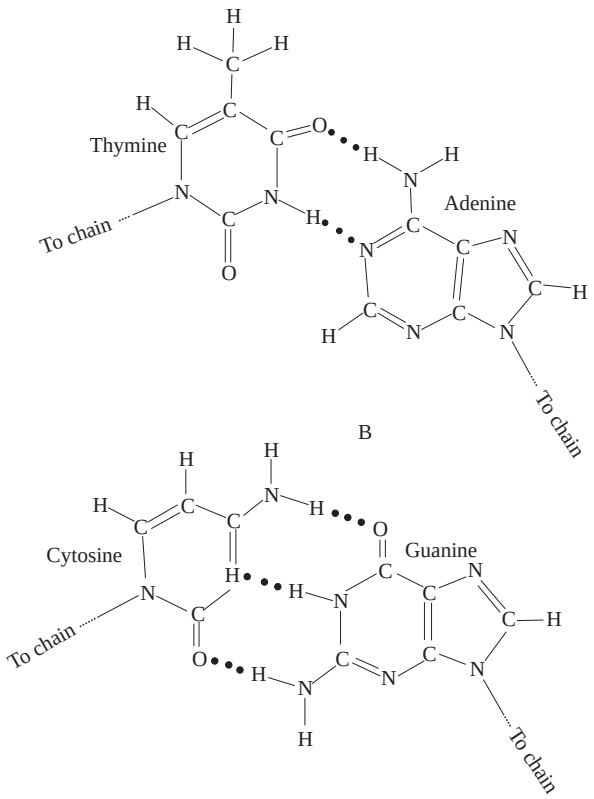


Fig. 1.13 Base pairing: (a) Thymine-Adenine; (b) Guanine-Cytosine.

adenine-thymine, cytosine-guanine, the established bases of one strand in DNA will determine the sequence on the partner strand. For instance, if the order of bases in one strand are A G C A G G T, the base sequence on the complementary strand will be T C G T C C A. This property of specific base pairing in DNA is very important in the replication process of DNA, and in the preservation and transfer of species characters to the offspring.

While DNA is helical in all organisms, it differs from species to species due to changes in the sequence of nucleotides in the strand and also due to the variation in the length of the strand. Because of these differences in DNA, the genes and consequently the characters of species differ.

One of the important functions of the DNA is the replication of the genetic information coded in its strands. Replication process is initiated at one end of the helix by the separation of its polynucleotide strands in a linear and unidirectional fashion. Each one of the separating strands acts as a template and captures the corresponding bases from the nuclear fluid. The bases thus picked up are similar to those lost in the separation. These newly acquired bases are joined together lengthwise to form a strand, and for this process energy-rich phosphate of the nucleotide is required. The energy-rich phosphates are triphosphates. Since there are four types of bases which are required to be connected lengthwise, evidently four types of triphosphates exist. These are collectively known as nucleotide triphosphates and individually they are deoxy ATP, TTP, GTP and CTP. Each of these nucleotide triphosphates is joined to its neighbour, in the presence of specific nucleotide polymerase, with the liberation of two inorganic phosphate molecules. With the linking of the bases the formation of a complimentary strand is completed.

Another function of DNA molecule is, it acts as a template in the formation of messenger RNA (mRNA).

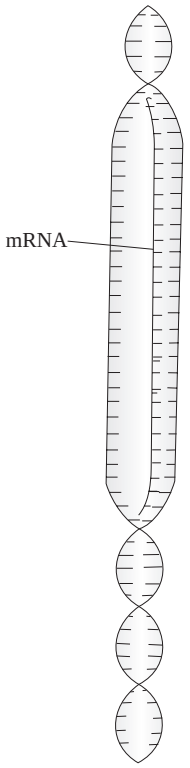


Fig. 1.14 Formation of the messenger RNA.

RNA: The information required to carry out cellular activities is codified in DNA. Most of the cellular activities, such as metabolic and synthetic processes take place in the cytoplasm under the influence of enzymes. Since the code for the proteins is in the DNA (genes), it must be brought to the cytoplasm by a form that can carry out protein synthesis. This is accomplished by a form of ribonucleic acid, which because of its function is called messenger RNA.

There is yet another major type of RNA known as tRNA, or soluble RNA. The function of tRNA is to carry and assemble the amino acids for the synthesis of polypeptide chains. Knowledge of the structure of both the RNAs is necessary for an understanding of their role in the polypeptide synthesis. The structure of tRNA has been described in chapter 5. Before we go in for the mRNA structure let us know how an RNA differs from the DNA.

The RNA differs from DNA in that, its pentose sugar is ribose instead of deoxyribose; one of its bases is uracil* instead of thymine; the polynucleotide is single stranded chain rather than double stranded helix as in DNA. RNA strand contains only hundreds of nucleotides while DNA has them in thousands. DNA contains genetic code and is present in the cell nuclei whereas RNA contains message encoded from DNA template, and migrates to cytoplasm to be associated with the synthesis of proteins with the aid of ribosomes.

Messenger RNA: The process of formation of mRNA in the nucleus is not completely clear. However, it is presumed that mRNA is formed using one of the strands of DNA as a template (Fig. 1.14), firstly because it has a base sequence complimentary to one of the DNA strands, and secondly because much of the newly synthesized RNA is closely associated in the form of a hybrid with DNA. It should be remembered that uracil of RNA is, complimentary to adenine of DNA. Enzyme RNA polymerase, and four, triphosphates (ATP, UTP, CTP, and GTP) are, of course, required in the synthesis of RNA.

While the enzyme is needed in making the complimentary copies of bases line up in sequence with those of DNA, the energy rich phosphates are required to join the RNA nucleotides together to form a strand. The mRNA with a transcribed nucleotide copy of the message from the DNA travels to the protein forming sites. i.e, the ribosomes.

Nucleolus: Nucleolus is very large and conspicuous in growing cells but disappears during cell division. It can be seen as a dense area within the nucleus and has no membrane separating it from karyoplasm. It is thus exposed for interaction with nuclear material. The RNA and proteins which make up ribosomal particles are formed in the nucleolus. While some ribosomal particles remain within the nucleus to synthesize nuclear proteins, the other migrate to the cytoplasm. These nuclear ribosomes synthesize enzymes required in the nucleus, such as DNA-polymerase, etc.

*The structure of uracil is very much like that of thymine but for the lack of CH_3 (methyl) group which thymine possesses.

CHAPTER

2

Foodstuffs

As we know well, food is a matter other than oxygen and water which animals take into their body. It has three principal uses: First as fuel to supply energy, second as a material for building new tissues (i.e. in growth) and for repair, and third as substances which with little or no change take part in the various biochemical reactions without being part of the structure of the body.

The food of animals chiefly consists of varied complex organic substances such as carbohydrates, fats and proteins. These basic foodstuffs get oxidized to meet most of the energy requirements of the animals. The amino acid units of proteins are required for growth and repair of tissues, and also for the synthesis of various secretions of the body. Besides these, there are a number of substances such as vitamins and inorganic compounds, which the animals cannot synthesize and still essential in carrying out both energy yielding and anaerobic growth promoting reactions. Such substances form part of the food and are obtained from outside. Finally the body also obtains through food, minerals required for the regulation of osmotic pressure and acid-base balance, for incorporation in the structure of certain tissues, and for the activation of several enzyme reactions. All the above mentioned substances are taken in by the animals through the food. Since they are the constituents of the food they are conveniently called foodstuffs or nutrients. The ensuing portion of this chapter gives an account of the nature of various foodstuffs.

2.1 CARBOHYDRATES

Carbohydrate is the chief nutrient for the animal kingdom. Animals procure carbohydrates from plants. Plants synthesize carbohydrates through a process of photosynthesis, a process in which the chlorophyll would utilize solar energy to synthesize carbohydrates from the carbondioxide of the air and the water from the soil. Carbohydrates serve as main source of energy. Their energy value is four kilocalories per gram of carbohydrate nutrient and it is provided to the various synthetic needs of a cell. Carbohydrates include sugars and starches.

Carbohydrates are organic compounds in which most of the carbon atoms carry the elements of water. In a simple carbohydrate unit, there are six carbon atoms arranged in a chain with atoms of hydrogen and oxygen attached to the carbons in the same ratio as found in water, (Figure 2.1). In other words, in carbohydrates carbon, hydrogen and oxygen are generally found in the proportion of 1 : 2 : 1.

Classification of Carbohydrates

Compounds of carbohydrates are classified into the following simple groups, viz. monosaccharides, disaccharides, oligosaccharides and polysaccharides, each group containing one, two or more carbohydrate units respectively.

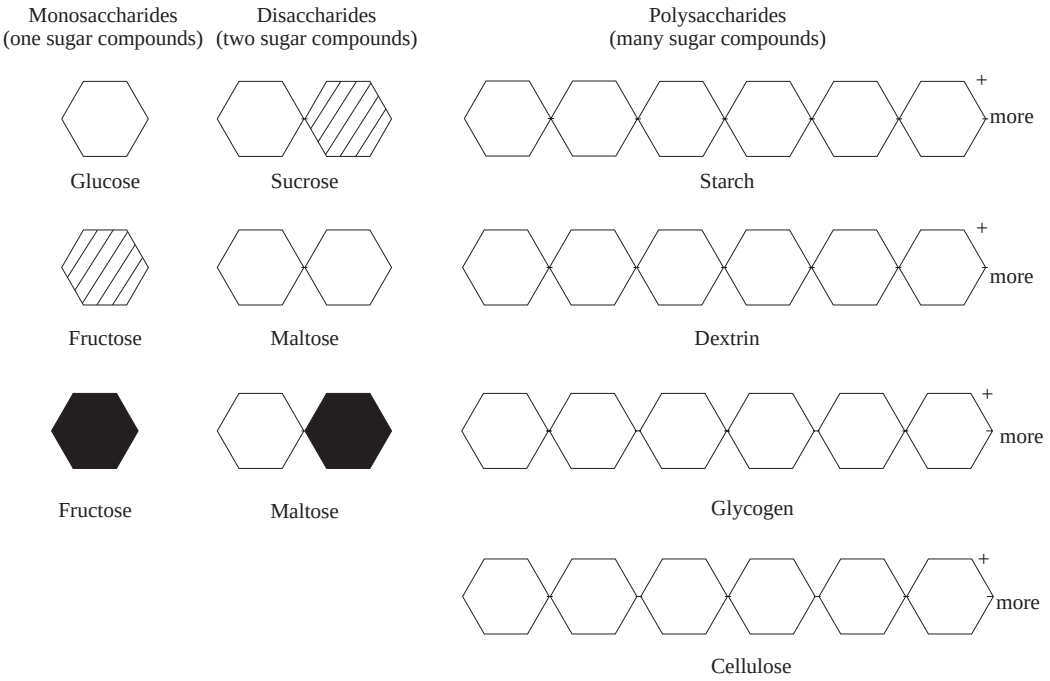
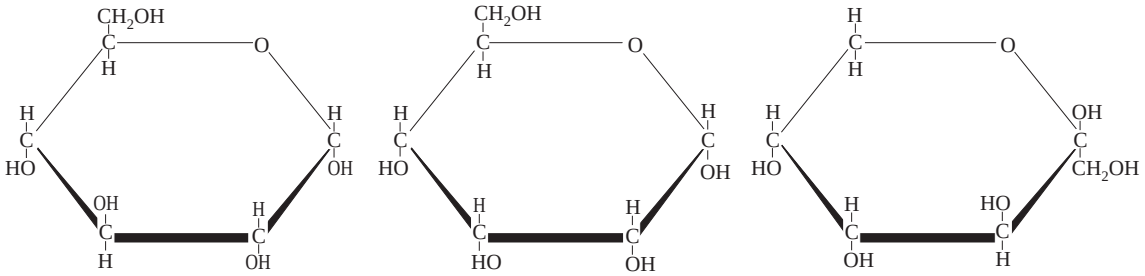


Fig. 2.1 Figure showing the structural moieties of monosaccharides, disaccharides and polysaccharides.

From the above illustrated chart it is apparent that the three monosaccharides—glucose, galactose and fructose are the units contained in the structure of di-and polysaccharides. Mono-and disaccharides have common characters in that they are soluble in water, crystalline, and sweet. They are sugars and their names end with characteristic-ose. In contrast to this the polysaccharides are insoluble in water, and are neither crystalline nor sweet. Their names do not have a characteristic ending.

Monosaccharides: The monosaccharides are grouped according to the number of carbon atoms in their structure, i.e. trioses ($C_3H_6O_3$), tetroses ($C_4H_8O_4$), pentoses ($C_5H_{10}O_5$), hexoses ($C_6H_{12}O_6$),

heptoses ($C_7H_{17}O_7$). Of these only the pentoses and hexoses play fundamental roles in cellular nutrition. Glucose, fructose and galactose are the hexoses. The glucose and the galactose are aldoses (aldehydes} while fructose is a ketose (ketones). Though pentoses and hexoses are often depicted as open chain compounds they are normally arranged in a continuous ring in their biologically active state called hemiacetals.



Ring structure of glucose, galactose and fructose. Glucose and galactose are isomers.

Disaccharides: As already mentioned these are compounds joined with two monosaccharide units. Disaccharides are formed by a chemical reaction called condensation. In this process an OH group of one monosaccharide joins with one of the carbon atoms of the other to form a *glycoside bond* with the elimination of water.

Sucrose, lactose and maltose are the common disaccharides. Disaccharides are split into their constituent monosaccharides in the process of digestion before being absorbed. Both beet and cane sugars are known as sucrose and consist of glucose and fructose units. Lactose which is present in milk has in it glucose and galactose monosaccharides. Maltose contains two molecules of D-glucose and is not available free in nature. It is present in malt products. It is produced in the body as a result of the action of maltase on starch.

Polysaccharides: Polysaccharides consist of a long chain of monosaccharide units. Polysaccharides are also accomplished by condensation. Polysaccharides have a general formula $(C_6H_{10}O_5)n$—where *n* is the number of groups that a molecule may include. Because of the insolubility and large size, they form colloidal solutions and solids, and will not pass across natural animal membranes. They are chemically inert and do not ionize and for this reason very much suited as reserves—as starch in plants and glycogen in animals. Starch, dextrin, glycogen and cellulose are the four polysaccharides that are important as nutrients. Polysaccharides consisting of only one type of monosaccharide units are called *homopolysaccharides,* while, those with different types of monosaccharides or monosaccharide derivatives are *hetero-polysaccharides.*

Homopolysaccharides

Starch: Plant products such as roots, tubers, fruits and seeds contain primarily starch. Starch is found in the cells of plants in the form of granules. The size, shape and markings of starch granules are typical of the plant in which they occur—oval shaped in case of wheat starch; small, rounded and angular in case of corn starch. The composition of starches also differs to some extent, however, all

types have both amylose and amylopectin. The former is a straight chain polymer and the later a branched chain. Starch is hydrolyzed in the intestinal tract yielding dextrins and maltose, and eventually glucose.

In animals, starches are digestible polysaccharides whereas celluloses are indigestible ones. The end product of starch hydrolysis in the body is glucose. Unwanted or excess glucose is stored in liver and muscles as glycogen. When the cell need glucose, the glycogen (also known as animal starch) is hydrolyzed by amylase to form maltose, which is further hydrolyzed by maltase to form glucose.

Dextrins: They are the intermediate compounds formed as a result of hydrolysis of starch to maltose and finally to glucose, both in the process of preparing food (heating) and digestion of starch. They are available particularly in the germinating seeds. The presence of dextrin medium in the alimentary canal is favourable for the growth of acidophilic organisms.

Glycogen (or Animal Starch): Glycogen is present in invertebrates as well as vertebrates. It is a branched chain polymer having 6,000 to 30,000 glucose units. It gives a brown to red colour with iodine. On hydrolysis both yield glucose as the end product.

Cellulose: The cell walls of plants are made up of cellulose which consists of a linear chain of glucose units. These units may range from 900-2000 in different celluloses. It is not acted upon by digestive enzymes secreted by mammals, but bacteria break it down. Cotton has a pure form of simple cellulose.

Heteropolysaccharides

These are complex polymers containing several different monosaccharides or monosaccharide derivatives. In combination with proteins polysaccharides form mucopolysaccharides. They occur in mucous. Many mucopolysaccharides tend to be highly viscous and are responsible for the viscosity of body mucous secretions. These are of no dietary significance.

Functional Significance of Carbohydrates

Carbohydrates serve variety of functions. They supply energy for the body functions and form part of the structure of the mucous membranes, supporting tissues, and the central nervous system. Carbohydrates are antiketogenic and aid in the utilization of body fats. Carbohydrates have also protective function.

The main function of carbohydrate is to supply energy for the body processes. Glucose, a monosaccharide sugar, is the ultimate product into which the entire starch (polysaccharide) and half of the sucrose and lactose (disaccharides) are converted. The other products formed in this conversion are fructose and galactose, but they are present in smaller quantities. The fructose and most of the galactose are converted into glycogen by the liver and a large amount of it is stored in it. Similarly it is also stored in other tissues notably muscles. Glycogen is broken down to glucose by the process of glycogenolysis and the glucose is carried out by blood and gets burnt to provide energy for the body processes.

Structural Function of Carbohydrates

Galactose, which is present in small quantities in the blood of milk drinking people, serves an important structural function, i.e. forming a covering to nerve fibres.

At the time of birth of a child its nerve fibres, especially those of the spinal cord lack the necessary covering of the myelin sheath. The child needs galactose in its blood in order to synthesize myelin, which is a complex fat like compound. Myelin sheath acts as an insulator around nerve fibres and prevents the nerve impulses from the leakage from one fibre to another.

Thus small amounts of carbohydrates and their derivatives form the structural elements in certain tissues.

Pentoses are constituents of nucleic acids. Various carbohydrates are present in many conjugated proteins. Cartilage, bones and tendons have an aminopolysaccharide. The formation and the destruction, of these carbohydrate—containing structural elements is a phase of carbohydrate metabolism.

Formation of Fats from Carbohydrates

When excessive carbohydrates are absorbed into the body, the liver transforms the excess amount into glycogen and stores them. However, if the carbohydrate intake is more than the limited transformation capacity of the liver, they are then converted to fats and stored in the tissues. Formation of fats from glucose is brought about by the synthesis of two components, i.e. fatty acids and glycerol. Acetyl coenzyme A (acetyl CoA), the last compound in the glycolytic pathway acts as a starting substance in the synthesis of fatty acids. In case of glycerol synthesis, the dihydroxyacetone phosphate, also a compound of the glycolytic pathway acts as a starting substance. Though the mechanism involved in the formation of fats is a complicated one, it may be summarised in the following two paragraphs.

The first reaction requiring energy from ATP, is the carboxylation of acetyl CoA to malonyl CoA in the presence of coenzyme, biotin. After this malonyl CoA complex reacts with another molecule of acetyl CoA to form butyryl CoA. Thus by a series of repeated condensations the long chain fatty acids are formed.

For the synthesis of glycerol, the starting substance is dihydroxyacetone phosphate. In the first step this substance is reduced to β-glycerolphosphate. The β-glycerolphosphate is then esterified by two acetyl CoA molecules resulting in phosphodiglycerides. Next to this triglycerides are produced under the action of another acetyl CoA molecule.

Antiketogenic Role

Ketones are formed from the degradation of lipids when the organism suffers from carbohydrate starvation or diabetes. In such circumstances sugar which provides energy is not available to the organism, and hence the energy is supplied by extensive oxidation of lipids. The ketones (acetone, acetoacetic acid, β-hydroxybutyric acid) formed during the catabolism of lipids are so large that they exceed the oxidative capacity of the tissues, hence they get accumulated in the blood (ketonemia) and pass into the urine (ketonuria). Since some of the ketones are strong acids their excessive production alters akalinity of blood to acidity, thus producing hyperacidemia. In normal conditions hyperacidemia does not occur because the small amounts of ketones formed can be neutralized by the alkaline reserve of the blood, but during carbohydrate deficiency ketone bodies become overloaded.

Glucose has an antiketogenic role because it is found that the administration of carbohydrates in the case of carbohydrate starvation reduces ketogenesis. Similarly, carbohydrates and insulin administration prevents diabetic ketogenesis.

Protection of Proteins

Another important function of carbohydrates is to spare protein for building and repairing of body tissue which is its primary duty. The energy need of body is to be first satisfied before the nutrients are used for other functions. When the carbohydrate and fat content of the diet is below the desirable caloric level, protein stops its primary duty of tissue building and repairing and starts degradation. Certain amino acids, leucine, phenylalanine, tyrosin, which are products of this degradation are considered to be ketogenic since they can form acetoacetic acid. Normally acetoacetic acid is utilized in lipogenesis but during carbohydrate starvation this does not happen.

2.2 PROTEINS

The term protein was suggested by Gerardus Mulder in 1840. This term is taken from Greek and it means "to come first". Truly enough the proteins form the most important constituents of the animal body and account for about one half of the total dry weight of the body. A liberal supply of protein is necessary to the body and without it no life is possible. It must be constantly supplied to the body for growth and repair.

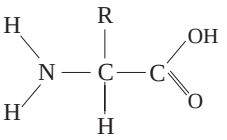
There are different types of proteins. These types are closely related yet they are distinct physiologically. Plant proteins differ from each other and are convertible to animal proteins. Each species in animals has its specific proteins. Further, a given animal has many different proteins within its organs, fluids and tissues. No two proteins are exactly alike in their physiological behaviour. Some proteins serve as structural components, some others are important components of extracellular fluids. The proteins function within the living cells as biological catalysts. Almost every reaction taking place within the cell requires a specific catalyst. Such catalysts are called enzymes and account for about 90% of the total functional protein in the cell. A single cell may contain in it as many as 1,000 different enzymes.

Carbon, hydrogen, oxygen and nitrogen are the elements which are present in each protein molecule. Many proteins also contain sulphur, a few others contain, phosphorus, iron iodine and cobalt. Nitrogen is the distinguishing element in the proteins because it is present in these compounds whereas it is absent in carbohydrates and fats.

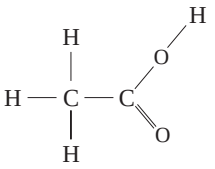
The individual molecules of proteins have high molecular weight and for this reason they are often referred to as macromolecules. Since a large number of similar units, known as macromolecules, are joined to form chain-like molecules they are characterized as bio-polymers.

Amino Acids

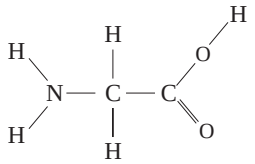
A complete hydrolysis of various proteins yields about 20 different amino acids, showing thereby that the proteins are made up of amino acid subunits. All these amino acids are α-amino acids, i.e. the amino group is attached to the α-carbon. Each amino acid has one part which is same for all and one part which is specific only to that particular amino acid. Taking these points in view the general formula of an amino acid is sketched as follows:



The part labelled R varies for each amino acid: whereas rest of the molecule is the same for all. The simplest of the amino acid is amino-acetic acid or glycine.



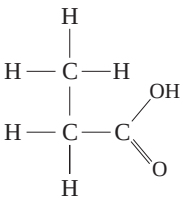
Acetic acid



Amino acetic acid (Glycine)

Alanine, the next simplest amino acid, is amino-propionic acid. There are two amino derivatives of propionic acid and their formation is based on the attachment of the amino group either to α- or β-carbon atom.

Propionic acid



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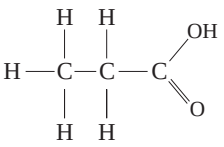
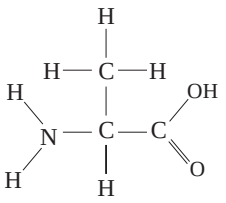


α-amino-propionic acid (alanine)



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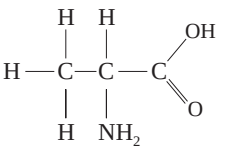
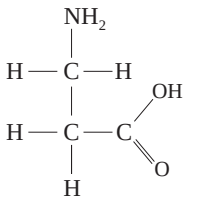


β-amino-propionic acid (β-alanine)



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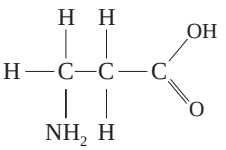


In the same way three possible amino acids can be derived from butyric acid. But the proteins are constituted only with α-amino acids and not with other amino acids.

In all amino acids, with the exception of glycine, the α-carbon atom is attached to four different atoms or groups. The α-carbon atom is therefore asymmetric. The amino acids containing asymmetric α-carbon are optically active and can exist in two stereo-isomeric forms (Fig. 2.2).

The naturally occurring ones belong to L-form. But some amino acids such as alanine are dextrorotator although they belong to L-form. They are amphoteric electrolytes because they have both amino, and carboxyl groups which react as acids in the presence of bases and as bases in the presence of acids.

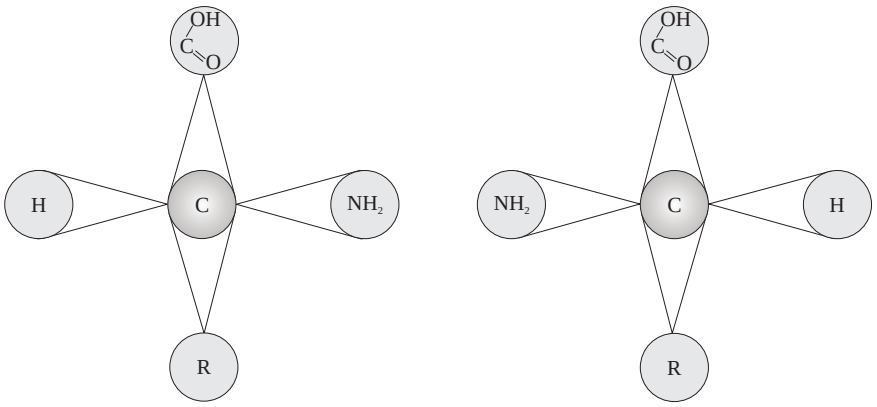
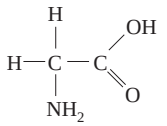
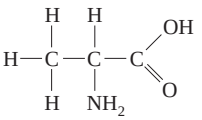
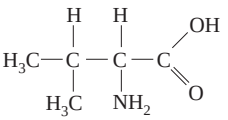
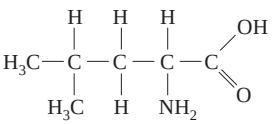
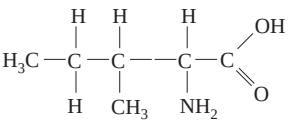


Fig. 2.2 Two isomeric forms of typical amino acid.

Structure and Classification of Amino Acids Available in Proteins: The classification of amino acids, obtained by the hydrolysis of proteins is given below and it is based upon the composition of the side chain, i.e. R-group which varies from, one amino acid to another.

Table 2.1 Amino Acids and Their Structural Formulate

Name	*Structural formula*
I. Side chains with no functional groups—simple amino acids.	
Glycine (Gly) $C_2H_5NO_2$	
Alanine (Ala) $C_3H_7NO_2$	
Valine (Val) $C_3H_{11}NO_2$	
Leucine (Leu) $C_6H_{13}NO_2$	
Isoleucine (Ilc) $C_6H_{13}NO_2$	

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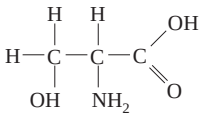
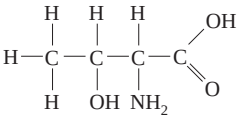
II. *Side chains* with hydroxylic (OH) groups—Hydroxy-amino acids.

Serine (Ser)
$C_3H_7NO_3$

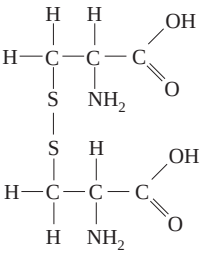


Threonine (Thr)
$C_4H_9NO_3$

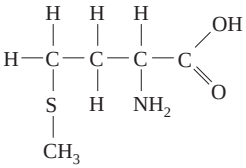


III. Side chains with sulfur atoms.

Cystine (Cys)
$C_6H_{12}N_2O_4S_2$

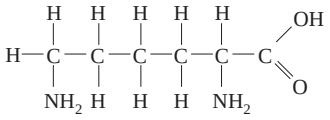


Methionine (Met)
$C_5H_{11}NO_2S$

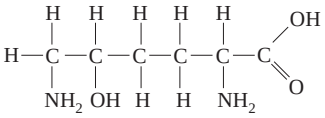


IV. Side chains with a basic group.

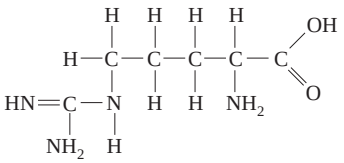
Lysine (Lys)
$C_6H_{14}N_2O_2$



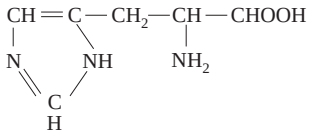
Hydroxylysine (Hyl)
$C_6H_{14}N_2O_3$



Arginine (Arg)
$C_6H_{14}N_2O_2$



Histidine (His)
$C_6H_9N_3O_2$



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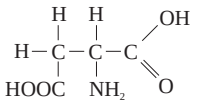
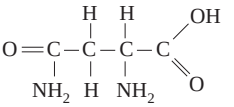
V. Side chains with a carboxyl group—Acidic amino acids—or their amides

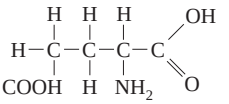
Aspartic acid (Asp)
$C_4H_7NO_4$



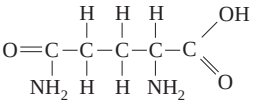
Asparagine (Asn)
$C_4H_8N_2O_3$



Glutamic acid (Glu)
$C_5H_9NO_4$

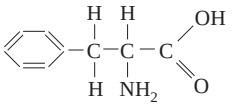


Glutamine (Gln)
$C_5H_{10}N_2O_3$

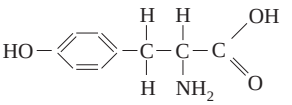


VI. Side chain with aromatic (Benzene-like) group.

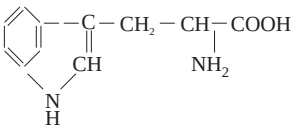
Phenylalanine (Phe)
$C_9H_{11}NO_2$



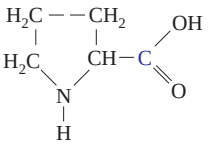
Tyrosine (Tyr)
$C_9H_{11}NO_3$



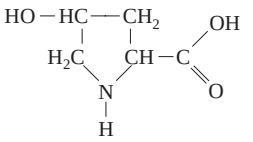
Tryptophan (Trp)
$C_{11}H_{12}N_2O_2$



Proline (Pro)
$C_5H_9NO_2$

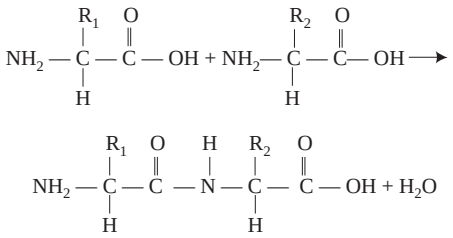


Hydroxyproline (Hyp)
$C_5H_9NO_3$



The amino acids are described as the building blocks of proteins, and may be conveniently grouped under two broad categories, i.e. essential and nonessential. The essential amino acids are to be obtained by the animal through its diet. The nonessential amino acids are, however, synthesized by the animal tissues.

Peptides: The amino acids (subunits) are joined together in the following manner.



The above reaction shows the amino acids as linked through the carboxyl () and amino groups. The carbon and nitrogen bond between each pair of amino acids shown above is a special kind of amide bond and is referred to as a *peptide bond.* The compound formed is a dipeptide. Like amino acid, it still has an amino group at one end and a carboxyl group at the other end. Therefore it can still react with another amino acid to form a longer chain (tripeptide), which in turn can react in this way to accept amino acid again and again in order to form polypeptides. These complex polypeptides have properties resembling that of proteins. Each polypeptide has in it about 100 to 200 various amino acids in the form of amino acid residues. A protein may have two or more of such peptide chains. Several such chains join to form macromolecules of proteins.

Protein Structure

For a better understanding of the structure of protein the following information is essential.

(a) Molecular size of protein.
(b) Relative proportions of various amino acids.
(c) Amino acid sequence in the chain.
(d) Three-dimensional arrangement of the chain.

Molecular Size

The estimation of the size or molecular weight of protein is made from the accurate measurements of osmotic pressure, or of sedimentation velocity and sedimentation equilibrium in the ultracentrifuge.

Primary Structure of Proteins: the Sequence of Amino Acids

The term primary structure is used to designate the sequence in the arrangement of amino acids in proteins. The sequence remains same in a specific protein and it is an important character in protein function. Any change in sequence of amino acid is enough to disqualify the protein from discharging its function. The substitution of only one amino acid for another in the haemoglobin molecule is sufficient to cause sickle-cell anemia. The sequence of amino acids in insulin molecule was first established in the year 1955. The molecule has two polypeptide chains, and arranged in them are 51 amino acids. The two chains, one with 21 and other with 30 amino acid, are joined by sulphide bond.

The complete amino acid sequence of several other proteins is known. These include enzymes such as ribonuclease, lysoyme and the subtilisin of *Bacterium subtilis,* etc.

Thus, under the primary structure we have considered the amino acid sequence. Each amino acid is linked with its immediate neighbour by peptide bonds. The peptide bonds are the primary and strongest linkages.

Secondary Structure of Proteins

In addition to the above mentioned primary structure the biological activity of protein also depends on the secondary structure. Under the secondary structure of protein we consider the structural aspects like folding of the polypeptide chains into a specific coiled structure arising as a result of the linkage of amino acid units which are not far from each other, as in the α-helix. These amino acid units are linked by means of hydrogen bonds and disulphide bonds (Fig. 2.3).

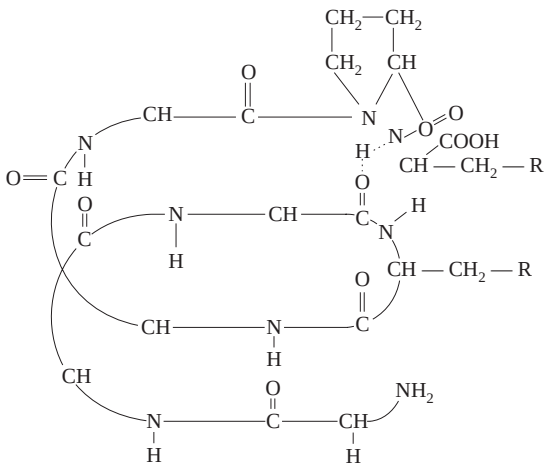


Fig. 2.3 Alpha-helix (after Pike and Brown, *Nutrition*, 1967, p.40).

Tertiary Structure of Proteins

Tertiary structure deals with the arrangement and interrelationship of twisted chains of proteins. Such a structure enables the proteins to form specific layers, crystals or fibres. This tertiary structure is maintained by weak hydrogen bonds and electrostatic forces. The tertiary structure is important and found in globular proteins. If this structure is disrupted, the biological activity of protein would be lost. The tertiary structure of the myoglobin which was established is illustrated in Fig. 2.4.

Quaternary Structure of Protein

In addition to the above three structures, a fourth level of structure has been recognized in some proteins. This structure is essential for the activity of enzyme protein. There are also a number of globular proteins having the quaternary structure, i.e. they are composed of *subunit peptide chains*

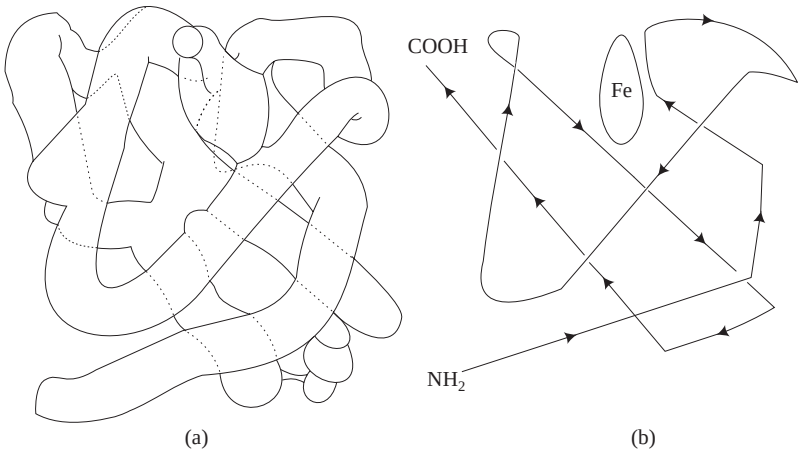


Fig. 2.4 (a) Tertiary of the myoglobin; (b) the course of the polypeptide chain.

linked together by any or all of the forces that can act between amino acid side chains. Haemoglobin, a protein capable of carrying oxygen, is a fine example of quaternary structure. It has four peptide chains and each chain (subunit) is complexly folded and resembles myoglobin to some extent. Chains fall under two types, each type consisting of one pair. These peptide pairs of haemoglobin interact with each other resulting in a quite stable and compact bundle, which is the active protein.

Classification

During the early stages of the protein chemistry a number of operational classification systems were suggested. The situation is not much different even today. Presently there are three classifications of proteins. They are as follows:

A. Classification based on the composition of proteins.
B. Classification based on the shape of the protein molecule.
C. Classification based on the solubility of proteins.

Still none of the above systems of classification is satisfactory. The present information on the precise structure of proteins is insufficient. A precise system of classification aiming at correlating structural characteristics with biochemical function should be possible with the availability of more and more information on the exact nature of protein. Till that time, to lessen confusion it is necessary to follow one. Given below is an arbitrary classification for descriptive purposes. The proteins are mainly classified into two broad groupings, viz. (a) simple proteins, and (b) conjugated proteins. The simple proteins would give rise to only amino acids on hydrolysis. In case of conjugated proteins some other substance—a prosthetic group—is attached to the protein.

(*a*) *The simple proteins:* Proteins such as albumins, globulins, glutelins and gliadins, scleroproteins, protamines and histones come under this group.

(i) *Albumins:* They are soluble in water, dilute acids and bases. They coagulate when heated. If a solution of albumin is heated near the isoelectric point the protein gets denatured and then

precipitates as coagulum. This property is utilized to test the presence of albumins in urine. Albumins are precipitated (usually without denaturation) by saturating the solution with ammonium sulphate. In natural state they are frequently associated with small quantities of mucopolysaccharides (refer to mucopolysaccharides under the title polysaccharides). Common examples are albumins of egg-white and, of blood serum, and lactalbumin.

(ii) *Globulins:* They are insoluble in water but soluble in dilute solutions of salts. They are also coagulable when heated. From solutions they are precipitated by half-saturating with ammonium sulphate. Common examples are the globulins of egg-white; of blood serum; of variety of seeds, including squash, soybean, hemp, and others.

(iii) *Glutelins:* They are soluble in dilute acids and alkalies, and insoluble in neutral solvents (distilled water and alcohol), but when pressed and squeezed (kneaded) with water they form a tenacious sticky mass. They are coagulable when heated. They are plant proteins and found in the cereals. Glutelin from wheat is one of the examples.

(iv) *Scleroproteins:* They are chiefly the fibrous proteins, insoluble in water or in other common solvents. They are partially or highly resistant to digestive enzymes. They are the important constituents of the connective tissue and external coverings of animals. Keratin, collagen, elastin are examples of scleroproteins. Keratins are the principal constituents of skin, hair, bones; collagens are present in tendons, bones and also in the skin. Elastins are the main constituents of arteries, elastic tissues and also tendons.

(v) *Protamines:* They have relatively low molecular weights ranging from 2,000 to 4,000. They are very soluble, small, stable proteins and cannot be coagulated by heat. They contain only a few amino acids but a large proportion of them are basic in nature. The protamines are exceptionally rich in arginine, hence strongly basic in nature. By virtue of this basic, character they readily form salts with mineral acids, acidic proteins, and nucleic acids. These salts are either insoluble or partially soluble. During the process of purification of proteins in order to eliminate the unwanted nucleic acids, they are made to combine with protamines to form insoluble nucleic acid salts. Insulin in the form of protamine-insulin is less soluble and more stable towards heat. Further, it is absorbed more slowly and hence effective over a longer period of time. Protamines are found in the ripe sperm cells of certain fish.

(vi) *Histones:* Like protamines, histones have low molecular weight, are basic in nature and very soluble in most common solvents. However, histones greatly differ from protamines in that they (histones) are somewhat weaker bases and are insoluble in ammonium hydroxide solutions. Histones are found associated with nucleic acids in the nucleoproteins. They are obtainable from the spleen, thymus; nucleated R.B.C. of birds.

(b) Conjugated proteins: Phosphoproteins, glycoproteins, chromoproteins, and lipoproteins are included under this major group.

Phosphoproteins: In these the protein molecule is linked to phosphoric acid. The phosphoproteins when treated with dilute sodium hydroxide yield inorganic phosphate. Examples of phosphoproteins are casein of milk and vitellin of egg-yolk.

Glycoprotein: They contain small quantity of carbohydrate, bound to protein molecule. The carbohydrate in these is usually the mucopolysaccharide. Mucin of saliva, chorionic gonadotropins and some of the pituitary hormones such as follicle stimulating hormone (FSH) and the luteinizing hormone (LH) are glycoproteins.

The nucleoproteins: These are formed by the combination of nucleic acid with protein.

The chromoproteins: These are the compounds consisting of protein and the coloured material—a non-protein. Haemoglobin contains protein globin and red pigment haem. Other examples are haemocyanins, flavoproteins and cytochromes. Haemocyanins are the respiratory pigments found in the blood of invertebrates and they have copper content.

The lipoproteins: They are compounds of protein and lipids. Lipoproteins have solubility properties of proteins, hence the lipid portion is insoluble in ether. Extraction of lipid portion is possible only when the protein is denatured. Lipovitelline of egg-yolk is a lipoprotein.

2.3 LIPIDS

Lipids are important group of organic compounds extractable from biological materials with usual fat solvents such as (ether-alcohol mixtures, chloroform, benzene, acetone, etc.). They are insoluble in water. Lipids, like carbohydrates, also contain carbon, hydrogen, and oxygen, the former two in larger quantity than oxygen. Some lipids contain phosphorus and nitrogen. The lipids in the body, serve as condensed reserve of energy. Some have structural functions yet others are hormones essential for various reactions in intermediary metabolism. Lipids play very significant roles in nutrition and physiology. Lipids are transported in biological fluids when they are in combination with proteins (e.g., fatty acids with plasma albumin in vertebrates) or when they exist as derivative of a protein (e.g. lipoproteins).

Classification of Lipids

A brief classification presented below is limited to lipids of importance in animal nutrition.

A. **Simple Lipids:** These are esters of fatty acids with various alcohols.

1. Fatty acids.
2. Neutral fats (mono-, di-, and triglycerides)
3. Waxes (esters of fatty acids with higher alcohols)
   (a) Sterol esters (cholesterol esters with fatty acids)
   (b) Nonsterol esters (i.e. vitamin A esters, etc.)

B. **Compound Lipids or Complex Lipids:** These are lipids with additional group mentioned below.

1. *Phospholipids:* Any lipid containing phosphorus is included under phospholipids
   (a) Phosphatidic acids (phosphoglycerides) lecithins, cephalins. etc.
   (b) Plasmalogens
   (c) Sphingomylins

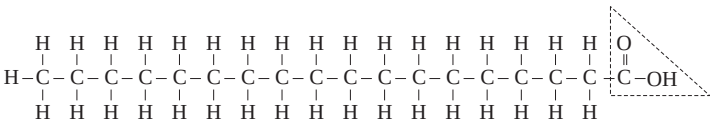
2. *Glycolipids:* These are carbohydrate containing lipids.
   (a) Cerebrosides
   (b) Gangliosides
3. *Lipoproteins:* These are lipids in combination with proteins.

C. **Derived Lipids** (alcohols, including sterols, hydrocarbons).

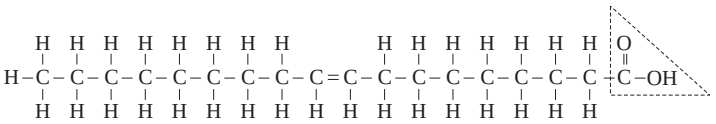
These are products derived by hydrolysis of above mentioned groups, and still having some general properties of lipids.

SIMPLE LIPIDS

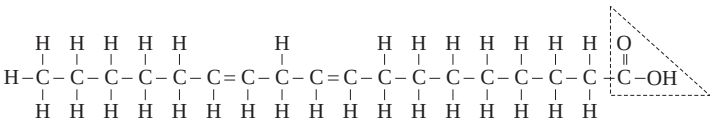
Fatty Acids: Fatty acids are the simplest of all the lipids. These are constituents of most of the lipids. Fatty acids are monocarboxylic acids. The molecule of a fatty acid has a polar carboxyl group soluble in water and a non-polar hydrocarbon chain soluble only in common organic solvents. They have greater solubility in organic solvents than in water. The fatty acids with short chain length (under 12 carbon atoms) have greater solubility in water while the solubility decreases appreciably with the increase in their chain length (more than 16 carbon atoms). The short chain fatty acids are present in coconut oil, milk fat and butter fat. Most of the long chain fatty acids (16 to 18 carbon atoms) are found in the average diet. Fish oils, peanut oil contain fatty acids having more than 20 carbon atoms.



Stearic acid (saturated) $C_{18}H_{36}O_2$ $CH_3 - (CH_2)_{16}–COOH$



Oleic acid (monounsaturated) $C_{18}H_{34}O_2$ $HC - (CH_2)_7–CH_2$
$HC - (CH_2)_7–CHOOH$



Linoleic acid (polyunsaturated) $C_{18}H_{32}O_2$ $HC–(CH_2)_4–CH_3$
$HC–CH_2–CH$
$HOOC–(CH_2)_7–CH$

The fatty acids found in foods are classified under three groups based on their degree of saturation or unsaturation. A saturated fatty acid contains as many hydrogen atoms as its carbon chain can hold. The saturated fatty acids have the general formula $C_nH_{2n-1}O_2$ or C_nH_{2n-1} COOH.

Chemical Structure of Saturated and Unsaturated Fatty Acids Certain other fatty acids have a single double bond in their carbon chain resulting in the loss of two hydrogen atoms. These are called *monounsaturated fatty acids* and their general formula is $C_nH_{2n-2}O_2$ or $C_nH_{2n-1}-COOH$. There are yet other fatty acids, viz. *polyunsaturated fatty acids,* which have 2, 3, 4 or more double bonds in the carbon chain with the consequent absence of 4, 6, 8 or more hydrogen atoms. The polyunsaturated fatty acids, linoleic, linolenic and arachidonic are often termed as *essential fatty acids* since they must be supplied in adequate amounts in the diet of mammals. The absence of linoleic acid in diet of rats, pigs and the like develops the characteristic dermatitis.

A limited types of unsaturated fatty acids can be synthesized by the animal cell. For this purpose addition of any new double bonds must be carried out between the carboxyl group and the first double bond of the fatty acid molecules. The conversion of linoleic acid into arachidonic acid in animal cells is an example of this.

The existence of different isomeric forms of unsaturated fatty acids is due to the occurrence of double bonds in its molecule.

In the modern chemical nomenclature, the names of the fatty acids have suffixes which denote the state of saturation, i.e. *-anoic,* saturated; *-enoic,* one double bond; *-dienoic,* two double bonds and so forth. These names are given in parenthesis in the following table.

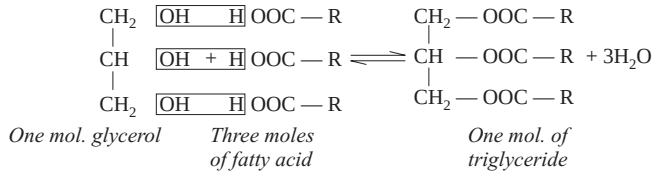
Table 2.2 A Few Common Fatty Acids Found in Lipids

Fatty acids	*Formula*
Saturated acids	
Butyric (butanoic)	$C_4H_8O_2$
Caproic (hexanoic)	$C_6H_{12}O_2$
Palmitic (hexadecanoic)	$C_{16}H_{32}O_2$
Stearic (octadecanoic)	$C_{18}H_{36}O_2$
Arachidic (eicosanoic)	$C_{20}H_{40}O_2$
Unsaturated acids	
Oleic (hexadecenoic)	$C_{18}H_{34}O_2$
Linoleic (octadecadienoic)	$C_{18}H_{32}O_2$
Linolenic (octadecatrienoic)	$C_{18}H_{30}O_2$
Arachidonic (eicosatetraenoic)	$C_{20}H_{32}O_2$

As regards properties, the saturated fatty acids have higher melting points and are less reactive than the unsaturated ones having equal number of carbon atoms. For example, stearic acid (saturated) melts at 70°C, whereas oleic, linoleic and linolenic acids are liquid at room temperature in spite of the fact that they all have 18 carbon atoms. These characteristics are important in physiology as they influence the properties of fats and other media in which the fatty acids are combined.

Neutral Fats: The chemical term for a neutral fat is triglyceride. Triglycerides are esters formed by a combination of the trihydroxy alcohol (glycerol) with three molecules of fatty acids. The

trihydroxy alcohol bears three side groups each of which terminates in an —OH radical. In the illustrated reaction given below the R in the general formula of fatty acid stands for the side chain; whereas the carbon at the other end bears a group which is typical of all organic acids, i.e. —COOH. As a result of reaction, the carboxyl group of three molecules of fatty acid units with the alcoholic group in the glyceryl molecule to form a molecule of triglyceride on one hand, while on the other hand, the hydroxyl groups from the three arms of the glycerol units with each of the hydrogen atom in the acid radical of the three molecules of fatty acid to form three molecules of water.



The triglycerides may be esters of one, two, or three fatty acids with glycerol. If all the 3 moles of the fatty acids that go in the formation of a triglyceride are the same, it is known as simple triglyceride. A mixed triglyceride is one, in the formation of which, different fatty acids are involved. The commonest fatty acids found in fats are palmitic, oleic and stearic acids.

As already stated triglycerides are neutral fats; neutral, because the acid ions are neutralized during their unification with glycerol; fats, because the bulk of the molecule is devoid of electro-negative elements which can unite with hydrogen to form water molecules. Because of neutral and inert nature of triglycerides, they mainly serve as a storage medium for carbon compounds such as fatty acids and glycerol. These latter compounds can be broken down in order to liberate energy to be used by the cell.

Whether a triglyceride is a liquid or solid is based on the kind of fatty acid residues in its structure. The triglyceride which is liquid below 20°C is termed oil in industrial classification and contains residues, more of the unsaturated fatty acids; whereas the glyceride which is solid above this temperature is called a fat and contains more of the saturated fatty acid residues in its structure. The oils are predominant in plants, and the fats are predominant in animals.

The fats of animals differ from species to species. Even within the same species fats in the different parts exhibit variation. For example, melting point of lard (the typical body fat of swine) is 28°C, whereas the fat of its kidney melts at 43°C. This indicates that the kidney fat has firmer consistency and higher saturated fatty acid composition. Because of this difference in the property, the fat around the kidneys acts like cushions to protect them from shock injuries. The fats in the more active parts of organisms have lower melting point and are more unsaturated. This means they would be more easily oxidized and constantly utilized than those stored as fatty tissues elsewhere.

Body Fats: Animals living in cold zones generally possess fats having more unsaturated components than those of animals from tropical regions. Further, there is variation in the type of fat present in poikilotherms and homeotherms. The poikilotherms, i.e. cold blooded animals, are softer and thus have more unsaturated fats than warm-blooded animals. The meat eating animals have softer fats than the vegetable feeders.

Fats are hydrolized into glycerol and fatty acids by dilute mineral acids, enzymes such as lipase, or steam. Enzymatic hydrolysis takes place during digestion by pancreatic lipases.

Saponification: Boiling the fats with alkali such as sodium hydroxide would yield glycerol and the alkali salt of the fatty acid, which is known as soap.

$$\begin{array}{l} CH_2.O.CO.C_{17}H_{35} \\ | \\ CH.O.CO.C_{17}H_{35} \\ | \\ CH_2.O.CO.C_{17}H_{35} \end{array} + 3NaOH \rightarrow \begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} + 3C_{17}H_{35}COONa$$

Tristerin *Glycerol* *Sodium stearate*

This process is known as saponification, and it occurs during the digestion under the action of sodium salts in the bile. Fats are also hydrolyzed by superheated steam.

The saponification value of a fat is the number of milligrams of alkali, the potassium hydroxide, required to saponify one gram of fat.

Hydrogenation: The fats (triglycerides), liquids at ordinary temperature; are mentioned as oils containing a large proportion of unsaturated fatty acids. The oils may be hardened to make solid triglycerides or soft fats may be hardened to increase the melting point by a chemical process known as hydrogenation. In this process the triglycerides are treated with hydrogen under certain pressure, in the presence of finely divided nickel as a catalyst. As a result, the hydrogen is added on the double bonds of the unsaturated fatty acids to form a more saturated fat. The process of hydrogenation is not allowed to reach completion as it makes the fat too hard and brittle making it undesirable.

The saturation of double bonds due to hydrogenation, makes the fat less reactive and thus tends to prevent the oxidative changes. Thus, the hydrogenation is used for improving the storage qualities especially of certain vegetable oils.

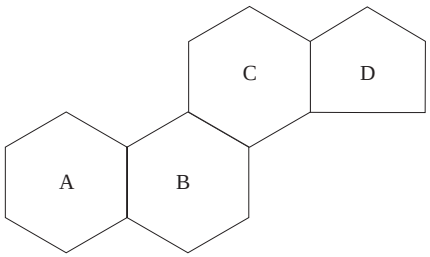
Role of Fats and Oils: The fats and oils are important constituents of the dietary requirements because they have higher energy value. By calorimetric studies it is found that one gram of carbohydrate would yield 4.1 calories, whereas one gram of fat yields 9.3 calories. Lipids contain fat soluble vitamins and the essential fatty acids which are found in the naturally occurring fats. The fat reserves (adipose tissue) in the body are the rich store houses of energy and it would be released when the body needs energy. The adipose tissue found in the subcutaneous tissues serves as an insulating material. As already mentioned, they serve as protective cushions for the viscera and certain organs in the body such as the kidneys.

Essential fatty acids, viz. linoleic, linolenic and arachidonic help preventing certain disorders in mammals like mouse, dog and man. Linolenic acid has an essential role in reproduction and lactation. It also serves as a protective agent against radiation effects and prevents heavy loss of water from the body by acting against the development of permeability of skin capillaries.

Waxes: The wax is an ester of a fatty acid with one of the higher monohydroxy or dihydroxy alcohols. The waxes have high melting points. The fat splitting enzyme lipase cannot react on waxes and for this reason these are not suitable as food. However, many animals, insects in particular, synthesize and secrete the waxes. Beeswax is an ester of palmitic acid with myricyl alcohol.

Spermaceti is an ester of palmitic acid with cetyl alcohol. It is formed from the head of the sperm whale.

Steroids: These are often associated with fat and are separable from it by a process of saponification. The steroids occur in unsaponifiable residue. Like lipids they are soluble in fat solvents and generally insoluble in water. All steroids have similar cyclic nucleus of the type shown below. The rings A, B, and C in the figure represent phenanthrene and to this structure the ring D representing cyclopentane is attached.



It should be noted that this cyclic nucleus is not uniformly unsaturated, but the actual parent substance is completely saturated and is termed as perhydro-cyclopentanophenanthrene. Steroid compounds are the derivatives of this ring. The steroids include such substances as cholesterol and other sterols, the bile acids, the male and female sex hormones, the hormones of the adrenal cortex, etc.

Sterols: Certain steroids are characterized by a free hydroxyl group and as such behave chemically like alcohols. Such alcohol-like steroid compounds which do not contain carboxyl or carboxy groups common to other steroids are referred to as *sterols* (the term *sterols* means solid, and *ol* represents the ending, 'ol' of alcohol). The sterols are classified into: (*i*) zoosterols, if they are available from the animal tissues, i.e. cholesterol, corticosterone, etc., (*ii*) phytosterols, if available from vegetable tissues; (*iii*) mycosterols, if available from fungi, yeast, etc.

Functions: Sterols form esters with fatty acids and act as carriers of fats for absorption and transport. They go into the formation of nerve shealth (myelin), and cell membrane. The sterols, which are present as esters in the fatty secretions of the sebum, cerumen, etc., act as lubricants to skin and hair.

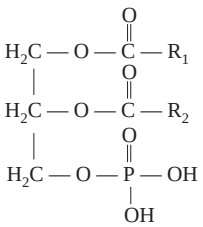
COMPOUND LIPIDS

Phospholipids: Lipids containing phosphorus are phospholipids. They are also referred as phospholipins and phosphatides. They differ from the neutral fats in containing phosphoric acid and an organic nitrogenous base. The phospholipids are widely distributed in animal and plant cells. They are abundant in brain and nervous tissues. They have a polar group which can combine with protein to form biologically important lipoproteins.

The different types of phospholipids described below have the same essential structure but they vary only in the nature of one of the groups linked to the phosphate portion of the molecule. Further,

most of the compounds are of phosphodiesters nature. One type of phospholipid, viz. phosphatidic acid is indicated below with a general formula.

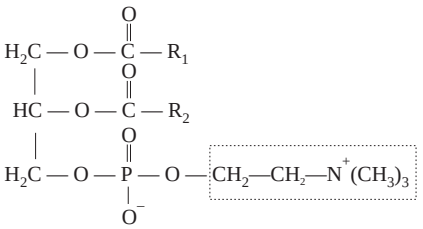
Phosphatidic Acids: Phosphatidic acids are compounds consisting of glycerol, two fatty acids, and a phosphate group.



The R_1 and R_2 in the above formula represent the residues of the molecules of fatty acids. The phosphatidic acids should be viewed as fats in which one of the fatty acids is replaced by phosphoric acid. The structure suggests, these acids could easily give rise to triglycerides or to phospholipids. The phosphatidic acids are not present in significant amounts in the tissue extracts. They play important role as active intermediates in the biosynthesis of other lipid compounds.

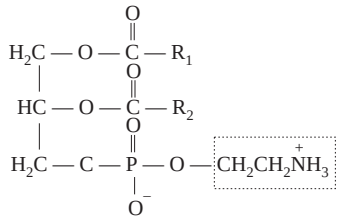
Lecithins (phosphatidyl cholines): Lecithins are choline esters of phosphatidic acid. They are fats in which one of the fatty acids is replaced by phosphoric acid and the nitrogenous choline. They are best known and probably the most common form of phospholipids in animals. On hydrolysis, lecithins break up into glycerol, fatty acids, phosphoric acid and choline.

Lecithins are soluble in the fat solvents except acetone and by this property they are distinguished from the fats. These are required for the transport, and utilization of other lipids especially in the liver. If the synthesis of choline, an important component of lecithin is interrupted, the synthesis of lecithin also will be stopped. With the result, the transport of fat to and from the liver is interrupted. Consequently, the lipids accumulate in the liver giving rise to a condition called *fatty liver.* The lack of dietary choline also leads to a variety of troubles such as growth failure, hemorrhagic kidneys, and slipped tendons, etc.

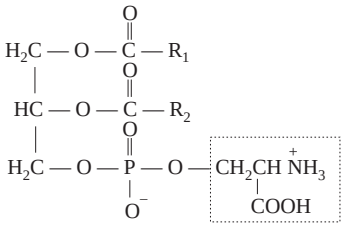


The enzyme, lecithinase A, present in the venoms of snakes attacks lecithins and removes one of the fatty acid residues leaving a product known as lysolecithin. This product has the ability to haemolyze the red blood corpuscles.

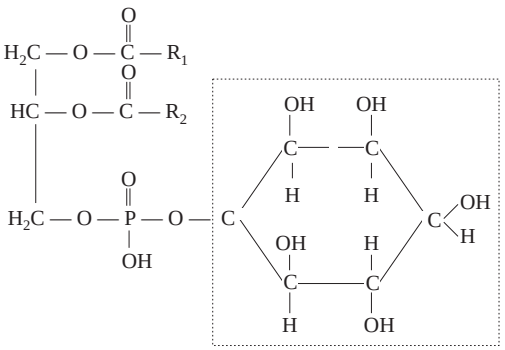
Cephalins (Phosphatidyl ethanolamines): These are phospholipids which resemble lecithins in most properties, but differ in containing aminoethyl alcohol (NH_2—CH_2—CH_2—OH) in place of choline.



Phosphatidylserine is a cephalin like compound and contains the amino acid serine in place of ethanolamine.

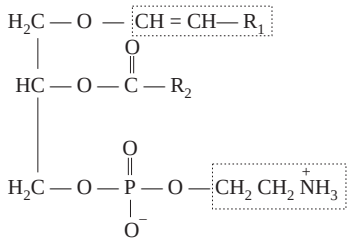


Phosphatidyl inositol is a phospholipid similar in structure to the lecithin but has inositol in place of choline.



These are mainly found in plants and in nervous tissues.

Plasmalogens: These phospholipids are abundant in brain and muscle. They resemble licithins and cephalins in structure but possess an aldehyde group in place of one of the fatty acids in typical phospholipid molecule. The structure shown below represents a typical plasmalogen. The portion of the formula enclosed by line is an ethonolamine residue.

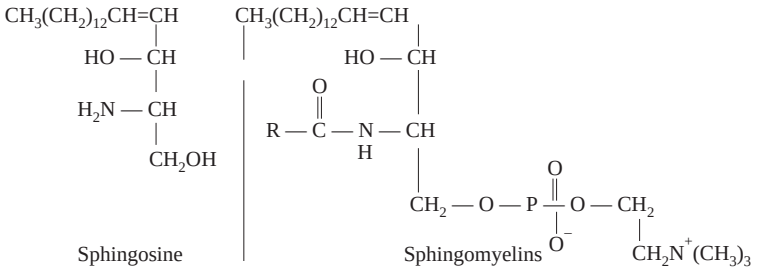


Structure of plasmalogen (phosphatidal ethanolamine)

Sphingosine lipids: These are more complicated phosphatides containing sphingosine, a long-chain amino alcohol in place of glycerol.

Sphingomyelins (sphingosine phosphatides): These phospholipids contain sphingosine and a fatty acid attached in the form of amide linkage to the nitrogen of carbon 2, and phosphorycholine attached to the terminal carbon atom of sphingosine. On hydrolysis they yield fatty acids, phosphoric acid, choline and sphingosine. The sphingomyelins are found in large quantities in the nerve tissue, particularly in the myelin sheath of the nerve.

GLYCOLIPIDS: Cerebrosides and gangloisides are the chief glycolipids.



Sphingosine Sphingomyelins

These are particularly abundant in the brain and in the myelin sheath of nerves. There are the lipids containing carbohydrate radicals and nitrogen in the molecule. In structure these are related to sphingomyelins, but contain a hexose instead of phosphorycholine. On hydrolysis they give rise to fatty acids, sphingosine and galactose.

2.4 VITAMINS

In the year 1911 Funk, a Polish biochemist, coined the term "vitamine" to designate the antiberiberi factor. In the term vitamine *vita* suggests the essential nature of the factor, and *amine* indicates the chemical structure. Later the same term was used for several unknown dietary factors. Not all the known factors have the amine structure. Therefore in 1919 the last "e" from the vitamine was discontinued from use thereby removing the implication of chemical structure.

Vitamins can be described as accessory food factors which are essential for some metabolic reactions within the cell and which must be provided in the diet in minute amounts. In many animals

they cannot be synthesized by the body. Certain animals do synthesize a few vitamins in very minute quantities but they fall short of the requirements. In any case, the animals should procure them through food. Vitamin deficiency results in several diseases characteristic of each vitamin deficiency.

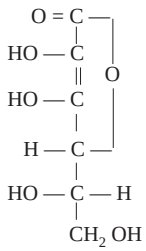
Vitamins are a special group of organic substances which are chemically unrelated. They may be organic acids, amines, amino acids, esters, alcohols, steroids, etc. These vitamins are traditionally divided into two subgroups on the basis of their solubility properties, such as water soluble vitamins and fat soluble vitamins.

Water-soluble Vitamins

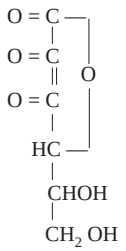
Vitamins C and B complex are water soluble substances.

Ascorbic Acid or Vitamin C: Ascorbic acid is a hexose derivative and is properly classified as a carbohydrate. It is a white crystalline substance highly soluble in water. It is easily oxidized in solution and cooking destroys it. The vitamin is highly unstable in alkaline solutions.

This vitamin is required by primates and the guinea pig. All other vertebrates and some invertebrates as well as plants and most micro-organisms can synthesize ascorbic acid from carbohydrates. It is an essential dietary factor, the deficiency of which causes painful disease of the joints and gums called *scurvy.*



L–Ascorbic acid (reduced form)



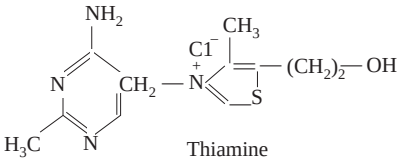
L–Dehydroascorbic acid (oxidized form)

Hydroxyproline is an unusual amino acid found in the collagen and is essential for maintenance of normal tissues. It is formed by hydroxylation of proline and this process requires vitamin C. In the absence of this vitamin the hydroxyproline production is very much reduced. Therefore, the collagen formed during ascorbic acid deficiency, contains negligible amount of hydroxyproline and this brings about structural abnormalities observed in scorbutic tissues.

Vitamin C is contained in fresh vegetables and fruits.

Vitamin B Group: Vitamin B group contains about twelve known vitamins. Each one performs specific function. They are collectively called vitamin B complex and are generally found together.

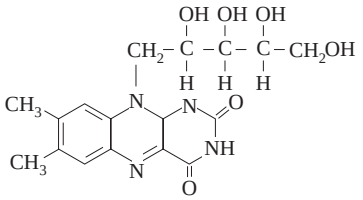
Thiamine or vitamin B: This vitamin is contained in yeast, milk, egg, peas and beans hence richly distributed in plants, animals and certain microorganisms. A deficiency of this vitamin causes a serious disease known as *beriberi.* Its symptoms are inflammation of nerves, muscular weakness and paralysis of limbs.



Thiamine

Thiamine functions as part of the coenzyme cocarboxylase which is the coenzyme for pyruvic decarboxylase and for several other enzymes which are necessary for the oxidation of pyruvic acid to acetyl—CoA and α—Ketoglutaric acid to succinyl—CoA (see Chapter 4).

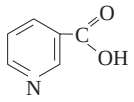
Riboflavin or vitamin B_2: This vitamin is widely distributed and the rich sources are liver, yeast, wheat germ, milk, eggs, and green leafy vegetables. Its deficiency causes cracks in the corners of the mouth and dermatitis of the face. The eyes become inflamed and there is dimness in vision. This vitamin is a part of two coenzymes, flavin mononucleoide (FMN) and flavin adenine dinucleotide (FAD) which are linked to proteins as flavoproteins.



Riboflavin

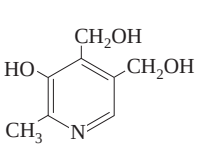
Nicotinic acid or niacin: Nicotinic acid is an essential dietary requirement. Tryptophan is its precursor which is converted into nicotinic acid through a series of biochemical reactions. Its deficiency in diet causes a disease pellagra which is characterized by dermatitis diarrhoea and nervous disorders. This vitamin (niacin) is a component of coenzymes like DPN and TPN which take part in oxidation-reduction of carbohydrates, fats, proteins and nucleic acid metabolism.

This vitamin is widespread, but the chief sources are meat, liver, yeast, beans and wheat germ.

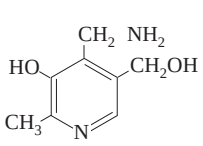


Nicotinic acid

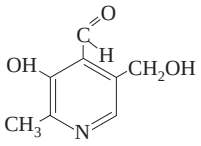
Vitamin B_6: This vitamin occurs in three forms; pyridoxine, pyridoxal and pyrodoxamine. It is an essential component of the coenzyme pyridoxal phosphate which is an important cofactor in the transmination and decarboxylation of all naturally occurring amino acids. The dietary requirement of vitamin B_6; in human beings is not yet fully established. It is synthesized by green plants and a number of microorganisms. The vitamin is found in many foods like liver, pork, kidney, yeast, egg-yolk, grains and various seeds.



Pyridoxine

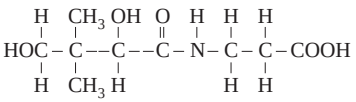


Pyridoxamine



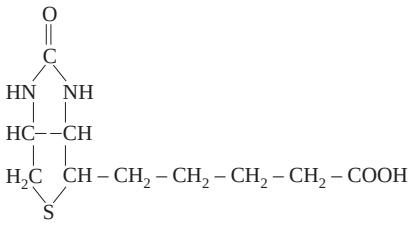
Pyridoxal

Pantothenic acid: The requirement of this vitamin in human beings has not been established so far, although it is essential for many other animals. Pantothenic acid is essentially a portion of the coenzyme. A molecule which has a key role in the metabolism of fats, carbohydrates and amino acids. Its role has been fully determined in rats where the lack of this vitamin induce retardation of growth, reproduction impairment and greying of black hair. The rich sources of this vitamin are liver, yeast, eggs and royal jelly. This is synthesized by green plants and some microorganisms.



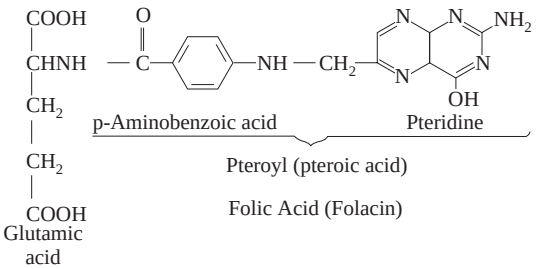
Pantothenic acid

Biotin: Biotin requirements in man have not been determined so far, but its importance in other animals has been amply demonstrated. However, recently it has been shown that this vitamin is necessary in fatty acid synthesis. It is also necessary for growth and respiration of certain species of bacteria. Rich sources of biotin are liver, yeast, kidney and egg-yolk. Some animals obtain their biotin requirements from the biosynthetic activity of their intestinal bacteria.



Biotin

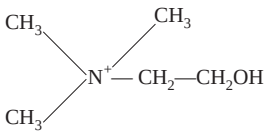
Folic acid: This vitamin exists in several different forms depending upon the biological source. Its importance has been established in a number of animals. Simplest type is the folic acid molecule called pteroylglutamic acid, which is isolated from liver. This contains glutamic acid, para-aminobenzoic acid and peteridine. The importance of folic acid is found in the enzymatic synthesis of serine from glycine. It is also necessary in the metabolism of tyrosine, ascorbic acid, biotin and vitamin B_{12}. It is chiefly found in green leafy vegetables, liver, yeast and kidney.



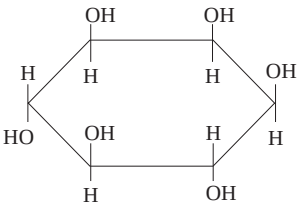
Vitamin B_{12}. This vitamin occurs in nature in a variety of forms. Its chemical structure is complex and contains a metal cobalt, cyanide, ribose sugar and other components. This vitamin has been

shown as a very important growth factor for many animals, man and microorganisms. A deficiency of this vitamin causes a blood disease called pernicious anaemia in higher animals. The vitamin is found in liver, kidney, meat and milk, etc., and takes part in the metabolism of proteins, fats, carbohydrates and nucleic acids.

Other B vitamins: Choline, inositol, lipoic acid, carnitine and pantothenic acid are important constituents of group B vitamins. Choline is a portion of lecithin, one of the cell phospholipids. A dietary deficiency of choline produces symptoms like deposition of excess of fat in the liver of mammals and shortening and thickening of bones in birds. One of the most important functions of choline is to provide a source for methyl groups (CH_3) in cell metabolism. It is abundantly found in egg, liver, meat, kidney and *Inositol* is a growth factor for several yeasts and fungi and also for human cell brain when in culture medium. In the intact organism (man) its necessity has not been determined so far. This is usually found in substances rich in calcium, phosphorus and magnesium. Its rich sources are milk, cereals, liver, sharks, etc.

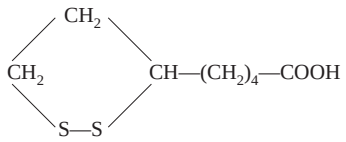


Choline

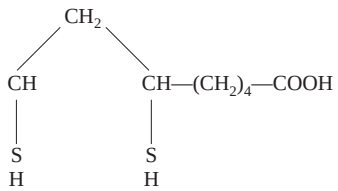


Inositol

Lipoic acid has not been shown to be required by animals in their diets although it is a growth factor for certain microorganisms. It is an 8-carbon compound containing sulphur. However, it functions in plants, animals and microorganism as hydrogen carrier in the metabolism of pyruvic acid.



Lipoic acid (oxidized form)



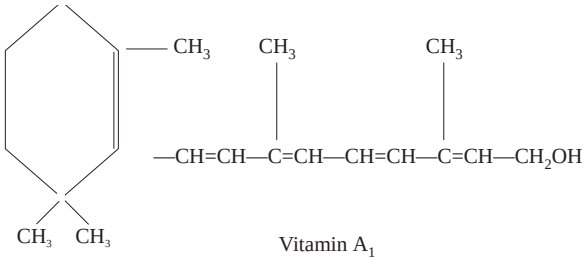
Lipoic acid (reduced form)

Carnitine is found to be necessary in certain insects and is responsible for the completion of their life cycle. Its functions are still not known.

Fat-soluble Vitamins

Vitamins A,D,E and K are fat-soluble vitamins. Their enzymatic role has not been elucidated so far due to their fat-soluble nature.

Vitamin A: It is available in several chemical forms and was recognized as early as 1913 by McCollum and Davis. It is determined to be a growth factor in rats. The carotenes of plants upon ingestion are converted into this vitamin. In most of the animals, a deficiency of this vitamin causes retardation of growth, drying out of epithelial cells and deposition of horny substances in the corner of the eye. The latter symptom leads to conditions of blindness (xeropthalmia). In man, its deficiency causes night blindness causing depletion of a red pigment called rhodopsin. Fish, liver, milk, cheese and vegetables are rich sources of this vitamin.



Vitamin A_1

Vitamin D: The vitamins D are all sterols and in nature they are chiefly found in animal organisms. These vitamins are formed from their provitamins which are also sterols. In mammals vitamin D can cure or prevent rickets—a disease in which bones fail to calcify. For this reason they are also called antiricketic vitamins.

The provitamin D_2 (ergosterol) occurs in plant kingdom (i.e., ergot and in yeast) and as such is available to animals through food. Man and other animals can synthesize provitamin D_3 (i-dehydrocholesterol). The provitamins D_2 and D_3 are then activated to form vitamin D_2 (calciferol) and vitamin D_3 (cholcalciferol) when the animal is exposed to ultraviolet rays. The activation takes place in the skin and the vitamins are subsequently transferred to various organs for utilization. A part of the vitamins D is stored chiefly in the liver, though skin, brain, lung, spleen, and bones also contain small amounts of stored D-vitamins.

Pure vitamins D are white, crystalline, odourless substances soluble in fats and fat solvents such as ether, chloroform, acetone and alcohol. They are resistant to oxidation, alkali and to temperatures below 140°C. In acid media these vitamins D are relatively unstable.

Functions of vitamins D: Vitamin D is essential for the normal growth of the bone. In case of deficiency of this vitamin, deposition of inorganic bone minerals fail to occur in the newly formed bone matrix, but the matrix continues to form. The provisional zone of calcification would no longer be clearly demarcated, but is irregular and deformed. In children rickets is a skeletal deformation and

can be noticed in the form of bowlegs, knock-knees, rachitic rosary (beaded appearance on ribs at the juncture of the rib bones and the costal cartilage), and pegion-breast. In adults it leads to late rickets (osteomalacia).

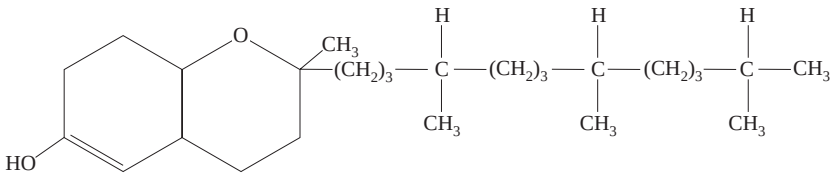
Another important function of vitamin D is to increase the intestinal absorption of calcium. To explain the mechanism by which the vitamin aids calcium absorption, two explanations have been given. According to the first, the vitamin is involved in active transport of calcium. The second explanation is that the vitamin increases permeability of cells of the mucosa to the mineral. It is believed that the vitamin aids not only absorption of calcium but also that of various other minerals such as magnesium, beryllium, zinc, iron, etc. Later these would be deposited in the bone.

In parathyroidectomized animals, vitamin D facilitates the excretion of the phosphate by the kidney and consequently the high serum phosphate level is lowered.

Sources of vitamin D: The main sources of vitamin D are fish, liver, oils and milk. Vitamin D is not widely distributed in nature but both provitamins are widely distributed. Although provitamin D_2 is a common occurrence in vegetation, vitamin D_2 is not present in living plants. Animals are the only source of 7-dehydrocholesterol. These provitamins require sunlight, to be transformed into active form.

Vitamin E: Vitamin E was discovered in the year 1922 and it has been known as antisterility vitamin. First discovered as an essential compound for the normal reproduction in male and female rats. Its absence causes death and resorption of foetuses, and testicular degeneration in rats. Compounds possessing vitamin E activity are chemically known as tocopherols. This name is derived from the Greek in which *tokos* means child birth; *perhos* means to bear; and the *suffix-ol* signifies an alcohol.

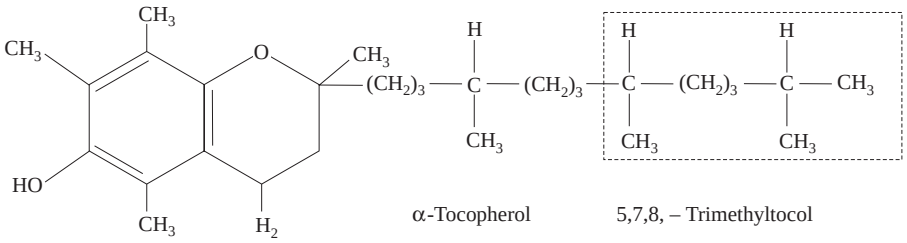
There are seven forms of tocopherols and each form, despite its difference due to the position of methyl groups, is called vitamin E. All these different E-vitamins are derived from the compound -*tocol*



The difference between the various forms of tocopherols is in the structure of the molecule. The various tocopherols are:

α-tocopherol:	5, 7, 8-trimethyltocol
β-tocopherol:	5, 8-dimethyltocol
γ-tocopherol:	7, 8-dimethyltocol
δ-tocopherol:	8-methyltocol
∈-tocopherol:	5-methyltocol
ζ-tocopherol:	5, 7-dimethyltocol
η-tocopherol:	7-methyltocol

Of these tocopherols, the α-tocopherol is most widely distributed among animals.



α-Tocopherol 5,7,8, – Trimethyltocol

Function of vitamin E: The absence of vitamin E in the diet hampers the normal reproduction in both the sexes in rats. In females it causes death and resorption of foetuses and in males it brings degenerative changes in the testes. In the mouse, only the female seem to suffer from this vitamin deficiency resulting in death and resorption of foetuses. However, in case of hamsters only the males affected and result in testicular degeneration. In cattle, sheep, or goats neither the deficiency nor the concentrated doses of this vitamin seem to have influence on the reproductive performance.

Vitamin E has a powerful antioxidant property and protects vitamin A, carotene and ascorbic acid from oxidative destruction both in the digestive tract and in the body tissues. As a result of this protection these vitamins retain their properties long enough to facilitate the body to use them more efficiently.

Vitamin E functions as a cofactor in the electron transfer system operating between cytochromes *b* and *c.* Its deficiency leads to uncoupling of oxidative phosphorylation.

In some species of animals vitamin E deficiency produces muscular distrophy primarily in the skeletal muscles. Intake of trocopherol-rich diets cures this trouble. The diets deficient in both sulphur-containing amino acids (particularly cystine) and vitamin E produced hepatic necrosis in experimental animals. Such a dietary liver necrosis can be prevented by providing the animals with adequate amounts of sulphur-containing amino acids, and vitamin E along with selenium.

Vitamin E increases the nucleic acid turnover rate in skeletal muscles.

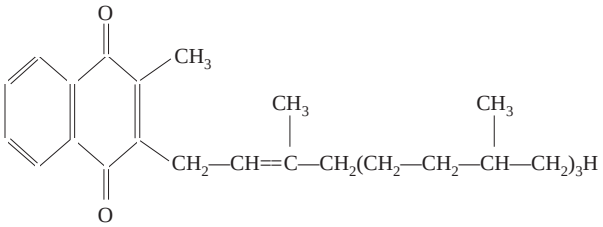
There is no clear evidence to suggest that vitamin E is essential to man. Even though its level in the serum is considerably reduced for 10 to 22 months, no significant clinical or physiological effects have been noticed. In human beings the administration of this vitamin neither brought any relief from sterility, nor cured human muscular distrophy.

A deficiency of vitamin E is unlikely to occur because of the ability of the body to store it. Storage is mostly in the liver though small quantities do exist in other organs and tissues.

Sources of vitamin E: Richest sources of this vitamin are vegetable oils such as wheat germ oil and cotton seed oil. Leafy-green plants and vegetables as well as whole grain cereals are also rich sources of vitamin E. Among animal products liver, heart, kidney, milk, and eggs are the best sources of vitamin E.

Vitamins K (Naphthoquinones): In 1929 Dam, a Danish scientist observed that the chicks raised on synthetic diet suffered from hemorrahage under the skin. In the year 1935 he identified this as due to the absence of an antihemorrhagic factor. When these chicks were fed with green leaves, the hemorrhagic syndrome disappeared. He therefore reasoned that these foods contained the

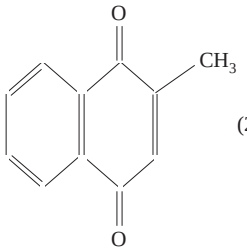
antihemorrhagic factor and named it as vitamin K—symbolizing the Danish term, "Koagulation Faktor". The vitamin was isolated in a purified form from alfalfa by Dam and his associates in the year 1939. In nature it occurs in two forms—as K_1 in green leaves and as K_2 when produced by bacterial synthesis. These K_1 and K_2 are derivatives of 2-methyl-l, 4-naphthoquinones and soluble in oils.



Vitamin K_1

The compound 2-methyl-1, 4-naphthoquinone is a synthetic form of this vitamin and is called menadione. It is also termed as vitamin K_3. It is water soluble and is more potent than the naturally occurring vitamins K.

Functions of vitamin K: The parenchyma in normal liver produces prothrombin which is one of the factors required for blood clotting. The function of vitamin K is to catalyze the synthesis of prothrombin. In the absence of vitamin K, hypoprothrombinemia occurs and this greatly delays the blood clotting. Administration of vitamin K alleviates hypoprothrombinemia if the hepatic parenchyma is healthy enough to produce prothrombin. In cirrhosis the parenchyma fails to produce prothrombin and in such a case vitamin K has no effect.



(2–methyl–1, 4–naphthoquinone
Vitamin K_3;(menadione)

It is known that vitamin K functions in electron transport and oxidative phosphorylation system in mitochondria. The exact location of vitamin K in the electron transport chain is not clear. Martius (1956-1961) suggests that first the cytochrome *b* oxidizes vitamin K and the latter, then, is reduced by a specific enzyme called the vitamin K reductase.

The vitamin K_1 is altered by the action of ultraviolet radiation. Rats when fed with sterilized food developed vitamin K deficiency and as a result the activity of oxidative phosphorylation was impaired. When supplied with vitamin K, oxidative phosphorylation was restored as usual. This suggests the important role of this vitamin in oxidative phosphorylation.

Normally, dietary vitamin K deficiency is unlikely to occur firstly, because it is fairly well distributed in foods and, secondly because the microorganisms in the intestinal tract synthesize considerable amounts of it. However, deficiency of this vitamin can occur when liver or gall bladder fail to secrete or pass bile fluid, the vitamin K like other fat soluble vitamins, cannot be absorbed through the intestine and this results in deficiency of vitamin K. Excessive use of sulpha drugs can destroy intestinal microorganisms helpful in synthesizing considerable vitamin K.

Sources of vitamin K: Green leafy tissues of plants are a good source of vitamin K.

2.5 MINERALS AND WATER

In addition to proteins, carbohydrates and lipids animal body requires mineral elements which serve structural and physiological functions. Calcium, phosphorus, sodium, potassium, magnesium, iron, selenium, molybdenum, manganese, copper, cobalt, zinc, sulphur, chlorine and iodine are the mineral elements which conduct certain essential functions in the animal body. A nutrient is said to be essential if its absence in the diet of the concerned animal prevents its growth, survival or the normal functioning. The essentiality of the above elements has not been tested for all species; however, all these are required by all higher animals. Besides the above mentioned, there are also other mineral elements in the body tissues. All these are available to animals through diet. Some of the elements are merely retained in the body while the essential function of a few others is yet to be discovered. Recent investigations have been bringing to light the useful functions of flourine and chromium. These two elements may also be entitled to be classified under essential elements.

Though all these elements are needed by the animal body, the quantity of requirement of certain elements such as calcium, phosphorus, sodium, potassium, magnesium, sulphur and chlorine is fairly large. Iron, manganese, copper, cobalt, zinc, iodine and molybdenum are required in minute quantities. Elements like selenium and chromium appear to serve certain functions in metabolic systems.

Minerals from organic complexes can be removed either by dry ashing or by wet ashing. In the former process the material is heated to high temperatures in muffle furnace, and in the latter process materials are dissolved in strong acids. In addition, there are other techniques for assaying trace minerals in organic complexes (Sandell 1959, AOAC 1960, and Oser 1965).

Calcium

Calcium goes in the formation of hard structures like bones and teeth and about 90 per cent of all body calcium is concentrated in these structures. Small amounts of calcium are present in blood, inter- and intra-cellular fluids, playing a fundamental role. About half of the calcium in these fluids is present in the form of free ions and this is essential for a variety of processes. A major role of calcium is in the regulation of ion transport across the cell membranes. Calcium exerts a profound effect upon neuromuscular irritability. High concentration of calcium stimulates the contraction of heart muscle.

Calcium level in the blood is maintained independent of its intake through diet. Serum calcium is maintained at the normal level by the parathyroid. If calcium level is low, the calcium from the bones is added into the blood. Certain enzymes—lipases, ATPase of actomyosin and myosin cholinesterase,

and succinic dehydrogenase—require calcium for their activation. Calcium is necessary for blood coagulation.

Phosphorus

Phosphorus accounts for 12 gm/kg of fat free tissue in the human body. Out of this about 85 per cent is present in skeletal tissues in the inorganic form. The total phosphorus content in both the plasma and the RBC may range from 30 to 45 mg/100 ml blood.

Organic phosphates are very much involved in the cellular functions in all cells. The high energy compound ATP supplying energy to all cellular activities contains phosphorus. Phospholipids in cellular membranes help in the permeability.

Phosphorus is a widely distributed mineral in the foodstuffs. Hence dietary deficiency of it is unlikely to occur in the human body. Grazing livestock depending on grass and herbage of the phosphorus deficient soils, lose appetite and appear emaciated. Such animals resort to eating materials such as bones, wood, clothing, etc. depending on accessibility.

Magnesium

The body magnesium is about 0.5 gm/kg of fat-free tissue. Of this, bones hold about 60 percent. Small amounts of magnesium is present in the extracellular fluid. The normal amount of magnesium is 1-3 mg/100 ml of serum.

Next to potassium, the concentration of magnesium is greater in the cells of the soft tissues, and a loss of magnesium would mean tissue breakdown and cell destruction. Magnesium is necessary in oxidative phosphorylation leading to the formation of ATP.

All enzymatic reactions requiring thiamine pyrophosphate (TPP) and the various reactions in the lipid and protein metabolism also need magnesium. Magnesium deficiency does not appear to occur in human beings because of their universal distribution in foodstuffs. Green vegetables contain fairly good amounts of magnesium. Severe diarrhoea or excessive vomiting however, cause magnesium deficiency in human beings.

Magnesium deficiency brings about personality changes, muscle tremor, gastro-intestinal disturbances. Decrease in magnesium also brings down the levels of serum calcium and potassium.

Sodium, Potassium and Chlorine

These, unlike the previously described minerals, are largely present in the fluids and soft tissues. Maintenance of osmotic pressure and acid-base equilibrium, regulation of the movement of nutrients into the cells, and participation in the water metabolism are some of the functions carried out by these minerals. These minerals need to be regularly taken through diet because the body has limited storage capacity. When the availability of these minerals to the body is limited, they are excreted in lesser quantities. The body thus conserves them. The deficiency of any one of these elements is followed by lack of appetite, loss of weight and production in the adult, reduction in growth, and decreased blood levels.

Sodium: The body contains approximately 1.8 gm of sodium per kg fat-free body weight. Although a larger proportion of it is found in the extracellular fluids, studies indicate that some of the sodium is bound in the bones. Sodium together with calcium, magnesium and potassium in the extracellular fluid are basic in reaction. Sodium forms about 93 percent of the bases in the blood serum and hence is highly concerned in maintaining neutrality.

Sodium is capable of passing across the cell membrane. During the process of nerve transmission and muscle contraction, a temporary exchange of extracellular sodium and intracellular potassium takes place. Subsequently this sodium is pumped out of the cell.

A dietary deficiency of sodium does not occur in human beings. His diet generally contains more sodium than necessary. Sodium is readily absorbed and it circulates through the entire body. It is excreted through the kidneys as chlorides and phosphates. Aldosterone, a hormone of the adrenal cortex is responsible for the reabsorption of sodium from kidney tubules. Absence of this hormone increases sodium excretion and brings out deficiency symptoms. A major portion of sodium is lost in man at hard work, particularly in summer. Vomiting, diarrhoea, or profuse sweating would result in increased loss of sodium. A lack of this mineral would also reduce the utility of digested protein and energy and prevents reproduction.

Potassium: The human body contains about 2.6 gm of potassium/kg fat-free body weight. Unlike sodium a larger proportion of potassium is present as a chief cation in the intracellular fluid. The body conserves more potassium than sodium. Potassium is concentrated mainly within the cells. Potassium aids in the maintenance of osmotic pressure and acid-base balance in the cells-sodium and chloride mostly located outside the cells, potassium inside the cells.

Potassium is required in carbohydrate and protein metabolism, in the formation of glycogen, and in the degradation of glucose. Investigations suggest that potassium is an activator of enzymes. It may play an important role in the amino-acid uptake by the cell.

Potassium transfer across the membrane takes place more easily than that of sodium. Most of excessively absorbed potassium is normally excreted through urine and sweat.

Like that of sodium, the potassium deficiency does not occur in human beings under normal conditions of health. Hypopotassiemia results due to excessive excretion of potassium through the kidney. Body burns, excessive vomiting and diarrohea also result in the loss of potassium. Such a loss is supplemented by the depletion of body potassium.

Potassium deficiency is characterized by muscular weakness; and weakness of skeletal muscle results in paralysis. Its deficiency in chicks retards growth, incapacitates legs and finally leads to death.

Chlorine: Unlike sodium and potassium, chlorine is distributed in large concentrations both in intracellular and extracellular fluids. It is the chief anion of the extracellular fluid and a greater part of it occurs in combination with sodium. A small amount. i.e. about 15 to 20 percent of the chlorine is in combination with protein and other organic substances. Chlorine with phosphate and sulphate groups, and protein is acidic in reaction. The chlorine transfer between the serum and erythrocytes is easily performed and this phenomenon is termed as the chloride shift (Chapter 10). This is an example of homeostatic mechanism by which the pH of the blood is maintained. In addition, chlorine is an

essential component of the gastric hydrochloric acid, and activates the amylase of saliva for the starch splitting process.

Dietary deficiency of chlorine is unlikely to occur owing to its abundance in the normal diet. Moreover, the body is capable of storing certain amount of chlorine in the skin and subcutaneous tissues. The chloride content of a teaspoon full salt is about 4.2 gm. The chlorine transfer across the membranes generally takes place by passive diffusion. However, in gastric and intestinal mucosa the transfer is by active transport.

The same factors causing sodium loss are responsible for chloride loss. However, its loss due to vomiting is high because of excessive loss of hydrochloric acid from the stomach.

Iron

The iron content in human body is about 75 mg per kg of fat-free body weight. It is widely distributed throughout the body. Though this element is present in small quantities, it plays a key role in life processes. It is a constituent of the respiratory pigment, the haemoglobin. The haeme molecule is composed of ferrous or ferric iron at the centre of a porphyrin ring. *Four* porphyrin units are bound to the protein globin forming haemoglobin. The haeme molecule is also a component of cytochrome C peroxidase, catalyse, and other enzymes. Iron is also present in the plasma bound to a specific globulin called transferrin. About 55 to 60 percent of the body iron is in the blood.

Some iron is also present in the myoglobin-a compound present in skeletal and heart-muscle; and it has greater affinity to oxygen. A considerable amount iron (about 26 per cent of total body content) is stored in the liver and secondarily, in the spleen, in the kidneys and in the bone marrow.

Intestine, excepting the colon part, is capable of absorbing iron. The absorption is highest in the duodenum and it decreases progressively towards ileum (Brown, 1963; Moore and Dubach, 1962). The absorption is efficient in the ferrous (reduced) state. The absorbed iron goes directly into the blood. Once iron enters the blood stream it would be held by the body. The excretion of iron through the blood in the intestine is very minute. Iron excretion through urine is less than 0.2 mg per day. The iron released from the breakdown of RBC is saved and reused.

The absorbed iron would leave the body in significant quantities as a result of loss of blood. Iron deficiency anemia occurs in women and children due to the lack of building stone necessary for haemoglobin synthesis. Iron deficiency anemia is the commonest of the nutritional anemias. Its deficiency may be due to dietary inadequacy or due to poor absorption, or due to excessive loss of blood. The haemoglobin level of a person with iron deficiency is lower than the normal, and the size of the RBCs are smaller than normal (hypochromic nicrocytic anemia). As a result of this condition the oxygen carrying capacity is lessened, and the tissues receive less oxygen resulting in fatigue.

Iron requirement through diet varies in various animals. Chickens require 80 mg per kg of diet, while pigs need 80 mg per kg. Ruminant's requirement varies between 25 to 40 mg per kg. Young people between 15 and 18 years of age and women between 18 and 55 of age require an intake of 15 mg of iron per day. Adults require 10mg per day.

Liver, as food, is an excellent source of iron. Meat products and eggs also obtain iron in generous amounts. Iron content of leafy-green vegetables is fairly good.

Sulphur

The body has about 0.15 percent of sulphur. Sulphur is principally located in the sulphur-containing amino acids, i.e. cystine and methionine. This element is also present in saliva, bile, glutathione and insulin, but these are synthesized in the body with the help of cystine and methionine.

Sulphur is present as chondroitin sulphate in the cartilage. It is also present in minute quantities in the blood. Thiamin and biotin also have small quantities of sulphur but these vitamins are not synthesized inside the body.

Sulphur is excreted through faeces and urine. In urine it is present as inorganic sulphates, ethereal sulphur and neutral sulphur. Neutral sulphur occurs in the form of cystine, thiosulphates, and other compounds.

Little is known about the effects of sulphur deficiency in man and animals. Wool contains about 13 percent of cystine. However, cystine feeding did not, improve the wool production in sheep. There is also no evidence of any relationship between the dietary deficiency of sulphur and the lack of hair growth in humans.

Body acquires sulphur in the form of organic complexes, i.e. amino acids. These amino acids are constituents of proteins and hence available to the body through protein diet. Wheat germ, cheese, kidney beans are very rich in sulphur.

Trace Elements

Body contains many trace elements, of which only a few are known to take part in cellular metabolism. Dietary deficiency of iodine and cobalt are known to obstruct normal physiological functioning. Trace elements are required in minute quantities. They are widely distributed in most of the foods and hence are available to the body in sufficient quantities. The very fact that these trace elements are in small amounts in the body suggests that they play primarily catalytic roles in the cellular metabolism.

Deficiencies of some trace elements have been reported in cattle grazing on pastures grossly deficient in these elements. Excess intake of certain trace elements may result in toxic effects. Studies on trace mineral metabolism were handicapped by two basic problems. First being the precise determination of infinitesimal quantity of trace element in foods, blood and other tissues. Second being the total purification of the diet from the trace element. Essential nutrient is that substance which by its absence in the diet of experimental animals would affect growth, survival or the normal functioning.

Copper

The human body has about two mg of copper per kg of fat-free body weight. Though present in all body tissues, copper is observed in highest amounts in brain, heart and kidneys.

Copper deficiency causes an anemia in which the erythrocyte synthesis and the level of total body iron is reduced. Inadequate copper in sheep, cattle and pigs results in abnormalities in bone structure. Extreme loss of protein under certain pathological conditions would result in low copper level in serum. Evidence on specific dietary deficiency of copper in human body is lacking. However, there are reports when anemia in children has responded to the administration of copper but not to iron.

Manganese

Very low concentrations of this mineral are found in all animal tissues, but high concentration of it is present in pituitary, liver, pancreas, skin, bones and muscles. Highest concentration of manganese is in the bone.

Manganese is required in the body since it can replace magnesium as cofactor for certain phosphorylations. It is probably also required to incorporate acetate into fatty acids, and in the conversion of mevalonic acid to squalene in the cholesterol synthesis.

Dietary deficiency of manganese is unlikely to occur in humans, but such a deficiency has been demonstrated in rats, chicks, pigs, etc. In rats, its deficiency effects the normal process of reproduction and lactation. In such cases the females fail to suckle their young, and the males suffer degeneration of reproductive organs. In chicks the deficiency results in an abnormality of leg bones known as *perosis* or slipped tendon. The leg bones shorten and undergo physical and chemical changes.

High intake of manganese retards growth in rats; and in dogs it causes only gastric disturbances. Men inhaling ore dust containing manganese oxide develop manganese toxicity, the symptoms of which are a peculiar mask-like expression of the face, involuntary laughing, low voice with indistinct speech, spastic gait, and tremors of the hands.

Manganese requirement in man is not known. The average diet of man contains about 4 mg of this substance per day. Wheat bran, blueberries, whole wheat are the richest sources of manganese.

Iodine

Iodine is an important dietary nutrient required for a normal functioning of thyroid gland. Body contains infinitesimally small quantity of iodine. The quantity of total iodine in the body varies from 20 to 30 mg. One-third of this is concentrated in the thyroid gland. Next to thyroid, highest concentrations of iodine have been found in ovary, muscles, and blood. Small quantities of the remaining iodine are present in the other tissues.

Though this element is observed as iodine, in the thyroid gland it quickly gets oxidized to iodine and goes in the formation of thyroglobulin. The thyroid gland serves as a store house for iodine. The body conserves some iodine when thyroxine, one of the iodine containing compounds breaks down in the normal process. This is again reused by the body. Iodine is excreted chiefly through the kidney. It is also excreted through intestine, and through the skin by way of perspiration. Iodine is secreted into the milk during lactation.

Thyroxine and other compounds of thyroid gland which contain iodine as an essential component, serve important physiological functions. The function of thyroxine is therefore attributed to that of iodine. Increase in the secretion of thyroid hormone would speed up the rate of oxidation in the cells. In the absence of thyroxine, the rate of energy metabolism is retarded. Thus the rate of metabolism or the basal metabolic rate is an indicator of the normality of thyroid function. Hypersecretion of the hormone increases the basal metabolic rate and hyposecretion results in low rate.

Thyroxine is also essential for the normal growth and development. Undersecretion of thyroxine retards growth and a prolonged undersecretion prevents physical and mental maturity. In children

thyroxine deficiency causes cretinism. Its symptoms are retarded growth; arrested development; coarse and swollen facial features; thick, dry wrinkled skin; enlarged tongue; thickened lips and partly opened mouth. In adults the deficiency causes myxoedema which is symptomized by the thickening of subcutaneous tissues, in particular that of face and extremities. The face is expressionless and the person becomes lethargic. Besides, iodine is also necessary for normal reproduction. Absence of iodine supply for a prolonged period may result in sterility or the birth of deformed progeny. Lack of iodine in the foods is due to deficiency of this mineral in the regional soil where they are grown. This causes simple goiter which is endemic. Endemic goiter can be cured by constantly providing iodized table-salt in the food.

Iodine content in foods is extremely small and a quantitative determination of this element is possible only by sensitive chemical method. Its content in foods varies greatly and is dependent on the soil condition. Marine or deep-sea fishes and shell fishes have high iodine content. Anadromous fishes (salmon, sea trout, etc.) have higher iodine content than those fishes that live all the time in fresh water. The leaves of vegetables such as spinach, turnip, and broccoli have higher iodine content than in their roots.

Zinc

Animals and plants have small quantities of zinc in their body. Most of this mineral is present in the liver, bones, and blood. The exact function of this element in the body is unknown, though its presence is reported in several enzymes and hormones. The respiratory enzyme carbonic anhydrase present in the RBCs contains zinc. The zinc in this enzyme hastens the breakdown of carbonic acid in lungs in the process of exchanging carbon dioxide for oxygen. Zinc is an essential component of one of the protein splitting enzymes of the pancreas. Several dehydrogenases present in the liver also have zinc in their structure. Zinc is present in the crystalline structure of insulin. Of the several types of insulins manufactured, the protamine-zinc insulin is in wide use, because in this form the insulin is absorbed more slowly into the tissues.

Zinc deficiency in rats and mice results in the reduction of growth rate, and loss of hair around neck and shoulders. In pigs it causes parakeratosis—the symptoms of which are retarded growth, a lesion of the horny layer of the skin, and lowered feed utilization. In chicks its deficiency symptoms are slow growth, shortened and thickened leg bones and poor feathering. Calves supplied with low zinc ration develop alopecia, parakeratotic skin lesions.

High level of zinc in the body would cause zinc toxicity, the symptoms of which are growth depression, anemia, and decreased copper level in the liver. When in excess, it interferes with the function of copper in the formation of iron-porphyrin compounds, and thus leads to anemia. Excessive amounts of zinc also interfere in the iron metabolism. Zinc deficiency is unlikely to occur in animals and man, firstly because of its presence in most natural diets and secondly because of its retention power. Oysters, wheat germ, and brain are richest in zinc. Fruits and vegetables contain only small amounts.

Cobalt

Animal body requires cobalt in small amounts and gets it through the diet. Cobalt is present as a part of the vitamin B_{12} and this is synthesized in the rumen with the help of bacteria. This vitamin is not

present in the plant foods consumed by the ruminants. Since vitamin B_{12} is synthesized in the body it is not a dietary essential vitamin and thus cobalt is necessary in the formation of RBCs.

The cobalt requirement in animals varies. The requirement of cobalt in mg per kg of ratio is 0.05-0.07 in cattle; 0.08 in sheep and 5-8 in the case of horses. Animals grazing in deficient soils do not get cobalt and consequently they fail to synthesize vitamin B_{12}. In case of cattle and sheep, absence of vitamin B_{12} leads them to restlessness, loss of appetite and weight, weakness and anemic, and finally to death.

If cobalt intake exceeds the normal requirement, the RBC number in blood increases. This increase is called *polycythemia* and has been observed in rats, guinea pigs, rabbits, dogs, pigs, children and man. Polycythemia is a normal occurrence in people living at high altitudes and this helps them cope with the lower percentage of oxygen there. The daily need of cobalt in different animals has not been established. The average diet normally supplies the required amounts of cobalt to man.

Molybdenum

Molybdenum is another trace element which is found essential in nutrition. Molybdenum is an essential factor for the formation and maintenance of xanthine oxidase of some animals. In man the function of this mineral is yet to be known. This enzyme, essential in the oxidation of aldehydes and purines, is present in liver and intestinal tissue, and also in milk. Molybdenum is an essential nutrient and is always available to the animals through their diet. For this reason neither animals nor man show the symptoms of molybdenum deficiency. However, excess intake of this element causes reduced growth rate and death in rats; retarded growth, loss of weight, low haemoglobin and RBC counts, alopecia and malformed leg bones in rabits; loss of weight and change in hair coat in calves.

Legumes, cereal grains, dark green vegetables liver and kidney, are rich in molybdenum.

Selenium

Dietary intake of traces of selenium is required in certain animals.

An unidentified factor found in certain foods (milk, brewer's yeast, meat, and some kinds of cereals) is capable of preventing ill effects caused by vitamin E deficiency in rats and chicks. This is termed factor 3. From this factor, Schwarz and Foltz (1957) isolated selenium. Minute amounts of sodium selenite are found to be as effective as the vitamin E in the prevention of liver necrosis in rat, mouse, and pig. Selenium salts are also effective in the prevention of exudative diathesis in chicks, and the muscular distrophy in lambs.

The mechanism of selenium function is not completely established, but it clearly has a role in metabolism of tocopherol compound.

Selenium toxicity in farm animals grazing on selenium rich soils is well known. The symptoms of selenium poisoning are emaciation, loss of hair and hoofs, cirrhosis of liver, and skeletal erosions.

The toxicity seems to be due to inhibition of certain enzyme systems. Linseed oil meal, arsenilic acid, and organic arsenicals effectively counter the selenium toxicity.

Chromium

Schwarz and Mertz (1959) suggested that trivalent chromium is an essential dietary requisite in rats. Chromium probably acts as a cofactor with insulin in carrying out the glucose metabolism. Chromium deficiency retards growth in male and female rats and results in a syndrome similar to that caused by diabetes mellitus (Schroeder, 1966).

Water

Water is an essential liquid present in all cell structures. About 65 percent of the body weight is water. Two-thirds of this is contained within the cells and the remaining is present in spaces outside the cell. It is the medium in which chemical reactions required in cellular metabolism take place and hence it is the most essential of the nutrients in all animals.

Water is available to the body through the mineral water and solid foods taken in by the animals. About 15 percent of the daily requirements of water is gathered by the body as a result of the oxidation of foodstuffs within its cells.

Water is the solvent for more substances than any other liquid. It is an ideally suited medium for the transportation and distribution of nutrients to all the cells in the body. Water regulates the body temperature by conducting and distributing heat energy to the entire body. It removes, by vaporization, the excess heat of the body generated by metabolic reactions.

Water is lost from the body due to profuse sweating, diarrhoea, and prolonged and frequent vomiting. As a result of water loss, dehydration and loss of electrolytes would result. Animals can live longer periods without food, but without water they die soon.

CHAPTER

3

Biological Oxidations

All living systems require energy to carry out life processes. The most common and cheapest source of energy is the sun. The solar energy is initially utilized by the chlorophyll of green plants and some bacteria to synthesize carbohydrates in the form of starch. This process is known as photosynthesis. Green plants are eaten by animals and the stored carbohydrates serve as the basic source of energy.

Cells of plants, animals and bacteria are primarily composed of polymers such as carbohydrates, proteins, fats and fatty acids. These biopolymers are large molecular complexes which upon oxidation yield energy. The maintenance and growth of cellular structures is dependent upon the energy-requiring or *endergonic* reactions by which these large polymer complexes are synthesized. Endergonic reactions store up energy in a potential form in the products of the reaction. Photosynthesis is an ideal example of such endergonic reactions. Energy is, however, required for other functions as well, such as endergonic chemical reactions. Biological systems also require *exergonic* reactions which are energy-yielding processes. Energy thus liberated is used up for growth, chemical synthesis, muscle contractions, maintenance and repair of body parts and other protoplasmic activities. Therefore, a biological system must provide the energy necessary for its maintenance, growth, and various other processes. Biological oxidations are the most important exergonic reactions of the living matter which furnish chemical form of energy to build and maintain structure.

3.1 BIOENERGETICS

Energy is defined as the capacity to do work. It is a common knowledge that energy can neither be created nor destroyed, but can be transformed from one form to the other. Various forms of energies exist, such as thermal, mechanical, electrical and chemical and it is the transfer of energy through one of these means that work can be performed. Muscular work, conduction of nerve impulse and synthesis of complex food molecules are some of the examples in which energy transfer is involved. Living organisms, therefore, cannot consume energy, but they can transform it involving oxidation,

reactions. Such transformations are accomplished at rather constant temperature and under constant pressure-volume relationships.

Before discussing energy in biological systems, it would be appropriate to discuss the general laws of thermodynamics which govern all energy transformations. The laws which govern the behaviour of all energy in the universe are the first and the second laws of thermodynamics. The physical and chemical events taking place in the universe are under the control of energy contained in the universe. Both matter and energy must be exchanged between the system and surroundings.

The first law of thermodynamics states that *the energy content of the universe must remain constant* since it can neither be created nor destroyed. Biological systems absorb from their environment useful form of energy under constant temperature and pressure and return the same amount of energy of less useful form to the environment. Thus useful form of energy absorbed by biological systems is called the *free energy* which is capable of doing work.

Biological systems have a high molecular complexity and orderly structure, whereas the non-living matter is in a state of disorder or randomness. The second law of thermodynamics states that the *randomness or entropy of the universe always increases*. Living organisms maintain their orderliness at the expense of their environment and in return increase its entropy.

Living organisms are called *open systems* since they exchange both matter and energy with the environment. Although apparently a living system may seem to be in equilibrium, but it is not; it is actually in a steady state. Steady state is that condition of an open system in which the rate of transfer of matter and energy from the environment into the system is balanced by the rate of transfer of matter and energy out of the system.

Living organisms are unable to use heat as a usable energy, since they are essentially isothermal. In man-made machines, such as steam engines, heat energy is transformed into work. This may be expressed as follows:

$$\Delta E = q - w$$

Where ΔE is the change in the energy of the system, q is the increase in heat and w is the amount of work done by the system. The energy change accompanying a chemical reaction may be measured in the form of heat gained or lost and is called *enthalpy* change. This can be expressed as H or the heat of reaction. In living cells heat is not used as a source of energy because heat can do work at constant pressures only. If in a reaction there is no change in pressure, no work is accomplished, then

$$\Delta H = \Delta E$$

Organic molecules like carbohydrates have a heat of combustion which is characteristic of any given molecule. If 1 mole of glucose is completely oxidized aerobically, the heat evolved or the molar enthalpy can be measured. This is expressed as

$$\Delta H = -686{,}000 \text{ cal/mole}$$

In this case ΔH is negative since heat is lost during the reaction

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 686 \text{ kcal}$$

We have discussed earlier that the entropy content of the universe always Increases. If the entropy of a system increases during a process, the amount of useful energy contained in the system decreases.

Thus higher the degree of order, lower the entropy. The free energy which does useful work is designated is ΔG after Willard Gibbs, who developed the concept. The equation representing relationship among free energy, enthalpy and entropy may be expressed as:

$$\Delta G = \Delta H - T \Delta S$$

where

ΔG is change in free energy.

ΔH is change in enthalpy.

ΔS is change in entropy.

T is absolute temperature.

A drop in free energy is followed invariably by an increase in entropy. The change in free energy of a reaction is an index of its ability to do useful work. Exergonic reactions proceed spontaneously and have a negative ΔG value. Endergonic reactions do not proceed spontaneously, require chemical energy input and have a positive ΔG value.

3.2 TYPES OF REACTIONS

A most accurate definition of oxidation is rather impossible. However, a compound is said to be oxidized if the following take place:

(a) when it loses one or more electrons, for example

$$Fe^{++} \xrightarrow{-e} Fe^{+++}$$

(b) when the compound loses one or more atoms of hydrogen, for example

$$CH_3CH_2OH \xrightarrow{-2H} CH_3CHO$$

(c) When one or more atoms of oxygen are added to the compound, for example

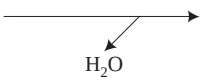
$$CH_3CHO \xrightarrow{+O} CH_3COOH$$

Reduction reactions are reverse of oxidations.

All the chemical reactions in the cells are varied and frequently complex, but are restricted to these three simple classes. In order to understand them better, we may classify them into five general groups: digestion, synthesis, transfer, oxidation and reduction.

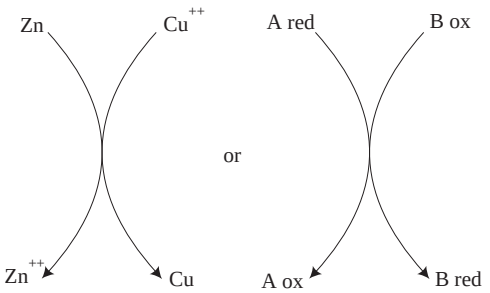
(a) A digestion reaction is actually a hydrolytic reaction in which complex molecules are broken down to smaller sub-units. Degradations of carbohydrates, fats and proteins are common examples of such digestion reactions.

(b) In synthetic reactions smaller molecules are combined into larger ones with loss of water:



(c) In transfer reactions a portion of one molecule is transferred to another molecule.

(d) Oxidation and reduction reactions always occur together involving change in the number of electrons. If the number of electrons decreases, the atom is said to be oxidized, whereas increase in the number of electrons indicates reduction. If oxidation occurs, reduction must follow it. An example of this reaction is when metallic zinc is added to an aqueous solution of copper sulphate. The zinc is oxidized to zinc ions at the expense of copper ions which are reduced to metallic copper.



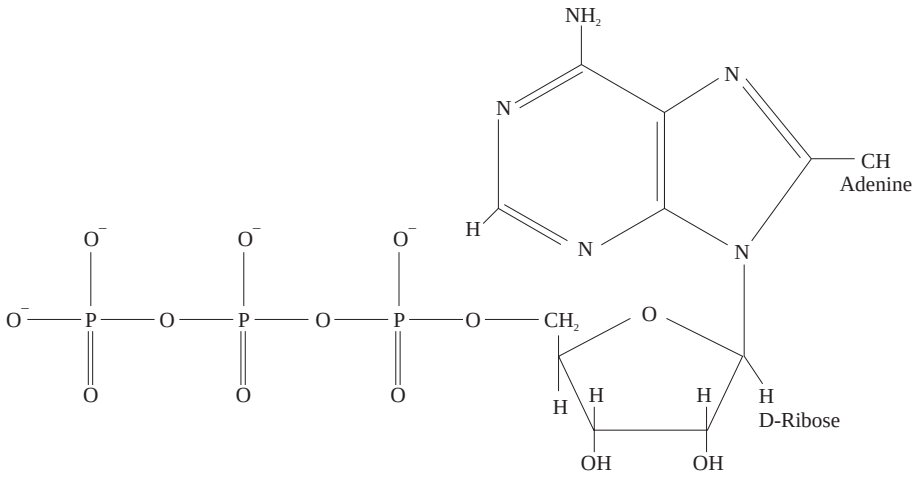
3.3 COUPLED REACTIONS

The biological systems are dependent upon coupling of energy between oxergonic and endergonic reactions. In coupled reactions there is always a molecular form which donates energy and another molecular form which accepts energy. It is necessary that we examine some of the exergonic reactions which provide usable energy to the living system to derive endergonic reactions.

It should be stated here that the carbohydrates, fats and proteins are not the immediate fuels that run the living system, instead ATP (adenosine triphosphate) performs this function. ATP is the most common and universal energy donor belonging to the class of nucleoside triphosphates. The chemical structure of ATP is given in Figure 3.1. ATP consists of a nucleoside (adenine 5-carbon D-ribose) and three phosphate molecules attached to it. The phosphate ester linkage (P—O—P) between the two terminal phosphate groups of ATP is relatively weak. This terminal phosphate group breaks spontaneously from the kinetic energy of the molecule when ATP is in complex with an enzyme. The breakage of the phosphate bond releases chemical energy causing an immediate shift in the bond energies within, giving rise to ADP (adenosine diphosphate). About 10 kcal/mole of energy is released.

$$\text{ATP} \underset{\Delta G = +10 \text{ kcal/mole}}{\overset{\Delta G = -10 \text{ kcal/mole}}{\rightleftharpoons}} \text{ADP} + \text{Pi}$$

When the terminal bond of ATP is broken down, the phosphate becomes inorganic phosphate (Pi) which is endowed with low energy. The reaction is reversible and the formation of ATP requires ADP



Adenosine Triphosphate (ATP)

Fig. 3.1 Structure of ATP.

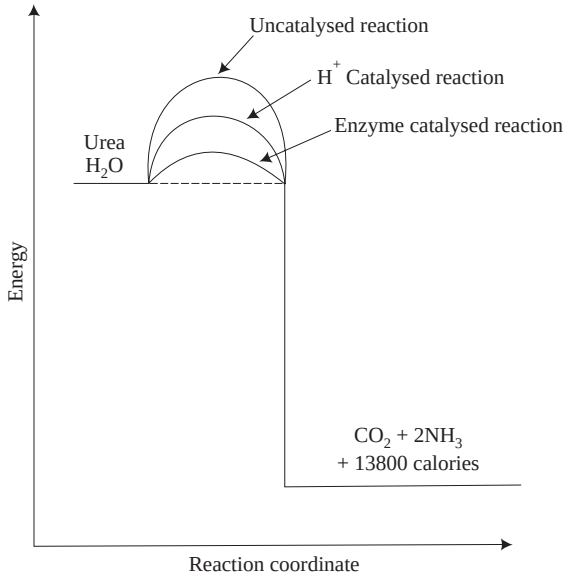


Fig. 3.2 Enzyme catalysed reaction.

and inorganic phosphate. The regeneration of ATP requires 12 kcal/mole and occurs in the coupled reactions.

ATP is universal energy donor, hence it should be regenerated constantly when any mechanical work is done. In the coupled reaction as shown above, 10 kcal/mole of energy is released from one

ATP molecule, showing thereby a loss of 2 kcal/mole of energy during regeneration. When a phosphate bond is broken, 10 kcal/mole of energy is released, but only 8 kcal/mole is converted to work and the rest is lost. Thus ATP can derive most of the metabolic reactions requiring 8 kcal/mole or less.

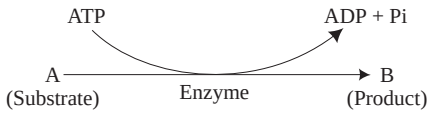
3.4 ENERGY EXPENDITURE IN METABOLIC PROCESSES

The cell machinery functions on maximum economy basis, hence the energy expenditure is to be minimized in a metabolic process. This is achieved by the use of enzymes which help in lowering the energy of activation (Fig. 3.2). Enzymes are referred as biological catalysts which obey certain general rules. The enzyme-catalyzed reactions take place at physiologically low temperatures and require extremely small amounts of enzymes.

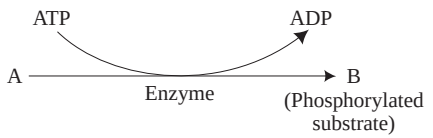
If a glucose molecule is subjected to a series of enzyme-catalyzed reactions, the molecule is broken down with minimum expenditure of energy. Sometimes the energy required for the reaction is present in the kinetic form in the reaction itself, but more frequently, the enzyme acts in combination with ATP requiring low activation energy. Both synthetic and degradative pathways require the use of ATP. In synthetic reactions the ATP molecule combines with the enzyme at one of the active sites and the substrate combines with other sites. This can be shown as:

$$\text{Substrate} + \text{enzymes} + \text{ATP} \rightarrow \text{ATP} - \text{enzyme} - \text{substrate} \rightarrow \text{ADP} + \text{Pi} + \text{enzyme} + \text{product}$$

Since the reaction is enzyme mediated, it can be simplified thus:



In degradation reactions, the terminal phosphate group of ATP (A – P – P – P) is usually transferred to the substrate which gets phosporylated. This may be shown as:



Since ATP is used both in synthetic and degradative reactions, its regeneration is essential for efficient functioning of the biological systems (Fig. 3.3). Regeneration of ATP is possible in two ways: (i) by transfer of a phosphate group from a high-energy molecule (substrate-level phosphorylation) and (ii) by electron transport system.

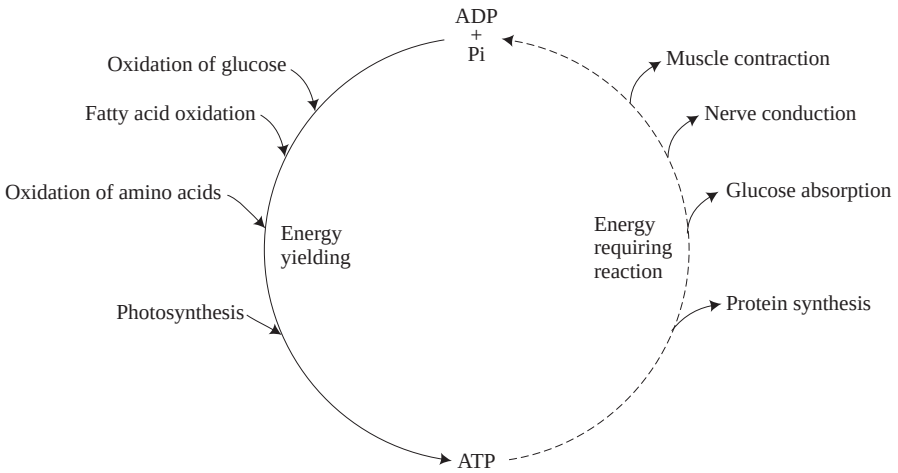


Fig. 3.3 ATP cycle in energy-transfer processes.

3.5 OXIDATION-REDUCTION REACTIONS

One of the major classes of reactions is the oxidation-reduction reaction in which electrons are transferred from one atom to another. We have already noted that oxidation involves loss of electrons whereas reduction involves gain of electrons. This may be shown as:

$$Fe^{++} \underset{\text{Reduction}}{\overset{\text{Oxidation}}{\rightleftharpoons}} Fe^{+++} + e^{-}$$

In some of the redox reactions, the values of ΔG are very high, hence they may serve as ATP regeneration reactions. In all redox reactions, a reducing agent (electron donor) and an oxidizing agent (electron acceptor) are present. During the reaction, reducing agent gets oxidized and the oxidizing agent gets reduced and this is dependent upon the ability of reducing agent to furnish electrons and the tendency of oxidizing agent to accept them. This relative ability to donate and accept electrons is called *redox potential*. It is represented by the following equation:

$$E_h = E_o + \frac{RT}{nF} \ln \frac{(\text{oxidant})}{(\text{reductant})}$$

where

E_h = the redox potential
E_o = the standard emf of the system
R = gas constant in joules/mole/degree
T = absolute temperature
n = valence of the ion or number of electron equivalents

F = Faraday (96,500 coulombs)

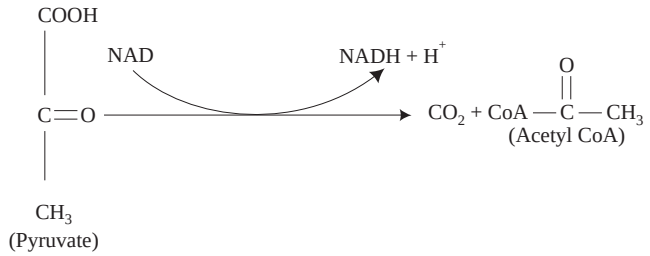
ln = logarithm to the base e

When the concentration of oxidant and reductant are equal, In (oxidant/reductant) becomes 0 and $E_h = E_o$. The redox potential values as cited above are applicable when the pH value is 0, otherwise it would vary with the change in pH. The E_o of the hydrogen at pH 7.0 is –0.420 V (redox potential of hydrogen at pH 0 is 0.000).

3.6 THE CYTOCHROME SYSTEM

The transfer of electrons occurs on an atomic level. Whether the atom should act as a reducing or oxidizing agent depends on the structure of the molecule. The cytochromes are a group of complex types of molecules belonging to the class of porphyrins. They have an atom of iron (Fe) held in the porphyrin ring structure. An important property of the cytochromes is their ability to undergo reversible oxidation involving a change in the valency of iron.

A variety of cytochromes have been identified from plants and animals. In mammalian cells, five types of cytochromes have been identified: Cytochromes b, c_1, c, a and a_3. These have important roles in the oxidation reactions. In biological systems, reactions involving the removal of hydrogen atom occur most frequently and this hydrogen atom is transferred to the hydrogen acceptor. NAD (nicotinamide adenine dinucleotide) is the common hydrogen acceptor which acts as an oxidizing agent. In the conversion of pyruvate to CO_2 and acetate, role of NAD can be indicated:



In the above reaction, the carboxyl group is lost by the pyruvate and the remaining 2-carbon portion is joined to coenzyme A. When NAD accepts a hydrogen, it also involves ionic bonding to a second hydrogen, giving rise to product NADH + H^+:

$$\text{NAD + pyruvate} \rightarrow \text{NADH} + H^+ + 2e^- + CO_2 + \text{acetate}$$

Regeneration of ATP is dependent upon reactions involving removal of hydrogen. The oxidizing agent NAD has an E_o *value of* –0.320, whereas the reducing agent pyruvate has an E_o value of –0.700. There are many compounds in biological systems which have more positive redox potentials than NAD and NADH. When NAD reacts with such compounds, it serves as a reducing agent in its NADH + H^+ form. NADH (reduced form) enters into a series of reactions involving transfer of electrons. In the course of reactions great deal of energy is generated. Series of such reactions are called electron transport system. In the scheme of reactions, oxygen is the final hydrogen acceptor (Fig. 3.4).

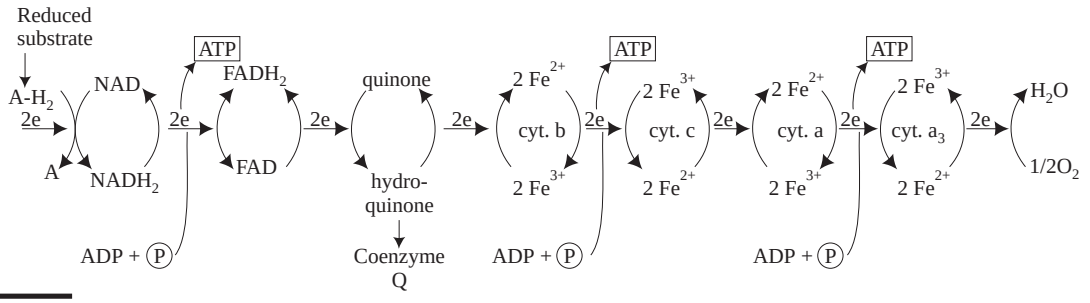


Fig. 3.4 Electron transport system.

Electron transport system in different organisms may differ in certain steps. However, the major components of the system are:

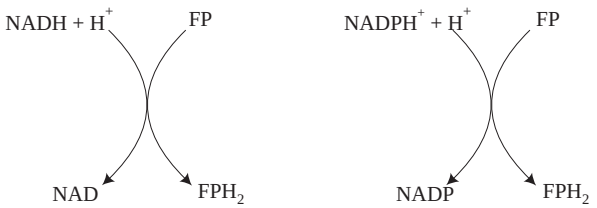
(a) an enzyme—NAD (nicotinamide adenine dinucleotide)
(b) a flavoprotein
(c) coenzyme Q
(d) cytochrome compounds

3.7 THE FLAVOPROTEINS

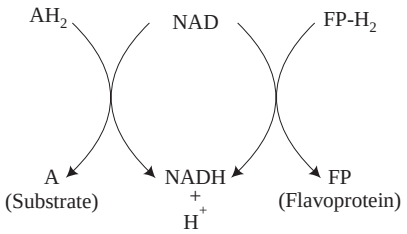
In biological systems, many metabolites undergo oxidation by the loss of hydrogen through catalyzed reactions. The specific enzymes involved are known as *dehydrogenases* and the reaction can proceed with the aid of a hydrogen acceptor. Dehydrogenation of a substrate depends upon the availability of NAD^+ or $NADP^+$. The oxidized forms of these enzymes are present in the system in minute quantities, and in order to maintain a constant supply, NADH and NADPH are reoxidized to NAD and NADP. The mechanism to reoxidize these enzymes is furnished by certain *flavoproteins*. These are proteins and act as coenzymes. They contain a prosthetic group FMN (flavin mononucleotide) or FAD (flavin adenine dinucleotide). These coenzymes catalyze oxidation-reduction reactions:

$$FP \underset{2H}{\overset{+2H}{\rightleftharpoons}} FPH_2$$

Two co-enzymes of this group, $NADH_2$ and $NADPH_2$ reoxidize NADH and NADPH. This can be shown as:



The flavoprotein enzymes act upon certain metabolites that function as substrates. Flavoproteins take hydrogen from NAD and thereby get reduced. The same flavoprotein molecule is reoxidized to become active again to participate in further reactions. This can be shown as:



Reoxidation of flavoproteins is accomplished by cytochrome system.

3.8 DEHYDROGENATION

The initial step of the electron transport system is the oxidation of the organic substrate by NAD, and in turn NAD gets reduced to NADH. It may be shown as under:

$$\text{Metabolite} + \text{NAD} \rightarrow \text{oxidized metabolite} + \text{NADH} + H^+$$

In the above reaction, the oxidizing agent NAD is reduced to NADH by accepting two electrons and one hydrogen, the other hydrogen atom is released as hydrogen ion (H^+). Such reactions involving loss of hydrogen and two electrons by the molecule to be oxidized are called dehydrogenations. The enzymes which catalyze such reactions are called *dehydrogenases*. Specific dehydrogenases are required for different organic molecules.

The NADH thus formed is reoxidized to NAD by a flavoprotein enzyme. Flavin functions as a coenzyme. The NADH is oxidized to NAD and flavin accepts two electrons, a hydrogen atom and the H^+ from the solution.

$$H^+ + \text{NADH} + \text{Flavin} \rightleftharpoons \text{Flavin } H_2 + \text{NAD}$$

Thus flavin is reduced, and further undergoes reoxidation by coenzyme *Q*. The reaction involves oxidation of reduced flavin by the transfer of two electrons and two hydrogens to a molecule of coenzyme *Q*.

In the next reactions, reoxidation of coenzyme *Q* takes place by cytochromes which act as electron carriers. There are a number of such compounds which occur in the mitochondria of the cells, and differ from each other on the basis of their structure with respect to the protein molecule. Owing to their structural differences, they also differ with respect to their ability to accept or donate electrons. They react in a particular order, cytochrome *b* → cytochrome *c* → cytochrome *a* →

cytochrome a_3. Cytochrome *b* accepts an electron from coenzyme *Q* and thus gets reduced. Since one electron is accepted by one molecule of cytochrome *b*, two molecules are necessary to reoxidize coenzyme *Q*. The hydrogens of the reduced coenzyme *Q* are not accepted by cytochromes and they are released as hydrogen ions in the medium.

An electron thus accepted by cytochrome *b* is next passed on to *c*, then to *a*, and finally to cytochrome a_3 with alternate oxidation and reduction of the iron atom of the cytochromes. Cytochrome a_3 is commonly known as cytochrome oxidase which is capable of undergoing direct oxidation by molecular oxygen. Cytochrome oxidase is an important enzyme showing a specific behaviour. In the presence of oxygen it works efficiently and continuously transfers electrons in one direction, that is to oxygen. In the absence of oxygen, it becomes inactive. The sequence of reactions is shown in Fig. 3.4.

3.9 ENERGY RELEASE AND OXIDATIVE PHOSPHORYLATION

If one mole of glucose is completely oxidized, 686 kcal of energy is released. This can be shown thus:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 686 \text{ kcal}$$

In order to produce ATP from ADP and Pi in a biological system, about 10 kcal of energy per mole of ATP is required. It is apparent from the above equation that 68 moles of ATP would be produced for each mole of glucose aerobically oxidized. However, it is not true. Actually, 38 moles of ATP are produced per each mole of glucose oxidized. One might ask as to what happens to the rest of energy?

Rest of the energy is lost in the form of heat. The typical reaction of glucose oxidation can be shown as follows:

$$C_6H_{12}O_6 + 6O_2 + 38 \text{ ADP} + 38\text{Pi} \rightarrow 6CO_2 + 6H_2O + 38 \text{ ATP} + 310 \text{ kcal (heat)}$$

The above equation summarizes the oxidation of glucose in one step. In actual oxidation process, however, several steps are involved so that the energy is released bit by bit in a stepwise fashion. Thus only one mole of ATP is synthesized in one single reaction. In biological systems glucose is the chief fuel to be oxidized for the production of ATP. A series of oxidation reactions are employed by the cell to oxidize glucose to CO_2 and water. We shall describe these reactions with respect to oxidative phosphorylation.

3.10 GLUCOSE OXIDATION

Carbohydrates are fuels which are first broken down to 6-carbon sugars before they are oxidized. Glucose is the simplest 6-carbon sugar which must be first converted into glucose-6-phosphate through phosphorylation by ATP. This is the common early stage of *glycolysis.* It is further transformed to pyruvic acid as summarized in Fig. 3.5.

In the next reaction glucose-6-phosphate is converted to fructose-6-phosphate under enzymatic control. Fructose-6-phosphate is phosphorylated again on the first carbon and leads to the formation of fructose-l, 6-diphosphate. This conversion is accomplished by ATP which acts as both energy and

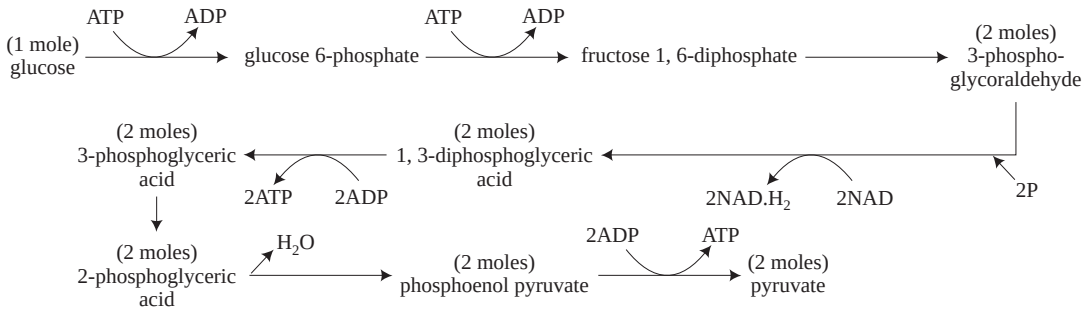


Fig. 3.5 Scheme of glucose oxidation (glycolysis).

phosphate donor. In the next series of reaction, the 6-carbon diphosphorylated sugar is split into two 3-carbon phosphorylated sugar, phosphoglyceraldehyde (PGA) and dihydroxy acetone phosphate (DHAP). Next reaction is very important for two reasons. Firstly, one more molecule of phosphate is added, and secondly in the course of this reaction a hydrogen is removed from the aldehyde group which is picked up by NAD. In the next reaction transformation of the two molecules of 1, 3-diphosphoglyceric acid and 2 molecules of ADP to two molecules of 3-phosphoglyceric acid and 2 molecules of ATP takes place. This is an enzyme catalyzed reaction. Up to this stage the energy yield has been balanced by the energy expenditure. Any energy derived from the process after this step will be the net gain.

The next two reactions rearrange the phosphate group of 3-phosphoglyceric acid resulting in the formation of 2-phosphoglyceric acid which is then converted to phosphoenol pyruvate by losing one hydrogen. For each glucose molecule oxidized, two phosphoenol pyruvates are formed. The phosphate group of phosphoenol pyruvate is transferred to ADP molecule to form ATP. After transferring the phosphate from phosphoenol pyruvate, the resulting compound pyruvic acid or pyruvate is formed. Four ATP molecules are formed in this glycolytic process, two pay back for the two expended in the beginning of glycolysis. Therefore the net gain is of 2 ATP molecules.

The importance of glycolysis can be enumerated as follows:

1. About 10 per cent of free energy available in glucose molecule is released.
2. Glucose molecule is changed to form pyruvic acid which can enter the citric acid cycle to release more energy.
3. A net synthesis of 2 molecules of ATP takes place.
4. Two molecules of NADH are generated by dehydrogenase action.

The remainder of energy contained in the glucose molecule is released during the course of citric acid cycle and oxidation by the electron transport system. The product of glycolysis, pyruvic acid enters the mitochondria where it undergoes oxidative phosphorylation with the help of electron transfer chain to generate more of ATP. The complex reactions, their sequence and products are summarized in Fig. 3.6.

At the beginning of the cycle, pyruvic acid is acted upon by an enzyme pyruvic dehydrogenase and acetyl CoA is formed. The enzyme also transfers hydrogen from pyruvic acid to NAD which

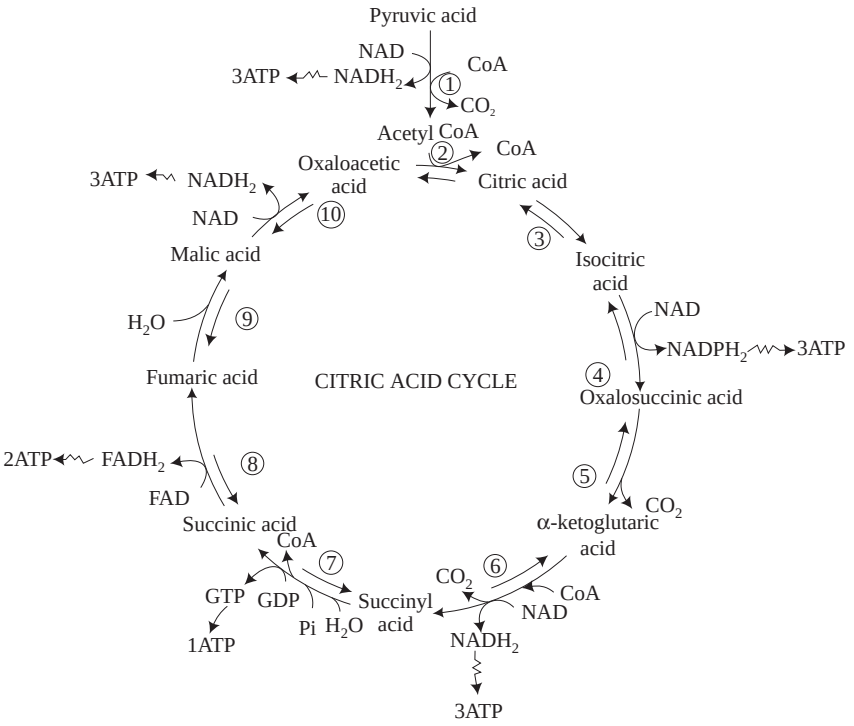


Fig. 3.6 The citric acid cycle (Krebs cycle).

forms NADH, a reduced compound. The compound acetyl CoA enters the citric acid cycle by reacting with a 4-carbon molecule, oxaloacetic acid, forming citric acid.

In the next reaction isocitric acid is formed involving minor changes in the citric acid molecule. In the next step dehydrogenation of isocitric acid to oxalosuccinic acid takes place. The coenzyme which acts as the electron acceptor is NADP. Oxalosuccinic acid is a keto-acid and is capable of undergoing decarboxylation. The product of this reaction is α-ketoglutaric acid. α-ketoglutaric further undergoes decarboxylation and forms a high energy complex, succinyl CoA. NAD acts as an oxidizing agent removing the hydrogen. Succinyl CoA is rapidly cleaved to succinic acid and CoA becomes free. This reaction leads to the synthesis of one molecule of ATP.

In the next reaction, an enzyme succinic dehydrogenase oxidizes succinic acid to fumaric acid. In this reaction 2 molecules of ATP are formed. An addition of a water molecule to fumaric acid yields malic acid. Malic acid is then finally oxidized by NAD to oxaloacetic acid, thus completing the cycle. There ATP molecules are formed in this step. The most important conclusion drawn from the cycle is the entrance of an acetyl group which is oxidized to CO_2, and H_2O, by four oxidation reactions. The balance sheet of the ATP yield from glycolysis and various steps of oxidative phosphorylation is given in Table 3.1.

Table 3.1 Net ATP Gain During Complete Glucose Oxidation

Sequence of reactions	*Net ATP yield*
1. 1 glucose → 2 pyruvic acid moles	2
2. NADH + H^+ from conversion of 3-phosphoglyceraldehyde to 1, 3-diphosphoglyceric acid (electron transport system)	6
3. 2 pyruvic acid → 2 acetyl-CoA + $2CO_2$	6
4. 2 acetyl-CoA → $4CO_2$ (citric acid cycle)	24
Glucose → $6CO_2$	38

CHAPTER

4 Enzymes—The Biological Catalysts

In the previous chapter it has been mentioned that the enzymes catalyze metabolic reactions in a precise way by lowering the energy barrier (see section 3.4 of Chapter 3). In living systems chemical reactions proceed at physiological temperatures which are quite low (in most cases 37°C). The same reactions *in vitro* will proceed at considerably higher temperatures and pressure. But the remarkable property of the cell to carry out its reactions at mild temperatures, low pressure, and in dilute solutions is due to the influence of enzymes.

Chemically, enzymes are complex protein molecules synthesized in the cells where they act as biocatalysts in carrying out various physico-chemical reactions. These proteins have their own specificity and kinetics. By definition a catalyst is a substance which speeds up the rate of a given reaction and at the end of the reaction it remains unaltered. Further, the catalyst helps in attaining a reaction in a state of equilibrium. Many noncatalyzed reactions remain in a nonequilibrium condition since their rate of reaction to reach the state of equilibrium is very slow. For example, glucose and oxygen may remain together in a solution for years without reacting with each other. However, if a suitable catalyst is added to the solution, both will react readily to attain the state of equilibrium by forming carbondioxide and water. This may be represented as,

$$6CO_2 + C_6H_{12}O_6 \rightleftharpoons 6CO_2 + 6H_2O$$

The above reaction has an equilibrium favouring the product formation since no detectable amount of glucose will be formed if CO_2 and H_2O are mixed together.

4.1 GENERAL PROPERTIES OF ENZYMES

All enzymes are proteins and synthesized within the cell. Owing to their protein nature their physical and chemical properties conform to the nature of proteins. Action of strong acids, bases, organic solvents, heat and agitation will denature and render them biologically inactive. An essential property

of these biological catalysts is to speed up the rate of chemical reactions and while doing so they remain unchanged without loss of activity. An enzyme recognizes its specific *substrate* and reacts with it to form *product* and gets regenerated at the end of the reaction. The enzyme lowers the activation energy and allows a larger number of molecules to react at a given temperature. The efficiency with which an enzyme acts on its substrate is known as its *turn over rate* which is the number of substrate molecules converted into the product by a single molecule of enzyme per unit time.

Enzyme catalysis follows the same general rules as observed for nonenzymatic catalysis. Both catalyze forward and backward reactions to reach a state of equilibrium. However, the main difference lies in the fact that the enzyme delicately controls and regulates the cellular processes at considerably low temperatures with maximum economy.

Enzyme Specificity

Enzymes have a preference for their specific substrate on which they act. The phenomenon is known as enzyme specificity. Some enzymes have absolute specificity, i.e. the enzyme can act on only one substrate. Urease hydrolyzes urea and any modification in urea molecule will render it ineffective for enzyme attack:

$$CO\begin{matrix}NH_2\\NH_2\end{matrix} + H_2O \rightleftharpoons 2NH_3 + CO_2$$

Certain enzymes are capable of acting on a specific organic group, thus showing group specificity. For example, alcoholic dehydrogenases act only on alcohols. Similarly, carboxyl esterases will act on carboxylic acid esters only.

$$R - OH + NAD^+ \rightleftharpoons R - CHO + NADH + H^+$$

In the above reaction the R and H groups are immaterial.

Some enzymes act on a range of substrates showing a *broad specificity.* Trypsin will attack peptide bonds in a protein chain only between certain amino acid residues involving lysin and arginine.

Certain enzymes show *optical specificity,* i.e. they are able to discriminate between their optical isomers. An L-amino oxidase will not act on D-amino acids or vice versa.

Enzyme-substrate Interaction

An enzyme reacts with its substrate at various concentrations. When the rate of enzymes catalyzed reaction is studied at various substrate concentrations, a hyperbolic curve is obtained (Fig. 4.1). When the initial substrate concentration is low, the rate is directly proportional to the substrate concentration. As the substrate concentration is increased the velocity of reaction reaches a maximum $V_{max.}$ At high concentrations the rate of reaction becomes independent of substrate concentration (Fig. 4.1).

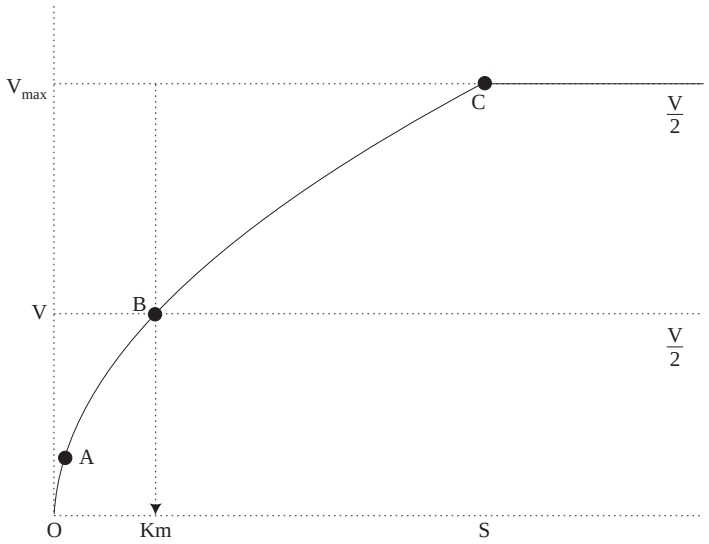


Fig. 4.1 Effect of substrate concentration on the velocity of enzyme catalyzed reaction.

The enzyme catalyzed reaction is a two-step process which can be shown thus:

$$[E] + [S] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [ES] \quad (1)$$

$$[ES] \underset{k_4}{\overset{k_3}{\rightleftharpoons}} [E] + [P] \quad (2)$$

In this process the enzyme first combines with the substrate [S] to form enzyme-substrate [ES] complex. This complex breaks down into the product [P] and the enzyme [E]. The enzyme catalyzed process was quantified by Michaelis and Menten who applied the law of mass action to the formation of ES complex. Following equation was obtained to express the relationship:

$$V = \frac{V_{\max}[S]}{K_m + [S]}$$

where

V = velocity of reaction

[S] = substrate concentration

V_{max} = maximum velocity

K_m = Michaelis-Menten's constant

K_m represents the substrate concentration at which the velocity of reaction is half of the maximum velocity (V_{max}).

4.2 THE MECHANISM OF ENZYME ACTION

Activation Energy

An exergonic reaction will take place very slowly, but the rate of reaction is determined by how many molecules have the activation energy (E_A) to react together at anyone moment. In a slow reaction only a small percentage of the molecules involved have the necessary amount. The activation energy therefore represents a kind of barrier which must first be overcome before the reaction can proceed (Fig. 4.2).

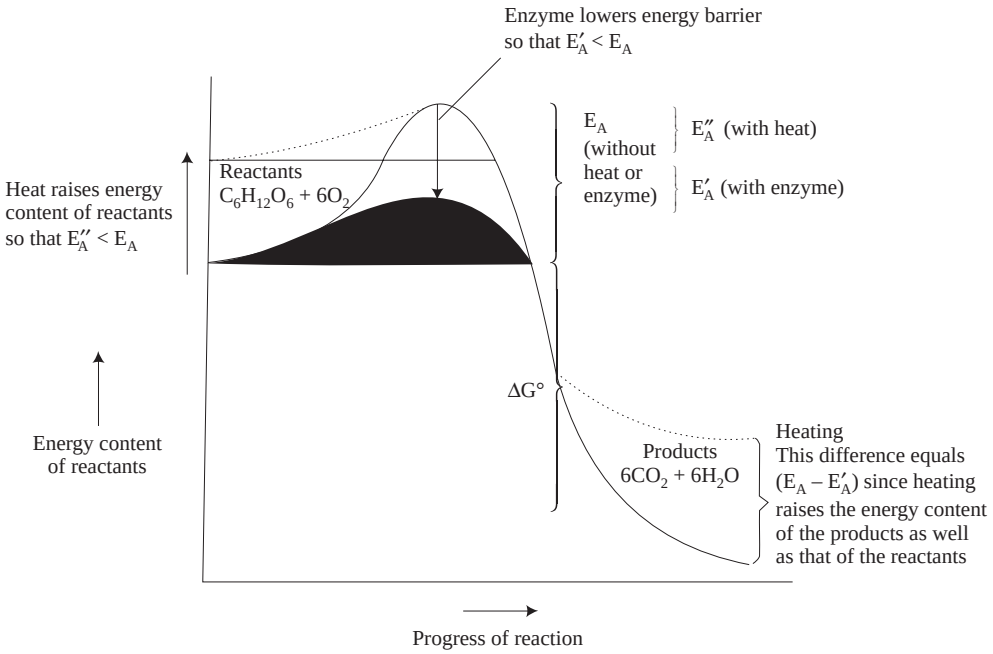


Fig. 4.2 Activation energy: enzyme lowers the energy barrier. Heat produces a secondary effect, increasing the kinetic energy of the reactants.

In principle, reactions can be speeded up in two ways:

(a) by supplying the reactants with more energy, for example by heating;

(b) by lowering the activation energy by means of a catalyst.

In living organisms heat from any external source cannot be applied, because of limitations. Besides, heat also denatures proteins which include enzymes also. However, enzymes are biocatalysts

which lower the activation energy barriers dramatically. How these enzymes lower the energy barrier? Before we answer this question, let us have a closer look at the concept of the *active site* or the *catalytic site.*

Lock and Key Hypothesis

Fischer postulated a lock and key hypothesis to explain the interaction between the enzyme and the substrate. The model visualizes the enzyme molecule as a rigid structure having a fixed substrate binding site (Fig. 4.3). For a key to work it must be provided with the right lock and so is with enzyme and substrate.

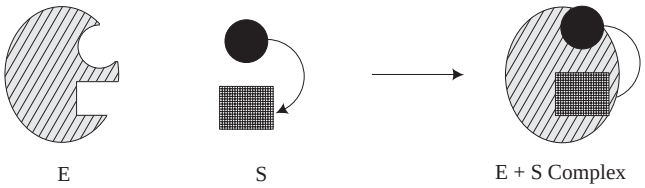


Fig. 4.3 Fischer's lock and key hypothesis to explain the formation of enzyme-substrate complex.

The concept of active site. The active site of an enzyme molecule is the catalytic site which reacts with the substrate. It has been suggested that there must be one or more active sites of an enzyme which are the centres of catalytic activity. Enzymes are huge molecules with a high molecular weight, but substrates are often small. It has been suggested that one or two substrate molecules bind at a time to specific points on an enzyme, showing that enzymes possess specific active sites for catalysis. If the active site is only a small part of the enzyme molecule, what is the function of the rest of the molecule? In 1963, Koshland suggested that an enzyme consists of essentially four categories of amino acids (Fig. 4.4):

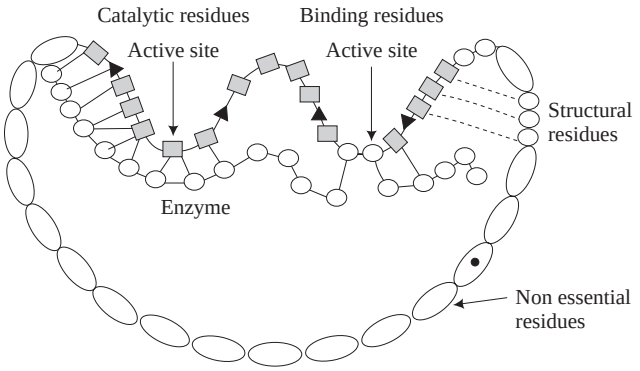


Fig. 4.4 Amino acid residues in an enzyme molecule showing four sites: catalytic site, binding site, structural residues and non-essential residues.

(i) *Catalytic residues:* These are the amino acids at the catalytic site which make and break chemical bonds. They participate in the catalytic activity.

(ii) *Binding residues:* These amino acids hold the substrate in place while catalysis is taking place.

(iii) *Structural residues:* These amino acids hold the active site in the correct shape so that it can function properly.

(iv) *Non-essential residues:* These amino acids have no specific function. They are often near the surface of the enzyme and can be removed or replaced without loss of function.

Induced-fit Theory

The idea of an enzyme 'wrapping around' a substrate to form a more stable structure is called *induced-fit* hypothesis. These and other considerations led Koshland to postulate that essential functional groups on the active site of the free enzyme molecule are not in their optimal positions for promoting catalysis when the active site is unoccupied, but when the substrate molecule is bound by the enzyme, the binding affinity forces the enzyme molecule into a conformation in which the catalytic groups assume a favorable geometric position to form the transition state. This is the induced-fit model of enzyme-substrate interaction (Fig. 4.5).

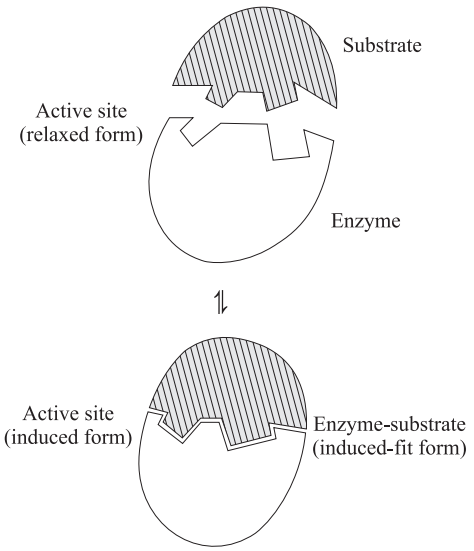


Fig. 4.5 Induced fit model of enzyme molecule as proposed by Koshland.

Molecular Basis of Enzyme Action

According to thermodynamic considerations, enzymes lower the energy barrier or activation energy through several steps, each step with reduced activation energy, including the formation of ES-complex. On the other hand, in molecular terms, few major factors appear to participate and probably

operate simultaneously at the active site, all of which contribute to a lowering of activation energy, and hence to the large rate accelerations produced by the enzyme (Table 4.1).

Table 4.1 Mechanisms which Contribute to the Catalytic Efficiency of Enzymes

Mechanisms	*Description of catalysis*
Proximity effects	Temporary binding of reactants close to each on an enzyme increases the chance of a reaction.
Orientation effects	Reactions are held by the enzyme in such a way that the bonds are exposed to attack and a transition state is readily achieved.
Strain effects	Enzyme may induce strain or distortion in the susceptible bond of the substrate molecule, making the bond easier to break.
Acid-base catalysis	Acidic and basic amino acids in the enzyme facilitate transfer of electrons to and from the reactants.
Covalent catalysis	Enzyme may combine with the substrate to form an unstable covalent intermediate that readily undergoes reactions to form the products.
Microenvironmental effects	Hydrolytic amino acids create a water-free zone in which non-polar reactants may react more easily.

4.3 CLASSIFICATION OF ENZYMES

The International Enzyme Commission has adopted a system of classification of enzymes recognizing six major classes (Table 4.2).

Table 4.2 Major Classes of Enzymes and the Types of Reactions Catalyzed by Them

Enzyme class	*Nature of reaction*	*Major type of enzymes with their specific reactions*
1. Oxido-reductase	Biological oxidation and reduction	1. Dehydrogenases: catalyze removal of 2 atoms of hydrogen 2. Oxidases: these catalyze reduction of O_2 3. Oxygenases: which catalyze incorporation of molecular O_2 into the substrate 4. Oxidative deaminases: catalyze the oxidation of amino compounds with the formation of NH_3 5. Hydroxylases: these introduce OH groups 6. Peroxidases: they use H_2O_2 as oxidant
2. Transferases	Effecting exchange of groups groups between two substrates: AB + CD AC + BD	1. Aminotransferases: catalyze exchange of amino & Keto group between amino and Keto acid 2. Kinases: catalyze the transfer of a PO_4 radical 3. Acyltransferases: catalyze the transfer of acyl/acetyl group to a suitable acceptor 4. Glycosyltransferases: they transfer glycosyl groups

Contd.

Contd.

3. Hydrolases	They catalyze hydrolysis reactions: AB + H_2O A OH + HB	1. Peptidases: catalyze hydrolysis of peptide bonds 2. Glycosidases: catalyze glycosidic bonds 3. Esterases: carry hydrolysis of carboxylic esters 4. Phosphatases: hydrolyse phosphoric acid esters 5. Phosphodiesterases: 6. Deaminases: catalyse hydrolysis of amines 7. Deamidases: catalyze hydrolysis of amides
4. Lyases	Remove groups from substrates non-hydrolytically: AB A + B	1. Decarboxylases 2. Aldolases 3. Dehydratases
5. Isomerases	Catalyze isomerization of substances (substrates)	1. Racemases 2. Epimerases
6. Ligases	Catalyze joining together of two molecules coupled with the break down of a pyrophosphate bond in ATP	Synthetases; bring about the formation of C-O, C-S, C-N or C-C bonds. Reactions require expenditure of energy with simultaneous cleavage of ATP.

4.4 FACTORS INFLUENCING ENZYME ACTIVITY

There are many factors which influence the activity of enzymes. These may be physical or chemical in nature.

A. Substrate and Enzyme Concentration

An enzyme catalyzed reaction is dependent on the enzyme concentration.

Usually the enzyme is present in much lower concentration than the substrate. In vitro experiment if we take increasing enzyme concentration in the presence of an excess of the substrate, a linear relationship is observed showing increased utilization of the substrate (Fig. 4.6). However, with fixed enzyme concentration and increasing substrate concentration a different relationship is observed (Fig. 4.7). First of all a rapid rise in velocity of reaction is observed and subsequently the reaction rate slows down until no change in velocity is observed. The enzyme at this stage is saturated with the substrate. Three situations are observed at points A, B and C.

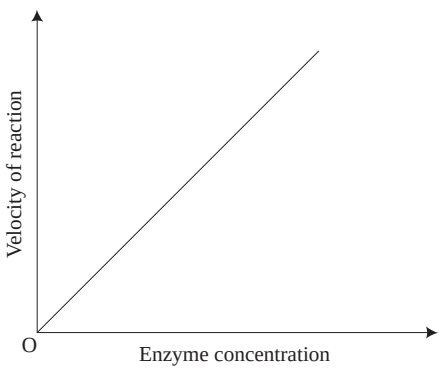


Fig. 4.6 Effect of enzyme concentration on the rate of reaction.

1. At point A (Fig. 4.1) the [S] is much less than K_m, hence the velocity V is dependent upon the substrate concentration.
2. At point B the substrate concentration is equal to K_m, hence the velocity is half of V_{max}.
3. At point C the substrate concentration is much greater than the K_m value, hence the velocity of reaction is maximal (V_{max}).

B. Temperature

Temperature has a marked effect on enzyme-catalyzed reactions as enzymes are very sensitive to elevated temperatures. At high temperatures the enzymes undergo denaturation resulting in complete loss of their biological activity. For most enzymes, optimal temperatures are close to that of the ambient temperature of the cell. In homeotherms this temperature is around 37°C. However, the enzymes show their activity over a limited range of temperature.

According to vant Hoff law, a rise of 10° in temperature will double the velocity of a reaction. If we assume that at a given temperature T^{o} the rate of reaction is V, the latter becomes $2V$ at temperature $T \neq 10°$. This is expressed in terms of the ratio of velocities of the reaction at two temperatures 10° apart and is indicated by Q_{10}. This holds true for an enzyme-catalyzed reaction at low temperatures. The temperature range for most of the enzymes lies between 30° and 50°C.

C. pH

The enzymes are influenced by pH changes since they are proteins and have an ionic character due to amino and carboxylic groups. Each enzyme has an optimum pH at which the velocity is maximal provided all other conditions like temperature, substrate concentration etc, are ideal. In a typical curve showing the effect of pH on an enzyme-catalyzed reaction it is observed that the maximal catalytic activity is seen at the optimum pH, while on either side of the curve it is low (Fig. 4.7). Within a narrow range of pH (i.e. slightly alkaline or acid condition), the changes in the reaction are reversible. However, if the pH is either too low or too high, the changes are irreversible due to denaturation of the enzyme protein.

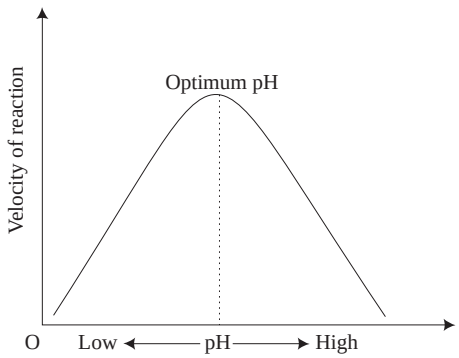


Fig. 4.7 Effect of pH on the enzyme catalyzed reaction.

D. Redox Potential

Many enzymes are sensitive to oxidizing and reducing agents and the comparative ability of oxidation or reduction of an enzyme is known as redox potential. It is the electromotive force (measurable in millivolts) developed by the solution when in physical contact with the platinum electrode as compared to the normal hydrogen electrode at zero potential. The redox potential of an enzyme is either positive or negative owing to its relative oxidizing or reducing ability in comparison to hydrogen.

E. Inhibitors

There are certain compounds, also known as antimetabolites, which when added to the substrate combine with the enzyme reversibly or irreversibly to block the production of end products. Such substances are known as inhibitors which include drugs, antibiotics, poisons and certain metabolites. Inhibition occurs in a variety of ways, but broadly they may be classified into two categories: reversible and irreversible inhibitions.

(a) Irreversible Inhibition: Some enzymes have a thiol (SH) group at the active site. The compounds like iodoacetate (CH_2 I.COOH) or mercurials react with the functional—SH group of the enzyme and form covalent derivatives. This kind of binding with the active site of the enzyme molecule causes more or less inactivation of the enzyme. The inhibitor cannot be released by any means, hence called irreversible inhibitor. The inhibition is proportional to the concentration of the inhibitor.

$$E—SH + ICH_2COOH \rightarrow E—S—CH_2COOH + HI$$

(b) Reversible Inhibition: Many inhibitors reversibly bind with the enzyme molecule affecting the equilibrium constant of the reaction. Three types of reversible inhibitions are known.

(i) Competitive Inhibition: In this type of inhibition both the inhibitor and the substrate compete for the same active site of the enzyme, but the inhibitor has greater affinity. The effect of inhibition can be overcome by increasing the substrate concentration. In such cases the inhibitor is structurally related to the substrate and bind with the enzyme decreasing the effective concentration of the enzyme. An example of this type of inhibition is succinic dehydragenase which converts succinate to fumaric acid. If malonic acid (it is analogous to the structure of succinic acid) is added to the reaction, the activity of succinic dehydrogenase falls but the activity can be restored by increasing the concentration of succinic acid (Fig. 4.8).

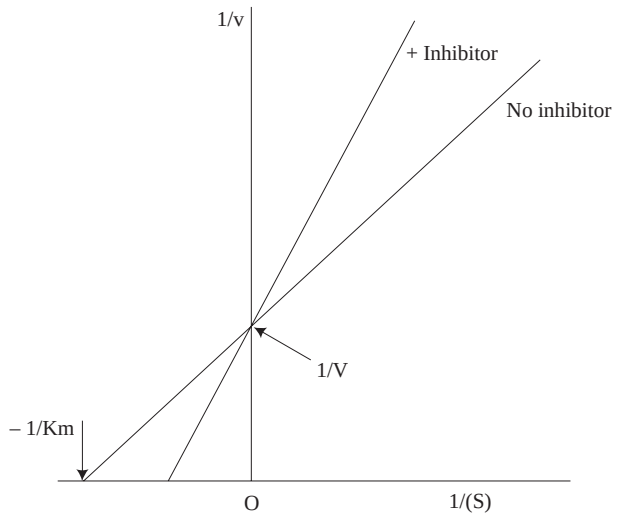


Fig. 4.8 Graphical representation showing competitive inhibition.

(ii) Noncompetitive Inhibition: In this case there is no competition between substrate or inhibitor and the inhibitor either combines with the enzyme with the ES complex (Fig. 4.9). Noncompetitive inhibition cannot be fully reversed even at high substrate concentrations. Noncompetitive inhibitors lower the V_{max} but do not affect the K_m value. From the Fig. 4.10

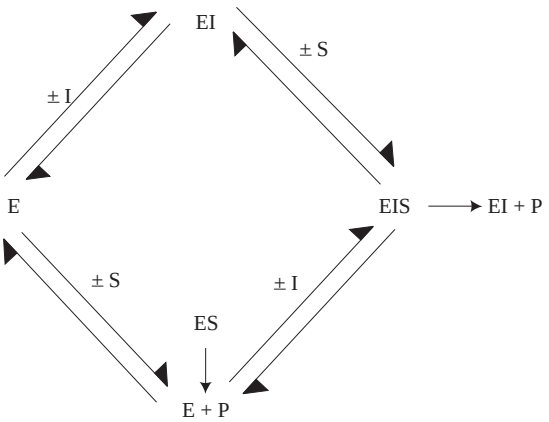


Fig. 4.9 Mechanism to show binding of inhibitor with enzyme or with ES complex.

it can be seen that the substrate has an affinity both for enzyme and for EI complex. When the results are plotted with $1/V$ against $1/S$ at various concentrations of the inhibitor V_{max} is lowered (Fig. 4.10).

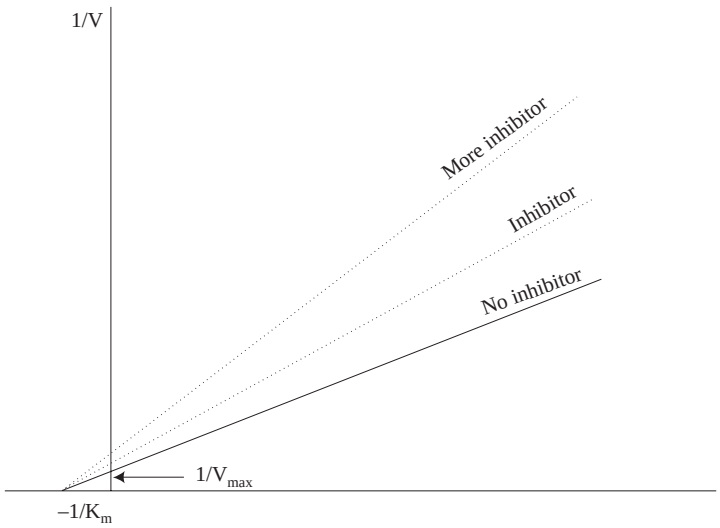


Fig. 4.10 A plot showing non-competitive inhibition.

(iii) **Uncompetitive Inhibition:** Some inhibitors combine with ES complex, hence are called uncompetitive inhibitors. Such inhibitors have no affinity with the substrate and bear no resemblance with it. This type of inhibition can never be reversed. A variety of poisons such as iodoacetamide and metal ions (Ag, Hg) cause this type of inhibition.

4.5 ISOENZYMES

Certain enzymes occur in multimolecular forms in the same organism. These enzymes have different physical properties but catalyze the same type of reactions. Lactate dehydrogenase (LDH) is an example of an isoenzyme which can be distinguished into 5 distinct types. These five different forms differ in their behaviour, amino acid composition and relative rates of reaction. They have been reported from different organs of vertebrates, chiefly from the heart and skeletal muscles. Two basic subunits of LDH enzymes are known, the H type predominant in the heart and the M type in the skeletal muscles. Both have same molecular weights (35,000) and both are produced in the same cells by two, separate genes. LDH is composed of M and H subunits in 5 different combinations. These are: H_4, H_3M, H_2M_2, H_1M_3 and M_4. M_4 and H_4 are pure tetramers. Besides LDH, many other enzymes like alkaline phosphatase, glutamate-oxaloacetate transaminase and creatinine phosphokinase also occur in the form of isoenzymes.

4.6 ALLOSTERIC ENZYMES

There is an important class of enzymes usually known as allosteric or regulatory enzymes. They have distinct regulatory and catalytic sites and their activity can be enhanced or inhibited by organic compounds that occur as intermediates in the sequence of reactions. The inhibitor of the regulatory enzyme bears no structural similarity with that of the substrate and usually occurs at the end of the pathway.

Allosteric enzymes do not follow Michaelis-Menten kinetics and behave atypically in their relationship with the substrate concentration. Normally, an enzyme possessing independent binding sites will yield a hyperbolic curve to represent their velocity of reaction. However, in case of allosteric enzymes, binding of one substrate molecule will induce structural changes in the enzyme to facilitate affinity of the substrate with the remaining binding sites. In such cases the enzyme-substrate relationship yields a sigmoidal curve (Fig. 4.11). This is also known as positive cooperativity. In order to explain this behaviour example of haemoglobin may be cited. Haemoglobin molecule is composed of 4 polypeptide chains linked to the haeme molecule and the binding of a molecule O_2 would induce structural changes in the haemoglobin molecule which would facilitate further binding of O_2 to reach saturation point. The relationship is sigmoidal. On the other hand, myoglobin, which has only one

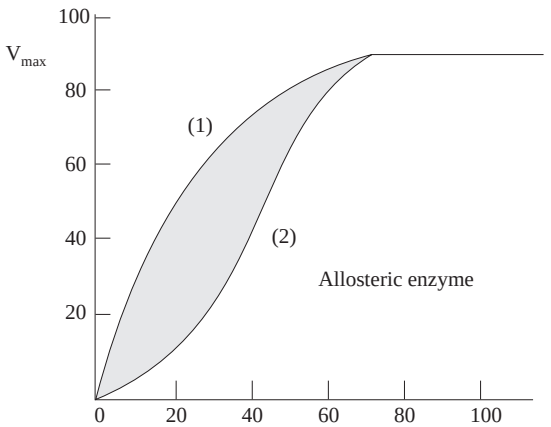


Fig. 4.11 Effect so substrate concentration on the velocity of (1) an enzyme showing Michaelis-Menten kinetics and, (2) allosteric enzyme.

polypeptide chain, can bind with one molecule of oxygen and shows a hyperbolic curve. Michaelis-Menten kinetics is not applicable to allosteric enzymes, hence Km has no meaning. Sometimes the binding of substrate molecule to one catalytic site obstructs the binding at another site and this behaviour is known as *negative cooperativity.*

Two types of allosteric responses are known, homotropic and heterotropic, In case of homotropic response the substrate (effector) is a second molecule. Many, enzymes have several active sites per molecule. When the substrate is bound at one site, the affinity at other sites changes markedly due to conformational change in the enzyme molecule. In case of heterotropic response, the effector or the co-substrate (other than the substrate) binds at the regulatory site affecting the affinity of other vacant binding sites. Most heterotropic effectors that bind with the regulator sites are unrelated to substrates or products, but are usually identified as terminal products of the metabolic pathway exhibiting a feedback control.

4.7 COENZYMES

Some enzymes such as pepsin and ribonuclease occur as pure proteins, while others have a non-protein component associated with the protein molecule. This non-protein component of the enzyme molecule is known as *prosthetic* group and the protein moiety is called the *apoenzyme* which together constitute *holoenzyme.*

The prosthetic group may be either in the form of a metal ion (cofactor) or as a coenzyme which may be firmly or loosely bound to the apoenzyme. Enzyme activity depends upon the coenzyme which is often regarded as a second substrate. Some important coenzymes along with their functional characteristics are given in Table 4.3.

Table 4.3 Coenzymes and Their Functional Characteristics

Name of coenzyme	*Functions*
Nicotinamide adenine dinucleotide (NAD)	Transfer of hydrogen atoms (electrons)
Nicotinamide dinucleotide phosphate (NADP)	Transfer of hydrogen atoms
Thiamine pyrophosphate (B_1)	Decarboxylation and aldehyde group transfer
Flavin mononucleotide (FMN)	Transfer of hydrogen atoms
Flavin adenine dinucleotide (FAD)	Transfer of hydrogen atoms
Lipoic acid	Transfer of acyl groups
Biotin	Transfer of CO_2
Pyridoxal phosphate (B_6)	Participates in transamination, decarboxylation and racemization, reactions of amino acids
Tetrahydrofolate	Transfer of methyl, methylene, formyl or formimino groups.
Cyanocobalamine (vitamine B_{12})	Transfer of alkyl groups in alkylation reactions
Coenzyme Q	Transfer of hydrogen atoms
Coenzyme A	Transfer of acyl groups

Regulation of Enzymes

Cells contain a large number of enzymes all of which do not function simultaneously. They have to be regulated and their functioning coordinated according to the requirements of the cell. Some enzymes remain in inactive form and unless they are activated by proper conditions they will not participate in catalytic activity (examples: pepsin and chymotrypsin). A few hydrolytic enzymes are found to be trapped in lysosomes, and if found loose in the cytoplasm they may cause self destruction of the cell machinery.

A number of enzymes form enzyme complexes and have spatial arrangement in the cell. These are known as multienzymes and catalyze a series of reactions. The enzymes within the mitochondria and chloroplasts are so organized in the membrane system of the organelle that they establish a chain of reactions. Enzymes of the respiratory chain are membrane bound and transfer electrons from the substrate to oxygen.

A number of enzymes remain in physical association with each other in form of clusters (fatty acid synthetase). So long the enzymes are physically bound together, their catalytic activity remains intact. Some enzymes occur as independent molecular entities in the cytoplasm and convert a substrate to an end product by producing a number of intermediate metabolities and if the product of the system accumulates in the cells, it specifically inhibits the activity of the first enzyme of the reaction system. The first enzyme of the sequence which is inhibited is known as the regulatory enzyme and the product is known as the modulator (Fig. 4.12). On the other hand, if the initial substrate accumulates in the cell it activates the enzyme E5 so that the conversion of initial substrate (A) continues.

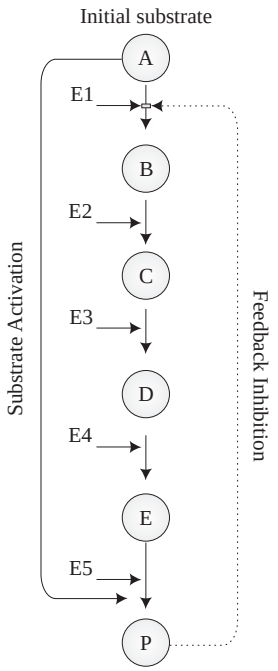


Fig. 4.12 Feedback inhibition showing self-regulation to control concentration of metabolites.

CHAPTER

5

Animal Calorimetry

The organisms should be considered as chemical laboratories where energy transformations are continually taking place. Life of organisms is intricately woven with their environment and as long as they live, they continually exchange matter and energy with their environment. Thus, the organisms constitute a dynamic system exhibiting biological activity at cellular and organismic levels. The complex pattern of activity involves capture of food, digestion and absorption of food, transport of nutrients and elimination of wastes.

The food supplies the energy needed for the maintenance of body temperature, for muscular movements of the heart and respiration, and for other physical activities. The packets of energy liberated from utilisation of various foods are different. The quantity of energy available from 1 g of carbohydrate is 4.1 kilocalories: from 1 g of fat, 9.3 kilocalories are liberated, and from 1 g of protein only 5.3 kilocalories are obtained in the form of heat of combustion.

5.1 ANIMAL CALORIMETRY

To maintain normal physiological functions, physical activity, growth, and tissue repair, the body needs an ongoing supply of energy from oxidation of foodstuffs. Oxidation of dietary carbohydrates, fats and proteins supplies the required energy for these body functions. Recommended dietary allowances (RDA) have been established for required energy needs, including vitamins and minerals. However, RDA does not apply to people with conditions such as chronic illness, infections or metabolic disorders. In these cases nutritional requirements are determined on individual basis.

Animal calorimetry deals with the measurement of energy requirement of the body under various physiological conditions and the fuel value of foods which supply the energy. In animals the chemical source only need be considered and we may confine our attention to that form of combustion in which the substance finally appears in the completely oxidised form.

Unit of Heat

Since oxidation of food involves combustion in the body, heat is evolved as recognised by temperature which is due to the energy of molecular motion. In animal calorimetry the unit of heat is the large calorie (kilocalorie), which is defined as the amount of heat necessary to raise the temperature of 1 litre of water from 15°C to 16°C.

When certain substances are combusted in a *bomb calorimeter* (an apparatus for measuring heat), we can obtain the values of heat of combustion:

1 g of H_2 gas produces 34.5 kilocalories

1 g of charcoal produces 8.0 kilocalories

1 g of glucose produces 3.7 kilocalories

1 g of sucrose produces 3.96 kilocalories

1 g of starch produces 4.2 kilocalories

Fuel Value of Foods

The potential energy of different foods is determined by combusting them in an atmosphere of oxygen in a metal chamber known as *bomb calorimeter*. The amount of heat produced in the instrument is measured in terms of calories, which is slightly more than the food actually oxidised in the body. The caloric content of the three principal foodstuffs measured in a bomb calorimeter is given in Table 5.1.

Carbohydrates and fats are completely oxidised in the body to CO_2 and water. However, proteins do not undergo complete oxidation, therefore the energy obtained is a bit less.

Table 5.1 Fuel Values of Food Obtained by Burning 1 g of Food

	In bomb calorimeter	*In the body*	*Metabolic water*
Carbohydrates	4.2 kcal	4.1 kcal	0.55 g
Proteins	5.6 kcal	4.1 kcal	0.41 g
Fats	9.3 kcal	9.3 kcal	1.07 g

Types of Calorimetry

The amount of heat produced in the body is dissipated and is measured as the amount of energy expenditure in the animal. There are two methods of measuring energy expenditure.

Direct Calorimetry

Direct calorimetry measures the total heat production in a living organism. The animal is placed inside a large chamber having double insulated walls with a provision to remove water evaporated from the lungs and skin, and exchange of oxygen and carbondioxide. A constant flow of air is needed which enters through a saturator and then passes through a heat exchanger. The air then circulates in the calorimeter and comes out through another heat exchanger. The flow rate of both the exchangers is identical. The circulating water in heat exchangers is warmed by absorbing the heat given out by

the animal. The temperature of water is recorded which gives an indication of the body heat production. Though direct calorimetry is a cumbersome process requiring expensive equipment, it permits precise and continuous measurement of heat produced.

Indirect Calorimetry

When carbohydrates are oxidised in the body, oxygen is consumed involving heat production and carbondioxide formation. In such a case, the rate of oxygen consumption can be measured to find out the heat evolved. The equipment used for the purpose is known as *spirometer***.** The spirometer is filled with oxygen and also contains a vessel full of soda lime. The animal is placed inside the respiratory chamber. When the animal inhales oxygen, water and carbondioxide are expired, which are absorbed by soda lime. The chamber is airtight but since it is not insulated, heat production cannot be obtained directly. The animal remains in the oxygen chamber for several hours and its oxygen consumption and carbondioxide output are measured at regular intervals. A writing pen is attached to the spirometer connected with a revolving drum. There is an upward stroke for inspiration and a downward stroke for expiration. The slope indicates the rate of oxygen consumption. The relationship between oxygen consumption and heat production varies with the type of food consumed, hence with the help of a calibration chart one can find out the amount of heat produced in relation to the oxygen consumed. To know heat production of the body from the oxygen consumed, it is necessary to know the nature of food (carbohydrate, fat or protein) being consumed. The information can be obtained from the respiratory quotient (Table 5.2).

Respiratory Quotient (RQ)

The respiratory exchange of gases is dependent upon the type of food consumed and the physiological state of the animal. For this purpose, the amount of O_2 consumed and the amount of CO_2 evolved are used as a measure of the extent of the type of fuel oxidized by a particular animal. The ratio of the volume of CO_2 produced to the volume of oxygen absorbed is known as the respiratory quotient (RQ). This has different value for each of the major food components and serves to determine what substances are being burned.

Table 5.2 Energy Relationships and Respiratory Quotients

	Carbohydrate	Fat	Protein
O_2 utilised/g (in litres)	0.75	2.03	0.97
CO_2 produced/g (in litres)	0.75	1.43	0.78
RQ	1.00	0.71	0.80
Kcal yield/g	4.10	9.30	4.10
kcal equivalent of 1 litre of O_2	5.47	4.60	4.23

We can further explain the concept of RQ by taking an example of glucose oxidation where the quantity of CO_2 evolved is equal to the amount of O_2 consumed:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

$$\text{The RQ for glucose (carbohydrate)} = \frac{\text{volume of } CO_2 \text{ evolved in time } t}{\text{volume of } O_2 \text{ consumed in time } t}$$

$$= 6/6 \text{ or } 1.0$$

1 litre of oxygen represents a liberation of 5.011 kcal. In animal calorimetry the heat equivalent of 1 litre of oxygen is generally accepted as 5.047 kcal when carbohydrates are burned in the body.

As noted above, the ratio is known as RQ, which is different for different foodstuffs. In other foods, however, this RQ is less because relatively less oxygen is consumed. In case of fat (tristearin for example), the RQ is about 0.71:

$$C_{57}H_{110}O_6 + 163O_2 \rightarrow 114CO_2 + 110H_2O$$

$$= \frac{CO_2}{O_2} = \frac{114}{163} = 0.71$$

Proteins too have a lower RQ value ranging between 0.8 and 0.82. Based on the analytical figures for the average protein, it is estimated that 1 g of urinary nitrogen represents the metabolism of 6.25 g of protein, the absorption of 5.91 litres of oxygen, the production of 4.76 litres of carbondioxde, and the liberation of 26.51 kilocalories.

5.2 BASAL METABOLISM

Energy requirement of the animal body may be studied with the help of two functional parameters: energy required for basal metabolism and the energy needed for active work. Basal metabolism includes the energy expended in respiration, blood circulation, intestinal contractions, activities of various organs, maintenance of muscular work, thermal equilibrium, etc. The basal metabolic rate (BMR) is influenced by the amount of protoplasmic mass, height, weight, surface area, age, sex, composition of the tissues, general health etc., and is governed by endocrine organs, particularly the thyroid and pituitary glands.

The energy consumed in active work and indeed all forms of voluntary activity, imposes an additional requirement for fuel over the basal metabolism, which depends upon the nature and extent of the muscular work. Ordinarily an average man expends about 100 kcal per hour while sitting at rest, his metabolism may increase to about six times with extreme physical effort. In a healthy person, energy requirements are determined by basal energy expenditure, physical activity, and the energy used for digestion. Digestion related energy expenditure is known as *calorigenic effect of food*, which generally equals about 10% of the basal energy expenditure.

Basal Metabolic Rate (BMR)

Basal metabolic rate is a measure of the heat production of the body in complete mental and physical repose, and in the post absorptive state. It represents the lowest energy expenditure consonant with minimal physical activity and reflects the amount of energy needed to maintain basic physiologic functions and is expressed as the heat produced per hour per meter square. It is determined 12 hours after the last meal. The subject should have had complete rest at 20°C before the BMR estimation is done. The BMR is expressed in kcal/hr per surface area (in sq metre) and is determined from the RQ values obtained over a known period of time.

Factors Affecting BMR

The BMR values differ with age, sex, surface area, climate, racial variations, state of nutrition, disease and hormonal balance. Smaller individuals have higher rate of metabolism. It is lower in women and higher in children (Table 5.3). From birth to the age of one and a half years the basal metabolism increases at a remarkable rate, and is followed by a gradual decline until full growth and development is reached. Constancy characterises the rate in adult life, with a slight decline as old age advances. In normal healthy males around 20 years of age, the BMR is about 40 kcal/hr/m^2. In women the BMR averages about 12% below that of men.

Table 5.3 Oxygen Consumption (in litres) and Heat Production Calories/Hour/M^2 (in Humans)

Age (yrs)	*Males*		*Females*	
	O_2 consumed	*Calories*	*O_2 consumed*	*Calories*
14–15	9.53	45.9	8.91	42.9
16–17	8.91	42.9	8.29	39.9
18–19	8.50	40.9	7.88	37.9
20–29	8.19	39.4	7.67	36.9
30–39	8.19	39.4	7.57	36.4
40–49	7.98	38.4	7.46	35.9
50–59	7.77	37.4	7.25	34.9
60–69	7.57	36.4	7.05	33.9

In many diseased states such as leukemia, hypertension, anaemia and fever, which involve increased cellular activities, the BMR is increased. Thyroid malfunctioning influences BMR to a great extent, hyperthyroidism increases BMR due to excessive O_2 consumption, while hyperthyroidism lowers it. The rate of heat production is also influenced by epinephrine. It is lowered in conditions of under-nutrition, deficiency of adrenal cortex and in some pituitary disorders.

Measurement and Calculation of BMR

The BMR is expressed in kilocalories per sq meter of the body surface area per hour. Surface area may be obtained from the Du Bois' standard chart. However, the average surface area of women is about 1.6 m^2, and for men about 1.8 m^2. A simple method for calculating surface area is circumference of midthigh (in cm) × height (in cm). A simple method to calculate BMR from the oxygen consumption is largely in use. The diet consists of a mixture of carbohydrates and fats, and the RQ of this mixture of foods is taken to be 0.82, a value obtained after 12 hours of fasting by the subject. If the O_2 consumption over a period of 10 minutes is 2.5 litres, then the hourly heat production under defined conditions at RQ 0.82 will be:

$$2{,}500 \times 4.825 \times 60/10, \text{ i.e. } 72.36 \text{ kcal.}$$

If this subject has a surface area of 1.8 m^2, the heat production would be 72.36/1.8 = 40.2 kcal/m^2/hr. The surface area of the body bears a relationship with height and weight.

Although there are different systems available for calculating normal heat production, Du Bois system is more favoured which is based on the height and weight:

$$A = Wt^{0.425} \times Ht^{0.725} \times 71.84$$

where A equals the area in m^2, Wt is in Kg and the height (Ht) in cm.

5.3 CALORIC REQUIREMENT

Caloric Requirements in Man

The heat production of normal individuals under basal conditions largely depends upon the factors of age, height and weight. The normal standards are based upon thousands of determinations (Table 5.4).

Table 5.4 Standard Values for Energy Production in Relation to Age and Sex

Age year	*Kilocalories/m^2/hour*	
	Males	*Females*
6	53.0	50.6
7	52.4	49.1
8	51.8	47.0
10	48.5	45.8
12	46.8	44.3
14	46.4	41.5
16	45.7	38.9
18	43.3	37.0
20	41.8	36.2
25	40.4	35.9
30	39.6	35.8
35	38.9	35.7
40	38.2	35.0
50	37.0	34.5
60	35.8	33.0

The above table is kg intervals for the height range of 110 to 200 cm and the weight range of 20 to 110 kg.

Caloric Requirements of Animals

The energy requirements of animals are varied depending upon their metabolic capacities. In cold-blooded animals most of the energy released from food is used to perform physical activities. Generally they need greater amounts of oxygen with increasing ambient temperature. Therefore, in such animals the metabolic rate is measured under specific conditions. This is known as *standard metabolic rate* (SMR). In warm-blooded animals such as birds and mammals, a high constant internal temperature is maintained. Their energy requirements vary with the change in ambient temperature in either direction. There is greater consumption of oxygen at low temperature associated with muscular activities. Thus in homeotherms, the basal metabolic rate represents the minimal metabolic energy required and is determined by measuring oxygen uptake by a resting animal.

After food ingestion the metabolic rate of an animal is enhanced resulting in more heat production. Increase in metabolism above the basal level is due to calorigenic effect of food and is called *specific dynamic action* (SDA) of food and its effect is known as *specific dynamic effect* (SDE).

There is undoubtedly a close relationship betwen body surface and the metabolism. In this regard greater attention has been paid to mammals as compared to other animals. In mammals oxygen consumption and therefore heat production varies with the body weight, approximating to about two-thirds. The relationship between weight and volume is 1:1, hence the heat loss is directly proportional to the surface area, because body surface determines heat loss.

Generally the BMR values have not been found to be related to climatic conditions. This is well illustrated by the fact that birds and mammals living in low temperatures of arctic, when exposed to temperatures much below their core body temperatures (50°C), do not show significant difference in the BMR as compared to birds and mammals of tropical regions.

Daily rhythm is another parameter which can hardly be over-emphasized in energy relationships. Physiologic measurements show striking relationship with the daily cycle. Factors such as oxygen consumption, body temperature, locomotor activity and blood sugar, etc., show variations in a cyclic manner. These daily cycles, called *circadian rhythms*, influence heat production to an appreciable extent.

CHAPTER

6

Metabolism

Metabolism can be defined as the sum total of chemical reactions necessary for the foodstuffs to be utilized by the body. We have already seen in Chapter 2, the foodstuffs fall under six categories: proteins, carbohydrates, lipids, water, minerals, and vitamins. These are broken down to simpler substances before being absorbed by the body tissues. During the process of biochemical reactions a number of intermediate products are formed which participate in a variety of interactions. The subject of metabolism is very vast and falls in the purview of biochemistry. However, we shall attempt to describe in brief such reactions that have relevance to the understanding of physiological processes.

Metabolism is absolutely essential to the maintenance of homeostasis of the body chemistry. During metabolism energy is required to breakdown the foodstuffs, which in turn yield more energy to derive other vital life processes. Some of the energy is evolved as heat necessary to maintain a constant body temperature (especially in warmblooded animals). A good deal of energy is utilized in the synthesis of new protoplasm during growth and tissue repair, in impulse transmission, in muscle contraction, etc. In general, these processes fall under two categories: (a) *anabolism,* in which simple substances are converted into complex substances; and (b) *catabolism,* involving degradation reactions converting complex substances into simpler molecules during which energy is released.

A. PROTEIN METABOLISM

Protein metabolism consists essentially of transformations of amino acids which are more readily absorbed from the intestine into the portal blood to be conveyed to the liver. Some proteins can be synthesized in the body from amino acids ingested in food as proteins, while a number of them are synthesized from amino acids not present in the diet. Amino acids are also oxidized for energy and utilized for non protein nitrogenous compounds. The body is not capable of storing large amounts of amino acids and proteins, hence interconversion of amino acids to other compounds like carbohydrates, fats, etc., takes place. Most of the absorbed amino acids are removed from the blood

by the liver and the muscles so that the average concentration of amino nitrogen is about 6 mg per 100 ml. This level is maintained almost constant, although the blood urea nitrogen may be somewhat increased.

Proteins a re indispensable and supply the required amino acids for growth, repair and maintenance of the body. In all, there are about 20 naturally occurring amino acids which fall under two categories: essential and non-essential (Table 5.1).

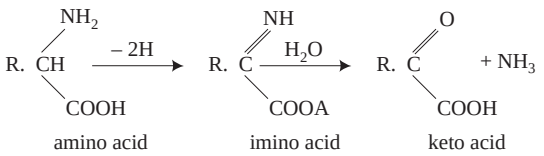
Table 6.1 Essential and Non-essential Amino Acids

Essential	*Non-essential*
Threonine	Glycine
Valine	Alanine
Leucine	Serine
Isoleucine	Aspartate
Methionine	Glutamic acid
Phenylalanine	Proline
Histidine	Hydroxyproline
Tryptophan	Arginine
Lysine	
Arginine	
*Cysteine	
*Cystine	
*Tyrosine	

*Replaceable amino acids.

6.1 OXIDATION OF AMINO ACIDS

The process of *deamination* takes place in the liver, kidney and intestinal mucosa, although urea formation is confined to the liver only. In this process ammonia is liberated in the intestine and the kidney, and goes into circulation in the form of glutamine. Very little amount of ammonia is found in the systemic circulation. The ammonia produced as a result of deamination of amino acids is converted into urea in the liver which is then excreted out.



Transamination involves interconversion of a pair of amino acids and a pair of keto acids catalyzed by transaminases or amino-transferases. The reactions are reversible.

Various amino acids enter the citric acid cycle in different ways. Valine, threonine and alanine can be converted to pyruvic acid. The conversion of alanine takes place in the following way:

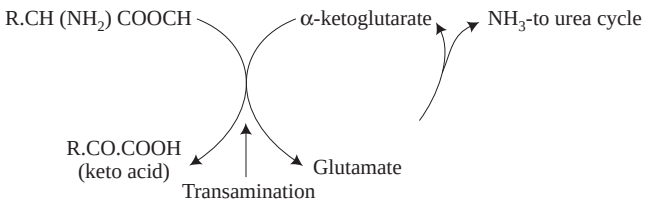
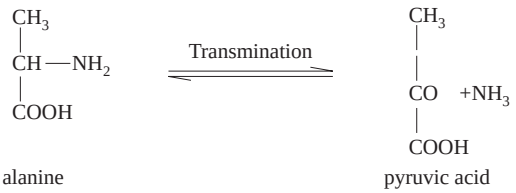


Fig. 6.1 Transamination reaction.



Aspartic acid is converted to oxaloacetic acid, while glutamic acid is changed to alpha ketoglutaric acid.

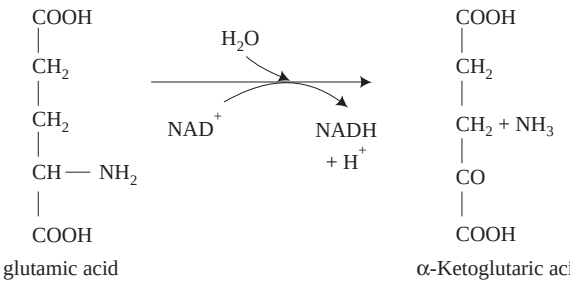


Fig. 6.2 Conversion of glutamic acid to α-ketoglutaric acid by glutamate dehydrogenase.

6.2 UREA SYNTHESIS

Ammonia is formed in the tissues by oxidative deamination, some of which is excreted in the form of ammonium salts (ammonium salts are excreted in metabolic acidosis). However, much amount of ammonia is excreted in the form of urea. The conversion of ammonia into urea takes place in the liver. The formation of urea is a complex process and takes place via ornithine cycle as proposed by Krebs. In the scheme of urea formation arginine is hydrolyzed by an enzyme arginase to yield one molecule of urea and one of ornithine. The details of the process are described in Chapter 14.

6.3 DECARBOXYLATION

Decarboxylation is a process in which certain amines are formed by the removal of CO_2 from the COOH (carboxylic group) of amino acids. Amines are physiologically important. Decarboxylation is catalyzed by amino acid decarboxylases in the presence of coenzyme pyridoxal phosphate. The resulting amines, for example, histidine yields histamine, tyrosine tyramine, and serine yields ethanol amine. Tyramine gives rise to adernaline.

6.4 REACTIONS OF SOME AMINO ACIDS

Although glycine is the simplest amino acid, it is a precursor of the ring systems in purine and porphyrins. Glycine can be converted into serine when it combines with formaldehyde (HCHO), a single carbon compound. Glycine, serine, alanine and glucose are interconvertible (Fig. 6.3).

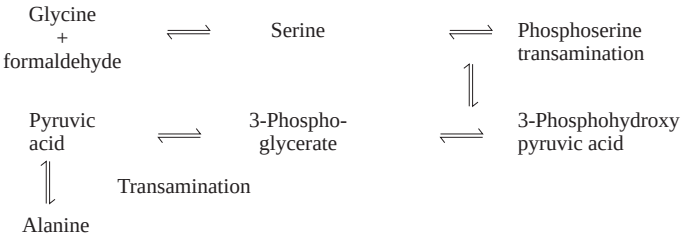


Fig. 6.3 Interconversion of glycine, serine and alanine (schematic).

Some amino acids have tremendous physiological importance. Phenylalanine and tyrosine fall in this category which have aromatic nuclei. These two amino acids form the precursors of the hormones thyroxine, noradrenaline and adrenaline. Phenylalanine can give rise to tyrosine in the body, but this is an irreversible reaction. Defective metabolism of these amino acids causes a disease *alcaptonurea* which is due to an inborn gene error. In this disease the urine turns black when exposed to air. A gene mutation causes the absence of *homogentisate oxygenase* from the cells, with the result, homogentisic acid accumulates in the cells and appears in the urine. In another disease, *phenylketonuria,* the conversion of phenylalanine to tyrosine is blocked, consequently the pigment melanin is not produced. Children deficient in phenylalanine suffer from mental imbecility (for more details refer to chapter on Physiological Genetics).

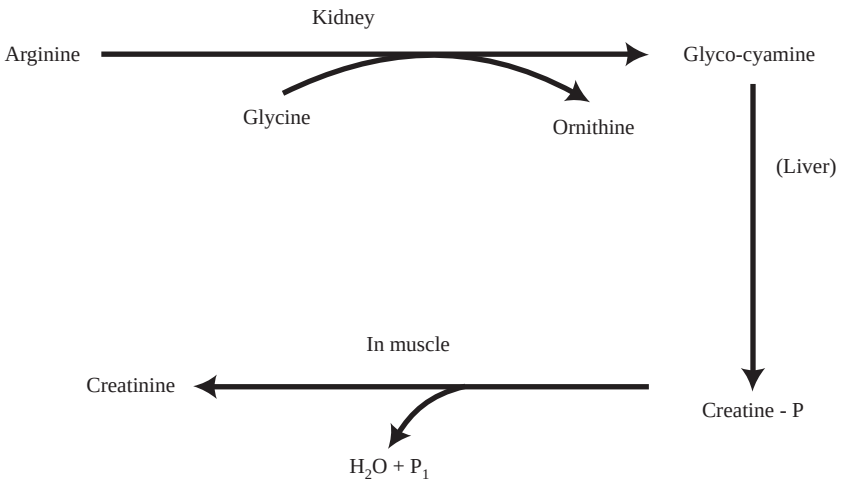
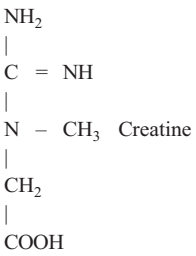


Fig. 6.4 Formation of creatine from arginine in the liver (schematic).

6.5 METABOLISM OF CREATINE AND CREATININE

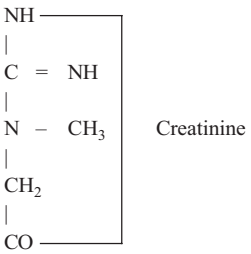
Creatine and creatinine are metabolically important compounds which are related to each other. Creatine is methyl guanidine-acetic acid, and its formula is:



Creatine is found abundantly in muscle, which is probably the site of its formation. It is composed of amino acids glycine, arginine and methionine.

Creatine is useful in the body in many ways. Hydrolysis of phosphocreatine in the muscle provides energy for muscle metabolism, for resynthesis of adenyl pyrophosphate, supplies energy for muscle contraction. Creatine accepts phosphates during glycolysis. When combined with phosphoric acid, creatine serves as a buffer.

Creatinine is an anhydride of creatine. Its structural formula is:



Creatinine is an excretory product and solely derived from creatine (Fig. 6.4). It is found in the muscles where it is synthesized and excreted in the urine. About 1.0 to 1.5 gm of creatinine per day is excreted in the urine and it is independent of the protein intake. After heavy muscular exercise creatinine output is temporarily accelerated which soon stops during the recovery period.

6.6 SULPHUR METABOLISM

Sulphur is a constituent of sulphur containing amino acids cysteine, cystine and methionine. It is also found in glycoproteins as mucoitin-sulphuric acid in mucine, sulpholipids in nervous tissue, or as inorganic sulphates. Sulphur is present in tissue proteins, in hairs, horns and feathers, in mucin as mucoitinsulphuric acid, in some glycoproteins of tendons, cornea and connective tissues.

Small amounts of sulphur-containing amino acids are utilized for the synthesis of the insulin. The tripeptide glutathione and the β–mercaptoethylamine, a constituent of coenzyme A. Important pathways of sulphur metabolism are shown in Fig. 6.5.

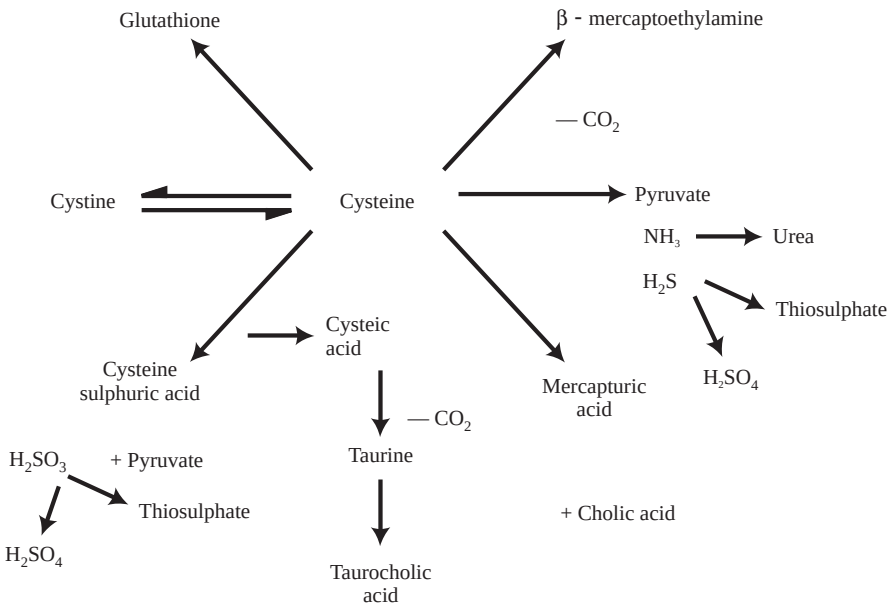


Fig. 6.5 Sulphur metabolism (schematic).

Majority of sulphur-containing amino acids are catabolized in the liver producing urea, and sulphur is oxidized as sulphuric acid to be excreted as sulphates in the urine. The sulphur compounds are useful in the following ways:

(1) Synthesis of tissue proteins like hair, feathers, etc.
(2) Synthesis of glutathione, taurine, insulin, sulphatides, etc.
(3) Production of sulphuric acid in the liver which is used for detoxication of compound like phenol.
(4) Formation of heparin.
(5) Help in the activity of several enzymes where free SH group is available.

6.7 METABOLISM OF NUCLEOPROTEIN

Nucleoproteins are complex compounds that are present in the chromosomes of the nucleus and the cytoplasm. Chemically they are composed of simple proteins like protamines, prolamines or histones conjugated with nucleic acids and are rich in basic amino acids. They are made up of basic building blocks called nucleotides containing purine and pyrimidine bases, pentose sugars and phosphoric acid. The purines are converted to uric acid and pyrimidines are oxidized to produce CO_2 and NH_3. The pentose sugars are in the form of deoxyribose or ribose resulting in deoxyribose nucleic acid (DNA) or ribose nucleic acid (RNA) respectively. The purine bases comprise adenine and guanine, and pyrimidines consist of thymine and cytosine. All these four bases are present in DNA, whereas in RNA thymine is replaced with uracil.

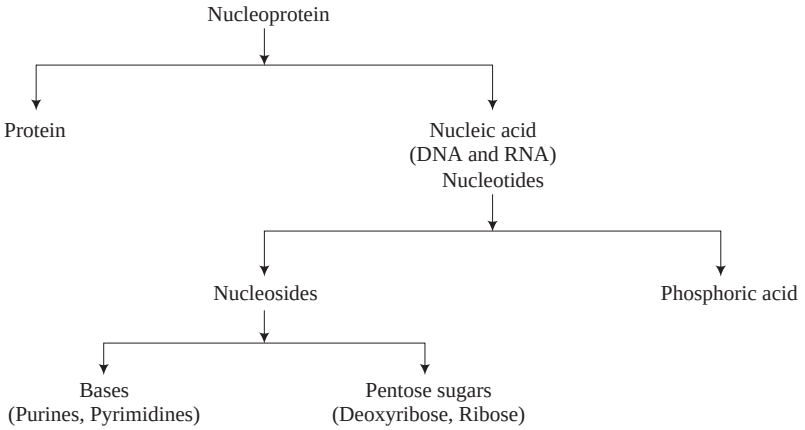


Fig. 6.6 Hydrolysis of nucleoproteins (schematic).

Nucleotides are nucleoside phosphates. Nucleosides are formed when the phosphoric acid component is removed. They are moderately or entirely soluble in water.

Nucleoproteins are of two kinds: exogenous and endogenous. The exogenous sources are muscles and tissues like pancreas, testis, kidney, thymus, etc. Endogenous sources are various cells that undergo breakdown during the metabolic process.

Several nucleotides function as coenzymes which are derivatives of 5-adenylic acid. Some of the important coenzyme nucleotides which take part in the metabolism are: nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP) and flavin adenine dinucleotide (FAD).

Ribonucleic Acid

About 80 percent of the RNA in a cell, is associated with ribosomes. They are primarily found in the cytoplasm of the cell. Very little RNA is found in the nucleus. RNA plays the key role in protein synthesis. RNA is labile to alkali and is hydrolyzed by ribonuclease (RNAse).

Deoxyribonucleic Acid

The nucleus of the cell contains almost all the DNA of the cell. It is the primary component of the genes which are the carriers of heredity. The amount of DNA remains constant in the somatic cells; however, just prior to cell division this amount is doubled so that each daughter cell receives the same amounts as that of the somatic cell. The DNA is a double stranded helical structure, and each strand is complementary to the other. These strands from the templates for the transcription of RNA molecules. The synthesis of DNA is found to be most active in bone marrow, thymus and embryonic tissues where the cell proliferation is maximum and rapid.

Catabolism of Nucleic Acid

The ingested nucleo-proteins are hydrolyzed into protein and nucleic acid in the digestive tract by the action of proteases. The specific enzymes, deoxyribonuclease and ribonuclease break the DNA and

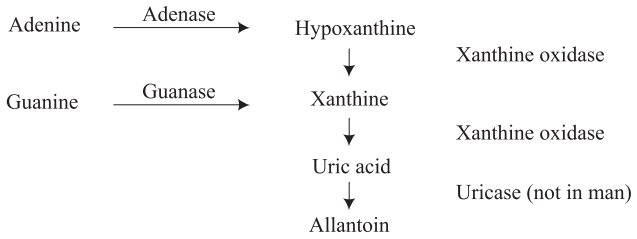


Fig. 6.7 Catabolism of purine bases (schematic).

RNA respectively into oligonucleotides and tetranucleotides. Nucleotidases act on nucleotides and nucleosidases act on nucleosides. The nucleosides upon hydrolysis form adenylic and guanylic acids. The nucleotidase acts upon nucleotides to form adenosine or hypoxanthine and guanosine or xanthine respectively. By specific enzymes, adenase and guanase, adenine and guanine are converted to hypoxanthine and xanthine respectively. An enzyme oxidase then acts upon hypoxanthine to convert it to xanthine; xanthine is further converted to uric acid by the action of xanthine oxidase. The catabolism of purine bases is shown in Fig. 6.7.

The purine bases are absorbed in the blood and one of the final wastes in man is uric acid. In other animals, uric acid is converted to allantoin. The fate of pyrimidines is quite complicated. However, it is known that they are catabolized to CO_2 and NH_3.

Protein Biosynthesis

Proteins are continuously degraded to amino acids, and side by side amino acids continually participate in protein formation. Proteins are essential for the body and form structural proteins, and multiple enzyme systems and hormones that are necessary for chemical reactions. Proteins cannot be synthesized from any other source except amino acids and thus they form an essential component of the diet. Protein synthesis is under genetic control, that is gene directed. As a result of current researches, a fairly good picture has emerged as to how genetic information stored in the DNA is translated and expressed in specific protein molecules. This aspect falls under the field of biochemistry. The reader is therefore advised to look for this in a textbook on biochemistry.

B. CARBOHYDRATE METABOLISM

Carbohydrates are the main foodstuffs which are synthesized by plants and utilized by animals for their energy requirements. We have discussed in efficient details the different classes of carbohydrates in Chapter 2. In this chapter attention will be given to the manner in which carbohydrates are employed as sources of energy in different tissues and the way they are distributed and stored in the body.

The food of organisms contains large amounts of carbohydrates in the form sugars and starches which are hydrolyzed in the course of digestion into their monosaccharide moieties or simple sugars. These simple sugars are absorbed by the small intestine and utilized in many ways which may be enumerated as follows:

(1) Simple sugars like glucose, fructose, and galactose are absorbed by the intestine and may circulate as blood sugars.
(2) Sugars (glucose) are absorbed into the portal blood and converted into glycogen for storage and future use.
(3) Simple sugars may be changed into glycogen in the muscles.
(4) They may be transformed into fat and deposited as adipose tissue.
(5) A good portion of the absorbed glucose is oxidized as an immediate source of energy.
(6) Some amount of sugars is excreted in the urine.

6.8 BLOOD SUGAR

Glucose is the free sugar which circulates in the blood. After a meal, the circulating blood sugar level is elevated quickly after absorption. In a fasting human being the glucose concentration in blood is about 80 mg per 100 ml. However, the blood sugar level is maintained almost constant and varies within narrow limits only unless during abnormal conditions like hyperglycemia or hypoglycemia.

Several regulatory mechanisms are responsible for maintaining blood sugar level. Much of the glucose absorbed from the gut is passed on to the tissues for oxidation or converted into glycogen in the muscles (Fig. 6.8). Quite a good amount is still converted to glycogen in the liver through the process of glycogenesis. In case the blood sugar level falls below the required level, glycogenolysis occurs. Thus these two processes have a marked regulatory effect.

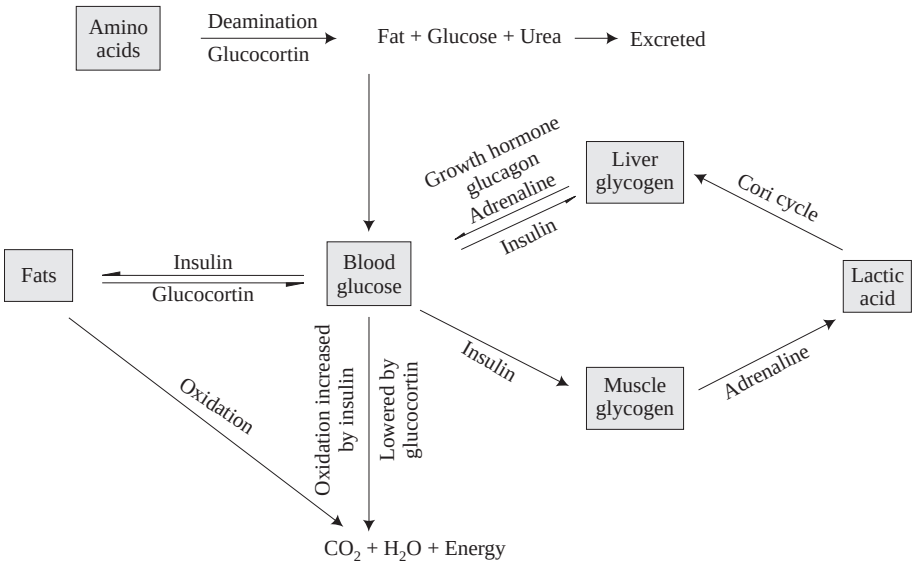


Fig. 6.8 Regulation of blood sugar.

A number of hormones are responsible for glucose regulation. There are three endocrine organs involved in carbohydrate metabolism. These are: pancreas, adrenals and anterior pituitary. We shall discuss in brief the role of each of these (for more details see Chapter 19). The islets of langerhans of the pancreas secrete the hormone *insulin* which is closely linked with carbohydrate utilization in the body. Increase or decrease in the amounts of circulating glucose depends upon insulin efficiency. Insulin speeds the movement of glucose from the blood into tissue cells, thereby lowering the blood glucose level. If too much of insulin is present in the circulation, the blood sugar level will drop below normal causing hypoglycemia. On the other hand, if enough insulin is not present, then the transport or mobilization of blood sugar is slowed down causing rise in blood sugar level. This condition is known as hyperglycemia. Deficiency of insulin causes the disease, *Diabetes mellitus.* Insulin may also help in the process of phosphorylation during glycolysis by acting as a coenzyme to the enzyme glucokinase.

Another hormone that affects the blood sugar level is adrenaline and produced by the adrenal medulla. This hormone increases the concentration of glucose in the blood by facilitating the breakdown of liver glycogen. Noradrenaline probably performs the same function to a very limited extent.

The steroid hormone from the adrenal cortex, hydrocortisone, stimulates the liver to convert proteins and fats to carbohydrates (gluconeogenesis) resulting in an increase blood sugar level.

The anterior pituitary (adenohypophysis) secretes some hypoglycemic hormones which include thyroid stimulating hormone (TSH), adrenocorticotropic hormone (ACTH) and growth hormone (STH). These hormones antagonize the action of insulin and elevate the blood sugar.

6.9 GLYCOLYSIS

After absorption the glucose molecule is subjected to a series of reactions and is completely oxidized into CO_2 and H_2O plus some energy. The splitting of glucose molecule is referred to as glycolysis. One mole of glucose when oxidized yields 686 kilocalories of kinetic (active) energy. Much of the energy released during carbohydrate metabolism is stored in the form of high energy phosphate compounds such as ATP. In the process of glucose oxidation several chemical steps are involved and each step is catalyzed by specific enzyme (Fig. 6.5). In the first step each molecule of glucose produces two molecules of pyruvic acid:

$$\text{Glucose} + 2\text{ADP} + 2\text{PO}_4 \rightarrow 1 \text{ pyruvic acid} + 2\text{ATP} + 4\text{H}$$

The net yield of energy is 2 molecules of ATP. The series of reactions involved in glycolysis are collectively known as *Embden-Meyerhof Pathway*. The entire process is an anaerobic process and does not require oxygen.

After glucose has been converted to pyruvic acid, the next step is the conversion of pyruvic acid to acetyl coenzyme A.

$$\text{Pyruvic acid} + \text{Coenzyme A} \rightarrow \text{Acetyl coenzyme A} + 2\text{H}$$

The reaction neither requires ATP, not is ATP generated. Now acetyl coenzyme A undergoes another series of reactions which is referred to as *citric acid cycle* or *Krebs cycle.* This cylcle is

aerobic and the end products are CO_2, H_2O and energy in the form of ATP. The details of the steps are given in Fig. 4.6. A total of 38 molecules of ATP are produced utilizing only about 55 per cent of kinetic energy released during the process.

Besides Embden-Meyerhof Pathway, there is yet another pathway for glycolysis known as *hexose monophosphate shunt* (HMP). This pathway occurs in the liver, lactating mammary glands and the adipose tissue of mammals. Considerable fraction of glucose is oxidized in this way. In the HMP-pathway, first glucose-6-phosphate undergoes dehydrogenation and decarboxylation to yield ribulose-5-phosphate (a pentose). In the next phase ribulose-5-phosphate is converted back to gIucose-6-phosphate by a series of intermediate reactions. In case of HMP-pathway, NADP (nicotinamide adenine dinucleotide phosphate) is used instead of NAD, as hydrogen acceptor. The shunt works as a source of chemical rather than energy.

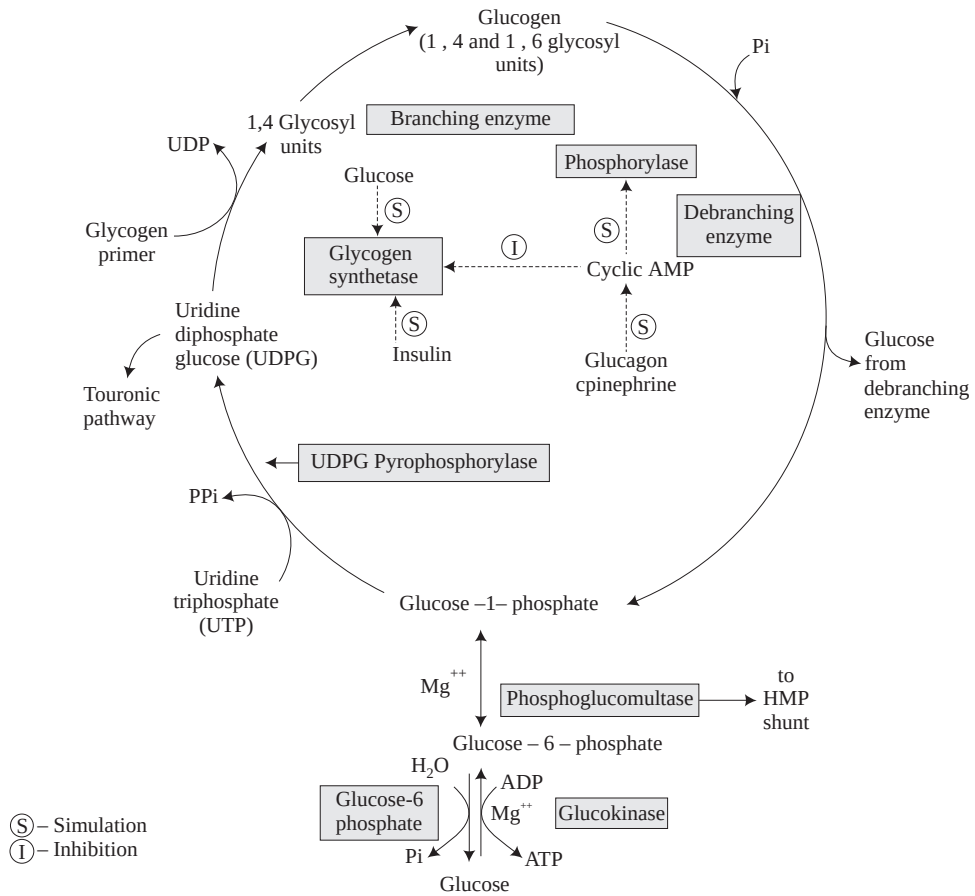


Fig. 6.9 Glycogenesis.

6.10 GLYCOGENESIS

The formation of glycogen is called glycogenesis which occurs both in the liver and the muscles (Fig. 6.9). First, glucose is phosphorylated to glucose-6-phosphate, which is then converted to glucose-1-phosphate, a reaction catalyzed by phosphoglucomutase. Then glucose-1-phosphate reacts with uridine triphosphate (UTP) to form uridine diphosphate glucose (UDPG). After this, enzyme glycogen synthetase reacts with UDPG and forms a glycosidic bond between 1 carbon of activated glucose and 4 carbon of the glucose residue of glycogen releasing uridine diphosphate (UDP). The reactions showing interconversion of glucose and glycogen in the liver are given in Fig. 6.12.

Liver glycogen is formed not only from simple sugars, but also from the lactic acid that is produced in the muscles (Fig. 6.8). Lactic acid produced during muscle contraction goes to the liver through the circulating blood and is converted into glycogen. Glycogen in the liver can be converted into glucose, and glucose can be changed to muscle glycogen which in turn is converted into lactic acid, some of which is later transformed back to liver glycogen. The cyclic pathway involved in this process is known as *Cori cycle* (Fig. 6.10).

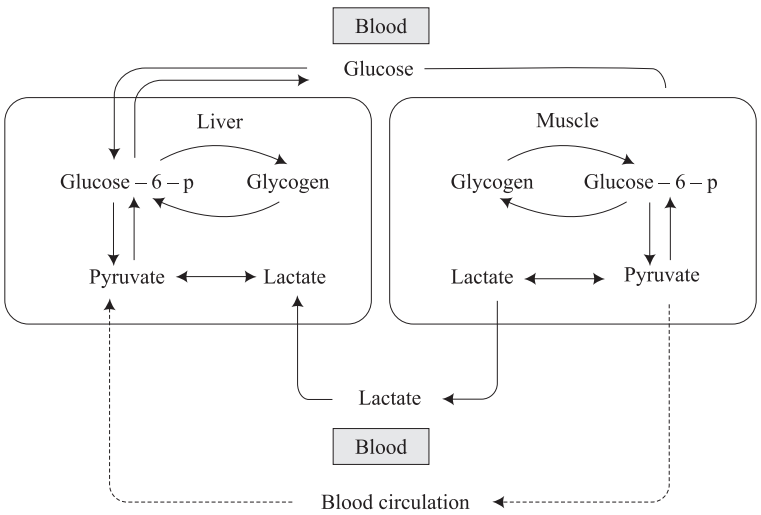


Fig. 6.10 Cori Cycle.

6.11 GLUCONEOGENESIS

Liver glycogen is also formed from non-carbohydrate sources such as proteins and fats. The conversion of protein into glycogen is known as gluconeogenesis. Formerly it was believed that gluconeogenesis occurs only in special circumstances. However, lately it has been proved that it occurs simultaneously with glycogenolysis. The excess amounts of protein are metabolized by the carbohydrate pathway and can be converted into glucose or glycogen by reversal of glycolysis. Gluconeogenesis is most important because it usually occurs when the glycogen store is exhausted.

In the process, the excess of protein is first hydrolyzed to amino acids which are then deaminated and later metabolized through either carbohydrate pathway or fat metabolism.

In addition to these sources of glucose or glycogen in the liver, glucose may derived from fat also, although this conversion takes place to a very limited extent. In this conversion the essential part of the process consist of the oxidation of long chain fatty acids in the mitochondria. Then the glycerol component of the fat reacts with ATP to form glycerol phosphate which is then oxidized to glyceraldehyde 3-phosphate, This may be further oxidized to pyruvic acid or may be converted to glycogen by reversal of the part of the glycolytic pathway.

6.12 MUSCLE GLYCOGEN

Muscles are no less important than liver in carbohydrate metabolism. Normally the concentration of glycogen in the muscle ranges between 0.5 and 1.0 per cent of the weight of the muscle. In this way muscle glycogen is far greater in amount than the liver glycogen. Liver is the storage organ for glycogen, whereas muscle glycogen acts as a source of energy during contraction. Starvation does not affect the muscle glycogen. Synthesis of glycogen in the muscle takes place in the same manner as in case of liver. Muscle cannot convert glycogen to glucose since the specific enzyme required for its conversion is absent in the muscle. However, glycogen is broken down to lactic acid in the muscle from where it is carried to the liver through the bloodstream to be converted into glycogen.

6.13 METABOLISM OF OTHER SUGARS

Glucose, galactose and fructose are sugars of great metabolic importance. These sugars enter the metabolic pathways after being phosphorylated. Phosphorylation of glucose has already been described in connection with glycolysis. The source of galactose is mainly the lactose content of the milk. Galactose is first phosphorylated in the presence of a specific enzyme *galactokinase,* and then it reacts with uridine diphosphogalactose to form uridine diphosphoglucose which may participate in glycogen synthesis. Large quantities of galactose in blood are known to be toxic and cause a disease *galactosemia.*

Fructose is obtained from sucrose and fruits and is readily converted into glucose or glycogen in the liver. It is phosphorylated in the presence of fructokinase into fructose-1-phosphate. The sequence of events is shown below (Fig. 6.11).

Occasionally glyceraldehyde is oxidized to glycerol. Glyceraldehyde can also be reduced to glyceraldehyde-3-phosphate (triose), and two molecules of triose can be converted to fructose-1, 6-diphosphate.

C. FAT METABOLISM

Fats and lipids are important constituents of the protoplasm. They may be present in the foods or may be formed in the body. We have described the chief classes of fats and lipids in Chapter 2. The

structural fats are very complex compounds, whereas reserve fats or depot fats are the ordinary fats which make up the bulk of the body. Depot fats are in the form of neutral triglycerides which can be hydrolyzed to form monoglycerides and free fatty acids. Triglycerides may be resynthesized from the fatty acids in the cells of intestinal mucosa.

In the metabolism of fat, three major processes are involved:

(1) Mobilization of fat from storage fat depots of the body, which later take part in catabolism.

(2) Absorption of digested fats.

(3) Synthesis of fats in the liver, from intestinal muscosa and the adipose tissue carbohydrate and protein sources.

Mobile fat is in the form of minute oily droplets known as *chylomicrons* which travel in the bloodstream. Chylomicrons are made up of neutral fats, and also consist of phospholipids (fat plus phosphate), cholesterol and cholesterol esters of fatty acids. The chylomicrons are absorbed by the liver where they are hydrolyzed giving rise to free glycerols and fatty acids. Mobilization of fats can be conveniently observed in case of starving animals. After starvation for a short period, the glycogen reserve of the liver is depleted. Since no more of carbohydrates are synthesized (except by way of gluconeogenesis), the liver does not receive its carbohydrate supply. Under such conditions, large, amounts of fats are transported to the liver which take part in metabolism.

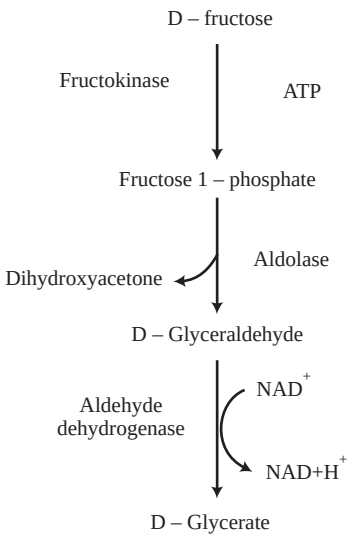


Fig. 6.11 Metabolism of fructose.

6.14 ROLE OF LIVER IN FAT METABOLISM

Liver has a key role in the metabolism of fats. It has been proved beyond doubt that in conditions of carbohydrate depletion, most of the fat of the body is mobilized to the liver to provide an alternative source of energy. Liver is not normally an accumulator of fats as it is for carbohydrates. The fat content in the liver is maintained uniformly constant between 3 and 8 per cent. Thus excess of fat deposits is transformed by the liver into useful substances through various interconversions (Fig. 6.12).

Besides interconversion of proteins, fats and carbohydrates, which goes on in the liver, it is also responsible for transformations of lipids into phospholipids and cholesterol, desaturation of fatty acids, oxidation of fatty acids, etc. The sluggish function of the liver results in several metabolic disorders, which may be normally due to the effect of certain poisons, fat-rich diet, protein-poor diet, deficiency of vitamins and a host of other causes. Therefore, we can say that liver is the predominant organ which maintains the healthy state of an individual.

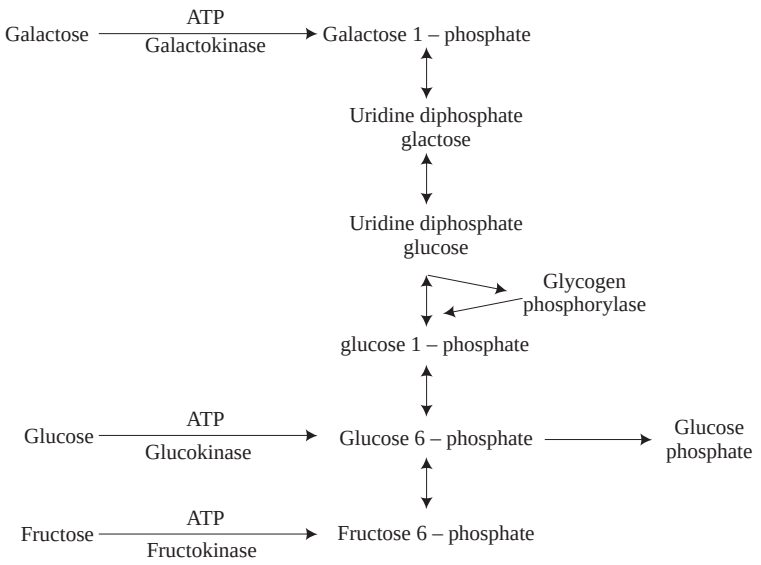
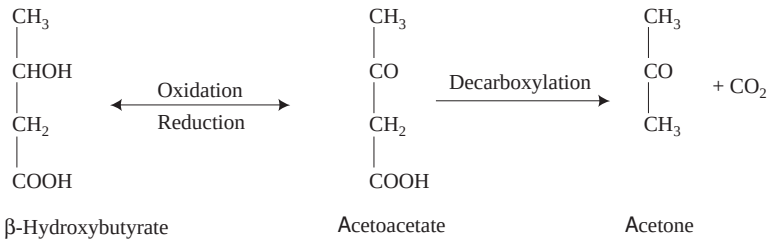


Fig. 6.12 Interconversion of carbohydrates in the liver.

6.15 OXIDATION OF FATTY ACIDS

Fats, especially triglycerides, are hydrolysed to their constituents fatty acids and glycerol before they proceed to catabolic pathway. Much of the fat hydrolysis takes place in adipose tissue, where free fatty acids (FFA) are produced to be carried into the plasma (Fig. 6.13). The FFA acids reach the tissues (liver, kidney, heart, muscle, testis, brain, and adipose tissue) where oxidation takes place. The long chain fatty acids are systematically broken down to 2-carbon units in the form of "active acetates". The acetate and the long chain fatty acids are metabolised through a common pathway, requiring ATP and CoA enzyme (Fig. 6.14).



The acetyl CoA can either combine with oxaloacetate before entering the citric acid cycle, or it may be directly oxidised to acetoacetate, the first ketonc body. Acetoacetate may further breakdown to β-hydroxybutyrate and acetone, which accumulate in the liver in small amounts. The liver tissue cannot oxidise acetoacetate. Acetone arises from acetoacetate by spontaneous decarboxylation.

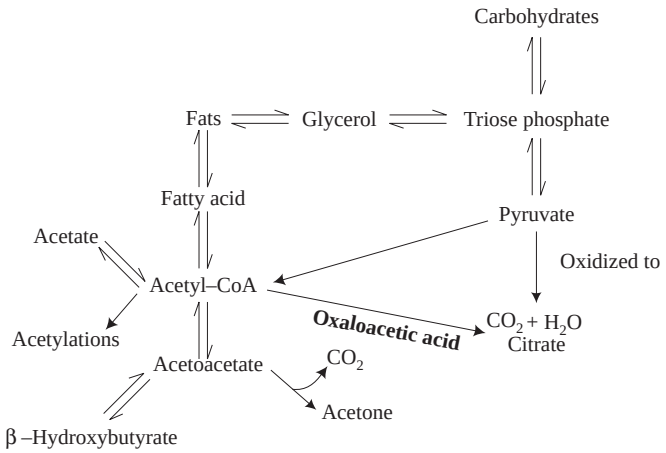


Fig. 6.13 Schematic representation of metabolism of fats and carbohydrates.

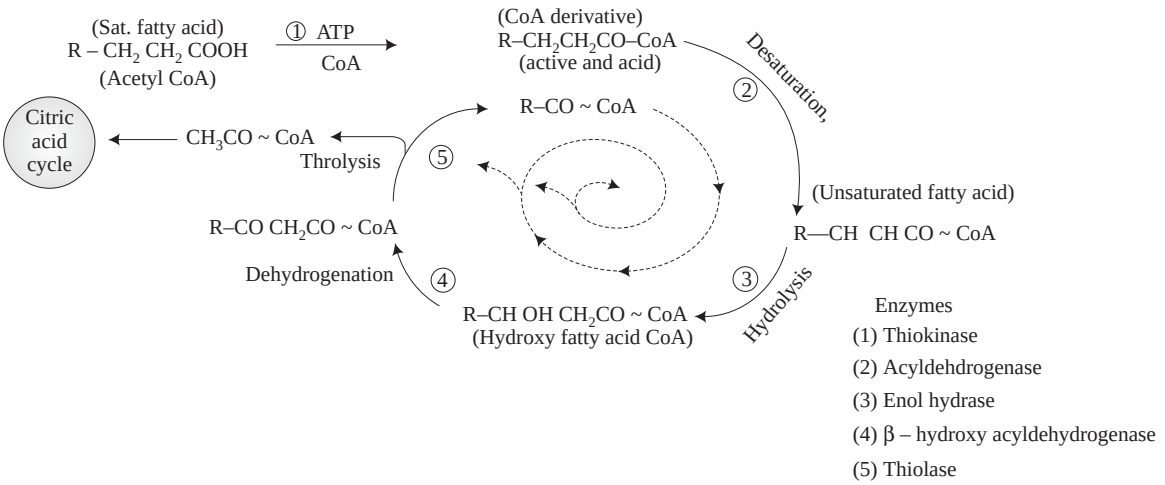


Fig. 6.14 Metabolism of fatty acids.

The utilisation of glycerol is dependent on the activating enzyme, *glycerol kinase,* which is found in sufficient amounts in liver, kidney, intestine, brown adipose tissue and lactating mammary glands.

6.16 β-OXIDATION OF FATTY ACIDS

Fatty acids are mainly oxidised by a process called β-oxidation, a scheme proposed by Knoop in 1904. He postulated that since majority of neutral fats contain even number of carbon atoms, β-oxidation is the more likely process in which the fatty acid molecule is broken down in a stepwise manner, removing 2-carbon atoms from the carboxyl end in each step, yielding acetate equivalents.

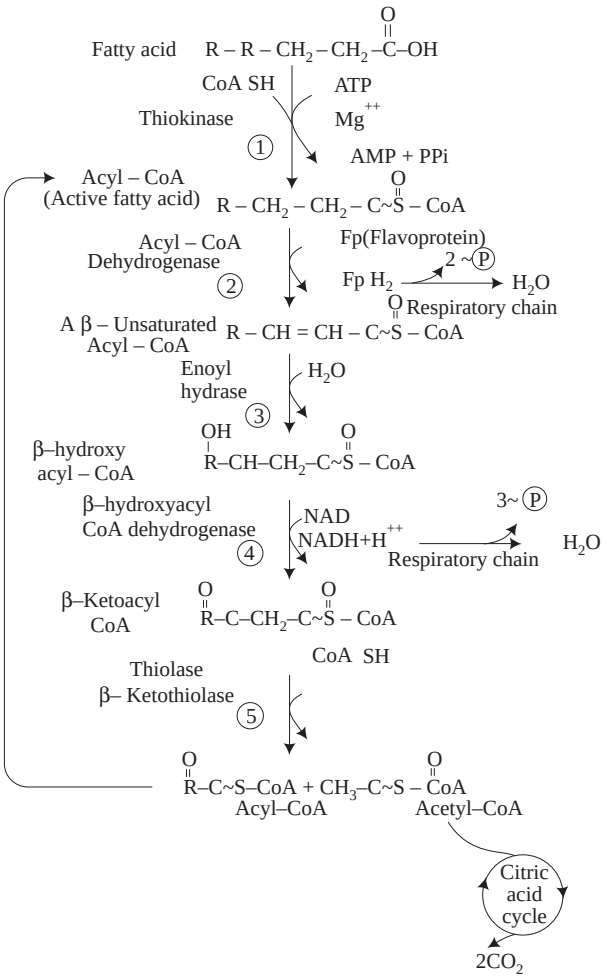


Fig. 6.15 β-oxidation.

The acetate molecules can be completely oxidised via citric acid cycle or may be utilised to synthesise glucose and other complex carbohydrates as per needs of the animal. Some important steps of β-oxidation scheme are shown in Fig. 6.15.

It must be borne in mind that fatty acid oxidation takes place in the mitochondria, but before FA enters the mitochondria it has to be made ready for oxidation reactions. The FA in the cytosol is activated by a molecule of ATP in the presence of acylcoenzyme A(CoASH). The reaction occurs either in the endoplasmic reticulum or at the outer mitrochondrial membrane, resulting in the formation of fatty acyl CoA derivative. The fatty acyl CoA derivative is then transported inside the mitochondria with the help of *carnitine,* a carrier molecule. This reaction is catalysed by an enzyme, acyl CoA transferase. Once the fatty acyl CoA enters the mitochondrial matrix, there follows the removal of 2 hydrogen atoms from the α and β carbons, catalysed by a dehydrogenase, resulting in

the formation of unsaturated acyl CoA. The unsaturated fatty acyl CoA derivative is subsequently hydrated and dehydrogenated at the expense of specific enzymes to form corresponding β-keto-acyl CoA compound. Finally, β-keto-acyl CoA undergoes thiolytic cleavage by *thiolase* producing an acyl-CoA unit and the remaining acyl-CoA chain containing 2-C less than the original fatty acyl CoA molecule. In this way, a long chain fatty acid may be degraded completely to acetyl-CoA (2-C fragments), which can be oxidised to CO_2 and water through citric acid cycle.

In case of fatty acids with odd number of carbon atoms, oxidation takes place through β-oxidation scheme, leaving behind propionyl CoA, a 3-carbon unit. This compound can enter the citric acid cycle after conversion to succinyl CoA.

Energetics of β-oxidation

Let us consider the oxidation of one mole of palmitic acid ($C_{16}H_{32}O_2$), entering the mitochondria in the form of palmitoyl CoA. Initially one mole of ATP is required to activate the acid, and at the end of each oxidative spiral, one $FADH_2$ and one NADH are formed along with an acetyl CoA fragment. In order to oxidise palmitoyl CoA, 8 acetyl CoA will be formed and the energy gained in terms of ATP will be as follows:

8 acetyl CoA + 7 $FADH_2$ + 7 NADH + H^+	35 ATP formed
8 acetyl CoA oxidised via citric acid cycle	96 ATP formed
ATP initially used for activation	01 ATP consumed
Net gain of ATP	130 ATP

The overall equation is represented as:

$$C_{16}H_{32}O_2 + ATP + 7\ FAD + 7\ NAD^+ + 8\ CoASH + 7\ H_2O \rightarrow AMP + PPi + 7\ FADH_2 + 7\ NADH + H^+ + 8\ CH_3CO—S—CoA.$$

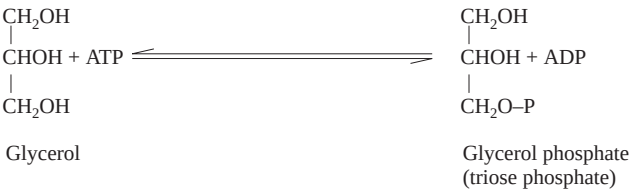
Since each ATP molecule has 7.6 kcal of bond energy, the net gain would be $130 \times 7.6 = 988$ kilocalories. The calorific value of palmitic acid is 2340 kcal/mole, the system receives at least 42% of high phosphate bond energy ($988/2340 \times 100$) of the total energy of combustion of the fatty acid.

Oxidation of Unsaturated Fatty Acids

Body lipids are rich in unsaturated fatty acids and these are oxidised more slowly. Some examples are: palmitoleic acid (16:1), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3) and arachidonic acid (20:4). All double bonds in naturally occurring unsaturated fatty acids are in *cis*-configuration. Palmitoleic and oleic acids are not essential as they can be formed in the body, but the remaining three acids come under the *essential fatty acids* category and have to be supplied in the diet. Oxidation of unsaturated fatty acids proceeds the usual β-oxidative pathway until the double bond is reached. The double bond in *cis*-configuration is not vulnerable to enzymic attack unless it is isomerised to *trans*-configuration. Polyunsaturated fatty acids, such as linoleic, arachidonic etc. are more complex and require additional enzyme for oxidation. They are normally found as structural components in association with cholesterol and phospholipids (e.g. membranes and reproductive organs). In mammals, arachidonic and some related C-20 fatty acids are known to give rise to unique compounds like *prostaglandins* which have hormone-like activity.

6.17 METABOLISM OF GLYCEROL

One of the hydrolysis products of triglycerides is glycerol which is metabolised or utilised in organs/tissues where specific enzyme glycerol kinase is abundantly present. Certain organs such as liver, kidney, intestinal mucosa and lactating mammary glands are rich in the enzyme, while muscles and adipose tissue contain very little activity. Glycerol is predominantly converted into carbohydrate through glycerol phosphate, formed by a specific glycerol kinase at the expanse of ATP.



Glycerol phosphate is then oxidised to triosephosphate by a glycerol phosphate dehydrogenase which ultimately forms glycogen through glycogenesis. Triosephosphate may be, however, oxidised to pyruvic acid by way of glycolysis (Fig. 6.16). In diabetic or phlorrhizinised animals, glycerol is converted almost quantitatively to glucose.

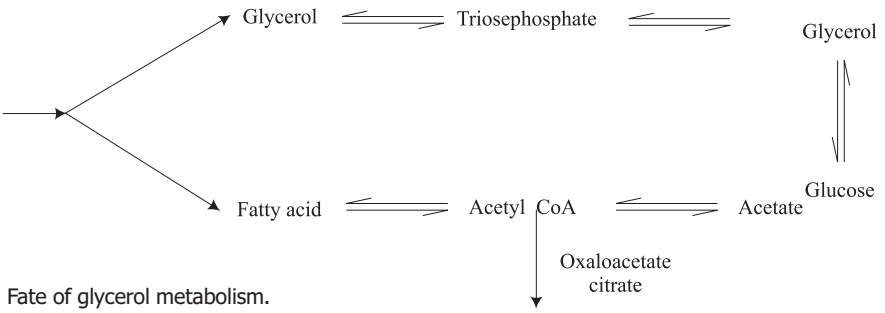


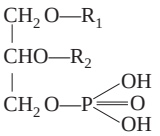
Fig. 6.16 Fate of glycerol metabolism.

6.18 SYNTHESIS OF GLYCERIDES AND FATTY ACIDS

It has been known for a long time that fats are synthesised from metabolites such as acetate and acetoacetate. Fats are also synthesised from protein and carbohydrate sources. All naturally occurring fatty acids possess even number of carbon atoms; thus it is logical that fatty acids must be synthesised from 2-carbon fragments. It has been shown that the starting material is acetyl-CoA which can be derived from pyruvate. Alternatively, pyruvate can also be derived from free, acetate when it reacts with coenzyme A and ATP.

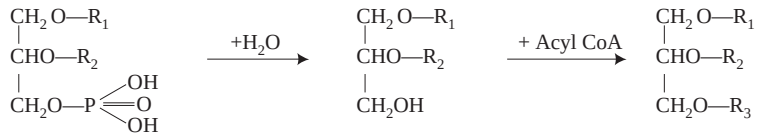
Synthesis of Glycerides

Triglycerides are formed by reactions between acyl-CoA compounds and ∝-glycerophosphate, which is formed by specific glycerol kinase. There are other enzymes which catalyse the formation of mono—and diphosphatidic acids at the expense of fatty acyl CoA derivatives:



Phosphatidic acid

In the next stage the phosphate group is removed by a specific phosphatase followed by replacement by a third fatty acyl residue:



Phosphatidic acid Diglyceride Triglyceride

Synthesis of Fatty Acids

Fatty acid oxidation occurs in the mitochondria, but fatty acid synthesis takes place not only in mitochondria but also in mitochondria-free systems. The pathway for synthesis is not exactly reversal of β-oxidation scheme, but it involves, some modifications. Under anaerobic conditions, mitochondria catalyse the incorporation of acetyl-CoA units into long chain fatty acids (viz. stearic acid, palmitic acid), requiring ATP, NADH and NADPH. Synthesis in extra-mitochondrial system, especially in the liver, brain, kidney etc., acetyl-CoA units are incorporated into fatty acids, catalysed by cytosolic enzymes and cofactors such as ATP, NADPH and Mg^{2+} or Mn^{2+} ions. This system is dependent on CO_2 supplied by bicarbonate. Chain elongation usually takes place in the microsomes.

6.19 METABOLISM OF PHOSPHOLIPIDS

Phospholipids are found in all cells and are synthesised either from phosphatidic acid or phosphatidyl choline. Lecithin is the most important phospholipid in the body and is synthesised in the liver. Synthesis of phospholipids from fats involves mobilisation of fats in and out of the cells. Hence the actual sites are liver, intestine and kidney. Phospholipids are largely found in combination with proteins and are transported in the blood in the form of protein complexes.

The most common among phospholipids are lecithins and cephalins, while where are others in which bases are replaced by serine or inositol. Catabolism of lecithin is accomplished in the following manner (Fig. 6.17):

Sphingomyelins are phospholipids containing a fatty acid, phosphoric acid, choline, and a complex of amino alcohol, sphingosine, but are devoid of glycerol. Abnormal quantitities of phospho- and sphingolipids in certain tissues, especially in the nervous system, cause diseases like sphingolipidoses, and demyleinating diseases that are inherited.

6.20 METABOLISM OF CHOLESTEROL

Cholesterol occurs in various body tissues and in the plasma either in the free form or storage form as long chain fatty acid esters belonging to the class of sterols. It is also an important component of the

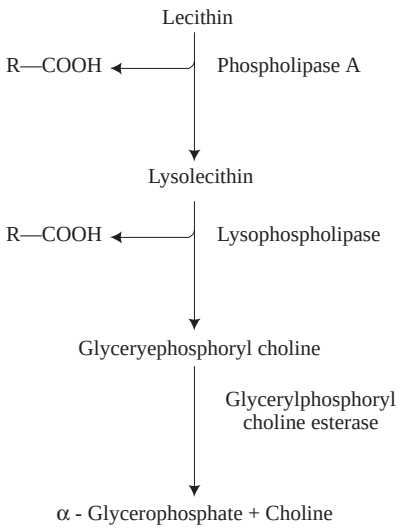


Fig. 6.17 Catabolism of lecithin.

membrane system of the cell and is the precursor of steroid hormones, excreted from the body in the form of bile acids (salts). The cholesterol content of the blood ranges from 150 – 250 mg/100 ml.

Sources of Cholesterol in the Body

While major part of the body cholesterol (about 1 g/day) is synthesized in the body, a small portion is (about 0.3 g/day) provided by various foods intake such as egg yolk, meat, liver, and brain and its uptake is through low density lipoproteins (LDLs). Synthesis occurs from acetyl-CoA (two-carbon units) produced by beta oxidation of fatty acids in the liver, intestine and almost all cells. About 700 mg/d is synthesized by a well regulated mechanism. Free cholesterol is removed from the body by plasma high-density lipoproteins (HDLs).

Synthesis of Cholesterol

Although cholesterol is synthesized by many tissues such as adrenal cortex, skin, intestine testes etc. liver is considered to be the main site. Two molecules of acetyl-CoA condense to form acetoacetyl-CoA, which again reacts with another molecule of acetyl-CoA to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), leading to the synthesis of mevalonate (Fig. 6.18). It must be noted that all carbon atoms of cholesterol originate from acetyl-CoA enzyme. Mevalonate is the crucial compound and through a series of reactions, gives rise to cholesterol.

Transport and Excretion of Cholesterol

Cholesterol, in association with other lipids, is absorbed in the intestine and thereafter incorporated into chylomicrons and very low-density lipoproteins (VLDLs). A greater part of cholesterol (about 80%) in the lymph undergoes esterification with long-chain fatty acids and transported as lipoproteins in the plasma. Highest proportion of cholesterol is found in the LDL. Free cholesterol in the plasma is

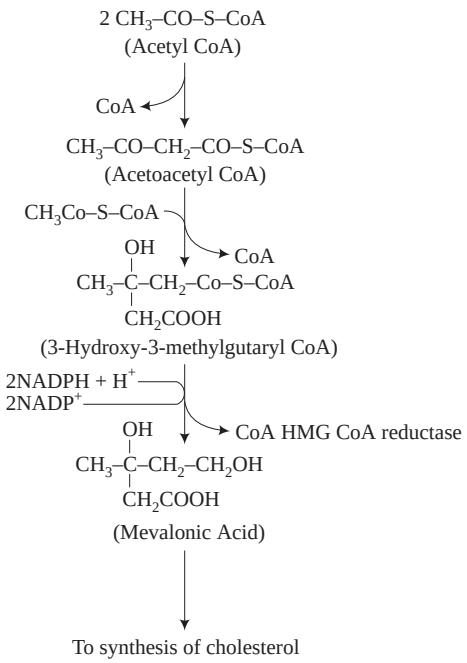


Fig. 6.18 Steps in the synthesis of mevalonic acid, the precursor of cholesterol synthesis (steps not shown).

equilibrated with the cholesterol esters and about half of the free cholesterol is eliminated by excretion in the faeces, while remainder is excreted as neutral steroid.

The liver contains a pool of unesterified cholesterol from where it is transported to the intestine and then back to liver through chylomicrons. Cholesterol is excreted from the body as cholesterol or bile acids. *Coprostanol* is the main sterol secreted in the faeces, a product of cholesterol, arising from the intestinal bacteria activity. Much of the cholesterol secreted in the bile is either reabsorbed or gets deposited in the arteries in the form of esters or may participate in steroid synthesis.

6.21 KETOGENESIS

During conditions of starvation and diabetes, oxidation of free fatty acids is enhanced in the mitochondria. In vertebrates, the acetyl-CoA originating from the β-oxidation can leave the mitochondrion in the form of citrate. However, a major part of this acetyl-CoA is used for the synthesis of *ketone bodies* in the liver (*ketosis*). There are three types of ketone bodies: acetone, acetoacetic acid, and 3-hydroxybutyric acid, which are generated through a process called *ketogenesis*, taking place in the mitochondria.

Formation of Ketone Bodies

Long-chain fatty acid are transported to the inner mitochondrial membrane through carnitine derivatives. β-oxidation of fatty acids takes place in the mitochondrion, releasing acetyl-CoA, with

the consequent production of large amount of ATP. The initial steps are similar to those of cholesterol synthesis (Fig. 6.19). The 3-hydroxy-3-methylglutaryl-CoA formed is split into acetyl-CoA and free acetoacetic acid.

The acetoacetic acid is either spontaneously decarboxylated to acetone or reduced to 3-hydroxybutyric acid:

$$\underset{\text{(Acetoacetic acid)}}{CH_3CO{-}CH_2{-}COOH} \longrightarrow CO_2 + \underset{\text{(Acetone)}}{CH_3{-}CO{-}CH_3}$$

$$CH_3{-}CO{-}CH_2{-}COOH + NADH + H^+ \longrightarrow \underset{\text{(3-Hydroxybutyric acid)}}{CH_3{-}CHOH{-}CH_2{-}COOH} + NAD^+$$

Long-chain fatty acids (even number) produce acetyl-CoA units through beta oxidation, but odd chain fatty acids produce acetyl-CoA and propionyl-CoA, which is glucogenic. Peroxisomes oxidize long-chain fatty acids.

Ketone Bodies Serve as Energy Source

Acetoacetic acid and 3-hydroxybutyric acid are produced in large amount and are utilized as energy source by various tissues such as muscles, kidney and the brain (extrahepatic tissues). Acetoacetic acid diffuses freely across the cell membranes and cannot be reactivated unless they reach the cytosol where they can participate in cholesterol synthesis as happens with acetoacetate.

Acetoacetate is reactivated in extrahepatic tissues:

Acetoacetate + Succinyl-CoA → Succinic acid + Acetoacetyl-CoA. Acetoacetyl-CoA splits by thiolysis into two molecules of acetyl-CoA which enters the Krebs cycle. Ketogenesis is an extremely important physiological process and is exclusively hepatic.

Ketogenesis is regulated chiefly by two hormones: insulin and glucagon. When blood glucose levels are decreased, insulin is depressed, thereby raising glucagon levels, resulting in arresting glycolysis, increase in gluconeogenesis and inhibiting fatty acid synthesis. As a consequence, hydrolysis of triglycerides is affected by hormone-dependent lipase in adipose tissues. The fatty acids thus liberated are transported to the liver.

The hormonal imbalance inhibits acetyl-CoA carboxylase, depressing malonyl-CoA concentration, which is a repressor of acetylcarnitine synthetase, hence it gets activated. This favours penetration of fatty acids into the mitochondria where they are catabolized by beta oxidation, increasing the NADH/NAD ratio. This will lead to reduction of oxaloacetate to malate which leaves mitochondria to participate in gluconeogenesis. The reduction of oxaloacetate prevents transformation into citrate of acetyl-CoA arising from β-oxidation. Thus the only fate of acetyl-CoA is to synthesize ketone bodies to be transported to other tissues capable of utilizing them.

Oxidation of ketone bodies takes place in the extrahepatic tissues because of the absence of key enzymes in the liver to oxidize them, i.e. 3-oxoacid-CoA transferase. This enzyme is present in the kidney, red muscle, brain and other peripheral tissues. Normally ketone bodies are continuously oxidized in muscles, therefore only traces of these may be found in the blood and urine. In fasting mammals these accumulate in blood and the levels may rise to 20-25 mg/100 ml after a week's fasting. 3-hydroxybutyrate apearing in the blood reaches the muscles (skeletal as well as the cardiac), where a mitochondrial enzyme, 3-hydroxybutyrate dehydrogenase oxidizes the compound to

acetoacetic acid. The latter compound in the muscle mitochondria is activated by 3-oxoacid-CoA transferase, catalyzing the transfer of CoA from succinyl-CoA to acetoacetic acid. Excessive production of ketone bodies by the liver leads to *ketonemia*.

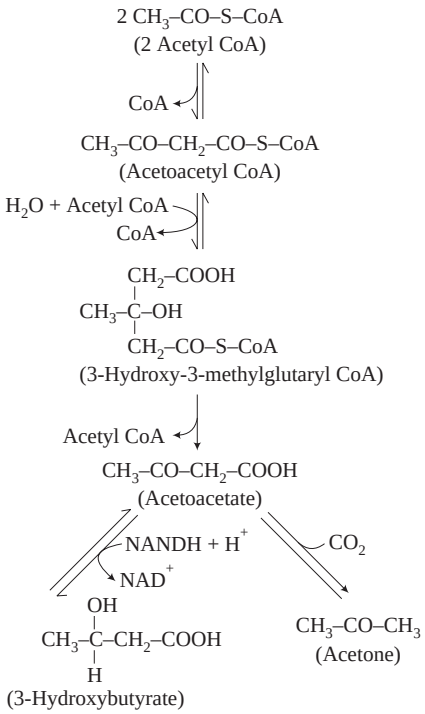


Fig. 6.19 Formation of ketone bodies from acetyl CoA.

CHAPTER

7

Digestion and Absorption

In the previous chapter, we have given an account of the classification and nature of nutrients required by animals. The food materials available to the animals are, as such, rarely in a form suitable for cellular consumption and to achieved when the foods are subjected to mechanical processes such as mastication, swallowing and movements of gastrointestinal tract, and chemical processes such as the enzymatic reactions in the digestive tract.

During the process of digestion, the complex nutrients are split into their simpler substances, i.e. the proteins into amino acids; the polysaccharides into monosaccharides; and the fats into their constituent fatty acids and glycerols. In addition to hydrolysis of proteins, carbohydrates and fats, the process of digestion renders food in a soluble form for abosrption and helps in separating the required from those not required.

7.1 MODES OF NUTRITION

Based on their method of food procurement the living organisms (plants, animals and microorganisms) are broadly classified into two major groups, viz. *autotrophs* and *heterotrophs.*

Autotrophs

The autotrophs synthesize all essential organic compounds from inorganic constituents. They include *phototrophs* and *chemotrophs.* The phototrophs are chlorophyll bearing plants and make the essential organic compounds by photosynthesis. For this process they require sunlight, and inorganic substances like carbon dioxide and nitrogenous compounds. The chemotrophs are bacteria making their food by chemo-synthesis. Since the autotrophs synthesize the required organic substances within their body, digestion is not required.

Heterotrophs

The heterotrophs require organic substances as food and their synthesizing capability is limited. For this reason they depend on organic substances like carbohydrates, fats and proteins to carry out their life processes. They obtain their food from plants and animals. Such a food is in a form unsuitable for direct absorption. The food may be in the form of tiny particles (food of protozoans and other lower organisms), large chunks or whole animals (as in higher animals), or in the liquid form (leeches, certain insects, etc.). Therefore these food particles are to be reduced to sizes suitable for entrance through the cellular membranes to take part in metabolic activities. This process as we have mentioned earlier is called digestion and only in heterotrophs it is a necessary process with the exception of certain parasites (cestodes, etc.) and commensals. The cestodes directly absorb the digested food through its body surface, from its surrounding medium in the intestine. The male echiurid derives its nourishment from the female echiurid. Such organisms are devoid of a digestive tract of their own.

The organisms are thus broadly divided into two groups. However, there are living beings between the typical chemotrophs and the typical phototrophs, and also between the autotrophs and the heterotrophs. Such living beings are dependent on both the processes and hence are grouped under mesotrophs. The *Euglena,* for example, is a mesotroph and can synthesize essential organic compounds but it still requires certain growth factors or vitamins for which it is dependent on organic sources.

7.2 INTAKE OF FOOD MATERIALS

In order to capture different kinds of food and prepare it for absorption by the cells of the body, the heterotrophs have suitable anatomical structures and devices.

Among animals the feeding mechanisms exhibit some relation to the type of food they take. It is reasonable to presume that food of the most primitive organisms is organic matter in solution. Similarly organic matter in dissolved form is directly absorbed by protozoan parasites, tapeworms and a few other animals. Studies on the absorption of dissolved organic substances in bacteria as well as in the cells of higher animals revealed the existence of a number of active sites, each of which receives the specific compounds to lead them into the cell. Dissolved substances are absorbed by yet another process. In this process minute droplets of dissolved food are engulfed by the cell through *pinocytosis.*

Protozoans prey upon parts of other organisms smaller than or equal to themselves. To ingest this type of food the protozoans have a variety of mechanisms. *Amoeba,* for example, has a mechanism which may possibly be an extension of pinocytosis and upon coming in contact with its food particle the amoeba surrounds the particle and engulfs it. Another distinct mechanism of feeding can be observed among ciliates. The ciliates produce water currents by ciliary movements and these currents carry the impaled organisms or the particulate food to the mouth or gullet. From here the food is taken into the body. In sponges the method of collecting food particles by water currents and their subsequent engulfing by cells for digestion exists in a specialized form. In this method the food particles are carried along the water currents into the paragastric cavity through body pores.

There are several coelenterates which, like sponges, are sessile animals. However, they procure food by trapping method rather than the current method noted in sponges. The important structure involved in the trappin mechanism is the cnidoblast (nematocyst). The tentacles in *Hydra* and some other coelenterates are armed with cnidoblasts and these would burst when small animals brush or bump over the cnidocil which is a small hair-like process serving as receptor of contact stimuli. As a result of this burst a poisonous thread from the nematocysts is thrust into the victim to impale it. The impaled organism is then carried to the mouth by one or more of the tentacles.

Filter feeding is characteristic of sessile animals and sedentary feeders such as bivalve molluscs, *Amphioxus, Ammocoete* larva, etc. These animals have varied types of filtering and trapping devices. Cilia and setae produce water currents which carry the food particles to the the feeding surfaces. These particles get entangled in the mucus cord which is carried into the digestive, tract by the aid of ciliary movement. Certain sea-cucumbers have sticky tentacles which trap small organisms. These tentacles are then thrust into the pharynx in order to wipe off food from them.

Although filter feeding is a characteristic feature of sessile and sedentary group of animals, it is also present in a few other animal groups. Small active copepods, sock-eye salmon, the huge basking shark and the whalebone whale are all filter feeders. The filter feeders do not select their food, hence they are known as nonselective feeders. However, they respond to certain chemicals and stimuli by operating, or by preventing the filter mechanism, depending on favourable or hazaradous conditions.

In other nonselective feeders such as many annelids, some echinoderms and hemichordates, food enters the body by water currents. From this whatever is needed by the body is absorbed and the rest discarded. The animals of this group are omnivorous provided those which come in their way should be sufficiently small to be collected in their filters or traps. Thus collected food is subjected in their filters or traps. The collected food is subjected to grinding when such organs are present. If absent, the food material is passed directly for chemical digestion.

Selective feeders employ various methods for the capture and effective utilization of bulky foods or for withdrawing the juices from animals and plants. Each mechanism is supported by suitable anatomical modifications and physiological alterations. While the fishes, amphibians, reptiles and many birds swallow their food without chewing, the mammals masticate food and supply it to the digestive system in the form of small size particles. For efficient mastication, changes have been brought about in the jaw bones and muscles. The air passages are separated and guarded by soft epiglottis and pharyngeal folds in order to facilitate breathing during mastication. These changes are directly related to the changes in the feeding habits.

The dependence of selective feeders on specific types of food is so great that in its absence they suffer from functional irregularities. The female mosquito, for instance, needs a meal of blood for the development of eggs. The rabbit flea, *Spilopsyllus cuniculi,* requires the blood of breeding, or pregnant rabbit, containing reproductive hormones, to carry out its own reproductive cycle.

Selective feeders have neurosensory and neuromuscular specializations to enable them to locate and capture specific foods. The neurosensory abilities may be in the development of visual, chemical and other stimuli.

A. DIGESTION

The digestion is classified into two main types — i.e. the intracellular, and the extracellular digestion. Between these two, there exists a transitional type of digestion known as contact digestion.

Intracellular Digestion

The digestion is intracellular in protozoans and sponges. The particulate food matter enters the cell by pinocytosis or phagocytosis and both these processes require energy. In these processes a portion of the cell's plasma membrane encapsulates the particle and the capsule is then pinched off in the form of a vesicle from the membrane to form pinosome or phagosome which is then carried into the cell for digestion. The lysosomes fuse with these vesicles and the digestive enzymes react with food to hydrolyze them to simple substances. After digestion is complete the remnants are excreted from the cell as excretory products.

Contact Digestion: The digestion in some coelenterates (such as sea-anemones) shows a transition between primitive intracellular type and the highly specialized extracellular type. When a sea-anemone swallows a large organism or a large food particle, the digestion is carried out in the gastrovascular cavity itself. The gastrovascular cavity contains sea water which makes the enzymes ineffective if secreted into it. To overcome this a specialized process of contact digestion has developed in sea-anemones. In this process, the filaments of endothermal cells lining the coelenteron are applied closely to the surface of the large food particle and secrete digestive enzymes from the attached points directly on to its surface. The food particle gratually disintegrates, and the same cells absorb the resulting products as soon as they are formed. Intracellular digestion, however, still persists in coelenterates when small food particles are digested.

Extracellular Digestion

Animals with complete digestive tracts carry on their digestion extracellularly also. The enzymes in these animals are secreted into the lumen of the digestive tract. The muscular movements of this tract bring about mixing of the food material with the enzymes and direct the entire mass posteriad. As a result of these processes, the enzymes split the foodstuffs within the lumen to a form suitable for absorption.

7.3 DIGESTION OF FOODSTUFFS

The foodstuffs are digested into a form suitable for absorption by the action of specific enzymes. These enzymes are produced by the organism which consumes the food. There are certain foodstuffs the digestion of which is performed by the bacteria present in the digestive tract of animals. In the following paragraphs, some basic enzymatic activities involved in the digestion of major foodstuffs have been described.

Major enzymes, their sources and specific actions on different foods are presented in Table 7.1.

Table 7.1 Principal Enzymes of the Digestive Tract and Their Activity on Specific Foodstuffs (in mammals)

Source of enzyme	*Enzyme and optimum condition of activity*	*Substrate*	*Products of digestion*
Salivary gland	Salivary amylase pH 6.6-6.8	Starches	Maltose
	Ptyalin		Maltotriose, dextrin
Stomach: Chief cells and parietal cells	Pepsin pH 1.0-2.0	Protein	Proteoses Peptones
	Rennin pH 4.0	Casein of milk	Coagulates milk proteins
Pancreas	Trypsin pH 7.9	Protein, Proteoses	Polypeptides, Dipeptldes
	Chymotrypsin pH 8.0	Proteoses, Proteins	Peptides
	Carboxypeptidase	Polypeptides having free carboxyl groups	Peptides, amino acids
	Amylase pH 7.1	Starch	Maltose
	Lipase pH 8.0	Lipids (primary ester linkages)	Fatty acids, monoglycerides, diglycerides, glycerol
	Cholesterol esterase	Free cholesterol	Esters of cholesterol with fatty acids
	Ribonuclease	RNA	Ribonucleotides
	Deoxyribonuclease	DNA	Deoxynucleotides
Small Intestine: Brunner's glands & the glands of Lieberkuhn	Maltase pH 5.8-6.2	Maltose	Glucose
	Lactase pH 5.4-6.0	Lactose	Glucose, galactose
	Sucrase pH 5.0-7.0	Sucrose	Glucose, fructose
	Aminopeptidase pH 8.0	Polypeptides with free amino groups	Peptides, free amino acids
	Dipeptidase pH 8.0	Dipeptides	Amino acids
	Phosphatase pH 8.6	Organic phosphates	Free phosphates
	Polynucleotidase	Nucleic acid	Nucleotides
	Nucleosidases	Purine or Pyrimidine nucleosides	Purine or pyrimidine bases, pentoses
	Lecithinase	Lecithin	Glycerol, fatty acids, phosphoric acid, choline

Digestion of Carbohydrates

Starch grains are polysaccharides having a central core of amylose covered over by a husk of amylopectin. Both amylose and amylopectin contain chains of glucose units. However, amylopectin has branched chains, each branch containing about twenty four glucose units joined to each other. These chains are not broken by any of the digestive enzymes. Glycogen is another polysaccharide consisting of branched chains, each containing twelve glucose units.

The digestive enzymes cannot act on starch unless it is broken down. The starch grains are broken down either by boiling, by chewing, by bacteria or by enzymes. The saliva of most mammals is free from enzymes and hence in these animals starches cannot be split by saliva. However, pigs, elephants, and primates have an enzyme *ptyalin (endoamylase)* in their saliva and this attacks the simple links of amylose and amylopectin. As a result of this attack a series of maltose, maltoriose, and dextrin molecules are formed. Ptyalin also hydrolyzes glycogen to form maltoses. The optimum pH 6.2-6.8 required for the activity of digestive amylases is maintained by chloride ions.

The digestion of starch by ptyalin goes on as long as it is in the mouth. When the starch reaches stomach the gastric acidity stops further action of ptyalin; however, in case of solid foods the starch hydrolysis continues into the stomach so long as the acid diffusion is not complete. Acid diffusion would be complete in about 40 minutes and by this time the starch would be split to dextrins and maltoses.

Inulin is a storage compound of certain plants (compositae) and is often consumed by mammals. It is not hydrolyzed by ptyalin. The stomach does not produce any enzymes to digest either inulin or other carbohydrates such as cane sugar. However, these are hydrolyzed by the acid in the gastric juice.

The pancreatic juice secreted into the small intestine has *amylopsin,* which is similar in action to ptyalin of the saliva. Amylopsin attacks to split raw starch grains to dextrins, maltotrioses and maltoses. The ptyalin also hydrolyzes dextrins and maltotrioses, already produced by the salivary digestion, to yield maltose. Further, several enzymes which hydrolyze various disaccharides to the appropriate hexoses are found in the lumen of small intestine. These enzymes are called *glucosidases* or *disaccharases.* Of these enzymes, *maltase* and *lactase* are brought into the intestinal lumen by both the pancreatic juice and the intestinal juice. The lactase which splits milk sugar (lactose) to glucose and galactose is prominent in young mammals. The maltase splits maltose to form two glucose molecules. In addition to maltase and lactase, another sugar-splitting enzyme present in the intestinal juice in *sucrase.* Sucrase hydrolyzes sucrose (a storage or transport sugar of several nutritious plants) to one molecule of glucose and one of fructose.

Cellulose Digestion in Ruminants

The ruminants ingest large quantities of fodder rich in cellulose or lignin. Neither substances can be digested directly by mammals. But the cellulose is attacked in the rumen by the enzymes produced by symbiotic bacteria. Sheep and cattle initially swallow food without masticating. In the rumen food is mixed with water. The rumen has millions of bacteria of several species, some of which attack the cellulose walls of the fodder liberating the cell contents of the food. The bacteria also breakdown carbohydrates and proteins to simple substances. In the rumen main products of fermentation are acetic, propionic, butyric, and other acids, along with small quantities of other substances such as ethanol. Besides, symbiotic organisms in the rumen synthesize much more riboflavin and pantothenic acid than is normally procured through diet. In addition to bacteria, a number of protozoan ciliates belonging to spirotrichida and holotrichida have been observed in the rumen. Some of these can break down cellulose. The stored carbohydrates and proteins within these ciliates are believed to serve as reserves for a short time when fodder is not available.

Cellulose breakdown by bacteria also takes place in the stomach of kangaroo *(Stronix brachyurus),* and sloth *(Choloepus).* In kangaroo the stomach is enlarged, and in the sloths it is a compound stomach. Cellulose splitting bacteria are present mostly in the caecum and ventral colon of horses, and in the caecum of rabbits. The rabbits, like other lagomorphs, and many rodents, have developed a habit of reingestion or pseudorumination. When rabbit eats fresh food, it directly reaches the caecum, remains there for one or two days undergoing fermentation, and is then expelled as soft faeces. These are then eaten and this time they reach the cardiac stomach, but the freshly eaten food goes straight into the caecum as usual. After digestion and absorption the twice swallowed food is excreted as hard faecal pellets without letting it in through caecum.

Digestion of Proteins

As a result of enzymatic action the peptide links of proteins are hydrolyzed. Consequently the simple proteins yield amino acids, and the conjugated proteins yield amino acids as well as nonamino groups. The enzymes responsible for splitting proteins are called proteolytic enzymes. Of the most familiar ones are pepsin, trypsin, chymotrypsin, aminopeptidase, carboxypeptidase, tripeptidase and dipeptidase. Though these enzymes are present in several animals they are, like proteins, highly specific. The pepsins from various species of vertebrates are found to act differently on certain substances, and have different reaction optima.

The proteolytic enzymes of the digestive tract in vertebrates fall under two distinct groups; the *endopeptidases* and the *exopeptidases.*

Endopeptidases: The endopeptidases, also known as proteinases under an old name, attack polypeptide chains at peptide bonds away from the ends and prefer very specific links in the protein chain. *Pepsin* is secreted in the gastric juice as an inactive precursor, *pepsinogen.* Pepsin hydrolyzes peptide bonds in which the amino function is contributed by phenylalanine, tyrosin, tryptophan, leucine, aspartic acid or glutamic acid. Pepsin is active in acid medium (optimum pH 1.5-2.5) depending on the substrate. It is inactivated in neutral or alkaline solutions and is devoid of a prosthetic group. Pepsin, like rennin, has the property of coagulating milk by converting caseinogen to casein which forms and insoluble complex with calcium.

Trypsin is secreted by the pancreas in the form of an inactive precursor, *trypsinogen.* Trypsinogen is activated by the enzyme *enterokinase* secreted by the intestinal mucosa and then autocatalytically by trypsin itself. Trypsin catalyzes the hydrolysis of peptide bonds in a protein chain in which the carbonyl function is contributed by lysine or an arginine residue. Trypsin has no prosthetic group and its pH optimum lies between 7-9. It is relatively stable to heat in acid solution and less so in alkaline solution.

Chymotrypsin is secreted from the pancreas in an inactive form, *chymotrypsinogen.* Activation of chymotrypsinogen to its active form is brought about by the trypsin. The optimum pH is 7-8. Chymotrypsin attacks the peptide bonds in which the carbonyl group is furnished by the aromatic amino acids, phenylalanine, tyrosine or tryptophan.

The endopeptidases described above hydrolyze large protein molecules to smaller peptides which are further broken down to smaller peptides by the action of exopeptidases and dipeptidases.

Exopeptidases: Exopeptidases, also known as peptidases under an old name, catalyze the removal of terminal amino acids and require a metal ion as activator for their catalytic activity. These are either secreted by the pancreas or the intestinal mucosa.

There are two *carboxypeptidases* which are secreted as precursor *procarboxypeptidases* and activated by trypsin. Carboxypeptidase A (Zn^{++} containing) hydrolyzes terminal amino acids with free caboxyl groups with the exception of lysine or arginine. Carboxypeptidase B will hydrolyze peptide with free carboxyl terminal of lysine or arginine. Both do not attack dipeptides.

Aminopeptidase prefers the terminal amino acid with free amino group. This is rather nonspecific enzyme and does not act on dipeptides.

There are a number of *dipeptidases* which are quite specific for specific dipeptides. *Prolidase* is an exopeptidase which hydrolyzes proline peptides derived from the breakdown of collagen.

Endopeptidases as well as exopeptidases are required both for intracellular and extracellular types of digestion. The pepsin, trypsin and chymotrypsin are for extracellular digestion. Their counterparts in intracellular digestion are *cathepsin* A, B and C. Other than the endopeptidases the cells also possess exopeptidases corresponding to those of extracellular enzymes. The intracellular enzymes do not usually require an activator.

Digestion of Fats

Because there is no lipase in the saliva nor in the gastric juice, the digestive activity of lipase in the stomach is inhibited, although some pancreatic lipase may be found in the gastric contents through regurgitation. When the chyme passes through duodenum, the bile and the pancreatic juices are released and these get mixed up with the chyme. The bile salts lower the surface tension of the fat droplets in the chyme and emulsify them to facilitate their digestion. The fat or triglyceride particles in the emulsion have a diameter of 0.5 to 1.0 μm. The emulsification of triglyceride exposes it to the action of *pancreatic lipase* which hydrolyzes triglycerides to form a mixture of free fatty acids (FFA) and monoglycerides and diglycerides (Fig. 7.1). The pancreatic lipase reacts most readily with triglycerides having long fatty acid chains, and for this the required optimum pH lies between 7 and 9. In artiodactyls the hydrolysis of fats is done by the action of bacteria present in the rumen, and the glycerol is converted to propionic acid.

The fatty acid esterified in position-2 is not fit for the lipolytic attack, hence the fatty acid residue is transferred to 1-position. As a result of hydrolysis the principal products, free fatty acids and monoglycerides along with bile salts and phospholipids are dispersed into fine particles known as *micelles.* The micelles contain small amounts of di-and triglycerides. The fats in the form of micelles enter the mucosal cells and get surrounded by endoplasmic reticulum. During their entry through the mucosal cells, the products of lipase action are resynthesized into triglycerides.

Resynthesis of triglycerides may take place either by reacylation of 2-monoglycerides or by the acylation of 3-glycerophosphates. Free glycerol is not used for resynthesis. The short-chain fatty acids are absorbed into the portal blood, while the majority of long-chain fatty acids escape esterification and become bound to plasma proteins. A layer of protein and phospholipids is formed around the resynthesized triglycerides before they pass through the mucosal cells. The protein coated droplets of fats are known as *chylomicrons* having a diameter of about 0.5 μm.

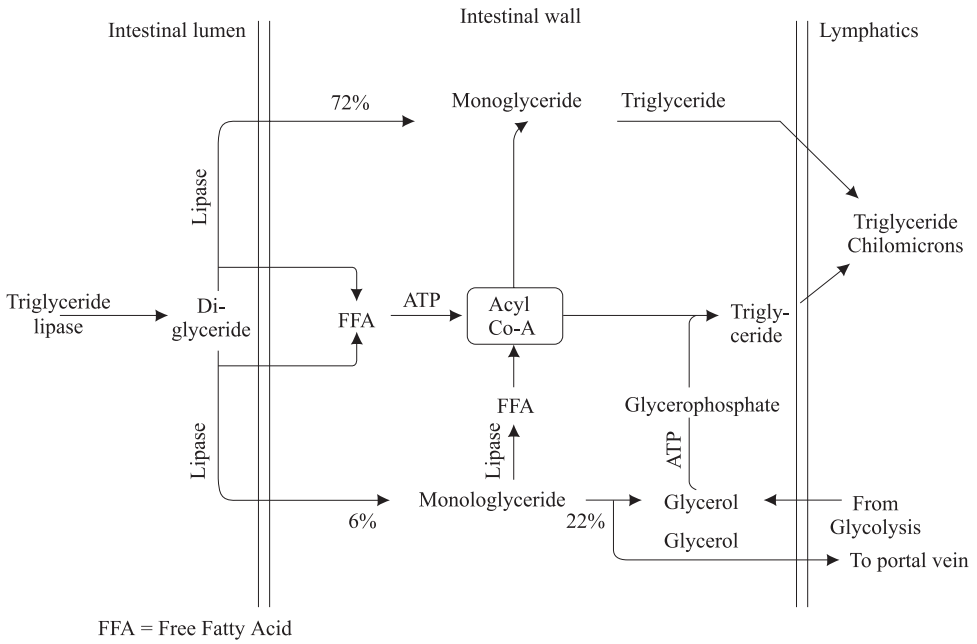


Fig. 7.1 Schematic diagram showing the digestion and absorption of fats in the intestinal lumen.

Digestion of Nucleic Acids

The enzymes hydrolyzing nucleic acids are present in the pancreatic tissue and can be divided into endonucleases and exonucleases. Endonucleases carry on the hydrolysis of polynucleotide chain in the middle, and the exonucleases remove the terminal nucleotides. Deoxyribonuclease I, an endonuclease, hydrolyzes between all pyrimidine-purine pairs in a DNA chain, whereas ribonuclease hydrolyzes RNA chains into nucleotides. Phosphodiesterase hydrolyzes nucleotides successively from the 3'end of both DNA and RNA oligonucleotides. Nucleosidases are quite specific in nature. There is a type which attacks only the purine containing nucleosides, while another type breaks the pyrimidine nucleosides liberating uracil, cytosine and thymine.

7.4 DIGESTION IN MAMMALS

There are certain variations in the mechanism of digestion among vertebrates, but the digestive process in man and the mammals, which is generally studied in the laboratory, is typical of all such processes. The digestive process in mammals falls under three distinct divisions:

(i) digestion in the mouth;
(ii) digestion in the stomach; and
(iii) intestinal digestion.

Digestion in the Mouth

The food material captured has to be passed through the mouth which in the vertebrates is helped by a pair of apposed movable jaws. These jaws are used in a variety of ways to collect and hold food or in certain vertebrates to reduce the food to small particles.

Three pairs of salivary glands are present opening into the mouth by way of ducts and their secretory products are collectively called saliva. These glands, according to their position, are termed as the parotids, the submaxillaries, and the sublinguals. Besides, the mouth also has a number of so-called buccal glands pouring their secretions into the mouth. The chief product of these glands is the mucus. The food taken into the mouth is cut and ground by the teeth. By this process the food not only is broken to pieces but also mixed with saliva. The saliva dissolves the food to give a sense of taste to the taste buds. The enzyme ptyalin initiates the digestion of carbohydrates, and starch in particular. The mixing of saliva with food eases the swallowing process.

Digestive Control Mechanisms

Digestion is a coordinated process wherein proper amounts of digestive juices must be secreted to reach the food at desired times. This naturally involves some kind of control mechanism to coordinate various components of the process. Numerous studies have revealed that both nervous and hormonal controls are involved in the process. However, a secretogogual mechanism (presence of food in the alimentary canal also acts as a stimulant) to stimulate secretions has also been demonstrated to be operative in the mouth and stomach. Nervous mechanisms dominate in the anterior part of the digestive tract and as nervous controls decline, hormonal controls take over to assume greater importance.

Salivary Control: The secretion of saliva is generally coordinated with the intake of food, but olfactory and gustatory stimuli normally initiate the nervous reflex resulting in the stimulation of salivary secretion. Conditioned reflexes resulting in the flow of saliva are due to olfactory stimulation. Salivary glands receive both sympathetic and parasympathetic nerve fibres, hence either of the systems or both may exert control over normal secretion of saliva.

Gastric Control: In many cases the presence of food in the stomach has a stimulatory effect on the gastric secretion (secretogogual). Besides this, a nervous stimulation is also necessary which is followed by a hormonal phase. A nervous stimulus is necessary to produce the hormone gastrin from the pyloric end of the stomach which is liberated in the bloodstream and reaches back to the stomach via circulation. Gastric stimulates the secretion of hydrochloric acid and probably helps in the secretion of bile and pancreatic juice.

Control of Pancreatic Secretion: The digestive enzymes of the intestine mainly originate from the pancreas which are entirely secreted under hormonal stimulus. The presence of chyme in the duodenum also initiates secretion of pancreatic juice mediated through the hormones. The presence of hydrochloric acid and partially digested foodstuffs stimulate the duodenal mucosal cells to secrete hormones which are carried to the pancreas, liver and gall bladder through general blood circulation.

Bayliss and Starling in the year 1902 suggested that the hormone responsible to stimulate the pancreatic tissue originates from the duodenum and they named it *secretin.* Recent work, however, has shown that secretin contains five factors: (1) *secretin* which stimulates a bicarbonate rich pancreatic secretion and poor in enzymes; (2) *pancreozymin* which stimulates pancreatic acinar cells to produce viscous fluid low in bicarbonates and rich in enzymes; (3) *hepatocrinin* which stimulates liver to secrete a salt-poor bile; (4) *cholecystokinin* that causes evacuation of gall bladder; and (5) *enterocrinin* which stimulates the flow of intestinal juices (succus entericus).

Control of Secretion: Excitation of salivary glands is dependent upon the nerve impulses. The glands receive the impulses from the autonomic nervous system. There are three types of stimuli which arouse reflex secretion of saliva. These are: (1) tasting and smelling of food (excitation of chemoreceptors); (2) chewing, or the presence of solid substances in the mouth (excitation of pressure receptors); and (3) seeing or even thinking of good food (psychic reflex).

Digestion in the Stomach

The food entering the stomach pushes the already existing food towards the sides and itself remains away from the gastric walls. This prevents the gastric secretions from mixing with immediately. The newly entered food, therefore, continues to react with ptyalin for some time till the gastric juices penetrate into the food and stop the ptyalin action. The stomach produces hydrochloric acid, and certain enzymes, viz. pepsin, rennin, and gastric lipase The stomach acts as a reservoir for mixing the food with its enzymes and from here the food is slowly passed into the intestine.

Chemistry of Gastric Digestion: The enzyme pepsin as such is not secreted directly. The cells of the gastric glands produce a proenzyme, pepsinogen, which requires an acid pH for its desired action. This is accomplished by the hydrochloric acid secreted into the stomach by the, parietal cells of the fundic glands (Fig. 7.2). Hydrochloric acid removes the inhibitory polypeptide from pepsinogen converting it into active pepsin. Small amounts of pepsin also covert pepsinogen into pepsin and this process is auto-catalytic. Pepsin is a proteinase and hydrolyzes proteins (proteoclastic) to acid metaproteins, proteoses, and peptones. If pepsin is allowed to continue its action for long time, the proteins would get hydrolyzed into traces of amino acids. The pepsin attacks the peptide bonds which are formed from the α-carboxyl of an L-dicarboxylic acid and the α-amino group of an L-aromatic amino acid, and a few others. Consequently, the chief amino acids, viz. L-tyrosine and L-alanine are liberated. Pepsin attacks the cell walls and breaks them liberating the contents.

In man, calf, and pig, pepsin is produced from two regions in the stomach. One produced near the fundus has pH optima between 2.0 and 3.6; another produced near the pylorus has pH optima between 1.5 and 3.2. Peptic digestion would cease at pH $\leq$ 5, because at this pH the inhibitory polypeptides recombine with pepsin and inactivate it.

Rennin is an important enzyme in the digestive system of some young mammals. The enzyme is secreted by cells in the fundus as zymogen prorennin. Prorennin as such is inactive and requires acid for its activation. The hydrochloric acid transforms prorennin into an active rennin. Rennin acts rapidly upon caseinogen, the chief protein of milk. In milk caseinogen is present in a soluble form. The nature of action of rennin on caseinogen is not definitely known, but it is believed that it mildly

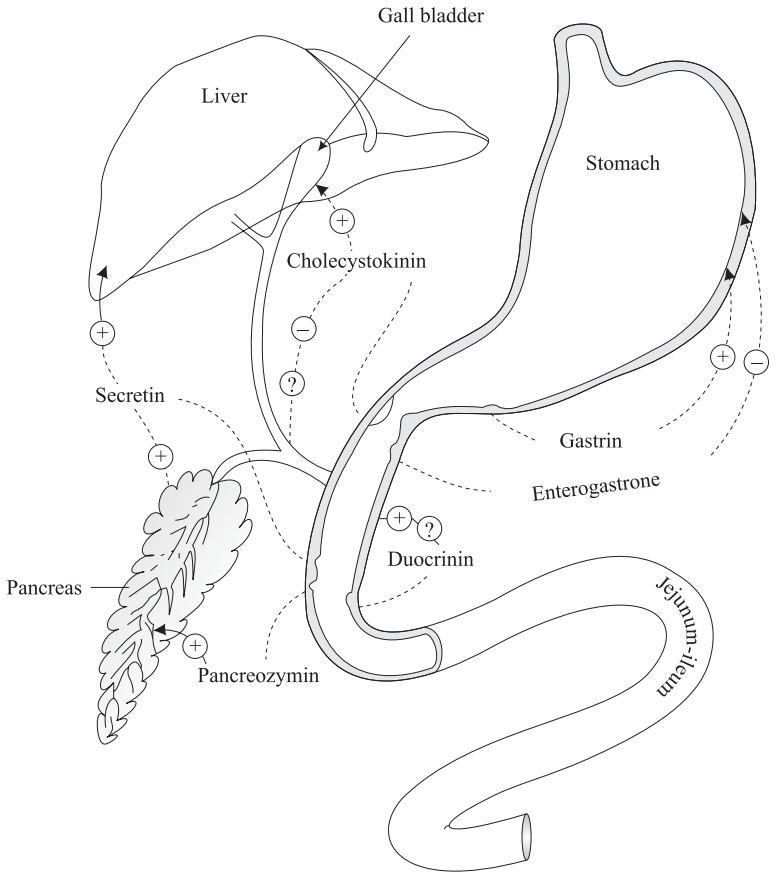


Fig. 7.2 Secretory activity of the stomach and the intestine and its associated structures.

hydrolyzes caseinogen. As a result of the action of rennin on caseinogen, casein is produced. The calcium ions present in milk combine with casein which is precipitated in the form of calcium caseinate. Rennin thus prepares milk suitable for gastric digestion. The curdled milk is retained in the stomach where calcium caseinate would be attacked by pepsin. The optimum pH for its action is 4.

The fat splitting enzyme lipase present in the stomach is inactive at the normal acidity of gastric contents. But, highly homogenizd fats such as those of milk, and egg-yolk would, however, undergo partial hydrolysis. There is disagreement over the secretion of this enzyme in the stomach. Some account its presence due to regurgitation of intestinal contents into the stomach. But, the milk lipolytic activity observed in extracts of gastric mucosa suggests that the gastric glands do secret traces of lipase.

Gastric Movements: The stomach is chiefly composed of smooth muscles. The movements of the stomach are controlled by the autonomic nervous system which are increased by parasympathetic

stimulation, and inhibited by sympathetic activity: The gastric movements are of three main types: they are hunger contractions, filling, and emptying. Of these filling and emptying are important in view of digestion.

(a) *Filling:* The muscle of the stomach wall progressively relaxes as more and more food enters it and as a result the stomach gets expanded. When food is present, the stomach muscles bring about vigorous movements facilitating thorough mixing of food with digestive juices.

(b) *Emptying:* The peristaltic waves in the stomach propel the food towards the pyloric sphincter. However, the emptying of material across the pylorus depends upon the relative pressure in the stomach and duodenum, the opening of the pyloric sphincter, and the fluidity of the gastric contents.

Passage of Chyme Through Pylorus: The stomach contents are passed through the pylorus into duodenum. By opening at brief periods, the pyloric sphincter allows materials from the stomach to pass into the intestine at a slow pace and this process prevents overcrowding of the intestine. This material has a thick soup-like consistency and is called the chyme. It is acidic in nature. Pyloric sphincter needs chemical stimulus for the requisite relaxation and contraction. Acidity of chyme may lead to the opening of the pyloric sphincter. When the acid chyme comes in contact with mucosa of the pylorus, the sphincter gets contracted. As a result, peristaltic waves are set in that carry forward the acidified gastric contents towards the sphincter which then allows chyme to pass bit by bit into the duodenum. Duodenum also plays a role in the evacuation of stomach contents through pylorus. It functions in two ways, i.e. by physical and by chemical means.

In the first case, the motility of stomach is at its optimum when the duodenum is empty, and as a result the chyme is pushed from stomach to duodenum. Entry of chyme increases pressure in the duodenum arid this prevents gastric contractions. Once again, motility of stomach would be resumed as and when the duodenal contents are emptied.

In the second case it is by chemical means. Enterogastrone, liberated into the blood by the duodenal cells under the influence of lipids or high concentration of sugars, prevents gastric motility. The gastric motility would be resumed once the duodenum is empty and secretion of enterogastrone is prevented.

Digestion in the Intestine

As the chyme enters the duodenum, its HCl content excites the mucosal cells of that region to actively secrete a hormone, *secretin.* This hormone is a polypeptide and being relatively simple in structure it easily diffuses into the blood. It is then carried through the blood to the pancreas where it excites the active secretion of pancreatic juice.

The pancreatic juice contains a series of enzymes which progressively hydrolyze proteins to polypeptides, dipeptides, and amino acids. These enzymes are collectively known as *pancreatic proteases.* Two such enzymes are *trypsin* and *chymotrypsin.* The former acts more rapidly than the latter and both are present as zymogens (inactive forms) in the pancreas, i.e. in the form of *trypsinogen* and *chymotrypsinogen.* The chymotrypsinogen is activated by trypsin. The trypsinogen is prevented from being active by an inhibitor protein. When trypsinogen is extracted from the pancreas, the inhibitor also accompanies it. In such a case the inhibitor would be slowly destroyed if left for

some time. It can also be destroyed rapidly in acid solution (pH1). Trypsinogen, free from inhibitor, can be activated rapidly by traces of trypsin. It thus undergoes an autocatalytic reaction. But during the intestinal digestion trypsinogen is normally activated to trypsin by *enterokinase.*

When proteins reach the intestine they are already in a partly hydrolyzed state due to the action of pepsin of the stomach. On such proteins the pancreatic proteases act bringing out complete proteolysis. Trypsin activity is optimum in alkaline medium between pH 8 and 9. The pH is brought about in the intestinal contents by the alkaline salts of the pancreatic juice and the bile. These salts neutralize the acidity of the chyme and bring the required alkalinity. Trypsin attacks on partly hydrolyzed proteins and causes them to pass through several intermediate products, viz. alkali metaproteins, proteoses and peptones. Thus trypsin acts upon proteins arriving in the intestine and splits them into polypeptides. Another enzyme known as *erepsin,* found in the intestinal mucosa, splits proteoses, peptones and peptides. It is now known that this splitting is not by a single enzyme erepsin, but is done by a group of enzymes called peptidases. One of these enzymes, viz. *carboxypeptidase* is from the pancreatic juice. This enzyme disrupts the peptides by attacking at the end of the amino acid chain where the free – COOH group is placed.

The action of pancreatic enzymes on food is dependent upon the emulsifying action of bile. This action of bile is due to bile salts. When bile is not secreted into the intestinal lumen, as it happens in case of jaundice patients, the fat from the food forms a coating over proteins and carbohydrates of the chyme and prevents them from being digested by pancreatic enzymes. This is because the water soluble pancreatic enzyme cannot penetrate the fatty, coating over the nutrient materials in the chyme. The presence of bile also hleps in the absorption of products of digestion. The fatty acids and the insoluble calcium soaps are dissolved in bile and in this state they are easily absorbed by the mucosal cells of the small intestine. The alkalinity of bile helps to neutralize the acidity of chyme.

Composition of bile: The bile, soon after secretion, contains as much as 97 per cent of water and is usually transparent. The bile is then conveyed to the gall bladder for storage. The walls of gall bladder absorb water from the bile. The secretory cells of gall bladder add some of the constituents into the bile. The bile is released by the gall bladder only upon receiving the proper stimulus. When released, bile consists only about 80 per cent of water and abundant suspended matter. It contains chiefly proteins, bile salts, cholesterol, bile pigment, inorganic salts, lecithin and other lipoid substances.

Bile Salts

About 1 g of cholesterol is eliminated from the body per day through the faeces either in the form of cholesterol or bile acids (bile salts). Bile acids are synthesized in the liver from cholesterol, and the principal constituents include cholic acid and chenodeoxycholic acid. Other physiologically important bile salts are the sodium salts of glycholic and taurocholic acids. These salts are soluble in water and alcohol and they are perfectly alkaline. The bile salts have remarkable power of lowering the surface tension and emulsify fats. Emulsification is also partly due to the power of bile salt solutions to dissolve fatty acids and the water-insoluble soaps. The solvents power of bile is further augmented in the presence of cholesterol. Both cholesterol and lecithins are lipoid compounds, of which cholesterol is present in greater amounts in bile than in any other body fluid.

In the intestine, the bile acids further undergo modification to form secondary bile acids such as deoxycholic and lithocholic acids by the activity of intestinal bacteria. Cholesterol is procured by the animals through the plant or animal food, or synthesized in the liver from its precursor, that is acetyl-CoA. Cholesterol is the precursor of all steroid hormones in the body and plays an important role in the membranes. Excess of cholesterol is excreted in the bile. Elevated levels of cholesterol is present in VLDL (very low density lipoproteins) or LDL (low density lipoproteins) and is associated with the disease atherosclerosis.

Bile Pigments

The bile has two chief pigments which are *bilirubin* and *biliverdin*. Bilirubin is yellowish whereas biliverdin is green in colour and this variation is due to the presence of different pigments. Bilirubin, a breakdown product of haemoglobin, is the predominant pigment in the bile. It is estimated that in an adult human being, 1-2 $\times$ 10^8 erythrocytes are destroyed per hour, making a turnover of about 6 g of haemoglobin. Conversion of haeme to bilirubin takes place by reticuloendothelial cells and then transported to the liver by plasma albumin. Further metabolism of bilirubin takes place in the liver (Fig. 7.3).

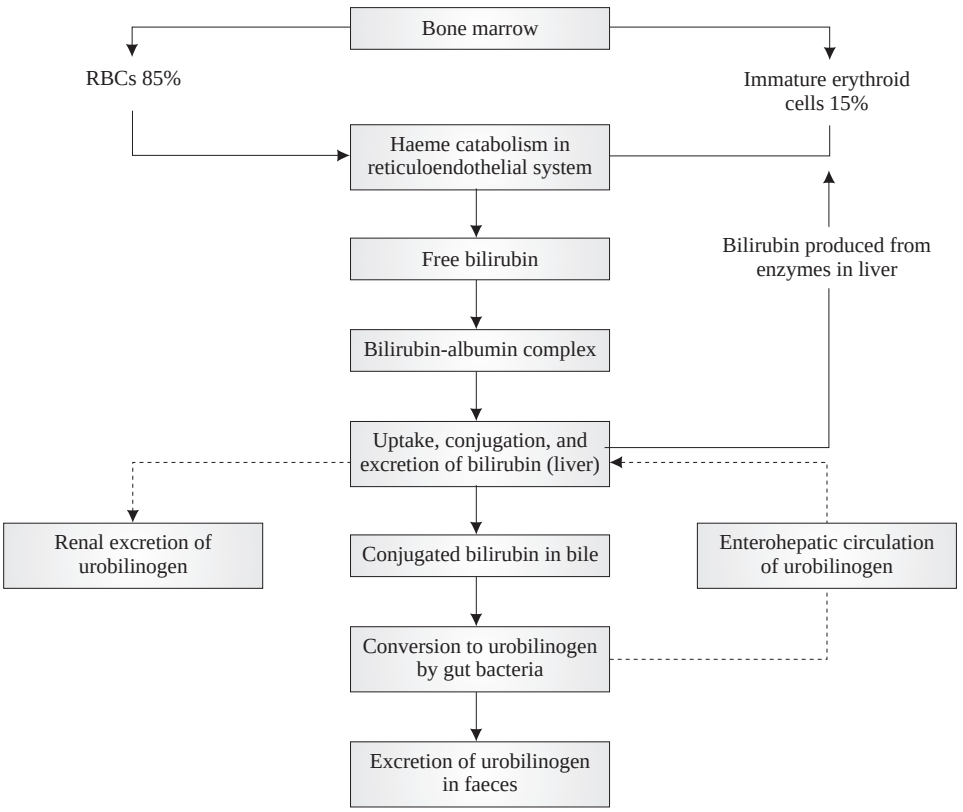


Fig. 7.3 Bilirubin metabolism schematic.

Bilirubin is slightly soluble in water, but when bound to albumin it becomes water-soluble. However, binding with albumin increases the solubility of bilirubin in the plasma. Total bilirubin content of the serum include conjugated and unconjugated amounts of bilirubin. Conjugation of bilirubin with glucuronic acid renders it water-soluble by an enzyme glucoronosyltransferase residing in the endoplasmic reticulum. Unconjugated bilirubin is not filtered by the glomerulus, travels to the liver, where it is separated from albumin, conjugated bilirubin with glucuronic acid (diglucuronide) is actively secreted into the bile, which is filtered by the glomerulus (Fig. 7.3). Increases in the serum albumin results in jaundice and haemolytic anaemias.

Conjugated bilirubin is secreted into the bile through active transport mechanism. When it reaches the large intestine, the glucuronides are removed by the enzymes secreted by intestinal bacteria and broken down to *urobilinogens*, a portion of which is reabsorbed and resecreted through the liver. Normally, the urobilinogens are colourless.

Control of Bile Secretion: The secretion of bile from the liver cells is controlled by nervous and chemical stimuli. The nervous stimulus on bile secretion is indirect, in that it first stimulates the flow of more blood into the liver which in turn activates the liver cells. The continuous secretion of the bile is due to the presence of chemical stimulants. The presence of bile salts and acid in the intestine stimulates the secretory activity of the liver cells. The bile salts coming into the intestine are absorbed through the walls of the intestine and travel to the liver via portal circulation.

The acid from the stomach, upon reaching the intestine, stimulates the mucosal lining to secrete something in addition to pancreatic secretin, which would aid the secretory activity of the liver cells (Fig. 7.3). Protein diet influences bile secretion. In the stomach protein increases the secretion of HCL which in effect stimulates the duodenal mucosa to produce secretin. This effect of acid in stimulating secretin production has been explained to stimulate liver activity.

Flow of bile: The bile fluid, although continuously produced by the liver would not be allowed to flow into the intestine. Its flow is prevented by the contraction of a small sphincter present at the point where the common bile duct opens into the intestine. It therefore finds its way into the gall bladder. The wall of the gall bladder has thin layer of nonstriated muscle fibres. These muscles when excited contract to force the contents into the intestine. The excitation for this contraction of bladder muscles and the simultaneous relaxation of the sphincter are provided either by the nervous stimulus or by the presence of acids or fats in the intestine. The presence of the latter in the intestine is believed to stimulate the production of a hormone, *cholecystokinin*, in addition to secretin. Cholecystokinin when carried to the gall bladder stimulates it to contract and forces the bile into the intestine.

Intestinal Movements: In the intestine the chyme is mixed with the intestinal juices and passed posteriad. Two types of intestinal movements facilitate this process. The mixing is facilitated by the *segmenting* or *dividing movements* and propulsion of the chyme is carried out by *peristalsis.* Dividing motions along the length of the intestine are performed by repeated alternate constrictions and relaxations of the circular muscles. At equidistant regions, contractions appear dividing the intestine into a number of sac-like compartments (Fig. 7.4). In the next step the circular muscles at the constrictions relax but those at the adjacent sac-like compartments constrict. Such alternate movements are repeated rhythmically and the number of repetitions may range between 20 and 30 per

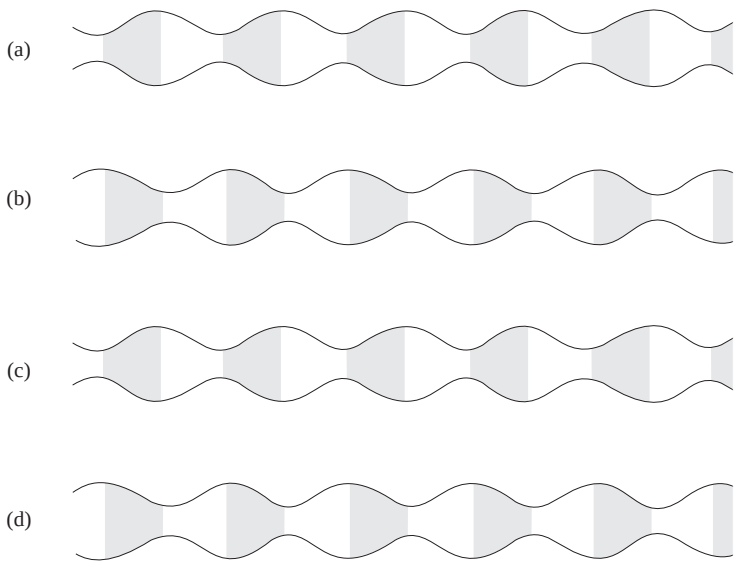


Fig. 7.4 Dividing movements of the intestine. The constricted portions seen in the first phase (a) are due to the contractions of circular muscles. The portions between the constrictions appear as sacs. In the second phase (b), the constricted muscles relax and the muscles at the sacs constrict. Such alternate contractions and relaxations of the muscles are repeated several times and this brings about the mixing of food.

minute. The changing pressure of the intestinal contents against the walls, perhaps, stimulates the nerve net present in the intestine. This nerve net is believed to regulate the rhythmic movements.

Peristalsis: The dividing movements of intestine are from time to time interrupted by peristaltic waves. This wave is called *diastalsis.* The wave proceeds towards the posterior end of the intestine gradually forcing the food in that direction. The dividng motion is resumed, once the peristaltic wave dies out. Diastalsis is carried out by the myenteric reflex action.

Anastalsis: The movement of large intestine, especially that of the descending colon, consists of a wave of contraction which moves upward preventing the contents of colon from reaching the rectum. Such a movement of the large intestine is called anastalsis or antiperistalsis.

Mucosal Structure: To facilitate absorption of the digested food, the surface area of the epithelial lining of the small intestine is enormously increased by *villi* and *microvilli* (Fig. 7.5). The villi are finger-like projections extending into the intestinal lumen. All the epithelial cells on their free surface are covered over by microvilli. The microvilli are presumed to be the excrescences of the plasma membrane occurring on the free surface of each epithelial cell. These microvilli form the brush border over the epithelial cells (mucosal cells). It is estimated that each epithelial cell has 2,000-4,000 microvilli. The epithelial cells of the villi have a short life-span of about 3 days. The older cells are replaced by new cells which are formed at the proliferative zones lying at the bases of the villi. These zones are called crypts of Lieberkuhn. The villi are supplied with capillary network and a lacteal.

Mucosal Function: *Intestinal juice* or *succus entericus* includes a number of enzymes secreted into the intestine by the various glands of the intestinal mucosa. Intestinal mucosa has glands and their secretions aid in the digestive process. *Brunner's glands* present in the submucosa at the upper end of duodenum secrete mucus which protects the intestinal wall from the acid as well as from the digestive enzymes. The secretion of these glands is alkaline. One of the important constituents of the intestinal juicel *is enterokinase,* and it is secreted by *crypts of Lieberkuhn.* In the intestine enterokinase catalyzes the transformation of pancreatic trypsinogen to trypsin. Enzymes like peptidases, sucrase, maltase, lactase and lipase are present within the mucosal cells of the intestine and they are not secreted into the intestine. The action of these enzymes on the food is interlocked with the absorptive mechanism. The action of the peptidases in the mucosal cells was attributed to that of a single enzyme *erepsin.* But now erepsin is known to contain a group of peptidases. The *carboxypeptidase* and *aminopeptidase* belong to this group.

The activation of parasympathetic fibres greatly increases secretion of intestinal juice. The presence of chym in the intestine stimulates the mucosa to secrete a hormone *enterocrinin,* which stimulates the intestinal glands to secrete the intestinal juice.

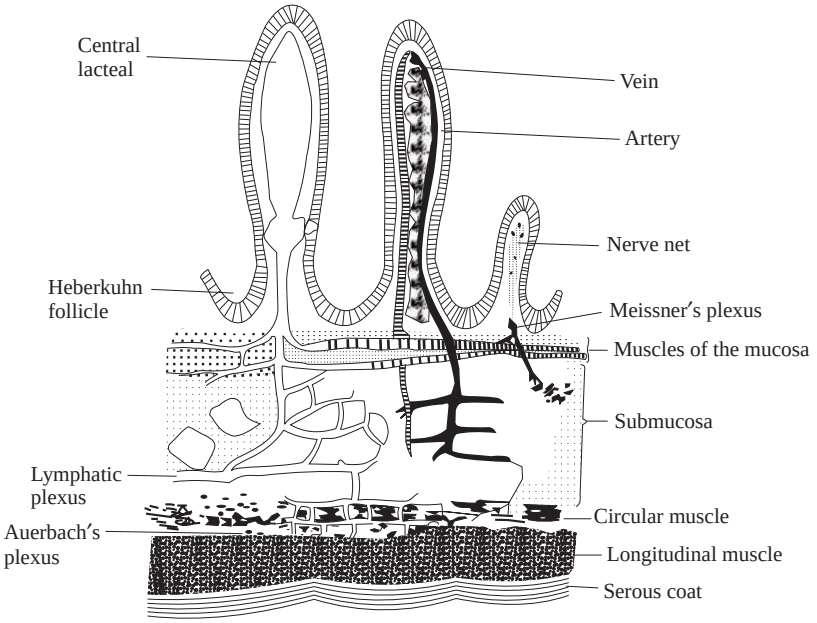
Carbohydrates, proteins, and fats are believed to be completely digested in the intestinal lumen to form their constituent units. But the presence of disaccharidases, dipeptidases, aminopeptidase, alkaline phosphatase, lipase, and esterase in the mucosal membrane indicated that the last of the digestive reactions occur in the membrane and not in the intestinal lumen. The ultimate hydrolysis on or in the brush borders of the mucosal cells ensures abosorption of the products the moment they are formed. It is presumed that the nutrients enter the cell via membrane transport, pinocytosis, or through membrane pores directly into the endoplasmic reticulum.

A large number of transport systems and mechanisms have been explained as responsible for the transport of multiplicity of nutrietnts across the membrane of the mucosal cell facing the lumen. These have been explained under the section ABSORPTION. After entering the mucosal cell, the nutrients are transported to the various organs to satisfy their metabolic needs. To achieve this, the nutrients first travel through the mucosal cell, then leave through the serosal membrane, they next enter the interstitial fluid, then squeeze through their walls of the lymphatic and vascular capillaries, and finally reach the sites where they are required for metabolism.

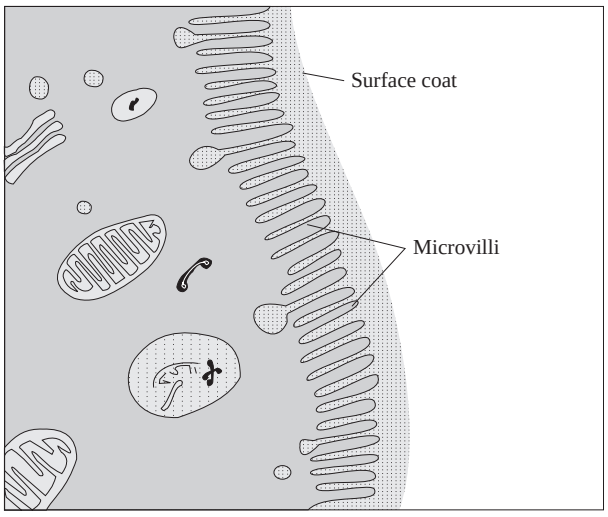
Movement of Substances Through Ileocecal Valve: By the time the intestinal content reaches the ileocecal valve, most of the digested proteins, carbohydrates, fats, vitamins, and minerals are absorbed in the intestinal mucosa. At this stage the residue containing unabsorbed nutrients enters the large intestine through the ileocecal valve.

Secretions from the Large Intestine: The large intestine does not secrete digestive enzymes and the secretion is mostly mucin. This secretion is alkaline and has a pH of about 1.8. It is rich in bicarbonate and potassium. The secretory activity of the large intestine is under parasympathetic control, but there is no evidence of hormonal regulation. The appendix spontaneously secretes a large quantity of fluid which is poured into the large intestine.

Colonic Movements: The colon performs all those movements discussed under intestinal movements. As a result of segmenting, and peristaltic movements, the semisolid material that has



(a)



(b)

Fig. 7.5 The absorptive surface of the intestinal lumen is greatly increased due to the presence of finger-like projections of the intestinal mucosa (a) villi and (b) microvilli of the plasma membrane of the epithelial cells.

entered the colon gets mixed with the secretions and the micro-organisms of the large intestine. The antiperistaltic movements of the large intestine prevent the residue from reaching the rectum.

Most of the micro-organisms swallowed along with the food, are killed in the stomach and in the upper part of the intestine. However, a few yeasts, some acid producing bacteria, and some bacterial spores remain alive. There is a gradual increase of these micro-organisms towards the posterior end of the intestine. These acid producing bacteria impart acidity to the undigested or partly digested food and subject it to acid fermentation as it passes farther and farther along the intestine. The intestines of animals have various types of micro-organisms which are specific to the species they live in.

In the colon the food stays for a considerably long time of 3 to 4 hours, and this facilitates the micro-organisms to further carry out fermentative and putrefactive effects. As a result of *fermentation* complex substances such as polysaccharides and fats are converted to simpler substances. The conversion of proteins by bacteria into simpler substances is known as *putrefaction* and this term is specific for proteins.

Enzymic hydrolysis of organic substances is compensated in the large intestine by the bacteria. The bacteria split the polysaccharides, fats and proteins to yield partial or complete hydrolysis products. The cellulose consumed by herbivores is broken down due to fermentation by the intestinal bacteria and as such none of the higher animals has enzymes capable of hydrolyzing it. In such animals the cecum can retain food for a long time to complete the fermentative process.

The bacteria in the large intestine produce enough quantity of vitamin K and some components of the vitamin B complex. These vitamins are absorbed to meet the body requirements. Generally, adequate amounts of these vitamins reach the intestine through the ingested food and as such the body does not suffer from their deficiency.

While some of the products of fermentation are harmless, there are substances such as indole, skatol, phenol, and hydrogen sulphide that have a pungent odour and toxic nature. Of the toxic gases, CO_2 and methane are produced chiefly from carbohydrate fermentation, whereas H_2S and N_2 are produced from protein putrefaction. The toxic products are absorbed into the portal blood and are taken to the liver for further oxidation or transformation to relatively harmless substances.

Faeces: After fermentation and putrefaction the material in the large intestine contains indigestible matter as well as undigested foods. A large proportion of the faeces, however, constitutes bacteria and intestinal epithelial cells. The bilirubin, present, in the faeces, is converted to *urobilinogen* which is then oxidized to pigment *urobilin.* The colour of the faeces is due to pigment. In addition, the faeces contain inorganic constituents that entered through food and remained unabsorbed. About 75 per cent of the faeces is water. This percentage is maintained in the faeces because of the secretory and absorptive ability of the colon. The quantity of the faeces formed is mainly dependent upon the quality and quantity of ingested food.

Defecation: The accumulation of faeces distends the rectum and stimulates the proprioceptors. The impulses from the proprioceptors are propagated to the sacral portion of the spinal cord. Impulses strong enough to stimulate the motor nerves innervating the smooth muscles of the rectum are created only when the pressure in the rectum reaches the required level. In humans, the required pressure level is about 40 mm Hg. This defecation reflex, coordinated at the sacral level, may be inhibited or enahanced by higher control centres. The defecation reflex may be inhibited by the voluntary

contraction of anal sphincter. The enhancement of defecation reflex is caused by contracting the abdominal muscles and increasing the pressure in the abdomen. Abdominal pressure may be further increased by increasing the intrapleural pressure and by forcing the diaphragm to descend. The pressure resulting from the combined activty of diaphragm and the abdominal muscles is sufficient to squeeze the rectum and push the faeces through the anal sphincter.

7.5 DIGESTION IN OTHER VERTEBRATES

Salivary digestion in general is wanting among the vertebrates, but the saliva of frogs and toads, and of the fowl contains the enzyme amylase. The enzymes such as amylase and disaccharidases are also present in the intestine of vertebrates. In bony fishes both amylase and maltase are secreted by the pancreas. Enzymological studies on a gold fish, a lizard and certain birds have revealed the presence of chitinase in the saliva.

Stomach is absent in prochordates and consequently there is no production of pepsin. In other vertebrates, except holocephali, dipnoi and many teleosts, a stomach is present and serves both storage and digestive functions. The stomach in these groups secretes pepsin-like enzyme acting in acid medium. In amphibia, pepsin is secreted more in the oesophagus than in the stomach. In fishes, amphibians, reptiles and mammals, individual proteases are similar, if not identical. The acidity of the stomach contents in vertebrates is generally lower than that of man and dog. The pH values range between 2.5 and 4.5 in fishes, amphibia, and birds. However, in some rays and bony fish, the stomach content is alkaline in spite of the presence of pepsin. In contrast to this, the sharks have an acidity twice that of man.

A small amount of gastric lipase is secreted in the stomach of some birds and fishes. In their intestine the proteases are generally distributed as in mammals. Secretion of lipase and the presence of bile salts in the intestine is a general feature of all vertebrates. Honey-guides are a kind of birds that feed on bees-wax but do not produce esterases required for its digestion and are dependent on their microflora. Autolytic and bacterial digestion has been observed in the crop of fowl.

There are scattered references on hormonal and nervous control of digestion among vertebrates. In some animals (selachians) neither nervous nor hormonal control was evident. Studies on control of pepsinogen secretion revealed that the nervous (vagus) stimulation was not needed in *Rana,* whereas in the toad *Bufo,* some nervous control is required. There is an indication of the presence a hormone gastrin in amphibia. In some (salmon, dog-fish, frog, tortoise, etc.) secretin is present but its activity is not established.

The problem of intracellular digestion has been solved by the presence of food vacuole. The vacuole enclosing the food is spherical and consists of fluid and a surrounding membrane. The food in the vacuole gradually breaks down and disappears, which is due to the secretion of appropriate enzymes into the vacuole from the surrounding cytoplasm. The food constituents diffuse into the cytoplasm and get assimilated. The leucocytes of higher animals, which engulf foreign particles (phagocytosis), also have similar intracellular digestive mechanism. The food vacuole in these is analogous to the gastrointestinal tract of higher animals and for this reason, it is appropriately known as *gastric vacuole.*

7.6 DIGESTION IN INVERTEBRATES

Our knowledge of the digestive processes among invertebrates is based on the morphological and anatomical features of their digestive organs and enzymes present in them. The alimentary canal of invertebrates is relatively simple and all the enzymes are often secreted by a single gland. Further, these enzymes nearly always digest the food in one part of the alimentary canal which is anatomically simple and undifferentiated into functional zones.

In general, all animals possess enzymes necessary for splitting starch, glycogen, proteins and fats. Some of the enzymes in unicellular organisms are present in the lysosomes, while others are found attached to the cell membrane.

In many echinoderms and lamellibranchs intracellular digestion is performed by wandering cells. In lamellibranchs, these cells may even enter the mantle cavity and ingest food particles. The wandering cells can split all three classes of food, viz. carbohydrates, proteins and fats.

In most animals, extracellular digestion takes place completely within the digestive tract. There are some animals which partly digest certain types of food outside the body. Such an external digestion has been observed in some species of rhabdocoelidae and in blowfly maggots. The latter excrete proteases through the faeces in order to liquefy the meat in which they live. Their liquefied meat is then ingested.

The power of digestion of food is very much limited among certain parasites since they have weakly developed digestive tract. The *Fasciola hepatica,* feeding on blood, has a proteinase, but most of the other nutrients are absorbed through the body surface. *Ascaris,* however, does not absorb nutrients through its body surface but ingests both digested and undigested foods and in its digestive tract they are attacked by a group of enzymes, viz. amylase, maltase, protease, peptidase, and lipase. Parasite like *Taenia* has no digestive tract and it sustains itself on predigested food from the host.

Among invertebrates, the carbohydrases have not received as much attention as proteases. The amylase from a number of groups is very similar to vertebrate amylase. It can attack starch to split it to maltose, but it fails to split pure starch grains, and usually has a pH optimum slightly on the acid side of neutrality. Many invertebrates can hydrolyze the three common disaccharides –maltose, sucrose and lactose. In some animals, snail in particular, the disaccharidases can hydrolyze at least seventeen sugars and related substances. The snail produces enzyme inulase to digest the inulin of the compositae into fructose. The snail can also digest cellulose and chitin with the help of symbiotic bacteria. The occurrence of *cellulase* has been reported among a few protozoans, earthworms, some wood boring beetles, and the wood boring bivalve *Teredo,* etc. *Chitinase* has been found in soil amoebae, in coelenterates, nematodes, earthworms, and in all animals consuming arthropods as food.

The proteinase of *Maia,* like vertebrates, requires an activator and in fact, it is secreted along with it. The gut of a crab *(Maia),* and a marine snail *(Murex)* has been found to contain four proteases—proteinase, carboxypeptidase, aminopeptidase, and dipeptidase. All these proteases resemble those present in vertebrates. The pH optima for these enzymes are of the same orders those for vertebrate enzymes. The *enterokinase* which activates the proteinase in the vertebrates can also activate the purified proteinase from *Maia,* suggesting its resemblance with that of vertebrates. In several insects the protease is known to be consisting of a tryptic proteinase, a carboxypeptidase, an aminopeptidase

and a dipeptidase. The proteinase and its activator from caecum of *Sepia* have similarities with those of trypsin and its activator enterokinase of vertebrates. Thus in general the protein digesting enzymes of invertebrates are activated by the same series of enzymes that activate in vertebrates. The proteases of *Pheretima* and larvae of *Calliphora* and other meat-eating flies react in a slightly acid medium and are comparable to pepsin.

The lipases among invertebrates largely differ from those of vertebrates. Many invertebrate lipases hydrolyze esters of lower fatty acids more easily than oils and fats, and for this reason, they are more aptly called esterases than lipases. Bees-wax consumed by wax-moth is partly digested by bacterial action and partly by enzymes.

B. ABSORPTION

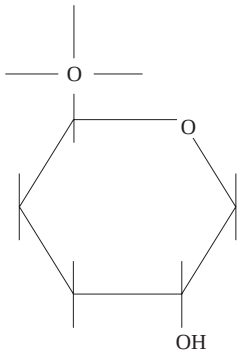
The process of absorption takes place chiefly in the small intestine. To a limited extent, absorption of ordinary foods also takes place in the stomach. Nontypical foods such as pepper, mustard, condiments, and alcoholic drinks seem to be absorbed in the stomach.

Small intestine is most suitable for absorption. For this purpose, the epithelial lining of the small intestine is well adapted. The small intestine has an extensive absorbing surface formed due to the enormous number of small papilliform villi. The intestinal contractions as well as the contractions of the villi constantly expose the entire surface of the small intestine to the liquid material for maximum absorption.

The digested material from the intestine is absorbed either into the blood capillaries or into the lymphatics, both of which are present in the villi. The material absorbed into the capillaries is then carried to the mesenteric veins and the portal vein. From the latter, it gets into the liver before being sent to the general circulation of the body. In another pathway the absorbed material would reach the blood indirectly. In this case the material is absorbed into the lymph spaces of the villi and the lymph vessels of the intestine. It is then transported into the large lacteal vessels and the thoracic duct. The material transported through the lacteals is termed *chyle.* The thoracic duct empties the chyle into the venous system near the heart.

7.7 CARBOHYDRATE ABSORPTION

Carbohydrates are chiefly absorbed by the small intestine, in the form of monosaccharides. The absorbed sugars are utilized for the metabolic needs. The monosaccharides for the most part are absorbed by active transport mechanism. Intestinal membrane has selectivity in absorbing simple sugars. Cori, in 1925, observed that the sugars administered into the stomach of rats disappeared from the intestine at different rates. The pentoses have smaller molecules and therefore diffused more rapidly than hexoses. Of the hexoses, galactose and glucose are more rapidly absorbed than fructose. This rapid intake is due to their active absorption against the concentration gradients. It has been suggested that for active transport the sugars must contain six carbon atoms in the form of a D-pyranose ring with an intact—OH at the 2-carbon position as illustrated in Fig. 7.6. Fructose does not conform with this minimal required structure and for this reason it does not enjoy active transport.



D-pyranose ring

Fig. 7.6 D-pyranose ring with an intact—OH at the 2-carbon position can be readily absorbed by active transport.

Still, its rate of absorption is faster than mannose, xylose, and arabinose. This is so because of its conversion in the epithelial cell to lactic acid (Wilson and Wiseman, 1954) in rat and hamster, and to glucose (Hers and Kusaka, 1953) in guinea pig and hamster. In rat and human intestinal cells, the glucose-6-phosphatase, required for the conversion of fructose into glucose, is absent.

The precise mechanism for the transport of sugars is not known. However, all sugars transported actively are believed to be attached to the common carrier on the lumenal border of the epithelial cell membrane. Since they all have a common carrier, some of the sugars having greater attraction to the carrier compete with others to gain attachment for their transport into the cell. Because of this competition, the rate of intake of some sugars is higher than the others. Though the following hypothesis is with reference to the absorption of glucose. the same is believed to hold good for other sugars also.

Glucose transport involves two systems (Fig. 7.7). The first system consists of a carrier or translocase with two binding sites; one for attachment with glucose and the other for Na^+. The carrier with both Na^+ and glucose attached moves to the membrane surface facing cytoplasm. In this process, the carrier is subjected to the forces of two gradients, an outward glucose gradient and an inward Na^+ gradient. The Na^+ gradient exerts greater force on the carrier complex than the glucose gradient, and results in the inward movement of the carrier complex. On reaching the inside surface, glucose and Na^+ dissociate from the carrier and enter the cytoplasm. Thus the first transport system brings glucose and Na^+ ions from the lumen into the cytoplasm. If this continues, Na^+ ion concentration in the cell increases and the gradient diminishes. However, this does not occur. The second transport system removes Na^+ brought in by the first transport system and thus maintains the inward Na^+ gradient—a high concentration outside and low concentration inside the cell. Since the second transport system is involved in the pumping of Na^+ against the gradient, it requires energy. The energy for this process, comes from the hydrolysis of ATP.

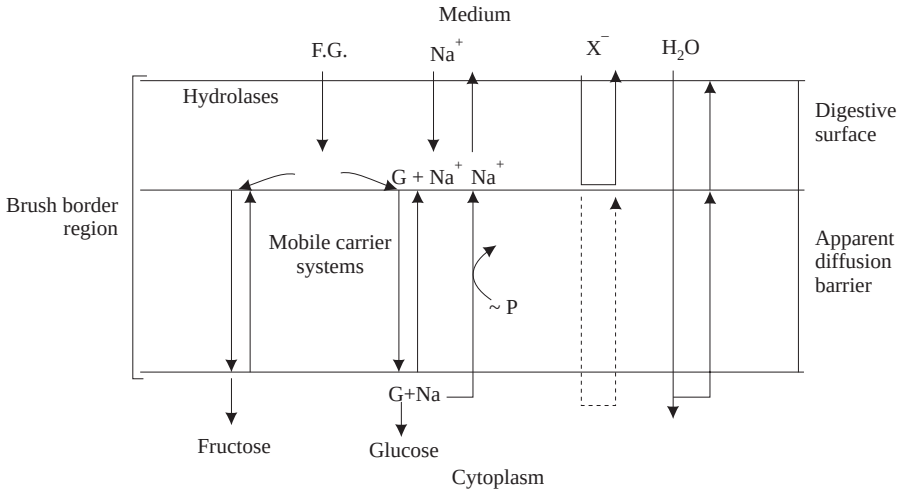


Fig. 7.7 The mechanism of glucose absorption.

7.8 PROTEIN ABSORPTION

The proteins enter the blood only in the form of amino acids. The inward movement of amino acids across the membrane is similar to that of glucose absorption. The transport system contains a complex of carrier its specific amino acids, and Na^+.

There are four types of systems each of which is specific for a group of closely related amino acids. Amino acids such as glycine, alanine, and leucine are transported by a specific type of transport system. Similarly there is a transport system specific for acidic amino acids like glutamic acid and aspartic acid. Still other types of transport systems are specific for basic amino acids. Proline and hydroxyproline have a common transport system. One of the significant and common properties of these transport systems is the requirement of the presence of high concentration of Na^+ in the lumen of the intestine.

The absorption of amino acids is thus a selective process rather than simple diffusion. Their absorption is rapid and active. After studying the rates of absorption, Gibson and Wisemen (1951) observed that in case of 13 amino acids the intestinal wall preferred the L-form (natural form) to its D-form (optical isomers). The amino acids thus absorbed enter the blood and are rapidly removed from there to the liver. In the transport of amino acids, the lacteal system of the intestinal wall plays no significant role.

The neutral amino acids appear to share a common earlier for their transport across the membrane of the mucosal cell (Fig. 7.8). For active transport of these amino acids the transport systems must possess the required optical specificity (the L-stereoisomer), an intact carboxyl group (COOH), an α-amino group, an α-hydrogen, pyridoxal phosphate, and the fat soluble side chain (R).

The carrier system which transports cystine also transports the basic amino acids, viz. lysine, arginine and ornithine. The existence of this common transport system came to light during

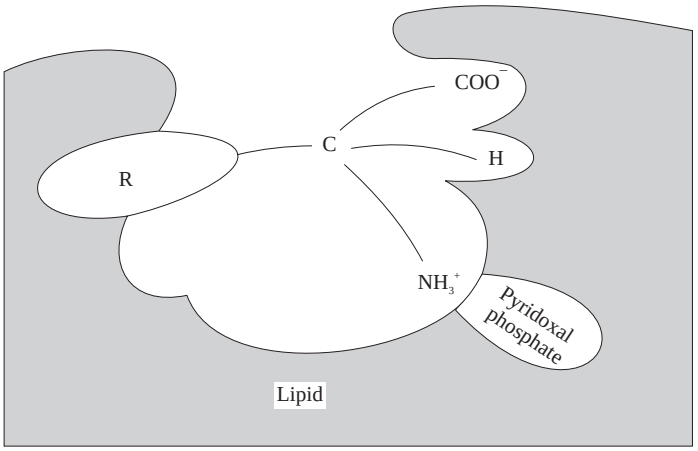


Fig. 7.8 Transport of neutral ammo acid across the membrane of mucosal cell.

investigations on cystinurea. Cystinurea (existence of cystine in urine) is a disease caused by a genetic defect. Due to this defect, the carrier responsible for the absorption of cystine does not functionally exist, and in such cases the absorption of not only cystine but also that of lysine and ornithine is prevented from the intestine. It is believed that a similar carrier exists in both the kidneys and the intestine for these amino acids.

Amino acids proline and hydroxyproline have a common (betaine) transport system, and the existence of this system eliminates these amino acids from competing with the neutral amino acid transport system. Only small quantities of glutamic and aspartic acids are recoverable in the portal blood after their absorption from the intestine and this has been explained to be due to transamination during absorption.

7.9 ABSORPTION OF FAT

The digestion of fat is never so complete to form only glycerol and fatty acids. The digested fat is a mixture of triglycerides, diglycerides, monoglycerides, and free fatty acids along with glycerol. All these can be absorbed by the intestinal cells. However, monoglycerides, fatty acids, and glycerol form a significant part of the material absorbed by the intestine. The bile salts act as detergents and bring the fatty acids, cholesterol and monoglycerides in contact with the microvilli. The bile salts are dissociated from the digested fats as they are absorbed and become available once again to bring the digested fats in contact with the microvilli. Some suggested that dissociation is taking place in the lumen (Lack and Weiner, 1963). In such a case, the bile salts would have the mucosal cell and come back into the lumen.

The glycerol is water soluble, and being a small molecule, it is speedily absorbed by the intestine. The fatty acids and glycerides are said to be taken into the cell by pinocytosis (Palay and Karlin, 1959). But biochemical and electron microscopic studies did not reveal any significant increase of

pinocytic inclusions between fed and fasted rats. Whatever might be the process, these fatty acids enter the mucosal cells, and are transformed to triglycerides within the endoplasmic reticulum.

Two pathways exist for the transport of fats from the mucosal cells. The triglycerides formed by long chain (about, or more than 12 carbon atoms) fatty acids are coated with a protein to form *chilomicrons.* These are then secreted by the mucosal cells into the lymph spaces. The chilomicrons are lipoproteins, about lμ in diameter, and impart a milky colour to the lymph spaces and lymph yessels, which for this appearance are called *lacteals.* The chilomicrons are then carried along with the lymph from the lymphatics into the venous blood via thoracic duct.

In the other pathway, the short chain fatty acids are directly absorbed into the blood capillaries of the intestinal villi. They then enter the portal bloodstream, from where they are quickly removed by the liver and for this reason plasma would not appear milky.

Of the cholesterol present in the lumen of the intestine, about half is derived from the bile. The cholesterol is said to pass into the cell through solution from the lipid portion of membrane. The fatty acids, especially oleic acid, as well as the pancreatic juice, stimulate and enhance the absorption of cholesterol into the mucosal cells. From the mucosal cell they are passed into the lymph. The following postulation explains the process. The cholesterol enters the chilomicrons along with triglycerides. The bile helps in esterification and the chilomicron formation. These chilomicrons are then secreted into the lymph for transport (Wilson, 1962).

7.10 ABSORPTION OF OTHER SUBSTANCES

Absorption of Nucleic Acids

As a result of the hydrolysis of nucleic acids by ribonuclease and deoxyribonuclease, the nucleotides and nucleosides are formed. These products are then absorbed by the mucosal cells inside the mucosal cells the nucleotides are split to nucleosides to enable them to diffuse through the serosal side into the intestine.

Absorption of Water Soluble Vitamins

The relatively low molecular weight of the water soluble vitamins would enable them to enter the mucosal cells of the intestine by simple diffusion. The vitamin B_{12} molecule is, however, the largest of all the B group of vitamins. To be absorbed it requires a gastric intrinsic factor (IF), which is also a large molecule. It is secreted by the stomach. Due to the presence of acid medium in the stomach, the vitamin B_{12} is released from the ingested animal protein. The IF then gets attached to the vitamin B_{12} (Fig. 7.9) and travels down through the digestive tract and is absorbed in the ileum. The cells in the ileum have specific capacity to absorb vitamin B_{12}. The IF-B_{12} complex in the presence of Ca^{++} gets attached to the receptor sites on the surface of mucosal cells of the ileum. For adequate absorption of vitamin B_{12}, there should be enough IF and it should form the complex (IF- B_{12}) before it gets attached to the receptor site. In sufficient or exclusive quantities of IF would lead to inadequate absorption of vitamin $B_{12.}$

After this attachment, it is not clear how the vitamin enters the cell. One view is that the entire IF-B_{12} complex enters the cell by pinocytotic process. According to another view the B_{12} is split from the

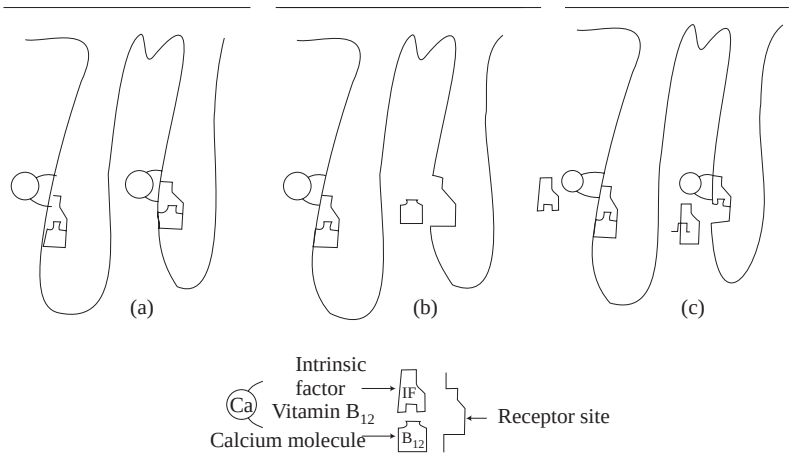


Fig. 7.9 Absorption of vitamin B_{12}.

complex by a releasing factor and is then taken into the cell. Whether vitamin B_{12} enters the cell alone in association with IF, it must get dissociated from the IF and bind to a protein within the mucosal cell. This protein molecule is small enough to pass though the capillary wall into the blood-stream. The vitamin B_{12} protein complex in the plasma is called *transcorrin* and it is an α 1-globulin. In this form, the vitamin B_{12} is transported.

Absorption of Fat Soluble Vitamins

The bile is essential for the absorption of these vitamins which are found in association with lipids (e.g. as esters) in the diet. The vitamin A ester (retiny lester) is first hydrolyzed in the intestinal lumen to form retinol (a free vitamin alcohol). The retinol then enters the mucosal cell and gets re-esterified with palmitic acid (Mahadevan and Ganguli, 1961) to form retinyl palmitate, and this form it enters into the chilomicrons. These chilomicrons are then secreted into the lymph and transferred to liver via blood. In the liver, it is stored as palmitate ester. Similarly some of the β-carotene (a provitamin) is converted to retinyl ester on its entry into the mucosal cell and it is transported to the liver as retinyl pamitate.

Inadequacy of protein in diet has adverse effect on the absorption of vitamin A and carotene. What precise role do the proteins play in preventing the vitamin A and carotene absorption is not known.

Water and Electrolyte Absorption

Water and electrolytes reach the intestinal lumen from two sources, i.e. through the ingested diet and water through the intestinal juices. The quantity of from water source is greater than from the former. Most of the water is absorbed throughout the length of the intestine and passed into the blood. This process of movement of water in the intestine has been referred to as *gastrointestinal circulation.* The transfer of water and salts through the mucosal wall is partially dependent on the active metabolism of

the mucosal cells. Generally, the rate of absorption of divalent ions is slower than that of monovalent ions. The absorption of Ca^{++} is 50 times slower than that of Na^{++}.

Calcium Absorption

Calcium is very much needed for young animals to meet the requirements for bone growth and for this reason calcium is efficiently absorbed from the intestine. The calcium absorption has little to do with age and in young and old its absorption is need based. When the body needs more calcium, active transport system adjusts to this need and enters the mucosal cell more efficiently. The calcium absorbed by the mucosal cells is derived from the ingested food and the gastrointestinal secretions. Since the quantity of calcium released along with the secretions is difficult to ascertain, the quantity of calcium absorbed by the intestinal mucosa is not possible to determine accurately. However, the difference in the quantity of calcium between ingested food and excreted matter is taken as the quantity of calcium absorbed. The calcium absorption is greater in the duodenum and jejunum than in the distal portions of the small intestine. The studies to clarify mechanism of calcium absorption were initiated with the availability of Ca^{++}. In these studies, the calcium is observed passing into the mucosal cells against the concentration gradient, thus indicating active transport across the membrane.

Lactose is believed to act directly on calcium so as to maintain it in a form suitable for transport across the membrane of the mucosal cell. Vitamin D also plays an important role in the increased absorption of calcium. However, its action is not directly upon calcium but upon the intestinal mucosa. The precise action of vitamin D is not yet definitely known. There is some evidence that vitamin D helps in the synthesis of a protein which is necessary for the membrane transport of calcium, but is not well received. The fact that inhibition of oxidative phosphorylation does not prevent vitamin D influenced calcium transport suggests that this transport system does not require energy.

Another view is that the parathyroid hormone along with vitamin D regulates the total amount and the ratio of calcium and phosphate ions in the internal environment. However, the exact mechanisms of this combined effort are not satisfactorily explained.

Absorption of Iron

It is well established that the excess iron, which may enter the body either by injection, or by the disruption of the normal regulation of iron absorptive process, is not excreted through faeces or urine. The fact that the intestinal mucosa absorbs iron but does not excrete suggests that its movement is unidirectional. Excess of iron in the body accumulates in the cells as iron-containing hemosiderin.

There are four important views regarding the mechanism of regulation of iron absorption.

Mucosal Block Theory of Granick: According to this theory, the quantity of iron absorption is determined by the ferritin content within the mucosal cells of duodenum and upper jejunum. If the body has already absorbed adequate iron, high levels of ferritin are formed in mucosal cells. High ferritin content in the mucosal cells would block further iron absorption; and low ferritin content permits iron absorption and its combination with the protein *apoferritin* in the cell to form ferritin. Iron is absorbed more readily its divalent form and therefore Granick proposes first the transformation

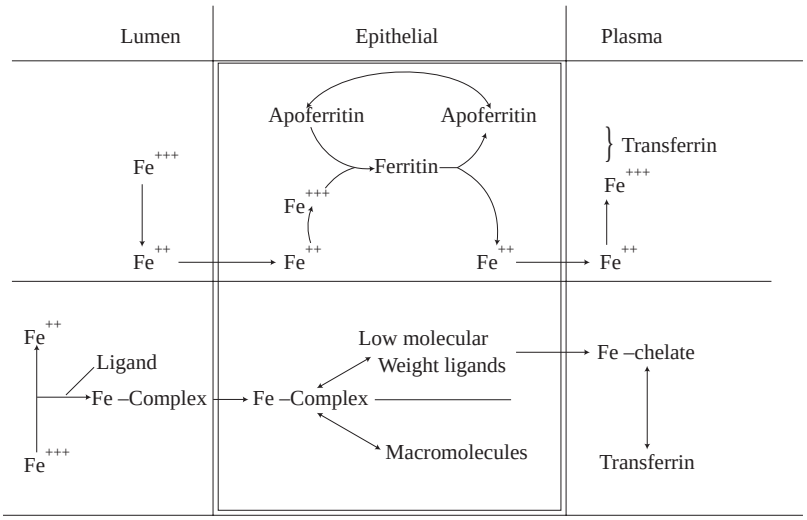


Fig. 7.10 Diagram illustrating Granick's hypothesis for iron absorption.

of ferric ion (Fe^{+++}) to ferrous form (Fe^{++}) in the intestinal lumen and then its absorption into the mucosal cell (Fig. 7.10). Once in the mucosal cell, the ferrous iron is oxidized to ferric form which combines with apoferritin to form ferritin. If the body needs iron, ferritin in the mucosal cell is split to form apoferritin and ferric iron. The ferric iron is reduced to ferrous form, which then passes through the serosal membrane into the blood. Upon entering the blood, the iron is reoxidized and attached to the protein *transferrin* which is an iron-binding 1-globulin.

Chelation and Equilibrium Binding Theory: Charley et al. (1963) suggested that in the lumen the ferrous ion combines with a ligand such as sorbitol or fructose to form a soluble uncharged complex. This complex enters the cell and the iron combines with other ligands of low molecular weight as well as apoferritin. The passage of iron across the serosal membrane into circulation depends upon the availability of unbound transferrin.

Manis and Schachter's Iron Absorption Theory: According to Manis and Schachter (1962) both the mucosal cell iron and its serosal transfer are energy dependent. The former action is rapid and proportionately increases with the concentration of iron in the lumen. The serosal transfer of iron is maintained constant and maximal and requires the energy derived from oxidative metabolism within the cell. According to these authors, both ferric and ferrous ions pass through the mucosal membrane facing the lumen, whereas for its transfer through the serosal membrane only the ferrous form is preferred.

'Messenger Iron' Absorption Theory: Conard, Weintraub and Crosby (1964) suggested that the iron administered orally was incorporated in the mucosal cells of the small intestine as *messenger iron.* If mucosal cells contain large quantities of messenger iron, this would mean more nutritive iron in the body and consequently they reject iron absorption. If the body is deficient in iron, messenger iron content in mucosal cells is very little or totally absent, and such mucosal cells would freely absorb iron.

CHAPTER

8 Water Relations and Ionic Regulations

Life began in a medium, the physical forces and chemical factors of which were quite congenial to permit its multiplicity and continuity. Such factors existed in seawater. The first organisms which arose in this medium contained in themselves a fluid whose concentration is similar to seawater. But the fluid in the organism has to be something different from seawater in order to carry out life activities. For this the organisms contain a balanced fluid of organic and inorganic substances. In this biochemical processes take place, and to facilitate these processes the composition of the internal fluid must be meticulously maintained at the required level. For this a barrier exists between the life sustaining internal fluid and the life giving external medium (seawater). Such a barrier is in the form of a semipermeable membrane. The importance of the membrane barrier is dealt under Section 8.1.

Many of the living organisms moved from the seawater to other habitats in the course of evolution. A great many organisms even left the aquatic habitats and chose extremely dry terrestrial habitats. These habitats tend to bring changes in the water and salt content of the organisms. Water continues to be an essential requirement for all organisms and is the universal biological solvent, hence is the most suitable medium to supply the necessary substances to carry out the biochemical reactions.

In the very primitive and the simplest of the living marine organisms the procurement of food, oxygen, and the elimination of wastes are carried out directly between the intracellular fluid and the seawater. The more complex multicellular animals have developed tissues which are not accessible for direct contact with seawater. In these animals the tissues are directly bathed in the extracellular fluid which is in osmotic equilibrium with the surrounding seawater. These body fluids supply the tissues with nutrients and oxygen, and the tissues in turn eliminate waste products into them. Since the body fluids subserve the function of sea, as medium around the tissues, it is said that they, act as *internal sea.* The volume of these body fluids is usually smaller than that of cells and hence the composition of the fluids is maintained constant to meet the needs of the cellular requirements of the tissues. The depleted levels of oxygen and nutrients in the body fluids are soon replenished by respiration and digestion.

The volume of water and the composition of solutes in the body fluids are controlled by the excretory organs which either remove or conserve substances already present in the body fluids. However, at the other two sites, i.e. gut lining and body surface in aquatic animals, the salts and water are transported in either direction. The development of an internal medium, i.e. the body fluids, helped the maintenance of cellular composition of not only the complex marine animals but also that of freshwater and terrestrial animals. All these animals regulate their body fluid concentrations at levels specific to the various groups. In freshwater animals the volume of water and the composition of salts in the internal medium is maintained remarkably at the required level in spite of the disturbing effects of diffusion and osmosis. In terrestrial animals the internal medium loses water and salts through the body surface and kidneys. Water is lost by evaporation mainly through the lungs. They developed mechanisms for conserving water and salts. These animals still lose certain amounts of water and salts in spite of conserving mechanisms. Such a loss is compensated only by absorbing them from the food and water ingested from outside into the digestive system.

8.1 ROLE OF MEMBRANES IN OSMOTIC AND IONIC REGULATIONS

The unit membrane, besides enclosing the cytoplasm and offering some sort of protection from minor collisions, serves for the transport of selected substances across it.

A number of multicellular and a cellular organisms live in an environment that is different in some respects from their internal environment. Even if the cells are bathed in a medium that has an osmotic concentration similar to seawater, a difference in the ionic composition between the internal and external environments of the cells is always maintained. Within the cells there is sort of balance in the amount of water, salts and organic substances. The entry of substances into the cytoplasm and expulsion into the external medium is meticulously regulated.

The cell membrane is permeable to many substances in either direction. For this the membrane possesses a structure, the chemical composition of which is suitable for transport of selected substances. The exact chemical composition is still under investigation. However, based on important observations certain hypotheses regarding membrane structure have been made and these were discussed in Chapter 1.

For convenience, the mechanisms involved in the transport of substances across the membrane can be treated under eight types. Of these, four are physical mechanisms in which the forces that drive substances across the membrane are supplied from the environment of the cell. The transport by these mechanisms is often termed as passive transport which does not involve chemical covalent bond breaking and bond making reactions. These physical mechanisms are *diffusion, osmosis,* events leadings to *Donnan distribution* and *solubilization.* The remaining four mechanisms comprise complex enzymatic reactions and include processes such as *pinocytosis* or *phagocytosis, facilitated diffusion active transport* and *cellular secretions.* These four mechanisms make use of the energy produced by the cell's own metabolism.

Passive Transport

Diffusion: In a solution the major component is termed solvent and the substances dissolved in the solvent are termed solutes. Initially, if a solute in a solution is in unequal distribution, in time due to random movements of the solute particles they get distributed uniformly and produce a homogenous solution. When a concentrated solution of a substance is separated from the same quantity of a dilute solution of the same substance by means of a membrane permeable to solute molecules, the solute molecules would then move from the concentrated solution towards the dilute one until an equilibrium is reached. The movement of solute particles down the concentration gradient is a physical phenomenon and does not depend on cellular energy. In this case the solute particles diffuse down the concentration gradient (see Chapter 1, Section 1.2).

Diffusion Down the Electrical Gradient: In solution the molecules of the solute are usually dissociated into ions that carry an electric charge. The fluid media inside and outside the cells have charged particles and the cell membranes have the ability to maintain potential difference between inside and outside. The potential difference of the membrane is measured with microelectrodes and it has been found to range from 50 mV to 100 mV. The existence of such a potential difference is due to the asymmetrical distribution ions between the inside and outside of the membrane.

The ions involved in the formation of potential gradient are potassium and chloride. The intracellular concentration of potassium ions in most cells of higher animals is higher than its concentration in the extracellular medium. Likewise, the concentration of sodium ions is higher in the extracellular medium. These K^+ and Na^+ ions tend to move down their concentration gradients across the pores in the membrane and if these movements continue uninterrupted the potential difference would collapse. But the cellular membranes maintain the potential gradient by transferring the Na^+ and K^+ ions against their concentration gradients. The movement of the charged materials is influenced by the electrical charge existing on either side of the membrane hence the materials diffuse against their concentration gradients.

Osmosis: When two aqueous solutions of different concentrations are separated by a membrane permeable to water but impermeable to solute molecules, water diffuses through the membrane from the solution of low solute concentration to that of high solute concentration until the molal concentrations on either side are the same (Fig. 8.1)

This process of solvent movement is called osmosis. In an artificial system the movement of water by osmosis increases the *hydrostatic pressure* of the high concentration solution to a level at which no further movement of water in that direction is allowed. The hydrostatic pressure required to prevent the movement of water from pure water to the solution side of the semipermeable membrane is known as the *osmotic pressure* of the solution. In symbols, the osmotic pressure of a solution can be expressed as

$$P = RTC$$

where R is 0.0825 litre-atm/moles-degree, T is absolute temperature, and C is the molal concentration of the solute.

While this equation holds good, the direct determination of osmotic pressure of solutions is technically a difficult process because it requires a relatively large volume of solution to estimate the

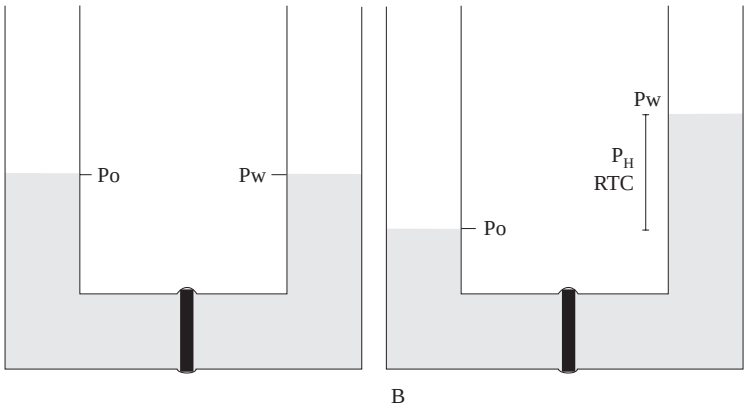


Fig. 8.1 Osmosis
P_w — Vapour pressure of pure water; P_0 — vapour pressure of the solution; P_H — osmotic pressure. The osmotic pressure is calculated by the formula; P_H — RTC, where C is molal concentration; R is 0.0825 1-atm. per degree; T is absolute temperature.

number of solute particles. However, since the osmotic pressure of solution is dependent on properties such as the depression of the vapour pressure, elevation of the boiling point and depression of the freezing point, which are all directly proportional to one another, it would be easier to determine the *osmotic concentration* of a solution by taking one of these colligative properties into account. Of these, the depression of the freezing point is the property that is often utilized to express the osmotic concentration of the solution concerned. With this process the osmotic concentration of even minute quantities of solutions can be measured. A depression in freezing point by 1.858°C would indicate one molal solution of an ideal nonelectrolyte. Most of the investigators in this field prefer to express osmotic concentrations in the form of *freezing point depressions* rather than the molal concentrations of solutions. For convenience, the term freezing point depression is abbreviated as Δ.

The relation between depression of the freezing point and concentration can be explained in the following equation:

$$\Delta t_f = K_f C$$

where Δt_f is the change in freezing point for a given solvent, K_f is a cryoscopic constant, and C is the molal concentration. The K_f equals to 1.858°C per mole of ions of neutral compounds in 1,000 gm of water. The value of K_f would change with the type of solvent. If the solvent is cyclohexanol the K_f value would be 41.8. The value of K_f for a given solvent is determined as the slope of plot of freezing point versus molal concentration of a soluble solute.

Body fluids contain both strong electrolytes and weak electrolytes such as phosphates or magnesium salts and due to their complete and incomplete dissociation their osmotic coefficients differ. Such a difference is partly the result of incomplete dissociation and partly due to the departure of the particles from ideal behaviour. Due to this reason, it is difficult to make an accurate estimation of osmotic pressure of body fluids from its chemical composition. The accurate method of

determining osmotic pressure of biological solution of unknown concentration would be by measurement of the freezing point depression or one of the other colligative properties.

The value of t_f is determined by measuring the freezing point of pure water. Then the value of t_f is divided by K_f to get the *osmolality* of the solution. The osmotic pressure of biological solutions is expressed in terms of *osmoles.* A solution is said to contain one osmole if the amount of solute in one litre of water exerts the same osmotic pressure as does one molal solution of ideal nonelectrolyte.

A solution of one-gram molecular weight of glucose in one litre of water is equal to one osmole (1 osmole/litre). On the other hand a solution of one gram molecular weight of sodium chloride in one litre of water is approximately equal to two osmoles.

Exchange Diffusion: There are some membranes which are impermeable to ions, but contain carrier units for ion exchange material. When solutions are separated by such membranes the ions fail to diffuse freely across the membrane. However, the membranes have within themselves carrier unit materials which have high affinity to ions in the two solutions. These ions carrying units travel within the membrane from one surface to the other and due to their affinity they are always saturated (Figure 8.2).

Ion carrying units saturated with the ions of the solution-1 travel to the surface of the membrane facing solution-2 to have 1:1 exchange of ions with the solution-2. The ions carrying units, now saturated with the ions from the solution-2 would move to the surface of the membrane facing sclution-1 to have a 1:1 exchange with the ions of solution-1. Thus in exchange diffusion process, a 1:1 exchange of ions would take place on either side of the membrane, so that the total flux in both the directions remains same and hence the concentrations of the two solutions would remain unchanged.

The exchange diffusion is carried out only if a significant concentration exists on either side of the membrane. If the solution-1 is replaced by a pure solvent, obviously there would be no flux of ions by way of exchange diffusion and would occur from solution-1 to solution-2, and therefore the

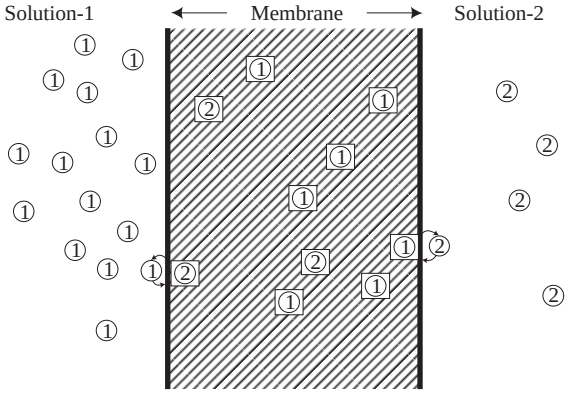


Fig 8.2 Exchange diffusion: The carrier in the membrane forms complexes with the ions. When the carrier with the ion of solution-1 reaches the membrane surface facing the solution-2. a 1:1 exchange of ions lakes place between the carrier-ion complex of that solution. Similar 1:1 exchange of ions would take place if the carrier carrying ion of solution-2 reaches the membrane surface facing solution-1.

flux of ions from solution-2 to solution-l also would cease. This is the case in exchange diffusion, and it differs from the simple diffusion, in which the flux would be from the solution to the pure solvent.

Active Transport of Ions

All diffusible substances enter or leave the cell down their concentration gradients, and if allowed uninterrupted the organization in the living system would be jeopardized. The cell membrane of all living cells has the capacity to transport some substances against the gradient and such a transport is called the active transport.

The active transport can be distinguished from the passive transport by an important criterion, i.e. as a result of the active transport of solute the entropy decreases and the free energy of the system increases, whereas in passive transport the free energy in the system would decrease. But the second law of thermodynamics says that in the universe no spontaneous process occurs that would result in a decrease of entropy or a net increase in free energy of the system. Therefore, in the active transport free energy cannot increase by itself.

The increase is due to the production of free energy by a process that is coupled with the active transport. A change in the free energy of a system does occur after transport which may be active or passive. There are equations to calculate the change in free energy of the system. If the free energy change is positive the process is an active transport. On the other hand, if the change is negative the process is passive transport. The equation to calculate the change of free energy when 1.0 mole of an uncharged solute is transported from one compartment to another is:

$$G^{o} = 2.3\ RT \log_{10} \frac{C_2}{C_1}$$

C_1 and C_2 are the concentrations of the free solute at the beginning and end of the transport process, R is the gas constant, and T is the absolute temperature.

Let us assume that one gram molecular weight of glucose is to be transported from a compartment in which its concentration is 0.001 *M* to a compartment in which its concentration is 0.1 *M*, i.e. up along the concentration gradient. Then the change in free energy can be calculated with the above equation.

$$G = 2.3 \times 1.98 \times 298 \times \log_{10} \frac{0.100}{0.001}$$

$$= 2.3 \times 1.98 \times 298 \times 2.00 = 2680 \text{ cal}; \; G = 2.68 \text{ kcal}$$

The free energy change is 2.68 kcal. Since it is positive in sign it indicates increase in free energy.

If the movement of glucose is from a compartment in which its concentration is 0.1 *M*, to a compartment in which its concentration is 0.001 *M,* i.e. down the concentration gradient of 100 – 1, the free energy change will be of the same magnitude but in negative sign i.e. – 2.68 kcal.

The above cited formulas hold good to uncharged solute molecules.

For the active transport of Na^+, which is a charged molecule, it is required to move against two gradients. These gradients are: (a) the concentration gradient; and (b) the electrical gradient. Since there are two gradients, more work is required to move such a charged ion up.

Important Features of Active Transport System: Active transport systems have the following characteristics; (*a*) these systems are dependent on the metabolic processes yielding ATP; (*b*) these are solute specific; (*c*) their activity depends on the concentration of the substances being transported; (*d*) these are direction specific; (*e*) these transport systems may be selectively poisoned; (*f*) as a result of the integrated action of active transport mechanisms the internal solute and ion composition of the cells is maintained at a remarkably constant level, despite fluctuations in the external composition.

(a) The active transport is dependent on the source of metabolic energy. This phenomenon has been explained with reference to the transport of K^+ and Na^+ between the RBC and the plasma.

The RBC has high K^+ and relatively little Na^+. The plasma surrounding the RBC has very little K^+ and high Na^+. Since the RBC membranes is permeable to both Na^+ and K^+, they tend to move down their concentration gradients. But the Na^+ and the K^+ diffusing, down their concentration gradient, are pumped back. Since the pumping in both the cases is against the concentration gradient, energy is required. For this active transport, the energy comes from the glycolytic pathway.

If glycolysis is stopped there would be no production of ATP and the intracellular concentration of K^+ will gradually fall and that of Na^+ will rise until both K^+ and Na^+ concentrations are equalized on both sides of the RBC membrane. This indicates that the transport of Na^+ and K^+ across the membrane of RBC is energy dependent. In other kinds of cells such as liver or kidney cells, energy requirement is met from the oxidative phosphorylation.

(b) There are many transport systems pumping substances against the gradients. Each system has a specific substance or substances which it can pump. The active transport of only certain specific substances across the membrane of some cells suggests that they contain transport system specific to those substances. For example, the RBC of some mammals transport D-glucose inward rapidly, whereas they transport D-fructose only very slowly.

Some other cells have a pump specific for neutral amino acids like glycine and alanine which have uncharged R groups, but these cells fail to transport glutamic acid or lysine since R groups of these have an electrical charge.

(c) The movement of glucose into the cells is carried out by active transport. The rate of this process depends on the external concentration of glucose. The rate of glucose influx increases with the rise of its external concentration until a peak is reached when no further increase is possible. This can be interpreted as due to complete saturation of its active transport system. Such a property has also been found in case of enzyme activity.

(d) The active transport has a specific direction. For example, the K^+ in most cells is actively pumped only in the inward direction. Similarly, glucose and amino acids are pumped in the inward direction by respective systems. On the other hand, there are active transport systems which are directed outward. The system responsible for pumping Na^+ always transports Na^+ in the outward direction.

(e) The active transport specific to various substances can be prevented by selective poisoning. For example the active transport of glucose in the kidney can be poisoned by *Phlorizin* which is a glucoside obtained from the bark of pear tree. In another example the active transport of Na^+ can be inhibited by glucoside *ouabain.*

(f) Integrated actions of the active transport mechanisms maintain the intracellular solute and ion composition remarkably constant even when their composition in the external medium fluctuates widely. In this respect the yeast and bacteria can be taken as examples having remarkable ability to adjust their internal solute composition compatible with cellular function under variable pH.

Active transport of cell membranes resembles enzymes in that (*a*) they show substrate specificity; (*b*) they can be inhibited; and (*c*) they can be saturated by their substrates. These characters suggest that the active transport system contains two major components. The first is a carrier or porter molecule with a binding site specific to the substance to be transported and it is a protein. The second component is a protein or group of proteins which transfers energy to the first component in order to transport the substrate against the concentration gradient.

Active Transport of K^+ and Na^+: The active transport systems fall under two general types. Of these, one type maintains a balance of K^+, Na^+, and water in the cell. The other type pertains to the transport of organic nutrients such as glucose and amino acids into the cell and this we have included in Chapter 3. The first type, i.e. the active transport of K^+ and Na^+ is described here.

In most of the vertebrate cells the K^+ concentration is relatively high and constant and it ranges between 100 and 150 mM. The Na^+ concentration in the cells is quite low. K^+ concentration in the cells is high because of its role in carrying out vital enzymatic reactions at a fast rate. Such high and low concentrations of K^+ and Na^+ in the cells of higher animals are made possible because of the presence of an active transport system (Figure 8.3) that can pump K^+ into the cell and Na^+ out of the cell. The carrier component of the active transport system, responsible for the transport of K^+ and Na^+, is an enzyme called ATPase which is present in the cell membrane. It has a large particle weight and consists, of two or, more component protein molecules. This enzyme hydrolyzes ATP to form ADP and phosphate when activated by $Na^+ + K^+$ and for this reason it is called $Na^+ – K^+$ dependent ATPase. $Na^+ – K^+$ dependent ATPase system is fixed in the membrane in such a way that it always transfers Na^+ ions out of the cell and K^+ ions into the cell, both moving again their concentration gradients. It has been found that for each molecule of ATP hydrolyzed, three Na^+ ions are removed from the cell and nearly an equal number of K^+ ions are brought inward. The hydrolysis brings about two events; it decreases the free-energy causing a configurational change, or possibly a rotation of the carrier (ATPase) so that the attached Na^+ ion is brought in contact with the surface of the membrane facing outside and that K^+ is brought to the inside surface. The second event causes a transfer of terminal phosphate from ATP to functional group to the carrier. Since the functional group receives this phosphate it is said to have undergone phosphorylation and this process would take place before the enzyme completes the transport of Na^+ and K^+.

Most of the ATP produced in the cells is used by the ATPase present in the cell membrane. The epithelial cells of the kidney and nerve cells in the brain consume most of the cells ATP.

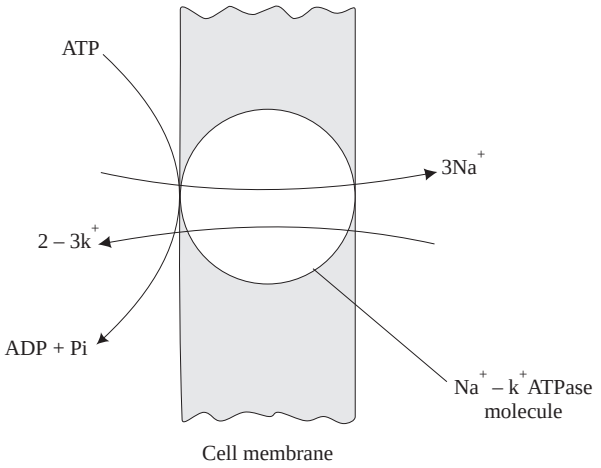


Fig 8.3 Active transport system for K^+ and Na^+.

Water Balance by ATPase: The Na^+– K^+–stimulated ATPase in the membrane is also responsible for the maintenance of water balance in the cell. If K^+ is constantly pumped into the cell without the loss of cation from the cell. Water would enter the cell along with the K^+ ions as a result of which the cell swells. This event is prevented by the simultaneous pumping of Na^+ along with an equal amount of water. Thus the cell maintains internal K^+ and water balance.

8.2 SOME DEFINITIONS

Before a discussion of the osmotic and ionic regulations in animals is made it would be necessary to define certain technical terms often used in connection with osmoregulation. In osmoregulatory studies our interest is mostly centred on the concentrations of fluids, inside and outside the cells and even outside the organism. The concentrations of these fluids are expressed either in terms of the quantity per unit weight of solvent, i.e. water.

The quantity of solute can be measured either in terms of grams (g), milligrams *(*mg = 10^{-3}g), and micrograms (g = 10^{-6}g), or in terms of moles (M), i.e. gram-molecules, millimoles (mM = 10^{-3}M), or micromoles (M = 10^{-6}M).

In body fluids the quantity of a solute may usually be expressed in moles or millimoles because in this way the number of particles present in a litre of solution or in a Kg of water is perceived.

A molar solution is one in which the molecular weight of a substance in grams (mole) is dissolved and made up to one litre with water.

A one-molar solution contains the molecular weight of a substance in grams (mole) dissolved in 1,000 gm of water.

In fairly dilute solutions like water in nature and the body fluids of many animals there is little difference between concentrations expressed by molarity or molality. This lack of significant

difference is due to the absence of substances with high molecular weight in these fluids. In more concentrated solutions like body fluids and the blood of higher animals, and the protoplasm of most cells, substances of high molecular weights are present and therefore a significant difference exists between molal and molar concentrations.

For this reason the concentrations of these fluids are expressed in molalities. The concentration of body fluids is of the level of miliimoles and hence they are conveniently expressed in millimoles rather than in moles.

Osmotic Pressure

One of the very important physical properties of solutions in which we are mainly concerned at this juncture is the osmotic pressure. The osmotic pressure of a solution is related more directly to the molal than to the molar concentration. Besides, osmotic pressure, the other colligative properties of solutions are the depression of the vapour pressure, the elevation of the boiling point, and the depression of the freezing point. The colligative properties are directly proportional to one another. The direct measurement of osmotic pressure of solutions, whether artificial or obtained from organisms, is technically difficult and requires large volumes of fluid. For this reason, the osmotic pressure is calculated indirectly from one of the colligative properties, i.e. the depression of the freezing point. Though the determination of freezing point depression in biological fluids is beset with several difficulties, it is the most convenient method because it requires only minute quantities of fluid.

Pure water has a freezing point of 0°C. Freshwater and the seawater have solute particles and hence their freezing temperature falls below that of pure water. Such a freezing point depression is directly proportional to the molal concentration of the solution. Freezing point depressions are used very frequently to determine the concentrations, and the depression is usually denoted by the Greek capital Δ. The greater the depression in freezing point, the higher is the solute concentration. Seawater with its high concentration of solute particles has a freezing point depression of – 2.2°C (2.2Δ°C). The freshwater has far less solute particles and has freezing point depression between – 0.03 and – 0.05°C (0.03Δ and 0.5Δ°C). A one-molar solution freezes at –1.86°C (1.86Δ°C).

Tonacity and Osmoticity

A solution is said to be *isoosmotic* with another if it exerts the same osmotic pressure. Solutions of similar osmotic pressure have the same vapour pressures, freezing points and boiling points.

An *isotonic solution* is one which neither swells nor shrinks the cell that is not immersed in it. An isotonic solution is generally also isoosmotic, but this need not necessarily be so. A slight difference in the osmotic pressures of the medium and the cellular fluid does not bring about change in the volume of the cell because of the rigidity of the cell wall. This should not lead to the inference that the two solutions are isoosmotic. Since this solution did not bring about change in volume of the cell it is said to be an isotonic solution.

A solution which is more dilute than another is termed *hypoosmotic* and the one which is stronger is *hyperosmotic.* Animals which have isoosmotic and hypoosmotic body. Fluids exist in marine habitat, whereas those with hyperosmotic body fluids live in freshwaters. In Table 8.1 the freezing point depression of the body fluids of different groups of animals in relation to their habitats is given.

Table 8.1 The Relationship between the Osmotic Pressures of Body Fluids of Animals and Their Habitats

Sea (2.2Δ°C)	*Freshwater* (0.03Δ°C)	*Terrestrial environment*
INVERTEBRATES		
Blood roughly isoosmotic with medium	Most freshwater invertebrates: 0.4Δ to 0.8Δ Molluscs: 0.2Δ	Insects 0.6Δ to 0.8Δ
VERTEBRATES		
Teleosts: 0.8Δ to 1.1Δ	Teleosts: 0.5Δ to 0.7Δ	
0.8Δ — Eel (migratory) —	— 0.6Δ	
	Amphibia: 0.4Δ to 0.5Δ	
Turtles: 0.6Δ —	— Reptiles: about 0.5Δ	Birds: 0.6Δ
Whale: 0.7Δ —	—	Mammals: 0.5Δ to 0.6Δ

Adapted from *Principles of Animal Physiology* by D. W. Wood: with slight modifications

Marine invertebrates and hagfish are the true saltwater animals and descended from marine ancestors and their body fluids are isoosmotic with the seawater. They have the same freezing point depression as that of seawater. There are also other animals such as lampreys and teleost fishes, which invaded sea from freshwater. Their body fluids are hypoosmotic with the seawater. In hypoosmotic forms, the water from the body fluids ends to move into the hyperosmotic medium.

The freezing point depression of the body fluids of all freshwater animals given in Table 8.1 suggests that they are hyperosmotic in relation to the freshwater and therefore they tend to gain water. The freezing point depression of terrestrial animals is very near to that of freshwater animals suggesting their origin from freshwater animals. The problems that are posed by the terrestrial environment are quite different from those of aquatic environment. The land animals tend to lose water through evaporation.

8.3 AQUATIC AND TERRESTRIAL HABITATS

The animals which migrated to different environments during the course of evolution developed suitable physiological adaptations. What problems did these environments impose on other inhabitants and how effectively the species living there had made physiological adjustments to flourish there can best be understood if we first know the ideal relationship between the true marine forms and their environment, i.e. the sea. In fact, there always exists a constant interaction between the organisms and their environments due to certain physical forces and chemical factors acting upon them.

Seawater: The physical and chemical factors of these water such as mineral concentration, temperature, density, and acidity remain fairly constant except for limited variations during all seasons of the year. Even these slight variations tend to appear slowly giving adequate time for the animals living there to bring about necessary physiological adjustments.

Brackish Waters: Brackish waters are mixohaline having their salinity between 30 per cent and 0.5 per cent as per Venice system. Open seas such as Arctic ocean, have salinities as low as 30 per cent. The partially enclosed seas such as Baltic or Bay of Bengal have significantly low salinities, i.e. below 30 per cent. The lower limit of brackish water salinity is not so clearly defined and it is above 0.5 per cent, which is the salt content of freshwater lakes and rivers. The brackish water is the physiological bridge between sea and freshwaters. It has a gentle salinity gradient through which marine animals, in course of evolution, migrated to freshwaters. The salinity gradient at the estuaries provided an opportunity for the gradual adaptation of marine animals to lower salinity of brackish waters during their migration to freshwater.

Freshwater: The physical and chemical factors are very much variable. The concentration of minerals is much lower than that of the seawater and varies considerably. Even the ionic composition of freshwater varies from place to place and season to season. The climatic factors such as rain and temperature bring about quick changes in mineral concentration, density, acidity and temperature of the freshwater. The swift flowing waters of the rivers contain more oxygen than the sea. The oxygen content in stagnant waters is very less.

Terrestrial Environment

This environment is filled with air which is a mixture of gases. The temperature and humidity of this environment fluctuate very often. The terrestrial environment has a less dense atmosphere compared to the aquatic one. The radiation from the sun vaporizes the water from the seas and ponds, and the vapour remains in the air. The amount of water vapour in the air increases with temperature. Not only the water of the seas and ponds but also that present within the terrestrial organisms is subjected to evaporation. However, since the organisms are covered by nonaqueous material evaporation is very much reduced. The climate of the terrestrial environment varies from place to place and time to time. The temperature and humidity near the seashores are different from that of the desert. The climate at high altitudes is different from that of the sea level. Similarly the climate at the equator is not the same as the one prevailing at the polar regions.

8.4 PROBABLE MOVEMENTS OF ANIMALS BETWEEN DIFFERENT ENVIRONMENTS

We have mentioned earlier in this chapter that the animals moved from the sea to other habitats. Such a movement did not occur directly from sea to freshwater. The body fluids of the marine animals are isoosmotic to sea medium, but compared to freshwater, they are hyperosmotic. The true marine animals when transferred to freshwater would die because they have no mechanisms to pump out water that enters into their body by osmosis. Therefore any movement of marine animals into freshwater would have been accompanied by the gradual development of suitable physiological mechanisms to remove the influxed water. Such a movement is a step by step process and occurred through the brackish water that exists near the estuaries.

To suit the conditions in freshwater, true marine animals in course of evolution developed such drastic changes in their nature of life, that their return to sea seemed inconceivable. However, during

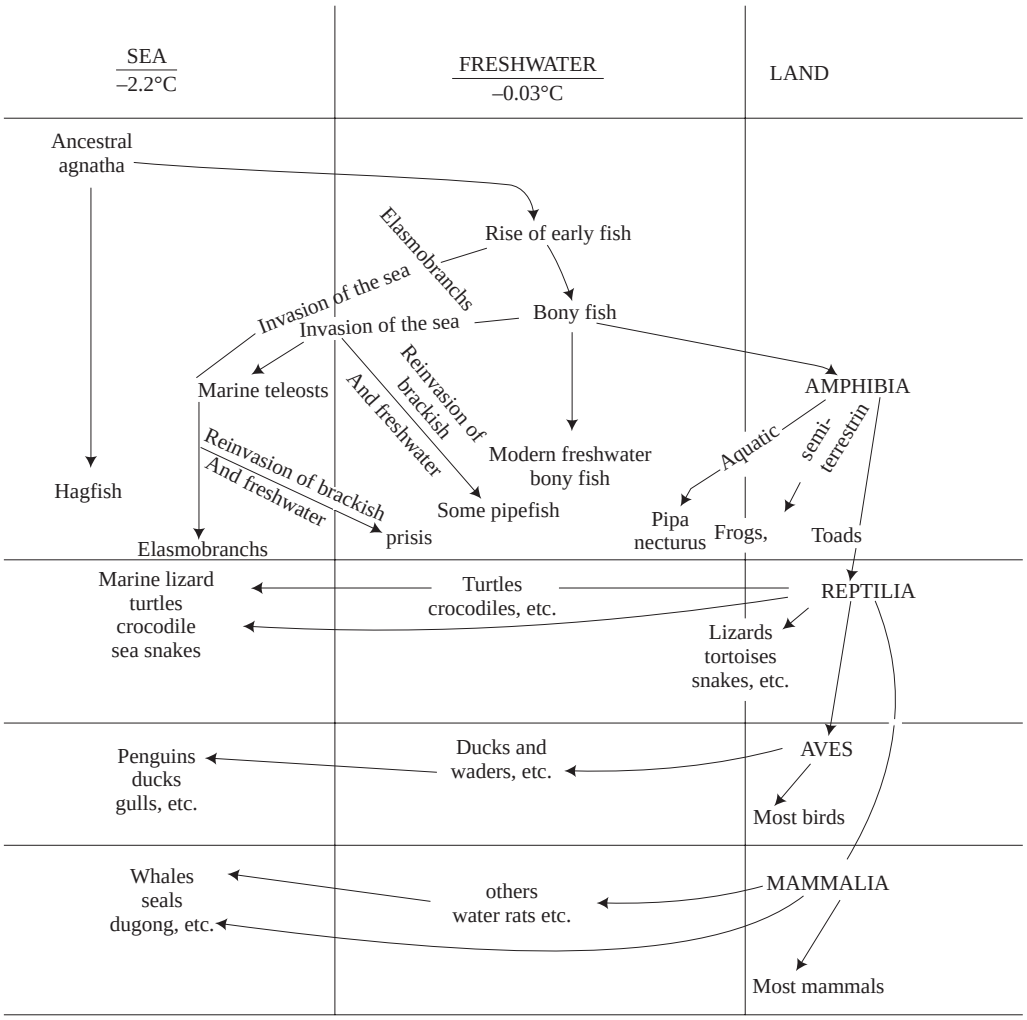


Fig 8.4 Schematic presentation of the probable movement of the vertebrate groups into different media during evolution.

evolution, many vertebrates moved from the freshwater back to the sea. A schematic presentation of probable movement of vertebrates into different media is given in Fig. 8.4.

In the early history of the vertebrates, the ancestral agnatha (jawless fishes) developed capacity to live in freshwater. From these agnatha the jawed fishes evolved in freshwater. Elasmobranchs and bony fishes evolved from the early jawed fishes. The forms giving rise to elasmobranchs migrated to the sea. Some of the marine elasmobranchs like *Pristis* reinvaded freshwaters. Three offshoots arose from the bony fishes. One offshoot consists of fishes which invaded the sea to form the ancestors of the modern marine teleosts. These marine teleosts also believed to have migrated back to freshwater leading to the evolution of a number of freshwater teleosts of today including some pipefishes.

Another offshoot of bony fishes continued in freshwater. The third offshoot invaded land resulting in the origin of amphibians. The higher vertebrates, i.e. amphibia, reptilia, aves and mammalia evolved from non-marine forms, and all but amphibian include some species which are associated with seawater. Marine turtles, sea snakes from among reptiles, penguins and gulls, etc., among birds, and whales, seals, dugong, etc., from mammals are the familiar examples that migrated to the sea.

The invertebrates too migrated from sea to freshwater and land. The terrestrial invertebrates, like vertebrates, evolved mostly and directly from freshwater ancestors. However, a few terrestrial invertebrates such as land crabs, wood-lice and some molluscs have invaded the land directly from the sea. Several groups of invertebrates directly colonized the freshwater at different times during evolution. The pulmonate molluscs and aquatic insects belong to the group that migrated from land to freshwater.

Such an invasion from one environment to another is possible only when the animals acquired adaptive mechanisms to maintain the composition of their body fluids at a level required by the cells to carry out their normal physiological activities. In the absence of such adaptive mechanisms, the cellular functions would succumb to the dominating physical forces of the new habitats. The cells would therefore shrink if transferred to a habitat containing highly concentrated medium, and would swell if kept in low concentration. In either case the cells cannot carry out their physiological activities and would reach a perfect equilibrium state with the medium, i.e. they become inanimate.

In order to perform normal physiological activities, the cells require following conditions:

(a) The cells should be surrounded by a water medium.

(b) The cells should excrete water as quickly as it enters.

(c) The cells should be able to adjust their internal concentration to match the variations that might occur in the external medium.

In higher invertebrates and vertebrates the cells are surrounded by body fluids. The concentration and composition of the body fluids bathing the cells are dependent on the forces imposed by the environment. The blood concentration of sea invertebrates is similar to that of seawater and hence sea poses no threat to the concentration of blood. The freshwater medium has ion concentration much less than that of seawater. Blood concentration of the freshwater animals is above that of freshwater and it must be maintained against the forces from the environment in order to provide ideal bathing medium for the cells. Because blood is in higher concentration than that of the environment, water tends to enter inside by osmosis. If this movement continues, blood gets diluted and consequently the cell volume and the composition of the cellular fluid change abnormally impairing the normal physiological functions of the cells and the organism as a whole. The air breathing aquatic forms have a body surface which is almost impermeable to water and ions. But in case of animals with aquatic respiration the body surface is permeable to oxygen as well as water and inorganic ions. Therefore, in these aquatic animals the water entering by osmosis is removed as urine, and the inorganic ions escaping into the water medium are actively transported back to the blood.

The brackish water is an environment that very often varies in concentration. Further, the concentration fluctuations are very high. Under such variations the true marine forms cannot survive. In order to live successfully in such an environment, the animals must be equipped with special adaptations. Unlike the true marine forms, the brackish water animals have developed tolerance to the

concentration fluctuations in their body fluids. These animals allow fluctuations in the concentration of their body fluids to suit the change of concentrations in brackish waters. Besides this tolerance, the animals, like all other organisms, have the capacity to increase or decrease the rate of active transport of specific electrolytes in relation to the concentration of the medium. At the same time a change in the blood concentration of these brackish water dwellers brings about a suitable alteration in the solute concentration of the cells. However, in the absence of such a modification the body cells would either shrink or swell jeopardizing their physiological activities.

Animals living in water have direct access to the salts and the dissolved gases in addition to water. The terrestrial environment lacks water and salts, but has only the gases. This poses problems. Firstly, these animals do not directly receive water and salts from this environment, and secondly, the heat and the dryness of the environment causes desiccation. Such problems were overcome to a limited extent by land animals through modifications of many of the mechanisms already acquired by their aquatic ancestors and by evolving a few new ones of their own.

The mechanisms falling under the first category are: (a) tightening up of water permeable coverings, (b) drinking of water, (c) extrarenal excretion of salts, (d) reduction of glomerular filtration, and (e) the ability of protoplasm to carry out cellular activities with altered amounts of electrolytes and water. Under the second category the new mechanisms developed by these animals are: (a) the ability to recover large amounts of water from urine (hypertonic urine), (b) the capacity to absorb significant amounts of water at the surfaces, (c) the ability to depend largely upon the metabolic water, and (d) the behavioural responses of avoiding desiccating microhabitats, and living not far from the water source.

8.5 ROLE OF BODY FLUIDS

There are two major types of body fluids—intracellular and extracelluar fluids. The single celled organisms have only the intracellular fluid and these organisms osmoregulate directly with the external medium in which they live.

Higher metazoa have extracellular body fluids with which the cells of the interior tissues perform osmotic and ionic regulations. These extracellular body fluids are similar in concentration to that of seawater. Therefore, the body fluids, bathing the tissue cells, can be described as being surrounded by, what we may call, an internal sea. The extracellular fluids in higher metazoa are separated into two compartments, viz. a *primary body cavity* or *haemocoele,* and a *secondary body cavity* or *coelome.* The fluids of the primary body cavity are blood and lymph and they are constantly circulated throughout the body. There are other specialized body fluids such as *cerebrospinal fluid* in the central nervous system, *occular fluids* in the eye, *endolymph* and *perilymph* in the ear, etc.

The coelome usually receives the genital and excretory products and forms gonoducts or excretory ducts. There exists a reciprocal relationship between the size of the coelome and that of haemocoele. In echinoderms, vertebrates, annelids and cephalopods the coelome forms large perivisceral spaces, whereas it is very much reduced to pericardium, gonoducts and excretory system in the arthropods, and in gastropod and lamellibranch molluscs it is large.

The blood drawn from the blood vessels is called *whole blood* and it contains blood cells surrounded by the fluid. Fluids like *plasma* and *serum* can be separated from the whole blood. Plasma is separated from the blood cell and other solid particles by centrifuging the whole blood. Serum can be obtained by allowing the whole blood to clot. The serum, however, has similar electrolyte composition to that of plasma.

Invertebrates lacking a circulatory system possess tissue fluids or lymph. This fluid forms minute lakes which surround the cells. It contains substances required by the cells for their nutrition and osmoregulation, and carries away the wastes from the cells. The lymph has blood cells which are phagocytic in nature. The lymph in higher vertebrates occurs in a special system of channels called lymphatics. These would commence blindly in the tissues and join the veins to empty the lymph into them. Thus, in these animals with closed circulatory system, the blood capillaries and the lymphatics have fluids distinctly separated from the tissue fluids. The tissue fluids are formed as a result of the filtration through the walls of capillaries into the tissue spaces. The lymph is derived from the tissue fluids and it enters the lymphatics either by diffusion or by flowing through the terminal openings of the lymphatics.

In animals with open circulatory system the blood, the lymph and the tissue fluids do not exist as separate entities. The fluid of the open circulatory system is called hemolymph because it moves through vascular channels and the tissue spaces.

8.6 ADAPTATION TO MARINE HABITAT

Analysis of the body fluids from a number of marine animals has resulted in the following generalizations:

(a) The body fluid concentrations are similar to that of seawater.

(b) They differ from seawater in relation to the ionic composition.

(c) Considerable variation exists in the ionic regulation by various groups of animals.

(d) In related animals the ionic regulation does show similarities. The body fluids of most marine invertebrates and the hagfishes are in osmotic equilibrium (isoosmotic) with seawater. In some groups of animals, i.e. coelenterates and echinoderms, the degree of regulation of ions is not great. Their individual ionic concentrations do not vary significantly from that of the seawater, which has the following standard concentrations of common ions (mM/kg).

	Sodium	Potassium	Calcium	Magnesium	Chloride	Sulphate
mM/kg	478.3	10.13	0.48	54.5	558.4	28.77

However, majority of the marine members of other invertebrate groups, viz, crustaceans, molluscs, etc., maintain high concentration of potassium and calcium; and often low concentrations of sulphate and magnesium, in their blood as compared to seawater. Thus they differ in ionic composition from that of seawater. From the above observations it is evident that while coelenterates and echinoderms maintain roughly similar ionic composition to that of seawater, the crustaceans and molluscs maintains an ionic composition that is different from that of seawater.

Regulatory Mechanisms

The maintenance of differences in ionic composition between the animal and its environment is vital and depends on the passive and active factors. The passive factors are: (1) the permeability of the body wall that is in contact with the medium; (2) the presence of protein in the body fluids which would produce Donnan effects, and bind some of the ions, particularly calcium in indiffusible complexes. The active factors in ionic regulation are: (i) the excretion of salts and water, and (2) the active absorption of salts and water.

Permeability Of the Body Wall: The body wall of marine invertebrates is permeable to salt and water and it is because of this that these animals are isoosmotic with seawater. The soft marine invertebrates when placed in a different salinity of seawater, become isoosmotic with this new medium. Acquisition of this new equilibrium is partly due to the osmotic movement of water and partly due to the movement of salt.

Distributions of Ions due to Protein in Body Fluids: Protein in the body fluids affects the distribution of ions between the body fluids and the outside medium in the following ways; that is, (1) by forming indiffusible complexes with some of the ions, particularly calcium; (2) by inducing a Donnan equilibrium affecting the distribution of all the ions.

Excretion and Ionic Regulation: Excretory organs play an important role in reducing the concentrations of some ions in the blood. In the invertebrates, the excretion of salts is performed by renal or antennary glands. The blood of decapods is poor in magnesium and sulphate, but rich in potassium and calcium. That is because the renal organs tend to remove magnesium and sulphate and conserve potassium and calcium. By this conservation process the concentration of potassium and calcium ions is maintained at a level not above that of seawater. But as stated above, decapods have potassium and calcium in higher concentration than in seawater, and such concentrations are due to active uptake of these ions from seawater.

Uptake of Salts and Water: The differential permeability, the effects of the presence of protein in the body fluids, and the excretion may account for some degree of ionic regulation. But higher concentrations of ions in body fluids than in the seawater are possible only if they are actively transported. In addition to active uptake of ions, marine invertebrates need water to compensate its loss in the production of urine. For this, the water is absorbed through the gut. Active uptake of ions at the body surface produces very localized concentration difference and this prompts water uptake at that region.

Hagfishes, of which *Myxine* is an example, are the only group of vertebrates which remained marine throughout their evolution (Fig. 8.1). The body fluids of these hagfishes are isoosmotic with seawater and the major part of the osmotic pressure is accounted for by the inorganic ions. As in many crustaceans, concentrations of magnesium and sulphate ions of the *Myxine* blood are less than that of the seawater. Potassium and calcium concentrations are also lower, yet the osmotic pressure of its blood is maintained by the strongly concentrated sodium. The osmotic regulation in these hagfishes is not very different from that of marine invertebrates.

In marine teleosts and lampreys, concentration of blood is a little higher than that of freshwater forms. Since these forms are hypoosmotic to the seawater, they tend to show dehydration as a result of

the osmotic loss of water through the epithelial lining of the mouth, pharynx and gills. Loss of water through the general body surface is greatly reduced due to the adaptive changes such as thickening of the dermis, presence of mucous glands on the body surface and the growth of scales. The osmotic water loss from these hypoosmotic forms can be replaced by drinking, and in fact this is one mechanism adapted by teleosts. In the gut, water is absorbed by osmosis, provided that a major part of ions is also absorbed from the seawater.

The fish may drink seawater as much as 50-200 ml/kg day. Drinking seawater creates a new problem. The salts go on accumulating and the NaCl in particular increases in the blood if not continuously removed. Therefore, salt should be eliminated without losing water. Salt elimination takes place through the gills and this was demonstrated by heart-gill preparation. The experiment has revealed that the salt composition of blood returning from the gills and that of the medium bathing the gills had undergone definite changes. This, as well as recent experiments using isotopes demonstrated that the gills of the teleost fish would secrete salts into the medium against the concentration gradient. The active extrusion of sodium and chloride seems to have been performed by all the cells in the gill epithelium.

In these marine teleosts considerable amount of salts is also lost by way of urine. The kidney of these fishes cannot produce urine that is hyperosmotic to the blood but it is slightly hypoosmotic to the blood. Increased amounts of salts cannot be eliminated by the kidneys, as this would require the formation of more urine. Marine teleosts cannot afford to lose more water, and therefore they reduce the production of urine to the bare minimum. The urine produced in these teleosts contains magnesium, calcium and sulphate that force their entry into the blood. Thus the kidney plays an important role in the maintenance of the ionic composition of the blood.

8.7 ADAPTATIONS TO BRACKISH WATER HABITAT

Brackish water is defined as dilute seawater with concentration anywhere between 1.5 per cent and 90 per cent of that of pure seawater. Marine fauna cannot survive in dilutions below the upper limit of brackish water concentrations. Brackish waters exist in restricted coastal regions, such as estuaries or salt marshes where the freshwater from the rivers mixes with the seawater. The landlocked seas such as Baltic, the Caspian and the Aral Sea are also brackish water.

Marine animals living in shallow water near the shores and particularly those near the estuaries are constantly subjected to changing concentrations. The marine animals that cannot tolerate concentration variations must bring about suitable modifications in their biochemistry, physiology and behaviour to live under these varying concentrations. The gentle salinity gradient existing from sea to freshwater provides an opportunity for the gradual adaptation of marine animals to lower salinities.

The fauna living between the salinity ranges of the brackish water are of three types: (a) marine animals with tolerance to low salinities existing at the upper end of brackish water salinity range; (b) freshwater animals with tolerance to moderate salinities existing at the lower end of the brackish water salinity range; and (c) true brackish water animals which are not found in either seawater or freshwater although they are able to survive in them.

Marine animals are isoosmotic at 100 per cent seawater and most of them cannot maintain their normal vigour at lower salinities of the brackish water and for this reason, the number of marine species declines with salinity *Carcinus maenas* (a shore crab) and *Mytilus edulis* (a mussel) are characteristic marine animals living in brackish water. A considerable number of these animals can penetrate down to about 15 per cent of seawater.

Palaeomonetes varians and *Nereis diversicolar* are the true brackish water animals which can tolerate seawater, but do not live there. Variations in the body fluid concentrations of a few species dwelling in brackish water are given in Figure 8.5 which indicates that they conform to the fairly wide fluctuations in the medium. The concentrations of body fluids are expressed in this diagram in terms of depression in freezing point. This change in the concentration of the body fluid of each animal under experimentation is measured for every change in the concentration of the medium and plotted. The animals respond to the change in the external medium by suitably altering the concentrations of their body fluids. A graph is obtained for each animal that indicates the ability or otherwise of the animal to regulate its salt and water content in media of changing concentrations. The graph so obtained is a straight line, which if extrapolated passes through zero. The blood concentration of the animal concerned varies directly with the change in that medium. In such a case the animal is

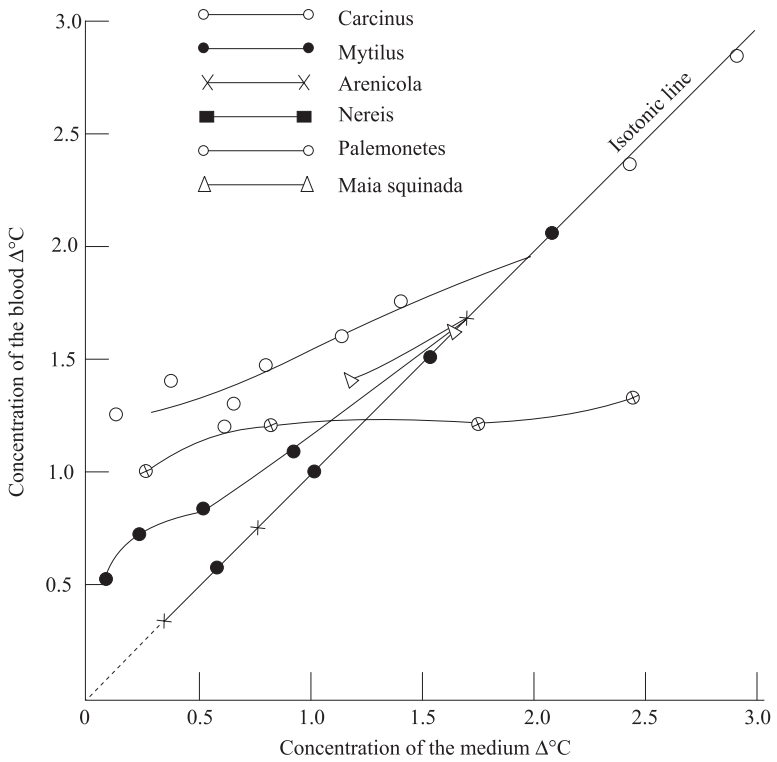


Fig 8.5 Brackish water animals have tolerance to the changing concentrations of the medium. The blood concentration of these animals varies directly with the changes in the concentration of the medium.

incapable of any degree of regulation. If the curve obtained deviates from the line representing isotonicity (i.e. line passing through zero), the animal concerned has the ability to regulate to changes in the concentration of external medium. The greater the degree of deviation of the curve, the greater is the animal's ability to regulate its blood concentration against the osmotic effects of the dilute medium.

The linear curves obtained in case of *Arenicola* (Annelida) and *Mytilus* (Lamellibranchiata) suggest that they are isoosmotic species with no ability to regulate their blood concentrations against changes in the concentrations of the medium. However, a fall in blood concentration in tune with that of external concentration poses regulatory problem between the blood and the body cells in general. In these animals the blood is hypoosmotic to that of cells. Hence the osmotic and ionic regulations are confined between the cells and the blood.

Brackish water animals such as *Nereis diversicolar* (Annelida). *Palaemonetes varians,* and *Carcinus* (Decapoda) have the ability to regulate their blood concentration in order to maintain it at hyperosmotic level despite wide changes in the brackish water concentration. In spite of these regulations, the blood concentration of these animals falls below that of true marine forms. In Fig. 8.5, the graphs for two crabs, *Carcinus* and *Maia* suggest the relation between the concentration variations of the medium and the body fluids. The former, which is commonly found in estuaries, can osmoregulate and the latter, the blood of which is isoosmotic with its marine medium, cannot osmoregulate if wide variations occur in medium concentration. The blood concentration of *Carcinus* corresponds to about 60 per cent seawater when it is living in 15 per cent seawater. In *Nereis* the ability to regulate is less than *Carcinus* but the animal is capable of living even in 5 per cent seawater because it can tolerate osmotic effects of low salinity by suitably reducing blood concentration to a level equivalent to 30 per cent seawater. The blood concentration of the brackish water prawn, viz. *Palaeomonetes* is rather different from those of marine animals living in brackish waters. *Palaeomonetes* maintains its blood concentration corresponding to 50-70 per cent seawater even when the concentrations of the medium range anywhere between 5 and 110 per cent seawater. It appears that it can also survive in concentrations below 5 per cent seawater, but below 0.5 per cent seawater it dies. The graphs of *Carcinus, Mytilus, Arenicola,* and to some extent that of *Nereis* indicate that these animals tend to act in line with the fall in the contraction of the medium by reducing their concentration to the extent possible, thereby reducing active work required to maintain ionic and osmotic regulation. This is the general feature for all salt water animals that penetrate into freshwater; and, in fact, all freshwater species do have blood concentrations lower than their nearest marine relatives.

Mechanisms of Osmotic Regulation

Most of the brackish water arthropods maintain their blood concentrations, greater than that of the medium and hence water tends to enter by osmosis and ions tend to escape by diffusion. To maintain hyperosmotic condition of blood, the water entered is returned to those medium and the ions escaped are actively transported back into the blood. The water is removed partly as urine and partly as extrarenal Water. The active uptake of solutes involves expenditure of energy, which should be minimized.

The *Carcinus* is much less permeable to both salts and water than strictly marine relatives like *Cancer* and *Hyas*. The urine produced by *Carcinus* is approximately isoosmotic to the blood but hyperosmotic to medium. This suggests that the salts are lost along with the elimination of water. Tracer studies have revealed that loss of salts also takes place along with the extrarenal excretion of water. The antennary organ of crustaceans is now thought to be primarily an organ for ionic regulation rather than osmotic regulation. The fluid produced by the antennary organ is high in magnesium as compared with the medium. This is then excreted through the excretory pore. However, the salt loss is compensated by the active transport of salts through the gills.

8.8 ADAPTATION TO FRESHWATER HABITAT

Freshwater animals have body fluids hyperosmotic to their medium. They have osmotic problems similar to those facing the brackish water animals, but more extreme in their nature. Freshwater animals meet these osmotic problems by improving upon some of the osmotic and ionic regulatory mechanisms of the type existing in brackish water animals. The permeability of the body surface of these animals is far less than that of brackish water animals. But freshwater molluscs have more permeable body surface, a feature favourable for the influx of water. However, the influx of water is greatly reduced because the blood concentration of these molluscs is far lower than that of other freshwater animals.

The entry of water inside would reduce blood concentration. In order to stabilize the blood concentration, either the water or the salts are to be removed from blood. Freshwater animals conserve salts by producing urine which is generally less concentrated than blood. In a few animals the urine may be isoosmotic to the blood. Even though salt loss through urine is minimized, it continues to be the main avenue through which significant amount of salts is lost. The formation of very dilute urine is the way by which the body gets rid of excess of water. In 24 hours the crayfish, *Astacus,* produces urine which may be as much as 4 per cent of the body weight. The production of urine is thus essential for the osmotic and ionic regulation and such a function is performed by the antennary glands. These glands have a coelomic sac, a labyrinth, a nephridial canal and a urinary bladder (Fig. 8.6). The urine is formed by filtration in the coelomic sac.

Studies on the antennary glands of freshwater *Astacus* and that of the marine *Homarus* revealed an important morphological difference. *Astacus* has a long nephridial canal with ample increase in surface area. The blood supply to antennary organ is greater in freshwater form than in the marine form. In the freshwater, form the number of the blood vessels and the lacunae supplying the antennary organ is much greater than in the marine form. The longer nephridial canal absorbs the chlorides and secretes water. The freshwater crayfish compensates the amount of water and salt loss, by a continuous uptake of the substances through the gills even if their concentration in the external medium is very low. It is believed that apart of the loss of water and salts is also compensated easily through the food.

We have already learnt that the freshwater animals have blood concentrations less than brackish water forms. The small difference in the gradient between blood and freshwater medium requires minimum work to be done for active transport of substances and hence expends less energy.

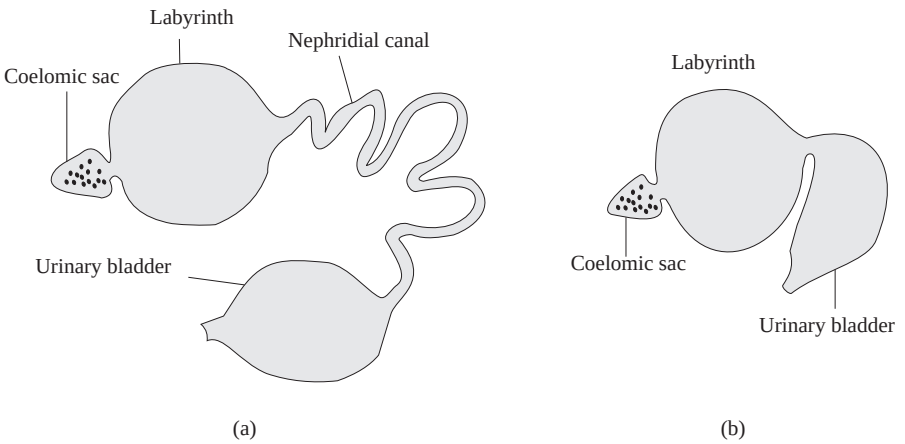


Fig 8.6 The antennary glands of (a) freshwater crayfish, *Astacus* and (b) marine crayfish, *Homarus.*

In case of aquatic insect larvae, the osmotic and ionic regulations are performed by the gut. To facilitate this certain areas of the gut are specialized for this purpose. In *Aedes aegypti* the anal-papillae and the rectal wall are responsible for the conservation of the salts.

Freshwater Teleost and Lampreys: The freshwater fishes and the freshwater invertebrates have similar osmotic conditions and regulatory mechanisms. Both Lampreys and teleosts have hyperosmotic blood. The concentration of blood in the freshwater species is maintained at a fairly constant level. Due to hyperosmotic nature of these forms, water tends to enter their body via general body surface, gill and mouth epithelia. The permeability of skin in lampreys is greater than that of the teleosts. The lampreys have skin which is naked whereas the skin of teleosts is covered by scales. The scales greatly reduce the rate of water diffusion. Lampreys living in freshwater take up water through the skin but in teleosts, the water uptake is mainly through the gills. This way in 24 hours the teleosts may take water equivalent to about 30 per cent of their body weight and this is secreted copiously in the form of urine. Most of the salts in the urine is reabsorbed and concentration of urine is about 0.04°C which is hypoosmotic to blood. Though the kidney conserves salts by reabsorbing them, still the amount lost through urine is considerable. The salt uptake in the food is far less than the salt loss from the body. The salt loss is, however, compensated by the active uptake of them through the gills. There is also some evidence suggesting the uptake of ions from the freshwater medium directly by the body surface. The freshwater fishes do not normally drink their medium to compensate water and salt loss.

8.9 ADAPTATIONS TO TERRESTRIAL HABITAT

Various animal groups have invaded land at different times. Insects, terrestrial arachnids and tetrapods, for example, are found first in the Devonian period. At a later time animal groups such as the operculate gastropods, the opisthobranchiates, the isopods and crabs have colonized the land. Similarly a few other groups such as earthworms, onychophorans, triclads, etc., also took to terrestrial

life though they live in damp habitats. Many of the animals that migrated to terrestrial environment continue to live in damp areas or nearer to water source. However, there are other animals which later migrated to semiarid and arid environments. These environments are hazardous, as they could desiccate the animal and cause quick death. The development of air breathing system has helped in procuring oxygen directly from the air. But breathing dry air has increased the danger of desiccation. The water, which is essential to maintain cell volume and to act as a medium in which cellular activities proceed uninterruptedly, gets evaporated through the breathing organs as well as through the general body surface. Besides these, water is also lost by way of urine and faeces. However, during the transition from aquatic to the terrestrial life the animals have evolved suitable adaptations to withstand the furies of the terrestrial environment. The animals have brought about morphological, physiological, and behavioural adaptations to continue their vital activities on land. All these adaptations help in reducing the loss of water and salts. The skin of mammals is less keratinized than that of reptiles. Yet the loss of water through the skin of mammals is as low as in the reptiles. Despite reduced water loss through the skin, the overall loss from the mammals such as rats is much higher as compared to reptiles of comparable size. This increase in loss is mainly due to the removal of more water in the respired air. Two factors influence this loss and they are high metabolic rate and higher body temperature.

1. High metabolic rate raises water loss. The metabolic rate is higher in homeotherms than in reptiles. Such a high metabolic rate necessitates rapid rate of breathing and hence increased loss of water. The metabolic rate per unit mass is related to the body size of the animals. In smaller animals the metabolic rate per unit mass is higher than in larger animals. Accordingly the water loss from small animals such as rates is higher than man.

2. The mammals loose water by way of respiration even if they inhale air saturated at the ambient temperature. This is possible if the body temperature of the mammals is higher than the ambient temperature. The exhaled aim of such mammals would have a temperature higher than ambient temperature. At higher temperature, the air holds more moisture. Mammals are liable to suffer from heat stroke if they are exposed to higher temperatures. When the body temperature of these animals is increased 4-5^{o}C above normal, the body sweats which then evaporates making the body cool. Though cooling is essential to bring down temperature, water loss in this process brings about an undesirable viscosity of the blood. Increased viscosity of blood reduces the speed of blood circulation. Due to reduced speed the blood cannot remove all the heat from the body. Therefore, the body heat increased and when the animal loses about 10 per cent of its body water it dies. The hot and dry climate of deserts is not ideal for comfortable living and would cause heat stroke. But certain animals such as kangaroo rat, camel and donkey have colonized deserts by developing suitable physiological and behavioural adaptations to desert climate.

The kangaroo rat (*Dipodomys*), which is well adapted to desert life, has developed novel mechanisms to conserve water. The water loss is considerably reduced due to the following factors:

(a) Reduction of evaporative loss through skin and lungs.

(b) Production of concentrated urine.

(c) Production of dry faeces.

Absence of sweat glands is another factor in reducing the loss of water through the skin. Because of the absence of sweat, glands, the cooling mechanism is inefficient but the animal developed tolerance to temperatures upto 41°C, i.e. 6°C above its normal temperature. However, higher day temperatures are avoided by the kangaroo rat by deve1oping behavioural habits such as living in relatively cool and humid burrows during the day, and foraging during nights (nocturnal) when the ambient temperature falls down to a comfortable level.

Much of the evaporative loss of water in kangaroo rat is by way of respired air. In dry air, the evaporative loss in kangaroo rat is estimated to be more than 70 per cent of the total loss. But in the relative humidity of 80 per cent existing in the burrows, the water lost is only 40 per cent of the total water loss. At 10 per cent humidity, the water loss by way of respiration is compensated by the metabolic water produced. At all relative humidities above 10 per cent the animal maintains the water balance by losing lesser amount of water; and excessive water that is taken in through the hygroscopic food as well as the excess of metabolic water is excreted in the form of more urine.

While a greater part of conservation is responsible for the existence of humidities in the burrows, the success in reducing evaporative loss through lung is also partly due to the cooling effect of the nose. From the nose a small quantity of water evaporates and as a result the nasal passages are cooled to a temperature of only 24°C. Moisture from the expired air condenses on the mucosa of nasal passages. Thus, the water that is escaping is recovered.

The mammals including kangaroo rat produce hyperosmotic urine. The kangaroo rat's urine is a 20 per sent solution while that of man is only 8 per cent solution. Therefore the kangaroo rat can excrete more nitrogen without much loss of water.

In kangaroo rat, the water from the faeces is absorbed at the posterior end of the gut and the remaining is voided as dry faeces. The amount of water that is lost by this way is very less and accounts for one third the dry weight of faeces.

As a result of such meticulous conservation of water by kangaroo rat, the water content of the blood is maintained at a constant level.

Camel can go without water for long periods. The old tales that they store water in the reservoirs in stomach or hump are no longer true. Camel loses water by evaporation through the skin and the lungs. Water is also lost through the faeces and urine, from digestive tract and the kidney respectively. However, it has evolved mechanisms to minimize water loss through these organs. Unlike the desert rat, camel is large and hence cannot burrow and is exposed to hot and dry climate of the deserts. For this reason, the methods of conservation in camel are different from those of kangaroo rats which also live in deserts.

During winter months camel meets its water needs by browsing on bushes and succulent plants. Therefore it goes without drinking water for periods longer than two months and still shows no signs of dehydration. However a camel fed on completely dry fodder could go without drinking for several weeks, but it loses water steadily through lungs and skin and through the formation of urine and faeces and consequently the animal loses weight that is equal to the weight of water lost. When accessible to water, the camel drinks enough of it within minutes and regains the weight lost. In 10 minutes the camel gains its normal appearance. One might wonder how the camel fed on dry fodder can go without water for several weeks. The first explanation is that camel obtains metabolic water by

the oxidation of foodstuffs and the reserve fat stores. The metabolic water produced by the oxidation of foodstuffs is less than even the water lost through the expired air. Foodstuffs contribute different amounts of water and the amount of water produced by way of oxidation depends upon their hydrogen content. One gram of fat yields 1.07 gm of water; 1 gm of carbohydrate, 0.60 gm of water; and 1 gm of protein, 0.3 gm of water.

Camel obtains water also from the oxidation of fat stored in the hump. The camel with 100 pounds of fat can obtain 110 pounds of water, i.e. more than 13 gallons. However, water obtained by oxidation is less than the amount of water the camel loses by evaporation from the lungs.

The second explanation is that these animals have developed water conserving mechanisms. These, however, differ from those of kangaroo rat. In spite of these water conserving mechanisms, camel still suffers water loss. But it can go without water for prolonged periods.

During summer in desert the temperature soars to 65°C or more. Such an ambient temperature tends to raise the body temperature of higher animals. Since they cannot tolerate change in their body temperature, they sweat in order to cool their body. In this process, they lose the precious water which is scarce in the desert. They then die of heat stroke. But camel has remarkable powers to withstand dehydration and rise in body temperature. Though it tolerates dehydration better than man, the process of dehydration is very much slow. Such a slow process is due to (a) the excretion of small quantity of urine which may be as low as a quart a day during summer; and (b) the reduction of sweating.

Further, the water loss in camel does not bring about a significant reduction in its blood volume. If 50 litres of water is lost from camel, its blood volume decreases only by 1 litre.

Camel's kidney is highly efficient. When it is fed on dry food, the urine flow is considerably reduced; it may be around 500 ml/day. If the dry food of camel is rich in proteins one might expect a high urea content in its urine as in desert rat. But in camels the urea output is greatly reduced. The exact mechanism of the kidney in this reduction process is not known. Further the maximum concentration of urea in camel is not known with certainty but it appears that it is a little over half that of kangaroo rat.

In camels water loss by sweating is greatly reduced in spite of the prevailing high temperatures in deserts. They have the ability to raise their body temperature from a minimum of 52°C to a maximum of 58°C, a difference of 6°C, without sweating. During the hot day, camel absorbs heat from the rising temperature of the environment. But only when its body temperature rises above 58°C does the sweating initiate to cool from further rise. The body, however, attains this maximum temperature only after much of the hot day is over. Thus the need to cool the body by sweating is restricted for a short period and this ability of camel conserves significant amount of precious water.

8.10 RETURN TO THE SEA

The terrestrial animals which return to sea or depend on sea for their food, receive high doses of salts either by drinking seawater or by eating isoosmotic sea-animals. The terrestrial air-breathing animals though live on marine forms cannot tolerate high concentrations of salts. The salt concentration in their body fluids should be limited to about one per cent, i.e. less than one third of the salt

concentration of seawater and this is possible if the excess salt is removed by some means. The reptiles, birds and mammals that returned to marine life did possess efficient way of removing salts. If man drinks salt water, it would cause diarrhoea and consequently the tissues suffer dehydration. The marine reptiles and sea birds do drink seawater; their kidneys are less efficient than those of man and yet they suffer no ill effects. This is because the sea birds have a special organ, viz. the salt gland which is much more efficient in eliminating salts than the kidney. It appears that the marine reptiles also possess such a salt eliminating gland.

When sea gull is given seawater equal to a tenth of its body weight, nearly all the salt content of it has been excreted within three hours. The salt glands are responsible for the removal of 90 per cent of the salt with less loss of water. The remaining 10 percent of the salts is removed by kidney with greater amount of water loss. Obviously, this would mean that the nasal fluid is more concentrated and the urine less concentrated.

The nasal fluid is five times as salty as the bird's blood and other body fluids. In order to understand how the salt gland produced such a concentrated solution, it is necessary to understand the anatomy of the gland. A cross section of the gland reveals that it consists of a number of parallel cylindrical lobes (Fig. 8.7), each lobe consisting of several thousand branching tubules which radiate from a central duct. Thus each lobe of the gland appears like that of a bottle brush. The tubules secrete salty fluid. Each lobe is supplied with a network of capillaries which run parallel to the length of the tubules (Fig. 8.8). The blood flow in these capillaries is opposite to the direction of the flow of

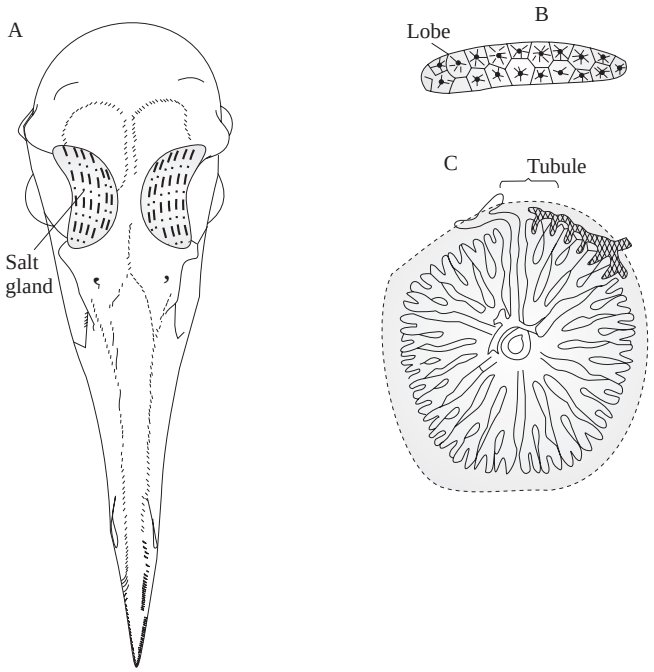


Fig 8.7 Salt gland (A) showing location of the salt gland, (B) cross section of salt gland showing lobes, (C) cross section of the lobe showing tubules.

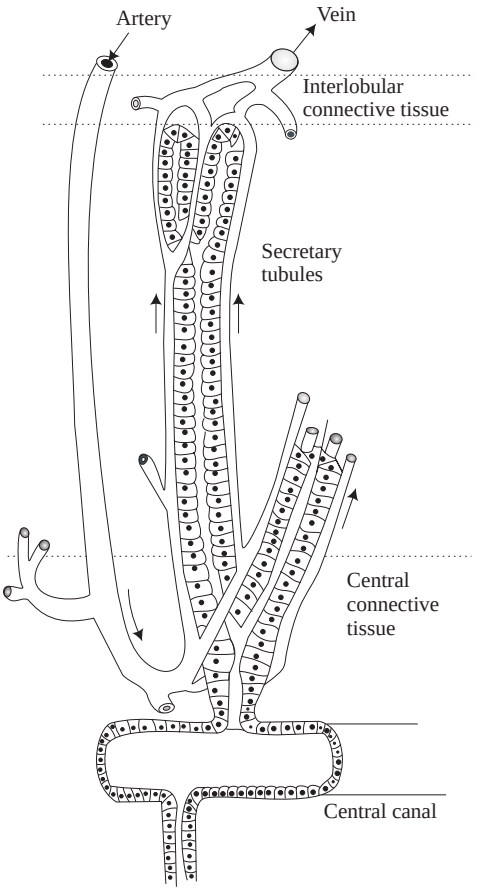


Fig 8.8 Enlargement of a single tubule surrounded by capillaries. (Redrawn from Sci. Amer., 1959, vol. 200, No. 1, pp. 109-116).

salt solution in the tubules. Such a counter current flow amplifies the transfer of salt from the blood in the capillaries to the fluid in the tubules. A similar arrangement existing in mammalian kidneys is responsible for their efficiency in producing concentrated urine. The reptilian kidney has no such counter current system, but in the birds it is only slightly developed. Besides counter current flow, there is another factor which is involved in salt concentration and it is an increase in the area of secretion of the tubule by forming folds.

The cells of the tubule have some physiological mechanisms to pump sodium and chloride ions from the dilute salt solution of the blood to the more concentrated solution in the lumen. The mitochondria of the cells of the tubule appear to have involved in the transport of sodium and chloride ions.

Despite certain similarities, the salt gland differs from mammalian kidney in some important features. They are: (a) The salt gland is a simpler organ. (b) Compositionwise the secretions of the salt

gland contain only sodium, chloride and water except for traces of potassium. This is the sole function of the salt gland. In contrast to this the kidney performs a variety of conservative and eliminative tasks. It produces a complex fluid which varies in composition based on the physiological activities. Some of the substances from this complex fluid are reabsorbed to conserve them at that particular time. (c) The salt gland's ability to remove salt is greater and in one minute, it can produce salt solution equivalent to its own weight. The human kidney can produce only about twentieth of its weight of urine in one minute. (d) While the salt gland functions intermittently whenever needed to eliminate salt, the kidney functions continuously and secretes at varying rates.

The functioning of salt gland depends on salt concentration in blood. When the concentration of salt in bloodstream increases, some centre perhaps in the brain responds and sends impulses through central nervous system.

Salt glands are also present in marine reptiles. In marine turtles, the glands are positioned behind the eyeball and pour out secretions through a duct that opens into the eye. When these turtles come to the shore, the secretion can be clearly seen pouring out from the eye. This led people to believe that the marine turtles weep. No one until recently knew that the marine turtles "weep" to eliminate salt from their body. In composition, the tears of sea turtles are very much like the secretions from the salt glands of marine birds. Anatomical studies of marine crocodiles and sea snakes have revealed the presence of large glands in their heads. The function of these glands seems to be similar to that of the salt gland.

Marine mammals such as seals satisfy their water needs with the fluids of the fish on which they feed. Since these fluids also contain salts, the seals eliminate them through the kidneys. The whales feeding on plankton, squid, etc., are required to eliminate large quantities of excess salt. For this, the whales seem to possess more powerful kidneys than those present in human beings.

CHAPTER

9

Membrane Physiology

Biological membranes, also called plasma membranes, surround all individual cells and form closed compartments. The membrane is asymmetric, highly viscous and dynamic in nature to provide selective permeability owing to the presence of ion-channels and ion pumps. In plants, it lies internal to the cell wall and encloses the ctytoplasm of the cell. Besides transport of cellular materials, ions, water and macromolecules, the membranes also function in transmembrane signaling and cell-to-cell interactions. Deficiencies in cell membrane result in a variety of diseases, which will be dealt within chapter 23 on physiological disorders.

9.1 CHEMICAL COMPOSITION OF MEMBRANES

Membranes are found to consists of mainly lipids, proteins, some carbohydrates, and water. The protein to lipid ratio varies greatly. For example, the inner mitochondrial membrane has 76% protein, while the myelin membrane of neurons possess only 18% protein. The difference in protein-lipid ratio accounts for the specific functions the organelles have to perform.

Nature of Membrane Lipids

Lipids in the membrane are a complex mixture of cholesterol and fatty acids, mainly in the form of glycerides and phospholipids. Cholesterol is widely distributed in the membrane, though phospholipids predominate. Because of its high phospholipid content, the myelin of the nerve cells can electrically insulate the cell from its enviornment. The lipid composition varies among different membranes.

Five types of Phospholipids Found in Membranes

Five important types of phospholipids include phosphatidic acid, lecithin, phosphatidyl inositol, phosphatidyl serine, and phosphatidyl ethanolamine (Fig. 9.1). Though all membranes contain a

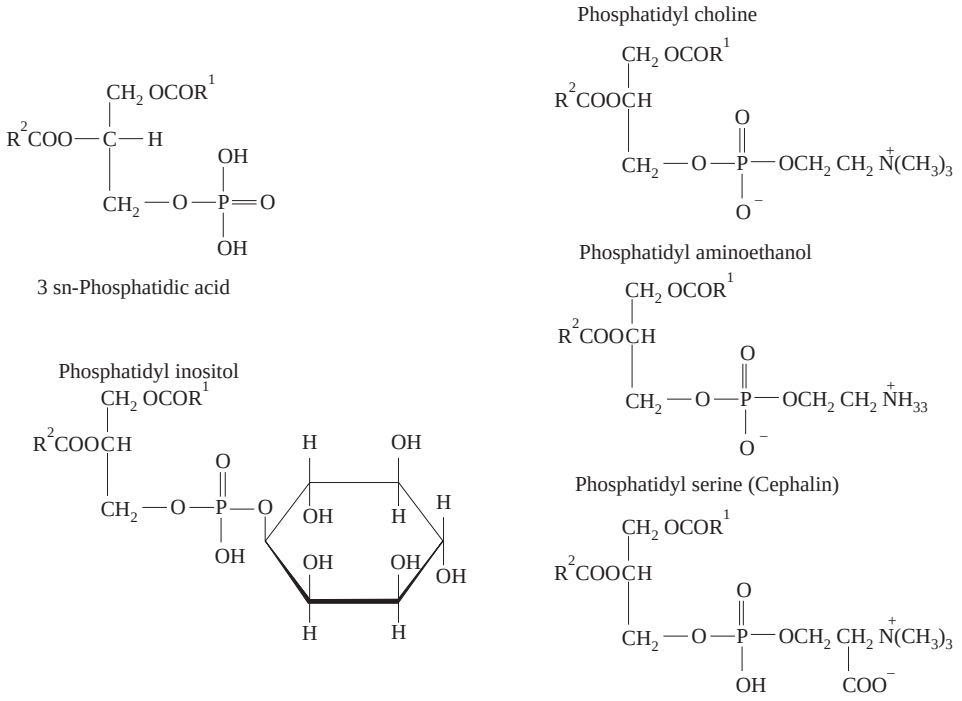


Fig. 9.1 Amphipathic nature of phospholipids: showing hydrophilic and hydrophobic regions.

substantial proportion of phospholipids, predominant being phosphoglycerides having a glycerol backbone. All membrane phospholipids are *amphipathic*, having both hydrophilic and hydrophobic portions (Fig. 9.2). Other lipids include cholesterol, glycolipids, phosphatidylcholine and sphingomyelin. Sphingomyelin lacks a glycerol backbone, but quite common in plasma membranes. Instead of a glycerol backbone it contains sphingosine, an amino alcohol with a long unsaturated hydrocarbon chain. A fatty acyl side chain is linked to the amino group of sphingosine by an amide bond to a ceramide. The terminal hydroxyl group of sphingosine is esterified to phosphocholine, making the hydrophobic head of sphingomyelin similar to that of phosphatidylcholine.

A major difference among phospholipids concerns the charge carried by the polar head groups at neutral pH. Some phosphoglycerides (phosphatidylcholine, phosphatidyl ethanolamine) have no net electric charge; others (cardiolipin, phosphatidylserine) have a net negative charge. A few rare lipids carry a net positive charge at neutral pH. Nonetheless, the polar head groups in all phospholipids can pack together into the characteristic bilayer structure. Sphingomyelin and glycolipids are similar in shape and can form bilayer with them.

Cholesterol an Important Constituent of Membrane Lipids

Cholesterol an its derivatives constitute another important class of membrane lipids, the steroids. The basic structure of the steroids is the four-ring hydrocarbon (Fig. 9.3). Cholesterol is the major

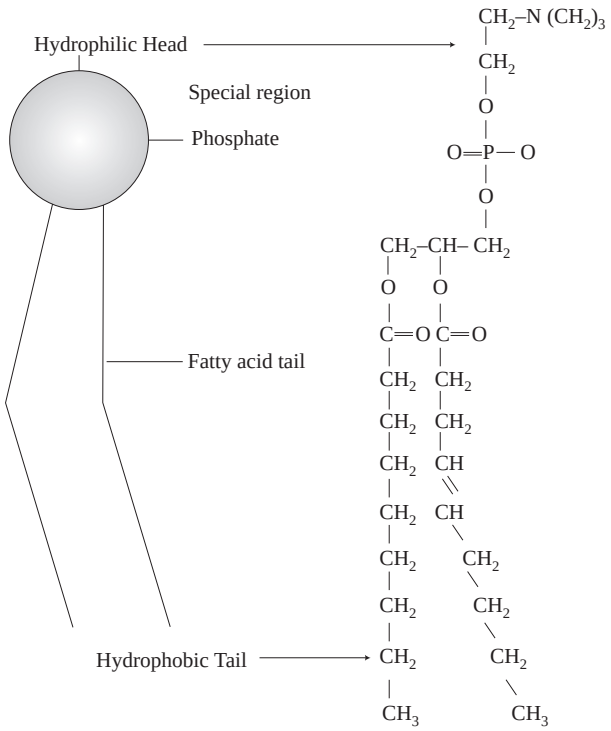


Fig. 9.2 Structure of phospholipids present in the plasma membrans.

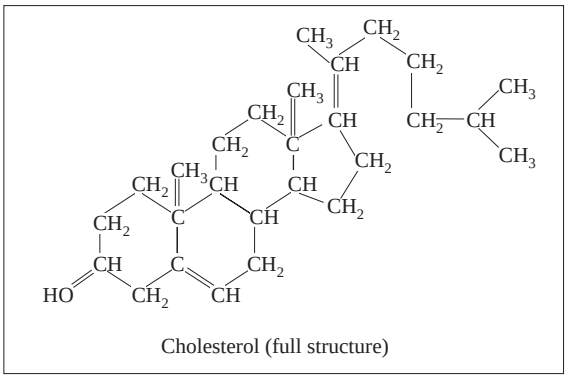


Fig. 9.3 Structure of cholesterol whose derivatives are present in the membrane.

constituent (a steroid) of animal tissues; other steroids play important roles in plants. Although cholesterol is entirely a hydrocarbon in composition, it is amphipathic because it contains a hydroxyl group that interacts with water. Cholesterol is especially abundant in plasma membrane of mammalian cells, but found to absent from most prokaryotic cells.

Proteins are also found in Membranes

Proteins form the bulk of cell membrane, existing as *integral* or *peripheral* proteins.

Integral Membrane Proteins

Many of the proteins associated with the plasma membrane are tightly bound to it, while some are attached to lipids in the bilayer. In others-the *transmembrane proteins*, the polypeptide chain actually traverses the lipid bilayer. All G-protein coupled receptors (e.g. receptors of peptide hormones, and odours), each span the plasma membrane seven times (Fig. 9.4). In all these cases, the protein with the lipid bilayer consists primarily of hydrophobic amino acids. These are usually arranged in an alpha helix so that the polar –C = O and –NH groups at the peptide bonds can interact with each other rather than with their hydrophobic surroundings. Those portions of the polypeptide that project out from the bilayer tend to have a high percentage of hydrophilic amino acids. Furthermore, those that project into the aqueous surroundings of the cell are usually glycoproteins, with many hydrophilic sugar residues attached to the part of the polypeptide exposed at the surface of the cell. Some transmembrane proteins that span the bilayer several times form a hydrophilic channel through which certain ions and molecules can enter or leave the cell (Fig. 9.5).

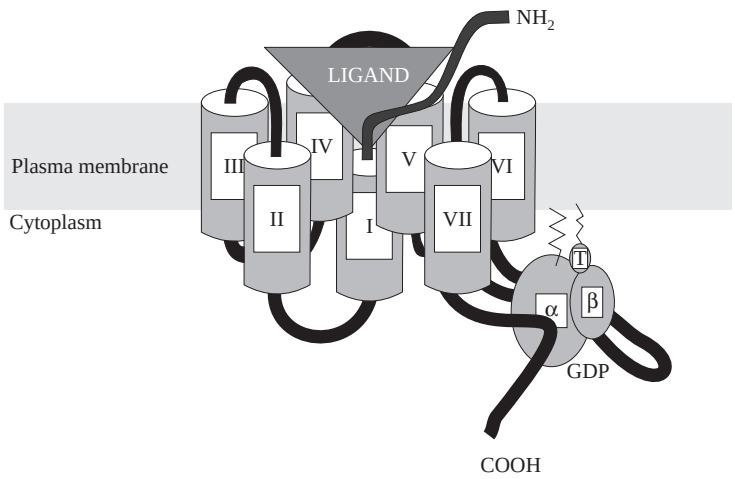


Fig. 9.4 A G protein-coupled receptor. It consists of seven transmembrane proteins with N-terminal projecting to the exterior.

Peripheral Membrane Proteins

Peripheral membrane proteins are those that adhere only loosely to the membrane with which they are associated. These molecules do not span the lipid bilayer core of the membrane, but attach indirectly, typically by binding to integral membrane proteins. Therefore, the so called regulatory protein subunits of many ion channels and transmembrane receptors, for example, may be defined as peripheral membrane proteins. These proteins, in contrast to integral membrane proteins, tend to

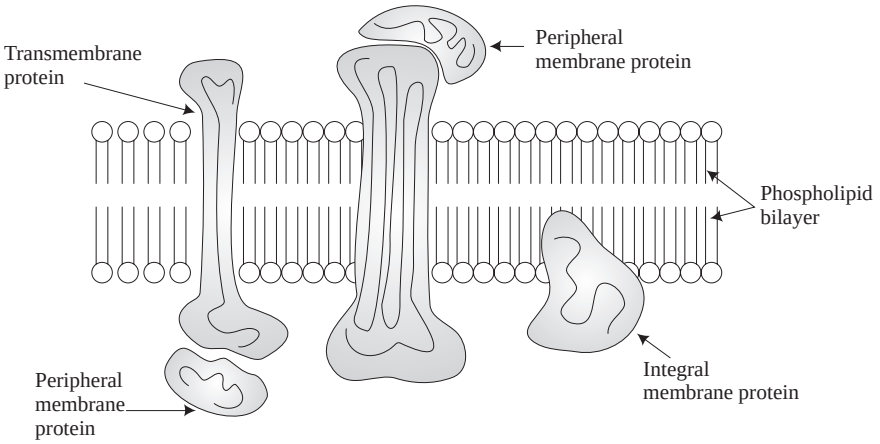


Fig. 9.5 Topographical arrangement of proteins in the plasma membrane.

collect in the water-soluble fraction during protein purification. These are more loosely associated with the membrane. They are usually attached noncovalently to the protruding portions of integral membrane proteins. Membrane proteins are often restricted in their movements (Fig. 9.5).

Carbohydrates are Also Present in the Membrane

Carbohydrates in the plasma membrane are present in the form of covalently-linked molecules with proteins and lipids. There two types: *glycoproteins* and *glycolipids*. The common sugars associated with the proteins are D-glaaucose, D-gactose, D-mannose etc., which are oligosaccharide complexes. Besides simple sugars, sugar derivatives such as N-acetyl-D-glucosamine and N-acetyl-neuraminic acid (sialic acid) are also present. Almost all proteins present on the outer surface of the membrane have not got carbohydrate component, as will be seen from the Table 9.1.

Table 9.1 Variation in Protein, Lipid and Carbohydrate Composition of Some Membranes

Membrane	*Protein %*	*Lipid %*	*Carbohydrate %*
Myelin	18	79	3
Human erythrocyte	49	43	8
Mitochondrial inner membrane	79	24	0
Amoeba plasma membrane	54	42	4

9.2 MEMBRANE ARCHITECTURE

Membrane has a dynamic structure. Although there are large variations in the composition of membrane, the basic structural unit of virtually all biological membranes is the *phospholipid bilayer*. This bilayer is a sheet-like structure composed of two layers of phospholipid molecules whose polar head groups face the surrounding water and their fatty acid chains form a continuous hydrophobic interior and 3 nm thick.

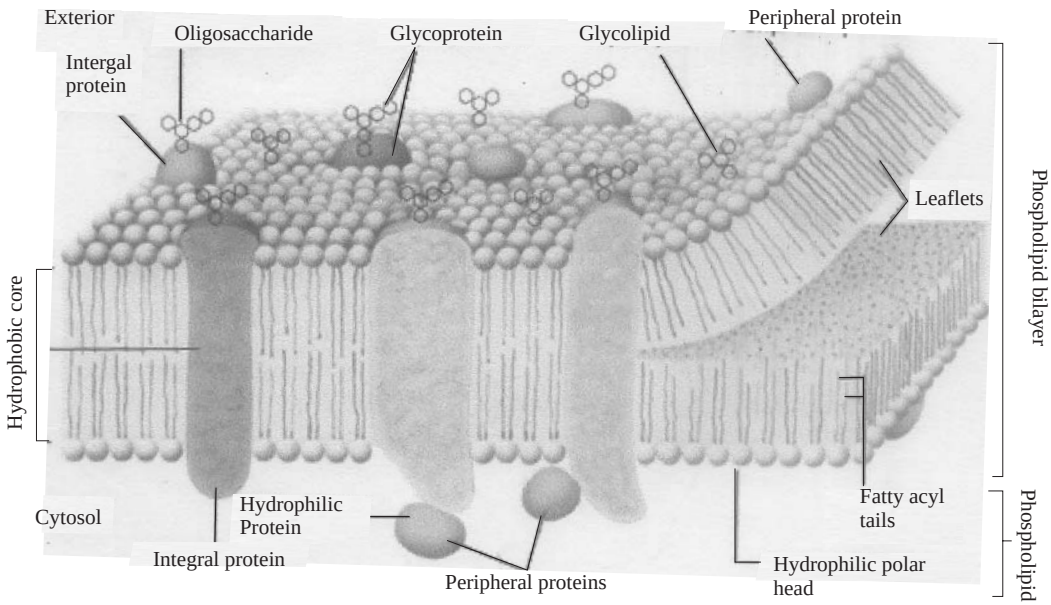


Fig. 9.6 Bileaflet medel of the membrane showing arrangement of proteins and their sugar components.

Each phospholipid layer in this lamellar structure is called a *leaflet* (Fig. 9.6). The major driving force for the formation of phospholipid bilayers is the hydrophobic interaction between the fatty acyl chains of glycolipid and phospholipid molecules. Van der waals interactions among the hydrocarbon chains favour close packing of these hydrophobic tails. Hydrogen bonding and electrostatic interactions between the polar head groups and water molecules also stabilize the bilayer. Micelles are generally not formed by phospholipids in aqueous solutions, since the fatty acyl chains in sphingomyelins, glycolipids, and all phospholgycerides are too large to fit into the interior of a micelle (Fig. 9.7).

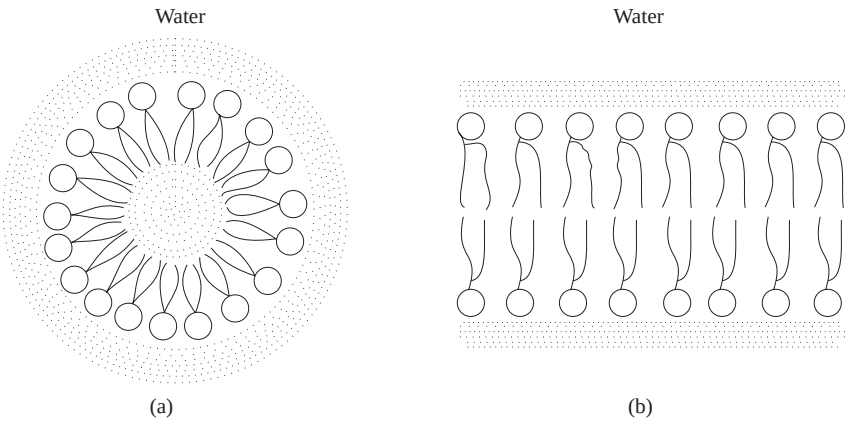


Fig. 9.7 Amphipathic phospholipid molecules in the aqueous phase: (a) formation of micelles in which polar heads are directed towards water; (b) formation of bilipid layer.

Fluid-mosaic Model of Plasma Membrane

The *fluid-mosaic model* of the plasma membrane is the universally accepted model, which was proposed by Singer and Nicolson in 1972. The model suggests that the lipids form a viscous, two-dimesional fluid matrix into which proteins are inserted and integrated more or less deeply. The peripheral proteins are superficially located and many of them function as enzymes, while the integral proteins are associated with lipids and penetrate into the interior of the membrane along with fatty acid side chains (9.8). They are tightly bound to the lipids and constitute functional proteins that are not easily separable. All membrane-bound enzymes and carriers are included in this category. Peripheral proteins have a loose affinity and can be easily displaced. Such a membrane is more dynamic and explains the intricate semi-permeable phenomenon.

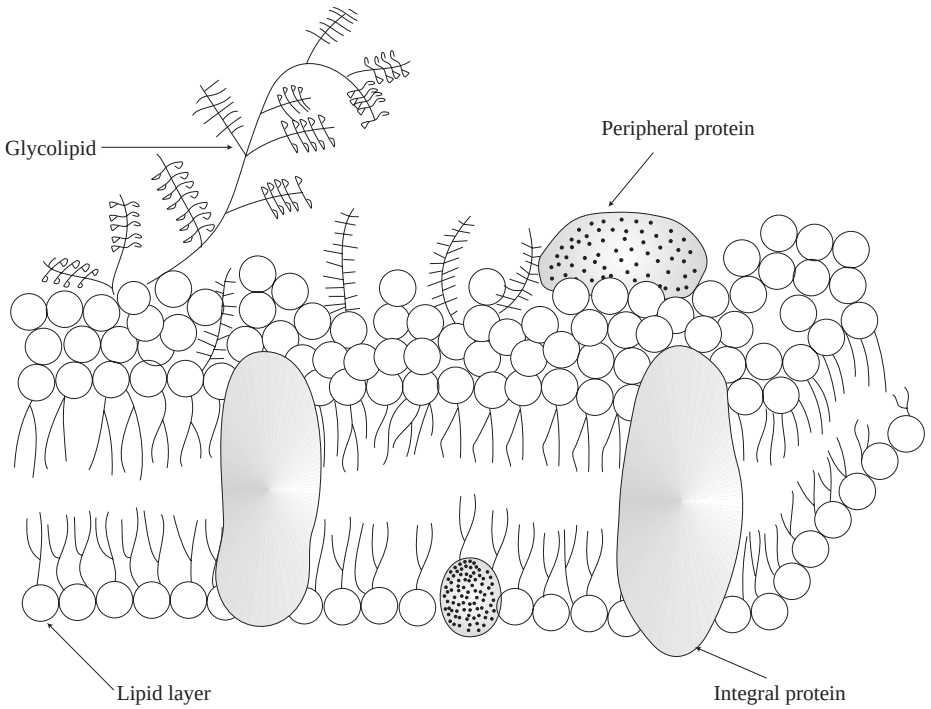


Fig. 9.8 Fluid-mosaic model of Singer.

Orientation of Proteins in the Membrane

Orientation of the proteins is important in the functioning of the membranes. The N-terminus is always at the side that is turned away from the cytosol. Usually the hydrocarbon residues get attached to the N-terminus. Typical membrane proteins can therefore be counted among the glycoproteins. The C-terminus of proteins can either lie at the outside, within the lipid layer or protrude into the cytosol, i.e. the site of protein synthesis (Fig. 9.9).

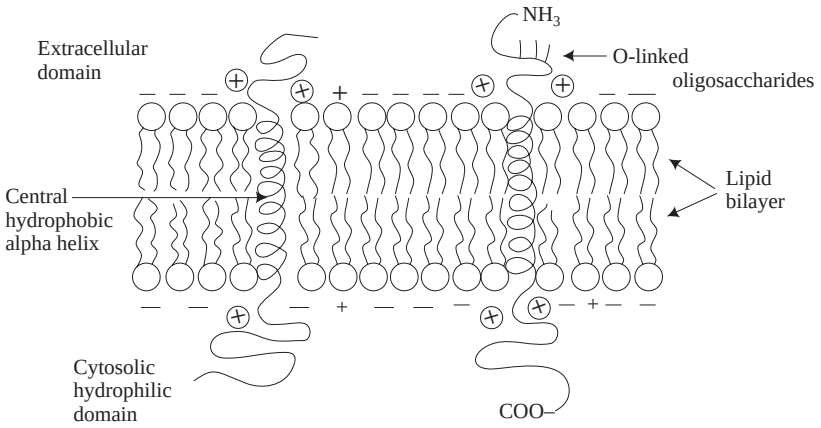


Fig. 9.9 Integral membrane proteins showing two membrane-spanning regions, i.e. glycophorins.

Integral Proteins Bind Asymmetrically to the Lipid Bilayer

Every integral membrane protein has a single, specific orientation with respect to the cytosolic and exoplasmic faces of the membrane. Molecules of integral membrane protein, such as *glycophorin*, lie in the same direction, providing asymmetry to the two membrane faces. In contrast to phospholipids, proteins have never been observed to flip-flop across a membrane, because such movement would be energetically unfavourable and would require a transient movement of hydrophilic amino acids and sugar residues through the hydrophobic interior of the membrane. The asymmetry of membrane proteins is established during their biosynthesis and maintained throughout proteins' lifetime.

Membrane asymmetry is most obvious in the case of membrane glycoproteins. In the plasma membrane, all the O-and N-linked oligosaccharides of glycoproteins and oligosaccharides in glycolipids are on the exoplasmic surface. In the endoplasmic reticulum, they are found on the interior or lumenal membrane surface.

Lipid Composition of Different Membrane Leaflets

All the plasma membranes have different lipid composition in the two leaflets. In the human erythrocyte, all the glycolipids and almost all of sphingomyelin and phosphatidyl choline are found in the exoplasmic leaflet. In contrast, the cytosolic leaflet contains lipids with neutral or negative polar head groups, such as phosphatidylethanolamine and phosphatidylserine. The relative abundance of a particular phospholipid in the two leaflets of a membrane can be determined on the basis of its susceptibility to hydrolysis by phospholipases when added to the cell exterior, because phospholipases are unable to penetrate the cytosolic face (Fig. 9.10).

Movement of Molecules is Two-dimensional

In both natural membranes and pure phospholipid bilayers, thermal motion permits phospholipid and glycolipid molecules to rotate freely around their long axis and to diffuse laterally within the membrane leaflet. Sine these movements are lateral or rotational, the fatty acyl chains remain in the hydrophobic interior of the membrane (Fig. 9.11).

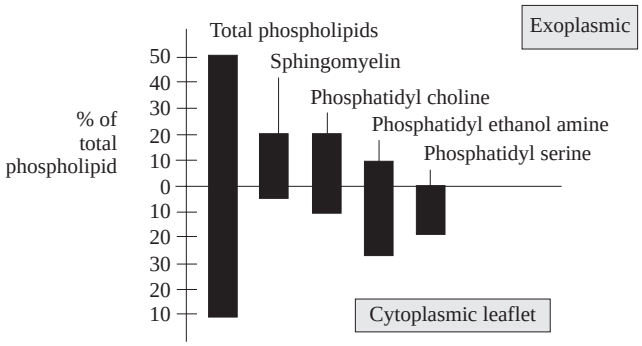


Fig. 9.10 Schematic showing lipid composition in erythrocyte membrane. The relative abundance of phospholipids in each leaflet accounts for their specific functions.

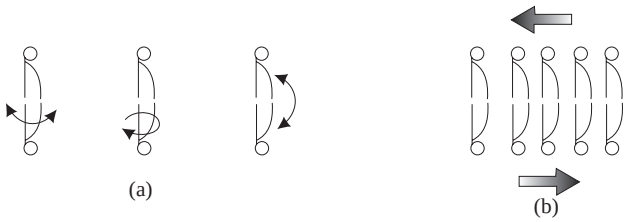


Fig. 9.11 Membrane lipids are constantly mobile: (a) rotational movement; (b) lateral movement.

The phospholipids in pure lipid bilayers do not flip-flop or migrate from one leaflet to the other. However, in some natural membranes, occasional movement is observed owing to catalysis by membrane proteins. Energetically, such movements are extremely unfavourable, because the polar head of phospholipid must be transported through the hydrophobic interior of the membrane.

Artificial Membranes can be Formed by Mechanical Dispersion of Phospholipids

Two systems of pure phospholipid bilayer are *liposomes* and planar bilayers. Liposomes are spherical vesicles upto l μ in diameter consisting of a phospholipid bilayer that encloses a central aqueous compartment, formed by mechanically dispersing phospholipids in water. Planar bilayers are formed across a hole in a partition that separates two aqueous solutions. When a suspension of liposomes or a planar bilayer composed of a single type of phospholipid is heated, it undergoes an abrupt change in physical properties over a narrow temperature range. This phase transition is due to increased motion about the C-C bonds of the fatty acyl chains, which pass from a highly ordered, gel-like state to a more mobile fluid state. During gel-to-fluid transition, a relatively large amount of heat is absorbed over a narrow temperature range that is the melting temperature of the bilayer. Lipids with short chain or unsaturated fatty acyl chains undergo phase transition at lower temperatures than lipids with long or saturated chains.

9.3 MEMBRANE TRANSPORT FUNCTIONS

The plasma membrane functions as a barrier between the cell and its extracellular environment but ensures transport of essential molecules, such as glucose, amino acids, lipids, and ions etc., into the cell and allows wastes to leave the cell.

Membrane is Selectively Permeable

Selective permeability of the plasma membrane allows the cell to maintain a constant environment in the interior. Similarly, organelles within the cell often have a different internal environment from that of the surrounding cytosol, a different maintained by the organeller membranes. For example, the lysosomes within an animal cell are involved in the digestive and scavenging roles, where the concentration of protons is 100-1000 times that of the cytosol. This proton gradient is maintained by proteins in the organelle membrane.

Permeability of Artificial Membranes

An artificial membrane composed of pure phospholipid or of phospholipid and cholesterol is permeable to gases, such as CO_2 and O_2 and small molecules, such as ethanol. These molecules can cross cell membranes unaided by transport proteins. Absolutely no metabolic energy is expended because movement is from high to low concentration of the molecules, down the concentration gradient.

In contrast, a pure phospholipid membrane is only slightly permeable to water and is essentially impermeable to most water soluble molecules, such as hydrogen, sodium, calcium and potassium. Proteins play important role in the transport of such molecules and ions across all cellular membranes because different cell types require different composition of these low molecular weight compounds, since the plasma membrane of each cell type contains a specific set of transport porteins that negotiate only certain ions or molecules to cross, as does the membrane surrounding each type of organelle (Fig. 9.12).

Some Transport Proteins Function as Ion-channels

Some membrane-located proteins function as ion-channels to transport water or specific types of ions down the concentration gradient. They form a protein-lined passageway across the membrane through which multiple water molecules or ions move simultaneously in a single file at a very rapid rate (10^8/second). For example, the plasma membrane of all animal cells is rich in K^+ and its movement downhill through always-open channels generates an electrical potential across the membrane. Many other types of channel proteins are usually closed, and open only in response to specific signals (Fig. 9.13).

Another class of membrane proteins, called *transporters*, move a wide variety of ions and molecules across the membrane. These transporters bind only one or a few substrate molecules at a time, undergo a conformational change to transport molecules across the membrane. Conformational change of proteins requires energy for movement, hence their movement is slow, about 10^2–10^4 molecules per second.

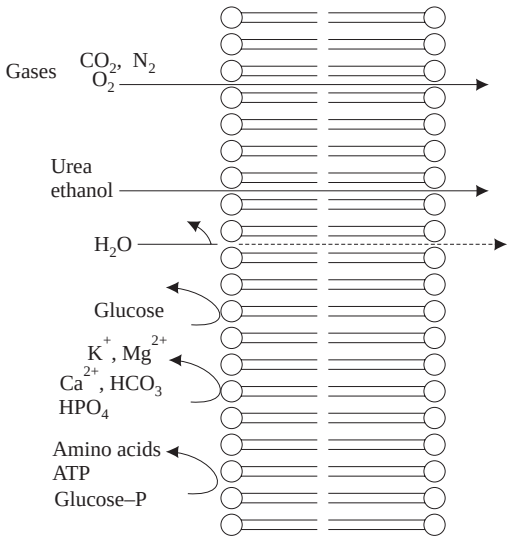


Fig. 9.12 Major types of transport proteins. One type couples ATP hydrolysis for up-hill movement of ions; the other two types, which are not ATPases, transport ions down-hill.

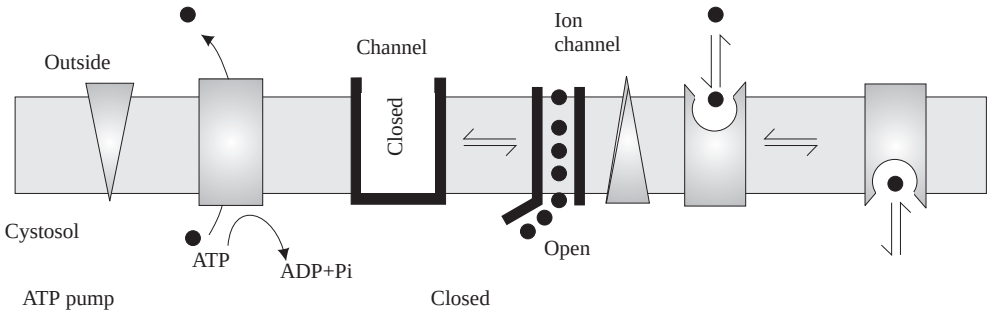


Fig. 9.13 Transport proteins can be classified into various types; channel proteins transport water or specific ions down the concentration gradients or electrical gradients down-hill. Ions or water molecules move single file at a rapid rate.

Three types of transporter molecules have been identified, which include *uniporters*, *antiporters*, and *symporters*. Uniporters transport one molecule at a time down the cocentration gradient (e.g. glucose and amino acids). The antiporters and symporters catalyze movement of one type of ion or molecule against the concentration gradient, coupled to the movement of different ion or molecule (Fig. 9.14). These are often referred to as active transporters but without hydrolysis of ATP.

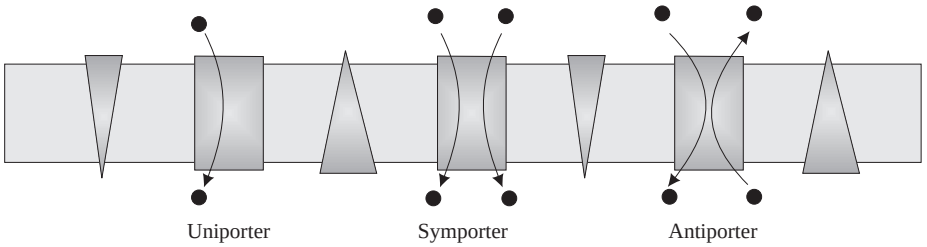


Fig. 9.14 Transport proteins are either symporter, uniporter or antiporter type, named according to the directional movement of molecules.

9.4 MECHANISMS FOR TRANSPORT OF MATERIALS ACROSS MEMBRANES

Several transport mechanism have been proposed.

Passive Diffusion

Passive diffusion is movement of a molecule from the aqueous solution into the hydrophobic interior of the phospholipid bilayer. The hydrophobicity of a substance is measured by the *partition coefficient K*, the equilibrium constant for its partition between oil and water. Since the composition of the interior of the phospholipid bilayer resembles that of oil, the partition coefficent of a substance moving across a bilayer equals the ratio of its concentration just inside the hydrophobic core of the bilayer C^m to its concentration in the aqueous solution C^{aq}:

$$K = C^m/C^{aq}$$

The partition coefficient is a measure of the relative affinity of a substance for lipid versus water.

Once a molecule moves into the hydrophobic interior of a bilayer, it diffuses across it; finally, the molecule moves from the bilayer into the aqueous medium on the other side of the membrane. The hydrophobic core of a typical cell membrane is 100-1000 times more viscous than water, hence the diffusion rate of all substances across phospholipid membrane is much slower than the diffusion rate of the same molecule in water. Thus, movement across the hydrophobic portion of a membrane is the rate-limiting step in diffusion.

Glucose Transport is Uniporter-Catalyzed

Very few molecules enter or leave cells, or cross organelle membranes without the aid of proteins. Transport proteins frequently accelerate transport of molecules such as water, and urea that can freely diffuse through phospholipid bilayers. It is essential to understand the properties of various kinds of membrane proteins and their roles in organismic physiology. This has been possible with the help of liposomes (artificial membrane). Liposomes having a single type of transport protein can be used to examine properties of transport protein (Fig. 9.15). A non-ionic detergent, such as octyglucoside, solubilizes the integral proteins of an erythorocyte membrane. The transport protein, a uniporter, can be purified and then incorporated into liposomes made of pure phospholipids.

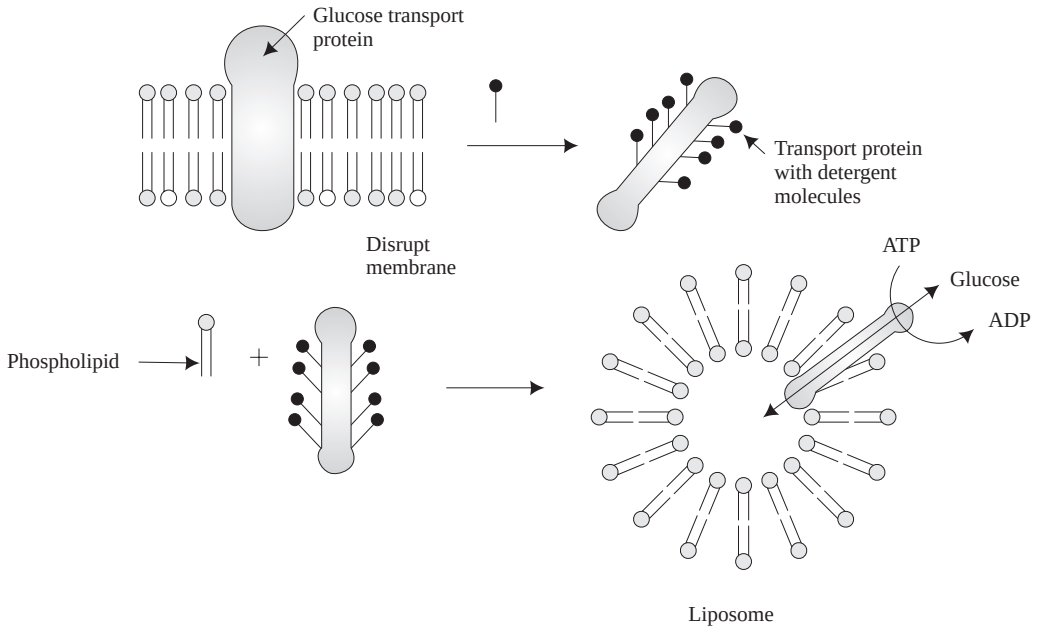


Fig. 9.15 Uniporter catalyzed transport of glucose. Liposomes help to examine the functional properties of membrane proteins. The figure shows a liposome containing a single type of transport protein. A nonionic detergent solubilizes the integral proteins of erythrocyte membrane. A uniporter type of glucose transport protein can be incorporated into the liposome made of pure phospholipids.

Uniporters catalyze movement of one glucose molecule at a time down a concentration gradient. Similar to enzymes, uniporters accelerate a reaction that is thermodynamically favoured, and the movement of a substance across the membrane down the concentration gradient will have a negative ΔG. This type of movement is referred to as *facilitated diffusion*.

Important properties of uniporter-catalyzed transport:

1. The rate of uniport transport is quite high.
2. Uniport transport is specific.
3. Uniport transport occurs via a limited number of transporter proteins, rather then throughout phospholipid bilayer. Consequently, there is a maximum transport rate, which is achieved only when the concentration gradient across the membrane is very large.

Two Models for Transport

The first model is the *carrier model*, in which the transporter protein binds the molecules to move at one face, moves through the membrane, and releases the molecule at the other face (Fig. 9.16).

The second model envisages use of too much energy. For example, the antibiotic valinomycin increases the transport of K^+ ions across biological membranes by forming a sphere around each K^+ ion (Fig. 9.17). The hydrophobic amino acid side chains of the antibiotic lie on the outer surface, and six or eight oxygen atoms on the inside coordinately bound to the K^+. The hydrophobic exterior

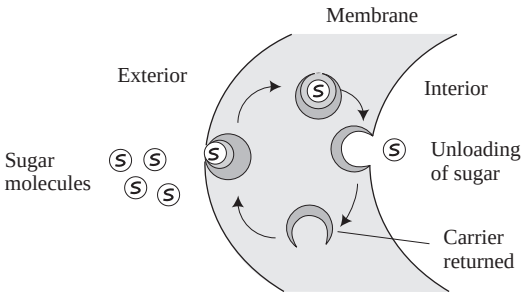


Fig. 9.16 Carrier-mediated transport of sugar molecules.

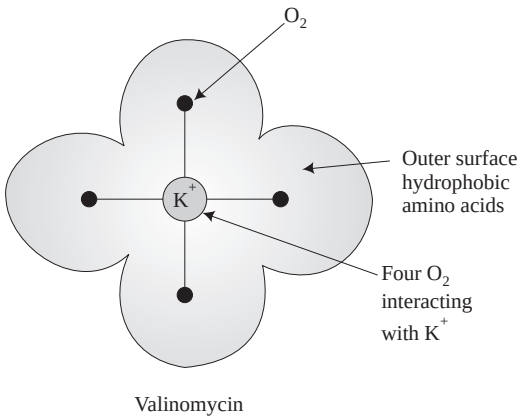


Fig. 9.17 Antibiotic valinomycin-facilitated transport of K^+ ions.

makes the K^+ carrier complex soluble in the lipid interior of the membranes. It is beleved that membrane transporters undergo conformational changes that permit bound ions or molecules to pass through the membrane.

Transport of Glucose

The glucose transporter alternates between two conformational states:

(a) Glucose-binding site faces the outside of the membrane.

(b) In the other glucose face the inside of the cell.

Unidirectional transport of glucose from outside to inside occurs when the transporter with glucose from outside undergoes a conformational change so that the outward facing site is inactivated and the bound glucose moves through the protein and becomes attached to the newly-formed inward facing site. The glucose is released into the cell interior, the transporter undergoes the reverse conformational change, inactivating the inward-facing glucose binding site and retreating the outward-facing glucose binding site (Fig. 9.18).

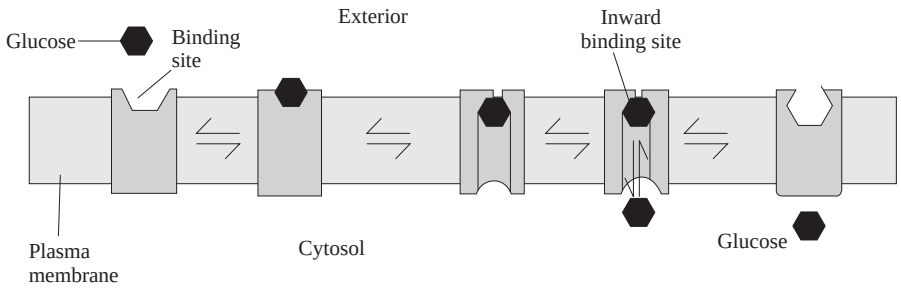


Fig. 9.18 Glucose transporter protein alternates between two alternative states.

Passage Through Ion-channels

The movement of ions across the plasma membrane and organeller membranes is also mediated by transporter proteins; symporter and certain antiporter co-transport ions together with specific small molecules. Ion-channels, ion pumps and some antiporters transport only ions, but the rate and extent of ion transport is influenced by the ion concentrations on the two sides of the membrane as well as by an electric potential that exists across the membrane.

Ionic Gradients Maintain the Electric Potential

Ionic composition of the cytosol differs from that of the surrounding fluid in almost all types of cells. The cytosolic pH is near neutral (pH 7.0) and the cytosloic K^+ ion concentration is always higher that the Na^+ ions. In both invertebrates and vertebrates, K^+ ion concentration is 20–40 times higher in the cell than in the blood, while Na^+ ion concentration is always lower. However, the concentration of free Ca^{++} ions in the cytosol is generally less than 1 micromolar, about a 1,000 times lower than the blood.

The plasma membrane is provided with channel proteins that allow the principal ions (Na^+, K^+, Ca^{++} and Cl^-) to move through at rates determined by their concentration gradients. The selective movements of these ion through the channels create a difference in electric potential between the inside and outside of cell. The magnitude of this potential is –70 mV with respect to inside. The plasma membrane is an electrical device called capacitor. The ionic gradients and electric potential are responsible to drive many biological processes, hence opening and closing of ion channels are essential to conduction of electrical impulses.

K^+ Channels Generate Electric Potential

The distribution of K^+, Na^+ and Cl^- ions is almost similar in animal cell and its environment and if the membrane is impermeable to all ions, no ions will flow across the membrane and there will be no potential difference. As noted above, the membrane potential across the plasma membrane is about –70 mV due to negative charge on the cytosolic face. The membrane contains many open K^+ channels, but very few open Na^+ and Ca^{++} channels, resulting in major ionic movement of K^+ from the inside to outward, leaving an excess of negative charge on the inside and positive charge on the outside.

The $Na^+ – K^+$ ATPase ion pump moves K^+ ions into the cytosol from the extracellular medium, generating K^+ concentration gradient. Movement of K^+ ions through potassium channels from the cytosol down the concentration gradient generates the inside negative membrane potential. The potassium channels have now been cloned and sequenced.

Active Transport Machanisms

The active transport of molecules and ions requires an input of metabolic energy, which can be derived either from direct coupling to the hydrolysis of ATP or by coupling to the movement of an ion down its concentration gradient.

Active Ion Transport

ATP-powered pumps transport ions against their concentration gradients. For instance, the $Na^+ – K^+$ ATPase pumps K^+ into the cell and Na^+ outwards, establishing a high cytosolic concentration of K^+ that is essential for generation of negative potential across the plasma membrane. The Ca^{++} ATPase pumps Ca^{++} out of the cytosol into the extracellular medium or into the intracellular organelles in order to maintain concentration of Ca^{++} into the cytosol much lower than in the extracellular medium. The pump derives its energy from the hydrolysis of ATP (Fig. 9.19).

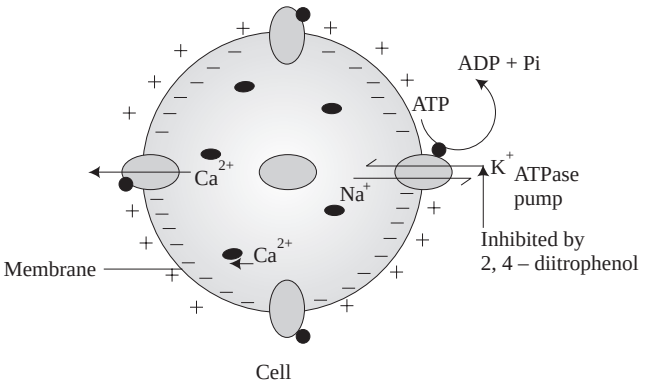


Fig. 9.19 ATP-powered pumps transport ions against concentration gradients. Na^+–K^+–ATPase pumps out K^+ and Na^+ into the cell. The Ca^{2+} – ATPase pumps out Ca^{2+} of the cytosol. The pumps can be inhibited by 2, 4-dinitrophenol.

Existence of these pumps came from studies in which aerobic production of ATP in cell was inhibited by 2, 4-dinitrophenol. The ion concentration inside the cell gradually approached that of the external environment as the ions moved through the membrane channels down the concentration gradient, ultimately leading to the cell death because K^+ requirement of the cell could not be met.

Three Classes of Ion Pumps

Three classes of ATP-powered pumps have been recognized, called P, V, and F. Class P pumps are simplest and composed of four transmembrane subunits, such as two alpha and two beta polypeptides (Fig. 9.20). The larger alpha subunit is phosphorylated during transport of ions. $Na^+ – K^+$ ATPase and Ca^{++} ATPase pumps are included in this category.

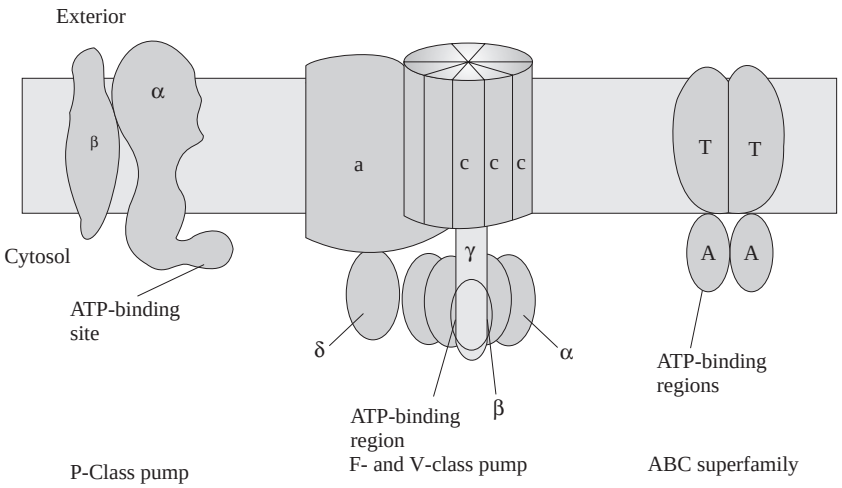


Fig. 9.20 Three classes of pumps have been recognized. V-class pumps are found in plant vacuoles and lysosomes, while F and V-class pumps are found in mitochondria and bacterial plasma membranes.

The V and F types of pumps, though unrelated to class P pumps, transport only protons. F-class pump contain three types of transmembrane proteins and V-class pumps contain atleast two kinds of proteins. V-class pumps are ATP powered pumps that maintain low pH of plant vacuoles and of lysosomes and other vesicles in animal cells by using energy from ATP hydrolysis to pump protons from the cytosol to the exoplasmic face of the membrane. F-class pumps are found in mitochondria, chloroplasts and in the plasma membrane of bacteria.

Glucose Transport Into Intestinal Cells is ATP-Driven

The lumenal cell of the intestine have two domains, differentiated on the basis of lipid and protein composition of the membrane. The membrane with cells having brush border face the lumen carrying microvilli, and the basolateral surface is in contact with neighbouring cells and blood capillaries. The microvilli help in increasing the cell surface for absorption of nutrients.

Glucose, other sugars as well as amino acids are transported across the membrane from low concentration in the lumen of the intestine to a higher concentration in the cytosol of the epithelial cells by a symporter protein, which is ion-driven active transport process. The energy comes from the movement of Na^+ down its concentration gradient. The basolateral side of the epithelial cells, in contact with the blood capillaries, maintain a concentration gradient of glucose across this membrane to allow glucose to move out of the cell by facilitated diffusion through a transporter. The low concentration of Na^+ inside the epithelial cells is maintained by Na^+–K^+–ATPase pump on the basolateral membrane (Fig. 9.21).

9.5 BULK TRANSPORT SYSTEMS

Cells have also developed mechanisms to transport large molecules across the plasma membrane, which otherwise cannot diffuse through the membrane barrier. Such molecules include proteins,

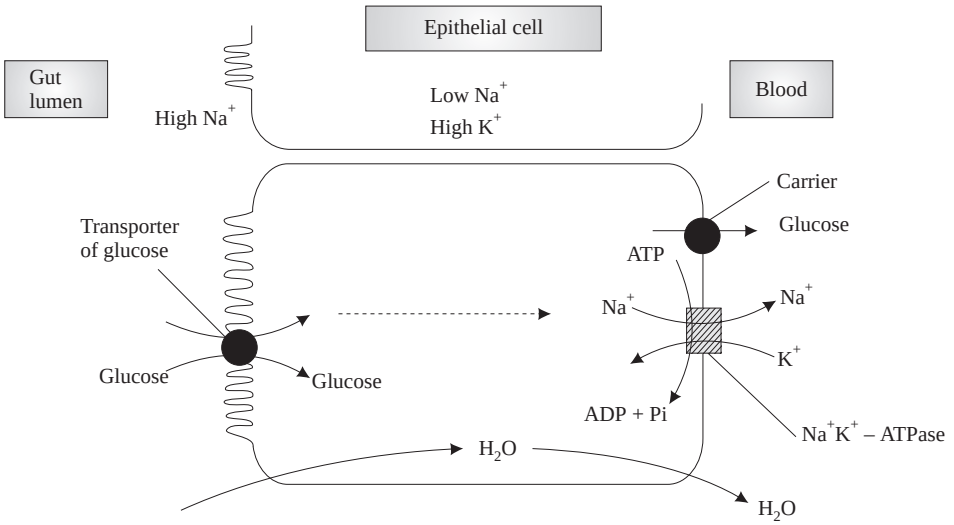


Fig. 9.21 Mechanism of glucose transport in intestinal epithelial cells.

polysaccharides, polypeptides and polynucleotides etc. This is done by a process called *endocytosis*. Similarly particulate matter, secretory molecules likes hormones, and proteins can be transported out of the cell by a process called *exocytosis*. Both the processes involve the formation of vesicles.

Endocytosis

Endocytosis is essentially an energy dependent process and occurs in almost all eukaryotic cells. Extracellular macromolecules are engulfed and transported to the cell interior where it is progressively enclosed in a small portion of the plasma membrane. Three distinct types of endocytotic processes have been observed, which include *phagocytosis*, *pinocytosis* and *receptor-mediated* endocytosis.

Phagocytosis

Phagocytosis is a major mechanism where particulate matter is engulfed without fluid, such as viruses, bacteria, and macromolecules. The process is particularly observed in protozoans, and in multicellular organisms it is carried out by specific cells such as macrophages, polymorphs, lymphocytes and neutrophils, where it is a form of feeding process. The particle to be ingested is engulfed in a large endocytic vesicle called *phagosome* (Fig. 9.22). The particle then binds to the surface of the phagocyte aided by a surface receptor and the vesicle enclosing the particle is engulfed with its plasma membrane. The phagosome then fuses with a lysosome so that ingested particle is broken down and digested in the cytosol, while undigested material is found in the form of residual bodies to be transported out of the cell.

Pinocytosis is the Process of Fluid-intake

Pinocytosis or cell-drinking is a constitutive process, occurring continuously in almost all kinds of cells. A cell sipping away at the ertracellular fluid by pinocytosis acquires a representative sample of

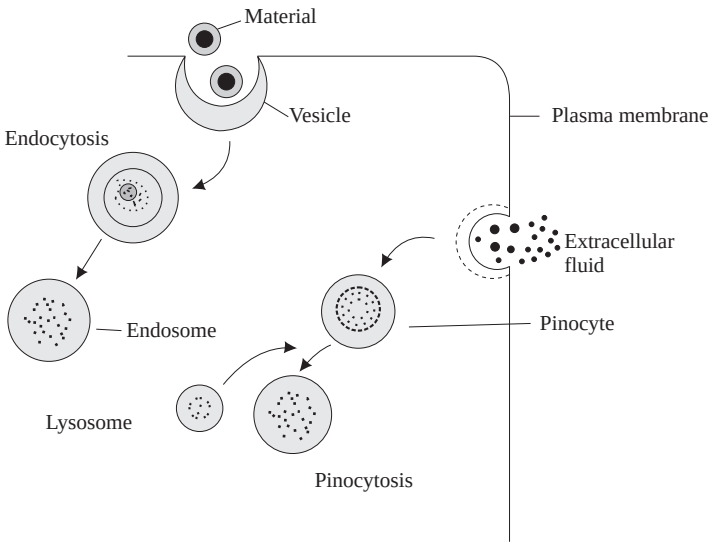


Fig. 9.22 Two modes of endocytosis: phagocytosis and pinocytosis.

molecules and ions dissolved in the extracellular fluid. Small areas of the plasma membrane are ingested in the form of vesicles which are returned to the cell surface. These are pinocytic vesicles which enclose a small amount of extracellular fluid. This is a non-specific endocytosis, but provides a much more elegant method for cells to pick up critical components of the extracellular fluid that may be in scant supply.

Receptor-mediated Endocytosis

Some integral membrane proteins displayed at the membrane surface function as receptors for extracellular fluid components. For instance, iron in the blood is transported complexed with a protein called *transferrin* whose specific receptor is located on the surface. When a receptor encounters transferrin, binding takes place and the receptor with the transferrin is endocytosed, releasing iron in the cytosol while the receptor is returned to the surface for recycling (Fig. 9.23). Cholesterol is also taken up by the cells by receptor-mediated endocytosis. The most abundant cholesterol carriess in humans are the low-density lipoproteins or LDLs. People who inherit defective genes for LDL receptor have poorly functioning receptors, which create excessively high levels of LDL in their blood, predisposing them to atherosclerosis and heart attacks. This is a familial disease called *hypercholesterolemia*.

Exocytosis

Exocytosis is reverse of endocytosis in which membrane-bound vesicles move to the cell surface where they fuse with the plasma membrane. Exocytic vesicles are formed in various ways. Some vesicles are simply endosomes traversing the cells, while some are either pinched off from endosomes before they fuse with the lysosomes. Some vesicles may be formed from the endoplasmic reticulum

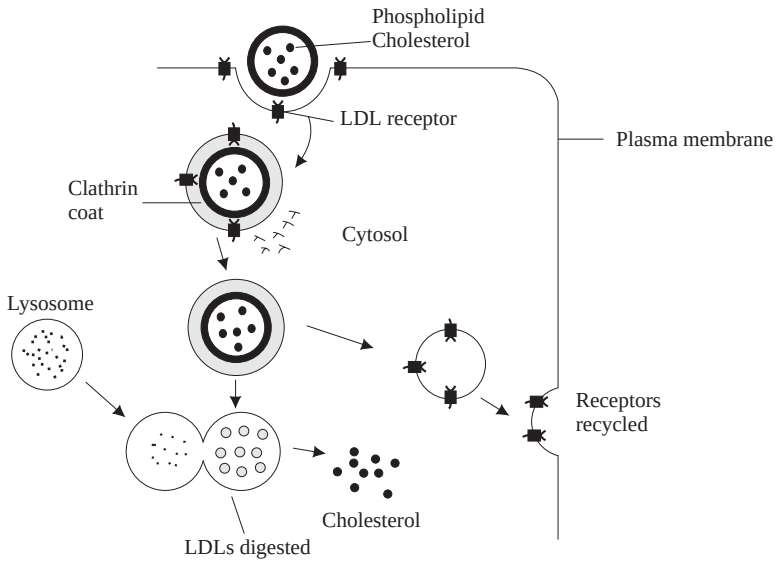


Fig. 9.23 Receptor-mediated endocytosis.

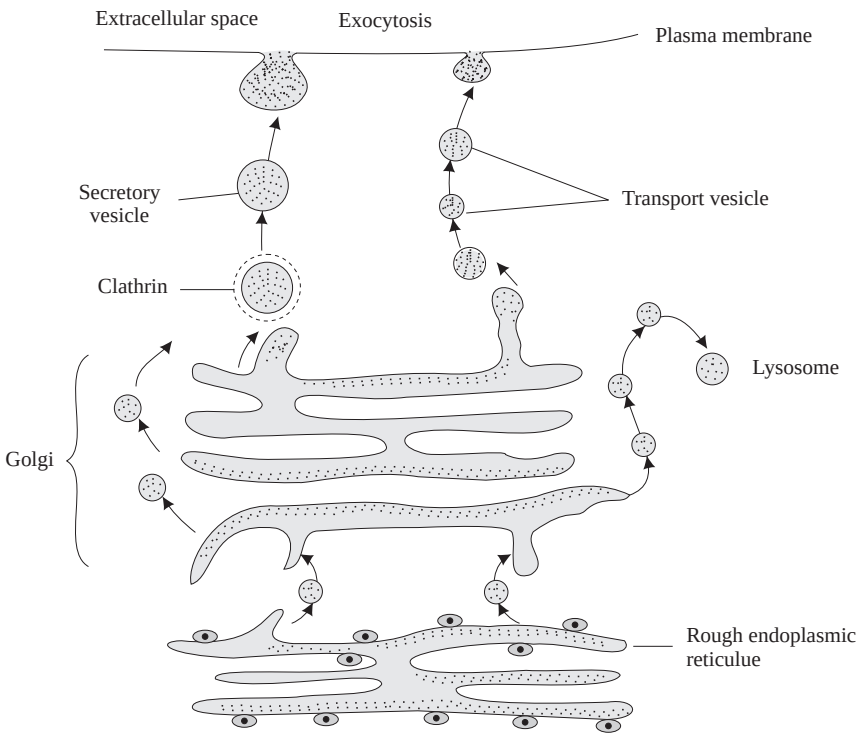


Fig. 9.24 Elimination of substances (wastes) through exocytosis.

and the Golgi to take their products to the cell surface. Formation of exocytic vesicles is shown in Fig. 9.24. Exocytosis is essential to restore the normal amount of plasma membrane, and ensures display of its characteristic cell-surface proteins. It also helps in the secretion of various components of the extracellular matrix. The exocytosis of lysosomes supplies the membrane much needed material to repair the wounds in the plasma membrane.

CHAPTER

10

Temperature Regulation

Different forms of energy manifested in the living matter are a result of biochemical reactions. All biochemical reactions come under the field of metabolism which constitute degradative as well as synthetic reactions. Synthetic processes require energy which is made available to the system through oxidations. All the energy released during the oxidative process is not utilized; however, some of it is dissipated out of the body in the form of heat. Thus metabolism and heat production are intimately related. Many biochemical reactions are extremely temperature sensitive. A 10°C rise in temperature accelerates the rate of reactions twofold, whereas low temperatures have an opposite effect. Since life of organisms is dependent on chemical reactions, it follows that all biological processes may be profoundly influenced by temperature fluctuations.

Biological systems have predominance of carbon compounds which are stable within a temperature range of 40-45°C. The lower limit of the temperature range is close to the freezing point of water which is – 1°C and the upper limit lies close to the 45-50°C range beyond which proteins get denatured. A few algae have been known to thrive at 70°C. Although the environmental temperatures have a wide range, the biological activity exists in only a small part of the total range lying close to the lower limit.

10.1 HABITATS OF ANIMALS

The habitats of animals can be divided into three categories, namely, terrestrial, aquatic and aerial. Animals living in terrestrial environment have an acute problem of temperature. Because of the radiant heat of the sun, environmental temperature may reach an upper lethal limit. Air has a low specific heat and so it gains or loses heat rapidly. After sunset considerable heat gained by the environment gets lost so that lower lethal limit is reached. Terrestrial animals have acquired greater adaptability since they have to live under wide range of temperatures. In deserts, the temperatures exceed the biological limits and sandy tracts attain temperatures as high as 70°C, while the air temperature may

be around 50°C. In the tropics and subtropics, sometimes the temperature reaches below freezing point (−65°C to −50°C).

Animals living in aquatic habitats do not face acute thermal problems as faced by terrestrial ones. Water has a high specific heat and it gains or loses heat slowly, thus making little changes in temperature. Thermal adjustments are not a problem with aquatic animals. Aerial animals like birds have a higher limit of thermal tolerance (35-42°C) due to higher rate of metabolism (Fig. 10.1).

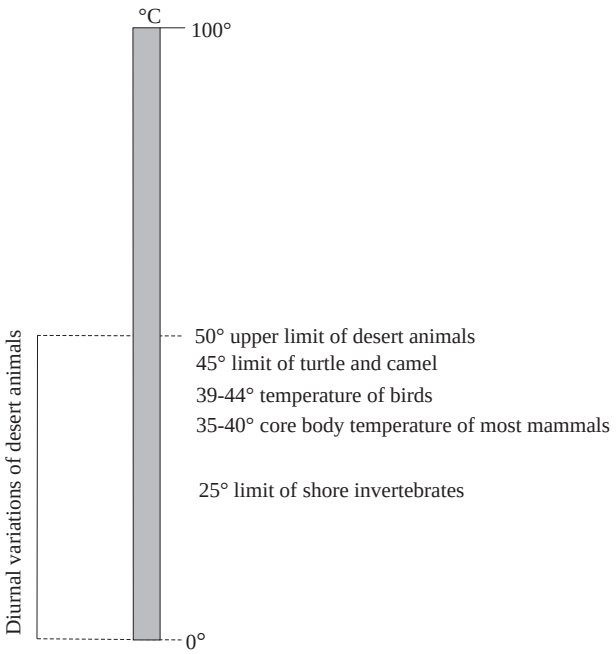


Fig. 10.1 Temperature ranges of various animals.

Temperature adjustments are related to physiological adjustments. Aquatic animals have a low rate of metabolism and cannot adjust to extremes of temperature. Nevertheless, terrestrial animals have the capacity to step up or step down their metabolic rates in accordance with the thermal changes.

10.2 NOMENCLATURE OF THERMOREGULATION

On the basis of body temperatures animals are classified as *warm-blooded* or *cold-blooded.* These terms are rather vague and have been replaced by more appropriate terms such as *homeothermic* and *poikilothermic.* Animals which are capable of maintaining a relatively constant body temperature in spite of great variations of external temperature are said to be homeothermic, whereas animals in which the body temperature varies with that of the environment are called poikilothermic. Poikilotherms include invertebrates and aquatic animals like fishes and amphibians. Some animals

have a high rate of thermal conductance and low rate of heat production. Such animals acquire heat from the environment and regulate their body temperatures quite independent of the heat produced in the body. These animals are known as *ectothermic* and include vast majority of animals species. In contrast to this, a few animals produce sufficient heat due to their own oxidative metabolism and maintain body temperature at a constant level. Such animals are called *endothermic* which include homeotherms like birds and mammals.

There is yet another category of animals which do not maintain constant body temperature like prototheria, but during activity they show endothermic regulation. These are called *heterothermic* animals. They are also called facultative endotherms since they are capable of regulating physiological temperature at certain times only.

10.3 ENERGY RELATIONSHIPS OF ANIMALS

The temperature relations between the organisms and the environment are dependent upon the water contents of the individuals. Terrestrial animals have a complex environment, hence it is difficult to make an accurate measurement of their thermal environment. However, thermal relationships of aquatic animals are easier to determine. Water has a low heat conductivity and it gets heated up slowly. Therefore, aquatic animals maintain their body temperature close to the ambient temperature. Terrestrial animals, on the other hand, are faced with much greater problem of thermal regulation. Most of the heat produced by the body is lost to the environment through conduction, convection, radiation or evaporation. Mammals have efficient thermoregulatory physiological devices to maintain their body temperature. When the ambient temperature exceeds the body temperature, the body temperature is not allowed to rise by evaporation of water through body surface. Evaporation lowers the temperature of the body. The skin and the respiratory system of animals have tremendous thermoregulatory significance.

Q_{10} law

The outside temperature affects the metabolism in the same way as it does for biological reactions. The heat production of an individual is directly related to the body metabolism. The biochemical reactions are extremely sensitive to temperature; an increase in temperature accelerates the rate of reactions. This fact was explained by van't Hoff who stated that the biochemical reactions are approximately doubled by 10°C rise in temperature. **This generalization was known as Q_{10} law and can be quantitatively expressed as:**

$$Q_{10} = \frac{k_t + 10}{k_t}$$

Where k_t is the velocity constant at temperature t and k_t + 10 the velocity constant at 10°C higher, and the value is calculated by the formula

$$Q_{10} = \left(\frac{k_1}{k_2}\right)^{10(t_1 - t_2)}$$

$$\text{or } \log Q_{10} = \frac{10(\log k_1 - \log k_2)}{t_1 - t_2}$$

Where k_1 and k_2 are velocity constants corresponding to temperatures t_1 and t_2 respectively. The relationship is not a linear one, but logarithmic. In case of chemical reactions, Q_{10} is fairly constant and lies between 2 and 3, but at higher and lower temperatures this relationship is not followed. Homeotherms do not follow this law since they can adapt to the heat production when the heat is lost. Enzyme catalyzed chemical reactions are not linear in function, hence Q_{10} law is not applicable. Influences of temperature on biological reactions were explained by Arrhenius equation:

$$K = A_c - E_a/RT$$

where K is velocity constant, A_c is a constant relating to molecular collision frequency, E_a is activation energy, and R and T are gas constant and absolute temperature respectively. However, because of extreme thermal fluctuations a straightforward law an animals cannot be formulated.

10.4 LOW TEMPERATURE EFFECTS

Majority of living organisms face environments where temperature fluctuates both diurnally and seasonally. Only birds and mammals are able to regulate their internal temperature, while other living organisms conform to prevailing external temperatures. Protoplasm can exist in living state between 0°C and 45°C, and very few organisms can withstand such a wide range temperatures.

Animals respond to cold in several ways. Many try to avoid cold by migrating to warmer regions. Migration in birds from colder regions to warmer regions is quite a familiar phenomenon which is, however, seasonal. Such animals develop cold tolerance and adapt to changed environments by resorting to hibernation, spending their periods of inactivity in burrows.

Generally, low temperatures have an adverse effect on the life processes of animals. If an animal is slowly subjected to low temperature, metabolic rates become feebler and feebler and ultimately death ensues. The protoplasm of the cells is an aqueous solution and freezes a few degrees below zero. Slow freezing causes formation of ice crystals which cause lethal effects. On the other hand, fast cooling does not allow the formation of ice crystals and the tissues are preserved in a *chill comma* stage. This is called *supercooling*.

Vinegar nematodes and certain species of protozoa survive temperatures as low as –197°C when placed in liquid air. Protozoa in the encysted stage and also some insects can withstand prolonged sub-zero temperatures. This is due to the effect of supercooling. Winterhardy species of insects can survive supercooling since they can tolerate temperatures –23°C to –30°C. Slow freezing has obviously some disadvantages:

(a) Freezing causes formation of ice crystals in the cell and disturbs the cell organization.
(b) Metabolism is greatly lowered and as such the rate of oxygen consumption is also very low. This is because the diffusion of O_2 and CO_2 through the ice is very slow.
(c) The enzymes become inactive.

Poikilotherms have their body temperature generally lower than the surroundings, but severe cold induces a factor of acclimatization. The lethal effects of cold or low temperature may be avoided by altering the freezing points. The freezing point of any solution is lower than that of the pure solvent.

Any increase in the osmotic content of the body fluids will lower the freezing point and protect the organisms from being frozen. Hence, poikilotherms avoid cold by acquiring an *antifreeze* phenomenon or may avoid cold by supercooling. Insects sometimes face temperatures lower than the freezing point of the body fluids. A parasitic hymenopteran, *Bracon cephi,* can withstand supercooling at −47°C. It has been found that in such insects the haemolymph normally contains glycerol which lowers the freezing point and offers protection to the frozen tissues from damage.

Vertebrates do not have tolerance to freezing or supercooling as compared to invertebrates. Fishes in the Arctic are not able to survive when frozen completely. The temperature which kills a poikilotherm is not fixed and depends upon its previous thermal history. Acclimatization can, however, alter the lethal limits to a small extent. It is generally believed that acclimatization involves either the synthesis of new forms of enzymes which are able to operate under new temperature zone or quantitative changes in the amounts of existing enzymes.

10.5 TEMPERATURE RELATIONS IN POIKILOTHERMS

The activity of poikilotherms depends on the prevailing ambient temperature, and in doing so they do not expend any energy on thermoregulation since their metabolism is at a lower level with little or no heat production. It has been discussed already that the rates of biological processes approximately double for each 10°C increase until a lethal limit is reached. However, when comparing the rates of metabolic processes during summer and winter, one may find Q_{10} less than 2. Thus, living organisms have the ability to compensate for environmental temperature changes by altering their metabolic rate. In cold conditions their body temperature is low and in hot weather the body temperature rises. The body gains heat from the environment and the metabolic rate is accelerated. Thus there is no fixed metabolic rate in poikilotherms and it varies with the ambient temperature. Poikilotherms, however, regulate their temperature by physical mechanisms only:

(a) Poor insulation allows greater heat loss and prevents conservation of heat.
(b) Core body temperature (measured through the rectal region) is lower than the ambient temperature.
(c) In hot environments body heat is removed through evaporation.
(d) In low ambient temperatures, there is no regulated way of heat production since chemical regulation is wanting.

Aquatic Poikilotherms

Thermal regulation in aquatic poikilotherms is a simple phenomenon. Heat exchanges in aquatic animals are largely by conduction and convection. Thermal environment of aquatic animals is relatively stable, still seasonal variations do occur in the surface layers of the sea and lakes. For aquatic animals which do not possess cold-hardiness, even the temperature above the freezing point may prove lethal. On the other hand, some poikilotherms cannot tolerate high temperatures. In such cases, death may occur even below temperatures at which proteins are usually denatured.

Aquatic Invertebrates: Aquatic invertebrates can tolerate wide ranges of temperature fluctuations as compared to poikilothermic vertebrates. *Chironomid* larvae in hot springs can tolerate

temperatures as high as 50°C, whereas in some overwintering species of insects survival at sub-zero temperatures for prolonged periods is possible. Such adaptations are, however, species specific having a temperature range of their own.

Aquatic Vertebrates: Fishes are gill-breathing aquatic poikilotherms whose body temperature is maintained close to that of water. Their metabolic rate is very low; hence their rate of heat exchange is also low. A fish while swimming may produce some heat due to muscular activity which may increase the body temperature temporarily, yet there is no appreciable difference between its body temperature and surrounding water. This is because the heat produced due to muscular activity is immediately transferred to the blood eventually reaching the gills which are in immediate contact with water. Gills are extremely efficient organs for respiratory exchanges and also help in equilibrating temperature of the blood and the surrounding water. Physical factors like large surface areas of the fish, counter-current mechanisms and thin-walled blood vessels facilitate exchange of heat between water and the fish so that the body temperature remains equal to that of water. There is, however, one notable exception to this generalization. In case of a large and fast swimming fish tuna, the temperature of the axial muscles is about l2^{o}C higher than the surrounding water. The heat produced due to muscle activity is regulated by the counter-current mechanism to a limited extent and the dissipation of heat is reduced.

It has been found that the matching between body temperature and water temperature will be closer in smaller species than in large ones. Under conditions of sustained activity, large forms will show transient increases in body temperature. Fishes are generally more susceptible to drastic changes in environmental temperature. Fishes living in shallow waters or in the surface layers of the sea experience drastic temperature fluctuations seasonally. These fishes are relatively independent of temperature in their physiological adjustments. On the other hand, fishes living in tropics or in deep waters at any latitude do not face temperature fluctuations, hence are extremely sensitive to changes in environmental temperatures. Fishes that normally experience seasonal changes in temperature are known to have evolved biochemical adjustments to maintain normal functions despite changes in body temperature.

Terrestrial Poikilotherms

Terrestrial poikilotherms maintain their body temperature almost equal to that of environment. However, terrestrial animals face greater temperature fluctuations. The heat balance of such animals is more related to their water balance as compared to aquatic animals.

The problems of terrestrial poikilotherms are varied. Desert animals are subjected to daily temperature variations. The days are warm and the nights are cool, showing a temperature range between 10^{o}C and 45^{o}C. In hot summer days, the temperature may exceed even 50^{o}C. In arctic region also the temperature varies from 20 to 60^{o}C.

Owing to low specific heat air exchanges heat quickly. On land, animals absorb the radiant heat with readily and give up heat with the same rapidity. This is because air is a poor conductor of heat. Poikilotherms lose heat readily by evaporation of water from the body surface.

Terrestrial Invertebrates: Invertebrates are perhaps the most important group of animals which have acquired maximum adaptability to their environment. Their habitats are varied and so also

their thermal requirements. In this context, our description will be restricted to the arthropods and insects in particular since they are found to be active in extreme thermal environments. The body temperature of insects may vary from that of the air due to three reasons: (a) heat loss by evaporation of water from the body surface, (b) absorption of heat by radiation; and (c) heat production by metabolic activity of the body.

(a) *Heat loss by evaporation:* Many insects are able to maintain their body temperature 3-5^{o}C lower than the air. This is possible by loss of heat from the body by evaporation. Water is generally lost from the tracheal system through spiracular openings. In dry air, excessive water loss from the body poses the risk of death due to desiccation. The cuticle of insects is impermeable to water because of the presence of a waxy layer in it. However, if temperature of the body exceeds 40°C, the waxy coat melts rendering the cuticle permeable to water causing dehydration of the body.

(b) *Absorption of the radiant heat:* Insects absorb radiant heat of the sun and raise their body temperature. The amount of heat absorbed by an insect depends on the pigmentation, surface area of the body, and orientation of the body in respect to the sun. Dark coloured insects absorb more heat as compared to light coloured insects. Orientation of the body in respect to the sun's rays is an important parameter, *Schistocerca,* a desert locust, is active at 17-20°C and orients itself perpendicular to the sun's rays to get maximum heat.

(c) *Heat production due to metabolism:* Heat production in insects increases during flight activity. At low temperature the flight muscles remain inactive and sustained flights may not be possible at all. Many insects warm up by fanning their wings before commencing actual flights. Warming up period is longer at low temperatures. Observation on *Vanessa* indicates that it takes about 6 minutes at 11^{o}C, $1\frac{1}{2}$ mintues at 23°C, 18 seconds at 34^{o}C and none at 37^{o}C to warm up. In social insects like honeybee and termites, metabolic heat is useful for temperature regulation in the colonies. Ideal temperature for brood development in honeybees is 34.5-35°C. Excessive heat of summers creates uneasy situation for the brood which is overcome by transporting and spreading water in the hive by workers. On the other hand, low temperatures during winter force the honey bees to cluster together to increase the temperature of the hive a little above the air temperature.

Terrestrial Verterbrates: Amphibians are a unique group of animals which show a remarkable example of temperature adjustment in relation to the environment. The skin of amphibians, although ineffective for physiological regulation, offers a good protection in extreme situations. In dry and warm surroundings, water is lost from the skin by evaporation. When on land, the wet skin functions like a wet-bulb thermometer and the constant evaporation of water from the skin keeps the body temperature below that of the environment. Generally the amphibians are very sensitive to high temperatures and in this respect they have a poor adaptability as compared to reptiles, birds and mammals. Amphibians cannot withstand high temperatures on land owing to, inadequte physiological mechanisms. However, they regulate their body temperature through behavioural adjustments and thermal acclimatization (see Sections 10.9 and 10.10).

10.6 TEMPERATURE RELATIONS OF HOMEOTHERMS

Although reptiles have some environmental constraints, they are the first group of terrestrial vertebrates which mark the beginning of homeothermy by exhibiting thermoregulatory mechanisms at the primary level. Birds and mammals maintain their body temperature independent of the environment and have evolved efficient thermoregulatory devices. Heat is produced and conserved in cold environments, while heat is lost in hot environments. Heat exchanges between the body and the environment are regulated by thermoregulatory centres located in the hypothalamus which functions like a thermostat. Regulation of the body temperature is brought about in the following ways:

(a) Heat production and heat loss are rapidly adjusted in relation to body and environmental temperatures. This is physical heat regulation.

(b) Heat production is regulated by chemical heat regulation. This is done by accelerating metabolic rate when heat requirements of the body are high.

Physical Heat Regulation

Homeotherms maintain a constant body temperature. In order to do so, it is necessary that the heat production must be equal to heat loss. When the ambient temperature is lower than the body, the body loses heat to the environment (Fig. 10.2). In order to compensate this heat loss, homeotherms are capable of producing heat by stepping up their metabolic rate.

According to Newton's law of cooling, the change of heat in a body per unit time is proportional to the difference between its temperature and the ambient temperature. The relationship can be expressed thus:

$$\frac{dH}{dt} = C\ (T_B - T_A)$$

where C is thermal conductance, T_B is body temperature and T_A is ambient temperature. Since heat loss = heat gain = heat production = metabolic rate, heat loss = $C(T_B - T_A)$. Therefore, C = metabolic rate/$(T_B - T_A)$.

Heat loss: Heat is lost from the body by skin, lungs and excretions. Skin forms by far the most important source of heat loss. Homeotherms dissipate heat by conduction, convection, radiation and evaporation. Thermal conductance of the body is of utmost importance since accumulation of heat in the animal may produce death due to overheating. The problem of overheating has been observed in fur seals, *Callorhinus ursinus,* which live in the arctic region. Fur seals live in water and dissipate heat to the environment, the difference between core body temperature and that of the environment being about 30°C. This is overcome by a heavy and waterproof insulation and a subcutaneous layer of fat. When fur seals are in water, large quantity of heat is generated due to swimming which is lost to the water through large and richly vascularized flippers. While on land, the flippers do not allow heat loss as efficiently since air has a low specific heat. This creates a problem for fur seals when air temperature rises above 12°C. Heat loss is not facilitated, hence death may follow.

The external factors that determine the amount of heat loss are temperature, humidity of the air, velocity of air currents, and temperature of surrounding objects. Generally homeotherms maintain

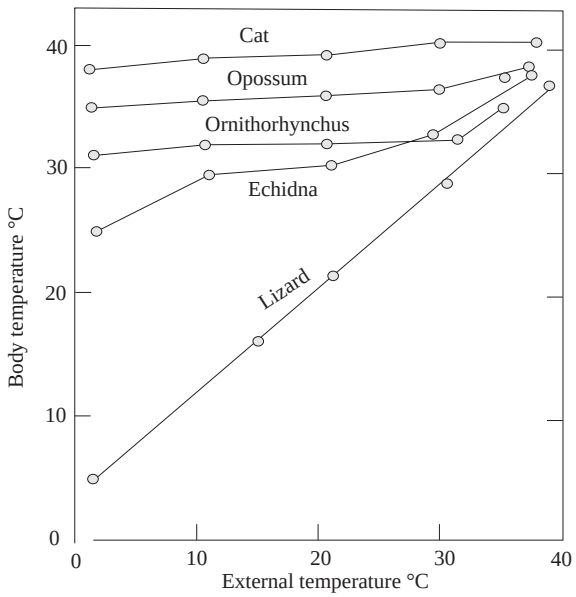


Fig. 10.2 Relationship between body temperature and the ambient temperature for certain animals.

their core temperature higher than that of the ambient temperature allowing heat loss through skin to cool the body. This establishes a temperature gradient from the core to the skin surface. If thermal conductivity of the subcutaneous fat is changed by altering the blood flow, the direction of temperature gradient is also reversed.

Heat lost from the skin is linked with two problems, i.e. the blood flow in the skin, and the external insulation.

Blood flow in the skin: Blood flow in the skin is responsible for the regulation of heat loss. During conditions of *hypothermia* (low core temperature) the flow of blood to the surface of the body is restricted minimizing the heat loss. Consequently, the temperature of the skin surface falls. On the other hand, during *hyperthermia* (high core temperature) the blood flow through the skin is augmented so that there is a greater loss of heat through the skin. In such circumstances the difference between the core body temperature and the skin surface is minimized.

Blood flow in the skin is controlled by the sympathetic nervous system. In conditions of hyperthermia dilation of the blood vessels takes place to increase the blood flow. This is brought about by:

(i) Relaxation of the activity of the nerves which cause vasoconstriction:

(ii) Increase in the activity of sympathetic vasodilator fibres; and

(iii) Release of chemical substance, *bradykinin* from the sweat glands having a vasodilatory action.

External insulation: In homeotherms the skin is provided with structures like feathers, fur, hair, etc., which function as insulators. The effectiveness of these insulating structures is enhanced by the sympathetic nervous system. Air, which is a bad conductor of heat, is trapped between the feathers or furs of the skin and acts as a barrier for the loss of heat.

Chemical Heat Regulation

Practically all the heat in homeotherms is derived from the oxidation of foodstuffs. Although every tissue contributes to heat production by oxidative mechanisms, the skeletal muscles contribute the largest amount. Heat production mechanisms involve two processes.

Heat Production Due to Muscular Activity: In poikilotherms the chemical action varies directly with the temperature of the reacting agents. The heat production is correspondingly reduced followed by a fall in the temperature. In cold environments, the rate of metabolism gradually declines in cold blooded animals. In cold environments, homeotherms show muscular activity to increase heat production. Cold produces "shivering" which may increase heat production between 2 and 5 times the basal level. This involves the somatic nervous system. On the other hand, exercise is able to remove shivering by producing more heat and increasing the rate of heat loss. However, additional heat produced as a result of exercise does not have a thermoregulatory significance.

Nonshivering Thermogenesis: In many mammals production of heat is stepped up without involving muscular exercise. In resting or fasting state also, heat is produced at a steady level. Nonshivering thermogenesis helps in acclimatization of mammals to low temperatures. Exercise has no effect on nonshivering thermogenesis.

Heat production during nonshivering thermogenesis involves some change in the intermediary metabolism which may be brought about by calorigenic action of hormones or brown fat.

Calorigenic action of hormones: Cold acclimated rats are able to utilize and synthesize more glucose which is a consequence of hormonal regulation. Injection of norepinephrine in cold acclimated rats shows a calorigenic action by enhancing body temperature and oxygen consumption. Thyroxine also augments oxygen consumption increasing the heat output by accelerating the metabolic rate.

Brown fat: In the young of many mammalian species (especially primates and rodents), brown fat tissue occurs which is highly vascular and multilocular. It is well developed in hibernating mammals and is an important site of nonshivering thermogenesis. The brown fat deposits are located around the neck, thorax and major blood vessels. During prolonged low temperature exposures of the body, brown fat deposits are increased. The brown fat has a rich blood supply and owing to this the average oxygen consumption of it is higher than the rest of the tissues. The heat produced in the brown fat is transported to the brain and head through blood circulation.

10.7 TEMPERATURE RELATIONS OF HETEROTHERMS

Certain mammals belonging to the class Prototheria and Metatheria (e.g *Echidna, Ornithorhynchus,* Armadillos, Opposums, three-toed sloths, etc.) have low body temperatures in relation to their environments and show a wide range of temperature and metabolic fluctuations. Such animals are called heterotherms. Some parts of the body like legs, tail, ears, etc., have poor insulation as compared to other parts and as such the temperature of these parts is lower than the core temperature. *Echidna* has its body temperature at 34°C in relation to its environmental temperature which is 35°C. In contrast to homeotherms, if the external temperature falls, the body temperature of

Echidna also falls considerably. Some endotherms show diurnal variations in their body temperature. Small mammals and birds come under this category. In herring gulls, the core temperature ranges from 38°C to 41°C, but temperature of some peripheral parts ranges between 6 and 13°C. The gulls can walk on ice at temperature –30°C, but if the gulls are acclimatized to warm laboratory conditions and then allowed to walk on ice, their feet are frozen. In some birds the diurnal variations are correlated with the activity during day as compared to the activity at night.

10.8 THERMOREGULATORY CONTROL CENTRE

Endotherms maintain a stable core body temperature and in order to achieve this a thermoregulatory control centre becomes operative to balance heat production with heat loss. This is controlled by the nervous system. Voluntary muscular activities or shivering enhance heat production and both these activities are effected through motor nerves. Heat loss can be altered by varying the amount of blood flowing through the skin or it can be increased by sweating. These activities are under the control of sympathetic nervous system. Blood flow through the skin is able to maintain small adjustments in the body temperature; however, larger adjustments are possible through shivering or sweating.

In homeotherms thermoregulatory control centres are located in the hypothalamus which integrates the incoming sensory information through temperature receptors. There are two kinds of thermoreceptors, viz. the peripheral thermoreceptors, and the central thermoreceptors. Peripheral thermoreceptors are distributed all over the body surface and in certain portions of the alimentary canal. Central thermoreceptors are situated in the body core.

The Hypothalamus

The hypothalamus is a small part of the brain situated below the thalamus. It forms the floor and part of the lower central walls of the third ventricle. It is important in the internal regulation and contains temperature sensitive cells constituting the thermostat of birds and mammals. It also contains osmoregulatory receptors. Hypothalamus also exerts control on the endocrine activity of the body by influencing the pituitary function. Thus it is obvious that the hypothalamus controls the autonomic and endocrine functions of the body. Food and water intake, sexual behaviour, sleep and emotional responses are also under the control of hypothalamus.

Stimulation Of The Hypothalamus: Hypothalamus contains thermoregulatory centres which can be stimulated by electrical or thermal stimulus. Techniques have been evolved by placing fine electrodes in the hypothalamic areas to localize temperature sensitive centres. It has been demonstrated that there are two centres in the hypothalamus concerned with temperature regulation. These are an anterior "heat loss" centre and a posterior "heat production" centre.

The anterior part of the hypothalamus behaves as a thermotaxic centre. It receives afferent impulses from temperature-sensitive receptors in the skin and probably in the muscle in response to which it is able to control loss of heat by sweating. Electrical stimulation of the anterior heat loss centre causes vasodilation in the skin, vigorous panting and cessation of shivering. Stimulation of the posterior "heat production" centre causes vasoconstriction of blood vessels in the skin, enhances shivering and inhibits panting.

The two regions of the hypothalamus concerned with the responses to hyperthermia and hypothermia are anatomically interconnected. Hyperthermia activates the heat loss centre whereas hypothermia activates heat production centre. Thermoregulatory centres in the brain can be activated by thermal receptors in the skin or by changes in the temperature of the blood.

A study of the electrical recordings of the hypothalamus has shown that there are at least three types of cells which show thermal sensitivity. These are:

(a) *Heat receptors*—cells which increase their activity when the hypothalamic temperature increases, but skin temperature does not affect them.

(b) *Cold receptors*—cells which increase their discharge when hypothalamic temperature is lowered and remains unaffected by changes in the skin temperature.

(c) *Mixed receptors*—cells which respond to increase in skin temperature, but further increase their discharge when the hypothalamus also gets heated.

Thermal receptors in skin: Skin contains heat and cold receptors. Heat receptors are situated deeper in the skin while the cold receptors are superficial in location and generally more abundant. Many of these receptors are in the form of naked nerve endings. By increasing environmental temperatures, the skin temperature is also raised causing increased discharge from the heat receptors momentarily (about 2-3 seconds) and then settles down to the frequency corresponding to the temperature. Upon withdrawal of the heat stimulus, the heat receptors reduce their frequency of discharge. Similarly cold receptors can be made to discharge at a higher frequency by lowering the temperature until they acquire adjustment to altered temperature. When the cold stimulus is withdrawn, the cold receptors are temporarily withdrawn.

10.9 TEMPERATURE REGULATION IN ENDOTHERMS

Birds: Generally birds have a higher core temperature than the mammals. This is advantageous to birds in hot weather, especially for those living in arid climate. However, the temperature range of desert and non-desert species falls within the same range. The upper lethal limits for desert as well as non-desert birds show marked diurnal cycles in body temperature with a narrow variation of 2-3°C. Muscular activity increases body temperature temporarily.

In hot surroundings birds lose more water through respiration. In certain cases (American Cardinal) at higher ambient temperature increased respiratory activity results in water loss at least four times between 34°C and 40°C. This is *evaporative cooling.*

Heat transfer in birds: Besides evaporative cooling, there are several devices which facilitate heat transfer to the environment. Such devices include holding the wings away from the body to expose body parts, compression of plumage, increased blood flow to the legs and to the combs which increase thermal conductance. In addition to this there are certain behavioural aspects involved in temperature regulation. Many birds move into the shady area during day and reduce heat gain. Soaring birds ascend to high altitudes to escape the heat of lower altitudes. Diurnal birds keep their activities at a bare minimum in hot summer days to reduce metabolic heat production.

Mammals: The core body temperatures of most of the mammals fall between 35°C and 40°C, which is generally higher than the prevailing ambient temperature. Therefore, temperature regulation

in mammals mainly concerns with ecological and morphological adaptations. Mammals continuously lose heat to the environment by heat transfer mechanisms. The thermoregulatory process concerns with (1) control of rate of heat loss to the environment and, (2) increasing heat production.

Mammals living in cold regions maintain a uniformly high temperature by controlling heat loss and increasing metabolic heat production. The greatest contrast between poikilotherms and homeotherms is in the heat production in relation to external temperatures (Fig. 10.3). During intense cold the body temperature of mammals is maintained fairly constant. This is made possible by (a) efficient body insulation consisting of hair, fur, subcutaneous fat, etc, (b) effective vasomotor control and counter-current heat exchanges in the vascular system, and (c) decreasing cold sensitivity of peripheral regions.

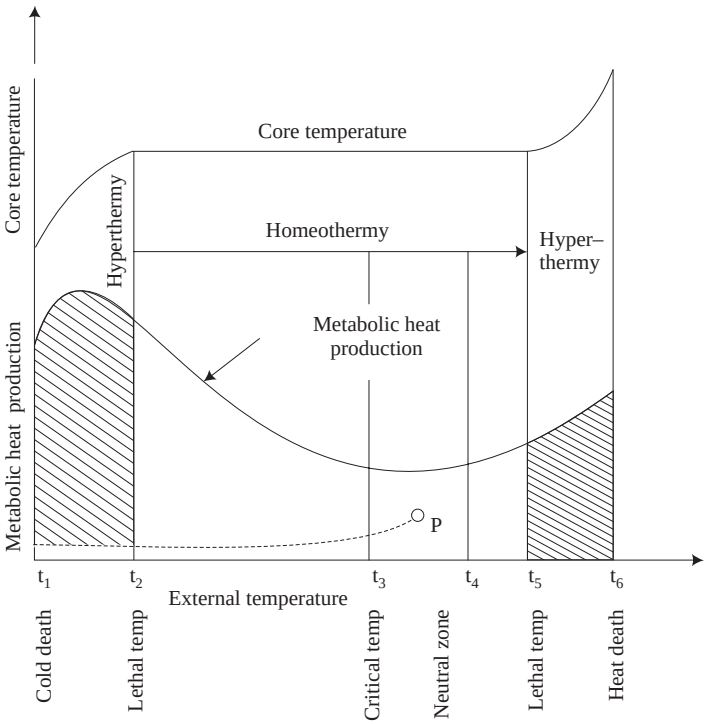


Fig. 10.3 Pattern of heat production in respect of variations in body temperatures of homeotherms in relation to variable external temperatures. The dashed line P shows the heat production in poikilotherms (Adapted from Hoar, 1966).

In desert mammals, the problem of temperature is rather acute since the ambient temperature exceeds body temperature. Under such circumstances heat moves from the environment to the body. Since heat transfer takes place in a reverse direction, cooling of the body is brought about by evaporation.

Adaptations to High Temperatures

Thermoregulation is a problem for endotherms especially in desert regions where animals are faced with intense heat. The ambient temperature exceeds the core body temperature and under such circumstance heat moves from the environment to the body. Two physiological mechanisms are said to be functioning:

1. Control of the rate of heat loss.
2. Transfer of endogenous heat against the thermal gradient from the body to the hot environment by evaporation to keep the body cool. In deserts, water is in short supply, hence animals cannot afford to lose water. The physiological solutions to meet this handicap are, however, not yet available. Deserts abound in herbivorous mammals which support many carnivorous birds, reptiles and mammals.

Adjustments of animals to intense heat of the desert is a problem of adaptation. Deserts are usually located far away from the equator and experience marked seasonal changes in temperature. Besides, the animals face a diurnal temperature cycle, that is during the day the temperatures are high, after sunset quite low. Nights are cold. Thus the animals do not face continuous stress of heat. Therefore, vertebrate animals living in the tropical deserts adjust to the extreme climatic conditions by behavioural means according to their physiological capabilities aided by the complex nervous system. Thermoregulatory problems of desert animals fall under three categories:

(a) Relaxation of thermal limits during which homeostatic control is maintained.

(b) Behavioural adjustments predominate thermoregulatory devices.

(c) Special structural and functional adaptations develop.

These aspects will be considered here in respect of desert mammals and birds.

The kangaroo rat (*Dipodomys*) is a small nocturnal desert mammal. This rat cannot withstand high temperatures of the day, hence spends the hot day time underground retreating in the burrows where humid conditions prevail. After sunset when the temperature comes down, the rat comes out of the burrow. In this way, this small animal avoids evaporative cooling. This adjustment in the kangaroo rat is of utmost importance since it cannot obtain its water requirement by drinking due to non-availability of water. Water requirements, however, are partially met with from dry seeds and plants on which it feeds, or from the metabolic water by oxidation of foods.

Camel is yet another example exhibiting desert adaptations and physiological mechanisms which enable it to survive most successfully in high temperatures of desert. Camel is famous for travelling long distances without drinking any water for days together. Schmidt-Nielson has explained certain physiological mechanisms existing in the camel. According to him, and contrary to the popular belief, camel does not have any storage space for water in its body.

Camel can tolerate very high environmental temperatures. This is because it has an unusually high core temperature. When camel has no access to drinking water, the core temperature during the day time may exceed 40°C, and when it has access to water the core temperature may be around 34°C. Variation in core temperature is a means to conserve water by storing heat during the day. It has been estimated that in a camel weighing about 500 kg, a rise of 6°C in core temperature would help in storing about 2500 kcal. Besides this thermoregulatory mechanism, camel's fur also acts as an

effective barrier to heat transfer. It helps conserve water and retards heating up of the body. As stated earlier, the camel does not have any storage capacity of water and avoids evaporation of water by diurnal variations in the core temperature. Nevertheless, it does lose some water by way of urination and respiration. It can withstand dehydration up to 25-30 per cent during long journeys across the desert and avoids heat stress. When water is available camel can recover from dehydration by drinking large quantities within a short time (see Chapter 8 also).

Birds have excelled themselves from the mammals in matters of adaptation to high temperatures and high rate of heat production. They are more prone to meet the challenges of the environmental temperatures. Birds have a temperature range of 39-45°C and therefore can sustain in areas of intense heat. In certain cases when the ambient temperature is higher than the body temperature, birds can lose heat by physical processes like conduction, convection and radiation. Although birds have a high body temperature, they neither lose heat nor resort to sweating. However, evaporative cooling, as it occurs in vertebrates, is facilitated. The thick covering of feathers acts as an insulator and affords minimum water loss. Nevertheless, water is lost from the body from the buccal cavity and the respiratory system while the bird pants. Panting increases breathing movements. In tropics and temperate zones during summer, humidity is high owing to which birds lose about half of their body heat by evaporation. In moderately high humid environments the body temperature of birds rises resulting in hyperthermia, facilitating passive heat loss from the body. In avian hyperthermia there is heat loss, whereas in the camel hyperthermia is caused by heat storage. In extremely low humid environments, the body temperature of some birds is maintained below the ambient temperature. This results in panting, and in order to sustain this some birds have evolved a novel method of *gular flutter* requiring less expenditure of energy.

Effect of Cooling

Cold receptors upon stimulation evoke reflex responses to conserve heat. As a result of stimulation of cold receptors, constriction of blood vessels supplying to the skin takes place reducing heat loss considerably. Cold may bring about erection of hairs, feathers and increased muscular activity. The blood temperature is lowered consequent upon which heat regulating centres become operative followed by shivering.

Shivering steps up metabolic rate to produce more heat. It is believed that the adrenal cortex is stimulated by exposure to cold which liberates noradrenaline. Increased metabolic response is due to the combined calorigenic action of adrenaline and the thyroid gland.

Adaptation to Cold Environments

In order to cope up with energy requirements, endotherms consume more food in cold climate. However, a rich food supply cannot be always ensured, hence certain adaptive patterns have been evolved by small mammals and such patterns are grouped under adaptive hypothermia.

Hibernation: Hibernation or winter dormancy is a phenomenon in which the body temperature falls to a low level in accordance with the ambient temperature during cold periods. It is a pattern of adaptive hypothermia usually found in small mammals like rodents, insectivores and bats. In these animals, the climatic stress and food shortage poses a problem of survival and as such the animals

behave as poikilothermic during cold weather. The hibernants show a number of physiological attributes which are as follows:

(a) The core body temperature falls 1-2°C below the environmental temperature.
(b) Oxygen consumption is reduced to as low as 5 per cent of the basal metabolic rate.
(c) Breathing rates are considerably reduced, sometimes prolonged suspension of respiration takes place.
(d) Heart rate of the animal is markedly reduced, as low as 5-6 beats per minute. However, the blood pressure remains adequate.
(e) A condition of *torpor* or profound sleep occurs during hibernation.
(f) Spontaneous arousal from torpor is possible in re-establishing high body temperatures of the endotherms by stepping up heat production.

The adaptive responses described above are found in various types of hypothermia which have been evolved independently in different orders under the influence of ecological and physiological stresses. We can take the case of small birds and mammals which maintain normally high body temperatures while active. During periods of inactivity, body temperature and oxygen consumption drop to a low level. These animals have restricted feeding habits and as such undergo daily periods of torpidity (birds feed during the day time and remain inactive during night). In regions of low temperatures small mammals undergo prolonged periods of hypothermia (dormancy) in contrast to mammals having daily torpor. Hibernation is an adaptive challenge requiring a previous preparation before entering hibernation. Before winter dormancy starts, the animals store large amounts of fats and experience periods of lethargy followed by long periods of dormancy.

Arousal from hibernation: It is a complex process. The hibernants arouse from their winter sleep several times and the period of arousal may last from a few hours to several days. This period is utilized for elimination of metabolic wastes and sometimes to consume food previously stored in the hibernating site. The cause of arousal has been attributed to shivering and nonshivering thermogenesis resulting in spurts of heat production and oxygen consumption.

In contrast to hibernation, many animals respond to periods of drought or high temperature and aestivate to avoid hot climatic stress. Ground squirrels of the genus *Citellus,* which have a cosmopolitan distribution, hibernate during winter and aestivate when serious drought prevails. Many endotherms such as rodents, insectivores, marsupials, etc., aestivate.

10.10 ACCLIMATIZATION

Acclimatization is the term often used to denote adjustments to temperatures in the field in natural environment, whereas *acclimation* is the term used for adjustment in laboratory conditions. Changes in the climate are always associated with the changes in the metabolic rate of the animal. An animal's life can be studied in relation to its environment including temperature, oxygen supply, availability of food and water, etc. If an animal is kept in a new environment other than its own, it may show adjustments for its survival, failing which the animal may die. Some poikilotherms show an abrupt increase in their metabolic rate when external temperature is raised and on cooling show a sudden decline. These changes in the metabolic rates are described as *compensation* which are a

consequence of acclimation. When the animal is returned to its normal temperature, the rate of reactions does not return to the original level, but may be higher or lower as the case may be depending on the direction of acclimation.

Thermal Acclimatization

The problem of thermal acclimatization has a compensatory influence of temperature on the metabolic rate. Amphibians can tolerate high temperature which is in essence a consequence of acclimatization.

Reptiles: Reptiles enjoy a transitory position in the animal world. They are believed to have given rise to birds and mammals. With the exception of a few aquatic reptiles (crocodiles and turtles), they are undoubtedly terrestrial in habits and show beginnings of some physiological thermoregulatory mechanisms. Although thermal regulation of reptiles resembles that of amphibians, they are capable of varying their temperature by habitat selection. They show many behavioural adaptations and evolution of physiological capabilities anticipating thermoregulatory mechanisms of birds and mammals.

Lizards kept in laboratory under constant conditions acquire body temperature equal to that of the environment and thus behave as poikilotherms. However, if the lizard is kept in its natural habitat it may behave differently by employing a thermoregulatory behaviour. Many snakes and lizards move into the sun if the air temperature is lower than the body and gain heat. When the body temperature rises above the preferred temperature, they again move in the shade and lose heat. Thus they maintain their body temperature within a narrow limit by altering their behaviour. In desert reptiles thermoregulatory behaviour is more efficient to suit various preferred temperatures. The American desert iguana, *Disosaurus,* can adjust to temperature as high as 48°C which is more than birds and mammals.

Physiological mechanisms involved in temperature regulation of reptiles are still at infancy stage. Poor insulation of the skin allows a quick heat loss or heat gain. The heating and cooling rates are under cardiovascular control. During eating, the heart rate is faster than at cooling. Nevertheless, the rate of heat exchange between the lizard and the environment depends on the blood volume flowing per unit time between core and the surface. Obviously, the circulation is faster during heating and slower during cooling. The Galapagos marine iguana *(Amblyrhynchus cristatus)* maintains its body temperature at about 37°C by behavioural regulation.

CHAPTER

11

Body Fluids

About 70-90 per cent of the total body weight of organisms consists of body fluids which circulate in the body. The fluid remains in a state of motion and flows through a network of vessels (open or closed) so as to reach every part of the body. Motion of the body fluid remains in a state of dynamic equilibrium so long the animal is alive. The composition as well as the volume of these body fluids is also maintained in a steady state. In aquatic animals, which are living constantly under water, volume of the internal fluids remains constant irrespective of changes in the pressure of air or water. Therefore the internal pressure and the volume of the fluids inside the body remain constant.

11.1 MAJOR TYPES OF BODY FLUIDS

Within the body of animals, body fluids occur either as *intracellular fluids* or *extracellular fluids.* The intracellular fluids are present within the cell which are responsible for metabolic reactions. Extracellular fluids, which appear outside cells and within tissues, provide nourishment to the cells and also serve to eliminate waste products. In protozoa, only intracellular fluid is present which is responsible for all the exchange mechanisms. However, the metazoans possess extracellular fluids because of their increasing complexity in structure. In metazoans, extracellular spaces occur prominently in the form of lumen and cavities and the fluid circulating in these spaces is compartmentalized. In acoelomate animals, the spaces appear as interstitial channels, whereas in coelomates additional circulatory system is present.

The extracellular fluid can be classified into several types.

In many pseudocoelomates like nematodes, entoprocta and rotifers, the extracellular fluid is known as *coelomic fluid.* It is a watery fluid which supplies nourishment to different parts of the body. It is also responsible for removing the wastes, but has no role in respiration.

In arthropods and molluscs (except Cephalopods), the extracellular fluid is called the *haemolymph* which circulates in open channels. Although watery in consistency, it possesses some

pigments also. Owing to the presence of these pigments, this fluid serves for nutrition as well as for respiration. It is rich in proteins.

Lymph is a colourless fluid which is found in all vertebrates except cyclostomes and elasmobranchs. It circulates in a well developed network of lymph channels which are extremely thin-walled with a true endothelial lining. The channels originate in connective tissue spaces.

The lymph is similar to the plasma of blood in composition and contains lymphocytes and granulocytes. The lymphatic system is an open system consisting of anastomosing channels and lymph sinuses occurring beneath the skin, in the muscles, in the walls of the digestive system and around the nervous system. Lymph fluid from the sinuses flows into the lymph vessels and from there enters the blood veins. At the junctions where the lymph vessel joins the vein there are small contractile *lymph hearts* which ensure a continuous flow of lymph into the blood vascular system. Lymph hearts are present in lower vertebrates, such as fishes, amphibians and reptiles. They are absent in birds and mammals.

In mammals, lymph nodes or *lymph glands* are present which are solid bodies made up of connective tissue and placed in the way of lymph vessels. These nodes contain aggregations of lymphocytes which are formed inside them, and as the lymph flows past these nodes they are carried away into the lymph circulation. Lymphocytes are a type of white blood corpuscles. Lymph nodes also act as "filter" organs which prevent harmful substances from reaching the blood vascular system.

The flow of lymph is regulated by valves which allow it to go from smaller to larger vessels. Movements of muscles, viscera, etc., pressing upon the delicate walls of the lymphatics, squeeze the lymph.

Lymphatic system has many functions. One of the important functions of lymph is to convey proteins to the blood plasma. The lymphocytes produced in the lymph nodes are responsible for the destruction of foreign bodies and harmful bacteria. They also produce antibodies. Another function of lymph is to convey lipids from the intestine to bloodstream.

Transcellular Fluids

These are certain specialized cavities or spaces in the body which contain fluids differing from each other in their composition. Such cavities are distinguished as pleural, pericardial or cerebrospinal cavities containing transcellular fluids. These fluids lubricate the organs contained in the cavities and provide protection against shocks and injury.

11.2 BLOOD

Blood is a liquid tissue which flows through a network of closed circulating channels. The chief liquid component is the blood plasma containing blood cells. Because of its physiological significance, blood of humans will be dealt with in somewhat greater details.

Functions of Blood

Since the blood is a circulating fluid and almost every organ receives a blood supply, it performs a number of vital functions in the body which are as follows:

(1) *Respiration:* Transportation of oxygen and carbon dioxide is the fundamental function of the blood. Transport of oxygen from the lungs to different tissues, and the transport of carbon dioxide from the tissues to lungs is mainly effected by the blood.

(2) *Transport of food materials:* Blood is the only medium by means of which the absorbed food materials are transported to various parts of the body.

(3) *Excretion:* Metabolic wastes like urea, uric acid, creatine, water, carbon dioxide, etc., are transported by blood, to kidneys, lungs, skin and intestine for removal.

(4) *Regulation of body temperature:* The blood has an important role in the regulation of body temperature by distributing heat throughout the body. This heat is generated in the muscles by the oxidation of carbohydrates and fats.

(5) *Maintenance of acid-base balance:* The blood has buffering capacity and maintains normal acid-base balance in the body.

(6) *Regulation of water balance:* Blood serves to maintain water balance in the body by exchanging water between the blood and the tissue fluid.

(7) *Defense:* Blood affords protection to the body against infections and the antibodies.

(8) *Transport of hormones:* Blood is the only medium which serves to distribute hormones to different parts of the body.

(9) *Clotting:* Loss of blood from the body through injury is prevented by the action of thrombocytes of the blood.

(10) *Transport of metabolites:* Blood is responsible for the supply of chemicals and essential metabolites.

11.3 GENERAL PROPERTIES OF BLOOD

Colour: Colour of the blood is generally red which depends on the nature of the haemoglobin, a red pigment within red corpuscles. The venous blood has less redness and more blueness as compared to the arterial blood which is oxygenated.

Blood is a liquid tissue mainly consisting of the *plasma* and the blood corpuscles floating in it. Normally in a healthy person total blood volume varies from 6 to 8 per cent of the body weight. About two-thirds of the total blood is plasma and one-third is the corpuscles. The volume of blood in human body is about 8 per cent of the body weight. Thus, if a man weighs 80 kg, his body would normally contain about 6.4 kg of blood.

Specific Gravity: Specific gravity of the blood largely depends upon the number of red cells. The specific gravity of normal blood is 1.06, but may vary from 1.05 to 1.06.

Osmotic Pressure: Osmotic pressure of the blood is about 28 mm of mercury. This osmotic pressure is due to the presence of various salts, waste substances, proteins, and sugars dissolved in the plasma.

The pH: The pH of the blood is about 7.35, that is, it is a weak alkaline solution. Blood has a self-buffering capacity and the pH is maintained well within limits. A pH of 8 or much below 7 would be fatal for an individual.

11.4 COMPOSITION OF BLOOD

Oxygenated blood is about 3-4 times more viscous than water. If the blood is centrifuged, two distinct fractions can be removed: (a) the *plasma* and (b) blood cells called the formed elements.

The Plasma

The plasma is a homogeneous fluid, pale yellow in colour and alkaline in reaction. Under normal conditions, in man plasma forms 55-60 percent of the total volume of blood. It is composed of about 91 per cent water and 9 per cent of solid materials, out of which about 7 per cent are proteins only. The composition of the blood in man has been thoroughly worked out, hence the description presented here would relate to human blood only.

Plasma Proteins: The total protein concentration in the plasma is about 7gms/100 ml. All proteins may be separated by precipitation by different salt concentrations and the relative amounts of different proteins may be accounted for by paper electrophoresis. Four major categories of plasma proteins are known: albumins, globulins, fibrinogen and haptoglobins. Various fractions of these proteins are shown in Table 11.1.

Table 11.1 Composition of Plasma Proteins in GMS/100 ML of Fresh Blood

Plasma proteins	*Normal values gms/100 ml*
Total proteins	6.3-7.8
Albumins	3.2-5.1
Globulins	2.5
α_1- Globulins	0.06-0.39
α_2- Globulins	0.28-0.74
β- Globulins	0.69-1.25
γ- Globulins (Immunoglobulins)	0.8-2.0
IgA	0.15-0.35
IgG	0.8-1.8
IgM	0.08-0.18
IgD	0.003 approximately
Fibrinogen	0.2-0.4
Mucoprotein	0.135 approximately
Haptoglobins	0.03-0.19

The concentration of plasma proteins remains constant even during dietary variations and abnormal conditions. Prolonged malnutrition, however, affects the protein concentration. All types of plasma proteins can be isolated and quantitatively determined by paper electrophoresis. This method commonly employed in clinical work is known as immunoelectrophoresis. This is a semiquantitative method, but zone electrophoresis in starch or polyacrylamide gel gives a better and accurate separation.

Albumins: These have the lowest molecular weight (69000), and are synthesized mainly in the liver. Albumins have a half-life of 17-20 days and about 10-12 gms of these are produced every day in a healthy person. In certain abnormal conditions the albumin content of the plasma is lowered; concentrations below 2 gms/100 ml are always associated with oedema.

Globulins: The molecular weight of globulins varies between 90,000 and 100,000. They are separable into a number of sub-fractions (Table 11.1). α-and β-globulins carry the lipid fraction of proteins, while gamma globulins contain antibodies for generating immune responses. Variation in various fractions of globulins is of diagonostic importance. Globulins are synthesized in the reticuloendothelial system, in macrophages and in the lymphocytes.

Fibrinogen: This is essentially a type of globulin of a high molecular weight, 400,000. It is a precursor of fibrin in the blood coagulation process. Fibrinogen is exclusively formed in the liver, but its increased concentration in the plasma is associated with a rapid erythrocyte sedimentation rate.

Haptoglobins: Four types of haptoglobins are known so far that are present in varying combinations. They have the property of binding small amounts of haemoglobin, about 1.35 mg/100 ml of haemoglobin can be bound in this way.

Plasma proteins have several important roles. Firstly, they help in maintaining colloid osmotic pressure of the plasma. The osmotic pressure of the blood is about 28 mm of mercury under normal circumstances. Secondly, plasma proteins are essential in immune reactions of the body. Thirdly, protein deficiency of food is made good by utilizing the plasma proteins. And, above all, plasma fibrinogen is essential in clotting of blood.

Plasma carbohydrates and fats: There are small amounts of glucose and fats dissolved in the blood plasma. Glucose concentration in plasma varies considerably due to metabolic disorders. There are, however, several devices which maintain the glucose concentration in blood within normal limits. The fats are in the form of neutral fats.

Inorganic ions: Inorganic salts of iron, calcium, potassium, magnesium and sodium in the form of chlorides, sulphates carbonates and phosphates are present in concentrations which, under normal circumstances, do not vary much and behave as electrolytes.

Plasma Nitrogen: Plasma nitrogen is present in the form of urea, uric acid and other non-protein compounds. Besides these, fairly good amount of nitrogen is present in plasma proteins.

Other substances: Gases like oxygen, carbondioxide are found in the plasma. Certain hormones are also present in the plasma which include adrenaline, nonadrenaline, androgens, oestrogens, insulin, adrenocorticotrophic hormone, thyroxine and pituitary hormones. A very large number of enzymes are found in the plasma. Some important enzymes are: alkaline phosphatase, aldolase, dehydrogenase, glucose-6-phosphatase, lipase, maltase etc. All the vitamins are also present in the plasma.

Non-protein constituents of the whole blood and the plasma are given in Table 11.2.

Blood Electrolytes: Blood is rich in anions and cations which function as electrolytes. The average concentrations of these in the plasma and cells are given in Table 11.3. The plasma should be electrically neutral, that is, the number of anionic charges must be equal to the number of cationic charges. Besides, the ionic concentration should be independent of strong electrolytes, that is to say, the presence of Na^+, CI^- and HCO_3^- ions should be independent of sodium chloride and sodium

Table 11.2 Non-protein Organic and Inorganic Constituents of Whole Blood and Plasma Values in mg/100 ml

Constituents	*Whole blood*	*Plasma or Serum*
Non-protein nitrogen		
Total - N	28 - 39	22 - 29
Urea - N	8.9 - 15.2	9.6 - 17.6
Amino acid - N	4.6 - 6.8	4.3 - 7.7
Creatine - N	1.0 - 1.6	0.3 - 0.4
Creatinine - N	0.4 - 0.6	0.4 - 0.5
Uric acid - N	0.3 - 1.3	0.3 - 1.3
Nucleotide - N	4.4 - 7.4	0.2 - 0.4
Ammonia - N	0.01 - 0.02	0.01- 0.02
Bilirubin (total)	—	0.26 - 1.4
Bilirubin (direct)	—	0.1- 0.5
Carbohydrates		
Glucose	60 - 90	120 (maximum)
Glycogen	1.6 - 16.2	—
Hexosamine	—	83.4
Pentoses	—	1.80 - 3.29
Hexuronic acids	4.1 - 9.3	0.4 - 1.4
Neuraminic acid	—	60
Lipids		
Total, ether soluble	397 - 722	400 - 700
Neutral fats	85 - 237	0 - 450
Fatty acids	290 - 420	200 - 450
Non-esterified fatty acids	—	10 - 17
Cholesterol total	129 - 228	120 - 250
Free cholesterol	80 - 110	30 - 60
Bile acids	2.5 - 6.0	0.2 - 3.0
Bile salts	—	5 - 12
Phospholipids total	—	150 - 250
Metabolic products		
Acetone bodies	0.5	0.3 - 0.9
Pyruvic acid	0.41- 1.11	0.5 - 1.0
Lactic acid	4.7 - 15.1	6.1 - 16.9
Citric acid	1.3 - 2.3	1.6 - 3.2
Malic acid	0.24 - 0.75	0.01 - 0.9
Inorganic materials	0.8	0.9

bicarbonate. In the blood sodium ions are mainly extracellular and potassium ions intracellular, the distribution of which are maintained by active transport. The kidney is also responsible in controlling the cations in the blood through acid base balance and hormonal influence.

Table 11.3 Principal Blood Electrolytes in One Litre of Whole Blood (1 Litre of whole Blood = 550 ml Plasma Plus 450 ml Cells).

	Plasma constituents		*Cell constituents*		*Total*
	mEq/1Plasma	*mEq/1 whole blood*	*mEq/1 cells*	*mEq/1 whole blood*	*mEq/q whole blood*
Cations					
Na^+	135 - 155	79.8	16 - 25	9.5	89.3
K^+	3.1 - 5.5	2.3	92 - 100	42.8	45.1
Ca^+	2.1 - 2.7	1.4	—	—	1.4
Mg^+	1.3 - 1.8	0.9	2.5	1.1	2.0
Total	153.3	84.3	118.5	53.3	137.6
Anions					
CI^-	100 - 107	56.7	58.9	26.5	83.2
HCO_3^-	27.0	14.9	16.7	7.5	22.4
Phosphate	2	1.1	1.5	0.7	1.8
Sulphate	1	0.6	—	—	0.6
Plasma proteins	15.6	8.6	—	—	8.6
X^-	4.7	2.4	4.7	2.1	4.5
Haemoglobin	—	—	36.7	16.5	16.5
Total	153.3	84.3	118.5	53.4	137.6

There are two types of anions in the blood: These are anions of mineral acids like Cl^- and SO_4^{2-} which do not combine with hydrogen at the blood pH. Other anions pertain to organic acids which include lactate and pyruvate. The second type of anions comprise of the plasma proteins, haemoglobin, oxyhaemoglobin, HCO_3^- and PO_4^{2-} (phosphate). These act as blood buffers. Plasma proteins carry negative charges, hence called anions. Similarly, haemoglobin and oxyhaemoglobin also act as anions since their isoelectric point is lower than the blood pH.

11.5 FORMED ELEMENTS OF BLOOD

The formed elements or the structural components of the blood include the *erythrocytes* (red blood cells), the *leucocytes* (white blood corpuscles) and the *thrombocytes* (blood platelets) which are suspended in the plasma.

Haematologic Parameters of Red Blood Cells

A. *Red Blood Cells.* In mammals and the humans, erythrocytes are circular and biconcave, without a nucleus. Their size averages about 7.6 μ in thickness and about 8.8 μ in diameter. The stroma of red cells is permeated with haemoglobin, estimated to be about 90% of the weight of the cells. The red blood cell (RBC) count gives the number of RBCs found in a cubic millimeter (mm^3) of whole blood and provides an indirect estimate of the blood's haemoglobin content. Normal values are 4.5 to 6.0 million/mm^3 of blood for males: 4.0 to 5.5 million/mm^3 for females.

B. *Haematocrit* (Hct) or *packed cell volume* (PCV). It measures the percentage by volume of packed RBCs in a whole blood sample after centrifugation. It is determined by putting uncoagulated whole blood in a Wintrobe tube which is calibrated (Fig. 11.1). The tube is centrifuged for 30 minutes at a speed producing 1500 g and the percentage is calculated for the volume of red cells. The Hct value is usually three times the haemoglobin value and is given as percent. A low Hct indicates such conditions as anaemia or overhydration; a high Hct denotes such conditions as polycythemia or dehydration.

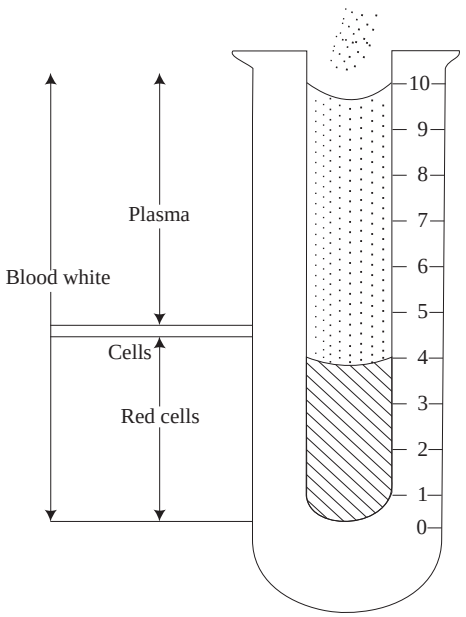


Fig. 11.1 Determination of Haematocrit. Whole blood is centrifuged in a tube and allowed to settle. The haematocrit, that is, the volume of red cells per 100 ml of whole blood, is obtained by multiplying the number at the top of the red cell fraction by 10.

C. *Haemoglobin* (Hb). Haemoglobin, a complex of protein (globin) and iron, is produced in the red cells and synthesised from acetic acid and glycine. The product is called a *porphyrin,* which combines with iron to produce a *haeme* molecule. Four haeme molecules then combine with one molecule of globin to form haemoglobin (Fig. 11.2). The molecular weight of haemoglobin is about 64,500. The haemoglobin content can be measured in a haemoglobinometer which measures the grams of haemoglobin contained in a 100 ml of whole blood and provides an estimate of the oxygen-carrying capacity of the RBCs. Normal values range from 14 to 18 g/100 ml for males and from 12 to 16 g/l00 ml for females. The high value depends on the number of RBCs and the amount of haemoglobin in each RBC. A low Hb value indicates anaemia.

D. *Wintrobe Indices* or *RBC indices.* They provide important information regarding RBC size, Hb concentration and Hb weight. They are primarily used to categorise anaemias.

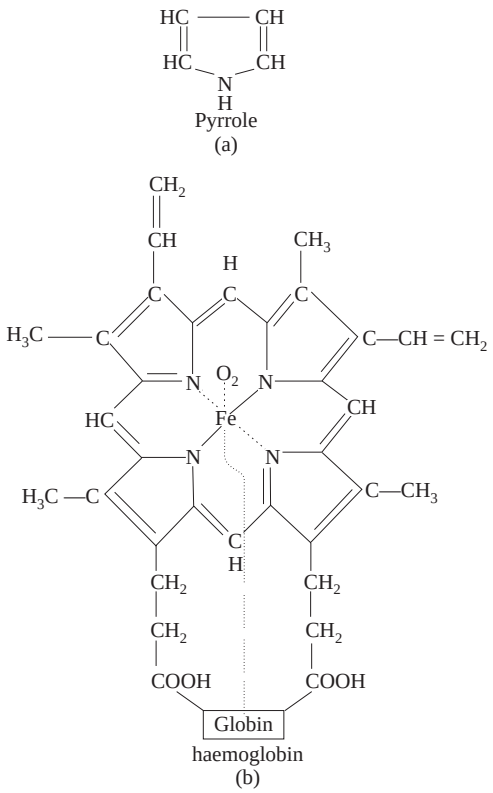


Fig. 11.2 Chemical structure of haemoglobin. (a) pyrrole ring, (b) haemoglobin molecule—the carbon atoms of the pyrrole rings carry groups which are either methyl (CH_3—), vinyl (CH_2 = CH—), or propionic acid ($HOOC{\cdot}CH_2$—CH_2) groupings.

(a) *Mean corpuscular volume* (MCV) is the ratio of the Hct to the RBC count:

$$\frac{\text{Hct (\%)} \times 10}{\text{RBC count (in millions)}} = \text{MCV}$$

The MCV value essentially evaluates average RBC size and reflects any variation in RBC size. A low MCV indicates *microcytic* (undersized) RBCs, as occurs in iron deficiency. A high MCV indicates *macrocytic* (oversized) RBCs, as occurs in vitamin B_{12} or folic acid deficiency. The normal value of MCV is 90 ± 10.

(b) *Mean corpuscular haemoglobin concentration (MCHC)* represents the average concentration of Hb in an average RBC, defined as:

$$\frac{\text{Hb} \times 100}{\text{Hct}} = \text{MCHC}$$

The normal value for MCHC is 34 ± 2. A low MCHC indicates *hypochromia,* resulting from decreased Hb content.

(c) *Mean corpuscular haemoglobin* (MCH) represents the amount of Hb in an average RBC and is defined as:

$$\frac{\text{Hb} \times 10}{\text{RBC count (in millions)}} = \text{MCH}$$

The normal range of MCH is 30 ± 4.

E. *Reticulocyte Count.* It is a measure of immature RBCs (reticulocytes), which contain remnants of nuclear material. They circulate in the blood for about 1 to 2 days in this form hence, this provides an index of bone marrow production of mature RBCs. Reticulocytes normally comprise 0.5% to 1.5% of the total RBC count, or 25,000 to 75,000/mm^3. An increased count occurs during haemolytic anaemia, acute blood loss and iron deficiency. Aplastic anaemia occurs due to a decreased count.

F. *Erythrocyte Sedimentation.* Sedimentation effect results from alterations in plasma proteins. Sedimentation rate can be found out from the RBC settling from whole, uncoagulated blood over time. Normal rates range from 0 to 15 mm/hr for males and from 0 to 20 m/hr for females. Erythrocyte sedimentation rate (ESR) increases in acute or chronic infections, rheumatoid etc. ESR is useful in demonstrating the presence of occult organic disease.

White Blood Cells (WBC)

The WBC are called *leucocytes,* which are slightly larger than the red cells. They are colourless or transparent and can be easily counted under microscope after proper staining. They are nucleated possessing one, two or more nuclei, and move about with pseudopodia. There are five main classes of WBC (Fig. 11.4). The normal range of WBCs is 5,000 to 10,000/mm^3. An increased WBC count signals infection, and a decreased count indicates bone marrow depression, resulting from viral infection or toxic reactions to chemical agents (Table 11.4).

Fig. 11.3 Types of leucocytes.

The WBC differential evaluates the distribution and morphology of five major types of WBCs—the neutrophils, basophils and eosinophils *(granulocytes)* and the lymphocytes and monocytes *(nongranulocytes).*

A. *Neutrophils* include polymorphonuclear leucocytes, also known as PMNs, and immature *bands.* Neutrophils stain with neutral dyes and phagocytize and degrade many types of particles. They serve as the body's first line of defense when tissue is damaged or foreign materials gain entry. They congregate at the sites of infection in response to a specific stimulus through a chemotactic action. Certain bacterial, viral and fungal infections cause an increase in their number resulting in *neutrophilic leucocytosis.* A decrease in the number of neutrophils may cause *neutropenia.*

Table 11.4 Percentage Values of WBC Cell Differential Count Under Normal and Infection Conditions

Cell type	*White blood cell count*	
	Normal percentage value	*With infection*
Total white blood cells	8,000 (100%)	15,5000(100%)
Neutrophils		
Polymorphonuclear leucocytes	50-70%	82%
Segmented bands (immature)	3-5%	6%
Lymphocytes	20-40%	10%
Monocytes	0-7%	1%
Eosinophils	0-5%	1%
Basophils	0-1%	0%

B. *Basophils.* They stain deeply with blue basic dyes and are few in number (1%). They are small and contain large granules; in tissues they are referred to as *mast cells.* An increased number of basophils *(basophilia)* may occur with chronic myelogenous leukemia.

C. *Eosinophils.* They stain deep red with acid dyes and are associated with immune reactions. They contain a bilobed nucleus and show phagocytic behaviour. An increase in the number of eosinophils, known as *eosinophilia,* occurs during acute allergic reactions (asthama, drug allergy etc.) and parasitic infestations.

D. *Lymphocytes.* They have a large bean-shaped nucleus with dominant role in immunologic activity. They produce antibodies. Two main classes are B lymphocytes and T lymphocytes; T lymphocytes are further subdivided into helper (Th) and suppresor (T_s) cells. An increased number of lymphocytes, called *lymphocytosis,* usually accompanies a normal or decreased total WBC count, caused by viral infections. A decrease in number, *lymphopenia,* may occur due to severe illness, immunodeficiency or the AIDS virus.

E. *Monocytes.* These are largest of all white blood cells, ranging from 16 to 22 μ in diameter, and characterised by the presence of a large, indented or horseshoe shape nucleus. They are phagocytic and destroy or devovour infectious agents. Monocytosis, i.e. increase in number of monocytes occurs during acute infections.

Thrombocytes or Platelets

The platelets, smallest formed elements of the blood (about 2 to 3 μ in diameter), are very fragile, irregularly shaped, containing distinct granules but no nucleus. They are involved in blood clotting and are vital to the formation of a haemostatic plug after vascular injury. The normal range of platelet count is 150,000 to 300,000/mm^3. However, their number may vary from time to time, usually increasing after exercise and haemorrhage. The platelets, formed inside the bone marrow, are derived *from megakaryocytes* by budding while passing through the walls of sinusoids. The life of platelets is about 10 days in the circulating blood, and in case of transfusion into a patient having no or low platetlets, their life-span is reduced to one or two days. A decrease in platelet count causes

thrombocytopenia, which can occur in conditions, such as idiopathic thrombocytopenic purpura, *dengue fever* (haemorrhagic fever caused by *Aedis aegypti),* or occasionally from drugs like quinidine and sulfonamides.

Besides their participation in clotting, platelets are also believed to possess the property of adhesion owing to which they are able to stick to foreign surfaces and plug up holes in capillaries. The platelets contain a thromboplastin precursor and quantities of histamine and 5-hydroxytryptamine, which are released during coagulation. Anticoagulants reduce the adhesion property of platelets.

Origin of Formed Elements

The process by which all types of blood cells are formed is called *haemopoiesis* and the organs where these are formed are called haemopoietic organs.

Erythropoiesis. The formation of erythrocytes or red cells is known as erythropoiesis. In the embryonic life, red cells are produced by liver, spleen and thymus and after birth they cease to produce them and the function is taken over by the red marrow of bones, ribs, vertebrae, and to some extent, with the exception of children, in the ends of the limb bones. The red cells pass through several stages of development before they enter the peripheral circulation. The RBCs originate from megaloblasts which are devoid of haemoglobin in early stages, but possess a nucleus. At a later stage, it acquires haemoglobin and undergoes reduction in size, a stage called proerythroblast, followed by normoblast stage (Fig. 11.4). Normoblast still has a nucleus which is gradually expelled in course of maturation. The cells, now called reticulocytes, are discharged in the bloodstream, and within few hours, lose their reticulated pattern and transform into mature erythrocytes.

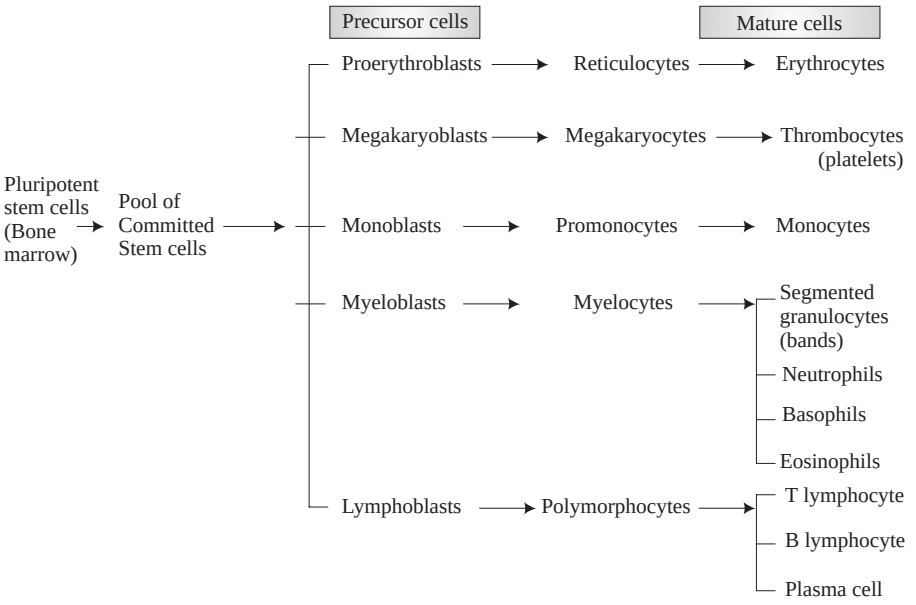


Fig. 11.4 Formed elements of the blood derived from the stem cells of bone marrow. Mature cells are found in the peripheral blood circulation.

Erythropoiesis or red cell production is controlled by a kidney-produced humoral factor called erythropoietin, whose primary action is considered to be the stimulation of cells in the marrow, producing increased number of erythroid elements. However, the kidney is not the only source of erythropoietin, since very small amounts of the hormone with the same marrow stimulating effect have been detected in the plasma of nephrectomised patients.

Erythrocytes have a short life-span, about 110 days, after which they are destroyed in the liver. Upon destruction, haemoglobin is liberated and undergoes changes to form *bilirubin,* that is finally excreted in the faeces. The iron component is set free and a good part of it is reused in the formation of new haemoglobin. Approximately 2.5 million cells are destroyed per second and almost equal number is produced each second. This mass destruction of erythrocytes produces about 250 mg bile salts per day.

Production of Leucocytes. The three varieties of granulocytes are formed in the bone marrow, whereas small lymphocytes are produced in the lymph nodes and lymphoid tissue, generally throughout the body. Reticulocytes are elements of *reticuloendothelial* system, consisting of primitive cells normally present in the general connective tissue, lung, spleen, liver, lymph nodes, bone marrow etc. There are many varieties of these, some of which are motile and possess phagocytic properties. Monocytes are also derived from reticuloendothelial system.

The reticuloendothelial cells in the blood sinuses in the liver are known as *Kupffer cells.* These as well as those of the bone marrow and connective tissues possess the ability to convert haemoglobin into bile that is liberated from erythrocyte disintegration. Spleen also contains *macrophages,* also called neutrophils, which are phagocytic. In embryonic life granulocytes are also formed in the spleen, but its ability ceases just before birth.

11.6 BLOOD GROUPS AND TRANSFUSIONS

The transfusion of whole blood into persons who have lost a great deal of blood due to haemorrhage or accidents brings up the problem of blood grouping or blood typing. Blood cannot be transfused indiscriminately and the donor's blood has to be matched with that of the recipient needing a transfusion. This is called *compatibility of blood.* If the whole blood of a wrong type is given, the red cells stick together or *agglutinate* causing plugging up of blood capillaries resulting in death of the recipient. Such a blood is incompatible.

The most outstanding land mark in the history of blood transfusion was the discovery of isoagglutination and A-B-O blood groups in human beings by Karl Landsteiner in 1900. His sensational discovery won him a Nobel prize. Subsequently, in 1902 Decastello and Sturli discovered the fourth and the rare blood group AB.

In 1927 Landsteiner and Levine discovered the M and N blood groups by injecting human red cells into rabbits. This produced antibodies M and N, resulting in the identification of M, N and MN types of human populations. In the same year, another blood group P was reported. In 1937 Landsteiner and Wiener discovered a blood group factor by injecting the red cells of Rhesus monkey into rabbits and designated it as *Rh-factor.* Subsequently, about a hundred of different blood factors belonging to about 12 blood group systems were described of which only a few are clinically important.

Inheritance of Blood Groups

Blood group factors are highly specific and follow a typical Mendelian inheritance pattern. In the A-B-O blood groups, suppose the three alleles A, B and O occur in one locus of a chromosome. The occurrence of the alleles is in such a way that only two alleles can combine in one individual. The possible gene combinations that may arise are: A A, B B, O O (homozygous) and A O, B O, and A B (heterozygous), assuming A and B are dominant and O recessive. The gene O is called an *amorph* since it does not produce any recognizable affects. The possible genotypes and phenotypes in the A-B-O system are shown in Table 11.5.

Table 11.5 Blood Groups-A-B-O and Possible Genotypes and Phenotypes

Blood groups	*Genotypes*	*Phenotypes*
A	AA or AO	A
B	BB or BO	B
AB	AB	AB
O	OO	O

Table 11.6 gives the possible genotypes of the children arising from matching in different blood groups.

Table 11.6 Blood Groups of the Offspring in A-B-O Matgsing

Blood group of parents	*Blood group of children*	*Blood groups that cannot be*
O × O	O	A,B, AB
O × A	O, A	AB, B
O × B	O, B	A, AB
O × AB	A, B	O, AB
A × A	A, O	B, AB
A × B	A, B, AB, O	None
A × AB	A, B, AB	O
B × B	B, O	A, AB
B × AB	A, B, AB	O
AB × AB	A, B, AB	O

Antigen-antibody Reactions

It has been found that the blood proteins are species specific which behave as foreign substances when introduced into the body of a different species. Such differences between proteins of different animal species can be detected by means of *precipitin* test. Introduction of a serum protein into the body of an animal to whom it is foreign has been studied in relation to immunological reactions. For example, if the blood of a patient suffering from pneumonia containing *Pneumococcus* is given to a healthy person, the normal reaction of the tissues is to produce antibodies which are specific to the

proteins of the infecting organisms. If the healthy person survives the disease, he is said to be immune from the infection. His blood contains antitoxins which neutralize the toxins produced by the invading bacillus.

There are two types of substances in the blood known as *antigens* (agglutinogens) and *antibodies* (agglutinins). The antigens are complex chemical substances which are present in the red blood cells. Chemically, the antigens are a combination of a protein component called *hapten* which cannot produce antibodies but can combine with them. The red cell antigens are present on the surface.

An antibody is a protein present in the serum globulin that is formed in response to an antigenic stimulus. Human serum globulins contain many antibodies of different types.

Almost all proteins act as antigens which react with antibodies if injected into an animal who does not possess specific proteins in its tissues. The reaction between a given antigen and its corresponding antibody is known as *antigen-antibody reaction.* Both antigen and antibody cannot be present in the same blood, for the red cells of a donor do not agglutinate by the serum of the recipient. There are two types of red cell antibodies present in the human sera: (1) Naturally occurring antibodies like anti-A and anti-B isoagglutinins (isoagglutinin is a agglutinin which reacts with the agglutinogen found in members of the same species) and, (2) Immune antibodies which are produced as a result of antigenic stimulus. The process of antibody formation is known as *immunization.*

If the antigen is derived from a member of the same species, it is known as *iso-immunization,* and on the other hand, if antigen is derived from a member of another species, the process is called *hetero-immunization.*

Agglutination

The red cell antigens when mixed with their corresponding antibodies on a slide cause clumping (agglutination) or haemolysis of the red cells. The antibody causing agglutination is called *agglutinin* and the antigen producing it is referred as *agglutinogen.* When the red cells burst open, haemolysis results and the antibody causing it is called *haemolysin,* and the antigen as *haemolysinogen.*

The A-B-O Blood Group System

Under this system, there are four blood groups designated as A, B, AB and O. In this system the serum of a healthy individual is tested with the red cells of other healthy individuals. These tests depend upon the presence of two antigens called A and B present in the human red cells and their corresponding antibodies anti-A and anti-B present in the serum (Table 11.7).

Table 11.7 Blood Groups, Their Antigens and Antibodies in Human Blood

Blood group	*Antigen (agglutinogen) in red cells*	*Antibody (agglutinin) in serum*	*Frequency in Indian population*
A	A	Anti-B	22%
B	B	Anti-A	33%
AB	A & B	None	5%
O	None	Anti-A and Anti-B	40%

The table indicates that the blood group of an individual is named after the presence or absence of one or both antigens A and B in the red cells. In a person of group B or group O, antigen A is absent, but naturally occurring antibody anti-A is present. In persons with group A or group O, antigen B is lacking while the serum contains anti-B. The serum of group AB contains antigens A and B, but none of the antibodies. The blood group AB is rare and was discovered in 1902 by decastello and Sturli. Wiener suggested that the blood group O serum contains an isoagglutinin anti-C in addition to anti-A and anti-B.

In active blood transfusions, if the red cells of the transfused blood agglutinate by the recipient's serum, the transfusion cannot be made and the blood is said to be incompatible. In order to determine compatibility of blood, a small amount of the blood is diluted with the normal saline and then a drop of this mixture is added to the test sera belonging to group A and B separately. Thereafter the blood is examined under a microscope to observe if agglutination has taken place or not. If agglutination does not occur, the blood is said to be matching. Individuals belonging to group O are called *"universal donors"* because their red cells lack antigens A and B and are not agglutinable by the sera of other blood groups. Individuals of group AB are said to be *universal recipients* since their sera lack antibodies anti-A and anti-B. Persons of this group can receive blood from all other groups (Table 11.8).

Table 11.8 Determination of Blood Groups

Donor	*Recipient*			
	Group O Red Cells O; plasma AB	*Group A Red cells A; plasma B*	*Group B Red cells B; plasma A*	*Group AB Red cells AB; plasma O*
Group O	–	–	–	–
Group A	+	–	+	–
Group B	+	+	–	–
Group AB	+	+	+	–

Note: – denotes absence of agglutination
+ denotes agglutination

The Rh System

It has been observed that an individual of blood group A can donate his blood to individuals of group AB. This is possible since AB is a universal recipient group. Sometimes it was observed that agglutination occurred in such cases which must have been due to some unknown factor. This was discovered by Landsteiner and Wiener in 1937 and was named *Rh factor* or *Rhesus factor.* The factor was named after the rhesus monkey, *Macacus rhesus* in which it was discovered first. Landsteiner and Wiener injected rhesus monkey's blood (red cells) into a rabbit to produce an immune serum or anti-rhesus serum. This anti-serum agglutinated red cells in 85% human population and rhesus monkey red cells as well. This suggested the presence of a new antigen in human erythrocytes called 'Rh' in the antiserum that produces anti-Rh antibody. The human, population possessing Rh antigen are called Rh positives, and those lacking it are Rh negatives. The distribution of Rh antigen in man is

independent of the blood groups and the Rh antibody appears in the serum of an individual after immunization only. The Rh-negative individuals can be immunized against the Rh factor in the following ways:

1. By transfusing Rh-positive blood,
2. By intramuscular injection of Rh-positive blood, and
3. By pregnancy with a Rh-positive foetus.

The incidence of Rh-positive and Rh-negative individuals in different racial populations is given in Table 11.9.

Table 11.9 Percentage of Rh-negative and Rh-positive Population in Different Races

Races	*Rh-negative*	*Rh-positive*
Whites	85.0	15.0
Negroes	95.5	4.5
Indians	93.0	7.0
Japanese	99.4	0.6
Chinese	98.5	1.5

While studying specific cases it was observed that transfusion of Rh-positive blood to Rh-negative individuals results in the formation of anti-Rh bodies (antibodies) without any apparent ill effects. If the individual is subsequently transfused with Rh-positive blood even after a lapse of several years, a haemolytic reaction follows that often proves fatal.

A similar situation arises if an Rh-negative woman conceives a Rh-positive foetus. If the baby's Rh-positive cells get into mother's circulation at the time of delivery, these cells cause the mother to produce Rh-antibody, although no harm is done to the mother since she does not possess Rh cells. First baby will be unharmed. However, if the mother becomes pregnant second time with an Rh-positive foetus, the antibodies from maternal circulation enter the foetal circulation through the placenta and build up a high concentration destroying the red cells. If by chance, the mother is given a second transfusion of Rh-positive red cells, the Rh antibodies will be formed and agglutination will occur causing haemolysis of Rh-positive cells proving fatal. Hence, Rh-negative girls and women of child-bearing age must never be injected with Rh-positive blood.

Other Blood Groups

Besides A-B-O and Rh systems, there are other blood groups of little clinical importance which are inherited independently of A-B-O groups according to Mendelian laws. Some of these well known groups are listed below along with the names of their discoverers:

M-N-S	Landsteiner and Levine	(1927)
P	Landsteiner and Levine	(1927)
Rh-Hr	Landsteiner and Wiener	(1937)
Kell	Coombs, Mourant and Race	(1946)
Lutheran	Callender and Race	(1946)

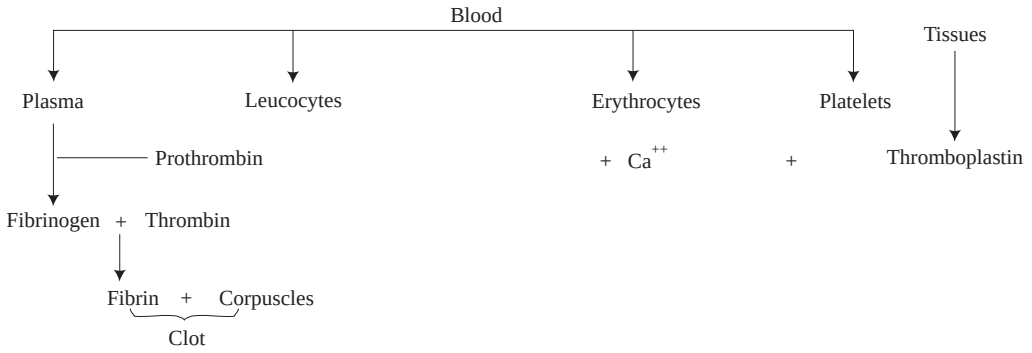
Lewis	Mourant	(1946)
Duffy	Catbush, Mollison and Parkin	(1950)
Kidd	Allen, Diamond and Neidzeila	(1951)
I-i	Wiener and Unger	(1956)

Haemolytic Disease

The study of blood groups is important in the field of haemolytic disease of the newborn. Sometimes the newborn babies with blood Groups A and B born of mothers of Group O, start showing clinical symptoms of jaundice. The symptom occurs soon on the first or the second day after the birth. This is caused due to erythroblastosis. The cause is yet not fully known. It has been shown that the placenta is permeable to certain antibodies present in the maternal blood which cross over to the foetal circulation. These antibodies are called "immune" antibodies and cause haemolysis of foetal red cells. Although most children possess blood group antigens which are inherited from the father (mothers usually lacking them), mothers generally do not produce immune antibodies. However, in rare cases when immunization of mothers occurs, the foetus is affected and develops haemolytic disease. The most important antibody which may cause haemolytic disease of the newborn is that of Rh antigen D.

11.7 COAGULATION OF BLOOD

When a sample of blood is drawn from the blood vessels, it turns into a jelly-like mass within 3 to 10 minutes. This process is called *clotting* or *coagulation* After some time the clot shrinks and a fluid called *serum* is expelled from it. Upon microscopic examination, the clot is found to consist of corpuscles of blood enmeshed in a network of thread-like substance called *fibrin.* Fibrin is a gel which is not present as such in the circulating blood, but is formed from fibrinogen which remains dissolved in the plasma. The conversion of fibrinogen into fibrin is catalyzed by an enzyme *thrombin* which is produced from it precursor *prothrombin.* The conversion of prothrombin to thrombin takes place with the help of *thromboplastin* in the presence of calcium. Thromboplastin is released from damaged tissues (blood vessels) and the blood platelets. The reason that blood does not clot while in circulation is that this enzyme is absent from circulating blood and is released only when the blood vessels get cut or injured. Calcium ions are normally present in the plasma. This process has been summarized in the following scheme:



The thromboplastic activity originates from two sources, the extrinsic and intrinsic systems. The extrinsic factors are present in the tissues and the intrinsic factors in the plasma itself (Table 11.10). Tissue thromboplastin is present in the tissue extracts of lung, brain, placenta and testis which is liberated due to injury.

Table 11.10 Clotting Factors and Their Synonyms

Factor	*Name*	*Synonym*
I	Fibrinogen	
II	Prothrombin	
III	Thromboplastin	
IV	Calcium	
V	Labile factor	Proaccelerin, accelerator globulin (Ac-globulin)
VII	Proconvertin	Stable factor, Serum prothrombin conversion accelerator (SPCA); Cothromboplastin, autoprothrombin
VIII	Antihaemophilic factor (AHF)	Antihaemophilic globulin (AHG)
IX	Christmas factor	Plasma thromboplastin component (PTC)
X	Stuart-Power factor	
XI	Plasma thromboplastin anticedent (PTA)	
XII	Hageman factor	
XIII	Fibrin-stabilizing factor (PSF)	Laki-Lorand factor (LLF), Fibrinase

Thromboplastin (Stage I)

Thromboplastin catalyzes the conversion of prothrombin to thrombin. Substances for thromboplastin activity are released from the plasma, platelets and the tissues. The factors which contribute to the thromboplastic activity include Antihaemophilic globulin (AGH-factor VIII), plasma thromboplastin anticedent (PTA-factor XI, Christmas factor—factor IX), Hageman factor (factor XII), Stuart-power factor (factor X), and labile factor (factor V). Thromboplastic precursors are also contributed by the tissues as well as from the platelets.

Prothrombin (Stage II)

Prothrombin is a globulin and an inactive precursor of thrombin which keeps on circulating in the plasma. Prothrombin is formed in the liver and released into the bloodstream. The conversion of prothrombin to thrombin requires interaction of thromboplastic factors which include factors VII, IX and X. This process requires sufficient quantities of vitamin K since calcium is necessary in clotting. Lack of any of these factors would inhibit the conversion of thrombin from prothrombin.

Fibrinogen (Stage III)

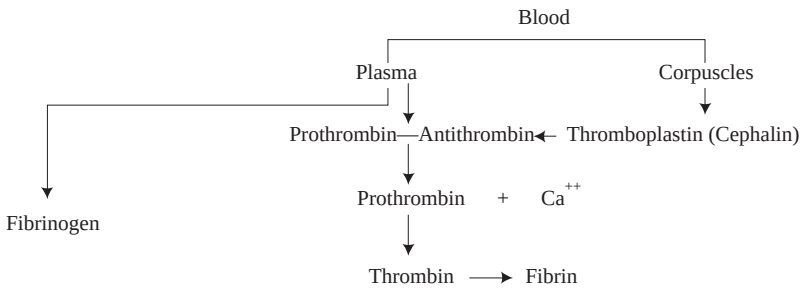
Fibrinogen is another protein which is essential in the process of coagulation. Fibrinogen is also formed in the liver which functions under the influence of thrombin. The conversion of fibrinogen to fibrin requires factor II.

11.8 THEORIES OF COAGULATION

Blood coagulation is a complex chemica1 process. Several theories have been advanced to explain the mechanism; however, no single theory is complete in itself. Some of the theories proposed by various workers are given below.

Howell's Theory

Howell studied the blood clotting mechanism in *Limulus*. He extended his observations to the clotting mechanism in the mammalian blood which were later modified by Ferguson. According to Howell's theory, thromboplastin is released by the dying cells and the platelets, although small quantity of thromboplastin is also present in the blood plasma. In the next step, this thromboplastin reacts with a prothrombin-antiprothrombin complex to release prothrombin. Prothrombin is then converted into thrombin in the presence of calcium ions. Thrombin now reacts with fibrinogen to form fibrin. It was suggested that an enzyme tryptase is responsible to convert prothrombin to thrombin. The theory is, however, incomplete in the sense that it does not explain the exact nature of thromboplastic substances. The exact role of tryptase in the clotting process and the chemical reaction involving fibrinogen to fibrin have not been explained satisfactorily. The process can be summarized as shown below.

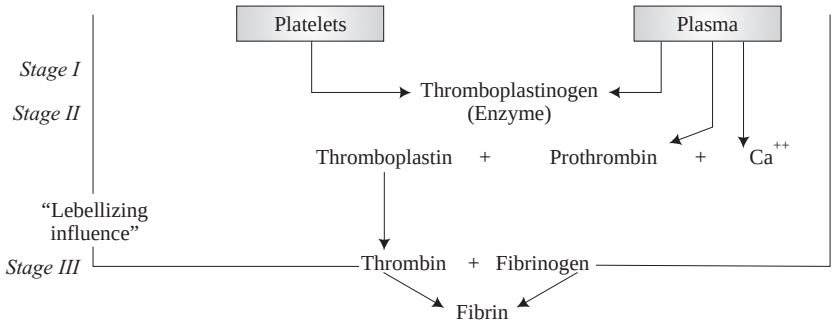


Fuld and Spiro Theory

The theory put forward by Fuld and Spiro differs from that of Howell in one important respect. They showed the presence of an enzyme *thrombokinase* which is produced by the blood platelets. This enzyme activates prothrombin to thrombin in the presence of calcium ions. Thrombin then reacts with fibrinogen to produce fibrin.

Theory by Quick and Coworkers

Quick and his coworkers proposed a theory which is quite different from Howell's theory. According to this theory, the blood platelets release an enzyme *Thromboplastinogen.* A labellizing influence is also exerted on the platelets so that the clotting process is hastened. The scheme can be summarized as follows:



11.9 HAEMOLYSIS

Disintegration of red blood corpuscles and subsequent release of haemoglobin from them is known as *haemolysis.* The chief cause of haemolysis is the changes in the osmotic pressure inside the red cells. The osmotic pressure inside the red cells is equal to the osmotic pressure of the plasma. If the corpuscles are placed in hypotonic salt solutions, swelling up of the corpuscles takes place which leads to their bursting and consequent release of haemoglobin. Haemolysis can also occur if the corpuscles are kept in distilled water. Certain chemical agents can also cause haemolysis. Dilute acids and alkalies and bile salts can cause haemolysis. Mechanical shaking of blood with ether and chloroform destroys the corpuscles by dissolving the lipids of the stroma. Snake venoms have a haemolyzing influence which is due to specific substances called *haemolysins.* When foreign blood is injected into the body of an animal, haemolysis occurs. Bacterial infections produce specific haemolysins in the blood (e.g.. *streptococci*, *Bacillus tetanus,* etc.) causing haemolysis. Haemolysis is also brought about by whipping the blood, by freezing or thawing. Haemolyzed blood becomes transparent and acquires a deep red colour due to the release of haemoglobin.

Clot Retraction

During the clot formation the fibrin threads are formed. The clot then undergoes contraction which brings the fibrin threads closer due to shortening. The texture of the clot becomes hard and it adheres to the vessel in which the blood is contained. This phenomenon of clot contraction is termed as clot retraction. During clot retraction serum is squeezed out. Blood platelets are necessary for clot retraction.

Anticoagulants

Intravascular clotting is prevented by certain substances present in blood which act as natural inhibitors of coagulation. Such substances are called anticoagulants. Intravascular coagulation of blood can take place due to injury of blood vessels, or presence of foreign substances like bacteria. It may also be induced by injecting large amount of tissue extracts. In the normal course, anticoagulants present in the blood would retard the coagulation process. There are at least three inhibitors present in the blood, which are heparin, anticephalin and antithrombin.

Coagulation may be inhibited by removing free calcium ions by adding neutral salts like sodium sulphate (Na_2SO_4) or magnesium sulphate ($MgSO_4$). Vigorous strirring of blood also prevents

coagulation. In this process fibrin is deposited on the stirring rod which can be removed from the blood.

Real problem of preventing blood coagulation is faced in laboratory experiments while doing blood analysis. For such experiments, chemical substances are used which act as anticoagulants, such as sodium citrate, sodium oxalate and disodium ethylenediamine tetraacetic acid (EDTA). Two important anticoagulants, *heparin* and *coumarin* are obtained from animal and plant sources. Heparin is a normal constituent of blood which is formed chiefly in the liver, lung and muscles. Heparin inhibits coagulation at two points; the formation of thrombin and reaction between thrombin and fibrinogen are interfered.

Another chemical, coumarin of plant origin, is an active anticoagulant which interferes with the production of prothrombin, factor VII and factor X in the liver. Coumarin, which is generally known as *dicumarol,* acts as a vitamin K antagonist and inhibits the release of factor VII. Thus the action of coumarin can be reversed by administration of vitamin K. Thus, coumarin and heparin appear to be of much value in preventing intravascular clotting.

Thrombosis

Earlier it has been seen that the life-span of blood platelets is for four days only. It is thus clear that the platelets are constantly undergoing destruction, and also new ones are being formed simultaneously. One would expect that due to destruction of these platelets coagulation process may set up in the vessels. However, this does not happen since an anticoagulant is present in the circulation. Yet there may be conditions when intravascular clotting may take place. Due to some injury or other reasons, a clot may be formed intravascularly which gradually becomes hard. This clot may be detached from its position and may enter in circulation, sometimes occluding the blood vessels causing thrombosis. Intravenous clotting is more common in veins due to sluggish flow of blood. Intravascular clotting is accompanied by prolonged rest in bed and varicosity of the veins. In certain cases, thrombosis may occur 8-12 days after surgical operations owing to increase in the number of platelets.

Pulmonary thrombosis or *embolism* is another case of thrombosis which usually occurs after surgical operations. In spite of the normal wound healing, suddenly the patient develops a serious condition and expires. This is due to the blood clot clogging the pulmonary blood vessels. Usually the clot is formed elsewhere, but is carried away in the circulation to the pulmonary vessels. Pulmonary embolism occurs due to overloading of blood with thromboplastin, exceeding the amount of heparin.

Haemostasis

The clotting of blood may cause a number of diseases including occlusion of pulmonary blood vessels leading to death. Disturbances in blood coagulation may lead to bleeding diseases. These processes are responsible for *haemostasis.*

Failure of blood clotting mechanism causes a disease *haemophilia* in which the blood is incoagulable due to the absence of fibrinogen. The disease is a genetic one which is sex-linked. In this disease the females act as the carriers whereas the males are always the sufferers. Due to this genetic deflect, factor VIII, and probably some other factors are not produced in sufficient quantities.

There is another haemorrhagic disease, *thrombocytopenia* in which the plateet count is very low. Whenever there is a small wound the bleeding occurs continuously for a long time. Coagulation fails to occur owing to the failure of the small blood vessels to contract. If at all the clots are formed, they fail to retract. As a result of small haemorrhages in skin and mucous membranes, bleeding occurs in subcutaneous spaces giving the area a blue or purple tinge. This disease is known as *thrombocytopenic purpura* wherein the blood loses its oxygen content.

11.10 HAEMATOLOGICAL ABNORMALITIES

Interference of drugs or chemicals results in abnormalities of blood cell components or their numbers. Some drugs affect the bone marrow, thereby disrupting all formed elements of the blood, whereas others may interfere with only a particular type of blood component.

Anaemia

Anaemia is characterised by a decline in the RBC count. There are several types of anaemia attributed to various causes.

(1) *Aplastic anaemia* develops when a drug suppresses or destroys bone marrow stem cells, causing deficiencies of all formed elements of the blood. Benzene derivatives, insecticides, and chloramphenicol are some compounds that can cause aplastic anaemia.

(2) *Megaloblastic anaemia* stems from impaired synthesis of DNA by RBCs, resulting in slow cell division and production of large, immature, dysfunctional RBCs.

(3) *Pernicious anaemia* is due to considerable reduction in the RBCs; cells become nucleated and assume a large size without appreciable loss of haemoglobin.

(4) *Disease related anaemia* is caused by diseases such as malaria, kalaazar, sepsis, ankylostomiasis (hookworm disease) etc. These diseases cause large scale destruction of RBCs.

(5) Anaemia due to *nutritional deficiency* results when the rate of formation of RBCs is much slower than the rate of destruction, or there may be a lack of haemopoietic factor.

(6) *Secondary anaemia* occurs due to the loss of blood by haemorrhages. Bleeding causes blood loss lowering blood pressure, and to restore the blood volume tissue fluid migrates into it, suppressing the blood count significantly.

(7) *Haemolytic anaemia,* characterised by premature RBC destruction, may be induced by two different drug-related mechanisms.

   (a) Certain oxidant drugs such as antimalarials and sulphonamides interfere with RBCs in patients with glucose-6-phosphate dehydrogenase deficiency.

   (b) A drug-induced *immune haemolytic anaemia* results from certain antigen-antibody reactions. For example, with penicillin administration, IgG antibodies may react with the RBC-pencillin complex, resulting in haemolysis.

(8) *Sickle-cell anaemia,* characterised by sickle-shaped RBCs, generally locked together obstructing the flow of blood in capillaries. This is a genetic abnormality prevalent in Negro

populations, often proves to be fatal, caused by substitution of *valine* in place of *glutamic acid* in the protein chain of haemoglobin.

Leucopenia

It is an abnormality in which white blood corpuscles (WBC) are considerably reduced.

(1) *Agranulocytosis* is a severe reduction in the number of granulocytes (basophils, eosinophils, and neutrophils), caused by drug-induced hypersensitivity reactions or by bone marrow suppression at the myeloblast level.

(2) *Neutropenia* is characterised by a decreased neutrophil level caused by oncologic drugs.

(3) *Disease-related* leucopenia is caused by fever, sore throat, chills, rash, and secondary infections.

Leucocytosis

Increased number of leucocytes beyond the normal range causes *leucocytosis.* Internal infections, appendicitis and certain viral infections may lead to abnormally high number of WBC (more than $10{,}000/mm^3$). During pregnancy and in the newborn also the number may increase. In contrast to this, a decrease in number of WBC results in a condition known as *leucopenia.* Sometimes in the absence of any infection, the WBC count becomes abnormal, a situation arising from excessive proliferation of bone marrow myeloblasts. This condition leads to blood cancer or *leukemia,* which is associated with low RBC count.

Thrombocytopenia

It is a condition characterised by abnormal reduction in the platelet level, may occur if a drug or disorder causes platelet destruction or decrease platelet production.

CHAPTER

12

Circulation of Blood

In the preceding chapter, we discussed the body fluids and their functions. Of all the body fluids, blood is the major transport medium in all the vertebrates which circulates in closed vessels. In order to transport nutrients, gases, hormones and antibodies, the blood flows through a definite and elaborate circulatory system. The blood is propelled by a well organized pumping organ into the peripheral parts of the body through the arteries, veins and capillaries. The fine capillary network allows exchange of water, electrolytes and nutritive materials across their thin walls.

The efficient system of blood circulation is responsible for the maintenance of homeostatic mechanisms in the body. It is essential that the volume and the composition of the intracellular and extracellular fluids are maintained constant since the proper fluid balance would help the animal maintain its steady state. The volume of water and the concentration of the electrolytes are regulated through the circulatory system.

Lower groups of animals have very simple structure and do not require an elaborate circulatory system. In such animals, the metabolic rate is low and the surface area/body volume ratio is very high. In simple animals like Coelenterates, the transport of food particles and dissolved oxygen is achieved by water currents created by slow flagellar movements of endoderm cells. In small crustaceans blood is pushed through tissue sinuses by the movements of legs and internal organs. In large crustaceans, there is a dorsal tubular heart which pumps blood to different parts of the body through arterial vessels. The abdominal vessels open into the cavities or sinuses which are filled by the blood. The blood comes in direct contact with the tissues. Such circulatory systems are called *open systems.*

Vertebrates, on the other hand, have a closed circulatory system and the blood does not come in direct contact with the cells and tissues; however, the diffusion of gases, water and other smaller molecules is possible through the walls of the fine capillary network.

12.1 THE BLOOD VOLUME

The volume of blood remains constant. In adult human beings, the total volume of blood is about 5 litres. The average volume of blood is calculated on the weight basis, i.e. approximately 70 ml of blood for each kilogram body weight. The fluid volume of blood is not affected even when large volume of water is taken. The excess amount of water is got rid of by enhanced urine output. Similarly, during heamorrhage, considerable amount of blood is lost which is soon restored to normal volume. After haemorrhage, the fluids from the tissues move into the blood vessels to restore the blood volume. At the same time the urine output is also lowered considerably to make good the losses. Apart from restoring the fluid volume of blood, the loss of erythrocytes is also compensated by their increased rate of production in the spleen and the bone marrow.

Several methods are in use for determining the total blood volume in the body. The most reliable and efficient method is the radio isotope I^{131} method. In this method, albumin is combined with I^{131}. Albumin does not diffuse through the capillaries, and I^{131} can be determined easily. If the plasma volume and the percentage of the blood corpuscles are known, the total amount of blood can be calculated as follows:

$$\text{Blood volume} = \frac{\text{Plasma volume} \times 100}{100 - \text{haematocrit}}$$

Generally, blood volume remains constant since the normal blood pressure has to be maintained at all times. However, under certain conditions the volume may vary within narrow limits. The plasma volume of mammals decreases at high altitudes; whereas the volume of the red blood cells increases.

Laws of Circulation

Three major factors are involved in circulation and maintenance of pressures within circulatory channels:

1. Active state of the animal.
2. Cardiac output.
3. Peripheral resistance.

In small and primitive animals, the general activity of the animal is largely responsible for the blood flow. In higher animals too, the blood flow in certain specialized areas depends on this factor (for example, in small veins of birds and mammals). The cardiac output is largely responsible for the circulation since the blood volume and the force of the heart vary considerably. Peripheral resistance is an important factor in the vertebrates and larger invertebrates with closed circulation. The velocity and pressure of blood vary considerably within different areas governed by physical principles.

12.2 THE COMPONENTS OF CIRCULATORY SYSTEM

The blood circulation has two purposes: it serves to supply nutrients and oxygen to the tissues, and removes wastes like carbon dioxide and others from the tissues. The essential components of the circulatory system are the heart, arteries, veins and the capillaries. Some information about their anatomy would be useful for the purpose of a better understanding of the circulatory process.

Arteries: The arteries are thick-walled and muscular vessels that carry the blood away from the heart. Structurally arteries are made up of three layers: intima, media and externa. They may be large, medium and small. The medium sized arteries contain well developed musculature and are further divisible into small arteries or arterioles. The walls of the arterioles also possess muscle layers which cause these vessels to constrict or dilate. This property of *Vasoconstriction* of arterioles is responsible for increasing the blood pressure to enable it to flow with a greater velocity. Arterioles are supplied with neurons which respond to sympathetic stimulation. In response to sympathetic stimulation, chemical agents like norepinephrine are secreted which produce vasoconstriction. Other chemical agents like epinephrine, serotonin and, angiotensin also produce the same effect. If the vasoconstrictor neuron is severed, the arteriole dilates. Vasodilation can also be caused by the action of chemicals like acetylcholine, bradykinin, histamin, etc.

Veins: Veins are thin walled with fewer elastic fibres. The three layers present in the arteries are also present in the veins, but they are much thinner. Small veins are called venules. The larger veins of the abdomen and lower limbs possess valves which open in the direction in which the blood is flowing, i.e. towards the heart.

Capillaries: Capillaries are very fine blood vessels which are composed of a thin wall made up of a single layer of flat endothelial cells. The cells have a basement membrane which is continuous. Capillary wall is so thin that it allows transfer of gases or substances through it. Two types of capillaries can be distinguished:

1. True capillaries.
2. Sinusoidal capillaries.

True capillaries are present in most tissues and have a lumen diameter ranging from 4 to 8 μ. Sinusoidal capillaries are channels with irregular diameter ranging from 5 to 30 μ. Such capillaries are generally found in blood forming tissues like thymus, lymph nodes, bone marrow, liver, spleen and adrenal cortex.

The capillary walls are permeable, but the permeability is not the same the throughout the body. The capillaries of the liver are most permeable. In conditions of trauma permeability increases to the extent that even cellular elements can also pass through it. Although the capillary walls are very thin, yet they are capable of withstanding pressures as high as 90 mm Hg or even higher.

Heart

The heart is a muscular organ which propels blood to various parts of the body. It is responsible for maintaining the direction of the flow with the help of the valves present in it. The heart contracts periodically to ensure continuous circulation and its stoppage would mean the death of the animal. In order to study the physiology of the heart, it is necessary to consider the action and the control mechanism of the heart.

12.3 HEART OF INVERTEBRATE

Acoelomates do not possess blood vessels and hearts (exception Nemertines). Coelomic invertebrates have hearts which can be classified into three types:

1. Tubular hearts.
2. Pulsating hearts.
3. Ampullar hearts.

Tubular Hearts

In arthropods, the systemic hearts consist of long, tubular contractile structures. The heart may be suspended in a large *pericardial chamber* by means of elastic ligaments or it may be free without any support. The heart is bathed in the surrounding haemolymph. In most arthropods (insects), the heart is held in position by special *alary* muscles and receives blood by means of lateral paired openings called ostia. The ostia are guarded by valves. These ostia openings close when the alary muscles contract, and the blood is pushed through the artery. Consequent upon the contractions of the heart, a negative pressure is created within the pericardial chamber thereby forcing the fresh supply of blood from the haemocoel into the heart through the ostia. The entire heart may show wave of contraction. In case of crustaceans, the blood passes from the heart into the arteries and arterioles and from there to the gills. The veins then bring back the blood to the pericardial chamber. The heart of tunicates is a convoluted tube situated in the pericardium. The heart pumps blood in one direction for some time and then the direction of the flow is reversed.

Pulsating Hearts

Pulsating hearts are characteristic of annelids which have a closed circulatory system. These pulsatile hearts contract in a peristalic fashion. In the earthworm, rhythmic pulsatile movements are observed in the dorsal tubular vessel from the posterior to the anterior end. The lateral vessels, commonly known as hearts, also beat rhythmically independent of each other. In *Hirudinaria,* there are two lateral channels which show alternate contractions.

Ampullar Hearts

In certain animals ampullar hearts or accessory booster hearts are present which function as booster pumps to force the blood with increased pressure. Such accessory hearts are commonly found in cephalopods and insects. In cephalopods, these hearts help in forcing the blood into small peripheral vessels. In insects they are situated at the base of antennae, wings and legs. In aphids, booster hearts force the extracellular fluids into the legs.

12.4 HEART OF VERTEBRATES

The hearts of vertebrates are known as chambered hearts. Chambered hearts are also found in molluscs where one or two auricles and one ventricle are present. In the vertebrate series, fishes have two chambers in the heart, the auricle and the ventricle. In addition, two antechambers, the sinus venosus and the conus arteriosus are also present. The sinus venosus opens into the auricle and the conus arteriosus springs from the ventricle. The venous blood entering the sinus venosus is brought into the auricle from where the blood comes into the ventricle which distributes arterial blood through the conus arteriosus. The two chambered heart attained more specialization in its structure with the evolution of land vertebrates. In reptiles for the first time the ventricle became incompletely divided

by an incomplete ventricular septum. This ventricular septum became complete in birds and mammals. Birds and mammals have four chambered hearts, having two auricles and two ventricles. These hearts are highly specialized in their structure and function.

Structure of Mammalian Heart

The heart of mammals has attained a high functional efficiency. In order to understand the physiology, we can examine the human heart. In man, the heart is situated in the thoracic cavity slightly displaced towards the left side. The wall of the heart is composed of three layers, namely, the *endocardium, myocardium* and the *epicardium.* The endocardium consists of connective tissues lined with a thin layer of endothelium. The myocardium is the principal muscle layer which is thin in the auricles and thick in the ventricle. The epicardium is made up of epithelial cells and connective tissue. The heart is enclosed in a pericardial membrane. The space between the pericardium and epicardium is known as pericardial space which contains a fluid. The pericardial fluid lubricates the heart.

The heart is four chambered. There are two auricles and two ventricles ensuring complete separation of oxygenated and deoxygenated blood (Fig. 12.1). The blood enters the right auricle from systemic circulation through the vena cavae. The left auricle receives blood from the pulmonary circulation. The blood is then pushed into the two ventricles. The right ventricle receives deoxygenated blood from the right auricle and pumps it into the pulmonary circulation. The left ventricle receives oxygenated blood from the left auricle and pumps it into the systemic circulation through the aorta.

The circulation of blood through the heart is guided by four valves. The left auricle opens into the ventricle guarded by a *mitral valve* which is bicuspid, and the opening of the right auricle into the ventricle is guarded by a *tricuspid valve* (Fig. 12.1). There is an *aortic* valve between the left ventricle and the aorta. The opening of the pulmonary artery into the right ventricle is guarded by a pulmonary valve.

12.5 PHYSIOLOGICAL PROPERTIES OF CARDIAC MUSCLES

The cardiac muscles are syncytial in nature and show a number of properties resembling the skeletal muscles. The physiological properties of the cardiac muscles are discussed below.

Excitability and Contractility

Cardiac muscles are excitable and respond to various stimuli like thermal, chemical, mechanical or electrical. In response to a specific stimulus physical changes are caused in the heart muscle. The physical change is responsible to bring about contraction followed by a concomitant release of energy which is proportional to the length of the fibres. A stimulus which causes contraction is called the *threshold of the stimulus.* While undergoing contraction the heart muscle will not respond to any stimulus. This state of the heart muscle is called refractory state. When the phase of contraction is over, a subthreshold stimulus (feeble stimulus) fails to evoke an appreciable response; hence, a strong stimulus would be necessary to make the heart responsive. This period is known as relative refractory period.

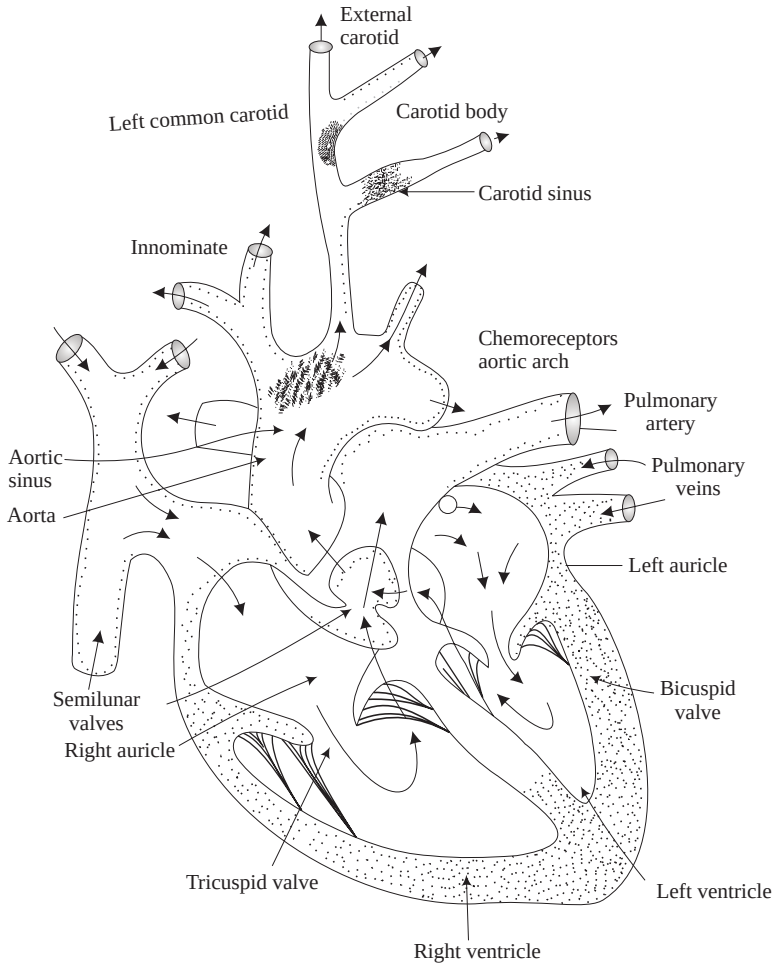


Fig. 12.1 Internal structure of the mammalian heart.

Automatic Rhythmicity

The heart beats are regular and occur in an orderly manner. The frog's heart shows regular beats starting from the sinus venosus followed by auricles, ventricle and the truncus arteriosus. The heart has the inherent power to initiate its own heart beat without the help of any external agency. If the heart of frog is removed from the body and kept in Ringer's* solution, it continues to beat regularly for some time. The muscle tissue beats faster than that of the auricles, and the auricular muscles beat faster than those of the ventricle. This has been shown conclusively by applying Stannius ligatures. If the ligature is tied round the sinuatrial junction, the sinus continues to beat, while rest of the heart

*Ringer's solution of physiological saline consists of 0.6 gm of sodium chloride, 0.0075 gm of potassium chloride, 0.01 gm of calcium chloride, 0.01 gm of sodium bicarbonate in 100 ml of water.

stops and remains in a relaxed condition for some time and later regains beating at a slower rate. If a second ligature is applied round the heart between the atria and the ventricle, the ventricle begins to beat but at a slower rate. It could be concluded from this experiment that the rate of heart beats diminishes in the descending order. The higher rhythmic activity of the sinus venosus is due to the *sinu-auricular node* consisting of special cells that trigger the impulse. This is called the *pacemaker,* Rhythmicity of the heart does not depend on the central nervous system. Although the frog's heart is governed by the vagus and sympathetic nerves.

In the frog's heart, sinus venosus sets the pace since it beats faster. This property has been studied by giving electrical stimuli to the ventricle to allow it to beat faster than the sinus. It has been observed that in such a case the other chambers contract in a reverse order, i.e. the ventricle, the auricles and then the sinus. If the sinus is warmed, it starts beating much faster, whereas the ventricle remains unaffected.

Conductivity

The cardiac muscles are syncytial in nature. Due to this remarkable property has been studied by giving electrical stimuli to the ventrical to allow it to beat faster than the sinus. It has been observed that in such a case other any part of the heart is stimulated, the wave of excitation spreads over all parts uninterrupted.

All-or-None Law: The muscles of the heart are all interconnected. If an effective stimulus is applied to the heart, it produces a maximum response. If the stimulus is weak, it will not elicit any response. Thus, when an effective stimulus is provided to the heart, it will produce a maximum response making all the fibres contract. This is called "all-or-none law" (also refer Chapter 12).

Refractory Period: In skeletal muscle, the refractory period is very short, less than the contraction period. For this reason, repeated stimuli can be given at short intervals producing sustained contraction. In cardiac muscle the refractory period is quite long, almost equal to the contraction period (Fig. 12.2). For this reason, the heart muscle normally does not show tetanic contractions. In skeletal muscle, the refractory period lasts for 0.0015 sec whereas in cardiac muscle it lasts for about 0.4 sec. During the contraction phase of the cardiac muscle any stimulus will prove

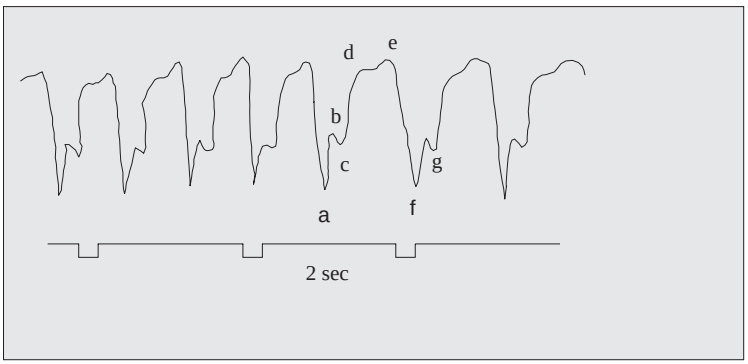


Fig. 12.2 Diagram showing the tracings of the heart beat.

ineffective, hence this is called *absolute refractory period.* After the phase of contraction is over, the muscle undergoes relaxation. In order to elicit another contraction, a stimulus greater than the usual is necessary. This period is called *relative refractory period.* In other words, the heart muscle will not show any response before relaxation has taken place.

Staircase Phenomenon: If the stimuli are applied repetitively at short intervals, gradual increase in the height of four to five contractions is recorded (Fig. 12.3), after which the amplitude of contractions remains constant. This phenomenon is called the *staircase phenomenon* or *treppe.* This is probably due to accumulation of products that are beneficial. During contraction, lactic acid is produced as a result of which the pH and the temperature rise slightly.

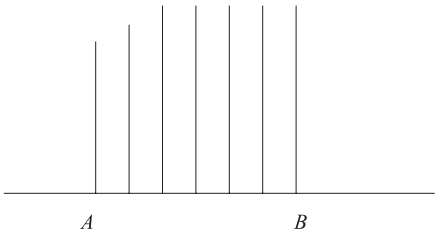


Fig. 12.3 Staircase phenomenon.

Effects of Inorganic Ions: If the excised heart of a frog is kept in Ringer's solution, it keeps on beating in a normal way for some hours. This is due to the fact that the composition of the Ringer's solution is similar to the frog's plasma. The sodium, calcium and potassium ions are very essential to maintain the muscular responses of the heart. Sodium ions contribute to the maintenance of the excitable properties of the heart muscle. Withdrawal of sodium ions from the Ringer's solution weakens the contractions. Perfusion experiments with solution containing sodium salt only (calcium and potassium ions absent) brings about the weakening and ultimately failure of the heart beats; when calcium ions were added to the solution, the heart beats restored rapidly. Presence of excess of calcium ions is found to produce vigorous contractions. Removal of potassium ions causes relaxation of the heart to become feeble, ultimately stopping the systole. On the other hand, potassium-rich solution facilitates relaxation.

Conduction Through The Heart: The conduction system of the heart initiates two electrical sequences that cause the heart chambers to fill with blood and contract. These are: (a) *impulse formation,* the first sequence, takes place when an electrical impulse is generated automatically, and (b) *impulse transmission,* the second sequence, occurs once the impulse has been generated, signalling the heart to contract.

The conduction system consists of four main structures composed of tissue that can generate or conduct electrical impulses.

(1) *The sinuatrial or sinuauricular node (SA node),* in the wall of the right auricle, contains cells that spontaneously initiate an action potential. Serving as the heart's main pacemaker, the SA node initiates 60 to 100 beats per minute. Impulses generated by the SA node trigger auricular

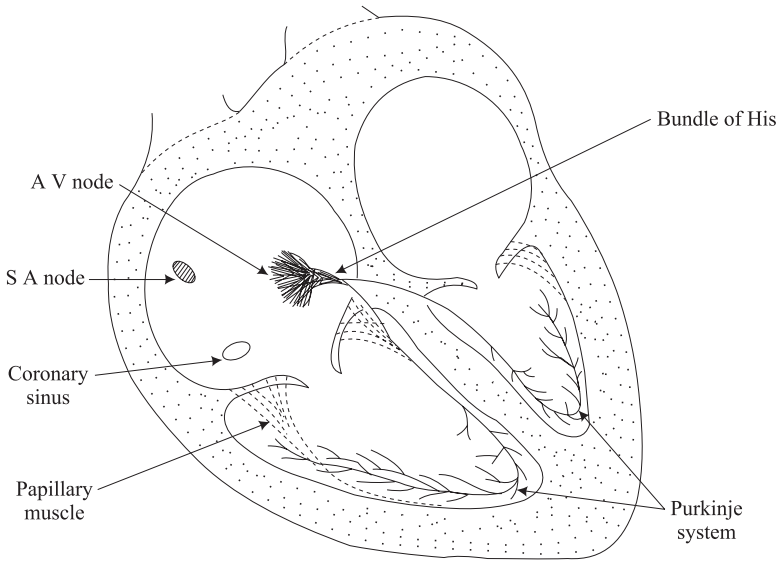


Fig. 12.4 Conducting system of the mammalian heart.

contraction. The impulses travel through internodal tracts–the anterior tract, middle tract, posterior tract, and anterior interatrial tract (Fig. 12.4*).*

(2) At the *atrioventricular (A V) node,* situated in the lower inter-arterial septum, the impulses are delayed briefly to permit completion of auricular contraction before ventricular contraction begins.

(3) At the *bundle of His,* the muscle fibres arising from the AV junction, impulses travel along the left and right bundle branches, located on either side of the intraventricular septum.

(4) The impulses reach the Purkinje fibres, a diffuse network extending from the bundle branches and ending in the ventricular endocardial surfaces. Ventricular contraction then occurs.

The AV junction, bundle of His, and Purkinje fibres are *latent pacemakers;* they contain cells capable of generating impulses. However, these regions are having a slower firing rate than the SA node. Consequently, the SA node predominates except when it is depressed or injured.

Before cardiac contraction can take place, cardiac cells must depolarise and repolarise, resulting from changes in the electrical potential across the cell membrane, caused by the exchange of sodium and potassium ions. The *action potential* which reflects this electrical activity has five phases (Fig. 12.5).

(1) *Phase 0 (rapid depolarisation)* takes place when sodium ions enter the cell through fast channels; the cell membrane's electrical charge changes from negative to positive.

(2) *Phase 1 (early rapid repolarisation)* occurs when fast sodium channels close and potassium ions leave the cell. The cell rapidly repolarises returning to resting potential.

(3) *Phase* 2 *(plateau)* is reached when calcium ions enter the cell through slow channels while potassium ions exit, stabilises the membrane's electrical activity temporarily.

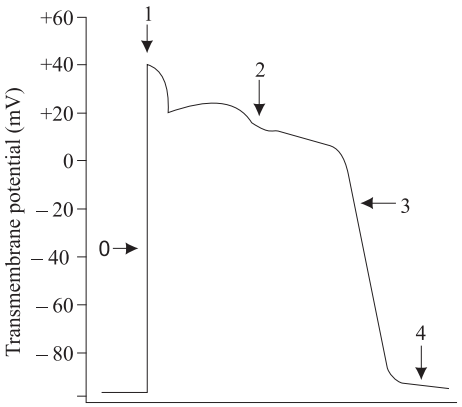


Fig. 12.5 Myocardial action potential curve. The curve represents ventricular depolarisation/repolarisation. 0–phase 0 (rapid depolarisation); 1–phase 1 (early rapid repolarisation); 2–phase 2 (plateau); 3–phase 3 (final rapid repolarisation); 4–phase 4 (slow depolarisation).

(4) *Phase* 3 *(final rapid repolarisation)* takes place when potassium ions are pumped out of the cell as the cell rapidly completes repolarisation and resumes its initial negativity.

(5) *Phase* 4 *(slow depolarisation).* The cell returns to its resting state, with potassium ions inside the cell and sodium and calcium ions outside.

During depolarisation/repolarisation, a cell's ability to initiate an action potential varies. The cell cannot respond to any stimulus during the *absolute refractory period* (beginning during phase 1 and ending at the start of phase 3). A cell's ability to respond to stimuli increases as repolarisation continues. During the *relative refractory period,* occurring at phase 3, the cell can respond to a strong stimulus. When the cell has been completely repolarised, it can again respond to stimuli. It has been observed that cells in different cardiac regions depolarise at various speeds, depending on whether fast or slow channels predominate. Sodium flows through fast channels, and calcium through slow channels. In cardiac muscle cells where fast channels dominate, depolarisations occurs quickly. Slow channels dominate cells of the SA node and AV junction, consequently they show slow depolarisation.

Abnormal impulse formation and conduction may give rise to arrhythmias. Abnormal impulse formation may stem from depressed automaticity (escape beats and bradycardia) or increased automaticity as in premature beats, tachycardia and extrasystole. Impulse conduction may become abnormal when there is a delay or block in the conducting system, or unidirectional conduction. Arrhythmias may decrease cardiac output, reduce blood pressure, and disrupt perfusion of vital organs.

Control of the Heart Beat: The rhythmic activity of the heart is an inherent property. As we have discussed above, the pacemaker of the heart is responsible for initiating and spreading the wave of heart beats. In some invertebrates, the wave of heart beats starts at the posterior end and spreads anteriorwards. In earthworm, the beating of blood vessel starts from behind.

In certain insects also, the heart muscles themselves show the rhythmic contractions and no nerve cells or ganglia are associated as in *Belostoma, Aeschna,* etc.

In all cases, the heart is a specialized muscular tissue which keeps on beating periodically. However, the exact triggering mechanism has not yet been known. Physiologists have recognized two types of hearts.

Myogenic hearts: The myogenic hearts show their rhythmic activity due to the muscles themselves. The vertebrate heart is myogenic type. The heart beat is initiated at the sinus venosus in case of fishes and amphibians. In birds and mammals, heart beat starts from the sinuauricular node. Some invertebrates also possess myogenic hearts in which the heart beats may originate from any point.

Neurogenic hearts: In certain animals, the heart muscles are innervated by nerves. In such cases regulation of heart beats is dependent upon nervous regulation. Majority of arthropods and invertebrates in general, possess neurogenic hearts. Among insects, especially the orthopteran species have neurogenic hearts. The nerve endings, upon stimulation, produce a chemical transmitter substance called acetylcholine which seems to accelerate the heart beats.

The heart of *Limulus* is supplied with a ganglion cell on the dorsal surface of the heart. The ganglion cell is made up of mutipolar neurons having connection with the lateral nerves. This median ganglion is responsible for initiating heart beats. When the median ganglion is removed heart stops beating. Among some annelids too, such as *Lumbricus* and *Arenicola,* nerve cells have been located in the hearts which act as pacemakers. All neurogenic hearts are accelerated by the action of acetylcholine.

The Cardiac Cycle: Two phases can be recognized while the heart is beating: (1) *systole*–the contraction phase, and (2) *diastole*–the relaxation phase. In mammals, the heart beats originate in the sinuauricular node which functions as pacemaker. The two auricles simultaneously contract and force their blood into the ventricles (auricular systole). After a slight pause, the ventricles contract simultaneously (ventricular systole) and force their blood into the pulmonary artery and the aorta. After each contraction phase the auricles as well as the ventricles relax for a while. This relaxation period is called the diastole. The sequence of contractions and relaxations of the auricles and the ventricles constitutes the cardiac cycle of the heart. The human heart normally beats about 70 to 80 times per minute and each cycle lasts about 0.8 sec. The auricular systole lasts about 0.1 sec. The time taken by ventricular systole is 0.3 sec. and for the joint diastole, it is 0.4 sec (Fig. 12.6).

Cardiac Dynamics: When blood enters the right auricle, the pressure of blood is almost zero as compared to the atmospheric pressure. The pressure of blood leaving the right ventricle is at 25 mm of mercury, whereas the pressure of blood leaving the left ventricle is at 120 mm of mercury.

Starling's law: Starling and his associates postulated a law of the heart which states that the initial length of the cardiac muscle fibre determines the force of contraction. In other words, greater the initial length of the muscles, greater will be the force of contraction. The law of the heart has been summarized by Starling as "The energy of the contraction is a function of the length of the muscle fibres". The initial length of the heart muscle depends upon the quantity of blood that flows into the ventricle during diastole. The law proves that the heart is governed by a self-regulatory mechanism

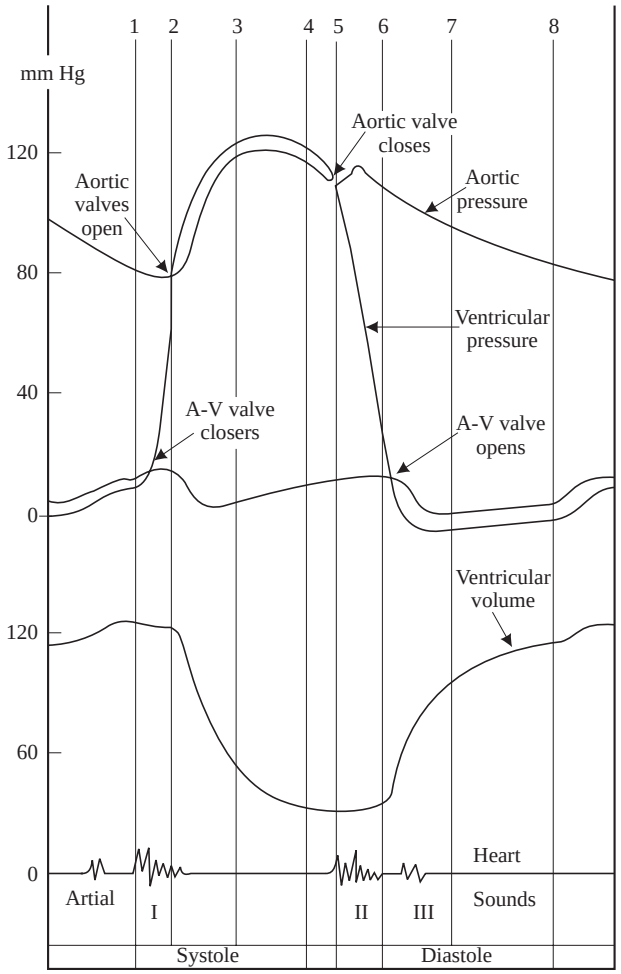


Fig. 12.6 Diagram showing the cardiac cycle.

which permits the heart to adjust automatically to the changes in the volume of blood during diastole (Fig. 12.7).

Cardiac Output: Normally the volume of blood expelled by each ventricle is the same. However, under exceptional circumstances blood volume expelled by both the ventricles may differ for a short time. Hence, we can define the cardiac output as the volume of blood expelled by one side of the heart per minute. The quantity of blood expelled out of the heart may depend on the following factors:

1. The force of contraction.
2. Amount of blood entering the ventricles during diastole.
3. The heart rate.

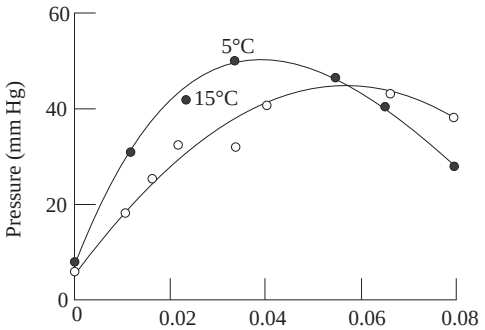


Fig. 12.7 Relation between tension developed by a frog's heart contracting isometrically and its initial contained volume.

The cardiac output in man is approximately 5.5 litres per minute which may increase during violent exercise. The quantity of blood expelled out from one side of the heart per beat is called the *stroke volume.* In man, the actual volume at rest is about 80 ml. The stroke volume as well as the cardiac output varies a great deal with the amount of muscular exercise.

Several methods are used to determine the cardiac output in man. Some of them can be briefly mentioned here.

Fick's method: Fick's principle states that in a given time the total amount of any gas gained or lost in the lungs should be equal to the difference between the amounts of the gas brought to the lungs in the arterial blood and the amount leaving the lungs in the venous blood. If we measure (a) the amount of oxygen taken up by each ml of blood passing through the lungs, (b) the amount of oxygen consumed per minute, and (c) the oxygen content of the venous blood, the cardiac output can be calculated as follows:

$$\text{Cardiac output (ml/min)} = \frac{\text{Oxygen consumed per minute (ml/min)}}{\text{Arterial } O_2 \text{ content (ml } O_2\text{/blood)} - \text{Venous } O_2 \text{ content (ml } O_2\text{/ml blood)}}$$

On an average, at rest 250 ml of oxygen are consumed per minute. The arterial oxygen content is about 0.195 ml/ml and the venous oxygen content is about 0.150 ml/ml. By using Fick's equation: .

$$\text{Cardiac output} = \frac{250 \text{ ml/min}}{0.195 \text{ ml/min} - 0.150 \text{ ml/ml}} = \frac{250}{0.045} = 5{,}556 \text{ ml/min}$$

Radioisotope method: In this method, a known amount of a radioisotope is injected into the arm vein. Usually I^{131} combined with protein is used. One ml of standard isotope solution is injected. Now 1 ml of standard solution is diluted to a known volume. After 10 minutes, a known volume of blood is drawn. Now 1 ml of the diluted standard is counted in a scintillation detector and similarly 1 ml of blood is also counted and the equilibrium value is determined.

$$\text{Cardiac output} = \frac{C_{eq} \times \text{Blood volume}}{C_{eq} \times \text{Time}}$$

where C_{eq} = counts per minute at equilibrium, and C_{av} = average count per minute during first circulation.

The blood volume is calculated as:

$$\text{Blood volume} = \frac{\text{Standard (c/min)} \times 500}{\text{Blood (c/min)}}$$

where 500 is the dilution of the standard solution.

The cardiac output may vary under certain conditions. At rest, the cardiac output in man is about 5 litres per minute, whereas under abnormal conditions it may increase or decrease.

12.6 REGULATION OF THE HEART

From the preceding description, it is clear that the heart possesses automatic rhythmicity and is governed by a self-regulatory mechanism. The greater the elasticity, the greater is the force of contraction. However, the force of contraction depends upon nervous regulation and a number of other factors such as temperature and hormones.

Electrical Activity of the Mammalian Heart

The functioning of the mammalian heart (human) can be recorded in the form of electrical activity with the help of electrocardiography. The recording instrument is provided with electrodes which are placed on the surface of the body. In all, there are four leads (electrodes) connected to the instrument which are placed on the surface of the body in the following manner:

(1) Lead I connected to the right and the left arm
(2) Lead II connected to the right arm and the left leg
(3) Lead III connected to the left arm and the left leg
(4) Lead IV is placed on the chest over the heart.

The electrical activity of the heart is recorded on the chart recorder with the help of a moving stylus. The typical electrocardiogram of a cardiac cycle (Fig. 12.8) consists of a series of waves arbitrarily designated by Einthoven as the P wave, the QRS complex, the T wave and the U wave.

The P Wave: The P wave represents depolarization of the arteial musculature which spreads radially from the sinuauricular node to the auriculo-ventricular node. It is ordinarily upright in lead I and II (Fig. 12.8), but may be diphasic and inverted in lead III depending upon the direction of depolarization. Its duration is about 0.11 second and amplitude about 2.5 mm.

The PR Interval: The delay in transmission of impulse at the AV-node on the electrocardiogram is the *PR* segment of about 0.2 second. This is measured from the beginning of the P wave to the beginning of QRS complex.

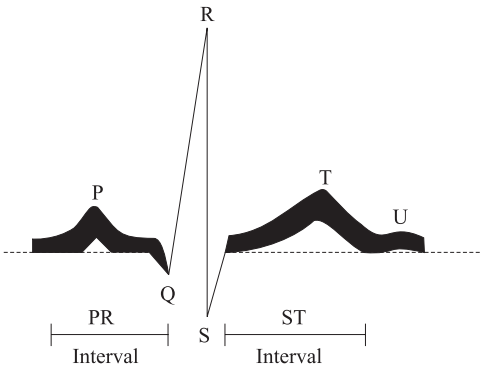


Fig. 12.8 Electrocardiogram of the human heart showing the sequence of events during the cardiac cycle.

The QRS Complex: The QRS complex is measured from the beginning of the Q wave to the S wave and represents the wave of depolarization of the ventricle spreading through the auriculo-ventricular bundle of His and the ventricular fibres. The normal range of QRS complex in adults is from 0.06 to 0.1 second. Rarely it is less than 0.06 and the duration greater than 0.1 sec is indicative of some cardiac disease.

The Q wave is about 3 mm in depth and is due to depolarization of the septum (about 0.03 sec duration). The R wave is very high and varies between 4 and 22 mm in height with a duration of about 0.07 second. The S wave is about 6 mm in depth.

The ST Interval: The duration of the ST interval is a measure of the duration of the depolarized state plus that of repolarization. In most electrocardiograms, there is no true ST segment.

The T Wave: The T wave represents the wave of ventricular repolarization. The deflections above the base line are positive waves and those below are negative. The T wave may be altered due to many physiologic states other than those found in cardiac diseases. The physiologic states that may alter the T wave are:

1. Drinking of cold water prior to ECG recording.
2. Smoking
3. Extreme emotional upset
4. Variation in the position of the heart
5. Under the medication of digitalis

A negative T wave is an indication of some myocardial disease. Normally the T wave (Lead 1) is about 0.5 mm.

The U wave: The U wave represents the positive after potential and the period of greater excitability of the ventricles. In most of the electrocardiograms, it is not discernible, hence its interpretation is difficult.

The wave form of the ECG is an indication of the state of heart and cardiac abnormalities can be detected by distortion and irregularities in the normal wave form. Some abnormalities are discussed here.

Atrioventricular block: This is caused by some disease affecting the auriculo-ventricular bundle interfering with the blood supply of the heart. In such a case, the ECG would show two auricular deflections against one ventricular deflection.

Tachycardia: This is a disease in which the heart rate is rapidly increased enhancing the blood flow. The rate ranges from 150 to 250 per minute. In normal heart the impulses arise from the sinu-auricular node, but in tachycardia they are generated elsewhere (in the auricles or auriculo-ventricular node or the ventricle). The rapidly occurring extra systoles impose a limitation on the filling time. The drug atropine induces tachycardia.

Bradycardia: It is a disease causing the slowing of the heart beats.

Auricular fibrillation: This disorder is due to the defect in the muscle contraction of the heart. The walls contract more rapidly, usually 400-600/minute and the auricles are never emptied. The normal P waves are replaced by small waves (F waves).

Nervous Regulation

The force of contraction is governed by the nerves innervating the heart. In many invertebrates, such as molluscs and arthropods, the heart beats are regulated by certain nerve centres. These nerve centres are located in the centrally placed ganglia and may have acceleratory or inhibitory effect.

In vertebrates too, the heart beat and the blood flow are controlled by the nerves. The heart is innervated by the autonomic nerves and the integration is achieved through medulla oblongata.

The Vagus Nerves

The heart of mammals has a dual control. The vagus nerve (X Cranial nerve) contains both parasympathetic (motor) and sensory nerve fibres. The heart receives outgoing branches of the vagus and sympathetic nerve fibres from the upper thoracic region of the spinal cord coordinated through the medulla where the *control centres* are located. These control centres are made up of a number of cell bodies which are of two types: (a) cardioinhibitor centre, and (b) the cardioaccelerator centre. The inhibitor centre gives rise to parasympathetic nerves that travel to the heart and produce inhibitory effect. The accelerator centre gives rise to accelerator nerves that travel down the spinal cord and have an accelerator effect on the heart. The cardiac activity is regulated by these centres through vagal and accelerator functions. Both the centres also send short neurons to each other, so that the activity of inhibitory centres can depress the accelerator centres and vice versa. The cardiac functions are also influenced by other parts of the brain such as thalamus and hypothalamus. These parts of the brain contain such centres which, upon stimulation, affect the emotional states of individuals and increase rate of heart beat, blood pressure during sleep and exercise.

The sinu-auricular and auriculo-ventricular nodes are innervated by the parasympathetic neurons. When the vagus neurons are stimulated, the force of contraction is decreased showing slow heart beats. The heart may stop completely, if stronger stimulation is given. Continued stimulation, however, induces the ventricles to region contractions. This phenomenon is called *vagus escape.* In other words, the ventricles continue to beat independently without getting impulses from the auricles. This shows that the vagus has no direct effect on the ventricular activity, since the ventricles escape from the inhibitory influence of the vagus. The vagus has an inhibitory influence in suppressing the

sinu-auricular and auriculo-ventricular nodes which stops the ventricle. But the continued beating of the ventricle is due to the action of the ventricular pacemaker.

The vagus nerves always send impulses to the heart which have a retarding influence on the rate of heart beat. This is called the *vagal tone.* When the vagus nerves are cut, the heart rate is accelerated owing to the loss of the inhibitory action of the vagus.

The Sympathetic Nerves

The sympathetic nerves or *accelerator nerves* arise from the second, third and fourth thoracic segments of the spinal cord and reach the cervical sympathetic ganglia. From these ganglia postganglionic sympathetic nerve fibres travel to the heart and innervate the sinu-auricular, auriculo-ventricular nodes and the muscle fibres of the heart. The sympathetic fibres upon stimulation, accelerate the heart rate and increase the force of contraction. If the sympathetic nerves are cut, there is decrease in the heart rate, but if the parasympathetic nerves are cut, the heart rate increases. The two systems have an antagonistic function.

Blood Pressure

Blood pressure is measured in terms of systolic reading which is 140 mm Hg and diastolic reading of 90 mm Hg. Elevation of blood pressure causes systemic hypertension in which systolic reading is greater than 140 mm Hg and a diastolic reading greater than 90 mm Hg. Blood pressure is, therefore, equal to (stroke volume × heart rate) × total peripheral resistance. Alteration of any of the factors on the right side of the equation will result in a change in blood pressure, as shown in Fig. 12.9.

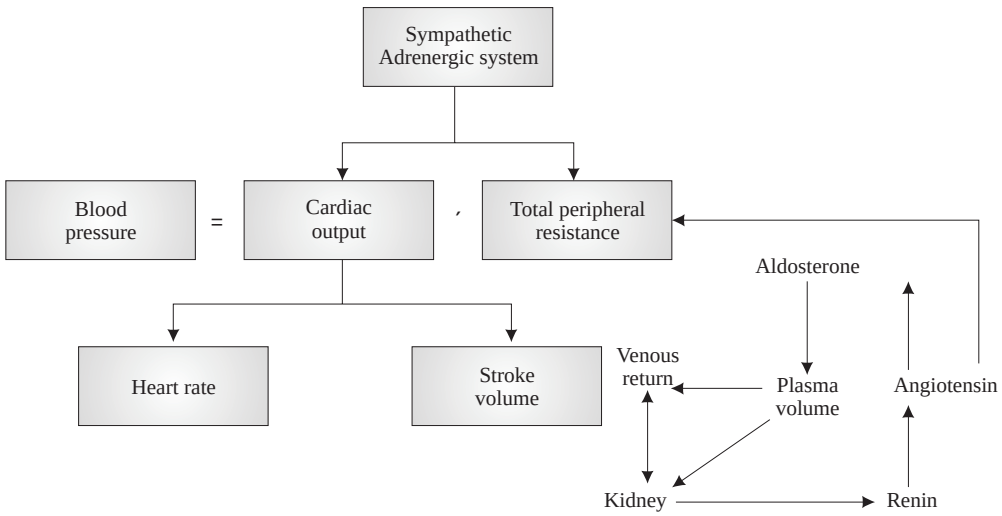


Fig. 12.9 Blood pressure regulation.

There are various determinants of blood pressure associated with cardiac output and total peripheral resistance. *Angiotensin,* a vasopressor, not only increases total peripheral resistance but, by

stimulating aldosterone release, leads to an increased plasma volume, venous return, stroke volume, and ultimately an increase in cardiac output. Factors that bring about the regulation of blood pressure are:

1. *Sympathetic nervous system.* In carotids and aortic arch pressure receptors, known as *baroreceptors,* are located which respond to changes in blood pressure influencing vasodilation and vasoconstriction. During vasoconstriction, the contractile force strengthens, increasing the heart rate and augmenting peripheral resistance, thus increasing cardiac output. If pressure remains elevated, then baroreceptors reset at the higher levels, sustaining hypertension.

2. *Renal pressor system.* Kidneys, besides excretory functions, have a blood pressure regulating function. Occlusion of the renal artery and arterioles by atherosclerotic changes causes reduction in the renal supply. Decreased renal perfusion pressure in afferent arterioles stimulates the juxtaglomerular cells (renal cortex) to release an enzyme called *renin.*

The renin reacts with a specific substrate, *angiotensinogen,* circulating in the blood to produce angiotensin I which is a weak vasoconstrictor. Another enzyme in the blood, a converting enzyme, acts on angiotensin I to convert it to angiotensin II having a very powerful vasoconstrictor effect about 200 times that of noreptinephrine. The angiotensin increases the force of heart beat and constricts the arterioles, and this often results in diminished renal blood flow even though peripheral blood flow may remain unchanged. In addition to raising blood pressure, angiotensin also causes the constriction of smooth muscles. This vasopressor also stimulates aldosterone release, with a resulting increase in sodium reabsorption and fluid volume. Normal kidneys contain an enzyme *angiotensinase* capable of destroying angiotensin. The reactions of renin–angiotensin–aldosterone system are shown in Fig. 12.10.

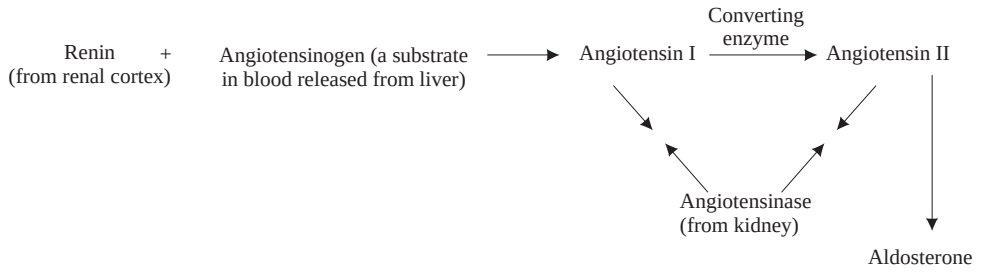


Fig. 12.10 Reactions of the renal pressor system.

3. *Fluid volume regulation.* Increased fluid volume increases venous system distension and venous return, affecting cardiac output and tissue perfusion. These changes lead to alterations in vascular resistance, increasing the blood pressure.

12.7 CHEMICAL REGULATION

The cardiac functions are greatly modified by chemical substances which are either administered, or found in the blood. They may be secreted by the nerves innervating the heart muscles. These chemical substances may be classified as follows;

(a) Neurotransmitters.
(b) Drugs acting on the heart.

Neurotransmitters

The parasympathetic nerve fibres innervating the heart muscles secrete acetylcholine; hence, they are called cholinergic nerves. Acetylcholine reduces the frequency and force of contraction of the heart. When injected, acetylcholine brings about ventricular arrest, but the auricular contractions continue as usual.

The sympathetic nerve fibres upon stimulation secrete noradrenaline which serves to accelerate the rate of heart beat and force of contraction. When injections of noradrenaline or adrenaline are given, they serve to increase the blood pressure and reflexly slow the heart. The smooth muscles of coronary arterioles in the viscera, muscles and the skin are also innervated by the sympathetic fibres. Upon stimulation by adrenaline or noradrenaline vasodilation occurs in the coronary arterioles, but in the arterioles of the skin and muscles vasoconstriction occurs.

In resting condition very little amount of adrenaline is present in the blood. Additional amounts of adrenaline are released in the blood when more energy is needed. Increased amounts of adrenaline cause vigorous supply of blood to the heart and muscles and elevation of blood suger level. More blood supply to the heart is owing to accelerated heart rate and vasodilation in the heart and the muscle. Enhanced adrenaline secretions increase the breakdown of glycogen reserves; hence, increase in blood sugar is noticed.

Effect of Drugs on the Heart

1. *Digitalis:* It acts directly on the heart muscles and peripheral circulation. The drug has been in use for long since it increases tonicity of heart muscles, contractility and irritability. Hence the drug serves as a powerful cardiac tonic in increasing the force of contraction.
2. *Pilocarpine, Muscarine. etc:* When administered, cause slowing of the heart by acting on the heart muscles or vagal terminations. The effect of the drugs can be removed by atropine.
3. *Atropine* causes acceleration of the heart beat.
4. *Serotonin* (5-hydroxytryptamine) influences the blood pressure. In dogs, it has a pressor influence whereas in cats, it acts as a depressant.

Effect of Temperature

The rate of heart beat is profoundly influenced by temperature. In most terrestrial poikilotherms, increase in the rate of heart beat is recorded with the increase in ambient temperature. This is owing to large amounts of blood needed for circulation. In homeotherms, however, the temperature of the body is maintained constant irrespective of change in the ambient temperature. The rate of heart beat remains constant, although while sweating and panting increased blood flow in the skin regions may be caused.

CHAPTER

13

Respiration

Animal life on earth is dependent upon its ability to utilize oxygen and eliminate carbon dioxide. About 20 per cent of oxygen is present in the atmospheric air, but the efficiency of animals to utilize oxygen varies with their physiological state. The atmospheric oxygen must be transported to blood and reach every cell of the body so that the foodstuffs may be oxidized. The foodstuffs of animals comprise carbohydrates, fats and proteins which contain the chemical form of energy. This energy can be released when the foods are burnt or oxidized. As described in Chapter 4, oxygen is essential in the biological oxidations as the terminal acceptor of electrons and hydrogen ions during electron transport system.

Oxygen reaches the cells and carbon dioxide is given out in exchange through a complicated physiological process called *respiration.* The oxygen requirements of the cells are also variable. Most cells of the central nervous system, and the heart are extremely sensitive to low blood levels of oxygen. Lack of oxygen renders most brain and heart cells as dead within 3-5 minutes.

From the standpoint of the physiologist, respiration is accomplished in two major processes: one involving breathing and the other associated with the release of energy from the foodstuffs within the cells. The former process is called *external respiration* and the latter is called *internal* or *cellular* respiration. Besides obtaining sufficient quantity of oxygen and eliminating carbon dioxide formed in the body, respiration serves the following purposes:

1. It helps in keeping the functions of the blood normal by adjusting changes in the pH of the blood.
2. It helps in maintaining proper oxygen tension of the blood.
3. It helps in maintaining normal body temperature.

The oxygen supply and discharge of wastes like carbon dioxide are effected through the extracellular fluids in which all the tissues are bathed. The terrestrial and aquatic vertebrates are faced with wide range of respiratory problems. Terrestrial vertebrates draw their oxygen requirements from atmosphere through the process of diffusion and by a similar process carbon dioxide is discharged out

of the body fluids to the atmosphere. The oxygen content of water is about 20 times less than air and as such a huge volume of water must be passed over the respiratory surfaces to get the required oxygen.

13.1 RESPIRATORY DEVICES

In majority of organisms belonging to the lower phyla, especially the aquatic ones, exchange of gases takes place through the body surface. In larger animals such as vertebrates, respiration is a much more elaborate process since the body has attained high complexity. There are special organs to carry out the exchange process. Gaseous exchange taking place between blood and air (water in case of aquatic animals) is called *external respiration.* In this case, no chemical process is involved. Gaseous exchange between blood and the active cells of the organism comprises *internal respiration* involving chemical process.

There are a number of organisms like protozoans, coelenterates and flatworms where specialized respiratory organs are wanting and as such the gases enter and leave the body by a process of slow diffusion. Several respiratory devices are found in higher organisms some of which will be described here.

Integumentary Respiration

Diffusion of gases through the protoplasm is a very slow process and as such it is unlikely that the metabolic demands of animals are satisfied through diffusion alone. According to Krogh (1941), the metabolic demands of animals larger than 1 mm in diameter may not be satisfied through this process. Hence respiratory devices are so modified that they facilitate sufficient exchange of gases through the body surface. In a large number of aquatic animals, the integument is highly vascular and readily permeable to gases. Earthworms, leeches and larvae of many fishes have a vascular skin which blows diffusion of oxygen through it and the entire metabolic demand is satisfied through it. However, larger animals like amphibians, and fish also rely on cutaneous respiration occasionally or continuously in addition to pulmonary or gill respiration.

Cutaneous respiration is possible only when the skin is thin, vascular and moist at all times. However, animals with thin skins are more prone to predation. Cutaneous respiration takes place in small crustaceans where chitinization is not very strong. Highly chitinized crustaceans do not exchange their gaseous requirement through the skin.

Branchial Respiration

Special respiratory appendages are developed in a number of aquatic animals. These are called *gills*. Such organs have originated independently and vary in structure from animal to animal. They range from simple filamentous epithelial structures to complex structures comprising hundreds of filamentous lamellae enclosed in a gill cavity or branchial chamber. Gills are supplied with blood vessels and are continuously flushed by water flow so that gaseous exchange is possible between water and blood of the gills. According to position, gills may be external or internal.

External Gills: These are most simple and primitive structures which develop as hollow evaginations of the body wall. In echinoderms, variety of such hollow strucutres develop, which are

papilliform in starfishes and branched in case of sea urchins. These gilliform structures subserve respiratory function in addition to the tube feet which are also used for exchange of gases. In annelids usually the skin is used in respiration, but there are specialized respiratory structures too. In some polychaetes, there are highly vascularized gills attached to the notopodium (*Glycera, Eunice* and *Nereis).* In *Arenicola* a burrowing polychaete, and *Ozobranchus,* a leech, the gills are paired, segmentally arranged branchial tufts along sides of the body. The tantacles of sabellids and serpullids are also considered respiratory structures.

Among vertebrates, the gills occur in the larva of frogs (tadpole) or as a neotenic feature of the adult in salamanders (axolotl, *Necturus).* In some fishes also, external gills are present during the larval condition (Elasmobranchs, Dipnoi). The larvae of *Protopterus* and *Lepidosiren* have four pairs of external gills in early life which are replaced by internal gills when the operculum develops.

Internal Gills: External gills have obviously a number of disadvantages. They are a hindrance during locomotion and a source of attraction for predators. During the course of evolution, the gills were drawn into partially closed chambers providing protection to the delicate structures. This also afforded streamlining of the body. One of the greatest advantages of the internal gills is the continuous flow of water currents to ventilate the gill chambers. In majority of aquatic animals internal gills are present.

In bivalves, tunicates and some echinoderms, ciliary activity is responsible for the circulation of water through the branchial chamber. The animals receive their oxygen requirements and also the food supplies from circulating water. In crustaceans, various types of gill structures are present which are enclosed in well developed gill chambers. The gills are made up of fine vascularized lamellate structures. In case of gastropod molluscs, the gills are situated inside the mantle cavity which receives continuous water currents.

Aquatic vertebrates have developed a very efficient branchial respiration. Available information on the subject is quite exhaustive; however, some salient features of the branchial respiration of teleost fish have been described here. The gills are housed in a chamber known as opercular chamber. The oral cavity draws in water to irrigate the gills and the water is forced back over the gills to come out from the opercular cavity.

The flow of water over the respiratory epithelium is continuous and the respiratory current is produced by muscular activities which pump the water. To ensure this, a double pumping mechanism is said to be in operation (Fig. 13.1) simultaneously. The buccal cavity functions as a pressure pump that forces water across the gills and the opercular suction pump draws the water through them. Thus a continuous flow is maintained by simultaneous action of these two pumps. The buccal cavity and the opercular opening are guarded by valves which are passive but move according to the pressure gradients across them.

In many aquatic animals, especially fishes, an important feature is that the flow of water over the gill is in one direction and the flow of blood in the opposite direction. This is called *counter-current* principle and ensures a constant gradient of oxygen tension between the blood and water.

Tracheae

Aquatic habitat is considered to be the most primitive. Modifications in the respiratory structures were consequent upon the change from aquatic to terrestrial habitat. Terrestrial arthropods, especially

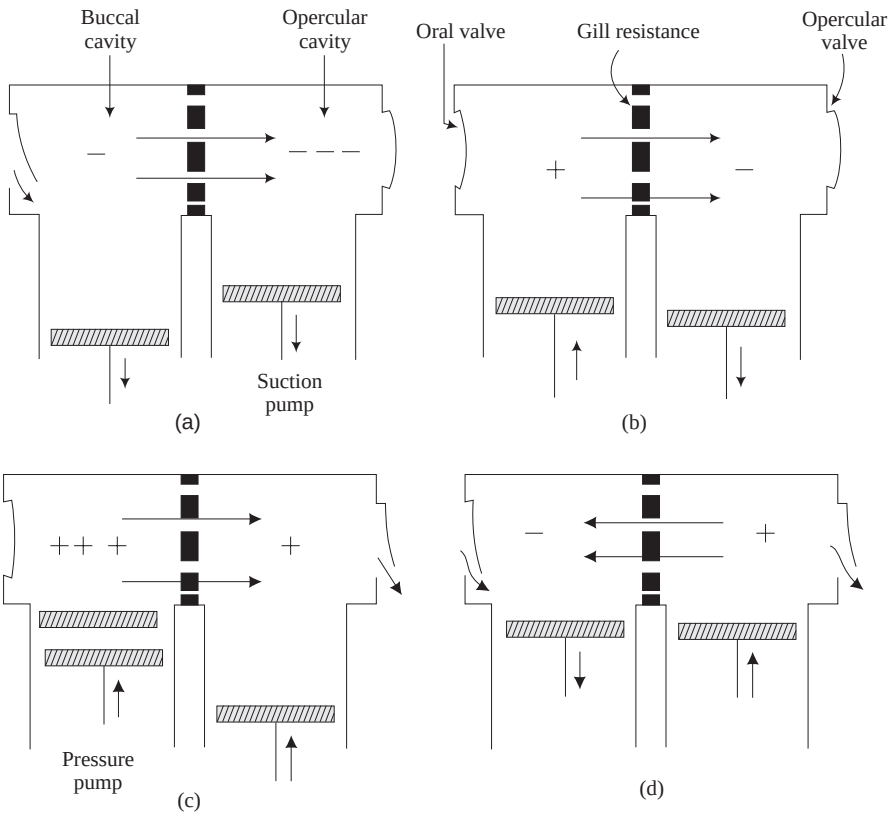


Fig. 13.1 Double pumping mechanism illustrating the ventilation of gills in teleost fishes: (a) showing the phase in which suction pumps are predominant; (c) showing buccal pump forcing water across the gills; (b) and (d) showing transition phase. Transition phases take about one-tenth of the whole cyele. (After J.D. Jones, Comperative Physiology of Respiration, Edward Arnold, 1972).

insects, have a tracheal system which is comparable to the highly developed lung system. Tracheal system has two functions: (1) it brings air into the body; and (2) distributes it to the cells. Hence, no other transport system is necessary.

Tracheal tubes are ectodermal derivatives which develop as invaginations of the body wall and open to the exterior through spiracles. In most cases, spiracles are provided with opening and closing mechanisms controlled by valves. The tracheae have a cuticular spiral, forming a layer, so that the tubes can be distended when full of air. However, when the air is drawn out, the chitinous spiral does not allow the tracheae to collapse. Larger tracheae branch into smaller anastomosing tracheoles which penetrate into the cells of the body (Fig. 13.2). These tracheoles are the important physiological units of gaseous exchange.

Ventilation of the tracheal system is dependent upon the gaseous diffusion and is brought about through the movements of the body wall. In small and large sluggish insects the oxygen demand is met adequately by the tracheal ventilation. However, in large insects which are actively running or

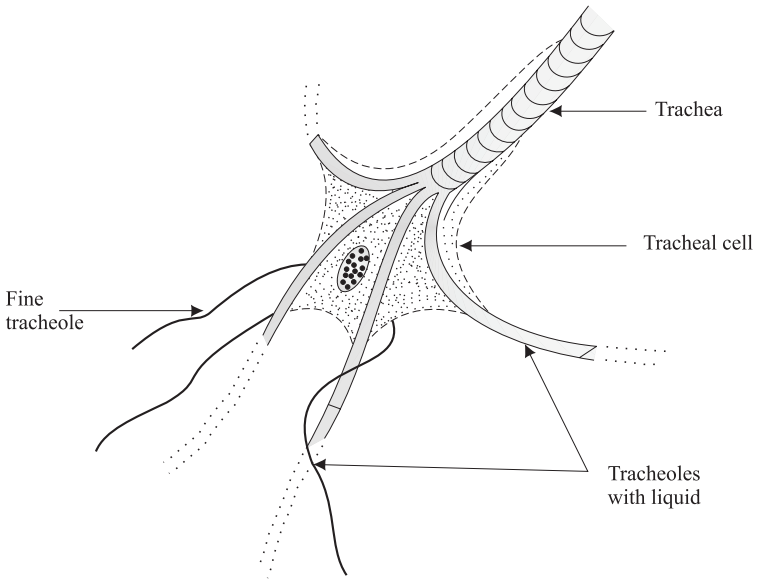


Fig. 13.2 Structure of tracheae in insects.

flying, the rate of metabolism is very high and as such additional oxygen demand is met by some amount of mechanical ventilation. For this purpose, large air sacs or dilations of the tracheal trunks are present in body cavity (as found in honey bees, wasps, etc.) and these carry large air stores.

The respiratory movements in majority of insects are confined to the abdomen consisting of dorso-ventral flattening movements (as in case of grasshoppers and beetles). In a few cases (as in bees and flies), longitudinal telescoping movements of the abdomen are recognized. The inspiratory and expiratory movements are controlled by the spiracles. Spiracles have two functional differences, i.e. some spiracles are inspiratory while others are expiratory. In the locust, *Schistocerca,* the thoracic spiracles, serve for inspiration while the abdominal spiracles are for expiration. The respiratory movements are regulated by impulses from nerve centres. Such respiratory centres lie either in the segmental ganglia to control movement of their own segments or may be in the secondary centres controlling movements of the whole insect.

Lungs

In most air-breathing animals, the need of oxygen is greater and cannot be met by cutaneous respiration. Hence, such animals are provided with special respiratory organs having vascular epithelium called the lungs. There are many different forms of lungs whose efficiency depends upon their structural complexity.

Diffusion Lungs: The simplest types of lungs are found in many terrestrial invertebrates. Small tubular lungs in the form of book lungs are found in spiders and scorpions. Gastropods have simple pulmonary structures for gaseous exchange. These lungs of terrestrial invertebrates are devoid of a

ventilating system and the gaseous exchange depends only on diffusion. Therefore such lungs are referred to as *diffusion lungs.*

Ventilation Lungs: Terrestrial vertebrates have developed air-breathing structures which perform regular coordinated movements for ventilation. The gaseous exchange mechanisms ensure transport of oxygen from lungs by circulating blood. Ventilation lungs permit greater exchange of oxygen to facilitate higher metabolic rate.

Amphibians were, perhaps, the first terrestrial vertebrates to successfully utilize their lungs to supplement cutaneous and buccal respiration. The lungs of amphibia are quite simple with relatively less elasticity and alveolar spaces as compared to birds and mammals. Ventilation of lungs is due to a buccal force-pump aided by sternohyoid muscles.

The avian lung is a more specialized organ. Three important features of this specialization are enlargement of the respiratory epithelium, efficient mechanisms for ventilation, and an efficient circulation. The avian lung has attained a great deal of structural complexity. Instead of alveoli, its lung is provided with air capillaries so that atmospheric air can efficiently circulate through the lung. The lungs lead to non-respiratory structures called the air sacs which act as reservoirs of air. These air sacs provide buoyancy to these flying animals.

The air capillaries have their walls with diameters of about 10 μ and these are separated from the pulmonary capillaries by thin endothelial wall. It is in these air capillaries that the gas exchange takes place. Ventilation of the lungs and air sacs is due to a costal suction-pump principle. During inspiration, enlargement of lungs and air sacs takes place. Expiration is due to relaxation of the external intercostals and lowering of the sternum. When the birds are in flight, ventilation is largely achieved by raising and lowering of the sternum. The lungs combined with the air sacs allow more uptake of oxygen with relatively less area for gas exchange.

Mammalian Lungs: The lung structure in mammals shows enormous development of air passages, owing to which an efficient exchange of gases is possible. In man, the right lung has three lobes whereas the left one has only two. The lungs are placed in the thoracic cavity and covered by a double fold of coelomic epithelium called *pluera.* This divides into two bronchi, each of which further subdivides into the tissue of the lung into bronchioles. The bronchioles are minute air capillaries ending into dilated vesicles called infundibula, each of which has its inner surface thrown into a number of rounded pockets called alveoli or air sacs. The blood capillaries of lungs lie just outside the thin epithelium of the alveoli. Exchange of gases between the air and blood takes place in the alveoli.

13.2 MECHANISM OF BREATHING

The movement of air into and out of lungs is accomplished by expansion and contraction of the thoracic cavity. During the act of inspiration diameter of the thoracic cavity increases so that the volume of the pleural cavity is increased by about 500 cubic centimeter. Owing to this, lungs and the contained air spaces tend to increase correspondingly in volume, resulting in the reduction of pressure inside the lungs. The pressure of the atmosphere which is now higher exerts its pressure and air rushes in through the nostrils in order to restore the pressure. This constitutes inspiration. During

expiration the thoracic walls contract and pressure is exerted upon the inflated lungs forcing the air to move out the way it entered. In the inspiration of mammals, the air is sucked in.

The expansion of the thoracic cavity is caused by movements of ribs, sternum, intercostal muscles and the diaphragm. When the external intercostals contract, each rib is pulled anteriorwards so that the sternum is also pushed ventralwards (Fig. 13.3). Simultaneously the diaphragm is also stretched, resulting in expansion of thoracic cavity. The contraction of thoracic cavity is brought about by the movements of the same structure in opposite manner. The internal intercostal muscles contract pulling the ribs back to their original position. At the same time the diaphragm also attains its original arch shaped position exerting pressure on lungs to force out the air.

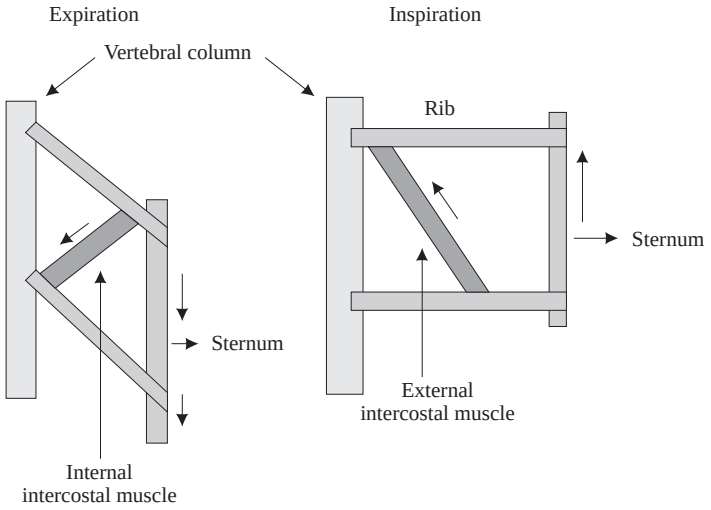


Fig. 13.3 Respiratory structures involved in breathing mechanism of man.

Air in Lungs

During normal respiration, a man takes and discharges about 500 cc of air with each act of inspiration and expiration. This is known as *tidal* air. The lungs are never deflated after expiration and about 2,500 cc of air is left behind which is known as *stationary* air. In deep breathing about 1,500 cc of supplemental air is inhaled making the total capacity of the lungs 4,500 cc. About 3,500 cc are expelled out during expiration. This exhaled air constitutes 500 cc as the tidal air, 1,500 cc as the supplemental air and remaining 1,500 cc as *complemental,* about 1,000 cc of air still remains in the lungs which cannot be expelled. This is called *residual* air. The alveoli contain less oxygen and more CO_2. This is because oxygen is exchanged for CO_2 in the alveoli. Table 13.1 gives an analysis of inspired and expired air.

Table 13.1 Analysis of Inspired and Expired Air (in Man)

Gas	*Inspired air*	*Expired air*	*Alveolar air*
Oxygen	20.96	16.40	14.00
Nitrogen	79.00	79.50	80.50
Carbon dioxide	0.04	4.10	5.50

Transport of Oxygen

The atmospheric air contains 20.9 per cent oxygen, 0.04 per cent carbon dioxide, and 79 per cent nitrogen. There are trace amounts of other gases which, however, are of no physiological importance. The expired air contains about 15 per cent less oxygen and about 5 per cent more carbon dioxide as compared to the inspired air. The percentage of nitrogen, however, remains unaltered. About one-fourth of the inspired oxygen passes into blood and is utilized for respiratory purposes. Diffusion of oxygen in water and body fluids of the organism takes place at a slow rate.

In almost all vertebrates and many invertebrates, the transport of oxygen is achieved by coloured proteins called *respiratory pigments.* Respiratory pigments have the capacity to loosely combine with oxygen when exposed to high pressures, and releasing the gas easily at lower pressures. The pressures or tensions in the tissues are always less so that gas can be readily released for biological oxidations.

13.3 RESPIRATORY PIGMENTS

Respiratory pigments differ in their chemical constitution in different groups of animals and even in the same phylum, there may be several types of pigments. These pigments include the cytochromes, the flavoproteins and other coloured molecules of proteins called chromoproteins. Here we shall restrict our attention to those chromoproteins, which are important in the transport of gases and impart a definite colour to the body fluids. These are circulating pigments which mediate transfer of oxygen at the extracellular and intracellular levels. Four important classes of such respiratory pigments have been recognized: the haemoglobins, the haemocyanins, the chlorocruorins, and the haemerythrins. All of these have a metallic atom in their constitution. The distribution of these pigments and their oxygen carrying capacity has been given in Table 13.2.

Haemoglobin

Haemoglobin is the most characteristic respiratory pigment found in blood of the vertebrates. This pigment is found in the erythrocytes. Besides, haemoglobins are found generally in muscle cells of birds and mammals, and occasionally in teleosts and elasmobranchs. The chemical composition and structure of haemoglobin has been described in Chaper 9. Haemoglobin is also found freely distributed in the plasma of many annelids and molluscs *(Terebella, Planorbis).*

Haemoglobin has the ability to combine reversibly with oxygen:

$$Hb + O_2 \rightarrow HbO_2 \text{ (oxyhaemoglobin)}$$

Table 13.2 Distribution and Oxygen Carrying capacities of Respiratory Pigments (Adapted from Prosser and Brown, 1961)

Pigment	*Colour*	*Melallic atoms*	*Site*	*Animal*	*Oxygen volume percent*
Haemoglobin	Red	Fe^{++}	Corpuscles	Mammals	15–30
				Bird	20–25
				Reptiles	7–12
				Amphibians	3–10
				Fishes	4–20
			Plasma	Annelids	1–10
				Molluscs	1–6
Chlorocruorin	Green	Fe^{++}	Plasma	Annelids:	*
				Polychaetes	9
Haemocyanin	Blue	Cu^{++}	Plasma	Molluscs:	
				Gastropods	1–3
				Cephalopods	3–5
				Crustaceans	1–4
Haemerythrin	Red	Fe^{++}	Corpuscles	Annelids	2

The oxygen combines loosely with haemoglobin and its release is dependent on the oxygen tension in the medium surrounding the haemoglobin. Such relationships have been described in more detail in Section 13.4.

Haemoglobin Function in Invertebrates: The function of haemoglobin in invertebrates is rather interesting. It has been found that in some invertebrates, haemoglobin transports oxygen at atmospheric pressures, whereas in others at low pressures. Yet in some invertebrates, haemoglobin stores oxygen during conditions of hypoxia. Thus the property of haemoglobin may vary between groups and sometimes between species. In Fig. 13.4, the oxygen dissociation curves of three animals have been given where the respiratory pigment is haemoglobin. The oxygen dissociation curves of *Arenicola,* man and pigeon show that the haemoglobins of the three animals become saturated at different oxygen tensions, thereby showing different properties of the three haemoglobins.

Chlorocruorin: Chlorocruorin is also a metallo-porphyrin which is closely related to haemoglobin and cytochromes. This pigment is found in the plasma only and its distribution is restricted to four families of polychaeta (Annelida); Sabellidae, Serpulidae, Chlorhaemidae and Ampheretidae. The oxygen combining capacity of chlorocruorin is as great as that of haemoglobin.

Haemocyanin: Haemocyanin pigment is of wide occurrence and is a non-haeme respiratory pigment. It is dispersed in the plasma solution and has never been found in the corpuscles. The pigment is found in many molluscs and arthropods. The metallic atom present in the haemocyanin molecule is copper which gives it a characteristic blue colour.

Haemerythrin: Haemerythrin occurs only in a few groups of animals and is known of to be restricted to *Magelona* (a polychaete), most sipunculids, *Priapulus* and *Halicryptus* (priapulids), *Lingula* and *Glottidia* (brachiopods). These are all unrelated phyla and bear no phylogenetic

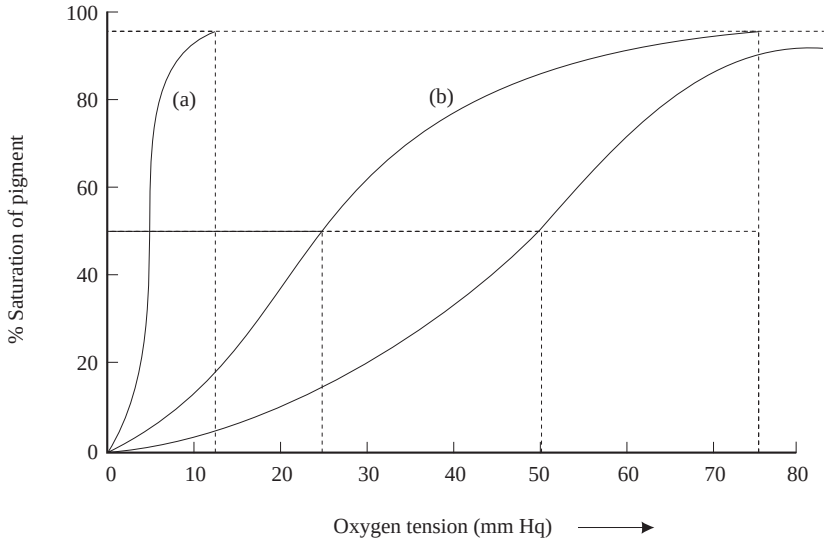


Fig. 13.4 Oxygen equilibrium curves of (a) Arenicola, (b) man, and (c) the pigeon (modified from Comparative Animal Physiology, Prosser and Brown, 1961, 2nd ed.).

relationships. The pigment circulates in the corpuscles and contains iron. It has been found that three atoms of iron are necessary to combine with one molecule of oxygen. Although haemerythrin is considered a close relative of haemocyanin, its oxygen transport capacity is very low. Probably, it is a storage pigment.

13.4 PROPERTIES OF RESPIRATORY PIGMENTS

Oxygen Transport by Haemoglobin

In almost all vertebrates, haemoglobin of the blood is responsible for the transport of oxygen. Haemoglobin is contained in the red blood corpuscles which are fully packed with this pigment. The oxygen affinity of the pigment is quite high and is governed by certain gas laws. It would be appropriate here to discuss briefly the laws governing absorption of gases by liquids.

If the pressure of a gas in a volume of water is kept constant, solubility of the gas is lowered as temperature is increased. Since the temperature of the body remains constant, other factors would modify the solubility of gases. The solubility of a gas in water depends directly on its pressure, temperature remaining constant. If the gas is mixed with other gases, its solubility will depend upon its partial pressure. The moment an equilibrium is established between gas and water, the number of gas molecules leaving it equals the number of gas molecules entering. The property of a gas to leave the liquid is called its tension which is measured by finding the pressure or partial pressure (in case of mixed gases) of the gas in the atmosphere.

The partial pressure of a gas (P) in a mixture of gases is calculated by multiplying the pressure of the mixture by the percentage of the gas in it. Therefore, the partial pressure of oxygen (PO_2) in atmosphere at 760 mm Hg is $21/100 \times 760 = 160$ mm Hg, where 21 is the percentage of oxygen in air. At 38°C, 100 ml of water can hold 2.3 ml of oxygen, 51 ml of carbon dioxide and 1.2 ml of nitrogen when the pressure of each gas is 760 mm Hg. The approximate volume of gases in 100 ml of blood and their tensions are given in Table 13.3.

Table 13.3 Volume And Tension Of Gases In Human Blood

	Oxygen		*Carbon dioxide*	
	ml/100 ml of blood	*Tension (mm Hg)*	*ml/l00 ml of blood*	*Tension (mm Hg)*
Arterial blood	20	100	50	40
Venous blood	15	40	54	46
Tissues	–	30	–	50

The oxygen-carrying capactiy of blood shows marked variations in different groups of animals. It has been seen that greater amounts of oxygen can be held by blood of mammals and birds as compared to cold blooded vertebrates. The amount of oxygen dissolved in blood, i.e. its percentage saturation is dependent on the partial pressure of oxygen (PO_2) in the atmosphere. At high tensions, the haemoglobin absorbs oxygen, and at low tensions oxygen is dissociated from the haemoglobin.

The relationship between tension and percentage saturation of the blood is shown in Fig. 13.5 which shows that the relationship is not a linear one. The graph shows sigmoid shaped curves ensuring the amount of oxygen given up for small changes in tension is very great. These curves are called *oxygen equilibrium curves* or *oxygen dissociation curves*. The curves are drawn by determining the amount of oxygen which combines with blood exposed to oxygen at various pressures. At equilibrium the amount of gas combined with the blood is expressed as the per cent saturation of oxygen. From the sigmoid curve, it is clear that at high tensions of oxygen found in lungs, almost complete saturation occurs at about 95/100 mm Hg. In the tissues, where the oxygen tension is as low as 40 mm Hg, oxygen is given up. This shows loading of oxygen at high pressures and rapid unloading of oxygen at low tensions in the tissues so that the oxygen is made available to the cells.

13.5 FACTORS AFFECTING OXYGEN DISSOCIATION

A number of factors influence the dissociation of oxygen from haemoglobin which are briefly stated here.

Temperature: Haemoglobin saturation is decreased by a rise in temperature. At a temperature of 38°C and at an oxygen tension of 100 mm Hg, 93 per cent haemoglobin saturation is obtained, whereas at 25°C and at the same oxygen tension about 98 per cent saturation is possible. It is interesting to know that the per cent saturation of haemoglobin at 10 mm Hg tension and at 25°C is about 88 per cent. whereas at 37°C it is about 56 per cent. The interesting fact demonstrates that in warm-blooded animals oxygen dissociates from haemoglobin more efficiently and rapidly than in the cold-blooded animals.

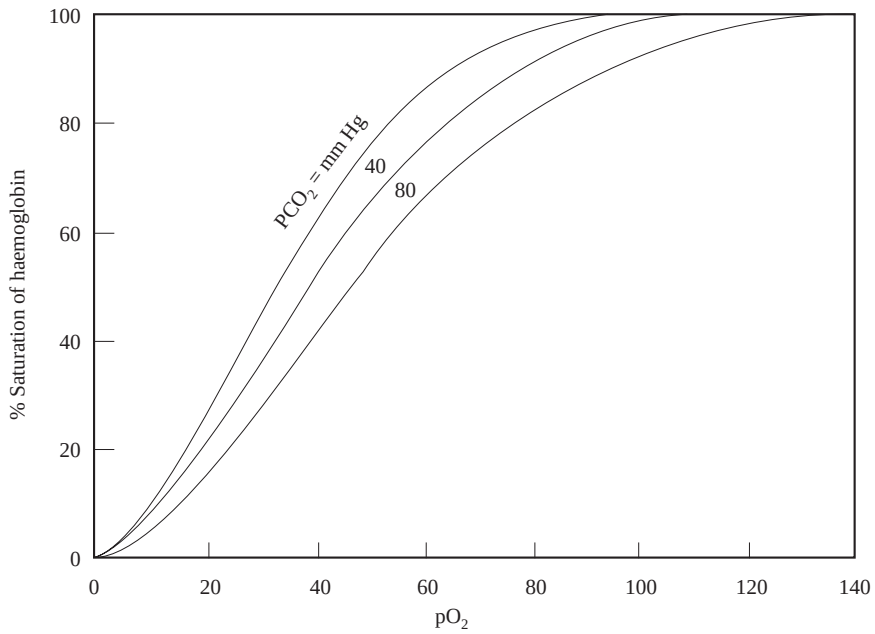


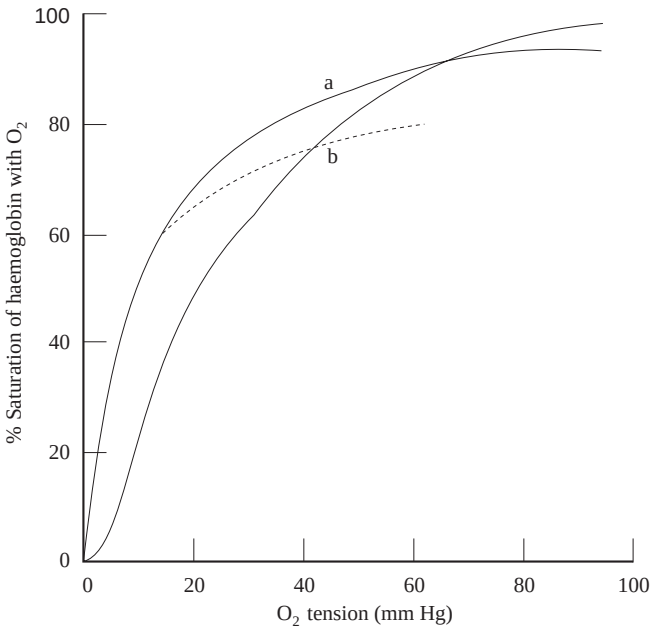
Fig. 13.5 Relationship between oxygen tension and percentage saturation of blood.

Electrolytes: It has been found that oxyhaemoglobin releases oxygen more readily at low tensions in the presence of electrolytes (Fig. 13.6). This has some significance when the blood passes through systemic capillaries where the oxygen tension is very low, the salts present in the blood influence the release of oxygen.

Hydrogen-Ion Concentration: Dissociation of oxyhaemoglobin is favoured by increase in the pH. The presence of carbon dioxide in the blood increases acidity. Fig. 13.7 shows that increase of carbon dioxide tension enhances the release of oxygen from oxyhaemoglobin. Carbon dioxide tensions shift the slope of the oxyhaemoglobin dissociation curve to the right side and this phenomenon is discussed in the following paragraph.

The Bohr Effect

It has been noted that an increase in temperature and the hydrogen-ion concentration causes a shift of the oxygen dissociation curve of haemoglobin to the right. Thus the oxygen binding capacity of the haemoglobin is lowered. Variations in the pressure of CO_2 (PCO_2) alters the pH, thus affecting the oxygen affinity of the haemoglobin. This is called *Bohr effect.* The Bohr effect may be normal or negative depending on the rise or fall in the pH. When enhanced tissue activity takes place, as in muscular contraction, CO_2 and lactic acid are produced which lower the pH. In Fig. 13.7, increasing pressures of CO_2 upon oxygen dissociation curves of haemoglobin at constant temperature 37°C has been shown. On the contrary haemocyanins from many gastropod molluscs show either a reverse Bohr effect or no effect. In many fishes, which show the Bohr effect, increased PCO_2 (acidity) exerts a secondary effect on the properties of haemoglobin. This effect is termed *root effect* in which



(a) Curve in absence of electrolytes (b) Curve in presence of electrolytes

Fig. 13.6 Oxygen dissociation curves in the presence of electrolytes.

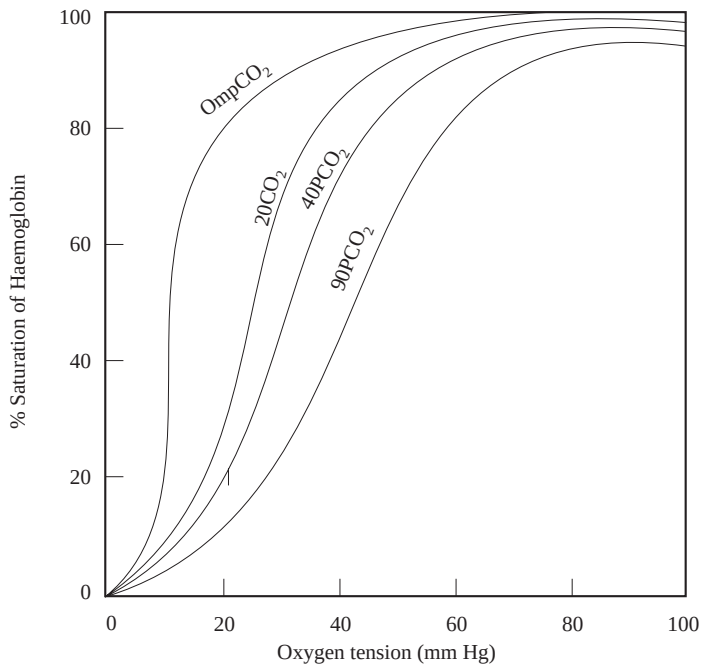


Fig. 13.7 Effect of carbon dioxide pressure (PCO_2) on oxygen dissociation curves of haemoglobin at 37°C.

haemoglobin cannot be saturated with O_2 at any PO_2. This means that at lower pH values less O_2 is carried per unit volume of blood than at higher pH values.

13.6 TRANSPORT OF CARBON DIOXIDE

Blood pigments, besides transport of oxygen, also transport carbon dioxide either directly or in the form of buffers. In a number of cases, blood pigments also serve to maintain the osmotic pressure of blood colloids. Solubility of CO_2 in water is far greater than the oxygen. However, the amount of CO_2 transported in this way is not adequate for most animals. Generally, about one-tenth of the requirements of CO_2 in a mammal can be met with in this way. Most carbon dioxide is carried as sodium bicarbonate, though about one-third of total CO_2 is transported in dissolved condition in the blood (Fig. 13.8). In this way, CO_2 is combined with amino groups of the haemoglobin molecules, ($HHbCO_2$).

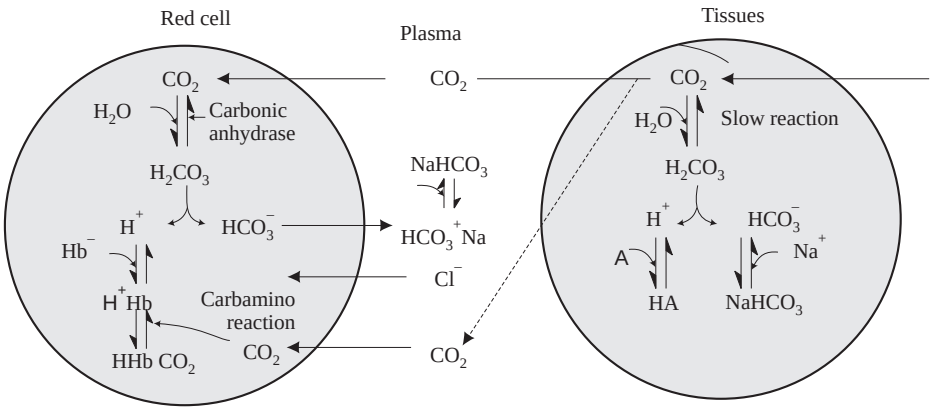
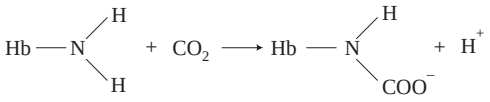


Fig. 13.8 Diagram to explain chloride shift (A – protein; Hb – haemoglobin).

Carbon dioxide is carried by blood both in the cells and the plasma. Large quantities of carbon dioxide are taken up by blood and in spite of this the pH of blood remains almost constant varying within very narrow limits. The human arterial blood has a pH 7.35 and it may change to 7.32 or 7.34 as it becomes venous. Hence blood has a remarkable self-buffering capacity.

Since large quantities of CO_2 can be held by the blood, it is necessary that CO_2 must exist in other forms besides gaseous state. These are: (a) in the form of carbamino compounds; (b) small amounts of carbonic acid; (c) in the form of bicarbonates of sodium or potassium.

Carbamino Compounds: In haemoglobin solutions, about 20 percent of the total blood CO_2 forms links with amino groups in the protein portion of the haemoglobin molecules forming carbamino compounds.



The formation of carbamino compounds is very rapid.

Carbonic Acid: The amount of CO_2 physically dissolved in the blood is not very large. When CO_2 reacts with water carbonic acid is formed in the presence of carbonic anhydrase—a zinc containing enzyme. Carbonic anhydrase is present in erythrocytes but not in the plasma.

The reaction is reversible and can be shown as:

$$2H_2O + 2CO_2 \underset{\text{anhydrase}}{\overset{\text{carbonic}}{\rightleftharpoons}} H_2CO_3 + H^+ + HCO_3^-$$

It takes place slowly in the absence of the enzyme and moves in either direction, but when CO_2 enters the capillaries, catalytic action of enzyme hastens the reaction, thereby favouring more CO_2 to enter the blood corpuscles.

Formation of Bicarbonates: CO_2 tends to accumulate in the blood in the form of carbonic acid, but very little CO_2 can be transported in this way since the presence of carbonic acid has a unfavourable effect on the pH. Any major shift in the blood pH would be harmful. Therefore most of the carbonic acid is converted into bicarbonate compounds of Na^+, and K^+ ion needed to form the bicarbonate comes from the NaCl of the blood, and K^+ comes from the haemoglobin itself, since it is a potassium salt. The reaction would take place in the following manner:

$$KHb + H_2CO_3 \rightarrow KHCO_3 + HHb$$
$$NaCI + H_2CO_3 \rightarrow NaHCO_3 + HCl$$

13.7 BUFFER SYSTEMS OF BLOOD

Haemoglobin Effect

Haemoglobin has remarkable buffering capacity. The carbonic acid and HCl are both transported in the erythrocytes without producing an acidic effect. How could this be achieved? The behaviour of haemoglobin and oxyhaemoglobin is responsible for this curious phenomena. Haemoglobin occurs as a potassium salt and therefore K^+ ions are abundantly present in it. This protein compound reacts with acids like HCl and H_2CO_3 and gets neutralized. Thus haemoglobin and oxyhaemoglobin react to form neutral KCl and alkaline $NaHCO_3$ and haemoglobin and oxyhaemoglobin are set free. H^+ ions so liberated combine with free proteins which act as buffers within the corpuscles. These reactions are illustrated in Fig. 13.9.

The buffer action transforms oxyhaemoglobin to haemoglobin which is weakly acidic. The buffering effect of plasma proteins has immense importance since they release cations for the transport of at least 10 percent of the total CO_2.

The phosphates present in the erythrocytes also exert some buffer action and account for about 25 per cent of the total CO_2 carried. However, the buffering role of haemoglobin and oxyhaemoglobin

has a major share in the transport of CO_2 and accounts for about 60 per cent of its transfer. Under low oxygen tension in the tissues, reduction of oxyhaemoglobin takes place by giving off O_2 to the tissues. Thus, reduced haemoglobin is formed. At this stage CO_2 of the tissues enters the blood forming H_2CO_3. This H_2CO_3 dissociates into H^+ and HCO_3^-. H^+ ions are accepted by reduced haemoglobin forming HHb (acid-reduced haemoglobin). At this point, there is no significant change in the pH. However, when the blood goes back to the lungs, these H^+ ions are released owing to the formation of oxyhaemoglobin which is a stronger acid. H^+ ions are again released which are quickly neutralized by HCO_3^-. This reaction is of utmost importance in the liberation of CO_2 in the lungs.

Chloride Shift

About 85 per cent of the CO_2 transport capacity resides in the red blood cells, about 60 per cent contributed by the haemoglobin and about 25 per cent by the red blood cell phosphates. This would mean that the buffering capacity of the whole blood should be far greater than the plasma alone.

When blood is loaded with CO_2, the bicarbonate ions are present in large quantities in the red blood cells. These HCO_3^- ions from the red cells diffuse through the capillaries into the plasma and Cl^- ions enter into the red cell to maintain electrical neutrality. This phenomenon is called *"chloride shift"* or *Hamburger phenomenon.* We shall see here how this is achieved.

The movement of the ions across the membrane of the red cells takes place by Donnan equilibrium. The membrane is impermeable to protein ions, and cations like K^+ and Na^+, whereas anions like Cl^- and HCO_3^- can diffuse across the membrane. The movement of the anions take place in such a way that equal distribution of Cl^- and HCO_3^- ions is achieved with respect to inside and outside the cell membrane. The shift takes place rapidly and is effected within a second.

The haemoglobin, like all other proteins, is negatively charged. The negatively charged haemoglobin combines with H^+ ions formed due to ionization of H_2CO_3 giving rise to haemoglobinic acid (HHb).

$$H_2CO_3 \rightarrow H^+ + HCO^-_3$$
$$H^+ + Hb^- \rightarrow HHb$$

Thus HCO_3^- ions accumulate in the red cells and tend to diffuse away into the plasma. For every bicarbonate ion that comes out of the red cells, one Cl^- ion moves into the red cell from the plasma to balance the electrical neutrality. The chloride shift has been illustrated in Fig. 13.8.

Acid-base Balance

It has been noted above that blood has a remarkable buffering capacity, and as such a proper balance in the ratio of H^+ and OH^- ions in blood is maintained (Fig. 13.9). This is known as the *acid-base balance.* It has been seen that the only free acid in the blood is the carbonic acid (H_2CO_3) which is formed by the union of CO_2 and H_2O. Besides this, the only alkali present in the blood is in the form of $NaHCO_3$. The standard bicarbonate can be calculated from the Henderson-Hasselbalch equation which is defined as HCO_3^- ions at a PCO_2 of 40 mm Hg when the haemoglobin of the blood is fully oxygenated at 38°C. The pH of the blood may be determined by the ratio HCO_3^-/ H_2CO_3.

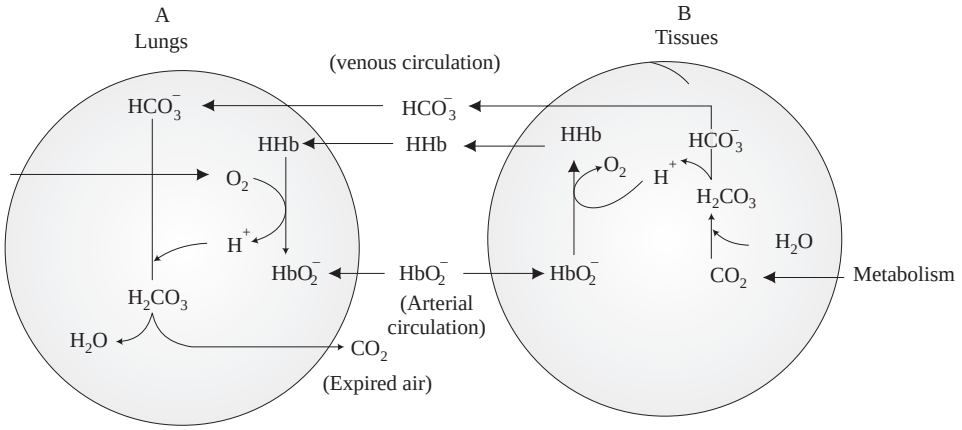


Fig. 13.9 Buffering action of haemoglobin: (A) in lungs—higher pH releases CO_2, and (B) in tissues—lower pH releases O_2 in tissues.

Any change in the pH may be due to alteration of HCO_3^- ions with H_2CO_3 being normal or due to alteration in H_2CO_3 when HCO_3^- ions remain normal. These changes are due to alteration in H_2CO_3 concentration or PCO_2 during respiration. The ratio of these two substances is 20:1 which is represented by pH 7.4. The pH of blood may vary within narrow limits, i.e. variations from 7.3 to 7.5 may be considered normal.

Disturbances in the acid-base balance lead to specific disorders which are not tolerated by the tissues since they are highly susceptible to such changes. Three departures from the normal condition have been recognized:

(a) A fall in pH causes respiratory acidosis, whereas rise in pH causes respiratory alkalosis.
(b) Bicarbonate ions are either raised or lowered.
(c) The carbonic acid content (PCO_2) of the blood may be raised or lowered.

13.8 ACID-BASE DISTURBANCES OF RESPIRATORY ORIGIN

Acidosis

Lungs have a key role in acid-base regulation. Respiratory acidosis is generally caused by defective pulmonary excretion of CO_2 leading to an increase in the H_2CO_3 of blood. Consequent upon this, a fall in the pH of blood is recorded. However, if the HCO_3^- and H_2CO_3 are somehow balanced, the pH would be normally adjusted. In case the removal of CO_2 from the lungs is insufficient, return to normal condition will not be restored. In such circumstances return of pH towards normal is accomplished by the kidneys. The kidneys reabsorb more of HCO_3 ions and Na^+ ions and excrete H^+ ions. Thus in respiratory acidosis CO_2 content of blood increases with consequent increase in the bicarbonate ions. This would result in a situation where both carbonic acid and bicarbonates will be higher than normal. The respiratory acidosis is then "compensated".

Respiratory Alkalosis

Due to excessive losses in the CO_2 content of blood, the pH is raised causing alkalosis. The rate of elimination of CO_2 far exceeds the rate of CO_2 production in the tissues so that a fall in the H_2CO_3 content of the arterial blood is noticed. The partial pressure (PCO_2) in the alveolar air also falls. As a result of these disturbances, the acid-base ratio is altered. Respiratory alkalosis occurs due to overbreathing, either voluntary or forced.

13.9 ACID-BASE DISTURBANCES OF NON-RESPIRATORY ORIGIN

Metabolic Acidosis

Disturbances in the acid-base balance due to changes in the bicarbonate of blood are said to be metabolic in origin. Metabolic acidosis is caused by a decrease in the bicarbonate fraction without any change in the carbonic acid fraction. This occurs very commonly in uncontrolled *diabetes mellitus.*

Metabolic Alkalosis

Metabolic alkalosis occurs when excess of bicarbonates accumulate in the blood without any change in the carbonic acid content. In alkalosis vomiting may result due to loss of hydrogen ions resulting from loss of gastric secretions. In pyloric stenosis (intestinal obstruction), persistent vomiting results in rapid loss of electrolytes causing alkalosis.

Table 13.4 Disturbance of $_pH$ and Acid-Base Ratio of the Blood

Condition	*pH*	*Base-acid ratio*	*Respiratory disturbance*	*Normal acid-base balance*	*Non-respiratory disturbance*
Normal	7.40	$\frac{20}{1}$	—	$\frac{24}{1.2}$	—
Acidosis	7.0	$\frac{8.0}{1}$	$\frac{24}{3}$	—	$\frac{9.6}{1.2}$
Alkalosis	7.60	$\frac{70}{1}$	$\frac{24}{0.34}$	—	$\frac{84}{1.2}$

13.10 REGULATORY PROCESSES IN RESPIRATION

The respiratory movements have to be regulated to ensure proper supply of oxygen whose composition varies with composition of blood. All such movements have to be coordinated without which oxygen supply to the tissues and elimination of carbon dioxide from the tissues cannot be effectively regulated. A number of regulatory processes are at work, some of which will be described in the following paragraphs.

Chemical Regulation

As shown by Haldane, O_2 content of blood and alveolar air is largely responsible for the regulation of respiration in mammals. If oxygen does not reach the blood, *asphyxia* occurs. Reduction in the supply of oxygen to the tissues causes *hypoxia,* whereas if the tissues are completely deprived of oxygen, the condition is known as *anoxia.* The inhalation of air containing 5-10 percent CO_2 causes increase in ventilation. Respiratory movements are increased and become violent. This causes increased effort on part of the muscles resulting in many symptoms like vasoconstriction, salivation, contraction of pupils, etc.

Effect of Carbon Dioxide

Several mechanisms operate to maintain the composition of alveolar air. Small increases in the CO_2 fraction of the blood have a definite effect on the medullary respiratory centres. There is normally 0.03 per cent carbon dioxide present in the inspired air. If the medulla is cut off from the sensory input, and the carbon dioxide content of the inspired air is raised to 6.5 per cent, medulla shows well defined respiratory potentials. The effect of CO_2 on ventilation is shown in Fig. 13.10. At higher concentrations ventilation increases sharply. Many individuals can tolerate about 8 per cent carbon dioxide in the inspired air, but higher concentrations produce depression and unconsciousness and ultimately respiratory paralysis may occur. In mammals, breathing is generally increased due to the action of CO_2 on chemoreceptors located centrally in the medulla and peripherally in the carotid and aortic bodies (See Chapter 10).

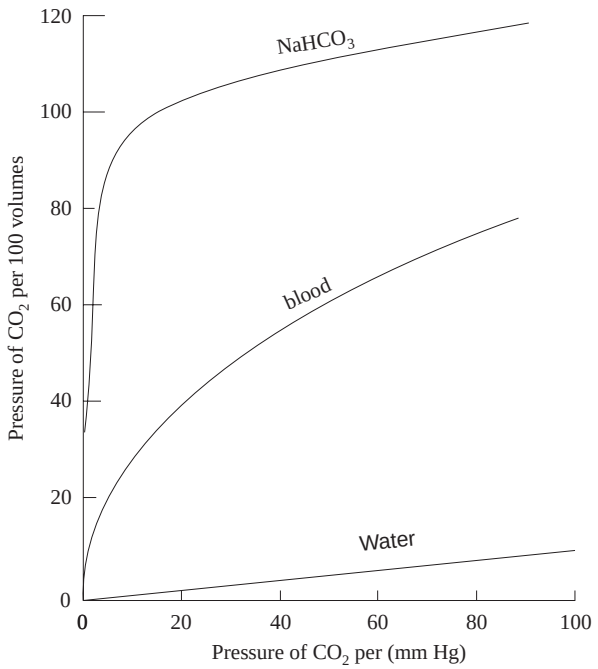


Fig. 13.10 Effect of CO_2 on ventilation.

Low CO_2 tension of the blood causes a decrease in lung ventilation and an increase raises ventilation. The partial pressures of carbon dioxide express the ventilation of lungs more precisely. The normal PCO_2 is 40 mm Hg and if it increases to 60 mm Hg, ventilation increases at least ten times. If the tension of CO_2 is lowered, such as by voluntary deep breathing, CO_2 is lowered in the blood, as a result of which the respiratory centre is temporarily inhibited. Low CO_2 tension in the blood causes *acapnia* and in this condition the excitability of the respiratory centre is blocked causing cessation in breathing.

Effect of Low Oxygen Supply

Oxygen tension in the blood is the determining factor for regulation of breathing. It depends largely on the percentage of O_2 in the atmosphere and upon the atmospheric pressure. At sea-level the percentage of oxygen is less but the breathing remains normal. If the oxygen tension is further lowered, breathing is stimulated in order to inhale more oxygen into the alveoli. A simple experiment can be done to demonstrate this principle. If a person is subjected to breathe in an air-tight collapsible bag through a soda lime container, the person becomes blue. The outgoing CO_2 is absorbed by the soda lime but renewal of oxygen is not possible. Thus oxygen lack causes unconsciousness and even death.

Effect of Acidity

Carbon dioxide influences the respiratory centre to increase the acidity (H^+) of the cells. Increased acidity of the blood enhances ventilation, although the pH may not vary extensively. However, it seems quite likely that to some extent, increase in pH is due to CO_2 tension. According to Gesell (1939), intravenous injection of $NaHCO_3$ will increase the CO_2 tension and thus stimulate respiration. On the other hand, injection of Na_2CO_3 help in lowering CO_2 tension in the tissues making blood alkaline. This depresses respiration.

Neural Control of Respiration

We have already noted that the most important factor in the regulation of respiration is the carbon dioxide tension. Carbon dioxide of blood stimulates the respiratory centre whose activity is modified by afferent impulses arriving at the medulla. The impulses originate from the receptors in the chest-wall and influence the vagus nerves, and the nerves from the carotid body and the carotid sinus. It is, however, emphasized that the act of breathing is quite complicated and involves the movement of voluntary muscles. The control of all these muscles is exerted by the respiratory centre in the brain stem or medulla. The voluntary muscles contract when they receive impulses from the respiratory centre. The intercostal muscles are supplied by motor nerves arriving from the spinal cord. The diaphragm is served with the phrenic nerves which take off from the fourth and fifth spinal nerves. From the respiratory centre, impulses arrive at the diaphragm through phrenics. Certain other fibres carry impulses to the external-intercostal muscles which contract to cause respiration. Relaxation of intercostals causes expiration.

The control of breathing by respiratory centre has been studied in mammals and it is believed that there are three stepwise controls in the process:

(1) pneumotaxic centre; (2) expiratory centre; and (3) inspiratory centre. Respiratory control involves:

1. Adjustments due to variations in the composition of environmental gases or body fluids.
2. Central nervous system.
3. Centrally mediated acts of respiration.

The medulla contains two groups of neurons that control inspiratory and expiratory centres in all vertebrates (Fig. 13.11). It controls all rhythmic respiratory activities and is also dependent on the higher centres. If the medulla is sectioned from the spinal cord, all respiratory activities will cease. If the brain is cut between the medulla and the lower pons, respiratory activity becomes abnormal showing the influence of higher centres.

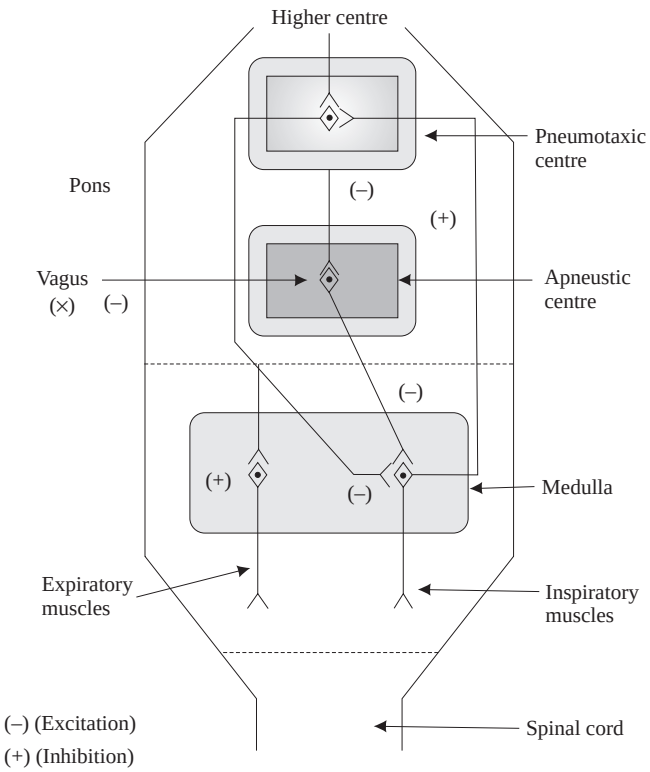


Fig. 13.11 Diagram showing respiratory control centres of the brain and their possible interconnections.

The pneumotaxic centre is the controlling centre situated in the cranial part of the pons which controls activity of neurones of the lower part. The expiratory centre exerts control over the muscles of expiration. The inspiratory centre is said to be in a constant state of excitability and it sends impulses to inspiratory muscles, but it also sends impulses to the pneumotaxic centre (Fig. 13.11). The pneumotaxic centre in turn transmits impulse to the expiratory centre which will also send inhibitory impulses to the inspiratory centre.

Chemoreceptors

Certain chemoreceptors sensitive to changes in the partial pressures of oxygen and carbon dioxide also control the respiratory activities. These are present in the carotid bodies located at the bifurcation of internal and external carotids and in the aortic bodies on the dorsal aorta. If the PO_2 in the blood and tissues falls, there is increase in the frequency of respiration. Both carotid and aortic bodies receive a profuse arterial blood supply. They can perceive changes in the PO_2 of water, especially in aquatic vertebrates. Low PO_2 acts as stimulant of respiratory activities. In mammals a fall in PO_2 to 50 mm Hg increases the firing rate of chemoreceptors. During asphyxia, when PCO_2 is more and PO_2 low, the receptors are activated.

Vagal Control

There are numerous *stretch receptors* or inflation receptors scattered throughout lungs which respond to the distension of lungs during inspiration. The vagus nerve supply to the lungs has some afferent fibres which end up in these receptors. During the inspiratory cycle these receptors are stimulated and send impulses to the expiratory centre through vagus fibres. This centre than sends inhibitory impulses to the inspiratory centre to stop inspiration. This is a reflex termed *Hering-Breuer reflex* in which the vagus centre during inspiration is affected indirectly in the opposite direction by the impulses which inhibit respiratory centre. The lungs get deflated mechanically. This, however, happens in quiet breathing. During laboured breathing, sudden collapse of lungs stimulates deflation receptors which send impulses to the inspiratory centre causing forced respiration. This reflex is an important mechanism for the control of breathing in many animals and also for a short time in the newborn baby. In adult man, it is weak or even absent.

CHAPTER

14

Excretion

As a result of metabolic activities certain waste products are formed. The major waste products are carbon dioxide, water and nitrogenous compounds. These wastes, if retained in the body, will have harmful effects. Hence their removal becomes necessary. Removal of these wastes from the tissues of the body to the outside is called excretion.

14.1 ORGANS OF EXCRETION

The organs or the tissues responsible for the elimination of waste products are called excretory organs. These organs eliminate the wastes in the following ways:

(i) by eliminating nitrogenous wastes.

(ii) by adjusting water balance of the body.

(iii) by maintaining ionic composition of the extracellular fluids.

Major organs of the body which help in the excretion process are: integument, gills, liver, intestine, lungs and kidneys. In certain lower animal groups such as protozoa and porifera, excretion of wastes takes place directly through the cellular membranes. In such cases, simple mechanisms like osmosis and diffusion may be found very effective. In certain species, however, excretion is done through contractile vacuoles as in *Amoeba* and *Paramecium.* In higher invertebrates and vertebrates, definite excretory organs are found which do the specialized job of excretion. Integument or the skin helps in the elimination of urea through the sweat glands. Along with the urea, certain inorganic salts are also removed by the skin. The gills and the lungs are helpful in removing gaseous products like carbon dioxide. Liver is one of the most important glands in the body of vertebrates which helps in the removal of cholesterol, bile salts and excess of calcium and iron salts. These are generally eliminated by the intestine along with the faecal matter. The intestinal epithelium also excretes some inorganic salts which are in excess. Rubidium, potassium, calcium and magnesium, etc., are excreted in part through the intestinal wall. Kidneys are the major excretory organs in all vertebrate groups and

also in some invertebrates which eliminate urea, excess of water, salts and other nitrogenous wastes. The renal mechanisms are responsible for maintaining ionic regulation or fluid balance in majority of animals.

14.2 TYPES OF EXCRETORY PRODUCTS

The end-products of metabolism fall under two major categories: (i) carbon dioxide; and (ii) compounds containing nitrogen. Besides these products, water forms an important product which requires to be eliminated if found in excess, and also it forms a vehicle for the transport of waste products to the exterior. Here we shall give our attention mainly to the production of nitrogenous wastes and their elimination.

Nitrogenous Wastes

In living organisms, nitrogen is never eliminated in the form of free nitrogen but results in the formation of nitrogenous end-products. Proteins are the main nitrogen containing compounds which are metabolized to form end-products like ammonia, urea and uric acid. These end-products are derived from the degradation of proteins, amino acids, pyrimidines and purines.

Proteins are important dietary constituents needed for the building, growth, and repair work of the body. Proteins are broken down to smaller protein molecules (peptides and dipeptides) upon hydrolysis. These subunits can be further hydrolyzed to yield amino acids which are metabolized to yield ammonia and urea as nitrogenous end-products.

Normally, adults are said to be in a state of *nitrogen balance* when the nitrogen loss equals to the nitrogen intake. If the nitrogen balance is disturbed in an animal, it experiences a new situation and tries to adjust itself to a new nitrogen level. This new level can be achieved by an adjustment between increase or decrease of nitrogen excretion. Nitrogen excretion losses from the body are generally measured by analysis of the urine and faeces.

Ammonia

Ammonia is the chief breakdown product of amino acids and is removed by oxidative deamination process. Deamination chiefly occurs in the liver, but kidney also helps in the process.

Ammonia is a toxic substance, and is constantly being produced in the tissues by deamination of amino acids. It is removed very rapidly from the body by one of the following reactions:

(1) amination of keto acids.

(2) amidation of glutamic acid.

(3) formation of urea in the liver.

The rapidity with which ammonia is removed from the body ensures a very low concentration of it in the blood of most animals. Mammals cannot withstand ammonia in their blood in concentrations more than 0.0001 to 0.0003 mg/100 ml. However, the blood of amphibians, reptiles and fishes can withstand a higher concentration of ammonia (less than 0.1/100 ml). Many invertebrates show a higher tolerance for ammonia.

Ammonia is highly soluble in water and in majority of aquatic animals, it is lost by diffusion in the surrounding water. In a number of animals ammonia does not form the excretory waste, but helps in maintaining acid-base balance. In mammals, ammonia is obtained chiefly by deamination of glutamine of blood by glutaminase (Fig. 14.1). This happens in kidney where ammo-nitrogen is increased, which reacts with hydrogen ions so that more ammonium ions (NH_4^+) are secreted. This aspect of acid-base balance will be treated in more details while discussing the role of kidney. Ammonia is also formed from urea by the action of urease.

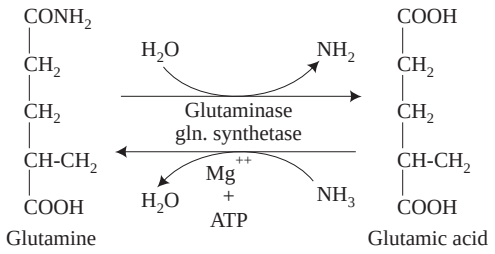


Fig. 14.1 Deamination of glutamine results in the production of NH_3. Glutamine is synthesized from glutamic acid by glutamine synthetase.

Urea

Urea is derived from organic compounds like amino acids and purines, and liver is believed to be the chief organ capable of making it. It is highly soluble in water and less toxic than ammonia. The human blood normally contains 18 to 38 mg of urea per 100 ml. However, higher concentrations of urea can be tolerated by man which, of course, indicates uremic condition.

Urea formation in liver has been studied by Krebs and Hanseleit and has been explained in a series of reactions (Fig. 14.2).

Ornithine, citrulline and arginine are the three amino acids which participate in the formation of urea. Liver contains an enzyme arginase which hydrolyzes arginine to ornithine and urea is formed as a byproduct. This is also known as *Ornithine cycle.*

The ornithine cycle has been studied mostly in the mammalian liver. In many vertebrates which lack arginase, urea is not formed and instead uric acid is the chief end-product of nitrogen metabolism.

The steps involved in urea formation are as follows:

1. Citrulline is formed by the addition of CO_2 and ammonia to ornithine. This CO_2 and ammonia come from *Carbamyl phosphate.*
2. Citrulline gives rise to arginine in two intermediate steps. In the first step, citrulline and aspartate form arginosuccinate in the presence of ATP and magnesium ion. It is a reversible reaction. In the second step, arginine is formed from arginosuccinate by splitting of fumarate. The fumarate is later converted to malate and oxaloacetate in citric acid cycle, and gives rise to aspartate (Fig. 14.3).

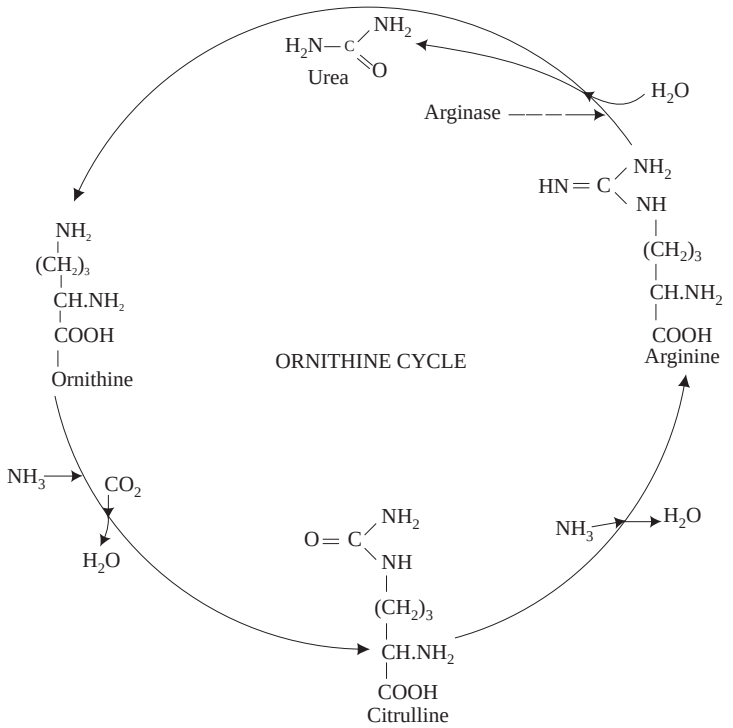


Fig. 14.2 Urea formation in the liver by ornithine cycle.

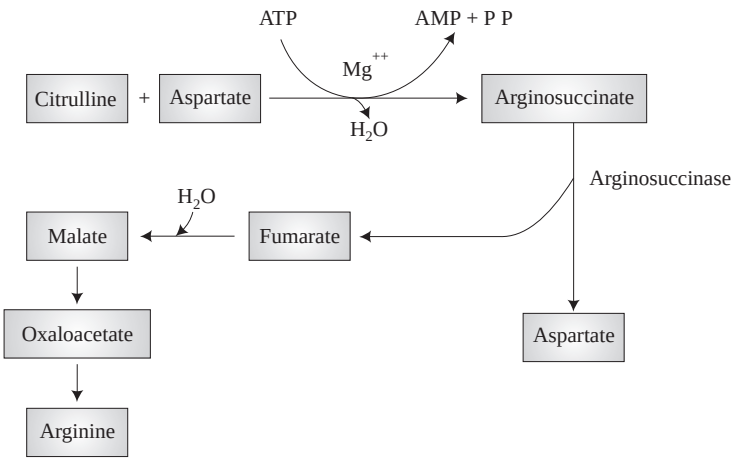


Fig. 14.3 Metabolism of citrulline.

Uric Acid

Uric acid is the most important nitrogenous waste in the urine of birds, reptiles, some snails and insects. It is formed from ammonia and contains less hydrogen than any other nitrogenous waste. Uric acid is less toxic and being insoluble in water, may be stored or excreted in crystalline form. Formation of uric acid is an adaptation for the conservation of water since its elimination requires very little water.

Uric acid is formed in the liver of birds, and in insects it is made in the Malpighian tubes. Uric acid is formed either as an end-product of purine metabolism or as a product of waste nitrogen derived from the protein. In man, uric acid is the end-product of purine metabolism. In subprimate mammals, as also in a number of insects, uric acid is further oxidized to allantoin. Thus allantoin is the main end-product of purine metabolism in such animals (Fig. 14.4). Further breakdown products of allantoin are derived as follows:

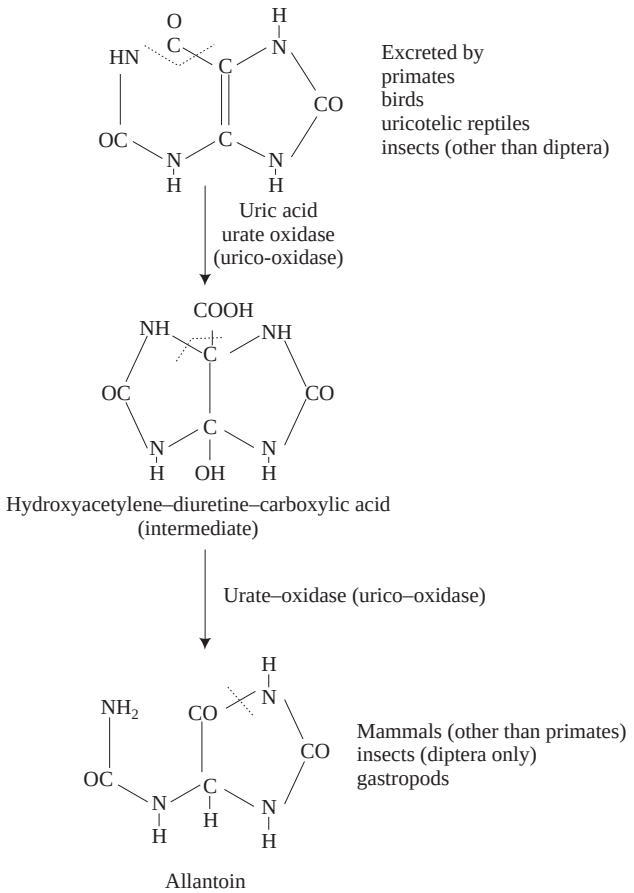


Fig. 14.4 Conversion of uric acid to allantoin.

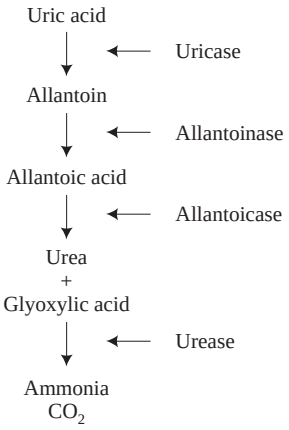


Fig. 14.5 Breakdown of uric acid to ammonia and CO_2.

Other Nitrogenous Constituents

The excretory nitrogenous products come from nucleic acid metabolism. There are purine compounds, viz. adenine and guanine. Pyrimidine nitrogen is excreted as urea or ammonia. Traces of pyrimidine may be excreted as such also.

(a) *Guanine:* Guanine is the main nitrogenous excretory product in some arthropods (spiders), but is conspicuously absent in insects. It is faintly soluble in water and its mode of formation is rather unknown.

(b) *Xanthine and hypoxanthine:* In a number of insects *(Melophagus, Galleria* and *Pieris,* etc.) xanthine and hypoxanthine are excreted.

(c) *Trimethylamine oxide:* Marine teleost fishes, which have a diet rich in trimethylamine, excrete trimethylamine oxide.

(d) *Hippuric acid and ornithuric acid:* Hippuric acid is formed in mammals. The diet of mammals contains traces of benzoic acid which is a toxic substance. This benzoic acid combines with amino acid glycine to form a less toxic substance hippuric acid. In case of birds, dietary benzoic acid combines with ornithine and is excreted in the form of ornithuric acid.

(e) *Creatine and creatinine:* Creatine is present in the muscle, brain and blood in the free state as well as combined state (as phosphocreatine). Traces are present in the urine also. Three amino acids–glycine, arginine, and methionine are involved in the synthesis of creatine. Some of the creatine is converted into creatinine which is an anhydride of creatine. It is formed largely in muscles and occurs in the blood and urine in free state.

(f) *Pterydines:* Pterydines are also regarded as excretory products which are important pigments in insects. The synthesis of pterydines resembles that of uric acid. In some insects *(Oncopeltus)* traces of pterydines are excreted in the faeces, whereas in butterflies *(Pieris brassicae)* pterydines are deposited in the wings, fat body, etc. Only traces are excreted in the urine.

14.3 PATTERNS OF EXCRETION

We have seen that diverse types of nitrogenous excretory products are formed in animals, and more than one type of such products may be excreted in an individual. The dietary proteins are digested in the stomach and the intestine by way of enzymatic hydrolysis which liberates amino acids. Most of the amino acids are absorbed as such, while some may be lost by way of excretion. Thus the loss of amino acids from the body may prove injurious to the health of the organism. Amino acids are required as the building blocks for the synthesis of the blood and tissue proteins. Due to transamination reactions nitrogen is formed which may be eliminated in several forms (urea, ammonia, etc.) as described above. Based upon the type of nitrogenous compound excreted, animals have been classified into several broad categories.

Ammonotelic Animals

Animals in which ammonia is the chief metabolic waste are called ammonotelic. Ammonia is highly soluble in water and diffuses rapidly from the body surface into the surrounding aquatic medium. Aquatic vertebrates excrete large amounts of ammonia which is formed by hydrolysis of urea present in the blood. The presence of ammonia in blood is toxic, hence requires plenty of water for rapid elimination. The amount of ammonia liberated in the body varies with the diet and the species of the animal. Certain protozoans like *Tetrahymena* and *Paramecium* excrete large quantities of ammonia. Sea anemones excrete about 52.7 per cent ammonia in the form of nitrogenous waste. Echinoderms and polychaetes are also ammonotelic. Cephalopods and pelecypods, both freshwater as well as marine forms, excrete large quantities of ammonia.

The crustaceans excrete ammonia predominantly although they form amino acid nitrogen also. In aquatic insects also ammortotelic behaviour is found. In *Sialis* larvae, for example, about 90 per cent of nitrogen in the form of ammonia is liberated. Aquatic habitat of animals appears to be an important requirement for ammonotelic behaviour and may be said to be an aquatic adaptation. An interesting behaviour in earthworms has been described by Bahl (1947). It was experimentally demonstrated that earthworms kept in natural moist surroundings produce more urea than ammonia. However, if the earthworms are kept immersed in water they start excreting ammonia (Delaunay, 1934).

Freshwater fishes let out more ammonia than urea, a major proportion of which diffuses out through the gills. The amount of nitrogen excreted out through urine is comparatively less.

Ureotelic Animals

Ureotelic animals excrete most of their nitrogen in the form of urea. It is the predominant organic substance present in the urine of animals. The problem of water conservation in adult mammals has necessitated reabsorption of water in the kidney tubules, thus excreting urea in a concentrated form. In desert animals, low water intake has resulted in active tubular secretion of urea. In ruminants, urea excretion is greatly reduced and is retained in the rumen which acts as a protein source. It has been suggested that low protein diet leads to a marked decrease in urea output, whereas a high protein diet increases urea production.

Amphibians are predominantly ureotelic, as also the elasmobranch fishes. The synthesis of urea in frogs takes place in the liver, and in elasmobranchs all tissues except brain and blood are capable of synthesizing it by the same cycle as occurring in mammals.

Uricotelic Animals

Terrestrial animals like insects, lizards, snakes and birds excrete their nitrogen in the form of uric acid. As already described, uric acid is formed by deamination and oxidation of purine bases (guanine and adenine). Uric acid production is also related to the problem of water conservation in these animals.

In insects uric acid is the most important nitrogenous constituent of urine. When plenty of water is available to insects, uric acid remains in solution in the Malpighian tubules. However, during scarcity of water, uric acid crystallizes in the form of crystalline spheres. In *Rhodnius,* the urine is dried and consists of about 64-84 percent of uric acid (Brown, 1937). In lizards, snakes and birds, the urine is in the form of a solid or a semi-solid mass and contains large quantities of uric acid.

Guanotelic Animals

In some arthropods such as spiders, guanine is a predominant excretory product elaborated by the Malpighian tubules and cloacal sacs. The formation of guanine from protein nitrogen is still not adequately known.

Trimethylamine Oxide

Marine teleosts excrete trimethylamine oxide as the major nitrogenous product which is soluble in water and nontoxic in nature. It is, however, absent in marine elasmobranchs. Marine teleosts are faced with the problem of maintaining osmotic balance by retaining water in the body which is aided by trimethylamine oxide. This compound is present in small quantities in the muscle, and blood of the marine fish which diffuses out through the membrane. This compound has a foul smell and is probably derived from the breakdown products of lipoproteins. Considerable quantities of trimethylamine oxide are formed in octopus, squids, crabs and barnacles. It occurs in traces in the urine of certain animals like echinoderms, oysters, gastropods and tunicates. Its presence in marine teleosts is, however, related to the maintenance of concentration in the body. It has been suggested that this substance is not produced endogenously, and rather comes from the food of the fish.

Table 14.1 Percentage of Nitrogen Products Excreted in Different Forms

Animal	*NH_3*	*Urea*	*Uric acid*	*Aminoacid*	*Purine*	*Others*
Annelids						
Sipunculus	50	9.7	0	16.6	4.1	19.4
Lumbricus (fed)	72	5	1.4	–	–	16 undetermined
Pheretima	42	50	0	0.6	–	Creatine 7.8
Echinoderms						
Asterias	39.3	11.7	trace	23.8	3.6-10	16-26

Contd.

Contd.

Molluscs						
Sepia	67	1.7	2.1	7.8	4.9	
Limnaea (F.W., summer)	42	14	5	–	39	
Crustaceans						
Carcinus	68	3	0.7	8.7	3.2	
Astacus	60	11	0.8	10.1	4.4	
Insects						
Rohdnius (1 day post feeding)	0	trace	90.92	0	–	+ creatine
Aedes aegypti	6.4	11.9	47.3	4.4	–	19
Culex pipens	10	7.9	46.9	5.5	–	20
Fish						
Cyprinus	60	6.2	0.2	6.5	–	22
Torpedo	1.7	85.3	–	1.7	–	
Amphibians						
Bufo (larva)	80	–	–	–	–	–
Bufo (adult)	15	–	–	–	–	–
Birds						
Hen	3.4	10	87	–	–	–
Mammals						
Dog	2.7	88	0.4	–	–	Allantoin 3.6
Man	4.8	86.9	0.65	–	–	Creatine 3.6 undetermined 4.0

*Based on Prosser and Brown, *Comparative Animal Physiology,* 2nd ed. (1961).

14.4 CHANGES IN NITROGEN EXCRETION WITH LIFE CYCLE

Principal wastes come from the metabolism of purines, pyrimidines and amino acids. It is a known fact that the production of nitrogenous wastes may vary during the development and adult life; variations may be due to environment and diet also. In amphibians, for example, urea is a predominant waste in the adults, whereas larval amphibians are ammonotelic. Environmental influence is perhaps more important in shifting the production of nitrogenous substances. If animals producing ammonia are transferred to a medium containing more salt, their capacity of urea production is enhanced. Higher concentrations of urea are helpful in maintaining osmoregulatory functions.

Embryonic history of animals reveals that the patterns of metabolism change with the differentiation of organ systems. The metabolic machinery of the individual is geared to suit the individual to its environment. In case of the tadpole of *Bufo,* 80 per cent of nitrogen is excreted as ammonia which gradually declines to 15 per cent until the adult stage is reached. Needham has proposed existence of recapitulation in patterns of nitrogen excretion on the basis of excretory pattern in the chick. In a three-day old chick embryo, ammonia is the chief excretory product. The percentage of ammonia gradually declines. On the fifth day, ammonia declines considerably and the percentage

of urea increases to a maximum until the eighth day. After this, urea also starts declining and uric acid increases rapidly. About the eleventh day uric acid concentration in the urine is maximum with a concomittant drop in urea. The cycle of events in the embryonic life of the chick shows that up to four days the chick was ammonotelic, later on it became ureotelic, and about the eleventh day, it became uricotelic and continued to be so throughout its adult life.

14.5 DIETARY INFLUENCE ON NITROGEN EXCRETION

Diet influences the pattern of excretion to a great extent. Insects provide the best examples to demonstrate this fact. In a reduvid bug *Rhodnius,* immediately following a blood meal, considerable quantity of urea is excreted in the urine which contains excess of water and salts. After a few hours urine becomes rich in uric acid. In the meat eating larvae of *Calliphora* and *Lucilia* (blowfly) the excreta is rich in ammonia. However, in the pupal stage excretion of ammonia is replaced by uric acid. Thus the excretion depends on the substances present in excess in the diet and also on the production of waste substances in metabolism.

Earthworms furnish yet another example where the excretory wastes depend on the nutritional state. In a normal well-fed *Lumbricus,* urea appears in small quantities, i.e. 8-15 per cent of the total excretory nitrogen, while ammonia is predominant. During the state of starvation urea increases up to 85 per cent and percentage of ammonia declines considerably.

14.6 EXCRETORY DEVICES IN INVERTEBRATES

Excretory devices met with in the organisms are essentially the adaptive capabilities evolved in relation to their habitat. Animals may reside in one of the following surroundings: freshwater, marine and land. Diverse excretory devices have been developed in animals in order to ensure ionic regulation of the body fluids.

Protozoans

Although protozoa do not have specialized excretory organs, the wastes are discharged through cellular membranes. Several mechanisms like osmosis, diffusion, etc. are responsible for waste elimination through the membranes. However, in a number of species, contractile vacuoles serve as excretory organelles. The function of contractile vacuoles has been extensively studied in *Paramecium.* The vacuoles are membrane bound vesicles formed temporarily, which collect excess amount of water and discharge on the surface of the organism. These vacuoles may be of wandering type and may follow a definite path for elimination. Only freshwater protozoans possess such vacuolar mechanisms for waste regulation.

Coelenterates

Coelenterates also do not possess specialized excretory organs and processes like diffusion osmosis and active transport to regulate the fluids in the body. The need for organs of excretion in coelenterates is greatly restricted.

Platyhelminthes

The animals of this phylum are characterized by having a specialized *flame cell system.* The flame cell is a large cell blinded at one end and bearing many cytoplasmic processes. There are series of such cells which open in an excretory duct. The nucleus is displaced generally towards the blind end side and the cytoplasm bears many secretory droplets. A bunch of cilia arises in the hollowed out cytoplasmic region which keep on moving to produce a directed flow of fluids. The excretory products enter the flame cells in a fluid state from the parenchymatous cells by diffusion. Excess of water alongwith metabolic wastes are thus discharged by the flame cells.

Annelida

The excretory organs are in the form of tubular and coiled structures called *nephridia* which are metamerically arranged. These nephridia are open at both ends, hence known as metanephridia. In some annelids, protonephridia are present in place of metanephridia, which are branched and open blindly in the coelom. A metanephridium differs from a protonephridium in having a ciliated funnel or nephrostome. These nephridia receive fluid waste from the blood and the coelomic fluid and eliminate urine rich in urea and ammonia.

Mollusca

In molluscs, the excretory organs are in the form of *kidneys* and *pericardial gland.* The kidneys are mesodermal organs which communicate with the coelom, whereas the epithelial lining of the pericardium containing glandular tissue serves as pericardial gland. In cephalopods, the nitrogenous wastes are eliminated in the form of guanin, while uric acid and urea in case of opisthobranchs and bivalves respectively.

Arthropoda

Excretory organs in arthropods are of several types and include nephridia, coxal gland, green gland, shell gland and Malpighian tubules, etc. Except Malpighian tubules, these organs are derived from coelomoducts. In the present context, we shall deal with the mechanism of excretion by Malpighian tubules as they have proved to be the most efficient organs of excretion in terrestrial arthropods. These tubules open into the lumen of the intestine through their proximal end and their distal blind end remains suspended within the haemocoelomic spaces. The excretory products pass out through the alimentary canal.

The Malpighian tubules collect and transport solutions from the haemolymph into the hindgut where water and some physiologically important compounds are absorbed by the hindgut epithelium. These tubules are characteristic organs of insects which help in removing wastes and sometimes to conserve water. The physiology of these tubules has been excellently described in mosquito larvae by Ramsay (1953) and in *Rhodnius* by Wigglesworth (1965). The tubules are bathed in the haemolymph from where they absorb potassium ions. The absorption of potassium ions takes place by active transport mechanism which also helps in the diffusion of water and substances of low molecular weight such as inorganic salts, glucose and urea into the tubules. A continuous flow of such substances from the haemolymph into the hindgut takes place via these tubules. In the hindgut,

recovery of essential compounds and water takes place by reabsorption and thus only wastes are eliminated. The rectal glands of insects are responsible for conservation of water. Generally the Malpighian tubules lie freely in the body cavity, but in certain insects the terminal portions are intimately attached to the wall of the rectum (Fig. 14.6). This condition is found in lepidopterous larvae, coleoptera and some tenthredinid larvae and is associated with the conservation of water in dry habitats.

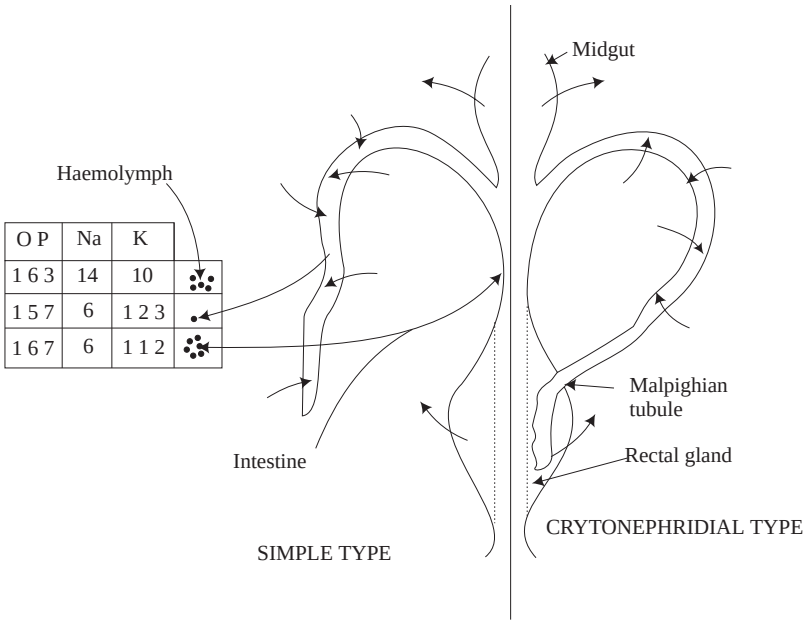


Fig. 14.6 Malpighian tubule of an insect.

In *Rhodnius,* a clearcut regional distinction has been found in the Malpighian tubules. Wigglesworth (1942) found that the upper portion is concerned with the secretion and the lower portion with reabsorption. For this reason, the lower portion contains uric acid granules and the upper portion contains clear fluid.

14.7 EXCRETION DEVICES IN VERTEBRATES RENAL PHYSIOLOGY

Kidney

Kidneys are the chief organs for excretion of wastes in vertebrates and, therefore, deserve special attention. Besides their excretory function, kidneys function in a significant manner in the maintenance of internal environment of the body.

Structure of Kidney: Mammalian kidney could be taken as an example to explain the structure and function of a typical vertebrate kidney. The kidneys are paired organs which are generally bean-shaped structures. When seen in a sagittal section, it shows two main divisions. The outer portion is called the *cortex,* and the inner region forming the main mass of the kidney is called the *medulla.* The medulla is composed of several pyramids containing renal tubules projecting into a cavity towards the inner region of kidney, called the *pelvis.* Pelvis is the region where the renal artery and vein enter kidney. The cortex contains a large number of Malpighian bodies and convoluted tubules (Fig. 14.7).

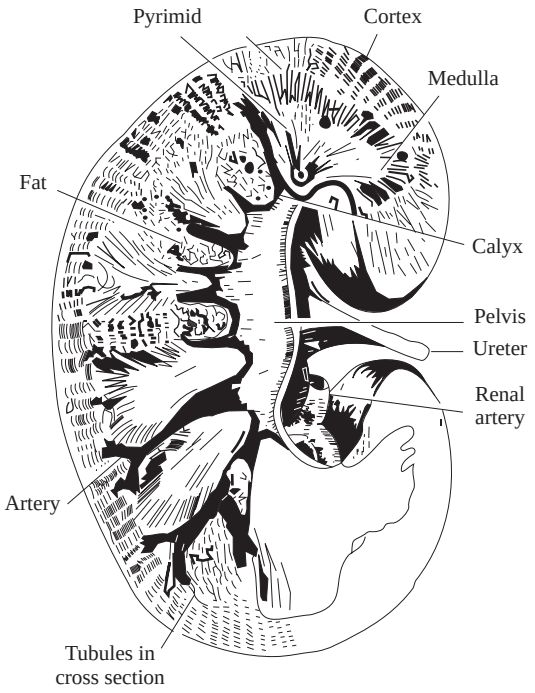


Fig. 14.7 L.S. of vertebrate kidney.

Histological examination of kidney shows that it is made up of a large number of secreting units, called *nephrons.* Each nephron is composed of a spherical structure known as the Malpighian body and a convoluted tubule. Malpighian bodies have a double-walled capsule enclosing a network of capillaries called *glomerulus.* The capsule opens into a long tubule through a narrow neck and takes a rather tortuous course in the cortex, and later descends down in the medullary region. Here it makes a *loop of Henle* and through ascending and descending loops terminates into a collecting tubule (Fig. 14.8). The tubules are surrounded by a network of blood capillaries which helps in the exchange of materials between the blood and the cells of the tubules. The capsule has very thin layer of endothelial cells, while the ascending and descending loops are lined with cuboidal cells. The lumen of the tubules is very narrow.

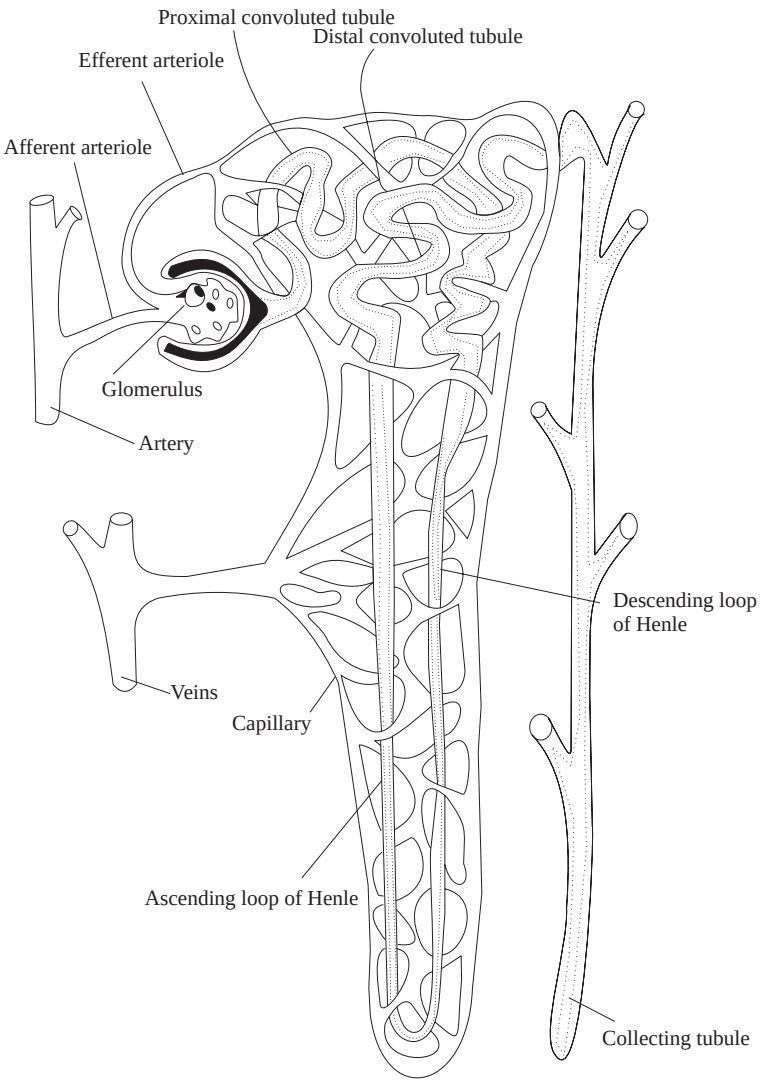


Fig. 14.8 Structure of a nephron.

Blood Supply: Kidneys receive a very rich supply of blood. An adult kidney receives about 1.3 litres of blood per minute. The blood supply of kidney comes from a short renal artery which arises from the abdominal aorta. After entering the kidney, the renal artery divides into a number of arterioles—*afferent arterioles.* The *afferent arterioles* further branch into capillaries and enter into each glomerulus. These capillaries then join to form another arteriole called *efferent arteriole* which further opens into another set of capillaries, called *peritubular capillaries* surrounding the proximal tubule, the thin loop and the distal tubule of the same nephron. Having taken this tortuous course, this

capillary opens into avenule which joins with other venules to form finally the renal vein. The renal vein opens into the inferior vena cava.

At this stage, it would be interesting to know as to how the pressure for blood flow is maintained through afferent and afferent arterioles, and peritubular capillaries. The renal artery is thick and short and thus the pressure drop in reaching the blood to the kidney is small. The afferent arterioles which are larger than other arterioles in the body lower the pressure to some extent to about 60 mm Hg which is close to the hydrostatic pressure in the glomeruli. The efferent arterioles bring the pressure further down to about 15 mm Hg. This causes further decrease in the hydrostatic pressure in the peritubular capillaries and allows movement of fluid into the capillaries. Further, the afferent and efferent arterioles are subjected to vasomotor changes to alter the blood pressure in the glomeruli. Constriction of the afferent arterioles decreases the pressure within the capillaries and allows less blood flow. On the other hand, dilation of these arterioles causes more blood to flow through them. Efferent arterioles, upon contraction, raise the blood pressure in the glomerulus capillaries and consequently decrease the blood flow.

Functions of kidneys: The principal functions of kidney are:

(1) To eliminate certain nonvolatile waste products of the body like urea, sulphates, etc.
(2) To regulate hydrogen-ion concentration of blood by eliminating any excess of nonvolatile acids and bases.
(3) To remove excess of certain nutrients such as sugar and amino acids when their concentration increases in the blood.
(4) To remove foreign or injurious substances from the blood, such as, iodides, pigments, drugs and bacteria, etc.
(5) Maintenance of osmotic pressure of the blood by regulation of the excretion of water and inorganic salts, thus keeping constant the volume of circulating blood.
(6) Kidneys regulate the arterial blood pressure by secreting the hormone *renin.*

Urine Formation: In mammals each kidney is composed of tens of thousands of uriniferous tubules which form the urine. Urine is formed from the blood circulating in the glomerulus (Fig. 14.9). Thus urine is a filtrate of the blood which goes into the tubules as a dilute fluid resembling the plasma deficient in colloids. This dilute fluid is concentrated in the tubular region by reabsorption of the excess portion of the fluid and certain salts and thus replaced in the blood stream. Besides, certain susbstances may also be secreted by the tubular epithelium into the urine. Formation of urine by the kidneys is considered to be due to three types of activity–glomerular filtration, selective secretion and tubular reabsorption.

Function of the Glomerulus: In the middle of the nineteenth century, Ludwig proposed a theory of physical filtration and diffusion. According to him, noncolloidal constituents of blood were removed by the thin membranes of the glomerulus by filtration and only clear dilute filtrate was allowed to enter the tubules where excess amount of water was reabsorbed making the urine concentrated. Later, Cushny in 1914 proposed a partial modification of Ludwig's theory and suggested that alongwith water, the noncolloidal constituents of the plasma filter through the glomerulus. As the liquid passes through the tubular region, some of the water, salts, glucose, amino acids and certain other constituents useful for the body are reabsorbed by the tubular epithelium and sent back to the blood.

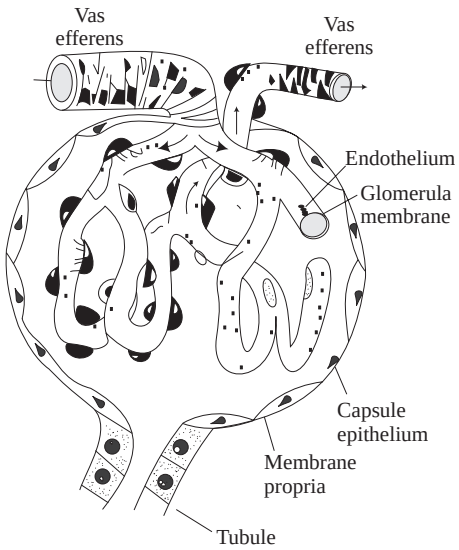


Fig. 14.9 Structure of a Bowman's capsule.

We shall now consider the process of filtration in the light of the current ideas. The structure of the glomerulus suggests that it is best suited for the purpose of filtration which would depend on three conditions: (1) semipermeable nature of the glomerular membranes; (2) osmotic pressure exerted by the contents on either side of the membrane and; (3) blood pressure in the glomeruli.

Filtration: The semipermeable nature of glomerular membrane would allow to pass through it proteins of low molecular weights. Egg albumin (MW*:* 35,000), gelatin (MW: 35,000) and haemoglobin (MW: 64,500) and substances around these molecular weights can be expelled through it. Proteins of high molecular weight such as casein (200,000), serum globulin (160,000) and serum albumin (72,000) are not excreted. Such membranes exert osmotic pressure to effect filtration. The colloids of the blood exert an osmotic pressure, against filtration and may cause diffusion of water into the blood. However, the hydrostatic pressure of blood would try to force the water from the blood into the tubules. This suggests that blood pressure is higher than the osmotic pressure to cause filtration and the energy of filtration is derived from the hydrostatic pressure. The hydrostatic pressure of blood in the afferent glomerular artery is about 75 mm Hg, whereas the osmotic pressure exerted by the plasma proteins is about 20 to 30 mm Hg. The interstitial pressure acting on the capillaries is 10 mm Hg. Therefore the final pressure or the actual driving force is 25-30 mm Hg which may be expressed as follows:

$$P_b - P_o - P_c = P_f$$

where P_b = hydrostatic pressure of the blood, P_o = osmotic pressure of proteins, P_c = total of interstitial pressure and movement pressure, and P_f = final driving force.

Glomerular filtration rate (GFR): About 1 litre of blood is filtered per minute by both the kidneys. When the net filtration pressure is about 25 mm Hg, about 120 ml of glomerular filtrate is formed at the Bowman's capsule. Therefore GFR is about 120 ml per minute, which is actually the volume of plasma filtered per minute. It may be expressed as:

$$GFR = \frac{UV}{P}$$

where U = mg of the filtered substance per ml urine, V = ml of urine per minute, and P = mg of filtered substance per ml of plasma.

Function of the Tubule: The composition of urine is quite different from the glomerular filtrate. The glomerular filtrate contains some essential substances like water, glucose, amino acids, chlorides, sodium and other wastes like urea, creatinine, and uric acid. The essential substances are retained to carry on normal metabolism, and this selective function is carried out by the tubules of the kidney. Thus by secretion and reabsorption the glomerular filtrate is transformed into urine.

Tubular Reabsorption: Certain substances which appear in normal quantities are reabsorbed completely, but appear in the urine when normal levels are exceeded. Such substances are known as *threshold substances.* Amino acids and glucose are such threshold substances which are efficiently reabsorbed by the tubular cells (Fig. 14.10).

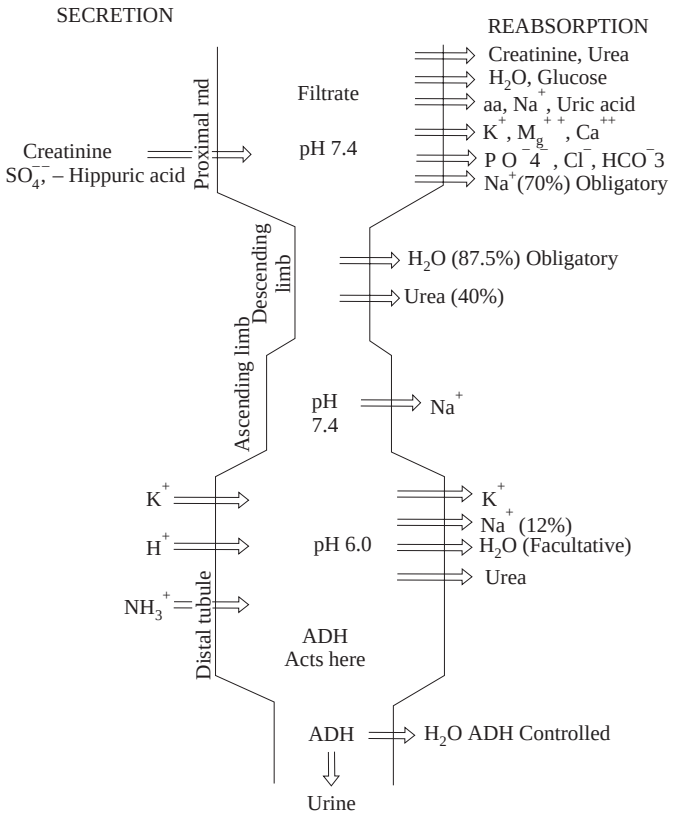


Fig. 14.10 Schematic diagram of a urinary tubule showing functional regions of secretion and reabsorption of various substances.

Reabsorption of glucose: In a normal adult, when the GFR is 120 ml/minute, about 120 mg of glucose are transferred into the filtrate per minute. Normally, except only a few mg, the entire quantity of glucose is reabsorbed. The absorption takes place in the proximal part of the tubule by active transport mechanism associated with phosphorylation. In men, the maximum rate at which glucose can be reabsorbed by the tubule is 350 mg/minute. This is known as the tubular maximum for glucose (TmG). In women TmG is about 300 gm/minute. Sometimes considerable amounts of glucose are found in the urine, a condition known as glycosuria. Glycosuria can be artificially caused by administering *Phlorizin* which inhibits phosphorylation.

Reabsorption of water: The osmotic pressure of the plasma remains more or less unchanged and is due to the presence of inorganic salts which act as electrolytes. If large quantities of water are taken, this might cause dilution of the plasma. This causes reduction in the osmotic pressure which results in the excretion of larger amounts of water in the urine. Thus the kidney defends osmolarity of the plasma by excreting excess amount of water. Increased rate of urine secretion is known as *diuresis* and the substances which produce this effect are called diuretics. Urea has a diuretic effect. Certain salts such as NaC1 and Na_2SO_4 also have diuretic effects.

Normally 150 to 180 litres of glomerular filtrate is produced every day, and out of this approximately 80 per cent of the filtrate is reabsorbed in the proximal part of the tubule. This is called obligatory reabsorption.

Reabsorption of inorganic salts: Sodium, chloride and bicarbonate ions are selectively reabsorbed in proximal tubular portion. Along with the reabsorption of Na^+ there is parallel reabsorption of it so that regaining of NaCl helps in the return of water. Reabsorption of Na^+ is aided by the adrenal cortical hormone.

Potassium is also present in small quantities in the glomerular filtrate. Normally all the potassium filtered is reabsorbed by the proximal tubule. However, if any potassium appears in the urine, it originates as a tubular secretion from the distal tubule which is responsible for acid-base equilibrium. The secretion and reabsorption of sodium and potassium are controlled by a hormone, *aldosterone,* which is secreted from the adrenal cortex. Sodium reabsorption is lowered during osmotic diuresis and *glucocorticoids* (costisol and corticosterone) increase the tubular reabsorption of Na^+ in exchange of H^+ and also reabsorption of Na^+ with Cl^-.

Diuresis: When the rate of urine secretion is increased, the condition is called is *diuresis* and the substances which cause this are known as diuretics. Glucose and urea have marked diuretic effects. Besides these, other compounds like caffeine and deoxycorticosterone acetate also act as diuretics.

Increased water content in the blood results in diuresis. The solutes of the glomerular filtrate which are not absorbed in the proximal tubule exert an osmotic pressure so that more water passes out of the tubules thereby increasing the concentration of solutes in the blood. Such is the state when glucose and urea are present in higher concentrations in the blood. If sufficient water is available to the organisms, the excretion of water runs parallel with that of urea. Animals consuming a high protein diet will form more urea in the body and would require larger volumes of intake of water, otherwise a mild diuresis occurs. Diabetic patients also excrete larger volumes of urine so that the filtered load of glucose exceeds TmG resulting in a condition known as polyuria.

In some cases, diuretics bring about an increased blood flow through the kidney which is mainly the function of vasomotor nerves. Although no direct nervous control of the kidney has been demonstrated, yet sometimes under emotional stress, diuresis or decrease in urine excretion are observed. This may be caused by vasomotor nerves which bring about vasoconstriction or dilation of renal arteries and arterioles, thus altering the kidney secretion.

Clearance: The maximum amount of the blood plasma that can be cleared by the kidney in one minute is called the clearance.

Clearance tests are done with reference to the plasma. Mathematically, calculation of plasma clearance of any substance can be represented as:

$$C = \frac{UV}{P}$$

where C = plasma clearance in ml per minute, U = concentration of the substance in urine in gm/100 ml, V = volume of urine passed in ml/minute, and P = concentration of the substance in the plasma in gm/100 ml.

To take a specific example, if the plasma contains 0.05 gm of inulin per 100 ml, the urine 6.25 gm inulin per 100 ml, and the rate of urine excretion is 1 ml per minute, then inulin clearance rate would be

$$\frac{6.25 \times 1}{0.05} = 125 \text{ ml/minute}$$

Homeostasis

Life is an extension of non-living processes. The physico-chemical laws which are applicable to the non-living systems are applicable to the living systems as well in many ways, a living process can be considered as a kind of super-chemistry and is an example of interactions between matter and energy to produce a highly complicated and well organised self-duplicating automatic system. Thus living system is not a *closed* system but an *open* system with matter and energy that flow into the system being in a *steady state* with the matter and energy that flow out of the system. Living organisms create and maintain their essential orderliness at the expense of their environment and this tendency to maintain themselves in a steady state condition is known as *homeostasis.*

In the nineteenth century, physiologist Claude Bernard developed an idea that the cells and tissues of the body which are bathed in an internal fluid constitute the internal environment or *milieu interior,* whereas the external environment of the whole organisms constitutes *milieu exterior.* The internal environment is maintained at a constant, irrespective of the organism's external environment. In animals a number of homeostatic mechanisms are at work which serve to maintain internal environment such as osmotic and ionic regulation, temperature regulation, buffer mechanisms, active transport and excretion. The details of these homeostatic mechanisms have been described in appropriate places of this book.

14.8 COMPOSITION OF URINE

The volume and composition of urine varies remarkably and such variations are governed by the type of food consumed by the individual, and volume of fluid intake. In a normal adult about 1-1.5 litres of urine is formed daily. In warm climates, urine volume is less since a good amount of water is lost through perspiration. If the volume of water intake is increased, urine volume is also increased.

The normal urine is transparent and straw coloured. Pale yellow colour is due to the presence of pigment called urochrome. Specific gravity of urine varies from 1.005 to 1.040 and it is generally acidic with a pH 6.0. Upon standing, urine becomes alkaline because conversion of ammonia from urea takes place.

The concentration of the constituents in urine varies with individuals. It consists of a number of solids, half of which is urea. Average concentration of principal constituents is given in Table 14.2.

Table 14.2 Composition of Normal Human Urine (Volume of urine 1.250 ml/24 hours)

Constituents	*Grams per 24 hours*
Water	12,140
Total solids	58.5
Total nitrogen	15.5
Urea	25.0 – 28.5
Uric acid	0.6
Creatine	1.5
Ammonia	0.7
Hippuric acid	0.60
Allantoin	0.01
Carbohydrates	0.90
Oxalates as oxalic acid	0.015
Lactates as lactic acid	0.01
Ketone bodies, acetone	0.01
Free amino acids, etc.	3.0
Chlorides	12.0
Phosphates as phosphoric acid	2.3
Sulphates as H_2SO_4	1.8
Potassium	2.0
Sodium	6.0
Calcium as CaO	0.2
Magnesium	0.2
Iron	0.006

The constituents of urine may be broadly classified into three categories: (1) nitrogenous wastes; (2) nitrogen-free organic compounds; and (3) inorganic salts.

Principal Constituents

(1) **Urea:** It is diamide of carbonic acid and is the most abundant substance of the urine. The urea content depends on protein catabolism of an individual. Animals having low protein diet produce less urea, whereas in animals whose diet is rich in protein, urea content increases. It normally contains 60-90 per cent of total nitrogen.

(2) **Ammonia:** Fresh urine contains little ammonia. However, on standing urea is converted into ammonia which occurs mainly in the form of salts such as urates and chlorides.

(3) **Creatinine and Creatine:** Creatinine contents of urine are fairly constant. The concentrations of creatinine are as follows:

20-26 mg/kg body weight/day in normal man.

14-22 mg/kg body weight/day in normal woman.

Creatine is also present in the urine of infants.

(4) **Uric Acid:** It is the most important end-product of purine metabolism in the body. In certain diseases, like leukemia, liver disease and gout, the uric acid output is increased.

(5) **Amino Acids:** Glycine, histidine, glutamine and cystine are principal amino acids found in the urine. Other amino acids are also present, but in very small quantities. About 200 mg of amino acids are excreted per day in urine of a normal man.

(6) **Allantoin:** This compound occurs in very small quantities in human urine. However, in other mammals its content is more where it is formed due to purine metabolism. This is also derived from the partial oxidation of uric acid.

(7) **Hippuric Acid:** Small quantities of hippuric acid are found in the urine. It is formed by conversion of benzoic acid, which otherwise cannot be oxidized easily. Conversion is generally accomplished by the intestinal bacteria.

In addition to the above mentioned substances, small amounts of chlorides, sulphates, phosphates, oxalates, minerals, vitamins and steroid hormones and gonadotropins are also present in the urine.

Table 14.3 Some Constituents Urine and Plasma Compared

	Plasma mg %	*Urine mg %*	*24 hour urine gm*
Glucose	80.0	0.0	0.0
Urea	2.5	1,600	24.0
Uric acid	4.0	53	0.8
Creatinine	1.5	100	1.5
pH	7.41	6.0	6.0

14.9 COUNTERCURRENT MECHANISM

Generally, with variations in the intake of fluid and solutes, the osmotic pressure of the plasma remains unaltered. The osmolarity of the plasma of many animals is around 285-300 mOsm/litre of water which depends on its inorganic salt contents. If large volumes of water are taken, the kidney adjusts the osmolarity of the plasma by excreting larger amount of water. However, in some animals hyposmotic or hyperosmotic urine may be produced under certain conditions.

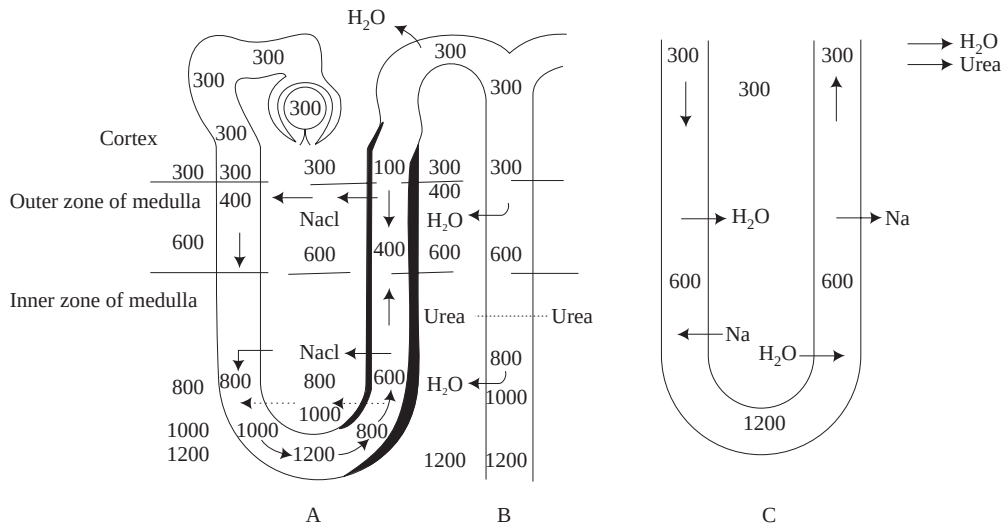


Fig. 14.11 A scheme of the countercurrent mechanism.

In order to achieve these conditions, certain mechanisms are at work. Hyposmotic filtrate may be produced if sodium chloride is reabsorbed in the ascending limb of the tubule. On the other hand, the tubular filtrate becomes more concentrated, as much water without solute is reabsorbed in the descending limb, thus producing hyperosmotic urine. Fishes and amphibians are capable of producing hyposmotic urine whereas reptiles produce hyper-osmotic urine. It is only in case of birds and mammals where hyposmotic as well as hyperosmotic urine may be produced. The mechanism by which these conditions can be achieved is called *countercurrent mechanism.*

The countercurrent mechanism has certain special features which are as follows:

(1) The descending limb of Henle's loop is permeable to water, whereas the ascending limb has poor water permeability.

(2) The ascending limb of the nephron is the site for active transport of sodium.

(3) The regulatory influence of the antidiuretic hormone (ADH) is exerted on the permeability of the distal tubules and the collecting ducts. High hormone levels increase water permeability and its absence reduces it to a low level.

The countercurrent mechanism is a process in which urine is rendered hyposmolar as it passes through different portions of the tubules.

From the structure of the urinary tubules, it is seen that the descending, ascending and collecting tubule are very close to each other. The flow in the ascending tubule runs counter to that in the descending and the collecting tubule. Sodium and other ions are transported out of the filtrate in the proximal tubule where water also moves out. By the time urine reaches the loop of Henle, about 80 per cent of total filtrate is out of the tubule. The loop of Henle absorbs lot of sodium and chloride and hands over to the surrounding tissue, making the urine concentrated or hyperosmolar.

In the ascending tubule and the loop of Henle, there is no movement of water consequent upon the active transport of sodium, making the filtrate less concentrated. The fluid returned to the distal tubule is hypotonic, but this segment is able to reabsorb water and the fluid reaching the collecting tubules is isotonic. There is further transport of sodium from the collecting duct, but as the fluid passes through the medulla, water diffuses out of the filtrate and the fluid reaching the pelvis of the kidney is the concentrated form of urine. From Fig. 14.11 it will be seen that all along the system the concentration gradient is always about 200 mOsm/litre and never greater than this. However, the concentration of the filtrate progressively increases from 300 mOsm/litre to 1200 mOsm/litre by the time it passes out of the collecting tubule.

The countercurrent mechanism is also maintained by the vasa rectae. In vasa recta the flow of blood run counter to the flow of urine. The flow of blood in the medullary tissue is downward and in the cortex it is upward. As the blood flows through the medulla, water diffuses out and sodium diffuses in, whereas reverse movement takes place in the cortex, leaving most of the sodium to remain in the medullary interstitial fluid circulation.

14.10 ACID-BASE REGULATION

The kidney regulates both volumes of water and mineral salts. Apart from this, another important function performed by the kidney is acid-base regulation. Kidneys provide a self-correcting device by means of which cations like sodium are conserved. Excess of sodium ions is exchanged for hydrogen ions in the proximal tubules, whereas exchange of sodium and potassium ions for hydrogen ions takes place in the distal tubule.

During metabolism hydrogen ions and ammonia are produced in excessive amounts which must be buffered to maintain the balance. In protein metabolism, certain nonvolatile acids, such as lactic acid, the ketone bodies, sulphuric acid, etc., are produced which must be eliminated by some mechanism. In the course of metabolism of phospholipids, phosphoric acid is produced. These acids are buffered by sodium ions mainly and eliminated by filtration in the glomerulus. Sodium ions thus lost are recovered by tubular reabsorption in exchange for hydrogen ions.

In the proximal tubule, carbonic acid is formed from CO_2 and water in the presence of carbonic anhydrase. This carbonic acid is ionized to release H^+ ions which are mobilized out of the tubule and is exchanged for sodium bicarbonate as shown in Fig. 14.12. This results in the formation of carbonic acid in the tubular filtrate, which again decomposes to set free CO_2 and water; CO_2 thus set free again diffuses back into the cells of the tubule and hydrogen ions are reutilized.

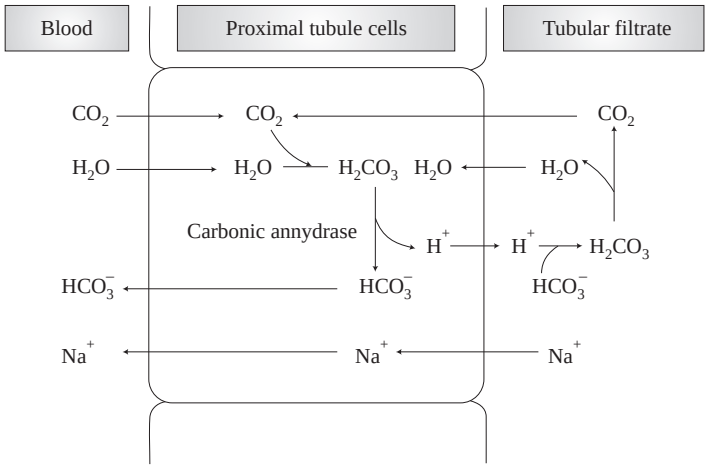


Fig. 14.12 Mobilization of hydrogen ions in the proximal tubule.

Almost all bicarbonate ions are reabsorbed in the proximal tubule and the remaining are absorbed in the distal tubule. In the distal tubule, the hydrogen ions are exchanged for sodium ions of Na_2HP_4 increasing the acidity of urine. Figure 14.13 gives a summary of the secretion of hydrogen ions in the distal tubule.

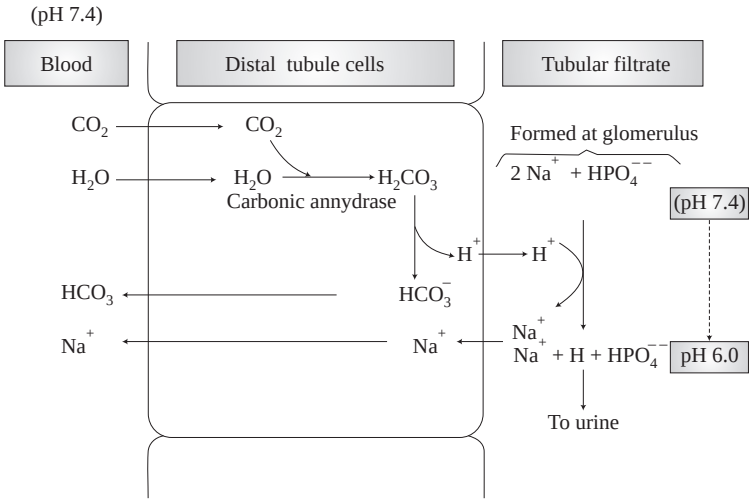


Fig. 14.13 Secretion of hydrogen ions in the distal tubule.

The distal tubule helps in the elimination of hydrogen ions by yet another process. The cells produce ammonia by deamination of amino acids which combines with the hydrogen ions so that ammonium (NH_4^+) ions are produced. Such a mechanism operates when sodium chloride is present in sufficient amount in the tubular filtrate. The mechanism is summarized in Fig. 14.14.

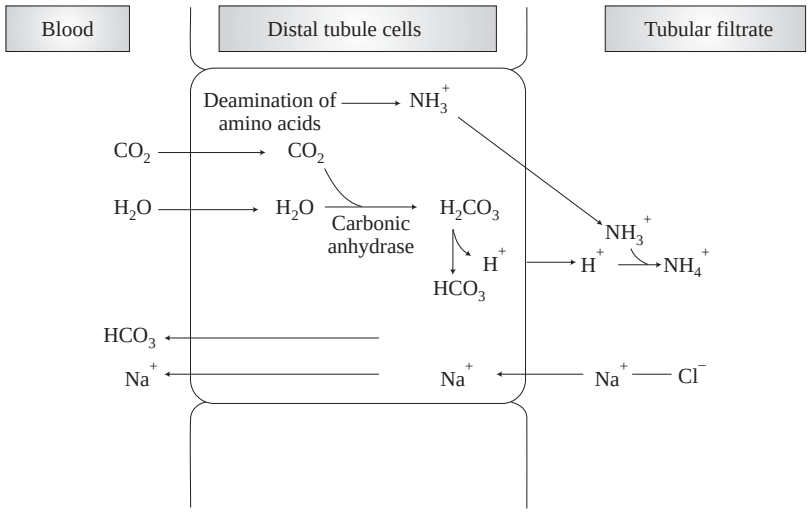


Fig. 14.14 Sectetion of ammonia ions in the distal tubule.

14.11 RENAL CONTROL MECHANISMS

Several control mechanisms operate to maintain constancy of the composition and the volume of the extracellular fluid.

The osmolarity of urine changes without any variation in the amount of salt excreted. Any gain or loss in the amount of water is dependent upon the action of the antidiuretic hormone (ADH) secreted from the posterior lobe of the pituitary. The ADH level is controlled by changes in the osmotic pressure of the extracellular fluid. There are special 'osmoreceptors' situated in the hypothalamus or in the walls of the blood vessels going in it. These receptors are sensitive to changes in the osmolarity of the blood. ADH secretion is thought to be due to nervous impulses originating in the hypothalamus and passing into the posterior lobe of the pituitary. When the extracellular osmolarity is lowered, the ADH secretion is suppressed which in turn decreases water reabsorption. However, higher levels of ADH stimulate water reabsorption. The ADH has a specific site of action acting at the distal tubule and the collecting ducts.

This control mechanism can be exemplified by a disease, *Diabetes insipidus*. The disease is caused due to damage of certain centres in the brain stem inhibiting ADH secretion. Such patients dischange large amounts of urine, sometimes exceeding 40 litres a day. In order to compensate the excessive loss of water, the patients drink large amount of water. Administration of ADH injections cures this disease.

The mechanisms which maintain the volume of extracellular fluids are poorly understood. However, the hormone of the adrenal cortex, aldosterone, a sodium conserving hormone, is considered to be significant in this regard. The site of action of this hormone is the distal tubule. Aldosterone remains in circulation and its levels are influenced in two possible ways:

(1) Low sodium concentration in the extracellular fluid, provides a stimulus to the adrenal cortex for aldosterone secretion.

(2) The extracellular fluid volume influences the secretion, increase in volume enhances, while decrease in volume suppresses aldosterone secretion.

The possible mechanism of aldosterone secretion may be explained as follows: special cells of the renal cortex (ischemic region) secrete a proteolytic enzyme, renin, that goes into the blood by renal vein. The impact of renin reduces the glomerular filtration rate and consequently delivery of sodium to the distal tubule is decreased. Loss of sodium induces shrinkage in the fluid volume. Whenever there is a fall in the fluid volume, the glomerular filtration rate is also lowered. Renin acts upon angiotensinogen, a globulin present in the plasma, and converts it to angiotensin. Angiotensin, increases the rate of heart beat and arterial blood pressure thereby stimulating aldosterone secretion. Aldosterone promotes sodium reabsorption by the renal tubules, and to maintain proper osmotic balance, water is retained to keep up the fluid volume. Thus sodium chloride is an important electrolyte which regulates the extracellular fluid volume.

CHAPTER

15

Nerve Physiology

Living organisms have a unique property to respond to changes in the environment. The information received by the organism is coordinated through the nervous system so that the organism can act effectively under any circumstance. The information is obtained in the form of a stimulus. A stimulus is a change in the environment which evokes a response from the organism. The types of stimuli are many and diverse, such as caused by light, heat, sound, temperature, pressure, gravity, chemical, etc. All such information is responsible to evoke a response from the organism by chiefly affecting the receptor organs and may be perceived simultaneously. The mechanism of perception is a highly complicated one involving a number of sensory structures in the body.

Some of the information is used consciously by the organism so as to influence its behaviour. Information may be gathered and used unconsciously as well, such as contraction of muscles, blood pressure, posture of the body, etc. All these information influence the behaviour of the organism involving nervous system. In order to process diverse information from an animal's environment, the nervous system has to function in three principal ways:

(1) It should receive external and internal stimuli to provide sensory information about itself and its own environments.

(2) It should integrate all this information so that meaningful interpretation of the data fed in by sensory organs may be made.

(3) It should regulate intracellular activity to maintain coordination of movement of the whole organism (This may involve stimulation of muscles, glands and neurons concerned with perception, learning and memory, etc.).

In order to understand the functioning of nervous system, it is necessary to consider the basic structure and properties of its structural components.

15.1 UNITS OF THE NERVOUS SYSTEM

The nervous system may be divided for the sake of convenience into: (1) the central nervous system including the brain and the spinal cord; and (2) the peripheral nervous system consisting of cranial nerves, spinal nerves and (3) the autonomic nervous system. The functional units of the nervous system are called *neurons* which are distributed throughout the body (vide infra). They have a large number of different kinds of *synapses* through which information can be transmitted.

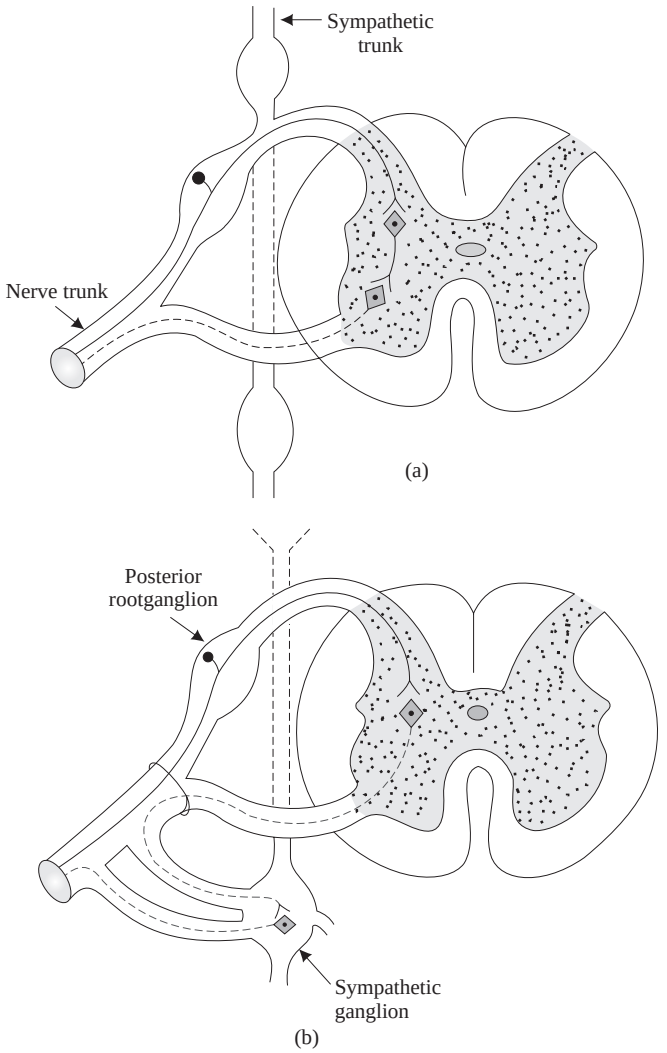


Fig. 15.1 Diagram showing the anatomical relationships of (a) somatic and (b) autonomic nervous system.

In higher organisms, the process of cephalization has resulted in the concentration of nerves as well as neurons which has given rise to brain. Cephalization of the highest order is found in mammals and birds, where a well developed central nervous system (CNS) consisting of a spinal cord, brain stem, medulla oblongata, cerebellum and the cerebrum. The peripheral system consists of nerves that are directly connected with the brain and supply to the peripheral organs.

In the central nervous system, there is a somatic division or somatic nervous system and the autonomic nervous system. The somatic nervous system is characterized by the presence of certain special connections called synapses lying within the spinal cord, whereas in the autonomic system the synapses lie outside the spinal cord (Fig. 15.1).

Main functions of the central nervous system are to receive impulses, to give rise to various patterns of responses, and also to modify them. As soon as the stimulus is given, a response is conducted which is spontaneous. In order to transmit impulses, nervous system has certain basic functional units which have been referred to as neurons. These are a part of the grey matter.

Neurons

The existence of neurons was first discovered by His and Forel. Neurons are elongated nerve cells; a vast majority of them are located in the spinal cord and brain. A neuron is a microscopic structure consisting of a main cell body, dendrites, a long axon fibre and a number of terminal fibres (Fig. 15.2). The axon is a hollow cylinder filled with cytoplasm which differs in its chemical composition from the surrounding fluid. The axon takes part in the formation of a nerve.

The part of the neuron containing the cell body alongwith the nucleus is not needed to conduct waves of impulses, but it is mainly needed for growth, maintenance and repair. If an axon is severed off from the nucleated cell body it would be regenerated. If the cell body is destroyed, the axon cannot be regenerated.

The axon is generally covered with a myelin sheath, which acts as an insulator. This sheath is highly developed in vertebrate neurons. The myelin sheath over the axon is not continuous but leaves small areas naked which occur at regular intervals of about 1 mm. These naked areas are called nodes of *Ranvier*. The cell body and also the terminal fibres are not enveloped by this sheath. These two naked areas are the regions of input and output respectively (Figure 15.3).

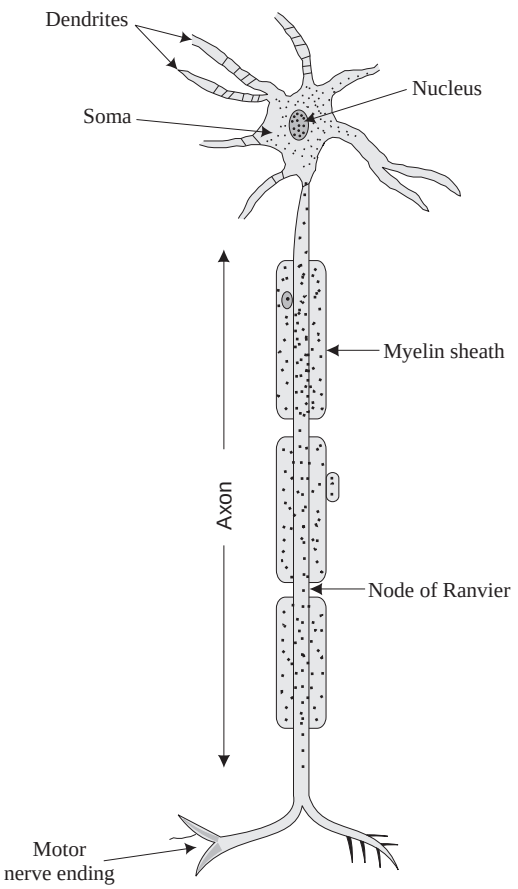


Fig. 15.2 Diagram of motor neuron with its axon.

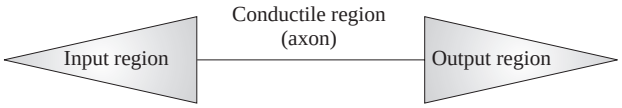


Fig. 15.3 Schematic diagram to show the functional components of a neuron.

In invertebrates, the neurons are usually enclosed by a single layer of sheath cells called *Schwann cells* (Fig. 15.4). However, some invertebrate neurons are naked. A nerve is made up of several such neurons. The cell body contains protoplasm and a well defined nucleus. Within cytoplasm are minute *nissle bodies.*

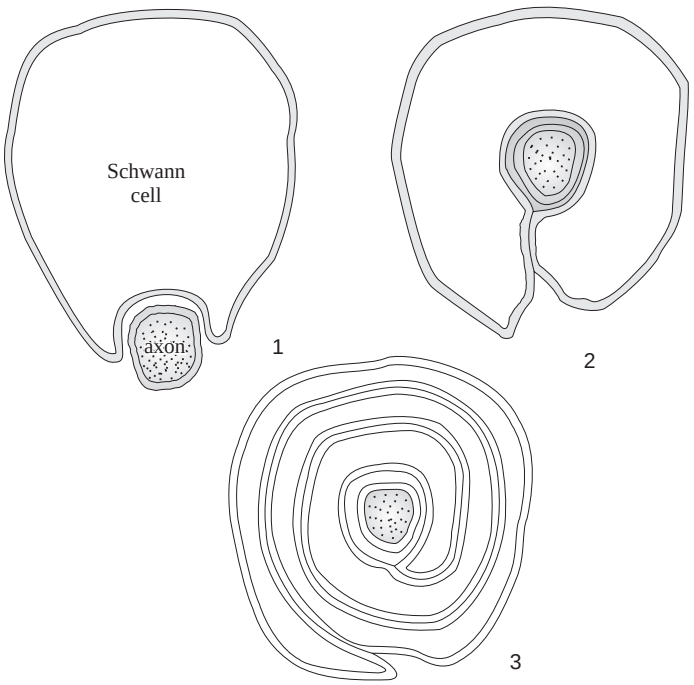


Fig. 15.4 Diagram of schwann cells.

Axon can be distinguished from dendrites as it is longer and carries neurosecretory vesicles within its distal tip. Dendrites branch in the immediate vicinity of the cell body and provide an increased surface area for reception of stimuli from the external environment or from other neurons. Physiologically speaking, axons differ from dendrites in two ways. Firstly, the dendrites receive stimuli from the external environment and from neighbouring neurons and convert them into impulses. Secondly, the dendrites usually carry the stimuli toward the cell body, whereas the axons carry impulses from their origin (in the cell body) to the terminal fibres at the distal end.

Neurons exist in a bewildering variety of shapes and sizes, but all share common features. Neurons can be classified on the basis of the number of cell processes, functions and whether they are myelinated. A myelinated neuron is enclosed in a sheath which is composed of one to several layers of membranes derived from schwann cells. The membrane contains cholesterol and phospholipids.

Structurally, the neurons may be classified on the basis of the number of cell processes leaving the cell body. A *unipolar* neuron has one axon that carries the impulse towards or away from the axon. A *bipolar* neuron has two processes arising from the cell body, and a *multipolar* neuron has several dendrites at one end, an axon on the other.

On the basis of function, neurons can be classified into four major types: (1) motor neurons which convey information from the central nervous system to the effector organs, such as muscles, glands, etc.; (2) *sensory neurons*, which transmit sensory information from the peripheral parts of the body to the central nervous system; (3) *internuncial neurons* which lie between motor (efferent) and sensory (afferent) neurons, transmit signals in several directions by modifying them in a way best suited to the need of the animal; and (4) *neurosecretory neurons* that are specialized for the production of hormones.

Functioning of the nerves depends on the neurons and this can be demonstrated by cutting the nerve from the neuron which renders the cell functionless. Cajal propounded his famous neuron theory and suggested that the dendrites arising from the neuron form a complex network in the grey matter and all of them are in contact with each other. Sherrington (1897) suggested that the dendrites are not continuous but continuous. Cajal's theory was later discarded in favour of Sherrington's.

The Neuroglial Cells

In some invertebrates, all the axons are devoid of the surrounding sheath and remain naked. In most invertebrates and all vertebrates, neurons are fused with satellite cells or neuroglial cells. These are huge cells with large membrane potentials and low electrical resistance. Although their functions are poorly understood, they are supposed to serve many important functions in influencing the nervous activities.

A primary function of these neuroglial cells seems to be exchange of nutrients and waste products since they are rich in glycogen deposit and lipid granules. They also help in giving structural support for neurons and appear to be involved during degeneration and regeneration of nervous elements. They do not produce any action potentials and are not involved in the electrical activity of the central nervous system.

15.2 IRRITABILITY

Irritability can be defined as the capacity to react to changes in the environment, both internal and external. Irritability is always expressed in some kind of response. Organisms possess certain mechanisms of irritability and respond to various stimuli. Generally strong stimuli produce a response which is spread over a large area. This is due to formation and conduction of wave of excitation passing along the cell membrane. The sequence of events is; (a) stimulation; (b) excitation; (c) conduction; and (d) response.

When a stimulus is given, there is a delay (latent period) before the response is observed. This delay is due to the time taken for conduction of the wave of excitation from the point of stimulation.

15.3 ELECTRICAL PHENOMENA OF NERVES

Resting Potential

The inside of a neuron, when not excited is negative to the outside. This difference in charge is known as the *membrane potential* or *resting potential.* The resting potential may be defined as the net difference in the charge between inside and outside of a neuron requiring little or no expenditure of energy. In a neuron this resting potential is about –70mV, so long as the neuron is in a non-excitable state, and is maintained almost constant. The following paragraphs would explain the mechanism involved in the resting potential.

The neuron, like all other living cells, is enclosed by a semipermeable membrane containing large quantities of protein molecules. These molecules are unable to penetrate through the membrane, and bear a net negative charge. However, smaller ions of potassium (K^+), sodium (Na^+) and chloride (Cl^-) can diffuse across the membrane. Na^+ ions are found in higher concentration outside the cell, and in low concentration inside the cell. Conversely, K^+ ions are distributed in high concentration inside the cell and in low concentration outside the cell. The relative concentrations of some of these ions inside and outside the squid axons and frog muscle are found to be as follows (in mM concentration):

Table 15.1 Ionic Concentration of K^+, Na^+ and Cl^-, on Either Side of the Membrane of Squid Axon and Frog Muscle Fibre

	Squid axon		*Frog muscle fibre*	
Ion	*Inside*	*Outside (blood)*	*Blood*	*Muscle*
K^+	400	20	2.5	140
Na^+	50	440	120	9.2
Cl^-	40	560	120	3.4

These relative concentrations given in Table 15.1 suggest that Na^+ behaves differently as compared to K^+. Na^+ ions are more outside the fibre (or membrane) making it positively charged in relation to the inside. How is it that. the Na^+ ions do not migrate inside along their concentration gradient to take the place of K^+? German physiologist Bernstein (1902) propounded a hypothesis to explain the reason for this phenomenon (ionic equilibrium) which exists during resting potential. He suggested that the membrane is selectively permeable to K^+ ions and impermeable to Na^+ ions during this state. Potassium ions which are slightly smaller can however, flow out to make the outside more and more positively charged thereby building up a negative potential inside (Fig. 15.5). As a result of this an ionic equilibrium would be attained, when the potential difference reaches to about 60 mV between inside and outside. Such an equilibrium would prevent a further outflow of K^+ ions.

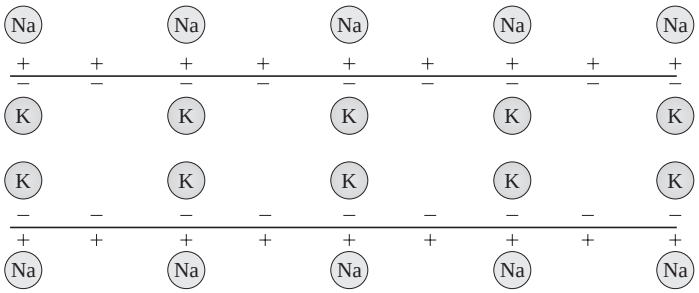


Fig. 15.5 Distribution of ions in a nerve fibre during resting potential.

Electrical Activity in a Stimulated Neuron

A stimulus could be in the form of an electrical, chemical, physical, electromagnetic or pressure change. Once a stimulus is given to a nerve cell, it produces certain electrical events which bring about a potential change and this could be measured with the help of a cathode ray oscilloscope. For this purpose, microelectrodes are placed on the nerve and connected to the cathode ray oscilloscope through an amplifier. Changes in the potential are recorded in the form of vertical deflections as they move on the oscilloscope screen.

Chronaxie

There are two parameters which determine the excitability of the tissue: (1) minimal strength of the current when allowed to flow for any length of time will produce excitation, and (2) a definite amount of current should flow for a minimal time to produce excitability. In order to evoke a response, the stimulus must act for certain duration. This was shown by Lapicque who demonstrated the existence of a definite relationship between the strength of the stimulus and its duration. The minimal time required to evoke a response is called *excitation time.* The stronger the stimulus, shorter is the excitation time. This could be shown with the help of Fig. 15.6.

A strong stimulus of 300 mV requires a brief length of time, but for a weaker stimulus greater length of time is needed. In Fig. 15.7, 100 mV is considered to be the threshold strength of the stimulus which is applied for a longer duration. A weaker stimulus than this would be ineffective and will not evoke a response. If the strength of the current is increased, the excitation time is lowered. Lapicque gave a term *chronaxie* to explain this principle. Chronaxie defined as the time required for a current twice the *rheobase* to excite a nerve. Thus the efficiency of a stimulus is determined by its intensity, its duration, and the speed with which it is applied.

Action Potential

The most familiar activity of the excited neurons is the action potential. The equilibrium condition of the resting potential can be disturbed by applying an outside energy source.

When the neuron is excited, there is a marked change in the potential from the resting level zero and then it becomes 20 or 30 mV. This reversal of polarity called the *overshoot* (Fig. 15.7). Suddenly the potential then reverts to the resting level, that is about –70 mV. In certain cases, it may fall even

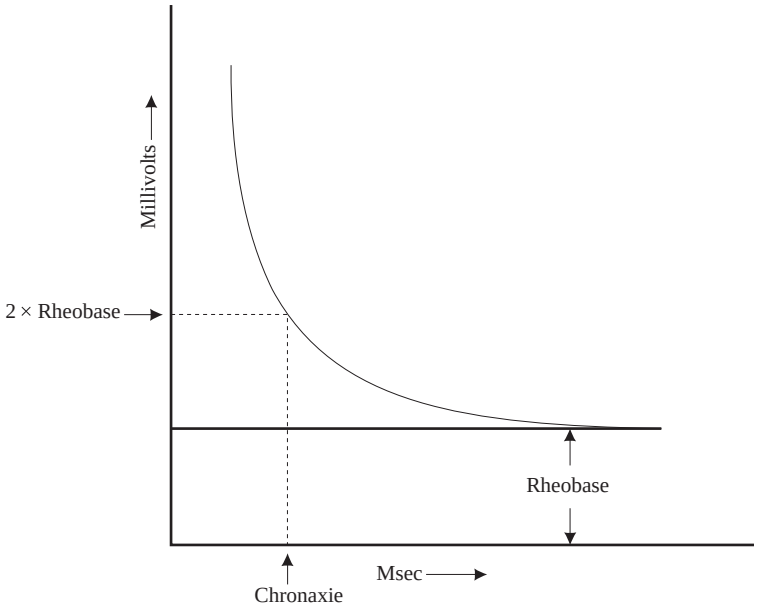


Fig. 15.6 Strength-duration curve of sartorius muscle of frog (chronaxie).

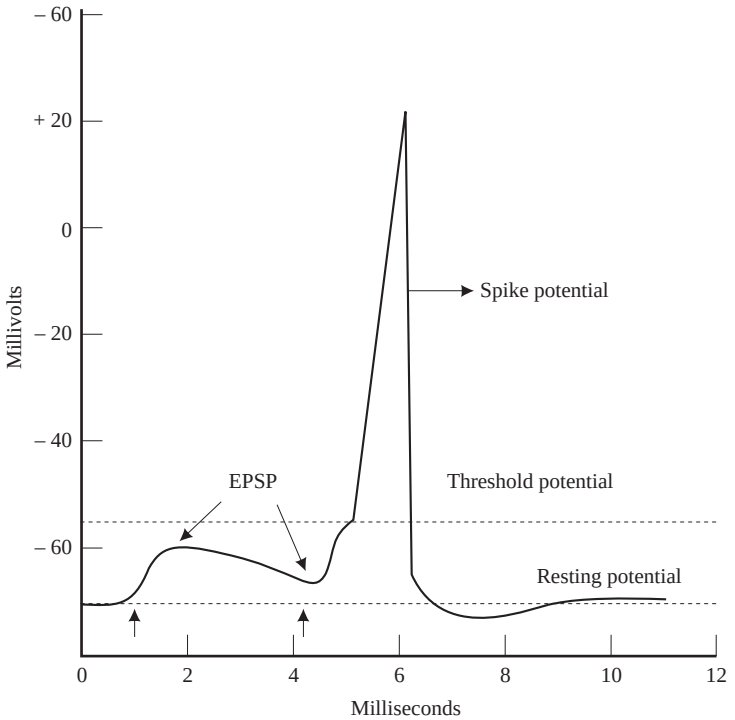


Fig. 15.7 Diagram of the action potential.

beyond that. All these changes together cause an action potential. The rise and fall of the action potential is called the *spike potential,* and the short phase of the action potential before reaching the resting potential is called the *negative after-potential.* The portion of the curve which falls below the resting level is called the *positive after-potential.*

The reversal of the membrane polarity that occurs due to a stimulus provided to it, has been explained on the basis of *sodium theory* or *membrane potential theory.* Both the K^+ and the Na^+ ions of the neuron and the surrounding fluid, take part in reversing the polarity. During the resting potential stage, Na^+ ions are ten times more in concentration outside than inside the cell, whereas K^+ ions are twenty times more inside than outside. When a stimulus is provided, there is a rapid inward movement of Na^+ ions to make the inside positively charged. Consequent upon this sudden inward surge of positively charged ions, the resting potential is disturbed causing the charge differential to disappear, resulting in depolarization of the membrane (Fig. 15.8).

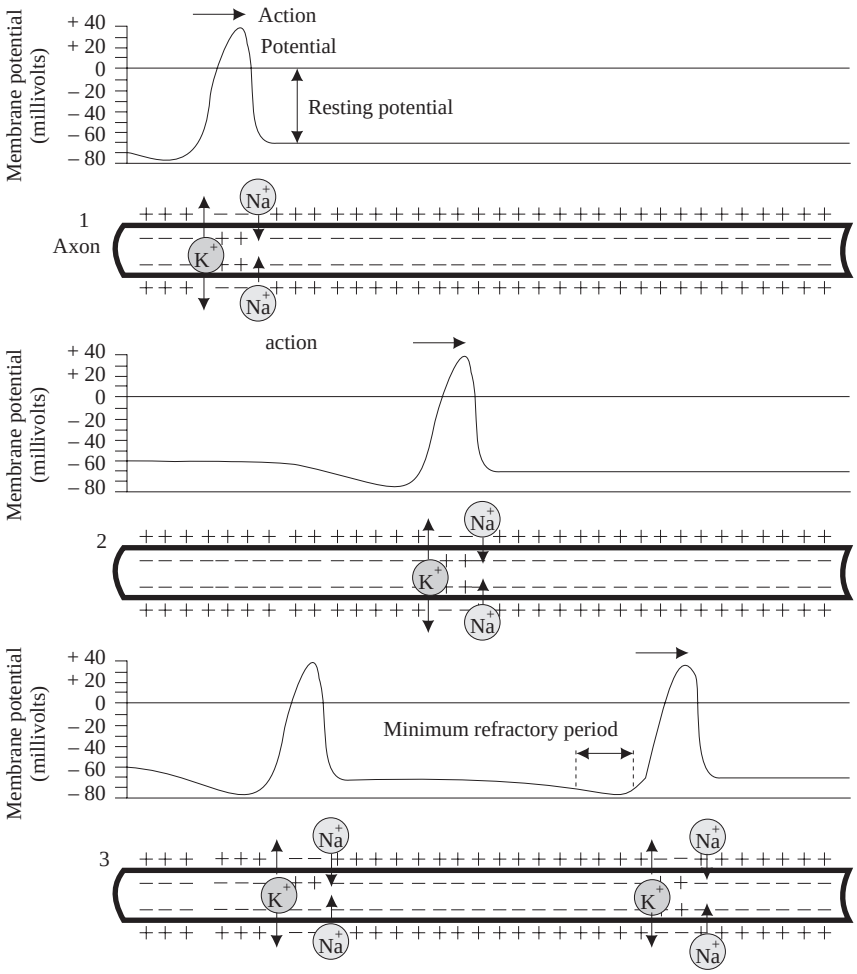


Fig. 15.8 Sequence of events in a nerve fibre during the conduction of an impulse.

However, soon after the reversal of polarity, penetration of sodium ions stops altogether followed by an overshoot of about 50 mV in the nerve cell. At this stage K^+ ions now begin to flow till such time the state of resting potential is restored. This process of *action potential* lasts for just 5 to 10 milliseconds (Fig. 15.9).

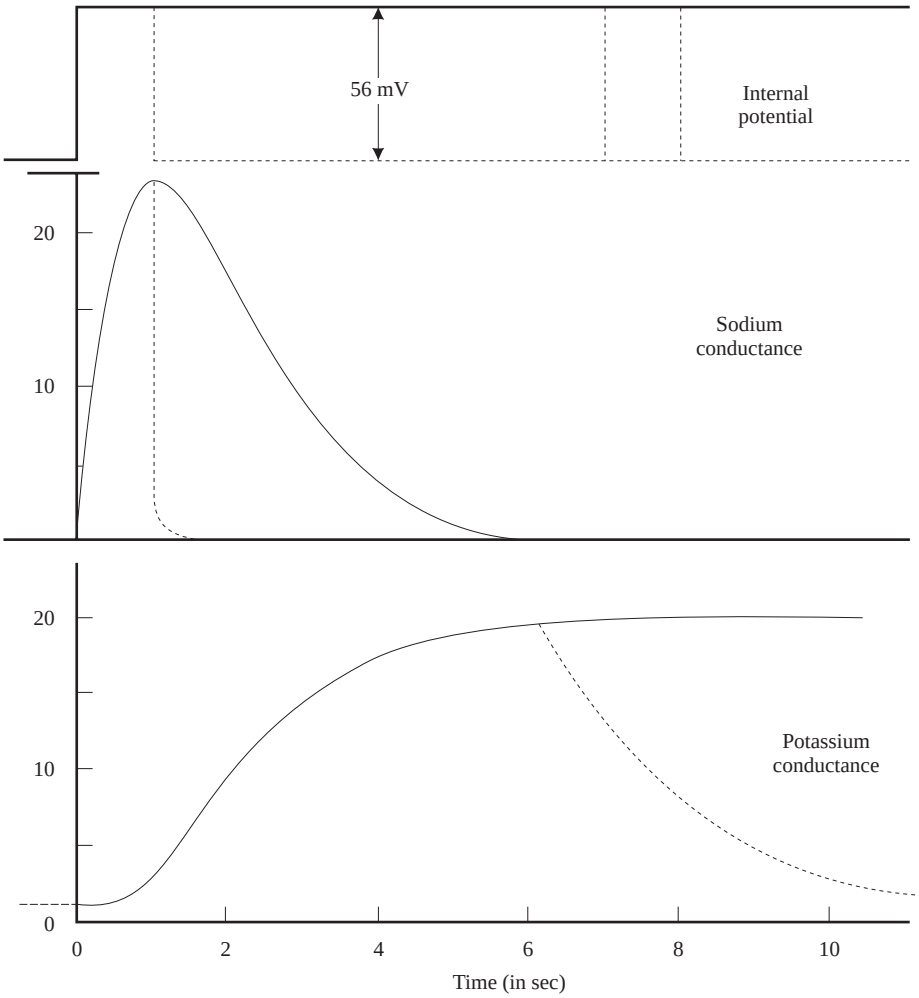


Fig. 15.9 Effect of depolarization on the conductance of Na^+ and K^+ ions. (a) Changes in Na^+ and K^+ conductance resulting from a step depolarization of 56 mV applied at time 0. Na^+ conductance reaches a maximum and then declines. K^+ conductance rises after some time delay and remains hight.

The best explanation of the action potential was given by Hodgkin, Huxley and Katz. When the neuron is stimulated, sodium ions suddenly move into the cell and cause a positive potential. Since the sodium ions move into the cell due to excitation, the resting potential changes from –70 mV towards the positive side accelerating the movement of sodium ions. This influx is due to an increase in the membrane permeability. The explanation given by Hodgkin and Huxley is summarized below.

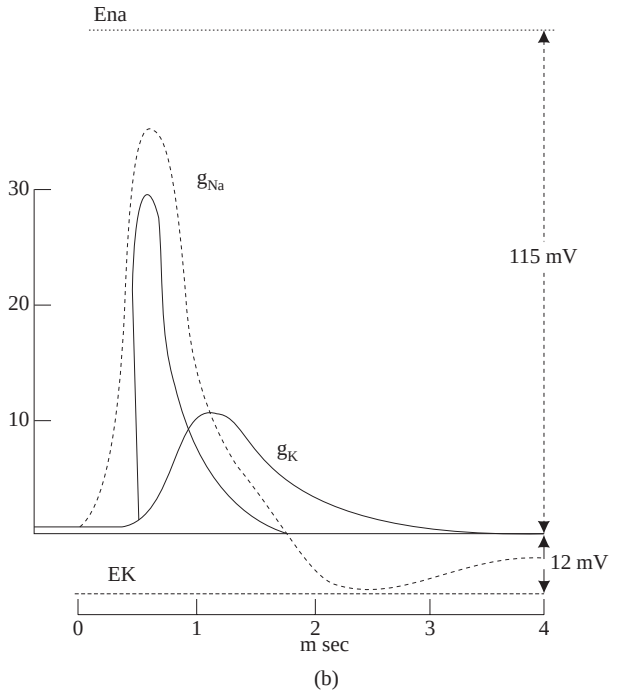


Fig. 15.9 (b) Changes in sodium (g_{Na}) and potassium (g_K) conductance in a squid axon. (Adapted from M.S. Gordon, Animal Function: Principles and Adaptations, 1968).

(1) An electrical stimulus depolarizes the membrane increasing its sodium permeability. Consequently, influx of Na^+ ions takes place. If the inward flow is slow, it is counterbalanced by the outward flow of K^+ ions, which are more mobile. If the initial depolarization is large enough, that is, if the membrane becomes sufficiently depolarized, Na^+ ions move faster than K^+ ions leaving the fibre. This causes the membrane potential to drop still further (about 10-20 mV) when the outflux of K^+ ions can no longer keep pace with the faster influx of Na^+. This causes an action potential. The relationship between sodium permeability arid the membrane potential is shown in Fig. 15.10.

(2) The influx of sodium ions does not continue indefinitely and for some reasons, it is halted near the peak of action potential. As a result of this Na^+ permeability falls to its resting level and K^+ ions also move outward. The stoppage of sodium entry may be partly due to sodium *inactivation* and partly because the membrane potential reaches a level where the net inward driving force acting on Na^+ ions becomes zero.

(3) Soon after attaining the peak of the spike, sodium permeability stops and potassium permeability of the membrane rises above the resting state. Consequently, efflux of K^+ ions takes place restoring the membrane potential. At the end of the spike, original level of potential is restored but the sodium permeability is still in an inactive state. This is *absolute refractory period* when it is impossible to initiate another action potential. After this negative

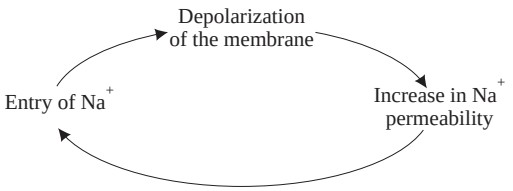


Fig. 15.10 Relationship between Na^+ permeability and the membrane potential.

after-potential is reached where the membrane can be retriggered to elicit another action potential response (Fig. 15.9).

Electrotonic Potentials

Subliminal stimulus fails to elicit a spike potential, although there is a small change in the potential difference between inside and outside the cell, and the potential may be altered in either direction. The direction of change depends upon the current used. An anodal current causes the potential to become more negative, whereas the cathodal current makes it less negative. Potentials produced in this way are called *electrotonic potentials.*

Ionic Basis of Resting and Action Potentials

It is well established that the interior of all nerve and skeletal muscle fibres is negative with respect to outside. Bernstein's (1902) hypothesis postulated that the membrane is selectively permeable to K^+ ions and impermeable to Na^+, chloride and other organic ions. As such the excitation is developed due to depolarization of the membrane caused by increased permeability of cations. Lately, Bernstein's hypothesis has been modified in view of certain objections to it. Excitation does not cause simply depolarization. A reversal of potential is developed. The modern concept presupposes a "cation pump" in the membrane which allows rapid entry of K^+ than Na^+ ions and the leakage of Na^+ into the cell is controlled by the sodium pump (Fig. 15.11). Hence most physiologists believe that the bio-electric potentials arise from a combination of concentration gradients and membrane permeability to generate action and resting potentials.

In a system like the one explained above, the membrane separating the two solutions is specifically permeable to only one type of ions. The potential difference across the membrane is given by Nernst equation.

$$E_m = \frac{RT}{F} \frac{\text{(Penetrating ion outside)}}{\text{(Penetrating ion inside)}}$$

$$= 58 \log_{10} \frac{\text{(ion outside)}}{\text{(ion inside)}} \text{ millivolts}$$

E_m is the equilibrium potential at which the force exerted by the concentration gradient is exactly balanced, RT/F denotes the conversion factor of gas constant (absolute temperature, valence and Faraday units). For a positively charged cation, E_m is the inside potential. It has been demonstrated

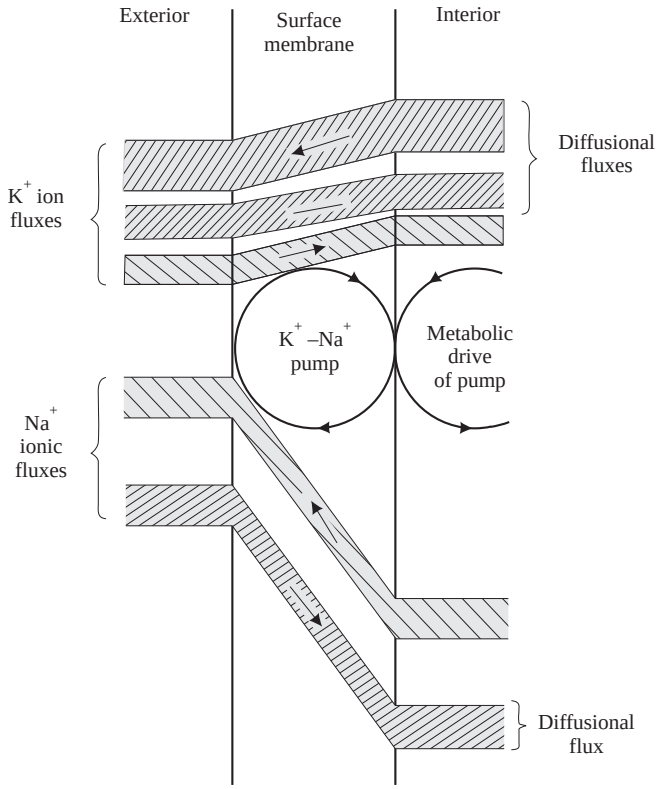


Fig. 15.11 Model illustrating the sodium pump showing ionic composition inside and outside a resting nerve cell (After Eccles. Scient. Amer. 212 (1) 55, 1965)

now that the resting membranes of nerves and muscle fibres are not only exclusively permeable to K^+ ions, but also permeable to Cl^- ions and partially to Na^+ ions. According to Goldman, the potential developed across a membrane permeable to all these ions can be formally described thus:

$$E_m = \frac{RT}{F} \log_e \frac{P_K\,(K)_o + P_{Na}\,(Na)_o + P_{Cl}\,(Cl)_i}{P_K\,(K)_i + P_{Na}\,(Na)_i + P_{Cl}\,(Cl)_o}$$

where *i* and *o* refer to inside and outside respectively and *P* denotes the permeability constant; Cl^- refers to ions for which there is no active transport.

The ionic composition and equilibrium potentials have been extensively worked out for frog muscle and for squid axons (Table 15.2).

The resting potential arises from the potassium concentration gradient and the membrane behaves like a potassium electrode.

Table 15.2 Concentration of Ions in the Cytoplasm and in the Blood Plasma of Squid Axons and Frog Muscle and Equilibrium Potentials Calculated from Nernst Equation

	Squid axon	*Frog muscle*
	m—mole/kg of water	
(K^+) inside	410	125
(K^+) outside	22	2.6
E_K	– 74 mV	– 98 mV
(Na^+) inside	49	15
(Na^+) outside	460	110
E_{Na}	+ 56 mV	+ 50 mV
(Cl^-) inside	123	1.2
(Cl^-) outside	560	77
E_{Cl}	– 38 mV	– 104 mV

(Table adapted from Hodgkin (1951)*,* Biol. Rev. 26; *Keynes* (1964)*,* J. Physiol. 169.)

15.4 THEORIES OF EXCITATION

A number of theories have been propounded which attempt to explain the phenomenon of excitation. However, not a single theory is complete in itself and all explain the intricate phenomenon partially only. Some of these theories will be considered here.

Nernst Theory of Excitation

This is the oldest theory which accounts for the quantitative formulation of the excitation phenomena. In a resting cell, free movement of ions does not take place, but when the cell is stimulated electrically, ionic movement into and out of the cell takes place. Nernst excited the cell by a constant current and noted that a current of high frequency does not produce excitation at very high intensity. This led him to suggest a relationship of strength-duration by a formula,

$$i\sqrt{t} = K$$

where i is the strength of the current enough to excite, t is the time duration, and K is the constant for the cell which is excited. Nernst formula has been improved upon by several workers. Nevertheless, it has failed to account for some changes taking place in the electrical potentials.

Membrane-permeability Theory

This theory takes into account the fact that whenever a cell is excited, there is a change in the permeability of the surface membrane of the protoplasm. Studies performed on the giant axon of squid revealed that by using electrodes to measure the impedance to alternating current across the axon, impedance was greatly reduced in a reversible manner. After cessation of the stimulus the resistance was restored. This means that the axon sheath permits an increased permeability to ions when excited.

Calcium-release Theory

Heilbrunn proposed a theory that excitation causes certain calcium-protein compounds to release Ca^{++} ions that induce changes in the protoplasm. The Ca^{++} ions so released near the cell surface cause liquefaction of the gel-cortex of the cell so that Ca^{++} ions diffuse to the cell interior, liquefaction is temporary and is followed by gelation.

Acetylcholine-production Theory

When the cells are excited, acetylcholine is released and its release is linked with the calcium-release theory. When a neuron is excited, acetylcholine is liberated from some complex which changes the properties of the cell membrane causing movements of ions. Na^+ and K^+ are mainly concerned with this. Na^+ ions start moving into the cell to increase the concentration and are responsible for the rising phase of the action potential Conversely, K^+ ions start moving out of the cell across the membrane which corresponds to the falling phase of the action potential. Acetylcholine cannot stay longer and immediately, it is hydrolyzed by an enzyme acetylcholinesterase marking the refractory period, after which the membrane is again ready for another excitation. The calcium ions are largely responsible to activate the enzyme acetylcholinesterase.

Osterhout's Theory

Based on the study of excitation phenomenon in the aquatic plant *Nitella,* Osterhout postulated that K^+ ion concentration of the sap and the surrounding solution is responsible for an action potential. Excitation induces movement of K^+ ions. This theory takes into account the movement of K^+ ions only and it has been suggested that the movement of other ions must also be considered.

15.5 FACTORS INFLUENCING EXCITATION AND PROPAGATION

The process of excitation of a nerve, and the propagation of the impulse is influenced by a number of factors, some of which are briefly described here.

Repetitive Stimuli

The excitability and conductivity of a nerve is hampered by repeated stimulation provided the interval between two stimuli is not less than relative refractory period. If the interval between two stimuli is shorter than the refractory period, the subsequent stimuli produce progressively smaller spikes as compared to the first. By continuous stimulation the negative and positive after-potentials increase. The excitability and velocity of conduction become subnormal during the positive after-potentials.

Temperature

The excitatory process as well as the propagation of the nerve impulse are greatly influenced by temperature changes. Lowering of temperature brings about blockade of impulse transmission. In mammals and frogs, a temperature of 0°C always suspends the excitatory process.

Drugs

Administration of certain drugs like cocaine, novocaine, etc., do not alter the spikes, but the excitability and after-potential are greatly affected.

15.6 IMPULSE PROPAGATION

When a stimulus is provided in nerve tissues, a local depolarization is initiated and it spreads out rapidly over the cell membrane in the form of a wave known as the nerve impulse. The conduction of an impulse is a bioelectrical phenomenon. This impulse is propagated along the axon undisturbed. The nerve impulse obeys *all-or-none* principle; once initiated it is propagated down the axon without any change in its intensity or velocity.

The impulse is initiated at the dendritic end of a cell and it travels along the axon towards the terminals (output zone).

There are certain aspects of the nerve conduction which should be taken into account. These are excitability threshold, refractory period and accommodation. Excitation causes depolarization of the cell membrane and the magnitude of the stimulus required to activate a neuron varies with the state of the cell. Excitability threshold is the critical level when the membrane potential is developed at which the net rate of entry of Na^+ becomes exactly equal to the net rate of exit of K^+ ions. During the spike potential, the neuron will not respond to any stimulus, however strong it may be. This period is the absolute refractory period (Fig. 15.7). Thereafter, until the beginning of the negative after-potential, there is relative refractory period. During this period excitability is higher than at rest. During the negative after-potential excitability is low, whereas during the positive after-potential it is higher than at rest. Physiologically speaking, during the refractory period sodium permeability is inactivated, but the K^+ ions permeability is greater than the normal.

Nerves also show the power of accommodation. If the nerves are stimulated by gradually increasing currents, they do not show a response because of the accommodation of the stimulus. Accommodation may be either due to prolonged potassium permeability or to inactivation of sodium permeability mechanism.

Action Potential Conduction

We have outlined before that action potential is initiated with the depolarization of the membrane and the Na^+ ions start diffusing inside the nerve fibre. This brings about alteration in the membrane polarity making outside of the cell negative in relation to inside. Such changes are first initiated at the point where stimulus is provided and at this particular point the polarity of the remaining area of the membrane is undisturbed. Because of the attraction of positive charges towards the negative ones, positive charges flow in towards the negative setting up a flow of current (Fig. 15.12). Since the positive charges are removed from the point, the potential difference between the two sides of the membrane is lowered, making the resting potential less negative at that point. This increases permeability of the membrane to Na^+ ions which quickly move in. Again this portion of the membrane undergoes reversal in polarity making the outside positive. The entire sequence is repeated and a sequence of action potentials is developed which moves in either direction from the point of

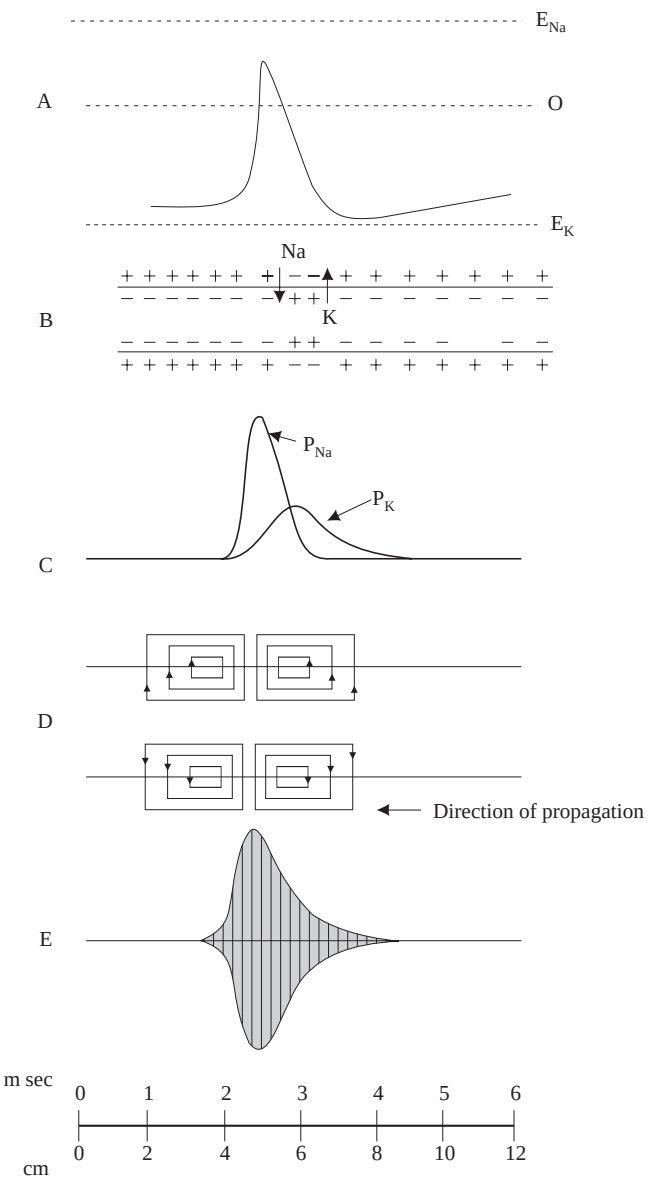


Fig. 15.12 Conduction of action potential in nonmyelinated squid axon fibre. Horizontal scale represents conduction velocity of 20 m/sec.

(A) Change in membrane potential, broken lines showing zero potential and Nernst potentials for Na^+ and K^+; (B) Polarity of potential difference across the membrane and ionic move ments; (C) Variation in Na and K permeabilities; (D) Local circuit current flow; (E) Variation in total membrane conductance; (A to C, after Hodgkin and Huxley (1952), J. Physiol, 117, 530; D to E, after Cole and Curtis (1939) J. Gen Physiol, 22). E_m for K ions = E_K, E_{Na} for Na ions = E_{Na}

stimulation. This is how the impulse is generated in a nonmyelinated neuron. However, in case of myelinated neurons, the situation of impulse propagation is somewhat different.

Conduction in Myelinated Nerves

The mechanism of impulse propagation in myelinated fibres is different from that of unmyelinated ones.

The conductivity of nonmyelinated nerve fibres is lower because of the absence of insulating material. In myelinated nerves, there are points of *nodes of Ranvier* where medullary sheath is almost absent. These points are highly excitable. In the process of conduction, the excitation jumps from one node to another along the length of the nerve (Fig. 15.13).

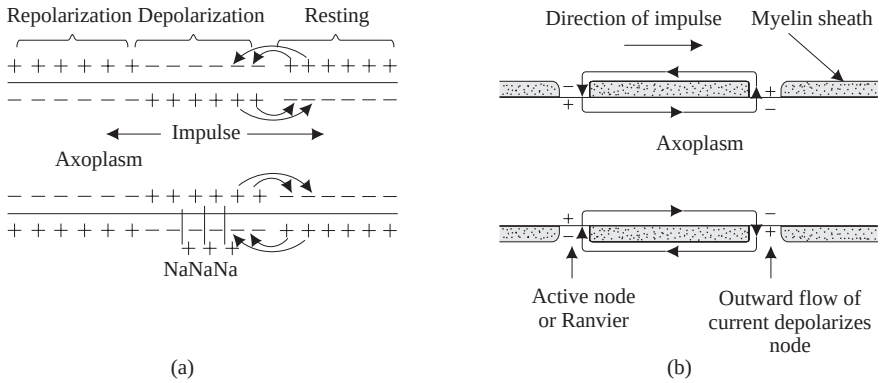


Fig. 15.13 Diagram illustrating the local circuit theory in (a) nonmyelinated fibre and (b) myelilnated fibre (saltatory conduction). (After A.L. Hodgkin (1957), Proc. Roy. Soc. B. 148)

This jumping mechanism has been called saltatory condition. In this process, active generation of the current is confined to the nodal points due to the entry of sodium ions, whereas the internodal parts of the nerve are depolarized by local circuit action. However, the local circuits act much ahead of the region of activity since the conduction activity of internodal portions is quite high. Saltatory conduction was demonstrated by Huxley and Stampfli (1949).

Events in Impulse Propagation

The action potential is characterized by a series of events that can be studied with the help of two sets of electrodes. One set is used to give a stimulus and the other is used to record the voltage across the cell membrane.

When a stimulus is applied, a short latent period occurs which is actually the time lapse between the application of the stimulus and the response. Following the latent period, the nerve membrane is depolarized with the consequent reduction of transmembrane potential. If a subthreshold stimulus is passed through a nerve, the impulse propagation fails to occur, but only a local potential appears. Local potentials are non-propagative and graded. By gradually increasing the strength of the stimulus, a threshold level of the stimulus is reached which produces a *critical potential.* When the critical

potential is reached, the impulse is propagated in the form of spike. At this moment, depolarization of the membrane occurs due to the inflow of sodium ions. The duration of the spike varies with the speed of conduction and fibre size.

After depolarization the membrane potential declines in its negative value, passes through zero potential and becomes positive. The moment spike height attains its peak, the potential reaches its maximum positive value. This is how action potential is caused. After this the membrane potential is restored causing repolarization. This is due to diffusion of potassium ions to the outside. A *positive after-potential* is soon reached in which case voltage is more negative than the resting potential. Following this, another event of *negative after-potential* is reached when the voltage is less negative than the resting potential (Fig. 15.7).

During the positive after-potential the nerve has a greater threshold excitability. All these events described above constitute an action potential. After the cell membrane has been depolarized, a *refractory period* occurs which continues until the resting potential is restored, and during this period the nerve recovers. The chemical changes accompanying the electrical event are probably connected with the recovery process. During refractory period the portion of the fibre through which the impulse has already been propagated is not responsive to stimuli. But, after the recovery process the nerve is again excitable and stimulus at this time would create another pulse. All such impulses are conducted along the nerve at a constant amplitude and velocity. Thus, the propagation is in the form of a series of pulses which are all alike. This principle is called *pulse-coded analog* principle since a nerve fibre conducts an impulse intermittently.

Velocity of the Nerve Impulse

The velocity of the nerve impulse differs in nerve fibres of different animals. The nerve impulse velocity can be measured with the help of a cathode-ray oscilloscope utilizing two parameters: (1) the difference between the duration of two latent periods, and (2) the length of nerve between the two points of excitation. The latent period can be determined by knowing the time lapse between the application of nerve stimulus and the moment the muscle begins to contract.

In the sciatic nerve preparation of frog at room temperature, the velocity is about 30 metres per second, whereas in the human motor nerve it is about 120 metres per second. In nonmedullated nerve fibres, the velocity of conduction is much slower, as low as l cm per second in some invertebrates. Variations in the velocity are due to several parameters. Diameter of the nerve fibres is the most important factor. The velocity of impulse conduction is proportional to the diameter (v/d ratio). The velocity of conduction is very slow in the sensory nerve fibres of the skin as compared to the motor nerves having larger calibre. For this reason, the nerve fibres carrying sensation of pain are very slow in their conduction.

Other factors which affect the velocity are temperature, conductivity of the medium, sheath thickness, etc.

Nature of a Nerve Impulse

Any disturbance along the length of a nerve may cause a change in its potential at any point. Physiologists believe that the change in the potential may be either a (1) physical change brought about by movement of ions, or a (2) chemical change accompanied by liberation of chemical energy

(viz. oxygen consumption, formation of CO_2 and liberation of heat are some evidences of chemical change), or (3) a physicochemical change.

The passage of nerve impulse causes chemical changes in the nerves affecting their metabolic rate. It has been demonstrated that the nerves under stimulation produce more CO_2 as compared to the resting ones. Excitation also increases oxygen consumption by nerves. This has been shown experimentally by keeping nerve preparations in oxygen-free atmosphere when they lose their excitability. Excitation of nerve also results in greater heat production.

15.7 SYNAPTIC TRANSMISSION

The Synapse

As already noted, the neurons are the basic units for communication. The communication may not be possible unless the information induces an effector organ or cell to act by secreting a chemical messenger or a transmitter substances across a gap. The gaps between neurons are called *synapses.*

The discovery of synapse was made by Cajal at the end of the nineteenth century. Following this discovery, a controversy was raised over the transmission of impulse across the gap. Some thought that the synaptic transmission was electrical, while others thought that it is mediated by a chemical messenger. Synapses exhibit three important characteristics: (1) the mode of transmitting the impulse across the synapse; (2) one-way transmission pattern; and (3) modification of impulse transmission.

The nerve endings are bulb-like having a synaptic membrane investing in the terminal region synaptic vesicles. For conduction, the impulse reaching the presynaptic membrane must be strong enough to depolarize the presynaptic membrane to start an impulse in the postsynaptic neuron (Fig. 15.14).

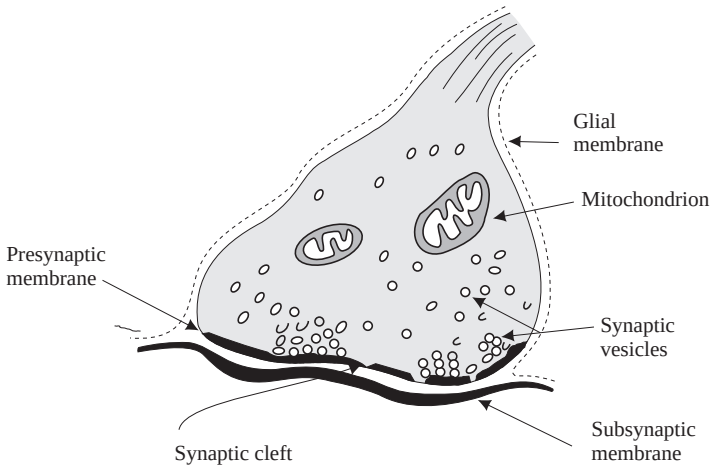


Fig. 15.14 Diagram to show the structure of a synapse.

An impulse may be propagated in the neuron in either direction. However, if the neurons are arranged in a chain, the impulse can travel in only one direction because of the synapse formation. Usually at a synapse the impulse is transmitted from the terminal knobs of the axon of one neuron to the dendrites, or cell body of the secondary neuron. It cannot be transmitted in the opposite direction since the dendrites and the cell body do not release any transmitter substance.

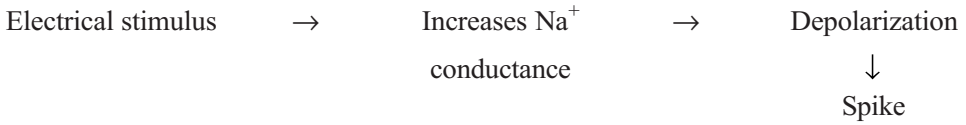
If a postsynaptic neuron is stimulated, the impulse will be conducted to the dendrites and the presynaptic neuron will remain unaffected. Synaptic transmission will continue as long as there is adequate supply of oxygen and ATP. In the absence of these, the synapse will fatigue. On the contrary, the neurons do not undergo fatigue and the nerve constituting them will continue to produce action potentials. The synaptic fatigue is due to the deficiency of transmitter substance.

Drugs also affect synapses. There are some drugs which stimulate, while there are others which have inhibitory influence. The inhibitory drugs block the release of transmitter substance, and the excitatory drugs block the release of inhibitory transmitter substance.

Types of Synapses: Although synapses vary between different types of cells, they have certain common properties. Generally, the presynaptic fibres divide into fine branches which terminate in synaptic knobs. These knobs are not in intimate contact with the membrane or the dendrites of the postsynaptic cell but lie very close to them. Electron microscopical studies have revealed that the pre- and postsynaptic membranes are separated by narrow gaps or clefts about 250 Å wide. The synaptic clefts offer very little resistance when electrically excited and there is very little spread of the current through the postsynaptic membrane. Two types of synapses have been recognized: (1) electrical synapses; and (2) chemical synapses.

Electrical synapses: Electrically transmitting synapses have several features worth noting. They have a large synaptic area of contact, the junction between the two cells is very tight and there is almost no resistance to the flow of the current. Consequently, there is no synaptic delay and the current flow is almost instantaneous across the synaptic cleft. The electrical coupling between the presynaptic cell (PRC) and the postsynaptic cell (PTC) is an intimate one so that enough current flows from the presynaptic cell to the postsynaptic cell to cause depolarization. The depolarization caused by an electric current is regenerative and produces an all-or-none effect which is propagated in the form of a spike without any decrement. Electrical synapses can normally transmit in either direction unlike chemical synapses and are generally non-amplyfying types.

Electrical synapses were considered a property of invertebrates only, but lately they are found to occur in several vertebrates also.



These synapses can be divided into two types on the basis of the depth of the synaptic clefts. First type of synapses are *excitatory* in which case the synaptic cleft is about 300 to 400 Å deep. The synaptic membrane is usually thicker and provided with dark staining dense patches. In other types of synapses, the synaptic cleft is about 150-200 Å deep. The *subsynaptic* membrane is thin and enlarged by the presence of folds. The neuromuscular junctions are of the latter type and are called *inhibitory*

synapses. Although it is difficult to distinguish between excitatory and inhibitory synapses on morphological grounds, the gamma-aminobutyric acid (GABA) acts as the inhibitory transmitter in vertebrates. The probable mode of action of GABA is similar to that of acetylcholine. The release of transmitter substance causes a temporary increase in permeability to K^+ and Cl^- ions (Na^+ ions are not affected) so that the resting potential is equal to the equilibrium potential.

Chemical synapses: Majority of the synapses that have been investigated fall in the category of chemically transmitting types. A simple synapse involves two neurons in a chain. In case of knee-jerk reflex two neurons play the part from reception of the stimulus to causing the response. This is called a *monosynaptic reflex.* In a number of cases, many internuncial neurons are involved so that the situation becomes complicated. This is, however, accomplished by integrating the information at the synaptic junctions.

There is a clearcut distinction between transmission of an impulse along nerve and transmission across a synapse. The passage of an impulse across the synapse is unidirectional and is accomplished by the release of a chemical transmitter substance. The synaptic knobs contain a large number of synaptic vesicles which are probably the sites of manufacture and release of the transmitter. For the impulse to travel across the synapse, there is some time delay caused by the release and diffusion of the transmitter substance across the synaptic cleft. The depolarization of the presynaptic membrane generates an action potential which is transferred to the postsynaptic membrane after a delay of about 0.4 to 10 msec.

In almost all chemical types of synapses, synaptic cleft is present. The neuromuscular junctions resemble such synapses in all essential details where the impulse transmission is much faster. In 1936, Dale and coworkers demonstrated that acetylcholine is the neuro-transmitter substance which is released at the motor nerve terminal in the neuromuscular junction. Consequently, a postsynaptic potential or an end-plate potential (EPP) is developed which is localized to the end-plate only and begins about 1 msec after the arrival of the impulse in the nerve terminal.

Very little is known about the mechanism of formation of the transmitter substance. However, it has been established that many of the synapses are chemically transmitting types and of the nature of neuromuscular junctions.

The entire mechanism of chemical transmission is very complex, and is only partially understood. The transmitter substance alters the permeability of the postsynaptic membrane reversing the polarity thereby making it more permeable to Na^+ ions. Such synapses are called *excitatory synapses,* which release an inhibitor substance enhancing the permeability to K^+ ions which tend to diffuse outward. This makes the inside of the postsynaptic membrane more negative resulting in the increase of potential across the postsynaptic membrane. This is called *hyperpolarization.*

Most of the synapses have acetylcholine as the transmitter substance. When a nerve impulse reaches the presynaptic bulb, the vesicles burst open and release acetylcholine into the synapse which diffuses in all directions. The chemical causes depolarization in postsynaptic membrane and thus the excitation travels in one direction only. As soon as acetylcholine is released in the cleft, it is quickly hydrolyzed by an enzyme *acetylcholinesterase.* Thus acetylcholine does not remain in the cleft for more than a millisecond.

$$\text{Acetylcholine} + H_2 \xrightarrow{\text{acetylcholinesterase}} \text{Choline} + \text{Acetic Acid}$$

Acetylcholine is not the only chemical transmitter in all the neurons. Acetylcholine is the transmitter substance in case of vertebrate central nervous system. Other transmitter chemicals are adrenaline (epinephrine) and serotonin (5-hydroxytryptamine). Adrenaline is secreted by the synaptic bulbs of the nerves supplying to smooth muscles, gland cells and heart Gamma-amino-butyric acid (GABA) is an inhibitory neurotransmitter and prevents antagonistic muscles from contracting.

CHAPTER

16

Sensory Mechanisms

Information about the external world is received through the receptors or sense organs and integrated in a way so as to produce a meaningful response. In the integration of sensory information, all specialized parts including the nervous system and the receptor-effector system are involved. This information is processed through elaborate neural mechanisms. The receptors are responsible for gathering more precise and complete sensory information, whereas the effectors are concerned with the effective execution of the reactions of the nervous system to such information.

Each sense organ is an organized group of cells around a group of receptor cells which are specialized to receive specific type of stimulus. There are two types of sense organs: simple and complex. Simple types of sense organs such as *sensilla* occur in arthropods which contain a few nerve cells. Complex type of sense organs are composed of several nervous and non-nervous tissues in a single organ. These include the organs of special senses of vertebrates such as the vision, audition (hearing), taste and olfaction. Our eyes respond to light waves of specific wavelengths, our ears to vibrations in the air, our taste buds and olfactory (smell) organs to chemical substances. But the perception of specific stimuli is not an independent function of receptors alone. The receptors have to communicate with the nervous system as well so that a stimulus is translated into a nerve impulse.

A study of sensory mechanisms is of great interest to us since the function of the nervous system is dependent on such mechanisms. The nervous function is adapted to the sensory input which is also responsible for regulating the overall activity of the central nervous system. In this chapter, our aim will be to study the sensory mechanisms involved in the perception of various kinds of stimuli. In this context, the reader is advised to go through the neuron structure and the physiology of the impulse transmission through a nerve which would be of immense value in the understanding of receptor mechanism.

16.1 CLASSIFICATION OF RECEPTORS

The point of contact between the nervous system and the environment is the receptor cell. A receptor

cell may exist independently or outside a sense organ and may detect most readily only certain stimuli for which it is specialized. When an effective stimulus impinges upon a suitable sensory cell, it generates an impulse to be communicated to the nervous system. This is ca1led sensory *transduction*. The impulse travels in a certain direction with a specific speed depending upon the quality and duration of the stimulus.

There are several types of receptors in an animal body and these have been classified variously by different authors. The oldest classification, based on their position in the body, recognized two types of receptors-*exteroceptors* and *enteroceptors*. Exteroceptors respond to stimuli like light, temperature, and pain, whereas the enteroceptors located inside the body respond to internal changes such as hydrogen-ion concentrations, osmotic pressure, and oxygen tension.

Receptors may also be classified according to their source of stimulus. These are: *exteroceptors* located at the surface of the body responsive to stimuli emanating from the environment; *proprioceptors* located in the deeper regions of the body sensitive to the position of the body; and *enteroceptors* located in the lining of the digestive system and responsive to stimuli coming from within the body of the organism. The classification of receptors as proposed by Parker, based on the nature of stimulus, is given below:

(1) *Chemoreceptors:* Receptors which respond to stimuli by chemicals are termed as chemoreceptors. They include: (a) olfactory receptors; and (b) gustatory receptors (taste).

(2) *Mechanoreceptors:* They respond to mechanical stimuli of various modalities and include: (a) muscle receptors; (b) pressure receptors of the skin; (c) gravity receptors; (d) audio receptors; and (e) nociceptors.

(3) *Radioreceptors:* These are excited by the effects of radiant energy such as heat and light, and are: (a) photoreceptors; and (b) thermoreceptors.

16.2 CHEMORECEPTORS

The chemoreceptors respond to chemical stimuli and help the animal in locating the food, finding mates and escaping the enemies. There are two types chemical receptors-olfactory receptors and taste receptors. In the simplest animals such as invertebrates, the chemical senses are widely distributed over the body and their definite role in chemoreception can be ascertained by studying the nature of response to specific chemicals.

Olfactory Receptors

Olfactory receptors are primarily concerned with the sense of smell. In vertebrates the olfactory receptor is innervated by the first cranial nerve and consists of a group of highly specialized receptors responding to chemicals that are volatile. These sense organs vary in structural details in different animals, but in general they are composed of a group of neurons located near the body surface. Each neuron sends out a long dendritic filament into some space which may be a pit, peg-like or hair-like papilla. Pit-like sense organs are present in mammals whereas peg-like or hair-like sense organs are found largely in arthropods.

Olfactory Receptors in Vertebrates: The olfactory sense in vertebrates is located in the nasal region. We may describe here the human sense of smell providing a good basis of understanding. The olfactory passage in the nose is lined by the olfactory epithelium or mucosa. The mucosa contains basal cells, supporting cells and the olfactory bipolar cells (Fig. 16.1). The bipolar cells are the actual neurosecretory olfactory cells having at their free ends hair-like processes. These cells possess a dendrite, called the *olfactory rod*, which extends beyond the olfactory epithelium.

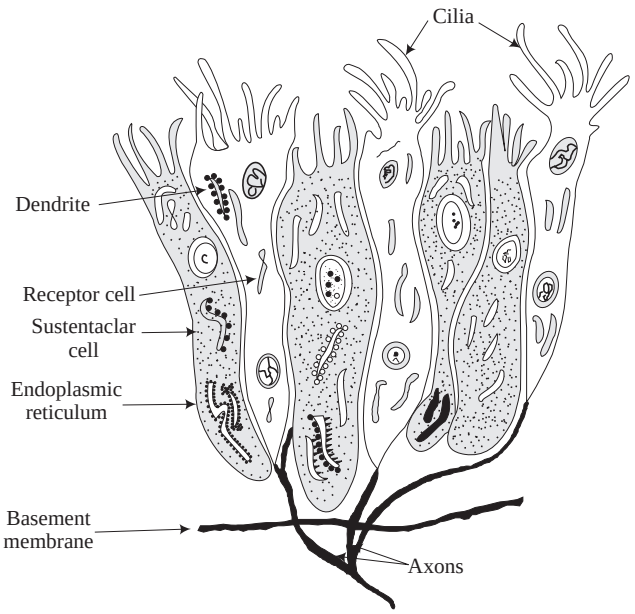


Fig. 16.1 Diagram showing the bipolar cells of the olfactory epithelium in vertebrates.

Smell receptors receive stimulation from odours coming from distant sources which develop slowly generator potentials in the bipolar cells. The bipolar cell has a dual function: it functions as a receptor cell as well as a ganglion cell. But, how are different odours discriminated? Very little is known about the sense of discrimination in olfaction. Seven categories of primary odours have been identified: these are camphoraceous, musky, floral, peppermint, ethereal, pungent and putrid. Although each receptor has a certain degree of specificity in identifying a particular odour, yet overlapping is possible. However, the specificity of the receptor cell is able to provide enough clues to the brain for discrimination. A *stereochemical theory* has been proposed to explain odour discrimination. The theory postulates that the odoriferous substance is a volatile one and each substance has a specific molecular configuration. In order to perceive seven different primary odours, there are at least seven types of olfactory cells each of which is capable of perceiving a molecule of a specific configuration. Of course, morphologically such receptors are difficult to be identified.

Olfactory Receptors in Invertebrates: Olfactory receptors are found in lower invertebrates also but they are scattered all over the skin. Such receptors are always of primitive kind and are devoid of any synapse between the receptor cell and the nerve fibre joining the nervous system. Smell receptors of sea anemones are of this nature which show sensitivity to foods and other substances present in water. However, primitive receptors of this type are found in all animals.

Gustatory Receptors

The organs of taste *or gustation* consist of *taste buds* located chiefly on the dorsum of the tongue. They are also found in the epiglottis, soft palate, and the pharynx. The structure of taste buds is fairly uniform in vertebrates, and each consists of a cluster of neurosensory cells and supporting cells arranged in a goblet-shaped structure embedded in a stratified epithelium (Fig. 16.2), The supporting cells form the outer covering while neurosensory cells remain in the interior. Each neurosensory cell is long and narrow with a thin taste hair at its free end and a sensory nerve fibre at its base. The taste hairs are cilia-like and project into a depression called *taste pore* in mammals. In some animals, the hairs project above the surface of the epithelium. In such cases, the taste pores are absent.

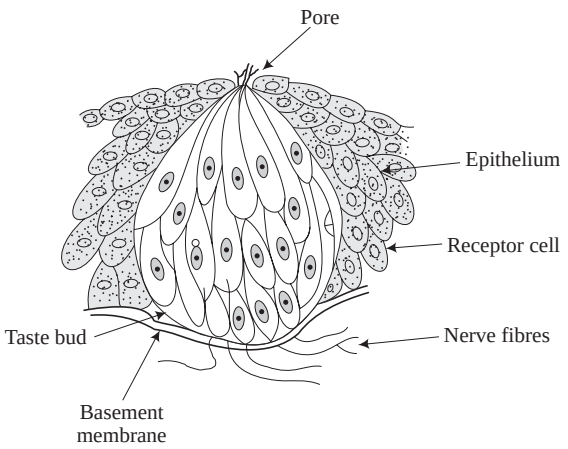


Fig. 16.2 Taste buds in vertebrates.

Taste buds are widely distributed in fishes, located in the mouth, pharynx, branchial cavities, outer surface of the head, and in some even occurring over the entire body surface. In vertebrates, the taste buds are innervated by eighth, ninth and tenth cranial nerves that carry the taste fibres to the medulla. Some of the taste fibres end up in a nucleus outside of the solitary tract within the medulla. The taste sensations are carried to the thalamus and the cerebral cortex.

In mammals various kinds of papillae are found on the tongue containing taste buds. The taste buds can perceive taste when substances are in solution. In man, four primary sensations of taste have been recognized; they are sweet, salty, bitter and sour. These qualities are dependent upon the function of specific receptors which are distributed in specific areas of the tongue. The receptors for

sweet taste are located mostly on the tip of the tongue, for bitter taste at the root, and for sour and salty taste on the sides of the tongue.

Since the taste buds are innervated by neurons that enter from the base, it is probable that the nerve fibres are stimulated directly by dissolved substances that enter the taste pore. The inside of the taste bud is negative to the outside with a potential difference of about 70 mV. Stimulation of taste buds occurs when the hair-like processes are sensitized which is possible when substances in solution come in contact with the taste buds changing the potential to less negative. The magnitude of the receptor potential is directly proportional to the intensity of the stimulus; i.e. the concentration of the substances which increases the rate of firing. The rate of firing of the sensory neurons increases with the increasing rate of stimuli.

16.3 MECHANORECEPTORS

The mechanoreceptors respond to mechanical stimuli of different modalities. These include muscle sense, sense of equilibrium, touch and hearing. The simplest kind of mechanorceptores are the pressure or touch receptors located in the skin and responding to stimuli emanating by movement or pressure exerted by an object against the receptor. The sense of hearing or audition is adapted to record mechanical disturbances from any source through a medium consisting of air, water or earth. The gravity receptors respond to any deviation in the orientation of the body in relation to the field of gravity.

Muscle Receptors

The vertebrate muscles have a special kind of receptor cells which convey responses to the central nervous system when the muscles are stretched or relaxed. In addition to their occurrence in muscles, the receptors may also occur in joints or tendons. The receptors of the joints have a position sense, whereas those of the tendon help keep the muscles within functional limits. Both of these do not have the muscle sense. The muscle receptors are important since they lie in the body of the muscle close to the intrafusal muscle fibres (Chapter 17). Intrafusal muscle fibres are striated fibres but differ from them in having sensory nerve endings and a motor supply. A typical muscle spindle of vertebrates consists of intrafusal muscle fibres with sensory nerve endings (Fig. 16.3).

The muscle spindles show a ready response to stretching. When a receptor is stretched the motor neurons are activated to generate impulses so that the muscle exhibits a shortening response. As the muscle shortens, the activation of the muscle diminishes, causing diminution of the excitation in the motor neuron. Simple rcflexes of vertebrates are the best examples of this mechanism in which the spinal cord exerts a control. However, this system is also under the control of higher centres to produce voluntary movements. In such cases, impulses from the higher centres are relayed to the motor neurons of the intrafusal muscle fibres (gamma motoneurons) which cause the fibres to contract in a graded manner. The spindles also contain extrafusal muscle fibres (alpha motoneurons) which are influenced by the activity of gamma motoneurons. Slow voluntary movements are produced by activation of both gamma and alpha motoneurons. Violent muscular activity is due to alpha motoneurons, whereas in steady state position gamma motoneurons control the muscular activity.

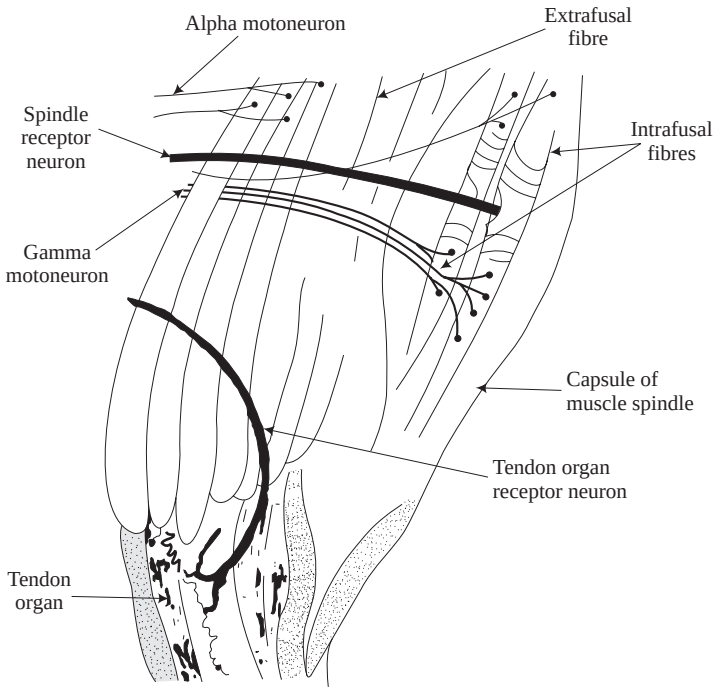


Fig. 16.3 Intrafusal muscle fibres with sensory nerve endings.

Pressure Receptors

The pressure receptors are of two types: external and internal. The external pressure receptors occur as small bodies distributed all over the skin and possess afferent nerve endings. They are found in the outer layers as well as in the deeper layers of the skin. Internal pressure receptors have many branched nerve endings extending to the muscle fibres. The fibre terminations are the pressure receptors which are excited by muscle contractions.

Several types of pressure receptors have been described in animals which include crustacean stretch receptors, amphibian muscle spindles and mammal-ianpacinian corpuscles.

Pacinian corpuscles are typical pressure receptors found in the deeper layers of the skin, in the mesentary of the viscera, in the connective tissues of the tendons, muscles and joints. Each corpuscle is a large structure, about 1 mm long, and consists of a number of connective layers surrounding a thin granular mass having a free terminal of nonmyelinated nerve (Fig. 16.4) The spaces between the connective layers are filled with a fluid which is helpful in transmitting pressure changes to the non myelinated nerve ending. The myelin sheath extends for some distance in the corpuscle and one node occurs the cell.

The naked nerve ending in the pacinian corpuscle can be excited by a very small movement such as 0.5 μ for a duration of 0.1 msec. The corpuscles can be excited by electric currents also and electric responses are in the form of generator potentials which are graded. In case a mechanical disturbance

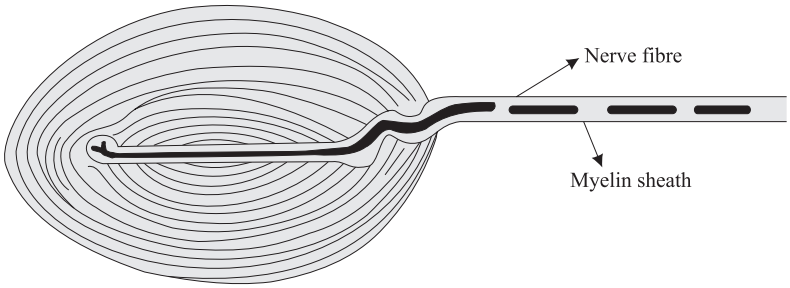


Fig. 16.4 Pacinian corpuscle.

is applied at a point, the generator potentials that are produced go on decaying when measured at a distance from the point of stimulus. If the naked fibre is stimulated at two points separated by some distance (about 0.5 mm), a single large generator current is produced as an additive effect.

Gravity Receptors

Gravity receptors are also termed as equilibrium receptors which respond to change in position or orientation of the body with respect to the gravitational field. Statocysts of crustaceans are specialized mechanoreceptors concerned with reactions to gravitational field. A statocyst is a sac-like organ lined by sensory cells and has a central core made of statolith (otolith). The sensory cells have sensory hairs or transducing membranes at their free ends. By mechanical stimulus the statoliths produce vibrations in the hairs and this information is transmitted through the sensory cells to the nervous system and the animal becomes aware of its position with respect to the gravity. Statocysts are phylogenetically very primitive organs found in coelenterates, flatworms and crustaceans. Formerly, they were supposed to have an auditory function, but experiments have proved that these are gravity receptors which may be likened to structures like saccule and utricle of the inner ear of higher animals.

The membranous labyrinth of vertebrates, besides functioning as an organ of hearing, also acts as an organ of equilibrium. It consists of two sacs, the *sacculus* and the *utriculus* together with three semicircular canals originating from the utriculus (Fig. 16.5). These semicircular canals are oriented perpendicular to each other. The sacs are interconnected by ducts and are filled with an endolymphatic fluid. Each semicircular canal has an ampulla at one end provided with rows of cells carrying cilia which are embedded in a single gelatinous cupula. Rotational movements cause the cupula to move, displacing hairs which in turn stimulate the nerves. In the chamber below the semicircular canals, there are three more patches of hair cells, each bearing otolith. The otoliths of utriculus are concerned with gravitational stimuli and body position.

Audio Receptors

The auditory or sound receptors respond to vibrations produced in the surrounding medium consisting of air, water or earth. The *neuromast* organs of fish and the lateral line canals are the most primitive sound receptors which receive vibratory stimuli through the liquid medium. In the course of evolution, the anterior lateral line system is supposed to have given rise to the membranous labyrinth

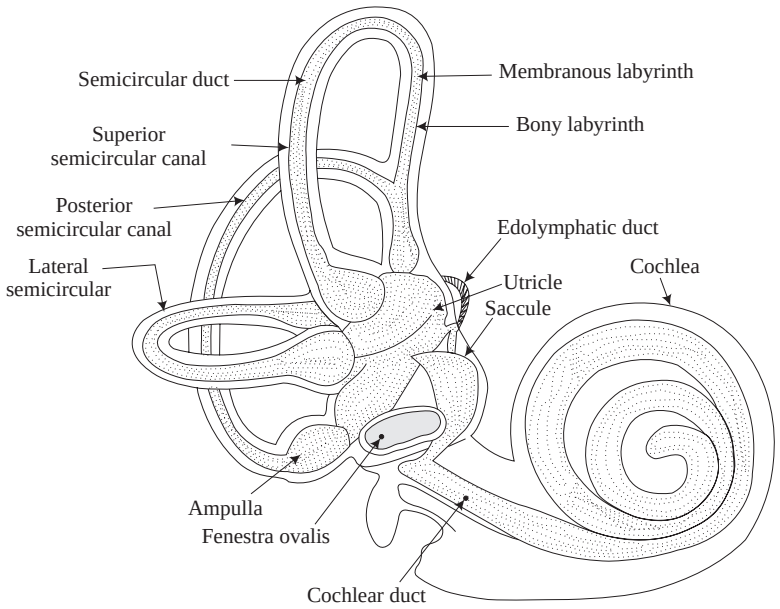


Fig. 16.5 Diagram showing membranous labyrinth.

or the internal ear which is however, highly evolved in mammals both from the structural and functional points of view.

Lateral Line System: The lateral line system is an elaborate mechanism of hearing in fishes, cyclostomes, and aquatic amphibians. Fishes living in great depths of the ocean are unable to rely on vision, hence are provided with a system of hearing with which they can perceive sound from a distance. The receptor organs are neuromasts composed of neurosensory cells and supporting cells. Each neurosensory cell has a thin process of hair at its free end and a nerve fibre at the base. In most cartilaginous and bony fishes the neuromasts sink with in the skin into depressions, grooves and canals, whereas in cyclostomes, a few fishes and aquatic amphibians the neuromasts lie on the surface of the skin, especially on the head. The lateral line canals contain mucus and open on the surface of the skin by minute pores. In addition to neuromasts, the lateral line system has other receptors, such as pit organs and ampulla of Lorenzini (elasmobranchs). The lateral line receptors detect movements of water currents and furnish information regarding the position of the body in relation to the environment. It is quite probable that they detect vibrations in water.

The lateral line system is so constructed that the water-borne sound is able to activate the receptors. In order to do so, the receptors are linked with such structures that would allow sound perception by increasing the specific gravity of the receptors. Fishes with air bladders are able to utilize them for hearing. Sound waves will stimulate the air bladder wall to produce considerable motion. The actual auditory receptors are located in the labyrinth which receive the sound vibrations from the bladder. The transmission of vibrations is made possible either by the extension of bladder or through Weberian ossicles which are modifications of the anterior vertebrae.

The Mammalian Ear: The auditory systems of mammals are highly evolved and consist of an external ear, middle ear, and an inner ear. The external ear is merely a fold of skin that helps in catching and concentrating the sound waves. The middle ear is responsible for conducting the sound waves from the air to the labyrinth of the internal ear.

The external ear includes an expanded portion namely the pinna and the external auditory canal which is also known as the *auditory meatus*. The pinna serves to collect sound waves and directs them towards the auditory meatus. The auditory meatus is a tubular passage which leads to the tympanic membrane.

The middle ear or tympanum is an ovoid structure separating the auditory canal from the tympanic cavity. It is innervated by a branch of the mandibular nerve. The tympanic cavity is an irregularly shaped cavity in the temporal bone containing three movable *ossicles*. namely, the *malleus*. the *incus* and the *stapes*. Sound vibrations in the tympanum are communicated to the malleus, then to incus and finally to stapes which fits into the *fenestra ovalis* (Fig. 16.6). A eustachian passage connects the cavity of the middle ear with the pharynx. This passage maintains an equal pressure of air on either side of the tympanum. The middle ear transfers sound waves from the air to the labyrinth, otherwise it is not absolutely essential for hearing. It also helps in protecting the labyrinth from injury.

The internal ear consists of an osseous labyrinth which is composed of a vestibule, the cochlea and semi-circular canals. The osseous labyrinth has in it a membranous labyrinth which is separated from the bony walls by the fluid called *perilymph*. The membranous labyrinth also contains a fluid, *endolymph*, and fine branches of acoustic nerves on its walls. A vestibule is situated behind the cochlea and communicates with the middle ear by means of fenestra ovalis. The membranous labyrinth comprises two sacs, known as the *sacculus* and the *utriculus*. A small canal, *ductus endolymphaticus*, comes off the posterior walls of the sacculus. The inner walls of the two sacs have

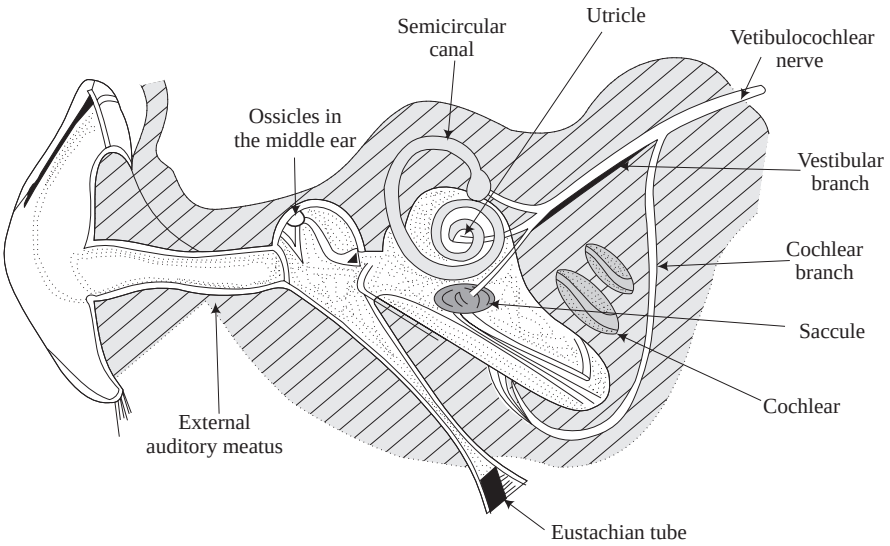


Fig. 16.6 Structure of the internal ear.

columnar cells with specialized hair like processes projecting into the endolymph. The utriculus contains small crystals of calcium carbonate called *otoliths*.

The cochlea is the main component of the internal ear which receives sound waves through the middle ear. It resembles a snail shell having a spiral canal. The cochlea is divided by a basilar membrane into two chambers, the upper one known as *scala vestibuli* and the lower chamber known as *scala tympani* (Fig. 16.7). The apex of the cochlea has a small opening called the *helicotrema* which joins the two scalae. The helicotrema helps in equalizing pressure difference between the two cochlea canals. Lying in between the two scalae, there is a membraneous cochlea whose lower part supports a sound sensitive structure, the *organ of corti*. The organ of corti consists of rodshaped cells and hair cells numbering about 1700 arranged in four rows all along the length of cochlea. These extend into the endolymph of *scala media*. The hair cells are not in contact with the tectorial membrane. As the basilar membrane vibrates, the hair cells are alternately stretched and relaxed. The hair cells also have synaptic contacts with a number of neurons lying in the spiral ganglion which sends off axon fibres to the brain in the vestibulocochlear nerve. Sensory cilia from the free ends of the hair cells are also in close contact with the organ of corti.

There are three *bony* semicircular canals lying above and behind the vestibule and communicating with it by five openings. One end of each tube is enlarged to form an *ampulla*. The sensory receptors are actually located in the ampulla which are in the form of columnar cells having flexible hairs projecting in the endolymph. The cells are covered with a gelatinous substance

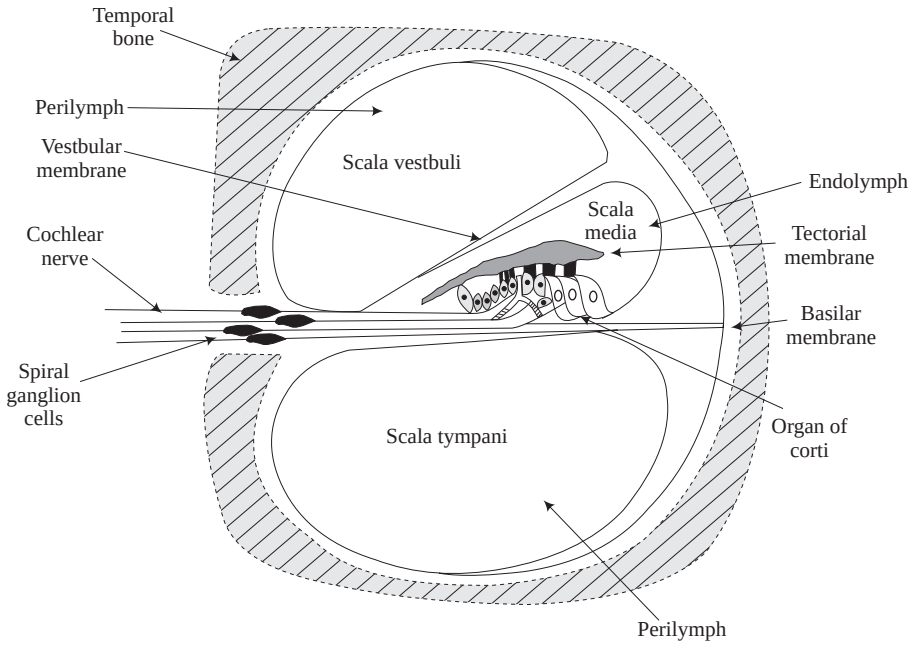


Fig. 16.7 Diagram showing the cross section of the mammalian cochlea.

containing otoliths. Otoliths are fine particles of calcium carbonate which vibrate on the hairs. Hair movement initiates reflexes that maintain balance against gravity. The hair cells are connected to the fibres of the vestibular branch of the vestibulocochlear nerve (VIIIth branch).

Process of hearing: Sound waves travel in the air at approximately 1,100 feet per second. There are three important properties of sound: pitch, intensity and quality. The pitch is determined by the number of vibrations per second; greater the vibrations, greater is the pitch. The intensity or loudness of the sound depends upon the amplitude of the waves. Higher intensity is related to greater wave amplitude. The third property of the sound is the quality or timbre which depends on the overtones. The quality varies with the source. For example, the quality of the sound produced by a violin can be readily distinguished from the sound of a guitar by its overtones.

All sound producing bodies vibrate and transfer their vibrations to the air with which they are in contact. The vibrations are picked up by air and enter the auditory meatus and set the tympanic membrane vibrating. The sound vibrations then communicate with the ear ossicles, then to perilymph and finally to endolymph of the membranous labyrinth. The movements in the fluid stimulate the nerve endings in the organ of corti from where the impulses are conveyed to the acoustic centre.

Cochlea is remarkably sensitive in detecting and discriminating various sound frequencies. It has been demonstrated that nerves are unable to conduct impulses greater than 1000 per second, but some mammals as well as humans are able to discriminate frequencies ranging up to 20,000 cps. Experimental proofs have been obtained suggesting the function of cochlea as a frequency analyzer which sorts out high frequency sounds to excite specific receptors responding to specific frequencies.

Theories of hearing: The cochlea has been suggested as an organ of sound frequency analyzer. The function of cochlea is associated with the basilar membrane which responds to variations in the frequency range. Several theories have been suggested to explain this principle.

(1) *Resonance theory:* This theory was proposed by Helmholtz in 1868 utilizing the principle of resonance. Since the cochlea functions as a sound analyzer, the basilar membrane is the one which is affected by the range of pitch. The basilar membrane has a number of fibres, as many as 24,000 running across it and varying in length. Short fibres are at the base and the long ones at the apex of the cochlea. Sound vibrations at the tympanum induced by high notes cause the short fibres to vibrate, whereas low tone vibrations vibrate the long fibres at the apex. The human ear can distinguish about 10,000 pitches of tones. This is possible because the basilar membrane with its nearly 15,000 fibres has the most efficient mechanism for differentiation of tones. The resonance theory suggests that varying pitch of the sound can be discriminated on the basis of sensory stimulation of basilar membrane at specific points by different frequencies of sound.

(2) *Travelling wave theory:* In the resonance theory, it was seen that the fibres in basilar membrane resonate at different frequencies. But recent researches have established that each fibre in the membrane cannot resonate alone, because each one is joined to the adjacent one. Hence it was suggested that sound waves arising from the perilymph set up a travelling wave along the basilar membrane from the base towards the apex. The travelling wave then reaches its maximum amplitude to cause displacement of the membrane depending on the frequency, and finally fades out as the wave reaches the upper part of the cochlea where the membrane is more flexible and suited to respond to lower frequencies (Fig. 16.8).

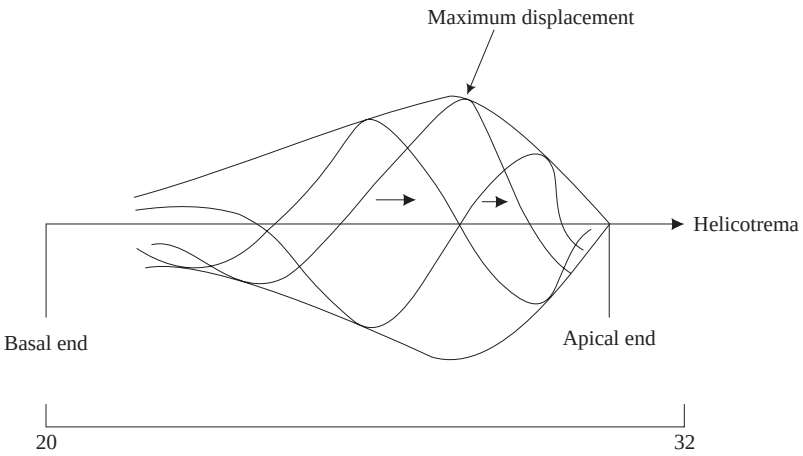


Fig. 16.8 The patterns of maximum displacement of the basilar membrane caused by a travelling sound wave.

(3) *Place theory:* The resonance theory of Helmholtz does not explain any correspondence between the frequency and the response. This has given room to another theory, what is known as the Place theory. Experiments with guinea pigs have demonstrated that there are specific regions for sound perception along the basilar membrane which respond to specific sound frequencies. Higher frequencies (4100 cps) may be able to damage the membrane in part of the basal turn of the cochlea, whereas low frequencies (200 cps) may cause damage in the third turn of the cochlea. The damage is caused at the site where the sound frequency shows a greater effect. The theory postulates that frequency discrimination is due to excitation of cochlear fibre nerve endings in the specific regions of the organ of corti on the basis of pitch discrimination and the input is recognized by the central nervous system.

Hearing in other vertebrates: The ear of mammals is highly specialized, while in other vertebrates it is quite simple. Fishes have neither a middle ear nor a cochlea and the auditory receptor in these animals is the labyrinth which is supposed to be a modification of lateral line system. Many fishes that have air bladders, utilize them for sound reception. The bladder is connected to the labyrinth either by an extension or by mechanical linking of a chain of three vertebrae known as *Weberian ossicles*. A water-borne sound can stimulate the air in the bladder which transmits sound to the labyrinth. A puncture in the bladder lowers the frequency range of sound perception. In many cases, the sound waves are transmitted to the statocyst (sacculus) which is a part of the labyrinth. Elasmobranch fishes are nearly deaf.

The ear of amphibians, reptiles and birds contains only one bone in the middle ear which is *columella auris*. In amphibia, cochlea is represented by a *lagena*, a small structure projecting from the sacculus. Frogs can hear tones of 50 to 10,000 cps and can poorly discriminate some sound frequencies.

Reptiles too have a small projection from the sacculus. Lizards respond to sound fairly well. Snakes, however, have no tympanum and a columella is present which is attached to the quadrate.

Snakes are unable to hear any air borne sound but respond to ground-borne sound waves reaching the skull.

Birds can hear sounds of different frequencies and intensities with the same efficiency as the mammals do, although the middle ear of birds has only one ossicle.

Bats and porpoises have developed a most remarkable mechanism of echolocation, a method of acoustic orientation. The principle is identical in both the cases. The principle of hearing in bats is likened to a radar system. They make use of echoes, in the absence of vision, not only to detect the presence of objects in the environment but also to determine the distance and direction with a remarkable accuracy. The sound emitted by bats is ultrasonic which is beyond our hearing power since they have short wavelengths and reflect strong echoes. The bats also find their way by listening to the echoes of their own voices (18,000 to 30,000 cps).

Hearing in invertebrates: Among invertebrates, insects possess well developed auditory organs. Their location on the body varies in different groups. The mechanical sense organs for sound reception are located in the proximal part of antenna of the male mosquitoes in the form of Jhonston's organ. These are excited by the pressure changes in the air coupled with sound waves. The antenna is set in vibration by the sound produced by the female which provides a stimulus to mating. In some grasshoppers, crickets and moths, the tibia of each front leg consists of a membrane covering a series of sensory cells or scolopodia. These insects produce sounds which are recognized by their own species. They can detect sounds of higher frequencies which are not detected ordinarily by the human ear. In some hemipterans (cicadas and corixids) similar organs are found on the abdomen which are sensitive to perception of very high-pitch sounds (high frequencies). Beetles, cockroaches and butterflies can detect ground-borne sounds through their legs.

Nociceptors

These are responsible for nocuous stimuli. Although nociceptors are not specialized receptors, they are in the form of finely branched naked nerve endings located in the deeper layers of the skin which provide a means of protective mechanism in response to excitation. These nerve endings have a punctiform distribution and were called by Sherrington as nociceptors. Painful sensations are caused when fine nerve endings are excited by any means chemical, mechanical, injury, pinching, etc. Consequent upon some damage to the body tissues, pain is caused. The receptors for pain may be located in the skin, muscles, joints, tendons, etc. Pain is also caused by distension of the intestine, bile duct, ureter, and spasm of the muscle and digestive tract by interfering with the blood supply.

When pain is caused, the impulses are transmitted to the thalamus through lateral spinothalamic tracts. The type and intensity of pain is felt by the cerebral cortex. Hypothalamus also participates in feeling the pain. In certain cases, visceral pain is not localized in the organ but is felt on the surface of the body. This type of pain is called *referred pain*.

Hunger: The feeling of hunger is a sensation that is projected to the region of the stomach, normally before the meal. The *pangs of hunger* are felt due to the contractions of the empty stomach. In order to arouse the sensation of hunger, the receptors present in the mucous membrane are stimulated. In case food is not taken the hunger increases in intensity and may cause fatigue and headache.

16.4 RADIORECEPTORS

Radioreceptors are located on the surface of the body and include receptors of vision and temperature stimulated by direct environmental forces such as light, heat and cold.

Photoreceptors

Almost all animal species have the capacity to respond to light. Light is in the form of electromagnetic radiations travelling in a wave-like motion. The visual organs of animals show sensitivity to different wavelengths. For example, many insects are sensitive to the near ultraviolet light, whereas the visual organs of most vertebrates, including man, respond to wavelengths which are in the visible range (0.4 to 0.8 μm*)*.

The simplest type of visual sensitivity has been found in certain protozoans which possess photosensitive pigments. *Euglena* possesses a photosensitive spot, the stigma, which is composed of a mass of pigment granules. Even the protoplasm has the general property of responding to light. In many animals, specialized cells or organelles sensitive to light are found. These include the chromataphores of echinoderms, crustaceans, fishes, amphibians and reptiles.

Besides showing sensitivity to light, majority of the animal phyla have a well organized visual organ—the eye, which helps in the perception of an image of the object. Eyes are found in many species of the phyla Platyhelminthes, Nematehelminthes, Annelida, Mollusca, Arthropoda and in all vertebrates. An eye is made up of photoreceptor cells which perceive certain qualities of light such as intensities and colours.

The image forming visual systems of all animals are similar in principle, although differing in structural details. To elucidate the principle of image formation, we shall first describe the mammalian eye.

Structure of the Mammalian Eye: The mammalian eye is a specialized organ which has almost spherical eye ball situated in the orbit. The movement of the ball is controlled by a set of six extrinsic muscles which are named according to the region where they are inserted. The eye ball is composed of three coats. The outermost coat is the *sclerotic coat* (Fig. 16.9), which is made of dense fibrous connective tissue. It is a protective and opaque coat. In the front the sclera is modified into a transparent glassy part *cornea* which is covered by a thin transparent and vascular conjunctiva. The middle coat is called the *choroid coat*. It is composed of highly vascular connective tissue with some pigment cells. In certain nocturnal animals, the choroid is made up of reflecting layer which shines in the night. In the front, the choroid separates from the sclerotic coat forming an *iris*. The iris is pigmented in mammals. The innermost coat of the eye is called the *retina* which is a thin, light-sensitive nervous layer containing sensory epithelium. The optic nerve fibres originate from the retina. The images are formed on this coat and so it is comparable to the film plate of a camera.

Behind the iris is a transparent biconvex lens which is attached to the ciliary body. The lens divides the eye into two chambers, the space between the cornea and the lens is the anterior chamber and the space behind the lens is the posterior chamber. These chambers are filled with a watery fluid; the anterior chamber contains *aqueous humour*, and the posterior one contains transparent jelly-like *vitreous humour*.

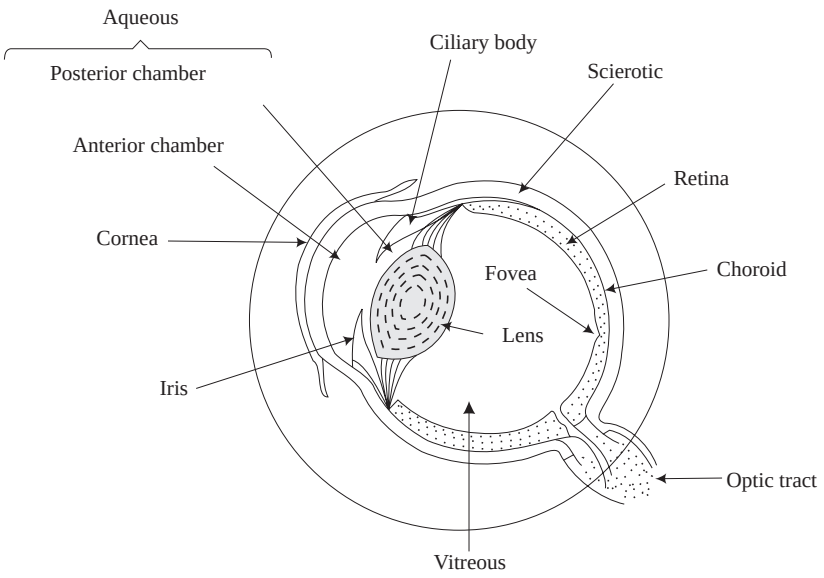


Fig. 16.9 The structure of the mammalian eye.

Structure of the Retina: The retina is quite delicate and most sensitive to the light rays. It receives the image of the external object and transfers the impression to the visual centre in the cerebral cortex. The retina consists of three sets of neurons arranged in such a way that the cell bodies arid their processes form seven layers (Fig. 16.10). It has two limiting membranes, one in contact with the vitreous layer, and the second layer marking the external limit of the rod and cone layers. There is one pigmented layer between the layers of rods and cones and the choroid coat. The last layer (seventh) is called the layer of rods and cones which is light sensitive. This layer transforms light energy into nerve impulses which are transmitted to the branch of the optic nerve.

In order to reach the light-sensitive receptor layer (seventh layer), light must pass through cornea, aqueous humour, lens, vitreous humour and retinal layers. The receptor layer has a small special region called *fovea* which contains only rods and cones. The rods and cones constitute two different types of visual receptors differing in their structure as well as function. Each one of them has an important role in vision. Electron microscopy has revealed the fine structure of these rods and cones (Fig. 16.11). Beginning from the outside of the eye, each rod has five parts: (1) an outer segment containing visual pigment; (2) a stalk; (3) the inner segment containing the nucleus, oil droplets and mitochondria; (4) stalk with neurofibrils, and (5) the synaptic peduncle. The cones have the same histological make up as that of the rods, but differ physiologically having specialized for receiving lights of different wavelengths.

The distribution of rods and cones in the retina is not uniform. In man, as we go from fovea to the periphery of the retina, the cones diminish in number while the proportion of rods increases until at the periphery only rods are present. In nocturnal animals, the retina is mainly composed of ‘rods, whereas in certain animals which are active during the day only cones are formed.

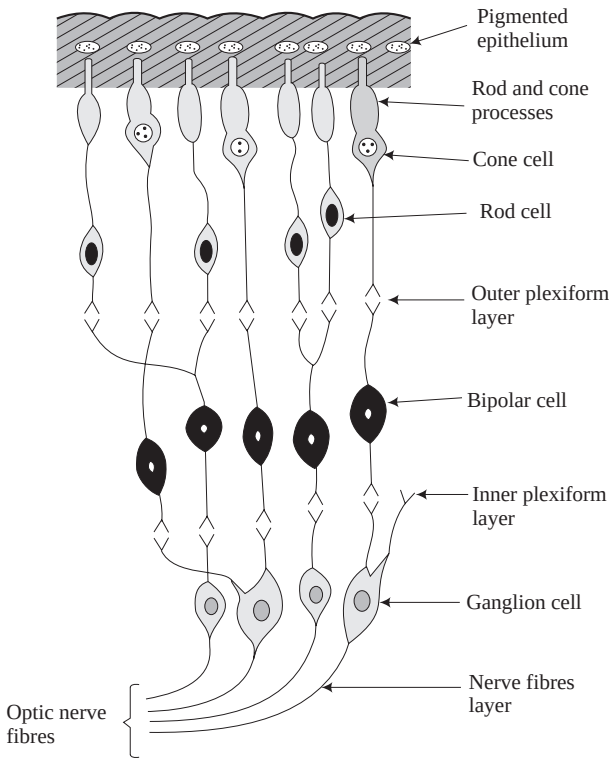


Fig. 16.10 Structure of the retina displaying seven layers with their cell bodies.

The rods are important for vision in dim light as they are stimulated by very little light and by most of the wavelengths of the visible spectrum. They are, however, unable to produce a colour sensation. The photosensitive pigment rhodopsin is present within the rods which are decolourized by light and regenerated in the dark. Due to slow regeneration, the photosensitivity of the rods gradually increases giving rise to dark adaptation. For regeneration of rhodopsin, vitamin A and nicotinamide are essential. In the absence of these two vitamins, night blindness is caused.

The cones are particularly important for colour vision in bright light illumination. In contrast to rods, the cones need bright light in order to get excited. In the fovea region of the retina, only cones are present and each one has its own nerve fibre. The cones also contain a photosensitive pigment which has not been isolated so far in man. However, this pigment is considered to be akin to rhodopsin. There are three types of canes, each one having a definite pigment which makes them sensitive to red, green or blue colour.

Physiology of Vision: The eye functions like a camera in which the retina acts as a film plate where the image is formed by refraction. The light rays are reflected from the visible objects and get focussed on the rods and cones of the retina from where impulses are transmitted to the optic nerve. The information from the optic nerves is relayed to the visual centres in the occipital lobes of the

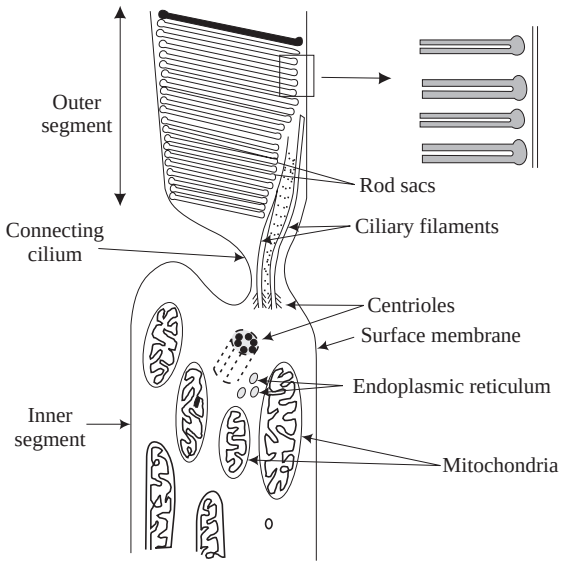


Fig. 16.11 Electronmicroscopic structure of the fine structure of the rods and cones.

cerebrum via several relay stations. When the eye is focussed for near objects, the cones of the fovea are employed. The fovea is the most important part for acute vision. In case of bright light, the object is focussed directly on the fovea. In dim light, however, the eye shows divergence in order to focus the image into the peripheral and sensitive part of the retina.

Image Formation: The rays of light from the objects are focussed clearly on the retina so that the photoreceptive rods and cones get stimulated. A dioptric apparatus is formed by the cornea, aqueous humour, lens and vitreous humour which are all transparent. The dioptric apparatus is concerned with the focussing of image on the retina. The most important role in vision is contributed by the cornea and the lens. The cornea helps in placing the image on the retina, while the lens is responsible in making adjustments for sharp focussing.

In the resting condition, the normal eye is focussed to see things at a distance of 20 to 30 feet or far. Such an eye is called *emmetropic* eye. If an emmetropic individual wants to see near objects, he has to make some efforts to adjust the lens to focus near objects. This process of adjustment is called *accommodation*. To achieve this the convexity of the lens, is changed by means of ciliary muscles. For nearer objects the lens becomes more convex. An inverted image is formed on the retina.

Vision by both the eyes is responsible for binocular vision. This creates a stereoscopic effect. Binocular vision is possible due to certain qualities of the eyes, viz. convergence (moving the eye inward), accommodation, change in the size of the pupil, and refraction. Change in the size of the pupil is respopsible for the accommodation. Because of refraction, the image that is formed on the retina is an inverted one. But to the eye, the objects do not appear upside down. This is possible by the visual sensations that take place in the brain.

Colour vision: Colour vision in vertebrates depends upon three pigments, each one responding to a definite wavelength corresponding to blue, green and red regions of the spectrum. This has given rise to a *trichromacy theory* according to which these pigments reside separately in the cones. The pigments individually or collectively are responsible for the range of colour visions.

Several theories have been put forward to explain the colour vision which include theories of negative after images, colour mixing and colour blindness, etc. However, none of these theories so far formulated is able to explain the phenomenon of colour vision satisfactorily. Different types of colour blindness have been explained on the basis of trichomatic theory. There are three types of individuals with partial colour blindness; some are blind to red, some to green and others to blue colours.

The concept of colour vision has been most satisfactorily explained by Young Helmholtz theory. According to this theory, there are three types of cones. Some cones are sensitive to red, some to green and others to blue colours. These primary colours can be combined in various proportions to produce all the colours of the spectrum. It has been demonstrated that the retina contains three types of opsins confined to different cones. The absorption spectra of each opsin have been found out with peaks at wavelengths of 435, 540 and 565 nanometers (n) which are close to those of three primary colours. The light of a particular wavelength will stimulate the cones of one particular type to produce a specific colour vision. The rods are, however, deficient in producing a colour sensation. The colour vision is possible in high illumination so that the cones are engaged. In dim illumination colour sensation is not produced because the cones are not utilized in the vision.

As has been said, the rods and cones are the photoreceptor cells which convert the electromagnetic radiations into neural impulses. How is this accomplished? We have now a fairly good picture as to how the photoreceptors behave when the light rays fall upon them.

The rods and cones contain four types of opsins. The opsins are proteins which give rise to *rhodopsin* and three kinds of *iodopsin* after combining with a chromatophore called 11-cis retinal. The rods contain rhodopsin and the cones contain iodopsins. When light falls on the retinal chromatophore, it is isomerized from the 11-cis to the trans form. The change is able to excite the rods to evoke a generator potential in the receptors to generate impulses in the afferent neurons. Rhodopsin is converted to *lumirhodopsin* which is unstable and readily changed to *metarhodopsin*. Metarhodopsin splits into *scotopsin* and *retinene* which interact and regenerate rhodopsin. This step requires vitamin A. If the vitamin deficient condition prevails the regeneration of rhodopsin will be small and the vision will be impaired.

Vision in Invertebrates: Many invertebrates possess simple eyes with, lenses (e.g. dinoflagellates, medusae, worms, etc.), but an evidence whether an image is formed in them is rather wanting. Cephalopods and arthropods are, however, provided with eyes with an absolute mechanism of image formation. Some insects are even provided with a colour discriminating vision (e.g. honey-bees, butterflies and many dipteran flies). Here we shall describe the structure of an arthropodan eye. Although it is different from that of vertebrates, the visual system are, however, alike in principle.

The adults of higher arthropods have compound eyes. Each eye is made up of a large number of eyelets or ommatidia having a refractive cone and a group of receptor cells consisting a retinula (Fig. 16.12).

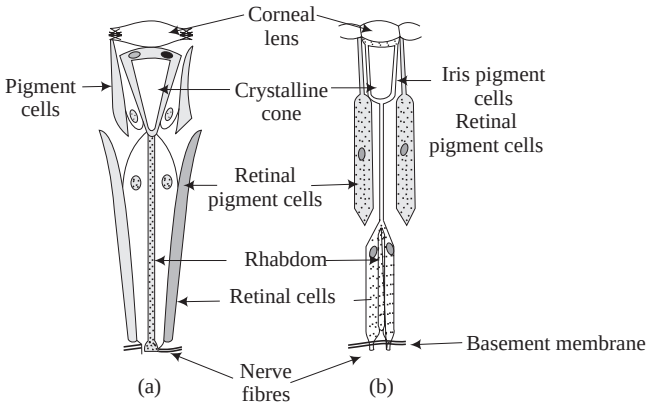


Fig. 16.12 Structure of the compound eye of arthropods.

In arthropods the light which falls on the cornea passes into a crystalline cone from where it is directed to the photoreceptors called rhabdomeres. The crystalline cone is surrounded by pigment cells which together with the crystalline cone act as the pupil. The pigment cells help in adjusting the intensity of light that should fall upon the rhabdom. Each ommatidium consists of several rhabdoms and the compound eye is formed by a collection of a large number of ommatidia. The rhabdom is a component of a sensory cell. Each ommatidium is cut off from its neighbouring one by a sheath of movable pigment cells arranged in two series. The outer series lying along the crystalline cone is called *iris* and the inner series separating the rhabdom is called the retinal pigment cells.

In the working of the compound eye, each ommatidium provides a seperate image of a small part of the object in view. Several such bits of images of the object are pooled to form a mosaic image and such a vision is called *mosaic vision*. In bright light, pigment cells move out in a way to isolate adjacent ommatidia so that the rays of light striking the cornea obliquely, are absorbed by the pigment cells without producing a visual effect. However, rays of light falling perpendicularly on the cones reach the rhabdomeres producing a visual effect, and the image formed is a mosaic of several components. Since each ommatidium responds to a small fragment of the total object, the fragmentary images are put together to form a single image by apposition method.

In dim light, the pigment cells migrate in such a way that they do not cut off neighbouring ommatidia and even the oblique rays falling upon them are capable of forming a point of image. As a result superposition image is formed which of course, lacks in sharpness.

Thermoreceptors

Although temperature receptors are found in all animals, they have beer best studied in human beings. They occur within as well as outside the body and are distributed in the form of scattered patches. Two types of thermo receptors, viz heat sensitive receptors or end organs of Ruffini and cold sensitive receptors or end bulbs of Krause have been identified. Heat sensitive receptors are activated by temperature higher than the skin and develop maximum frequency in impulse propagation between 37 and 40°C. Cold sensitive receptors are activated by temperature lower than the skin and generate action potentials of higher frequencies as the temperature is brought down to 25°C.

In invertebrates, the thermoreceptors are usually found on the antennae, mouthparts, or legs and show wide fluctuations in temperature sensitivity. In vertebrates, they are mostly distributed over the skin surface. Among homeotherms, the temperature receptors are distributed over the body surface as free nerve endings. They are, however, present in heavy concentrations on the tongue. For effective temperature regulation in homeotherms, the external temperature receptors integrate information with the internal receptors and this is accomplished through the hypothalamus which allows thermal adjustments involving the whole body.

CHAPTER

17

Nervous Coordination

In Chapter 15, we have considered the basic units of the nervous system, i.e. neurons. Organisms normally respond to selected stimuli only. They can instinctively choose a particular stimulus and specifically respond to it. The selective behaviour of the organism depends on the environmental situations. Such responses come under the category of behaviour which include both innate (instinctive) and learned (acquired) types. However, there are certain stimuli which evoke certain responses regardless of the environmental situations. These responses are programmed.

The most important operational units of the nervous systems are reflexes. A reflex apparatus usually involves a receptor, sensory pathway, modulator, motor pathway and the effector, and the path traced by the reflex action is known as *reflex arc* (Fig. 17.1). The receptors are specialized sensory cells that initiate nerve impulses carrying information. The information is transmitted to modulators such as brain and the spinal cord through sensory nerves. The brain and spinal cord interpret the sensory information to the effector organs through the motor nerve fibres. The effectors are generally muscles and glands which carry out the responses and may be recognized as forms of coordinated behaviour.

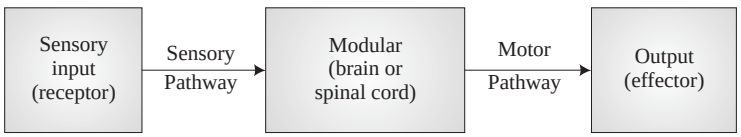


Fig. 17.1 Diagramatic representation of the receptor and effector relationship.

This coordinated activity involves judgement and interpretation of the data fed by sensory receptors. All animal behaviour is due to the coordinated activity of various structures outlined above which function harmoniously under a well regulated mechanism. The nervous system thus forms the most efficient means of communication and the impulse being propagated as a molecular change.

The basic components of the central nervous system are the neurons which have been described in sufficient detail in the previous chapter. These neurons are part of the *grey matter* of the brain and the spinal cord having been provided with long nerve fibres for intercommunications. The *brain* and *spinal cord* act as modulators and coordinate behavioural activities. There is another component of the nervous system: the autonomic nervous system (ANS) performing autonomic functions of various organs concerned with more delicate type of work such as visceral reflexes.

17.1 INTEGRATION

We have so far considered in some details the neuronal organization, conduction and transmission of nerve impulse. Now we shall consider here the ways in which the nervous system is able to utilize the sensory input to determine the output of any cell or group of cells by selecting appropriate response. This mechanism is called *integration.* In the process, the receptors and the effectors both perform integrative functions.

The mechanism of integration can be considered at three levels: (1) neuron, (2) organized groups of neurons; and (3) whole animal level, its behaviour.

The neuron has integrative properties, and obeys *all-or-none* principle in order to trigger an impulse. A certain amount of depolarization is necessary before an impulse can be propagated. The rate of firing of neurons depends on the tension or stretch on the dendrites. Neuronal integration falls under three classes: (1) neurons which receive convergent inputs from several sources; (2) those which receive input from one source only; and (3) such neurons which exhibit spontaneous activity.

At the neuronal level responsiveness is to be distinguished from the sensitivity. Sensitivity is a measure of the intensity of a stimulus to produce a threshold response, whereas responsiveness measures the response emanating from a threshold stimulus. Responses of an integrative neuron may be of several kinds.

In some cases the response is in the form of a *pacemaker potential* which gradually increases depolarizations leading to a local potential or spike. Pacemaker neurons always show spontaneous activity and occur in hearts (both neurogenic and myogenic), central neurons and stretch receptors.

Many neurons show *all-or-none* activity while propagating an impulse and the signs are in the form of *spike potentials.* The spike potentials are generally not involved in integration, except when they arise and terminate. They are continuous and graded and convey information over long distances.

17.2 SYNAPTIC INTEGRATION

Two or more independent impulses in the same neuron may not be efficiently integrated and as such two or more neurons can integrate the impulse effectively. Special structures called synapses are developed which have sites of contact or integration. The synapse is considered a functional unit which is the seat of all labile nervous functions. When the neurons function in an aggregate manner a pattern of behaviour of the animal is produced. The behavioural response will be dependent on the quality of the integrating system, the efficiency of the receptors, and the organization of the CNS.

The Reflex Arc

Almost all behaviour responses involve a sequence of receptor-CNS effector organs, suggesting thereby a definite anatomical pathway. Behaviour may be *learned* which is suggestive of some past experience of the animal, or it may be *innate* in which case the anatomical pathway is genetically predetermined. However, in either case a definite nervous pathway is involved. In animals such coordinated pathways are called the *reflex arcs.*

In the reflex arc, a nerve impulse enters a sensory nerve axon and is transmitted through several connecting neurons (internuncial neurons) to the motor neuron which ultimatley transfers the information to the effector organs (normally muscles; Fig. 17.2). The terminating fibres of the axon of one neuron and the dendrites of the cell body of another form a synapse. The transmission in the synapse is strictly one-way traffic.

Four principal forms of synaptic integrarion have been recognized. These are: summation, facilitation, recruitment, and inhibition.

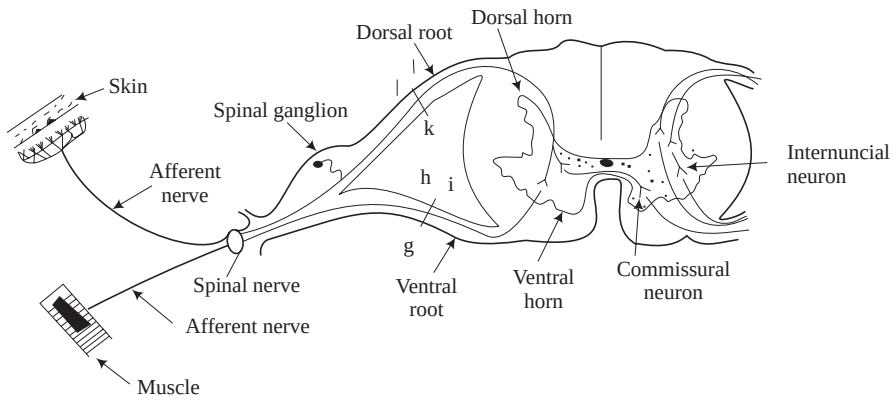


Fig. 17.2 Diagram of the reflex arc.

Summation: Certain patterns of neuronal activity are derived from a stimulus input irrespective of the actual stimulus or response. For example, if a sensory nerve is given a weak stimulus but adequate enough to transmit the impulse as far as the synapse, the motor neuron may not discharge since the impulse has not bypassed the synapse. By giving repetitive impulses of the same intensity the motor nerve will finally discharge. The train of repetitive impulses has an additive effect which causes the immediate discharge of the motor neuron. This is called *temporal summation.* Another type of summation is known as *spatial summation.* In many cases, different sensory neurons may be in communication with a motor neuron. If two afferent nerves (sensory nerves) are given a stimulus separately, the individual response of the motor nerve may not be produced, but if the two nerves are stimulated simultaneously, the motor nerve will discharge appreciably. Thus, by simultaneous stimulation of the two sensory neurons, there is a summation of the excitatory state of the motor neuron.

Facilitation: An important property of most synapses is facilitation. Facilitation refers to an increase in the excitability of a synaptic junction without showing any summation of electrical responses. Increase in excitability produces larger postsynaptic potentials. Therefore one afferent impulse may not elicit a response across the synapse, but more than one impulse in succession is needed to produce a postsynaptic response. The duration of facilitation is unexpectedly long, sometimes lasting for hours and days. Several explanations have been put forward to explain the causes of facilitation. It has been suggested that slow hydrolysis of the transmitter substance after the response has been elicited, may be the cause of facilitation. However, this may not be able to explain facilitation lasting for hours or days. Another cause has been attributed to changes in the membrane constants of the postsynaptic neuron which seems quite plausible.

Recruitment: Recruitment is a property of the synaptic activity across afferent-efferent synapses in a reflex arc. When motor neurons are stimulated directly, a condition of tetanus results instantaneously and upon withdrawal of the stimulus contraction of the muscle stops immediately. This implies that direct stimulation of motor neuron causes contraction of all the muscle fibres that are innervated. On the other hand, if the motor neurons are stimulated through an afferent nerve, tetanus is achieved gradually and when the stimulus is withdrawn relaxation of the muscles also takes place gradually. This shows that afferent stimulation involves several motor units initially and later on large number of them are employed. This phenomenon is called recruitment.

Inhibition: Structurally inhibitory synapses are similar to excitatory synapses, but differ in their function only. The presence of inhibitory synapses can be recognized from the absence of a response by the effector organ when the inhibitory nerve is stimulated at the same time as the excitatory nerve. The incoming and outgoing messages are integrated through both excitatory and inhibitory synapses. In vertebrates, the information is integrated across one or more synapses in the dorsal region of the spinal cord and is sent out of the ventral region through motor neurons. In invertebrates, the incoming sensory information is integrated within the ganglia where one or more synapses occur. A stimulus to a skeletal muscle in vertebrates is always excitatory; and stimuli to smooth and cardiac muscles, or to another neuron may be either excitatory or inhibitory. The stimuli transmitted to a muscle in invertebrates may produce excitatory or inhibitory effect. When a stimulus elicits a postsynaptic response (excitatory or inhibitory) acetylcholine is liberated from the nerve endings. If the effect is excitatory, depolarization results, whereas if the effect is inhibitory, hyperpolarization is produced reducing the excitatory effects due to impulses from other nerve fibres. In central nervous system, excitatory effect is measured in the form of excitatory postsynaptic potential (EPSP), and inhibitory effect is measured as inhibitory postsynaptic potential (IPSP). It is the interplay of postsynaptic potentials that determine the excitability of the fibre to propagate an action potential. In vertebrates, presynaptic inhibition is also involved for integrating incoming and outgoing messages.

General Properties of Reflexes

Reflexes may involve either a single synapse or aggregations of synapses. In case of single synapses, following properties have been observed:

(1) Unidirectional transmission.

(2) Repetitive discharge presynaptically resulting in postsynaptic summation, achievement of threshold postsynaptically and generation of action potentials in nerve or muscle fibres.

(3) Failure of presynaptic fibres to discharge faithfully in response to a given stimulus.
(4) Susceptibility of the synapse to various drugs and oxygen deficiency.
(5) Occurrence of synaptic delay between presynaptic and postsynaptic fibres.
(6) May be inhibitory or excitatory in nature.

The Reflex Action

The reflex action is the simplest kind of activity which can be defined as a integrated activity occurring involuntarily in response to a stimulus applied to a receptor. The reflex arc is composed of the following: (1) receptor organ; (2) an afferent neuron; (3) synapse involving some cells in the CNS (modulator); (4) an efferent neuron; and (5) an effector organ.

A reflex may be triggered generally by a precise stimulus such as temperature, change in pressure or any other disturbance that may adequate enough to elicita response. The receptors which are sensitive to such stimuli transmit the information to the spinal cord or to brain through the afferent neuron which is sensory. The impulse thus generated reaches the synapse or synapses in the CNS (spinal cord or brain stem). The efferent limb of the reflex arc is provided with motor (efferent) neurons which have their cell bodies in the spinal cord or brain stem with axons innervating the peripheral muscles. Generally in the case of reflex movement, the effector organ is muscle. In some cases, however, reflex activity results in the secretion from gland.

A wide variety of movements in vertebrates are performed as reflex action. A strong light held in front of the eye makes it shut. If our hand comes in contact with a hot or cold object, we draw the hand away. The shot of a gun startles us. Some vital functions of the important organs of the body are also reflex actions. The gastric glands start pouring in their secretions as soon food reaches the stomach; the movement of the respiratory muscles due imperfectly aerated blood are some examples of the reflex actions.

It has to be understood that the spinal cord alone does not contain the nerve centres of reflex actions. They are present in the grey matter of the brain as well. Vertebrate reflexes are mediated by both. Many reflexes such as salivation, blinking, pupil reflex and respiratory reflexes are mediated by brain. Others such as stretch reflexes are mediated by the spinal cord.

Characteristics of Reflexes

Although the reflexes are involuntary functions, they have certain features which make them highly complicated. Some important characteristics are:

(1) *Predictabiliy*: Once the response of an organ to a specific stimulus is observed, one can predict that the same stimulus will always elicit the same response.
(2) *Purposefulness:* Generally all reflex actions are useful to the organism and are performed with a definite purpose.
(3) *Localization:* In performing a reflex action, a specific effector is involved in response to the stimulus applied to a specific receptor.
(4) *Delay: Reflex time* is the interval between the application of the stimulus to a receptor and the beginning of a response by an effector organ. A *synaptic delay* occurs due to latent period and reflex time at the synapse. This depends upon the number of synapses in the nerve pathway.

(5) *Unlearned:* In order to activate spinal effector mechanisms, no experience is needed to bring them in operation.

(6) *Adjustive* and *protective:* Reflexes serve adjustive and protective purposes and become an important part of the animal behaviour.

(7) *Fatigue:* Reflex responses are readily fatigued after prolonged and continuous work. As a consequence, the latent period of contraction becomes longer and the rise of tension smaller and more gradual.

17.3 TYPES OF REFLEXES

Stretch Reflex

The simplest kind of reflex is a stretch or myotatic reflex. A muscle when stretched responds by contracting. The receptors of the stretch stimulus are the spindle organs termed as *proprioceptors.* These are specialized microscopic capsules located in the muscles. Each muscle consist of 2-10 muscle fibres called *intrafusal fibres* which are elastic but non-contractile. The contractile fibres which are not part of the spindles are called *extrafusal fibres.* The ends of the intrafusal fibres are attached to extrafusal fibres or to tendons. A single intrafusal fibre has its two ends which are contractile, while the central region is non-contractile containing the nucleus. They contain two types of sensory nerve endings: the *annulospiral nerve ending* and the *flower-spray ending.* The fibres with annulospiral endings propagate more rapidly than the fibres with flower-spray endings. The contractile parts of the intrafusal fibres are innervated by small motor neurons called *gamma* motoneurons. When a muscle is stretched, the muscle spindles are elongated and due to this the annulospiral endings are also elongated. The distortion of annulospiral endings results in the increase of its permeability to Na^+ ions to cause an action potential (Fig. 17.3). Thus action potentials are generated when the muscles are stretched and are propagated via the reflex arc. This causes the stretched muscle to contract.

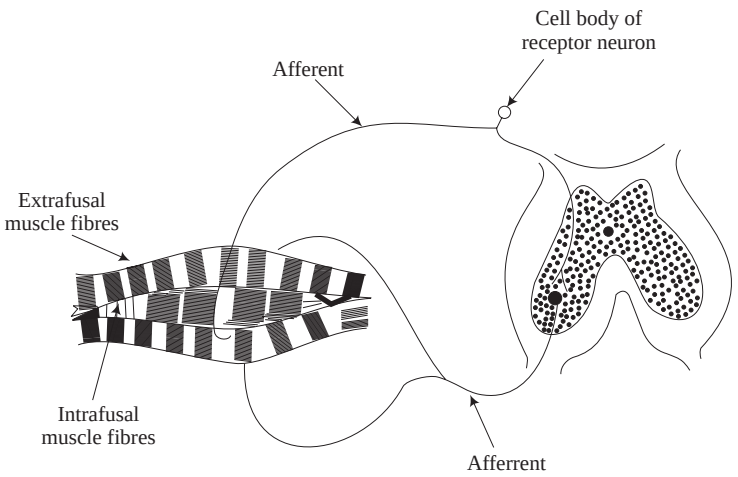


Fig. 17.3 Diagram of the stretch reflex.

The gamma efferent fibres cause the contractile portions of the intrafusal fibres to shorten. This may continue till the gamma neurons continue to fire. Therefore, if the muscle spindle is shortened, it will fire as soon as the extrafusal fibres are stretched. The gamma efferents are under the control of several centres in the central nervous system. We may, therefore, say that the response of a muscle to stretch is a function of gamma efferent activity.

Reciprocal Innervation: Movements of the body such as walking and running involve the coordination of two or more sets of muscles. The alternating contractions of the flexors and extensors of the limbs depend on the production and inhibition of the appropriate reflexes. To quote an example, in the movement of the forearm, the contraction of the biceps takes place and simultaneously the triceps relaxes. If both sets of muscles contract simultaneously, the arm will become rigid and immobile.

The impulses in the sensory afferent fibre cause discharge in the flexor efferent motor neuron via an interneuron causing inhibition of the extensor efferent motor neuron (Fig. 17.4). When a flexor is made to contract, there is inhibition of the antagonistic flexor muscle and vice versa. Joining the flexor and extensor inputs, there is an inter-neuron which functions as an inhibitory neuron. This involves reciprocal innervation.

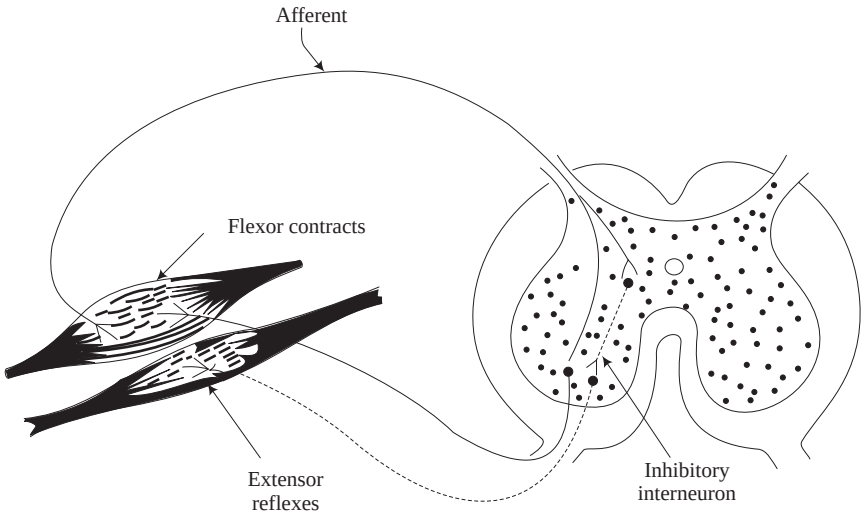


Fig. 17.4 Diagram showing reciprocal innervation.

Flexor Reflex

Certain stimuli which are of a painful nature result in withdrawal of the affected part involving flexor muscles. For example, in case of a decapitated frog, if the foot is pricked, the legs move away from the stimulus. This reflex is accomplished with the help of spinal cord. If the spinal cord is removed the withdrawal reflex does not occur. The flexor reflex is primarily a protective device to keep away the affected part from the harmful stimulus and the receptors that respond to such stimuli are called

nociceptors. In such reflexes, the tension of the flexor muscle rises suddenly suggesting simultaneous discharge from all motor neurons. There is no recruitment, of neurons.

Extension Reflex

The flexor reflexes are more complicated since more than one muscle is involved. In a spinal animal if the left lateral popliteal nerve is stimulated the right quadriceps femoris muscle (extensor of the leg) contracts. This is called *crossed extensor response.* For example, if by mistake the left foot is stepped on a thorn, the entire limb is withdrawn. Not only is the entire leg withdrawn, the other leg is also extended in order to provide support to the body for balancing.

Conditioned Reflexes

The simple reflex arc and its basic components have already been described. We have seen that such simple reflexes are carried out through the centres situated in the spinal cord. Although such reflexes are predictable, they are not dependent upon past experience or learning. Hence, these reflexes may be called *unconditioned reflexes.* In contrast to such reflexes, there are reflexes in which past experience, memory and training are utilized. These are called *conditioned reflexes* and are performed by the centres located in the cerebral cortex. A Russian physiologist, I.P. Pavlov, was the first to demonstrate conditioned reflexes.

In one of the famous experiments, a newborn pup was given milk to drink. Salivary secretion started as a result of stimulation of the taste buds. Drinking of milk is an inherent experience and does not require past training. However, if the pup grows older it becomes habituated and is able to associate with the sight of milk and its odour. If milk is brought in close vicinity of the grown up pup, the very sight of it stimulates the taste buds to secrete saliva. Such acts are called conditioned reflexes.

Conditioned reflexes can be inhibited. Any external disturbance such as sound, light, etc., cause distraction and will suppress the event. Inhibition is also possible if the original stimulus is applied repetitively. For example, if milk is brought in sight of the pup several times without allowing the animal to drink it, the conditioned reflex which causes salivary secretion becomes weaker and weaker and finally vanishes completely.

17.4 CLASSIFICATION OF REFLEXES

Reflexes can be classified in several ways. Generally they are classified according to the level of the nervous system involved. Accordingly, reflexes are: (1) spinal, (2) medullary, and (3) cerebellar types. Reflexes which involve skin, cornea, etc., are superficial reflexes; those which depend on proprioceptive impulses for muscles are called myotatic reflexes. Visceral reflexes are concerned with contraction and dilation of pupil, speeding and slowing the beating of heart, etc.

Spinal Cord

The spinal cord is cord-like in appearance and extends from the medulla oblongata, running all along the length of the vertebral column and ending at the lower border of the first lumbar vertebra. There is no trace of segmentation in the spinal cord, but 31 pairs of spinal nerves (in man) arise from it

giving an impression of thirty-one segments. Spinal cord is the seat of many important centres besides many reflex centres.

Spinal cord can be conveniently divided into two symmetrical halves by anterior and posterior fissures (dorsal and ventral fissures in animals). There is a central canal filled with cerebro-spinal fluid. The anterior root (ventral root in animals) is formed by the fusion of fibres that arises from the antero-lateral side of the cord. The posterior root (dorsal root in animals) arises from the postero-lateral side of the spinal cord. Both the roots combine to form the spinal nerve.

Histologically, the interior of the spinal cord around the central canal consists of grey matter composed of nerve cells. The peripheral part of the cord is occupied by white matter composed of nerve fibres. The white matter is divided into posterior, lateral and anterior columns (Fig. 17.5), while the grey matter appears as an H-shaped mass.

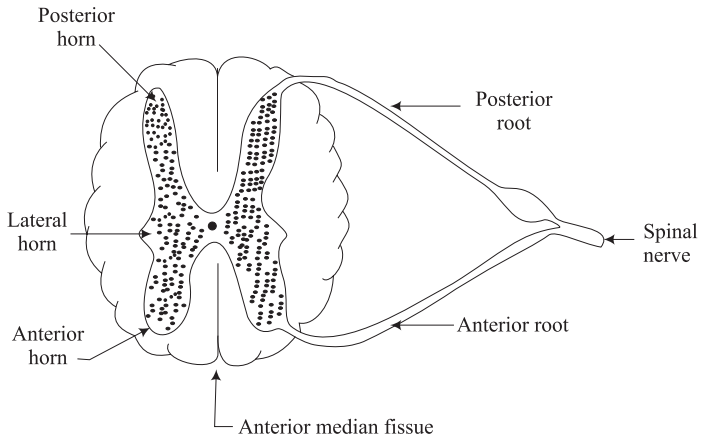


Fig. 17.5 A cross section of the spinal cord showing anatomical details.

The pointed apex of the grey matter is called the posterior horn, and the rounded end is known as the anterior horn. The anterior horns are occupied by large motor neurons whose axons leave the spinal cord through anterior or ventral roots. The innermost side of the base of the posterior horn is occupied by a group of cells called the *dorsal nucleus.* The outer side of the base of the posterior horn sends out white fibres and projections, combinedly known as *Reticular formation* which is in continuation of medulla oblongata. The rest of the posterior horn consists of the sensory nucleus. The apical portion of the posterior horn has a cap called *Substantia gelatinosa.*

The white matter of the spinal cord consists of many bundles of fibres arranged in afferent (tracts of ascending degeneration) and efferent (tracts of descending degeneration) tracts. In higher animals, the spinal cord has two important functions to perform: firstly, it serves as a line of communication between brain and the body through ascending and descending fibres, and secondly, it behaves as correlating and reflecting structure by which the information from the perifery is organized and returned to the perifery.

Effects of Sectioning of Spinal Nerve Roots and the Spinal Cord: Destruction or cutting of the posterior roots effectuates cutaneous anaesthesia and complete loss of sensations in all areas to which they are supplied. Cutting of the anterior roots produces paralysis of the muscles concerned with voluntary and reflex movements. When a spinal cord is cut transversely, the reflexes in the cord below the cut are depressed and those above the cut remain unaffected. Complete destruction of the spinal cord in man at the level of the thoracic region causes complete loss of voluntary movement in both legs with complete loss of sensation in the lower part of the trunk. When the spinal cord is cut entirely, the effects which follow are: complete paralysis on both sides, loss of sensation on either side, degeneration of fibres on both sides, exaggeration of reflexes such as knee-jerk and abolition of superficial reflexes and sphincter control.

Hemisection (only one half cut) of the spinal cord produces: (1) paralysis of all movements on the same side below the level of cut and loss of sensations (pain, touch, temperature) on the opposite side without loss of movement; (2) degeneration of the ascending and descending tracts on the same side; (3) vasomotor paralysis of the same side below the cut; and (4) rigidity of muscles of the same side, but if infection is established complete flaccid condition may be reached.

The Autonomic Nervous System: The autonomic nervous system is mainly responsible for controlling the internal environment of the organism. This system innervates the smooth muscle, cardiac muscle, intestine, uterus and glands, etc. These organs show spontaneous rhythmicity even when they are isolated and kept in a suitable medium. It is, therefore, a motor system. Thus the visceral functions are self-governing.

Anatomy of the autonomic nervous system: This system has two primary divisions of the motor outflow (Fig. 17.6):

(a) *Somatic division* which controls reflex and volitional activities of skeletal muscles.

(b) *Autonomic division* which controls the in idvoluntary activity of smooth muscles and glands.

Both divisions have many properties in common, but anatomical differences do exist. The postganglionic fibre of the somatic system has its cell body within the ventral horn of the spinal cord, whereas the cell body of the postganglionic autonomic fibres occurs in a ganglion lying outside the spinal cord. In the somatic system the peripheral nerves leave the spinal cord in an unbroken series, while the autonomic nerves arise in three distinctly separate groups:

(1) Cranial outflow which is asociated with III, VII, IX and X cranial nerves.

(2) Thoraco-lumbar outflow.

(3) The sacral outflow.

The autonomic systems are conventionally efferent systems and have two complementary parts: (1) s*ympathetic*, and (2) *parasympathetic* systems. The important difference between the two systems is that the parasympthetic fibres have their synapse in the effector organ, whereas in the sympathetic system the final synapse lies in a remote ganglion. The two systems are mutually antagonistic. Most tissues possess dual innervation, but some have only one (e.g. adrenal medulla).

Sympathetic system: The sympathetic outflow consists of ganglionated trunks together with terminal branches, communicates and subsidiary ganglia. The cell bodies that give rise to preganglionic fibres arise from the thoraco-lumbar segments of the spinal cord. The preganglionic fibres pass out in the ventral root of all the thoracic and the first 3 or 4 lumbar segments and enter the

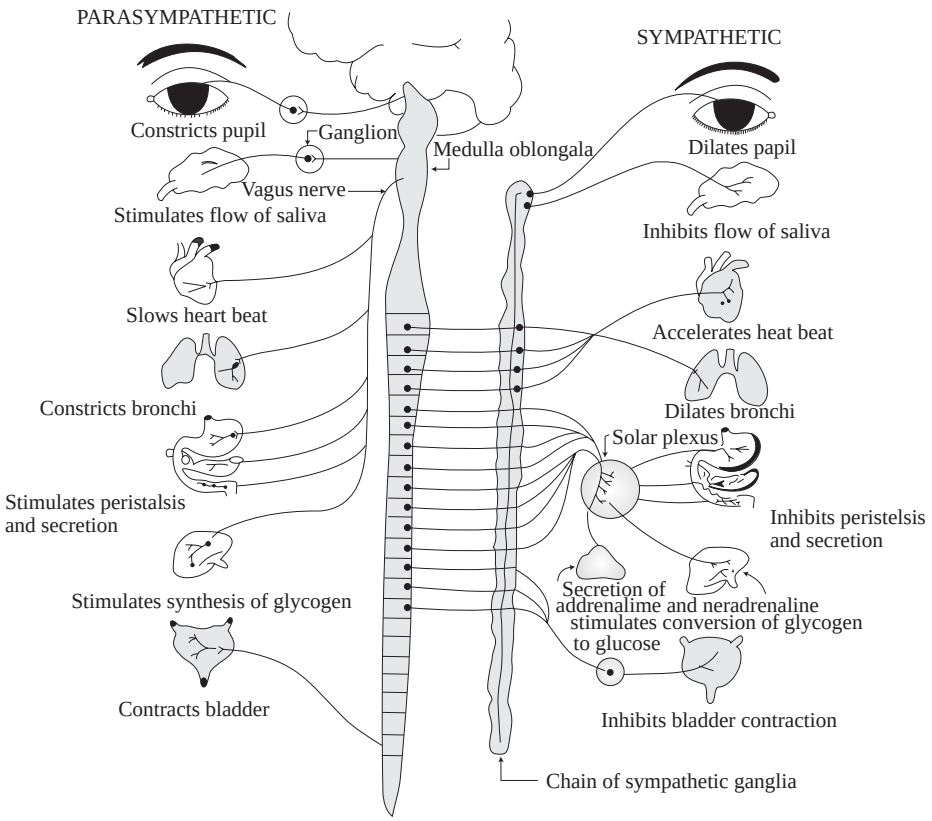


Fig. 17.6 Diagrammatic representation of the anatomical relationships of the autonomic nervous system.

spinal nerves. The spinal nerve sends out axons which connect with the respective sympathetic ganglia. The connections between the spinal nerves and the ganglia are called *white rami communicantes.* The preganglionic fibres may ascend or descend the chain or end in a special ganglion situated outside the chain. The postganglionic fibres may arise in two ways; (a) either from the ganglia of the sympathetic chains, or (b) from other ganglia. In the first case the postganglionic fibers may return to the corresponding spinal nerves through *gray rami communicantes.* The sympathetic chains lie on either side of the spinal cord extending the entire length of the cord. The supply to most parts of the body, whereas the preganglionic neurons are restricted to the thoraco-lumbar region of the cord.

Parasympathetic system: Anatomically, there is a long preganglionic and a short postganglionic fibre in the parasympathetic outflow and the ganglion is situated close to the organ supplied. The effects of parasympathetic activity are isolated and aid in conservation and restoration of energy. The parasympathetic neurons originate from three levels:

(1) *Sacral segments:* The preganglionic neurons arise from the sacral segments and innervate the visceral organs, and end in synaptic union with secondary neurons.

(2) *Brain stem:* Parasympathetic fibres originating from medulla oblongata are called bulbar parasympathetic fibres and the fibres pass in the VII, IX and X cranial nerves (which are facial, glossopharyngeal and vagus).

(3) *The ocular or tectal region:* The tectal fibres arise in the midbrain and are distributed along with the occulomotor nerve fibres which terminate in the ciliary ganglion.

Functions of autonomic nervous system: The autonomic system is indispensable for the body and brings about constancy of the internal environment for the efficient functioning of the organism.

The sympathetic system functions as a unit particularly under conditions of acute stress. Sympathetic stimulation accelerates the heart rate, increases blood pressure, raises blood sugar and decreases intestinal motility. The system is in constant activity and prepares the animal for any emergency.

The parasympathetic system is responsible for organised activity and is concerned with the conservation of energy. Parasympathetic stimulation slows the heart, lowers blood pressure and increases intestinal motility and glandular, secretions (Table 17.1).

Table l7.1 Functions of the Autonomic Nervous System

Effector system	*Sympathetic action*	*Parasympathetic action*
Heart		
rate	increased	decreased
stroke volume	increased	decreased
Blood vessel		
coronary blood vessel	dilated	constricted
skeletal muscle	dilated	no effect
pulmonary artery	constricted	dilated
gonadal organs artery	constricted	dilated
Eye		
iris	radial muscle constricted pupil dilated	contraction of circular muscle, pupil constricted
ciliary muscle	relaxed	contracted
Gut		
motility and tone	decreased	increased
secretion	decreased	increased
Glands		
sweat	secretion caused	
salivary	no secretion	secretion caused
liver	stimulation of glycogenolysis	no effect
pancreas	secretion decreased	secretion increased
adrenal medulla	stimulated	no effect
Urinary bladder		
wall	relaxed	contracted
sphincter	constricted	relaxed
Uterus	contracted in pregnant stage, relaxed during non-pregnancy	no effect

Chemical control: According to Dale's *Humoral theory,* when one nerve exerts its influence cover any viscera or any nerve, it does so by liberation of some chemical transmitters. All preganglionic and postganglionic parasympathetic neurons libertate acetylcholine at the fibre-endings. Such neurons are called cholinergic neurons. Acetylcholine is responsible for the activation of post-ganglionic fibres and also for the stimulation of the organ innervated by the postganglionic parasympthetic fibres. Most of the postganglionic sympathetic fibres going to the heart, intestine, etc., liberate norepinephrine at their endings, and hence are called as *adrenergic* neurons.

Brain: The brain is the central power station of the body. It consists of innumerable cells located in compartments with the capacity of performing several specialized functions. The brain is extremely complex (Fig. 17.7), especially in case of the mammals. We shall exemplify the human brain in the following paragraphs.

Brain Stem: The spinal cord is in continuation with the brain stem which includes the medulla oblongata, the pons and the midbrain. The medulla oblongata broadens rostrally and the central spinal canal opens into a lozenge-shaped space called the fourth ventricle.

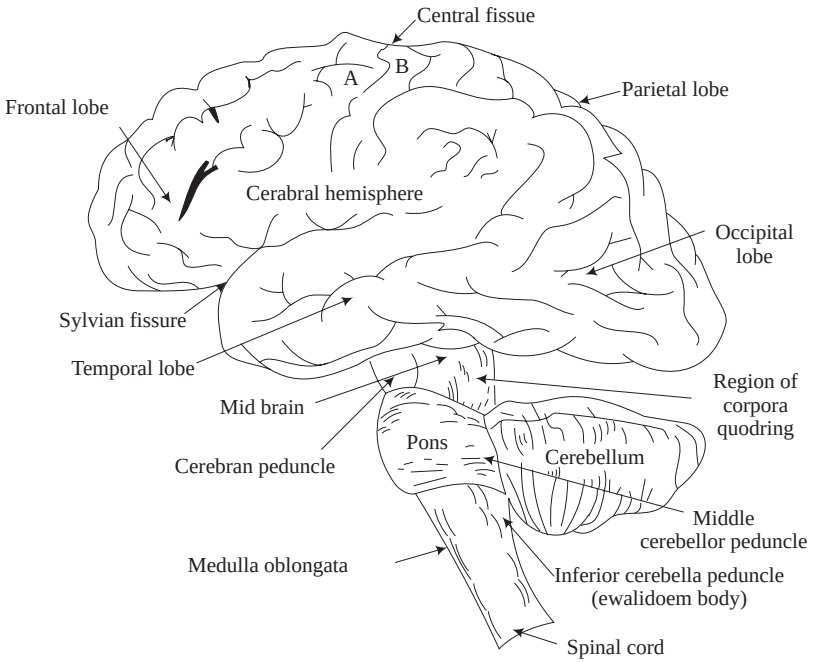


Fig. 17.7 Diagram showing the structure of the human brain.

The medulla oblongata: The medulla oblongata is the upward continuation of the spinal cord. It is divided into two halves by means of anterior and posterior median fissures. On the ventral side of the medulla, a pyramidal body and its fibres in the form of pyramidal tracts are present. A little anterior to this the pons are present forming a bridge-like structure. This contains transverse fibres arranged in the form of two cerebellar peduncles in the middle. These fibres go the cerebellum as

well. On the dorsal side of the medulla, the inferior cerebellar peduncles pass to the cerebellum. Anterior to the pons lies the midbrain. The structure of the spinal cord is still recognizable in the medulla oblongata. The grey matter contains several nuclei which give rise to cranial nerve functions of medulla.

The reticular formation: The central portion of the brain stem contains grey matter occupied by masses of nerve cells and intersecting fibres which run in all directions. This is called reticular formation. The reticular formation receives impulses from the cerebellum, brain stem nuclei, and the spinal cord, and in turn transmits them to other structures through definite pathways. Thus the reticular formation contains a network of afferent and efferent neurons. It is supposed to be containing important centres for the regulation of respiration, circulation gastrointestinal activities.

The reticular formation is responsible for motor activities. Stimulation of the anterior reticular formation results in the production of more generalized movements. It has been demonstrated experimentally that two systems of neurons are present in the reticular formation. Certain parts inhibit the movement whereas other parts facilitate it. Inhibitory neurons are concerned with the inhibition of movements which are induced reflexly or by cortical stimulation. If the lower part of the reticular system is damaged, its inhibitory influence is removed.

The reticular formation receives collaterals from the sensory pathways which helps in the coordination of motor activity. The impulses reach the higher centres of brain located in the thalamus and all parts of the cerebral cortex. Thus reticular formation helps in maintaining wakefulness of the animal. Damage to the reticular formation leaves the animal in a state of coma.

Functions of the medulla oblongata: The medulla oblongata contains important centres such as cardiac, vasomotor and respiratory centres. The centres for coughing, sneezing, vomiting are also located in the medulla.

Cerebellum: The largest part of the hind brain is cerebellum and it lies behind the cerebral hemispheres. In higher animals, especially the mammals, the cerebellum is highly evolved, whereas in lower group of vertebrates it is small and comparatively inconspicuous. The cerebellum is connected to the brain stem by three pairs of peduncles, namely, the superior peduncles, middle peduncles and the inferior peduncles. The afferent and efferent nerve fibres traverse through the peduncle.

The cerebellum consists of *Archicerebellum* situated below the *corpuscerebelli.* The two cerebellar hemispheres are connected to each other by means of central bridge called *vermis.* The cerebellum is the most important part of the brain for coordination of all movements. It acts as a computer using all available data and detects errors in information to take corrective measures. With the normal functioning of the cerebellum each act of movement can be performed with accuracy, speed and precision. Cerebellar function also involves the role of learning. Stretch reflexes also have a role in cerebellar function. Stimulation of one part of the cerebellum in the middle has an inhibitory influence on the stretch reflexes and stimulation on the lateral sides facilitates them.

Damages or lesions in the cerebellum would bring out important symptoms which are enumerated as follows:

(1) *Atonia* or loss of muscle tone.

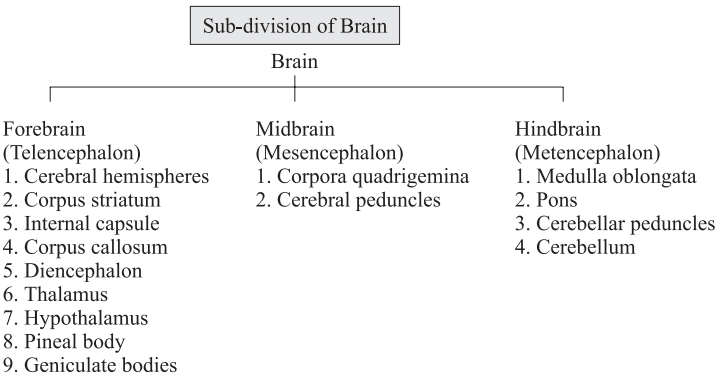
(2) *Atoxia* or incoordination of muscular action.

(3) *Astenia* or loss of muscle power.

(4) Tremors and rhythmical movements of the head and limbs due to widespread damage.
(5) *Dysmetria* in which there is difficulty in gauging the extent of muscular movements.
(6) *Dysdiadochokinesia* or loss of successive movements. It is a condition in which the rotation or movements of joints (pronation and supination) is very slow and clumsy.
(7) Rebound *phenomenon* in which the movement of a limb is unchecked.
(8) *Decomposition of movement* is exhibited by a patient with damaged cerebellum. In this case, the smooth and efficient pattern of a movement is broken down into several steps.

Midbrain: Midbrain is a narrow portion which connects the thalamus and the cerebral cortex with the pons and the cerebellum.

It is the smallest portion of the brain traversed by a narrow canal called *cerebral aqueduct* which communicates with the 3rd and 4th ventricles of the brain. The midbrain consists of the *cerebral peduncles* lying in front of the cerebral aqueduct. These peduncles are fused posteriorly to form *tegmentum* containing an oval mass of grey matter, called the *red nucleus*, and two smaller masses, called the nuclei of the oculomotor and trochlear nerves. The portion of the midbrain lying behind the aqueduct is called *tectum* and comprises two pairs of rounded eminences-*corpora quadrigemina.* Two of these eminences are called *superior colliculi* and the rest are *inferior colliculi.* The superior colliculus is the centre of visual reflexes, whereas the inferior colliculus serves as a centre of auditory reflexes. Each colliculus is associated with a small body, called *geniculate* body. The red nucleus is probably concerned with the execution of skilled muscular movements and has connections with the cerebral cortex, corpus striatum, thalamus, cerebellum and spinal cord.



Forebrain: This is the largest portion of the brain consisting of two halves called *cerebral hemispheres* separated by a longitudinal fissure. This portion is highly developed and most prominent in monkeys, apes and humans and represents highly evolved centre for wisdom. In case of lower vertebratesit is less prominent. Cerebral hemispheres are concerned with all volitional activities of the body, memory, intelligence and include centres for sight, smell, taste, hearing and sensations.

The cerebral hemispheres are occupied by a central mass of white matter and an outer covering of grey matter called the *cerebral cortex.* The cerebral cortex is provided with numerous characteristic foldings—the gyri or convolutions which have the semblance of the surface of a walnut kernel. By

these foldings the cortical area is greatly enlarged. Each cerebral hemisphere is divisible into four lobes: the prefrontal lobe, parietal lobe, temporal lobe and the occipital lobe.

Cortical lesions interfere with the memory and judgement. It has been suggested that memory is a function of the cortex as a whole rather than of any particular part of it. The frontal lobe consists of such brain tissues (motor areas) which are concerned with voluntary movements and personality. The parietal lobe is concerned with general sensations such as temperature, touch, pressure, pain and proprioception. The occipital lobe has centres of visual sense and the temporal lobe has cells that bring to consciousness the sensations of hearing and smell.

Some Important Functions of the Brain

Sleep: Sleep is the state when a person or an animal is unaware of his environment. This does not mean that the individual is unconscious. The difference between sleep and unconscious state lies in the fact that sleep is a normal recurring state during which the individual can be aroused, whereas in the unconscious state he cannot be aroused. There are two states of sleep: light sleep or slow-wave sleep and deep sleep or fast-wave sleep. Light sleep is characterized by some electrical activity in the cerebral cortex (although reduced considerably) and by the presence of some muscle tone. The deep sleep, also called as dreaming sleep, is a state characterized by loss of muscle tone, rapid eye movement and fast electrical waves.

The duration of sleep varies with age and a number of other factors. Infants sleep about two-thirds of the 24-hour period. The duration of sleep decreases with the advancing age. Cats and dogs sleep about two-thirds of the day, if undisturbed.

There are certain physiological factors that are involved in the sleep. During sleep, there is a slowing up of the heart rate, decrease in arterial blood pressure and the basal metabolic rate. The muscles also relax and become atonic. However, during fast-wave sleep while dreaming, there may be elevation of blood pressure and heart rate may also go up. Besides these, a number of other changes take place, such as increased blood flow to the cerebral cortex, elevation of brain temperature, increased gastric juice secretion, enhanced secretion in the adrenal cortex and changes in urine production. There is a high central nervous activity during fast sleep.

How does a person go to sleep? There is a definite mechanism which brings about this state of somnolence. Physiologically, sleep is very essential and a lack of it may lead to various psychological and physical disorders. Many theories have been advanced to explain the mechanism of sleep, some of which are described here. According to some, a sleep hormone is produced during the time we are awake and its accumulation induces sleep. Some waste substances of metabolism attack the central nervous system to cause this sate. It is now suggested that the impulses through thalamus and reticular formation of the medulla are relayed to the cerebral cortex to maintain the wakefulness. However, if the relaying of impulses is stopped, sleep occurs. Certain chemicals have also been known to induce sleep. Serotonin brings about light sleep, whereas catecholamines are responsible for fast-wave sleep. There are certain neurons in the brain which contain serotonin. Some drugs, such as acetylcholine and norepinephrine are known to act as inhibitors of sleep. However, their specificity is yet to be established.

Learning and memory: Learning and memory are seated in specific portions of the cerebral cortex, usually called *association areas* (Broca's area and Wernicke's centre). Learning involves

acquisition of knowledge, and memory is concerned with the retention of knowledge, and both are intimately related with each other.

There are two types of memory—short-term memory and long-term memory. Short-term memory may be due to a single experience; if not repeated, the memory fades away. Repetition of experiences causes long-term memory and brings about structural changes in the brain.

Although the mechanism of memory is not yet clearly understood, a few theories have been put forward to explain this. Neurons are rich in RNA. The electrical impulses impinging on the neurons bring about changes in the concentration and sequence of RNA and brain proteins. If the neuron is stimulated repeatedly, they build up memory by modifying the functioning of the neuron. The RNA molecule has a short life; hence the altered RNA should be replicated to preserve memory. How this is accomplished, is yet to be known. According to the well accepted hypothesis, RN A synthesis is gene-directed. Thus the main obstacle that still confronts us is to reveal how self-replication of RNA can be achieved.

In order to avoid the question of RNA self-replication, another hypothesis has been suggested which regards memory as an adaptation of neurons. The memory may thus be linked with gene regulatory mechanisms involving the operon concept, in which the genes are switched on and off.

A latest hypothesis developed to explain the mechanism of memory suggests the formation of an *engram* involving a change in the protoplasm in response to a stimulus. This requires synthesis of certain proteins in the brain. Lately some proof has been obtained suggesting a relationship between memory and protein formation in brain. Experiments were conducted in which some animals were given training to do a simple task. Then some substances were injected into the brain that interfere with protein synthesis before and after the training. *Puromycin,* an antibiotic, stops the synthesis of protein chain. In case when puromycin was injected after the training, it was found that memory of the training is jeopardized, thus showing a block in specific protein synthesis concerned with memory.

CHAPTER

18

Effector Organs

Organisms respond to various stimuli emanating from the environment outside the body through specialized systems consisting of cells or parts of cells. These are called effector organs. In the words of G.H. Parker, "Effectors are the parts by which the animals respond to changes in the world about them". There are various types of effectors, each one doing a specialized function upon some kind of stimulation through nerves or through chemical mediation (hormones). Some important effectors are: cilia and flagella, pseudopodia, muscles, electric organs, luminescent organs and chromatophores. Some of these will be described here.

A. MUSCLES

18.1 ANIMAL MOVEMENT

Movement is one of the basic characteristics of all living things. All animals can move and one of the important attributes of the movement is contractility which may occur in various ways. A living cell may show intracellular movements of its components, such as streaming movement of protoplasm or migration of chromosomes during cell division. However, cells may undergo locomotion also, which may be due to intrinsic behaviour of their specialized components.

Many unicellular organisms are capable of locomotion. For example, movement in *Amoeba* is due to streaming movements of cytoplasm observable in the fine processes called pseudopodia. Allen (1962) has described *Pseudopodial movement* in *Amoeba* due to movement of the molecules of the cytoplasm. In case of ciliates and flagellates, cilia and flagella form vibratile extensions of the cell surface which permit movements of the organisms. The theory of *ciliary locomotion* as advanced by Gray (1928) postulates the presence of a fibrillar system for explaining ciliary movement and muscular contraction of these unicellular organisms.

In higher organisms, movement of body parts or as a whole is due to the unique property of specialized tissues called the muscle tissue. Muscles are excitable tissues or effector organs which may respond to various stimuli, such as pressure changes, heat, light, etc. The functions of various systems, such as digestion, reproduction, excretion and the like, are also due to movements of muscles with which they are made of. Muscles are therefore the tissues which accomplish the movements in an organism.

Muscles have the properties of contractility, extensibility and elasticity. In a vertebrate body, muscles have two kinds of arrangements. Those muscles which move the appendages find their origins as well as insertions on the endoskeletal structures, are called *phasic muscles.* Phasic muscles function on a lever system and always occur in antagonistic pairs. Besides this, muscles occurring in soft organs like heart, urinary bladder, digestive tract and the body wall are called *tonic muscles.* These muscles do not have origins and insertions comparable to phasic muscles.

18.2 STRUCTURE OF MUSCLES

The function of the muscles is closely related to the structure, and therefore a detailed structure of the different types of muscles is necessary. In vertebrates, there are three distinct types of muscles which constitute about 60-75 per cent of the total body weight. They are: (1) skeletal muscles; (2) smooth muscles; and (3) cardiac muscles.

Skeletal Muscles

They are also called *striated* or voluntary muscles and are under voluntary control. These are multinucleate and display longitudinal and cross striations. Skeletal muscles are attached to the bones and help in the movement of the skeleton and the body as a whole. They comprise about 40 per cent of the total body weight.

Anatomically, a muscle is composed of a number of muscle fibres. Each fibre forms a basic unit of the muscles which is a cylindrical structure and may be many centimetres long. Length of the fibres is, of course, variable. Individual muscle fibres are held up together by means of connective tissue. They are abundantly supplied with blood vessels and efferent (motor) nerves. The fine microscopic structure of a muscle can be seen under the phase contrast microscope.

Each muscle fibre is a multinucleate cell. It consists of a semifluid *sarcoplasm* containing numerous longitudinal *myofibrils* which range from 10 to 100 μ in diameter. A bundle of myofibrils is called a *fasciculus.* The membrane enclosing each fibre is called *sarcolemma* beneath which are located numerous nuclei scattered throughout the fibre. The myofibrils are actual contractile elements which run all along the length of the fibre. A live myofibril is a transparent structure and when viewed under a polarized microscope, characteristic cross-striations with alternating light and dark bands are observed. The broad, dark bands are called *A bands* which are anisotropic, and the lighter areas are called *I bands* which are isotropic. Each *A* band is bound on each side by an *I band* and separated from the adjacent unit by a thin dark band known as Z *line* (Fig. 18.1). In the centre of the *A* band there is a less denser portion called *H zone.* The area between two Z lines is called a *sarcomere.*

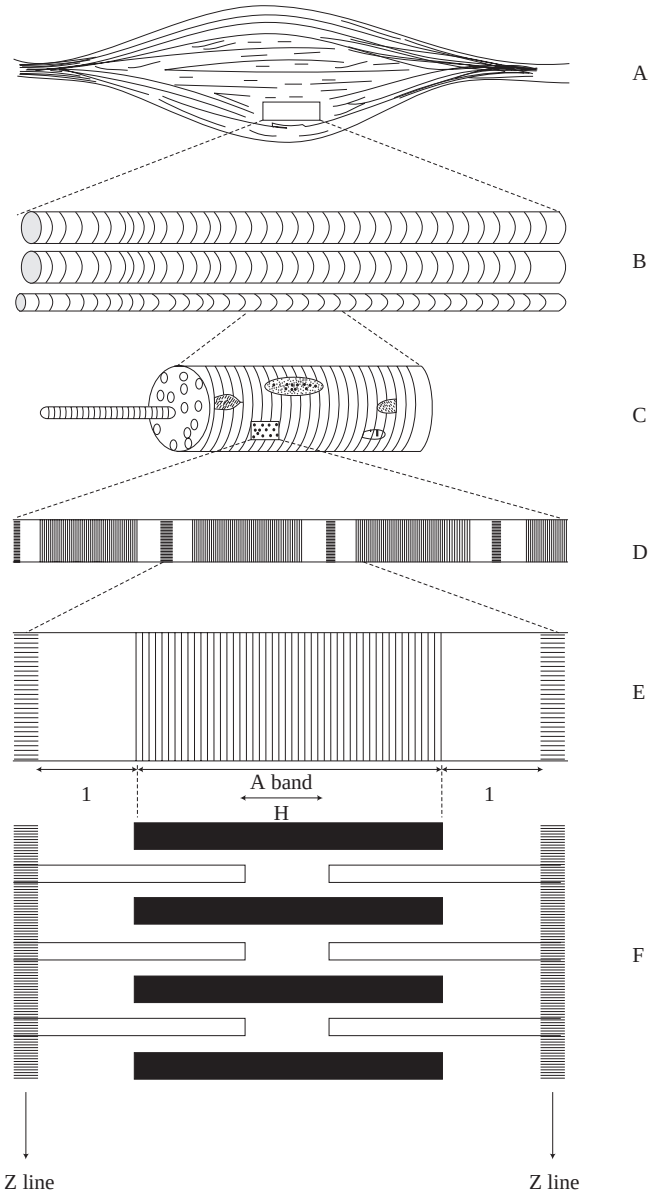


Fig. 18.1 Structure of striated muscle and myofibrils
A. bundle of muscle fibres; B. cross striations on the muscle fibres; C. a myofibril D. alternating dark and light bands on the myofibril E. a single sarcomere showing Z lines. *I* bands. *A* bands and *H* zone; F. single sarcomere magnified.

The myofibrils are the contractile units which undergo contraction and relaxation. They are made up of three types of proteins: *actin, myosin* and *tropomyosin.* Myosin is most abundant comprising about half of the dry weight of the muscle and is present in the primary filaments. Actin is found mostly in the secondary filaments; tropomyosin is probably present in the *I* bands.

Smooth Muscles

Smooth muscles are also called unstriated or involuntary muscles and are devoid of any cross striations. Anatomically, they are composed of spindle-shaped cells with long tapering ends and a centrally placed nucleus. They are generally found in the walls of the internal organs, such as digestive tract, respiratory passages, urinary bladder, arteries and veins, etc. They are slow in contraction and are not under voluntary control. Although these muscles contain actin and myosin, their mechanism of contraction is still not completely understood.

Invertebrates also possess smooth muscles, but in certain phyla they contain "paramyosin" filaments. The nonstriated muscle fibres of the molluscan adductor contains small actomyosin filaments and the large filaments contain paramyosin. Some smooth muscles have a spiral arrangement of fibrils as in case of Cephalopods.

Cardiac Muscles

These muscles are found in the heart only. They are made up of striated multinucleate fibres, thus resembling the skeletal muscles in many respects. The muscle fibres are arranged in a syncytial fashion, but do not appear to be fused with each other. These muscles are specialized in the sense that stimulation of cardiac muscle causes all the muscle fibres to respond initiating rhythmic beats of the heart. They are, however, involuntary in function and are innervated by autonomic nerves.

18.3 COMPOSITION OF MUSCLES

In order to understand the function of the muscles, an intimate knowledge of their composition would be very helpful. Each constituent has a functional significance. The principal constituents of a muscle include water, proteins, minerals and organic compounds.

Water

A muscle contains about 75-80 per cent water which plays a vital role in contraction. Water has unique physico-chemical properties and also provides a good medium for the inorganic ions and organic compounds present in the muscle. Considerable amount of water is present in the interspaces between the fibres. Muscle dehydration is a usual phenomenon which is regulated by osmotic forces. If an isolated muscle is kept in a hypotonic medium, the water tends to move inside and swells the muscle. In hypertonic medium water from the muscle moves out causing the muscle to shrink. For these reasons, in order to study the physiological properties of the muscle it is kept in a physiological saline whose osmolarity is identical with that of the muscle itself.

Muscle Proteins

The contractile property of the muscles is due to the presence of proteins. Most of the protein of the muscle is firmly attached with the fibrils and is not easy to be extracted. Of the total proteins, the skeletal muscles contain 20-25% water soluble proteins which is the *myogen* fraction rich in glycolytic enzymes such as aldolase, phosphorylase and glyceraldehydes 3-phosphate dehydrogenase. The myogen fraction can be obtained by squeezing the muscle or by dissolving them in dilute salt

solutions. Other insoluble proteins, which form about 90% of the total contractile proteins, can be extracted with 0.6M KCI solution. This fraction contains three major types: actin in the thin filaments, myosin in the thick filaments and tropomyosin B. Besides these, additional proteins are found in small amounts which include α-actinin, β-actinin, troponin and M-protein.

Actin is a globulin and structurally attached with the Z band. Its molecular weight is about 46,000 and consists of spherical molecules. Each globular unit is firmly bound to one molecule of ATP. Actin occurs in two forms: the globular G-actin and the fibrous F-actin. The G-actin is the monomeric form and in the presence of Mg^{++} ions it undergoes polymerization to form twostranded rope-like structure, the F-actin. G-actin can very tightly bind one Ca^{++} and also with one molecule of ATP or ADP. During polymerization, ATP molecule of G-actin is hydrolyzed forming ADP and inorganic phosphate ion is released. The reaction can be shown thus:

$$\text{G-actin} - \text{ATP} \xrightarrow[\text{hydrolysis}]{} \text{F-actin} - \text{ADP} + \text{Pi}$$

Myosin is complex and an asymmetric molecule (MW $\simeq$ 470,000) composed of two identical polypeptide chains coiled in a helical fashion (Fig. 18.2). The myosin molecule is composed of two portions: the head and the filamentous portions. The head of the myosin molecule consists of two smaller polypeptide chains (S_1) (Fig. 18.3). The filamentous portion is composed of two fragments. *heavy meromyosin* (HMM) and *light meromyosin* (LMM). The head of the myosin molecules is rich in ATPase activity having two catalytic sites. In the contractile mechanism, the actin molecules bind to myosin to form *actomyosin.* Pure on myosin requires Ca^{++} for ATPase activity while ATPase of actomyosin requires Mg^{++} ions for stimulation.

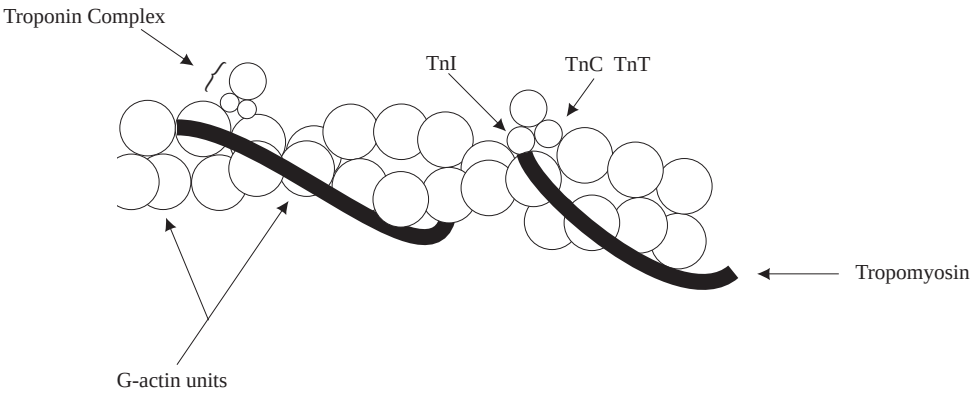


Fig. 18.2 Diagram showing two helical chains of G-actin monomers which give rise to F-actin filament. The rod-shaped tropomyosin molecules are also seen connecting several (seven in number) G-actin molecules. One troponin complex molecule is bonded to each tropomyosin molecule. Troponin complex is made up of three globular subunits: TnI: troponin- I; TnC : troponin-C; and TnT: troponin-T.

Another kind of muscle protein is known as *myoglobin* which is chemically similiar to haemoglobin. It is a conjugated protein and probably functions as oxygen carrier.

Actomyosin

The basis of the contractile mechanism can be explained by an interaction between F-actin and myosin. When mixed together, the two proteins form a complex known as actomyosin which is highly viscous. The actomyosin complex can dissociate in the presence of ATP and Mg^{++}, ATP undergoing hydrolysis.

$$\text{Actomyosin} \xrightarrow[Mg^{++}]{ATP} \text{Actin} + \text{Myosin} + \text{ADP} + \text{Pi}$$

When the hydrolysis of ATP is complete, actin and myosin reaggregate making cross-links between myosin and actin filaments.

Tropomyosin A and B

These two proteins resemble in structure to the tail portion of the myosin molecule. Tropomyosin B is water soluble and is the constituent of I zone of actin, whereas Tropomyosin A is water-insoluble and found in the catch muscles of the molluscs only. This is also known as *Paramyosin.*

Tropomyosin B is rod-like (MW—130,000) having two similar polypeptide chains (70,000 MW each) supercoiled in α-helical configuration. Both chains have free-SH groups. Tropomyosin B forms a complex with F-actin and occupies the groove of the coiled helix. The I-filaments contain another protein, *troponin* (MW ≈ 86,000) which forms a complex with tropomyosin B. This protein is necessary to effect relaxation and tightly binds with Ca^{++}. Other minor proteins like α-actinin and β-actinin are associated with actin filaments found in the Z bands.

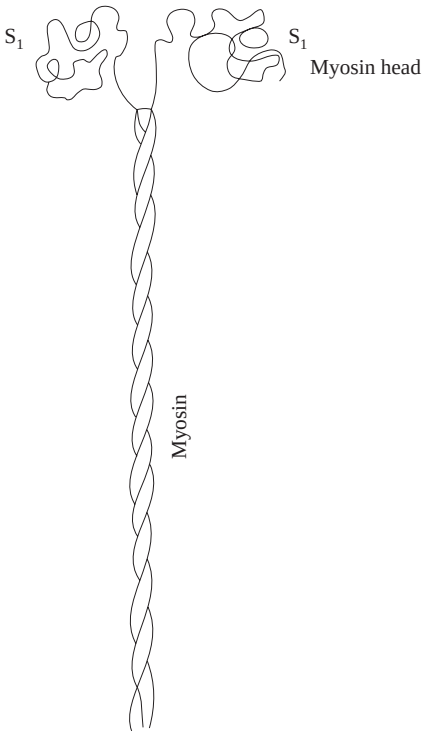


Fig. 18.3 Diagram of a single myosin molecule. The rod portion is composed of light meromyosin and heavy meromyosin portions, while the head of the molecule is made up of two S_1 portions which are rich in ATPase activity.

Inorganic Ions

Minerals are present in the form of inorganic ions. Potassium and sodium ions are the chief constituents of the muscles which are exceedingly important in setting up the action potential. Besides these, magnesium, phosphorus and calcium ions are also present in small quantities.

Organic Compounds

The organic compounds present in the muscle include glycogen, lipids, steroids and nonprotein nitrogenous compounds like ATP, creatine, phosphocreatine and urea. Glycogen forms a rich store in the muscles and is present in the forms of granules. Oxidation of glycogen yields energy to perform the muscular work. In anaerobic oxidation glycogen is converted, to lactic acid yielding less energy.

Lipids are also energy giving substances. Lipids are found as complex lipids in the form of phospholipids. Phospholipid content is related to the activity of muscles. The flight muscles of pigeons and the muscles of wild rabbits are said to have more phospholipids as compared to hens and domestic rabbits.

ATP is the most important source of energy in the muscle which is used by the contractile elements. The ATP molecules are bound up with G-actin molecules and take part in energy transformations. The ATP store of the muscle is about 3 MM/kg which is not much, but enough for 8 brief contractions. Thus ATP broken down during muscle twitch is quickly restored. Phosphocreatinine helps in restoring ATP supply. Traces of urea are also present in the muscle.

18.4 NEUROMUSCULAR JUNCTION

Skeletal muscles are activated by nervous impulses triggered by a mechanical or electrical stimulus. Activation of the muscle depends on the innervation of muscle fibres. A large motor nerve divides into a number of fine branches upon entering a muscle. These finer branches or nerve endings may be in close contact with the muscle sarcolemma (Fig. 18.4). The axon of the nerve terminates in a specially organized flattened structure called the motor *end-plate.* This is located at the surface of the muscle. The functional junction between the motor neuron terminal and the motor end-plate is called neuromuscular junction. In the motor *end-plate* the myelin sheath of axon terminates before it enters the muscle and the nerve endings remain naked being covered by plasma membrane only. The nerve endings are accommodated into specially formed crypts which are also lined by sarcolemma. The membrane of the muscle and the nerve remain in direct contact. The axoplasm and the sarcoplasm of the motor end-plate are rich in mitochondria.

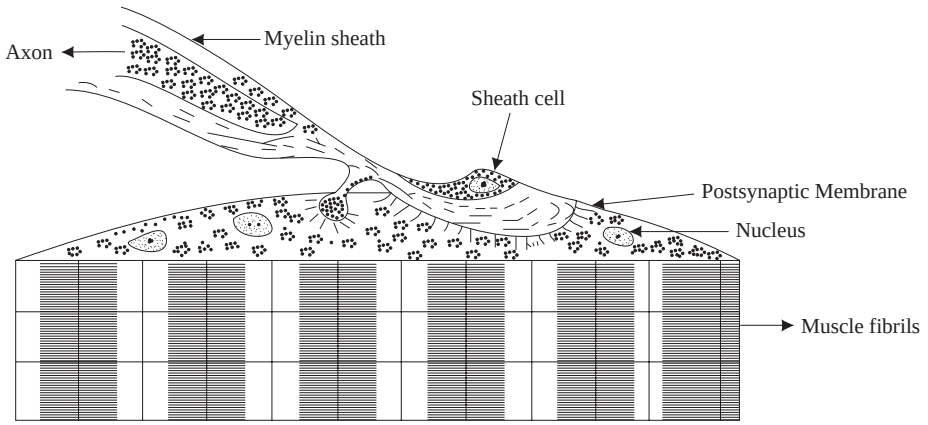


Fig. 18.4 Diagram of the motor end-plate in the skeletal muscle (neuromuscular junction).

The excitation from the nerve at the motor end-plate takes place through the release of neurotransmitters among which acetylcholine is most prominent. Acetylcholine is released at the nerve endings which increases the ionic permeability of plasma membrane, sending a wave of depolarization spreading from the end-plate to the muscle surface.

The motor end-plates are not known to occur in vertebrate smooth and cardiac muscles. In invertebrates, they have been recognized in a few insect skeletal muscles and segmental muscles of polychaetes.

18.5 EXCITABILITY OF MUSCLE TISSUE

A fundamental property of all living things is that they respond to various physical and chemical changes in their environments. Therefore, muscle tissue is no exception. Muscles are quite hardy and excitable tissues which may respond through the nervous system. Generally, studies of the muscle contraction are conducted on isolated muscles with its intact nerve supply. The gastrocnemius muscles of frog with the sciatic nerve forms the necessary preparation which is suspended to a clamp as shown in (Fig. 18.5). The muscle nerve preparation is to be kept moist by *physiological saline* to prevent drying up. The muscle is connected to a lever which moves up and down to record the response of the muscle to a specific stimulus. The lever records a tracing on a recording drum or a kymograph.

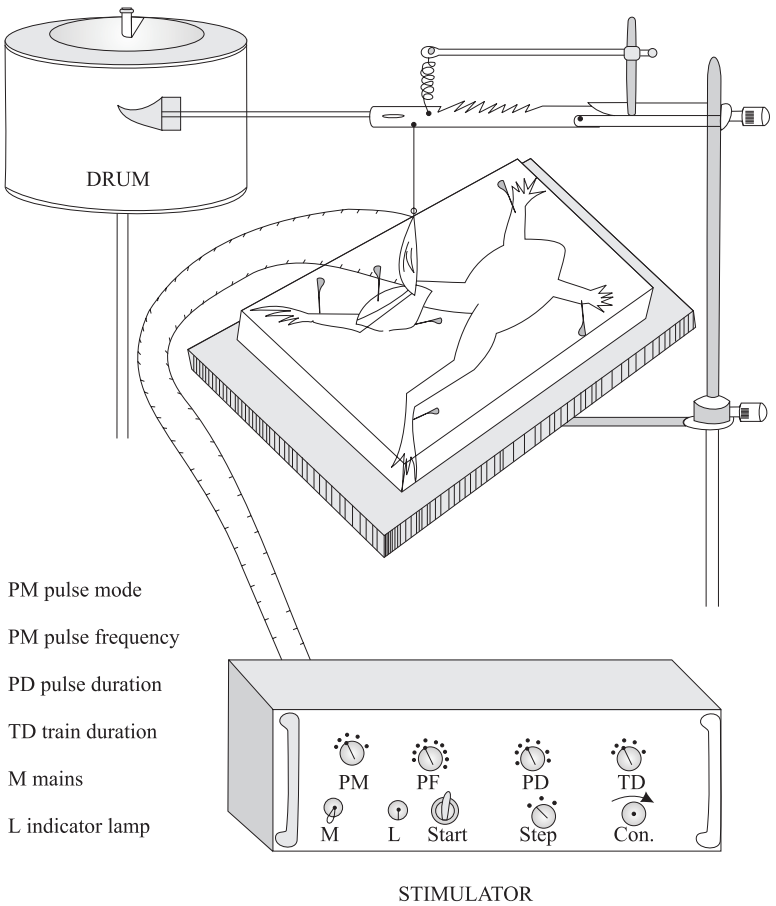


Fig. 18.5 Kymograph assembly for tracing the muscle twitch.

Stimulus

Changes in the environment constitute stimuli. A stimulus is quite specific and may be due to electrical, mechanical, electromagnetic, chemical, temperature or osmotic changes. In physiological experiments, electrical stimuli are generally used since they can be applied with greater accuracy. Electrical stimuli have several advantages over others; they are repeatable and controllable and the response is very quick. The tissue recovers very fast without any injury.

Action Potential

The skeletal muscle of mammals has a resting potential of –90 mV. Upon stimulation, the action potential is developed which persists for a longer time about 10 msec) in contrast to nerve action potential which varies from 0.5 to 2 msec. When a stimulus is provided, the membrane of the muscle fibre is depolarized and the impulse is propagated along the muscle. After about 2 to 3 msec the muscle undergoes contraction and develops action potential. The action potential is responsible for the release of the calcium ions.

Stimulus-response Relationship

The response of a muscle fibre is independent of the type, or strength of the stimulus. If there is any response, it is maximum. This is known as *all-or-none response,* and the minimum force or strength required to bring about a contraction is called the *minimum threshold.* The minimum threshold varies, with the type of stimulus applied. A subthreshold stimulus is unable to evoke a response and the muscle fails to give a twitch. However, a series of subthreshold stimuli are able to produce a muscle twitch.

Chronaxie

Duration and the intensity of the stimulus influence the rate of contraction a great extent. In case of electrical stimuli, the current which is just enough to excite is commonly called *rheobase* or the threshold strength of the stimulus. By varying the intensity and the duration of the current, strength-duration curves may be plotted (Fig. 18.7). Lapicque studied the relationship of the current and the excitation time and proposed a term c*hronaxie* for such measurements. Chronaxie is defined as *the time, that a current twice the strength of the rheobase requires to excite.*

18.6 MUSCLE CONTRACTION

Excitability or the power of responding to an adequate stimulus is an innate property of the muscle. When a brief stimulus is given, the muscle contracts followed by a wave of relaxation. This phenomenon is called a *muscle twitch.*

Fig. 18.6 shows a typical muscle curve of a skeletal muscle in response to single stimulation. The muscle curve can be recorded with the help of a kymograph. The curve indicates three phases: the latent phase, the contraction phase and the relaxation phase. The period between the stimulus and beginning of contraction is called the latent phase which lasts for about 0.01 second. During this period chemical changes take place as a result of the stimulus. Latent period is required for traversing

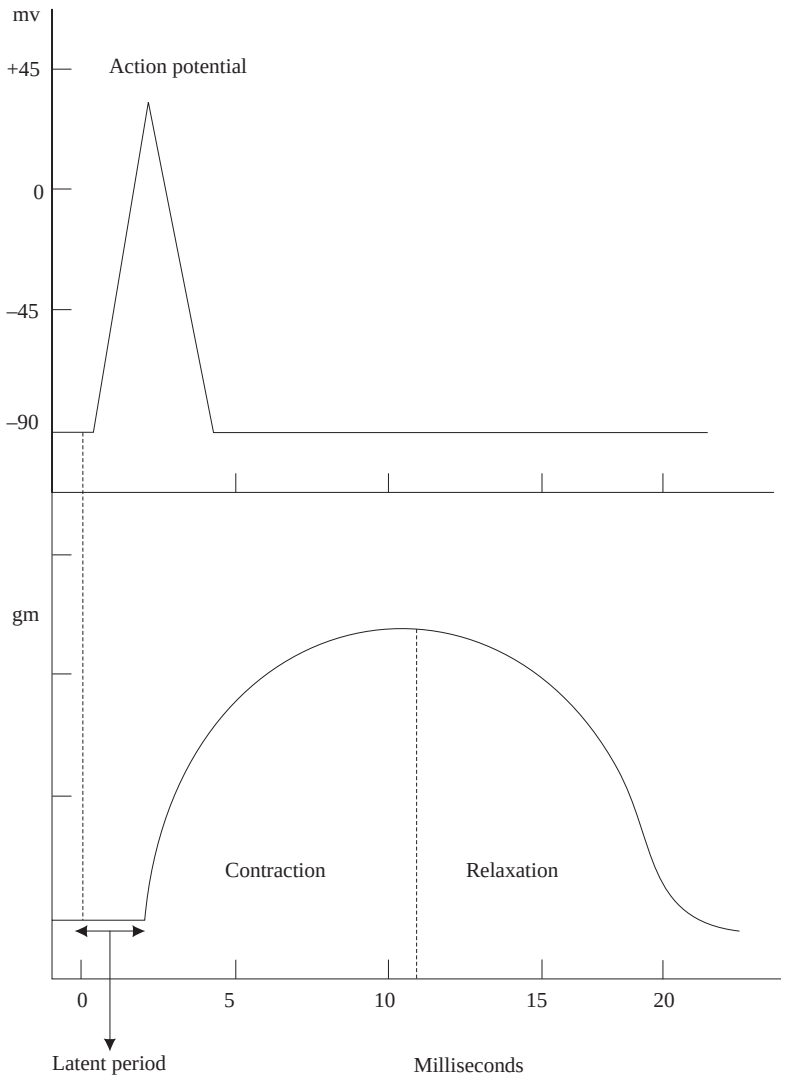


Fig. 18.6 Response of skeletal muscle to single stimulus showing phases in muscle contraction.

the excitation along the nerve and the neuromuscular junctions. The duration of the latent period varies with the species and depends on the type of muscle, temperature and condition of the muscle.

The contraction phase during which the muscle actually contracts lasts for bout 0.04 second in case of frog muscle. Shortening of the muscle takes place due to chemical events which will be described in some details later. The third phase or the relaxation phase lasts for about 0.05 sec. The total time taken by a single muscle contraction is about 0.1 sec which varies with the temperature. At low temperature contractions are prolonged, whereas with rising temperature the duration of contractions becomes shorter.

Force-velocity Relationship

The working efficiency of muscle depends on the amount of load which it moves. If a muscle contracts with no load upon it, it does no external work. However, when a load is attached to the muscle, it does external work. As the weight of the load is increased gradually the velocity of the contraction decreases till such time the load equals the optimum force the muscle can exert. At this stage there is no shortening of the muscle; hence the velocity of contraction is zero (Fig. 18.7).

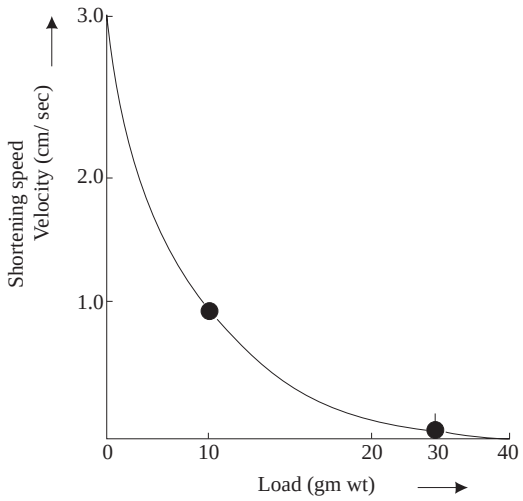


Fig. 18.7 Force-velocity relationship in octopus retractor muscle at 18°C (velocity—shortening speed, force — load).

Refractory Period

When a second stimulus is applied quickly after the first stimulus, there is no response to the second stimulus. This period during which the muscle shows no contraction is called refractory period. In skeletal muscles, the refractory period is very short, being about 0.05 sec. Two refractory periods are recognized: (1) absolute refractory period; and (2) relative refractory period. In the absolute refractory period no response can be elicited no matter how strong the stimulus is. However, a second response can be elicited immediately following this brief interval when a stimulus stronger than the threshold is applied.

Heat Production in the Muscle

Muscles continuously produce heat as a result of oxidation process while at rest or at work. However, heat production is more when the muscle is undergoing contraction. Muscular heat can be measured with the help of a thermopile and galvanometer and it is expressed in gram-calories. Thus in frog sartorius muscle heat production at 20°C is about 2 kcal/g/min during rest. In man, total weight of the muscle is about 30 kg. It has been estimated that at rest it would give off about 18 kcal/hr of heat. This heat energy is required to maintain the structure and electro-chemical gradients in the muscle.

When a muscle undergoes contraction, it produces heat in two phases:

(a) *Initial heat* or the heat of activation which is produced during the latent phase and the shortening period in small quantity in about 60 micro seconds of stimulation. It is less than half of the total heat produced (Fig. 18.8).

(b) *Delayed heat* is produced during relaxation and afterwards, and it is produced in greater quantum.

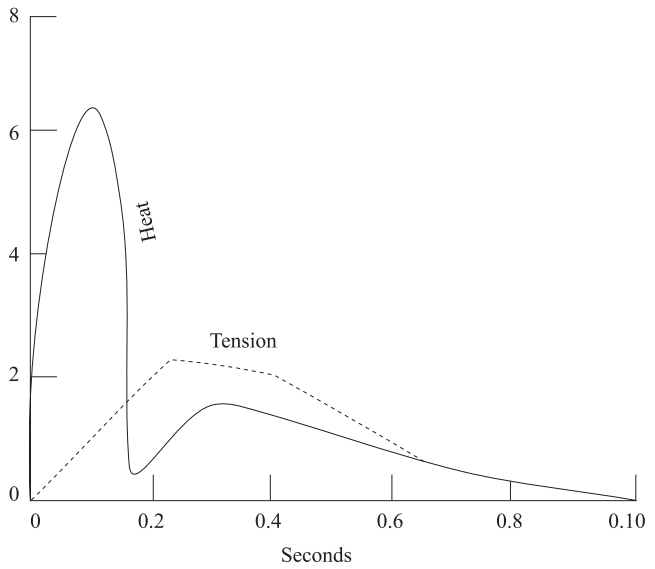


Fig. 18.8 Heat production in the muscle during contraction.

Muscle contraction can take place both in aerobic and anaerobic conditions. During oxygen block, less heat is evolved and lactic acid is produced. In oxygen-rich atmosphere more heat is evolved and lactic acid disappears during aerobic delayed heat production. About one-fifth of the lactic acid is oxidized and the rest is converted to glycogen. During contraction and relaxation, initial heat produced is independent of the oxygen availability and is associated with the breakdown of ATP and creatine phosphate. After, relaxation in the absence of oxygen a small amount of delayed heat appears due to the production of lactic acid from glycogen. On the contrary, in the presence of oxygen the aerobic delayed heat is due to the oxidation of lactic acid. Thus most of the heat evolved appears after the work is done.

External Work

The response of the muscle also depends on the shortening of the muscle which in turn is related to its weight lifting efficiency. With no load on the muscle, the contraction does no external work. If we take the gastrocnemius muscle of frog and suspend a weight to it (Fig. 18.9), it is seen that by increasing the weight a stage is reached when the muscle no longer can lift the load. Thus the load lifting capacity is decreased. In such a case, work done could be calculated as:

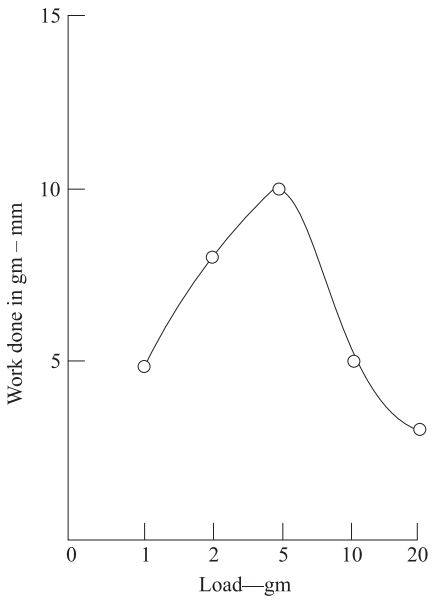


Fig. 18.9 Effect of load on the gastrocnemius muscle of frog.

Weight × Vertical distance through which weight could be lifted.

Duration of the stimulus is another important factor. A weak stimulus when applied for any length of time may not evoke any response. This is known as the *subliminal* stimulus. If a slightly stronger stimulus is applied for a shorter time, a response is observed. Thus stronger the stimulus, shorter is its duration.

Isotonic and Isometric Contractions

When a muscle is allowed to lift more load than its capacity, it does not show any visible contractions. There is no shortening in length, hence the work done also amounts to approximately zero. This is called *isometric contraction.* For studying isometric contractions, one end of muscle is fixed and the other end is connected to a strain gauge so that a very heavy weight is imposed on it. Upon stimulation the overall length of muscle remains unchanged although tension develops within it.

If the muscle is made to hold a constant load which it can easily hold, then the contractions will remain constant. This is called *isotonic contraction.* For studying isotonic contraction, the muscle is attached to a load through a simple lever. When the muscle is made to contract isotonially, its fibres shorten and maintain tension. This arrangement is useful for studying the amount of work that a muscle can do depending upon load.

Summation

If an isolated skeletal muscle is given a stimulus, it will undergo a single contraction. When a second stimulus is applied to muscle still in the contraction phase, further contraction or shortening in the

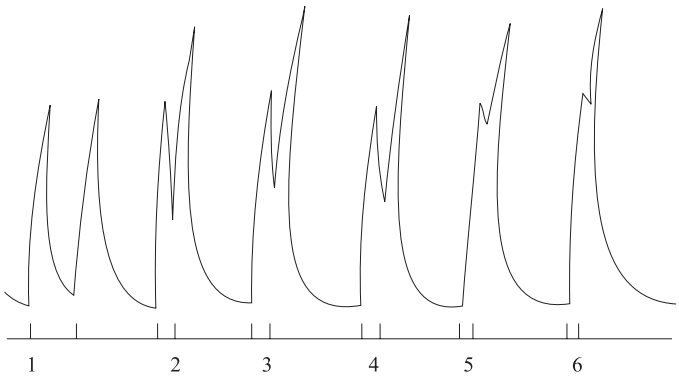


Fig. 18.10 Diagram showing summation.

fibres will take place. The second contraction adds to the first and causes a greater shortening of the fibres. This phenomenon is called summation (Fig. 18.10).

Tetanic Contractions

During normal activity such as locomotion, muscular contractions are not merely twitches lasting for a second or a fraction of it. They are sustained for a longer period during continued activity and exhibit compound or tetanic contractions. This can be experimentally demonstrated by applying a number of stimuli to a muscle-nerve preparation in rapid succession with little interval between successive stimuli, the resulting contractions tend to fuse to give a maximum contraction (Fig. 18.11). This sustained contraction is called *complete tetanus* which, however, varies with the kind of muscle and its condition. If repetitive stimuli are applied to muscle with long periods of interval, the individual contractions can be seen because of little relaxation. This condition is known as *incomplete tetanus.*

More interesting information is available about the tetanus. When a muscle is in tetany, a musical note is produced by it which can be heard with the help of a stethoscope. The pitch of the note is indicative of the vibrations that are produced at a rate corresponding to the rate of application of stimuli. Most of the voluntary contractions are of tetanus type which are produced by a series of nerve impulses arriving in the muscle from the central nervous system.

Muscle Fatigue

As a result of repetitive stimulation, at intervals not so close so as to produce tetanus, the muscle loses its ability to contract. This condition is known as muscle fatigue. Such a condition rarely occurs in the intact condition of the muscle and can be demonstrated with isolated muscles only. When repeated stimulations are given to an isolated muscle, the contractions become feebler and feebler and finally no response to stimulation is observed. The condition of fatigue is due to decreased level of phosphocreatine with concomitant acumulation of lactic acid in the muscles. Fatigue can be overcome by removal of lactic acid.

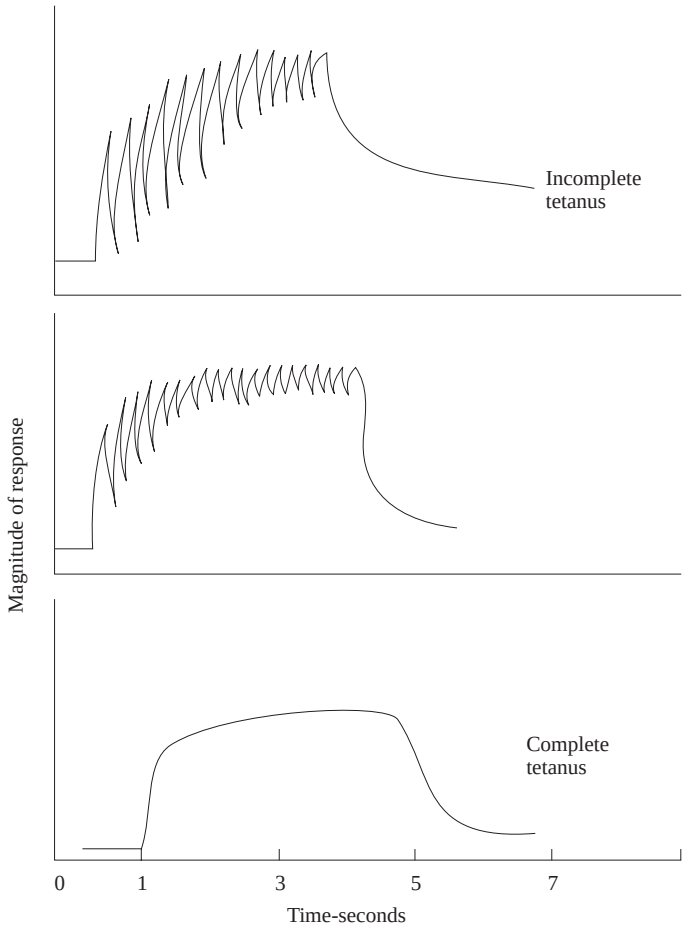


Fig. 18.11 Diagram showing the condition of tetanus.

Muscle Treppe

During contraction of muscle, some useful work is done. The amount of work done depends on certain conditions, such as size of the muscle, its type, its nutritive state and extraneous factors like temperature, rate of stimulation, intensity of stimulus, etc. Muscles with larger store of glycogen are capable of doing work for a prolonged period.

When a muscle is stimulated repeatedly at regular intervals, the first 5-15 contractions increase progressively in amplitude. This is called *treppe* or *staircase* phenomenon (Fig. 18.12). This state depends on certain internal conditions which affect the working capacity of the muscle, such as rise in temperature and depletion of glycogen store. Treppe, however, may not be observed in an intact healthy muscle in situ.

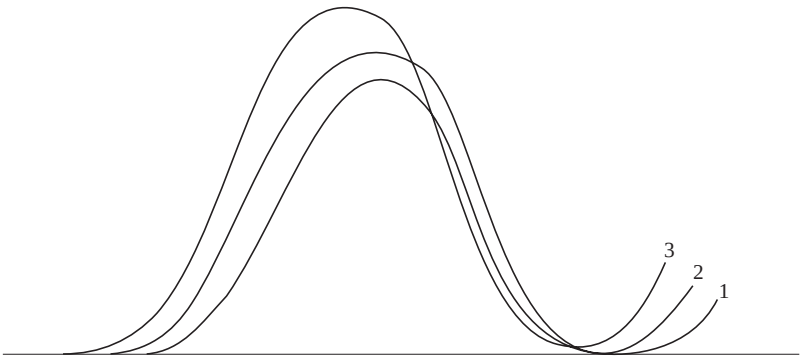


Fig. 18.12 Muscle treppe.

Muscle Tonus

During muscular activity, visceral muscles may be maintained in a shortened condition for quite some time. This condition is called muscle tonus. Tonus can be defined as involuntary resistance to passive stretch. When a muscle shows rhythmic activity, a maximal response is obtained during which all component muscle fibres act synchronously. Sometimes only a few fibres contract, while the remaining fibres contract some other time. In such a case, fatigue does not occur. During sleep muscles are almost completely relaxed, except that in this condition muscles remain in a state of partial contraction making the fibres firm with tension. These muscles possess a tone or tonus.

Muscles which are able to respond to a stimulus are said to possess a good tone which could be demonstrated by recording the action potential. A muscle whose motor supply has been severed of is devoid of the muscle tone and such fibres cannot be stimulated. Tones of muscle depend on its innervation. Denervated muscles are said to be *atonic.*

Although muscle tonus has been studied in relation to all kinds of muscles, the muscles of bivalve molluscs offer a good example of this phenomenon. In case of the bivalve, the adductor muscles closing the shells remain in a contracted state for a long time and any attempt to open them would be met with a strong resistance to stretch. Thus bivalve muscle can sustain heavy weight for long periods.

Muscle tone depends upon oxidative metabolism. In non-striated muscle, such as stomach muscle of the frog, increased oxygen uptake has been reported during muscle tone.

Contraction of Smooth Muscle

They are involuntary because the fibres are under control of autonomic nervous system and are found predominantly in visceral tissues such as digestive tract, respiratory passages, kidneys, arteries, veins, etc. They are slow in contraction since they are poorly organized.

The smooth muscles also display variety of variations. In vertebrates, they occur as bundles of fibres or as isolated fibres which respond to chemicals like acetylcholine, adrenaline, histamine and oxytocin, etc. The visceral muscles sometimes function as a syncytium and behave like cardiac muscles where the muscle contracts as a whole. Some of the smooth muscles structurally occur in the form of multiunits which show graded contractions.

In general, smooth muscles are controlled by the autonomic division of the central nervous system. However, there are some which are innervated by motor nerves and respond to impulses arriving through these nerves. In this way they, resemble the skeletal muscles and show quick repolarization. The resting potential also varies considerably. They have a resting potential varying from –30 mV to –75 mV.

Contraction of smooth muscle differs markedly from that of the skeletal muscle. The skeletal muscles contract immediately, whereas smooth muscle contract slowly and remain in this condition for a few seconds. These basic differences are due to release of calcium ions which form a complex with contractile proteins.

Contraction of Cardiac Muscle

Structurally cardiac and skeletal muscles appear to be similar; however there are some basic differences in their working. Cardiac muscle differs from the skeletal muscle in the following manner:

1. Cardiac muscle generates ATP aerobically and utilizes fatty acids for its generation instead of glucose.
2. Lactic acid produced by the skeletal muscle is transported to the heart by blood where it is further oxidized to generate more ATP.
3. During contraction, the action potential is seen to be prolonged in cardiac muscle and the process of repolarization is also prolonged. It is due to this reason that the action potential lasts about 100 msec as compared to 1 msec in skeletal muscle (Fig. 18.13).
4. The cardiac muscles show rhythmic contractions without any external stimulus and that is why the resting potential is not stable.

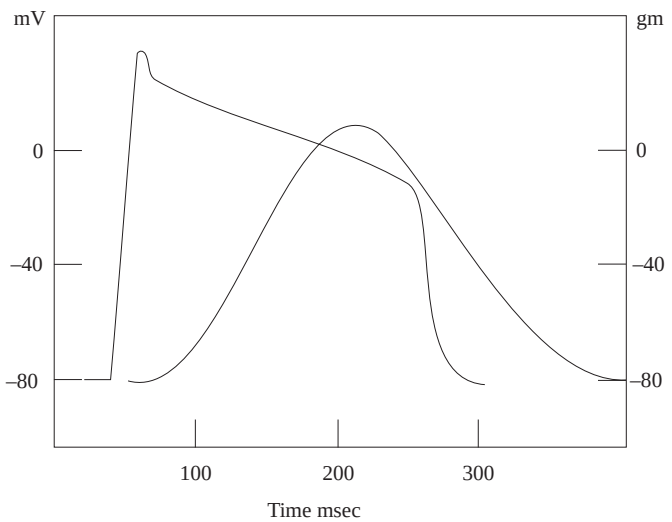


Fig. 18.13 Contraction of the cardiac muscle showing a longer duration of action potential.

5. During contraction cardiac muscle will not respond to a stimulus, hence the state of summation and tetanus never occur.
6. In cardiac muscle, the absolute refractory period is longer than the one recorded for skeletal muscle and lasts throughout the contraction phase.

Cardiac muscle possesses the unique property of inherent rhythmicity. The action potentials are generated by sinuatrial node and spread quickly to all parts of the heart. Thus sinuatrial node is called the pacemaker. The heart would continue to beat even after denervation.

18.7 THEORIES OF CONTRACTION

During contraction a muscle does some external work. How is this contraction accomplished? Several theories have been propounded, yet none of them is able to answer the question satisfactorily. One of the older theories postulates that sudden heating of the muscle fibres allows them to contract. Another theory suggested that sudden change of surface tension causes them to shorten. These theories were, however, unable to explain the complex phenomenon of contraction. Hence they did not meet with much support.

A more plausible theory was proposed by Meyer in 1929 and later supported by Weber and Fischer. The theory states that the submicroscopic muscle fibrils are composed of long, slender molecules of myosin which are capable of undergoing contraction. Myosin fibril is supposed to be analogous to a helical spring-like structure.

The latest theory about muscle contraction was furnished by H.E. Huxley on the basis of the ultrastructure of the muscle studied under electron microscope. As already discussed, the muscle is made up of thin fibres which in turn are made of long elements (about 1 micron in diameter) called myofibrils. A myofibril is composed of two kinds or filaments, one which is thinner and the other thicker, arranged in a definite pattern. The two kinds of filaments are linked by a system of cross-bridges responsible for contraction (Fig. 18.14).

Huxley's Sliding-filament Theory

On the basis of fine structure of the muscle, H.E. Huxley and his collaborators postulated a scheme which explains muscle contraction. During contraction and stretching of the muscle fibre, the length of the *A* band remains constant, whereas the length of *I* bands shortens. The length of the thick filaments is equal to the length of *A* band. The length of the *H* band increases or decreases with the length of the *I* band. This suggested that when the changes in the length of the muscle take place, the two sets of *H* and *I* bands slide past one another and when the shortening takes place, the ends of the filaments meet (Fig. 18.15). Both the thick and thin filaments remain constant in length when the muscle is at rest. Thus during shortening a new band pattern is observed. An earlier suggestion that contraction is due to extensive coiling of the filaments does not find any support in view of this theory.

Cross-linking of Filaments: Regular cross-bridges occur at regular interval from thick myosin filament in a helical pattern which link up the neighbouring thin actin filaments. These cross-links

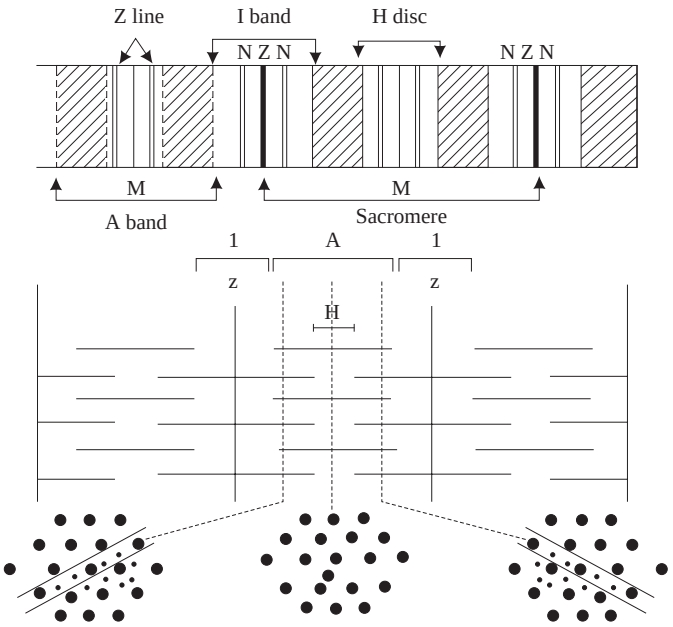


Fig. 18.14 Diagram to explain the mechanism of contraction of myofibrils, diagrammatic representation of cross band and striations of the myofibril of the skeletal muscle; also showing the arrangement of the sacromeres in longitudinal section and cross section of the filaments with two types of proteins.

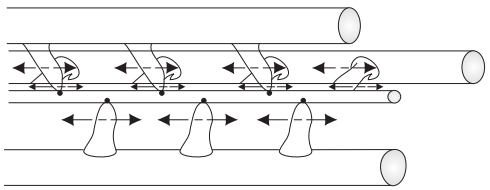


Fig. 18.15 Diagram explaining the sliding filament theory in skeletal muscle. Cross bridges on the myosin filament (thick) are able to engage on to the thin actin filaments; arrows indicating oscillatory movements of bridges.

probably help in the process of contraction of the muscle by making two types of filaments slide past one another. Each bridge is a part of a thick myosin filament and makes a contact at a specific site on the thin actin filament, thus maintaining a mechanical continuity all along the length of the muscle. These bridges are able to oscillate back and forth (Fig. 18.15). Each time a bridge slides past, a molecule of ATP is catalyzed to release a phosphate molecule to provide energy. During the relaxation phase, there is no linking of myosin bridges and ATP breakdown ceases (Fig. 18.16).

Szent Gyorgyi's Muscle Contraction Theory

According to Szent Gyorgyi, contraction is due to the role of myosin molecule. He suggested that pure myosin is a complex composed of protein subunits. The smaller subunits are called *protomyosins*

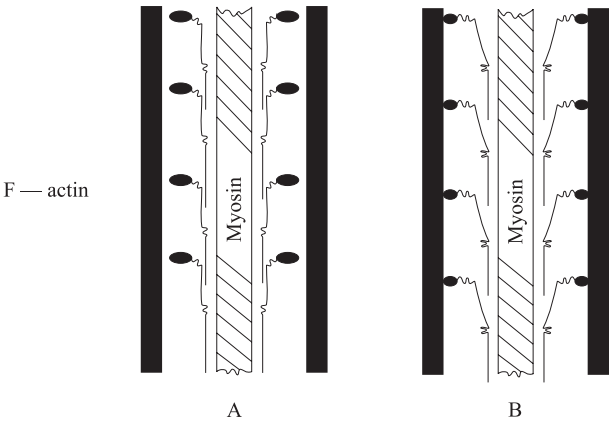


Fig. 18.16 Diagram showing the arrangement of F-actin and myosin filaments. (A) shows relaxed condition in which actin and myosin filaments do not form cross-bridges; (B) shows the contracted state in which the myosin heads favour the formation of cross-bridges with actin filaments.

which are held together by means of weak hydrogen bonds. About eight such protomyosin molecules when joined together form *light meromyosins* (L-meromyosin). The heavier units are called *heavy meromyosins* (H-meromyosin). If the muscle is excited in the presence of Ca^{++}, actin and myosin combine to form a complex known as actomyosin which is more rigid molecule. Myosin particle is kept in a stretched condition by the presence of water molecules, but contraction is accomplished when water molecules collapse or move out.

Actomyosin is highly sensitive to changes in the concentration of Ca^{++}, Mg^{++}, K^{+} and H^{+} as well as to ATP. Even small amounts of ATP would induce contraction of actomyosin. Heavy meromyosin is associated with ATPase which catalyzes ATP and the energy thus liberated is transferred to light meromyosin. Consequent upon this, the light meromyosin loses electrical charge and appears to undergo folding owing to contraction. Relaxation can be brought about by high concentrations of ATP.

18.8 CHEMICAL BASIS OF CONTRACTION

Excitation

When the vertebrate skeletal muscle is stimulated it undergoes contraction owing to the arrival of a nerve impulse at the motor end plate. The end plate membrane is depolarized and the electric potential gradients are spread over the entire surrounding area causing contraction. The result is observable in the form of shortening of muscle fibres. The major structural events associated with the contraction are: (1) the myosin heads involved in cross-linking change their orientation, and (2) the myosin heads make contacts with the actin filaments so that filament could slide.

Excitation-contraction Coupling

There are two basic questions that have to be answered. How contraction is triggered? How relaxation is caused? These questions could be answered in terms of chemical events taking place in the muscle.

The concentration of ATP in the resting muscle is quite high, hence it will not bring in a triggering action. The impulse arriving at the neuromuscular junction through the motor nerve spreads over the entire sarcolemma causing depolarization. This depolarization is caused due to increase in the permeability of cations, viz. K^+, Na^+ and Ca^{++} The communication of impulse with the interior of the muscle is initiated be diffusion of a chemical messenger from the sarcolemma to the interior of the myofibrils. How this is accomplished? Electron microscopic studies have revealed that there is a system of transverse tubules, called the T-*system* running across the Z lines of the myofibrils. An impulse arriving at the sarcolemma not only depolarizes it but also the T-system, and as a consequence all sarcomeres receive the communication. An experimental proof of the role of T-system was demonstrated by disrupting the tubules by treating the muscles with glycerol-ringer solution. The T-system communicates through a system of vesicles called sarcoplasmic reticulum. Electrical stimulus depolarizing the T-system causes increase in the permeability of the membrane forcing Ca^{++} ions to escape from the reticulum in the myoplasm. This event is electro-calcium coupling. This is followed by calcium-mechanical coupling involving interaction of ATP with actin and myosin filaments. Ca^{++} ions stimulate the hydrolysis of ATP by myosin. In the resting stage, however, ATP cannot be hydrolysed since free Ca^{++} ions are absent in the myoplasm.

Relaxation

Once the contraction phase is over, relaxation of the muscle follows. The mechanism of relaxation has been satisfactorily worked out and the sequence of events that follow are clearly the reversal of excitation-coupling process. The free Ca^{++} ions that were released during the contraction phase are withdrawn in the T-system. The reabsorption of Ca^{++} ions into the tubular system is brought about by the action of ATP-dependent Ca^{++} pump and each time when an ATP molecule is hydrolyzed to ADP-Pi, at least one Ca^{++} ion is withdrawn in the T-system. The Ca^{++} ions so accumulated in the tubules are discharged again into the sarcoplasm when another impulse arrives at the sarcolemma. Withdrawl of Ca^{++} ions is clearly responsible for the muscle to relax.

In muscles rich in mitochondria, removal of Ca^{++} ions during relaxation takes place in the mitochondria since they are capable of accumulating Ca^{++} ions at the expense of ATP.

Energy Source of Muscle Contraction

During contraction energy is released in the form of heat. Energy releasing chemical reactions are controlled by change in the length and the tension placed and the muscle while work is done. During activity muscles need more oxygen than at rest, although contraction can be induced in fully anaerobic conditions also. The muscles contain stores of glycogen which is decreased during contraction. In anaerobic contraction the decrease in glycogen is more since more glucose is oxidized to lactic acid yielding less energy. In aerobic contraction the energy yield is very high. Szent Gyorgyi has studied contractile mechanism of muscle *in vitro* which has yielded much valuable information. Myosin and actin together form actomyosin complex which can be drawn artificially in the form of fibres. These fibres, when immersed in a solution of ATP, contract showing that myosin, actin and ATP together form the basis of the contractile system.

Adenosine Triphosphate: It is obvious that the ATP is the main source of energy for muscle contraction. It ultimately comes from the oxidative metabolism of glucose (glycolysis). Anaerobically,

one molecule of glucose yields two molecules of ATP, whereas in aerobic oxidation the yield is 38 ATP molecules. Thus ATP yield will depend upon the glycogen reserve of the muscle.

Isolated muscles can be made to contract upon electrical stimulation and the amount of lactic acid formed can be estimated consequent upon glycogen depletion. Experimental evidence suggests that a muscle poisoned by injecting iodoacetate is still capable of contraction indicating that glycolysis is not essential for contraction. Iodoacetate inhibits the enzyme glyceraldehyde phosphate dehydrogenase. This evidence indicates that muscles have another energy-rich source which supplies energy for contraction in the absence of glycolysis. This observation again is not adequate since the amount of ATP present in the muscle is very small and remains unchanged before and after single contractions. An answer to this enigma was provided by high concentration of *phosphocreatine* present in the muscles.

Phosphocreatine: It is an energy rich-compound whose standard free energy of hydrolysis is very high ($\Delta G° = -14.80$ kcal) as compared to ATP ($\Delta G° = -7.30$ kcal). Although phosphocreatine cannot be used as direct source of energy, it, however, serves for the regeneration of ATP from ADP:

$$\text{Phosphocreatine} + \text{ADP} \underset{\text{phosphokinase}}{\overset{\text{Creatinine}}{\rightleftharpoons}} \text{Creatine} + \text{ATP}\Delta G° = -3.0 \text{ kcal}$$

Vertebrate skeletal muscles contain about 5 times more phosphocreatine than ATP. This explains the cause for no decline in ATP content of the muscle during single contractions.

In most active vertebrate muscles, regeneration of ATP from ADP is possible by rephosphorylation of ADP through oxidative phosphorylation in mitochondria (respiration). However, the muscles which are relatively poor in myoglobin and cytochromes, rephosphorylation of ADP is done at the expense of glycolysis. In muscles rich in mitochondria, myoglobin and cytochromes, contraction is accompanied by utilization of glucose and oxygen. As the muscle contracts, more ATP is broken down to ADP which is an acceptor of phosphate during electron transport chain.

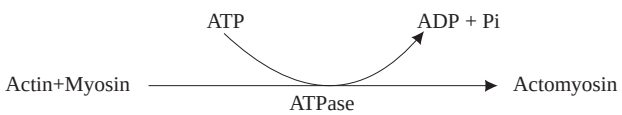
Chemistry of Contraction

Muscle contraction is accompanied by definite chemical changes involving the work of some enzymes. Each enzyme is associated with a definite reaction. During contraction energy is made available in the form of ATP, and more ATP is produced to make up the loss. This is obtained from the metabolism of carbohydrates and fats; during relaxation calcium ions are received from the cytoplasm.

The sequence of chemical processes taking place during muscular contraction may be summarized as follows.

1. **Interaction Between Actin and Myosin:** Muscular contraction depends upon interaction between actin and myosin when ATP is converted to ADP:

The initial breakdown of ATP by the specific enzyme ATPase results in sudden release of energy. During interaction, actomyosin ATPase requires mg^{++} for its activation and this mechanism is brought about by Ca^{++} ions released from the sarcoplasm.



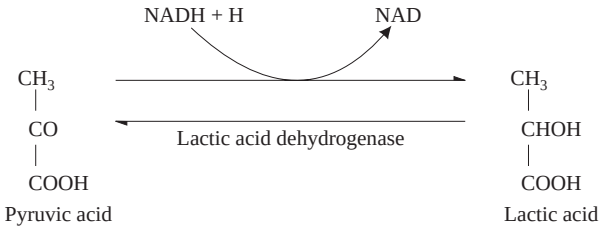
2. Phosphocreatine as a Source of Energy: During the interaction in the first step, the amount of ATP present in the muscle is depleted. In order to replenish the stock of ATP, phosphocreatine is broken down by the specific activity of phosphoryl creatine kinase in the following way:

$$\text{Phosphocreatine} + \text{ADP} \xrightarrow[\text{Creatine kinase}]{\text{Phosphoryl}} \text{Creatine} + \text{ATP}$$

This mechanism has limited scope and is operative during emergency needs only. However, this reaction can occur during anaerobic phase also. When a muscle is induced to contract over a prolonged period, fatigue develops owing to which glycogen, ATP and phosphocreatine levels decline, whereas ADP, AMP and lactic acid levels rise. In such cases, ATP may be regenerated in the following manner:

$$2\ \text{ADP} \xrightarrow[Mg^{++}]{\text{myokinase}} \text{ATP} + \text{AMP}$$

3. **Breakdown of Muscle Carbohydrate:** The third step is the breakdown of muscle glycogen by the glycolytic pathway yielding pyruvic acid (for glycolysis, see Chapter 6). In this process, eight molecules of ATP are synthesized for each unit of glucose. In the absence of oxygen supply, pyruvic acid is converted into lactic acid:



ATP breakdown and glycolysis proceed anaerobically. However, the accumulated pyruvic acid and lactic acid can be oxidized to yield CO_2 and H_2O in the presence of oxygen only via citric acid cycle (see Chapter 6). In this process, more molecules of ATP (30 mols) are generated for each glucose unit oxidized.

4. **Resynthesis of Phosphocreatine:** Contraction and relaxation are associated with the breakdown of ATP and phosphocreatine which are quite independent of oxygen supply. During relaxation and afterwards, lactic acid is produced from glycogen. The energy for resynthesis is derived from this process. One fifth of the lactic acid is oxidized and the rest is converted back to glycogen in the liver, muscles and other tissues. Glycogen formation is endothermic process requiring energy. Creatine combines with ATP to form phosphocreatine and the energy for its synthesis is derived from the breakdown of glycogen (Fig. 18.17).

Muscles cannot remain in a contracted state for a longer period, hence relaxation must follow. Relaxation is caused due to the absence of free calcium ions. We have seen before that Ca^{++} ions are necessary for ATPase of the actomyosin to hydrolize ATP. It has been suggested that the myosin bridges are reestablished when calcium ions are removed. Where do these Ca^{++} ions go? They are

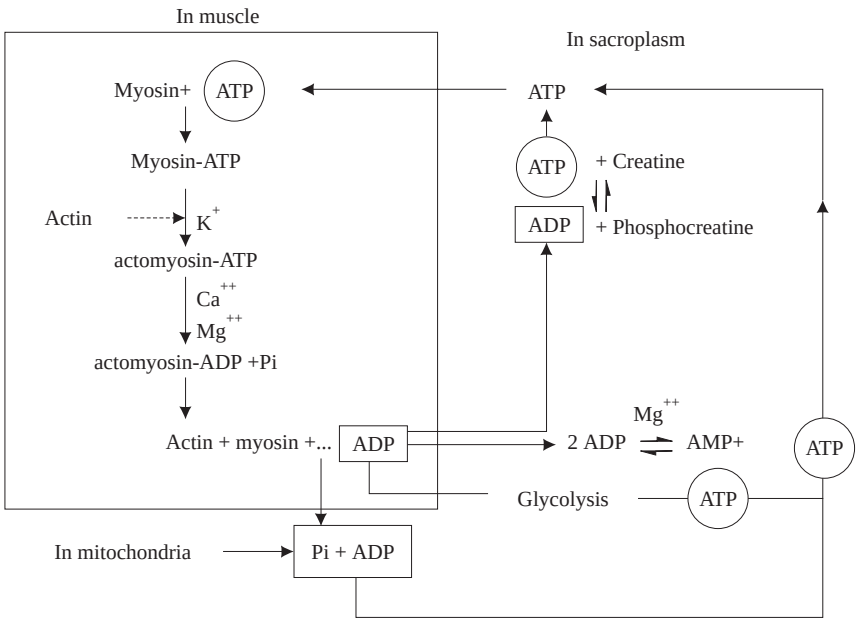


Fig. 18.17 Scheme of the chemical changes in the muscle showing synthesis and breakdown of ATP.

removed from the sarcoplasm to the endoplasmic reticulum by action of a releasing factor supposed to be a lipoprotein.

B. ELECTRIC ORGANS

18.9 BIOELECTROGENESIS

Fishes have specialized electric organs which exhibit bioelectric potentials. Although a large number of fishes possess these organs, they are best developed in electric catfish *(Malapterurus electricus),* torpedo *(Torpedo marmorata)* and electric freshwater eel *(Gymnotus electricus).* The electric organs generate electricity which is utilized for catching the prey and for self-defence.

The electric organ is made up of columns of electroplates or discs. Each electroplate is a unit structure and is also known as electroplax. Electroplaxes in each column are arranged in a long series, and the columns are arranged in parallel. In torpedo, electroplaxes are horizontally arranged in columns on dorsal and ventral surface; in electric eel they are vertical in longitudinal columns parallel to the spinal cord. The voltage of each electroplax varies from 0.4 to 0.1 volt and they function in series to produce a very high voltage. The total voltage attained from the whole organ is estimated to be about 600 volts in electric eel, 200 volts in catfish and 20 to 30 volts in torpedo.

The units of the electric organs are derived either directly from muscle fibres or the motor end-plates and remain embedded in a jelly-like material. Each organ is enclosed inside a compartment made of connective tissue. It was once thought that the electric organs of electric catfish are derived from skin glands, but according to Johnels (1956) they have been shown to arise from myoblasts.

The electroplaxes are innervated by the motor nerve fibres and the fine branches run on the surface only. The electric current in the organ flows from the innervated side to the other. Upon stimulation via the nerve, a postsynaptic potential is developed followed by an action potential and an overshoot. The nerve-organ preparations elicit the same results as obtained for a muscle preparation. In *Gymnotus,* the electroplax is just like that of a striated muscle fibre and the innervated surface consists of junctions like motor end-plates. The electric organ can be excited indirectly through acetylcholine or directly by an electric shock. In case of the electric catfish, electroplates are innervated and non-innervated, and both are excitable but varying only in amplitude.

The electroplate of torpedo is like a motor end-plate. In torpedo, the resting potential is 40-50 mV and the action potential 55-60 mV showing a little overshoot. The duration of the spike varies with temperature; at 8°C the spike duration is 3.5-4 msec. Since the electric organ of torpedo is like a motor end-plate, it can be excited only by the nerve. Drugs like tubocurarine or atropine reduce the response considerably, but acetylcholine can excite the organ.

The excitation from one plate spreads to the next by nerves only. The rhythmical discharge keep on stimulating series of successive discharges plate by plate; however, the successive discharges show decrement in their magnitude. The time taken by such series of discharges does not last more than half a second. The discharges are not continuous but periodical, and emitted to paralyze the prey or to keep away the enemies.

C. LUMINESCENT ORGANS

18.10 BIOLUMINESCENCE

There is a wide variety or organisms which have the ability to produce light. At least 40 orders of animals distributed over all the phyla have this property. It is rather surprising to notice that with a few exceptions, light producing organs are found either in marine forms or in terrestrial forms. Among the notable forms, luminescent forms are found in Protozoa (Radiolaria and Dinoflagellata); in Coelenterata and Ctenophora; in Polychaeta and Oligochaeta; in Ophiuroidea; in Mollusca; in Crustacea; in Chilopoda, in many orders of Insecta, in Hemichordata and Urochordata; and in Elasmobrachs and Teleostei. Among freshwater forms luminescent organs are found in glowworm and snail.

Bioluminescence or emission of light is a chemical phenomenon in which a substrate is oxidized through a series of reactions. Luminescence may be extracellular or intracellular depending upon the site of reactions. In extracellular luminescence the light producing reactants are expelled outside the body; and in intracellular luminescence the light producing reaction-proceeds within the cells. In the former case, luminescent organs take the shape of unicellular or multicellular glands pouring their secretions outside the body. However, in intracellular luminescence, specialized light producing or photogenic organs are developed. In all such cases, light production is generally in the form of intermittent flashes on receipt of some kind of stimulus. Some bacteria are also luminescent, wherein the luminescence is a continuous phenomena. Some squids, millipedes and insects also glow continuously.

18.11 CHEMISTRY OF BIOLUMINESCENCE

Biochemical aspect of luminescence in animals has been studied in some detail in several organisms. Generally there is some sort of biochemical similarity involved in carrying out similar functions in diverse species. Contrary to this general rule, we find significant diversity in bioluminescent systems among animals.

In spite of their diversity, these bioluminescent systems fall under five distinct biochemical patterns and they are referred to as types. The first four types were suggested by Cormier and Totter (1964), and the fifth was tentatively proposed by Hastings et al. (1966).

In this section, the first two types of biochemical reaction systems are described: the first type is an example of extracellular luminescence and the second, an example of intracellular luminescence.

Type I: Direct Oxidation-Simple Enzyme-Substrate System

Pholas: The bioluminescence system of this bivalve is an example of Type I reaction, the simple enzyme-substrate system. It is believed that in this simple reaction a reduced luciferin (LH_2) is oxidized by molecular oxygen. The resulting product is an oxidized luciferin molecule in the excited state.

Cypridina: C. *hilgendorfii* is a small ostracod crustacean inhabiting the ocean along the coast of Japan. Its luminescent phenomenon is most intensively studied. It releases luciferin and luciferase into the sea water where the reaction takes place resulting in luminescence. This reaction requires oxygen, and does not need cofactors, activators, or other components.

Table 18.1 Types of Biochemical Reactions Involved in Luminescence

Type I	Direct oxidation: Simple enzyme-substrate systems $LH_2+O_2 \rightarrow$ Light	*Pholas* (Mollusca) *Cypridina* (Crustacea) *Apogon* (Teleost) *Gonyaulax*
Type II	Substrate activation followed by oxidation: *adenine nucleotide linked* $\text{Pre-}LH_2 \xrightarrow{\text{activation}}$ $LH_2 \xrightarrow{O_2}$ Light	Firefly (Insecta) *Renilla*
Type III	Substrate reduction followed by oxidation: pyridine nucleotide linked $L \xrightarrow{\text{NADPH}} LH_2 \xrightarrow{O_2}$ Light	Bacteria Fungi
Type IV	Peroxidation reactions $LH_2 + H_2O_2 \rightarrow$ Light	*Balanoglossus* (Prochordata) *Chaetopterus* (Annelida)
Type V	Ion-activated: "Precharged" *systems* $P \xrightarrow[\text{or } H^+]{Ca^{++}}$ Light	*Gonyaula* *Aequorea*

Cypridina luciferase is a simple protein with a molecular weight of about 50,000. This enzyme has been extracted in a purified state by Shimomura et al (1961). It has neither prosthetic group nor a significant quantity of metals. The pH optimum for the bioluminescence is at 7.5.

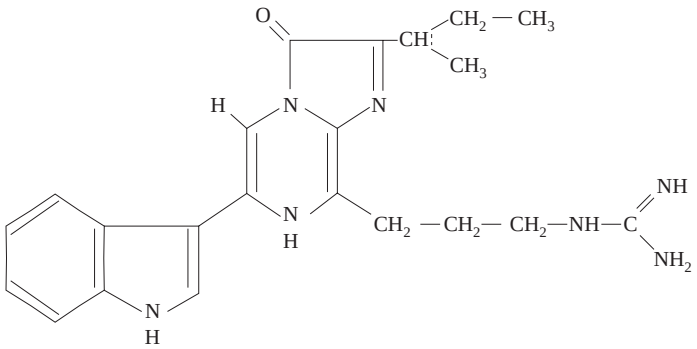


Fig. 18.18 Structure of Cypridina luciferin.

The structure of an active reduced form of Cypridina luciferin given in (Fig. 18.18.) is confirmed by synthesis (Kishi et al., 1966). Oxidation of Cypridina luciferin may result in the production of two forms, viz. oxyluciferin A and oxyluciferin B. In the former two hydrogens are removed; in oxyluciferin B, the isoleucine moiety, besides hydrogens, is lost. One important feature of this luciferin is its sensitivity to a spontaneous nonenzymatic oxidation. Thus luciferin can be oxidized by two different reaction pathways-enzymatic and nonenzymatic. The luciferin not utilized by enzymatic oxidation would be destroyed without producing light by nonenzymatic reaction pathway.

Cypridina reaction mechanism: Relatively little is known regarding the mechanism of the reaction. Cypridina luciferase functions as a catalyst like any other enzyme. The affinity between luciferin and luciferase is high and consequently, binding between them takes place in the absence of molecular oxygen.

The bioluminescence is blue with its maximum at 460 mμ. In phosphate buffer at pH 5.6, crystalline luciferin has three major absorption peaks, at 270, 312, and 425 mμ, and a fluorescence emission at 525 mμ. The luciferin loses both these absorption bands and the associated fluorescence due to enzymic and nonenzymic oxidation. This absorption loss is due to luciferin destruction.

Three possible reaction mechanisms have been suggested to explain the luminescent phenomenon in *Cypridina.* In all these reaction mechanisms, the luciferin is oxidized to yield light. But it is supposed that in all the reactions there is an intermediate product formed.

In the first reaction mechanism two hydrogens from the luciferin are extracted and the intermediate products are hydrogen peroxide and luciferin in an excited state (indicated by asterisk). The latter emits a photon *(hv).*

$$LH_2 + O_2 \rightarrow L^* + H_2O_2 \rightarrow L + hv$$

Second possibility is that the reaction is analogous to a hydroxylase, or to a mixed function oxidase type reaction with the luciferin molecule comprising both functions [and either L(O)* or

L*—OH as the electronically excited species, designated interchangeably L(O)*]. However, there is no evidence concerning this hypothesis.

$$LH_2 + O_2 \rightarrow L(O)^* + H_2O \rightarrow L(O) + hv$$

In the third reaction mechanism, the luciferin is split into two moieties called M and N. Of these, M is electronically excited. A hydroxylase, or a mixed function reaction might also be involved in this reaction mechanism. The presence of some kind of sensitized step is suggested to account for the transfer of excitation energy to yet another unspecified emitting species.

$$LH_2 + O_2 \rightarrow M^* + N \rightarrow M + hv$$

Type II: Substrate Activation Followed by Oxidation

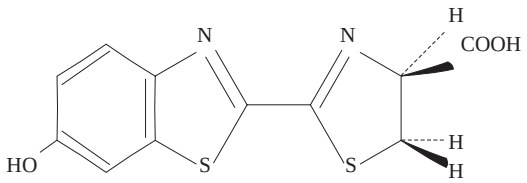
Firefly: Here we discuss the reaction mechanism involved in the production of light in firefly. In this type of reaction mechanism, the oxidizable compound (activated luciferin) is produced from an inactive precursor compound (luciferin). The luciferin (i.e. preluciferin) reacts with ATP forming an active luciferyl adenylate (LH, —AMP) and pyrophosphate (PP). The activated substrate (LH_2—AMP) is then oxidized to yield luminescence.

Pure and crystallized form of firefly luciferase was prepared by Green and McElroy (1956). In crystallized form it is pure. It contains significant quantities of magnesium and aluminium. It consists of 0.3 and 0.2 moles of magnesium and aluminium per molecule of protein. It is believed that the enzyme structure has influence upon the colour of the emitted light. The light emitted by different species of fireflies differ considerably in their spectral distribution. However, it is not known whether differences of the enzymes are in the primary or in the higher orders of protein structure. Changes in pH, temperature, and salts also bring about colour variations.

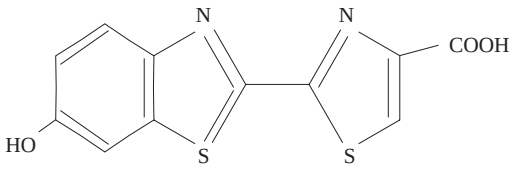
It has been established that the differences in the colour of light are not due to the luciferins, whether from various species or from the various sites of the same species *(Pyrophorus plagiophthalamus).* In this species, the dorsal organ emits a greenish light, while the ventral organ emits yellowish light. The fact that luciferins from different species are similar rules out their influence in the production of coloured light.

Firefly luciferin was extracted in its purified form by Bitter and McElroy (1957). Its structure was determined (Fig. 18.19) and later confirmed by synthesis (White et al., 1961, 1963). D(—)- and L (+)-luciferins are obtained as a result of the chemical synthesis. The formation of these two forms occurs in the last step of the synthesis due to a reaction with D(—)-and L(+)-cystine. Dehydroluciferin shown in the diagram is the product of nonenzymic oxidation and probably not a product of light emitting oxidation. Except for this process of formation and for the absence of oxygen atom (cf. figs. dehydroluciferin and L = 0), etc., the dehydroluciferin is similar in several respects to the oxidized luciferin. The dehydroluciferin readily reacts with luciferase, either in the form of its adenylate (L—AMP) or with ATP and Mg^{++} to form the adenylate.

Mechanism of light production in firefly: Various biochemical reactions involved in the production of light have received considerable attention. The biochemical reaction required in the production of light can conveniently be studied under two steps (Fig. 18.20). The first step includes all initial reactions resulting in the formation of enzyme-substrate complex. These reactions depend on the vital organization of cells.



d(-)Luciferin



Dehydroluciferin

Fig. 18.19 Structure of various luciferins of firefly.

The second step involves production of light and is independent of the vital organization of the cell. These materials, after extraction, can even be mixed outside the cells to yield light. This reaction is called *Photogenic* reaction.

In the first step both the luciferins, i.e. D(—)-and L(+) forms will react with luciferase and ATP in the presence of Mg^{++} to produce enzyme-active corresponding adenylates (E—LH_2—AMP, or E—L—AMP) and pyrophosphate (PP).

$$LH_2 + ATP + E \xrightarrow{Mg^{++}} E—LH_2—AMP + PP$$

However, the L(+) luciferyl adenylate is inactive for oxidation and hence does not yield light.

McElroy and coworkers have proposed that the primary product of light reaction is a dehydro compound containing an atom of oxygen ($\overset{O}{\overset{\|}{L}}$ —AMP). It has been found that one mole of luciferin utilizes one mole of oxygen during luminescent oxidation.

$$E—LH_2—AMP + O_2 \rightarrow E—\overset{O}{\overset{\|}{L}}—AMP + H_2O$$

$$E—\overset{O^*}{\overset{\|}{L}}—AMP \longrightarrow E—\overset{O^*}{\overset{\|}{L}}—AMP + h\nu$$

Product inhibition: After luminescent oxidation, the product remains tightly bound to luciferase and inhibits the enzyme from reaching a second time. This product inhibited luciferase reacts with pyrophosphate and as a result free enzyme and ATP are released.

$$E-\overset{\overset{O}{\|}}{L}-AMP + PP \rightarrow E + \overset{\overset{O^*}{\|}}{L} + ATP$$

The ATP and the enzyme thus released would once again take part in production of luciferase-ATP complex.

The control of flashing: To prevent flashing, it is necessary to stop the luminescent reaction. This can be achieved by separating or compartmentalizing the reactants. We have just now observed such a condition in case of product-inhibited luciferase (vide supra). The fireflies have a very potent pyrophosphatase activity at the luminescent organs and this would remove the PP, and as a result the slow turnover of flash reaction is completely prevented. This mechanism prevents the luminescent reaction from taking place. To explain the resumption of flash production, two possibilities have been suggested which have received experimental support.

1. The pyrophosphate released in the system is rapidly hydrolyzed by phosphatase resulting in decreased flash intensity. Repetitive flashing could readily occur as soon as pyrophosphate is produced as a product from acetylcholine-CoA-ATP cycle (Fig. 18.20).
2. It is suggested that the absence of oxygen supply prevents oxidation of enzyme-active luciferyl adenylate and results in its accumulation in large quantities. Upon the supply of oxygen a flash, at least 100 times brighter than at the steady-state level, is produced (Hastings et al., 1953) *in vitro* system. This experiment lends some support to theory that flashing in firefly is controlled by oxygen. However, the experiments conducted by Hastings and Buck (1956) did not support this theory.

D. CHROMATOPHORES

18.12 COLOUR PRODUCTION

Colour change in animals is a physiological phenomenon. Almost all multicellular animals are characterized by a distinct colour or colour pattern present in their skin. This skin colouration is due to special pigment cells known as *chromatophores.* In some cases pigmentation is static, whereas in some the colouration changes temporarily under certain selection pressures. The pigment cells or chromatophores are placed near the surface which upon movement impart the changing pattern of the skin.

Chromatophore Pigments

Several types of chromatophore pigments have been recognized: melanins, ommochromes, carotenoids, pteridines and guanine. Melanins are formed through oxidative phosphorylation of tyrosine in a sequence of reactions. The steps of tyrosine metabolism have been described in Chapter 19. In the embryo special melanin-synthesizing cells are present which are called melanoblasts that give rise to both melanocytes and melanophores. Ommochromes are black or brown pigments commonly found in Mollusca and Arthropods. Ommochrome pigments have been found in the insect

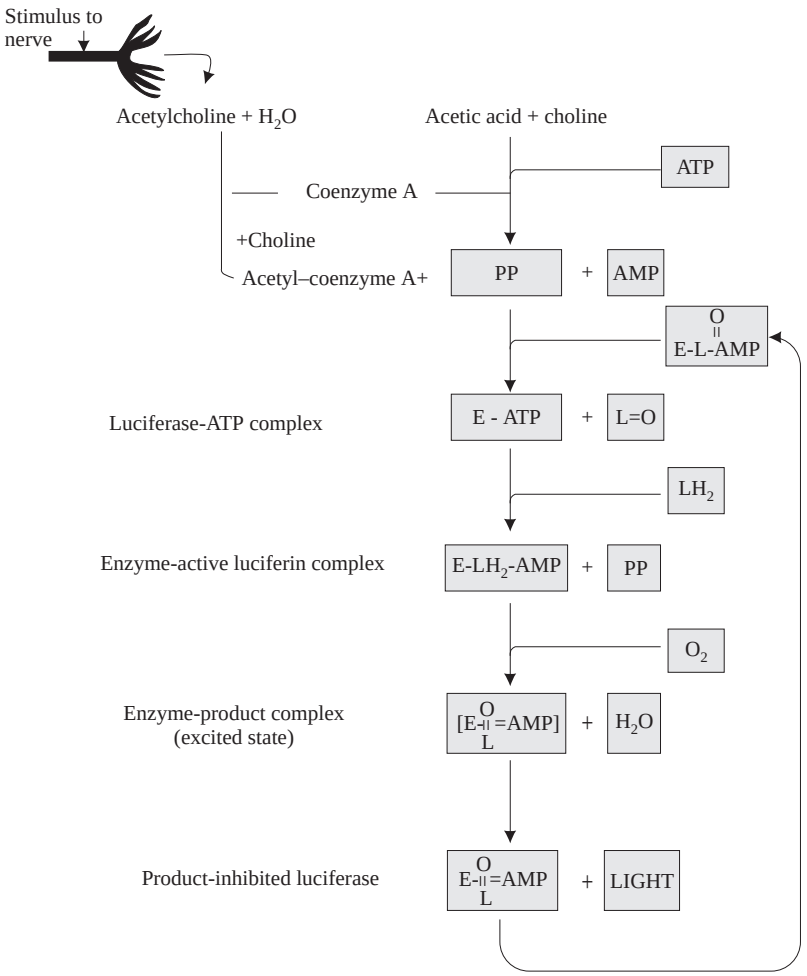


Fig. 18.20 Mechanism of firefly flash.

eyes and their formation is believed to be a genetic mechanism. These are derivatives of the amino acid tryptophan. Carotenoids have been extensively studied in plants as well as animals because of their ubiquitous distribution. Carotenoids are not synthesized in the animal body and they are obtained only from plant sources. However, the presence of carotenoids in the animal body is almost a certainty since they have a role in visual process and in integumentary pigmentation. Carotene is a yellow pigment, but in combination with different proteins it gives rise to range of colours, Pteridines or pterins are pigments which are commonly found in butterflies. Like carotenes, pteridines are also not synthesized in the animal body, but obtained indirectly from the plant sources. They are often associated with carotenoids and give rise to yellow, orange and red colours in fishes and amphibians. Guanine is universally present in animal and plant cells, and is a constituent of nucleic acids as one of the four nitrogenous bases. Besides having structural importance, guanine is responsible for

displaying a variety of beautiful colours in animals. It is also excreted as a waste product in some arthropods (spiders).

18.13 MECHANISM OF ACTION OF CHROMATOPHORES

Chromatophores are either located in the skin or sometimes in the deeper tissues of the body. The colouration of the body is influenced by dispersion or accumulation of the pigment. Two major types of chromatophores have been identified. One of these is confined to the cephalopod molluscs. Here the organ is a small sac-like cell containing pigment granules with radiating muscle fibres. The muscles are attached to the membrane of the cell. The shape of the pigment mass undergoes quick change by contraction and relaxation of the muscle to produce colouration. The second type of chromatophores are commonly found in colour changing species. In this case, there is an irregularly shaped cell or sometimes a syncitium containing pigment granules. The shapes of the chromatophores are also variable.

On the basis of colour and biochemical nature of pigments, chromatophores are of four kinds: (1) melanophores containing brown and black pigment; (2) erythrophores containing red pigment; (3) xanthophores with yellow pigment; and (4) guanophores containing silvery pigments. When the pigment remains concentrated in the cell, the colour pattern of the individual is not evident. However, dispersion of the pigment over a large area causes the colouration of the skin. This phenomenon is known as physiologic colour change. When the quantity of the pigment in the body is altered, colour of the skin changes. This mechanism is called morphological colour change.

Movement of Pigment

Colour changes have been studied in a large number of animals, both vertebrates and invertebrates. Generally, chromatophores are of fixed form, but the movement of the pigments takes place without changing the shape of the cell, and the movement is rather a physical process depending upon the sol-gel transformations of the protoplasm. For example, in *Fundulus*. dispersion of pigment granules takes place due to a decrease in the viscosity of the protoplasm, whereas clumping of the granules was observed when the protoplasm became more viscous. Electrophoretic migration of pigment granules also brings about colour changes. The movement of pigment granules is influenced by a number of factors such as temperature, humidity, tactile stimuli, light diurnal rhythms, pressure, ions, and hormones.

The chromatophores occasionally behave as independent effector organs in certain cases and in such cases they function under a definite control mechanism and the control may be nervous, hormonal or both. The chromatophores that are mediated by direct innervation show rapid movement and colour changes, and those controlled by hormones are regulated very slowly.

Control of Chromatophores

In cephalopods, the chromatophores are highly specialized effector organs and the movement of the pigments is brought about by stretching the cell membranes by muscle fibres. The contractions are very rapid and various tints and shades are produced. The chromatophores are innervated by nerves from suboesophageal ganglia of the brain.

The migration of pigment granules in crustacea is hormone-mediated. The effector cells are devoid of innervation and contractile elements, and the pigment movement takes place by streaming of protoplasm initiated by hormones produced in the different parts of the nervous system. Although it is difficult to give a general account of the actual process, it is suggested that dispersing and concentrating hormones are present which may have variable effects on different pigments. Pigment movement probably takes place under the influence of illumination or visual stimuli which makes the hormone more sensitive. In cyclostomes, elasmobranchs, teleosts and amphibians, a hormonal control of chromatophores has been established. In the cyclostome, *Lampetra,* a hormone from the posterior lobe of the pituitary produces colour changes. Darkening of the skin in majority of elasmobranchs is controlled by the hormone intermedin secreted from the intermediate lobe. In amphibians, the same hormonal mechanism is present; a melanophore stimulating hormone (MSH) secreted by the intermediate lobe of the pituitary causes dispersion and darkening of the pigment granules. In all such cases, general illumination, higher temperatures or humidity changes influence the neuro-secretory system to secrete hormones for inducing colour changes.

In some cases, a combination of both neural and hormonal control is exerted on the pigment effector cells. For example, the teleosts display an endocrine and a nervous control to produce colouration. In the eel, *Anguilla,* hormones dominate, whereas in *Fundulus* autonomic nervous system predominates the hormones. In reptiles chromatophores are regulated by hormonal control.

CHAPTER

19

Hormonal Regulation

INTRODUCTION

The overall metabolism of organisms is under the control of endocrine or ductless glands which discharge their internal secretions into the peripheral circulation. These exert physiological effects on distant tissues and organs. Every activity, viz. growth, differentiation, reproduction response, behaviour, and maintenance consists of exceedingly complex integrated biochemical reactions. Integration and regulation of all these activities within the organism are essential for the functioning and survival of the individual and are controlled by the internal secretions or hormones.

The internal state of the organism is maintained in a steady state by homeostatic mechanisms. Any disturbances in the external environment are communicated to the organism which affect the behaviour or functioning of various parts of the body. Coordination in the internal state and the external environment is also necessary. The nervous system and the endocrine system are two important coordinating systems which help in the function of integration.

The endocrine system communicates with the distant tissues and organs of the body through the circulatory system which helps in distributing the hormones–or chemical messengers. The whole endocrine system consists of a number of glands located in different parts of the body that have no anatomical continuity. However, the whole system forms a functional unit having a direct or reciprocal relationship.

The endocrine system is under the control of nervous system. The neuroendocrine relationships have been studied in a number of vertebrates. An intimate relation on endocrine system with the autonomic and central nervous systems via hypothalamus has been revealed. The hypothalamus serves as a relay station. Hormones also influence the nerve activities in many ways.

Hormones do not form any special class of chemical compounds. Some hormones are polypeptides in their chemical nature, such as hormones of the posterior pituitary (Table 19.1). Some hormones have the nature of amino acids (e.g. epinephrine). Another group of hormones is steroid in

Table 19.1 Important Endocrine and Their Hormones

Gland	*Hormone*	*Chemical nature*	*Major function*
Pituitary gland: (Hypophysis)			
Anterior lobe	1. Adrenocorticotrophic hormone (ACTH)	Polypeptide	Controls the synthesis and secretion of hormones from adrenal coretx.
	2. Thyroid-stimulating hormone (TSH)	Glycoprotein	Controls the synthesis and secretion of thyroid hormones.
	3. Follicle-stimulating hormone (FSH)	Protein	*Females:* Controls ovarian development; responsible for maturation of follicles. *Males:* Controls testicular development, spermatogenesis.
	4. Luteinizing hormone (LH): interstitial cell stimulating hormone (ICSH)	Glycoprotein	Interstitial cell-stimulating hormone; *Females:* Secretion of estrogens and progesterone; ovulation, transformation of the follicle to corpus luteum. *Males:* Regulates secretion of testosterone and androsterone.
	5. Luteotrophic hormone (LTH or Prolactin)	Protein	Active in mammary growth and lactation; supports corpus luteum maintenance and secretion in mammals. Induces migration to water in amphibians: stimulates production of crop milk in birds.
	6. Somatotrophic hormone (STH = GH) or growth hormone	Protein	Stimulates growth (protein synthesis), especially for bones of extremities and skull; raises blood sugar.
Intermediate Lobe	1. Melanocyte stimulating hormone (MSH) or Intermedin Vasopressin	Polypeptide	Pigment dispersion in melanophores; melanin synthesis; darkening of skin in all vertebrates.
Posterior lobe (Pars nervosa)	Oxytocin	Octapeptides	Antidiuretic activity.
		″	Causes milk ejection during suckling; stimulates uterine contractions during parturition and coitus; helps in sperm transport.
			Control of the release of anterior pituitary hormones.
Hypophysis (Median eminence)	1. Corticotropin releasing factor (CRF)	Peptides	Stimulates release ACTH.
	2. Thyrotropin releasing factor (TRF)		Stimulates release of TSH.
	3. Follicle-stimulating hormone releasing factor (FRF)		Stimulates release of FSH.
	4. Lutenizing-hormone releasing factor (LRF)		Stimulates release of LH.
	5. Prolaction-inhibiting factor (PIF)		Stimulates release of LTH.
	6. Growth-hormone releasing factor (GRF)		Stimulates release of STH.

(Table Contd.)

(Table Contd.)

Gland	*Hormone*	*Chemical nature*	*Major function*
Thyroid	1. Thyroxine	Iodine derivative of amino acid	Metamorphosis and differentiation in amphibians; moulting; Differentiation (maturation); regulation of body temperature in mammals.
	2 Triiodothyrorine (T_3)		Stimulates differentiation and metabolism.
	3. Calcitonin	Polypeptide	Decreases blood calcium.
Parathyroids	Parathormone	Polypeptide	Increases blood calcium, lowers serum phosphate and serum calcium.
Adrenals			
Medulla (Chromaffin tissues)	Epinephrine (Adrenaline)	Catecholamines	Increases cardiac output; rise in systolic pressure; accelerates conversion of liver glycogen to glucose; raises blood sugar level: converts muscle glycogen to lactic acid; increases oxygen consumption; raises body temperature and BMR; stimulates central nervous system.
	Norepinephrine (Noradrenaline)		Constriction of small arteries: vasoconstriction: raises both systole and diastole of the heart, raises blood pressure.
Cortex	Glucocorticoids (cortisol, corticosterone)	Steroids	Participate in carbohydrate. Fat and protein metabolism. Anti-inflammatory and antistress effects; diuretic action
	Mineralocorticoids (aldosterone)	"	Na^+ and K^+ regulation and carbohydrate metabolism; retention of Na^+ and Cl^- in the body.
Pancreas (Islets of Langerhans)			Increases level of blood glucose by stimulating the conversion of liver glycogen into glucose.
α cells	Glucagon	Polypeptide	
β cells	Insulin	Polypeptide	Enhances utilization of glucose by peripheral tissues: helps conversion of glucose into glycogen in the liver and skeletal muscles.
Ovary	Estrogens	Steroid	Stimulates female secondary sex characters and sexual behaviour.
	Progesterones	Steroid	Maintains pregnancy, development and growth of mammary glands.
	Relaxin	Polypeptide	Relaxes cervix, inhibits uterine contractions.
Testis	Testosterone	Steroid	Stimulates secondary sexual characteristics in males and sexual behaviour.

nature which usually consists of three hydrated benzene rings and one cyclopentane ring. The whole ring system is called cyclopentano-phenanthrene ring.

Generally hormones are not "species specific". However, they are specific in origin and are also specific in their activity. For this reason, hormones extracted from animals when administered in human, body are found to be quite effective.

Some of the principal endocrine functions may be enumerated below:

1. Hormones have the capacity to modify the metabolic processes by generally altering the rate of reactions.
2. Specific secretory stimuli are necessary for the secretion of hormones. However, the amount of secretion depends upon the nature and intensity of stimulus.
3. Hormones are generally secreted independent of each other.
4. Hormones are present in very minute quantities in blood and in many cases are bound to specific protein molecules which serve as carrier molecules.
5. When not required, the hormones in blood and tissues are rendered inactive by enzymatic mechanisms and then excreted from the body.
6. Hormones have a high degree of action specificity. The target glands secrete variable amounts of hormones which are responsible for producing, differential sensitivity in the end-organs. Certain tissues respond unusually to it particular hormone, whereas the same hormone is unable to evoke any response in other tissues. This shows that hormones have a high degree of target specificity.

Although invertebrates are also provided with endocrine glands, their distribution, location and function in vertebrates have been well established (Fig. 19.1). Most of the information on these glands pertains to mammals and the account that will follow here is reliable for mammals and humans in particular.

Mode of Action of Hormones

From the physiological standpoint, a hormone is a carrier of information the purpose of which is to modulate the function of certain tissues according to the needs of the body. These tissues are the *receptor* or the *target* tissues. In order to understand the mode of functioning of the hormones, we are confronted with certain fundamental questions. How does a given receptor cell selectively recognize the hormonal message intended for it? What is the mechanism employed by the cell to modify its own function to respond accordingly? It should be first appreciated that the hormonal concentrations in the body are exceedingly small, even then the magnitude of their response is quite big. How a signal is amplified to trigger a response is a matter of experimental study. The hormonal message is specifically recognized, and then translated into signals capable of modifying the programme of the receptor cell.

Discovery of the "Second Messenger"

In the nineteen-fifties, Sutherland and his associates made a crucial discovery while conducting research with adrenaline which is a hyperglycaemic hormone. The authors suggested that in order to increase hepatic glucose production, the hormone must play a part to increase the activity of the

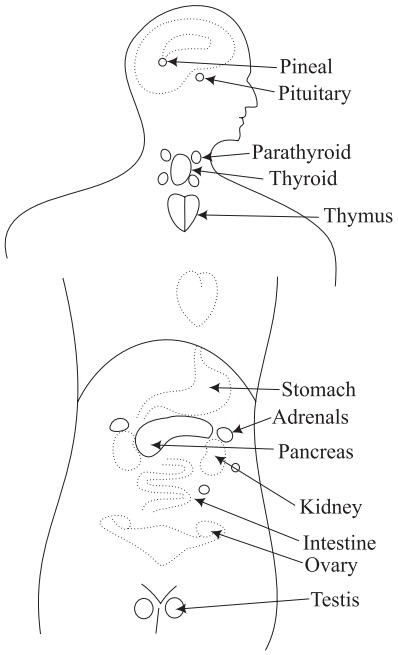


Fig. 19.1 Location of the endocrine glands in the human body.

enzyme responsible to convert glycogen to glucose. This enzyme happens to be phosphorylase which catalyzes hydrolysis of glycogen with the production of glucose-l-phosphate. Experimentation provided further proof that the activation of phosphorylase by noradrenaline required an intricate system in which the hormone did not activate the enzyme directly. The hormone triggers the formation of a substance which activates phosphorylase and this substance was identified as cyclic adenosine-3', 5' monophosphate (cyclic AMP) (Fig. 19.2).

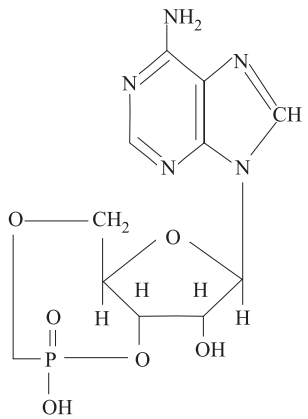


Fig. 19.2 Structure of cyclic AMP, the *second messenger.*

Now the question arises: where and how does the hormone elicit production of cyclic AMP within the cell, and by what mechanism does cAMP activate phosphorylase? It is known that the cAMP is formed from its precursor, ATP, under the catalytic influence of a specific enzyme, adenyl cyclase. The enzyme is ubiquitous in nature and has been found in many different types of cells. It is located chiefly in the cell membranes from which it cannot be easily separated. As for the mechanism by which cyclic AMP stimulates phosphorylase activity, we have got a clear understanding of the process. Phosphorylase exists in two forms, *a* and *b,* of which the former is more active. Transformation of *b* to *a* form is catalyzed by the enzyme phosphorylase-b-kinase, while the reverse process is catalyzed by posphorylase-phosphatase. Cyclic AMP does not act on phosphorylase directly, but on the kinase which converts it to more active form *a* (Fig. 19.3). It appears that there are receptors on the cell surface each of which is specific for a single hormone and the response of a given tissue to a hormone depends on the presence of a specific receptor site. There seems to be some kind of a coupling factor between the receptor and the enzyme adenyl cyclase.

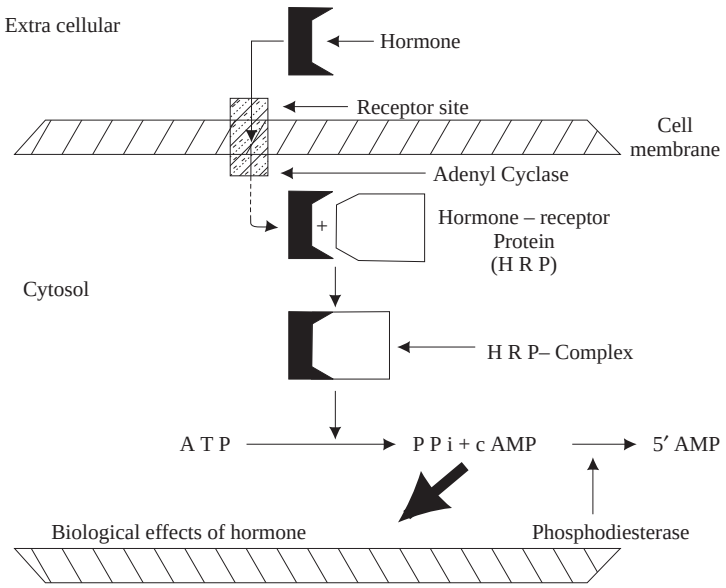


Fig. 19.3 Mechanism of action of a hormone showing the production of cyclic AMP. The hormone acts at a specific receptor site located in the plasma membrane.

Generalised Scheme of Action

The mechanism of action was discovered by Sutherland and his associates while working with adrenaline, a hyperglycaemic hormone. Many hormones, in fact, exert their action by way of formation of cyclic AMP in the receptor cells. Hence, the name "second messenger" was given to cyclic AMP owing to its role as intracellular intermediary. It is known that cyclic AMP is broken down in the cells by an enzyme, phosphodiesterase which converts it to 5'-AMP. This enzyme is inhibited by various members of the methylxanthine group, such as caffeine and theophyline. These

inhibitors counter the breakdown of cyclic AMP and favour the accumulation of the nucleotide (Fig. 19.3). Following steps may be clearly outlined:

1. The hormone must produce increased quantity of cyclic AMP present in the receptor cells.
2. Addition of exogenous cAMP to the system will reproduce specific biological effects of the hormone in all respects.
3. Addition of the inhibitor theophyline will potentiate the effects of both the hormone and the cAMP.

STRUCTURE AND FUNCTIONS OF ENDOCRINE ORGANS

19.1 THE PITUITARY

The pituitary gland or the hypophysis, as it is called, is a composite gland which is situated at the base of the brain. The pituitary is a small rounded body attached to the base of the brain by means of a hypophysial stalk and weighs about 0.5 gm in a normal individual. It is lodged in the cavity of the sphenoid bone, called sella turcica.

Anatomically, the pituitary is divided into three parts, namely the anterior lobe, posterior lobe and the intermediate lobe (Fig. 19.4).

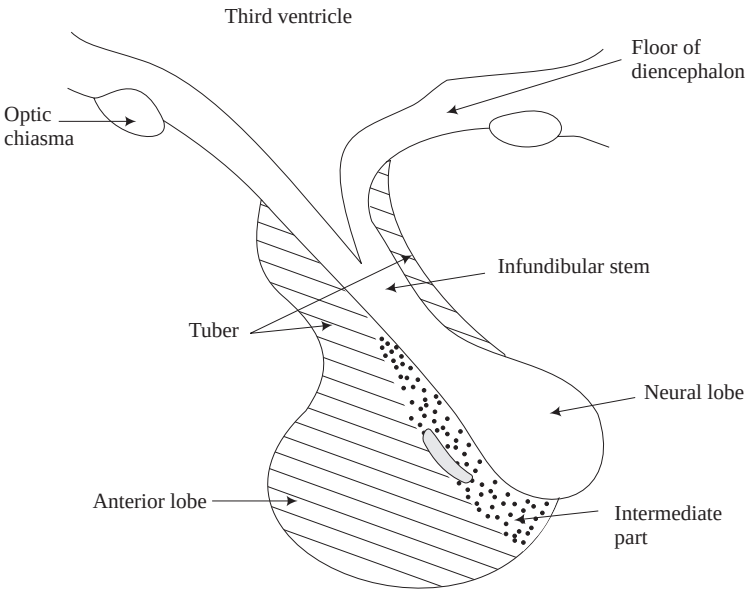


Fig. 19.4 Morphology of the pituitary gland.

The Anterior Pituitary

The anterior lobe of the pituitary is also called *adenohypophysis.* It is highly secretory in activity and consists of cords of epithelial cells surrounded by many large sinusoidal areas. Histologically, three different types of cells are distinguishable: (1) acidophils, (2) basophils, and (3) chromophobes. The following six hormones have so far been isolated:

1. Growth hormone or somatotrophic hormone (STH).
2. Adrenocorticotrophic hormone (ACTH).
3. Thyroid stimulating hormone (TSH).
4. Follicle stimulating hormone (FSH).
5. Luteinizing hormone (LH).
6. Luteotrophic hormone (LTH) or prolactin.

Out of these, the last five hormones are trophic hormones which act on other endocrine glands as their target organs.

Growth Hormone: Growth hormone or somatotrophic hormone stimulates tissue growth. It is proteinaceous in nature and secreted by acidophils of adenohypophysis. Excessive secretion of the growth hormone, before the union of epiphyses causes gigantism due to acceleration of bone growth. If the epiphyses are closed, the bones do not grow in length but thickening takes place. Growth hormone has striking effects on the bones, muscles, kidney, adipose tissue and liver. In addition to growth function, somatotrophic hormone has other functions also.

Somatotropin facilitates protein synthesis and retards amino acid catabolism. It also promotes transfer of blood amino acids into muscle cells resulting in a positive nitrogen balance. Administration of this hormone in young rate brings about accumulation of tissue proteins.

It also has some diabetogenic (anti-insulin) action. Administration of this growth hormone elevates blood-sugar level, which if prolonged, causes destruction of islets of Langerhans. This causes *diabetes mellitus* disease. Although the exact mechanism of its action on carbohydrate metabolism is not clearly understood, it, however, appears to slow down the rate of glucose utilization.

Lipid metabolism is also affected to a considerable degree. The hormone helps in mobilization of lipid to the liver and increased amounts of hormone may produce ketonemia and ketonuria. In females, full breast development is influenced by somatotropin.

Adrenocorticotropic Hormone: It is a long chain polypeptide containing 39 amino acid units. The hormone controls the function of adrenal cortex by adjusting the glucocorticoid output of the adrenal cortex. The adrenal cortex atrophies in the absence of ACTH, and administration of ACTH stimulates the release of cortical hormones, viz. cortisol, corticosterone, aldosterone, etc. ACTH also acts on adipose tissue and increases fatty acid concentration in the blood. It also regulates the ACTH secretion from the hypophysis. Larger doses of ACTH would inhibit its production from the adenohypophysis. ACTH is also associated with melanophore-regulating action.

ACTH secretion is regulated in two ways: There is evidence to show that the neurosecretory cells of the posterior part of the hypothalamus and median eminence secrete into the hypophysial portal system some neurohumoral substances which reach the sinusoids of adenohypophysis and stimulate the cells to produce hormones. These neurohumoral chemical substances are releasing factors which

are probably small polypeptide chains. One such factor that has been obtained in a highly purified form is called corticotropin releasing factor (CRF). Another way which controls the ACTH secretion is through glucocorticoids level in the plasma. Secretion of trophic hormones appears to be regulated by a feedback mechanism.

Thyroid Stimulating Hormone (TSH): Extirpation of anterior pituitary causes atrophy of the thyroid gland. The thyroid stimulating hormone controls thyroid function by stimulating formation of thyroxine and its release from the thyroid gland and the synthesis of thyroxine.

TSH is a glycoprotein with molecular weight of about 28,000, being secreted from the basophilic cells. The concentration of thyroid hormone is maintained at a constant level by circulating TSH. However, the secretion of TSH is controlled by a thyroid releasing factor (TRF) from the hypothalamus.

Follicle Stimulating Hormone (FSH): Follicle stimulating hormone is one of the three gonadotropins. In females, it leads to growth and maturation of graffian follicles with estrogen production. In males, it induces development of the germinal epithelium of the seminiferous tubules. Removal of hypophysis leads to degeneration or atrophy of gonads.

FSH has been prepared is a purified form from the hypophysis of sheep and swine. It is a protein in nature whose molecular weight varies depending on the source. FSH from sheep has molecular weight of 67,000. The hypothalamus secretes a follicle stimulating hormone releasing factor (FSHRF) which regulates the FSH levels.

Luteimzing Hormone (LH): This hormone is known as interstitial cell stimulating hormone (ICSH). In females, it promotes formation of corpus luteum which secretes progesterone. In the male, it stimulates the interstitial cells of the testis to produce testosterone.

LH is a protein whose molecular weight varies from 30,000 to 100,000 depending upon the source. Purified LH preparations have been obtained from the sheep and swine hypophysial glands. Regulation of LH is controlled by the blood level of gonadal hormones and luteinizing hormone releasing factors (LHRF).

Luteotrophic Hormone (LTH) or Prolactin: LTH or prolactin, also sometimes called lactogenic hormone, is responsible for maintaining corpus luteum of pregnancy. In rats the role of LTH has been fully established. However, in primates its function has not been demonstrated. It promotes development of mammary glands and the formation and secretion of milk during post-natal period. Its action in males is not known.

Prolactin is a protein with a molecular weight of about 25,000-30,000 containing glucose.

The trophic hormones secreted by the anterior pituitary (adenohypophysis) are regulated by the blood level of the concerning hormone (such as thyroxine blood level controls TSH). With the exception of prolactin, all hormones are secreted under the stimulus of specific releasing factors from the hypothalamus. These factors may be secreted under various stimuli which could be either nervous or chemical. Releasing factors are carried by the hypothalamic-hypophysial portal system which stimulate the adenohypophysis to release specific hormones.

Little or no secretion of adenohypophysis leads to a condition called *hypopituitarism.* This may bring about atrophy of the thyroid gland causing dwarfism or infantilism, and Simmond's disease

(Cachexia). Hyper-pituitarism causes disorders in the acidophil cells developing gigantism and acromegaly. Another disorder, *Cushing's syndrome,* is due to disorders in the basophilic cells.

Intermediate Lobe

The intermediate lobe has the same embryological origin as that of the anterior lobe. Sometimes it is difficult to identify it from the anterior lobe. This lobe secretes a hormone which is known as *intermedin or melanocyte-stimulating* hormone (MSH). In case of fish, amphibians and reptiles, hormone helps in darkening of the skin by dispersing melanocytes or pigment cells. In humans also this hormone is present which is a polypeptide consisting of 13 or 18 or 22 aminoacids. The darkening of the skin in humans is due to MSH. The regulation of the hormone is controlled by the sunlight. Lack of skin pigmentation is due to its absence. Experiments have proved that hypothalamus controls the secretion of MSH. If hypothalamus is damaged. MSH secretion takes place causing darkening of the skin.

Posterior Pituitary or Neurohypophysis

The neurohypophysis is an ectodermal derivative and originates as an outgrowth of the primitive brain, infundibulum, from the floor of the brain. Histologically it consists of nerve fibres and cells called pituicytes closely resembling nerve cells. Anatomically it is intimately associated with the hypothalamus via the hypothalamo-hypophysial fibre tracts, thus forming a component of the hypothalamic neurosecretory system (Fig. 19.5). The hormones are actually secreted from the hypothalamus from where they are transported and stored in the posterior lobe along the fibre-tracts for release.

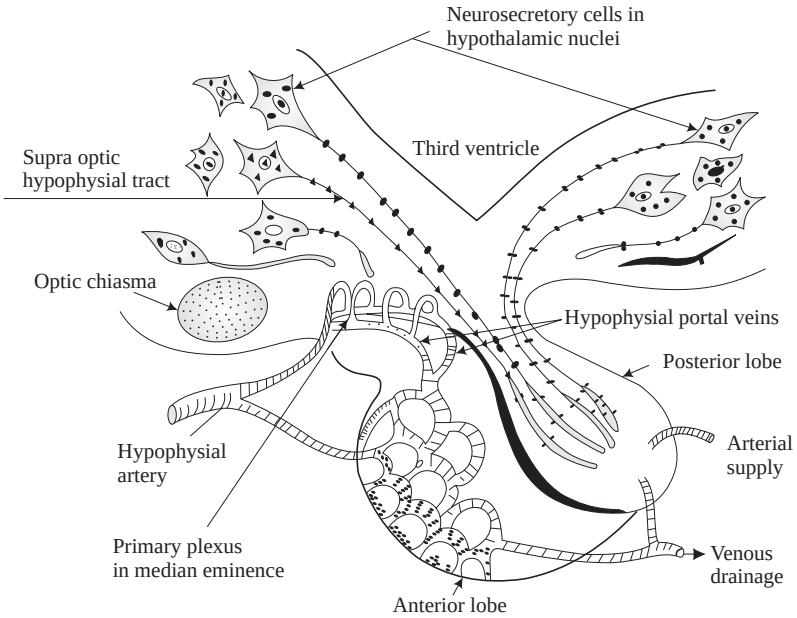


Fig. 19.5 Hypothalamic neurosecretory system showing hypothalamo-hypophysial fibre tracts.

Crude extracts of the posterior lobe reveal the presence of at least two important hormones which are octapeptides in nature (composed of eight amino acids).

Oxytocin: It is composed of 8 amino acids with a disulphide ring having a molecular weight of about 1,025. The hormone has a stimulating effect on the musculature of the uterus and promotes labour. Physicians sometimes use it during child birth. It also stimulates secretion of milk from the breasts and the stimulus is obtained from the suckling of the baby.

Vasopressin: This hormone is also called antidiuretic hormone (ADH) and is composed of 8 amino acids with a disulphide ring. The hormone promotes water retention in the kidneys, hence the name antidiuretic hormone. Secretion or inhibition of the antidiuretic hormone is controlled by "Osmoreceptors" present in the hypothalamus. Excessive intake of water would inhibit the secretion of ADH resulting in diuresis, whereas diminished quantity of body fluid would stimulate the secretion of the hormone. Removal of the posterior pituitary causes polyuria with symptoms of excessive thirst (polydipsia) but without glycosuria as in *diabetes mellitus.* Generally lesions of the posterior lobe and the adjacent hypothalamic parts cause this condition. The disease may have hereditary character; males generally suffer more than the females. The disease is commonly called *diabetes insipidus* and may be relieved by the administration of ADH suppressing the urine output.

The pressor action of the antidiuretic hormone is more pronounced by the increased secretion which elevates the arterial blood pressure by constriction of the arterioles and the capillaries. It also causes constriction of coronary arteries and possibly that of pulmonary vessels too. Its role in regulation of blood circulation is, however, doubtful.

Thyroid Gland

The thyroid gland consists of two lateral lobes situated on either side of the trachea just below the larynx, being connected with a narrow isthamus. In man, both the lobes weigh about 25 gm. The gland is supplied with right and left superior thyroid arteries. There are a large number of small closed vessels in the gland which are composed of columnar or cuboidal cells. The lumen of the vesicles is filled with the epithelial secretion which is colloidal in nature. The gland is innervated by the two branches of the autonomic nervous system. The postganglionic fibres come from the superior and inferior cervical ganglia, while the parasympathetic fibres come from the vagus. The innervation is responsible for the blood flow and not the secretions from the gland.

Thyroid Hormone: The exact nature of thyroid hormone is still obscure. It is, however, believed to contain at least four types of secretions, all of which are *iodothyronines.* These are: thyroxine (tetraiodothyronine), 3,5,3'—triiodothyronine, 3,3',5—triiodothyronine and 3,3'—diiodothyronine. Out of these four hormones, thyroxine is the principal hormone. However, 3,5,3'— triiodothyronine is most active and about 5 times more powerful than thyroxine (Fig. 19.6).

Iodine is the most essential component of thyroid hormones which is accumulated by the thyroid gland. Thyroglobulin, a mucoprotein, is hydrolyzed in the thyroid gland by a proteolytic enzyme releasing the hormone in the blood stream. Normally about 300 µg of thyroxine are secreted per day. The amount of iodine in the plasma is very small, approximately 6 µg, most of which are in the form of iodothyronines. Small quantity of thyroxine remains free, and the rest is bound with the plasma proteins.

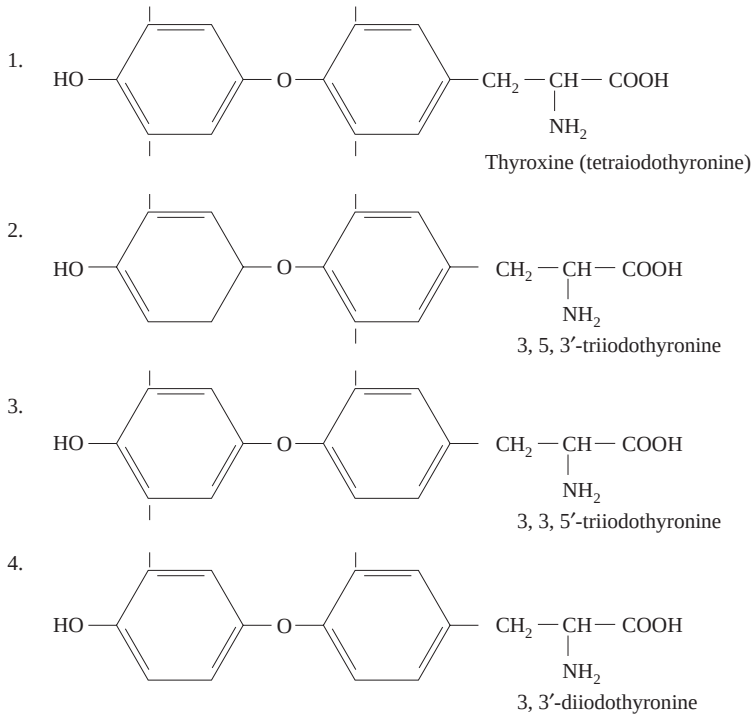


Fig. 19.6 Hormones of the thyroid gland.

Iodothyronines react in most cells of the body and are deiodinated. Small percentage of iodothyronine is excreted in the urine chiefly in the form of inorganic iodides which may be considered as the end, product of thyroid hormone metabolism. Thus some iodide is lost from the body through renal clearance. Hence the diet must contain about 25 µg iodine per day.

Synthesis of thyroid hormones: Dietary iodine is converted into iodide and absorbed in the blood from where it is actively transported into the gland. This process is called iodide trap. Inside the gland iodide gets oxidised and reacts with tyrosine giving rise to monoiodotyrosine. This is further iodinated to form 3,5–diiodotyrosine. Both mono–and diiodotyrosine form triiodothyronine, and two molecules of diiodotyrosine form tetraiodothyronine. The steps in the formation of the thyroid hormones from the serum iodine are given in (Fig. 19.7).

Thyroid function: Thyroid hormone, mostly in the form of thyroxine circulates in the body generally bound to protein. Various functions are as follows:

1. Thyroxine is essential for normal growth, skeletal maturation and mental development. Inadequate thyroid secretion retards growth and mental development. In tadpoles, it hastens metamorphosis. Children suffering from its deficiency have weak musculature, stunted growth and abnormal abdomen.
2. Thyroxine stimulates oxygen consumption of the body cells, except brain, testis, uterus, spleen, lymph nodes, etc.

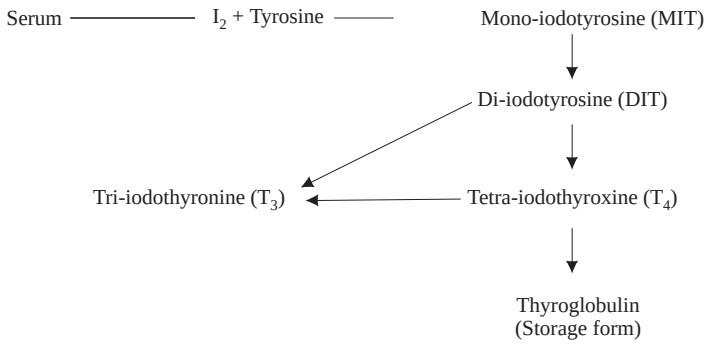


Fig. 19.7 Synthesis of thyroid hormones.

3. Thyroxine increases basal metabolic rate (BMR). Extirpation of the gland causes decrease in the BMR which can be recovered by administration of thyroxine.
4. Thyroxine increases protein synthesis and helps in regulating lipid metabolism. It stimulates cholesterol synthesis.
5. Thyroxine influences the cardiac output. Decreased amounts of the hormone decrease the heart rate and the force of contraction. Hyperthyroidism causes increased cardiac output which sometimes results in the cardiac failure.
6. Thyroxine enhances the rate of glucose absorption from the gastrointestinal tract. Hyperthyroidism causes blood sugar level to rise.
7. Deficiency in thyroid secretion retards the normal formation of myelin sheath and also reduces the number and size of the neurons.

Thyroid regulation: The thyrotropic hormone (TSH) secreted by the anterior pituitary regulates the thyroid function, TSH stimulates thyroid gland and elevates the thyroid hormones in the circulation. It may have the following functions:

1. The size of the gland may increase.
2. Secretion of thyroglobin may be accelerated.
3. Convert the thyroglobin to thyroid hormones.
4. Thyroid hormones may be secreted in enhanced quantities.

When the concentration of thyroid hormones in the blood decreases, TSH secretion is augmented which causes more iodothyronine to be secreted from the gland reaching a balance between two. Secretion of TSH from the anterior pituitary is under dual control. It is regulated by circulating iodothyronines and a neurohumoral substance, thyrotropic-releasing factor (TRF) secreted by the hypothalamus. A feedback mechanism exists between the thyroid hormone and TSH. Thyroid regulation may be explained diagrammatically (Fig. 19.8)

Thyroid Disorders: Clinical thyroid disorders are caused either due to too little (hypothyroidism) or too much (hyperthyroidism) hormone secretion.

Hypothyroidism: Hypothyroid states are caused by insufficient hormone secretion or reduced iodine intake. In a young animal damage to thyroid causes arrest of growth. In a child born without

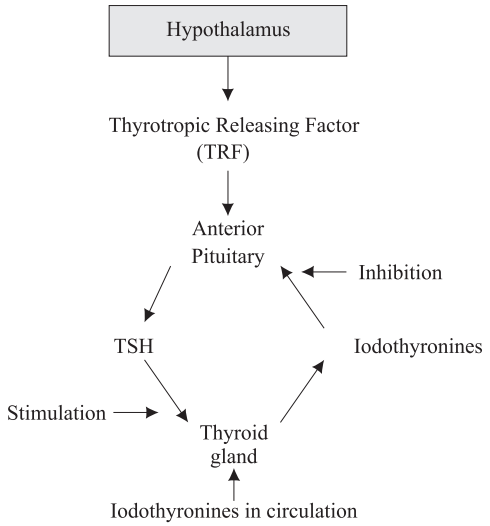


Fig. 19.8 Schematic representation of the regulation of thyroid activity.

any thyroid tissue, mental, physical and sexual developments are retarded. The child is docile and inactive. Other symptoms that follow are constipation, protruded tongue and dry, thick lustreless hair and skin. Such an individual is called a cretin and the disease is called *cretinism.*

Sometimes the thyroid gland functions normally in childhood, but becomes atrophied during adulthood. In such cases, the basal metabolic rate falls to a very low level. This condition in the adult is called *myxoedema.* The common symptoms of the disease are: (1) dry, coarse hair and skin, (2) puffiness of the lower eyelids and dorsum of the hands, (3) thick and coarse voice, (4) dull memory, (5) excessive lethargy, (6) bradycardia (abnormal electro-cardiogram patterns), and (7) marked intolerance to cold. For hypothyroid states, small doses of thyroid under careful watch are administered.

Hyperthyroidism: Hyperthyroid states may be caused by excessive thyroid secretion and may or may not be associated with enlargement of the gland. Hyperthyroidism is often accompanied by *exopthalmos* or bulging of the eyes. The disease is called *goitre* and is caused by non-inflammatory, non-neoplastic enlargement of the thyroid gland. Hyperthyroid states can be recognized by clinical symptoms, like high basal metabolic rate, sleeplessness, heart rate and blood pressure increase, profuse sweating, and emotional instability.

Goitre is developed as a result of a lack of iodine availability to the thyroid gland. Lack of iodine causes decreased thyroxine output and stimulates the anterior pituitary to secrete excessive thyrotropic hormone (TSH). TSH activates the thyroid gland to produce hyperplasia of the thyroid vesicles to manufacture adequate thyroxine. If iodine deficiency is corrected and thyroxine is formed in normal amounts, TSH activity is automatically reduced.

Hyperthyrodism is also associated with *primary* thyrotoxicosis or Grave's disease. It is one of the "stress" diseases. Excessive hypothalmic activity causes the anterior pituitary to secrete more TSH which stimulates the thyroid gland. Important symptoms are: high metabolic rate, excessive heat

production, sensitivity to heat, rapid breathing, fast but weak heart beat, weight loss, excessive eating, increased neural excitability, anxiety and emotional instability.

Parathyroid Glands

There are four parathyroids, two on each side placed over the thyroids. Their position varies in different animals. These are consisting of solid masses or columns of epithelial cells with vascular channels. The glandular cells are differentiated into two types: non-granular cells with large nuclei and clear cytoplasm, and the eosinophilic cells with distinct nuclei which appear after puberty. Non-granular cells are the chief secretory cells. Parathyroid glands are intimately involved in calcium and phosphorus metabolism of the body.

Parathyroid Hormones: Two hormones are now believed to be produced by these glands, which are *parathormone* (PTH) and *calcitonin*.

Parathormone: This hormone has now been purified and is known to contain about 83 amino acids in a single polypeptide chain with molecular weight of about 9,500. The polypeptide chain contains about 15.5 per cent nitrogen and traces of iron, sulphur and phosphorus. The secretion of the hormone is inversely proportional to the calcium concentration in the plasma. The level of ionized calcium acts directly on the glands to regulate parathormone secretion.

Calcitonin: This hormone has a hypocalcemic function. Whether it is secreted by the thyroid or parathyroid or by both is yet not decided. Its chemical nature is a polypeptide which is quite similar to thyrocalcitonin.

Functions of parathormone: Excessive secretion of the hormone is perhaps due to continued diuresis which causes hyperparathyroidism. In small doses, the hormone is responsible for calcification of bones by stimulating osteoblastic activity.

Parathormone is important in regulating the calcium concentration of the blood and a proper balance between Ca^{++} and Mg^{++} on one hand and Na^{+} and K^{+} on the other hand is maintained. About 10 mg of calcium per 100 ml of plasma is normally present, half of which is in the ionized form and the rest is bound to plasma proteins. Fall in the blood-calcium increases irritability, nervousness and spasmic activity. Major effects of parathormone can be elaborated as follows:

(1) Parathormone enhances calcium absorption by the gut. (2) Its major function is to cause demineralization of bone. Enhanced secretion mobilizes both calcium and phosphorus from the bones either by increasing the osteoclastic activity or by inhibiting osteoblastic activity. Thus the hormone increases the number as well as the activity of the osteoclasts. On the basis of is O topic studies it has been suggested that parathormone influences the removal of calcium from bones.

(3) Increased parathormone activity diminishes the phosphate resorption by the kidneys. Thus plasma phosphate concentration is reduced causing an increase in urinary phosphates. Removal of parathyroids reduces urinary phosphate levels.

(4) Parathormone increases the glomerular filtration rate.

(5) Demineralization of the bone material takes place with a consequent increase of blood calcium when large quantities of the hormone are administered. However, small doses help mineral deposits in the bone.

(6) Recent studies have demonstrated that the level of serum lipid is increased by parathormone administration, thus increasing total cholesterol.

Calcitonin: This hormone lowers blood calcium. This is probably achieved by increasing incorporation of calcium into bone.

Some Abnormalities of Parathyroid Glands

Hyperparathyroidism: When the gland secretes excessive amounts of parathormone, the level of calcium increases. This is achieved through the action of renal function. This results from hyperplasia of the parathyroid cells and this clinical disorder is known as *generalized osteitis fibrosa.* The disease is generally found in females between the age of 20 and 40 years. The patients suffer from weakness, muscle wasting and body pains. The voluntary muscles, are hypotonic, weak, painful and tender. Sometimes spontaneous fractures are also caused which heal up very slowly. Other symptoms may also develop, such as nausea, vomiting, loss of appetite and weight, difficulty in walking, severe attacks of abdominal or back pain, renal colic, deposition of calcium in kidneys, increased urine output and constipation, etc.

Hyperparathyroidism may also lead to calcium deposition in lungs causing dyspnoea. Sometimes cyanosis may also develop. Kidneys may develop calcium deposits causing calcinosis. Calcium may be deposited in the arterioles, thus elevating the blood pressure.

Good medical treatment is not yet available for hyperparathyroidism except for the surgical removal of the hyperactive parathyroid tissue.

Hypoparathyroidism: Extirpation or hyposecretion of the parathyroid results in decreasing blood-calcium level and a consequent increase in phosphate concentration. This leads to the clinical syndrome of *tetany* with symptoms like muscle spasms, convulsions and tremors often leading to the death of patients. Although tetany may not be solely due to hypoparathyroid function, it may occur due to severe alkalosis, severe malnutrition, pregnancy or lactation. In all the cases, a general fall in the level of ionised calcium results.

Cases of tetany can be controlled by administration of calcium gluconate orally. Mild cases are easily controlled with suitable treatment, but in chronic and recurrent cases intravenous injections of calcium gluconate solution prove more effective.

Regulation of parathyroid: Earlier suggestions that parathyroid function is controlled by the anterior pituitary have not been proved so far by experiments. However, there is a general agreement that the parathormone secretion is mainly influenced by blood-calcium level.

A fall in the blood-calcium level enhances the parathormone secretion, thereby increasing calcium absorption from the intestine. The hormone, however, increases osteoclastic activity causing demineralization of bone and increasing the calcium-blood level. Simultaneously calcium resorption in the kidney is also increased whereby blood-calcium level also increases. As a result of elevated blood-calcium level, parathormone levels are suppressed. The exact mechanism involved in the process is not well known. The following scheme is presented to explain this (Fig. 19.9).

The Adrenals

The adrenal glands are two in number, which lie just above the anterior surface of the kidney on either side. Anatomically, the glands consist of two parts, the cortex and the medulla. Histologically the

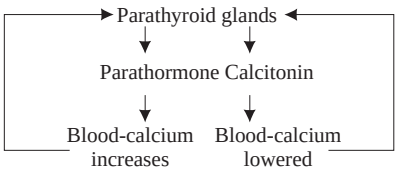


Fig. 19.9 Scheme of parathyroid regulation.

adrenal cortex consists of three *zones-zona glomerulosa, zonafasciculata* and the *zona reticularis.* The medulla consists of large irregularly arranged granular cells containing chromophil granules. The chromophil reaction is due to the presence of a hormone, *adrenaline* which stains yellow with chromic acid salts. Adrenal medulla is strewn with nervous tissue which cannot be regenerated upon destruction. On the other hand, the adrenal cortex when damaged can be regenerated. The adrenal glands also receive a copious supply of blood.

The Adrenal Cortex and its Hormones

Life cannot exist in the absence of adrenal cortex. If the adrenal glands are extirpated, death occurs soon. Death is caused due to the loss of the cortex and not that of medulla. Hence the cortical hormones are essential for survival. A crude cortical extract consists of two distinct fractions: the crystalline and amorphous fractions. About 30 steroid compounds have been identified from the crystalline fraction which control mineral metabolism, carbohydrate and protein metabolism and also the sex functions. Recently one hormone, aldosterone has been isolated from the amorphous fraction of the cortical extract.

Chemically, the cortical hormones are steroidal in nature having a basic cyclopentanophenanthrene ring. All cortical hormones are modifications of this basic structure.

Physiologically, the cortical hormones may be broadly classified as:

(a) *Glucocorticoids* (metabolocorticoids): those effecting the carbohydrate and protein metabolism of the body, such as cortisone, cortisol and hydrocortisone. Prednisone and prednisolone are synthetic analogues.

(b) *Mineralocorticoids:* They effect the metabolism of sodium and potassium, e.g. aldosterone.

(c) *Androgens:* These are sex and anabolic hormones.

Biosynthesis of Cortical Hormones: All the corticosteroids can be synthesized from cholesterol which forms the basic precursor. The overall scheme of biosynthesis is presented in (Fig. 19.10). Progesterone is an intermediate product which forms the major precursor for the synthesis of aldosterone, corticosterone, and cortisol.

Glucocorticoids: The naturally occurring glucocorticoids are cortisone and cortisol (compound F). They serve the following functions:

(i) *Carbohydrate metabolism:* Glucocorticoids cause hyperglycemia, that is, they tend to increase blood-glucose level as well as help in the deposition of liver glycogen. This is achieved by gluconeogenesis from proteins and fats. The corticosteroids, however, do not increase the rate of utilization of glucose by the body tissues.

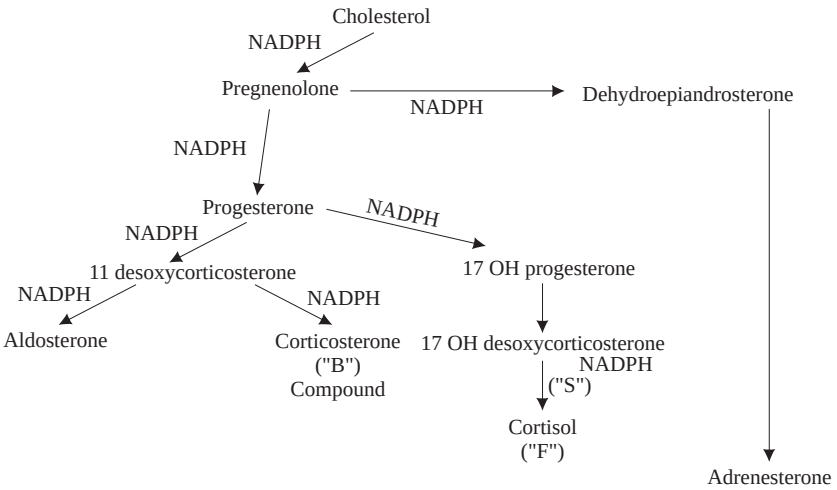


Fig. 19.10 Biosynthesis of corticial hormones from cholesterol.

(ii) *Fat metabolism:* Glucocorticoids increase fat metabolism by converting fat into glucose. They tend to selectively redistribute fat deposit. Excessive fat metabolism results in diseases like ketonemia and ketonuria.

(iii) *Protein metabolism:* Glucocorticoids like cortisol and corticosterone help in depleting the body proteins and convert them into glucose (gluco-neogenesis). Hence the action of the hormones is mostly antianabolic.

(iv) *Anti-inflammatory property:* The steroids help in suppressing acute inflammatory reactions without antibody formation.

(v) *Anti-leukaemic:* They reduce the number of circulating lymphocytes and cosinophils. However, the erythrocytes increase in number.

(vi) *Anti-allergic*

(vii) *Stress reactions:* The steroids help combat any form of "stress" situation in the body providing a defence in extraordinary circumstances.

(viii) *Anti-fibroblastic:* The growth of fibroblastic tissue is suppressed.

(ix) *Diuretic action:* If the glucocorticosteroids are withdrawn, diuresis occurs. They also increase the glomerular filtration and prevent water intoxication by checking the permeability of the tubules. Normally, this function is controlled by vasopressin, but in its absence glucosteroids do the function.

(x) *Euphoria:* They produce a sense of well-being and a gain in appetite is experienced.

Mineralocorticoids: Aldosterone is the chief mineralocorticoid hormone. Desoxycorticosterone is also secreted in minute quantities, but its synthetic form, desoxycorticosterone acetate (DOCA), is used extensively in clinical practice. Aldosterone or the natural salt hormone is several times more potent than DOCA (Fig. 19.11). The salt hormones mainly act on renal tubules and influence sodium,

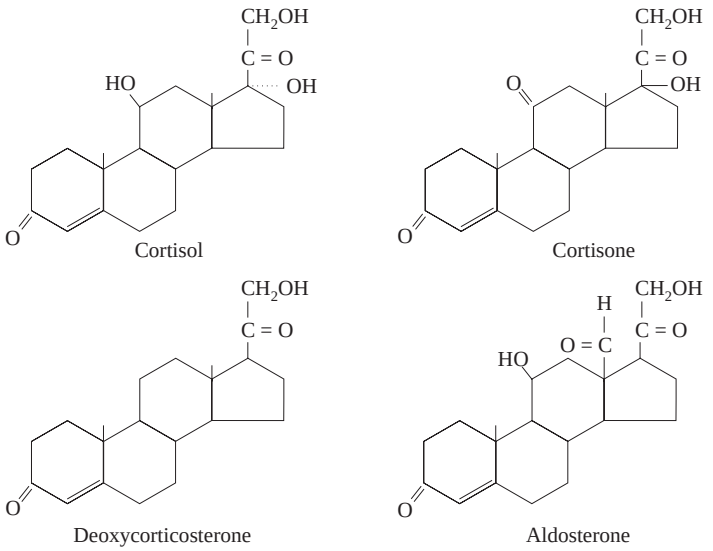


Fig. 19.11 Corticosteroids.

potassium and chloride metabolism. Sodium and chloride are retained by the body, whereas potassium is excreted under the influence of mineralocorticoids.

The following functions of mineralocorticoids can be enumerated:

(1) They increase the reabsorption of sodium from the urine, saliva, sweat and gastric juice.
(2) Potassium is excreted through the renal tubules causing potassium diuresis. On the other hand, sodium is reabsorbed under its influence.
(3) Deficiency of salt hormones causes salt loss from the body causing weakness, hypertension, tetany, polyurea, etc.

Androgens and Estrogens: Adrenal cortex secretes two types of sex hormones: testosterone and estrogens. Androgens of the adrenal cortex are concerned in the development of secondary sex character and promote protein synthesis. When secreted in the normal amounts they do not exert any masculinizing effects. Excess secretion before puberty, however, causes precocious development of secondary sex characters.

Estrogens are secreted from the cortex in very small amounts and may add to the effects of the hormones secreted from the ovary.

Regulation of Cortical Hormones

The secretion of adrenal cortical hormones is controlled by the *adreno-corticotrophic hormone* (ACTH) of the hypophysis. This trophic hormone regulates zona fasciculata and partly zona glomerulosa. There is a reciprocal relationship of glucocorticoids with ACTH. When the steroids decrease, more ACTH is secreted, whereas administration of steroids inhibits the ACTH secretion. Endogenous production of pituitary ACTH is depressed when ACTH is administered. Similarly, when

cortisone is administered for sometime, this causes atrophy of the adrenal cortex and the endogenous secretion of corticoids is suppressed.

There is a corticotrophin releasing factor (CRF) which is a neurohumoral substance secreted from the median eminence of the hypothalamus. This CRF induces the release of ACTH from the anterior pituitary. Thus the cortex activity is regulated. Evidence has been gathered suggesting inhibition of CRF by glucocorticoids (Fig. 19.12).

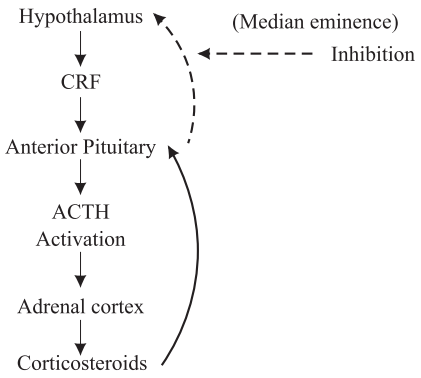


Fig. 19.12 Regulation of the activity of adrenal cortex.

It is to be noted that the secretion of aldosterone is not under the control of ACTH. Its secretion is probably controlled by the osmolarity and the sodium and chloride contents in the body fluids. Whenever the level of sodium in the blood is suppressed, the aldosterone secretion is stepped up. Aldosterone secretion is increased particularly in congestive heart failure, nephrosis, etc. Recent evidence suggests that a factor called *angiotensin* controls the aldosterone secretion. Angiotensin is formed in the blood from angiotensinogen and renin.

Disorders of The Adrenal Cortex: Hypoactivity and hyperactivity of the adrenal cortex gives rise to a variety of clinical syndromes.

Addison's disease: Addison's disease is caused due to chronic insufficiency of the secretion from adrenal cortex. Patients with Addison's disease are highly susceptible to various types of stress. Addisonians are chracterized by symptoms like vomiting, diarrhoea, collapse and pyrexia with rigour, mental clouding, hypoglycemia, low blood pressure, dehydration, acidosis, renal failure, progressive loss of weight and pigmentation. The treatment of the disease is carried out by the use of glucocorticoids and mineralcorticoids.

Cushing's syndrome: Cushing's syndrome is caused by hyper-function of the adrenal cortex with excessive secretion of glucocorticoids. Excessive steroids cause alterations in carbohydrate and electrolyte metabolism. The syndrome is more frequent in the female sex in the age group of 20 to 40 years. Patients suffer from obesity with deposition of fat confined to the face, neck, supraclavicular regions and the abdomen. The patient complains of fatigue and back pain. There may be mental depression or psychoses, accompanied by impotency in males and amenorrhoea in females. Effective treatment is generally adrenalectomy.

Adrenogenital syndrome: It is a disease which results from hyperplasia of the adrenal cortex, with excessive secretion of the androgens. The secretion of the gonadotropins of the anterior pituitary is inhibited blocking ovulation. The syndrome is generally found in female sex. The main symptoms are diminished menstrual flow, growth of hair over the beard area, breasts under developed with loss of fat and enlargement of the clitoris.

Aldosteronism (Conn's syndrome): This syndrome is characterized by excessive thirst, polyuria, weakness, hypertension, periodic muscle weakness, cramps and tetany. Physiologically, there is an excessive secretion of aldoste rone from the adrenal cortex. The excessive steroid causes sodium retention, low potassium in the blood with alkalosis and secretion of alkaline urine.

Treatment generally consists of large doses of potassium, but this provides only temporary relief. Effective treatment is surgical.

Adrenal Medulla and its Hormones

If the adrenal medulla is removed, without disturbing the cortex, the animal appears to be quite normal. The chromaffin cells, present in the medulla, are the source of the hormones that are present throughout the body which continue to secrete in the absence of the medulla (Fig. 19.13).

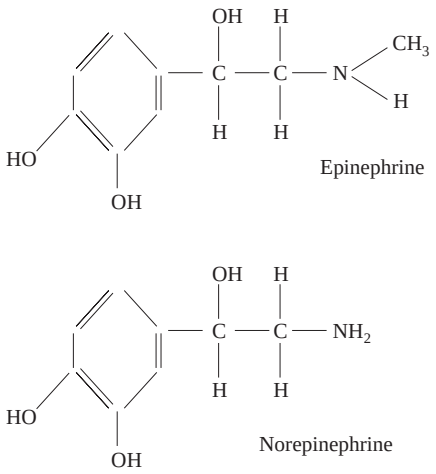


Fig. 19.13 Medullary hormones.

The medulla secretes two hormones: epinephrine (adrenaline) and nor-epinephrine (noradrenaline). Both the hormones are catecholamines and derivatives of the essential amino acid tyrosine. These hormones are excreted in the urine.

Functions of Medullary Hormones: The major functions are follows:

(1) Both these hormones raise the blood pressure epinephrine by raising the cardiac output and norepinephrine by increasing the peripheral resistance due to vasoconstriction.

(2) Epinephrine helps in raising the systolic pressure (diastolic pressure remains same), whereas norepinephrine raises both the systolic as well as diastolic pressures without changing the cardiac output.

(3) Epinephrine accelerates conversion of liver glycogen into glucose, with a consequent rise in the blood-sugar level. Muscle glycogen is broken down to lactic acid.
(4) Epinephrine increases oxygen consumption and the respiratory quotient is elevated.
(5) Epinephrine raises the body temperature and the basal metabolic rate.
(6) Epinephrine and norepinephrine stimulate the central nervous system and produce a state of excitation, alertness and awareness.
(7) Epinephrine inhibits the smooth muscles of the stomach, intestines, urinary bladder and uterus, whereas it excites the smooth muscles of ureter, pyloric, sphincter of bladder and anus.
(8) Epinephrine causes emotional conditions.

Regulation of Medullary Hormones: The two hormones are secreted from the medulla by separate cells under different stimuli. Adrenal cortex is innervated by neurons of sympathetic nature which when activated stimulate the section of these hormones. Medullary tissue is also stimulated by different centres in hypothalamus. Insulin is able to stimulate epinephrine secretion indirectly by lowering the blood sugar level of the body.

Disorders of the Medulla: Though the medullary secretion is not so essential as the cortical secretion is, it is supposed to be subservient to the cortical secretion in meeting with circumstances of "stress". However, sometimes medullary tumours develop which involve the chromaffin cells causing *phaeochromocytoma.* The tumour secretes both epinephrine and norepinephrine. In some instances, when the chromaffin tissue becomes involved in tumours, norepinephrine is secreted in abnormal quantities causing hypertension.

Excessive secretion of medullary hormones produces symptoms like sweating, tremor, headache, weakness, giddiness, diarrhoea, vomiting, abdominal colic and anxiety. These attacks are sometimes followed by emotional, upsets. In some cases glycosuria, and increased thyroid activity may lead to increased basal metabolic rate. Hypertension may cause cardiac failure or cerebral haemorrhage.

19.2 THE PANCREAS

The endocrine function of pancreas has been studied extensively. It consists of an acinar tissue having pyramidal epethelial cells. There are groups of isolated cells which are called *islets* of *Langerhans.* The cells of the islets of Langerhans are differentiated into three types: the *beta cells* which produce insulin, *alpha cells* produce the hyperglycemic factor glucagon, and the *gamma cells* whose function is still unknown. The acinar cells produce exocrine secretions, while the islets of Langerhans are endocrine secreting cells.

Insulin

Insulin is a hormone secreted by beta cells and is polypeptide in nature. Each insulin molecule is made up of two chains of amino acids which are connected by means of two disulphide bonds. One chain has 21 amino acids while the other has 30. Insulin is secreted into the portal blood flow and is bound to globulin. It is a hypoglycemic hormone which serves the following functions:

1. It enhances the utilization of glucose by the peripheral tissues.
2. It helps convert glucose into glycogen in the liver and the skeletal muscles.
3. It inhibits the conversion of fats and proteins into glucose, thus preventing gluconeogenesis.
4. It helps the formation of fat from glucose.

The site of insulin action is not yet exactly known. However, two theories have been put forth which explain its physiological action. According to one theory insulin increases the cell permeability for glucose and induces a rapid transfer of glucose through the cell membrane into the cell for further utilization. The other theory states that insulin prevents the inactivation of the enzyme hexokinase by the pituitary and adrenal hormones. It is just possible, that insulin serves both the functions.

Insulin secretion is principally controlled by the blood-glucose level. Hypoglycemia (low blood-sugar level) tends to decrease insulin secretion, whereas hyperglycemia stimulates it. Besides this, a nervous mechanism is also involved in the production of insulin. Stimulation of the vagus nerve increases insulin secretion.

Glucagon

Glucagon is a hyperglycemic hormone produced by the alpha cells of the islets of Langerhans. The hormone is a polypeptide consisting of 29 amino acid units in a single chain. Glucagon stimulates the secretion of glucose by liver and thus maintains blood-glucose balance. So far, no diseases are known to be caused by glucagon deficiency. However, glucagon is thought to increase glycogenolysis, Administration of glucagon increases metabolic rate. Insulin and glucagon have antagonistic functions. Low blood sugar level stimulates the alpha cells of the Langerhans to secrete glucagon and high blood sugar level has an inhibitory function (Fig. 19.14). Thus a feedback mechanism is in operation similar to that of insulin.

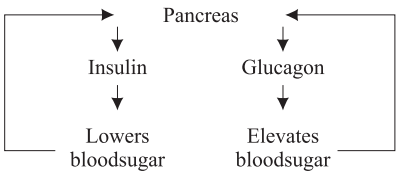


Fig. 19.14 Antagonistic functions of insulin and glucagon.

Disorders of Pancreatic Hormones: Insulin deficiency results in a syndrome called *diabetes mellitus.* This leads to serious disorders like alterations in carbohydrate, lipid, protein, electrolyte and water metabolism, often resulting in comma and death. Hypoinsulinism (insulin deficiency) causes accumulation of glucose in the blood. Glycogenesis is considerably reduced thereby elevating blood-sugar above renal threshold so that sugar appears in the urine. The disease is called *glycosuria.* The high blood-sugar alters the osmotic balance resulting in the diminution of water reabsorption in the glomerular filtrate. Consequently urine volume increases (polyuria). This causes dehydration, thirst and polydipsia.

Since glucose cannot be utilized by the cells, lipid and protein break-down increases. As a result, ketone bodies accumulate rapidly *(ketonemia)* and get distributed in the urine which is produced in larger quantities (ketonuria). The ketone bodies cause *acidosis* increasing dehydration.

The overproduction of insulin causes hypoglycemia.

19.3 HORMONAL CONTROL OF GROWTH

The process of growth and differentiation is under the control of hormones. Early embryonic development is controlled by genetic properties, but late embryonic and postembryonic developments are largely regulated by hormonal influences and partly by neural regulatory mechanisms. No one would mistake a tadpole for a fish. Similarly, caterpillar cannot be mistaken for a butterfly. The tadpole and the caterpillar both undergo a process of differentiation which is called metamorphosis. Metamorphosis is characteristic of many invertebrates and most orders of insects.

Control of Amphibian Metamorphosis

The most familiar example is found in amphibians and insects where the process of metamorphosis is intricately controlled by hormones. Metamorphosis is the period of postembryonic development when the larval features undergo gradual modifications until an adult is developed. In amphibians, hormones from the thyroid gland trigger the metamorphic changes and are continuously required as stimulants for the developmental processes.

The thyroid hormones are amino acids containing three or four atoms of iodine (tri-iodothyronine and thyroxine). If the thyroid gland of a tadpole is removed, the tadpole continues to grow but never metamorphoses. If thyroxine is administered to a young tadpole, it would show precocious metamorphosis. Sensitivity of tadpoles to thyroxines has been extensively studied and it has been found that extremely low concentrations of about I part per billion (10^{-9} molar concentration) are effective in inducing metamorphosis. The hormone acts directly on the transforming tissues. The thyroid function is controlled by the anterior pituitary gland which secretes a trophic hormone (TSH). TSH serves to stimulate the thyroid gland and regulates the hormone production-controlling metamorphosis.

19.4 HORMONAL CONTROL OF IONIC AND WATER BALANCE

Hormones have a decisive role in the control of metabolic processes, thereby maintaining. homeostatic mechanisms. Water is an important constituent of the protoplasm and extremely necessary for all the biochemical reactions that occur in the cells. It is a component of all the extracellular fluids of the body and helps in the transport of nutrients, wastes, maintenance of osmotic pressure and ionic environment of cells.

Ions of inorganic salts, such as sodium, calcium, potassium, chloride and bicarbonates, etc., are important constituents of the blood and tissue fluids. These function in a variety of ways: as activators of enzymes; in the production of electrical potentials across membranes; in the maintenance of osmotic concentration of body fluids. Osmotic and ionic gradients exist between the organism and its

environment which require mineral and water regulation. Normally in terrestrial vertebrates sodium and chloride are regulated by the kidney and are selectively retained whereas the unwanted ions are excreted as wastes.

Ionic Balance

The most important elements concerned with hormonal regulation are sodium, potassium, calcium, phosphorus and chlorine which are usually present in the form of inorganic salts in the tissue fluids. The hormones of the adrenal cortex exert their regulatory control over sodium and potassium. In adrenalectomized animals, sodium is not retained. There is a rapid loss of sodium and chloride ions causing salt depletion. This lowers the pH of the fluid. There is loss of potassium ions too, but its leakage is less rapid. The loss in the pH and consequent ionic disturbance can be restored by the administration of mineralocorticoids, and aldoesterone. In addition to their effects on the kidney function, mineralocorticoids also help in the greater absorption of salts by the intestine and reduce the salt loss through perspiration.

Besides the adrenal cortex, vasopressin or antidiuretic hormone (ADH) also prevents fluid loss by the kidney.

The water and mineral metabolism is also controlled by glucocorticoids. The glucocorticoids, such as cortisone and hydrocortisone, are gluconeogenic. They tend to promote the formation of carbohydrates from body proteins and fats. These hormones possess some salt retaining properties and constitute the main "stress" hormones.

Water Balance

In animals there are definite mechanisms to regulate water balance. In aquatic species the problem is not so acute as in case of terrestrial species. Desert animals are especially bestowed with such mechanism which enables them to utilize metabolic water. Loss of water is minimized by special morphological adaptations. Animals which have access to sufficient water supply regulate their water balance by regulating the volume of urine excreted.

The water balance and its regulation are under the control of a hormone vasopressin or antidiuretic hormone (ADH) which is found in the neurohypophysis of all the mammals except pig and hippopotamus. The glomerular filtrate which collects in the Bowman's capsule is passed into the tubular portion of the nephron where it is transformed into urine by reabsorption of useful substances like salts, sugar, amino acids, water, etc. The process of reabsorption is under hormonal control (see section 13.7). The antidiuretic hormone increases the permeability of the distal and collecting tubules of the kidney to water so that water is withdrawn from the urine by osmosis and becomes concentrated and less in volume. In the absence of ADH, the tubular walls become impermeable to water and the urine passes unaltered. Large doses of ADH cause constriction of blood vessels so that the blood pressure is elevated. However, the circulating ADH is rendered inactive very soon as it reaches the liver and kidneys. Special osmoreceptors are responsible for the stimulation of supraoptic and paraventricular nuclei of hypothalamus which secrete this hormone. There is a delicate feedback mechanism which controls the secretion. ADH deficiency causes a disease, *diabetes insipidus* in which large volumes of urine (polyurea) are produced with low salt contents and specific gravity.

19.5 PROSTAGLANDINS

Prostaglandins are an important class of biological compounds and by far the most important group of physiologically active substances. The prostaglandins comprise a family of lipid acids which exhibit highly potent and manifold effects in biological systems. The name "Prostaglandin" was given by von Euler in 1935 to a principal compound found in human seminal plasma and vesicular glands of sheep which induced contractions in isolated smooth muscle preparations. The prostaglandins are known to occur widely in almost all kinds of tissues, in very small quantites (nanogram to micrograms) and they have been identified from a great number of human tissues, such as seminal plasma, menstrual fluid, lungs, etc.

Prostaglandins are derivatives of a hypothetical C_{20} saturated fatty acid called *prostanoic acid.* It consists of a 5-carbon ring with two hydrocarbon chains attached to two neighbouring carbon atoms. All natural prostaglandins contain a hydroxyl group at C-13 and a trans double bond at C-13-14. Naturally occurring prostaglandins are of 4 types: PGE, PGF, PFA and PGB types.

Physiological Role of Prostaglandins

Prostaglandins influence numerous aspects of cell metabolism. This wide range of activity of prostaglandins has led to a number of clinical applications. They induce powerful contractions in smooth muscles like rat uterus muscle, gastrointestinal smooth muscle, respiratory tract smooth muscle, rabbit duodenum muscle, etc. When these isolated muscle trips are bathed in Tyrode solution and treated with prostaglandins, powerful contractions are observed. These can be recorded on a kymograph. The reaction of the isolated muscles differs with the type of muscle and it can also be influenced in many ways by experimental conditions. In several species PGE has been shown to relax the circular muscles of intestine but on the other hand induce contractions induce longitudinal muscles.

Prostaglandins are best known for their effects on the reproductive system. There seems to be some correlation between male fertility and the seminal prostaglandin content. However, there is not enough evidence to show the nature of prostaglandin influence in fertility. All prostaglandins of the PGE type have strong inhibitory action in vitro on strips of uterine muscle from nonpregnant females. The PGF compounds, on the other hand, exhibit stimulatory action that varies in degree during the menstrual cycle.

Prostaglandins of PGF_2 type are responsible for the regression of the corpora lutea when pregnancy does not occur. Some types of prostaglandins (PGE_1, PGE_2 and PGF_2) induce abortions and parturition in rats, mice and monkeys. This has led to use of prostaglandins to induce labour and bring about abortion in human pregnancies. It is possible that luteolysis is the mechanism of termination of early states of pregnancies.

Prostaglandins are natural constituents of nervous tissue. They are released from the brain upon stimulation of afferent pathways. The possibility of postaglandins acting as mediators of synaptic transmission has also been considered. An important metabolic effect is that the PGE_1 antagonizes the stimulatory effects of a number of hormonal compounds on the release of free fatty acids and glycerol from epididymal fat pads of rats.

Prostaglandins are known to have a unique role in the inflammatory response. Gastric acid is an important factor in causing gastrointestinal ulcers. Prostaglandins have some effect on the inhibition of gastric secretion to check ulcer formation.

There are reports suggesting the role of prostaglandins in renal functions. Intravenous infusion of norepinephrine has been shown to release prostaglandin like substances into the renal artery. It has been reported that the diuretic function of norepinephrine can be mediated by prostaglandin release. The presence of prostaglandins in the kidney and their release under various circumstances suggests a relationship with renal hypertension.

The numerous reports on the action of prostaglandins in the water flow and sodium transport across toad bladder, frog skin, guinea pig ileum, have suggested that prostaglandins function in the regulation of ion fluxes across epithelial membranes.

Thus it can be seen that prostaglandins are of great biological significance. It appears that prostaglandins are closely related to cyclic AMP, a compound of great biological importance. These two, prostaglandins and cyclic AMP, seem to be involved in the modulation of hormone action. Prostaglandins also appear to be linked to the function of Ca^{++} and cell membranes. However, the exact mode of action of prostaglandins is not yet clear and needs further elucidation.

19.6 HORMONAL REGULATION IN INVERTEBRATES

Biologists have long suspected that many activities such as moulting, colouration, and reproductive behaviour in invertebrates are controlled by hormones. Only in recent years, however, have the existence of such hormones and their sites of origin been verified. The neurosecretory cells of the brain are supposed to be the main sites of secretion of hormones in invertebrates. The neurosecretions are either discharged directly into the tissue fluids or through neurohaemal organs when present (as in insects). Besides neurosecretions from the brain cells, hormones in invertebrates are also produced by organs which are non-nervous in origin. Such organs are commonly found in arthropods. The invertebrate hormones are largely concerned with the timing of reproduction and regulation of growth which are dependent upon ecological factors. Investigators have identified hormone producing tissues and hormones in annelids, molluscs, a number of insects and crustaceans.

Annelida

The cerebral ganglia of annelids have neurosecretory cells which undergo seasonal secretory cycles related to growth and reproduction. The brain cells release a hormone whose chemical nature is unknown. It has been suggested that this brain hormone may inhibit the development of germ cells as in case of nereids, or it may stimulate sexual characters as in case of oligochaetes.

Mollusca

Neurosecretions influence the reproduction in molluscs also. Seasonal variations in the histology and secretions of neurosecretory cells have been demonstrated in certain molluscs where production of eggs or sperms is controlled by these secretions. In case of cephalopods (viz. *Octopus),* there are some optic glands that regulate the onset of sexual maturity in either sex. The optic glands are believed to secrete a gonad-regulating hormone under nervous control of brain.

Insects

Insects possess a highly organized endocrine system, and our understanding of their regulatory mechanisms is remarkably good. The processes of growth, development and metamorphosis in insects are controlled by hormonal mechanisms that have been successfully worked out in a number of insects. The system includes the neurosecretory cells present in the brain comprising *pars intercerebralis, corpora cardiaca* and sub-oesophageal ganglia. Besides these, other endocrine glands include *prothoracic glands* and *corpora allata.* The neural glands and the endocrine glands are intimately interrelated for coordinating secretory activities almost like that of vertebrates.

The neurosecretory cells of the brain lie in the dorsal and lateral parts of the brain which have neural connections. In the individual larval stages, the neurosecretory cells undergo periods of secretory activities, and produce an important substance concerned with the regulation of metamorphosis. The neurosecretory cells located in the pars intercerebralis produce a trophic hormone, *ecdysotropin* which is transferred to the corpora cardiaca (a neurohaemal organ) through axonic transport. The corpora cardiaca is situated close to the brain. The prothoracic glands are branched chain like organs penetrated by tracheae and nerves from the sub-oesophageal ganglion. The prothoracic glands degenerate during the post-pupation period.

The corpora allata are generally paired glands surrounded by connective tissue and lie behind the corpora cardiaca and receive nerves from them. There is a chain of activity of neurosecretory cells: in the larval instar, first the neurosecretory cells become active, then the prothoracic glands and lastly the cells of corpora allata. Although these events have been studied in many insects, the best experiments have been conducted on *Rhodnius* (Wigglesworth).

Control of Metamorphosis and Growth: The post-embryonic development of insects begins with hatching and extends through the period of growth, which is punctuated by a number of steps or moults. This period of *moulting* or *ecdysis* is called metamorphosis in which the characters of a sexually mature adult emerge from changes in external morphology and from the transformation of the external organs. In case of insects with incomplete metamorphosis (Hemimetabola) such as grasshoppers and cockroaches, the adult organs appear without a profound transformation of the larval organization. The larval characters disappear gradually and the rudiments of the adult organs, viz. wings, genital appendages, etc. appear. In insects with complete metamorphosis (Holometabola) such as moths and butterflies, early development of the larval stage leads to a nonfeeding pupal stage. In the adult leading to the pupa, the internal rudiments of the wings of the larva, genital appendages and other structures get everted; and during the pupal stage, the major events of metamorphosis are carried out. The demolition of larval organs is simultaneously accompanied by growth and differentiation of adult parts.

It has been amply established that moulting is caused by a circulating hormone produced in the region of the brain. Experiments with *Rhodnius* larvae have proved that the larvae fail to moult if their heads are removed shortly after a blood meal, although the headless larvae may live for more than a year. However, if the heads are removed after a week or more of the blood meal, moulting will not be prevented. A week's time after the meal is regarded as the *critical period* during which the moulting hormone has diffused into the tissues so that capacity to pupate gradually spreads from front to back.

The stimulus for pupation comes from a moulting hormone, *ecdysone* which is nonspecific. There is 3-stage process of the mechanism.

The hormone of the neurosecretory cells activates the prothoracic glands to secrete ecdysone which initiates the tissue alterations in metamorphosis. The actual moulting hormone comes from the prothoracic glands which requires continued stimulus from the secretions of neurosecretory cells until the critical period is reached. During larval instars, the action of ecdysone is controlled by a *juvenile hormone* (JH) secreted by corpora allata. The effect is due to its concentration. In high concentration, the JH makes the tissue more responsive to the prothoracic hormone. If the JH is completely withdrawn, adult transformation occurs, that is why in the last larval stage it stops entirely. Implantation of larval corpora allata into the young pupae prevents metamorphosis to the adult, and instead another pupal moult is initiated.

What would be the mechanism of regulation of these hormones? Several hypotheses have been advanced:

1. Certain nutritional condition of the larva is necessary for the neurosecretory cells to be active. Experiments with *Rhodnius* have shown that synthesis of brain hormone is followed by a few days of a blood meal sufficient to distend the animals.
2. Regeneration experiments show that from the growing organs of the larva signals are given off which initiate the activity of the neurosecretory cells of the brain.
3. Nerve impulses also influence the rhythmicity of secretions. The prothoracic glands receive nerves from the sub-oesophageal ganglion.
4. It has been suggested that the neurosecretory cells of corpora cardiaca serve for the transport and storage of the brain hormone.
5. The juvenile hormone secreted by corpora allata is nonspecific and is responsible for maintaining larval characters. Its other function is to maintain the secretory activity of the prothoracic gland.

An interesting effect of ecdysone has been observed in moths. Ecdysone, when applied to giant chromosomes, induces puffy areas. Sizes of the puffs are proportional to the amount of ecdysone administered. It was found that RNA and protein synthesis was accelerated in puffed regions due to activation of genes by ecdysone. Puffs can be caused by other chemical agents, which, however do not cause moulting. The model showing this mechanism is given in (Fig. 19.15).

Control of Diapause: In many insects, the course of development is temporarily arrested by a resting stage called *diapause.* This resting stage represents a physiological mechanism for survival during adverse conditions. Diapause can occur during embryonic stage, larval stage, or in pupa (mostly in Holometabola). During this stage, the metabolic rate is extremely low with a complete or partial cessation of growth.

The phenomenon of diapause is hormonally regulated. It has been suggested that the activity of the endocrine glands is controlled by environmental factors like temperature, photoperiodism, moisture, availability of oxygen and food. The adult development of pupa is also under hormonal control. It is indicated by experiments on the giant silk moth, *Hyalophora cecropia* which undergoes a long period of diapause. Pupation occurs during June to September and the pupae remain inactive

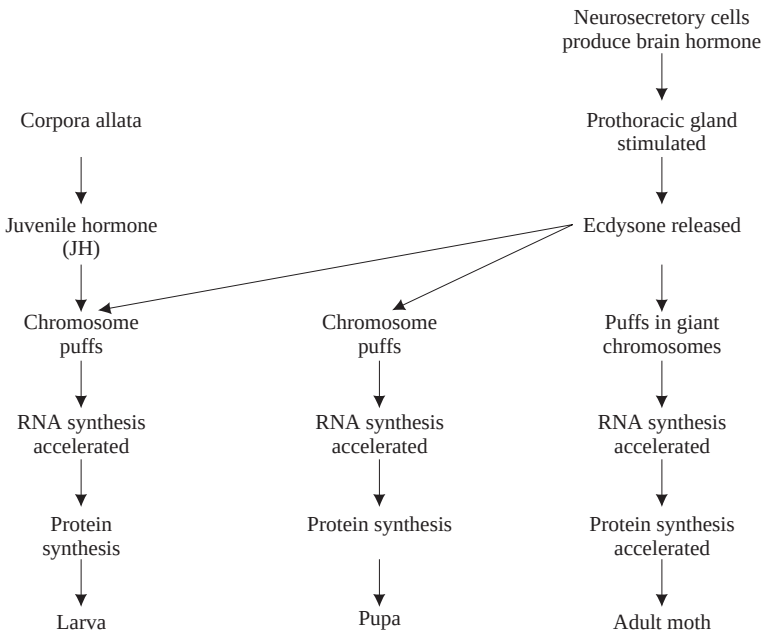


Fig. 19.15 Model illustrating the mechanism of hormone action in insects: ecdysone causes puffing in giant chromosomes, while juvenile hormone keeps the insect in its larval form before it becomes pupa.

for at least 8 months. The animals remain dormant at room temperature, but if the diapausing animals are held at 5°C for a while and then returned to room temperature, adult development is triggered and resumed in a few days. Ecological conditions are particularly responsible for the onset of diapause and reactivation of development, and it seems that, diapause is regulated through the same endocrine mechanism as described for metamorphosis. The arrest of development is regarded as due to temporary absence of the hormones necessary for the growth which are initiated in the brain. This hypothesis can be illustrated by an interesting experiment on *Hyalophora.* Diapausing pupae were cut into two parts, an anterior with the wings, and a posterior with the last six abdominal segments. The cut surface was sealed with paraffin wax and a hole was left in the centre of the sealed surface of the cut to introduce the implants. If a chilled brain is introduced into each half, the anterior half develops into the front half of the adult moth, and the posterior half fails to develop. Now, if the posterior half is tied to the rear end of the intact diapausing pupa and this intact pupa is allowed to develop by implantation of a chilled brain, the posterior half of the tied abdomen will also develop along with the intact pupa. If a prothoracic gland is also placed, in addition to the the chilled brain, in the isolated abdomen, a mature moth abdomen is formed. If a brainless diapausing pupa is connected to a diapausing abdomen by a glass tube, development will not proceed. However, if a chilled brain is introduced into the abdomen, diapause will be broken and both parts will develop into corresponding parts of the adult moth. This experiment fully illustrates that from the implanted brain an activating hormone is released to the anterior half which activates the secretion of prothoracic gland and diffuses throughout the body to cause changes of adult development.

Control of Reproduction: There is no direct evidence as to the control of reproduction and secondary sex characters by any hormones in insects. However, some kind of hormonal relationship has been observed with regard to the deposition of yolk in the oocytes. It has been suggested that yolk in oocytes is dependent upon the presence of hormone secreted by corpora allata. In *Rhodnius* and also in *Schistocerca,* removal of corpora allata induces the development of oocytes without yolk which later degenerate. In male *Rhodnius* and *Melanoplus,* the accessory glands maintain their activity under the influence of corpora allata. Allatectomy also depresses growth and activity of female accessory glands in *Calliphora, Melanplus,* etc. In the same way, considerable evidence exists to prove the effect of ovary on the activity of corpora allata. In *Melanoplus, Calliphora* and *Lucilia,* when the ovaries are removed, the corpora allata hypertrophies. The neurosecretory cells of the brain have also some importance in the development of oocytes. If the brain is removed in *Calliphora* the oocytes do not develop beyond a very small size. The brain hormone also influences the synthesis of protein needed for the developing oocytes.

Crustacea

Crustaceans possess an efficient endocrine system consisting of three components, viz. a sinus gland with an X-organ in the eye stalk, the post-commissural organ located behind the oesophagus, and the pericardial organs located around the wall of the pericardium. Besides these, there is a paired Y-organ located in the antennary or maxillary segment. This organ is concerned with moulting. In crabs, the outer covering must be shed periodically to allow the crab to grow. The moulting of the crab is controlled by ecdysone produced by the Y-organ. A moult inhibiting hormone (MIH) is produced by the X-organ and this prevents the formation of ecdysone. Normally, the MIH is produced while growth takes place. As the moulting time approaches, the MIH secretion declines so that ecdysone production is stimulated which is released into the body fluid by the Y-organ to cause moulting. If the eye stalks are removed from the juvenile or nonbreeding females, an increase in the weight of the ovaries is observed. The eye stalk cells secrete a gonad-inhibiting hormone and its absence initiates yolk production. The hormone from the sinus gland also inhibits vitellogenesis and moulting in egg-bearing females. The pericardial glands secrete a hormone that maintains the heart beats.

CHAPTER

20

Reproduction

Reproduction can be defined as a process by which an individual perpetuates its own kind. Of all living functions, reproduction may be said to be the most important since it makes room for the existence of more individuals and newer species in time and space.

20.1 LEVELS OF REPRODUCTION

Reproduction could be viewed to occur chiefly at three levels: molecular, cellular and organismal. All living cells have the tendency to grow and divide. In doing so they make use of nutrition available in their external environment and transform it into their cellular components. This results in accumulation of additional molecules which may be called the simplest form of molecular reproduction. Protein synthesis and DNA duplication are examples of this kind of reproduction.

Cellular reproduction is a process in which cells divide into two daughter cells having the same chromosomal complement as that of the original cell. This is accomplished by mitotic division. The cells of higher organisms, with the exception of their gametes, have chromosomes in multiple of two. Somatic cells of human beings have 46 chromosomes (23 pairs) and all such organisms representing this chromosomal condition are called *diploid* organisms.

Apart from molecular and cellular reproduction, the whole multicellular organism may also reproduce. Multicellular organisms may reproduce *asexually* or by sexual mode. In the asexual mode of reproduction quite a large part of the parent organism participates in the reproduction However, this process is of little evolutionary advantage and may be regarded as an environmental adaptation. Higher organisms, especially the mammals cannot reproduce asexually but produce specialized reproductive cells, the *gametes.* The gametes are of two types: male gametes are called *sperms* and female gametes as *ova.* The two must unite as a result of a sexual process and form a fusion product, the *zygote.* The fusion or the union is *called fertilization* after which the zygote can develop into a new individual.

20.2 PATTERNS OF REPRODUCTION

In some animal groups reproduction is accomplished by a nonsexual method, already referred to as asexual reproduction, occurring without the participation of sex cells. Some animals propagate by vegetative method known as *budding* in which a portion of the parent's body grows by repeated cell division to form a new or a group of new individuals. *Hydra* is a common example of this process. In some protozoans fission is a means of reproduction in which the nucleus divides mitotically followed by the division of the entire organism. Some organisms *regenerate* the parts which are lost as a result of some injury. However, this process is limited to a few groups of animals. Asexual reproduction is essentially a *mitotic* process involving one individual or parent and is far too simple. No mating is involved. The number of offsprings produced are generally numerous and all are exact copies of the parent.

Sexual reproduction is a complex process involving the fusion of two types of specialized sex cells produced by two different individuals or parents. The specialized cells are gemetes which are haploid in their chromosomal set-up, and upon fertilization they produce a zygote with diploid chromosoma complement. In contrast to asexual reproduction, sexual process has apparently more advantages. New organisms are produced which may be similar, but not identical to either of the parents. This variability has a great evolutionary significance.

Significance of Meiosis

At each generation, the number of chromosomes of individuals remains constant. This constancy is maintained by special division known as meiosis. Meiosis occurs, universally wherever a sexual process is involved.

The gametes have a haploid (*n*) set of chromosomes. During fertilization chromosomes from the paternal gamete pair with the chromosomes of the maternal gamete forming a zygote which is diploid (2*n*) in character. The 2*n* number of chromosomes is reduced to half (*n*) by two meiotic divisions (Fig. 20.1) in two successive steps. Meiosis takes place both in the testis and the ovary. In males all four haploid cells are found to be functional sperms. In females, only one cell becomes a functional ovum.

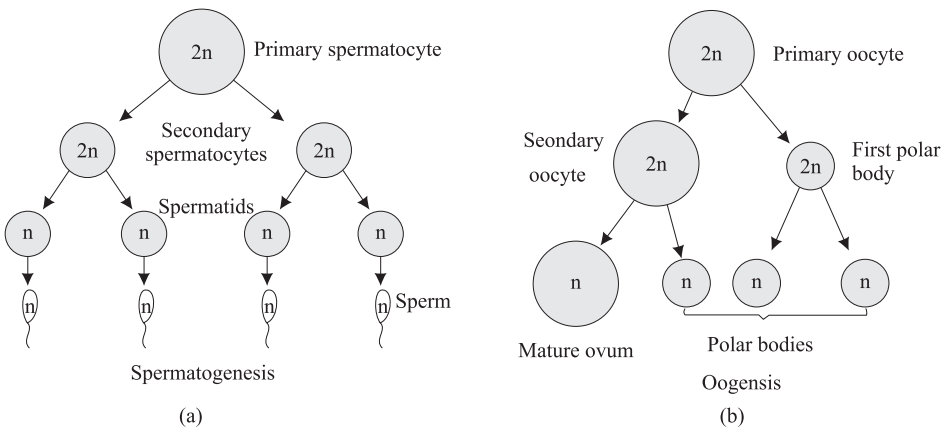


Fig. 20.1 Process of gamete formation: (a) spermatogenesis; (b) oogenesis.

20.3 MORPHOLOGY OF THE REPRODUCTIVE ORGANS

In some lower forms and primitive animals the gamete producing tissues art diffuse and do not form distinct localized organs. However, in higher animal and all vertebrates, the gonads are distinct and localized organs. The present account deals with the placental mammals. Hence, our treatment would be restricted to this taxonomic group only.

A male mammal possesses a pair of testis where the male gametes are produced as a result of *spermatogenesis.* In the female mammal, a pair of ovaries is present where *oogenesis* occurs so as to produce ova for ovulation. Besides testis and ovary, as the primary reproductive organs, there are secondary sexual organs present both in males and females, which help in the sexual act and union of sex cells. The following description applies to mammals in general.

Male Reproductive Organs

The male reproductive system consists of a pair of testes which are enclose in *scrotal sacs* lying generally outside the abdominal cavity in majority of the mammals. However, in some mammals like elephant, the testes lie inside the body cavity. Location of the testes outside the body cavity is particularly advantageous since proper temperature is available for maturation of sperms. Small tubular vessels, *vasa efferentia,* from each testes open into long convoluted *epididymis.* The epididymis is thrown into special coils so as to be accommodated into a small space. The walls of epididymis are muscular and help in the propulsion of sperms through it. It is divisible into three parts namely, *caput epididymis, cauda epididymis* and *epididymis proper.* According to some authors, the walls of epididymis secrete a substance which provides nourishment to the sperms. The epididymis of each side leads into *vasa deferentia* whose walls are again muscular and undergo rhythmic contractions. Vasa deferentia of either side enters the abdominal cavity through the *inguinal canal* and after taking a tortuous course open into sac-like *seminal vesicle* (Fig. 20.2).

Seminal vesicles are present in rats, squirrels, bats and man, but absent rabbit. The lower portion of the seminal vesicles and the urinary blade jointly form a long *urethral canal* as in case of rodents. All the mammals have external copulatory organ; hence fertilization is always external. In males, there is a penis which functions both for the exit of urine, and of sperms durin copulatory act.

Associated with the male reproductive organs are certain accessory glands: These are a pair of *prostate glands* and a pair of *Cowper's glands.* The prostatic secretion is a thin, white, mildly acidic fluid which contributes major share in the formation of *semen.* So long the sperms remain in the epididymis and vasa deferentia, they are inactive and nonmotile, but after coming in contact with the prostatic secretion they gain motility and can swim about. The Cowper's glands produce a transparent sticky fluid which protects the sperms from the acid reaction.

The Testes: Testes are the male gonads where the sperms are produced. The organ consists of a number of elongated *seminiferous tubules* (Fig. 20.3). Surrounding these tubules there are connective tissues containing large polyhedral cells with large nuclei and granular cytoplasm, called *cells of Leydig.* Each seminiferous tubule is lined with peripheral germinal epithelium which proliferates cells differentiating into *spermatogonia* and *sertoli cells.* The spermatogonia give rise to sperms which derive nourishment from the sertoli cells for further development.

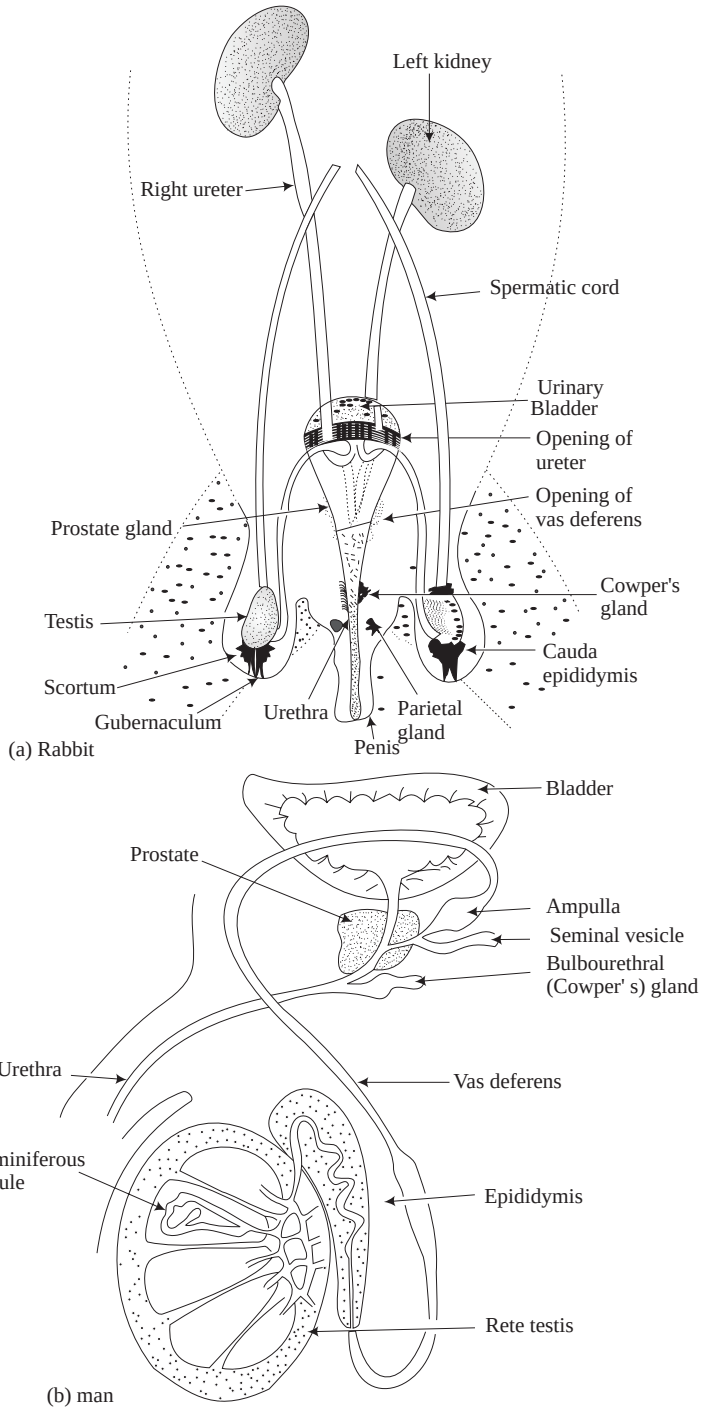
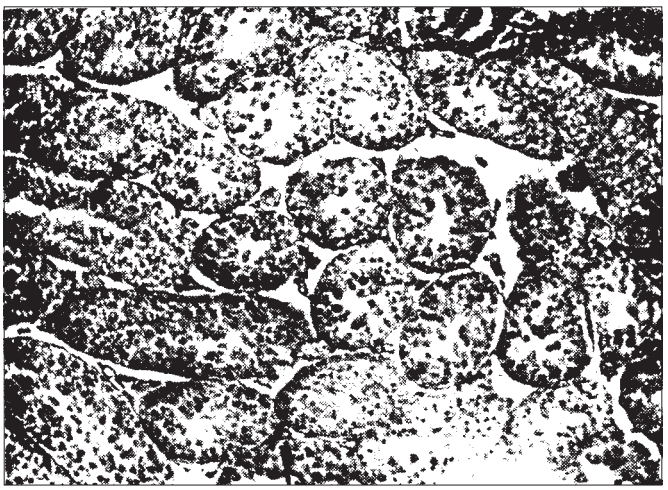
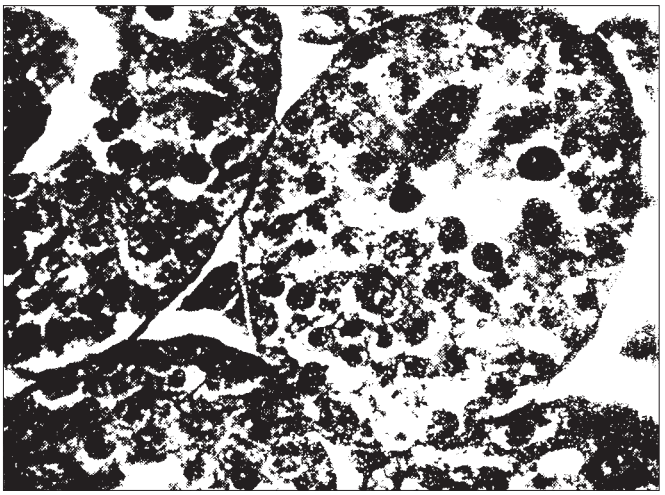


Fig. 20.2 Male reproductive organs.



(a) Seminiferous tubules in section.



(b) Same magnified to show spermatogonia and sertolli cells.

Fig. 20.3 Cross-section of a part of the testis of a male desert rat showing seminiferous tubules (microphotographs).

Spermatogenesis: The process of spermatogenesis involves differentiation and maturation of spermatogonia in the testes. The process is initiated and maintained by certain hormones (to be discussed later).

The male gametes or the sperm cells are formed in the seminiferous tubules of the testes, and the germinal epithelium. During the early stages of sperm formation, each sperm mother cell or primary spermatocyte has the full complement of diploid number of chromosomes. This number of chromosomes must be reduced to half, that is the haploid number, before maturation of the sperm is completed so that after fertilization the resulting zygote will have the normal diploid number. The first cells produced by the seminiferous tubules are called primary spermatocytes which are having dieloid number of chromosomes. The primary spermatocyte undergoes two meiotic divisions (Fig. 20.1a) in

succession so that the number of chromosomes is reduced to half. These resulting cells are called spermatids, each of which transforms itself into a sperm or spermatozoa. Thus each primary spermatocyte eventually gives rise to four spermatozoa and each containing haploid number of chromosomes.

Female Reproductive Organs

The female reproductive system consists of a pair of ovaries situated on either side of the vertebral column behind the kidneys. Each ovary is attached to the dorsal abdominal wall through a peritoneum. There is a pair of tubular *oviducts* distinguishable into three regions: the ciliated funnel, fallopian tube and the uterus. Fallopian tube is a portion where maturation, fertilization and division of the egg takes place. The uterus has undergone a progressive modification in mammals according to their evolutionary status (Table 20.1).

Table 20.1 Modification in the Uterus

Type of uterus	*Examples*
Uterus duplex	Guinea pig, rat, mouse, rabbit
Bipartile uterus	Cat, dog, cow, mare
Bicornuate uterus	Pig, insectivores
Uterus simplex	Primates, man

The uterus is a highly muscular organ where implantation of the embryo takes place. In case of rabbit there are two uteri, whereas in primates only one uterus simplex is present (Fig. 20.4). The uterus opens into the *vagina* through a narrow cervix, The vagina can be divided into two parts: the lower or posterior portion known as the *vestibule* and the posterior vagina. Attached to the vagina is a small structure called *clitoris* which is homologous to the penis in the male. The vagina is devoid of any glands; however, the mucus present in it, especially during the heat period, is secreted from the cervix. In case of rabbit, there is a pair of Cowper's glands attached to the dorsal wall of vagina (Fig. 20.5).

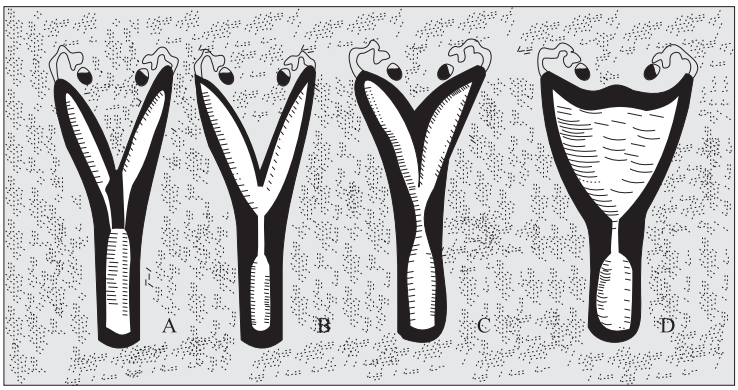


Fig. 20.4 Types of mammalian uterus.

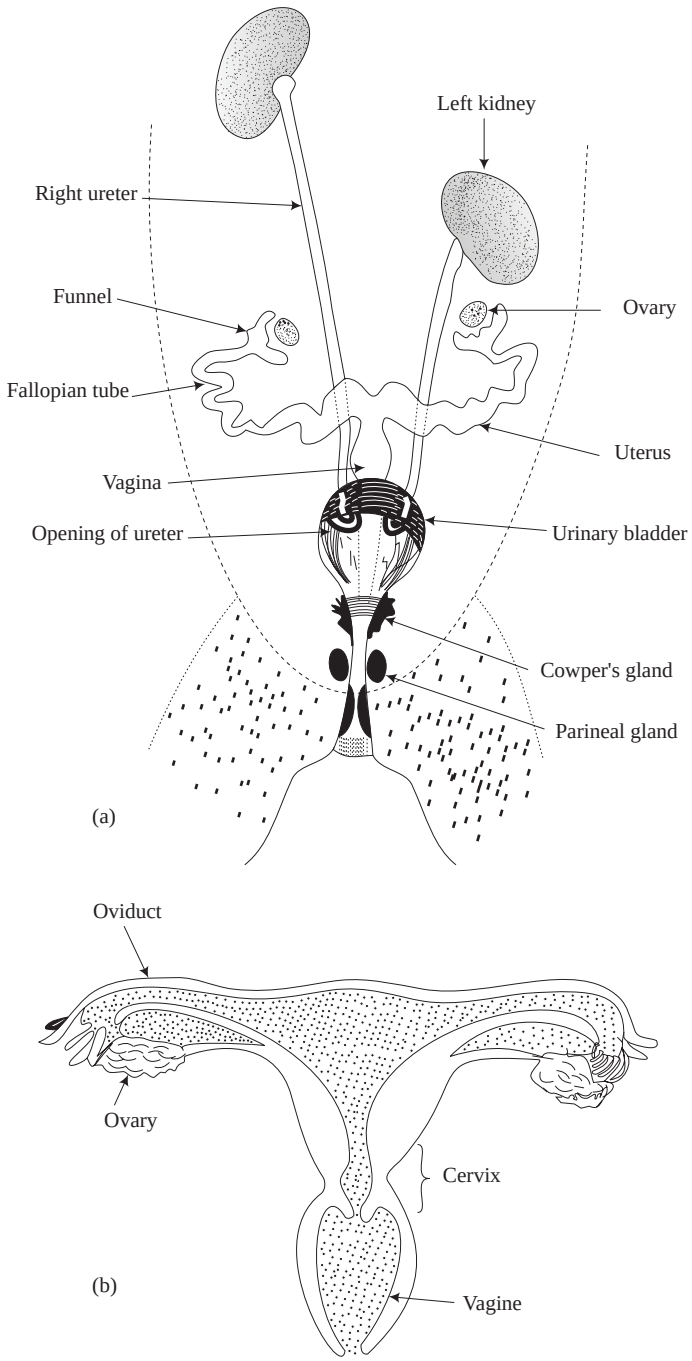
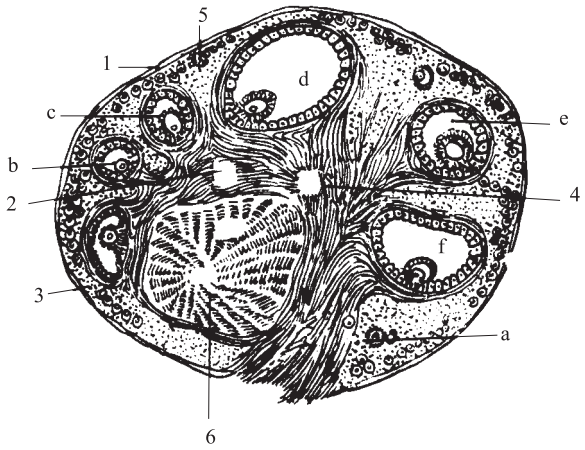


Fig. 20.5 Female reproductive organs of mammals: (a) rabbit; (b) woman.

Structure of the Ovary: Every ovary is enveloped by a connective tissue capsule and is attached to the uterus with ligaments. The ovary is lined by a germinal epithelium which proliferates thousands of primordial follicles during the embryonic life of an individual, and the remaining degenerate into *atretic follicles* (Fig. 20.6).



1. free border; 2. strome; 3. connective tissue; 4. blood vessel; 5. graafian follicles; (a to f—advanced forms of graafian follicles) and 6. corpus luteum.

Fig. 20.6 A cross-section of the mammalian ovary showing stages of egg maturation.

The ovary is differentiated into a cortex and a medulla. Within the cortex of the ovary, oocytes differentiate inside several layers of follicle cells forming *follicular epithelium* or *granulosa.* The stroma of the ovary surrounding the follicular epithelium develops into a connective tissue layer, called *theca* which differentiates into a glandular and estrogen-synthesizing layer called *theca interna* and the other layer called as *theca externa.*

Oogenesis: Inside the ovary the primary oocytes undergo two meiotic divisions (Fig. 20.1b). As a result, a secondary oocyte and a first polar body are produced. Subsequently, the secondary oocyte and the first polar body underlie reduction division and the secondary oocyte again divides to give rise to an ovum and another polar body. The ovum is haploid in nature and the polar bodies degenerate and become absorbed in the layer of cells just outside the egg. At sexual maturity the pituitary gland secretes FSH, which along with LH, regulates ovulation. In the stroma of the ovary, the ova are in various stages of maturation. Groups of germinal epithelial cells grow into the stroma to form follicles, one of which enlarges to become an oocyte while others form a layer around it. As the follicle grows, the follicular cells increase in number and size, and form several layers around the oocyte. The follicles move into the stroma and later on secrete a striated polysaccharide membrane called *zona pellucida* around the oocyte. Electrone microscopic study has shown, that certain microvilli from the follicular cells and the oocyte extend into the zona pellucida which serve for transport of metabolities. The layer of follicular cells surrounding the zona pellucida constitutes *corona radiata.* Soon after a cavity arises in the follicle cells containing *liquor folliculi* which separates an outer layer of follicle cells called *membrana granulosa* from the celis surrounding the oocyte known as discus proligerus (Fig. 20.7). When this condition appears, the follicle is fully

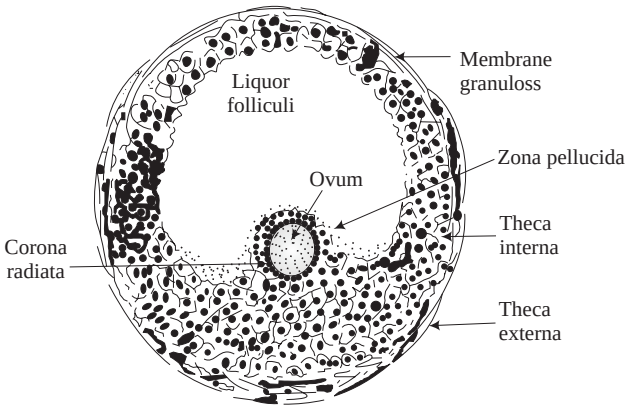


Fig. 20.7 Diagram showing a mature graafian follicle.

matured and called as *graafian follicle.* The graafian follicle migrates to the surface of the ovary and ruptures, releasing the oocyte (now the secondary oocyte) which has so far undergone one maturation division, making its way into the fallopian tube.

Within the graafian follicle, cells secrete estrogens which promote secondary sex characters and development of the uterus, mammary glands and courtship behaviour. Just before ovulation, progesterone is also secreted from the follicle. After the discharge of the oocyte, cells of the graafian follicle form a yellow body, the *corpus luteum,* in the follicular cavity and the space evacuated by the oocyte. Corpus luteum has an endocrine character and secretes progesterone and relaxin which maintain pregnancy, cause lactation and help in child birth. If pregnancy does not occur, the corpus luteum degenerates and becomes *corpora alibcantia.* Corpus luteum occurs in all vertebrate ovaries except birds.

20.4 BREEDING CYCLES

Animals can be broadly divided into two categories: (1) continuously breeding types, and (2) animals with specific breeding seasons. In continuous breeders no definite season is discernible. However, distinct peaks of proliferation in the gonads are observable. Monkeys, apes and man are year-round breeders. Most animals produce gametes during specific breeding seasons. Such seasons are generally annual and sometimes biannual, intervened by inter-mittent periods of sexual inactivity. Among seasonal breeders, some animals show regression of gonads in both sexes during the period of sexual inactivity, whereas in others the females become sexually inactive, while males show continuous spermiogenesis.

Breeding cycles are influenced by rhythmic annual changes in day lengths and temperatures. According to day length, the animals are classified as long-day and short-day breeders. Such photoperiodic stimuli affect the reproductive organs through nervous and endocrine systems. In mammals, the pituitary gland exerts chief endocrine control on reproduction. This gland begins to secrete gonad-stimulating or gonadotrophic hormones just at the start of the breeding cycle. This

stimulates the reproductive system which increases in size and becomes functional. During the cycle, there is always an enhanced level of gonadotropins. When the breeding season is over, the level of gonadotropins again falls to a low level, making the reproductive system nonfunctional and reduced in size.

20.5 HORMONAL CONTROL OF SEX AND REPRODUCTION

In complex animals there are a series of interlocking and synchronized events which must follow in a sequential manner. Events like shedding of germ cells, fertilization, gestation, parturition and lactation require accurate timing for the purpose of reproductive efficiency. In order to maintain this, all animals have evolved a sort of time-clock or a system of checks and balances comprising two interlocking systems, viz. an endocrine and the other nervous system. These two systems are able to control most of the reproductive events which are mutually controlling, cyclic and repeatable.

The primary hormones that regulate sex and reproduction are the sex steroids such as androgens, estrogens and progesterone which have masculinizing, femininizing and gestational effects, respectively. These hormones are secreted from the gonads called *target glands* which are in turn under the pituitary control. The tissues recognize their own trophic hormones and ignore others. The pituitary gland manufactures many hormones and their mechanism of action varies considerably. The gonadotropins are secreted from the anterior pituitary (adenohypophysis) and affect their target glands—the gonads. The existence of gonadotropins was indicated by crude pituitary extracts in hypophysectomized rats. In the male mammal reproductive behaviour and reproduction are mainly under the control of LH (luetinizing hormone) and FSH (follicle stimulating hormone). Not much is known about the role of FSH in males; however, it is supposedly concerned with gametogenesis. LH stimulates the maturation of interstitial cells (Leydig cells) and secretion of testosterone. It also causes depletion of interstitial cell's cholesterol in gonads of both sexes. These hormones are not known to have any extragonadial function. Besides FSH and LH, pituitary also secretes prolactin (lactogenic hormone) which exerts its control over some paternal behaviour of the males. LH stimulates the testes to produce androgens, the male sex hormones which increase at the start of the breeding cycle. Sperms are then actively produced. However, in man sperm production starts at sexual puberty and may continue throughout the life. If the androgens are present in excessive amounts, they have an inhibitory effect on the pituitary (Fig. 20.8).

20.6 HORMONAL CONTROL IN FEMALES

If the pituitary gland is removed at an early stage in rats, the ovaries fail to develop, while in adult condition, removal of pituitary leads to ovarian atrophy and the follicles do not develop beyond the antrum stage. The gonadotropins regulate gamete formation and the hormone secretion by the ovary is mediated by them. At the start of the breeding season, the pituitary becomes active and secretes prolactin and FSH. Prolactin induces maternal behaviour and FSH induces ovarian activity by stimulating follicle growth. The follicle cells around the egg produce female sex hormones, the estrogens which promote follicular growth and secondary sex traits. When estrogens increase beyond

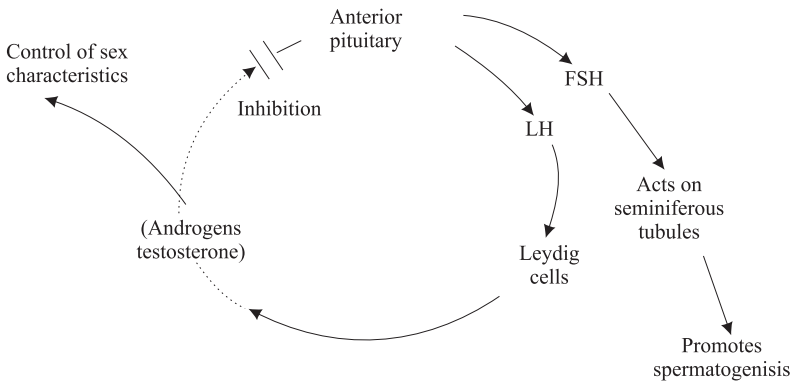


Fig. 20.8 Control of testosterone secretion.

a minimum required level, they exert an inhibitory influence on the FSH production. Pituitary also produces LH, a trophic hormone which causes ovulation. If the concentration of LH rises, the concentration of FSH falls, thus showing antagonistic behaviour (Fig. 20.9).

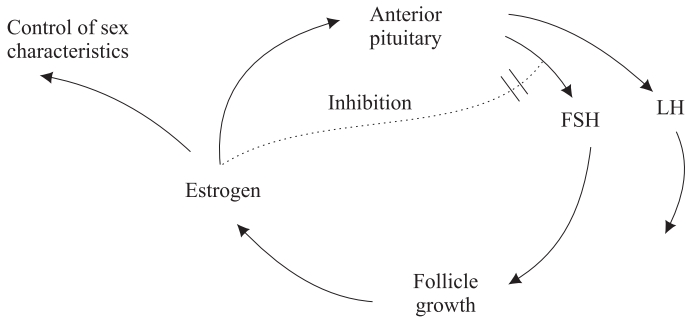


Fig. 20.9 Action of gonadotropins in mammals during egg maturation.

This shifting of hormonal balance induces ovulation. During ovulation the follicular wall ruptures and the mature ovum is released in the coelom. In case of rats, there is yet another trophic hormone-LTH (luteotropic hormone). Secretory activity of the corpus luteum is dependent on stimulation by the LTH which causes release of estrogens and progesterone.

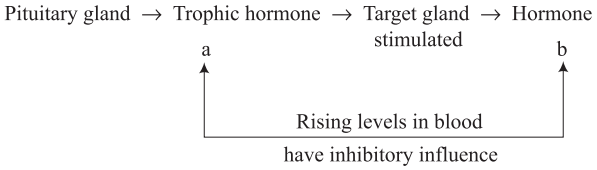
Neuroendocrine Integration

Release of gonadotropins is regulated by centres in the hypothalamus which mediate their effects by neurohumoral substances transported to the anterior pituitary lobe through portal vessels. There are a few hormones that are secreted in direct response to the concentration of particular metabolities in the blood or direct stimulation by secretory motor nerves. However, the pituitary is subject to a variety of environmental influences. The anterior pituitary produces trophic hormones which function primarily

as regulators of other endocrine glands called *target glands.* The secretion from the target glands continues so long the trophic stimulation continues.

In 1932, C.R. Moore and Dorothy Price gave evidence that there is a reciprocal relationship between the pituitary and its target glands, which is called negative feedback. However, feedback system alone is not enough to account for pituitary secretion.

Secretion of pituitary is subject to a variety of environmental influences that modify the rate of its secretion. The influences upon pituitary are communicated to it by the central nervous system (CNS). The neural control of posterior pituitary (neurohypophysis) is understandable, but the anterior pituitary poses actual problem since it has no direct neural connection. This problem was overcome by the discovery of a group of blood vessels called the *hypophysial portal system* which connect the hypothalamus with the anterior pituitary.



The afferent impulses of neuroendocrine reflexes may reach the brain through nerve pathways or circulation, or they may also be psychogenic. The efferent nervous signals to the pituitary are carried chiefly through the hypophysial stalk, but most of the stimuli to the anterior pituitary (adenohypophysis) are *humors* which are transmitted via hypophysial portal circulation. The afferent impulses may arise in one of the organs of special sense or in general the sensory nerve endings. The following kinds of effects explain the concept of humoral transmission: (1) Rabbits tend to ovulate only after coitus or sexual stiinulation (genital stimulation → CNS → anterior pituitary-ovaries-uterine changes); (2) Suckling is associated with the following cyclic changes- (suckling → CNS → pituitary → lactation). The demand for the release of a hormone of adenohypophysial origin in most cases is transmitted via the afferent nerve endings, and the hypothalamic neurosecretory cells respond by elaborating certain *releasing factors* that are transported through the portal system.

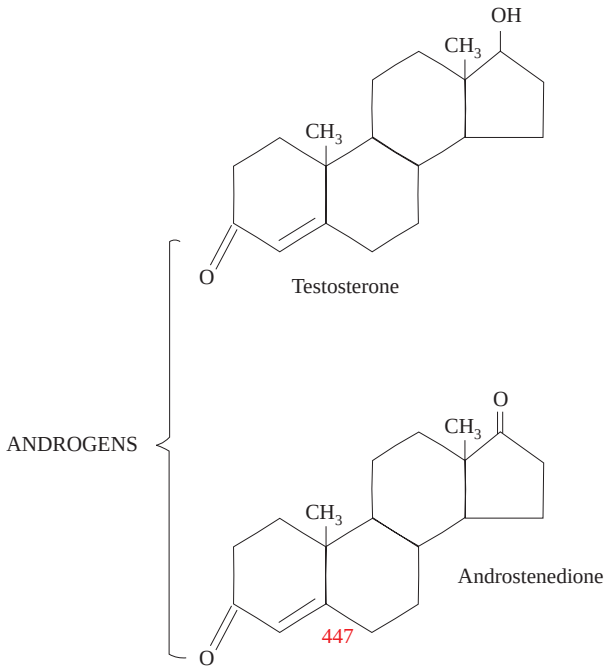
20.7 GONADAL HORMONES

Among all classes of vertebrates, gonadal hormones are responsible for the development of secondary sex characters. However, in certain animals the sex behaviour is also controlled by them. Hormones secreted from the testis and the ovary are steroid compounds, which, besides controlling secondary sex characteristics and sex behaviour, exert protein anabolic effects.

Testicular Hormones

Androgens are the male hormones secreted by the interstitial tissues of the testis. A number of androgens have been identified from the testis which are generally referred to as C-19 steroids. *Testosterone* is the principal male hormone synthesized by the interstitial cells (Leydig cells) of the testis. The physiologic functions of testosterone can be demonstrated by studying the effects on castrated animals. In a young castrated animal the accessory reproductive organs like epididymis, vas

deferens, seminal vesicle and penis do not develop, and the secondary sex characters also fail to appear. If an adult animal is castrated, the reproductive organs atrophy without much effect on secondary sex characteristics. However, injections of testosterone help in rebuilding the atrophied organs. Testosterone is synthesized from the cholesterol present in the interstitial cells.

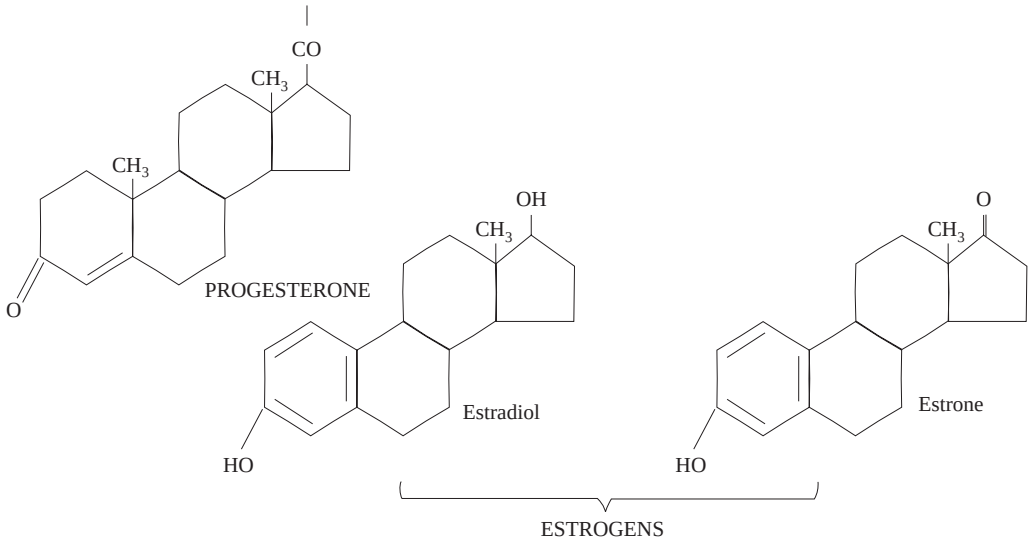


In a normal male, about 4 to 9 mg of testosterone are secreted per day. Besides promoting the growth and Junction of sex organs, testosterone also helps in muscular and skeletal growth spurt at puberty.

Ovarian Hormones

There are two main ovarian steroids in females: *Estrogens and Gestogens.* Estrogens are produced by the cells of the developing graafian follicles, and the gestogens (progesterone) are derived from the corpus luteum and certain other tissues in the mammals (Velle, 1963).

Estrogens: The estrogens are Cl8 steroids and differ from androgens in lacking one carbon. Estrogens are synthesized in the ovary, adrenal cortex, placenta and Leydig cells. The estrogenic group of hormones comprises three hormones: 17 β-estradiol is the most active hormone present in almost all vertebrates. Estrone is a circulating hormone. Estriol is found in the urine of pregnant women and in the placenta. The estrogenic or follicular hormones induce estrous cycle and help in maintenance of secondary sex characters and the accessory sex organs. Production of estrogen suppresses the FSH level.



PROGESTERONE: Progesterone is a luteal hormone which is synthesized by corpus luteum, adrenal cortex, placenta and the testis. It is, strictly speaking, a pregnancy hormone which acts on genital tissues and induces proper functioning of breast. The hormone has an antiestrogenic effect and blocks the continued growth of endometrium produced by estrogen. When progesterone begins to act, thickening of the endometrium stops and the endometrial tissue becomes secretory. In case the pregnancy is established, the progesterone levels decrease and corpus luteum is maintained. However, if fertilization does not occur, the estrogens and progesterone are suddenly decreased at the commencement of the next menstrual cycle.

In addition to progesterone, another hormone *relaxin* is also produced by the corpus luteum which causes softening of epiphysis and facilitates delivery. Small quantities of relaxin also occur in the placenta.

20.8 PUBERTY

Puberty is the period at which the organisms attain sexual maturity. Sex differentiation is complete and secondary sex characters are fully developed. During this period, there is rapid development of gonads and reproductive organs accompained by increase in body size. Different criteria are used in different animals for the recognition of pubertal age. Growth of pubic hair is one of the important criteria, but besides this, the organism experiences a number of morphological, physiological, endocrinological and psychological changes. In certain animals the testicles remain inside body cavity during immaturity and descend into the scrotal sacs at the pubertal age. In female rodents puberty is marked by the vagina opening and the occurrence of first estrous. It is at puberty that ripe spermatozoa first make their appearance in the seminal fluid. In temperate climate puberty begins in

boys at the age of 14 or 15 years, and in tropical climate puberty is reached somewhat earlier. Among women puberty occurs slightly earlier than men.

Physiological Changes

In males, there is spurt in muscular strength. Males can do more physical work than the females. Females experience deposition of subcutaneous fat layers which gives shape and contour to the body parts. At puberty the red cells in the blood increase in males. The morphological and physiological changes are dependent largely upon the functional development of the generative organs. The changes at puberty take place under hormonal control. It has been found that the gonads, adrenal and thyriod glands show gain in weight at the adolescent stage. In case of females, the volume of sella turcica increases as also the pituitary increases in size showing higher proportions of acidophils. Consequently, there is increased secretion of gonadotropins FSH and LH (ICSH). The seminiferous tubules of the testis and the follicles of the ovary develop due to increasing levels of FSH, and rising levels of LH cause Leydig cells to secrete testosterone.

It is striking to note that the events at puberty are initiated by the brain and not the pituitary. This has been successfully demonstrated in case of rats. If the pituitary of new born rat is grafted in place of an adult, pituitary begins to function immediately.

20.9 ESTROUS BEHAVIOUR

In a number of cyclically breeding animals the ripening of follicle or follicles marks the beginning of the breeding season which, of course, commences at sexual maturity or pubertal age. This event also marks the start of the estrous cycle. Three major events are observed in this process. These are: (i) the ripening of a follicle, (ii) rupture of the follicle and release of the ovum, and (iii) behaviour of the organism at the time of ovulation for achieving successful fertilization. The first two events are regulated by the gonadotropins secreted from the anterior pituitary, which are under the hypothalamic control. The last event is under the control of ovarian hormones.

Seasonal breeders are sexually inactive or less active during the anestrous period (non-breeding period). Continuous breeders undergo repeated cycles of sexual activity and permit copulation during a particular period, which is called the period of *heat* or *estrous.* The period from the beginning of one heat till the start of the next is called an estrous cycle. During this period certain physiological, morphological, and endocrine events take place which are all correlated.

Estrogen cycle is divisible into the following phases:

Estrous: It is the period of heat when the ovaries are considerably enlarged and deep red in colour due to maximal development of ovarian follicles. The ovarian follicles secrete increased quantity of estrogen which is responsible for bringing about psychological manifestations of heat. It is during this period the animal allows mating. In rats, only estrogen can bring about heat, whereas in guinea pigs traces of progesterone are also necessary. In certain domestic animals like cows and mares, *quiet heat* sometimes occurs which, is due to lack of enough estrogen quantity.

Table 20.2 Duration of Estrous Cycle and Heat in Some Animals

Animals	*Length of estrous cycle in days*	*duration of heat*
Cow	21	13-17 hours
Guinea pig	16	6-12 hours
Rat	4-5	13-15 hours
Rabbit	16	— —
Dog	3-4 months	7-10 days
Woman	28	12-25 days
Rhesus macaco	28	As in women

In the heat period many animals show increased activity which is caused by estrogen. Large amounts of mucus are secreted from the cervix during estrous period.

Metestrous: This is a stage when ovulation occurs and corpus luteum is formed. The events are under the influence of progestrones; hence it is progestional phase.

Proestrous: This is follicular phase or the stage when the follicles are ripening.

Diestrous: This is the resting phase or the period of sexual inactivity.

Vaginal and Uterine Changes During Estrous Cycle

The events during the estrous cycle are reflected in the changes taking place in the vagina and the uterus. These changes are, however, under the influence of ovarian hormones. The vaginal epithelium undergoes cyclical changes, it is torn down and rebuilt. The changes in the vaginal histology during the estrous cycle are tabulated (Table 20.3).

The uterine changes during the estrogen cycle are reflected in the striking changes taking place in the uterine endometrium and its glands. During follicular phase the uterine glands are simple and straight, often with few branchings. This stage is under estrogen effect. The endometrium is thin and perforated with holes. During the luteal phase, the endometrium increases in thickness and the glands grow rapidly in diameter showing abundant branching. This stage is under progesterone stimulation (Fig. 20.10).

Menstrual Cycle

Menstruation occurs in primates only and is characterized by sloughing of the uterine endometrium completely accompained by vaginal bleeding. In contrast to non-primates having short period of heats, the primates permit copulation throughout the whole cycle. Menstruation occurs due to the with- drawal stimulus of estrogen and progesterone. In menstruating women, ovulation generally occurs midway between two menstruating cycles or 12 to 15 days following the beginning of menstruation. However, individual variations in the cycle are also found where the normal cycle is prolonged upto 33 days.

Table 20.3 Histological Changes in the Uterus and Vagina During Estrous*

Phase	*Events of the estrous cycle (in woman)*			
	Pituitary	*Ovary*	*Uterus*	*Vagina*
Pre-ovulation	Secretion of FSH	The oocyte begins maturation, a cavity develops in the granulose, and the thecal cells secrete folliculin.	Repair of endometrium takes place mucosa thickens, glandular tubes are straight and arterioles short and slightly sinuous	Increase in the number of acidophilic cells (about 50%)
Ovulation	Simulataneous secretion of FSH and LH	Graafian follicle is voluminous.folliculin and small amount of progesteron are secreted. Towards the 13th day the follicle ruptures, ovulation takes place.	Glandular are hypertrophied cells full of glycogen, arterioes elongate and coiled arterioles elon	Acidophilic cells increase up to 60 to 80%
Pre-menstruation	Secretion of LH and also of LTH	The corpus luteum synthesizes proge sterone and 17 β-estradiol.	The endometrium is thick, glandular tubes numerous and convoluted, cells rich in glycogen, arterioles numerous and wound round the tubes.	
Menstruation			Spiral arteries undergo constrictions leading to ischemia of the surface of mucosa: this area breaks away and is eliminated with the blood of menses The mucosa will finally be reformed from the deeper layers.	

*Adapted from B. Rhyback (1968). Principles of Zoophysiology, Volume I

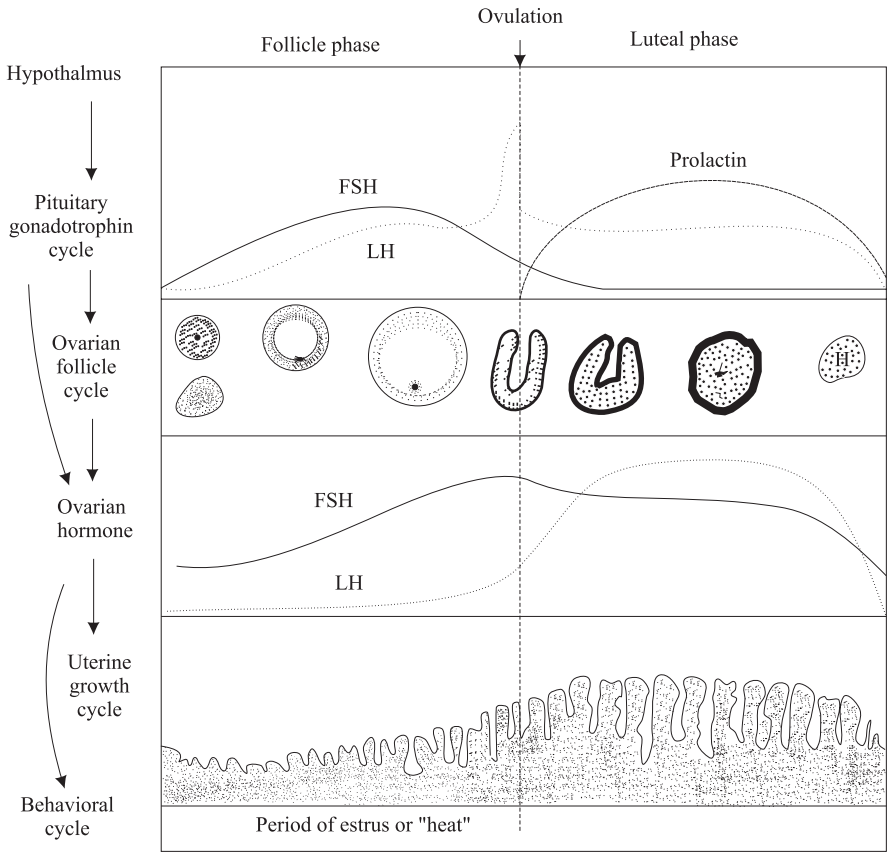


Fig. 20.10 Scheme of the ovarian events and change in the endometrium during the normal estrous cycle (menstrual cycle) in a female (woman).

20.10 OVULATION

The act of ovulation involves the rupture of ripe follicles and release of the ovum. These processes are related to the hypothalamic function. In majority of the animals, ovulation occurs shortly before or after the end of heat (e.g. mare rat, cow, etc.). Such animals are called *spontaneous ovulators.* On the other hand, in certain animals ovulation is induced by certain acts like genital stimulation, copulation, etc. These animals are called *induced ovulators.*

The hypothalamic-endocrine mechanism seems to be involved in the process of ovulation. To initiate the process, a sudden increase in the secretion of luteinizing hormone (LH) is required over a period of a few minutes. In rabbits, about 10-11 hrs elapse between LH release and ovulation. In women, ovulation takes place midway between the two menstruation periods, i.e. from 13th to 17th day of menstrual cycle. A number of theories have been advanced to explain the mechanism of ovulation. It has been suggested that the follicles rupture when they reach ovulatory size. Turgor

pressure created by liquor folliculi is also responsible for the ovulation. However, these views are held untenable. It has been suggested that ovulation is a two-stage phenomenon in the first stage, the follicular growth is observed under the stimulus of circulating gonadotrophic hormone. Growth continues till the follicles reach the ovulatory size. In the second stage, there is reduction in the concentration of gonadotrophic hormones which is not enough to maintain active follicular proliferation. Hence, the follicle becomes *physiologically atretic* and ovulates.

Formation of Corpus Luteum

The corpus luteum is formed in the ruptured follicles. After the ovum is discharged, the granulosa cells collapse and are transformed into luteal cells which secrete progesterone. If pregnancy is established the corpus luteum is maintained. In the absence of pregnancy, the corpus luteum degenerates and the waning corpus is known as *corpus albicans.* Regression of the corpus luteum is associated with the withdrawal of progesterone.

Transport of the Ovum

After the ovum has been shed, it must be transported through the oviduct (fallopian tube). The passage of ovum may take several days. Fertilized or unfertilized eggs are propelled through the oviduct aided by peristaltic waves set up in the oviduct walls. It has been suggested that the movement of eggs in the oviduct is influenced by the endocrine state of the animal. Progesterone accelerates progression of the ovum through the oviduct, whereas estrogen arrests the egg movement. In most animals fertilized ova pass into the uterus at stages ranging from 8-32 celled stage to early blastocyst stage after about 24-140 hours of ovulation.

20.11 SPERM TRANSPORT IN THE FEMALE GENITAL TRACT

During coitus, the sperms are deposited either in the vagina (rabbit, dog, ewe, cow, man, etc.) or in the uterus (mouse, rat, cow, mare, etc.). In the mammals so far studied, fertilization occurs in the oviduct. This fact shows that the sperms are transported to the fertilization site from the site of insemination. The sperms are transported rapidly to the oviduct by muscular contractions of the duct system. The genital tract itself is responsible for the rapid transportation by the muscular activity of the uterus, oviducts, and the ciliary activity of the epithelial lining of the oviduct. It has been suggested that oxytocin is essential for the rapid transport of sperms from the cervix to the oviduct, since oxytocin is known to be released during physical and psychological excitation of the females.

Although quite a large number of sperms are deposited in the female genital tract, only a few reach the fertilization site. It has been shown by experimental work that the fertilizing capacity of sperms is of much shorter duration than the duration of their motility (Table 20.4).

Sperms are deposited in the female tract in the seminal plasma which serves as a vehicle for transport. Besides this, the seminal plasma is a source of metabolic substrate for the spermatazoa and supplies requisite enzymes effective in fertility. An important fact has been discovered that the spermatozoa do not fertilize ova unless they have undergone a period of incubation in the female tract. This strange phenomenon has been called *capacitation.* The time for capacitation in rabbits and rats varies from 2 to 6 hours.

Table 20.4 Survival Times and Duration of Fertilizing Capacity of Sperms in the Female Genital Tract

Animal	*Duration of fertility* (*in hours*)	*Maximum duration of motility* (*in hours*)
Rabbit	30-32	—
Rat	14	17
Guinea pig	21-22	41
Cow	28-50	96
Man	28-48	48-60

Capacitation of sperms is influenced by hormonal conditions and exposure to cervical, uterine or tubal secretions before they acquire the ability to fertilize the ova. Due to capacitation, spermatozoa lose their microsomal cap allowing release of subtances necessary for penetration of egg. Capacitated sperms show a higher metabolic activity.

Transport of Ova

After ovulation, the ova are transported through the oviduct much slowly as compared to the transport of sperms. The exact mechanism is still uncertain. However, propulsion of the fertilized or unfertilized eggs takes place through the oviduct by the peristaltic waves and the ciliary beats towards the uterus. Movement of the eggs in the oviduct is also influenced by the endocrine state of the animal. Progesterone accelerates the rate of descent of the egg and estrogen arrests the progression in the tube. In most cases, fertilized ova pass into the uterus at stages ranging from 8 cell to early blastocyst stage. The fertilizable life of the ovum is also a variable one and ranges from 6 to 24 hours in different animals.

20.12 IMPLANTATION

In the absence of fertilization, the uterine endometrium undergoes regressive changes and degenerates at the end of luteal phase. However, following fertilization, the endometrium is retained in an extensive proliferative stage and is prepared for the reception of the fertilized ovum. Glycogen, lipids and other substances accumulate in the epithelium and the stroma, and are used up as nourishment during pregnancy.

For successful implantation, presence of luteal hormone is necessary. In rabbits, where the blastocysts remain for 3-4 days free in the uterus, progesterone has been found to be necessary for implantation. Besides this, there is an increased blood supply just below the uterine epithelium at the time of implantation. During blastocyst formation, *zona pellucida* layer gradually becomes thin and later gets dissolved so that the trophoblastic cells can come in contact with the maternal endometrium. Implantation is effected in one of the following ways: (1) trophoblast becomes the active agent, while the maternal tissue remains passive, and (2) the egg remains passive and the uterine wall becomes active. The trophoblastic wall of the blastocyst supplies nutrition to the egg and does not participate in the actual implantation.

The position of the blastocyst implants varies in different orders. It may be central as in case of platyrrhine and catarrhine monkeys, some insectivores, etc. Sometimes it may be eccentric as in many rodents, or it may be interstitial as in guinea pig, chiroptera, hedge-hog, man, etc.

Implantation could be initiated only under certain conditions; the uterus must be receptive and ovum should be mature enough at the same time. These events are precisely timed and determined by the secretion of ovarian hormones which function under the influence of hypothalamic-hypophysial axis. Any disturbance in the condition would either delay or prevent implantation.

Experimental studies on the endocrine influences on implantation have shown that implantation is a sequence of integrated mechanisms which are largely progesterone dependent. Attachment of the blastocyst in the uterine epithelium is progesterone dependent. In rodents (mouse or rat), implantation events have been studied to show that the blastocyst is passive and the endometrium is sensitized by progesterone. Although progesterone is responsible for implantation, estrogen appears to be indispensable. There is a definite progesterone-estrogen sequence required for implantation in rat.

20.13 PLACENTATION

The mammalian ovum (blastocyst) is small and can grow to a limited extent only since the food supply in the developing embryo is not sufficient. Therefore, a special tissue is formed by modifications of the uterine mucosa and a part of the blastocyst which helps in the transmission of nutrients from the maternal circulation to the growing embryo. This special tissue is called *placenta* which is formed by apposition or fusion of the foetal membranes to the uterine wall (mucosa) for physiological exchange.

Placenta is a complex structure which varies greatly in various orders (Table 20.5). Once the blastocyst has been positioned in the uterus, it appears to be compressed between the wall of the uterine mucosa. Shortly after attachment, the foetal membranes begin to appear and soon get organized in the form of chorion, amnion and allantois.

Functions of the Placenta

Placenta is a unique organ which performs several functions. The principal functions of placenta are: (1) to supply nutrition to the growing foetus derived from the mother's blood, (2) to provide a site of important hormones of pregnancy, some of which are stored while others are produced by it. In higher mammals where pregnancy goes beyond the luteal phase of the cycle, some control mechanisms are necessary that are mainly provided by the placenta. After establishment of the placenta, pituitary is no longer able to produce sufficient accounts of gonadotrophins; hence placenta takes over as an endocrine gland being located inside a target organ.

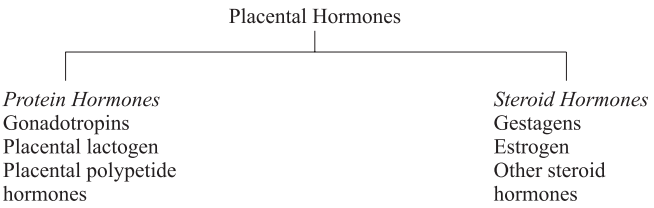
In rat, a luteotropin is produced by the placenta, which stimulates the corpus luteum to persist and produce gestagens and estrogens. In many animals, the placenta takes over the production of ovarian hormones which vary in quality and quantity from species to species. The placenta secretes protein as well as steroid hormones:

In case of women, a chorionic gonadotropin (CG) is secreted by the chorionic villi of the placenta. Functionally it helps in the maintenance of pregnancy and corpora lutea and also influences to some extent finer mechanisms of steroid synthesis and metabolism.

Table 20.5 Types of Placentae According to Grosser's (1909; 1927) Classification*

	Types of placenta	*Gross shape*	*Examples*	*Relation to endometrium*
1.	Epitheliochorial	Diffuse	Pig, horse and donkey	Non-deciduate
2.	Syndesmochorial	Cotyledonary	Sheep, goat, cow	Transitional
3.	Endotheliochorial	Zonary to discoid	Cat, dog, ferret	Deciduate or con-joined
4.	Haemochorial	Discoid, double discoid or Zonary	Man, monkey	"
5.	Haemoendothelial	Discoid, cup shaped or spheroidal	Guinea-pig, rat rabbit	"

* Adapted from Marshall's *Physiology of Reproduction,* Vol. II.



The human, placenta probably secretes a lactogenic hormone, *human placental lactogen* (HPL). Functionally it has a luteotrophic effect on the ovary. There is evidence for such a placental hormone occurring in other species, including rat, mouse and rhesus monkey.

Relaxin is yet another hormone secreted by the placenta and also by corpus luteum during labour pains.

The placenta produces gestagens (progesterone) and estrogens in large amounts during pregnancy to maintain conceptus for some time after nidation. However, it is not known as to which cells are responsible for their formation.

Among other steroid hormones, presence of androgens has also been claimed in the placenta, which probably are derived from the mother and the foetus.

20.14 PARTURITION

When the period of embroyonic growth is completed, the conceptuses are expelled from the uterine lumen. The gestation period in different mammals varies considerably as would be clear from the Table 20.6.

The process of parturition or child birth involves a series of events, some of which have not been understood so far. The process of parturition is initiated by rhythmic contractions of the uterus causing *labour pains.* The contractions are well pronounced which are supposed to be induced by

Table 20.6 Table Showing Gestation Period of Some Mammals

Animal	*Litter size*	*Period of gestation (in days)*
Mouse	Multiple	19-21
Rat	6-9	22
Rabbit (domestic)	Multiple	31
Guinea pig	2-4	64-68
Dog	Multiple	61-64
Cat	4	63
Rehesus Monkey	1	146-180
Sheep (domestic)	1-2	144-152
Lion	2-6	105-113
Man	1	280-9
Cow	1, rarely 2	277-290
Chimpanzee	1, rarely 2	202-278
Horse	1	330-345
Ass	1	365
Arabian Camel	1	370-440
Elephant	1, occassionally 2	641-676

nervous stimulation. It has been suggested by Feldman (1920) that the centres of uterine contractions lie in the cortex, medulla and cerebellum. On the other hand, it has been argued by some that the centre of uterine contractions is located in the lumbar region of the spinal cord, and thus parturition may be considered only a reflex act.

There has been considerable evidence in favour of hormonal control of the uterus during parturition. Under normal circumstances, labour is probably precipitated by alteration of the hormonal balance. Rhythmic contractions of uterine muscle fibres or bundles are under the influence of estrogen. It has been suggested that the uterine activity is enhanced by the release of oxytocin into the blood stream from the posterior lobe of the pituitary which ultimately leads to parturition. However, the role of oxytocin in precipitating labour has not been clearly understood. Experimental evidences are available which suggest that normal labour can occur in totally hypophysectomized rats, although the pituitary stalk and pars tuberalis were left intact. It may be just possible that oxytocin may still be present in the blood stream even after removal of the pituitary.

The duration of the gestation is almost fixed in anyone animal species, and may vary within narrow limits only. Under exceptional circumstances, the period of gestation may be unduly prolonged. Prolonged periods of gestation have been observed amongst women, cows, mares, rodents and pigs. Prolonged gestation may be due to large foetuses which are unable to be delivered in the normal course. However, hormonal disorders also seem to be the cause of this. Before parturition, the progesterone levels drop to a considerable extent with a concomitant elevation of estrogen titres, but in animals with prolonged gestation, the progesterone levels do not drop. The reasons for this disturbance are not yet clear.

Certain placental hormones are also necessary during parturition. A hormone, relaxin, which is secreted by the placenta as well as by the corpus luteum, helps the opening of the cervix which

otherwise remains closed during pregnancy. The cervix beocmes flaccid and relaxed so that the conceptuses can be expelled out through it.

20.15 LACTATION

The mammary glands are characteristic structures of mammals and form an important part of the sexual apparatus in the female. The glands secrete milk and provide nourishment to the newly born. The complex phenomena comprising the synthesis of milk, its flow through alveolar spaces and its ejection from the mammary glands is called lactation.

Mammary Glands

Mammary glands are present in both sexes. However, they are functional in females only. The number of glands is variable, but the average number is characteristic of species and related to the litter size. Their position also varies, situated as they are in the thoracic region in primates and elephants, whereas they are inguinal in position in ungulates and cetaceans.

The glands are ectodermal in origin and derived from sebaceous glands. In the embryo, a milkridge or a mammary crest arises on either side extending from the forearm to the abdomen. These are thickenings of the epidermis due to proliferation of the Malpighian layer. At several points in the milkridge, centres of proliferations appear as future teats.

At birth, the glands are rudimentary. During the period from birth to puberty, glandular growth is slow, but there is a general increase in mammary duct system. At puberty further growth of the glands takes place due to alveolar development under hormonal stimulation. Generally the extent of mammary development in sexually mature females is governed by the type of the ovarian cycle. In many species, ovarian cycles are accompained by cycles of mammary growth and regression. In primates, a gradual increase in the mammary gland and its alveolar tissue is seen with age. In women, especially, there are cyclic changes in the mammary parenchyma and alveolar system during luteal phase followed by complete regression after menstruation.

Endocrine Factors in Mammary Growth

Although there are no known neural mechanisms involved in the development of mammary glands, the process of lactation, particularly ejection of the milk from the glands, may be partially influenced by nervous mechanisms. Mammary growth is largely independent of the centrtal nervous system. Experimentally, it has been demonstrated that mammary glands regress after bilateral ovariectomy. It may be concluded from this observation that ovarian hormones are concerned in mammary growth. Studies of the mammary development associated with various types of estrous cycle and pregnancy demonstrate that estrogenic hormones stimulate duct development and alveolar proliferation during early pregnancy. However, a hormone from the corpus luteum also helps in the process. In rats, optimum growth of mammary duct system has been observed when the estrogen doses are kept within narrow limits. High doses develop stunted glands.

Progesterone also induces alveolar growth and the function of corpus luteum in rats, large doses of progesterone promote growth of mammary ducts and alveoli. However, in majority of the animals.

combined action of estrogen and progesterone promote the growth. The anterior pituitary has 'some mammogenic action. In hypophysectomized rats preparations of prolactin (LTH) with ovarian hormones (estrogen and progesterone) have been observed to promote mammary development. Prolactin is, of course, responsible for promoting secretion of milk in the breasts, which have already developed under combined influence of estrogen and progesterone.

Endocrine Factors in Lactation

There is no evidence on record to show the existence of any secretory nerves directly controlling the permeability or synthetic activity of the alveolar epithelium. Milk secretion is under hormonal control, although some kind of nervous function is also operative during lactation. During lactation period, hypophysectomy brings about complete cessation of secretion. Injections of the extracts of anterior pituitary were observed to initiate lactation process. In farm animals estrogens induce lactation. However, in rats estrogens have an inhibitory effect. Role of parathyroids is also important during lactation. In this process, there is a heavy drain of calcium and phosphorus which are precursors of milk formation. Circulating parathormone levels help in making up calcium and phosphorus deficiency. Insulin may influence the mammary glands in two ways: (1) by effecting general intermediary metabolism to maintaining milk formation; and (2) by its specific role in carbohydrate metabolism. Injections of posterior pituitary extracts are known to increase milk flow in goat by exerting a galactopoietic effect.

Lactation normally sets in after parturition. Several theories have been put forward explaining the role of hormones initiating lactation:

(1) Estrogens, probably of placental origin, suppress lactation during pregnancy by inhibiting the secretion of prolactin by hypophysis which has an inhibiting influence on the mammary gland. After parturition, estrogen levels decrease and prolactin block is lifted permitting release of prolactin by anterior pituitary. This initiates lactation.

$$\text{Estrogen} \xrightarrow{\text{inhibits}} \text{hypophysial prolactin}$$

(2) According to Meites and Turner (1942), estrogen stimulates secretion of prolactin by the anterior pituitary, but during pregnancy the presence of progesterone has an antagonizing influence on the estrogen function which decreases the prolactin potency of pituitary. Thus during pregnancy prolactin output of pituitary is so low that lactation cannot be initiated. After parturition the overriding influence of progesterone is removed leaving estrogen free to stimulate prolactin secretion.

(3) Low levels of estrogen stimulate the anterior pituitary to secrete hormones concerned in lactogenesis and galactopoiesis. High levels of estrogen have an inhibitory influence. After parturition, estrogen levels will naturally fall and activate anterior pituitary to secrete lactogenic hormones, initiating milk secretion.

(4) During gestation, mechanical distension of uterus suppresses lactation (Selye et al., 1934). Just prior to parturition this stops.

(5) Flow of milk following parturition is due to the beginning of the ejection of alveolar contents under the influence of oxytocin secreted during labour (Peterson, 1944).

Other Factors Influencing Lactation

Emotional upsets cause inhibition of milk secretion, perhaps through the hypothalamus. There is no direct nervous control over the milk secretion. Abdominal and thoracic sympathectomy cause interference in lactation. A suckling stimulus is closely associated with milk secretion. Suckling provides a nervous reflex which elicits a prolactin secretory response, maintaining constant flow. While milking a cow, tactile stimulation causes the milk flow and the pressure in the udder rises aiding in the flow. This phenomenon is called *let-down* of milk. This is an active process due to a nervous reflex excited by stimulation of the teat.

Nature and Chemical Composition of Milk

Milk is an opaque white fluid containing casein, lactose and milk fat as three major constituents. Milk is the only natural source of casein and lactose (Table 20.7). Milk solids are of two types: fatty solids and non-fatty solids. Milk fat is a complex mixture of glycerides, and fatty acids like oleic acid, myrstic acid, palmitic acid, steatic acid, butyric acid, capric acid, lauric acid and linoleic acid, etc. The non-fatty constituents include phosphoproteins, casein, lactose, lactoalbumin, lactoglobulin, pigments, and traces of urea, uric acid, creatine and creatinine. Besides these, enzymes and mineral salts like calcium phosphate and sodium chlorides, etc, are also present.

Table 20.7 Percentage Composition of Milk of Various Species of Mammals

	Water	*Fat*	*Sugar*	*Casein*	*Other proteins*	*Ash*
Man	88.50	3.30	6.80	0.90	0.40	0.20
Rat	68.30	14.80	2.80	9.20	2.60	1.50
Goat	82.34	7.57	4.96	3.62	0.60	0.84
Buffalo	86.04	4.63	4.22	3.49	0.86	0.76
Sheep	79.46	8.63	4.28	5.23	1.45	0.97

The milk produced from the breasts during the first 2 to 3 days after puerperium is called *colostrum.* In cattle, it is produced for about 6 to 12 days. Colostrum is rich in nitrogenous substances and globulins, and poor in sugar contents. In cattle, the ingestion of colostrum is of great importance. It has laxative property and also helps in the protection of the newborn against diseases. In many species like cow, horse and goat, the placenta is relatively impermeable to antibodies. In such cases, the colostrum acts as a means to transmit maternal antibodies to the offspring for a short period after parturition. In woman, colostrum is however, of less importance since placenta is permeable to maternal antibodies which are transmitted to the foetus before birth.

CHAPTER

21 The Genetic Code and Protein Synthesis

In the last few chapters, we have devoted our attention to a variety of physiological processes that are essential to maintaining the living machine. All these processes are intimately correlated and are characteristic of an individual with a high degree of organization. As stated earlier (vide chapter 3, Biological oxidations), the living matter represents a high degree of organization kept in a steady state by a continuous supply of free energy. Energy is the basic requirement for the maintenance of the cellular organization.

In the preceding chapter, we have seen that the organisms perpetuate their own kind through systematic behaviour of the nucleus. The offsprings are created according to the instructions provided by the genes located on chromosomes, and through the inheritance of genes by successive generations. Thus creation of new life becomes a controlled process. But, what is it that is inherited? The organisms do not inherit strong muscles, red blood, skin colour or any other trait. The organisms inherit genes which control all visible traits that determine the phenotype of an individual. The similarities between the offspring and the parent are due to genic control. The science of genetics deals with the study of such inheritance of characters from the parent to the offspring over generations through the mechanism of gene action. The science of genetics is vast and fast growing and it is not intended to present to the readers any information pertaining to the principles of inheritance. The scope of this chapter is rather limited. Our aim is to discuss the genic control of protein synthesis which is the basis of variation among organisms.

Recent years have witnessed a major breakthrough in the understanding of the concept of genes and their function. Genes are known by their effects and a knowledge of their pattern of inheritance and relationship to the behaviour of chromosomes during meiosis and fertilization provided biologists information as to how traits were passed on from generation to generation.

What is a gene? Are genes some particulate units residing on the chromosomes? These are intriguing questions which puzzled biologists for quite sometime. Biochemical and biophysical analyses of chromosomes have revealed that chromosomes are composed of DNA and protein and

that genes are DNA molecules themselves. Thus, it becomes necessary to discuss here the organization of chromosomes in order to understand the chemical nature of genes.

21.1 THE ORGANIZATION OF THE CHROMOSOME

The chromosomes are regarded to control all kinds of biochemical and physiological activities of the cell through tiny chemical units called genes. They replicate with precision during cell division and gametogenesis and help in the transmission of characters from one generation to another. Although chromosomes vary in number in different organisms, they maintain unique similarity in terms of their physical and chemical organization.

The genetic material of all cells is contained in the chromosomes which have a complex composition. The human chromosomes contain approximately 15 per cent DNA. 10 per cent RNA and 75 per cent protein, but the genetic material is almost always double stranded DNA. The viruses are the only exceptions which contain single stranded DNA and in certain cases the single stranded RNA functions as the genetic material. The chromosomes of viruses and bacteria are circular whereas in all other organisms the chromosomes are linear structures. A typical haploid chromosome of a complex organism is a cylindrical structure composed of two identical units, the *chromatids.* The chromatids are intimately twined around each other and each one is supposed to be containing about 8 fibrillar threads. Each fibril is composed of two double helices of DNA. Both the chromatids are attached with each other through a common point, the *centromere,* which represents a constricted region on the chromosome.

The chromosomes of complex organisms contain long stretches of non-informative DNA and some segments of DNA exist in multiple copies along a single chromosome. Although each species has a characteristic amount of DNA, the eukaryotes vary greatly in DNA content which is always more than the prokaryotes. One picogram of DNA, when properly stretched, would be equivalent to 31 cm of DNA. The chromatids are comprised of chromatin material which is heavily stained with uranyl acetate.

The chromatin is a viscous, gelatinous material which contains DNA, RNA, basic *proteins-histones* and non-histone proteins. Histone proteins and DNA are present in a fixed ratio of 1:1, but the non-histone proteins always vary in different tissues. Histones are small basic proteins rich in arginine and lysine, and bind intimately with DNA. There are four types of histones: H2A, H2B, H3, H4, each of which are present in equimolar amounts. There are certain regions in the chromosomes which remain condensed during interphase and early prophase. These are defined as *heterochromatin* regions which are genetically inactive. Besides these regions, the remaining chromosome remains in a non-condensed state and is called *euchromatin.*

What are Genes?

Cytogenetic observations suggested that the chromosomes contain DNA and that it is the hereditary material. Mendel gave the idea of discrete factors as hereditary determinants which are inherited. These ‘factors’ were later termed as *genes* and defined as particulate units arranged on a thread-like chromosome.

Morgan's work on *Drosophila melanogaster,* and later work with bacterial genetics on frequency of recombination led to the formulation of a correct definition of gene. Thus the gene is defined as a unit of recombination which cannot be subdivided by chromosomal breakage or crossing over and can be separated from its neighbouring segments by a crossing over event. As our knowledge of the gene advanced, it was discovered that the chromosomal unit functioning as the gene can undergo mutation and thus affect the physiological function or expression. It was soon realized that the effectiveness of a gene depends upon its relation to other neighbouring genes and as such there may be overlapping regions of gene function. From the studies of mutation and mutagenic agents it has been suggested that the unit of mutation could be much smaller than the functional unit, that is much smaller than the unit of recombination.

From the mutation studies in *Drosophila,* it has been found that the so-called white-eye gene exists in a number of alleles, hence a variety of mutations of this gene may give rise to various eye colours. From this, it has been surmised that a gene can exist in many forms with a difference in functions which account for distinct observable phenotypes.

21.2 REPLICATION OF DNA

From biochemical studies, it has been proved beyond doubt that DNA is the universal genetic material of all forms of life except certain viruses. The building blocks of DNA have been described in Chapter 1. In 1947, Chargaff demonstrated that DNA contains equal proportions of purine and pyrimidine bases, so that adenine and thymine, and cytosine and guanine are present in equimolecular proportions. These observations were quite significant which became clear from the elucidation of the *double helix* model of DNA by Watson and Crick in 1953.

The double helix model consists of two twisted polynucleotide chains in which the deoxyribose sugar units on adjacent nucleotides are linked with phosphodiester bonds to form a sugar-phosphate backbone. The purine and pyrimidine bases project inwards perpendicular to the sugar-phosphate backbone and are linked by hydrogen bonds. There is, however, a specific base pairing, occurring between adenine and thymine and between cytosine and guanine (Fig. 21.1). The polynucleotide chains are complementary to each other and the hydrogen bonds between the polynucleotide bases stabilize the double helix, The hydrogen bonds, however, are sufficiently weak and capable of breaking and reforming at room temperature. The genetic information in the DNA molecule depends on the sequence in which the four bases are arranged along the polynucleotide chains. In the complementary chains, the phosphate-sugar linkages run in the opposite directions. If in one chain, the sugar-phosphate links go from a 3'-carbon to a 5'-carbon, then in the complementary chain the sugar-phosphate linkages would run from a 5'- carbon to 3'-carbon.

The double helical structure of Watson and Crick suggests the manner in which the DNA molecule can undergo replication. By a variety of ingenious experiments it has been shown that DNA replication is *semi-conservative,* that is, each strand in the double helix serves as a template for the synthesis of a new strand simultaneously, while the original strand remains intact in daughter cells for several generations.

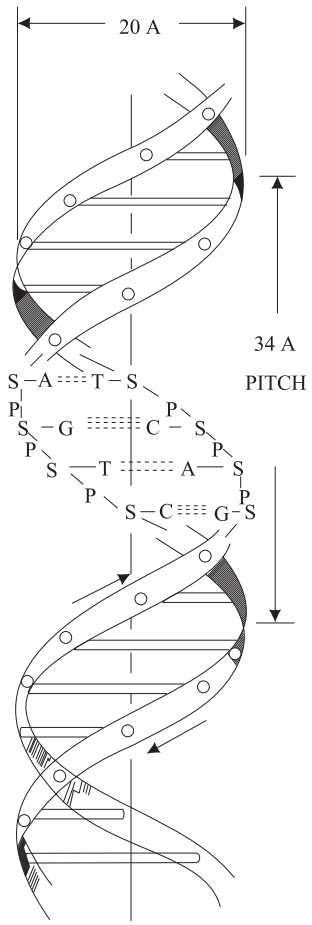


Fig. 21.1 Watson and Crick model of the double helix of DNA. The two chains are held together by hydrogen bonding between the bases.

Semi-conservative Replication of DNA

In 1958, Meselson and Stahl experimentally demonstrated in *E. coli* bacteria that DNA is replicated through semi-conservative mechanism. The chromosome of *E. coli* is a continuous DNA molecule, whereas in plants and animals the chromosomes are more complex in organization. Hence, the process of replication can be precisely elucidated in *E. coli.* Meselson and Stahl grew the bacteria initially in a medium containing labelled nitrogen (^{15}N). After growing them for several generations, it was found that all the DNA in bacteria was labelled. This was called heavy DNA. Then the bacteria with heavy DNA were grown in an ordinary medium for one generation only. Analysis showed that the next generation consisted of an intermediate form comprising of one heavy and one normal strand of DNA. When the bacteria were grown in the ordinary medium, in the next generation, half of the DNA was normal and another half of the intermediate form synthesized on the heavy strand (Fig. 21.2).

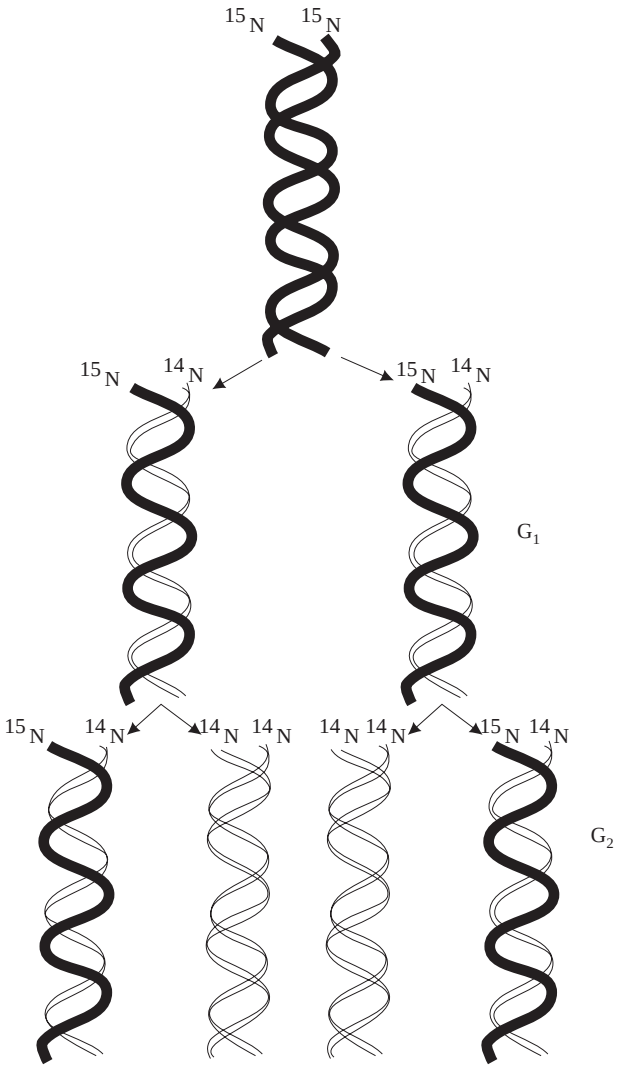


Fig. 21.2 The mechanism of semi-conservative replication of DNA.

The in *vitro* synthesis of DNA was demonstrated by Kornberg in 1956 and he showed that *DNA polymerase* catalyzes the replication of DNA molecule by addition of deoxyribonucleotides to the free —OH group at the 3' end of a chain. The enzyme has two outstanding properties. Firstly, it requires a mixture of the four types of DNA nucleotides (ATP, CTP, GTP and TTP) to function, and secondly, a DNA primer which acts as template. In the absence of DNA primer no DNA synthesis could occur.

$$\begin{matrix} \text{dTPPP} \\ \text{dGPPP} \\ \text{dAPPP} \\ \text{dCPPP} \end{matrix} \quad + \text{DNA primer} \quad \xrightarrow[\text{Polymerase}]{\text{DNA}} \quad (\text{–dTP –dGP –dAP –dCP})n + 4\,(n)\,\text{PP}$$

It was found that DNA polymerase acts on the strand which is copied in the direction from 3' to 5' end. Since there are two strands in each DNA molecule, it is envisaged that the two strands unwind which run in opposite directions so that the replication begins on each of the unwound strand. Experimental evidence suggests that both strands are copied at the same time. In that case, the strand which runs from 3' end to 5' end can be copied continuously. The other strand which runs in the opposite direction from 5' to 3' end is copied in the opposite direction, in the 3' to 5' direction. The synthesis cannot be continuous, hence newly synthesized DNA is formed in short segments which are later joined by DNA ligase as discovered by Khorana and his associates.

We have seen that the parent DNA molecule unwinds before replication takes place but the molecule does not unwind completely. Crick has pointed out that replication and unwinding take place simultaneously. As soon as unwinding takes place, the formation of two new chains starts. The mechanism is outlined in Figure 21.3.

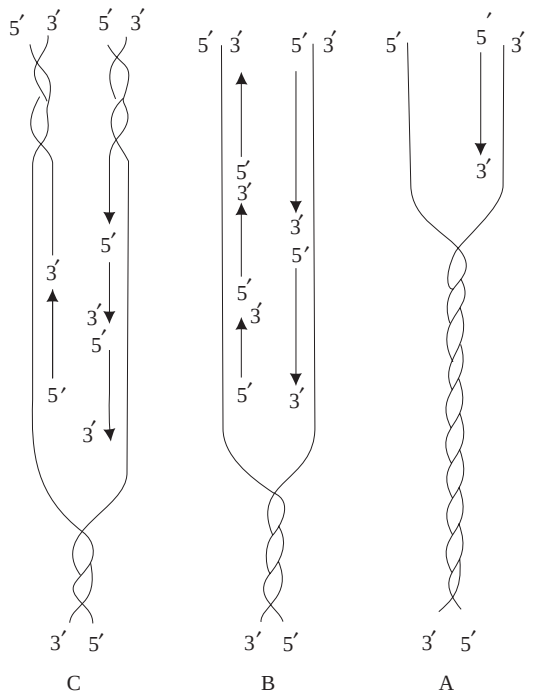


Fig. 21.3 Mechanism of discontinuous replication of DNA. (a) partial unwinding of the two chains showing synthesis at the 3′ end by the action of DNA polymerase. (b) newly synthesized DNA is in short length chains. (c) the new chains are joined by DNA ligase and reform new helixes.

The rate of DNA replication in *E. coli* is a fast process, but in higher organisms it is rather slow. In mammals, since the chromosome replication includes histone synthesis as well, the process is much slower. At the same time, autoradiographic studies show that in eukaryotic chromosome, there are several initiation points.

21.3 THE GENETIC CODE

While proposing the double helix model of DNA. Watson and Crick postulated that DNA is the information system in which the bases were incorporated in a manner so as to determine the sequence of amino acids in a protein molecule. This hypothesis visualizes that DNA utilizes a language of four letters, adenine, guanine, cytosine and thymille, in different combinations. Through these four bases DNA utilizes information to be transmitted to the cytoplasm for cellular control and its functions. But then, this code has three variants in the form of DNA, tRNA and mRNA. In the information theory, it was suggested that the code is operationally a system of codes and anticodes and if one was deciphered the other one could be automatically determined.

In the DNA molecule, there is a mutual attraction for adenine and thymine, and for cytosine and guanine which make the base pairs. DNA acts as the template for RNA synthesis. In the transcription of RNA the attractive forces would be between adenine and uracil since RNA does not contain thymine. Only one strand of double-helical DNA can act as a template for RNA synthesis. All types of RNA molecules (rRNA, tRNA and mRNA) are single stranded and complementary to the DNA template.

The messenger RNA (mRNA) contains a linear sequence of bases which dictates the sequence of amino acids of all polypeptide chains. The sequence of bases on mRNA is known as the genetic code. There are twenty different kinds of amino acids and therefore there must be a specific code for each amino acid. It was proposed by George Gamov that a combination of three bases seems to be most probable which would yield 64 different combinations (4^3). In 1964, Khorana synthesized a messenger RNA of known sequences though which he determined the triplet-base sequences that could code different amino acids. The codes on the mRNA are called *codons* specific for each amino acid.

There are a few generalizations about the genetic code (see Table 21.1). Several of the amino acids have more than one codon, hence redundancy is present in the codes. For example, leucine is coded by at least six codons, CUA, CUU, CUC, CUG, UUA, and UUA. The code is commaless and universal for all protein synthesizing organisms. There are three codons, UAA, UGA and UAG which do not code for any amino acid hence, specify termination of a peptide chain. The code is non-overlapping.

21.4 SYNTHESIS OF POLYRIBONUCLEOTIDES

RNA molecules differ from DNA in two respects. RNA is a long-chain molecule in which there is ribose sugar instead of deoxyribose and the fourth base thymine is replaced by uracil. Experimental evidence indicates that RNA is synthesized iron a DNA template by a process analogous to the replication of DNA and this process is known as *transcription.*

Table 21.1 The Genetic Dictionary: Scheme for the Genetic Code

First base	*Second base* *U*	*C*	*A*	*G*	*Third base*
	phenylalanine	serine	tyrosine	cysteine	U
	phenylalanine	serine	tyrosine	cysteine	C
U	leucine	serine	terminate	terminate	A
	leucine	serine	terminate	tryptophane	G
	leucine	proline	histidine	arginine	U
	leucine	proline	histidine	arginine	C
C	leucine	proline	glutamine	arginine	A
	leucine	proline	glutamine	arginine	G
	isoleucine	threonine	asparagine	serine	U
	isoleucine	threonine	asparagine	serine	C
A	isoleucine	threonine	lysine	arginine	A
	methionine	threonine	lysinee	arginine	G
	valine	alanine	aspartic acid	glycine	U
	valine	alanine	aspartic acid	glycine	C
G	valine	alanine	glutamic acid	glycine	A
	valine	alanine	glutamic acid	glycine	G

A specific enzyme called DNA-directed RNA polymerase is required to catalyze the synthesis of RNA on DNA templates. Like DNA polymerase, this enzyme also requires nucleoside triphosphates which align themselves on one of the DNA strands. When RNA synthesis starts, the DNA strands unwind. *In vitro* synthesis of RNA has revealed that the enzyme RNA polymerase is Mg^{2+} dependent and requires appropriate nucleoside triphosphates and a DNA template. Synthesis of RNA starts from the 5' end of the RNA chain, hence in this respect it resembles DNA polymerase. Chemical analysis has shown that RNA is copied as single strand from only one strand of the DNA molecule. Hence, the newly synthesized RNA strand should be complementary to the DNA strand which is copied. This has been amply proved with the help of hybridization experiments.

While a strand of RNA is synthesized on DNA template, the RNA molecule rapidly detaches itself and the two unwound DNA strands come together after the reformation of hydrogen bonds. The rate of formation of the phosphodiester bonds is rather slow (9000 per minute at 37°C) which is suggestive of a number of starting points along the DNA chain. It has been experimentally proved that a number of RNA molecules can be transcribed simultaneously on a DNA template. This shows that the enzyme RNA polymerase can attach to DNA at a number of places. The growth of RNA chains takes place from 5' to 3' direction as in case of DNA.

It is now known that RNA polymerase has a complex structure and functions on signals when to start synthesis of RNA and when to stop. A factor sigma (σ) gives the signals to start RNA synthesis and it is an integral part of the enzyme molecules as such. The termination message to stop synthesis of RNA is given by a protein factor rho (ρ) which is not considered as part of the polymerase.

As a general rule, RNA is formed on a DNA template. However, there are some exceptions to this general rule, for example, viruses whose genetic information is contained in a single RNA strand,

must first replicate a complementary RNA strand which may be copied to produce more. This synthesis is carried out by RNA-directed RNA polymerase.

According to the central dogma in molecular genetics the genetic information is transferred from DNA to RNA only. But in 1970, Temin reported that in some cancer producing RNA viruses copies of DNA are produced on RNA templates by an RNA-dependent DNA polymerase, called *reverse transcriptase.* The newly synthesized DNA may be incorporated in the host cell chromosome or it may be used to synthesize more of viral RNA. This discovery generated tremendous interest among the workers and the possible role of viruses in inducing cancer in humans was postulated. Nevertheless, it is not necessary that the presence of the reverse transcriptase activity may be the cause of malignancy of the virus to the host cells. Normal cells have also been shown to contain reverse transcriptase activity, which according to some, may play some role in gene amplification during differentiation of amphibian oocytes, and accidently the enzyme may produce new DNA sequences which may lead to malignant cancerous growth.

Function of Ribonucleic Acids

RNA is always found as a single stranded structure but the molecule may occur as secondary and three dimensional structures which are no doubt related to their functions. RNA is found to occur in several species as described below.

Transfer RNA

Transfer RNA (tRNA) is also referred as soluble RNA or adaptor RNA. The molecules are relatively small, about 75-85 nucleotides long. In a prokaryotic cell *(E. coli),* there are at least 60 different species of tRNA, while in eukaryotic cells, there may be as many as 100 tRNA molecules. Hence there may be two or three or more species of tRNA which can bind a single amino acid. Its molecular weight is about 25,000 to 30,000 The RNA chain is bent upon itself and this brings the compatible bases close to each other through hydrogen bonding. As a result, the tRNA molecule assumes a *cloverleaf* pattern (Fig. 21.4) which relates to its secondary structure. Another special feature of tRNA molecule is that it possesses a number of unusual bases.

A sequence of 3 bases is found on the middle lobe of the tRNA which is complementary to the triplet code on messenger RNA. All tRNA have a terminal base sequence cytidylic - cytidylic - adenylic acids (CCA) at the 3' terminal. The terminal nucleotide adenylic acid residue serves as an attachment site to the energy-rich enzyme-bound amino acid. Such an amino acid is attached to the 2nd or 3rd carbon of the ribose sugar of the terminal nucleotide. At the 5' end of the strand, the tRNA has an unpaired base guanine. The middle lobe with the sequence of three bases is known as the *anticodon* and the base sequence of this varies with the type of tRNA. The amino acyl-tRNA complex enters the ribosome and with the help of anticodon triplet recognizes its corresponding codon on the mRNA molecule. Thus the function of tRNA is to carry various amino acids from the pool and arrange them in the ribosomes as required by the messenger RNA.

Ribosomes and Ribosomal RNA

The ribosomes are submicroscopic particles occurring in every living cell (70 S ribosomes in prokaryotes and 80 S ribosomes in eukaryotes). These are the sites for protein synthesis which are

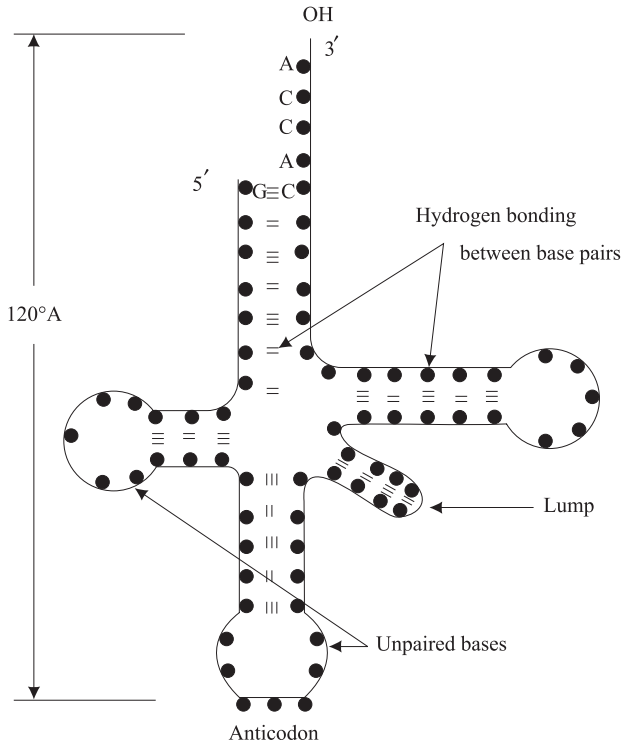


Fig. 21.4 The cloverleaf pattern of a typical tRNA molecule.

present throughout the cytoplasm in bacteria, whereas in higher organisms they are found attached to the endoplasmic reticulum. About 60 per cent of the ribosome is RNA and the remaining 40 per cent is protein. The ribosome is formed by the union of two subunits—a smaller 30 S* subunit, and a larger 50 S subunit. The smaller subunit has one large ribosomal RNA (rRNA) which is 16 S consisting of 1,600 nucleotides and 21 different proteins. The larger subunit has one large RNA (23 S) consisting of 3,200 nucleotides and 34 different proteins, and another smaller RNA (5 S) having 120 nucleotides. The task of ribosomal RNA is to join up the sequence of amino acids brought on the assembly line of the messenger RNA molecule to form a peptide chain.

Both the 30 S and 50 S subunits are heterogenous mixture of three groups of proteins viz., unit, marginal and fractional proteins. The ribosomes, owing to the existence of heterogeneous proteins in them, serve different functions and fall under different classes. Some of these types of proteins are known for their participation at various steps during protein synthesis. The 50 S subunit, in addition to the above three groups, has another type of protein known as functional repeat (FR) protein.

rRNA is mainly synthesized in the nucleolus on extrachromosomal DNA as template. rRNA acts as the framework on which the ribosomal particles are assembled during biosynthesis of ribosomes. They maintain ribosomal particles in a configuration permitting them to fulfill their role in protein biosynthesis.

*S is a Svedberg unit at which a particle sediments in a high speed ultracentrifuge.

Messenger RNA

In 1961, Jacob and Monod demonstrated that the species of RNA which carries genetic information from DNA to the ribosomes is the messenger RNA (mRNA). It has a thread like configuration which may be coded for protein synthesis in small segments. It is synthesized enzymatically on DNA template whose base sequence is complementary to DNA. In bacteria, messenger RNA has a short life span, probably about 2 minutes, which is evident from the rapid turn over of viral proteins when a phage infects the bacterium. The eukaryotic mRNA appears to be more stable, its half-life ranging from a few hours to perhaps weeks.

Isolation of mRNA is a difficult process because of its short life and small quantity available in the cell. However, techniques have been devised to isolate globin mRNA which synthesizes largely haemoglobin from rabbit reticulocytes. The globin mRNA sediments at a small peak 9 S whose estimated length is about 700 nucleotides. Messenger RNA has a base sequence complementary to DNA of the same cell. This has been demonstrated by DNA-RNA hybridization experiments. Out of the total-700 nucleotides of the globin mRNA, only 589 are coded by the DNA and the rest are attached to the mRNA after transcription to its tail end. The globin molecule (protein) consists of 146 aminoacids, hence it would require only 436 nucleotides on the mRNA chain. Thus it is evident that the mRNA has some extra nucleotides.

The prokaryotic mRNA is synthesized on one of the two strands of DNA acting as the template and functions as a template for protein synthesis. However, the eukaryotic mRNA has two noncoding regions which do not synthesize proteins. These regions are located at the 5' end and the 3' end. The 5' end has about 50 nucleotides whereas the 3' end has about 100 nucleotides. In some mRNAs the 3' end is extraordinarily large.

21.5 PROTEIN SYNTHESIS

We have briefly described the structure and role of different types of nucleic acids. DNA is the informational molecule which contains the genetic information in a coded form and this information is transcribed on the mRNA molecules before it is translated by ribosomes for the synthesis of specific proteins. Protein synthesis is thus a gene directed process which is carried out in the cytoplasm utilizing an elaborate protein-synthesizing machinery. For convenience, the polypeptide (protein) synthesis is discussed here under four stages: activation of amino acids; chain initiation; chain elongation; and chain termination. As far as possible, a brief comparison will also be made between protein synthesizing processes occurring in prokaryotes and eukaryotes to illustrate fundamental differences.

Amino Acid Activation

There are 20 different types of amino acids in the cytoplasm of the cell forming an amino acid pool and these are picked up one by one for assembly in a polypeptide chain. Activation of amino acid is a prerequisite for its attachment to the specific tRNA molecule. Each one of them is selected and bound by a specific enzyme, *amino-acyl tRNA synthetase* each of which is specific for each amino acid. Each of the enzyme-bound amino acid reacts with ATP from the cytoplasm and forms *amino*

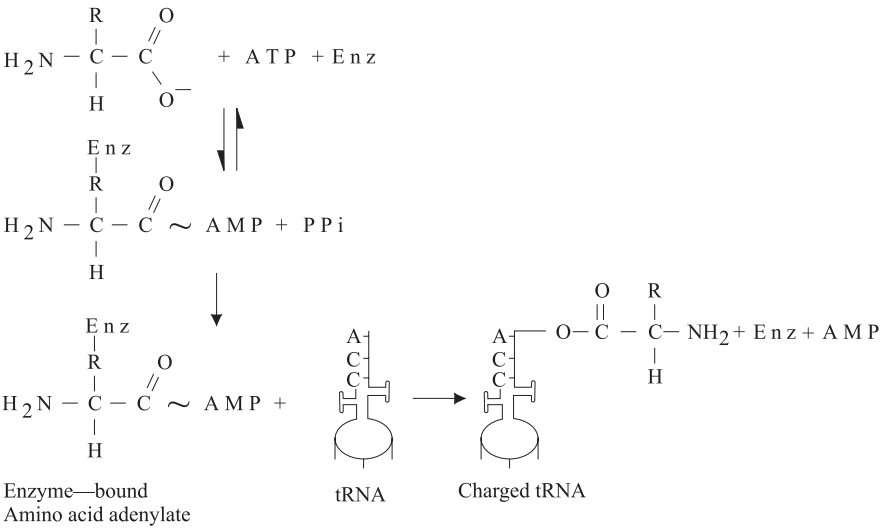


Fig. 21.5 Steps in the activation of an amino acid.

acid adenylate-enzyme complex (Fig. 21.5). The enzyme-bound activated amino acid would then readily react to bind with its specific tRNA. There is an amino acid specific for each tRNA, therefore there will be 20 different amino acid tRNA complexes.

The activating enzyme has two separate catalytic sites. The activation reaction occurs in two steps. The first reaction involves a reaction between ATP and the amino acid resulting in the formation of aminoacyl adenylic acid and pyrophosphate along with AMP. The carboxyl terminal of the amino acid binds with the 5'-phosphate group of the AMP through anhydride linkage (Fig. 21.5). The second step involves the transfer of aminoacyl group to the tRNA molecule releasing adenylic acid free.

Initiation of the Chain

The process of translation has to ensure that translation does not commence in the middle of mRNA chain and also correct alignment of the first anticodon with the first triplet codon of the messenger RNA. In case of wrong alignment the reading frame will be shifted to cause *frame-shift mutations.* Hence, chain initiation follows a series of events and includes a process of complex formation in the ribosome.

The following components are involved:

1. mRNA chain.
2. A special tRNA known as methionyl tRNA (fmet-tRNA).
3. Three protein factors that initiate protein synthesis: IF1, IF2 and IF3.
4. Guanosine trophosphate (GTP).

The process of protein synthesis is perhaps best understood in *E. coli, therefore the description presented here would pertain to this organism unless otherwise stated. It has been well established in*

E. coli and other bacteria in general that the amino acid at the NH_2 terminal of the nascent peptide chain is always N-formylmethionine which is initiated by a specific tRNA, fmet- tRNA. The reaction takes place as follows:

$$\text{met} + \text{f met} - \text{tRNA} + \text{ATP} \longrightarrow \text{fmet} - \text{tRNA} + \text{AMP} + \text{PPi}$$

$$\text{f} = \text{met} - \text{tRNA} + FH_4 - \text{CHO} \longrightarrow \text{CHO} - \text{fmet} - \text{tRNA} + FH_4$$

The formylation of methionine takes place enzymatically in which the formyl group of N-formyltetrahydrofolate is transferred. Formylation of methionine residue is necessary to block its amino a group so that it does not enter into a peptide bond formation.

The process begins by binding of the mRNA template to the 30 S ribosomal subunit in such a way that the first initiating codon of mRNA is positioned at the base of the peptidyl site (P-site) of the ribosome (Fig. 21.6). The initiating codon acts as a starting signal which is AUG. If the cells involved in protein synthesis are prokaryotic, the initiating codon would couple with a tRNA carrying methionine residue i.e., N-formyl methionine (fmet-tRNA). In bacteria the 50 S and 30 S subunits of the ribosome always dissociate after translating a segment of mRNA and the 30 S subunit attaches to a new mRNA containing AUG or GUG. The fmet-tRNA binds to the P-site in the ribosome. This binding requires GTP and some protein factors. These protein factors are called initiating factors which are loosely attached to the 30 S ribosomal subunit. The initiating factors are responsible for limiting the rate of protein synthesis. When the binding of 30 S subunit is complete with mRNA and fmet-tRNA, an initiation complex is formed which then picks up a 50 S subunit to complete the formation of a 70 S ribosome.

The initiating factor IF_1 helps in the interaction between IF_2 and the initiating f met-tRNA. The factor IF3 helps in the binding of mRNA and the 30 S subunit and also acts as a dissociating factor. Once the 50 S subunit is bound to the 30 S subunit, the initiation factors are released to be utilized again. At this juncture, the first peptide bond formation takes place.

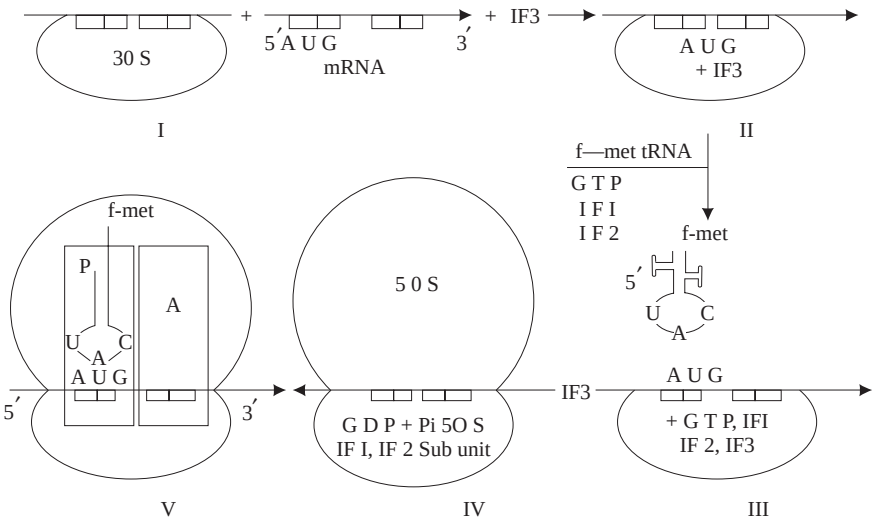


Fig. 21.6 Stages in the initiation of protein synthesis.

In eukaryotic cells, although the initiation of chain follows the same general pattern, there are some basic differences when compared to bacteria. First, there are 80 S ribosomes instead of 70 S types and the initiating tRNA does not carry formylated methionine. The initiating codon couples with methionine carrying tRNA through hydrogen bonding. The initiation factors involved are more than three and quite complex.

Chain Elongation

We have seen that the function of the tRNAs is to correctly align the amino acids on the messenger RNA and the essential process in elongation is the formation of peptide bonds. The peptide bond formation takes place between the—NH_2 group of the newly bound amino-acyl tRNA at the A-site and the carboxyl group of the amino acid at the P-site. The whole process of chain elongation is accomplished in three stages (Fig. 21.7).

First, an amino acid containing tRNA (aminoacyl tRNA) is selected if its anticodon triplets match with the codon of mRNA. The anticodon triplets of the selected aminoacyl tRNA are bound to the codon through hydrogen bonding at the A-site (aminoacyl site) of the 50 S subunit. For this hydrogen bonding one molecule of GTP is required besides a cytoplasmicprotein factor called T factor.

The second step involves peptide bond formation between the –COOH group of the initiating amino acid at the P-site and the $–NH_2$ terminal of the incoming amino acid through aminoacyl tRNA at the A-site. This is a nucleophilic reaction catalyzed by an enzyme *peptidyl transferase* present in the 50 S subunit. As a result of this reaction the initiating aminoacyl group from the tRNA of the P-site is transferred to the free $–NH_2$ terminal of the aminoacyl tRNA on the A-site forming a dipeptide. The dipeptide attached to the tRNA of the A-site an forming it into peptidyl tRNA. The tRNA at the P-site now devoid of initiating amino acid remains for a while.

The third step of the elongation process is translocation. In this process, the peptidyl tRNA from A-site is translocated to the P-site expelling the deacylated tRNA from the P-site, and at the same time the A-site is occupied by the next aminoacyl tRNA. It should be noted that the peptidyl tRNA remains attached to the mRNA while the ribosome moves in a 5' to 3' direction and the movement of the ribosome is facilitated by a molecule of GTP which is hydrolyzed to GDP and Pi and a special *translocase* factor which is called EFG factor. In fact, the EFG factor is required to split GTP into GDP and phosphate.

Elongation of the polypeptide chain required some elongation factors, out of which two factors namely, EFT and EFG were initially isolated. We have already referred to the EFG factor and its role. The EFT factor consists of two proteins: Tu (EFTu) and Ts (EFTs). The factors EFTu and EFTs facilitate GTP-dependent binding of aminoacyl tRNA to the ribosome. EFtu is released from the ribosome when GTP is hydrolyzed. The cycle of these three steps is repeated for addition of each amino acid to the growing polypeptide chain. Protein synthesis is an energy requiring process and for incorporation of each amino acid, one ATP and two ETP molecules are consumed.

The mRNA chain is associated with a number of ribosomes ranging from 4 to 40 and each structure is often termed as *polysome.*

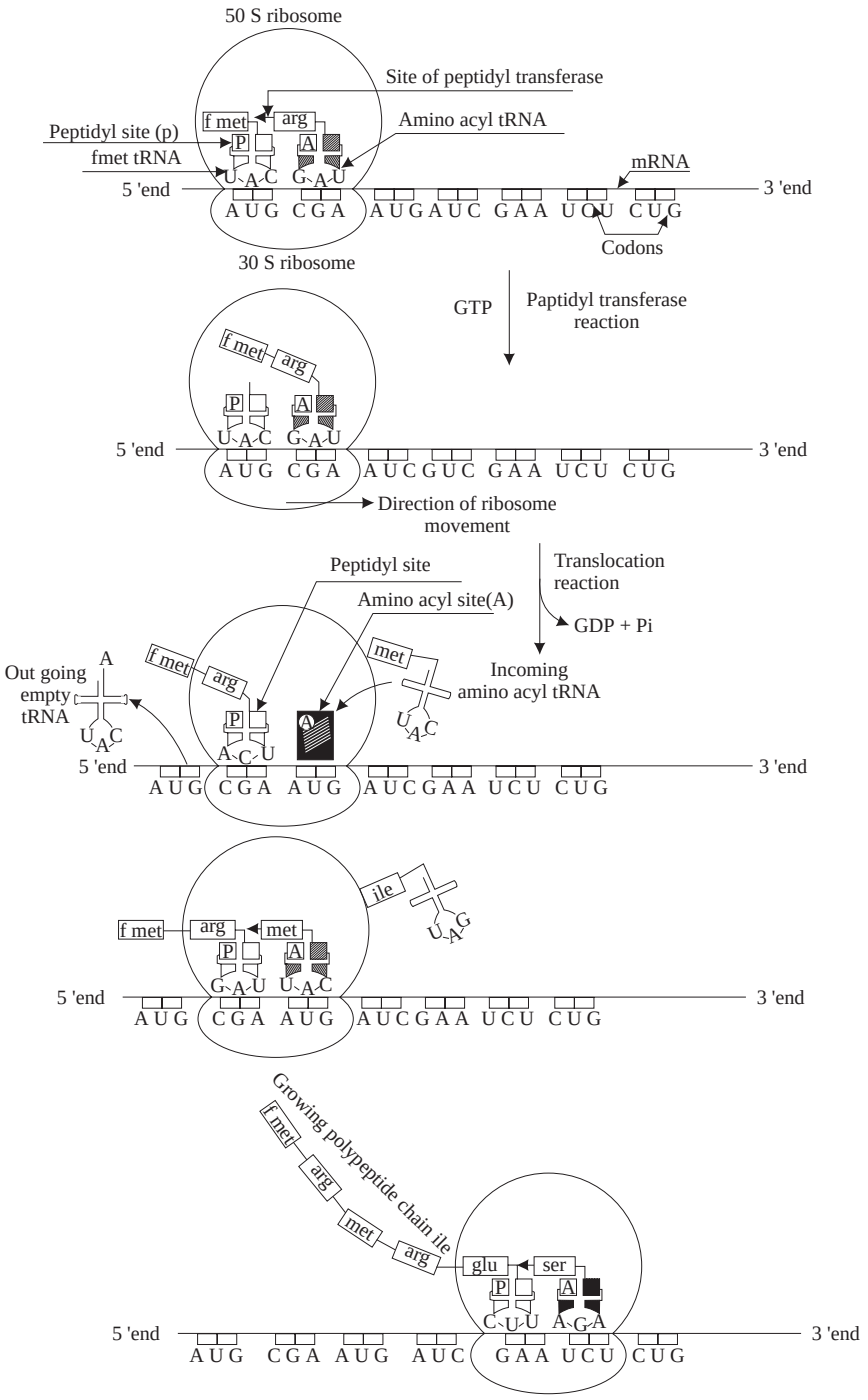


Fig. 21.7 The sequence of steps in the growth of a polypeptide chain on a ribosome.

Chain Termination

This is the last stage in protein synthesis in which the newly synthesized polypeptide chain is released from the peptidyl site with the aid of three protein release factors namely. RF1, RF2 and RF3. These factors become active in the presence of three terminating codons which are UAA, UGA and UAG. These terminating codons are recognized by the release factors and not by tRNAs. Termination of the polypeptide chain takes place when the 70S ribosome approaches the end of the cistron carrying terminating codon ultimately releasing the nascent protein and the tRNA. There are two events leading to the chain termination: first, recognition of the terminating codon on the mRNA cistron, and the second, hydrolysis of the peptidyl tRNA. While the recognition of the terminating codons is the function of the protein factors RF1 and RF2, the third factor RF3 helps in the recognition of the terminator condon. RF1 is specific for UAG and UAA and RF2 for UAA and UGA. There are two views regarding their mode of action. One view is that these factors are esterases which hydrolyze the peptidyl tRNA, and the other view is that they activate the enzyme peptidyl transferase to hydrolyze peptidyl tRNA.

After the chain has been terminated, the 70 S ribosome dissociates into its 30 S and 50 S subunits. These subunits are again ready to enter another cycle of protein synthesis. The terminated peptide chain has the formyl-methionyl residue at the NH_2 terminal which is removed before the peptide chain is completed. Removal of the formyl group is effected by an enzyme deformylase and the deformylated methionyl peptide is formed. Methionine residue is then removed by the action of a specific aminopeptidase.

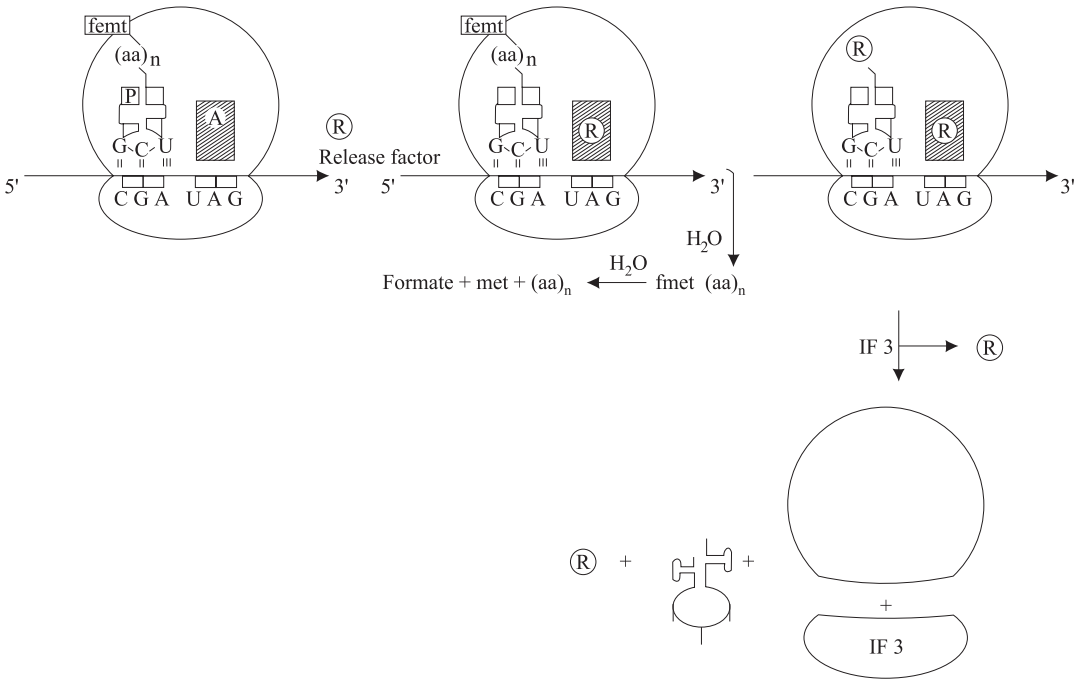


Fig. 21.8 Steps showing chain termination.

Role of Antibiotics in Protein Synthesis

Some antibiotics are known to play a specific role in studying the process of protein synthesis. *Puromycin,* which is an analouge of aminoacyltRNA, can bind to the A-site. In the elongation step 2, the peptidyl tRNA at the P-site is transferred to the amino group of the aminoacyl tRN A at the A-site to form a peptide bond. The peptidyl transferase helps in the transfer. However, if puromycin is put in the medium during the process, the enzyme transfers the peptidyl tRNA to puromycin to form peptidyl-puromycin complex which is released from the ribosome. Hence, puromycin inhibits the chain elongation process. Another antibiotic *streptomycin* causes misreading of the genetic message. Chloramphenicol inhibits protein synthesis in bacteria, chloroplasts and mitochondria but not in eukaryotes.

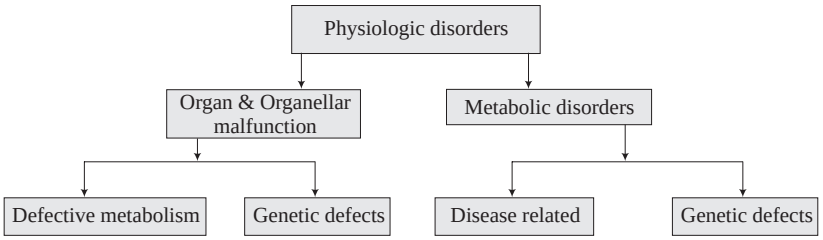
Tetracycline which is widely used in medicine inhibits the binding of aminoacyl tRNA to the ribosomes. Besides antibiotics, some toxins are also known to cause damage to the protein synthetic process. In eukaryotes, the diptheria toxin inhibits translocation by inactivating the elongation factor translocase. However, prokaryotes are not sensitive to the toxin. A fungicide, *cyclohexamide* interferes the peptide bond formation on ribosomes in eukaryotes and yeast cells. It has no effect in bacteria.

CHAPTER

22

Physiologic Disorders

Human body is prone to many diseases either caused by pathogens or by malfunctioning of various organs, organelles and metabolic processes. Most metabolic disorders and organelle dysfunction are rare and inherited in which clinical and biochemical abnormalities are fund to be caused by congenital deficiency of a particular enzyme. Sir Archibald E. Garrod in 1908 recognised them as "inborn errors of metabolism" caused by the presence of an abnormal and mutant gene. Physiological disorders can be broadly divided into two categories: Organ and Organelle malfunction and metabolic disorders.



22.1 ORGANELLE MALFUNCTION

A large number of diseases are caused by defective cell organelles. Diseases associated with the transport mechanisms, storage and defective metabolism in the cell are quite common which are produced by abnormal mitochondria, lysosomes or other organelles. For example, currently 46 abnormal conditions come within the category of lysosomal storage diseases. Many of these conditions are inherited and caused by mutations, leading to the disorders of various organelles. Here we discuss about the defective cell organelles and the diseases caused by them.

Lysosomal Abnormalities

Lysosomes are tiny organelles storing hydrolytic enzymes that carry out intracellular digestion. The enzymes are synthesized elsewhere in the cell and subsequently targeted to lysosomes. Several rare lysosomal diseases have been reported which account for defects due to lysosome biogenesis and trafficking, and storage disorders (Table 22.1). A syndrome called Hermansky-Pudlak (HPSI) is caused by a defective protein (HPSp), which is thought to transiently associate with the lysosomal membrane and mediates biogenesis and function of lysosomes. The abnormality leads to pigmentation defect, immunodeficiency and blood disease. The patients are chracterised by hypo-pigmentation due to defective melanosomes.

Table 22.1 Diseases Due to Lysosomal Disorders

Diseases	*Defective gene/protein*	*Abnormalities*
Diseases of lysosome biogenesis and trafficking		
Hermansky-Pudlak syndrome	HPSp	Pigmentation defect, immuno-deficiency, blood disease, lysosomal proteins appear to be transported and distributed. Bleeding disorder due to disorders of platelets storage defects.
Chedak-Higashi syndrome	LYST-lysosomal trafficking regulator protein	Pigmentation defect, immunodeficiency.
I Cell disease	N-acetylgluco samine 1-phospotransferase	Neurological disease, mannose-6-phosphate not added to lysosomal enzymes, hence lysosomal proteins are secreted and inefficiently transported to lysosomes.
Danon disease	Lamp 2	Heart disease, muscle disease, neurological disease; loss of lamp 2 leads to the development of a lysosomal storage disease, with normal acid maltase.
Lysosomal storage diseases		
Sphingolipidosis Gm I Gangliosidosis Sandhoff disease Fabry disease etc.	β-D-galactosidase β-hexo saminidase α-D-galactosidase acid sphingomye-linase	Neurological diseases; These enzyme defects cause inappropriate processing of sphingolipids, leading to accumulation of incorrectly modified sphingolipids in the lysosomes.
Mucolipidosis IV	Unknown	Neurological diseases, sphingolipids, phospholipids and acid mucopo/lysaccharides accumulate in lysosomes.
Sphingolipid activator protein deficiencies	Prosaposins	Nurological disease, the prosaposins are small acidic proteins required for hydrolysis of sphingolipids, and hence defects result in the accumulation of specific sphingolipids.
Batten disease	CLNI-CLN 8 (8 known alleles)	Neurological disease, Eight distict genes are responsible for the lysosomal storage disease. The defects cause a lysosomal accumulation of an auto fluorescent material that is resistant to analysis.

Lysosomal stroage diseases are clinically manifested in the form of neurological disorders caused by genic defects leading to inappropriate processing of sphingolipids that accumulate in lysosomes in incorrectly modified forms. Another rare disease, called I-cell disease results from faulty targeting of lysosomal enzymes. The chemical marker mannose-6-phosphate serves to target lysosomal enzymes to the organelle causing the defect. The I-cell disease is chracterised by progressive psychomotor retardation that proves fatal. Lysosomes become deficient in certain hydrolasses, hence do not function properly. The results in accumulation of incompletely digested material in the lysosomes.

Membrane-related Abnormalities

Membranes are located in most organelles and are involved in many cellular processes such as ion channel transport, receptor mediated responses, and enzymic functions. These processes are dependent on enzymes or membrane proteins whose functions may be jeopardised by mutations that generally affect glycosylation of these proteins. Examples of diseases caused by mutations in the plasma proteins and organellar membranes have been presented in the Table 22.2.

Table 22.2 Disorders Due to Membrane Abnormalities

Diseases	*Defective gene/protein*	*Abnormality*
Cystic fibrosis	CFTR	Lung disease, caused by mutation of gene encoding CFTR protein - a Cl^- transporter
Wilson' disease	ATPase	Gene encoding copper-dependent ATPase undergoes mutation
Hereditary hypercholesterolemia	LDL receptor	Mutation in the gene encoding LDL receptor
I-cell disease	Gl cNAc phosphotransferase	Gene encoding GLcNAc phosphotransferase mutated, causing deficiency of mannose-6-P that signals targeting of enzymes to lysosomes
Metastasis	Membrane glycoproteins and glycolipids	Presence of abnormalities in the oligosacc aharide chains of glycoproteins and glycolipids
Malignant hyperthermia	Ca^{++} channel protein	Ca^{++} release channel abnormality in skeletal muscles
Myotonia congenital	Cl^- channel	Abnormality of Cl^- channel in skeletal muscles
Hyperkalemic periodic paralysis	Na^+ channel	Abnormality of Na^+ channel in skeletal muscles

Abbreviations : CFTR = cystic fibrosis transmembrane regulator:
LDL = low density lipoprotein; GLcNAc = N-acetyl glucosamine

Ion-channel Defects

A growing number of human diseases have been discovered to be caused by inherited mutations in genes encoding channels, and hence are related to the functioning of cell membranes. Some examples are given below:

1. Chloride-channel diseases–cystic fibrosis is a disease of this type. There is also an inherited tendency to kidney stones (caused by a different kind of chloride channel than the one involved in cystic fibrosis.

2. Potassium-channel diseases–some inherited life-threatening defects in the heart beat are a result of potassium-channel disorders.
3. Sodium-channel diseases–there are some diseases related to the inherited tendency to certain types of muscle spasms. Liddle's syndrome is another disorder associated with inadequate sodium transport out of kidneys. This is because of a mutant sodium channel and leads to elevated osmotic pressure of the blood and resulting hypertension (high blood pressure).

Plasma Membrane Defects

The plasma membrane is a complex structure composed of lipids, carbohydrates and proteins. The basic structure of all membranes is the lipid bilayer, formed by two sheets of phospholipids in which membrane proteins are sandwitched. A number of primary disorders of plasma lipoproteins have been discovered, which include diseases like hypolipoproteinemias and hyperlipoproteinemias.

Mitochondrial Defects

Mitochondria are producers of energy from the oxidation of carbohydrate, fat and protein and the energy is made available in the form of reducing equivalents ($-H^+$ or e^-), which pass through the respiratory chain down the gradient of carriers to the oxygen as the final acceptor. A number of mitochondrial abnormalities have been found leading to fatal conditions. Mitochondrial encelopathy, lactic acidosis and stroke are inherited conditions resulting from cytochrome deficiency or complex I (ubiquinone oxidoreductase system), caused by mutation in mitochondrial DNA. Some clinicians have attributed this deficiency to Alzheimer's disease and diabetes mellitus. Sometimes absence of oxidoructases in the respiratory chain cause *infantile mitochondrial myopathy* and *renal dysfunction.* Lactic acidosis is a disease that is produced by abnormal mitochondris with defective aerobic metabolism. In this situation, lactic acid accumulates in the blood.

Abnormalities of Endoplasmic Reticulum

The membranous cytoplasmic material in the cell, called *endoplasmic reticulum* (ER), occurs in two forms: rough ER and smooth ER. The rough endoplasmic reticulum has a rough appearance because of the attachment of ribosomes which synthesise core proteins and subsequently targeted to Golgi and other destinations in the cell. Within the cisterae of the RER many other functions are performed such as glycosylation of proteins, protein sorting, fatty acid chain elongation, etc. Perhaps, the RER abnormalities give rise to many diseases and malfunctions related to lipid transport and cargo accumulation leading to ER signaling and stress (Table 22.3).

Peroxisomal Disorders

Peroxisomes are microbodies that contain catalases and oxidases and are involved in the metabolism of a variety of molecules such as fatty acids, cholesterol, purines, amino acids, lipids and hydrogen peroxide. There are about 50 enzymes in the peroxisomes, but urate oxidase and catalase are the marker enzymes. Originally discovered in mammalian systems and their membranes differ significantly from that of the mitochondria in having lower content of cardiolipin. An important

Table 22.3 Diseases Due to Endoplasmic Reticulum Disorders

Disease	*Defective gene/protein*	*Abnormality and clinical manifestation*
Cargo accumulation leading to ER signaling and stress		
Hereditary emphisema with liver injury	α1-Antitrypsin (PiZ) variant	Lung and liver diseases displaying accumulation and inefficient degradation of mutated α 1-Antitrysin, which leads to lung function,
Congenital hypothyroidism	Thyroglobulin	Endocrine disease and developmental defect; mutated thyroglobulin (Tg) accumulates in the ER leading to ER signaling and a marked expansion of the compartment.
Osteogenesis imperfects	Type I procollagen	Skeletal deformity, mutations disrupt folding and assembly of pro-collagen and delay formation of disulfide-linked trimers composed of two pro-α-1 and one pro-α-2 cahins, which are exported from the ER.
Nephrogenic diabetes insipidud	Water channel aquaporin-2	Diabetes caused by point mutations found in aquaporin2 leading to ER retention.
Charcot-Marie-Tooth syndrome	Peripheral myelin protein 22	Neurological disease, degenerative muscle disease; the mutant protein accumulates in ER. Signaling from the ER may contribute to abnormal growth and differentiation.
Alzheimer disease	Presenilin	Neurological disorder due to localization of presenilins in the ER.
Pelizaeus-Merzbacher disease	Proteolipid protein (PLP) gene	Neurological disorder caused by mutations that accumulate PLP gene products in the ER, leading to ER stress signaling and oligodendrocyte death by apoptosis.
ER transport machinery stress		
Combined factors V and VIII deficiency	ERGIC-53/p58	A blood disease caused by lack of ERGIC 53, due to missense mutations leading to a secretion block of coagulation factors V & VIII and development of bleeding disorder.
Spondylo-epiphyseal dysplasis a tarda	SEDL (sedlin)	A skeletal defect due to defective collagen transport that causes skeletal abnormalities
Lipid transport diseases related to ER		
Low plasma lipoprotein α	Apolipoprotein (*apo* α)	Vascular and liver disease caused by retention of *apo* α in the ER.
Apo β-lipoproteinemia	Microsomal triglyceride transfer protein (MTP)	A vascular disease caused by mutations in MTP, which associates with the ER-folding machinery, leading to a consequent loss of/Apo β from the ER.
Familial chylomicronemia	Lipoprotein lipase (LPL)	A vascular disease caused by mutations in the protein leading to prolonged ER retention and secretion block.

Contd.

Cargo retention and degradation in the ER		
Cystic fibrosis	CFTR protein	A lung disease caused by loss of chloride regulation due to mutation in the Cl^- conductance channel leading to retention and degradation of CFTR protein in the ER.
Protein C deficiency	Protein C	A blood disease caused by mutations in this coagulation factor leading to ER retention and degradation.
Oculocutaneous albinism	Tyrosinase	A pigmentation defect due to mutations in tyrosinase leading to its retention in ER.
Diabetes mellitus	Insulin receptor	Mutations in insulin receptor impair its transport from ER.

function of peroxisomes is their involvement in using substrate-linked hydrogen transport to shuttle reducing equivalents across the membrane. In this way, reduced NADH can be reoxidized without crossing the membrane by shuttling out its electrons on substrates that are freely permeated to the membrane.

Biogenesis of peroxisomes is highly debated. One school advocates the formation of peroxisomes by budding from the endoplasmic reticulum and the matrix enzymes enter the membranes during posttranslational uptake. Another view suggests their biogenesis by fusion or budding from the system of membranes in the cell. Absence or too few peroxisomes has been related to a profound neurological impairment as in *zellweger syndrome,* resulting in accumulation of long chain fatty acids and reduction in plasmalogens. The condition arises due to mutations in genes encoding *peroxins* which hampers peroxisome biogenesis. Besides zellweger syndrome, other peroxisome abnormalities, such as Acatalasemia, infantile Refsum disease, adrenoleukodystrophy, glutaryl-CoA oxidase deficiencies have also been observed.

22.2 METABOLIC DISORDERS

A number of biochemical abnormalities which are observed in humans can be attributed to the congenital deficiency of a particular enzyme, which in turn is caused by the presence of an abnormal or mutant gene. Usually these conditions have been found to be inherited as Mendelian recessive characteristics. It is, therefore, implied that the affected individual has received such an abnormal gene from each for the parents.Heterozygotes who receive the abnormal gene from only one parent and its normal allele from the other generally quite healthy though they usually show a partial deficiency of the specific enzyme which is grossly deficient in the affected patients. Metabolic disorders of this nature were characterized as 'inborn errors of metabolism'.

The term 'inborn errors of metabolism' was first introduced in medicine by Sir Archibald E. Garrod in 1908. It refers to certain rare inherited metabolic diseases in which the clinical and biochemical abnormalties can be attributed to a congenital deficiency of an enzyme activity. A defective enzyme is due to a gene mutation, which may lead to the synthesis of a structurally altered protein with defective or modified properties. This leads to the reduced activity of the enzyme. It is also possible that the mutation may completely inhibit the synthesis of the enzyme or cause

considerable reduction in the rate of synthesis. Table 22.4 gives a list of some well known metabolic disorders.

Types of The Enzyme Deficiencies

Specific deficiency of some enzyme activity is the root of inborn errors of metabolism. The deficiencies may be of three types:

1. A mutational change may lead to the synthesis of a structurally altered protein with defective or modified catalytic properties. A single amino acid substitution may lead to such an effect. Hemolytic anemia and pyruvate kinase deficiency are cases under this category.
2. The mutant gene may lead to the synthesis of a structurally altered protein whose catalytic properties are not necessarily altered, but whose inherent stability is much reduced due to modification of its three-dimensional conformation. Such an enzyme protein would tent to be more rapidly broken down *in vivo*, so that its life is much shortened. Glucose-6-phosphate dehydrogenase deficiency is an example of this effect.
3. The mutation may result in complete failure to synthesize the specific enzyme protein at all, or in a severe reduction in the rate of its synthesis so that very little is actually present at any time. Complete failure to from the enzyme might result from deletion of parts of the DNA sequence of the gene, or it could arise from a single base change causing premature chain termination.

Table 22.4 Some Heritable Diseases Due to Inborn Errors of Metabolism.

Diseases/Disorders	*Cause of the defect*
Disorders of carbohydrate metabolism	
Congenital haemolytic disease	Deficiency of hexokinase
Haemolytic disease	Deficiency of pyruvate kinase
Congenital haemolytic disease	Deficiency of 2,3-diphospho-glycerate mutase
Favism (primaquine sensitivity)	Deficiency of glucose-6-phosphate dehydrogenase
Glycogen disease Type I	Deficiency of glucose-6-phosphatase
Glycogen disease Type II	Deficiency of amylo-1-4-glucosidase
Glycogen disease Type III	Deficiency of amylo-1, 6-glucosidase
Glycogen disease Type IV	Deficiency of amylo-1, 4-1, 6-transglucosidase
Glycogen disease Type V	Deficiency of muscle phosphorylase
Glycogen disease Type VI	Deficiency of liver phosphorylase
Glycogen disease	Deficiency of phosphofructokinase
Juvenile cataracts	Deficiency of galactokinase
Benign fructosuria	Deficiency of fructokinase
Fructose intolerance	Deficiency of liver aldolase
Galactosaemia	Deficiency of glucose-1-phosphate uridyl transferase
Congenital pentosuria	Deficiency of L-xylulose reductase
Disorders of amino acid metabolism	
Phenylketonuria	Deficiency of phenylalanine hydroxylase

Contd.

Tyrosinaemia	Deficiency of p-hydroxyphenyl pyruvic acid oxidase
Alkaptonuria	Deficiency of homogentisic acid oxidase
Homocystinuria	Deficiency of cystathionine synthetase
Histidinaemia	Deficiency of histidase
Albinism	Deficiency of tysosinase
Argininaemia	Deficiency of arginase
Maple syrum urine disease	Deficiency of keto acid decarboxylase
Goitrous cretinism	Deficiency of iodotyrosine deiodinase
Hyperprolinaemia	Deficiency of proline oxidase
Hydroxyprolinaemia	Deficiency of hydroxyproline oxidege
Mucopolysaccharidoses and Sphingolipidoses	
Gaucher's disease	Deficiency of glucocerebrosidase
Fabry's disease	Deficiency of ceramidetrihexosidase
Generalized gangliosidosis	β-galactosidase
Tay-Sach's disease	Deficiency of acetylhexosaminidase
Fucosidosis	Deficiency of alpha fucosidase
Niemann-Pick disease	Deficiency of sphingomyelinase
Hurler's syndrome	Dermatan sulphate and heparan sulphate in urine and tissues, dermatan sulphate in fibroblasts; increased gangliosides in brain.
GM1 Gangliosidosis	Keratan sulphate like material in tissues and urine; very low β-galactosidase in tissues
Other disorders	
Xanthinuria	Deficiency of xanthine oxidase
Oroticaciduria	Deficiency of orotidylic pyrophosphorylase and orotidylic decarboxylase
Lesch-Nyhan disease (gout)	Hypoxanthine-guanine phosphoribosyl transferase is found to be deficient
Acatalasia	Deficiency of catalase
Methaemoglobinaemia	The enzyme methemoglobin reductase is found to be absent
Hypophosphatasia	Deficiency of alkaline phosphatase
Sheldon's disease	Deficiency of pancreatic lipase
Chronic granulomatous disease	NADH oxidase is found deficient
Failure of protein digestion	Severe deficiency of trypsinogen and enterokinase
Carnosinuria	Deficiency of carnosinase
Congenital haemolytic disease	Deficiency of adenosine triphosphatase
Hyperglycinaemia	Deficiency of propionyl Co-A carboxylase
Congenital erythropoietic porpyria	Deficiency of uroporphyrinogen III cosynthetase
Lysosomes fail to thrive	Acid phosphatase is deficient.

CHAPTER

23

Physiological Genetics

We have seen in the previous chapter that the genetic information encoded in the DNA molecule is transcribed into RNA and then translated into protein. In this chapter, we shall examine the ways in which a cell regulates its physiological functions through expression of genes. Much of the information on gene expression and regulation comes from the classical studies on *E. coli* where the chromosome structure is much simpler as compared to eukaryotes. A eukaryotic genome is highly complex, hence our knowledge about the function of genes in eukaryotes is not yet satisfactory. However, studies on polytene and lampbrush chromosomes have revealed a fairly good picture of the transcription process so as to visualize the molecular mechanisms involved.

The fundamental process which operates in prokaryotes for gene duplication and expression is applicable to eukaryotes as well as is revealed by the existence of the same genetic code. The process, however, becomes more complicated since the genetic information contained in the eukaryotic genome is much larger. Besides having a large quantity of DNA, the eukaryotic DNA contains large amounts of repetitive DNA. We shall now analyse how gene expression is controlled in organisms.

23.1 THE GENE AS A FUNCTIONAL UNIT

Genes operate in an environment that includes not only other genes and their subunits, but also the cytoplasm. Experimental evidences in favour of this have conclusively proved that the basic function of genes is to control protein synthesis. We can say that the gene is a chromosomal unit composed of DNA that controls the synthesis of a single type of amino acid chain. These chains form proteins which either function as enzymes or structural components, and these then contribute to the development of various secondary traits. It follows, then, that the visible traits of an organism are always a product of interaction between genes and the environment. However, the interacting genetic systems, considered at the chromosomal level, are complexes of genes and these always function in an integrated manner with the rest of the living material. It may be said that the smallest real unit of inheritance is one whole cell.

The activation of certain genes in the nucleus results in the production of chemical substances in the cell which in turn influence other developmental processes. These substances, produced as a result of genic activity, are restricted to cells where they are synthesized and influence the chemical, physical, morphological and physiological properties of these cells only, thus affecting the behaviour of the individuals. They may influence the phenotype. The mechanism by which the genes influence the reactions to control cellular metabolism have been studied in great detail. There is yet another aspect of physiological genetics which deals with the developmental events in organisms with a specified difference in the genotype. Certain events in the career of the cell during tissue and organ differentiation (morphogenesis) are controlled by genes and depend on the interplay between gene and cytoplasm. In the present chapter, we shall endeavour to discuss the effects of gene rather than their mode of inheritance in the control of metabolism and certain physiological processes.

23.2 CONTROL OF METABOLIC PROCESSES

Important discoveries in the field of biochemical genetics have demonstrated that the DNA within the chromosomes is the genetic material. The mechanism by which the chromosomes are transmitted over generations forms an integral part of the field of genetics. However, there is overwhelming evidence in favour of the DNA that it controls protein synthesis. A complete picture of gene action has emerged from such studies.

The problem of gene action can be studied at the molecular level. In this connection, we can proceed with the famous experiment of Beadle and Tatum on the mold, *Neurospora. Neurospora* is a bread mold which can be grown on a well defined medium containing inorganic salts, sucrose as the carbon source and the vitamin biotin. Beadle and Tatum postulated that the genic control is mediated through enzyme synthesis. If it is possible to induce a mutation to block the synthesis of an enzyme, its absence would be demonstrated by *Neurospora* requiring organic compounds that it was formerly not able to synthesize itself. *Neurospora* produces haploid spores (conidia) which grow into haploid plants. Spores were irradiated and then transferred to an agar medium to grow. The agar medium contained many organic compounds, some of which were not required by *Neurospora.* These spores germinated into filaments. Some of these were then transferred to another agar plate containing well defined medium having nutritional requirements for the normal strain. These plants did not grow showing thereby that a nutritional change has occurred in the mold. They needed something more to grow.

These plants were then transferred to media containing basic nutritional requirements plus an additional organic compound. One agar plate contained defined medium plus one vitamin; another contained defined medium plus an amino acid, and so on. If the plants grew, it was concluded that the specific nutritional requirement was present which was not synthesized by the individual. Beadle and Tatum reared thousands of plants from such irradiated pores and isolated a large number of mutants on the basis of their nutritional requirements. They found that one mutant strain grew only when vitamin para-aminobenzoic acid (PABA) was present in the medium *(mutant* 1633).

Now they took two strains of *Neurospora:* $PABA^+$ (normal) strain and $PABA^-$ (minus) mutant strain and crossed them. Both $PABA^+$ and $PABA^-$ plants were produced showing usual Mendelian inheritance. These experiment showed that additional nutritional requirements of vitamin were

needed by $PABA^-$ strain since it had lost the ability to synthesize the enzymes necessary for the production of the vitamin. The experimentors concluded that the genes present on the chromosomes control the synthesis of enzymes. Consequently, they postulated one gene-one enzyme hypothesis which says that each gene controls the synthesis of one enzyme. The hypothesis was later improved upon and a more accurate hypothesis, one gene-one polypeptide chain emerged.

In general two modes of action by genes have been recognized:

(1) Those genes whose action is confined to the cells in which they occur. The substances produced by the gene activity are restricted to cells in which they have been formed.

(2) Those genes which liberate chemical substances in the cells and these substances diffuse out of the cells to exert control in remote cells and tissues.

Sickle-cell Anemia

Sickle-cell anemia is a familial disease which is quite common among Negro populations in Africa and the United States. The inherited disease was shown by Neel (1951) to be derived from a rare single-gene recessive mutation which brings about a change in the primary structure of haemoglobin protein. The affected individuals have sickle-shaped red blood cells due to low oxygen tension. The individuals also suffer at times severe haemolytic anemia which may be lethal in early childhood. The condition is inherited as a Mendelian blending of two traits. The phenotypes were found with three genotype:

Normal	S/S	homozygous dominant
Sickle-cell trait	S/s	heterozygous
Sickle-cell anemia	s/s	homozygous recessive

The homozygous recessive (s/s) condition is fatal and the heterozygous condition (S/s) carries the trait of sickle-cell blood cells. Such heterozygotes are normal apparently but act as carriers of the sickle-cell trait. Haemoglobins from sickle-cell trait (sicklemia), sickle-cell anemia and normal individuals show different electrophoretic patterns. From the electrophoretic patterns, it is observed that the normal and sickle-cell anemia are different and the heterozygote has both types corresponding to haemoglobins A, S and a mixture of A and S respectively (Fig. 23.1). These are due to gene interaction so that the physical as well as the physiological properties of the haemoglobin are altered. The sickle-cell anemia appears to be due to S haemoglobin in relation to the content in the red cells.

Genes and Enzymes

Genes function in two ways. First, they are replicated and pass on their replicas to all cells in the next generation. The replicas are exact copies of the original gene. Second role of the genes is to control the cell activity through which they exert influence on each and every step of an organism's development.

The biochemical reactions of a cell are normally controlled by genes and it so happens that these reactions are catalyzed by specific enzymes. We have earlier discussed one gene-one enzyme hypothesis suggesting that each gene controls a specific protein or an enzyme. This gene-enzyme relationship has been studied in many organisms, including humans, where in a chain of reactions a

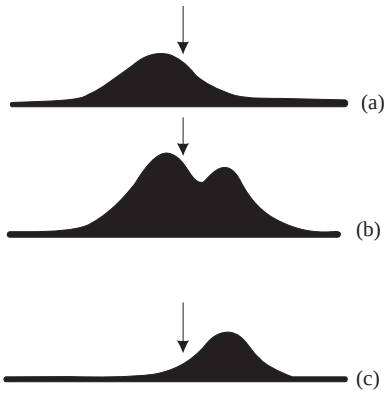


Fig. 23.1 Electrophoretic scanning pattern of haemoglobin from (a) normal, (b) sickle-cell anemia red blood cells.

biochemical step is blocked by a mutation. G.W. Beadle and E.L. Tatum studied this phenomenon in a mutant strain of *Neurospora.* We can conceive of a chain of reactions in which one compound is converted into another through a series of steps, each step being controlled by an enzyme (Fig. 23.2).

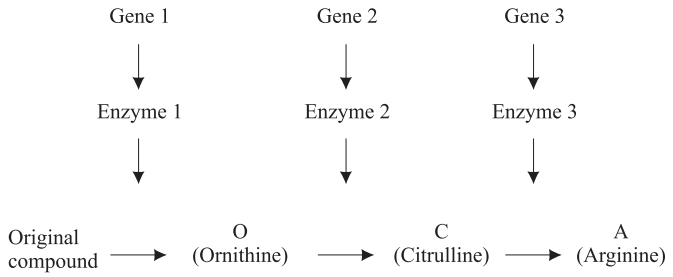


Fig. 23.2 Some steps in the ornithine cycle of Neurospora.

In this diagram, gene 1 converts the original substance to O; gene 2 converts O into C; and gene 3 finally converts C into A. If the normal functioning of gene 1 is disturbed by some means (by X-rays or ultraviolet rays), a mutation is caused blocking the first step of the overall reaction sequence. As a result of this, the mold *Neurospora* will not grow unless the functioning of the gene is restored to normalcy. Alternatively, if we could add the product O to the medium, growth would be resumed. Similarly, if the gene 3 mutates, C will not be converted to A. Hence in order to allow the sequence of reactions to go on in a normal fashion, the product A will have to be added to allow the growth of the mold.

Higher primates (chimpanzee and man) excrete uric acid in their urine, whereas monkey and other mammals excrete allantoin to a greater extent. Uric acid and allantoin are chemically related to each other (Fig. 23.3). The reaction is a complex conversion of uric acid to allantoin requiring number of steps, but only a single enzyme is required. A special variety of dogs, *Dalmation dogs,*

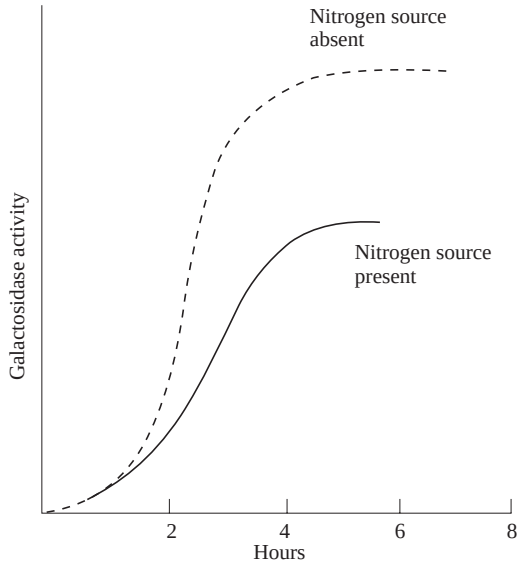


Fig. 23.3 Induction of galactosidase activity.

like man excrete uric acid, while other dogs excrete allantoin. It has been suggested that this may be due to a gene mutation owing to which uricase in such dogs was not produced.

23.3 NUCLEUS-CYTOPLASMIC INTERACTION

The nucleus-cytoplasmic interaction has been studied in a number of organisms. The most successful experiment has been conducted on the single- celled alga *Acetabularia.* Nuclear control of regeneration has also been demonstrated in *Acetabularia* and in amphibian embryos where the donor nucleus takes over the control of the recipient cytoplasm.

The effect of cytoplasmic changes on the nucleus have been demonstrated by experiments involving transplantation of nucleus from one type to another type of cell, leaving it there for some time and then transferring it back to the original type of cytoplasm.

In an experiment, two species of *Amoeba, A. proteus* and *A, discoides* were used. Both these species differ from each other in shape, nucleus size and fission rate. The nucleus of *A. discoides* was removed and the nucleus of *A proteus* was transplanted in the *discoides* individual. *A. discoides* produced a clone of individuals which were intermediate between the two species as regards shape, whereas in other characters they were similar to *A. discoides.* After spending a few generations in the *discoides* cytoplasm, the descendants of original *proteus* nucleus were transplanted back to *proteus* individuals. It was surprising to notice that longer the *proteus* nuclei remained in the *discoides* cytoplasm, the more they behaved like *discoides* nuclei.

Rana pipens and *Rana sylvatica* are two species of frog where interbreeding is possible, but their embryos die early at the gastrula stage. This shows that the DNA of both individuals is quite different and gives conflicting instructions. so that hybrid embryos do not develop at all.

Cellular Regulation

Cellular differentiation in organisms is under the influence of gene action. It has been demonstrated that all genes are never active at the same time in different cells. Therefore, the tissue characteristics are controlled by certain set of genes which are switched on. The switching on and off mechanism has been studied extensively in microorganisms, especially in *Escherichia coli,* and to the best of our knowledge the best workable model proposed so far is that of Jacob and Monod (1961).

A simple experiment of *E. coli* can be cited here. *E. coli* grows on a simple medium containing glucose as the sole carbon source in addition to other substances present in the nutrient growth medium in optimal amounts. If the culture is separated by gentle centrifugation and the nutrient medium is replaced by lactose instead of glucose, the growth of *E. coli* population will stop. Why does the growth stop? The simple explanation to this question is that *E. coli* is not able to utilize lactose. However, after a lapse of time the organisms start growing and reach a level of maximum growth. In order to utilize lactose, the cells need an enzyme β-galactosidase. Normally all the cells do not possess it. It has been found that during the initial lapse period the cells synthesizing this new enzyme β-galactosidase in response to a change in the environment. β-galactosidase appears in all cells even when a nutrient medium is replete or deplete with a nitrogenous source. Nitrogenous source is necessary for reproduction and growth. The new enzyme is produced by the newly synthesized protein and not by the pre-existing proteins (Fig. 23.3).

The relationship between enzyme induction and the inducing substance has been extensively studied by using an inducer substance (which is not a substrate). A repressor operator gene system has been developed using bacteria. Three classes of genes are involved in this system:

(a) the functional genes that guide the mRNA in protein synthesis;

(b) operator genes which control the functional genes; and

(c) repressor genes which control the operator genes.

In this connection two considerations must be borne in mind: (1) most operator genes are repressed at any point of time, making most functional genes inactive, and (2) repressor genes themselves are controlled by cytoplasmic differences such as substrate concentrations.

In the actual experiment, Jacob and Monod took the bacterium *E. coli* which utilizes sugars like β-galactosides by attacking them with β-galactosidase. When β-galactosides are absent from the medium, the enzyme β-galactosidase is not produced. This means that this enzyme is produced only when its specific substrate is present in the medium. However, other enzymes are produced irrespective of the presence or absence of their substrate. On this assumption, the authors proposed a unit of genes called *operon* which are supposed to function in collaboration with a *regulator gene.* The operon is a cluster of genes which regulates the synthesis of mRNA. In this model, let us assume that the operon consists of an operator gene and three structural genes, A, B and C. The DNA segment constituting gene A is responsible for the production of β-galactosidase, and genes B and C code for other associated, enzymes. The operator gene acts as a switch, which when open allows the structural genes to operate, and when it is closed the genes cease to work (Fig. 23.4). The regulator gene produces a repressor product which keeps the operator gene closed. If β-galactoside is present in the medium (environment), it reacts with the repressor substance and binds with it, making the operator gene functional. Thus the operator gene is switched on so that the structural genes start

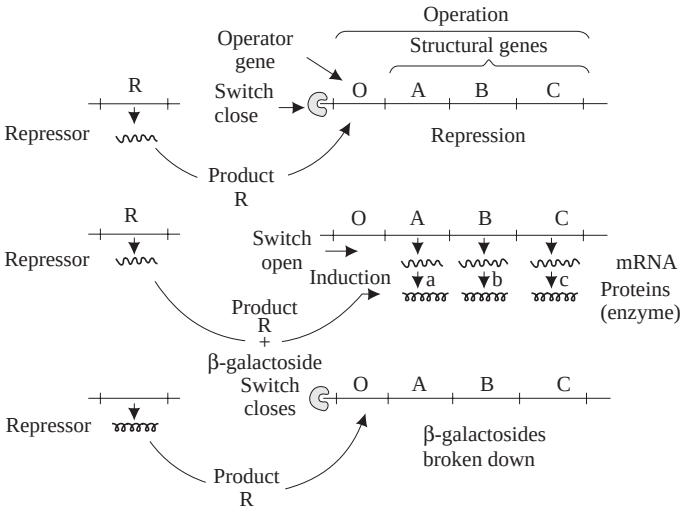


Fig. 23.4 Operon model showing gene regulatory mechanism.

coding for their characteristic mRNA. Thus β-galactosidase and two other enzymes are produced which help in the uptake and metabolism of β-galactosides. When the substrate is finished, the repressor substance starts accumulating and in turn closes the operator gene so that the structural genes no longer produce the enzymes.

23.4 TRANSPLANT EXPERIMENTS

Since the work of Beadle and Tatum (1941) on the biochemical genetics of *Neurospora,* there was a tremendous spur in the research activities on biochemical genetics. One of the famous experiments was that of eye transplantation in *Drosophila* conducted by Beadle and Boris Ephrussi in Paris.

In *Drosophila melanogaster* two types of mutants are found which are called vermillion (v) and cinnabar (cn) respectively. In these mutants, the eye colour is lighter as compared to the wild types. The eye colour in cinnabar mutant is deep red due to the combination of red and brown pigmentation. Brown pigment is lacking in both the vermillion and cinnabar mutants. Both these pigments are produced due to mutation of one gene. If in the beginning, eye primordia taken from the body of the larvae of these two genotypes are transplanted to the body of a normal larva, it is found that v-mutant develops the same red colour as was found in the wild type. The same effect was observed when the eye primordium of v-animal was transplanted into cn-individual. By transplanting (in a normal animal) the eyes of the cn-larva in a normal animal, its eyes also become deep red. If the eye promordia of cn-larva is transplanted in the body of v-individual, the colour of the eye is found to be light only. From these observations one can infer that both in the normal fly and in the cn-mutants a factor is developed which produces brown pigment in the eyes of v-mutants, which is otherwise wanting in them. In the same way, the factor necessary to produce brown colour in the eyes of cn-mutants is absent from v or cn-mutants although it is present in the wild type (Fig. 23.5). From

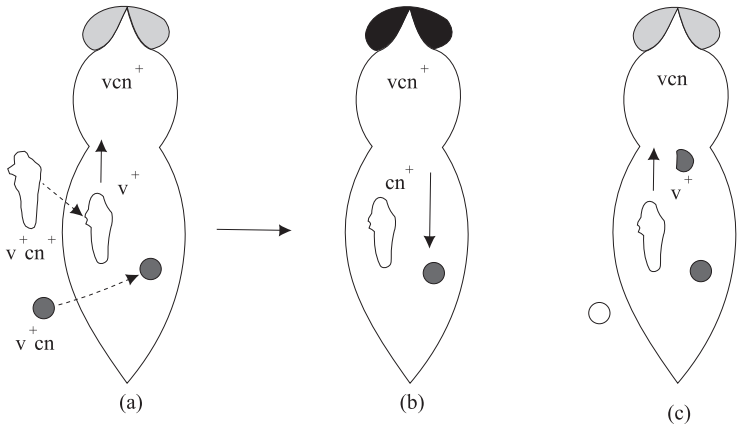


Fig. 23.5 Effect of vermillion (v) and cinnabar (cn) mutations in Drosophila. (a) a fat body of a normal animal (v^+ cn^+) is transplanted to a vcn^+ animal, where it secretes v^+ substance; (b) the host tissues transform this into cn^+ substance so that both the eyes of the host, and a grafted v^+ cn eye can develop normal pigmentation; (c) same transplantation into a vcn animal has no effect since the v^+ substance cannot be transformed into cn substance. (From Raven, An outline of Development Physiology, 1959).

experiments it has been known that these factors for eye pigmentation are produced by certain cells under the influence of genes and get diffused throughout the body by blood and lymph circulation.

It is known that quite a large number of mutants have negligible effects on the morphology, but reduce vitality markedly. However, traits like eye colour, pigmentation, etc. are instances which bring in some alterations in the normal physiological processes.

The genes are responsible for stepwise synthesis of certain products which produce the eye pigment. The synthesis takes place in the following manner. It is now known that recessive gene v blocks the synthesis of kynurenine and cn blocks the conversion of kynurenine to 3-hydroxy-kynurenine.

A single gene may influence several developmental processes. We can consider an example of this phenomenon in a moth *Ephestia* where one chemical substance influences the speed of development, viability of the embryo, the pigmentation of eyes and skin of the caterpillar, and the pigmentation of eyes, brain and sex organs of the imago. Most of the substances are not species specific. The v^+ and cn^+ substance of *Drosophila melanogaster* are found to exist in *Ephestia* and the wasp *Habrobracon* also. However, the end point of the reaction is not always the same.

23.5 GENETIC CONTROL OF EYE PIGMENTS

In *Ephestia,* the mutant allele suppresses pigment formation. In the normal wild type *Ephestia* (a^+/ a^+) the eyes are dark brownish black, the testes are brownish violet, and the skin is reddish, whereas in the mutant allele (a/a) individuals have red eyes, testes are colourless, and the skin has no pigment. In a transplantation experiment, when the male genital disc of wild-type larva was implanted into the

penultimate (next to last) larval in star of *a/a* individual, it was found that the skin was reddish in the next larval instar, the adult testes were dark.

The eye pigments of insects belong to a group of substances called ommochromes whose major precursor is tryptophan. The a^+ allele of *Ephestia* (= v^+ of *Drosophila)* produces an enzyme which converts tryptophan into kynurenine (Fig. 23.6). If kynurenine is added to *a* mutant, it takes the place of a^+ gene. However, for the formation of pigments additional genes are needed which participate in the chemical pathway. cn^+ is such a type of gene which influences the conversion of kynurenine into 3-hydroxykynurenine. Normal pigmentation will develop if hydroxykynurenine is injected in the cn mutants irrespective of the fact whether a^+ is present *or* not since hydroxykynurenine is the last step in the pathway. The eye pigment granules contain, besides pigments, protein and RNA as well and further steps in eye pigmentation can be blocked by mutations. The structural protein is made by wa^+, but its formation can be blocked by the mutation wa (white eyes), thus blocking pigmentation. In *wa*/*wa* individuals eye cells are devoid of pigment granules; in *a/a* individuals pigment granules like the wild type are present. If the normal implants are added to *wa/wa* individuals, the situation does not change since wa^+ gene has only one intracellular effect. The pigment precursors, kynurenine and 3-hydroxykynurenine, are released into the blood by various tissues of *wa* mutants as in case of normal individuals. The genetic blocks in ommochrome formation may be repaired by implanting *wa/wa* tissues into *a/a* host individuals. So it is possible to supply the missing precursor to the eye cells of a mutant which lacks the capacity to form a particular pigment. This can be done by a normal implant. In this way, certain intracellular defects of the mutant cells can be repaired by substances released from other cells.

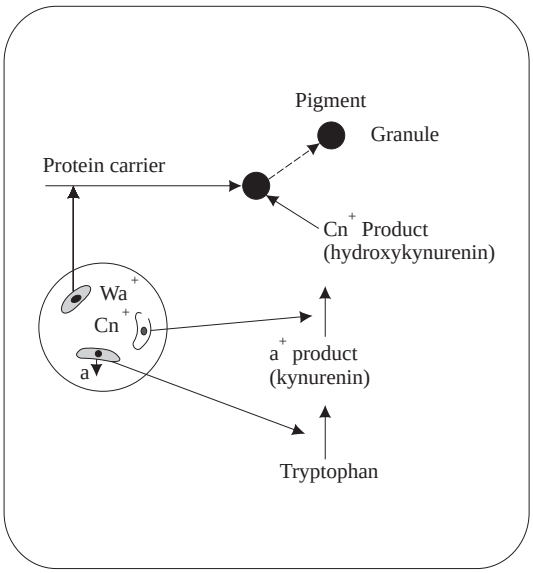


Fig. 23. 6 Diagram showing the activity of some genes in the formation of eye pigments in *Ephestia*.

23.6 GENIC CONTROL OF DEVELOPMENT

Important events during development, e.g. cleavage. cell differentiation and tissue-organ formation (morphogenesis) in multicellular animals are under the control of nucleus. Although these processes lie in the domain of developmental biology and do not come in the scope of this book, nevertheless, we shall try to describe only such processes that may be found useful in explaining the genic effects on certain developmental processes.

The role of nucleus is indispensable in development. It has been shown that, although cleavage of an egg is possible up to a few divisions only without a nucleus, normal development up to a significant stage will not take place in its absence. The behaviour of chromosomes in mitosis and meiosis is greatly influenced by the individual genes. Polarity and cleavage are also affected by genes. Such gene influences have been studied with the help of mutations.

Mutation may be defined as a sudden permanent heritable change* which may give rise to a new phenotype or it may be manifested in some way to cause a visible structural change in the organism. Mutations take place in all organisms and are the source of variations. When the genes undergo mutations, the effect may be either some visible or sometimes invisible changes in the phenotype. Some of the mutations are lethal which lead to the death of the embryo at any stage during the period of embryonic development. Some of these mutations concern single gene which are found to influence development at a particular stage, while in certain cases, a single gene mutation may influence a number of developmental processes.

Four kinds of phenotypic effects of gene mutations have been described: lethal, morphological, physiological and chemical. Lethal mutations may arise anytime during the life of an individual from egg to the adult. Morphological mutations may bring changes in shape, size and growth habits of the phenotype. Physiological mutations affect various functions of the organisms such as growth rates, pigmentation, susceptibility to environmental changes, and responses to various stimuli, etc. Chemical mutations are responsible for visible metabolic disorders.

The fertilized egg contains characters of both the parents in an undeveloped form. During cleavage, cleavage nuclei are formed. According to Weismann, there is a qualitative difference between the products of these nuclear divisions. Weismann's theory has been refuted since all the nuclei of the organism will contain all the genes typical of that organism. Interaction between genes and cytoplasm takes place influencing further development, and it is necessary that full complement of the genes should be present for the normal course of development. Absence of a chromosome or even a few genes of the chromosome will bring the development to a standstill at sufficiently early stage.

The genes have to function through interaction with the cytoplasmic factors Such interactions could be studied by combining nuclear material of one species of egg with the cytoplasmic material of the other species. The embryo of such a combination may give us some results of the nuclear cytoplasmic interaction producing hybrids. One example can be cited from amphibians illustrating this phenomenon.

*Mutations do not involve heritable changes arrived at by recombinations.

If a cross is made between *Rana esculenta* and *R. fusca,* a hybrid zygote is formed which does not undergo development beyond the gastrula stage. Such a hybrid is non-viable. It was found that in such hybrids the processes of respiration and carbohydrate metabolism were different from the normal ones. Brachet (1952) reported that in such gastrulae, RNA synthesis did not take place at all upto this stage. It is evident that foreign chromosomes inhibit the process influencing cell metabolism. When parts of such "lethal hybrids" were grafted into normal embryos, they continued their normal development accompanied by RNA synthesis. This shows that hybrids are unable to synthesize such materials which are indispensable to metabolism.

An interesting experiment was performed by Baltzer (1947) on the pigmentation of merogonic hybrids* of black and white axolotles. A single gene is responsible for the difference in pigmentation. The colour character depends on the epidermis of the species. The black-skin of axolotle secretes a substance (an oxidase) which produces melanin in the pigment cells. The white-skin axolotle is deficient in this substance. Baltzer's merogones, which had the cytoplasm of black axolotle and nucleus of the white axolotle, had a white pigmentation like the white race. It was found that when the skin of white merogone was transplanted into black larva early enough, a white pigmentation developed. It may be concluded that pigmentation was entirely due to nuclear factors. A plausible reason could be that during development certain genes are activated at certain time. This view has been strengthened by the experiments of Beermann (1963), and Kroeger (1968) on the giant chromosomes of dipterous larvae.

Certain organs, such as salivary glands, intestine, excretory tubules, etc. of dipterous larvae possess giant chromosomes which have alternating light and dark bands reflecting the sequence of genes on the chromosomes. Sometimes swellings or *puffs* appear on the dark bands. Appearance of puffs is due to alteration in the chemical composition in that region and accumulation of RNA. Puffing can be artificially induced by external factors, for example, by injecting ecdysone and juvenile hormone in the larva (Beermann, 1961; Kroeger, 1968). The puffs may be made to disappear by lowering the intracellular Na^+ concentration which is a consequence of low ecdysone concentrations. The puffs may reappear if the Na^+ concentration is increased. The cause of this is the electrolytic disturbance of the intracellular and intercellular environment. The appearance of RNA in the puffs directs the synthesis of such proteins that are able to alter the permeability functions of the cells. It was concluded that moulting depends on the activation of a sequence of genes.

The activation of genes produces chemical substances in the cell which influence the developmental processes. The role of genes during the course of development can be established by locating a particular development stage in which a departure from the normal arises by substituting a single allele. A phenotype of one genotype can be altered to the phenotype of another genotype by tracing the environmental influence on the phenotype. This process has been called *phenocopying* by Goldschmidt (1938). Temperature effect, X-rays and chemicals have been used to alter the phenocopies.

A mutant character in any part of the body may arise from an altered response of the cells or another part of the body may influence the particular character. This results in two types of defects: intracellular and intercellular genetic defects, also called as *autopheny* and *allopheny* by Hadorn.

* Merogone is an embryo from a haploid egg containing either a sperm or haploid egg nucleus.

23.7 EFFECTS OF GENE MUTATIONS

A mutation of a gene must involve some change in the base sequence of DNA, and there is enough indication to suggest that majority of spontaneous gene mutations that occur in nature involve no more than the change of one base at some point in the sequence of hundreds or thousands of bases contained in the DNA of a particular gene. In some cases, the base change alters a triplet coding for a particular amino acid to another triplet coding for the same amino acid. About 20-25% of base changes are of this type, hence many mutations are without any significant consequences. In about 70-75% cases base change results in altering the triplet leading to synthesis of an altered protein. A considerable variety of structurally altered forms of protein may arise in this way. In addition to this, about 5% of mutations involve single base changes which would alter the triplet coding for a particular amino acid at some point so as to give rise to a code for chain termination resulting in a shortened polypeptide.

Besides single base mutations, other kinds of mutations which have more drastic effect on DNA are also known to occur. These involve deletions, duplications and other rearrangements of base sequences. In majority of such cases a viable and functional protein is not likely to be produced. Such mutations can give rise to "inborn errors of metabolism". Thus a variety of mutant alleles may be produced by mutational events within any given gene, and each will be expected to have its specific effects on the synthesis and structure of the corresponding enzyme.

Gene mutations result in the formation of mutant phenotype in many cases. Mutant characters have been extensively studied in *Drosophila,* and it has been shown that lethal mutations can occur at any stage in the life of an individual. With different mutations, death occurs at different phases of development due to different causes. For example, in *Drosophila,* death will occur at the time of hatching from the egg due to *curly mutation,* whereas for *meander mutation* death will occur at the third instar larva. Poulson (1945) demonstrated that in *Drsophila* deficiencies of different parts of the X-chromosome are the root cause of different lethal effects. When all individuals carrying a particular lethal factor die at the same time, the gene is called a *monophasic lethal* factor. In contrast to this, a lethal factor may be *diphasic* or *polyphasic* in which case death may occur at two or more than two specific times.

Creeper Mutation

The creeper (Cp) gene in the fowl is a case of diphasic lethal gene factor. The creeper fowls (Cp / Cp) are homozygotes which show disproportionate body growth, and in the heterozygotes the bones of the extremities are either too short or bent. The homozygous embryos mostly die at the end of the third day or the beginning of the fourth day. Some creepers, however, survive this lethal death and continue to develop until the end of the incubation period and then die. These embryos are cripples and never hatch. In this case, the gene has an interesting action since lethality occurs at different phases of life. The heterozygotes also show some abnormalities like slow growth of wing bones and histological abnormalities and the like (chondrodystrophy).

Phenocopies of creeper mutant can be artificially produced either by injecting into the yolk sac or by giving selenium in the food of the normal layers (+/+). This chemical produces the same effect as the mutant gene does. The inhibition of growth in both the homozygotes and the heterozygotes

brings about bone dystrophy or crippling. The death, probably, is caused due to disturbance in the normal morphogenetic process.

Frizzle Fowl Mutant

Another mutant of the domestic chicken is known as *frizzle fowl* where the developmental process is upset. This fowl has twisted or curled feathers. The factor is strongly expressed in homozygotes as compared to heterozygotes. The down feathers are retained for about a year, and after that they become bald. The feathers of the mutant are devoid of hooks. The frizzles are quite sensitive to changes of weather conditions. They also suffer from many other defects. The homozygote frizzles are difficult to raise, and if at all they survive, are sexually immature. These fowls have a high rate of basal metabolism because of the unusual heat loss due to baldness.

In order to maintain body temperature within limits in relation to ambient temperature, the consumption of food is higher. Such fowls develop a secondary trait, that is increase in heart size which is needed for increased blood circulation to compensate the heat loss. The frizzle character is developed due to a mutant gene that is present in the feather rudiments.

23.8 INBORN ERRORS OF METABOLISM: GENIC DEFECTS

The term "inborn errors of metabolism" was first introduced in medicine by Sir Archibald E. Garrod in 1908. He recognized certain rare inherited metabolic diseases in which the clinical and biochemical abnormalities were observed in humans. Garrod attributed these diseases to the congenital deficiency of a particular enzyme, which in turn is caused by the presence of an abnormal and mutant gene. Usually these conditions have been found to be inherited as Mendelian recessive traits. The obvious implication is that the affected individual has received such an abnormal gene from each of his parents. Heterozygous individuals who receive the abnormal gene from only one parent and its normal allele from the other are generally healthy, though they usually show partial deficiency of the specific enzyme. Since the time of Garrod, a number of metabolic deficiencies in humans as "inborn errors of metabolism" are known (Table 23.1).

The essential feature of the inborn errors of metabolism is the deficiency of a particular enzyme activity resulting from gene mutations. The mutational change may lead to the synthesis of a structurally altered protein with defective or modified properties. A mutant gene may result in the synthesis of altered protein whose properties are not necessarily altered, but whose inherent stability is much reduced due to conformational change. This leads to the reduced activity of the enzyme. It is also possible that the mutation may completely inhibit the synthesis of enzyme protein or cause considerable reduction in the rate of synthesis.

Alcaptonuria

Sir Garrod recognized a disease alcaptonuria in man due to rare recessiye gene in which the urine turns black upon standing in air for some time. Persons carrying the disease are unable to break down a chemical substance, homogentisic acid which is excreted in urine. The inability to break down this acid is due to the absence of an enzyme which catalyzes this step.

Table 23.1 Some Diseases Due to Inborn Errors of Metabolism

Name of the disease	*Disorders in metabolism*
Phenylketonuria	Deficiency of phenylalanine hydroxylase leading to accumulation of phenylalanine; mental retardation
Alkaptonuria	Failure of breakdown of homogentisic acid due to deficiency of homogentisic acid oxidase: urine turns black
Cystinuria	Abnormal excretion of certain amino acids; kidney stone formation takes place
Albinism	Inability to produce melanin pigment in the skin; deficiency of tyrosinase
Goitrous cretinism	Insufficient secretion of thyroxine blocks the conversion of diiodotyrosine; deficiency of iodotyrosine deiodinase; mental development and body growth retarded
Haemolytic disease	Deficiency of hexokinase leading to congenital disorder
Haemolytic disease (congenital)	Deficiency of pyruvate kinase
Favism	Primaquine sensitivity; congenital haemolytic anaemia; glucose-6-phosphate dehydrogenase deficiency
Glycogen disease, Type I	Deficiency of glucose-6-phosphatase
Galactosaemia	Deficiency of galactose-1-phosphate uridyl transferase
Benign fructosuria	Deficiency of fructokinase
Gangliosidosis	Deficiency of β-galactosidase
TAY-SACHS' disease	Defect in lipid metabolism due to deficiency of β-acetylhexosaminidase; results in idiocy
Hypophosphatasia	Caused due to alkaline phosphatase
SHELDON'S disease	Deficiency of pancreatic lipase
Hyperglycinaemia	Deficiency of propionyl-Co-A carboxylase

Pleiotropic Effects of Genes

Pleiotropy may be defined as the effect of a single gene upon two or more characters in the same individual, not obviously related to each other. We know that genes produce inducing substances commonly called enzymes. In the same individual it is possible that a gene produces multiple effects through more than one biochemical steps. Pleiotropic effect of single gene is a widespread phenomenon and has been studied in a number of organisms, including plants and animals. In *Drosophila* alone at least seven mutant alleles have been described which prove pleiotropism. In this limited space, we cannot afford to describe the details of all such experiments. However, we shall illustrate this phenomenon by making reference to a few well known heritable diseases in man. These are phenylketonuria, goitrous cretinism and albinism. In order to understand pleiotropy, one should know the biochemical basis of these characters.

Phenylketonuria (PKU)

Phenylalanine is an essential amino acid which must be present in the diet of animals. In man, there is a special type of low grade mental activity (idiocy) caused by the failure to convert the amino acid phenylalanine into tryosine. Only a single biochemical step is necessary for this conversion. The mental defect is inherited as a simple recessive trait resulting in a difference of only one allele between idiots and normal persons. The enzyme responsible for the conversion of phenylalanine to tyrosine is produced in the liver which is however, absent in persons affected by idiocy.

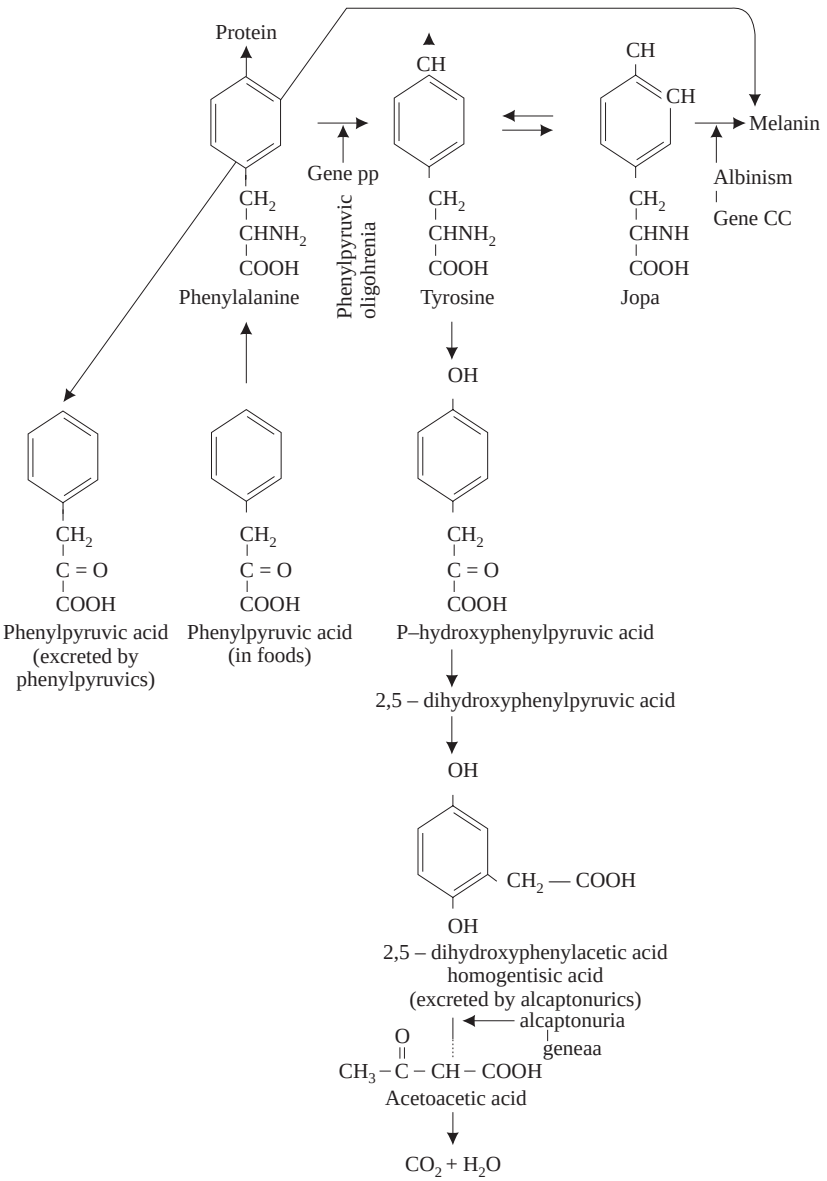


Fig. 23.7 Phenylalanine metabolism in man.

Some phenylalanine is absorbed in the body and incorporated into the protein molecules, while some is converted in the liver into tyrosine. Excess of phenylalanine is deaminated to form the phenylpyruvic acid, much of it is excreted in the urine. Phenylketonuria is induced by accumulation of phenylpyruvic acid which causes brain damage so that children become mentally retarded. The steps involved in phenylalanine and tyrosine are shown in (Fig. 23.7).

Tyrosine will not be synthesized if step A is blocked and the tyrosine requirements of the individual may be satisfied from the food source alone. Thus phenylalanine will accumulate in the body and some of it will be converted into phenylpyruvic acid and excreted in the urine of phenylpyruvics. Such phenylketonuric individuals are mentally retarded, but it is found that they are also deficient in the melanin pigmem in their hairs, eyes and skin. This is an excellent example where a single gere produces multiple effects.

Albinism

Albinism is a familiar phenomenon in animals and man which is caused by a homozygous recessive gene. Albinos are devoid of melanin pigment in their hair, skin and eyes. It has been shown that albinism is produced due to a genetic block is the tyrosine catabolism, although it is not yet known as to which step is blocked. Nevertheless, the genetic block prevents the formation of melanin in the hair, skin and eyes. In phenylalanine and tyrosine, metabolism, tyrosinase is missing which probably catalyzes the oxidation of dopa (3, 4—dihydroxyphenylalanine).

Goitrous Cretinism

Malfunctioning of the thyroid gland causes Goitrous cretinism. Thyroid secretes a hormone, thyroxine. If the secretion is insufficient, mental development and body growth are retarded. The deficiency of thyroxine is due to genetic block in its conversion from diiodotyrosine. Diiodotyrosine accumulates in the body most of it is excreted in the urine (Fig. 23.8).

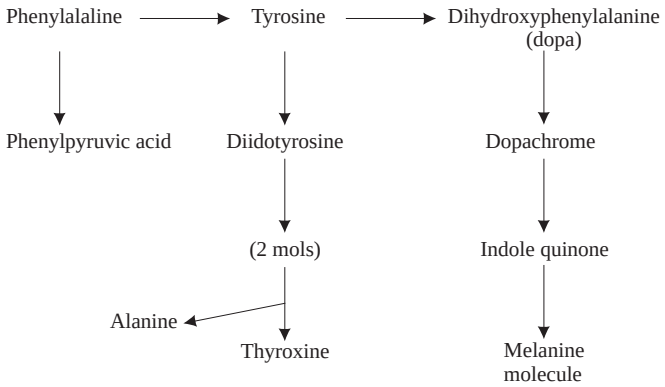


Fig. 23.8 Some derivatives of the amino acid phenylalanine.

Gout

Gout is a disease in which the patient suffers from elevation of uric acid in the blood (hyperuricemia) and excessive pain in the joints of lower extermities, especially of the foot and ankle. In extreme cases, there are deposits of uric acid crystals in the joints. The causes of gout are not yet fully known; however, it is suggested that purine metabolism is disturbed in the disease. The disease is thought to be inherited, finding its expression more in males rather than females in an autosomal dominant.

CHAPTER

24

Immune System

The body has to defend itself against infections caused by organisms or pathogens (foreign bodies). These foreign bodies may enter the body by crossing through barriers like skin, hair or open spaces (wound etc.). As a consequence, innate mechanism of the body comes into operation, but is inadequate to offer full protection in all cases, hence the system responds against foreign substances through immune system which, like other systems, is composed of several types of differentiated cells. This response is adaptive in nature and operates through the production of antibodies by lymphocytes.

Vertebrates have a unique position in the animal kingdom bestowed with the capacity to acquire immunity against foreign bodies. Specific immune system has been evolved for dealing with pathogens, a part of which is definitely inherited. An accidental entry of a foreign substance (macromolecule or a pathogen) into a mature animal evokes immune response to render the invader harmless. How does the body recognize the foreign material? What is the sequence of events that leads to development of immunity?

24.1 TYPES OF IMMUNITY

Immunity signifies all those properties of the host which confer resistance to a specific infectious agent. It depends on various factors, such as (a) host resistance, (b) dosage: a large dose of the pathogen will always overpower the host, and (c) virulence of the organism. The power of resistance may be inherited i.e., natural or innate, and acquired as a result of previous infection or inoculation.

Natural Immunity

This is an inherited resistance to infection and is concerned with species, races or individuals. Little is known about the mechanism of this kind of resistance. It is naturally present in an individual and is not acquired through previous contact with the infectious agent. Resistance to infection varies with

different individuals of the same species and with age also. Very young and elderly persons are more susceptible to microbial diseases than persons of other age groups.

Acquired Immunity

The immunity acquired during the life time of an individual is known acquired immunity. It is basically of two types:

1. *Active immunity:* Active immunity is an adaptive resistance that is built up in an individual following contact with foreign bodies (antigens) e.g., microorganisms and their toxic products. The mechanism involves production of antibodies by the host cells. Active immunity develops very slowly over days and weeks but persists over several years. The mechanism involves either *humoral response* or *cell mediated response.*

Humoral immunity: In this, many immunological reactions are especially directed against microorganisms and mediated through the circulating blood proteins known as antibodies. The antibodies are actively generated against antigens of microorganisms and their products. These antibodies may induce resistance by (a) neutralizing toxins or cellular enzymes, (b) killing bacteria or lysing with *complement,* (c) blocking the infective capacity of microorganisms, (d) making microorganisms susceptible to phagocytic action, and (e) combining with cellular antigens that interfere with phagocytosis (opsinizing).

Cell mediated immunity: This is a category of complex response requiring immunologically specific and non-specific features. The response may or may not be accompanied by humoral antibody formation and the principal agent is an immunologically active lymphoid cell. These are specific circulating cells which recognise foreign materials and initiate a chain of reactions. The reactions include mononuclear inflammatory responses, cytotoxic destruction of invading cells, activation of phagocytic macrophages and delayed hypersensitivity in tissues.

Passive immunity: It is a temporary resistance against an infective agent induced by the administration of antibody against that agent. The antibodies in question are derived from another host. Passive immunity lasts for a short time, usually a few weeks only, since the antibody molecules constantly break down without forming new ones. This has, however, one advantage, i.e. upon administration of antibody the immune response shows up immediately without any lag period.

24.2 WHAT ARE ANTIGENS?

Antigens are substances which, when injected parenterally into an animal lacking that substance, cause the production of antibodies foreign to those antigens. They are complex chemical substances, usually a combinatiion of a protein component and a non-protein or non-antigenic component called *hapten.* This hapten cannot by itself produce antibodies. but once the antibodies are formed, it cannot combine with it. Some polysaccharides and polypeptides can also act as antigens. Sometimes lipids and nucleic acids, in combination with proteins, also act as antigens. It seems that the size of a molecule provoking an immune response is an important requirement, for small molecules like glucose do not evoke an antibody response. Hence, an antigen molecule has to be a macromolecule. Nonetheless, all macromolecules do not behave as antigens. The macromolecules of our own body

that form the normal constituents do not have antigenic properties, but a macromolecule of a rabbit when injected into humans or vice versa will evoke an antibody response. The reason for this behaviour is due to the difference in the structure of molecule from two individuals belonging to different class of mammals.

What are Antibodies?

An antibody is an immune substance which is formed in the blood of an animal in response to an antigenic stimulus and which reacts specifically with the corresponding antigen in some observable way. An antibody is a protein, and forms part of serum globulin. The blood contains proteins such as albumin, globulins and fibrinogen which can be isolated by electrophoretic methods.

Antibodies may comprise 1-2% of total serum proteins and even more in certain abnormal conditions. Since the antibodies belong to the class of globulins, they are also called as immunoglobulins. Antibodies are characterized by their chemical, physical and immunological properties.

24.3 TYPES OF IMMUNOGLOBULINS

Immunoglobulins are functionally and structurally differentiated classes of globulins (globular proteins) that act as antibodies. A given antigen when injected into the body of an organism stimulates the formation of several antibodies which react with the antigen. Generally a more complex antigen stimulates the production of a greater number of antibodies.

There are five classes of immunoglobulins in human plasma: IgG, IgA, IgM, IgD, and IgE, the last two being in very small amounts. They have been identified and differentiated on the basis of their structure and electrophoretic behaviour. All immunoglobulins are composed of two types of polypeptide chains which are named as light (L) and heavy (H) chains. The light chains are linked to the heavy chains by disulphide (S-S) linkages and mostly form dimers $(LH)_2$. However, in certain cases the LH monomer is further polymerized to give a more complex structure. There are several classes of L and H chains. In humans, there are two major types of L chains: *Kappa (K)* and lambda (λ). These may be recognized by their amino acid sequences. So all immunoglobulins may have either kappa or lambda chains or both. Besides these, each class of immunoglobulins possesses an H chain. Light chains also have a constant and a variable region (Fig. 24.1). The carbohydrate portion is always present in the heavy chains. Immunoglobulins of different types have specific characteristics and special functions (Table 24.1) which are determined on the basis of structural differences.

Each antibody molecule is bifunctional and has two sites for combining with the antigen. The amino end, which is the variable region is responsible for recognizing and combining with the antigen (Fig. 24.1) Thus, both the heavy and light chains can recognize the antigen.

Different classes of immunoglobulins are as follows.

1. *IgG:* Most of the immunoglobulins belong to this class. IgG has four peptide chains comprising of two light and two heavy chains which are linked with S-S bonds (Fig. 24.2). There are two antigen binding sites in each IgG molecule located in the variable part of the light and heavy chains. The molecule looks like a Y shaped structure which can be subjected

Table 24.1 Classes of Immunoglobulins and Their Properties*

Description	*Classes of immunoglobulins*				
Type	*IgG*	*IgA*	*IgM*	*IgD*	*IgE*
Heavy chains M. W.	53,000	64,000	70,000	58,000	75,000
Light chains M.W.	150,000	180,000	950,000	160,000-185,000	190,000
Carbohydrate %	2.9	7.5	11.8	–	10.7
Sedimentation coefficient	7S	9-11 S	19 S	7 S	8 S
Half-life (days)	23	5.8	5.1	2.8	2.5
Synthetic rate (mg/kg/day)	33	24	6.7	0.4	0.016
Polymeric state	monomer	monomer, dimer, tetramer	pentamer	monomer	monomer
% of total immunoglobulin	75	14	10	1.0	0.003
Power to fix complement	+	–	+	–	–
Biologic properties	passes through placenta, enhances phagocytosis of micro-organisms	present in secretions	helps in agglutination and cytolysis	present on the surface of lymphocytes with IgM	causes allergy symptoms induced by helminth infections

*Compiled from various sources.

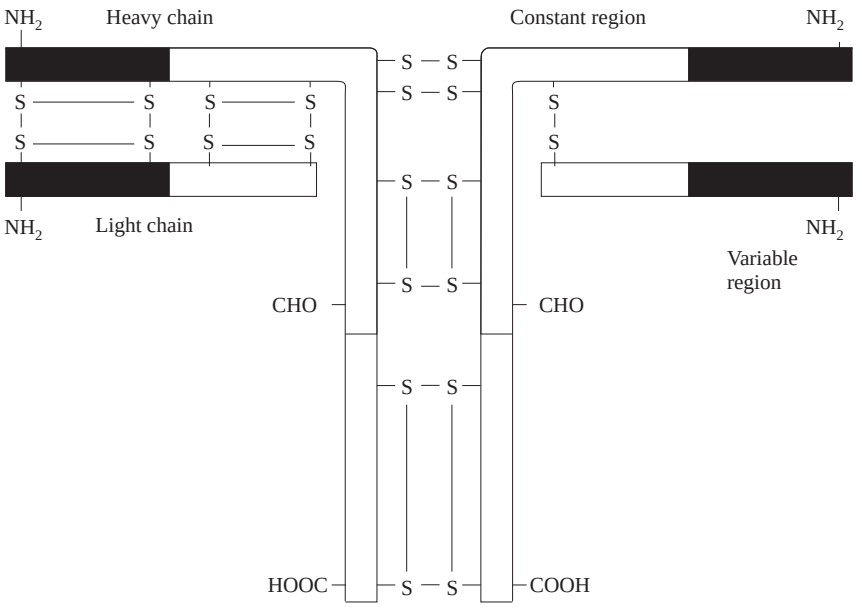


Fig. 24.1 The polypeptide chain structure of an antibody molecule of IgG with variable and constant regions.

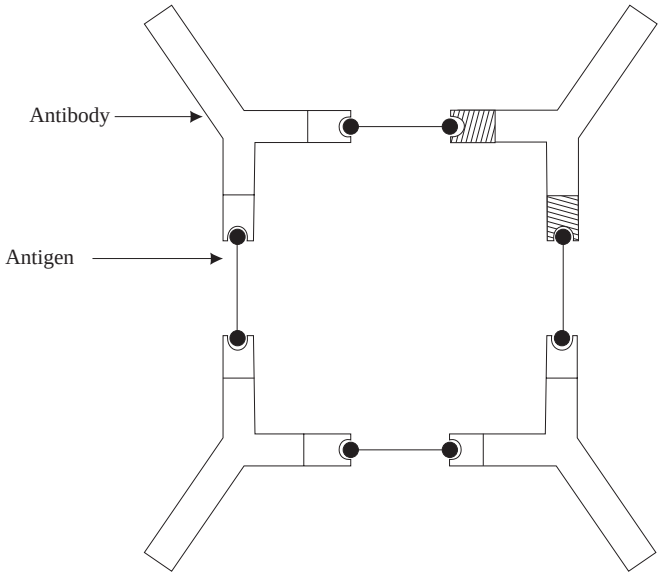


Fig. 24.2 A tetramer formed by binding of a 4 IgG antibody molecules showing the binding antigen molecules. Antigen is shown as a dumb-bell shaped structure.

to enzymic hydrolysis by papain at the hinge regions. The enzymic action results in the formation of three fragments, two antigen binding fragments (Fab) and one crystallizable (Fc) fragment. This particular class of immunoglobulin can traverse through the placenta so that the foetus also acquires immunity. The macrophages (monocytes) have receptors for IgG.

2. *IgA:* It is the second largest class of immunoglobulins present in the human plasma, and is composed of two light and two heavy chains. It is basically a monomer but can exist in dimer or tetramer state. This is abundant in the secretions of mucous membranes, salivary glands, respiratory and intestinal surfaces and in colostrum.
3. *IgM:* This antibody has a high molecular weight (MW-950,000) and occurs in the plasma in a highly polymerized state as pentamer (i.e., consisting of 5 molecules, each of 4 polypeptide chains). This antibody is synthesized in the early stages of immunization followed by IgG type of antibodies. It has the property of binding with the complement.
4. *IgD:* This antibody molecule is composed of four chains, two light and two heavy chains. The molecule exists in small amounts as monomer molecules where the heavy chains are referred as δ(delta) chains. The biological activity of IgG molecules are little known.
5. *IgE:* IgE class of antibodies are present in the blood in minute amounts. This is owing to a very small population of IgE synthesizing lymphocytes. These have a distinct biological role causing allergy symptoms, such as rash, hay fever, asthama etc. They also have cytophilic properties and bind themselves on the surface of mast cells in the skin and elsewhere. The IgE levels may be increased due to infection of helminth parasites.

24.4 LYMPHOCYTES AND THE LYMPHATIC SYSTEM

The cells that give rise to immunological response are located in a system of lymphoid organs which intercommunicate with the entire body through a system of lymphatic channels. These cells are called *lymphocytes.* They arise from the stem cells which are produced by the yolk sac, and foetal liver during embryonic life and from the bone marrow throughout the adult life. These cells multiply, differentiate and mature in the primary lymphoid organs, the thymus and the bursa.

Families of Lymphocytes

There are two families of lymphocytes in the body. These are:

(a) T-lymphocytes or T cells derived from the thymus

(b) B-lymphocytes or B cells derived from the bone marrow

The T lymphocytes mediate cellular immune responses such as destruction or tissue grafts or the tuberculin (delayed hypersensitivity response) reaction. The B lymphocytes are concerned with humoral immunity, i.e. antibody formation as such. There is yet another function attributed to T cells, i.e. they help B cells to initiate antibody production. However, this function is poorly understood.

Immunological Function of the Thymus

The thymus is a large organ which comprises, in man, 0.8% of the body weight at birth. It is a compound organ with endocrine and immunological function. It has an epithelial stroma which is embryologically derived from-the third branchial pouch adjacent to the primordia of parathyroids. In man, the developing epithelial thymus becomes infiltrated with lymphocytes at about 3 months of foetal development. Thereafter, the thymus consists of an epithelial stroma containing lymphocytes. Thymic lymphocytes are derived from the immigrant precursors originating in the haemopoietic tissues such as foetal liver and bone marrow. The lymphocytes, however, multiply within the thymus and from there some may migrate into the blood stream. The cortex of the thymus is densely packed with lymphocytes which have a high rate of mitosis and also show much cell death. The medulla of the thymus contains fewer lymphocytes and more prominent epithelial cells. The medulla also contains myeloid cells and differentiated epithelial structures called *Hassal's capsules.*

The thymus is accredited with the production of T cells which form a large proportion of a pool of recirculating small lymphocytes (Fig. 24.3). T cells have immunological specificity and participate in cell mediated immune responses as effector cells. T cells do not secrete humoral antibodies, but these are secreted by cells derived from the bone marrow independent of thymic influence (B cells). However, for many antigens, B cells require the presence of T cells before they can produce antibodies. Thus thymus is necessary for the development of immunity and humoral responses and it affects these systems by inducing within the thymus the differentiation of haemopoietic stem cells to T cells. This inductive influence is mediated by thymic hormones which can act outside the thymus since T cell differentiation can take place outside the thymus.

As a result of maturation of T cells within the thymus, T cells migrate from the thymus to the periphery. They go to the spleen where they undergo further maturation giving rise to various subpopulations of T cells. These cells develop certain surface markers. Some of the subpopulations produced are:

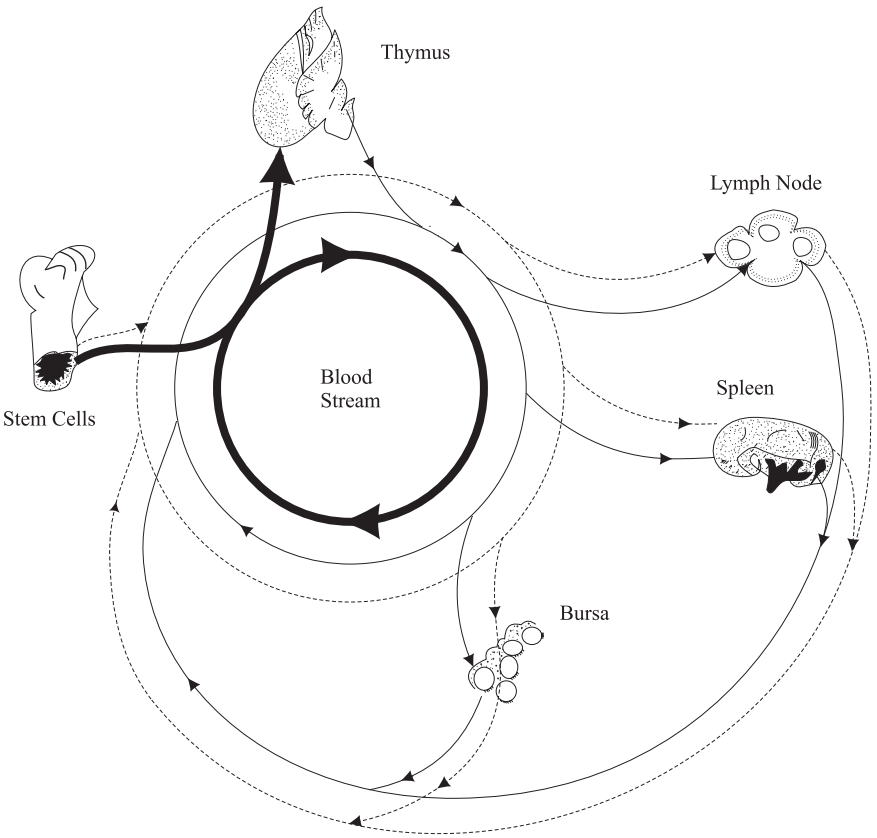


Fig. 24.3 Diagram showing the origin of stem cells from the bone marrow which reach the thymus to become differentiated to T cells and B cells. The immunocompetent T and B cells circulate between tissues, lymphatics and blood stream.

(a) precursors of cytotoxic cells which develop into *killer cells* participating in cell mediated immunity,

(b) cells that take part in mixed lymphocyte reactions and respond by proliferation to certain transplantation antigens, and

(c) cells which help in certain B cell's responses by producing antibodies.

The Bursa of Fabricius

The bursa is also a primary lymphoid organ in avians. There may be similar organ in mammals associated with the gut. The function of bursa is to process undifferentiated stem cells into immunocompetent B cells. Bone marrow stem cells reach the Bursa and differentiate into mature B cells. These cells are small lymphocytes with distinct immunoglobulin marker at the cell surface. These cells migrate to the spleen and remain there. However, a small proportion may migrate to other peripheral lymphoid organs also.

Some Properties of T and B Cells

The T cells carry distinct surface antigens and have receptor sites for binding with antigens. Human blood T cells have a specific property of forming rosettes when combined with sheep erythrocytes. T cells are long-lived and that is why thymectomy of adult animals does not affect the immune response.

The B cells have receptor sites for binding with antigens and have surface immunoglobulins. The B cells can be activated by lipopolysaccharide preparations from *Salmonella* and *Escherichia coli* (gram negative bacteria).

K-cells: This is yet another category of cells known as killer cells. The killer function is performed by a type of T cells and sometimes even B cells can also perform this function. Macrophages also perform this function, yet there is another category of cells which perform the killer function in the presence of antibody. These cells are devoid of surface immunoglobulins and form rosettes with sheep erythrocytes. They can be discriminated from others by their property of not adhering to plastic or glass sheets and they are nonphagocytic.

24.5 ANTIGEN-ANTIBODY INTERACTION

The plasma of a normal individual contains hundreds of distinct antibodies in very small amounts. If a new antigen enters the body, specific antibodies will appear in the blood which will react with the antigen. Repeated encounters with the antigen will increase the antibody titer in the plasma. If an individual is exposed to an antigen that had been previously encountered by the organism, large quantities of antibodies rapidly appear in the plasma. There are innumerable types of natural and synthetic antigens that will elicit such a response to synthesize specific antibodies. All such antibodies are proteins. A given antigen stimulates formation of several antibodies. If the antigen is a very large complex protein molecule, it will elicit a greater number of different antibodies. Each antibody molecule has two combining sites for an antigen, and if the antigen also has two sites, then its reaction with antibody may produce a *precipitin* reaction.

In order to understand antigen-antibody interaction, it would be necessary to know the structure of an antibody and its mode of reaction with the antigen. Whether many distinct proteins serve as antibodies to a single antigen and how does an antigen stimulate the production of specific antibodies are some of the fundamental questions to be answered for understanding the immune response.

Kinds of Antibodies

There are several categories of antibodies and most important among them are:

1. Antitoxins: these are produced against toxins
2. Agglutinins: the antibody causing agglutination if termed an agglutinin and the antigen producing it an agglutinogen
3. Precipitins: these form complexes, with antigen molecules in solution
4. Lysins: these are antibodies with complement which dissolve the antigenic cells.
5. Opsonins: these are antibodies which combine with surface components of microbes etc.

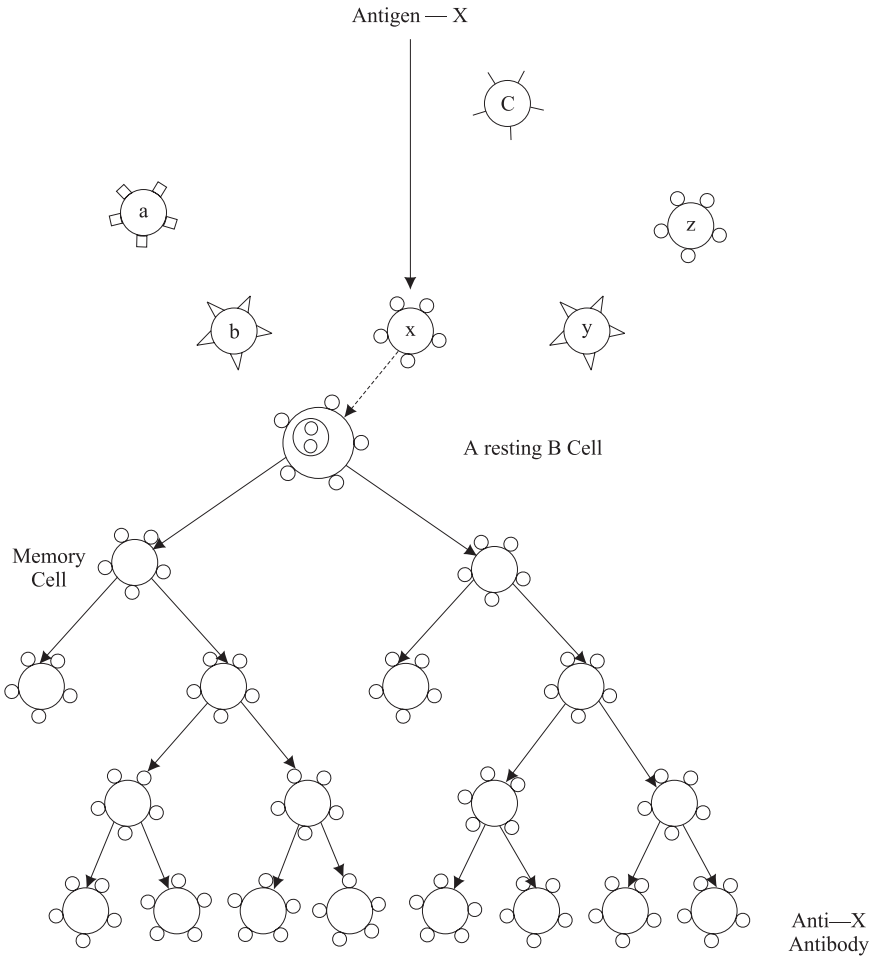


Fig. 24.4 Representation of the clonal selection theory of antibody formation. When an antigen meets a B cell with preexisting antibody receptor, the corresponding cell is stimulated to divide and differentiate. At least 8 cell divisions take place over 5 days until the cell is transformed into a *plasma cell* (antibody secreting cell). Some cells remain undifferentiated and retained as *memory cells.*

Induction of Immune Response

To develop an immune response an antigen molecule must come in contact with the lymphocyte surface. It is now widely accepted that lymphocytes capable of responding to an antigen have specific receptor molecules on the surface which are preadapted to respond to that antigen, and that different lymphocytes bear different receptors. The prevalent view is that each lymphocyte has only one kind of receptor which is an antibody molecule. All that the antigen has to do is to encounter a lymphocyte which has a corresponding antibody sticking out from its plasma membrane. This lymphocyte, one in thousands, is selected and specifically stimulated to divide a number of times and ultimately differentiated into a cell population that is actively secreting antibody (Fig. 24.4). This hypothesis of

antibody formation was put forward by Burnet in 1957 and is known as *clonal selection hypothesis.* A host of evidences have accumulated in favour of this.

Most antibody molecules have more than one antigenetically active patch (antigenic determinant) on their surface. When collaboration between T and B cells occurs, the T cell reacts to one determinant and helps a B cell react to another, second determinant on the same molecule. Such experiments are usually carried out with hapten-protein conjugates as antigens, e.g. dinitrophenyl-bovine serum (DNP-BSA). Haptens are substances which cannot elicit an immune response by themselves, but can bind with the antibody once it is formed. It has been seen that BSA-specific T cells help DNP-specific B cells to synthesize anti-DNP antibodies. In the process activated T cells release a special kind of antibody against the carrier which links to the carrier portion of the antigen molecule and perhaps transports to the *macrophage* surface for immune induction. The hapten portion, not covered by T cell antibody, is then free to stimulate its specific B cells.

Site of Antigen Trapping

What is the location of these events? Proliferation of lymphocytes occurs at the sites of infection or inflammation, but the bulk of antibody formation takes place in the secondary lymphoid organs which include lymph nodes, spleen, tonsils and appendix. The primary lymphoid organs do not have antigen trapping mechanisms, hence antibody formation does not take place in them. There are three distinct and specific cell types in lymphoid organs that are involved in antigen trapping. These are macrophages, the dendritic follicle cell and the lymphocytes.

1. *Macrophages:* Although the role of macrophages in antibody formation is highly controversial, there is no doubt that these constitute, both in lymph nodes and in spleen, one of the main depots of antigen persistence in the body. This has been demonstrated by autoradiographic studies. Within minutes of antigcn injection, sequential steps in antigen uptake can be elucidated. First, the antigen is attached to the outer cell membrane of the macrophage, and soon a vesicle is formed around the antigen engulfing it completely and finally a pinocytic vesicle is pinched off from the cell surface containing the antigen (Fig. 24.5). This occurs within minutes. Then the macrophage digests the ingested material and the digestion is accomplished by protolysosomes containing prestored catalytic enzymes that cluster around the pinocytic vesicle and fuse with it to form phagolysosomes. Following digestion, the breakdown products of various molecular weights are released by the macrophage,

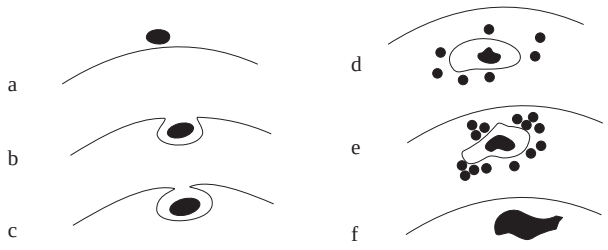


Fig. 24.5 Macrophage engulfing antigen particle.

presumably by reverse pinocytosis. Thus, there is overwhelming evidence suggesting that macrophages help antibody synthesis by processing large antigenic particles into low molecular weight forms, and by presenting antigen to surrounding lymphocytes. Some claim that macrophages manufacture an immunogenic RNA. However, the claim has not been accepted unanimously.

2. *Dendritic follicle cells:* In lymphoid follicles and germinal centres, a special type of cell is found which is a dendritic cell with long, complex and intertwining processes (Fig. 24.6). These can hold antigen on their surface for long periods without endocytosis and without denaturation or digestion, Lymphocytes which encounter antigen on the surface of a dendritic follicle cell may be stimulated. Next, they turn into large, fast dividing blast cells and create a nest of cells known as a *"germinal centre"*. These centres are largely B lymphocytes and it is likely that their progeny develops into antibody forming lymphocytes and plasma cells.

3. Lymphocytes are involved in antigen transport in two ways: first by absorption of antigen on the surface of lymphocytes with receptors specific for concerned antigen; secondly, by picking up antigen-antibody complexes and presenting them to circulation for antibody transport.

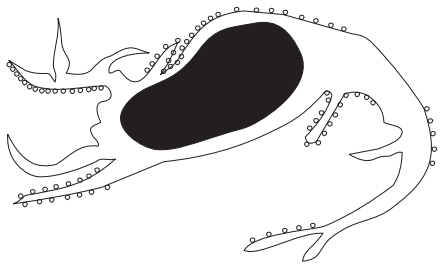


Fig. 24.6 Diagram showing a dendritic follicle cell with antigen particles attached to the surface of dendritic processes.

Synthesis of Antibodies: Primary and Secondary Responses

In order to know how antibodies are elicited, an antigen is injected in a test animal with a suitable *adjuvant.* An adjuvant is an agent in which the antigen is emulsified before injecting into the body of the test animal. *Freund's complete adjuvant* (FCA) is a most potent adjuvant commonly used which elicits both cell mediated as well as humoral responses.

Following an injection of antigen into the body of the animal, antibodies are detected in the serum after a few days (Fig. 24.7). The antibody titres gradually rise and then fall slowly and finally disappear from the serum. This is known as primary response or primary immunization. If a second injection or a booster dose of the same antigen is given to the animal at a suitable time while the antibodies are still present but at a low level, a rapid rise of antibodies to a higher peak is observed. The high antibody titres persist for months or years in many cases. This is *secondary response* or secondary immunization.

The above phenomenon reflects an episode in which there is an increase in the number of cells engaged in the production of one type of antibodies since one cell makes only one type of antibody.

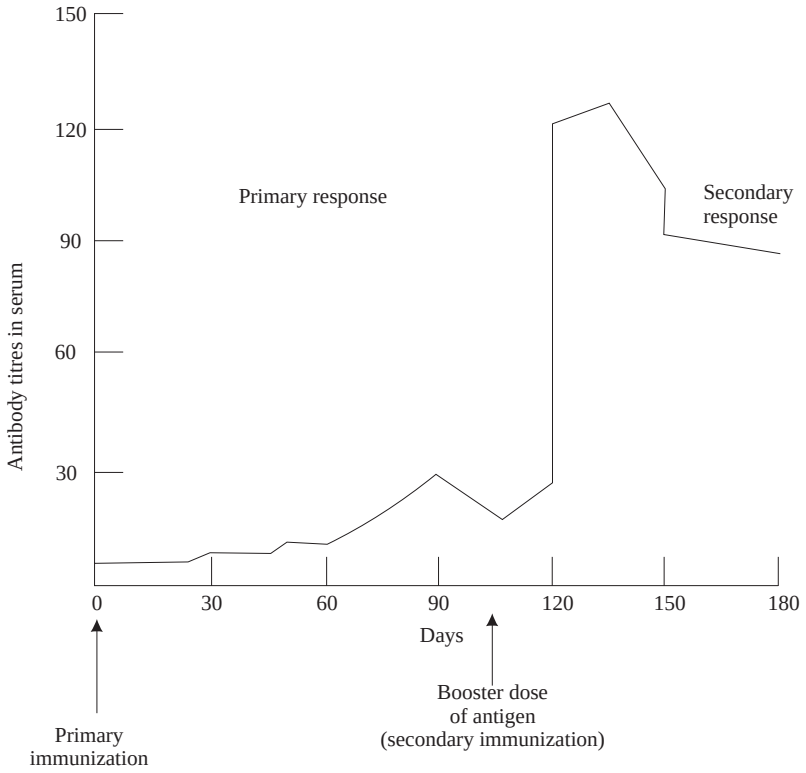


Fig. 24.7 Antibody production during a primary and secondary response produced by antigen administered to the animal.

Formation of antibodies can be explained on the basis of Burnet's *theory of clonal selection.* Once an antigen is attached to the receptor site of an appropriate lymphocyte, the cell is stimulated to undergo repeated mitotic divisions. Thus a clone of cells are produced which are capable of synthesizing particular antibodies. The B cells that become antibody cells carry on the surface immunoglobulin determinants which serve as receptors for binding with the antigen. The events leading to antibody forming cells can be summarized in a diagram (Fig. 24.4).

Clonal Selection

Antigens in some cases require processing in the macrophages. Macrophages may also serve the function of conserving the antigen at the surface so that it can be presented to the lymphocytes. Most antigens require T cell help for induction of immune response. The T cells recognize the carrier part of the molecule and B cells the haptenic end. In some cases, the T cells elaborate a product which helps in triggering a B cell directly or through the cooperation of macrophages (Fig. 24.8).

Upon interaction of the antigen with the antibody forming cell, the cell is activated or paralysed to a state of immunological tolerance. The triggering signal induces a chain of events leading to the prolifetation of the cell, i.e. cloning. The immunocompetent cell triggered by the antigen undergoes

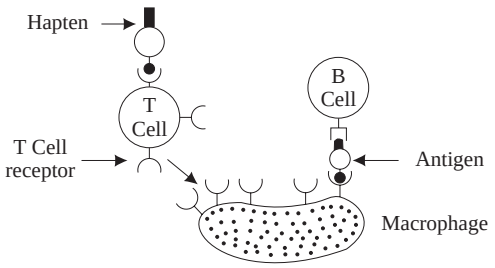


Fig. 24.8 Cooperation between T and B cell: (a) showing recognition of antigen molecule by both cell types; and (b) showing involvement of macrophage.

a series of divisions, each time dividing into two daughter cells. Besides division, the cells undergo a process of differentiation leading to the formation of a fully competent cell called a *plasma cell* (Fig. 24.9). However, a part of the population of daughter cells do not differentiate into plasma cells and are retained as *"memory cells".* The memory cells are triggered during secondary immunization. The differentiated plasma cells synthesize antibodies which are secreted from the cell by exocytosis. Antibody production occurs in the spleen, bone marrow and in the lymph nodes. A fully formed plasma cell is devoid of receptors but is rich in immunoglobulins in the cytoplasm.

The triggering stimulus, besides inducing division, produces a number of metabolic effects. These are:

1. Activation of membrane associated enzymes like adenyl cyclase, fatty acyl CoA ligase, cation (Na^+, K^+) dependent ATPase etc.
2. Increase in transport of monovalent and divalent cations.
3. Enhanced turnover of phospholipids.
4. Enhanced uptake of amino acids and energy substrates.

Cell Mediated Immunity

Thymus derived T cells are involved in cell mediated immunity. When an antigen comes in contact with a T cell, it undergoes divisions accompanied by differentiation and maturation. The scheme of events are almost similar to that of humoral immunity. The T cells synthesize mediators or *lymphokines* when they come in contact with the antigen. The mediators, mostly polypeptides inhibit the migration of macrophages and leucocytes. However, there may be a few peptides which may activate macrophages and cell proliferation. Some T cells function as *helper* cells for triggering B cells, while others may function to suppress the B cell activity.

Thus in cell mediated immunity the principal agent is not a soluble protein in the blood stream, but an activated lymphoid cell. The cell mediated immunities include delayed type of hypersensitivity (as in tuberculin reaction), transplantation immunity, tumour immunity and autoimmunity.

Role of Thymic Hormones in Immune Response

We have seen that the thymus is necessary for the development of cellular immunity and many humoral responses and it affects these systems by inducing, within the thymus, the differentiation of

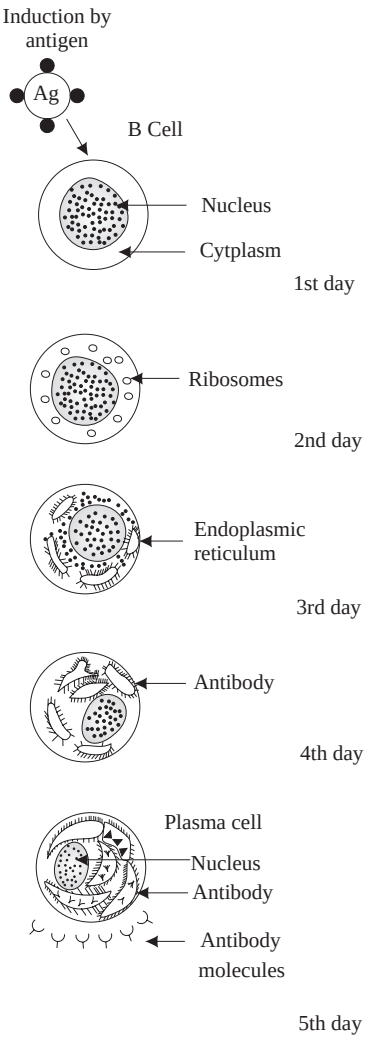


Fig. 24.9 Diagram showing transformation of a B cell into a plasma cell.

haemopoietic stem cells to T cells. The inductive influence is probably mediated through the thymic hormones (Fig. 24.10).

The evaluation of the role of thymic hormones on T cell differentiation is quite difficult. T cells have a long life, hence thymectomy of adult animals does not affect immune response. Therefore, in order to study the differentiation of stem cells to T cells one may use either thymectomized animals before the pool of T cells has been formed or X-irradiated thymectomized adults in which circulating T cells have been destroyed. X-irradiations damage the haemo-poietic stem cells. Another aspect worth noting is the localised action of thymic hormones in the thymus inducing differentiation of

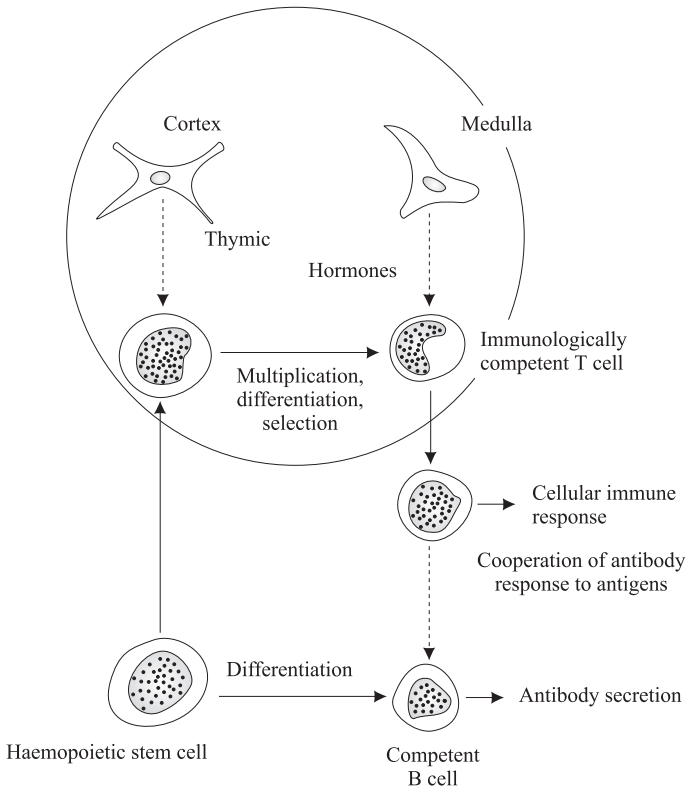


Fig. 24.10 Schematic diagram showing differentiation of stem cells under the influence of thymic hormones.

cells. It is suspected that, besides differentiation of T cells within the thymus, other potential cells reactive against analogous antigen are also eliminated here.

There is evidence to suggest that thymic hormones can act outside the thymus also. Experiments with thymus deprived rats have shown that thymic extracts improve delayed hypersensitivity responses, and the capacity to form haemolysin producing cells after immunization with sheep erythrocytes. All these responses suggest that T cell differentiation was stimulated by injecting thymosin (thymic hormone) in thymus deprived animals.

24.6 TRANSPLANTATION IMMUNITY

Transplantation experiments have been carried out since ages, but serious attempts to develop this technique in medical science began only in the beginning of twentieth century involving blood transfusions. Victims who had suffered blood loss were given transfusions, some of which proved successful while other were fatal for the patient. Landsteiner, a pioneer in blood transfusions, suggested failure in blood transfusions due to antigenic materials present on the red cell surfaces. Based on these antigenic substances, Landsteiner recognized A, B, O blood groups which are

inherited (see Chapter 11). It may be recalled that the blood group A has A antigens, B has B antigens, AB group has both, while O group has none. Then why should humans have antibodies against those antigens which they are lacking?

Blood is a liquid tissue, hence blood transfusions are excellent examples of tissue transplantation. Transplantation immunity is a form of cell mediated immunity (CMI) which deals with the prohibition of transplantation of living tissues between two individuals belonging to the same or different species.

Types of Grafts

Transplantation of a tissue between two animals of the same species but not of the same genotype are known as *"allogenic"* or *"homografts".* Grafts between two individuals belonging to different species are known as "*xenografts",* whereas grafting one part to another part of the same individual is known as *"autograft".* When two individuals are identical twins or genetically same, grafts between them are called *"syngeneic".*

Allograft Reaction

Transplantation experiments have been widely carried out using allografts. When a piece of skin tissue from the mouse of one strain is grafted on to the mouse of another strain, the graft survives well for a few days. The graft becomes highly vascularized by the growth of blood vessels and within seven days the blood vessels are engorged with blood and circulating lymphocytes penetrate all parts of the graft. But after a few days (11-14 days), suddenly the blood circulation stops, the graft disintegrates and finally comes off the body as on old scab. This shows that the graft is rejected after a few days indicating a lack of histocompatibility factors. This is called "first-set" reaction. If a second graft from the original donor is placed on the recipient's body, the graft rejection is more rapid (within 5-6 days) since it does not acquire a proper blood supply. This is called "second-set" reaction, a phenomenon considered equivalent to the secondary response (memory response).

What is the cause of graft rejection? Much of our information on tissue transplants comes from tumour transplant studies. In fact, the immunogenetic laws of tissue transplantation were first discovered by experimenting with transplantable tumours. These experiments suggested that Mendilian segregation of histocompatibility factor or dominant H-genes govern the transplantability of normal tissues.

The graft rejection is partly due to appearance of humoral antibodies in the serum of the recipient which are specific for the donor cells. These antibodies cause agglutination of red cells and transplantation antigens can be recognized in the individual. Humoral antibodies alone are not responsible for graft rejection. If the lymphoid cells of a donor who has previously rejected a graft are transferred to a recipient, the graft rejection is accelerated. This suggests that the sensitized lymphoid cells retain the memory of the first contact with graft antigens and kill the graft cells. In neonatally thymectomized animals graft rejection is not possible since they lack lymphocytes. However, if lymphocytes from a genetically similar animal are injected into the host, graft rejection is restored. The lymphocytes that are responsible for the graft rejection are thymus derived (not those which are precursors of plasma cells) bone-marrow cells (T cells) and participate in cell mediated immunity. Lymphocytes secreting antibodies are called B cells.

Prevention of Rejection

In human identical twins and inbred strains of mice graft rejection does not occur. Experiments with inbred strains of mice have contributed a wealth of information about the graft rejection and graft accepting process. Two inbred strains of mice (A and B strains) were bred and their F_1 offspring was given a skin graft from both parents. The grafts survived indefinitely. However, when grafts from F_1 offspring were placed on either of the parents, they were not found acceptable. In conclusion, genetic differences lead to antigenic differences. Both cellular and humoral components are involved in the organism's immune response against transplant associated antigens.

Immunosuppression measures are adopted to prevent graft rejection which drastically reduce the organism's antibody forming capacity to an antigenic stimulus. The immune response involves recognition of the antigen by immunologically competent cells (T cells) and, in some instances, participation of macrophages. Immunosuppressive techniques do not allow rapid cell proliferation.

Tissue transplants between identical twins are a perfect match, but in genotypically different individuals and strains, transplants become a problem. Besides tissue typing and blood group matching, immunosuppressive measures are adopted that weaken or minimize the immune response by grafts.

Immunosuppression

1. In transplantation practice combination of certain drugs, such as azathioprine (Immuran) with corticosteroids are used widely since the aim is to inhibit cell mediated immunity.
2. In specific cases the host response to a particular antigen is eliminated to achieve "tolerance". This is a case of specific immunosuppression where an antigen itself is an immunosuppressive agent.
3. Ionizing radiations applied to the whole body are responsible for suppressing lymphocyte proliferation in the haemopoietic system. The effect is nonspecific and impairs haemopoiesis, hence syngeneic bone marrow cells have to be periodically inoculated in the animal.
4. A category of endogenous corticosteroids: hydrocortisone, testosterone, corticosterone, prednisone and prednisolone are widely used to impair the immune response of the host, especially CMI.
5. Antiproliferative agents such as cyclophosphamide, DNA base analogues (6-mercaptopurine and its derivatives), actinomycin-D and mitotic poisons (mitomycin) are used.
6. Use of antilymphocyte serum is also common. It is a T-cell inhibitor of recirculating peripheral lymphocytes, but the lymphocytes within the lymphoid organs are unaffected.
7. Homologous antibody treatment: In transplantation practice the procedure of inhibiting cell mediated immunity by homologous humoral antibody is called 'enhancement'. The transplants are made to survive longer by infusing a specific antibody to impair CMI while the formation of humoral antibodies is protected.

Autoimmunity

The concept of autoimmunity was developed to explain the mechanism of self-tolerance in relation to a group of some poorly understood autoimmune diseases. According to Ehrlich's idea, the destructive potential of the phagocytes is not directed against tissues of the same organism. While studying somatic mutations of lymphocytes, Burnet proposed that among the variety of lymphocytes, there exists a group of lymphocytes known as "forbidden clones" which directed against self-components, have to be eliminated. The destruction of such lymphocytes by some antigen takes place by the time immune machinery of the body is properly established. It could be observed that an antigen administered prior to birth or at the time of birth develops tolerance to the antigen by the time immunologic machinery is established. By the time these ideas developed, different properties of T and B cells were also clearly understood.

Both T and B cells differ in their properties towards tolerance. T cells can become tolerant to an antigen even when the concentration is very low, whereas B cells require high doses of antigens to become tolerant. It is possible that normal individuals may have a population of B cells that are not tolerant to self antigens. There is evidence suggesting that the body can synthesize antibodies or sensitized lymphocytes against self-components.

Autoimmunity may be aroused in many ways. Autoimmune reaction may be expressed if self antigens undergo alterations in their chemical or physical form. In such cases, previously hidden determinants are modified and treated as foreign to produce a reaction. Introduction of foreign antigens into the body which are closely related to self antigens also arouse autoimmunity by producing cross reacting antibodies that trigger B cells directly.

24.7 ALLERGY

The term allergy refers to hypersensitivity marked by harmful responses to specific antigens such as pollen, dust, wool, hair and other substances. Survival without immune system is impossible. Allergic reactions are of a wide variety and are associated with specific immunologic reactions taking place tissues resulting in cellular injury. In immune mechanisms that give rise to allergies, both T cells and B cells may be involved.

Substances which give rise to allergic reactions are called *allergens* and they fall into a number of categories. Some allergens which are foreign proteins act as complete antigens, others are polypeptides, lipids or lipoid extracts, foods, microbial constituents possessing some antigenecity. The allergic response may be elicited by any of these substances and the development of hypersensitivity depends upon the contact of the individual with the allergens and its capacity for sensitization.

Allergic reactions fall under two categories: immediate and delayed. Immediate allergic reactions are acute systemic reactions caused by allergy to pollen, allergy to antibodies etc. The reaction starts within minutes of contact with the allergen and disappears within an hour. The reaction consists of dilation of capillaries and arterioles, swelling, redness and itching of the surrounding area of contact and is associated with circulating antibodies of IgE type.

Delayed type of reactions develop slowly and persist for quite some time. The reactions are not related to the serum antibodies, hence passive transfer of reactivity by means of serum is not possible. Such reactions develop when the body encounters bacteria, fungi, viruses, helminthes, parasites and chemicals. The reaction begins within several hours after contact with the allergen causing inflammations with lymphocyte infiltration. Thus delayed allergy is like cell mediated immunity requiring immunologically competent lymphocyte.

CHAPTER

25

Physiology of Aging

Aging is a general property of all eukaryotic organisms. Aging or senescence can be defined as a progressive decline of vital capacities of the organisms and in their abilities to adapt to environmental stresses, terminating in death. The response to stress at the cellular and organisational levels are the most likely factors which undergo alteration in senescent organisms. Aging processes are progressive and not reversible under physiological conditions. The science that studies biological causes of senescence is called *gerontology.*

All organisms have more or less a fixed life span and during this period a visible pattern of decline of various functions occurs after attainment of reproductive maturity. All round deterioration of functions may be attributed to plethora of causes at the cellular, molecular and environmental levels. Man has been interested in increasing his life span and to a great extent it has been possible to increase it by controlling and curing many diseases. The average life span has, therefore, been increased.

The life span of an animal is divided into two phases: growth and senescence. The phase of growth is marked with tremendous cellular activity and cell multiplication, which continues even after maturity until the age of adulthood is reached. The phase of senescence or aging is characterised by deterioration of several functions, but the onset of deterioration of each function and its rate occurs at different times. Different organs are responsible for different functions, thus functional decline of each organ has to be at different times. For example, skeletal muscles age earlier as compared to nerve cells. Therefore, in order to seek explanation to the causes of aging, we have to examine several factors such as nutrition, stresses of various kinds emanating in the environment, and genic.

25.1 AGING AT CELLULAR LEVEL

At the cellular level aging can be studied on the basis of three processes:

1. Possible decline in the final efficiency of nondividing highly specialised cells, such as neurons and muscle cells.
2. Progressive stiffening with age of the structural proteins such as collagens.
3. Limitation imposed on cell division as revealed by the studies on fibro-blast producing collagen and fibrin.

Finite Lifetime of Cultured Cells

As early as 1950, Alexis Carrel demonstrated that fibroblasts from chicken's heart could proliferate indefinitely in culture medium. Later, it came to be known that acquisition of this infinite property of division of cells is a property of abnormal cells resembling cancer cells. Since aging is a property of normal cells, it follows that normal cells cannot have an unlimited capacity of division. Around sixties, it was shown that normal human cells (diploid cells with a complement of 46 chromosomes), when cultured *in vitro* showed a limited capacity of proliferation, thus breaking the myth of *immortal* cells. It could be inferred that normal cells can not be maintained in a state of active proliferation in cell culture for a period beyond the specific age of the species from which the cells were obtained. Culture experiments with non-dividing and highly differentiated normal cells have shown that they cannot be maintained in their functional state *in vitro* in excess of the average life span of the species from which they were obtained. This is an important observation because functional decline in non-dividing cells is thought to be manifestations of age changes.

Studies were conducted on normal human embryonic diploid strains, which demonstrated that after a period of active proliferation, generally less than one year, these cells took longer time to divide (normally 24 hours), gradually lost mitotic activity, accumulated large amount of debris and ultimately died. When derived from normal human foetal tissue, the cultured fibroblast populations undergo about 50 population doublings over a period of about six months. This finite limit is an innate characteristic of all normal cells grown *in vitro* (Table 25.1).

Table 25.1 The Finite Lifetime of Cultured Normal Embryonic Human and Animal Fibroblasts

Species	*Range of population doublings for cultured normal embryo fibroblasts*	*Maximum life span in years*
Man	40-60	110
Chicken	15-35	30
Mouse	14-28	3.5

Senescence of Cultured Normal Cells

An important phenomenon that is fundamental to our understanding of the biology of aging is that interspecies differences in life spans are far greater than individual interspecies differences. *Drosophila,* fruit fly becomes ancient in 40 days, a mouse at 3 years, a horse at 30 and a man at 100 years. These differences are reflected in the doubling potential of cells cultured from animals of widely different life spans.

Progeria and Werner's Syndrome

A human condition, *Progeria,* in which a severe deceleration of growth takes place at a young age of about nine years, is considered to be a rare disease represented by precocious aging manifested in the physical appearance. Werner's syndrome, characterised by early greying and loss of hair, short stature, juvenile cataracts, atherosclerosis and calcification of the blood vessels, osteoporosis, high incidence of malignancy and proneness to diabetes, is a disease similar to progeria in many ways but these manifestations appear in later years. These examples relate to accelerated aging.

Mechanisms of Cell Aging

As seen from the foregoing account, it appears that normal cells have a finite limit for replication demonstrable *in vitro.* It. has been suggested that the functional losses occurring in cells prior to their loss of division capacity produce age changes in animals much before their normal cells have attained the limit of their ability to divide. It has been surmised, and now amply proved, that the loss in the dividing capacity of normal cells is due to genetic disturbances. Normal cells may cease to divide *in vitro* due to loss of genetic information either as a consequence of playing out of the genetic programme, the expression of *genes of aging,* or of the accumulation of errors in informational molecules, i.e. DNA.

In 1963, L. Orgel put forward a hypothesis suggesting that cellular aging results from impaired specificity of the translation step in protein synthesis. This hypothesis had an impact on experimental gerontology and it is gratifying to note that Orgel's predictions have been confirmed to a great extent by recent researches.

25.2 AGING AT THE MOLECULAR LEVEL

The past decade has seen rapid strides towards the understanding of the molecular approaches to the phenomenon of aging, especially on how genes and proteins regulate various processes. Many modern theories concerned with biochemical mechanisms of cellular aging centre upon the possible role of various types of nucleic acids and complex nucleoprotein structures in the aging process. The interplay of various nucleic acids and enzymes is also responsible for continuous regulation of somatic cell metabolism. If after a certain time, a cell loses the ability to maintain homeostasis, it is logical to assume that this is due to failure of nucleic acid functions resulting from impaired functioning of nucleic acids and their enzymes in the aging cell. Some possibilities are discussed below.

Quantitative Changes in Nucleic Acids

For a given species the amount of DNA per cell remains constant. Loss of DNA or RNA per cell or per organ may lead to decline in functional efficiency with increasing age. It is difficult to prove loss of DNA per cell. However, the RNA content of the cell is highly dependent on the prevailing functional state as well as on various physiological factors and circadian rhythms, and therefore it may not be a suitable parameter of the aging process. Loss of DNA per organ, arising out of the loss of cells, is far too small to explain declining functional efficiency.

This is true of the neurons in the central nervous system. Histological findings have shown that there is progressive loss with age of certain cells that may not be replaced at all.

Changes in Information Content

The information content of chromosomes containing DNA, histones and non-histone chromosomal proteins (known as chromatin) may undergo changes in properties and quantities. The DNA of aging animals has a higher melting point providing a greater stability. This property has been attributed to increased cross-linking of DNA with chromosomal proteins. DNA synthesis is also impaired with increasing age as suggested by Adelman.

It has been suggested that animals with longer life span have a higher proportion of repetitive DNA in the chromatin which is not transcribed, hence not responsible for protein synthesis. It may have some role in the regulation of the structural genes which are transcribed, thus providing greater stability and protection to the genome against mutation. Thus the higher amount of DNA enhances the life span of animals.

In the complex mechanism of protein synthesis, there are many ways in which information content can be hanged, falsified or diminished, and the methods that have been employed in recent years are equally numerous, This makes it more difficult to compare the results of many workers. The following mechanisms may be considered:

(a) It is assumed that in aging cell replacement of defective molecules of metabolic DNA gradually becomes impossible, and the defective molecules, therefore accumulate. When enough faulty DNA is present in the cell, functional impairment follows.

(b) According to another suggestion, aging is attributed to non-repetitive information. The highly repetitive DNA sequences in the genomes of the eukaryotes would not only represent a certain evolutionary reserve and a way of reinforcing the functional expression of the genetic information, but would also be a form of protection for this information against random molecular deteriorations which accumulate during ontogeny.

(c) It has also been reported that the number of methyl groups (5'-methylcytosine) in DNA decreases with age, thus information content of DNA is modified affecting protein synthesis.

Changes in Protein Regulatory Mechanisms

Transcription, translation and DNA replication are controlled by a complex system of enzymatic and protein factors produced by genes. A loss of enzyme specificity leading to incorporation of the wrong amino acids in the polypeptide chain is the progressive failure of the regulatory proteins at the level of operon regulatory mechanism. This would not give rise to faulty proteins, but would lead to loss of information.

According to Orgel's hypothesis, the ability of cells to produce functional proteins, i.e. enzymes, depended not only on the correct specification of the primary structure of the polypeptide chains in the genes, but also on the competence of the total protein synthesising mechanism. Errors due to reduced specificity of an information-handling enzyme lead to an increasing error frequency. Such processes are cumulative. Orgel, therefore, suggested that aging of cells may ensue as a result of cumulative errors of transcription and translation. Introduction of incorrect amino acids is bound to accelerate the aging process.

R. Holliday and his co-workers tested Orgel's generalisations and found that the life span of *Drosophila melanogaster* was appreciably reduced by incorporation of faulty amino acids. It has been

argued that cellular aging may be a result of swich-over of regulatory processes. Could there be an aging programme? Changes in the DNA pool in old age have been observed, and this has led to the formulation of a hypothesis that each spectes has species-specific programme that serves to maintain and prolong useful life. Random errors affecting metabolism interfere with this programme. It has been experimentally shown that there is a shift from an arginine-rich to a lysine-rich histone composition in the liver of old animals, suggesting functional impairment of intraregulatory process.

Changes in Enzyme Activity

Aging is accompanied by progressive decline in the activity of certain enzymes, while some enzymes show relatively more activity at old age. List of enzymes coming in this category is long, but we can take here some specific examples. Some of the liver enzymes such as glucokinase, malate dehydrogenase (both cytosolic and mitochondrial), and cathepsin show increased specific activities in old age. Alteration in the activity of some enzymes has been seen as a function of age. For example, the activity of acetylcholine esterase decreases significantly in aging animals and this may be the cause of decrease in functions like learning and memory. Failure of induction of acetylcholine esterase in old age is probably due to appearance of a specific repressor of the gene for the enzyme.

Changes in Hormonal Activity

The activity of several endocrine organs like thyroid, gonads, adrenal cortex and medulla decrease with old age, suggesting alteration in the hormonal status of the individual. The gonads and adrenals produce sex hormones which are dependent upon the activity of gonads. In females estrogen levels decrease with age. Similarly, both in males as well as females the androgens and 17-ketosteroids decrease after adulthood. However, these hormones do not appear to have any role in the life span.

It is interresting to know that the levels of FSH and LH of the pituitary increase with age while eastrogen and androgen decrease. Though the insulin level does not show any significant change, an age-dependent decline in the sensitivity of different organs to insulin is observed.

Accumulation of Modified Proteins

Recent discoveries have shown that modified and unstable proteins accumulate in aging cells and tissues which account for many health problems such as impaired cognitive function, immune responses, vision deterioration, wound healing and insensitivity towards environmental and metabolic stresses. Accumulation of modified proteins is believed to impede normal homeostasis. What is the source of modification of the proteins? In aging cells and tissues, modification results from post-synthetic covalent modifications by deamidation and oxidation reactions. These reactions produce conformational changes in proteins which may be degraded during course of time. They, however, get accumulated aging cells because of increased rates of modification and decreased ability of self-repairing or correcting mechanism. The accumulation of modified proteins may be due to several reasons:

(a) Environmentally induced damage to proteins caused by superoxide dismutase, catalase, antioxidants etc.

(b) Absence of a proper mechanism to repair modified proteins.

(c) Inability of the system to recognise and protect of native proteins.
(d) Decreased transport of modified proteins to the lysosomes, the site for degradation of proteins.

25.3 AGING OF CONNECTIVE TISSUE

Dermis, tendons, cornea, the vascular walls, cartilages, and the bones etc. are different forms of connective tissues, derived from the embryonal mesenchyme. The cells that synthesise these tissues are differentiated forms of the fibroblasts, such as the chondrocytes, fibrocytes, lipocytes etc. These differentiated connective tissues have large intercellular spaces containing four types of macromolecules: *collagen, elastin, proteoglycans* (acid mucopolysaccharides) and *structural glycoproteins* forming intercellular matrix macromolecules.

At this stage, it is necessary to understand the phenomenon of differentiation the course of embryonic development by which the tissues are formed. The morphogenetic events in the embryonic tissues and adult tissues are under the control of four types of intercellular matrix macromolecules described above. At the start of embryonic life, proteoglycans and structural glycoproteins actively participate in the differentiation process and later the role is taken over by collagen and elastin. In adult life, collagen functions as and when necessary, whereas elastin is suppressed. Synthesis of collagen, however, declines rapidly a relatively advanced age.

Aging of Elastic Tissue

During maturation and aging phenomena of elastic tissue, the elastic fibres play an important part in the elasticity and normal tone of the skin and the blood vessels. With the advancing age, the quantity of elastic fibres diminishes with age the skin, in the vessels, and to a much greater extent, during arteriosclerosis, most important disease of aging.

During maturation and aging of elastic tissue, the aortic mediacyte (muscle cell) synthesises microfibrils and proelastin. The microfibrils form the frame-work of future elastic fibre and the globular molecules of proelastin attach themselves to this primitive fibrous tissue in the form of a string of beads. Subsequently, the proelastin molecules undergo cross-linking initiated by the enzyme, lysine oxidase. During the maturation phase, the relative quantity of cross-linked proelastin increases, and that of the microfibrils decreases.

Lipids that are derived from the breakdown of lipoproteins or locally synthesised are deposited in the elastic fibres rendering them more rigid and susceptible to proteolytic digestion. This results in fragmentation of the elastic fibres with increased compensatory synthesis of micro fibrils. The breakdown of elastic fibres may start at a relatively young age, but at the age of about 45 years the degradation phenomena accelerates.

Aging of the Programme of Synthesis

It has been proposed that a genetic control regulatory mechanism exists to determine transcription and translation processes. In case the process is upset, the cells then either synthesise wrong molecules or simply stop synthesising. At the old age exhaustion of the genetic programme or the accumulation of errors seem to be quite logical. In addition to this, some *psycho-socio-economic factors*, contributed by the environment, nutrition and exercise also play a role in aging.

It has been mentioned earlier that the deposition of lipids in elastic tissue helps its degradation, but regular exercise keeps the tissues in good functioning.

25.4 AGING AND IMMUNOLOGICAL SURVEILLANCE

Aging is primarily related to the fate of less differentiated messenchymal cells responsible for the functions of defence and repair. Like all other proliferating somatic cells, these cells are subject to mutations. The mutations cause appearance of antigenically altered cell proteins which impose a limit on the number of cell generations in these cell lines. Genetic changes in somatic cells can modify cellular functions and with the appearance of altered antigens, immune responses against the changed cells become possible.

Somatic Mutation and the Hayflick Limit

Each species has certain life span beyond which genetically controlled programmes become progressively less effective until death. A normal diploid human fibroblast cell can undergo a limited number of generations. This limit in the number of cell generations is called *Hayflick limit.* Malignant or transformed cell lines show no such limits to growth. It has been suggested that continuing mutations in the somatic cells generate errors in the structure of enzymes concerned with protein synthesis, and thus generate a cascading error catastrophe rendering the cell incapable of further mitosis.

Role of Thymus

Thymus is an important 'biological clock' that allows phenotypic expression of genetically determined age. Thymus is the source of thymus-derived T-immunocytes and the serum antibody is produced by B-immunocytes. Natural antigens can elicit antibody production through cooperation of T-and B-immunocytes. T-immunocytes, without B-cell cooperation, and for clinically important segment of immunity against viral, mycobacterial and mycotic infections. The old age suffers from immunological inadequacies mainly arising from the weakness of T-cell functions. Therefore, with the atrophy of thymus in middle age, T-cell loses to recruit effective immunocyte clones against antigens not previously encountered.

Immune Surveillance

Somatic mutations are discontinuous inheritable changes in a cell line. This is perhaps responsible for differentiation and generation of diversity in immunocytes. What ever may be the nature of antigenic stimulus (viral or chemical), the development of neoplastic change, benign or malignant, must be regarded as somatic mutations. Such changes are generally associated with some; degree of antigenecity. Many incipient tumours are eliminated by immune responses against the new tumor-specific antigens as a part of immune surveillance mechanism.

If neoplastic cells produce new antigens resulting from somatic mutations, it is quite likely that any random mutatin involving cell surface components will also confer potential antigenecity. It has been suggested that common types of cancer can be regarded as diseases of old age. Aging may be presumed to act in two ways: (a) by allowing time for any necessary sequence of mutations to occur and (b) by a progressive weakening of immune surveillance with age.

25.5 MENTAL ASPECTS OF AGING

The mental aspects of aging involve intelligence, learning, memory and problem, solving. Aging brings about a cumulative deterioration in the human body, behaviour and psychological capacities. Various sorts of mental ability, e.g., vocabulary, memory span, and speed of mental work, tend to grow at much the same rate during the juvenile phase of development. But the effects of aging during the adult phase lead to what is known as differential decline of mental abilities, such that some abilities like vocabulary, commonsense, and general knowledge tend to remain intact or imporve, whereas others like spatial reasoning, creative output, and complex learning and memory functions tend to decline. Those that are maintained or improve with age are called "crystallised abilities" because they seem to depend mostly on experience and the exercise of well-practised mental skills.

The neurophysiological basis and many other psychological and behavioural functions is at present not well understood. There are indications which show the association between the degree of brain impairment in terms of loss of tissue, reduced blood flow, and pathological conditions such as senile plaques, softening and intracellular disorganisation, and the degree of mental impairment.

25.6 THEORIES OF AGING

The mechanism of aging seems to be a complex process that is influenced by a combination of several factors occurring at several levels. What determines the life span of an individual and what triggers the aging process? In the foregoing account, we have considered several biological aspects of aging based on which many theories have been propounded.

Free Radical Theory of Aging

The aging process may be divided into two categories of cumulative degradative changes: (a) more or less widespread damage produced by a variety of means, such as autoimmune reactions, ionising radiations and smog that are subject to inhibition to a greater or lesser degree, and (b) alterations in the so-called biological clocks-changes that can be altered little, under normal living conditions that largely determine the potential maximum life span of an individual.

D. Harman postulated that aging process is probably due to cumulative degradative changes brought about by free radical reactions, ubiquitous in living systems. Thus decreasing the level of deleterious free radical reaction in an organism may result in a decreased rate of biological degradation. Free radical reactions arise from the highly reactive intermediates that are present in the system. The high chemical reactivity of free radicals is due to the presence of a free electron. It is because of the magnetic field associated with a free electron that free radicals can be detected at low concentrations by the technique of electron spin resonance (ESR) spectroscopy. Free radical reactions are common place:

(1) The reaction of oxygen with gasoline, as in an automobile engine.
(2) Smog formation.
(3) Drying of linseed oil paints,

(4) The formation of many commonly used plastics, and

(5) The development of rancidity in butter.

Free radical reactions are invariably irreversible and give rise to a variety of products. In biological systems molecular oxygen is always present, which participates in free radical reactions involving oxidation of organic compounds (RH).

Initiation $RH + O_2 \xrightarrow{Cu} R + HO_2$

Propagation $R + O_2 \rightarrow RO_2$

$RO_2 + RH \rightarrow R + ROOH$

Termination $R + R \rightarrow R : R$

The rate of free radical reactions involving molecular oxygen is enhanced by catalysts such as copper, iron and manganese, and inhibited by antioxidents such as vitamin E, 2-mercaptoethylamine (2-MEA) and butylated hydroxytoluene, which are capable or removing the intermediate free radicals. These compounds are expected to minimise deleterious effect of free radicals, irrespective of their involvement with mucopolysaccharides, nucleic acids, lipids or proteins.

Influence of Diet

If free radical reactions contribute to the degradation of biological systems, it might be possible that dietary considerations might influence the life span. Studies on mice and rats have shown that increasing the amount and degree of unsaturation of dietary fat decreased the average life span in mice by 10%. It is possible that *in vivo* lipid peroxidation contributes to the deterioration of biological systems. Vitamin E is a natural antioxidant and it has a modest effect on the life span as it decreases the rate of formation of free radicals. In a similar study, on mice carried out with protein diet, it has been shown that lysine and histidine–rich protein food decreased the life span slightly, whereas soybean protein (low in easily oxidisable amino acids) has a moderate beneficial effect.

Free Radical Inhibitors

A number of free radical reaction inhibitors have been tested as antiaging agents: 2—MEA (an effective radiation protector), butylated hydroxytoluene (BHT), and ethoxyquine (antioxidant extensively used in animal feeds). These three antioxidants have been found to increase the life span in mice by about 25-45 per cent, depending on the strain of mice.

Some diseases like cancer, amyloidosis, senility, atherosclerosis and hypertension are associated with aging and these have been implicated with the accumulation of free radical reactions. Vitamin E, a natural antioxidant, has been reported to increase the life span of mice and *Drosophila.*

Somatic Mutation Hypothesis

An alteration that occurs in the genome of somatic cells and not that of the germ cells, is called somatic mutation. According to L. Szilard, aging is due to random mutations resulting in loss of chromosomes of somatic cells as in case of heart, skeletal muscles and brain. Cells of these tissues stop dividing after a certain stage. Each species has certain life span, beyond which genetically controlled programmes become progressively less effective until death. The most vulnerable material to random mutations is the genetic material, i.e. DNA. Changes in the base sequence of DNA alter the

template and consequently affect the regulatory and metabolic capacities of the cell. The genes of such cells produce defective proteins, rendering the cells inactive, followed by death.

Damage to the cell genome may occur in various ways, such as radiations, or replicating errors caused by mismatched nucleotides. Continuing somatic mutations will sooner or later involve errors in the structure of enzymes concerned with protein synthesis, and so generate a cascading "error catastrophe" that will render the cell incapable of further mitosis.

Mitochondrial Theory of Aging

Recently, Linnane *et al.* (1989) hypothesised that accumulation of somatic mutations in the mitochondrial DNA (mt DNA) is a major contributor to human aging and degenerative diseases. It has been proposed that mt DNA mutations are early molecular events associated with human aging and are responsible for the age-dependent decline of mitochondrial respiratory function in various human tissues. An impairment of the mitochondtial function and the subsequent reduced supply of ATP could have far reaching effects at the cellular level.

The mt DNA is highly susceptible to mutations, because it is small, highly compacted and unprotected with histones. Consequently, mt DNA mutations in mammals accumulate at least 16 times faster than in the nuclear DNA. Further, DNA repair and recombination mechanisms are lacking in mitochondria. In man, several degenerative diseases affecting muscle and nervous system are associated with mtDNA mutations. One of the causes attributed for mtDNA mutations is its susceptibility to free radical attack. The mtDNA is normally attached to the inner membrane of mitochondria where lipid peroxidation is carried out.

Since DNA repair mechanism in mitochondria is absent, mtDNA mutations are of the nature of deletions reported to occur in various tissues of old humans. It has been demonstrated that liver mitochondrial functions decline with age and at least three types of age-associated mtDNA deletions (5 kb, 6 kb, and 7.4 kb) in the liver of elderly persons are found. It has been found that aging is associated with an increase in lipid mitochondrial peroxidation and mtDNA deletion and there exists a correlation between the two in various tissues.

Genes for Aging: Gerontogenes

Whether or not there is any genetic programme that determines the exact time of death of an individual, evidence indicates a constraint in terms of achieving maximum life span within a species. If the life span of an organism is intrinsically limited, the possibility of the existence of certain genetic elements of regulation can not be ruled out. Such genetic elements that are involved in the regulation of aging are called *gerontogenes.* Gerontogenes are thought to be involved in the regulation of development and homeostasis.

What is the nature of gerontogenes? Are there any specific genes which carry a programme of self-destruction? It has been suggested that the concept of gerontogenes is linked with gene regulation involving genes for DNA repair, free radical scavenging genes and the genes for protein synthesis and turnover. As for the nature, gerontogenes may not have a definitive aging property as has been hypothesised, but may be a cluster of tightly coupled genes whose combined effect resembles that of a cryptic *gene for aging.* Therefore, gerontogenes have a functional rather than physical reality, hence called "virtual" gerontogenes. Experimental evidence to identify such genes comes from the studies

based on the extension of life span and slowing down of various age-related biochemical and functional alterations of *Drosophila melanogaster* by simultaneous over expression of *superoxide dismutase* and *catalase,* which indicate that these free radical scavenging and antioxidant genes belong to the family of gerontogens influencing aging and life span. A combined action and interaction of these genes probably influences maintenance and repair of the age-related changes, thus revealing their role as gerontogenes.

Orgel's Theory of 'Error Catastrophe' in Proteins

In 1963, Orgel put forward a theory of aging which explains the occurrence of faulty functional proteins, i.e. enzymes. Errors in the amino acid sequence of polypeptide chains may lead to loss of activity of the enzymes as well as the protein synthesising mechanism. Errors leading to reduced specificity of an information–handling enzyme such as RNA polymerase, may lead to an increasing error frequency. The concept of "error catastrophe" as postulated by Orgel suggested that the aging of non–or post-mitotic cells is a result of cumulation of transcription and translation errors in protein synthesis. Therefore, introduction of incorrect amino acids is bound to accelerate the aging process.

Theory of Gene Regulatory Mechanisms

It is a matter for serious consideration whether cellular aging is a result of failure or switch over of regulatory processes. We know that transcription and translation steps are regulated by genes, a fact that has been verified on the basis of 'operon model' in eukaryotes proposed by Jacob and Monad. Is it possible that an *aging programme* exists in the life of a cell, functioning in a manner similar to that operating in ontogeny which is strictly programmed. Cellular differentiation is best understood at the level of the regulatory processes, as sequential activation and inactivation of genes is programmed. Differential expression of genes at different times during the life of an individual takes place in response to its needs. Environmental stress also alter the gene expression. Organisms respond to elevated temperatures by activating specific genes to produce heat shock proteins that confer a degree of resistance to hyperthermia. Kanungo (1997) observed that alterations that occur in the expression of genes during aging are mediated through their promoter, and it may be possible to modulate the expression of genes through appropriate inducers and extend the adulthood period.

CONCLUSION

Aging is an inviolable phenomenon. Aging and death are biological events that are bound to take place. The life span of organisms is almost fixed, the human life span of about 70-75 years or so is similar in all societies. However, efforts have been to prolong this life span, but regardless to technological or medical progress, the life span appears to be fixed. By bringing various diseases under control, life expectancy at birth has increased but the life span remains almost constant. Gerontologists are, however, concerned with prolonging the life span or delaying the aging process. We have documented various theories and mechanisms, and it is hardly to be expected that there is one single universal mechanism. It can be asserted that the phenomenon of aging results only from a combination of several, mutually independent, mechanisms, both programmed and stochastic.

Self Assessment Questions (SAQs)

1. Which of the compounds below listed acts as an inhibitor of sodium dependent glucose transport across the plasma membrane?
   A. Ouabain
   B. Phlorizin
   C. Sodium azide
   D. Dicumarol
2. Which of the following enzymes is located in the inner mitochondrial membrane?
   A. Acyl CoA synthetase
   B. Fatty acyl CoA oxidase
   C. Succinate dehydrogenase
   D. Isocitrate dehydrogenase
3. Maintenance of Na^+ and K^+ gradients across the plasma membrane:
   A. Involves the enzyme ATPase
   B. Is an electrically neutral system
   C. Move Na^+ either into or out of the cell
   D. None of the above
4. Composition of the major fluid compartments of the body shows that:
   A. One of the major intracellular anions is Cl^-
   B. Plasma and intracellular fluid are similar in ionic composition
   C. One of the major intracellular anions is phosphate
   D. The major blood plasma cation is K^+
   E. The major cation of the intracellular fluid is Na^+
5. Which of the following compounds is least likely to be soluble in water?
   A. Nonpolar compounds
   B. Weak electrolytes
   C. Strong electrolytes
   D. Weakly polar compounds
6. During a breathing cycle:
   A. Gas exchange between alveoli and capillary blood can occur at all times
   B. There is net uptake of nitrogen by the blood
   C. The alveolar gases are completely exchanged for atmospheric gases

D. Gas exchange with the capillary blood occurs at the surface of all airways

7. Which of the following reaction (s) produces H^+?
   A. Formation of bicarbonate ion from CO_2 and water
   B. Binding of oxygen by haemoglobin
   C. Formation of carbamino compounds from CO_2 and Hb
   D. All the above
8. Where does the absorption of glucose take place?
   A. In small intestine
   B. In the stomach
   C. In duodenum
   D. None of the above
9. Disaccharides of the diet obtained from polysaccharide digestion:
   A. Are absorbed as such in the small intestine
   B. Are hydrolysed to constituent monosaccharides by pancreatic enzymes
   C. Are quantitatively unimportant since there are no enzyme deficiency diseases associated with their metabolism
   D. Are hydrolysed by specific enzymes of the small intestine
10. The hormone aldosterone:
   A. Is an antidiuretic hormone
   B. Increases the retention of sodium ions
   C. Is a glycoprotein
   D. Increases retention of potassium ions
11. In humans, fatty acids:
   A. Are not required at all in the diet
   B. Can be synthesised from excess dietary carbohydrate or protein
   C. Must be supplied entirely in the diet
   D. Containing double bonds cannot be synthesised
12. Primary bile acids:
   A. Are acids that are reabsorbed from the intestinal tract
   B. Are synthesised in the intestine by bacteria
   C. Are synthesised in the liver directly from cholesterol
   D. None of the above
13. Increased levels of acetoacetate are expected in urine when:
   A. Liver glycogen is depleted
   B. Acetyl CoA concentration is low
   C. Liver glycogen is normal
   D. None of the above
14. The major energy source for the brain is normally
   A. Ketone bodies in the blood
   B. Amino acids present in the blood
   C. Fatty acids present in the blood
   D. Glucose present in the blood
15. Chylomicrons are
   A. Transported from the liver
   B. Triglycerides present in the intestinal lumen
   C. Newly synthesised in the intestine
   D. None of the above

16. Ketosis is caused due to increased blood levels of
    A. Acetyl CoA
    B. Acetoacetate
    C. β-hydroxy-β-methyl glutarate
    D. None of the above
17. Ketone bodies are mainly produced from
    A. Phospholipids in the liver
    B. Cholesterol
    C. Triglycerides of fat cells
    D. None of the above
18. In the fasting state, oxidation of fatty acids or ketone bodies leads to a slower rate of glycolysis in muscles because
    A. The low insulin level reduces the uptake of glucose by muscle
    B. $NADH/NAD^+$ ratio decreases
    C. Pyruvate dehydrogenase is activated
    D. None of the above
19. Muscular weakness is characterised by
    A. Deficiency of sodium
    B. Deficiency of potassium
    C. Deficiency of calcium
    D. None of the above
20. Excess of iron in the body accumulates in the cells
    A. As haemoglobin
    B. As protein-iron conjugate
    C. As iron containing hemosiderin
    D. None of the above
21. *De novo* biosynthesis of triglycerides occurs mainly in the
    A. Brain
    B. Muscle
    C. Liver and adipose tissue
    D. None of the above
22. Which of the following occurs in non-shivering thermogenesis?
    A. Glucose is oxidised to lactic acid
    B. Ethanol is formed
    C. ATP is consumed for heat production
    D. Fatty acids uncouple oxidativephosphorylation
23. Components of bile that are active in the intestinal digestive process include all the following except
    A. Glycocholate
    B. Taurocholate
    C. Deoxycholic acid
    D. Bile pigments
24. For mammals some amino acids are essential, whereas others are not. Humans are capable of converting:
    A. Phenylalanine to tyrosine
    B. Arginine into lysine
    C. Aspartic acid into leucine
    D. None of the above
25. The urea cycle converts
    A. Urea into uric acid
    B. Urea into ammonia
    C. Urea to ammonia and CO_2
    D. All of above

26. Transcarbamylase is an important enzyme of the urea cycle which is concerned with
    A. The formation of urea from arginine
    B. The hydrolysis of ornithine
    C. The formation of ornithine from citrulline
    D. The formation of citrulline from ornithine
27. Which of the statements is true for alcaptonuria?
    A. The patient's urine turns dark on exposure to air
    B. Tyrosinase activity is absent
    C. The enzyme phenylalanine hydroxylase is absent
    D. There is too much activity of homogentisic acid oxidase
28. The non-protein nitrogenous compound present in blood is
    A. Ammonia B. Urea
    C. Uric acid D. None of the above
29. The major source of ammonia in fresh urine is due to
    A. Hydrolysis of glutamine by glutaminase B. Hydrolysis of urea by ureasse
    C. Deamination of amino acid aspartic acid D. None of the above
30. Creatine phosphate is:
    A. A waste compound B. Excreted by the kidney
    C. A source of ATP synthesis in the muscle D. A hormone
31. The important proteins that participate in muscle contraction are
    A. Actin B. Myosin alone
    C. Both actin and myosin D. All the above
32. Which of the following enzymes is most important in protein digestion?
    A. Chymotrypsin B. Pepsin
    C. Carboxypeptidase B D. Enterokinase
33. Which of the following amino acids is exclusively ketogenic?
    A. Isoleucine B. Phenylalanine
    C. Leucine D. Tryptophan
34. It is the key enzyme of the urea cycle:
    A. Urease B. Arginase
    C. Glutaminase D. None of the above
35. Hippuric acid
    A. Is an excretory product
    B. Is a conjugate of glycine and benzoic acid
    C. Is a source for elimination of excess nitrogen
    D. All the above

36. The Michaelis-Menten equation of enzyme reaction
    A. Assumes formation of enzyme-substrate complex
    B. Explains allosteric behaviour of certain regulatory enzymes
    C. Assumes the formation of a covalent intermediate between enzyme and substrate
    D. None of the above
37. Contraction of skeletal muscle is initiated by the binding of calcium to
    A. Tropomyosin
    B. Troponin
    C. Actin
    D. Actomyosin
38. Which of the following compounds accumulates in alcaptonuria?
    A. Homogentisic acid
    B. Tyrosine
    C. Phenylalanine
    D. All the above
39. Inactive zymogens (proenzymes) are the precursors of the following enzymes except
    A. Carboxypeptidase
    B. Chymotrypsin
    C. Pepsin
    D. Ribonuclease
40. A purely competitive inhibitor of an enzyme
    A. Increases Km without affecting Vmax
    B. Decreases Km without affecting Vmax
    C. Decreases Vmax without affecting Km
    D. Decreases both Km and Vmax
41. Which of the following proteins is found in the thick filaments of skeletal muscle?
    A. Troponin
    B. Myosin
    C. Actin
    D. Tropomyosin
42. Which of the following statements correctly describes allosteric enzymes?
    A. Michaelis-Menten kinetics describe their activity
    B. Effectors may enhance or inhibit substrate binding
    C. The regulatory site may be catalytic site
    D. None of the above
43. Lactate dehydrogenase is
    A. An allosteric enzyme
    B. An isoenzyme
    C. A coenzyme
    D. A regulatory enzyme
44. Desert animals have developed mechanisms to conserve water and include
    A. Production of concentrated urine
    B. Production of dry faeces
    C. Reduction of evaporative loss through skin and lungs
    D. All the above
45. The hypothalamus controls
    A. Heat loss and heat production centres
    B. The endocrine activity of the body by influencing pituitary function

C. Osmoregulatory receptors
D. All the above

46. In the endotherms, during hyperthermia
    A. The blood flow through the skin is minimised
    B. The blood flow through the skin is augmented and blood vessels dilated
    C. The blood vessels undergo constriction
    D. None of the above
47. The plasma proteins include
    A. Albumins
    B. Globulins
    C. Fibrinogen
    D. Haptoglobins
    E. All the above
48. Thrombocytes or platelets are
    A. The smallest cells about 2 to 3 μ in diameter
    B. The longest living cells
    C. Formed inside the liver
    D. Unimportant in blood clotting
49. Thrombocytes
    A. Are necessary for coagulation of the blood
    B. Possess adhesive qualities
    C. Contain precursor of thromboplastin
    D. All the above
50. The parasympathetic nerve fibres innervating the heart muscles secrete acetylcholine which
    A. Reduces the frequency of the heart beats
    B. Can bring about ventricular arrest but auricles continue to contract
    C. Is a neurotransmitter
    D. All the above
51. The heart muscles of a vertebrate
    A. Are striated and have a short refractory period
    B. Do not obey 'all-or-none-principle"
    C. Are nonstriated
    D. None of the above
52. Respiratory acidosis is caused due to
    A. Increase in the H_2CO_3 content in the blood
    B. decrease in the bicarbonate fraction
    C. Excess of bicarbonates that accumulate in the blood
    D. None of the above
53. At high partial pressues of oxygen (PO_2), haemoglobin

A. Does not combine with oxygen
B. Combines with oxygen covalently
C. Combines with oxygen loosely and releases the gas at low partial pressures
D. None of the above

54. Anoxia is caused when
A. Oxygen does not reach the blood
B. Oxygen supply to the tissues is reduced
C. Oxygen supply to the tissues is completely cut off
D. CO_2 supply is increased

55. In birds, uric acid is the chief nitrogenous product, which is
A. The end-product of pyrimidine metabolism
B. The end-product of purine metabolism
C. Highly toxic
D. Soluble in water

56. The mammalian kidney functions
A. To remove non-volatile waste products
B. To remove excess of non-volatile acids or bases
C. To regulate arterial blood pressure
D. All the above

57. Renin is
A. A hormone secreted by the kidney
B. An enzyme secreted in the intestine
C. Is a neurotransmitter which regulates water balance
D. None of the above

58. The distal part of the urinary tubule of the kidney
A. Helps in eliminating hydrogen ions in the form of NH_4^+ ions
B. Secretes hydrogen ions which are free ions
C. Secretes bicarbonate ions
D. None of the above

59. Gamma aminobutyric acid (GABA) is
A. An excitatory neurotransmitter
B. An inhibitory neurotransmitter
C. Responsible for increasing permeability of Na^+ ions
D. None of the above is correct

60. The metabolism of calcium is hormonally regulated by
A. Calcitonin
B. Parathyroid hormone and thyroid hormone
C. Calmodulin
D. Vitamin D

61. Insulin is synthesised in β cells of the pancreatic islets
    A. Is composed of two polypeptide chains
    B. Activates glycogen phosphorylase in the liver
    C. Is secreted in response to a fall in blood glucose concentration
    D. None of the above
62. Angiotensin II
    A. Is formed in the kidney
    B. Is derived from angiotensin I by proteolysis
    C. Regulates biosynthesis of cortisone
    D. None of the above
63. Glucagon
    A. Is composed of two polypeptide chains
    B. Is synthesised in the liver
    C. Is secreted in response to a fall in the plasma glucose concentration
    D. Promotes insulin secretion
64. Iron is
    A. Absorbed efficiently in the intestine
    B. Transported in the blood bound to albumin
    C. Excreted in large amounts in bile and faeces
    D. Stored in the body in the form of ferritin
65. Which of the following diseases is a result of dietary deficiency?
    A. Pernicious anaemia
    B. Addison's disease
    C. Simple goiter
    D. None of the above
66. All the following hormones use cyclic AMP as a second messenger except
    A. Estrogen
    B. Luteinizing hormone
    C. Epinephrine
    D. Glucagon
67. Following release of norepinephrine by sympathetic nerves and epinephrine by the adrenal medulla, all the following metabolic process are increased except
    A. Lipolysis
    B. Gluconeogenesis
    C. Glycolysis
    D. Glycogenolysis
68. Increased reabsorption of water from the kidney is due to which of the following hormones?
    A. Cortisol
    B. Vasopressin
    C. Glucagon
    D. Aldosterone
69. A deficiency of vitamin B_{12} causes
    A. Beriberi
    B. Scurvy
    C. Rickets
    D. Pernicious anaemia
70. Diet induced thermogenesis
    A. In humans, occurs primarily in skeletal muscles
    B. Can be increased by exercise and a high fat diet

C. May not be possible in obese people
D. None of the above

71. Plasma proteins
A. Are all synthesised in the liver
B. Function as blood buffers
C. Are important components of the body's defence mechanisms
D. None of the above

72. Which of the following muscle proteins contains ATPase activity for muscle contraction?
A. Actin
B. Troponin
C. Myosin
D. Tropomyosin

73. In the normal resting state, most of the blood glucose burned as fuel is consumed by
A. The liver
B. The brain
C. The kidneys
D. The muscles

74. Phenylketonuria is a disease caused by
A. Tyrosinase deficiency
B. An excess of tyrosine
C. Deficiency of hydroxylase involved in an amino acid conversion
D. Deficiency of a phenylalanine transport enzyme

74. Glycolysis is the only ATP producing pathway in
A. Erythrocytes
B. Hepatocytes
C. Lymphocytes
D. Adipose tissues

75. Which of the following tissues can metabolise glucose, fatty acids, and ketone bodies for ATP production?
A. Liver
B. Muscle
C. Brain
D. Red blood cells

76. The largest energy reserve in humans (in terms of kilocalories) is
A. Blood glucose
B. Liver glycogen
C. Muscle glycogen
D. Adipose triacylglycerols

77. Increased formation of ketone bodies during starvation is due to
A. Decreased levels of circulating glucagon
B. Increased levels of free fatty acids in the serum
C. Inhibition of β-oxidation in the liver
D. Decreased formation of acetyl CoA in the liver

78. Vitamin K
A. Is essential for clot formation
B. Increases coagulation time
C. Is a water soluble vitamin
D. Is not required in the body

79. In protein biosynthesis.

A. Each amino acid recognises its own codon on the mRNA template
B. Each amino acid is first attached to an anticodin specific for the amino acid
C. Each amino acid is added in its proper place to a growing peptide chain through the adaptor function of tRNA
D. A given codon and its anticodon must have identical base sequences in order for proper base pairing

80. Muscle glycogen cannot contribute directly to blood glucose levels because
A. Muscle glycogen cannot be converted to glucose-6-phosphate
B. Muscle lacks glucose-6-phosphatase activity
C. Musclecontains no glucokinase
D. Muscle lacks glucoisomerase activity

81. Upon depolarisation ions move through the membrane
A. By active transport
B. By polarity
C. By diffusion
D. By electrical attraction

82. Antibodies are
A. Foreign proteins that show immune response
B. Special proteins that destroy antigens
C. A class of cells that engulf invading proteins
D. Antigens that are partially inactivated

83. Neutral fats are accumulated in
A. Connective tissue
B. Adipose tissue
C. Nerve fibres
D. Brain

84. Oxidation of fatty acids takes place in the
A. Mitochondrial matrix
B. Intermembranal space
C. Inner membrane of the mitochondria
D. Cytosol

85. Biosynthesis of glucose from non-carbohydrate sources is called
A. Glycogenesis
B. Glycogenolysis
C. Gluconeogenesis
D. None of the above

86. Puromycin is
A. A hormone
B. An antibiotic that terminates protein synthesis
C. Inhibits chain elongation process in protein synthesis
D. An analogue of aminoacid

87. Which of the following statements is correct?
A. Trypsin is an exopeptidase that liberates free amino acids
B. Pepsin is an endopeptidase secreted by the pancreas
C. Chymotrypsin acts in the stomach on peptide bonds formed by glycin
D. Trypsin can act as an activator for all zymogens of pancreatic proteases

88. Catabolism of haemoglobin
    A. Occurs in red blood cells
    B. Results in the liberation of carbon dioxide
    C. Is the sole source of bilirubin
    D. Involves oxidative cleavage of porphyrin ring and results in liberation of haeme
89. Smooth muscle
    A. Is striated in appearance
    B. Contains the troponin system
    C. Contains tropomyosin
    D. None of the above
90. In the human body, iodine is
    A. Taken up by adrenals
    B. Taken up by the thyroid
    C. Not required
    D. None of the above
91. Hormone receptors that stimulate cyclic AMP production
    A. Cause release of the catalytic subunit upon binding the hormone
    B. Are proteins distinct and separate from those that catalyse the production of cAMP
    C. Are the outer projecting portion of a single protein that acts a receptor and the enzyme that produces cAMP
    D. None of the above
92. The genetic code
    A. Is universal
    B. Is read in the direction of 3'—5'
    C. Triplet CCA is found at the beginning of nearly all mRNA coding sequences
    D. Involves a number of minor bases associated with chain initiation
93. In the lymphatic system, antibodies for circulation in the serum are produced by
    A. B lymphocytes that have been transformed into plasma cells
    B. Macrophages
    C. Tlymphocytes
    D. Reticulocytes
94. Bile salts are
    A. Synthesised in the liver from cholesterol
    B. Reabsorbed from the gut
    C. Synonym of bile salts
    D. None of the above
95. Which of the following is involved in stopping the flow of blood from damaged capillaries:
    A. Heparin
    B. Paraaminobenzoic acid
    C. Platelets
    D. None of the above
96. Which of the following is not the blood coagulating factor?

A. Fibrinogen
B. Antihaemophilic factor A
C. Pepsinogen
D. Thromboplastin

97. Metabolic acidosis:
A. Is due to loss of anions
B. Is compensated by fall in plasma CO_2 and PCO_2
C. Is accompanied by hypokalemia
D. Is not found in patients suffering from ketosis

98. Increased plasma HCO_3^- is likely to develop in
A. Acute hyperventilation
B. Ammonium chloride ingestion
C. Respiratory failure due to neuromuscular paralysis
D. None of the above

99. Ingestion of a meal consisting exclusively of protein would result in
A. Hypoglycemia
B. Depletion of liver glycogen
C. Ketoacidosis caused by metabolism of ketogenic amino acids
D. An increased release of insulin

100. Which of the following statements concerning glucose metabolism is correct?
A. Conversion of glucose to lactate occurs in RBCs
B. An elevated level of insulin leads to decreased level of fructose
C. Pyruvate kinase catalyzes an irreversible reaction
D. None of the above

101. Which enzyme is directly responsible for the activation of chymotrypsinogen?
A. Aminopeptidase
B. Carboxypeptidase
C. Enterokinase
D. Pepsin
E. Trypsin

102. Which of the following reactions does not produce free ammonia?
A. Glutamate dehydrogenase
B. Glutamate-pyruvate transaminase
C. Glutaminase
D. Serine dehydratase

103. Which amino acid is responsible for production of neurotransmitter epinephrine?
A. Tyrosine
B. Methionine
C. Tryptophane
D. Histidine

104. Which amino acid gives rise to neurotransmitter serotonin?
A. Tyrosine
B. Histidine
C. Glutamate
D. Tryptophan
E. Methionine

105. During long–term starvation, most energy used by the brain is derived from which compound?
    A. Acetone
    B. 3–Hydroxybutyrate
    C. Oxaloacetate
    D. Malonic acid
106. Dopamine is derived from which amino acid?
    A. Methionine
    B. Histidine
    C. Tyrosine
    D. Tryptophan
107. Which of the following amino acids is released from the muscle and converted to glucose in the liver?
    A. Alanine
    B. Cysteine
    C. Valine
    D. Isoleucine
108. The kidney is unable to:
    A. Convert glutamine to ornithine
    B. Convert pyruvate to alanine
    C. Convert carbondioxide and ammonia into carbamoyl phosphate:
    D. Synthesize urea
109. Trypsin activates several proteases by partial hydrolysis of a precursor. Which of the following is exception?
    A. Carboxypeptidase
    B. Chymotrypsin
    C. Pepsin
    D. Elastase
110. Diabetic patients frequently suffer from *muscular fatigue,* despite being hyperglycemic. Which is the most likely explanation is for this fatigue?
    A. Inability to release free glucose from muscles
    B. Failure to utilize ketone bodies by muscle
    C. Inability to import sufficient quantity of glucose into muscles
    D. Inhibitation of TCA cycle by excess glucose
111. Which substance is the direct donor of nitrogen for the cytoplasmic phase of urea synthesis?
    A. Adenosine monophosphate
    B. Ornithine
    C. Glutamate
    D. Aspartate
112. Which is the most important determinant of the life span of haemoglobin in RBC?
    A. Age of the red blood cell
    B. Intracellular levels of cathepsins
    C. Intracellular levels of chymotryosin
    D. Degree of oxygenation
113. Human gastric juice contains:
    A. Aminopeptidase
    B. Chymotrypsin
    C. Papain
    D. Pepsin
    E. Trypsin
114. Phosphocreatine is:

A. A component of phospholipids
B. Used to import fatty acids into mitochondria
C. The major nitrogenous compound in urea
D. A reserve source of energy to replenish ATP in the muscle

115. Which enzyme is responsible for urea in the liver?
A. Arginase
B. Carbamoyl phosphate synthetase I
C. Glutamate dehydrogenase
D. Glutaminase

116. During starvation (more than two weeks), blood glucose is derived from:
A. Glycogen stores in liver
B. Glycogen stores in muscles
C. Liver protein amino acids
D. Adipocyte fatty acids

117. Which of the following can be converted to fuel for the brain in diabetes?
A. Muscle glucogen
B. Adipocyte troglycerides
C. Plasma fatty acids
D. Muscle-protein derived leucone

118. Patients of phenylketonuria suffer from:
A. Phenylalanine hydroxylase deficiency
B. Enhanced synthesis of phenylalanine in the body
C. Excess tyrosine in the body
D. Inadequate phenylalanine in the diet

119. Haemolytic anaemia is caused by a defect of which of the enzymes?
A. Transketolase
B. Glucose-6-phosphatase
C. Phosphoenolpyruvate carboxylase
D. Glucose-6-phosphate dehydrogenase

120. Galactosemia is characterized by all of the following except:
A. Failure to gain weight
B. Inhibition of an aldolase isoenzyme
C. Product inhibition of galactokinase
D. Reducing sugar in urine following milk ingestion

121. The presence of normal amounts of glycogen with abnormally long outer chains is characteristic of a deficiency of:
A. Phosphorylase
B. Debranching enzyme
C. Branching enzyme
D. Glucose-6-phosphatase

122. In sickle-cell anaemia, the basis of the malfunction of haemoglobin molecule is:
A. Incorrect secondary structure
B. Decreased tertiary structure changes
C. Reduced affinity for oxygen
D. Substitution of a single amino acid

123. Most CO_2 is carried from the tissues to lungs:
A. Bound to Hb as carbamino groups
B. As CO_2 bound to the haeme group of Hb
C. As HCO_3 in the plasma
D. As CO_2 gas in the plasma

124. Enteropeptidase is an important enzyme for digestion of proteins in the small intestine because
A. It is activated by bile salts so that it can cleave trypsinogen producing active trypsin which in turn activates other zymogens

B. It is the major protease found in the duodenum
C. It is produced in the stomach but activated by increased pH found in the small intestine
D. It is not a zymogen and therefore the only protease that is found in an active form at the time it is secreted by the pancreas

125. Most CO_2 is carried from the tissues to lungs
A. Bound to Hb as carbamino groups
B. As bicarbonate ions in the plasma
C. After enzymatic conversion to carbonmonoxide
D. As CO_2 in the RBC

126. Myoglobin and haemoglobin both
A. Bind haeme
B. Are alpha helical
C. Bind oxygen
D. All of them

127. The enzyme that directly breaks down fibrin for clot dissolution is
A. Urokinase
B. Streptokinase
C. Plasmin
D. Factor V

128. The function of acetylcholine esterase is to
A. Signal the release of acetylcholine into the synaptic cleft
B. Clear acetylcholine after it has been secreted into the synaptic cleft
C. Synthesize acetylcholine from acetyl-CoA and choline
D. Bind the receptors on the postsynapatic membrane

129. The form of beta galactosidase found in the plasma of patients with I-cell disease lacks a residue of
A. Asparagine-linked N-acetylglucosamine
B. N-acetylglucosamine
C. Glucose-6-phosphate
D. Mannose

130. Which coagulation factor serves at the junction of the extrinsic and intrinsic pathways?
A. Factor II
B. Factor V
C. Factor VII
D. Factor X

131. Which of the following is complexed with albumin in blood?
A. Chylomicrons
B. VLDL
C. Palmitate
D. HDL

132. The primary defect in cysctic fibrosis is:
A. Lack of vitamin C
B. Defective cystine transport
C. A problem with cysteine metabolism
D. Defective chloride channel

133. Which of the blood proteins does not require vitamin K to become fully functional?
A. Thrombin
B. Fibrinogen
C. Factor IX
D. Factor VII

134. The absence of which factor is responsible for haemophilia?
    A. Factor II
    B. Factor V
    C. Factor VIII
    D. Factor VII
135. Cystic fibrosis is what class of disease?
    A. Autosomal dominant
    B. Autosomal recessive
    C. X-linked dominant
    D. X-linked recessive
136. Lesch-Nyhan syndrome is due to the deficiency of
    A. Xanthine oxidase
    B. Pyrimidine biosynthesis
    C. De no vo biosynthesis of pyrimidines
    D. Hypoxanthine-guanine phosphoribosyl transferase
137. Deficient intake of beta carotene can result in
    A. Bleeding disorders
    B. Night blindness
    C. Kwashiorkor
    D. Marasmus
138. Tay-Sachs disease is the result of an inborn error of metabolism of
    A. Histidine
    B. Cholesterol
    C. Lactose
    D. Ganglioside
139. Where does haeme synthesis occur?
    A. In the liver
    B. In mitochondria
    C. In the cytoplasm
    D. None of the above
140. Iron is transported in the plasma primarily as
    A. Transferrin complex
    B. Free ferrous ion
    C. Haeme containing enzymes
    D. Ferritin complex
141. Jaundice is the result of
    A. Increased levels of iron
    B. Build up of bilirubin
    C. Genetic defect in the haeme biosynthesis pathway
    D. Excess excretion of bilirubin diglucuronide in the bile
142. Alzheimer disease is a neurological disorder due to
    A. Mitochondrial defect
    B. Degeneracy of muscles
    C. Disorders in the endoplasmic membrane
    D. Endocrine disorders
143. The disease hypercholesterolemia is caused by
    A. Accumulation of ketone bodies
    B. High concentration of acetyl-CoA in the body

C. Accumulation of excess of bile salts
D. None of the above

144. Phenylketonuria patients suffer from the deficiency of
A. Phosphofructokinase
B. Phynylalanine hydroxylase
C. Arginase
D. None of the above

145. Zellweger syndrome is characterized by neurological impairment resulting from:
A. Accumulation of long-chain fatty acid and reduction in plasmalogens
B. Accumulation of mutations in genes encoding peroxins
C. Absence of peroxisomes
D. All of above

146. Increased oxygen affinity for haemoglobin is due to
A. Sickle cell anaemia
B. Pyruvate kinase deficiency
C. Hexokinase deficiency
D. Carbon monoxide poisoning

147. Pernicious anaemia in elderly people is most often due to
A. Lack of folic acid in the diet
B. Deficiency of B_{12} in the diet
C. Autoimmune problem
D. Lack of intrinsic factor

148. The process of muscel contraction in *rigor mortis* is stopped due to
A. Calcium deficiency
B. Release of ADP and Pi
C. High energy configuration of myosin heads
D. Lack of ATP

149. Deficiency of which of the following enzymes is responsible for causing albinism?
A. Xanthine oxidase
B. Pyruvate kinase
C. Arginase
D. Tyrosinase

150. Congential haemolytic anaemia is caused by the deficiency of:
A. Hexokinase
B. Phosphorylase
C. Hexokinase and 2, 3-diphosphoglycerate mutase
D. Carbonic anhydrase

KEY TO SAQs

1. B	2. C	3. A	4. D	5. A	6. A	7. D	8. A	9. C	10. B
11. B	12. C	13. A	14. D	15. C	16. B	17. C	18. A	19. B	20. C
21. C	22. D	23. D	24. A	25. B	26. D	27. A	28. B	29. A	30. C
31. C	32. D	33. C	34. B	35. D	36. A	37. B	38. A	39. D	40. A
41. B	42. B	43. B	44. D	45. D	46. B	47. E	48. A	49. D	50. D
51. A	52. A	53. C	54. C	55. B	56. D	57. A	58. A	59. B	60. D
61. A	62. B	63. C	64. D	65. C	66. A	67. C	68. B	69. D	70. A

71. C	72. C	73. C	74. A	75. B	76. D	77. B	78. A	79. C	80. B
81. C	82. B	83. B	84. A	85. C	86. C	87. D	88. D	89. C	90. B
91. B	92. A	93. A	94. A	95. C	96. C	97. B	98. C	99. D	100. C
101. E	102. B	103. A	104. D	105. B	106. C	107. A	108. D	109. C	110. C
111. B	112. D	113. D	114. B	115. C	116. B	117. C	118. A	119. D	120. B
121. C	122. D	123. C	124. A	125. B	126. D	127. C	128. B	129. A	130. D
131. C	132. D	133. B	134. C	135. B	136. D	137. B	138. D	139. D	140. A
141. B	142. C	143. C	144. B	145. D	146. C	147. A	148. B	149. D	150. C

Review Questions

Chapter—1

1. "A cell is the locus of behaviour and that this behaviour needs a structural organisation." Justify this statement.
2. Though the plasma membrane limits the boundary of a cell, its activities maintain a dynamic equilibrium between the cell and its surroundings. How does it happen?
3. Give functional importance of various organelles found in the cell.
4. What are the physiological roles played by the endoplasmic reticulum?
5. Assign physiological functions to the following organelles:
   (a) Golgi complex
   (b) Mitochondria
   (c) Lysosomes
   (d) Plasma membrane
   (e) Centriole
6. The chemical constituents of DNA in every organism are same, yet the genetic message it carries differs from species to species. Comment upon this functional difference.

Chapter—2

1. Describe different classes of food materials and give functional importance of each class.
2. Explain what happens when:
   (a) fats are hydrolysed
   (b) a tetrapeptide is hydrolysed enzymatically
   (c) a sucrose molecule is hydrolysed
   (d) the diet is deficient in ascorbic acid
3. Name at least five compound lipids and describe their physiological importance.
4. Describe the B group vitamins and their nutritional role in animals.
5. Besides serving some structural functions, carbohydrates and lipids are important energy sources. Explain as best as you can.

6. What are functions served by proteins?
7. Choose the most appropriate word from the list to make the statements meaningful: (vitamin A, D, E, ascorbic acid, thiamine)
   (a) Vitamin ..... is essential for normal growth of bone.
   (b) ..... is an essential dietary factor in all mammals.
   (c) Beri-beri is caused by the deficiency of ..... in the foods.
   (d) A deficiency of vitamin ..... is unlikely to occur because of the ability of the body to store it.
   (e) A deficiency of vitamin ..... causes retardation of growth and blindness.
8. Make a table of minerals that are indispensable for the body. Give important physiological functions served by each.

Chapter—3

1. Define the term "free energy", and differentiate between endergonic and exergonic reactions in terms of ΔG values.
2. Distinguish between oxidation and reduction reactions and give one example in each case.
3. Relate the structure and properties of ATP to its function in living organisms. Illustrate your answer with reference to its involvement in biological functions that you know of. (Tip: synthesis of starch, muscle contraction etc.)
4. Define the term *redox reaction.* During an oxidation reaction the substance which accepts electrons is always:
   (a) oxygen (b) hydrogen (c) reduced, (d) oxidised.
5. Name the components of the electron transport system and the function of each one of them. What is actually accomplished by the cytochrome system?
6. Explain with examples:
   (a) How does NAD^+ oxidise an organic substrate?
   (b) How is NAD^+ regenerated?
7. What is oxidative phosphorylation? Identify the points where these occur in the Krebs cycle.
8. Answer the following:
   (a) How many moles of ATP are generated per mole of glucose oxidised?
   (b) What percentage of total energy is liberated during (i) glycolysis and (ii) fermentation?
   (c) What happens to the energy which is not captured as ATP?
   (d) How many ATPs per glucose molecule are needed to start glycolysis?
   (e) Why hydrolysis of C_6 sugar (glucose) has a positive DG*?*

Chapter—4

1. Name the types of enzymes which:
   (a) add CO_2
   (b) convert one isomer into another
   (c) remove hydrogen
   (d) break up large molecules into small molecules using H_2O

(e) make new amino acids by transferring amino groups from existing ones
(f) energise molecules by adding Pi from ATP

2. What are the factors which affect enzyme activity?
3. What happens when an enzyme is subjected to a pH change different from its optimum? How can the full activity of the enzyme restored?
4. How do enzymes work?
5. Distinguish between:
   (i) allosteric regulators, coenzymes, cofactors and prosthetic groups,
   (ii) irreversible, competitive and non-competitive inhibitors.
6. The enzyme reaction from the hyperbolic graph can be expressed as:

$$y = \frac{ax}{x - b}$$

where $a = V_{max}$ (maximum velocity of reaction)

b = Km (when substrate concentration is $\frac{1}{2}$ of the maximum velocity)

$x \approx$ substrate concentration [S]

$y \approx$ v (velocity of reaction)

Now substitute the symbols V, [S], V_{max} and Km into the above equation and show what you arrive at. Is it Michaelis-Menten equation?

7. What properties do enzymes share with inorganic catalysts? Mention the properties that are unique to enzymes along with their biological significance.

Chapter—5

1. What is animal calorimetry? Does direct calorimetry give better results compared to indirect calorimetry? For what purpose it is used.
2. How can you find out the fuel value of foods? What is the purpose?
3. Of what value is the respiratory quotient? How can it be calculated?
4. Define BMR. Explain the factors affecting it. Describe a method to measure BMR.
5. Write explanatory notes on:
   (a) Specific dynamic action of food
   (b) Caloric requirements of man

Chapter—6

1. Answer the following:
   (a) Where does deamination of amino acids take place?
   (b) What is the significance of creatine? Discuss its role in energy metabolism.
   (c) What is the importance of sulphur containing amino acids?
2. Name the organ responsible for detecting the blood glucose level and also the hormone this organ produces. What role does glucagon play in regulating blood glucose level?

3. What is glycolysis? Besides glycolysis, is there any other pathway for glucose oxidation? Explain.
4. What are different forms of storage carbohydrates? Where are they stored? Discuss various sources from which glycogen can be synthesised.
5. To what substances are fatty acids broken down? Discuss the route for further metabolism of this substance.
6. Ketone bodies are synthesised in the liver, but they cannot be oxidised the same organ. Why?
7. Explain the terms:
   (i) ketonuria, (ii) ketonemia, (iii) ketosis, and (iv) ketogenesis
8. What is respiratory quotient? What would be the RQ for the following:
   (a) glucose by respiration (b) oxidation of fats
   (c) oxidation of proteins

Chapter—7

1. Explain the process of digestion and absorption of fats.
2. Where is bile synthesised? How its secretion is controlled? Discuss role of bile and its constituents.
3. Differentiate between peristalsis and anastalsis with reference to the intestinal activity.
4. Describe the mechanism of glucose absorption in the intestine.
5. Can you suggest that why digestion and absorption mainly takes place in the intestine? Discuss the role of pancreatic secretion in digestion.
6. Describe the mechanisms of intestinal absorption of:
   (i) iron (ii) calcium
7. What would happen to the process of digestion if the gall bladder from a patient has been removed?

Chapter—8

1. What are the key functions performed by animal membranes (plasma membrane)?
2. Discuss important features of active transport system. How can you stop active transport of glucose in the kidney?
3. Distinguish between the following pairs of terms:
   (a) Isoosmotic and Isotonic (b) Hypoosmotic and Hyperosmotic
4. Animals living in terrestrial environment face certain problems that are overcome through modifications of some existing mechanisms and a few others by acquiring new specialisations. Explain such mechanisms.
5. How do aquatic animals carry out ionic regulations for adaptation to marine habitat?
6. What osmotic problems are faced by brackish water and freshwater animals? How do they overcome these?
7. How do the desert animals minimise water loss and conserve water?

Chapter—9

1. Describe different types of phospholipids and their properties as found in the membranes.
2. Classify membrane proteins and the purpose they serve in transport of substances.
3. How are proteins oriented in the plasma membrane? Is protein orientation a necessary prerequisite for their functions?
4. Describe various mechanisms of glucose transport across the membranes.
5. How many classes of ion pumps exist in the membrane?
6. Active transport of ions and other substances is dependent on the presence of ATPase pumps. Give an experimental evidence to suggest the presence of such pumps in the membrane.
7. Explain the following:
   (a) Receptor-mediated endocytosis
   (b) Bulk-transport mechanisms
   (c) Properties of hydrophobic tails of lipids.

Chapter—10

1. Explain Q_{10} law.
2. Discuss the mechanism of heat regulation in terrestrial poikilotherms.
3. Explain the specialised thermoregulatory devices developed by homeotherms to maintain body temperature constant, but independent of ambient temperature.
4. What is role of hypothalamus in endotherms to regulate body temperature?
5. Explain thermoregulatory devices acquired by endotherms while living in extremes of temperatures.
6. Explain the following:
   (a) Adaptations of endotherms to high temperatures
   (b) Adaptation of endotherms to cold environments
   (c) Acclimatization.

Chapter—11

1. Describe lymph and its functions.
2. Blood is a liquid connective tissue. What are its functions?
3. Write briefly about composition of the blood.
4. Differentiate between plasma and serum. Give an account of plasma proteins and their functions.
5. Describe various types of leukocytes and give their origin.
6. How can you determine A-B-O blood groups? Give the principle of blood transfusion.
7. What happens when an Rh negative woman concieves an Rh positive foetus? How can Rh negative individuals be immunized against Rh factor?
8. Describe different types of formed elements of the blood. Give their origin and functions.
9. Explain the clotting mechanism of blood and discuss the role of clotting factors.

10. Explain the following:
    (a) Break down of haemoglobin
    (b) Role of thrombocytes in clotting
    (c) Anticoagulants
11. What is haematocrit? How can you determine the same?
12. Differentiate between the following pairs of terms:
    (a) Neutropenia and lymphopenia
    (b) Leucocytosis and lymphocytosis
    (c) Haemolysis and erythropoiesis

Chapter—12

1. A patient lost good deal of blood due to haemorrhage. Explain how the body will restore the blood volume?
2. Describe different types of hearts in invertebrates.
3. What are the physiological properties of cardiac muscles?
4. Describe the conducting system of the mammalian heart.
5. Discuss the origin and propagation of beats in a mammalian heart.
6. Although mammalian heart possesses automatic rhythmicity and is governed by self-regulatory mechanism, yet the heart beats are regulated by certain nerve centres. Explain as best as you can.
7. Give an account of the modification of cardiac function when the heart is subjected to certain chemicals and drugs.
8. Define blood pressure and describe the factors that bring about its regulation.
9. Distinguish between the following pairs of terms:
    (a) Tachycardia and bradycardia
    (b) atrioventricular block and auricular fibrillation.

Chapter—13

1. Describe respiratory pigments found in the blood of animals.
2. The oxygen carrying capacity of haemoglobin is governed by certain gas laws. Describe them.
3. What are the factors that influence dissociation of oxygen from haemoglobin?
4. Describe the mechanisms that transport CO_2 in the blood.
5. Maximum transport capacity of CO_2 resides in the red blood cells. Explain how it is accomplished?
6. What are self-buffering mechanisms operating in the blood? Briefly explain.
7. Explain the mechanism of binding of haemoglobin with O_2. What is Bohr effect?
8. Describe the mechanism of neural control of respiration in mammals.
9. What happens when?
    (a) PCO_2 of the blood (H_2CO_3 content) is raised
    (b) a fall in the pH of the blood is noticed

(c) excess of bicarbonates accumulate in the blood without any change in the carbonic acid content.

10. Explain the following:
    (i) Chloride shift (ii) Partial pressure of oxygen
    (iii) acid-base balance (iv) Hering-Breuer reflex.

Chapter—14

1. Describe various nitrogenous wastes that are generated in animals. How are they produced?
2. Can you classify animals on the basis of patterns of excretion?
3. Describe the excretory organs met with in arthropods.
4. What are the principal constituents of urine?
5. Explain the following:
    (a) Glomerular filtration rate (G F R) (b) Diuresis
6. Describe physiology of excretion and urine formation in the mammalian kidney.
7. Discuss the process of ultrafiltration at the glomerulus.
8. How do kidneys regulate large volumes of water and acid-base balance?
9. Kidneys are described as homeostatic organs. Explain what control mechanisms operate to maintain constancy and composition of the volume of extracellular fluids in the body?

Chapter—15

1. Describe the ionic basis of resting potential.
2. Define action potential and show how is it generated?
3. Give the structure of a myelinated nerve fibre.
4. What is a nerve impulse and how is it propagated within a nerve fibre?
5. How does the pattern of conduction vary in a myelinated and an unmyelinated nerve fibre?
6. What is a synapse? Distinguish between excitatory and inhibitory synapses.
7. Describe the mechanism of synaptic transmission.
8. How can you explain the phenomenon that a nerve impulse is a self-propagating wave of depolarisation followed by repolarisation?
9. What is chronaxie? How is the strength of a stimulus to excite a nerve determined?
10. Explain the following:
    (a) Depolarisation (b) Saltatory conduction
    (c) All or none principle (d) Neurotransmitters.

Chapter—16

1. What are the different categories of receptors?
2. Describe olfactory and gustatory receptors of vertebrates.
3. Give the structure of an audioreceptor of mammals and explain the physiology of hearing based on various theories of hearing.
4. Describe the structure of vertebrate eye and explain the physiology of colour vision.

5. Write explanatory notes on the following:
   (a) Thermoreceptors (b) Pressure receptors

Chapter—17

1. Describe various types of synaptic integration.
2. Give general properties of reflexes. Explain how a reflex action is accomplished?
3. Describe various types of reflexes. What special features a conditioned reflex has?
4. Differentiate between myotatic and visceral reflexes.
5. Describe the functions of autonomic nervous system.
6. What are the different subdivisions of the brain? What functions are impaired when the medulla and cerebellum are damaged?
7. In what way the heart functions are influenced?
   (a) when sympathetic nervous system is stimulated,
   (b) when parasympathetic nervous system is stimulated.

Chapter—18

1. Describe different types of muscles and their properties in vertebrates.
2. Give the chemical composition of muscles.
3. An isolated muscle is electrically stimulated to produce a muscle twitch. Describe different phases the muscle has to undergo
4. Explain the following:
   (a) Nuromuscular junctions (b) Refractory period
5. Why should heat be generated when a muscle contracts? In which manner this heat is produced?
6. Write notes on:
   (a) Isotonic and isometric contraction (b) Tetanic contraction
   (c) Muscle fatigue (d) Summation.
7. Discuss the theories of muscle contraction and explain which one you consider the most plausible one?
8. Describe structural features of cardiac muscles and explain the basic differences in their working compared to skeletal muscles.
9. A muscle can undergo contraction both aerobically and anaerobically. What basic features are observed in either case?
10. The ATP store in the muscles is very small and remains unchanged before and after contraction. What are the sources of energy to sustain prolonged contraction?
11. Experimental evidence suggests that a muscle poisoned by iodoacetate is still capable of contracting, indicating that glycolysis is not essential for contraction. Then, what is the alternative source of energy?
12. Describe the chemical changes that are involved during muscle contraction. Explain how energy for contraction is obtained?
13. Explain the following:

(a) Mechano-chemical coupling during contraction
(b) Role of calcium during contraction

14. Describe the chemistry of bioluminescence
15. What is the mechanism of action of chromatophores during colour changes exhibited by animals?

Chapter—19

1. Describe the mechanism of hormone action on the membrane receptors and elaborate on the role of cyclic AMP.
2. Describe the biosynthesis of thyroid hormones and their action on metabolism.
3. What are the hormones secreted by pancreas?
4. Describe parathyroid hormones and discuss their physiological effects on various functions of the body.
5. Discuss the role of adrenal cortical hormones on carbohydrate and fat metabolism.
6. How the secretion of cortical hormones (adrenal cortex) is regulated? What abnormalities arise due to disorders of the adrenal cortex?
7. Give the mechanism of insulin secretion and discuss its effect on carbohydrate metabolism.
8. Explain how the hormones of the adrenal cortex and antidiuretic hormone exert a regulatory control over sodium, potassium and water balance in the body?
9. Discuss physiological functions of the following:
   (a) Prostaglandins
   (b) Mineralocorticoids
   (c) Glucagon.

Chapter—20

1. Describe male reproductive organs of a mammal.
2. What physiological role gonadotropins have in mammals during egg maturation?
3. Describe the effect of pituitary hormones on ovarian development.
4. What is estrous behaviour? Describe estrous cycle and the uterine changes that take place during estrous.
5. Give the development of Graffian follicle and trace the events from ovulation to implantation.
6. Placenta is a complex tissue in mammals. Explain its formation and physiological functions it carries out for the foetus.
7. What endocrine factors are involved in mammary growth and lactation?

Chapter—21

1. What are code triplets? On what reasoning the triplet code is postulated for amino acid specification?
2. How did Nirenberg show that there is redundancy in the genetic code?
3. Give important generalizations about the genetic code.
4. The flow of information from DNA to RNA shows that RNA is also an information system. How this information is utilised in protein synthesis?

5. What are the components of protein synthesis? Explain the activation step and the role of adaptor molecules.
6. Explain:
   (a) In what direction is ribosome translocated with respect to mRNA?
   (b) To which terminal of tRNA does the amino acid attach?
   (c) Which end of the protein is first synthesised? (C-terminal or N-terminal).
7. How do streptomycin and puromycin affect protein synthesis?

Chapter—22

1. Most physiologic disorders emanate from organelle malfunction. List the major organelles that are involved in physiologic disorders.
2. Describe defects due to ion-channels. Give specific examples.
3. Organelle malfunctions originate from some defective gene, hence tend to be hereditory. List at least two malfunctions that are attributed to endoplasmic reticulum abnormalities.
4. What are the disorders that are attributed to peroxisomal disorders?
5. Metabolic disorders are caused by enzyme deficiencies. What are the types of enzyme deficiencies that have been discovered?
6. Explain the following:
   (a) In-born errors of metabolism
   (b) Lysosomal storage diseases
   (c) Diseases due to membrane abnormalities.

Chapter—23

1. How do genes control the metabolic processes? Explain with reference to *Neurospora.*
2. The gene-enzyme relationship has been operating in organisms affecting metabolic pathways. Explain this concept with reference to the ornithine cycle of *Neurospora.*
3. Explain the mechanism of enzyme induction and repression.
4. Give the experimental proof that nucleus exerts control over cytoplasmic functions.
5. What is the contribution of Beadle and Tatum? What led them to formulate their famous one-gene-one-enzyme hypothesis?
6. Describe the negative feedback mechanism operative in the control of β-galactosidase in *E. coli.*
7. How are the eye pigments genetically controlled? Explain with reference to eye pigments in insects.
8. Certain genetic defects in human population are reflected in the development of inborn errors. Explain with the help of defective phenylalanine metabolism in man.
9. Write explanatory notes on:
   (a) Sickle-cell anaemia
   (b) Creeper mutation
   (c) Pleiotropic effects of genes

Chapter—24

1. Define immunity, autoimmunity and allergy.
2. What is the chemical nature of antibodies? Classify immunoglobulins and give their biological properties.
3. Discuss the origin of lymphocytes.
4. How lymphocytes generate immune responses?
5. Describe the role of thymus in immune functions.
6. What is clonal selection theory? Give the sequence of events that lead to differentiation of immunocompetent plasma cells.
7. What is the physiologic role of thymic hormones?
8. Give the mechanism of antibody synthesis. How are primary and secondary responses generated?
9. How is transplantation immunity generated?
10. Describe various types of grafts. What are the causes of graft rejection and how can it be prevented?
11. How the cells display antigen-antibody reaction?
12. Write about the following:
    (a) Macrophages
    (b) Haptens
    (c) Dendritic follicle cells
    (d) Immunosuppression
    (e) Origin of T and B cells.

Chapter—25

1. What reasons can you attribute to cells having a finite limit of population doublings?
2. Discuss various theories of aging.
3. Discuss age-related changes in the connective tissues. Can it be controlled?
4. What impact somatic mutation theory has on cell aging?
5. Is aging programmed? Give your comments
6. Write about the following:
    (a) Hayflick limit
    (b) Mitochondrial theory of aging
    (c) Orgel's theory of 'Error catastrophe'
    (d) Mental aspects of aging.

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Index