BIOCHEMISTRY

HIGHER SECONDARY - FIRST YEAR

Untouchability is a sin

Untouchability is a crime

Untouchability is inhuman

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INTRODUCTION

Scope of Biochemistry

Biochemistry is a branch of life science which deals with the study of chemical reactions that occur in living cells and organisms. Life is a chemical process involving thousands of different reactions occurring in an organised manner. These are called metabolic reactions.

The term Biochemistry was first introduced by the German Chemist Carl Neuberg in 1903. It takes into account the studies related to the nature of the chemical constituents of living matter, their transformations in biological systems and the energy changes associated with these transformations. Biochemistry may thus be treated as a discipline in which biological phenomena are analysed in terms of chemistry. The branch of Biochemistry for the same reason, has been variously named as Biological Chemistry or Chemical Biology.

Modern Biochemistry has two branches, descriptive Biochemistry and dynamic Biochemistry. Descriptive Biochemistry deals with the qualitative and quantitative characterization of the various cell components and the dynamic Biochemistry deals with the elucidation of the nature and the mechanism of the reactions involving these cell components.

Many newer disciplines have been emerged from Biochemistry such of Enzymology (study of enzymes), Endocrinology (study of hormones) Clinical Biochemistry (study of diseases), Molecular Biochemistry (Study of Biomolecules and their functions). Along with these branches certain other specialities have also come up such as Agricultural Biochemistry, Pharmacological Biochemistry etc.

Those who acquire a sound knowledge of Biochemistry can tackle the 2 central concerns of the biomedical sciences (1) the understanding and maintenance of health (2) the understanding and treatment of diseases.

Objectives of Biochemistry

The major objective of Biochemistry is the complete understanding of all the chemical processes associated with living cells at the molecular level. To achieve this objective, biochemists have attempted to isolate numerous molecules (Bio molecules) found in cells, to determine their structures and to analyse how they function. Biochemical studies have illuminated many aspects of disease and the study of certain diseases have opened up new therapeutic approaches. In brief the objectives can be listed as follows:

1. Isolation, structural elucidation and the determination of mode of action of biomolecules.
2. Identification of disease mechanisms.
3. Study of in born errors of metabolism
4. Study of oncogenes in cancer cells
5. The relationship of biochemistry with genetics, physiology, immunology, pharmacology, toxicology etc.

Biochemistry is related to almost all the life sciences and without biochemistry background and knowledge, a thorough understanding of health and well-being is not possible.

CHAPTER 1
LIVING CELL

1.1 Introduction

The cell is the basic structural and functional unit in all living organisms. Living forms vary in size but they are all made up of cells. In unicellular organisms, the cell is the organ. As an organism grow in complexity, the cells increase in number and similar cells aggregate into tissues and organs with specialized functions. The shape of the cell is often determined by its function and the size of the cell is determined by the optimum dimensions that will enable it to perform its duties more effectively.

The human body is composed of a multiple of specialized tissues which inturn consist of vast clusters of cells differentiated into specialised chemical factories which perform various biochemical reactions.

1.1.1 Types of cells

Two general types are recognised in nature. They are prokaryotic and eukaryotic cells.

1.1.1.1 *Prokaryotic cells*

Prokaryotes were the first cells to arise in biological evolution. They are very small and simple having only a single membrane. The cell membrane, is usually surrounded by a rigid cell wall.

They are devoid of nucleus and membranous organelles such as mitochondria and endoplasmic reticulum etc. (Fig. 1.1).

1.1.1.2 *Eukaryotic cells*

Eukaryotic cells are presumably derived from prokaryotes. They are much larger and much more complex than prokaryotic cells (Fig. 1.2).

They have nucleus and membrane bound subcellular organelles. Many of their metabolic reactions are segregated within structural compartments. The significant differences between prokaryotic and enkaryotic cells are:

	Eukaryotes	Prokaryotes
1.	Have nucleus with nuclear membrane	Do not have nucleus
2.	Have membrane bound subcellular organelles such as mitochondria, endoplasmic reticulum and golgi complex	Do not have organelles
3.	Have large ribosomes	Have small ribosomes
4.	Have many chromosomes which undergo mitosis and meiosis during cell division	Have a single chromosome, which consists of a single molecule of double helical DNA
5.	Reproduce by sexual division	Reproduce by asexual division
6.	They are present in fungi, protozoa, most algae, higher plants and animals	They are found in eubacteria, the blue green algae, the spirochetes and the mycoplasma

The eukaryotic cell structure is composed of (i) cell membrane, (ii) nucleus (iii) mitochondria (iv) endoplasmic reticulum (v) golgi apparatus (vi) ribosomes (vii) lysosomes and others. These specialised structural units are called as subcellular organnelles. Biochemistry today is increasingly concerned with the structure of cells and their organelles.

Fig. 1.2 The structural organisation of eukaryotic cells

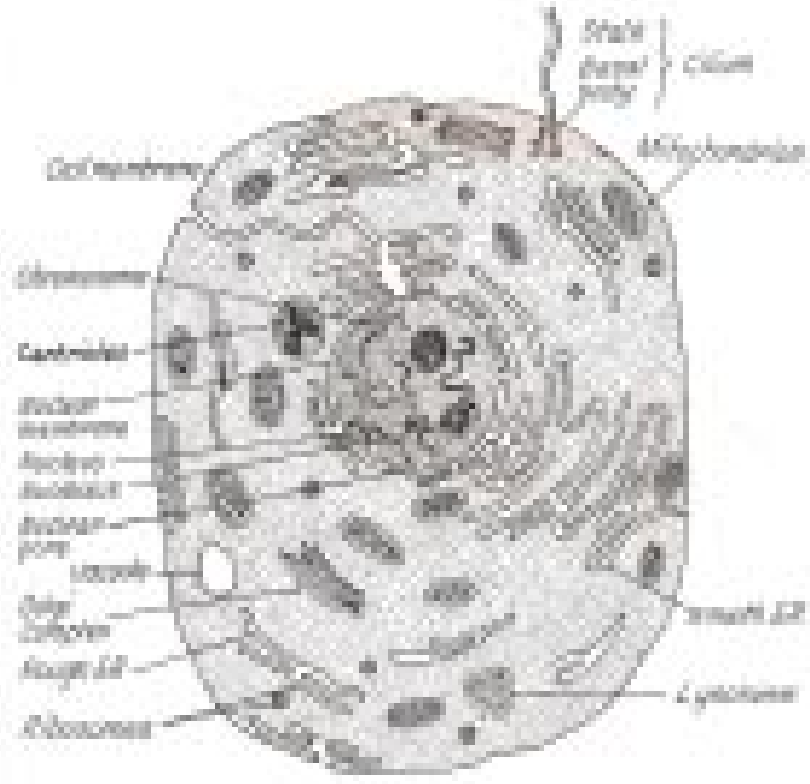
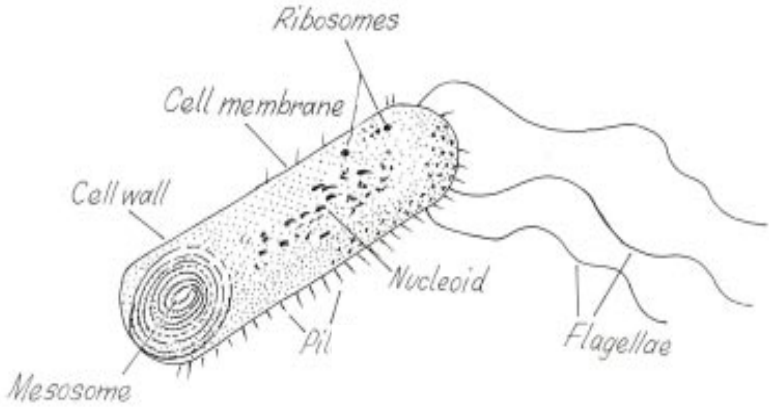


Fig. 1.1 The structural organisation of prokaryotic cells



1.2 Sub cellular Organelles

1.2.1 Cell Membrane

The cell is enveloped and thus separated from its surroundings by a thin wall contains a rigid framework of polysaccharide chains crosslinked with short peptide chains. Its outer surface is coated with lipopolysaccharide. Cell membrane is also called as plasma membrane (or) plasma lemma. The pili, not found in all bacteria have extensions of the cell wall. The cell membrane contains about 45% lipid and 55% protein. The cell membrane or plasma membrane have an average thickness of 75 A°. The principal lipids are phospholipids, sphingolipids and cholesterol. An important feature of these lipids is they are composed of hydrophobic (water - insoluble) hydrocarbon sections and hydrophilic (water soluble) units. The latter include charged units (eg. phosphate or amino groups) and uncharged units (eg. hexose). In water, such compounds orient themselves in such a way that only the hydrophilic section is exposed to water. The hydrophobic components of individual molecules tend to contact with other; this is accomplished either by arrangement into micelles or by the formation of bilayers.

Two types of poteins are found in cell membrane; viz. intrinsic or integral and extrinsic or peripheral (Fig. 1.3).Integral proteins are either partially or totally immersed in the lipid bilayer and difficult to remove by any means other than the distruption of the membrane with a detergent. Peripheral proteins are bound only to the surface of membrane and interact only with the hydrophilic groups and therfore are readily removed by extraction with an aqueous medium. The arrangement of lipids and proteins in the memberane is known as fluid mosaic model.

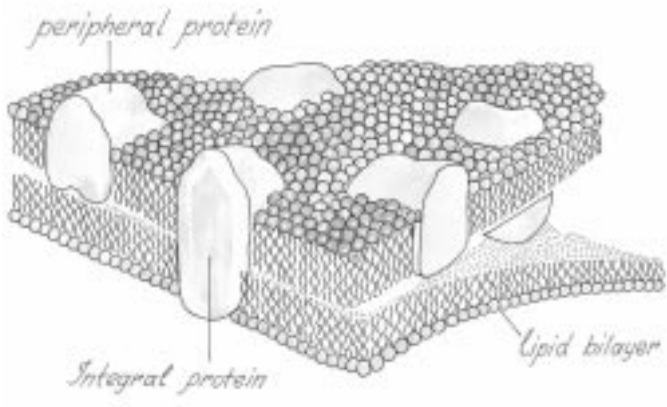


Fig. 1.3 Structural organisation of a cell membrane (Fluid mosaic model

Functions of cell membrane

The cell membrane performs a number of important functions

(i) It holds the cell together

(ii) The membrane is a selectively permeable boundary which allows water, certain required nutrients and metal ions to pass freely

(ii) It secrete waste products

(vi) It keeps out toxic materials

(v) It contain receptors to bind certain regulatory substances such as hormones which regulate the various metabolic activities.

1.2.3 Cell Wall

Plant and bacterial cell membranes are surrounded by a thick cell wall.

1.2.3.1 *Bacterial cell wall*

The bacterial cell is enclosed within a wall that differs chemically from the cell wall of plants. The cell wall contains a rigid framework of polysaccharide chain cross linked with short peptide chains and its outer surface is coated with lipopolysaccharide. The pili, found in some bacteria are extensions of the cell wall. In some bacteria the cell wall is surrouned by an additional structure called a capsule.

The cell wall and capsule confer shape and form of the bacteriam and also act as a physical barrier to the cell membrane. In the absence of cell wall and capsule is mechanically fragile and the bacteria would rupture.

1.2.3.2 *Plant cell wall*

The cell wall is a thick polysaccharide containing structure immediately surrounding the plasma membrane. In multicelllar plants, the plasma membrane of neighboring cells are separated by these walls, and adjacent plant cell have their walls fused together by a layer called the *middle lamella*. The cell wall serves both as a protective and a supportive unit for the plant. The degree to which the cell wall may be involved in the regulation of the exchange of materials between the plant cell and its surroundings is difficult to assess but is most likely restricted to macromolecules of considerable size. As in animal cells,most of the regulation of exchanges between the cytoplasm and the extracellar surrounding of plant cells is a function of the plasma membrane.

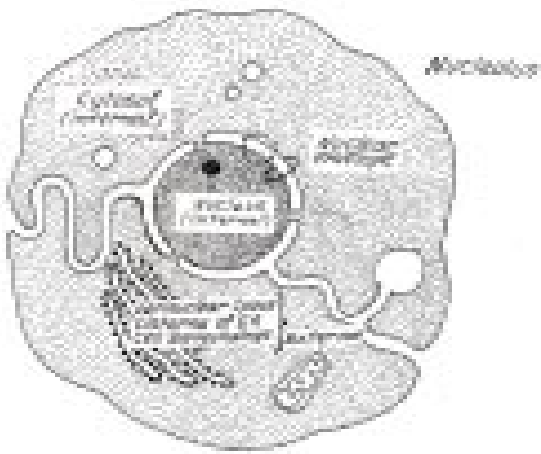
Functions

The cell wall protects bateria against swelling in hypotonic media. It is porous and allows most small molecules to pass. Some of the pili are hollow and serve to transfer DNA from sexual conjugation.

1.2.4 Nucleus

Nucleus is the heaviest particulate component of the cell. Except matured mammalian erythrocytes, nucleus is found in almost all cells. The nucleus about 4-6μm in diameter is surrounded by a perinuclear envelope. At various position the outer membrane of the envelope fuses with the inner membrane to form pores. Nuclear pores provide continuity between the cytosol and the contents of the nucleus (nucleoplasm). The electron microscope reveals that the nuclear content consist of granular or fibrillar structures. The nucleolus, a discrete body within the nucleus, contains ribonucleic acids (RNA). The most important component of the nucleus is an organised clumps of threadwork known as chromatin which is distributed throughout the nucleus and contains most of the cellular deoxy ribonucleic acids (DNA). Immediately before the cell division the chromatin organises into simple thread like structures known as chromosomes which will eventually be distributed equally to each daughter cell.

Fig. 1.4 A schemmatic diagram of Nucleus



Functions

1. Take part in cell division
2. Contain DNA molecules which are heriditary carriers.

1.2.5 Mitochondria

These are the largest particulate components of the cytoplasm and represent upto 15% -20% of the dry weight of the cell. They vary in shape (spherical, filamentous, sausage shaped) and size (0.5 to 3μ long 0.1 to 0.6μ wide).The number varies with the size and energy requirements of the cell. For eg. flight muscles in birds contain rich amount of mitochondria when compared to any other parts of the body.

Electron microscopic studies show that a mitochondrion has two membranes inner and outer which are separated from each other by 50 to 100°A. The outer and inner membranes differ in lipid composition and in enzyme content.

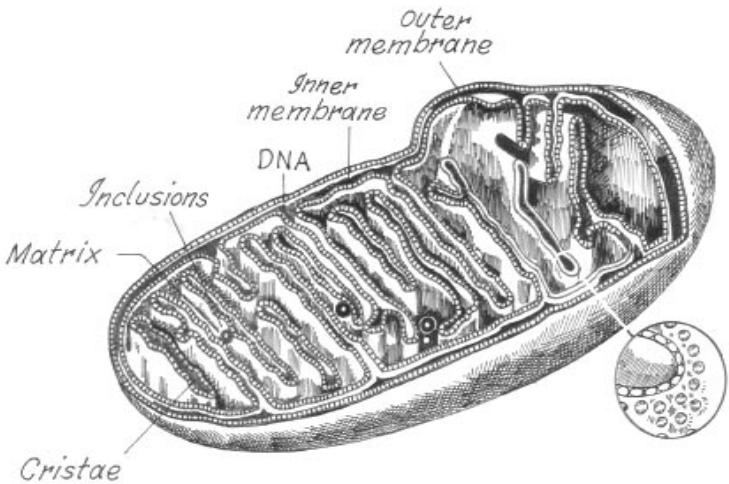


Fig. 1.5 A schemmatic diagram of mitochondria

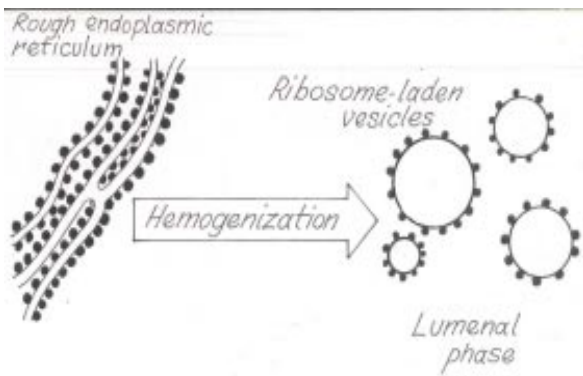
The inner membrane is very much folded to form shelf - like structures of varying width. These shelf - like structures, known as internal ridgs or cristae, extent into matrix of the mitochondrion structure. Thus two structurally different space can be distinguished, the intracristae space and the matrix space (Fig. 1.5). The matrix space is rich in enzymes. The inner membrane shows the existance of knob like structures, which are proteins involved in biological oxidations.

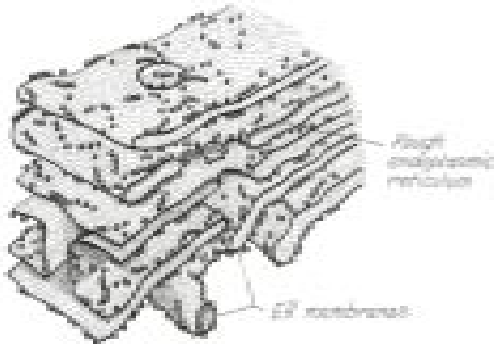
Functions : The mitochondria are the 'power houses' of the cell, where carbohydrates, lipids and amino acids are oxidised to CO_2 and H_2O by molecular oxygen, and the energy set free is stored in the form of adenosine triphosphate (ATP). The enzymes involved in this energy conversion are located in the inner membrane.

1.2.6 Endoplasmic reticulum

The endoplasmic reticulum consist of flattened single membrane vesicles. These have the same lipid bilayer structure but thinner than the cell membrane (about 7mm). The endoplasmic reticulum is of two types; rough (RER) and smooth (SER). Only the rough type has small granules known as ribosomes (Fig. 1.6).

Fig. 1.6 A schemmatic diagram of endoplasmic reticulum





Functions

RER is concerned with protein synthesis while SER is concerned with lipids and glycoprotein synthesis. The cisternae (enclosed spaces) of the endoplasmic reticulum play a role in the exchange of material between the cell and the extra cellular fluid. The exchange of material takes place by the process of pinocytosis.

1.2.7 Golgi apparatus

Golgi complex is a smooth membrane system consists of flattened, single membrane vesicles which are often stacked (Fig. 1.7).

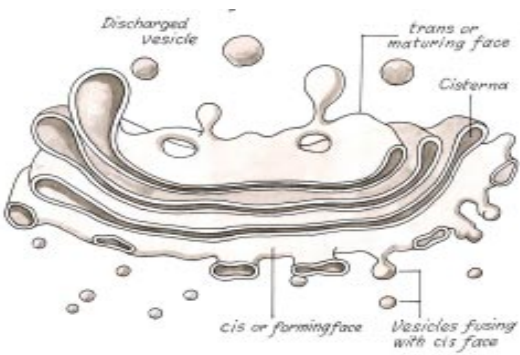


Fig. 1.7 A schemmatic diagram of golgi apparatus

Functions

These are organelles to which the newly synthesized proteins are transferred and temporarily stored.

Small vesicles arise peripherally by a pinching - off process. Some become vacuoles in which secretory products are concentrated.

1.2.8 Ribosomes

The outer membrane of the endoplasmic reticulum contain small granules commonly known as ribosomes, which are the smallest particulate components of the cytoplasm. They are rich in ribonucleic acids. Each ribosome has a large and a small subunit with a sedimentation constant of 50s and 30s respectively (Fig. 1.8). Each subunit contains about 65% RNA and 35% protein.

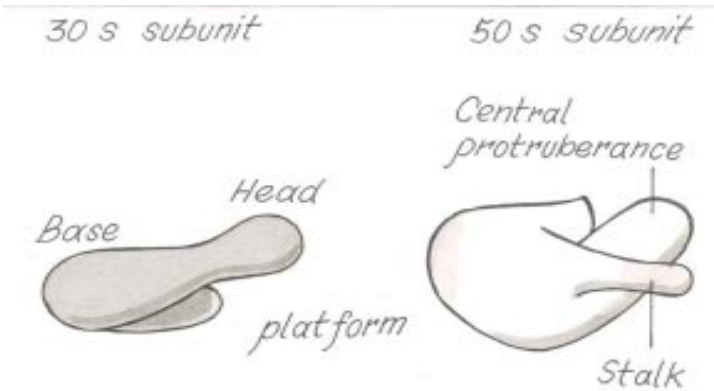


Fig. 1.8 A schemmatic diagram of Ribosomes

Functions

Ribosomes are the sites of protein synthesis. Messenger RNA binds in the groove between the subunits and specifies the sequence of amino acids in the growing polypeptide chains. The proteins synthesized on membrane bound ribosomes must pass successively through each of cytomembrane system.

Secretion may involve the fusion of the vacuoles with the plasma membrane followed by a discharge of the contents into the extra cellular space. This process is called exocytosis.

1.2.9 Lysosomes

Lysosomes are single membrane vesicles, having intermediate size between microsomes and mitochondria. These are surrounded by a lipoprotein membrane (Fig.1.9). Lysosomes are rich in many hydrolytic enzymes such as phosphatase and ribonuclease and because of this, they are named as lysosomes (Lyso means lytic action).

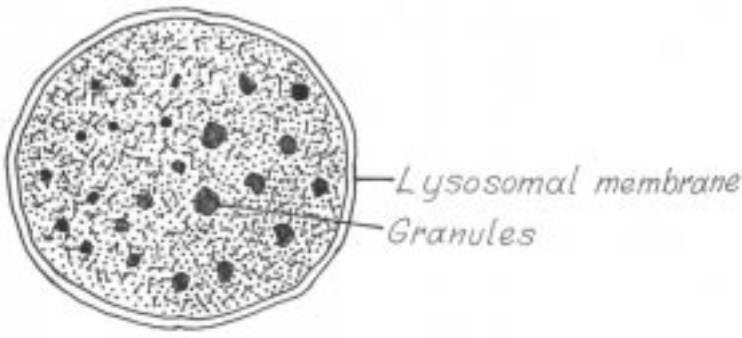


Fig. 1.9 A schemmatic diagram of lysosome

Functions

The hydrolytic enzymes of lysosome completely destroy the foreign materials like bacteria. They also serve to digest cell components after cell death. Inside the macrophages these lysosomes combine with vecuole which has engulfed the foreign particles and form phagolysosomes. Inside these phagolysosomes foreign particles are degraded or killed. The pathogen engulfed lysosomes are destroyed by the reticulo endothelial system. Due to this action lysosomes are called as 'Suicidal Bags'.

1.2.10 Peroxisomes

Peroxisomes are otherwise known as microbodies. They are single - membrane vesicles of about 0.5 mm in diameter. They contain catalase, D-amino acid oxidase, urate oxidase and other oxidative enzymes.

Functions

Microbodies participate in the oxidation of certain nutrients. Hydrogen peroxide, the toxic reduction product of oxygen is decomposed to form water in these organelles.

1.2.11 Cytoplasm

The intracellular cell content that posses both soluble and insoluble constituents is called cytoplasm.

Cytosol

The soluble liquid portion of the cytoplasm is known as cytosol in which the organelles are bathed. Cytosol is also known as cell sap. Cell sap contains water, proteins, lipids and numerous other solutes and is highly viscous (Fig.1.10).

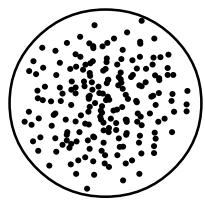


Fig. 1.10 Cytoplasm

Functions

Some important metabolic processes occur in the cytosol are glycolysis, gluconeogenesis, activation of amino acids and biosynthesis of fatty acids.

Plant Cells

Plant cells have cell wall made up of cellulose and the cytoplasm consists of big vacuoles and chloroplasts (Fig.1.11).

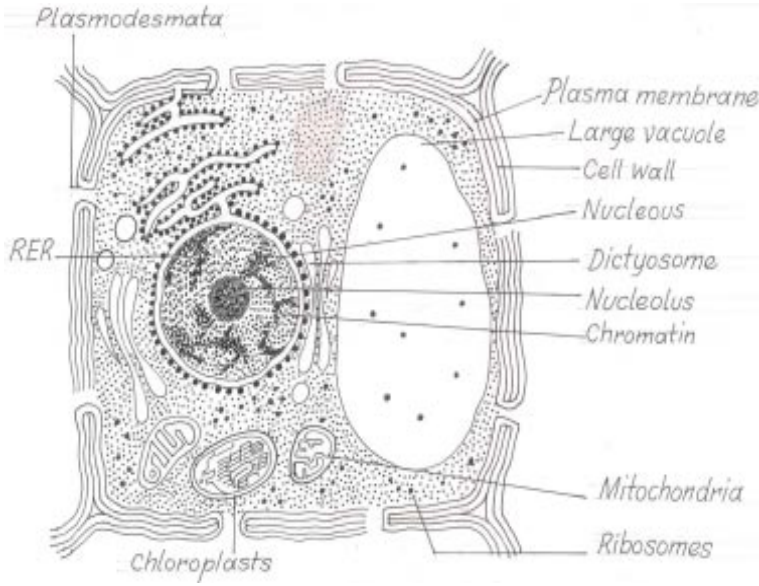


Fig.1.11 Structural organisation of a plant cell

1.2.12 Chloroplasts

The ability to use light as a source of energy for sugar synthesis from water and carbon dioxide is a special feature of certain plant cells. This process, termed photosynthesis is carried out in organelles called chloroplasts. These organelles are commonly ellipsoidal structures bounded by an outer membrane but also containing a number of internal membranes. Internally, the chloroplast consists of a series of membranes arragned in parallel sheets called lamellae and supported in a homogeneous matrix called the stroma. The membranes are arranged as thin sacs called thylakoids that contain chlorophyll and may be stacked on top of one another, forming structures called grana. Lamellar membranes connecting the grana are called stroma lamellae (Fig.1.12).

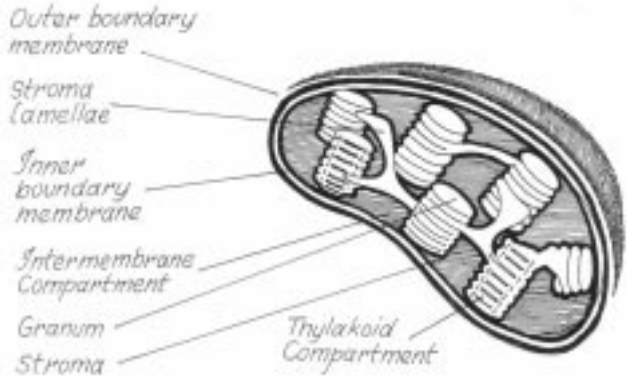


Fig. 1.12 A schemmatic diagram of chloroplast

1.2.13 Vacuoles

Although vacuoles are present in both animal and plant cells, they are particularly large and abundant in plant cells, often occupying a major portion of the cell volume and forcing the remaining intracellular structures into a thin peripheral layer. These vacuoles are bound by a single membrane and are formed by the coalescence of smaller vacuoles during the plant's growth and development. Vacuoles serve to expand the plant cell without diluting its cytoplasm and also function as sites for the storage of water and cell products or metabolic intermediates.

1.3 Blood and body fluids

1.3.1 Blood

Blood is a fluid which circulates in a closed system of blood vessels, in multicellular and highly complex vertebrate animals. Blood consists of a pale yellow fluid called plasma and solid elements such as red blood cells (erythrocytes) while blood cells (leucocytes) and platelets (thrombocytes).

1.3.1.1 *Composition*

Blood contains 55% of the plasma and 45% of the cellular fraction. The water constitutes about 80% of blood by weight. The cellular fractions composed of erythrocytes (5×10^6 cells/µl) leucocytes (4.5-11 $\times 10^3$ cells/µl) and thrombocytes (3×10^5 cells/µl). The plasma contains a large number of organic and inorganic substances in solution.

These subtances may be diffusible substances including various electrolytes, anabolic and catabolic substances formed during metabolism, vitamins and certain hormones. The non-diffusible constituents of plasma are albumin, globulin, fibrinogen, enzymes, lipids etc.

Functions

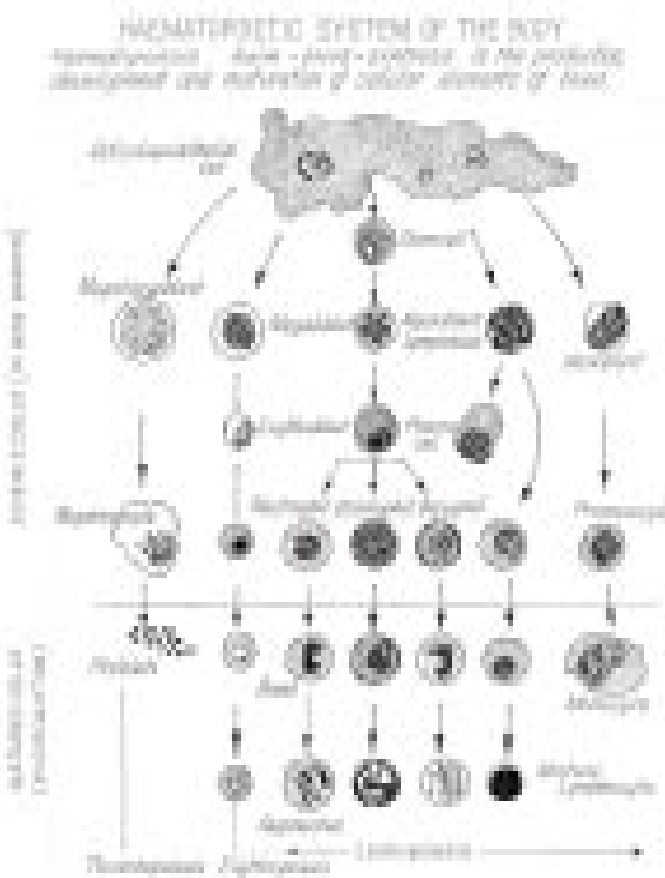
Blood perform the following functions:

1. Blood transport oxygen from lungs to the tissues and carbondioxide from the tissues to the lungs. Thus it is responsible for the important process of respiration.
2. It transports absorbed dietary nutrients from the digestive tract to all the body tissues.
3. It transports metabolic wastes to the kidneys, lungs, skin and intestine for removal.
4. It transports various hormones and minerals.
5. It maintains normal acid - base balance in the body.
6. It regulates water balance.
7. It regulates body temperature by the distribution of body heat.
8. White blood cells and antibodies in blood provide, defense against various type of infections.

1.3.2 Formation of blood

Blood biosynthesis is known as haematopoiesis. The process consist of production, development and maturation of cellular elements of blood (Fig. 1.13). In adults the production of blood cells is carried out by the bone marrow while in the developing fetus haematopoisis takes place in extra medullary regions till the bone marrow is matured and functional.

Fig. 1.13 A Schemmatic representation of haematopoiesis



1.4 Body Fluids

Cerebrospinal fluid and lymph are other important body fluids which are devoid of red blood cells and so they look colourless.

1.4.1 Cerebrospinal fluid (CSF)

CSF is formed by the choroid plexus of the brain and is an ultrafiltrate of the plasma. It is present in the central ventricles, spinal canal and subarachnoid spaces. The total volume of CSF is 100-150ml.

1.4.1.1 *Properties*

It is a clear, colourless and transparent fluid with a specific gravity of 1.003 to 1.008. It is alkaline and has the same pH as that of blood (7.35 to 7.40).

1.4.1.2 *Composition*

It's composition is more or less similar to that of plasma without the colloids.

Protein	-	20-45 mg /dl
glucose	-	40-80 mg /dl
Chloride	-	700-750 mg /dl
Non-protein Substances like urea and uricacid etc.	-	Urea - 20 - 40 mg/dl
		Uricacid -1 - 7 mg/dl
		Sodium - 300 - 350 mg/dl
		Potassium - 11-15 mg/dl
		Calcium - 4 - 5 mg /dl
		Phosphorous - 1 - 1.5 mg/dl

1.4.1.3 *Functions*

It acts as a protective jacket for the brain and spinal cord and maintains a uniform pressure on the nervous structures. It acts as a reservoir to regulate the contents of the cranium. To a limited extent it act as a medium for nutrient exchange in the nervous system.

1.4.1.4 *CSF in diseases*

The composition of CSF is altered in the following diseased conditions in human beings.

1. Meningitis
2. Neuro syphilis
3. Disseminated sclerosis
4. Encephalitis
5. Polio myelitis
6. Spinal and brain tumor
7. Cerebral haemorrhage

1.4.2 Lymph

The term 'lymph' denotes a fluid not only present in the lymphatic vessels but also the fluid which bathes in the cells, the tissue or interstitial fluid.

It resembles plasma in its components and their composition which can permeate the capillary wall but there are some differences in electrolyte concentrations.

About 2 litres of lymph are drained into the blood stream per day. Lymph flow is very slow. The protein present in entire plasma in an adult human is about 210 gm. About 1/3rd of this leaks out into the lymph through the interstitial fluid and is returned to the blood at the thoracic duct. If the thoracic lymph is drained off, the concentration of plasma proteins fall and the blood volume decreases.

1.4.2.1 *Functions*

Lymph is the main route of absorption of long chain fatty acids, partially digested fats, diacyl glycerol and cholesterol from the intestine

and their transport via the thoracic duct. Lymph also helps to keep the tissues from drying up by maintaining contact with the interstitial fluids.

1.5 Buffers

A buffer is a mixture of a weak acid and its salt with a strong base (eg) A mixture of acetic acid and sodium acetate.

$$HAC + NaAC \text{ -----> } Na^+ + H^+ + 2AC^-$$

where HAC = Acetic acid; NaAC = Sodium acetate.

A buffer solution is one which resists a change in its pH value (hydrogen ion concentration) on dilution or on addition of an acid or alkali. The process by which added H^+ and OH^- ions are removed so that pH remains constant is known as buffer action.

For (eg) if alkali is added to the above mentioned buffer it forms NaAC and no free H^+ or OH^- will be available.

$$[Na^+ + H^+ + 2AC^-] + NaOH \text{ ------> } 2NaAC + H_2O$$

If an acid is added to the buffer it will form NaCl and no free H^+ or OH^- will be available.

$$HAC + NaAC + HCl \text{ --------> } NaCl + 2\ HAC$$

In either cases there is no change in hydrogen ion concentration i.e. it resist the change in pH of the solution.

1.5.1 Examples of buffer - A mixture of

1. Glycine and HCl
2. Potassium dihydrogen phosphate and dipotassium hydrogen phosphate.
3. Sodium bicarbonate and sodium carbonate.

1.5.2 Uses of buffer

1. Bufers are used for preparing standard solutions in which it is always desired to maintain a constant pH.
2. These are used to maintain H^+ concentration which is necessary for optimal activity of enzymes.
3. Buffers regulate acid-base balance by restricting pH change in body fluids and tissues, since they are capable of absorbing H^+ ions and OH^- ions when an acid or an alkali is formed during metabolic activities.

1.5.3 Buffers of blood

The important buffers present in blood are

a. Bicarbonate buffer

b. Phosphate buffer

c. Protein buffer

d. Hemoglobin buffer

1.5.3.1 *Bicarbonate buffer*

It is the most important buffer in blood plasma and consist of bicarbonate [HCO_3^-] and carbonic acid [H_2CO_3]

This buffer is efficient in maintaining the pH of blood plasma to 7.4 against the acids produced in tissue metabolism (eg) phosphoric acid, lactic acid, aceto acetic acid and β-hydroxy butyric acid. These acids are converted to their anions and the bicarbonate is converted to carbonic acid a weak acid.

HCO_3^- + H^+ (obtained from acids) -----> H_2CO_3 ----->CO_2

CO_2 thus formed is expirated through lungs during respiration.

1.5.3.2 *Phosphate buffer*

The phosphate buffer consists of dibasic phosphate [HPO_4^{2-}] and monobasic phosphate ($H_2PO_4^-$). Its pKa value is about 6.8. It is more effective in the pH range 5.8 to 7.8. Plasma has a ratio of 4 between [HPO_4^{--}] **:** [$H_2PO_4^-$].

$$\text{Therefore pH} = \text{pKa} + \log \frac{[HPO_4^{--}]}{[H_2PO_4^-]}$$

pH = 6.8 + log 4 = 7.4 [7.4 is the normal pH of blood]

1.5.3.3 *Protein buffer*

The protein buffers are very important in the plasma and in the intracellular fluids but their concentration is very low in CSF, lymph and interstitial fluids.

They exist as anions serving as conjugate bases (Pr^-) at the blood pH 7.4 and form conjugate acids (HPr) accepting H^+. They have the capacity to buffer some H_2CO_3 in the blood.

$$H_2CO_3 + Pr^- \longrightarrow HCO_3^- + HPr \longrightarrow H_2CO_3 \longrightarrow CO_2\uparrow$$

1.5.3.4 *Hemoglobin buffer*

They are involved in buffering CO_2 inside erythrocytes. The buffering capacity of hemoglobin depends on its oxygenation and deoxygenation. Inside the erythrocytes, CO_2 combines with H_2O to form H_2CO_3 under the action of carbonic anhydrase. At the blood pH 7.4, H_2CO_3 dissociates into H^+ and HCO_3^- and needs immediate buffering. Oxyhemoglobin (HbO_2^-) on the other side loses O_2 to form deoxyhemoglobin (Hb^-) which remains undissociated (HHb) by accepting H^+ from the ionization of H_2CO_3. Thus, Hb^- buffers H_2CO_3 in erythrocytes.

$$HbO_2^- \longrightarrow Hb^- + O_2$$

$$Hb^- + H_2CO_3 \longrightarrow HHb + HCO_3^-$$

1.6 Acids and bases

According to the modern concept, an acid is defined as that species which can donate H^+ ions (protons) in solution and a base is that species which can accept H^+ ions. Since such transfer of protons is reversible any acid which gives up its proton becomes a base, while any base which accepts a proton becomes an acid. This theory was postulated by Bronsted and Lowry in 1923. The following general equation canbe written as

$$\text{Acid} \rightleftharpoons H^+ + \text{Base}$$

An acid and a base related in this manner are called conjugates

For (eg) in this reaction

$$HCl \rightleftharpoons H^+ + Cl^-$$

HCl is acid and Cl^- is its conjugate base.

Other examples

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$
$$H_2SO_4 \rightleftharpoons H^+ + HSO_4^{2-}$$

An acid which dissociates strongly and readily gives H^+ ions is known as a strong acid. The capacity of an acid to release its protons is known as acidity.

A base which has more affinity to combine with H^+ ions is known as a strong base. This property of a base is known as alkalinity (eg) HCO_3^-, HSO_4^{2-}, $H_2PO_4^-$ etc.

The acidity of a species is denoted by its pH value : larger the acidity of a species, lower will be its pH value.

1.6.1 pH - It is defined as the negative logarithm of hydrogen ion concentration (or) it is defined as the logarithm of reciprocal of the hydrogen ion concentration.

i.e. $pH = -\log [H^+]$ (or) $pH = \log 1/[H^+]$

Acidic - 1 - 6.9 Neutral 7.0 Alkaline 7.1 - 14.

1.6.1.1 *Determination of pH -Henderson - Hasselbalch equation*

Derivation

This concerns the dissociation of weak acid in equilibrium.

Let us consider HA a weak acid that ionises as follows:

$$HA \rightleftharpoons [H^+] + [A^-]$$

$$\text{ঙ} \quad K_a = \frac{[H^+]\,[A^-]}{[HA]} \quad \text{[where Ka = Dissociation constant]}$$

$$\text{ঙ} \quad H^+ = \frac{Ka \times [HA]}{[A^-]}$$

$$\log [H^+] = \log Ka + \log \frac{[HA]}{[A^-]} \quad \text{[Taking log on both sides].}$$

$$-\log [H^+] = -\log Ka - \log \frac{[HA]}{[A^-]} \quad \text{[Chaning sign on both sides].}$$

$pH = pKa + \log [HA] / [A^-]$ since $-\log H^+ = pH$ and $-\log Ka = pKa$

$$\mathbf{pH = pKa + \log \frac{[Salt]}{[Acid]}}$$

The above equation is known as Henderson - Hasselbalch equation. and can be used for the determination of pH of blood.

1.6.1.2 *Determination of pH of buffers*

The pH of buffers can be determined by Henderson - Hasselbalch equation.

pH = pKa + log [salt] / [Acid]

where pKa = log 1/Ka Ka = Dissociation Constant of an acid and

$$Ka = \frac{[H^+]\ [Ac^-]}{[HAC]}$$ for the reaction

$$HAC \rightleftharpoons [H^+] + [Ac^-]$$

$$\therefore H^+ = \frac{K_a \times [HA]}{[A^-]}$$

In case of blood, the ratio between $[BHCO_3] : [H_2CO_3]$ can be found out by applying the above equation to maintain average pH of blood 7.4. If the pKa value of H_2CO_3 is 6.1.
then

$$7.4 = 6.1 + \log \frac{[BHCO_3]}{[H_2CO_3]}$$

$$\therefore \log \frac{[BHCO_3]}{[H_2CO_3]} = 1.3$$

$$\therefore \log \frac{[BHCO_3]}{[H_2CO_3]} = \log 20$$

$$\log \frac{[BHCO_3]}{[H_2CO_3]} = 20/1$$

So $[BHCO_3] : [H_2CO_3] = 20:1$

1.6.1.3 *pH in living organisms*

The fluid in the cells and tissues of plants and animals is usually close to neutrality and the pH of extracellular fluids under normal conditions varies from 7.35 to 7.5.

1.6.1.4 *Regulations of acid-base balance (pH maintenance)*

The following mechanisms control the regulation of acid-base balance in the human body.

1. **Buffer system** : Hemoglobin, phosphate and carbonate - bicarbonate buffers are involved in the maintenance of pH since they are capable of neutralising H^+ or OH^- ions formed during metabolic activities.

2. **Respiratory regulation** : Lungs are actually the most effective organs for pH adjustment : One half of the H^+ ions drained by the cells to the extra cellular fluids combine with HCO_3^- to form H_2CO_3 which dissociates in to H_2O and CO_2. The CO_2 thus formed is expirated through lungs. So accumulation of H^+ ions is prevented.

3. **Renal regulation**

Kidney contribute for acid-base balance by excreting acids and ammonia. The excess of acids produced in the metabolic pathways are eliminated by kidneys in the form of urine. Ammonia is a base and formed in the tubular epithelial cells from glutamine. Glutamine is deaminated and then dehydrogenated to form two moles of ammonia. The ammonia so formed diffuses into the tubular urine and binds H^+ ions to form NH_4^+ and excreted as such in urine. If not excreted, ammonia accumulation in blood leads to acid-base imbalance.

If acids and ammonia accumulate in the blood, it lead to acidosis and alkalosis respectively.

Excercise

I. Choose the correct answer from the given four alternatives.

a. In the cell membranes, lipids are arranged as

i. Monolayers ii. Bilayers

iii. Random layers iv. Trilayers

b. Mitochondria are

i. Suicidal bags ii. Power houses of cells

iii. Carrier of genetic information iv. Site of protein biosynthesis

c. The fluid which maintains a uniform pressure on the nervous system is

i. Blood ii. Lymph fluid

iii. Cerebrospinal fluid iv. All the above

d. The organelle involved in photosynthetic activity is

i. Chloroplast ii. Mitochondria

iii. Nucleus iv. Ribosomes

e. The normal pH of blood is

i. 6.0 ii. 7.4 iii. 8.0 iv. 4.2

II. Fill up the blanks

a. Man is an example for _____ organism

b. Subcellular organelles _____ present in Eucaryotic and _____ present in procaryotic cells.

c. _____ is the most important buffer in blood plasma

d. _____ is an infectious state of CSF

e. _____is the negative logarithm of H^+ ion concentration.

III. Say true or false

a. Eukaryotic organisms are primitive type.

b. Lysosomes are knows as suicidal bags.

c. CSF maintains a uniform pressure on the nervous system.

d. Nucleolus contains DNA

e. Pili is present in eukaryotic cells.

f. Vacuoles are significantly bigger in plant cells.

IV. Match the following

1. Distinct vacuole	-	Plant cell
2. Pili	-	Henderson - Hasselbalch equation
3. pH	-	Suicidal bags
4. Lysosomes	-	Ribosomes
5. Endoplasmic reticulum	-	Prokaryotic cells

V. Give one word answer

a. What is the major function of ribosomes?

b. What is plasma?

c. What is the normal pH of blood?

d. Write Henderson - Hasselbalch equation?

e. What are intrinsic proteins?

VI. Answer the followings:

a. Explain the lipid bilayer structure of cell membrane.

b. Give the schemmatic diagram of an Eukaryotic cell.

c. Give the composition and functions of CSF.

d. How is acid-base balance maintained in human body.

e. Derive Henderson - Hasselbalch equation and mention its significance.

CHAPTER 2

BIOMOLECULES

2.1 Introduction

The human body is composed of major elements such as carbon, hydrogen, oxygen and nitrogen that combine to form a great variety of molecules called biomolecules. The four major complex biomolecules are carbohydrates, proteins, lipids and nucleic acids.It is very important to know the nature and functions of these biomolecules.

2.2 Carbohydrates

2.2.1 Importance

Carbohydrates are widely distributed in both plant and animal tissues. They are indispensible for living organisms and serve as skeletal structures in plants and also in insects and crustaceans. They occur as food reserves in the storage organs of plants and animals. They are the important source of energy required for the various metabolic activities of the living organisms.

2.2.2 Definition

Carbohydrates are defined as polyhydroxy aldehydes or ketones and are generally classified as follows.

2.2.3 Classification

Carbohydrates are generally classified into 4 major groups :

1. Monosaccharides
2. Disaccharides

3. Oligosaccharides and

4. Polysaccharides

2.2.3.1 *Monosaccharides*

These are carbohydrates that cannot be hydrolysed into more simpler form. These are otherwise known as simple sugars. The general formula is $C_n(H_2O)_n$. They may be subdivided into trioses, tetroses, pentoses and hexoses depending upon the number of carbon atoms they contain and also subdivided as aldoses and ketoses depending upon the presence of aldehyde or ketone groups (eg).

No.of Carbon atoms	Aldoses	Ketoses
3 (Triose)	Glycerose (or) Glyceraldehyde	Dihydroxy acetone
4 (Tetrose)	Erythrose	Erythrulose
5 (Pentose)	Ribose, Xylose Arabinose	Ribulose Xylulose
6 (Hexose)	Glucose, Galactose Mannose	Fructose
7 Heptose	Glucoheptose Galactoheptose	Pseudo heptulose

Simplest form of aldose - Glyceraldehyde
Simplest form of ketose - Dihydroxy acetone
Commonest aldose - Glucose
Commonest ketose - Fructose

Functions

1. Ribose is a structural element of nucleic acids and also of some coenzymes.
2. Glucose on oxidation yield energy which is required for various metabolic activities.
3. Fructose is found in fruits, honey etc.which are responsible for sweetness and can be converted to glucose and utilised in the body.
4. Galactose is a component of milk sugar-lactose, glycolipids and glycoproteins
5. Mannose is a constituent of mucoproteins and glycoproteins which are essential for the body.

2.2.3.2 *Disaccharides*

These are carbohydrates that yield two molecules of same or different types of monosaccharides on hydrolysis. The general formula is $C_n(H_2O)_{n-1}$ (eg) Lactose, Maltose and Sucrose. The monosaccharide units are united by a glycosidic linkage.

Functions

1. Lactose is otherwise called as milk sugar. It is present in milk and is made up of monosaccharides - glucose and galactose.

   Glucose + Galactose $\longrightarrow$ Lactose

2. Maltose is otherwise known as 'malt sugar' and is present in germinating cereals, malt etc.It is the intermediate product in the hydrolysis of starch by amylase in the alimentary canal. It is made up of 2 molecules of glucose.

   Glucose + Glucose $\longrightarrow$ Maltose

3. Sucrose is otherwise called as 'table sugar' or 'cane sugar'. It is the common sugar and is widely distributed in all photosynthetic plants. It does not exist in the body but occurs in sugarcane, pineapple, sweet potato and honey. It is made up of glucose and fructose.

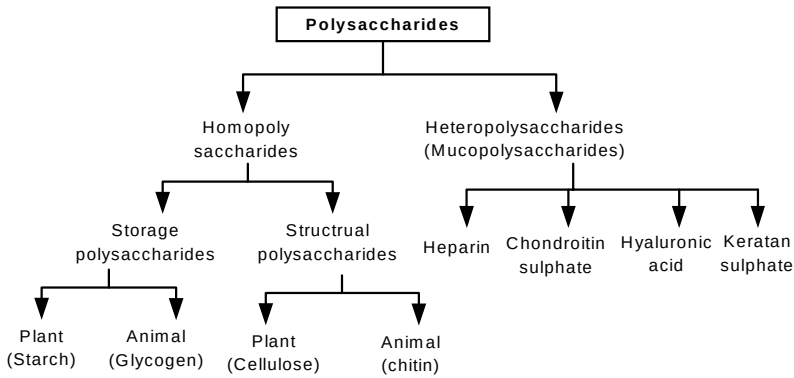
   Glucose + Fructose $\longrightarrow$ Sucrose

2.2.3.3 *Oligosaccharides*

These are carbohydrates that yield 2-10 monosaccharide units on hydrolysis.eg. Maltotriose.

2.2.3.4 *Polysaccharides*

These carbohydrates yield more than 10 monosaccharide units on hydrolysis. They are further classified into homopolysaccharides and heteropolysaccharides.



Homopolysaccharides

These on hydrolysis yield same type of monosaccharide units.

(eg). starch, glycogen, cellulose, inulin, pectin and hemicellulose yield only glucose on hydrolysis.

Heteropolysaccharides

These on hydrolysis yield a mixture of different types of monosaccharides. The heteropolysaccharides situated in extra cellular matrix are called as mucopolysaccharides.

(eg). hyaluronic acid, heparin, keratan sulphate and chondroitin sulphate.

Hyaluronic acid is made up of glucuronic acid and N-acetyl glucosamine

Hyaluronic acid $\xrightarrow{\text{Hydrolysis}}$ Glucuronic acid + N-acetyl glucosamine

Heparin is made up of glucosamine and glucuronic acid

Heparin $\xrightarrow{\text{Hydrolysis}}$ Glucosamine + Glucuronic acid

Chondroitin sulphate is made up of either glucuronic acid (A and C type) or Iduronic acid (B type) and 2-N-acetyl amino galactose.

Chondroitin sulphate $\xrightarrow{\text{Hydrolysis}}$ Gucuronic (or) Iduronic acid + Acetyl amino galactose

Keratan sulphate consists of N-acetyl galactosamine, galactose and sulphuric acid

Keratan sulphate $\xrightarrow{\text{Hydrolysis}}$ N-acetyl glucosamine + Galactose + Sulphuric acid

Functions

1. Starch is made up of repeated units of glucose moiety. It is the most important source of carbohydrate in our food. Such a compound which produces only glucose on hydrolysis is called a glucosan, and is found in cereals, potatoes, legumes and other vegetables.

2. Glycogen is the major carbohydrate reserve in animals and is often called animal starch. It is stored in liver and muscle of animals.

   It is also found in plants which have no chlorophyll system [eg. fungi and yeasts] but not in green plants.

3. Cellulose is widely distributed in plant sources. It occurs in the cell walls of plants where it contributes to the structure. It is the main

consituent of the supporting tissues of plants and forms a considerable part of vegetables.

4. Pectin and hemicellulose are present in fruits of many plants and serve as jelling agents.
5. Hyaluronic acid occurs in synovial fluid, in skin and in tissues. It acts as a cementing substance in tissues and also acts as a lubricant. It is also present in vitreous humor.
6. Heparin is used in medicine as an anticoagulant and prevents blood clotting.
7. Keratan sulphate is an important component of cartilage and cornea.

2.3 Proteins

2.3.1 Definition

Proteins may be defined as the high molecular weight polymers composed of α-amino acids united to one another by peptide linkage (-CO-NH-). Proteins are the major consitituents of all living organisms. They contain carbon, hydrogen, nitrogen, oxygen and sulphur.

2.3.2 Classification

They are classified into 3 main groups.

1. Simple protein : These proteins on hydrolysis yield only α-amino acids. (eg). albumin, globulin
2. Conjugated protein : These are proteins composed of simple proteins combined with non-protein part called as prosthetic groups. They are further subdivided into:
    a. Nucleo protein : Proteins present along with nucleic acids. (eg) Histones and Protamines.
    b. Phosphoprotein : These are protein containing phosphoric acid (eg) casein of milk.

c. Glycoprotein : These are proteins containing carbohydrate moiety as prosthetic group. (eg.) Gonadotropic hormone, mucous glycoprotein mucin (saliva) and osseomucoid (bone)

d. Chromoprotein : These proteins contain heterocyclic compounds like porphyrins as the prosthetic group. (eg) Hemoglobin and Myoglobin.

e. Lipoproteins : These are proteins conjugated with lipids (eg) chlyomicron, very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL).

f. Metalloproteins : These proteins contain metal as prosthetic group (eg) Siderophilin (Fe) and Ceruloplasmin (Cu).

3. Derived proteins : These are proteins derived from the simple and conjugated proteins by the action of acids, alkalies or enzymes. They are the products resulting from partial to complete hydrolysis of proteins. (eg.) proteoses, peptones and peptides.

2.3.3 Functions

1. Proteins are the essence of life processes.
2. They are the fundamental constituents of all protoplasm and are involved in the structure of a living cell and in its functions.
3. Enzymes are proteins which act as biocatalysts.
4. Proteins serve as components of the tissues holding the skeletal elements together.
5. Nucleoproteins serve as carrier of genetic characters and hence govern inheritance of traits.
6. They execute their activities in the transport of various compounds.
7. Some hormones are proteins which regulate numerous physiological functions.

8. They function in the homeostatic control of the volume of the circulating blood.
9. They are involved in blood clotting through thrombin, fibrinogen and other protein factors.
10. They act as the defense against infections by means of antibodies.

2.3.3.1 *Functional diversity of proteins*

Proteins are the fundamental constituents of all protoplasm and are involved in the structure and functions of living cells. Proteins have many different biological functions.

1. Catalytic proteins

   Enzymes are proteins which have catalytic power, far beyond that of man-made catalysts. They enhance the rate of biochemical reactions.

   (eg.) amylase, carbonic anhydrase etc.

2. Nucleoproteins

   Histones are basic proteins found in association with nucleic acids. They serve as carriers of genetic characters and hence govern inheritance of traits.

3. Hormonal proteins

   Some hormones are proteins which regulate numerous physiological functions. (eg) growth hormone, insulin and glucagon.

4. Storage proteins

   They have the function of storing aminoacids as nutrients and as building blocks for the growing embryo. (eg) casein of milk, ovalbumin of egg white.

5. Transport proteins

   They are capable of binding and transporting specific types of molecules via blood. (eg) hemoglobin and albumin.

6. Contractile proteins

   Proteins such as actin and myosin in skeletal muscle function as essential elements in contractile and motile systems.

7. Defensive proteins

   Some proteins have protective or defensive functions. The blood proteins - thrombin and fibrinogen participate in blood clotting. Antibodies or immunoglobulins are protective proteins which prevent the onset of diseases in the body.

8. Structural proteins

   Proteins such as collagen, keratin etc. serve as structural elements.

9. Toxic proteins

   Ricin of caster bean, diphtheria toxin and botulinum toxin represents another group of proteins which cause dysfunctions and disorders in the body.

2.4 Lipids

2.4.1 Definition

Lipids are the heterogenous group of compounds, actually or potentially related to fatty acids. Chemically, they can be defined as esters of fatty acids with alcohol.

Lipids are relatively insoluble in water and soluble in solvents like ether, chloroform and benzene.

2.4.2 Classification : Lipids are classified into :-

1. Simple lipids

These are esters of fatty acids with various alcohols. They are further classified into,

a. Fats : Esters of fatty acids with glycerol. A fat in the liquid state is known as oil.Simple lipids in animals are called as fats and in plants as oils.

   (eg) Triacyl glycerol.

b. Waxes : Esters of fatty acids with high molecular weight monohydric alcohols.

(eg) : Cholesterol ester, myricyl palmitate and cetyl palmitate.

2. Compound lipids

These are esters of fatty acids with alcohol, but it contains extra groups in addition to alcohol and a fatty acid. They are subdivided into the followings :

A. Phospholipids (Phosphatides)

They are esters of fatty acids with glycerol containing an esterified phosphoric acid and a nitrogen base. These lipids are present in large amounts in nerve tissue, brain, liver, kidney, pancreas and heart. Phospholipids are further classified into 3 types based on the type of alcohol present in the phospholipid

(i) Glycerophosphatides - In this, glycerol is the alcohol group.

(eg) Lecithin - Choline as base

Cephalin - Ethanolamine as base

Phosphatidyl serine - Serine as base

Plasmalogens - either Choline or Ethanolamine as base.

(ii) Phosphoinositides

In this phospholipid, inositol - an hexahydric alcohol is present along with the back bone glycerol (eg) Phosphatidyl inositol (lipositol)

(iii) Phosphosphingoside - in this phospholipids, sphingosine is the alcohol (amino alcohol)

(eg) Sphingomyelin.

B. Glycolipids - These lipids contain a carbohydrate moiety along with an amino alcohol. These are further classified into

(i) Cerebrosides : Contain galactose, a high molecular weight fatty acid and sphingosine.

(ii) Gangliosides - contain ceramide (sphingosine + fatty acid) glucose, galactose, N-acetyl galactosamine and sialic acid.

C. Lipoproteins - lipids like triacyl glycerol, phospholipids, cholesterol and cholesteryl esters and free fatty acids combine with protein in particular proportions and form a hydrophilic lipoprotein complex (eg) chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). The protein moiety in the lipoprotein is known as apoprotein.

(3) Derived lipids : These are lipids which are derived from the hydrolysis of simple and compound lipids. (eg) diacyl glycerol, fatty acids, glycerol and cholesterol.

2.4.3 Functions

1. Fats stored in adipose tissue serve as an efficient source of energy
2. Fats serve as a thermal insulator in the subcutaneous tissue and around the internal organs.
3. They also act as electrical insulator against nerve impulse transmission.
4. Cholesterol and phospolipids are important constituents of cell membranes.
5. Lipoproteins and glycolipids maintain cellular integrity and permeability.
6. Fats serve as the important source of fat soluble vitamins.
7. The phosphatides of blood platelets are involved in blood clotting mechanism.

2.5 Nucleic acids

Nucleic acids are a polynucleotides consisting of repeated units of mononucleotides.

2.5.1 Definition

Nucleic acids are the polymers of nucleotides. A nucleotide is a nucleoside containing phosphate group. A nucleoside is made up of a purine or pyrimidine nitrogenous base linked to a pentose sugar.

Pentose sugar + purine or pyrimidine base ⟶ Nucleoside

Nucleoside + phosphate ⟶ Nucleotides

Nucleotides (n) ⟶ Nucleic acids

2.5.2 Classification : Nucleic acids are classified into

1. Deoxyribonucleic acids (DNA)

   The most important consistuent of chromosome and exist as double stranded helix. The nitogenous bases present are adenine, guanine, cytosine and thymine and the pentose sugar is deoxyribose.

2. Ribonucleic acids (RNA)

   RNA is a polymer of purine and pyrimidine ribonucleotides linked together by 3', 5' phosphodiester bridges. The nitrogenous bases are adenine, guanine, cytosine and uracil and the pentose sugar is ribose.

   Types of RNA - There are 3 main classes of RNA molecules exist in all prokaryotic and eukaryotic organisms - They are

   a. messenger RNA (mRNA).

   b. transfer RNA (t RNA)

   c. ribosomal RNA (r RNA)

2.5.3 Functions of DNA

1. DNA serves as genetic material in cells.

2. DNA provides information inherited by the daughter cells and is also a source of information for the synthesis of proteins and it produces mRNA essential for protein biosynthesis.

2.5.4 Functions of RNA

1. It primarily functions in the cytoplasm of the cell as template for the synthesis of protein.
2. It carries genetic information from DNA to the site of protein biosynthesis.
3. It is an essential component of ribosome
4. Some RNA has enzymatic activity
5. It serves as genetic material for viruses such as tobacco mosaic virus, polio virus etc.

Excercise

I. Choose the correct answer from the given four alternatives

a. Glucose is a

i. Monosaccharide ii. Dissaccharide

iii. Oligosaccharide iv. Polysaccharide

b. An example for homopolysaccharide is

i. Hyaluronic acid ii. Cellulose

iii. Mannose iv. Starch

c. Chylomicron belongs to the group of

i. Metalloprotein ii. Chromoprotein

iii. Lipoprotein iv. Nucleoprotein

d. High molecular weight alcohols are present in

i. Waxes ii. Fats iii. Oils iv. Phospholipids

e. The differential base present in DNA and RNAis

i. Adenine ii. Guanine

iii. Cytosine iv. Uracil

II. Fill up the blanks

a. The general formula of disaccharides is _____

b. _____ is called as animal starch

c. The prosthetic group present in nucleoprotein is _____

d. _____ is a transport protein

e. DNA produce _____ which is the source of information for protein biosynthesis.

III. Say true or false

a. A disaccharide may contain different types of monosaccharide units.

b. Heparin is a homopolysaccharide

c. Glycoproteins exist in gastric mucosa.

d. Sphingolipid is a phospholipid

e. The genetic material in tobacco mosaic virus in RNA.

IV. Match the following

1.	Fructose	-	Disaccharide
2.	Ribose	-	Amylose
3.	Starch	-	Nucleic acids
4.	Hyaluronic acid	-	Ketose sugar
5.	Maltose	-	Heteropolysaccharide

V. Give one word answer for the followings

a. What is the functional group present in ribulose

b. Which sugar is known as milk sugar

c. How will you differentiate fats and oils.

d. Give an example for catalytic proteins

e. What are nucleosides.

VI. Answer the following

a. Give the classification of carbohydrates with examples.

b. Explain the functions of polysaccharides.

c. What are conjugated proteins? Explain their functions.

d. Give the classification of compound lipids. Explain their functions.

e. Explain the functions of DNA and RNA.

CHPATER 3

ENZYMES

3.1 Introduction

All the enzymes are proteins and they are produced by the living cells. They act as biological catalysts. Enzymes catalyse and enhance the rate of biochemical reactions occuring in various vital processes like breathing, digestion, pumping of heart, formation of body tissues, contraction of muscles, transport of ions across the plasma membranes etc.So without enzymes there is no life. They are inactive at O^oC and destroyed by moist heat at 100^oC.

The term enzyme was first used by Kuhne (1878) to designate these 'biological catalysts'. Sumner and My Back (1950) have defined the enzymes as "Simple or combined proteins acting as specific catalysts". They affect the life of an organism to such an extent that life has aptly called as an orderly function of enzymes.

The substances on which the enzymes act are called as "Substrates". Enzymes are highly specific in their action (i.e) an enzyme can act on a single or a small group of closely related substrates. During catalytic action, the enzymes do not undergo any permanent modification and regenerated at the end of the reaction. The general enzyme catalysed reaction takes place as per the equations

$$E + S \rightleftharpoons ES$$

$$ES \rightleftharpoons E + P$$

E - Enzyme; S = Substrate; ES - Enzyme-Substrate complex; P = product. ES complex is an unstable and highly energised complex.

For example.

α amylase acts on starch and produce maltose units. In this reaction α–amylase is the enzyme, starch is the substrate and maltose is the product.

$$\underset{(E)}{\alpha\text{- amylase}} + \underset{(S)}{\text{starch}} \rightleftharpoons \underset{(ES)}{\alpha\text{- amylase - starch}}$$

$$\underset{(ES)}{\alpha\text{- amylase - starch}} \longrightarrow \underset{(E)}{\alpha\text{- amylase}} + \underset{(P)}{\text{maltose}}$$

Most of the enzymes, synthesised by a living cell, function within that cell and hence are called as endo enzymes or intra cellular enzymes. Some enzymes are liberated from the living cells after synthesis, secreted to the environment and function in extra cellular regions. These enzymes are called as exoenzymes or extra cellular enzymes.

3.2 Chemical nature of enzymes

All the enzymes are proteins with no exception. Some enzymes are simple proteins i.e. their molecules consist of only amino acids. Many enzymes are conjugated proteins and their molecules consist of aminoacids and a non-protein part (not made up of amino acids). The non-protein part of the enzyme is known as coenzyme or prosthetic group, without which the enzyme is inactive. The protein part of the enzyme (in conjugated type) is known as the apoenzyme. The coenzyme and the apoenzyme complex is called as holoenzyme.

$$\text{Coenzyme} + \text{Apoenzyme} \longrightarrow \text{Holoenzyme}$$

For example, pyruvate decarboxylase is an enzyme which catalyses the decarboxylation of pyruvate to form acetaldehyde. The non-protein part of the enzyme is thiamine pyro phosphate without which the reaction can not be proceeded.

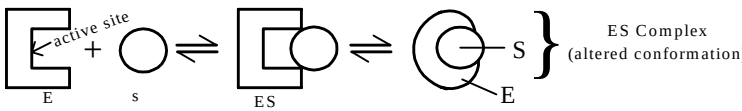
$$\underset{\substack{\text{TPP} \\ \text{(prosthetic group)}}}{\text{Thiamine pyrophosphate}} + \underset{\substack{\text{Protein} \\ \text{(apoenzyme)}}}{\text{Pyruvate decarboxylase}} \longrightarrow \underset{\substack{\text{decarboxylase - TPP} \\ \text{(holoenzyme)}}}{\text{Pyruvate}}$$

The non-proteinaceous part is either loosely or tightly bound with the apoenzyme. If the coenzymes are tightly bound with the apoenzymes the coenzymes are called as prosthetic groups. The losely attached coenzymes are simply called as coenzymes. A biologically active enzyme exist in 3-dimensional folded structure (conformation).

3.2.1 Active site

A restricted region of the enzyme to which the substrate comes and binds and concernes with the process of catalysis is called as the active site. In some enzymes, the active site is a deep groove into which the substrate binds. Specific amino acids are present in the active site which are responsible for the catalytic action. These amino acids are called as 'catalytic' or 'active' amino acids. For example lysozyme has glutamic acid and aspartic acid as catalytic aminoacids. Chymotrypsin which is a proteolytic enzyme contains serine and histidine as catalytic amino acids.

The active site and the other part of the enzyme undergo conformational modification when they come in contact with the substrate



Koshland's induced fit hypothesis of enzyme-substrate interaction postulates that the active site of the enzyme consists of a number of 'active' contact amino acids which permit the substrate to come close to the reactive groups of the enzyme which thereupon undergoes a conformational change, binding the substrate firmly to the enzyme and promoting catalytic activity.

3.3 Naming of enzymes

Except the enzymes ptyalin, pepsin, trypsin and renin, all the other enzymes are usually named by adding suffix - **ase** to the main part of the name of the substrate on which they act.

Examples :

Maltase acts on maltose

Lactase acts on lactose

Lipases act on lipids

Proteases act on proteins

Amylases act on starch (amylum)

3.4 Classification of enzymes

The most comprehensive system for the classification of enzymes was devised in 1961 by the Enzyme Commission of International Union of Biochemistry (IUB).

The 6 major classes of enzymes are

1. oxidoreductases

Enzymes catalyzing oxidoreduction reactions between two substrates A and B are called as oxidoreductases

$$\underset{\text{(reduced)}}{A} + \underset{\text{(Oxidised)}}{B} \rightleftharpoons \underset{\text{(Oxidised)}}{A} + \underset{\text{(Reduced)}}{B}$$

Example : (i) Cytochrome C-oxidase

$$\underset{Fe^{2+}}{\text{Cytochrome } (C_1)} + \underset{Fe^{3+}}{\text{Cytochrome (a)}} \rightleftharpoons \underset{Fe^{3+}}{\text{Cytochrome } (C_1)} + \underset{Fe^{2+}}{\text{Cytochrome } (C_1)}$$

In this reaction cytochrome C_1 is oxidised and cytochrome a is reduced simultaneously by the action of cytochrome C-oxidase.

This class includes several subclasses based on the group on which the enzymes act. The enzymes acting on

-CH -OH (1.1)

-C=O (1.2)

-C=CH (1.3)

-CH-NH_2 (1.4)

-CH-NH (1.5)

2. Transferases

Enzymes catalysing the transfer of a group (x) from one substrate(AX) to another (B) are known as transferases.

$$AX + B \rightleftharpoons A' + BX$$

Example

The reaction catalysed by alanine transaminase (ALT) is

$$\underset{(Ax)}{\text{L-Alanine}} + \underset{(B)}{\alpha\text{-Keto glutarate}} \xrightleftharpoons{ALT} \underset{(A')}{\text{Pyruvate}} + \underset{(Bx)}{\text{Glutamate}}$$

In this reaction the amino group from alanine is transferred to α-ketoglutarate to form glutamate.

These enzymes are further divided into subclasses on the basis of nature of the group transferred.

Transfer of (a) one carbon compounds (2.1)

(b) aldehyde or ketonic groups (2.2)

(c) acyl groups (2.3)

(d) glycosyl groups (2.4)

(e) Phosphate groups (2.7)

(f) Sulphur containing groups (2.8)

3. Hydrolases

The hydrolases are those enzymes which catalyse hydrolysis reactions i.e the direct addition of water molecule (s) across the bond, which is to be cleaved. The substrate for these enzymes are esters, ethers, peptides and glycosides.

Example : Pepsin. This enzyme is a gastro intestinal enzyme which is proteolytic in nature and involve in the hydrolysis of proteins present in the food.

$$\text{Protein} + \xrightarrow{\text{Pepsin}} \text{Smaller peptides and amino acids}$$

$$R - CH_2 - \underset{\underset{OH^-}{\|}}{C} - \underset{\underset{H^+}{|}}{N} - CH_2 - \underset{\underset{\|}{O}}{C} - \underset{\underset{|}{H}}{N} - CH_2 - CONH - R \xrightarrow{H_2O}$$

(Protein)

$$R - CH_2 - \underset{\underset{\|}{O}}{C} - \underset{\underset{|}{H}}{N} - CH_2 - \underset{\underset{|}{OH}}{\underset{\|}{O}}{C} + \underset{\underset{|}{H}}{N} - CH_2 - CO - NH - R$$

(C-terminal Peptide) (N-terminal Peptide)

The hydrolases are divided into several subclasses, depending on the nature of the group or bond being hydrolysed viz.,

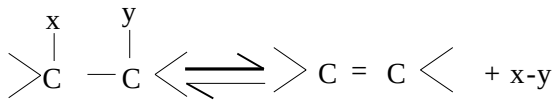
(a) esterases etc. - hydrolyse ester bonds (3.1)

(b) glycosidases - hydrolyse glycosidic bonds (3.2)

(c) peptidases - hydrolyse peptide bonds (3.4)

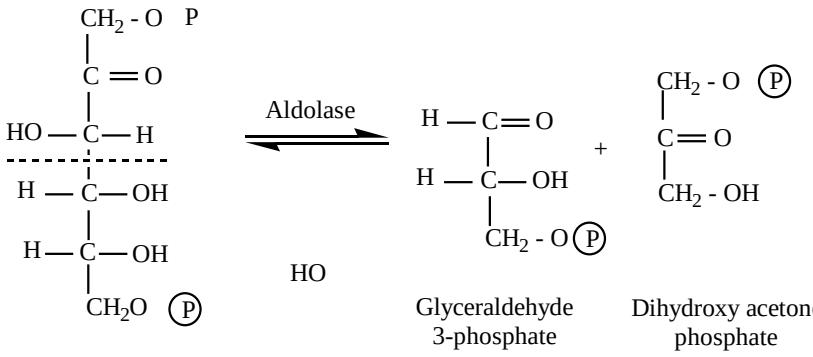
4. Lyases

The lyases are a smaller class of enzymes that catalyse the removal of a small molecule from a larger substrate molecule. Since the reactions are reversible, lyases may also be considered to catalyse the addition of small molecules to the substrate molecule



Example : Aldolase

Fructose-1-6-diphosphate ⇌ Dihydroxy acetone phosphate + glyceraldehyde 3-phosphate



Fructose 1,6 biphosphate

The lyases are further classified on the basis of the linkage they attack viz., acting on

a. C-C bond (4.1)

b. C-O bond (4.2)

c. C-N bond (4.3)

d. C-S bond (4.4)

e. C-halide bond (4.5)

5. Isomerases

This class includes all enzymes which catalyse isomerization reactions i.e. interconversion of optical, geometrical or position isomers.

Example

Retinene isomerase catalyses the conversion of

trans-retinene $\rightleftharpoons$ cis-retinene

Triose phosphate isomerase catalyses the conversion of

D - glyceraldehyde 3- phosphate $\rightleftharpoons$ Dihydroxy acetone phosphate

The isomerases are further divided into the subclasses

a. racemases (5.1)

b. epimerases (5.2)

c. cis-trans isomerases (5.3)

6. Ligases

These enzymes are otherwise known as synthetases. They catalyse synthesis reactions by joining two molecules, coupled with the breakdown of a phosphate bond of adenosine triphosphate. ATP cleavage provides energy for the new bond formation.

Example : Formation of malonyl CoA from acetyl CoA in the presence of acetyl CoA carboxylase.

ATP + acetyl CoA + CO_2 $\rightleftharpoons$ ADP + Pi + malonyl CoA

The subclasses of ligases are based on the nature of bond formed in the product. Formation of

a. C-O bond (6.1)

b. C-S bond (6.2)

c. C-N bond (6.3)

d. C-C bond (6.4)

Class, subclass and enzyme numbers of some enzymes

1. Alcohol dehydrogenase (ADH)

   Major class - oxidoreductase (1)

   Subclass - acting on CH-OH (1)

   Sub subclass - NAD or NADP as coenzymes (1)

   Enzyme no - 1.1.1

2. Hexo kinase

   Major class - Transferases (2)

   Sub class - Transfering phosphate group (7)

   Enzyme No. - 2.7.

3. Alkaline phosphatase

   Major class - Hydrolases (3)

   Subclass - acting on esterbond (1)

   Sub subclass - acting on phosphoric monoesters (3)

   Enzyme No. - 3.1.3

4. Fumarase

   Major class - lyases (4)

   Sub class - CO lyase (2)

   Sub subclass - acting on aldehyde (1)

   Enzyme No. - 4.2.1

5. Ribulose - 5 - phosphate epimerase

Major class - Isomerases (5)

Subclass - Racemases and Epimerases (1)

Sub subclass - acting on carbohydrates (3)

Enzyme no - 5.1.3

6. Acetyl CoA carboxylase

Major class - ligases (6)

Sub class - forming C-C bond (4)

Sub subclass - carboxyl group (1)

Enzyme No. - 6.4.1

3.5 Factors influencing enzyme activity

The activity of enzymes is markedly affected by several factors. These factors are

1. temperature 2. pH 3. substrate concentration 4. metal ions (activators) 5. inhibitors 6. enzyme concentration etc.

3.5.1 pH

All the enzymes have a particular pH at which their activity is maximal; above or below this pH the activity is low. The pH at which the enzyme shows maximum activity is known as optimum pH. Some of the enzymes and their optimum pH are

(a) Pepsin - 2.0 (b) Urease - 7.0

(c) Salivary amylase - 6.8 (d) Alkaline phosphatase - 9.9

Only in this optimum pH, ionisation of active amino acids in enzymes and substrate are favoured for ES complex formation.

The pH activity relationship is shown in the Fig. 3.1.

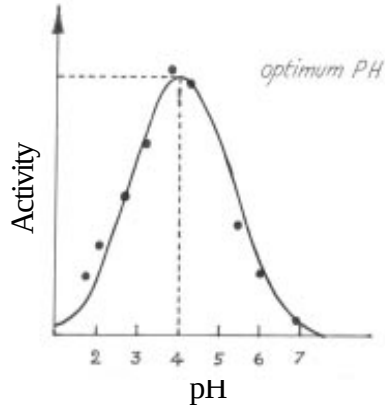


Fig. 3.1 The pH activity relationship

3.5.2 Temperature

Rise in temperature causes increase in the rate of enzyme catalysed reactions up to a certain temperature i.e about 45°C. Above which the activity declines due to denaturation of enzymes (due to their protein nature). As the enzyme is denatured and inactivated, the reaction which it catalyses slows down and ultimately stops. So the temperature at which the enzyme shows maximum activity is known as optimum temperature. The optimum temperature of most of the enzymes is found to be 37°C. The relationship of enzyme activity to temperature is shown below in Fig. 3.2:

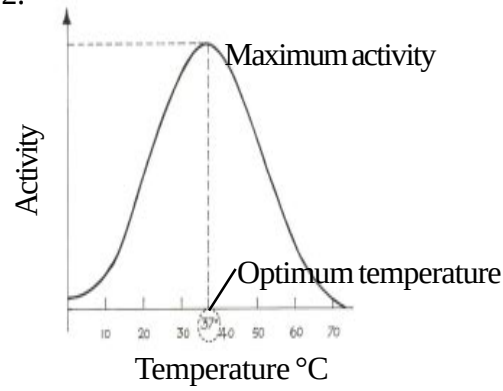


Fig. 3.2 The temperature activity relationship

3.5.3 Substrate concentration

With a fixed amount of enzyme, the reaction rate is proportional to the concentration of substrate. But this is true upto a certain concentration after which the increase in concentration of substrate does not further increase the velocity of the reaction.

Since the number of active sites on an enzyme molecule are limited, a stage will come when all of them have filled with the substrate molecules. This is known as saturation of enzyme. Now, since none of the active sites of the enzyme is free, further addition of the substrate molecule will not increase the product formation (Fig.3.3).

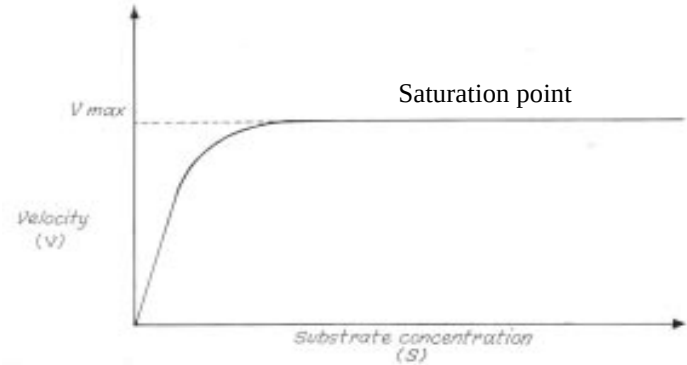


Fig.3.3 Substrate concentration - activity relationship

It was Michaelis and Menten in 1913, who proposed a successful explanation for the effect of substrate concentrtaion on the enzyme activity. According to them the enzyme 'E', and the substrate 'S' combine rapidly to form a complex, the enzyme substrate complex 'ES'. The complex then breaks down relatively, slowly to form the product of the reaction. The enzyme regenerated can involve in another round of catalysis.

$$E + S \rightleftharpoons ES$$

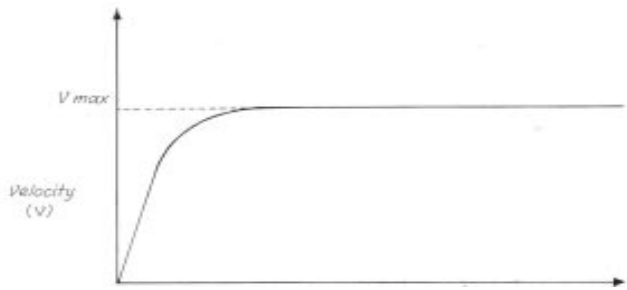
$$ES \longrightarrow E + P$$

3.5.4 Effect of activators

Divalent ions, like Mg^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+} and monovalent ions such as Na^{+} and K^{+} are required for the activity of many enzymes. For example, amylases need Cl^{-} ions, Zn^{2+} ions are required for carbonic anhydrase action, Fe^{2+} and Cu^{2+} ions are required for enzymes involved in redox reactions. Several peptidases are activated by Mn^{2+}, Zn^{2+} or Co^{2+}. Enzymes requiring metal ions or enzymes which contain metal ions in their structure are called as metallo enzymes.

3.5.5 Effect of concentration of enzyme

The velocity of an enzymatic reaction is directly proportional to the concentration of enzyme. In case the enzyme concentration is doubled then as much as twice active site become available to combine with the substrate, provided an excess of substrate is present and so the maximum velocity is also doubled. At a fixed concentration of the substrate a level is reached when all the substrate molecules are utilised and no more change in velocity of the reaction takes place (Fig. 3.4).



Enzyme concentration (E)

Fig. 3.4 Enzyme concentration - activity relationship

3.5.6 Inhibitors

Chemical substances which reduce the activity of enzymes are called as inhibitors. They may be small inorganic ions such as cyanide which inhibits the enzyme cytochrome oxidase or much more complex

molecules such as diisopropyl phospho fluoridate which inhibit acetyl choline esterase.

This phenomenon in which the enzyme activity is decreased by the presence of inhibitors is known as enzyme inhibition.

Types of enzyme inhibition

Enzyme inhibition may be of different types such as (a) competitive (b) uncompetitive (c) non-competitive and (d) allosteric inhibition.

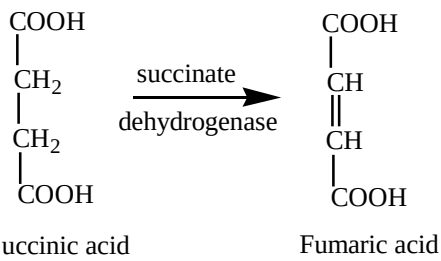
3.5.6.1 *Competitive inhibition*

This type of inhibition occurs when the structure of inhibitor resembles that of the substrate. The inhibitor competes with the proper substrate for binding at the active site of the enzyme. In this type of inhibition, both ES complex and EI complex (enzyme - inhibitor complex) are formed during the reaction. The relative amounts of the two complexes depend partly upon the affinity of the enzyme towards the substrate and inhibitor and partly upon the relative concentration of substrate and the inhibitor. Thus if the inhibitor is present in sufficiently high concentration, it can displace the substrate entirely and thus blocks the reaction completely (Fig.3.5).

$$E + S \rightleftharpoons ES$$

$$+$$

$$I \rightleftharpoons EI + \not\rightarrow P \quad EI = \text{enzyme inhibitor complex}$$



Succinate dehydrogenase catalyses the conversion of succinic acid to fumaric acid.

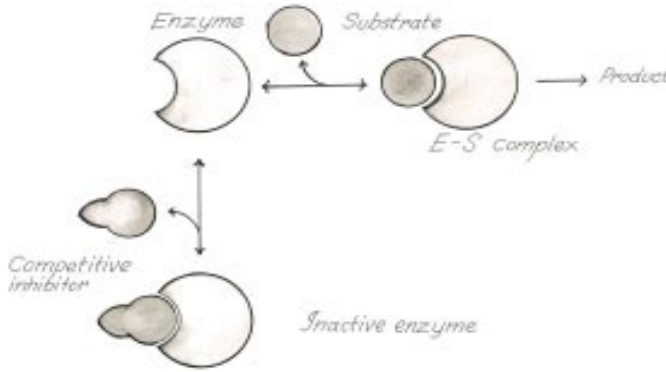
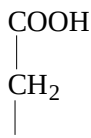


Fig. 3.5 Mode of action of competitive inhibitor

This reaction is completely inhibited by malonic acid which has structural resemblence with succinic acid.



- a competitive inhibitor of succinate dehydrogenase

Malonic acid

This type of inhibition can be reduced by increasing the concentration of the substrate and for this reason competitive inhibition is called as reversible inhibition. Many competitive inhibitors are used as drugs to block particular metabolic reactions.

3.5.6.2 *Uncompetitive inhibition*

In this type of inhibition, the inhibitor combines with enzyme - substrate complex to give an inactive enzyme - substrate - inhibitor complex which cannot undergo further reaction to yield the product (Fig. 3.6).

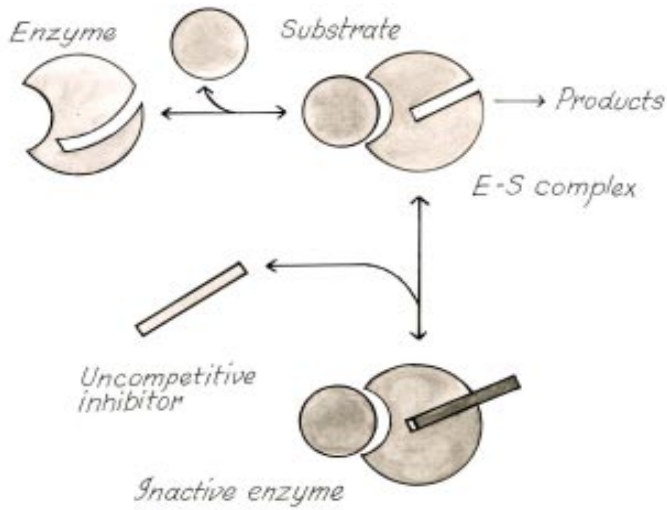
$$E + S \rightleftharpoons ES \longrightarrow E+P$$

$$+$$

$$I \rightleftharpoons ESI \not\longrightarrow P$$

In this type, the degree of inhibition may increase when the substrate concentration is increased. This inhibition cannot be reversed by increasing the concentration of substrate.

Fig. 3.6 Mode of action of uncompetitive inhibitor



3.5.6.3 *Non competitive inhibition*

In this type of inhibition, the inhibitor can combine with either the free enzyme or the enzyme substrate complex, interfering with the action of both. Non competitive inhibitor bind to the site on the enzyme other than the active site, often to deform the enzyme, so that it does not form the ES complex at its normal rate and once formed, the ES complex does not decomposes at the normal rate to yield products. These effects are not completely reversed by increasing the substrate concentration (Fig. 3.7).

$$E + I \rightleftharpoons EI \not\longrightarrow P$$

+

$$S \rightleftharpoons ES + I \rightleftharpoons ESI \not\longrightarrow P$$

$$\downarrow$$

$$E+P$$

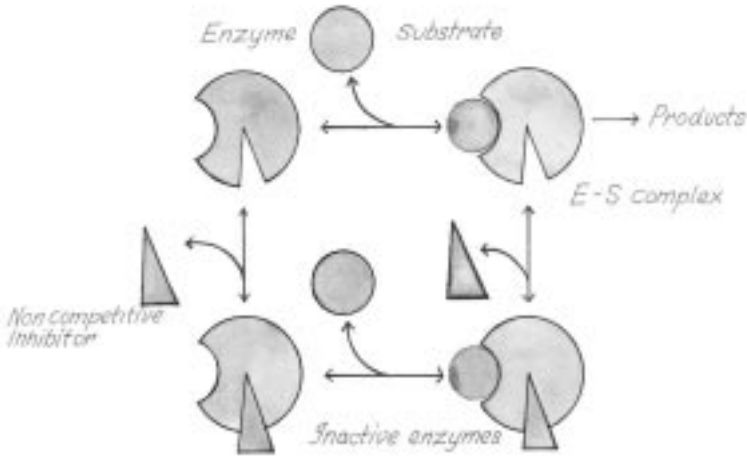


Fig. 3.7 Mode of action of non-competitive inhibitor

Examples

a. Effect of iodoacetamide on - SH group containing enzymes

b. Effect of diisopropyl phosphofluoridate on acetyl choline esterase.

These two inhibitors completety inactivate the respective enzymes. This inhibition can be partially reversible.

3.5.6.4 *Allosteric inhibition*

This type of inhibition is otherwise known as end product inhibition. The inhibitor binds with the modulator binding site (or) allosteric site of

the enzyme. The inhibitor present at the allosteric site may affect the conformation at the active site with the result it becomes difficult for the enzyme to take up the substrate molecule, and in the extreme case, the enzyme completely fails to take up the substrate molecule (Fig. 3.8).

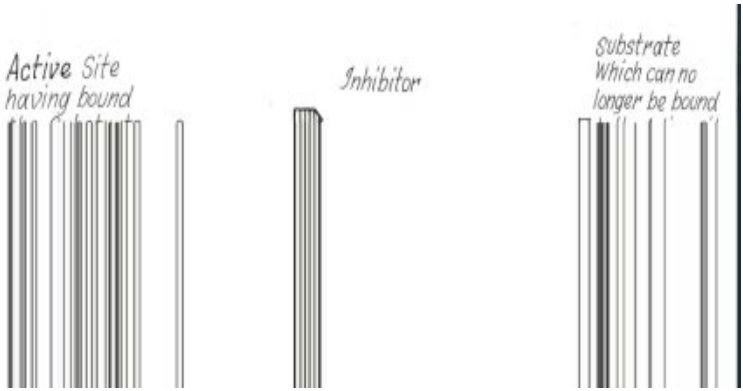


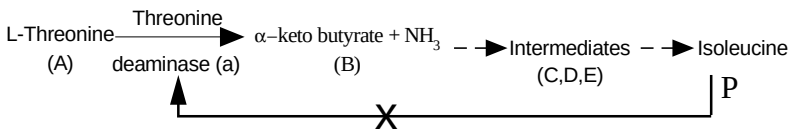
Fig.3.8 Mode of action of allosteric inhibitor

This type of inhibition is seen in multistep reactions in which each step is catalysed by different enzymes as shown below.

$$A \xrightarrow{a} B \xrightarrow{b} C \xrightarrow{c} D \xrightarrow{d} E \;\ldots\ldots\; \ldots P$$

where A is the starting substrate B,C,D,F are intermediates, a,b,c,d are enzymes and P the product. When the product concentration (P) increases, it binds with the enzyme 'a' which is the first enzyme in the reaction sequence. This enzyme which can be inhibited by the end product is known as allosteric enzyme.

Example



when isoleucine production increases, as a regulatory mechanism, it binds with threonine deaminase in the allosteric site and inhibit further binding of the substrate with the enzyme and ultimately production of isoleucine is stopped. This inhibition is otherwise known as feed back inhibition.

Many metabolic reactions in our body are regulated by means of allosteric enzymes.

3.6 Iso enzymes

Certain enzymes may exist in two or more forms which have the same catalytic activity but are distinct physically, chemically and electrophoretically. These forms exist in different tissues.

Example

a. Lactate dehydrogenase exist in 5 different iso enzymic forms LD1, LD2, LD3, LD4, LD5 and perform the same function of the conversion of lactate to pyruvate. LD1 is predominant in heart and LD5 in muscle and liver.

b. Creatine kinase exist in 3 different iso enzymic forms BB, MM and MB. These isoenzymes perform the same function of conversion of creatine to creatine phosphate.

   BB form exist in brain, MM form in muscle and MB form in heart.

3.7 Importance of enzymes

1. Enzymes catalyse many biological reactions and enhance the rate of product formation in metabolic pathways.

2. Some enzymes in blood are used as diagnostic indicators of various diseases. For example the level of transaminases are elevated in blood during jaundice - a liver disorder.

3. Some enzymes are used for therapeutic purposes

a. Penicillinase - to treat patients allergic to penecillin

b. Asparaginase - to treat leukemia

c. Diastase - to treat indigestion

Excercise

I. Choose the correct answer from the given four alternatives

a. Amylases act on

i. Starch ii. Lactose iiii. Sucrose iv. Glucose

b. The catalytic groups of lysozyme are

i. Glutamic acid ii. Aspartic acid

iii. Glutamic acid and Aspartic acid iv. Histidine

c. Fumarase belong to the major class of

i. Oxidoreductases ii. Transferases

iii. Hydrolases iv. Ligases

d. Cl^- ions act as activators of

i. Amylases ii. Peptidases

iii. Glycosidases iv. Lipases

e. The different morphological structures of the same enzyme are called as

i. Apoenzymes ii. Isoenzymes

iii. Coenzymes iv. Holoenzymes

II. Fill up the blanks

a. A holoenzyme contains apoenzyme and _____

b. The structural resemblence of substrate and inhibitor leads to _____ inhibition.

c. The termperature at which the enzyme shows maximum activity is known as _____

d. Allosteric enzymes posses an extra _____ site.

e. Cytochrome oxidase belong to the major class of _____.

III. Say true or false

a. Holo enzymes are complete and active enzymes.

b. Active sites are essential for enzyme action.

c. Competitive inhibition is reversible

d. Metal ions can activate enzymes.

e. Modulator binding sites are present in all the enzymes.

IV. Match the following

1.	Alkaline phosphatase	- Non protein part of the enzyme
2.	Coenzymes	- Hydrolase
3.	Allosteric inhibitor	- Enzyme activator
4.	Zn^{2+}	- Malonate
5.	Succinate dehydrogenase	- Feed back inhibition

V. Give one word answer

a. What is a coenzyme?

b. What are the catalytic amino acids of chymotrypsin?

c. How is competitive inhibition prevented?

d. Who proposed induced fit theory?

e. What is the action of ligases?

VI. Answer the followings

a. Explain the major classes of enzymes.

b. What are catalytic aminoacids? Give examples.

c. Explain allosteric inhibition with an example.

d. Write a note on the factors affecting enzyme activity.

e. Differentiate the action of competitive, uncompetitive and non competitive inhibitors.

CHAPTER 4

CARBOHYDRATES

4.1 Introduction

Carbohydrates are widely distributed in plants in which they are formed from carbon dioxide of the atmosphere and water by photosynthesis. Plants use carbohydrates as the precursor for the synthesis of proteins, lipids and other organic compounds. Animals obtain their carbohydrates from plants.

4.2 Functions of carbohydrates

Carbohydrates have a variety of functions in the animal and human body.

1. They supply energy for body functions and for doing work.
2. They are structural components of many organisms.
3. They exert a sparing action on proteins.
4. They provide the carbon skeleton for the synthesis of some non-essential amino acids and fats.
5. Some carbohydrates are present as tissue constituents.
6. Starch forms main source of carbohydrates in the diet.

4.3 Classification of carbohydrates

Carbohydrates are classified as follows

They are i. Monosaccharides ii. Disaccharides iii. Oligo saccharides and iv. Polysaccharides (Fig. 4.1).

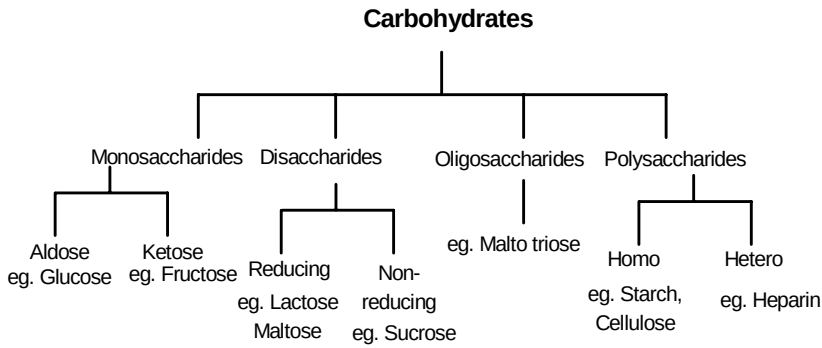


Fig. 4.1 Classification of Carbohydrates

4.3.1 Monosaccharides

Monosaccharides are defined as polyhydroxy aldehydes or ketones, which cannot be further hydrolysed to simple sugars. Monosaccharides are divided into two groups according to their functional groups (Fig. 4.2).

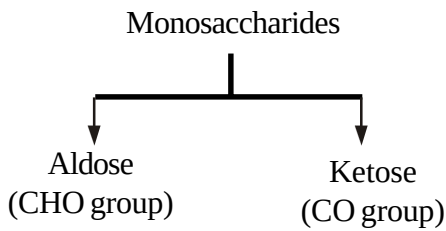


Fig. 4.2 Classification of monosaccharides

They are also classified based on the number of carbon atoms present in the monosaccharides

1. Aldotriose eg: Glycerose 1. Ketotriose :eg.Dihydroxy acetone

2. Aldotetrose eg : Erythrose 2. Ketotetrose : eg : Erythrulose

3. Aldopentose eg : Ribose 3. Ketopentose : eg. Ribulose

4. Aldohexose eg: Glucose 4. Ketohexose : eg. Fructose

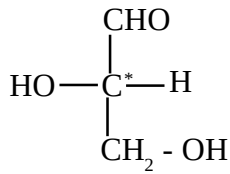
1. Aldoses are sugars containing aldehyde group

   eg : glucose, galactose, mannose.

2. Ketoses are sugars containing ketone group

   eg : fructose and sorbose.

Carbohydrates posses asymmetric carbon atoms. A carbon atom to which four different atoms or groups of atoms are attached is said to be an asymmetric carbon.



C* - Asymmetric carbon

Vant Hoff's Rule of 'n'

The number of possible isomers of any given compound depends upon the number of asymmetric carbon atoms the molecule posses.

According to this rule, 2^n equals the possible isomers of that compound, where n - represents the number of asymmetric carbon atoms in a compound.

4.3.1.1 *Hexoses*

Hexoses are monosaccharides containing 6 carbon atoms. The molecular formula of hexose is $C_6H_{12}O_6$. Aldohexoses contain asymmetric carbon atoms at position 2,3,4 and 5. Hence an aldohexose can exist in 16 isomeric forms. ($2^n = 2^4 = 16$).

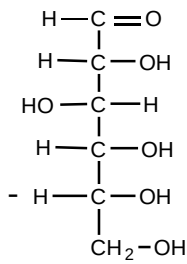
The ketohexoses contain 3 asymmetric carbon atoms at position 3,4 and 5. Hence, it exist in 8 isomeric forms ($2^n = 2^3 = 8$).

Structure of glucose and fructose

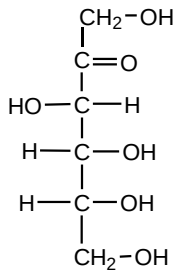
Glucose is a simple sugar. It is a monosaccharide. It cannot be hydrolysed further. Glucose is an important sugar of blood.

Human blood contains 60-100 mg of glucose in 100 ml of blood in fasting. It serves as the major metabolic fuel in cells and tissues. Oxidation of glucose quickly provides energy for the cells.Hence, glucose is described as the chief source of energy.

i. The empirical formula of glucose is CH_2O and the molecular formula is $C_6H_{12}O_6$. The molecular formula of fructose is $C_6H_{12}O_6$. Glucose has aldehyde group whereas fructose has ketone group.



Glucose



Fructose

4.3.1.2 *Physical properties of monosaccharides*

1. Colour and shape

   Monosaccharides are colourless and crystalline compounds.

2. Solubility

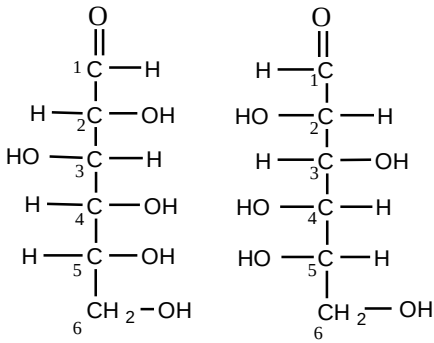
   They are readily soluble in water.

3. Taste

   They have sweet tase.

4. Stereo isomerism

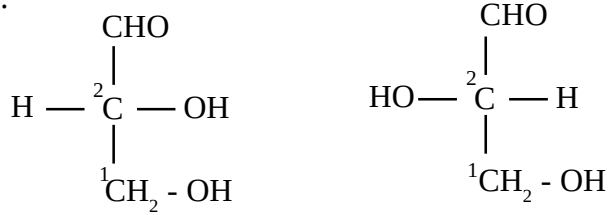
The presence of asymmetric carbon atoms in a compound give rise to the formation of isomers of that compound. Such compound which are identical in composition and differs only in spatial configuration are called "stereo isomers''. For example glucose can exist in two forms as shown below.



D-glucose and L-glucose are mirror images of each other.

D-series and L-series

The orientation of the H and OH groups around the carbon atom just adjacent to the terminal primary alcohol carbon, eg. C_5 in glucose determines the series. The D and L forms of glyceraldehyde are given below.



D-Glyceraldehyde L-Glyceraldehyde

when the -OH group of this C_2 is at the right, it belongs to D-series, when the -OH group is on the left it belongs to L-series.

5. Optical Isomerism

A beam of ordinary light may be regarded as bundle of electromagnetic waves vibrating in all directions perpendicular to the axis of the beam. When such a beam of light is made to pass through a nicol prism, all vibrations except those in one plane are eliminated. This is called as plane polarised light. When such a beam of plane polarised light is passed through a solution of an optical isomer, and if the plane polarised light is found to rotate to the left, it is described as levorotation. If the plane polarised light rotates to an equal number of degrees to the right, it is described as dextrorotation. This phenomenon exhibited by asymmetric compounds, is called optical isomerism (Fig. 4.3 & 4.4).

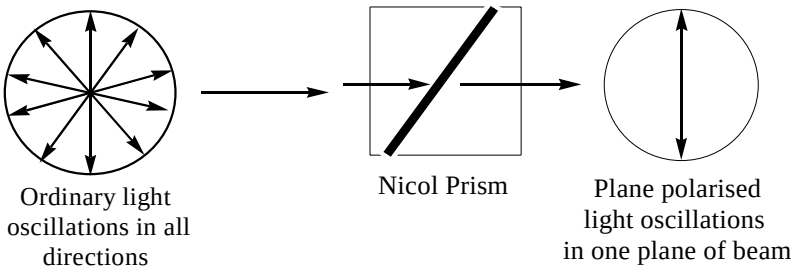


Fig. 4.3 Conversion of ordinary light to plane polarised light

Polarimeter is an instrument by which the specific rotations of optical isomers are detected.

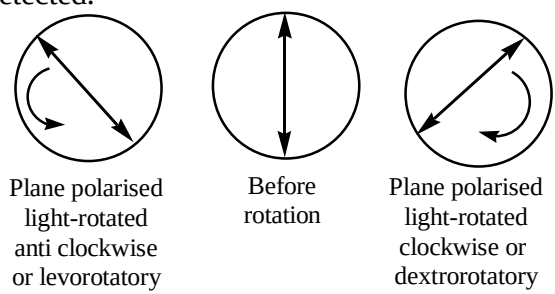


Fig. 4.4 Rotation of plane polarised light

Expression of optical activity

Optical rotation to the left i.e levorotation is expressed with a sign of l^- and rotation to the right i.e dextrorotation is expressed as d^+.

Racemic mixture

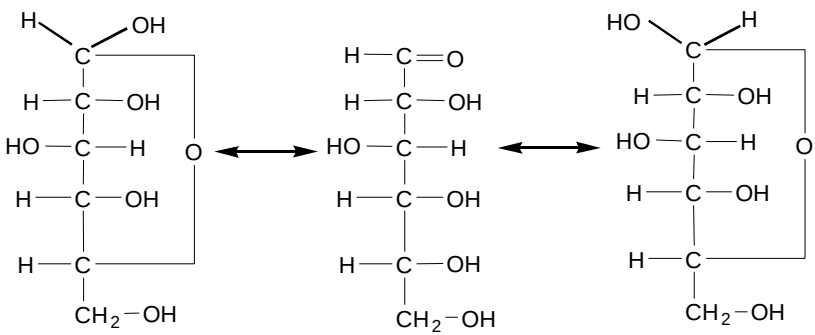
When equal amounts of dextrorotatory and levorotatory isomers are present, the resulting mixture has no optical activity, since the activities of isomers cancel each other. Such a mixture is said to be a **"racemic mixture"**.

Resolution

The separation of optically active isomers from a racemic mixture is called resolution.

6. Mutarotation

When an aldohexose is first dissolved in water and the solution is kept in optical path and plane polarised light is passed, the initial optical rotation shown by the sugar gradually changes until a constant fixed rotation characteristic of the sugar is reached. This phenomenon of change of rotation is called as "Mutarotation".



+ 112.2° ⟶ +52.7° ⟵ + 18.7°

The mutarotation is due to the existence of two optical isomers of glucose, namely α, D glucose with a specific rotation +112.2° and β, D glucose with a specific rotation +18.7°.

α and β isomers are called as anomers and the carbon atom responsible for this is the anomeric carbon atom. Anomers are isomers differing in configuration of a particular carbon atom alone.

A freshly prepared aqueous solution of α, D glucose has a specific rotation of +112.2°. When this solution is allowed to stand, the rotation falls to 52.7° and remains constant at this value. This gradual change in specific rotation is called mutarotation.

The value of mutarotation for α, D-glucose is +59.5°.

(+112.2°) - (52.7°) = +59.5°.

A freshly prepared solution of β, D glucose has a rotation value of 18.7°. It also gradually increases and reaches the same final value of + 52.7°.

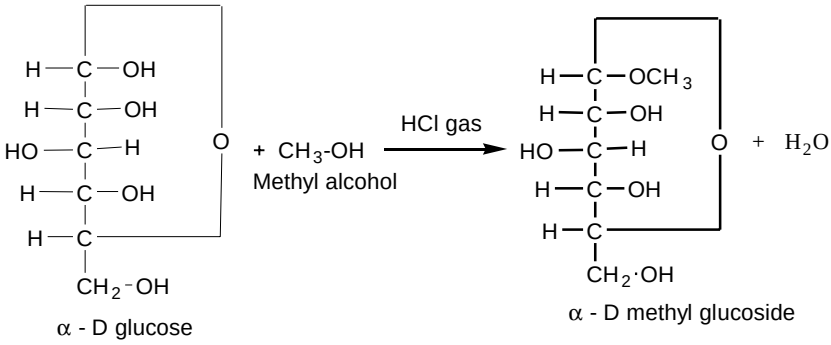
4.3.1.3 *Chemical properties of glucose*

Glucose contain active groups. The active groups are responsible for their chemical properties. There are three types of active groups in glucose. They are

1. Glycosidic -OH group
2. Alcoholic -OH group
3. Aldehyde - C(=O)-H group

1. Glucoside formation

Glucose reacts with methanol in the presence of HCl and gives α and β glucoside. Glucoside formation is due to the reaction of alcohol with glucoside -OH group of glucose.



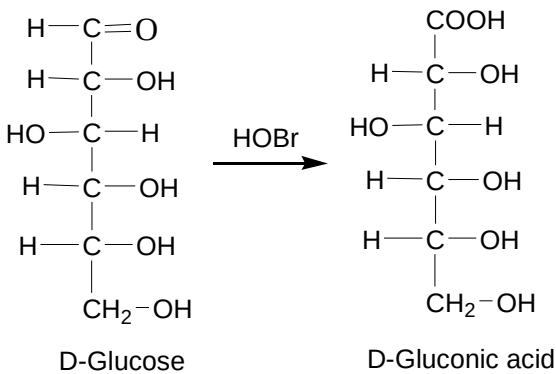
β, D glucose is forms β, D-methyl glucoside. In the same way, fructose forms fructoside.

2. Oxidation

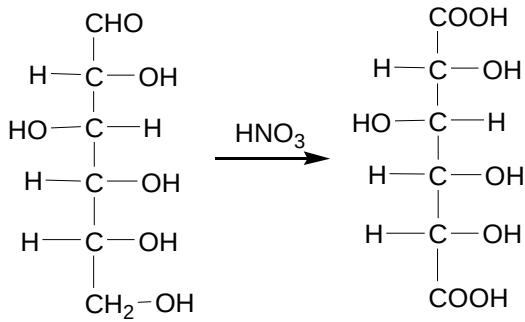
Glucose when treated with bromine water, forms gluconic acid. The aldehyde group is oxidised to carboxylic group.

$$Br_2 + H_2O \rightleftharpoons HOBr + HBr$$

First, bromine forms hypobromous acid (HOBr), with water and oxidises the glucose to gluconic acid.

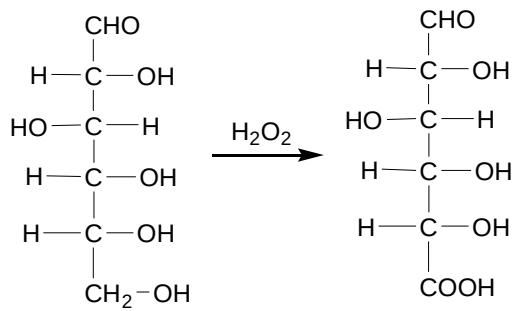


When glucose is oxidised with nitric acid, saccharic acid is formed.



D-Glucose Saccharic acid

When glucose is oxidised with hydrogen peroxide (H_2O_2), glucuronic acid is formed.



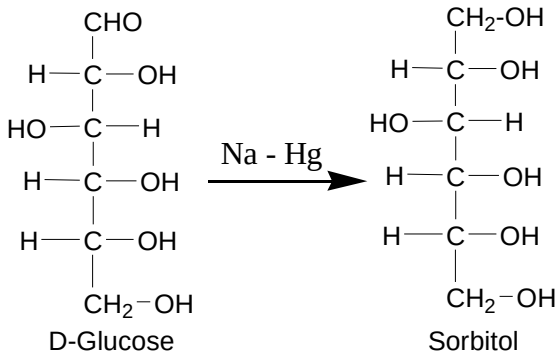
D-Glucose Glucuronic acid

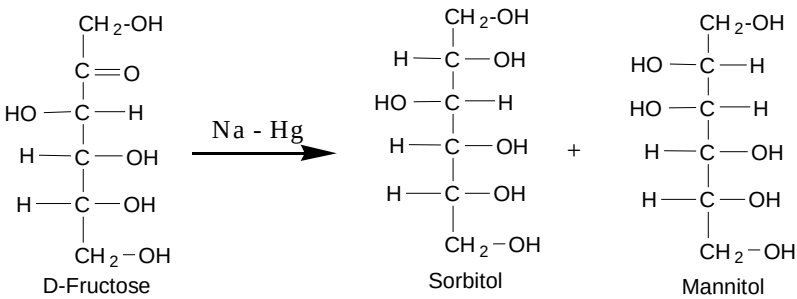
In this reaction only the primary alcohol is converted into carboxylic group, whereas the aldehyde remains unchanged.

3. Reduction

Monosaccharides can be reduced by various reducing agents such as sodium-amalgam or by hydrogen under high pressure in the presence

of catalysts. The reduction is due to the presence of aldehyde or ketone group. On reduction they yield alcohols.

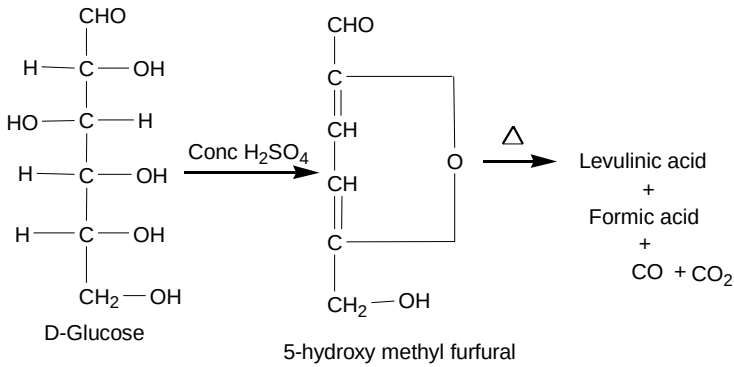




When glucose is reduced by sodium amalgam, sorbitol is formed. Mannose yeilds mannitol and fructose yeilds a mixture of sorbitol and mannitol because of the formation of new asymmetric carbon C_2 of fructose.

4. Reaction with concentrated H_2SO_4

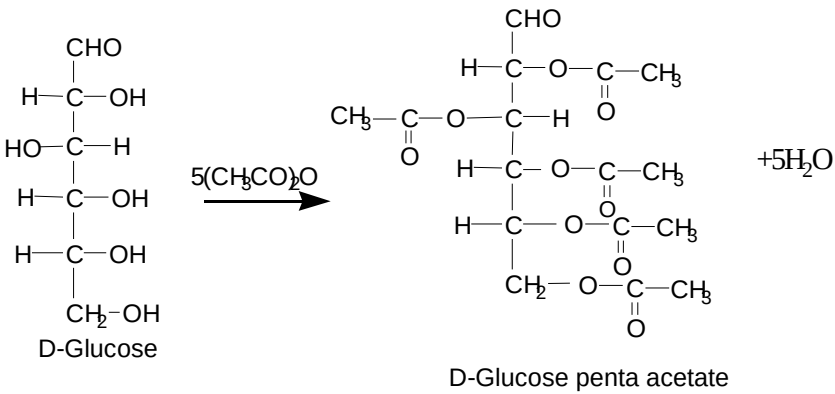
Glucose is treated with concentrated H_2SO_4 or HCl, and forms 5, hydroxymethyl furfural which on further heating yields levulinic acid and formic acid.



This reaction is the basis of the colour test, known as **Molish test** for sugars. When pentoses are treated with mineral acids furfural is obtained on heating.

5. Ester formation

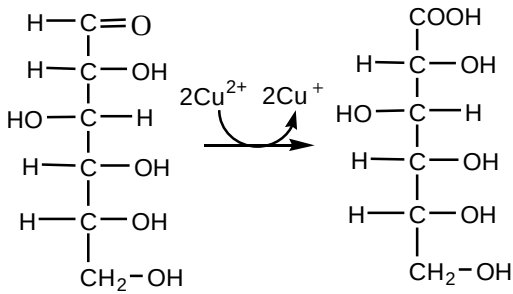
They can form esters with carboxylic acids due to the presence of OH groups. For eg. glucose reacts with five molecules of acetic anhydride to form penta acetate derivative. It obviously indicates that the glucose contain five OH groups.



6. Reducing property

Monosaccharides act as the best reducing agents. They readily reduce oxidizing agents such as ferric cyanide, H_2O_2 and cupric ion. In such reactions, the sugar is oxidized at the carbonyl group and the oxidising agent becomes reduced.

Glucose and other sugar capable of reducing certain compounds are called reducing sugars. Glucose reduces Tollen's reagent, Fehling's reagent, Benedict's reagent etc. At the same time glucose is oxidized to gluconic acid.

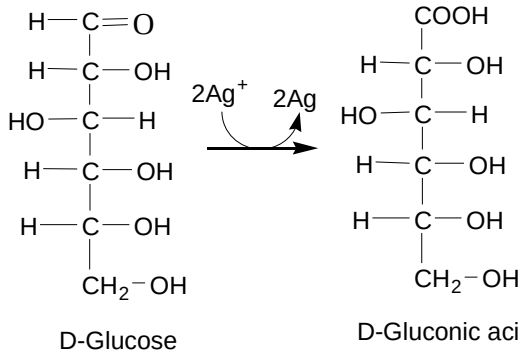


This property is the basis of Fehling's reaction (ammoniacal cupric sulphate), a qualitative test for the presence of reducing sugar.

Cu^{2+} is reduced into Cu^{+} and at the same time glucose is oxidised to gluconic acid. During this reaction the blue colour of the reagent changes to reddish orange colour. Benedict's reagent contains cupric ions which are reduced to cuprous ions by the reducing sugar and the colour change from blue to orange or red. It indicates the presence of reducing sugar.

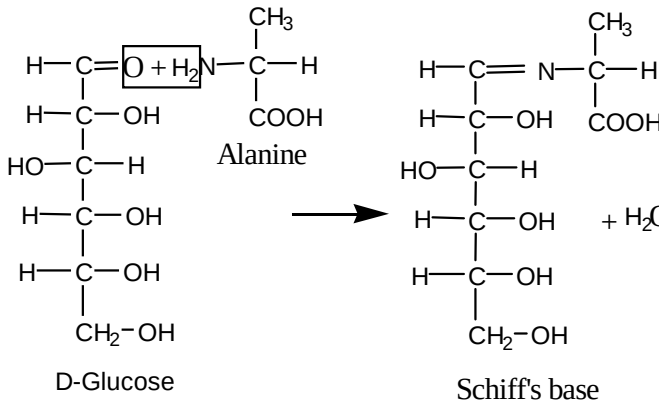
A standard test for the presence of reducing sugar is the reduction of Ag^{+} in ammonia solution (Tollen's reagent) to yield a metallic silver mirror lining on the sides of the test tube.

D-glucose $\xrightarrow{\text{Tollen's reagent}}$ D-Gluconic acid + silver mirror



7. Reaction with alanine

The aldehyde group of glucose condenses with the amino group of alanine to form **Schiff's base.** Fructose also gives Schiff's base with alanine.



The browning reaction occurs during baking of bread and other mixtures of carbohydrates and proteins is believed to be due to the formation of Schiff's base between the amino groups of proteins and the aldehyde groups of carbohydrates.

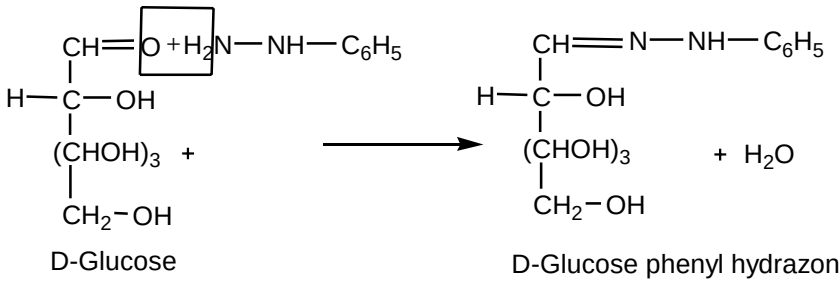
8. Osazone formation

An important reaction of reducing sugars, (monosaccharide and disaccharrides) having potential aldehyde or ketone group, is their action with phenylhydrazine to form phenyl hydrazones.

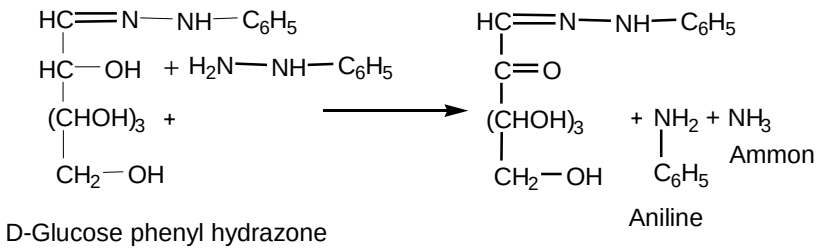
Reaction with phenylhydrazine involves only 2 carbon atoms namely the carbonyl carbon atom and the adjacent one.

The steps involved in phenylhydrazine reactions are

1. One molecule of glucose condenses with one molecule of phenyl hydrazine to form soluble glucose phenylhydrazone.

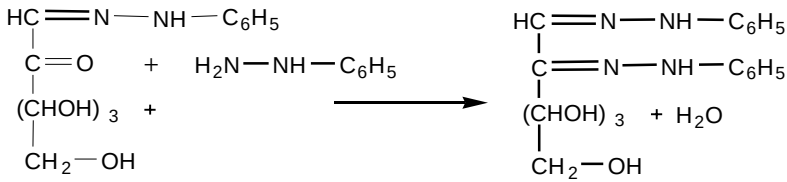


2. In the presence of excess of phenylhydrazine, another molecule of phenylhydrazine enters the reaction.



3. Now the third molecule of phenylhydrazine enters the reaction, giving rise to phenyl glucosazone which are yellow coloured crystals.

The shape of the crystals and the time of formation of osazone differ for various sugars.



Phenyl gucosazone

The reaction of phenyl hydrazine with fructose is similar to glucose. Here again 3 molecules of phenyl hydrazine take part in the reaction. Fructose gives fructosazone. Disaccharides such as maltose and lactose also exhibit the property of osazone formation. But, sucrose does not form osazone, since it does not contain free CHO or CO groups which are responsible for the reducing property.

9. Fermentation

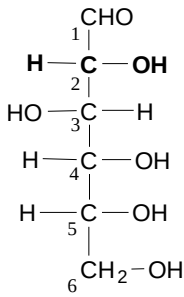
Fermentation is the process of converting a larger complex molecule into simple molecules by means of enzymes in an anaerobic condition. The products of the reaction are alcohol and CO_2.

$$\text{Glucose} \xrightarrow{\text{Zymase}} \underset{\text{Ethyl alcohol}}{2C_2H_5OH} + CO_2$$

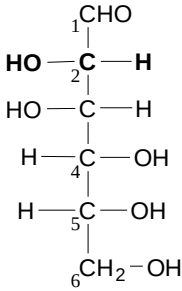
10. Epimerisation

Two sugars which differ from one another only in configuration around a single carbon atom are termed “epimers”

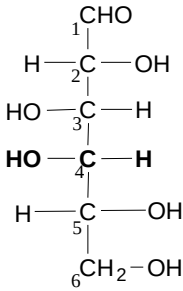
eg : Glucose and mannose are epimers in respect of C_2. Glucose and galactose differ only with respect to C_4. The process by which one epimer is converted to other is called as epimerization and it requires the enzyme epimerases in the living organisms. Galactose is converted to glucose by this manner in our body.



Glucose



Mannose

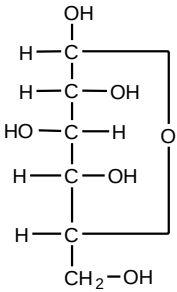


Galactose

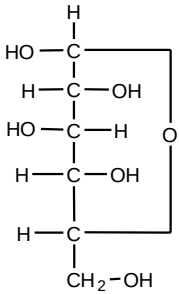
4.3.1.4 *Haworth projection formula of glucose and fructose*

According to Haworth, glucose exists in six membered ring structure called pyranoform (Fig.4.5) and fructose in five membered furano form. (Fig. 4.6)

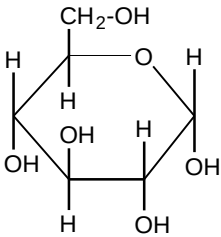
1. Glucose



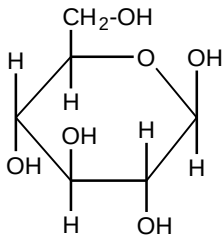
α D - Glucose



β D- Glucose



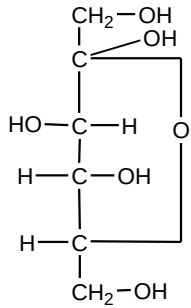
α D - Glucopyranose



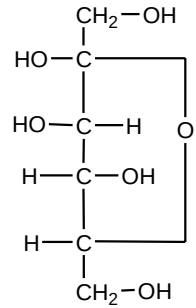
β D - Glucopyranose

Fig. 4.5 Pyranose structure of glucose

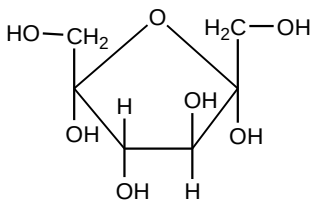
2. Fructose



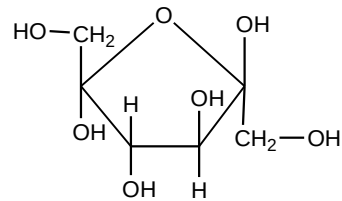
α D - Fructose



β D - Fructose



α D - Fructofuranose



β D - Fructofuranose

Fig. 4.6 Pyranose structure of fructose

4.3.2 Disaccharides

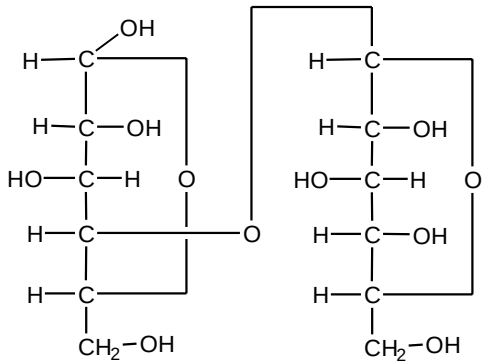
Disaccharides are sugars containing two molecules of monosaccharides. Disaccharides are formed by the condensation of two molecules of monosaccharides with the elimination of one molecule of water.

In disaccharides, monosaccharides are linked by the glycosidic bonds. The properties of the disaccharides depend to a great extent on the type of linkage. If the two potential aldehyde or ketone of both monosaccharides are involved in the linkage, the sugar will not exhibit

reducing properties and will not be able to form osazones. eg. Sucrose. But if one of them is not bound in this way, it will permit reduction and osazone formation by the sugars eg. lactose and maltose which are known as reducing disaccharides.

4.3.2.1 *Maltose*

Maltose is composed of two glucose molecules combined by α– 1,4 glycosidic linkage. It is commonly called malt sugar. Malt from sprouting barley is the major source of maltose. It is a rather sweet sugar and is highly soluble in water.



α D - Glucose (1) α D - Glucose (2)

(α 1,4 glycosidic linkage)

The structure of maltose shows that the potential aldehyde group of glucose -2 is blocked in the glycosidic linkage, whereas the potential aldehyde group of glucose -1 is free and can reduce alkaline copper solution. It is because of this free aldehyde in the first glucose molecule, maltose has reducing property.

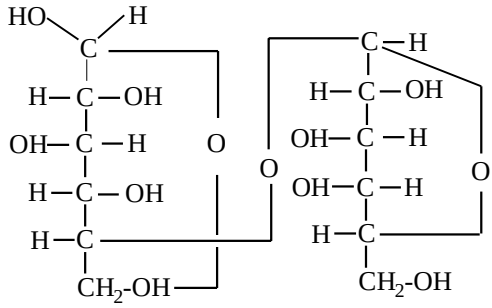
Metabolism

Maltose is the end product of digestion of starch by the action of salivary amylase, in the mouth and pancreatic amylase in the intestine. Maltose is formed as an intermediate product in the intestine. Maltose

is split into two molecules of glucose by the enzyme **maltase** of the intestinal juice before absorption.

4.3.2.2 *Lactose*

Lactose is commonly called as milk sugar. It is present in the milk of mammals. However lactose is found in the urine of pregnant and lactating women. It is less soluble in water and less sweeter than sucrose.



β D-glucose β D-galactose

(β 1-4 glycosidic linkage)

Just as in maltose, lactose has a free potential adehyde group in the glucose molecule, not involved in the glycosidic linkage between glucose and galactose molecules. Whereas, the potential aldehyde group of galactose molecule is blocked in the linkage. Because of the presence of free aldehyde group in the glucose molecule, lactose can reduce Fehling's solution and is therefore a reducing sugar.

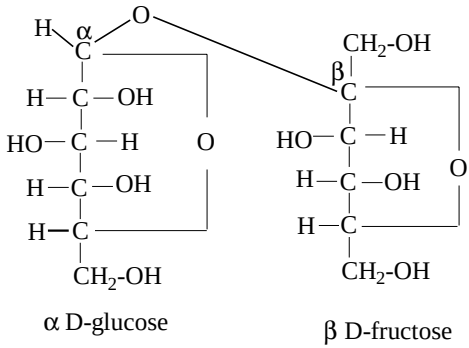
Metabolism

When lactose is hydrolysed by acids or by the enzyme **lactase**, one molecule of glucose and one molecule of galactose are formed. The intestine of milk sucking infants has the enzyme lactase, which converts lactose into glucose and galactose. Then only it is absorbed in the body. Excess of lactose ingested into the body causes diarrhoea,

abnormal intestinal flow and colic pain. Lactose is not fermented by yeast.

4.3.2.3 *Sucrose*

Sucrose is ordinary "table sugar". It is also called as "cane sugar" as it can be obtained from sugar cane. It is widely distributed in sugar cane, beet root, pine apple, honey, carrot and ripe fruits.



(α,β 1-2 glycosidic linkage)

Sucrose consists of one molecule of glucose and one molecule of fructose. The linkage between these molecules are formed between the aldehyde group of glucose and the ketone group of fructose. Thus, both the potential aldehyde group of glucose and the ketone group of fructose are blocked in the linkage and sucrose has no free reducing group. On account of this structural peculiarity sucrose is a non-reducing sugar. It does not reduce Tollen's and Fehling's solutions and does not form osazone.

Metabolism

Sucrose on hydrolysis by dilute acids or the enzyme **sucrase** or **invertase** gives a mixture of glucose and fructose. It is called as invert sugar.

Inversion

Sucrose is dextrorotatory (+62.5°) but it's hydrolytic products are levorotatory because fructose has a greater specific levo-rotation than the dextrorotation of glucose. As the hydrolytic products inverts the rotation, sucrose is known as invert sugar and the process is called as invertion. Honey contains plenty of 'invert sugar' and the presence of fructose accounts for the greater sweetness of honey.

4.4 Poly saccharides

Polysaccharides, which are also known as glycans composed of number of monosccharide units. They represent condensation products of several molecules of simple sugars or monosaccharides. Monosaccharides are linked together by glycosidic bonds in polysaccharides. They form linear chain or branched chain molecules. They contain only one type of monosaccharide units or many types of monosaccharide units. According to this nature polysaccharides are classified into two groups, homopolysaccharides and heteropolysaccharides.

Homopolysaccharides are composed of only one type of monosaccharides. On hydrolysis they yield only one type of monosaccharides Eg. starch, glycogen, cellulose etc. which yield only glucose on hydrolysis.

Heteropolysaccharides are composed of a mixture of monosaccharides. On hydrolysis, they yield a mixture of monosaccharides. Eg. Hyaluronic acid, Heparin, Mucopolysaccharides.

4.4.1 Starch

This is the storage form of carbohydrate present in plants. They are abundantly found in root, stem, vegetables, fruits and cereals. The bulk of our diet which consists mainly of rice, wheat and vegetables is a good source of starch.

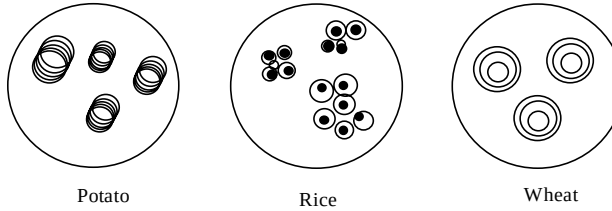


Fig. 4.7 Starch grains under microscope

Starch occurs in the forms of grain which may be spherical or oval in shape. Microscopically, the starch grains are found to differ in size and shape according to their sources (Fig.4.7).

Starch is made up of two structurally different homopolysaccharide units. They are amylose and amylopectin.

In amylose, glucose molecules are arranged in a linear form. Glucose molecules are arranged in a highly branched form in amylopectin.

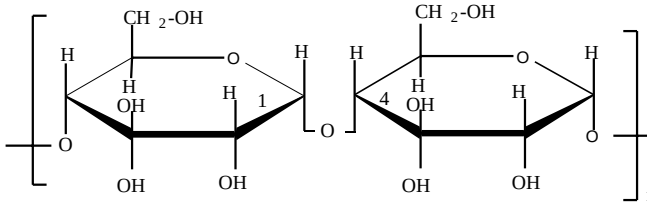


Fig. 4.8 Structure of Amylose (α 1-4 glycosidic linkage)

Amylose has 1,4 glycosidic linkages. The glycosidic - OH group of C_1 in one glucose unit is joined to that of C_4 of the next unit (Fig 4.8).

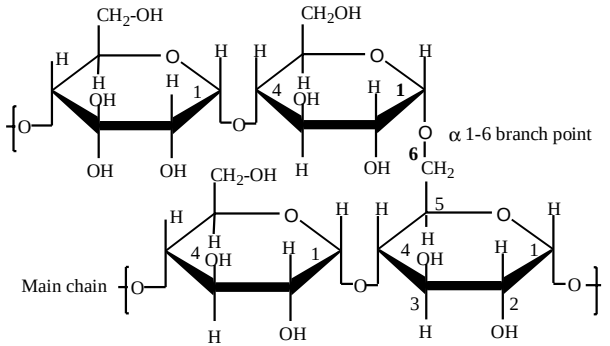


Fig. 4.9 Structure of Amylopectin (α 1-4 and α 1-6 glycosidic linkages)

The glucose unit present at each point of branching has subtituents at carbon atoms 1,4 and 6 i.e it has three points of attachment (Fig.4.9).

Reaction of starch with iodine

A solution of starch react with iodine to give blue colour. The blue colour formation is mainly due to the presence of amylose in starch.

Table 4.1 Difference between Amylose and Amylopectin

	Amylose	Amylopectin
1.	It has a simple unbranched structure	It has branched chain structure
2.	It is soluble in water	It is insoluble in water, can absorb water and swells up.
3.	It has α, 1-4 glycosidic linkages	It has α, 1-4 glycosidic and α, 1-6 glycosidic linkages
4.	Gives blue colour with dilute iodine solution	Gives yellow or orange colour with iodine solution
5.	The molecular weight ranges from 10,000 -50,000	The molecular weight ranges from 50,000 - One million

4.4.1.1 *Starch digestion occurs in stages*

Digestion of starch, the main carbohydrate source in the human diet, begins in the mouth. Saliva contains α amylase, which randomly hydrolyses all the α, 1-4 glycosidic bonds of starch except its outer most bonds and those next to branches.By the time thoroughly chewed food reaches the stomach, where the acidity inactivates α-amylase, the average chain length of starch has been reduced from several thousand to fewer than eight glucose units.

Starch digestion continues in the small intestine under the influence of pancreatic amylase which is similar to salivary amylase. This enzyme degrades starch to a mixture of disaccharide maltose and the trisaccharide maltotriose. These oligosaccharides are hydrolysed to their component monosaccharides by specific enzymes persent in the brush border membranes of the intestinal mucosa. The resulting monosaccharides are absorbed in the intestine and transported to the blood stream.

4.4.2 Glycogen

Glycogen is a homopolysaccharide since it gives only glucose units on hydrolysis. It is the major reserve carbohydrate in animals. Glycogen is present in all cells of skeletal muscle and liver and occur as cytoplasmic granules. Among plants, it is found in fungi and yeast. Primary structure of glycogen resembles that of amylopectin but glycogen is more highly branched, with branch points occurring every 8 to 12 glucose residues (Fig. 4.10). Glycogen is readily available as immediate source of energy. During starvation glycogen is mobilised from the storage tissue and converted to glucose by the enzyme glycogen phosphorylase. Glucose formed like this is oxidised to produce energy. The degree of branching is considerably more in glycogen when compared to starch.

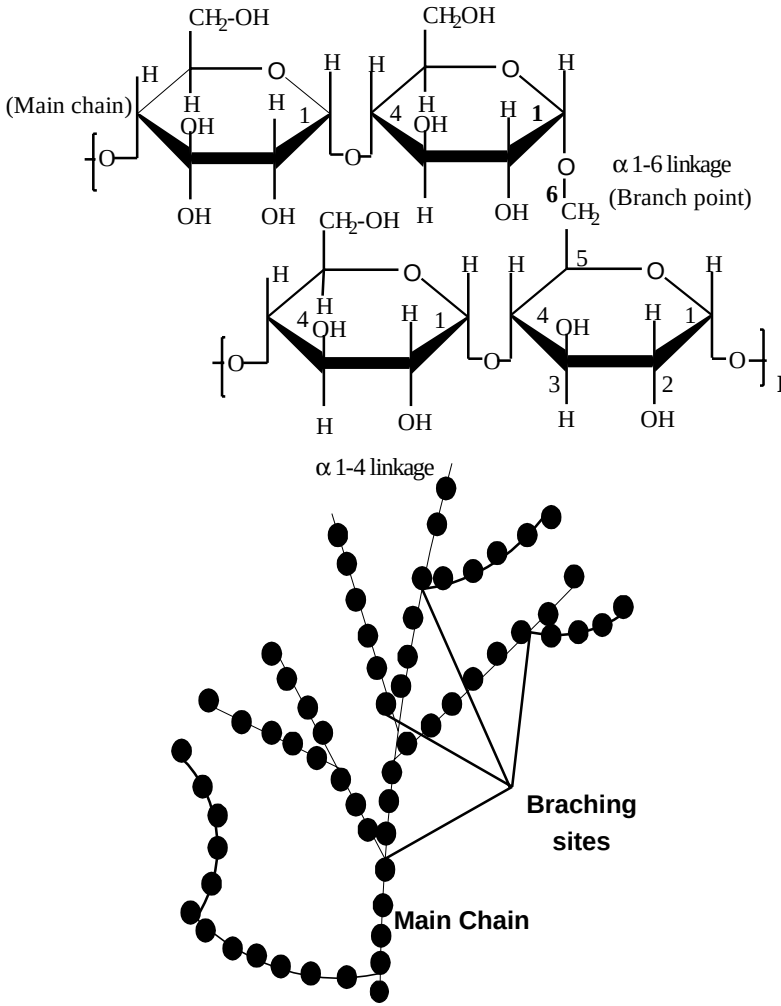


Fig. 4.10 Structure of glycogen

Formation of glycogen from glucose is called as **Glycogenesis** and breakdown of glycogen to form glucose is called as **Glycogenolysis.**

Excercise

I. Choose the correct answer from the given four alternatives

a. Glycogen is a

i. Monosaccharide ii. Disaccharide

iii. Homopolysaccharide iv. Heteropolysaccharide

b. Sucrose is a

i. Ketose sugar ii. Aldose sugar

iii. Dissaccharide iv. Polysaccharide

c. Glucose is oxidised by nitric acid and produce

i. Sorbitol ii. Saccharic acid

iii. Furfural iv. Glucuronic acid

d. The number of phenyl hydrazine molecules required for 1 molecule of glucose osazone formation is

i 1 ii. 2 iii. 3 iv. 4.

e. Iodine gives blue colour with

i. Glucose ii. Lactose

iii. Starch iv. Heparin

f. Starch is converted to maltose and dextrin by the enzyme.

i. Lipase ii. Protease

iii. Amylase iv. None of the above.

II. Fill up the blanks

a. Starch is composed of amylose and ----------

b. ---------- is known as animal starch.

c. Glucose on reduction with sodium amalgam produce ----------

d. Mannose is an epimer of ----------

e. Epimerisation reaction is catalysed by the enzyme ----------

f. Specific rotation of a optical isomer can be detected by --------

g. Separation of racemic mixture is called as ---------

III. Say true or false

a. Ribulose is a pentose

b. An aldohexose can exist in 16 isomeric forms.

c. In racemic mixtures d forms do not exist.

d. The basic monosaccharide units of lactose is glucose.

e. Glycogen yield only glucose on hydrolysis.

IV. Match the followings

a.	Glycogen	-	Invert sugar
b.	Asymmetric carbon atom	-	Starch
c.	Iodine test	-	Polysaccharides
d.	Sucrose	-	Animal starch
e.	Non sugars	-	Isomerism
f.	Lactose	-	Maltase
g.	Maltose	-	Milk sugar

V. Give short answer for the following:

a. What is the structural difference between glycogen and starch?

b. What is glycogenolysis?

c. Give the structure of sucrose.

d. What is epimerisation?

e. Mention the reaction between fructose and sodium amalgam.

f. What is racemic mixture?

VI. Answer the followings

a. Explain the classification of carbohydrates.

b. What are the functions of carbohydrates?

c. Explain the property of stereoisomerism in monosaccharides with two examples.

d. What are the reaction sequences of glucosazone formation?

e. Mention the difference between amylose and amylopectin.

f. Explain the mutarotation of glucose.

CHAPTER 5
PROTEINS

5.1 Introduction

Proteins are complex organic nitrogenous substances found in animal and plant tissues. The term protein is derived from Greek : Proteios means primary or holding first place.

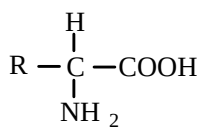
Protein is the essential constituent of living cells. Protein make upto 12% of the protoplasm. They are not only responsible for comprising the structure of the cell but are concerned with every function of the cell including those of respiration, catalysis of reactions by enzymes, transport of materials, regulation of metabolism, and defense actions. The foods rich in proteins are known as body building foods.

5.2 Sources of protein

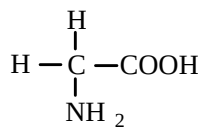
Proteins are obtained from animal and plant sources. The animal sources of proteins include milk, egg, meat, fish, liver etc. Plant sources of proteins are pulses, nuts and cereals.

5.3 Amino acids

Amino acids are the simplest units of a protein molecule and they form the building blocks of protein structure. The general formula of an amino acid can be written as,



R=H

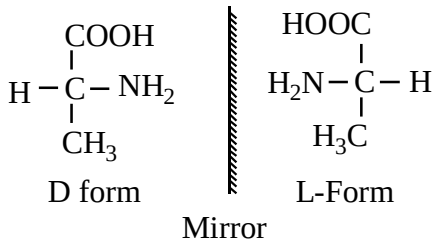


Glycine

An amino acid is an amino carboxylic acid. R is the side chain or residue and it represents the group other than $-NH_2$ and -COOH. It may be a hydrogen atom (H) or a methyl group ($-CH_3$) or an aliphatic group or an aromatic group or a heterocyclic group. The amino acids are classified based on the nature of R groups.

D and L amino acids

Based on the position of amino group on the asymmetric carbon atom, amino acids exist in two types. They are D and L amino acids.



The amino acid having the NH_2 group on the right is called D-amino acid. The amino acid having the NH_2 group on the left is called L-amino acid. These isomers are the mirror images of each other.

All amino acids are α amino acids because the NH_2 group is attached to the α carbon atom which is next to the COOH group. Examination of the structure of an amino acid except glycine, reveals that the α carbon atom has four different groups attached to it, thus making it asymmetric. Because of the presence of asymmetric carbon atom, amino acids exist in two optically active forms, dextrorotatory and levorotatory.

Dextrorotatory, they rotate plane polarised light in the clockwise direction. Levorotatory, they rotate plane polarised light in the anti clockwise direction. The direction of optical rotation of an amino acids indicated by the symbol + and - (+ indicates dextro and - indicate levo).

It has been found that L-amino acids are more common than D forms and most of the naturally occuring amino acids are L-amino acids. Therefore L-amino acids are called natural aminoacids. Since the L amino acids are more common, the letter "L" is usually omitted, while representing L-amino acids.

Amino acids are widely distributed in plants and animals.

5.3.1 Properties of amino acids

5.3.1.1 *Physical properties*

Amino acids are coloureless, crystalline, generally soluble in water, in acid and in alkali but sparingly soluble in organic solvents.

5.3.1.2 *Chemical properties*

i. Ionic forms of amino acids

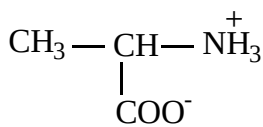
Amino acids bear atleast two ionizable weak acid groups, a - COOH and a $-NH_2$. In solution, two forms of these groups, one charged and another uncharged, exist in protonic equilibrium:

$$R\text{-}COOH \rightleftharpoons R\text{-}COO^- + H^+$$

$$R\text{-}NH_3^+ \rightleftharpoons R\text{-}NH_2 + H^+$$

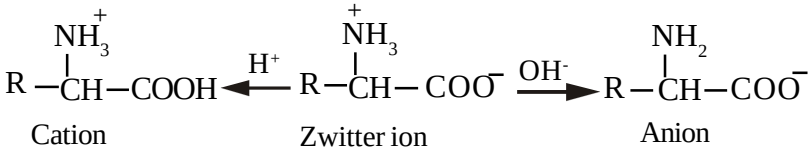
$R\text{-}COOH$ and $R\text{-}NH_3^+$ represent the protonated or acidic partners in these equilibria. $R\text{-}COO^-$ and $R\text{-}NH_2$ are the conjugate bases of the corresponding acids.

ii. Zwitterion



Zwitter ionic structure of alanine

Due to the presence of an acidic and a basic group in the same molecule, the amino acid exists largely as a dipolar ion or a zwitterion, which can react as an acid as well as a base. In zwitterion, the proton from the carboxylic group is transferred to the amino group. Thus a zwitterion carries both positive and negative charges.



In acidic solution an amino acid behave like a protonated derivative and therefore migrates to the cathode under electric field. In an alkaline medium, the same amino acid behaves like an anion derivative and therefore migrates to the anode.

iii. Isoelectric point

The net charge (the algebraic sum of all the positively and negatively charged groups present) of an amino acid depends upon the pH, or proton concentration of the surrounding medium.

The pH at which an amino acid bears no net charge and hence does not migrate to either of the anode or cathode under the influence of an electric current, is known as the isoelectric point or isoelectric pH.

iv. Reaction with ninhydrin

Ninhydrin oxidatively decarboxylates an amino acid to CO_2, NH_3 and an aldehyde. The reduced ninhydrin then reacts with the liberated ammonia forming a purple complex, which absorbs light at a wavelength of 570 nm.

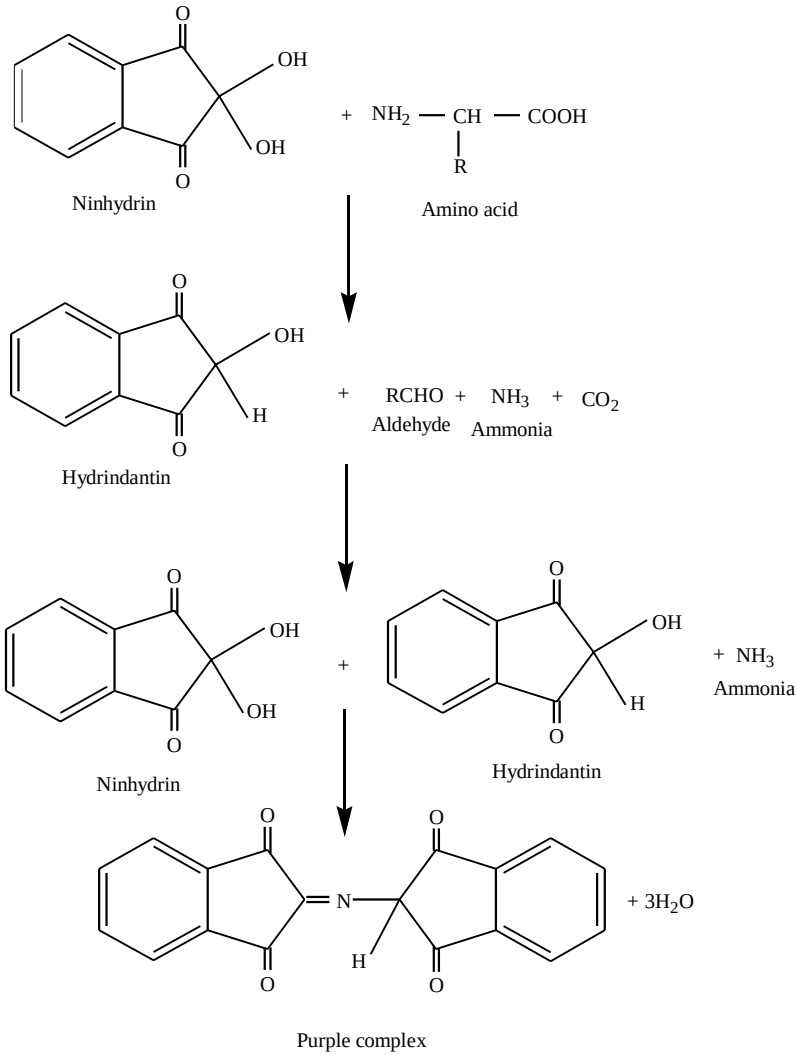


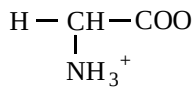
Fig. 5.1 Reaction of an amino acid with ninhydrin

5.3.2 Classification of amino acids

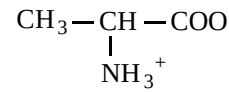
The amino acids are classified based on the nature of their R groups, in particular their polarity or tendency to interact with water at biological pH. The polarity of the R groups varies widely, from totally non polar to highly polar.

5.3.2.1 *Non-polar, aliphatic R-group*

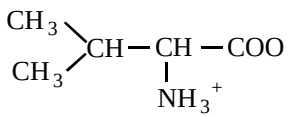
The R-group in this class of amino acids are non polar (or) hydrophobic. Six amino acids come under this class, which are **glycine, alanine, valine, leucine, isoleucine** and **methionine.**



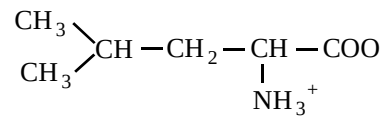
Glycine



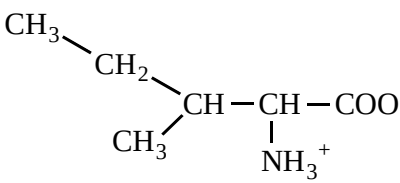
Alanine



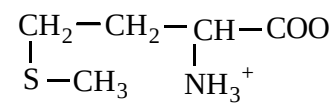
Valine



Leucine



Isoleucine

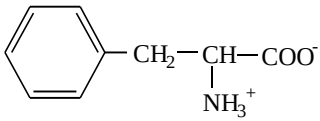


Methionine

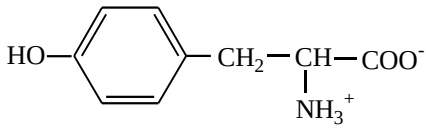
Glycine has the simplest structure. Methionine is one of the two sulphur containing aminoacids and has a non polar thio ether group in its side chain.

5.3.2.2 *Aromatic R groups*

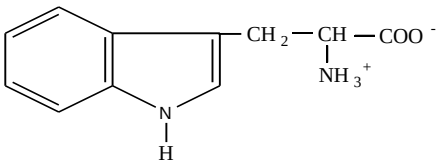
Phenylalanine, tyrosine and **tryptophan,** with their aromatic side chains, are relatively non-polar. All can participate in hydrophobic interactions. The hydroxyl group of tyrosine can form hydrogen bond with other compounds and it is an important functional group in some enzymes. Tyrosine and tryptophan are significantly more polar than phenyl alanine because of the hydroxyl group of tyrosine and the nitrogen of the indole ring in tryptophan.



Phenylalanine



Tyrosine



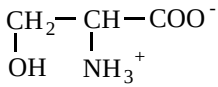
Tryptophan

5.3.2.3 *Polar-uncharged R groups*

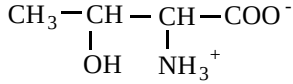
The R groups of these amino acids are more soluble in water or more hydrophilic, than those of the non polar amino acids because they contain functional groups that form hydrogen bonds with water. This class of amino acids includes **serine, threonine, cysteine, proline, asparagine** and **glutamine.**

The polarity of serine and threonine is contributed by their hydroxyl groups; that of cysteine by its sulphydryl (-SH) group; and that of asparagine and glutamine by their amide groups. Proline has a distinct cyclic structure and is only moderately polar. Proline has an imino group. Cysteine is readily oxidized to form a covalently linked dimeric amino

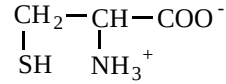
acid called **cystine**, in which two cysteine molecules are joined by a disulphide bond.



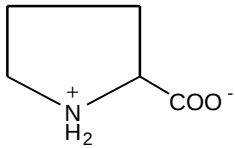
Serine



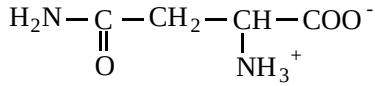
Threonine



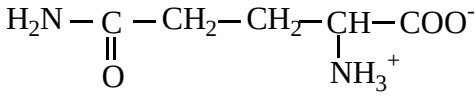
Cysteine



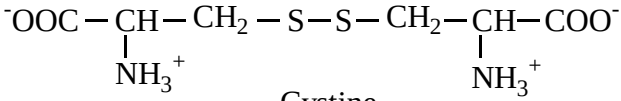
Proline



Asparagine



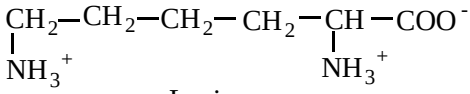
Glutamine



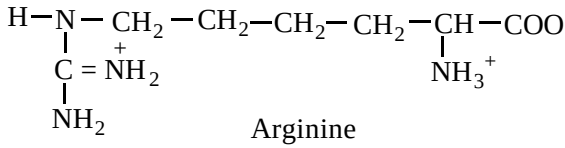
Cystine

5.3.2.4 *Positively charged (basic) R-groups*

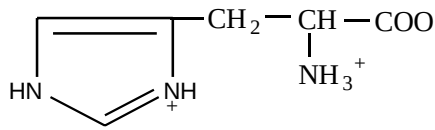
The most hydrophilic R groups are those that are either positively ($-NH_3^+$) or negatively ($-C00^-$) charged. The amino acids in which the R groups have significant positive charges at pH 7.0 are **lysine, arginine** and **histidine.**



Lysine



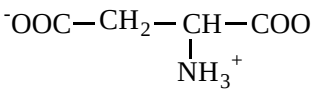
Arginine



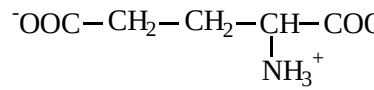
Histidine

5.3.2.5 *Negatively charged (Acidic) R groups*

The two amino acids having R groups with a net negative charge at pH 7.0 are aspartate and glutamate.



Aspartic acid



Glutamic acid

Based on their inclusion in the diet, amino acids are classified into two groups, namely essential amino acids and non-essential amino acids.

5.3.2.6 *Essential amino acids*

Certain amino acids can not be synthesized by the living organisms. They must be compulsarily included in the diet for normal health.These amino acids are called essential amino acids. For human being about 10 amino acids are considered as essential.eg,

1. Arginine
2. Histidine
3. Isoleucine
4. Leucine
5. Lysine
6. Methionine
7. Phenyl alanine
8. Threonine
9. Tryptophan
10. Valine

5.3.2.7 *Non-essential amino acids*

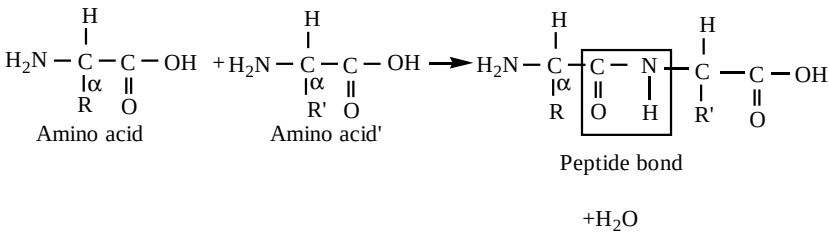
Certain amino acids can be synthesized in the cells from essential amino acids or from other compounds. So these amino acids need not be included in the diet. They are called non-essential amino acids.

5.3.2.8 *Non protein amino acids*

Certain amino acids which do not exist in proteins are called non protein amino acids eg. Ornithine and β-alanine etc..

5.3.2.9 *Peptide bonds*

In proteins, amino acids are linked together by linkages called peptide bonds. The carboxyl group of one amino acid is joined to the α amino group of another amino acid by a peptide bond.



The peptide bond is also called as the amide bond. The two amino acids, joined by a peptide bond, constitute a dipeptide. The dipeptide is formed by simple condensation reaction.

The product formed by a peptide bond is called a peptide. The compound formed by the linking of three amino acids is called as tripeptide. A peptide formed of less than 10 amino acids constitute an oligopeptide. More than 10 amino acids join together to form a polypeptide chain (Fig. 5.2).

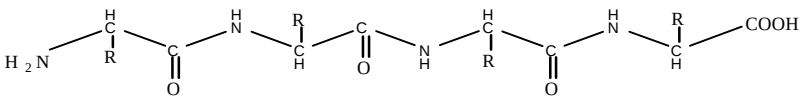


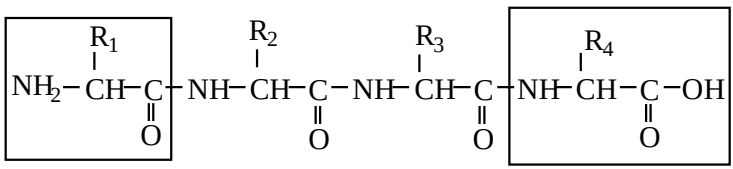
Fig. 5.2 Structure of a polypeptide

Protein is made up of one or more polypeptide chains

Many proteins, such as myoglobin, consist of a single polypeptide chain. Others contain two or more chains, which may be either identical or different. For example haemoglobin is formed of 4 polypeptide chains, of which two α chains are of one kind and the other two β chains are of another kind.

N and C terminal ends of protein

An amino acid in a polypeptide is called a residue. A polypeptide have two ends, namely amino and carboxyl terminal end. The end of the polypeptide chain containing amino group is called amino terminal or N-terminal. The end of the polypeptide chain containing carboxyl group is called carboxyl terminal or C-terminal. The terminal amino acid with the free amino group is called N-terminal amino acid and the terminal amino acid with the free carboyl group is called C-terminal amino acid.



N-terminal amino acid C-terminal amino acid

R_1, R_2, R_3 and R_4 - Side chains

5.4 Properties of proteins

5.4.1 Physical properties

1. Colour and taste

Proteins are colourless and usually tasteless. These are homogeneous and crystalline.

2. Solubility

Solubility of proteins is influenced by pH. Solubility is lowest at isoelectric point and increased with increasing acidity of alkalinity.

3. Optical activity

All protein solutions rotate the plane polarised light to the left i.e. these are levorotatory.

4. Colloidal nature

Because of their giant size, the proteins exhibit many colloidal properties are:

i. Their diffusion rate is extermely low.

ii. They may produce considerable light-scattering in solution, thus resulting in visible turbidity (Tyndall effect).

5. The comparatively week forces responsible for maintaining secondary, tertiary and quarternary structure of proteins are readily distrupted with resulting loss of biologic activity. This distruption of native structure is termed denaturation. Physically, denaturation may be viewed as randomizing the conformation of a polypeptide chain without affecting its primary structure (Fig.5.3).

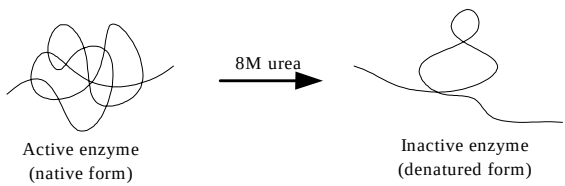


Fig. 5.3 Denaturation of protein

The biological activity of most proteins is destroyed by exposure to strong mineral acids or bases, heat, urea, acetone, alcohol and ionic detergents etc. Denatured proteins are less soluble in water.

5.4.2 Chemical properties

1. Hydrolysis

   i. By acidic agents

Proteins upon hydrolysis with concentrated mineral acids such as, HCl yield amino acids in the form of their hydrochlorides.

   ii. By proteolytic enzymes

Under relatively mild conditions of temperature and acidity, certain proteolytic enzymes like **pepsin** and **trypsin** hydrolyse the proteins. Enzyme hydrolysis is used for the isolation of certain amino acids like tryptophan. Two important drawbacks with this type of hydrolysis are:

   a. It requires prolonged incubation and

   b. Hydrolysis may be incomplete

2. Colour reaction with Biuret reagent

When a protein solution is treated with alkaline $CuSO_4$ reagent, the peptide bonds present in the protein interact with copper ions and forms **violet** coloured Biuret complex (Fig.5.4). The colour deepens which depend on the number of peptide bond present in the protein. The sturcture of the voilet complex is

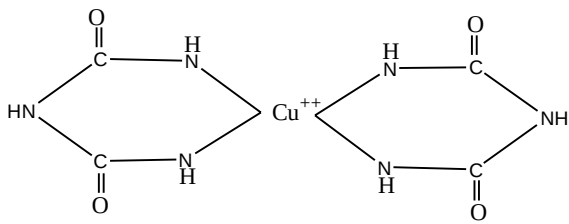
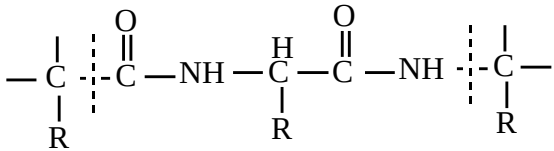


Fig.5.4 Voilet colour complex

All proteins except dipeptides react with Biuret reagent because a minimum of two peptide linkages are involved in this reaction.



This reaction is widely used both as a qualitative test for the detection of proteins and also as a quantitative test for the estimation of protein in biological materials.

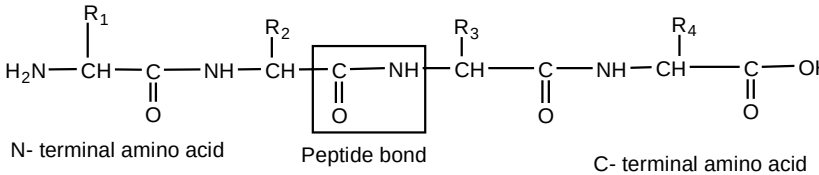
5.5 Protein structure

The architecture of protein molecule is complex but well organised. To understand this, a clear idea of certain basic details regarding the mode of arrangement of the structural units inside the molecule is necessary. Linder strom - Lang suggest four types of structural organisation for proteins. They are

1. Primary structure
2. Secondary structure
3. Tertiary Structure and
4. Quarternary strucutre

5.5.1 Primary structure

The primary structure of protein is defined as the sequence of amino acid residues making up its polypeptide chain. The protein may be formed of one or more polypeptide chains. The amino acids are arranged in specific sequence in these polypeptide chain. The amino acid residues are linked by peptide bonds. The peptide bond is formed between the carboxyl group of one amino acid and the amino group of adjacent amino acid. Some times the adjacent polypeptide chains are linked by disulphide bonds.



For example the primary structure of a protein can be written as

$$H_2N-\underset{1}{gly}-\underset{2}{lys}-\underset{3}{leu}-\underset{4}{val}-\underset{5}{ala}-\underset{6}{glu}-COOH$$

Each polypeptide chain of any length has at one end a N-terminal amino acid containing free amino group and at the other end a C-terminal amino acid containing a free carboxyl group. The amino acids in a polypeptide chain are numbered from the N-terminal end.

The primary structure has the following salient features

i. Primary structure refers to the linear sequence of amino acid residues.

ii. The proteins are linear and unfolded

iii. The protein is formed of one or more polypeptide chains.

iv. The amino acid residues are linked by repeating polypeptide bonds.

v. The adjacent polypeptide chains are linked by disulphide bonds.

vi. Most of the structural proteins which are in the form of fibres exhibit primary structure

vii. The primary structure provides informtion on the number and proportion of different amino acids in a protein.

Primary structures of a large number of proteins have been determined.

eg. i. human insulin has 51 amino acids distributed in two poly peptide chains. A chain-31 amino acids, B chain-20 amino acids and the polypeptides are linked by disulphide bridges.

ii. cytochrome C contains 104 amino acids.

iii. human serum albumin contains 584 amino acids.

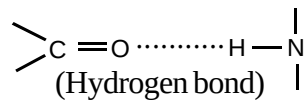
Primary structure is ultimately responsible for the native structure of the protein.

5.5.2 Secondary structure

The peptide chain thus formed assumes a two-dimentional secondary structure by way of folding or coiling consisting of a helically coiled, zig-zag linear or mixed form. It results from the steric relationship between amino acids located relatively near to each other in the peptide chain. The linkages or bonds involved in the secondary structure formation are hydrogen bonds and disulphide bonds.

i. Hydrogen bond

These are weak, low energy non-covalent bonds sharing a single hydrogen by two electronegative atoms such as O and N. Hydrogen bonds are formed in secondary structure by sharing H-atoms between oxygen of —C(=O)— and nitrogen of —N(H)— of different peptide bonds.



(Hydrogen bond)

The hydrogen bonds in secondary structure may form either an α-helix or β-pleated sheet structure.

ii. Disulphide bond

These are formed between two cysteine residues. They are strong, high energy covalent bonds.

Proteins exist in the two forms of secondary structure, α helix and β pleated sheet.

5.5.2.1 *α-Helix*

A polypeptide chain forms regular helical coils called α-helix. These coils are stabilized by hydrogen bonds between carbonyl oxygen of

first amino and amide N of fourth amino acid residues. Thus in α-helix intra chain hydrogen bonding is present. The α-helices can be either right handed or left handed. Left handed α-helix is less stable because of the steric interference between the carbonyl group and the side chains. Only the right handed α-helix has been found in protein structure (Fig.5.5).

Fig. 5.5 α-Helix structure

Each amino acid residue advances by 0.15 nm along the helix and 3.6 amino acid residues are present in one complete turn. The distance between two equivalent points on turn is 0.54 nm and is called a pitch.

Small or uncharged amino acid residues such as alanine, leucine and phenyl alanine are often found in α-helix. More polar residues such as arginine, glutamate and serine may repel and destabilize α-helix. Proline is never found in α-helix.

Hair, nail, skin contain a group of proteins called keratins rich in α-helical structure.

5.5.2.2 *β-pleated sheet structure*

A conformation called β pleated sheet structure is thus formed when hydrogen bonds are formed between the carbonyl oxygens and

amide hydrogens of two or more adjacent extended polypeptide chains. Thus the hydrogen bonding in β pleated sheet structure is interchain. The structure is not absolutely planar but is slightly pleated due to the bond angles. The adjacent chains in β-pleated sheet structure are either parallel or antiparallel, (Fig.5.6) depending on whether the amino to carbonyl peptide linkage of the chains runs in the same or opposite direction.

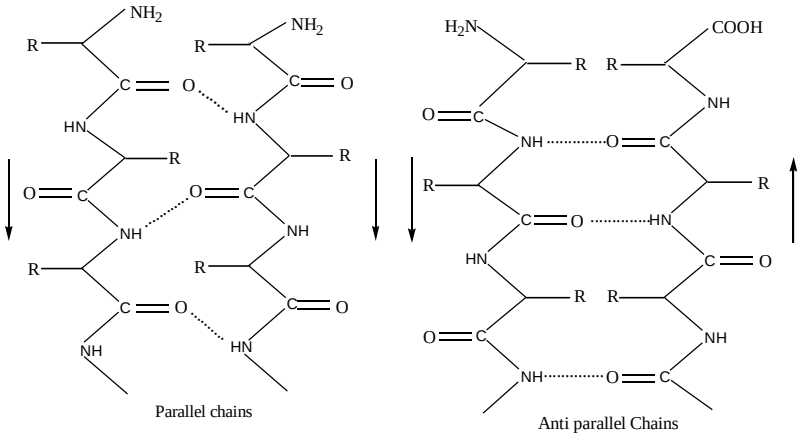


Fig. 5.6 β-pleated sheet structure

In both parallel and antiparallel β-pleated sheet structures, the side chains are on opposite sides of the sheet. Generally glycine, serine and alanine are more common to form β-pleated sheet. Proline occurs in β-pleated sheet although it tends to distrupt the sheets by producing links. Silk fibroin, a protein of silk worm is rich is β-pleated sheet.

5.5.3 Tertiary structure

The polypeptide chain with secondary structure may be further folded, super-folded, twisted about itself forming many sizes. Such a structural confirmation is called tertiary structure. It is only one such confirmation which is biologically active and protein in this conformation

is called as native protein. Thus the tertiary is constitued by steric relationship between the amino acids located far apart but brought closer by folding (Fig. 5.7). The bonds responsible for interaction between groups of amino acids are as follows.

i. Hydrophobic interactions

Normally occur between nonpolar side chains of amino acids such as alanine, leucine, methionine, isoleucine and phenyl alanine. They constitute the major stabilzing forces for tertiary structure forming a compact three-dimentional structure.

ii. Hydrogen bonds

Normally formed by the polar side chains of the amino acids.

iii. Ionic or electrostatic interactions

The interaction occurs between oppostively charged polar side chains of amino acids, such as basic and acidic amino acids.

iv. Vander -wall forces

Occurs between non polar side chains.

v. Disulphide bonds

These are S-S bonds formed between - SH groups of distant cysteine residues.

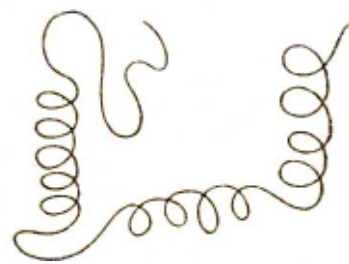


Fig. 5.7 Tertiary structure (eg. Myoglobin)

5.5.4 Quarternary structure

Some proteins are made up of more than one polypeptide chain These peptide chains held together by non-covalent interactions or by covalent cross - links it is referred to as the quarternary structure. The assembly is often called as an oligomer and each constituent peptide chain is called as a monomer or sub unit. The monomers of oligomeric protein can be identical or quite different in primary, secondary or tertiary structure (Fig. 5.8).eg:

Proteins with 2 monomers (dimer) eg. Creatine phosphokinase
Proteins with 4 monomers (tetramer) eg. Haemoglobin

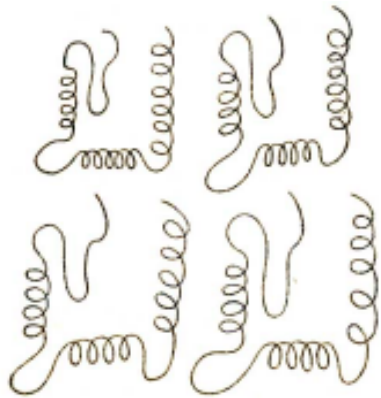


Fig. 5.8 Quarternary sturcutre (eg. haemoglobin)

5.6 Biologically important proteins

i. Glutathione is a tripeptide containing glutamic acid, cysteine and glycine. It is present in erythrocytes and several other tissues. It acts as a coenzyme and protects haemoglobin against oxidation.

ii. Insulin and glucagon are pancreatic hormones, involved in the regulation of glucose metabolism.

iii. Angiotensin is a peptide which stimulates the release of certain hormones from adrenal gland.

iv. Collagen, is a connective tissue protein rich in proline and hydroxy proline.

5.6.1 Plasma proteins

Plasma consists of many proteins such as albumin, globulin and fibrinogen. Total protein of the plasma is about 6-8gm /100ml. Plasma proteins comprise a major part of the solids of plasma. Albumin combine with substances of low solubilities such as cholesterol, triacylglycerol to form more soluble complexes, which can be transported in the an aqueous environment of the body fluids.

Excercise

I. Choose the correct answer from the given four altenratives

a. Phenyl alanine is a

i. Aromatic amino acid ii. Aliphatic amino acid

iii. Dipeptide iv. Glycoprotein

b. Essential amino acids

i. are synthesized in the body

ii. are not synthesized in the body

iii. are not used for protein biosynthesis

iv. are unstable

c. The primary structure of proteins is associated with this

i. Amino acid sequence

ii. β-pleated sheet structure

iii. Conformation

iv. Relative position of subunits

d. Protein denaturation results in

i. change of primary structure

ii. change of secondary structure.

iii. change of tertiary structure

iv. change in both secondary and tertiary structures.

e. The bonds responsible for the tertiary structure of proteins are

i. Hydrogen bonds

ii. Vander wall forces

iii. Disulphide bonds

iv. All the above.

II. Fill up the blanks

a. The simplest amino acid is ----------

b. All the amino acids exist in their ---------- form.

c. The functional group present in tryptophan is ----------

d. Guanidino group is present in the amino acid ----------

e. α helix nature of proteins tell about the ---------- structure of proteins.

f. ---------- amino acid is never found in α-helix

III. Say true or false

a. Proteins are not found in plants

b. Ornithine is a non-protein amino acid.

c. Glutamic acid has two carboxyl groups

d. Myoglobin can be analysed for its quaternary structure

e. A dipeptide has two peptide bonds.

IV. Match the followings

a.	Primary structure	-	Simplest amino acids
b.	Glycine	-	Guanidino group
c.	Zwitter ion	-	Amino acids sequence
d.	Arginine	-	Quaternary structure
e.	Hemoglobin	-	Tertiary structure
f.	Myoglobin	-	Denaturing reagent
g.	Urea	-	Isoelectric point

V. Give short answer for the followings

a. What is a peptide bond?

b. Give the D form of glycine.

c. Write the structure of Leucine.

d. How are proteins denatured?

e. Give the salient features of primary structure of a protein.

f. Name the any of two biologically important proteins with their functions.

VI. Answer the followings

a. Give the classification of aminoacids.

b. Give the structure of aromatic amino-acids.

c. What are essential amino acids? Give examples.

d. Explain the secondary and tertiary structure of proteins.

e. What are the salient features of primary structure of proteins?

f. Write the reaction of an amino acid with ninhydrin reagent?

g. What is the action of Biuret reagent on proteins?

CHAPTER 6

LIPIDS

6.1 Introduction

Lipids form a group of organic compounds which are widely distributed in living organisms. Chemically they are esters of fatty acids or are capable of forming esters. The important lipids are triacyl glycerols, phospholipids, sterols and fatty acids.

6.2 Fatty acids

Fatty acids are carboxylic acid with hydrocarbon side chains. They are the simplest form of lipids and they are water soluble. They exist in the body either as free acids or fatty acyl esters such as triacylglycerol. The fatty acids are released from these lipids on hydrolysis by lipases.

6.2.1 Classification

Fatty acids may be divided into (i) saturated fatty acids and (2) unsaturated fatty acids.

6.2.1.1 *Saturated fatty acids*

These are fatty acids which donot contain double bonds. They have general formula $C_nH_{2n+1}COOH$ (Table 6.1).

Table 6.1 Saturated fatty acids commonly found in natural fats

Acid	Formula	Carbon atoms
Acetic	CH_3COOH	2
Propionic	C_2H_5COOH	3
Butyric	C_3H_7COOH	4
Caproic	$C_5H_{11}COOH$	6
Caprylic	$C_7H_{15}COOH$	8
Decanoic	$C_9H_{19}COOH$	10
Lauric	$C_{11}H_{23}COOH$	12
Myristic	$C_{13}H_{27}COOH$	14
Palmitic	$C_{15}H_{31}COOH$	16
Stearic	$C_{17}H_{35}COOH$	18
Arachidic	$C_{19}H_{39}COOH$	20
Behenic	$C_{21}H_{43}COOH$	22
Lignoceric	$C_{23}H_{47}COOH$	24

6.2.1.2 *Unsaturated fatty acid*

These are fatty acids which contain double bonds. They have general formula ($C_nH_{2n-1}COOH$). They are subdivided into

(a) Monounsaturated fatty acid : These are fatty acids containing one double bond. (eg) Oleic acid.

$$CH_3(CH_2)_7CH = CH(CH_2)_7COOH$$

Oleic acid

(b) Polyunsaturated fatty acid : These are fatty acids that contain more than one double bond.

(eg) linoleic acid, linolenic acid, arachidonic acid.

Importance

1. They act as energy stores and fuel molecules.
2. They are the major components of cell membrane.

6.2.1.3 *Essential fatty acid (EFA)*

The fatty acids that cannot be synthesised by the body and therefore should be supplied in the diet are known as essential fatty acids. Chemically they are polyunsaturated fatty acids (PUFA), namely linoleic acid, linolenic acid and arachidonic acid.

Structure

$$CH_3\ (CH_2)_4\ CH = CH\text{-}CH_2\text{-}CH{=}CH(CH_2)_7\ COOH$$

Linoleic acid

$$CH_3\ (CH_2)_4\ CH = CH\text{-}CH_2\text{-}CH{=}CH\ \text{-}CH_2\text{-}CH = CH(CH_2)_7\ COOH$$

Linolenic acid

$$CH_3\ (CH_2)_4\ CH = CH\text{-}CH_2\text{-}CH{=}CH\ \text{-}CH_2\text{-}CH = CH\text{-}CH_2\text{-}CH{=}CH\text{-}(CH_2)_3\ COOH$$

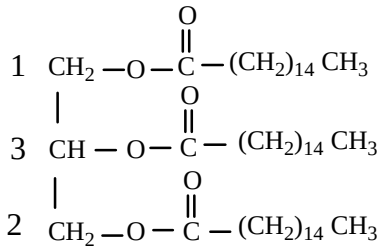
Arachidonic acid

Functions

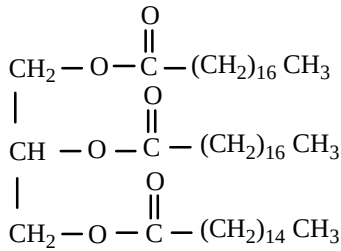
1. EFAs are requried for the membrane structure and functions.
2. They are necessary for the maintenance of growth, reproduction and good health.
3. They are important for the transport of cholesterol, formation of lipoportein and prevention of fatty liver.
4. They serve as precursor for prostaglandin biosynthesis.
5. They prolong clotting time and increase fibrinolytic activity.

6.3 Structure of triacyl glycerol

Triacylglycerols are simple lipids in which glycerol backbone is esterified with three fatty acids. This form the major part of dietary lipids. They are stored in adipose tissue and serve as concentrated fuel reserve of the body.



Tripalmitin (simple triacyl glycerol)



Distearopalmitin (mixed triacylglycerol)

If the three hydroxyl groups are esterified with same type of fatty acid then the lipid is called as simple glyceride. If the three hydroxyl groups are esterified with different type of fatty acids, the lipid is called as mixed glyceride.

6.3.1 Properties

6.3.1.1 *Physical*

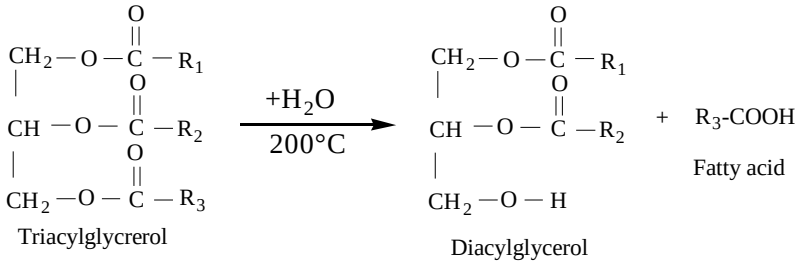
1. Triacylglycerols are non polar, hydrophobic molecules, insoluble in water, but soluble in organic solvents.
2. Specific gravity of fats is lower than water. Therefore fats and oils float on water.
3. Melting point of triacylglycerol is related to the chain length and degree of unsaturation of fatty acids. The longer the chain length, the higher the melting point and greater the number of double bonds, the lower the melting point.

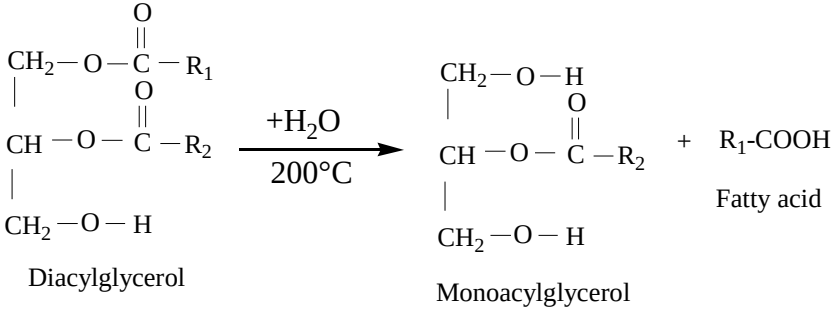
4. They are tasteless, odourless, colourless and neutral in solution.

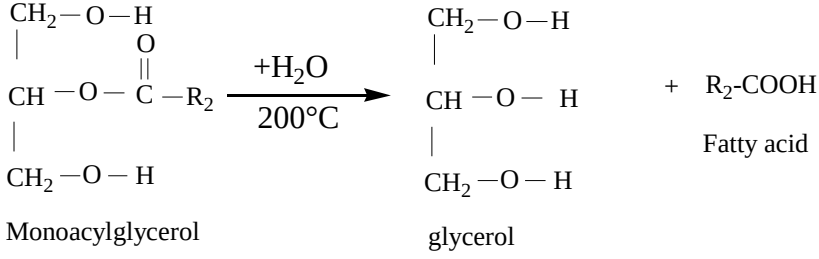
5. They are themselves good solvents for other fats.

6.3.1.2 *Chemical properties*

1. Hydrolysis : On boiling with water at 200°C, triacyl glycerols are hydrolysed to glycerol and fatty acids in a stepwise manner.







The reaction can also be catalysed by the enzymes lipases.

2. Hydrogenation

Hydrogenation of unsaturated fatty acids present in the fats, lead to the formation of saturated fats. Hydrogenation elevates the melting point. Thus an oil is converted to a solid fat.

$$\underset{\text{Oleic acid}}{CH_3(CH_2)_7CH = CH(CH_2)_7COOH} + H_2 \xrightarrow[\text{as catalyst}]{\text{Pt, Pd or Ni}} \underset{\text{Stearic acid}}{CH_3(CH_2)_7CH_2 - CH_2(CH_2)_7COOH}$$

This reaction is of great commercial importance since it permits transformation of inexpensive and unsaturated liquid vegetable fats into solid fats. The latter are used in the manufacture of candles, vegetable shortenings like vanaspathi and oleomargarine.

3. Saponification

Boiling with an alcoholic solution of strong metallic alkali hydrolyses triacyl glycerol into soap and fatty acid. This process is called as saponification.

$$\underset{\text{Triolein (a fat)}}{\begin{array}{l} CH_2 - OOC\,C_{17}H_{33} \\ | \\ CH - OOC\,C_{17}H_{33} \\ | \\ CH_2 - OOC\,C_{17}H_{33} \end{array}} + \underset{\text{Sodium hydroxide}}{\begin{array}{l} Na\ OH \\ Na\ OH \\ Na\ OH \end{array}} \xrightarrow{\text{Saponification}} \underset{\text{Glycerol}}{\begin{array}{l} CH2 - OH \\ | \\ CH - OH \\ | \\ CH2 - OH \end{array}} + \underset{\text{Sodium oleate (a soap)}}{3C_{17}H_{33}.\ COONa}$$

Soaps are important cleansing agents. Their cleansing property is due to their emulsifying action. This is accomphlised by means of negative charge the soap anion confers on oil droplets. The electrostatic repulsion

then prevents the coalescene of soap and results in the removal of dirt particles.

4. Halogenation

Unsaturated fatty acids in the triacyl glycerol take up chlorine, bromine and iodine atoms at their double bonds to form saturated halogenated derivatives.

$$\underset{\text{Oleic acid}}{CH_3(CH_2)_7CH=CH(CH_2)_7COOH} + I_2 \xrightarrow[\text{Room temperature}]{\text{Acetic acid / methanol}} \underset{\text{9,10 diiodo stearic acid}}{CH_3(CH_2)_7\underset{\underset{I}{|}}{CH} - \underset{\underset{I}{|}}{CH}(CH_2)_7COOH}$$

5. Rancidity

On storage, unsaturated fatty acids present in the fat are likely to undergo oxidation and hydrolytic cleavage in the presence of lipases present in the fat itself or secreted by the contaminating microorganisms. This leads to change of colour and odour of the fat. This change is called as rancidity. This occurs due to the formation of peroxides at the double bonds of unsaturated fatty acids. Rancidity can be prevented by certain antioxidants such as vitamin -E, gallic acid, butylated hydroxy toluene etc.Vegetable oils are less rancid because of the presence of natural antioxidants such as vitamin E and carotenoids.

6.3.2 Quantitative tests

There are certain chemical constants used for the characterisation of fats.

6.3.2.1 *Acid Number*

It is the number of milligrams of KOH required to neutralize the free fatty acids present in 1 gm of fat. The acid number, thus, tells us about the quantity of free fatty acid present in a fat. Obviously, a fat

which has been processed and stored properly has a very low acid number.

6.3.2.2 *Saponification number*

It is the number of milligrams of KOH requried to saponify 1 gm of fat. The saponification number, thus, provides information on the average chain length of the fatty acids in the fat. The saponification number varies inversely with the chain length of the fatty acids. The shorter the average chain length of the fatty acids, the higher is the saponification number.

6.3.2.3 *Iodine number*

It is the number of grams of iodine absorbed by 100 gm of fat. The iodine number is, thus, a measure of the degree of unsaturation of the fatty acids in the fat. The iodine number gives no indication as to the number of double bonds present in the fatty acid molecules.

6.3.2.4 *Polenske number*

It is the number of millilitres of 0.1 N KOH required to neutralize the insoluble fatty acids. This indicates the level of non volatile fatty acids present in the fat.

6.3.2.5 *Reichert - Meissl number*

It is the number of millilitres of 0.1 N KOH required to neutralize the soluble, volatile fatty acids derived from 5 gm of fat. The Reichert-Meissl number thus, measures the quantity of short chain fatty acids in the fat molecule.

6.3.2.6 *Acetyl number*

It is the number of milligrams of KOH required to neutralize the acetic acid obtained by saponification of 1 gm of fat after it has been acetylated. The acetyl number is, thus, a measure of the number of OH groups in the fat.

6.4 Phospholipids

Phospholipids are compound lipids containing phosphoric acid in addition to fatty acid, alcohol and a nitrogenous base.

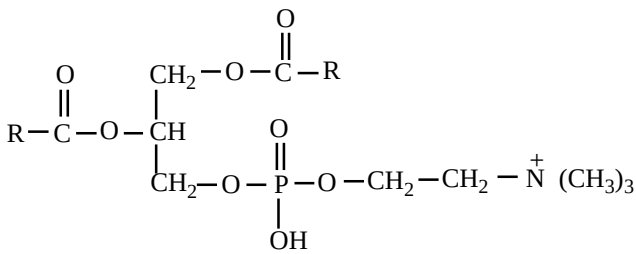
6.4.1 Classification

Phospholipids are classified into two types.

1. Glycerophospholipids (or) Phosphoglycerides that contain glycerol as alcohol.
2. Sphingophospholipids that contain sphingosine as alcohol.

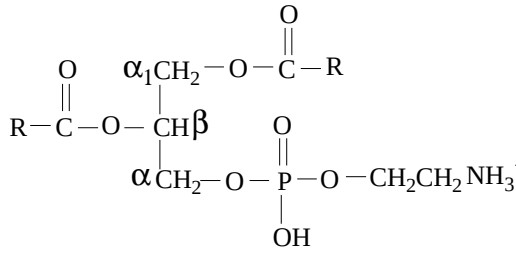
6.4.1.1 *Glycerophospholipids*

These are the major lipids that occur in biological membranes. They present in all plant and animal cells. They are abundantly present in heart, brain, kidney, egg yolk and soyabean. The important glycerophospholipids are lecithin, cephalin, phosphotidyl inositol, cardiolipin and plasmalogen.



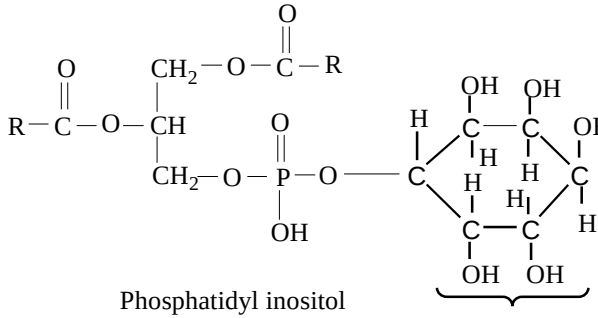
Lecithin

The lecithins contain glycerol, fatty acids, phosphoric acid and choline (nitrogenous base). Lecithins generally contain a saturated fatty acid at α_1 position and an unsaturated fatty acid at β postition. .



Cephalin

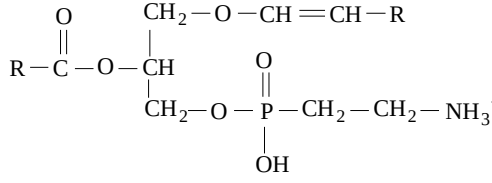
The cephalin contains glycerol, fatty acids, phosphoric acids and ethanol amine as nitrogenous base.



Phosphatidyl inositol

Inositiol

Phosphatidyl inositol contains a hexahydric alcohol called as inositol.

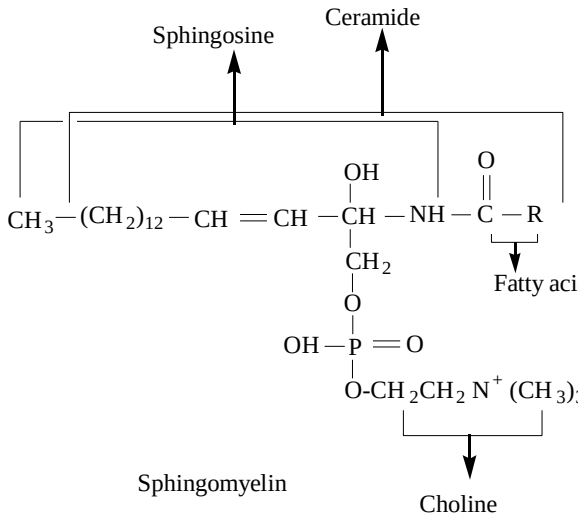


Plasmalogen

Plasmalogens posses an ether link in α_1 position instead of ester link. The alkyl radical is an unsaturated alcohol and they are found in brain and nervous tissue

6.4.1.2 *Spingophospholipids*

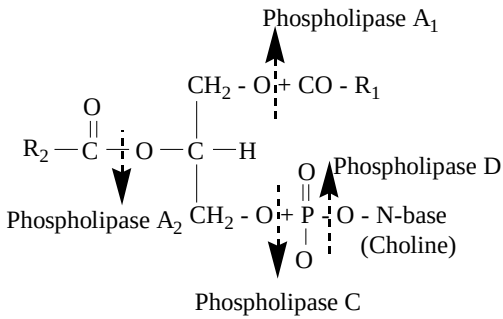
These are present in plasma membrane and myelin sheath. They are amphipathic lipids having polar head and non-polar tail. They contain an amino alcohol called shingosine. It is attached to a fatty acid by an amide linkage to form ceramide. Ceramide is linked to phosphoryl choline to form sphingomyelin, which is an important member of sphingophospholipids.



6.4.2 Properties of glycero phospholipids

1. Glycerophospholipids are white waxy substances, which become dark when exposed to air and light, owing to autoxidation and decomposition. This is due to the presence of unsaturated fatty acids in the molecules.

2. They are soluble in alcohol and other fat solvents except in acetone.
3. They are hygroscopic and mix well with water to form cloudy, colloidal and slimy solutions.
4. They donot have definite melting point and decompose when heated.
5. They are readily hydrolysed by boiling with acids and alkalies to their constituents.
6. They are hydrolysed by enzyme phospholipase to various components.



Phospholipase A_2 produces lysolecithin which is a potent hemolytic agent which degrade red blood cells.

6.4.3 Properties of sphingophospholipids

1. They are white crystalline substances
2. They form opelescent suspension in water.
3. They are insoluble in fat solvents like ether and acetone.
4. They are stable in air and light.

6.4.4 Importance of phospholipids

1. They form the structural components of membrane and regulate membrane permeability.
2. They play an important role in cellular respiration.

3. They participate in the absorption of fat from the intestine.
4. They act as surface tension lowering agent.
5. They are essential components of bile where they act as detergents and help in the solubilisation of cholesterol.
6. They also participate in blood clotting.
7. They protect and insulate the neuronal fibres of myelin sheath.
8. They are involved in the interaction of hormones with receptors.
9. They can act as lipotropic agents and prevent fatty liver formation.
10. They help in the reverse transport of cholesterol.

6.5 Sterols

Sterols are compounds containing a cyclic nucleus namely cyclopentanoperhydro phenanthrene (CPPP) and one or more hydroxyl groups. They are widely present in animal and plant tissue.

6.5.1 Cholesterol

Cholesterol is exclusively found in animals and is the most abundant animal sterols. It is widely distributed in all cells and is a major component of cell membrane and lipoproteins. In human beings, it is very important to control the normal level of cholesterol in blood.

6.5.1.1 *Structure*

Cholesterol is a C27($C_{27}H_{46}O$) compound. It has one hydroxyl group at C3 and a double bond between C5 and C6. An aliphatic side chain is attached to C17. Cholesterol contains a total of 5 methyl groups (Fig. 7.1).

Cholesterol is the precursor of various physiologically important compounds such as bile acids, vitamin-D, steroid hormones etc.

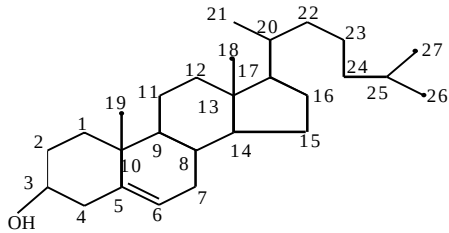


Fig. 7.1 Structure of cholesterol

6.5.1.2 *Properties*

1. They are white shining rhombic plate like crystals.
2. It is tasteless and odourless
3. It has a high melling point of 150°C.
4. It is insoluble in water and soluble in fat solvents.
5. It is a poor conductor of heat and electricity and serves as an insulator against electric charge. In brain, where it is present abundantly, it acts as an insulator against nerve impulse which are electrical in nature.
6. Cholesterol, when oxidised under suitable conditions, undergoes rapid oxidation to form a ketone-cholestenone.
7. The hydroxyl group of cholesterol readily forms ester with fatty acids, stearic acid etc.
8. It gives addition reactions such as hydrogenation and halogenation because of the presence of double bond.

6.5.1.3 *Physiological importance of cholesterol*

1. It is one of the essential constituents of cells.
2. It influences the permeability functions of the cell.
3. It controls the redcells from being easily hemolyzed.

4. It performs defensive action.

5. It assists the formation of bile acids and bile salts, 7-dehydrocholesterol, vitamin D3, corticosteroid hormones, androgens, estrogens and progesterone.

6. It acts as an antagonist to phospholipid.

6.5.2 Ergosterol

Ergosterol occur in plants. It is also found in yeast and fungi as the structural constituent of membranes. It is an important precursor for vitamin-D. When exposed to light, it is converted to ergocaliciferol, a compound containing vitamin-D activity.

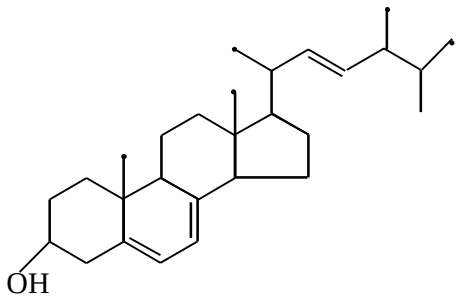


Fig. 7.2 Structure of ergosterol

Its structure is similar to that of cholesterol, but differs in the following aspects.

1. It has another double bond at C7-C8.

2. It has a double bond in the side chain.

3. It has an additional CH_3 group in the side chain (Fig 7.2).

6.5.3 Stigma sterol - It is structurally similar to that of ergosterol except at C7 (Fig. 7.3)

Stigmasterol and its derivatives sitosterols are probably the most common sterol of plants. The important sources are soya bean and calabar beans.

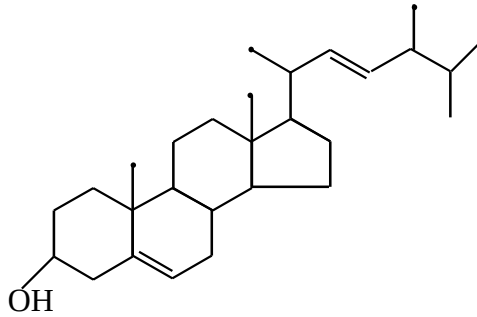


Fig. 7.3 Structure of stigmasterol

Excercise

I. Choose the correct answer from the given four alternatives

a. Triolein is a

i. mixed glyceride ii. simple glyceride

iii. fatty acid iv. phospholipid

b. Stearic acid is

i. an unsaturated fatty acid ii. a saturated fattyacid

iii. essential fatty acid iv. a simple lipid

c. The number of double bonds present in arachidonic acid is

i. 1 ii. 2 iii. 3 iv. 4

d. Cholesterol is present in

i. animals ii. plants iii. yeast iv. fungi

e. $C_nH_{2n-1}COOH$ is the general formula for

i. saturated fatty acids ii. unsaturated fatty acids

iii. simple lipids iv. phospholipids

II. Fill up the blanks

a. A fat with high acid number is said to be _____

b. Choline is present in _____

c. Cerebroside is a _____

d. _____ is an essential fatty acid.

e. Fats are _____ in water.

III. Say true or false

a. Oleodibutyrin is a simple glyceride

b. Iodine number denotes unsaturation in lipids

c. Sphingolipid contains glycerol

d. Rancidity of fats can be prevented by antioxidants.

e. Ergosterol can be converted to vitamin D.

IV. Match the following

1. Cholesterol	-	Unsaturated fatty acids
2. Oleic acid	-	Essential fatty acids
3. Lecithin	-	Simple glyceride
4. Tripalmitin	-	Phospholipid
5. Arachidonic acid	-	Animal sterol

V. Give one word answer

a. Name the nitrogenous base present in sphingolipids

b. Mention any one hydrolytic product of lecithin

c. What is the hydrogenated product of triolein

d. Name the parent compound present in sterols.

e. How many methyl groups are present in cholesterol.

VI. Answer the followings

a. Explain the structure and the properties of triacylglycerol.

b. Write a note on the structure and properties of phospholipids.

c. Explain the structure and functions of cholesterol.

d. What is ergosterol? Give the structure and functions of ergosterol.

e. Give the classification of fatty acids? Explain the functions of EFA and their structures.

CHAPTER 7

NUCLEIC ACIDS

7.1 Introduction

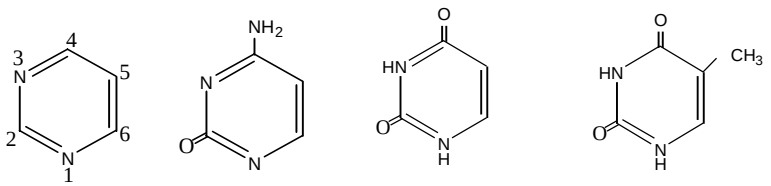
Nucleic acids are colourless, complex, amorphous compounds made up of three units : purine and pyrimidine bases, sugar and phosphoric acid. The nucleic acids are of two types DNA and RNA. It is very important to know the structure of nucleic acids and their components.

7.2 Pyrimidine bases

7.2.1 Structure of pyrimidine bases

Pyrimidine bases are derived from the parent compound pyrimidine.

The pyrimidine bases present in nucleotides are cytosine, uracil and thymine. It is 6 membered heterocyclic compound contains carbon, nitrogen, hydrogen and oxygen atoms (Fig 7.1).



Pyrimidine | Cytosine (2-oxy 4-amino pyrimidine) | Uracil (2,4, dioxy pyrimidine) | Thymine (2,4, dioxy 5methyl pyrimidine)

Fig. 7.1 Structure of pyrimidine bases

7.2.2 Properties of pyrimidine

1. Pyrimidine bases are soluble in water.
2. They absorb UV light at 260 nm. This property is used to detect and estimate pyrimidine nucleotides.

3. They are capable of forming hydrogen bonds with other purine bases
4. They exhibit keto-enol tautormerism.

7.3 Purine bases

7.3.1 Structure of purine bases

Purine bases are derived from the parent compound purine. Purine contains the heterocyclic ring system. Fusion of the pyrimidine ring with imidazole yields the purine ring (Fig. 7.2).

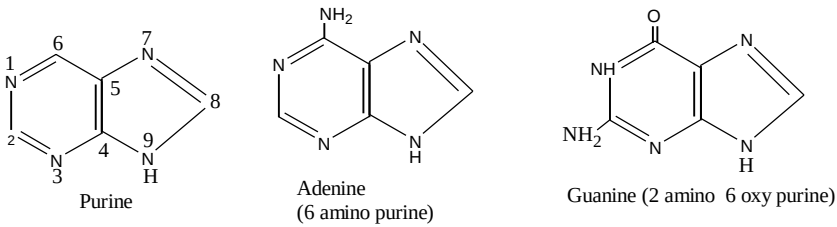


Fig.7.2 Structure of purine bases

The purine bases present in nucleic acids are adenine and guanine. Other purine bases are hypoxanthine and xanthine. They are intermediates in the formation of adenine and guanine nucleotides.

7.3.2 Properties of purine bases

1. Purine bases are sparingly soluble in water.
2. They absorb light in UV region at 260 nm. This property is used for the detection of and the quantification of purine nucleotides.
3. They are capable of forming hydrogen bonds.
4. They exhibit keto-enol tautomerism at body pH.

7.4 Nucleic acids

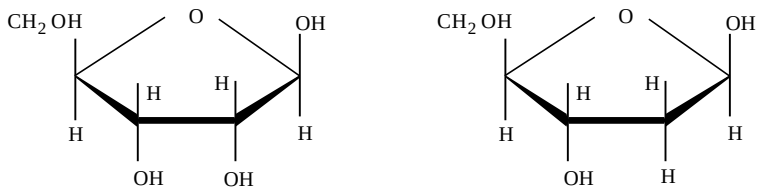
Two types of nucleic acids are present in all mammalian cells. They are DNA - deoxy ribonucleic acid and RNA- ribonucleic acid. DNA is present in the nucleus and mitochondria. RNA is present in the nucleus, risosome and cytoplasm.

Nucleic acids are acidic sustances containing nitrogenous bases, pentose sugar and phosphoric acid. Both DNA and RNA are polynucleotides. They are polymers of mononucleotides.

In nucleic acids, nucleotides are joined together by phosphodiester linkages.

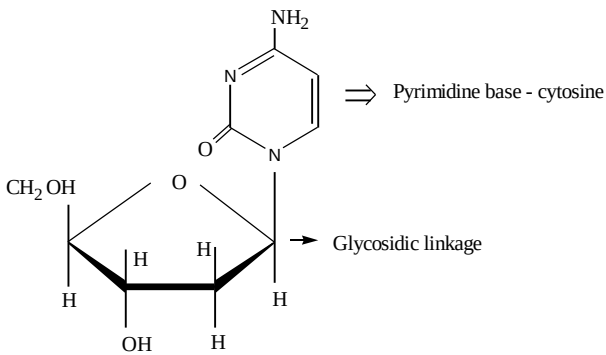
7.4.1 Nucleosides

A nucleoside is composed of purine or pyrimidine base and a pentose sugar. Two types of pentose sugar are present in nucleoside, they are ribose and deoxy ribose (Fig. 7.3). In the case of purine nucleosides, the sugar is attached to N-9 of the purine ring, whereas in pyrimidine nucleosides, the sugar is attached to N-1 of the pyrimidine ring. The type of linkage is N-glycosidic linkage (Fig. 7.4).



β-D-Ribose β-D-deoxyribose

Fig. 7.3 Structure of sugars in nucleic acids



A Nucleoside (cytidine)

Fig. 7.4 Structure of a nucleoside

7.4.2 Nucleotides

Nucleotides are phosphorylated nucleosides usually one or two of hydroxyl groups of ribose (or) deoxyribose are phosphorylated. Thus a nucleotide has three structural components. They are nitrogenous base, sugar and phosphate. Phosphate is attached to ribose (or) deoxy ribose through an ester linkage (Fig.7.5).

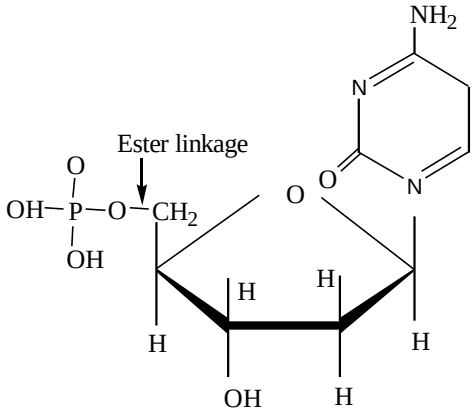


Fig. 7.5 Structure of a nucleotide (cytidylic acid)

7.4.3 Structure of DNA

7.4.3.1 *Primary structure*

Nucleotide sequence of a nucleic acid is known as its primary structure which confers individuality to the polynucleotide chain. Polynucleotide chain has direction. They are represented in 5'---> 3' and 3'----> directions. Each polynucleotide chain has 2 ends. The 5' end carrying a phosphate group and 3' end carrying an unreacted hydroxyl group (Fig 7.6).

In 1953, J.D. Watson and F.H.C. Crick proposed a precise three dimensional model of DNA structure based on model building studies, base composition and X-ray diffraction studies. This model is popularly known as the DNA double helix (Fig.7.7).

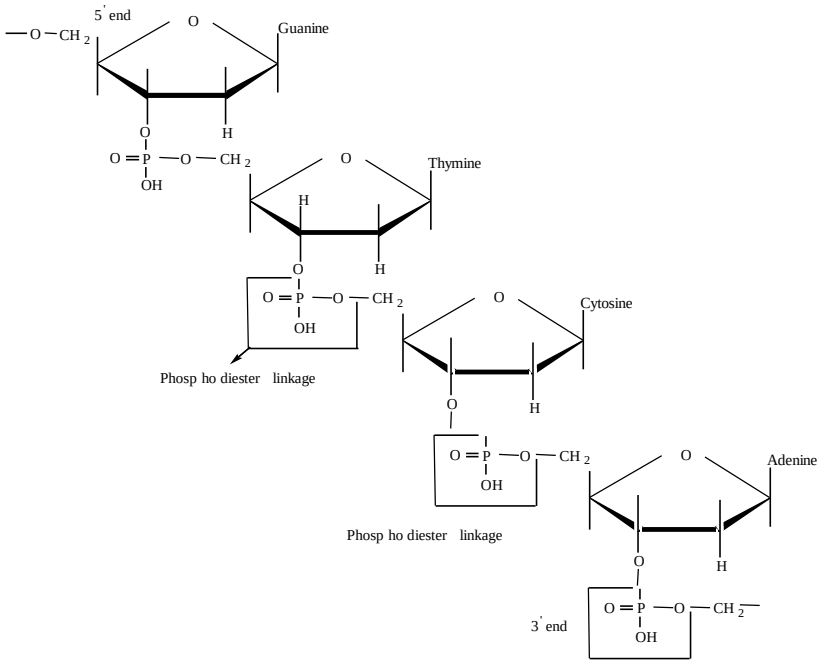


Fig. 7.6 Primary structure of DNA

The purine bases present in DNA are adenine and guanine and the pyrimidine bases present are thymine and cytosine. The purine and pyrimidine bases of DNA carry genetic information where as the sugar and phosphate groups perform the structural role.

7.4.3.2 *Salient features of double helix*

1. Two polynuleotide chains are coiled around a central axis in the form of a right handed double helix.

2. Each polynucleotide chain is made up of 4 types of nucleotides. They are adenylate, guanidylate, thymidylate and cytidinilate.

3. Each polynucleotide chain has direction or polarity. Further each polynucleotide chain has 5' phosphorylated and 3' hydroxyl ends.
4. The backbone of each strand consists of alternating sugar and phosphate. The bases project inwards and they are perpendicular to the central axis.
5. The 2 strands run in opposite direction (ie.) they are antiparallel.
6. The strands are complementary to each other. Base composition of one strand is complementary to the opposite strand. If adenine appears in one strand, thymine is found in the opposite strand and vice versa. When guanine is found in one strand, cytosine is present in the opposite strand and vice versa.
7. Bases of opposite strands are involved in pairing. Pairing occurs through hydrogen bonding and it is specific. Adenine pairs with thymine through two hydrogen bonds. Guanine pairs with cytosine with three hydrogen bonds.
8. Major and minor grooves are present on the double helix. They arise because glycosidic linkages of base pairs are not opposite to each other. Protein interact with DNA through the minor and major grooves without disrupting the DNA strands.
9. According to Chargaff's observation, the number of adenine base is equal to thymine base and the number of quanine base is equal to number of cytocine base ie. A = T and G = C. Also A + T = G + C and the ratio of A+T /G+C = nearly 1.0. The total number of purine bases = the total number of pyrimidine bases.

7.4.3.3 *Functions of DNA*

1. DNA is the genetic material of living organisms. It is the greatest super chip ever made by man.
2. DNA contain all the information required for the information of an individual organism.

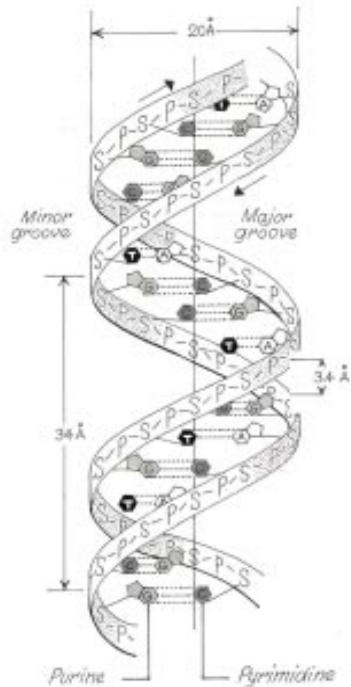
3. The genetic information in DNA is converted to characteristic features of living organisms like colour of the skin and eye, height, intelligence, ability to metabolise particular substance, ability to withstand stress, susceptibility to desease and ability to produce or synthesise certain substances.

4. DNA is the source of information for the synthesis of all cellular proteins. The segment of DNA that contain information for a protein is known as gene.

5. DNA is transmitted from parents to offsprings and hence transmit genetic information from one generation to another.

6. The amount of DNA in any given species or cell is constant and is not affected by nutritional and metabolic states.

Fig. 7.7 Double helical structure of DNA



7. Avery Macleod and Mc Carty in 1944 first demonstrated that DNA contained the genetic information and they referred DNA as 'transforming factor'.

8. The nucleotides present in DNA are deoxy adenylic acid, deoxy quanidylic acid, deoxy cytidylic acid and deoxy thymidylic acid.

7.4.4 Structure of RNA

RNAs are present in the nucleus, ribosomes and cytoplasm of eukaryolic cells. They are involved in the transfer and expression of genetic information. They act as primer for DNA formation. Some act as enzymes and as coenzymes. RNA also function as genetic material for viruses.

RNAs are also polynucleotides. In RNA polymer, purine and pyrimidine nucleotides are linked together through phosphodiester linkages. The sugar present is ribose. The nitrogenous bases present in RNA are adenine and guanine (purine bases), uracil and cytosine (pyrimidine bases). The nucleotides present in RNA are adenylic acid, quanidylic acid, cytidylic acid and uridylic acid.

7.4.4.1 *Types of RNA*

There are mainly three types of RNAs in all prokaryotic and eukaryotic cells. They are (1) Messenger RNA (mRNA) 2) Transfer RNA (tRNA) 3) Ribosomal RNA (rRNA). They differ from each other by size, formation and stability.

1. Messenger RNA

It accounts for 1-5% of cellular RNA. They have a primary structure. They are single standed linear molecules. They consist of 1000-10,000 nucleotides. They have a free or phosphorylated 3’ and 5’ end. They have different life span ranging from few minutes to days.

mRNA molecules are capped at 5’ end by methylated guanine triphosphate. Capping protects mRNA from nuclease attack. At 3’ end a polymer of adenylate (poly A) is found as the tail. Poly A tail protects mRNA from nuclease attack.

Intrastrand base pairing among complementary bases allows folding of the linear molecule. As a result, haripin or loop like secondary structure is formed.

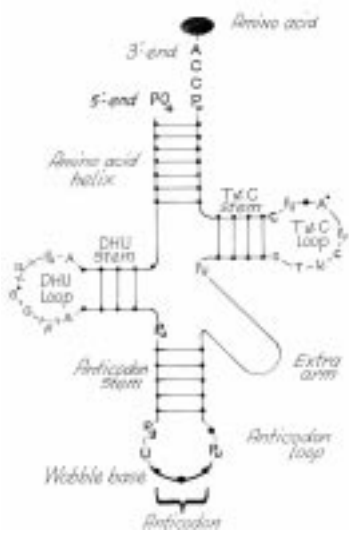
Functions

1. mRNA is a direct carrier of genetic information from the nucleus to the cytoplasm.
2. It contain information required for the synthesis of protein molecules.

2. Transfer RNA

It accounts for 10-15% of total cell RNA. They are the smallest of all the RNAs. Usually they consist of 50-100 nucleotides. They are single standard molecules. They contain unusual bases such as methylated adenine, guanine, cytosine and thymine,dihydrouracil and pseudouridine. These unusual bases are important for binding 6-RNA to intra chain base pairing. Further some bases are not involved in base pairing, resulting in loops and arms formation in tRNA. These folding in the primary structure generates a secondary structure (Fig.7.8).

Fig.7.8 Secondary Structure of t-RNA



Secondary structure of t-RNA is in the form of a clover leaf. The important feature of the clover-leaf structure are,

1. An acceptor arm with base sequence "CCA" 3'-OH of adenosine moiety of t-RNA.
2. An anticodon arm which recognises codon on mRNA.
3. TφC arm which contain unusual base cytosine.
4. D- arm which contain many dihydrouracil residues.

Functions

1. It is the carrier of amino acids to the site of protein synthesis.
2. There is at least one t-RNA molecule to each of 20 amino acids required for protein synthesis.

3. Ribosomal RNA

This accounts for 80% of the total cellular RNA. It is present in ribosomes. In ribosomes, r-RNA is found in combination with protein. It is known as ribonucleoprotein. The length of rRNA ranges from 100-600 nucleotides. rRNA molecules have a secondary structure. Intra strand base pairing between complementary bases generate double helical segments or loops.

Functions

1. They are required for the formation of risosomes
2. They are involved in the initiation of protein synthesis.

7.4.5 Differences between DNA and RNA

	DNA	RNA
1.	Sugar moiety is deoxy ribose	Sugar moeity is ribose
2.	Uracil is absent	Thymine is usually absent
3.	Double stranded molecules both run in opposite directions	Single stranded molecules
4.	Sum of purine and pyrimidine bases are equal G+C = A+T	Sum of purine and pyrimidine bases are not equal. G+C = A+T.
5.	Bases are not modified	Bases are modified
6.	Resistant to alkali hydrolysis	Highly susceptible to alkali hydrolysis.
7.	No catalytic activity	Some are catalytically active.
8.	Mostly DNA is present in nucleus and also in mitochondria	present in nucleolus and cytoplasm

7.4.6 Examples of nucleosides and nucleotides

Base	Nucleoside	Nucleotide
Adenine	Adenosine	Adenylic acid
Guanine	Guanosine	Guanidylic acid
Cytosine	Cytidine	Cytidylic acid
Thymine	Thymidine	Thymidylic acid
Uracil	Uridine	Uridylic acid

Excercise

I. Choose the correct answer from the given four alternatives

a. Thymine is exclusively present in

i. DNA ii. tRNA iii. mRNA iv. rRNA

b. Thymidynilate is a

i. purine base ii. nucleoside

iii. nucleotide iv. pyrimidine base

c. Anticodon arm is present in

i. DNA ii. tRNA iii. mRNA iv. rRNA

d. Adenine pairs with thymine

i. a single bond ii. double bond

iii. triple bond iv. none of the above

e. The alkali resistant nucleic acid is

i. DNA ii. tRNA iii. mRNA iv. rRNA

II. Fill up the blanks

a. Tφc arm is present in _____ nucleic acids

b. _____ nucleic acid is a genetic carrier

c. _____ is a nucleoside.

d. _____ RNA is derived from DNA.

e. Adenine is a _____ base.

III. Say true or false

a. Purine bases show maximum absorption at 260 nm

b. Uracil is a pyrimidine base.

c. The products formed when nucleosides are cleaved are nucleotides and nitrogenous bases.

d. Nucleases can cleave RNA.

e. DNA and RNA have similar structures.

IV. Match the following

1.	Cytosine	-	Purine base
2.	Thymine	-	Pyrimidine base
3.	t RNA	-	Adenylic acid
4.	Nucleotide	-	ATP
5.	Nucleoside	-	Amino acid activation

V. Give one word answer

1. what is a nucleoside?
2. What are purine bases?
3. What is phosphodiates linkage?
4. Which is the direction of DNa double strands?
5. Give the keto enol form of adenine.

VI. Answer the followings

1. Explain the structure of purine and pyrimidine bases.
2. Explain the primary structure of DNA.
3. List out the functions of DNa and RNA.
4. What are the salient features of Watson and Crick double helical DNA model .
5. Give the structure and the functions of tRNA.

CHAPTER 8
VITAMINS

8.1 Introduction

The vitamins are a group of complex organic compounds required in small quantities by the body for the maintenance of good health. They are not normally synthesized in the body and hence they should be supplied by the diet. The vitamins are present in foods in small quantities.

Unlike other groups of nutrients the vitamins are not chemically similar to each other. Each vitamin has a specific chemical structure and a specific function or functions in the living system. Most of the vitamins act as coenzymes in the body. Normally a well balanced diet will supply all the necessary vitamins in sufficient quantity.

8.2 Classification

Vitamins are generally classified into two main groups. (i) fat soluble vitamins (ii) water soluble vitamins.

8.2.1 Fat soluble vitamins

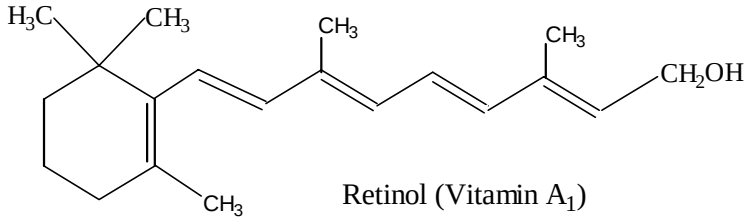
The members of this group are A,D,E and K. All these vitamins are not soluble in water and readily soluble in fat dissolving organic solvents. Inside the body for their transport and metabolism they need the presence of fats.

8.2.1.1 *Vitamin - A*

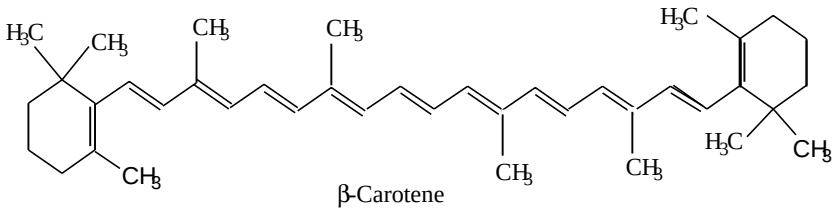
Vitamin A is found only in foods of animal origin. It is present in almost all species of fish, birds and mammals. The yellow plant pigments α, β and γ carotenes and cryptoxanthin are precursors of vitamin A. The body has the ability to convert these carotenoid compounds present in the diet into vitamin A.

The chemical structure of β - carotene is such that it oxidizes to form two molecules of vitamin A, the other provitamins form only one. β-carotene is more efficiently converted to vitamin A than α - or γ - carotene or cryptoxanthin.

There are two forms of vitamin A: Vitamin A_1 which occurs in the liver of marine water fish and Vitamin A_2 found in the liver of fresh water fish. The vitamin A which contain alcoholic group in the side chain is called as retinol (Fig. 8.1) and which contain aldehyde group is known as retinal. Though the two vitamins differ slightly in their chemical structures their physiological functions are the same.



Retinol (Vitamin A_1)



β-Carotene

Fig. 8.1 Structure of Vitamin A_1 and β-Carotene

Functions

Vitamin A is essential

i. for the growth and metabolism of all body cells

ii. for the formation of rhodopsin (visual purple) a complex substance formed from retinol and protein. Rhodopsin, a pigment found in retina is necessary for vision in dim light.

iii. for the maintenance of healthy skin, particularly mucous membrane of the cornea and the lining of respiratory tract.

Sources

The liver of any animal is a rich source of vitamin A. Fish liver oil is an excellent source. Whole milk, egg yolk, dark green leafy vegetables and deep yellow vegetables and fruits are rich in carotenes, which can be converted into vitamin A by the intestinal wall.

Requirements

Vitamin A requirement is based on the intake to maintain the normal blood level. Adults placed on a vitamin A free diet are found to show no change in the level for several weeks.The capacity of the body to store vitamin A provides for an effective emergency supply. Recommended amount of Vitamin A for different age group is as follows:

Infants	-	1500 IU / day
Children	-	2000-3000 IU / day
Adults	-	5000 IU / day
Pregnant and lactating women	-	6000-8000 IU / day
		(IU = International units)

Absorption and storage

Vitamin A and carotene are absorbed from the small intestine into the lymph system. The maximum absorption is reached 3 to 5 hours after consumption. The rate of absorption of vitamin A is more rapid than that of carotene. In the human being about 95% of the vitamin A stored in the body is found in the liver with small amount in the lungs, adipose tissue and kidneys.

Deficiency

The earliest sign of vitamin A deficiency is concerned with vision. Initially there is a loss of sensitivity to green light, followed by impairment to adapt to dim light. This condition leads to **night blindness**. More

prolonged or severe deficiency leads to the ulceration of cornea and this condition is known as **xerophthalmia** or **keratomalacia**.

8.2.1.2 *Vitamin D*

There are two distinct forms of Vitamin D

i. Cholecalciferol (Vitamin D_3) is the natural form of the vitamin occurring in foods (Fig. 8.2). It can be formed under the skin from 7 dehydro cholesterol by the influence of sun light (ultra violet (UV) radiation)

ii. Ergo calciferol (vitamin D_2) is a synthetic form of the vitamin which has the same activity as the natural vitamin. It is produced by the UV radiation of ergo sterol, a compound which can be extracted from yeast. This is the form of vitamin which is added to commodities such as margarine and baby foods.

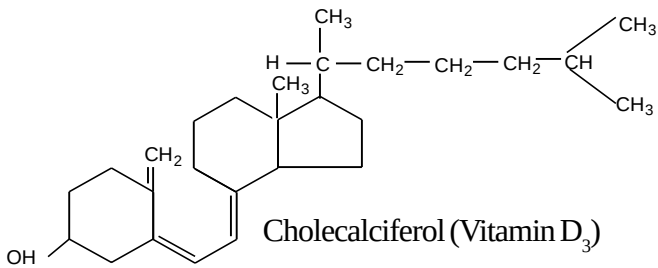


Fig. 8.2 Structure of Vitamin D

Functions

Vitamin D is necessary for the growth and maintenance of bones and teeth. Vitamin D helps in the absorption of calcium and phosphorus from the small intestine and for the uptake of calcium and phosphorus by the bones and teeth.

Sources

Vitamin D is not widely distributed in nature and the best sources are fish oil, especially liver oil. Milk, butter and egg yolk are the only foods in the ordinary diet that contain vitamin D.

Requirements

It is difficult to make standard recommendations for the dietary requirements of vitamin D since the amount of vitamin D produced in the body by the action of sunlight varies from person to person. Many people may obtain this vitamin from sunlight. It is, however certain that babies and growing children require more vitamin D than adults, due to rapid growth and bone development.

Infants and children	-	400 IU / day
Adults	-	200 IU / day
Pregnant and lactating women	-	400 IU/day

Absorption and storage

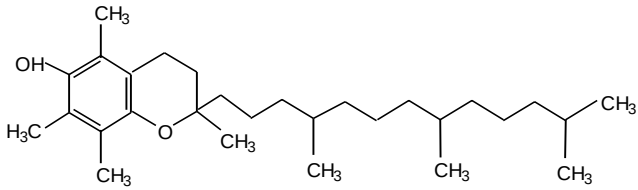
Fat helps in the absorption of vitamin D and bile is essential for its absorption. Vitamin D enters into the general circulation via lymph and stored largely in liver and kidneys.

Deficiency

Children receiving an inadequate supply of vitamin D develop **rickets**. Calcium and phosphorus are inadequately deposited in the bones. Premature infants are more susceptible to rickets than full term infants. In adults on inadequate supply of vitamin D causes **osteomalacia**, a condition in which the bones become soft, weak and painful.

8.2.1.3 *Vitamin E*

Vitamin E activity is possessed by a number of compounds known as tocopherols. Among these compounds α-tocopherol is known as vitamin E. Structurally vitamin E contains a dihydro benzopyran nucleus with an isoprenoid side chain (Fig. 8.3). Many of them are pale yellow oils soluble in fat.



α-Tocopherol

Fig. 8.3 Structure of Vitamin E

Functions

The principal role of vitamin E appears to be as an antioxidant. By accepting oxygen, vitamin E can prevent the oxidation of vitamin A in the intestinal tract, thus making vitamin A available for body use. Vitamin E also reduces the oxidation of the poly unsaturated fatty acids, thereby helping to maintain normal cell membrane. It protects the red blood cells against hemolysis. Vitamin E is required by animals, and presumably, by humans for the normal reproductive processes. It also plays an important protective role during ageing of cells.

Sources

Wheat germ oil and corn germ oil are the rich natural sources. Vegetable oils and fats are good sources. Cereals and animal foods are fair sources of tocopherol.

Requirements

It is difficult to establish vitamin E requirements. The requirement depends mainly on the intake of poly unsaturated fatty acids. It is generally accepted that the intake of vitamin E should be 0.4 mg α -tocopherol equivalents / g dietary poly unsaturated fatty acid. This does not present any problem, since all foods which are rich sources of poly unsaturated fatty acids are also rich sources of vitamin E.

There is some evidence that higher intake of vitamin E may have a useful protective effect against the development of ischaemic heart disease. This is because high concentrations of vitamin E inhibit the oxidation of poly unsaturated fatty acids in plasma lipoproteins and this oxidation which is responsible for the initiation of atherosclerosis (deposition of fat in the coronory artery walls). The levels that appear to be beneficial are of the order of 17-40 mg α -tocopherol/day, which is above what could be achieved by eating ordinary foods.

Adults : 25 - 30 mg / day

Absorption and storage

Vitamin E, like other fat soluble vitamins, is absorbed along with fat in the intestines. It is stored in the liver, muscle and body fat.

Deficiency

Vitamin E deficiency cause the following disorders in animals.

i. Reproductive failure

ii. Hemolysis of red blood cells

iii. Muscular dystrophy

8.2.1.4 *Vitamin K*

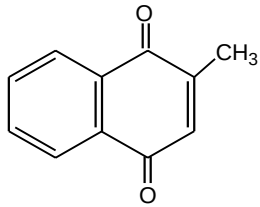
Vitamin K is known as the antihemorrhagic vitamin.

Three compounds which have the biological activity of vitamin K are

a. Phylloquinone, which is the normal dietary source, being found in green leafy vegetables.

b. Menaquinones, which are a family of closely related compounds synthesized by the intestinal bacteria, with differing lengths of the side chain.

c. Menadione, a synthetic compound which can be metabolised to yield phylloquinone (Fig 8.4).



Menadione

Fig. 8.4 Structure of Menadione (Vitamin K)

Functions

Vitamin K is needed for the formation of prothrombin, a substance necessary for blood clotting.

Intestinal bacteria normally synthesize substantial amount of vitamin K. Because vitamin K is fat soluble, its absorption is facilitated in the presence of bile. Small amount of vitamin k are stored in the liver, heart, skin, muscle and kidneys.

Sources

The best source of vitamin K are the green leafy vegetables eg. spinach, cabbage, kale etc. Good sources are cauliflower, wheat germ, etc. Carrots and potatos are fair sources. Milk, meat and fish are poor sources.

Requirements

Vitamin K requirement depends on the amount of vitamin K formed by the intestinal bacteria. The more the endogenous vitamin K formation less will be the dietary requirement.

Absorption and storage

Being fat-soluble, its absorption is enhanced by sufficient amount of bile salts mainly in the jejunum by the way of lymphatics. Liver stores appreciable amounts. It is present in blood stream in significant amount. All tissues contain small amounts of vitamin K.

Deficiency

The deficiency of vitamin K leads to a lowering of prothrombin level and increased clotting time of blood. This may lead to hemorrhagic conditions. Vitamin K deficiency causes hemorrhagic disease of the newborn.

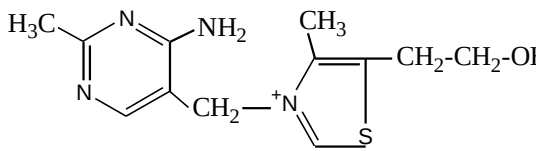
8.2.2 Water soluble vitamins

The members of this group are B complex vitamins and vitamin C. They are readily soluble in water.

8.2.2.1 *B Complex vitamins*

i. Thiamine (B_1)

The structure of vitamin B_1 is given in Fig. 8.5.



Thiamine (Vitamin B_1)

Fig. 8.5 Structure of Vitamin B_1

Functions

Thiamine is a coenzyme in many enzyme systems. These are involved principally in the breakdown of glucose to yield energy.

Thiamine also aids in the formation of ribose, a sugar that is an essential constituent of DNA and RNA, the carriers of the genetic code. The adequate level of thiamine provides healthy nerves, a good mental outlook, a normal appetite and food digestion.

Sources

Meats, especially pork and liver are rich in thiamine and account for about one fourth of the average intake. Dry beans, peanuts and egg are good sources.

Enriched and whole grain breads and cereals supply about one third of the daily thiamine intake.

Requirements

The requirement of thiamine depends on energy expenditure.

Infants	-	0.3 - 0.5 mg / day
Children	-	0.7 -1.2 mg / day
Adults	-	1.2 - 1.5 mg/day
Pregnant women and lactating women	-	1.3- 1.5 mg/day

Absorption and storage

Free thiamine is readily absorbed from the small intestine. Excess thiamine administered is not stored in the tissues. A part of the excess thiamine is excreted in urine and same of it is destroyed by the enzyme thiaminase.

Deficiency

The symptoms of thiamine deficiency occur because the tissue cells are unable to receive sufficient energy from glucose. Therefore, they cannot carry out their normal functions.

Early symptoms of thiamine deficiency include fatigue, irritability, depression and numbness of the leg and poor tone of the gastro intestinal tract together with constipation.

Beriberi, sometimes called “rice-eaters disease” is another deficiency symptom which is often seen in people whose chief diet is refined rice and is the most severe form of thiamine deficiency.

ii. Riboflavin (B_2)

Structurally vitamin B_2 consists of a ribitol moiety and a substituted isoalloxazine ring (Fig. 8.6).

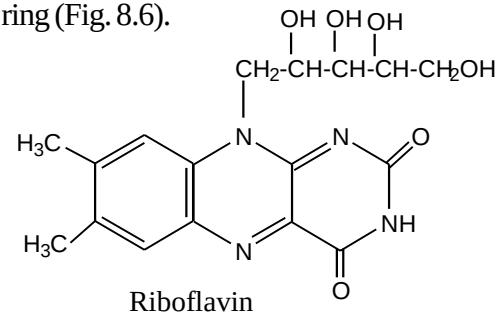


Fig. 8.6 Structure of Vitamin B_2

Functions

Riboflavin is a constituent of a group of enzymes called "flavoproteins". As with thiamine, the enzymes are necessary in the break down of the glucose to form energy. Riboflavin is essential for a healthy skin and for good vision in bright light. If the individual ingest more riboflavin than their body needs, the urinary excretion will increase, if the intake is inadequate, the body maintains its supply very carefully and the urinary excretion will practically stop.

Sources

About half of the intake of riboflavin daily is furnished by milk alone and cheese is a good source, although some of the vitamin has been lost in the whey.

Requirements

A more generous estimate of requirements is the level of intake at which there is normalisation of the activity of the red cell enzyme **glutathione reductase,** which is a flavoprotein whose activity is especially sensitive to riboflavin nutritional status.

Infants	-	0.4 - 0.6 mg / day
Children	-	0.8 - 1.2 mg / day
Adults male	-	1.5 -1.8 mg/day
Adults female	-	1.1 - 1.4 mg/day
Pregnant women	-	1.4 - 1.7 mg/day
Lactating women	-	1.6 - 1.9 mg / day

Absorption and storage

The vitamin is phosphorylated in the intestinal mucosa during absorption. It is absorbed from the small intestine through the portal vein and is passed to all tissues being stored in the body. The major part is excreted in urine and a small part is metabolized in the body.

Deficiency

Riboflavin deficiency leads to cheilosis, a cracking of the skin at the corners of the lips and scaliness of the skin around the ears and nose. There may be redness and burning as well as itching of the eyes, and extreme sensitivity to strong light.

iii. Niacin (B_3)

Niacin is pyridine-3-carboxylic acid (Fig. 8.7). It occurs in tissues as nicotinamide.

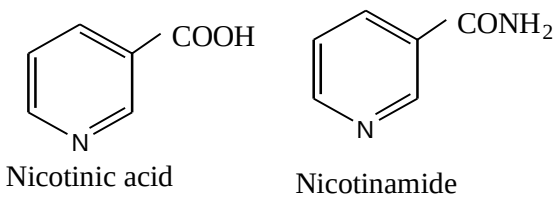


Fig. 8.7 Structure of Vitamin B_3

Functions

Niacin is required for the stepwise breakdown of glucose to yield energy. Niacin is essential for the healthy skin, normal functions of the gastro intestial tract and maintenance of the nervous system.

Sources

The meat group especially organ meats and poultry, is the chief source of preformed niacin. Dark green leafy vegetables, whole grain, enriched breads and cereals are fair sources.

Niacin is more stable to cooking procedures than thiamine or ascorbic acid (Vitamin C).

Requirments

The recommended niacin allowance is 6.6 mg/1000 k cal. This can be supplied by exogenous niacin in the diet and by the tryptophan, an essential aminoacid that is the precursor of niacin.

Infants	-	5 -8 mg / day
Children	-	9 - 16 mg / day
Adults male	-	16 - 20 mg/day
Adults female	-	12 - 16 mg/day
Pregnant women	-	14 - 18 mg/day
Lactating women	-	16 - 20 mg / day

Absorption and storage

Nicotinic acid and nicotinamide are absorbed from the intestine through the portal vein into the general circulation. Excess nicotinic acid is not stored in the body.

Deficiency

Pellagra is the major deficiency disease resulting from the lack of niacin. Dermatitis, especially of the skin exposed to the sun, soreness of the mouth, swelling of the tongue, diarrohea, and mental changes

including depression, confusion, disorientation, and delirium are typical of the advancing stages of the disease, which ends to death if not treated. (The disease is sometimes referred to as the "4D'S" - dermatitis, diarrohea, dementia and death).

iv. Pyridoxine (B_6)

Pyridoxine is 3-hydroxy 4,5 dihydroxy methyl 2- methyl pyridine (Fig 8.8) The metabollically active form of vitamin B_6 is pyridoxal phosphate.

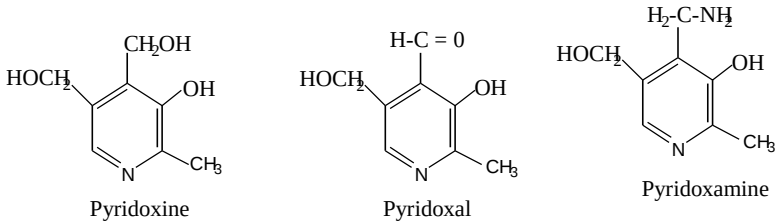


Fig. 8.8 Structure of Vitamin B_6

Functions

Three forms of vitamin B_6 exist in nature which are pyridoxine, pyridoxal and pyridoxamine. The functions of vitamin B_6 are closely related to protein metabolism, the synthesis and breakdown of amino acids, conversion of tryptophan to niacin, the production of antibodies, the formation of heme in hemoglobin, the formation of hormones important in brain function and others.

Sources

Meat especially organ meats, whole grain cereals, peanuts and wheat germ are rich sources. Milk and green vegetables supply smaller amounts.

Requirements

Although most of the body's vitamin B_6 is associated with glycogen phosphorylase in muscle, this is relatively stable and well conserved.

The requirement depends not on energy expenditure and glycogen metabolism, but on the intake of protein. The average requirement is 13 µg/g dietary protein.

Infants	-	0.3 mg / day
Children	-	0.6 - 1.2 mg / day
Adults	-	1.6 - 2 mg/day
Pregnant and lactating women	-	2.5 mg/day

Absorption and storage

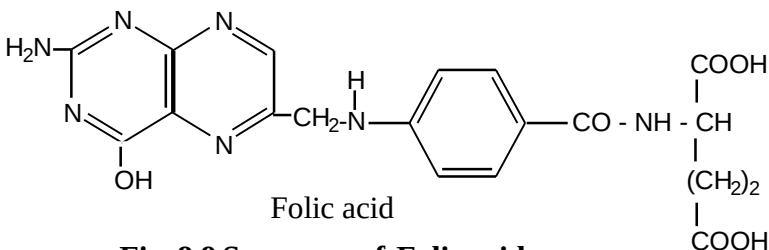
Pyridoxine is readily absorbed from the small intestine. The excess amount if ingested is not stored in the body but is excreted in urine.

Deficiency

Deficiency of vitamin B_6 is extremely rare. Nervous disturbances such as irritability, insomnia, muscular weakness, fatigue and convulsion have been recorded in infants. The cause of the convulsions severe impairment of the activity of the enzyme glutamate decarboxylase, which is dependent on pyridoxal phosphate. The product of glutamate decarboxylase is GABA **(γ–amino butyric acid)** which is a regulatory neurotransmitter in the central nervous system.

v. Folic acid

Folic acid contains a pteridine group linked to para amino benzoic acid and *l*-glutamic acid (Fig. 8.9). It is slightly soluble in water and stable to heat.



Folic acid

Fig. 8.9 Structure of Folic acid

Functions

i. Folic acid serve as coenzymes in reactions involving the transfer of one carbon units like formyl and methyl groups.

ii. It participates in the reactions concerned with the synthesis of purine, pyrimidine and nucleic acids.

iii. It is essential for maturation of red blood cells.

iv. Folic acid is required for the metabolism of amino acids like histidine.

v. Along with vitamin B_{12}, folic acid helps in the trans methylation reactions. eg: uracil to thymine.

Sources

Folic acid is particularly present in green leafy vegetables, cauliflower and dried yeast. Egg, liver and kidneys are rich animal sources.

Requirements

There is no definite requirement for normal human being. However, an increased amount is required during pregnancy and lactation.

Infants	-	50 µg / day
Children	-	100 - 300 µg / day
Adults	-	400 µg/day
Pregnant women	-	800 µg / day
Lactating women	-	600 µg/day

Absorption and storage

Absorption of folic acid takes place along the whole length of the mucosa of the small intestine. Folic acid about (5-15 mg/g) is in the liver and folate is also incorporated into the erythrocytes during erythropoiesis (Red blood cells production).

Deficiency

Deficiency of vitamin B_{12} also leads to functional folic acid deficiency.

i. Folic acid deficiency leads to megaloblastic anemia characterised by the release of large sized immature red blood cells into the circulation.

ii. Sprue and symptoms like glossitis and gastro intestinal disturbances have also been reported.

iii. Macrocytic anemia of pregnancy responds to treatment with folic acid.

vi. Vitamin B_{12}

Structure of vitamin B_{12}

Structurally, vitamin B_{12} consists of a corrin nucleus attached with 5,6 dimethyl benzimidazole moitey, an isopropanol unit, a ribose unit and a phosphate group. A cobalt atom (Co) is present at the centre of the corrin ring structure. One of the valencies of cobalt is filled by either CN^- (cyano cobalamin) or H_2O (aqua cobalamin) or OH^- (hydroxo cobalamin) or CH_3 (methyl cyano cobalamin).

Functions

Of all vitamins, vitamin B_{12} is the most complex. The trace mineral cobalt is an essential part of the molecule. Vitamin B_{12} is required for the maturation of red blood cells in the bone marrow and for the synthesis of proteins.

Sources

Milk, eggs, cheese, meet, fish and poultry supply ample amounts of vitamin B_{12}. Plant foods supply no vitamin B_{12} and use of an exclusively vegetarian diet for a long period of time will lead to symptoms of deficiency.

Requirements

Early estimates of vitamin B_{12} requirements were based on the amounts required to maintain normal RBC maturation in patients with pernicious anemia due to lack of intrinsic factor secretion. There is a

considerable enterohepatic circulation of vitamin B_{12}. It is secreted in the bile and re-absorbed in the small intestine. However, in patients with defective secretion of intrinsic factor, the vitamin cannot be re-absorbed, but is excreted in the feces. This means that patients with impaired secretion of intrinsic factor have much higher requirement for vitamin B_{12} than normal.

The average requirement of vitamin B_{12} is 3 µg/day.

Infants	-	0.3 µg / day
Children	-	1 - 2 µg / day
Adults	-	3 µg/day
Pregnant and lactating women	-	4 µg / day

Absorption and Storage

For the absorption of vitamin B_{12} from the intestines, a factor called "Intrinsic Factor" (IF) secreted by the stomach is essential. Vitamin B_{12} is stored in fair amounts in the liver.

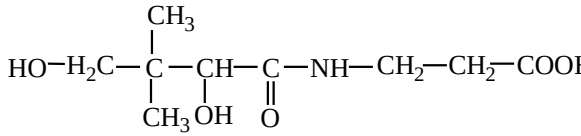
Deficiency

Pernicious anemia is the disease resulting from vitamin B_{12} deficiency. It is a genetic defect with the absence of intrinsic factor, hence the vitamin B_{12} in the diet cannot be absorbed. Since vitamin B_{12} is important for the maturation of red blood cells the deficiency of this vitamin leads to the formation of macrocytic red blood cells.

vii. Pantothenic acid (B_5)

The structure of pantothenic acid consists of an alanine chain in peptide linkage with dihydroxy, dimethyl butyric acid (Fig. 8.10).

Pantothenic acid is highly soluble in water.



Pantothenic acid

Fig. 8.10 Structure of (Vitamin B_5) Pantothenic acid

Functions

Pantothenic acid exists in the free form and in combination with β– mercapto ethylamine, adenine ribose and phosphoric acid. The later form is called as co-enzyme A (CoA).

The metabolic functions of pantothenic acid are due to its coenzyme derivative CoA, which participates in several metabolic reactions. CoA gains further importance after its conversion to form acetyl CoA.

i. Acetyl - Co A plays a key role in carbohydrate, protein and lipid metabolism.

ii. Acetyl - Co A is the precursor of cholesterol. It is the main source for the synthesis of cholesterol as well as steroid hormones.

iii. Acetyl - Co A combines with choline to form acetyl choline.

iv. Some of the amino acids require Co A for their activation.

Sources

Dried yeast, liver, royal gelly are the rich sources of pantothenic acid. Egg yolk, meat, fish, milk are good sources.

Requirements

The recommended daily allowance of pantothenic acid as follows:

Infants - 1.5 - 2.5 mg/day

Children	-	5 - 8 mg/day
Adults	-	5-12 mg/day
Pregnant and lactating women	-	10-15 mg/day.

Absorption and sotrage

Pantothenic acid and its salts are readily absorbed from the small intestine through the portal vein into the general circulation. If ingested in excess of the requirements, it is not stored in the body; but is excreted in urine or metabolised by the tissues.

Deficiency

Deficiency of this vitamin results in nausea, vomitting, certain gastro intestinal tract disorders, inadequate growth, anemia, fatty liver and failure in gaining weights.

viii. Biotin

Biotin is a heterocyclic, sulphur containing monocarboxylic acid (Fig. 8.11). Biotin is sparingly soluble in cold water and is freely soluble in hot water.

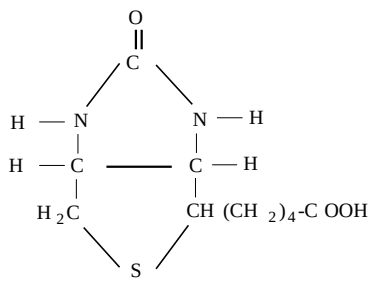


Fig. 8.11 Structure of Biotin

Functions

i. Biotin is required as the co-factor for a small number of carboxylation reactions, it acts as the carrier for carbon dioxide.

$$\text{eg. Acetyl CoA} \xrightarrow[\text{carboxylase}]{CO_2} \text{Malonyl CoA.}$$

ii. It helps to maintain the skin and the nervous systems in good condition.

iii. It assists in the deamination of amino acids like aspartic acid, serine and threonine.

iv. It helps in the synthesis of purine.

v. For the conversion of ornithine to citrulline in the synthesis of urea, biotin is required.

Source

Biotin occurs widely both in foods of vegetable and animal origin. Wheat germ, liver, peanut, and rice polishings are rich sources. Whole cereals, legumes, mutton and egg are good sources.

Requirements

Since, intestinal bacteria and diets supply biotin in adequate amounts the deficiency of this vitamin in human being is rare.

Infants	-	10- 15 µg/day
Children	-	20-40 µg/day
Adults	-	50-60 µg/day

Absorption and Storage

Biotin is readily absorbed from the small intestine through the portal vein into the general circulation. Excess of the requirements is not stored in the body but is mostly excreted in the urine.

Egg white injury factor (Avidin)

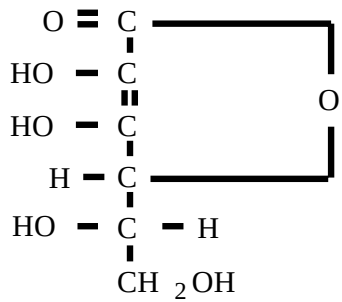
There is a protein in egg white called avidin which is responsible for producing egg white injury. Avidin binds with biotin tightly in the intestinal tract and prevents absorption of biotin from intestines. Avidin is denatured by cooking and then loses its ability to bind with biotin. The amount of avidin in uncooked egg white is relatively small, and problems of biotin deficiency have only occurred in people eating abnormally large amounts of raw eggs for many years.

Deficiency

Deficiency of biotin is rare in human beings.

8.2.2.2 *Vitamin C (Ascorbic Acid)*

Vitamin C is also called as ascorbic acid (Fig. 8.12).



L-ascorbic acid

Fig. 8.12 Structure of Vitamin C

Functions

i. Vitamin C is essential for building collagen the connective tissue protein which cements the cells and tissues together. The effect of this material is to provide firm tissues of all kinds. This vitamin helps forming strong blood vessels, teeth firmly held in their sockets, and bones firmly held together.

ii. It has a general antioxidant role, especially in the regeneration of oxidized vitamin E in membranes.

iii. Ascorbic acid reduces the ferric ion (Fe^{3+}) to ferrous (Fe^{2+}) ion and thus helps in the absorption of iron. It is also essential for rapid healing of wounds.

Sources

Raw fresh vegetables contain vitamin C, but some foods are more outstanding than others. Orange, grape, lime and lemon are especially rich in vitamin C.

Requirements

Recommended amount of vitamin C for different age group is as follows:

Infants	-	35 mg / day
Children	-	40 mg / day
Adults	-	45 mg / day
Pregnant women	-	60 mg / day
Lactating women	-	80 mg / day

Absorption and storage

Ascorbic acid is rapidly absorbed from the intestines and passed on through the portal vein to the general circulation. Liver and other organs and tissues have an optimal level of ascorbic acid. Excess intake do not increase further the optimal levels.

Deficiency

Severe deficiency of vitamin C leads to **scurvy.** This is characterised by easy bruising and hemorrhaging of the skin, lossening of the teeth, bleeding of the gums and distruption of the cartilages that support the skeleton.

8.2.3 Co-enzymic functions of Vitamins

Some of the B-complex vitamins act as co-enzymes for several enzymatic reactions as detailed below:

Table 8.1 The co-enzymic functions of Vitamins

S.No.	Name of the coenzyme	Name of the Vitamin	Name of the enzyme and the reaction involved
1.	Thiamine pyrophosphate (TPP)	Thiamine (B_1)	Pyruvate decarboxylase (Aldehyde group transfer)
2.	Flavin adenine dinucleotide (FAD)	Riboflavin (B_2)	Succinate dehydrogenase (Oxidation and reduction)
3.	Nicotinamide adenine dinucleotide (NAD)	Niacin (B_3)	Lactate dehydrogenase (Oxidation and reduction)
4.	Nicotinamide adenine dinucleotide phosphate (NADP)	Niacin (B_3)	Glucose-6-phosphate dehydrogenase (Formation of phosphoesters)
5.	Pyridoxal phosphate	Vitamin (B_6)	Aspartate amino transferase (Amino group transfer)
6.	Tetra hydro folate (FH_4)	Folic acid	S-adenosyl trans methylase (Methyl group transfer)
7.	Co-enzyme A	Pantothenic acid (B_5)	Pyruvate dehydrogenase (Acylation)

Exercise

I. Choose the correct answer from the given alternatives

a. Vitamin A is essential for the formation of

i. Rhodopsin ii. Bone

iii. Haemoglobin iv. Hormones

b. Cholecalciferol is

i. Vitamin D_3 ii. Vitamin B_1 iii. Biotin iv. Vitamin A

c. The vitamin which shows anti hemorrhagic action is

i. Vitamin K ii. Vitamin B_6 iii. Vitamin E iv. Vitamin C

d. The coenzymic form of pantothenic acid is

i. Coenzyme A ii. Tetrahydro folate

iii. Biotin iv. Pyridoxal phosphate

e. Avidin interferes with the absorption of

i. Biotin ii. Vitamin A iii. Vitamin C iv. Vitamin B_{12}

II. Fill up the blanks

a. ..... vitamin is essential for bone formation.

b. The compounds which posses vitamin E activity are known as .....

c. The vitamin essential for blood clotting action is .....

d. The main coenzyme which is involved in the transfer of one carbon compound is .....

e. For normal collagen biosynthesis the vitamin required is .....

III. Say true or false

a. Some vitamins act as coenzymes

b. Vitamin D is fat soluble

c. Vitamin B is water soluble

d. FAD is derived from vitamin B_{12}

e. Pyridoxal phosphate is involved in amino group transfering reactions

IV. Match the followings

a.	Vitamin A	-	Thiamine pyrophosphate
b.	Vitamin B_1	-	Pernicious anemia
c.	Vitamin B_{12}	-	Calcium absorption
d.	Vitamin E	-	Vision
e.	Vitamin K	-	Collagen synthesis
f.	Vitamin D	-	Antioxidant
g.	Vitamin C	-	Blood clotting

V. Give short answer for the followings

a. Differentiate the two types of vitamins.

b. What are the symptoms of vitamin A deficiency?

c. What are the effects of vitamin D deficiency?

d. What are the coenzymic functions of pyridoxine?

e. Give the functions of pantothenic acid?

VI. Answer the followings

a. Explain the co-enzymic functions of B complex vitamins.

b. Mention the fat soluble vitamins and their functions.

c. Give the classification of vitamins.

d. Explain the requirements and the deficiency symptoms of vitamin A and vitamin E.

e. Explain the requirements and the deficiency symptoms of vitamin C and vitamin B_{12}.

CHAPTER 9

MINERALS

9.1 Introduction

Mineral elements are inorganic substances. They are found in all body tissues and fluids. They are supplied by the diet as their salts; for example, sodium chloride. They may also be combined with organic compounds like iron in hemoglobin and sulphur in almost all proteins. Unlike carbohydrates, fats and proteins, mineral elements do not furnish energy but they may influence the rate of biological reactions through enzymes.

The mineral elements are not destroyed in food preparations un like vitamins. However, they are soluble in water so that some loss will occur if cooking liquids are discarded.

9.2 Classification

Minerals are classified into two main groups : macro elements and micro elements. Macro elements are required in large amount (>100 mg/day) and present in large quantities in the body, whereas micro elements are required in small quantity (<100 mg/day) and also present in small amount in tissues and body fluids.

9.2.1 Macro elements or Bulk elements

Some of the macro elements are calcium, phosphorus, sodium, potasium, chlorine (as chloride), magnesium and sulphur.

9.2.1.1 *Calcium*

Functions

i. About 99% of the body calcium is found in the bones and teeth where it is combined with phosphorus and other elements to give rigidity to the bone.

ii. The bones also serve as the store house for calcium which is needed for a number of cellular functions.

iii. Calcium is required for the complex process of blood coagulation.

iv. Together with other elements it regulates the passage of ions into and out of cells.

v. It controls the transmission of nerve impulses, brings about the normal contraction of muscles, including the heart.

vi. It also activate certain enzymes like adenosine triphosphatases, succinate dehydrogenase etc.

Distribution of calcium

Calcium is distributed in the body as follows:

Serum	-	9-11 mg/100 ml
C.S.F	-	4.5-5 mg/100 ml
Muscle	-	70 mg/100 gm
Nerve	-	15 mg / 100 gm

Calcium content of blood

The calcium content of plasma is fairly constant ranging from 9-11 mg/100 ml. This level is maintained constant in healthy individuals by the following factors: (i) the amount of calcium absorbed from food through the intestine and (ii) the secretion of parathyroid hormone which controls the level of calcium in blood.

Sources

Milk and cheese are rich sources of calcium. Egg yolk, cabbage, beans and cauliflower are good sources.

Requirements

Growing children require more amount of calcium, because of the increased rate of growth.

Infants	-	0.36-0.54 g/day
Children	-	0.8-1.2 g/day.
Adults	-	0.8 g/day.
During pregnancy and lactation	-	1.5 g/day

Absorption and excretion

Calcium is taken in the diet as calcium salts of phosphate, carbonate, tartrate and oxalate. Calcium is actively absorbed in the upper small intestine. Calcium is excreted in the urine, bile and digestive secretions.

Factors affecting calcium absorption

Some of the factors which influence the absorption of the calcium in the intestine are:

Vitamin D

Vitamin D promotes absorption of calcium.

Protein

High level of proteins in the diet helps to increase the absorption of calcium.

pH

Acidic environment in intestine favours calcium absorption.

Lactose

Lactose in chief increase the absorption of calcium. The beneficial effect of lactose is due to increased acidity (lactic acid) of the intestinal content which leads to increased calcium absorption.

Phosphates

Excess of phosphate in diet lowers the calcium absorption. The ratio of calcium and phosphorus in the diet should be 1:1.

Fats and fatty acids

Faulty absorption of fats leads to the presence of large amounts of fatty acids in the intestine were interferes with calcium absorption, as fatty acids form insoluble salt with calcium which are excreted in the feces.

Oxalic acid

Oxalic acid present in certain food forms insoluble calcium oxalate which is excreted in the feces and interfere with the calcium absorption.

Calcium balance

Dietary calcium which is not absorbed in the intestine is excreted in the feces. A small part of the absorbed calcium is excreted in urine. The calcium balance i.e. the difference between the quantity of calcium ingested and that excreted in urine and feces.

Deficiency

Calcium deficiency becomes evident only after a long period of inadequate intake. A dietary deficiency does not lower the blood calcium since the bones will supply the amount required. **Rickets** (in children) is a disease more directly related to vitamin D deficiency, but calcium and phophorus metabolism are also involved. **Tetany** and **osteomalacia** (in adults) are the diseases result from the deficiency of calcium.

9.2.1.2 *Phosphorus*

Functions

Probably no mineral element has more functions than phosphorus. It is essential for the formation of

i. bones and teeth.

ii. phospholipids that regulates the absorption and transport of fats.

iii. DNA and RNA, which are nucleic acids essential for protein synthesis and genetic coding.

iv. ATP and ADP, which are necessary for storing and releasing energy according to the body needs.

v. enzymes that are required to metabolise carbohydrates, fats, and proteins and

vi. buffer salts in the regulation of acid-base balance.

Blood content of phosphorus

The inorganic phosphorus content of blood in normal human adults ranges from 2.5 to 4.0 mg /100 ml and in children from 4 to 5 mg/ 100 ml.

Sources

This element is found in both animal and plant foods. Animal sources include fish, meat, egg, milk, liver and kidneys. Plant sources of phosphorus are nuts, beans, green vegetables and fruits. A diet which is adequate in calcium is usually adequate in phosphorus also.

Requirements

Infants	- 0.24-0.4 g/day
Children	- 0.8 -1.2 g/day
Adults	- 0.8 g/day
During pregnancy and lactation	- 1.5 g/day

Absorption and excretion

Moderate amounts of fatty acid favour absorption of phosphorus. High calcium content in diet decreases the absorption of phosphorus. Phosphorus is excreted in the urine and feces.

Deficiency

A deficiency of phosphorus leads to rickets. Low level of blood phosphorus characterised by defective bone and teeth formation.

9.2.1.3 *Sodium*

Functions

i. It is the major component of the cations of extracellular fluid and exists in the body in association with the anions like chloride, bicarbonate, phosphate and lactate.

ii. Sodium ion is mainly associated with chloride and bicarbonate in the regulation of acid-base equilibrium. It maintains osmotic pressure of the body fluids and thus protects the body against excessive fluid loss.

iii. It plays an important role in the absorption of glucose and nutrients from small intestine.

iv. Sodium ions are involved in the maintenance of heart beat.

v. It maintains the normal neuromuscular functions and it functions in the permeability functioning of the cells.

Blood content of sodium

The normal level of sodium in blood is 310-340 mg/100 ml of blood. Red blood cells contain no sodium ions.

Sources

Sodium chloride (Common salt) is the main source of sodium. Bread, cheese and wheat germ are rich sources. Cauliflower, carrot and milk are also good sources of sodium.

Requirements

For adults the daily requirement is 5 - 10 grams/day.

Absorption and excretion

Sodium is completely absorbed from the gastro intestinal tract. About 95% of the sodium leaving the body is excreted in the urine since sodium is easily absorbed in the intestine.

Deficiency

Adrenal gland secretes hormones called adrenocortical steroids which regulates the metabolism of sodium. In the insufficiency of adrenocortical steroids the serum sodium level is decreased with an increase in sodium excretion. Hyponatremia (Addison's disease) the condition in which sodium level of blood is below normal and in hypernatremia (Cushing's syndrome) the sodium level of blood is above the normal range.

9.2.1.4 *Potassium*

It was given the name potassium because it was obtained from potash. It is an indispensible constituent of the body cells.

Functions

i. Potassium is an important intracellular cation and needed for all cellular functions.

ii. It maintains the alkalinity of the bile and blood.

iii. It plays an important role in the regulation of acid-base balance in the cell.

iv. It influences cardiac muscle activity.

v. The glycolytic enzyme pyruvate kinase requires potassium for its maximal activity.

Sources

Most foods contain potassium. Animal sources include meat, fish , egg, milk, cheese. Vegetables like onion and carrot, fruits like banana, grapes and legumes such as beans also contain potassium.

Requirements

The normal intake of potassium in food is about 4 gm/day.

Absorption and excretion

Normally potassium is completely absorbed from the gastro intestinal tract and it is normally eliminated almost entirely in the urine and a small amount in the feces.

Deficiency

Deficiency of potassium leads to depression in cardiac and nervous system. Severe vomitting, diarrhoea, loss of appetite, fasting or starvation over a long period of time, may lead to deficiency of potassium. It also occurs during renal failure and shock. Fatigue, muscular weakness, heart and respiratory dysfunction are common signs of potassium deficiency.

9.2.1.5 *Chlorine*

Functions

i. As a component of sodium chloride (NaCl), chloride ion is an essential for acid-base equilibrium.

ii. Chloride ion regulates the water balance and osmotic pressure.

iii. It is also important in the production of hydrochloric acid in the gastric juice.

iv. Chloride ion is important as an activator of amylase enzyme.

Sources

Sodium chloride is the main source for chlorine.

Requirements

The requirements of sodium chloride depend on the climate and occupation and on the salt content of the diet. Animal foods contain more NaCl than those of plant foods. Normal requirement of chloride is 5-10g /day as NaCl.

Absorption and excretion

Chloride is completely absorbed from the gastro intestinal tract. Chloride is mainly eliminated in the urine and also in the sweat.

Deficiency

Chloride deficiency occurs when loss of sodium is excessive in diarrhoea, sweating and some endocrine disturbances.

9.2.1.6 *Magnesium*

Functions

About 70 percent of the total magnesium content of the body is combined with calcium and phosphorus in the complex salts of bone. The reminder is in the soft tissues and body fluids. It is the principal cation of the soft tissues. Magnesium ions act as an activators for many of the phosphate group transferring enzymes and it also functions as a co-factor for oxidative phosphorylation.

Blood content of magnesium

The normal level of magnesium in blood is 1-3 mg/100 ml.

Sources

Milk, egg, cauliflower, cabbage and fruits are rich sources of magnesium.

Requirements

Infants	-	100-150 mg/day
Children	-	150 -200 mg/day
Adults	-	200-300 mg/day

Absorption and excretion

Like calcium the salts of magnesium are rather insoluble and greater part of the daily ingested magnesium is not absorbed. Parathyroid hormone increases the magnesium absorption. Very high intake of fat, phosphate and calcium decrease the absorption of magnesium.

The major quantity of magnesium is excreted in the feces and remaining is excreted through urine.

Deficiency

Magnesium deficiency causes depression, muscular weakness, and liability to convulsions.

9.2.1.7 *Sulphur*

Functions

Sulphur is present in the amino acids such as methionine, cystine and cysteine. Thus, it is present in all proteins in the body. Especially connective tissue, skin, hair and nails are rich in sulphur. Also thiamine and biotin which are B-complex vitamins contain sulphur in their molecules.

Sources

Sulphur intake is mainly in the form of cystine and methionine present in protein. Other compounds present in the food contribute small amounts of sulphur.

Requirements

A diet that is adequate in protein supplies required amounts of sulphur.

Absorption and excretion

Inorganic sulphate (So_4^{2-}) is absorbed as such from the intestine into the portal circulation. Sulphur is excreted in the urine.

Deficiency

No specific sulphur deficiency has been observed in human beings.

9.2.2 *Micro elements or Trace elements*

The important micro elements essential for normal body functions are iron, copper, iodine, fluorine, zinc, cobalt, manganese, chromium, molybdenum, selenium etc.

9.2.2.1 *Iron*

Functions

The total content of iron in the adult body is only 3 to 5 grams. Most of the iron is present in hemoglobin, a protein that consists of an iron-containing compound, heme, attached to a protein globin. Hemoglobin is carried in the circulation by the red blood cells. It picks up oxygen in the lungs and transports the oxygen to the tissues so that oxidation reactions can take place in the cells. From the cells the hemoglobin carries CO_2 to the lungs to be exchanged.

Myoglobin is an iron-containing protein similar to hemoglobin and is present in muscle tissue. Iron is also a constituent of many enzymes that are required for oxidation of glucose and fatty acids for energy production.

Sources

Among the richest animal sources of iron are beef, liver, heart, kidneys, spleen, egg yolk and land snail. Plant sources of iron include whole wheat and its products, green vegetables, onions, coconuts and fresh fruits.

Requirements

Iron requriements are influenced by the availability of iron present in foods. Iron present in cereals, legumes and green leafy vegetables is available to a lesser extent than that present in egg, meat and fish. In view of this, iron requriements of persons consuming a predominantly cereal based diet, will be greater than those consuming large quantities of meat and egg. About 10 percent of the ingested iron is only absorbed.

Infants	-	10-15 mg/day
Children	-	15 mg/day
Adults	-	18 mg/day
During pregnancy and lactation	-	40 mg/day.

Absorption and excretion

Under normal conditions, very little dietary iron is absorbed which take place mainly in the stomach and the duodenum. Infants and children absorb a higher percentage of iron from food than adults. Iron deficiency in infants is due to a dietary deficiency. Iron deficient children absorb twice as much as that of normal children.

The body stores of iron are conserved very efficiently. Only lesser amounts are excreted in the urine, feces and sweat.

Factors affecting iron absorption

i. Impaired iron absorption takes place in patients who have total removal of stomach or a removal of the considerable amount of the intestine.

ii. A diet high in phosphate causes decreased absorption due to the formation of insoluble ferric phosphate.

iii. Phytic acid and oxalic acid interfere with absorption.

iv. Vitamin C increases absorption.

v. Alcohol ingestion favours iron absorption.

Deficiency

Insufficient iron in the diet causes iron deficiency **anemia.** This is common in growing children and pregnant women. Insufficiency may be brought about by blood loss during menstruation, inadequate intake of iron and hookworm infestation.

9.2.2.2 *Copper*

The importance of copper for the formation of hemoglobin was studied by Hart and co-workers in 1928. Later studies indicated that copper has many functions.

Functions

i. Copper is essential for the synthesis of hemoglobin.

ii. It is needed for the synthesis of collagen, melanin and phopholipids.

iii. It is a constituent of several enzymes.

iv. Three copper containing proteins namely cerebrocuprein, erythro cuprein and hepatocuprein are present in brain, RBC and liver respectively.

Sources

Copper is present in minute quantities in most foods, including liver, kidneys, shell fish and meat. Plant sources of copper are nuts and legumes.

Requirements

Daily requirement of copper is 0.05 - 0.85 mg/kg body weight for children and 2 mg/day for adults.

Absorption and excretion

Absorption of copper into the blood stream occurs via the villi of the small intestine. About 30 percent of the dietary copper is absorbed in the duodenum. Only 10 - 60 µg of copper is excreted in normal urine in 24 hours.

Deficiency

i. In human beings the only condition observed is anemia due to copper deficiency.

ii. Copper deficiency produces marked skeletal changes, osteoporosis and spontaneous fractures.

iii. Elastin formation is impaired in copper deficiency.

9.2.2.3 *Iodine*

Functions

Most of the body iodine is present in the thyroid gland, but all cells contain trace amount of iodine. Iodine is a constituent of two hormones, thyroxine (T_4) and triiodothyroxine (T_3). These hormones (i) regulate energy metabolism, (ii) synthesis of proteins and cholesterol and in the conversion of carotene to vitamin A.

Sources

The amount of iodine in plant food depends on whether or not iodine was present in the soil in which the plants were grown. Vegetables grown in iodine-rich soil will naturally be the good sources of iodine. The animal sources of iodine include milk, sea fish, shell fish and crabs.

Requirements

For adults are about 0.10 to 0.15 mg/day for infants and children 0.05 to 0.10 mg/day. This is normally supplied by an ordinary well balanced diet and by drinking water except in mountainous regions where the food and water are deficient in iodine content. Drinking and cooking water often contain iodine in sufficient quantities to provide the daily requirement. Iodised salt may be added when cooking foods in areas with insufficient natural sources of iodine.

Adults	-	100-150 μg/day
Pregnant women	-	200 μg/day

Absorption and excretion

Absorption is through the villi of the small intestine into the blood stream and 90 percent of the iodine of the thyroid gland is in organic combination and stored in the follicular colloids as thyroglobulin.

Inorganic iodine is mostly excreted by the kidneys, liver, skin, lungs and intestine and in milk. About 10 percent of circulating organic iodine is excreted in feces.

Deficiency

A deficiency of iodine leads to a decreased production of thyroxine, and inturn a lowered rate of energy metabolism. In an attempt to produce more thyriod hormones the thyroid gland enlarges. This condition is called **simple or endemic goiter**. In a mild deficiency the only symptom noted is slight enlargement of the thyroid gland visible at the neckline. However, if the condition persists, the women who has a simple goiter and who fails to get sufficient iodine during pregnancy will be unable to supply the fetus with its needs: thus, the baby is more severely affected than its mother.

People born in areas which lack of iodine in the water and soil have a tendency to develop 'goitre', a condition characterised by subnormal metabolic activities. The condition may be reversed if treated sufficiently with iodine at the early stage itself.

9.2.2.4 *Fluorine*

Functions

Fluorine exists in the body in compounds called fluorides.

i. Lesser amount of fluoride enter into the complex calcium salts that form tooth enamel.
ii. Fluorides may also be useful in maintaining bone structure. It is necessary for the prevention of dental caries.
iii. Fluoride ions inhibit the metabolism of oral bacterial enzymes and diminish the local production of acids which are important in the production of dental caries.
iv. It is in combination with vitamin D, required for the treatment of osteoporosis.

Sources

The chief source of fluorine is in the form of fluoride in drinking water.

Requirements

Fluorine is found is small amounts in normal bones and teeth. Since water containing 1-2 ppm (Parts per million) prevents dental caries and does not do any harm, the fluorine requirements of the body are met by the quantity normally present in drinking water (1-2 ppm) in most of the regions.

Absorption and excretion

Absorption of fluoride is via the small intestine into the blood stream. Fluoride is excreted in the urine and in the sweat, and by intestinal mucosa. Most of the fluorides that are not retained by the bones and teeth is excreted rapidly into the urine.

Deficiency

The absence of fluorine in the diet causes dental caries.

Toxicity

Excess of fluorine (above 5 ppm) causes chalky white patches on the teeth. If this is not treated in time, the patches change to a brown colour which later develop into holes.

9.2.2.5 *Zinc*

About 2 to 3 grams of zinc are found in the body. Like iron, zinc is absorbed according to the body needs.

Functions

i. Normal growth and sexual maturation.
ii. As part of an enzyme that transfers carbon dioxide from tissues to the lungs.
iii. The production of insulin by the pancreas.
iv. Synthesis of proteins.
v. Normal sensitivity to taste.

Sources

Zinc is widely distributed in animal and plant foods that are good sources of protein. Meats, egg, liver, sea food, legumes, nuts, milk wholegrain and cereals are good sources. People who eat a normal diet adequate in protein are not likely to develop zinc deficiency.

Requirements

Daily requirements of zinc are given below.

Infants	-	3-5 mg/day
Children	-	10-15 mg/day
Adults	-	15 mg/day.
During pregnancy and lactation	-	20-25 mg/day.

Absorption and excretion

Zinc present in animal foods are well absorbed in the small intestine, especially from the duodenum. Zinc present in plant foods are poorly absorbed due to the presence of phytic acid which interferes with its absorption. Zinc is mostly excreted in the feces.

Deficiency

The diet that has low zinc level leads to dwarfism and retarded sexual development. Zinc deficiency leads to diminished senstivity to taste (hypogeusia) and to a decrease in odour sensitivity (hyposmia).

9.2.2.6 *Cobalt*

Functions

Cobalt occurs in small amount in all tissues, higher concentrations occuring in liver and kidneys. Most of the cobalt is present in vitamin B_{12} which is necessary for red blood cells maturation.

Sources

It is largely available in food.

Requirements

Cobalt deficiency has not been observed in human beings. Cobalt requirements, if any, appear to be met by traces of cobalt found in foods. Human beings require a dietary source of vitamin B_{12} which is not synthesized by the body.

Absorption and excretion

Cobalt is readily absorbed from the small intestine. About 65% of ingested cobalt is excreted in the urine and the remainder in the feces.

Deficiency

Cobalt deficiency is rare in human beings.

9.2.2.7 *Manganese*

Functions

i. Manganese is essential for normal bone structure, reproduction and the normal functioning of the central nervous system.

ii. Manganese activates isocitrate dehydrogenase and phosphotransferases.

iii. Pyruvate carboxylase and superoxide dismutase contain tightly bound manganese.

iv. Manganese ions activate glycosyl transferases which is concerned with synthesis of muco polysaccharides of cartilages and also associated with the synthesis of glycoproteins.

v. Arginase an enzyme which is involved in the urea cycle is activated by manganese ions.

Sources

Nuts and whole grains are rich sources and vegetables and fruits are good sources of manganese.

Requirements

The average dietary intake of 2.5 to 9.0 mg/day is sufficient to meet the daily needs.

Absorption and excretion

Manganese is readily absorbed in the small intestine. Normally 3 to 4 percent of manganese present in the diet is absorbed. Large quantity of manganese is excreted mostly in the feces. Only very small quantities of manganese is excreted in the urine.

Deficiency

The deficiency of manganese leads to impaired growth and skeletal abnormalities.

9.2.2.8 *Chromium*

Chromium occurs in trace amounts in human and animal tissues.

Functions

Chromium plays an important role in carbohydrate, lipid and protein metabolism. It potentiates the action of insulin in accelerating the utilization of glucose.

Sources

It is highly available in average diets.

Requirements

The exact requirements are not known. Average diets can meet the chromium requirements.

Absorption and excretion

It is readily absorbed in the small intestine. It is mobilized from the tissues in response to glucose administration. Chromium is mainly excreted in urine, a small amount in bile and feces.

Deficiency

Chromium deficiency is characterised by impaired growth and disturbances in glucose, lipid and protein metabolism.

9.2.2.9 *Molybdenum*

Molybdenum also occurs in traces in human and animal tissues.

Functions

Molybdenum is an essential component of the xanthine oxidase. It is also present in nitrate reductase in plants, and nitrogenase, which functions in nitrogen fixation by micro organisms. Trace amount of molybdenum are requried for the maintenance of normal levels of xanthine oxidase in animal tissues.

Source

It is available in average diets.

Requirements

Adequate amounts of molybdenum are present in average diets. Hence, exact requirement is not known.

Absorption and excretion

About 50 to 70 percent of the ingested molybdenum is readily absorbed in the small intestine. About half of the absorbed molybdenum is excreted in urine.

Deficiency

Molybdenum deficiency is rare in human beings.

9.2.2.10 *Selenium*

Selenium is present in foods of plant origin grown in selenium rich soils.

Functions

Selenium is essential for normal growth, fertility and for the prevention of a wide variety of diseases in animals. Selenium is a constituent of the enzyme Glutathione peroxidase (GPx), a selenoprotein. This enzyme is the protective agent against the accumulation of H_2O_2

(Hydrogen peroxide), and organic peroxides within cells. It is involved in immune mechanisms, ubiquinone synthesis and mitochondrial ATP biosynthesis. Selenium has close metabolic relationship with vitamin E for curing certain diseases.

Sources

Selenium is largely present in different foods. The variation depends on the selenium content of the soil.

Requirements

Any normal diet can meet the daily requirement of selenium.

Deficiency

Selenium deficiency is very rarely seen in human beings.However, necrosis and muscular dystrophy are associated with selenium deficiency.

Exercise

I. Choose the correct answer from the given four alternatives

a. Minerals

i. Furnish energy ii. Enhance the rate of enzyme action

iii. Are insoluble in water iv. Are organic substances.

b. Calcium activates enzyme

i. Carboxy peptidase ii.Succinate dehydrogenase

iii. Hexokinase iv. Lipase

c. This mineral is involved in acid-base balance

i. Phosphorus ii. Calcium

iii. Selenium iv. Cobalt

d. Common salt is

i. Sodium chloride ii. Potassium chloride

iii. Magnesium chloride iv. Manganese chloride

e. Iodine deficiency leads to

i. Tetany ii. Hypernatremia

iii.Goitre iv. Anemia

II. Fill in the blanks

a. Iron deficiency leads to .....

b. ..... vitamin promotes calcium absorption.

c. Zinc is present in the structure of the enzyme .....

d. The micro element present in glutathione peroxidase .....

e. Cobalt is present in the vitamin .....

III. Say true or false

a. Minerals furnish energy to the body

b. Calcium is a micro element

c. Selenium is a macro element

d. Cereal rich food interferes with calcium absorption

e. Fluoride deficiency leads to dental caries.

IV. Match the followings

a.	Calcium	-	Hemoglobin formation
b.	Iron	-	Common salt
c.	NaCl	-	Bone formation
d.	Fluorine	-	Glutathione peroxidase
e.	Selenium	-	Goitre
f.	Iodine	-	Vitamin B_{12}
g.	Cobalt	-	Dental caries

V. Give short answers for the followings

a. What is osteomalacia?

b. What are the hormones connected with the maintenance of blood calcicum level?

c. Explain the functions of magnesium.

d. What is the form of calcium in bone?

e. What is the requirement of iron and phosphorus in growing children?

VI. Answer the followings

a. What are the biological functions of phosphorus?

b. Explain the factors affecting calcium absorption in the intestine.

c. Explain the functions of any four micro elements.

d. What is the importance of iron in the body?

e. How are sodium and chlorine important to the body functions?

CHAPTER 10

BIOCHEMICAL TECHNIQUES

10.1 Introduction

The major techniques applied for the qualitative and quantitative assessment of biomolecules are chromatography, centrifugation, electro phoresis and spectrophotometry.

10.2 Chromatography

One of the tasks of biochemists is to identify, separate and purify one or more biological components in a mixture of such compounds in a biological sample. One of the most important convenient methods for achieving such separation is the use of chromatographic techniques.

The term chromatorgraphy was originally applied by Micheal Tswett, a Russian Botanist, in 1906 to a procedure where a mixture of different coloured pigments (chlorophylls and xanthophylls) was separated from each other.

The basis of all forms of chromatography is the partition or distribution co-efficient which describes the way in which a compound distributes itself between two immiscible phases. For a compound distributing itself between equal volumes of two immiscible solvents A and B (Fig. 10.1), the value of distribution co-efficient is a constant at a given temperature and is given by the expression

$$\frac{\text{concentration in solvent A}}{\text{concentration in solvent B}} = Kd$$

where, Kd = distribution co-efficient.

Basically all chromatographic systems consists of two phases. One is the stationary phase which may be a solid, liquid or a solid liquid mixture which is immobilized. The mobile phase may be a liquid or a gas and flows over or through the stationary phase. Separation starts to occur when a compound to be separated is held more firmly by the stationary phase than the other which tends to move on faster in the mobile phase. Thus, the underlying principle of chromatorgraphy is to adsorb the components of the mixture on an insoluble material and then to differentially remove or elute these components one by one with suitable solvents.

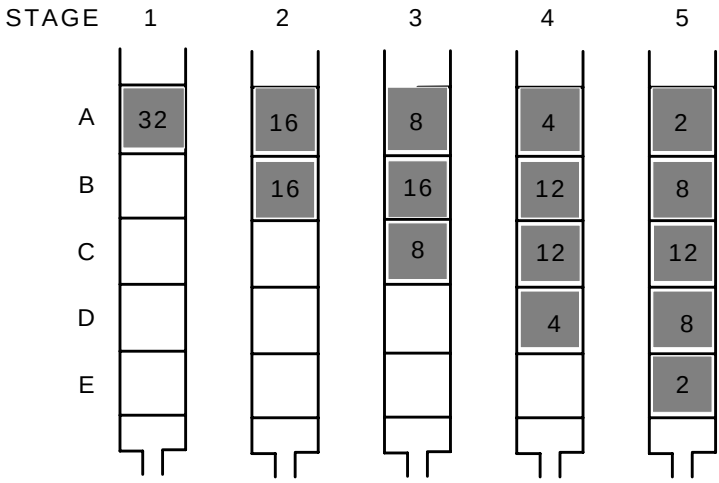


Fig. 10.1 Principle of distribution coefficient

The term effective distribution co-efficient is defined as the total amount as distinct from the concentration of substance present one phase divided by the total amount present in the other phase. Thus, a distribution co-efficient of a substance between alumina (stationary phase) and butanol (mobile phase) might be 0.25 which means that the

concentration of the substance in butanol is four times that in the alumina.The choice of stationary or mobile phases is made so that the compounds to be separated have different distribution co-efficient.

In practice separations may be achieved by using different types of chromatographic techniques

10.2.1 Types of chromatography

(a) Column chromatography: In this type, the stationary phase is packed into a glass or metal columns (wide tubes or cylinders). The mixture to be separated is layered on the top of the column in the form of a solution at particular concentration. After equilibration the components are eluted out of the column one by one using specific mobile phases (Fig.10.2).The solvent used to elute the separated components is known as eluant.

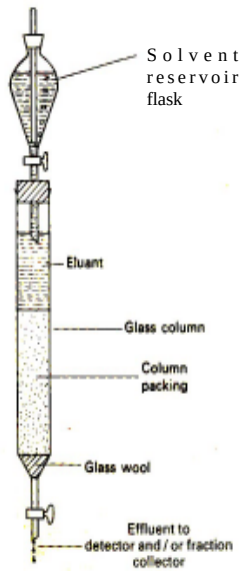


Fig. 10.2 Apparatus for column chromatography

(b) Thin layer chromatography: In this type, the stationary phase is thinly coated on to a glass, plastic or foil plates. The mixture to be separated is applied on the stationary phase at one end and kept vertical in the petridish containing the mobile phase. When the mobile phase reaches the other end of the plate the plate is removed from the petridish and the compounds separated are identified by using specific staining reagents.

(c) Paper chromatography: In this type the stationary phase is supported by the cellulose fibres of a paper sheet. The mobile phase flows through the stationary phase and effects separation.

Each of these three types of chromatography have their specific advantages, applications and method of operation.

10.2.1.1 *Column chromatography*: All the major types of chromatography are routinely carried out using column type (Fig. 10.3). The different types of column chromatography are

i. adsorption chromatography
ii. partition chromatography
iii. ion-exchange chromatography
iv. exclusion chromatography
v. affinity chromatography

i. Adsorption chromatography

Principle:

An adsorbent may be described as a solid which has the property of adsorbing molecules at its surface, particularly when it is porous and finely divided. Adsorption can be specific so that one solute may be adsorbed selectively from a mixture. Separation of components by the method depends upon differences both in their degree of adsorption by the adsorbent and solubility in the solvent used for separation.Adsorption chromatography can be carried out in both the column and thin layer modes.

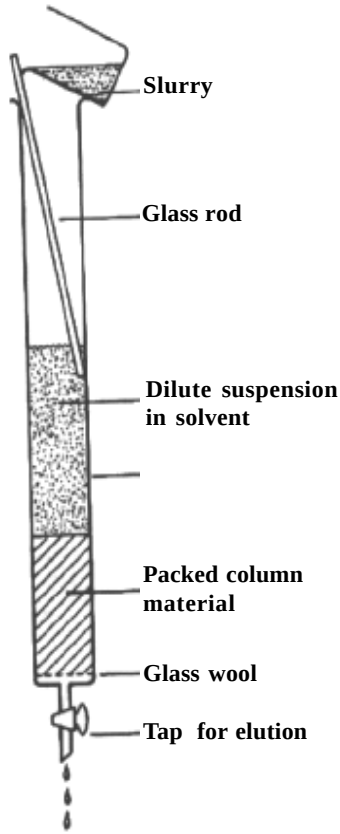


Fig. 10.3 The preparation of a chromatography column

ii. Partition chromatography

Principle: This technique is based on the partitioning of compounds between a liquid stationary phase and a liquid mobile phase. The liquid

stationary phase can be held on any solid support like paper. This technique is otherwise known as liquid- liquid chromatography.

iii. Gas liquid chromatography

Principle: This technique is based upon the partitioning of compounds between a liquid stationary phase and a gas mobile phase . It is a widely used method for the qualitative and quantitative analysis of a large number of compounds (eg. fatty acids) because it has high sensitivity, reproducibility and speed of resolution. A stationary phase of liquid material such as a silicone grease is supported on an inert granular solid . This material is packed into a narrow coiled glass or steel column 1 to 3 meter long and 2 to 4mm internal diameter. Through this column an inert carrier gas (the mobile phase) such as nitrogen, helium or argon is passed. The column is maintained in an oven at an elevated temperature which volatilizes the compounds to be separated.

The basis for the separation is the difference in the partition coefficients of the volatilized compounds between the liquid and gas phases as the compounds are carried through the column by the carrier gas. As the compounds flow, they leave the column and pass through a detector which is connected to a recorder and record a peak. The area of the peak corresponds to the concentration of the compound separated.

iv. Ion exchange chromatography

Principle: The principle of this form of chromatography is the attraction of oppositely charged particles . Many biological materials, such as amino acids and proteins, have ionisable groups and the fact that may carry a net positive or negative can be utilized in separating mixtures of such compounds. The net charge carried by such compounds depend on their pKa and on the p H of the solution.

Ion exchange separations are mainly carried out in columns packed with an ion exchanger, which contain the core matrix molecule with exchangeable ionic groups on its surface. There are two types of ion-

exchangers, namely cation and anion exchangers. Cation exchangers posses negatively charged groups and they will attract positively charged molecules. Anion exchangers have positively charged groups which will attract negatively charged molecules. The actual ion exchange mechanism composed of four steps;

a. selective adsorption of the molecules to be separated by the ion exchange resins.

b release of the exchangeable group from the matrix.

c. Elution of the absorbed molecule by specific eluants.

d. Regeneration of the matrix by recharging with the original exchangeable groups.

Cation exchanger

adsorbtion

RSO_3----------Na^+ + X^+, X, Y=====> RSO_3-----X^+ + Na^+

(mixture of compounds)

(elution)

RSO_3-----X^+ + H^+ =====> RSO_3------- H^+ + X^+

RSO_3------- H^+ + NaCl =====> RSO_3------- Na^+ + HCl

(regeneration)

Anion exchanger

R NH_3 ---------- OH^- + X^-, X, Y=====> RNH_3-----X^- + OH^-

(mixture of compounds)

RNH_3-----X^- + Cl^- =====> RNH_3-----Cl^- + X^- OH^-

RNH_3-----Cl^- + OH^- =====> RNH_3-----OH^- + Cl^-

Some of the ion exchange materials used in this technique are Amberlite IRC 50, Bio- Rex, Dowex 50, Sephadex etc.

v. Exclusion chromatography

This chromatography is otherwise known as gel permeation chromatography.

Princple: This technique is based on the separation of molecules on the basis of their molecular size and shape and the molecular sieve properties of a variety of porous materials which serve as the solid stationary phase.

A column of gel particles or porous glass granules is in equilibrium with a suitable solvent for the molecules to be separated. Large molecules which are completely excluded from the pores will pass through the interstitial spaces and smaller molecules will be distributed between the solvent inside and outside the molecular sieve and will then pass through the column at a lower rate . So the larger particles will come out of the column first followed by smaller particles (fig. 10.4).

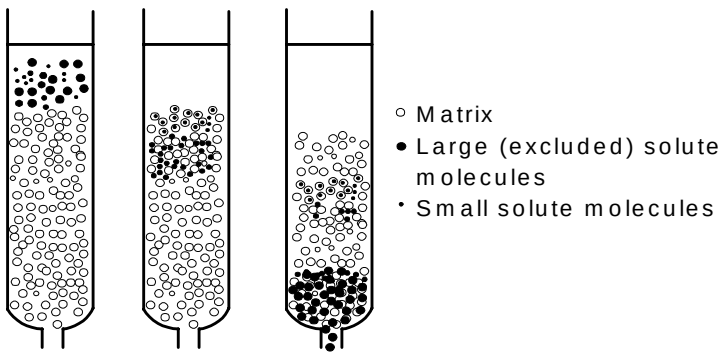


Fig. 10.4 Diagrammatic representation of separation by exclusive chromatography

The gel materials generally used for this technique are cross-linked dextrans, agarose, polyacrylamide, poly styrene etc.

vi. Affinity chromatography:

Principle: This technique is based on the specific biological interaction of the compounds to be separated with the special molecules attached on the stationary phase called as ligands. This technique requires that the material to be isolated is capable of reversibly binding to a specific ligand which is attached to an insoluble matrix (stationary phase).

$$\underset{\text{macro-molecule}}{M} + \underset{\text{ligand (attached to matrix)}}{L} \rightleftharpoons \underset{\text{Complex}}{ML}$$

Under suitable experimental conditions when a complex mixture containing the specific compound to be purified is added to the insolubilised ligand generally contained in a chromatography column, only that compound will bind to the ligand. All the other compounds can be washed away and the compound subsequently recovered by displacement from the ligand. The purification of an enzyme by this technique is shown diagrammatically in Fig. 10.5.

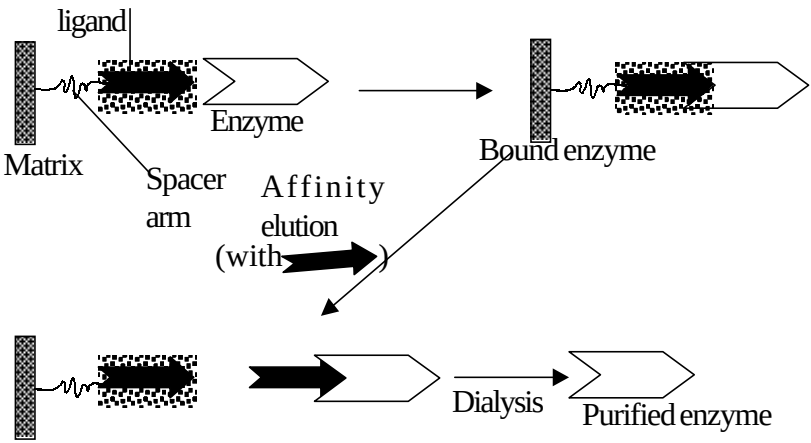


Fig. 10.5 Diagrammatic representation of purification of an enzyme by affinity chromatography

In practice , particles which are uniform , spherical and rigid are used as matrix materials are polystyrene, cellulose, porous glass and silica etc.

10.2.1.2 *Thin Layer chromatography*

Principle: Partition, adsorption, exclusion chromatography can be carried out in a thin layer mode. In this technique the stationary phase is made in the form of a slurry and applied as a thin coating on the surface of a glass plate.After activating the plate ,the sample to be separated is applied at one end of the plate . The plate is kept in a chamber specially designed for this purpose(TLC chamber) and allowed the sample and the mobile phase to raise through the stationary phase by capillary action. The whole procedure consists of.

a. Thin layer preparation : A slurry of the stationary phase , generally applied to a glass, plastic or foil plate as a uniform thin layer by means of a plate spreader starting from one end of the plate and moving progressively to the other. Calcium phosphate is incorporated into the slurry in order to facilitate the adhesion of the adsorbent to the plate. The plate is heated in an oven at 100 ^{0}C to activate the adsorbent.

b. Sample application: The sample is applied to the plate by means of a micropipette or syringe as spots or as a band on the stationary phase.

c. Plate development: Separation takes place in a glass tank which contains developing solvent to a depth of about 1.5 cm. This is allowed to stand for atleast an hour with a lid over the top of the tank to ensure that the atmosphere within the tank becomes saturated with solvent vapour.

d. Component detection: The components separated are detected by (i) spraying the plate with 50% sulphuric acid or 25% sulphuric acid in ethanol and heating; (ii) examining the plate under ultraviolet light; (iii) spraying of plates with specific colour reagents, for example ninhydrin for amino acids.

10.2.1.3 Paper chromatography

Principle: The cellulose fibres of chromatography paper act as the supporting matrix for the stationary phase. The stationary phase may be water, a non-polar material such as liquid paraffin or impregnated particles of solid adsorbent. The components get separated between the liquid stationary phase and the liquid mobile phase. The procedure consists of

a. Paper development: There are two techniques which may be employed for the development of paper, ascending and descending methods. In both cases, the solvent is placed in the base of a sealed tank or glass jar to allow the chamber to become saturated with the solvent paper. The sample spots should be in a position just above the surface of the solvent so that as the solvent moves vertically up the paper by capillary action, separation of the sample is achieved.

b. Component detection: The separated components can be detected by (i) examining the paper under ultraviolet light; (ii) spraying of papers with specific colour reagents, for example ninhydrin for amino acids and sulphuric acid for simple sugars.

The identification of a given compound may be made on the basis of its R_f value (retardation factor) which is the distance moved by the components during development divided by the distance moved by the solvent from the point of origin (Fig. 10.5).

$$R_f = \frac{\text{The distance moved by the solute from origin}}{\text{The distance moved by the solvent from origin}}$$

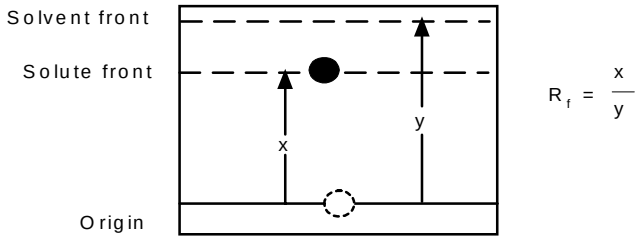


Fig. 10.5 The method of determination of Rf value in paper chromatography

The value of R_f is constant for a particular compound under standard conditions and closely reflects the distribution co-efficient for that compound.

10.2.2 Applications of chromatography

a. Thin layer chromatography is used for the separation of alkaloids, phospholipids and other lipids.

b. Gas liquid chromatography is applied for the separation of fatty acids in a lipid mixture.

c. Ion exchange chromatography can be used for the separation and identification of amino acids in a mixture of protein hydrolysate. This principle is used in auto-analyzer.

d. Exclusion chromatography can be applied for the determination of the molecular weight of the components separated.

e. Affinity chromatography is applied for the purification of a wide range of enzymes and other proteins like immunoglobulins.

10.3 Electrophoresis

10.3.1 General principle: Many important biological molecules such as amino acids, peptides, proteins, nucleotides and nucleic acids posses ionisable groups and can therefore be made to exists in solution as electrically charged species either as cations (+) or anions (-). When

a mixture of these components subjected to electric field, they migrate differentially and thus they can be separated.

10.3.2 Types of electrophoresis

(i) low voltage thin sheet electrophoresis; (ii) high voltage electrophoresis, (iii) gel electrophoresis – native poly acrylamide gel electrophoresis and sodium dodecyl sulphate (SDS) poly acrylamide gel electrophoresis, (iv) isoelectric focussing and (v) isotachophoresis.

10.3.2.1 Gel electrophoresis

The most commonly used electrophoresis is gel electrophoresis. In this technique either agarose or poly acrylamide is used as supporting media. Electrophoresis units are available for running either vertical or horizontal gel systems. Vertical slab gel units are commercially available and routinely used to separate proteins in acrylamide gels. The gel is formed between two glass plates that are clamped together but held apart by plastic spacers. Gel dimensions are mostly 12 cm x 14 cm with a thickness of 0.5 mm to 1.0 mm. A plastic comb is placed in the gel solution and removed after polymersization to provide loading wells for the samples. When the apparatus is assembled the lower electrophoresis tank buffer surrounds the gel plates and effect cooling of the gel plates.

In horizontal gel system, the gel is cast on a glass or a plastic sheet and placed on a cooling plate. Connection between the gel and electrode buffer is made by using a thick wetted filter paper (wick). The power pack supplies direct current between the electrodes in the electrophoresis unit. All electrophoresis is carried out in appropriate buffer to maintain constant state of ionization of the components being separated. Any variation in pH may alter the over all charge and so the mobility of the molecules being separated (Fig. 10.6).

Agarose Gels: Agarose is a linear polysaccharide made up of repeating units of agarobiose which contains galactose and 3, 6 anhydro galactose. This is isolated from seaweeds. Agaroses gel is usually prepared at the concentration of 1-3% solutions. The gels can be

prepared by suspending dry agarose in suitable aqueose buffer then boiling the mixture until a clear solution forms. This is poured and allowed to cool to room temperature to form a rigid gel. Agarose gels are used for the electrophoresis of both proteins and nucleic acids.

Polyacrylamide Gels: Electrophoresis in acrylamide gels is referred as **PAGE** being an abbreviation for **P**oly**A**crylamide **G**el **E**lectrophoresis. Polyacrylamide gels are prepared by dissolving required quantity of acrylamide with a small amount of N, N'-methylene bisacrylamide in suitable buffer. The polymerization is initiated by ammonium persulphate and N, N, N', N'-tetramethylene diamine (TEMED).The polymerization is free radical mediated reaction. Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis is the most widely used method for analyzing protein mixture qualitatively. It is particularly useful for monitoring protein purification. SDS is an anionic detergent. Samples to be run on SDS-PAGE are first boiled for 5 minutes in sample buffer containing beta mercapito ethanol and SDS. The mercapto ethanol reduces any disulphide bridges and cleave the protein into different sub-units. So, by this electrophoresis different units of proteins can be identified.

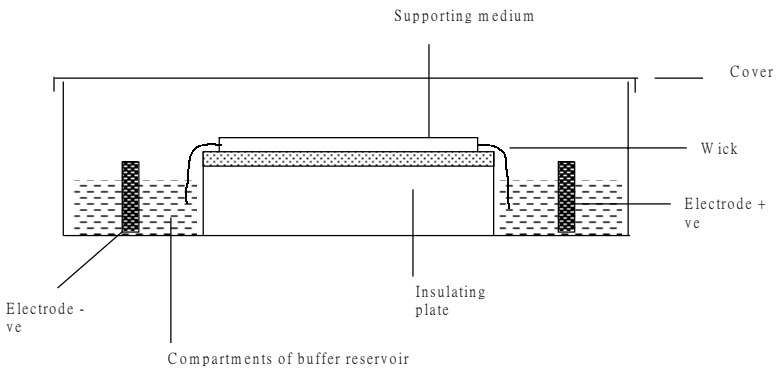


Fig.10.6 An horizontal electrophoresis unit

Detection of separated components

a. Proteins can be detected by using the dye-solution Coomassie Brilliant Blue R-250 (CBB). Staining is usually carried out using 0.1% CBB in methanol : acetic acid : water in the ratio 5:1:5. The protein bands will look blue in colour.

b. Glycoproteins are detected by using periodic acid – schiff (PAS) stain. The bands will appear in red colour.

c. Nucleic acids can be detected by using the fluorescent dye ethidium bromide. The nucleic acids bands will appear colourless in black background.

10.4 Centrifugation techniques

10.4.1 Principle

These techniques are based up on the behaviour of particles in an applied centrifugal field such as density, shape or size of the molecules being separated. The particles are normally suspended in a specific liquid medium held in tubes or bottles which are located in rotor. The rotor is positioned centrally on the drive shaft of the centrifuge. Particles which differ in density, shape and size can be separated since they sediment at different rates in the centrifugal field. Each particle sediments at a rate which is proportional to the applied centrifugal field. The rate of sedimentation can be expressed as rpm (revolutions per minute) or as g (gravitational force).

Basic principle of sedimentation: The rate of sedimentation is dependent up on the applied centrifugal field (G) which is determined by the square of the angular velocity of the rotor (w) and the radial distance (r) of the particle from the axis of rotation according to the equation $G= w^2 r$. The sedimentation rate or velocity (v) of a particle can also be expressed in terms of its sedimentation per unit centrifugal field, known as sedimentation co-efficient (s).

$$v = s\, w^2 r.$$

10.4.2 Types of centrifugation techniques: Two main types of centrifugation techniques are in general use.

a. Preparative centrifugation techniques: This technique is applied for the actual separation, isolation and purification of whole cells, plasma membrane, ribosomes, chromatin, nucleic acids, lipoproteins, viruses and many sub-cellular organelles. Large amount of materials may be involved for the bulk isolation.

b. Analytical centrifugation techniques: This technique is applied to study the characteristic features of pure macromolecules or particles. It requires only a small amounts of materials and utilizes specially designed rotors and detector system to continuously monitor the process of sedimentation of the material in the centrifugal field.

10.4.2 Centrifuges and their uses: The instrument used for this technique is known as centrifuge.Four major types of centrifuges are generally used. They are

a. small bench centrifuges

b. large capacity refrigerated centrifuges

c. high speed refrigerated centrifuges

d. ultracentrifuge (i) preparative (ii) analytical

a. **Small bench centrifuges:** These are the simplest and less expensive instruments.They are used to isolate erythrocytes from blood and other separations which require low centrifugal force. These centrifuges generally have a maximum speed of 4000-6000 rpm (revolutions per minute).The speed can also be expressed as g / min.

b. **Large capacity refrigerated centrifuges:** These centrifuges have a maximum speed of 6000 rpm/ min . Compounds to be separated can be taken in bulk.The instrment is provided with refrigeration facility . By this method biological materials can be isolated without any loss in their biological properties. Erythrocytes, coarse or bulky

precipitates, yeast cells, nuclei and chloroplasts can be isolated by using this centrifuge.

c. **High speed refrigerated centrifuges:** These instruments have maximum speed of 25000 rpm/min. They are used to collect microorganisms, cellular debris, large cellular organells and precipated proteins

d. **Preparative ultracentrifuges:** A maximum speed of 80000 rpm / min can be attained by this centrifuge. The rotor chamber is refrigerated , sealed and evacuvated to minimize excessive rotor temperature. These centrifuges are used for the separation of lipoprotein fractions and for deproteinisation of physiological fluids for aminoacid analysis

e. **Analytical ultracentrifuges:** These instruments are capable of operating at about 70000rpm/min. The rotor is present inside an evacuated , refrigerated chamber. An optical system is attached to observe the materials getting sedimented and to determine concentration distributions within the at any time during centrifugation.This technique finds applications for the separation and isolation of hormones, enzymes , ribosomal units, viruses and subcellular organells from animal and plant tissue homogenates.

10.4.3 Differential centrifugation technique

It is a type of preparative centrifugation. This method is based on the differences in the sedimentation rate of particles of different size and density. In differential centrifugation, the material (a tissue homogenate) to be separated in solution is centrifugally divided in to a number of fractions by the step wise increase of applied centrifugal field. The centrifugal field is determined by trial and error method so that the particular type of material sediments during predetermined time of centrifugation to sediment the particles in the form of pellet. The supernatant contains other materials which are unsedimented. At the end of each stage the pellet and supernatant are separated and the

pellet is purified by washing. Initially, all particles of the homogenate are homogenously distributed through out the centrifuge tube. During centrifugation particles move down the centrifuge tube at their respective sedimentation rates and start to form pellet at the bottom of the tube. Centrifugation can be continued till all the components are pelleted one by one by increasing the centrifugal field.

For example, the sub-cellular organelles (nucleus, mitochondria, lysosomes, microsomes) from a tissue liver homogenate can be isolated by applying this differential centrifugation techniques. The technique has the following steps:

a. Preparation of liver homogenate – 10% solution in 0.25 molar sucrose.

b. Centrifugation at 1000 g for 10 minutes.

c. Isolation of the pellet sedimented which is nucleus.

d. The supernatant decanted from step (c) is subjected to centrifugation at 3300 g for 10 minutes.

e. Isolation of the pellet sedimented which contains mitochondria.

f. The supernatant decanted from step (e) is subjected to centrifugation at 16300 g for 20 minutes.

g. Isolation of the pellet sedimented which contains lysosomes.

h. The supernatant decanted from step (g) is subjected to centrifugation at 105000 g for 60 minutes.

i. Isolation of the pellet sedimented which contains microsomes.

j. The supernatant obtained in the final step is the cell free cytosol.

The isolation of sub-cellular organelles is an essential procedure used in many biochemical research laboratories by using this differential centrifugation techniques. A schematic diagram of step-wise isolation sub-cellular organelles from a liver homogenate is given in Fig. 10.7.

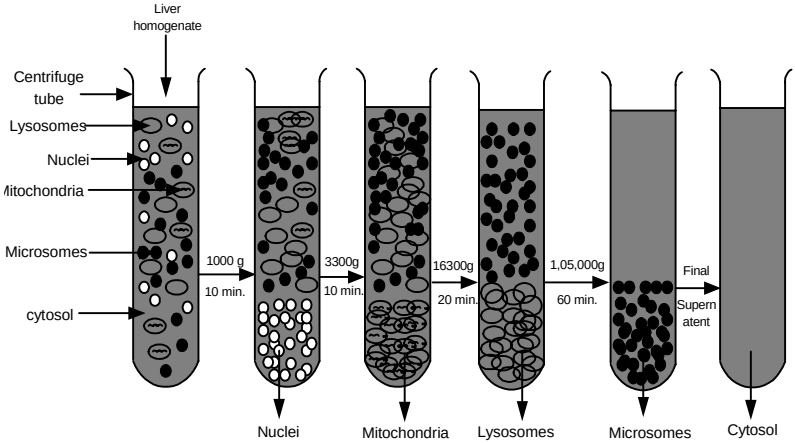


Fig. 10.7 Differential sedimentation of subcellular organelles

Applications of analytical ultra-centrifuge: The analytical ultra-centrifuge has found many applications in fields of protein and nucleic acid chemistry. This gives information about (a) determination of molecular weight of biomolecules, (b) estimation of purity of macromolecules and (c) detection of conformational changes in macromolecules.

10.5 Spectrophotometry

The present aim of the clinical chemists is the development of micro and ultramicro –methodology for the analysis of all the constituents of blood and body fluids. The study of functions of the body in both health and diseases critically requires the quantitative analysis of blood and body fluids for their various constituents.

Because so much of the quantitative methodology of biological chemistry is based on colour or light measurement, consideration must be given to the physical properties involved and to the fundamentals of the instrumental procedures. Many methods for the quantitative analysis of blood , tissue, urine, and other biological material are used on the separation of the substance in question and its chemical conversion to a compound which is capable of absorbing radiant energy. If the reaction

product in solution absorbs light in the visible region of the spectrum then the solution will be coloured. The intensity or depth of colour of such a solution can be used as a measure of concentration of the dissolved material. Determinations involving quantitative estimation of colour are known as colorimetric analyses.

Many biochemical experiments involve the measurement of the compound or group of compounds present in a complex. Probably, the most widely used method for determining the concentration of biochemical compounds is colorimetry which makes use of the property that when white light passes through a coloured solution, some wave lengths are absorbed more than others. Many compounds are not themselves coloured, but can be made to absorb light in the visible region by reaction with suitable reagents.These reactions are fairly specific and in most cases very sensitive, so that quantities of materials in millimolar quantities can be measured.

A knowledge of the physical nature of colour indicates that it is produced when specific regions or wavelengths of the visible spectrum are absorbed. To take a simple example , a solution has blue colour because , it absorbs a lesser proportion of the blue components of the mixed white light passing through it than any other coloured components. Thus the white light entering the solution will emerge in diminished intensity and have a preponderance of the blue wave lengths so the solution appears to be blue. The proportion of the various wave lengths of light absorbed is directly related to the concentration of light absorbing material. The intensity of the remaining transmitted colour is also a measure of the concentration of the material present in the solution .

Analytical procedures based upon the direct measurement of light absorption at specific wavelengths or regions of the spectrum are known as photometric procedures and the instruments used are photometers and spectrophotometers. In addition , there are methods which are dependent on the ability of insoluble particles to scatter light, called turbidimetric methods, which are dependent on the ability of

materials to emit light under specified conditions, called fluorimetric methods.

10.5.1 Principle: Spectrophotometric technique is based on the basic laws of light absorption. For uniform absorbing medium the proportion of the light radiation passing through it is called the transmittance, T, where $T=I/I_0$. I_0 = Intensity of the incident radiation, I= Intensity of the transmitted radiation. The extent of radiation absorption is more commonly referred to as the absorbance (A) or extinction (E) which are equal to the logarithm of the reciprocal of the transmittance,

i.e., $A = E = \log 1/T = \log I_0/I$

Transmittance is generally expressed on a range of 0-100% and used in certain type of turbidity measurement. Absorbance or extinction varies from 0 to ∞.

10.5.1.1 *The Beer –Lambert Law*

When a monochromatic light of initial intensity Io passes through a solution in a transparent vessel, some of the light is absorbed so that the intensity of the transmitted light I is less than Io .There is some loss of light intensity from scattering by particles in the solution and reflection at the interfaces, but mainly from absorption by the solution. The relationship between I and Io depends on the path length of the absorbing medium, *l*, and the concentration of the absorbing solution, c. These factors are related in the laws of Lambert and Beer (Fig 10.8).

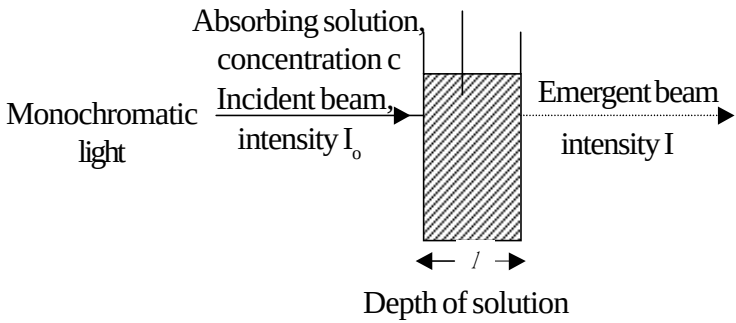


Fig. 10.8 The absorption of light by a solution

Lambert's law: When a ray of monochromatic light passes through an absorbing medium its intensity decreases exponentially as the length of the absorbing medium increases.

$$I = I_0 e^{-k_1 l}$$

Beer's law : When a monochromatic light passes through an absorbing medium its intensity decreases exponentially as the concentration of the absorbing medium increases.

$$I = I_o e^{-k_2 c}$$

These two laws are combined together in the Beer- Lambert law:

$$I = I_0 e^{-k_3 cl}$$

Transmittance: The ratio of intensities is known as the transmittance (T) and this is usually expressed as percentage

$$\text{Percent } T = I/I_0 \; 100 = e^{-k_3 cl}$$

Extinction: If logarithms are taken of the equation instead of a ratio then

$$\log_e Io/I = k_3 cl$$

$$\log_{10} Io/I = k_3 cl \; / 2.303$$

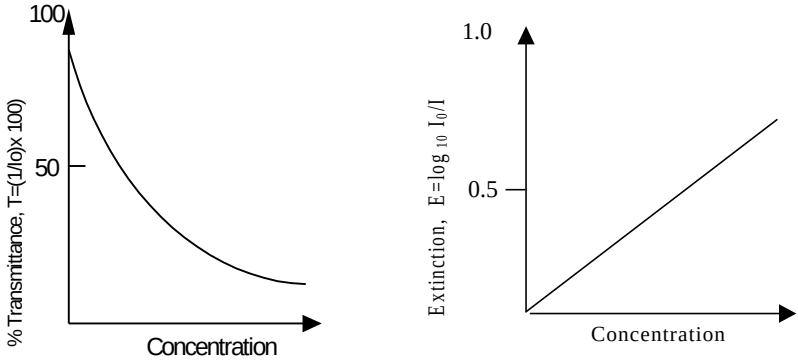
$$\log_{10} Io/I = kcl$$

The expression $\log_{10} Io/I$ is known as the extinction (E) or absorbance(A). The extinction is some times referred as optical density. Therefore

$$A \text{ (or) } E = k\,cl$$

where k is molar extinction co-efficient for the absorbing material at wave length l, c = molar concentration of the absorbing solution, *l* = path length in the absorbing material in cm. If the Beer- Lambert law is obeyed correctly and *l* is kept constant, then a plot of extinction against concentration gives a straight line passing through the origin (Fig 10.7)

Extinction, $E=\log_{10} I_0/I$



(a) Transmittance (b) Extinction

Fig 10.7: The relationship between the absorption of light and concentration of an absorbing solution

Some colorimeters and spectrophotometers have two scales, a linear one of percent transmission and a logarithmic one of extinction (Fig 10.8). The extinction scale is related linearly to the concentration and this scale is used in the construction of a standard curve. With the aid of such a curve the concentration of an unknown solution can easily be determined from its molar extinction.

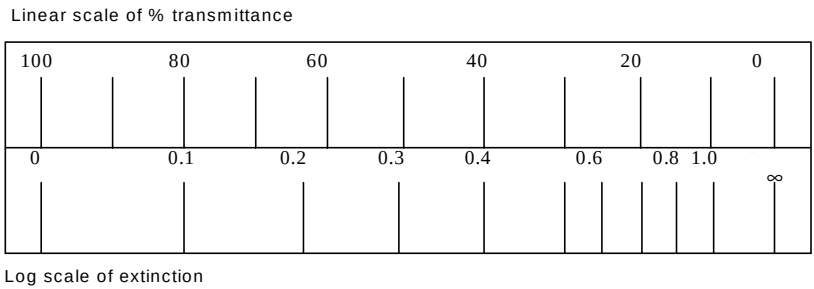


Fig. 10.8 The relationship between per cent transmittance and extinction

Molar extinction coefficient : If l is 1 cm and c is 1 mol/ litre then the absorbance is equal to k, the molar extinction coefficient, which is characteristics for a compound. The extinction coefficient k is thus the extinction given by 1 mol / lit re in a light path of 1 cm and usually written E_{1CM} , it has the dimention of mol^{-1} cm^{-1}. The instruments used for the measurement of extinction by the molecules to be quantified are spectrophotometer and photoelectric colorimeters.

10.5.2 The photoelectric colorimeter: A diagram of the basic arrangement of a typical colorimeter is given in Fig 10.9.

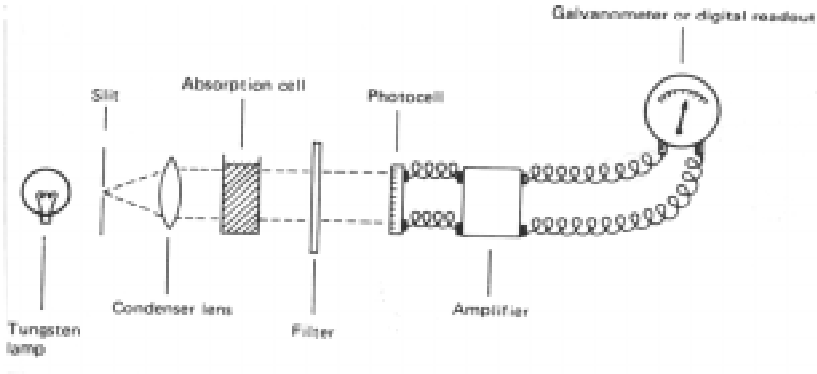


Fig 10.9 A diagram of a photoelectric colorimeter

White light from a tungsten lamp passes through a slit, then a condenser lense, to give a parallel beam which falls on the solution under investigation contained in absorption cell or cuvette. The cell is made of glass with the sides facing the beam cut parallel to each other. In most of the colorimeters, the cells are 1 cm square and will hold 5 ml of solution .

Beyond the absorption cell is the filter , which is selected to allow maximum transmission of the colour absorbed. If a blue solution is, then red is absorbed and a red filter is selected . The colour of the filter is, therefore, complementary to the colour of the solution under investigation (Table 10.1). In some instruments the filter is located before the absorption cell.

Table 10.1: The relationship between the colour of the solution under investigation and the filter chosen for colorimetric analysis.

Colour of solution	Filter
Red-Orange	Blue-Bluegreen
Blue	Red
Green	Red
Purple	Green
Yellow	Violet

The light then falls on to a photocell which generates an electrical current in direct proportion to the intensity of light falling on it. This small electrical signal is increased in strength by the amplifier , and the amplified signal passes to a galvanometer, or digital readout, which is calibrated with logarithmic scale and the extinction can be read directly. The blank solution (which does not contain the material under investigation) is first taken in the cuvette and reading adjusted to zero extinction and this is followed by the test solution and the extinction is recorded directly.

A better method is to split the light beam , pass one part through the sample and the other through the blank, and balance the two circuits to give zero. The extinction is determined from the potentiometer reading which balances the circuit..

10.5.2.1 ***Photometric analysis:*** There are four general steps in carrying out a photometric analysis:

a. separation of the substance from the complex mixture- for e.g., estimation of blood glucose requires the precipitation of lipids and proteins by deproteinising agents which otherwise interfere with the colour reaction of glucose

b. quantitative conversion to a coloured or light absorbing substance- for e.g., after deproteinisation as mentioned above for glucose estimation, the supernatant is made to react with orthotoluidine reagent to give a greenish blue coloured complex

c. measurement of light absorption- for e.g., the colour intensity of the above mentioned complex is measured by using a red filter.

d. calculation of the concentration of the substance - for e.g., by comparing the extinction with that of the standard solution of the same substance of known concentration.

$$\text{Concentration of unknown} = \frac{\text{absorbance of unknown}}{\text{absorbance of known (standard)}} \times \text{Concentration of known}$$

10.5.2.2 *UV Absorption Spectrophotometry*

A spectrophotometer is a sophisticated type of colorimeter where monochromatic light is provided by a grating or prism in the place of filter in ordinary colorimeter. The band width of the light passed by a filter is quit broad , so that it may be difficult to distinguish between two compounds of closely related absorption with a colorimeter. Some compounds absorb strongly in the ultra violet region and their concentration can be determined as with a colorimeter by using a more expensive type of spectrophotometer which operates down to 190 nm. For e.g.,

(i) The activity of enzymes requiring NAD as coenzymes can be determined by treating the enzyme source with the relevant substrate and measuring the NADH formed (colourless) which gives strong absorption at 340 nm. The increase in absorbance is proportional to the concentration of the enzyme.

(ii) the concentration of uric acid can be estimated by measuring the extinction of the solution at 293 nm before and after treatment with

an excess of the enzyme uricase. At pH 9.0, uric acid which absorbs at 293 nm, is oxidized by uricase to allantoin, which has no absorption at this wave length. The decrease in absorbance at 293 nm is a measure of uric acid level.

The main components of a simple spectrophotometer are shown in Fig 10.10.

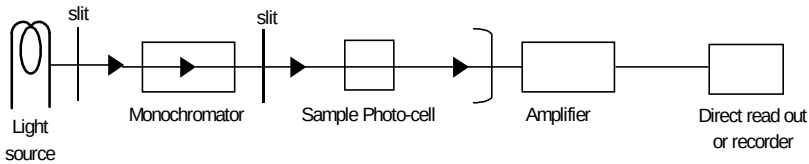


Fig. 10.10 The main components of a simple spectrophotometer

10.5.2.3 *Absorption spectra*

Many compounds have characteristic absorption spectra in the ultra violet and visible regions so that identification of those materials in a mixture is possible.

Proteins: Proteins absorb strongly at 280 nm according to their content of the amino acids tyrosine and tryptophan, and this provides a sensitive and non-destructive form of assay.

Nucleic acids: Nucleic acids and their component bases show maximum absorption in the region of 260nm. The extent of absorption of nucleic acid is a measure of their integrity, since the partial degraded acids absorb more strongly than the native materials.

Haem proteins: These conjugated proteins absorb in the visible region as well as in the UV region of the spectrum due to haem group. The visible spectra of the oxidized and reduced forms of cytochrome C are sufficiently different so that the relative amounts of these forms can be determined in a mixture.

Things to remember: The detailed operation of a particular instrument must be obtained by carefully reading the instruction manual. Few

important points concerning the use and care of calorimeters and spectrophotometers are given below.

a. Cleaning the cuvette:The cuvette should be cleaned by soaking in 50 per cent v/v nitric acid and then thoroughly rinsed in distilled water.

b. Using the cuvette: First of all, fill the cuvette with distilled water and check them against each other to correct for any small difference in optical properties. Always wipe the outside of the cuvette with soft tissue paper before placing in the cell holder. When all the measurement have been taken, wash them with distilled water and leave in the inverted position to dry.

c. Absorption of radiation by cuvettes: All cuvettes absorb radiation and the wave length at which significant absorption occurs depend on the material from which the cuvette is made. Silica cuvettes are the most transparent to U/V light but they are expensive. Glass cuvettes are much cheaper than silica, and so they are used whenever possible and invariably in the visible region of the spectrum.

d. Light source : A tungsten lamp produces a broad range of radient energy down to about 360 nm. To obtain the ultra violet region of the spectrum a deuterium lamp is used as the light source.

e. Blanks: The extinction of a solution is read against a reagent blank which contains all the reagents except the compound to be measured. The blank is first placed in the instrument and the scale adjusted to zero extinction before reading any solution . Alternatively, the extinction can be read against distilled water and the blank reading can be subtracted from that of the test solution

f. Duplicates: It is essential to prepare all blanks , standard solutions and unknown solutions in duplicates so that the accurate standard curve can be obtained.

g. Construction of standard curve : A series of concentrations of standard solution are taken in different test tubes and made to react with colouring agents. The blank tube is also treated similarly but by replacing standard solution with water. The absorbance are measured at the corresponding wavelength and a graph is plotted concentration of the standard versus the absorbance.

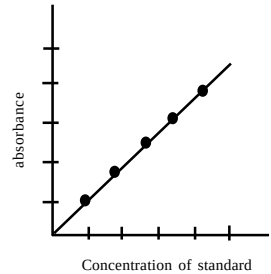


Fig. 10.11 Standard calibration curve

10.5.3 Applications of spectrophotometry

Colorimetry and spectrophotometry have widest application in biological sciences. These techniques are used for the determination of

a. glucose, proteins, lipids, nucleic acid etc

b. turbidity of solutions(bacterial cell mass)

c. absorption spectrum of a compound

d. purity of compound by knowing the molar extinction coefficient which is maximum for a pure compound.

Excercise

1. Choose the correct answer from the given four alternatives

a. Chromatography is based on the principle of

i. Conductivity ii. Distribution coefficient

iii. Counter balance of charges iv. Centrifugal force.

b. Agarose is a polymer of

i. Galactose ii. Agarobiose iii. Polyacrylamide

iv. Bisacrylamide

c. In analytical ultracentrifuges the maximum speed is

i. 70,000 rpm ii. 7000 rpm iii. 45000 rpm iv. 1000 rpm

d. Monochromators are seen in

i. Centrifuges ii. Potentiometers iii. Spectrophotometers

iv. Densitometers.

e. Amino acids can be identified by using the staining agent

i. Ninhydrin ii. Sulphuric acid iii. Ethydium bromide

iv. Coomasie brilliant blue

II. Fill up the blanks

a. The subcellular organelles of a cell suspension can be isolated by _____ technique

b. Polyacrylamide gel is prepared by mixing acrylamide and _____ at fixed proportions.

c. In exclusion chromatography _____ sized particles are eluted first.

d. According to Beer's law the intensity of the emerging light from a solution depends on _____ of the absorbing medium.

e. The molecular weight of porteins can be determined by _____ chromatographic technique.

III. Say true or false

a. In UV spectrophotometer, the lamp used for UV absorption measurement is tungsten lamp.

b. Transmittance is inversely proportional to extinction.

c. Monochromators have better resolution capacity of differnt light wavelengths than filters.

d. Nucleic acids and their component bases show maximum absorption at 260 nm.

e. In paper chromatography, the stationary phase is a liquid.

IV. Match the following

1.	Affinity chromatography	-	Fatty acid analysis
2.	Gas-liquid chromatography	-	Ligand
3.	Spectro photometer	-	Separation of proteins
4.	Differential centrifugation	-	Beer-Lamberts law
5.	PAGE	-	Isolation of subcellular organelles

V. Give one word answer

(a) What are the two phases involved in chromatographic technique?

(b) Mention any two materials used to prepare the slurry in TLC.

(c) What is agarose?

(d) Which type of ultra centrifuge is applied for the determination of molecular weight of compounds?

(e) How is transmittance and extinction are related?

VI. Answer the followings:

a. Explain the principle of affinity chromatographic technique.

b. How will you isolate subcellular organelles present in a liver homogenate?

c. What are the factors affecting electrophoresis?

d. Calculate the concentration of glucose present in 100 ml of the unknown solution by using the following data

Extinction read	Concentration of standard in a total volume of 4.0 ml (in microgram)
0.02	20
0.04	40
0.06	60
0.08	80
0.10	100
0.05	x (unknown) in a total volume of 4.0 ml

The volume of unknown used for the measurement of extinction is 0.5ml.

(e) Give the diagrammatic representation of photoelectric colorimeter and explain the principle.

PRACTICALS

Introduction

Biochemical analyses are of two types. (a) Qualitative analyses are done to identify and detect the presence of certain substances in a mixture of such compounds. (b) Quantitative analyses are performed to find out the exact amount of a particular substance in a pure solution or in a mixture. In biochemical laboratories both these analyses are performed to detect and to quantify some important metabolites in blood and body fluids.

Generally the qualitative tests can be conducted in transparent glass test tubes to observe the colour formation easily. The glass wares requried to conduct quantitative estimations are conical flask, standard flask, volumetric pipette and burette.

Qualitative tests for carbohydrates

Basically the qualitative tests used for carbohydrates can be catogorised in to the following groups:

(a) common tests for carbohydrates

(a) tests for reducing property of carbohydrate;

(b) tests for the functional groups (aldehyde/ketone);

(c) test for monosaccharide/disaccharide; and

(d) test for pentose.

It is suggested that 1 % solution of each of glucose, fructose, galactose, arabinose, ribose, lactose, sucrose, maltose and starch can be prepared and subjected to each test in a systematic manner.

Common tests for carbohydrates

1. Molisch' s test:

Principle- Sugars undergo dehydration in the presence of non- oxidizing acids like hydrochloric acid and sulphuric acid to form furfural or hydroxy methyl furfural. These compounds can react with aromatic amines such as α naphthol or phenol to give intensely coloured compounds. This reaction forms the basis of a general qualitative test for sugar.

Reagents- (1) 5% α naphthol in ethanol (2) Conc. Suphuric acid.

Test - Add 2.0 ml of the given solution in a test tube and add 2 drops of an ethanolic solution of naphthol. Carefully run down about 1.0 ml of Conc. sulphuric acid along the sides of the tube. Formation of a bluish violet coloured ring at the junction of two liquids or development of violet colour throughout the solution shows the presence of carbohydrate.

Anthrone test:

Principle- Carbohydrates form furfural with Conc. sulphuric acid and produce bluish green colour followed by the addition of anthrone.

Reagents- (1) 0.2% anthrone in Conc. sulphuric acid.

Test - Take 2.0 ml of the reagent in a test tube and add two drops of the test solution . Mix well . I f there is no colour change , boil in a water bath for 10 min. Formation of green colour indicates the presence of carbohydrates.

Tests for reducing property of carbohydrate

Fehling's Test

Principle- If the carbohydrate has reducing group, the cupric ions present in the Fehling's reagent will be reduced to cuprous ions and even copper and this will produce a rusty brown or red precipitate.

Reagent - Fehling solution is prepared by dissolving 7.0 gm of copper sulphate in water and making up to 100 ml. In a separate container 24.0 gm of potassium hydroxide and 34.6 gm of sodium potassium tartarate are dissolved and made up to 100 ml in water. Just prior to use, the two solutions are mixed.

Test - To 2 ml of this solution add a few drops of the given test solution and boil. Formation of red or brown precipitate shows the presence of reducing sugar .

Benedict's Test

Principle

When the reducing sugar is boiled with Benedict's reagent which is an alkaline solution of cupric sulphate, the blue coloured cupric sulphate is reduced gradually to form insoluble cuprous oxide which may be green ,yellow, orange or red in colour, depending upon the concentration of sugar in the solution.

Reagent – Benedict qualitative reagent: 17.3 gm of sodium citrate and 10.0 gm of sodium carbonate are dissolved in about 75 ml of water and filtered if necessary. Separately 1.73 gm copper sulphate is dissolved in about 20 ml water and this is slowly added to alkaline citrate solution with stirring. The volume is made up to 100 ml.

Test - Add 3 to 4 drops of the given test solution to 2 ml of the reagent and boil the contents. Formation of red precipitate shows the presence of reducing sugar.

Test for monosaccharide

(a) Barfoed's test: Principle- This test is used to distinguish monosaccharides from disaccharides as the monosaccharides immediately give cuprous oxide red precipitate on heating (for 1-2 min) with Barfoed's reagent which contains cupric acetate in acetic acid

Reagent – The reagent is prepared by dissolving 13.3 gm of copper acetate in 200 ml of water and adding 1.8 ml of glacial acetic acid.

Test - Add 1.0 ml of given test solution to 2.0 ml of the reagent and boil exactly for 1 minute. Formation of reddish orange precipitate shows the presence of monosaccharides. Excess boiling will give faults positive results., i.e., disaccharides also give red colour on excess boiling.

Test for ketonic group

Seliwanoff test: Principle- Keto hexoses like fructose form hydroxy methyl furfural derivatives with hydrochloric acid and form cherry red coloured compound with resorcinol present in the Seliwanoff 's reagent

Reagent- Seliwanoff's reagent – This reagent is prepared by dissolving 50 mg of resorcinol in 100 ml of dilute HCl.

Test – Add a few drops of the given test solution to 5.0 ml of this pre-heated reagent and boil the mixture. Formation of cherry red colour in three minutes shows the presence of ketose sugar.

Test for pentose

Bial's test: Principle- Pentose sugar forms furfural derivatives with hydrochloric acid and then reacts with orcinol to give green coloured products.

Reagent: Bial's reagent is prepared by dissolving 150 mg of orcinol in 50.0 ml of concentrated HCl.

Test : Mix 5.0 ml of this reagent and 2.0 ml of the given test solution in a tube and heat in a water bath. Note the time at which any colour change is observed. Formation of green colour within 10 minutes shows the presence of a pentose.

Tests for galactose

(1) Tollens phloroglucinol test: Add 1 ml of the test solution to 0.5ml of phloroglucinol solution. Formation of red colour indicates the present of galactose.

(2) Mucic acid test : Add 1 ml of the test solutoin to 0.5 ml of dilute nitric acid and heat in a boiling water bath for 90 mins. and let stand for

over night. Formation of crystalline white precipitate shows the presence of galactose.

Test for polysaccharides

Iodine test: Principle- Iodine forms blue coloured complex with 1,4 glycosidic linkages present in polysaccharides such as starch and glycogen.

Reagent - The reagent is prepared by dissolving 3 gm of iodine in 100 ml of 3 % potassium iodide.

Test – Add a few drops of the given test solution to 2 drops of 0.1N HCl followed by 2 drops of iodine reagent. Formation of blue colour shows the presence of starch and brown colour shows the presence of glycogen.

If the given carbohydrate solution is found to be a non-reducing one, the carbohydrate must be hydrolyzed to give reducing monosaccharide units and then all the tests should be performed to identify the components after hydrolysis.

Hydrolysis of non-reducing sugars

2.5 ml of the given solution is mixed with 5 drops of concentrated sulpuric acid and boil the contents for 5 minutes. Cool the contents and neutralize with saturated barium hydroxide. Remove the precipitate of barium sulphate by filtration and carry out all the tests in the filtrate.

Confirmatory test for carbohydrates

Phenyl hydrazine test: Principle- This is an important reaction of reducing sugars(monosaccharides and disaccharides). The aldehyde or ketonic groups present in sugar reacts with phenyl hydrazine and forms yellow crystalline products called osazones. The shape and the time of formation of osazone confirms the type of carbohydrate.

Reagent – The reagent is prepared by mixing 2 parts of phenyl hydrazine hydrocloride and 3 parts of sodium acetate by weight. These are thoroughly mixed in a mortar.

Test – To 2.0 ml of the given solution add about 1 spatula of the reagent mixture and boil the contents in a water bath. Note down the time taken for the formation of yellow coloured crystals. Allow the tube to cool slowly and examine the crystals microscopically. Better crystals can be obtained if the tubes are allowed to cool in a water bath.

Table 1: Time of formation and the shape of osazone

Carbohydrate	Time of formation of osazone	Shape of osazone crystals
Mannose	1-5 mins	Rapidly form an insoluble white mannosazone hydrazone even before heating. On heating form white broken glass like osazone particles.
Fructose	2-3 mins	Yellow needle shaped crystals
Glucose	5-7 mins	Yellow needle shaped crystals
Galactose	15-20 mins	Broken glass like crystals
Lactose	45-50 mins	Badminton ball shaped crystals, soluble in hot water.
Maltose	35-45 mins	Star shaped crystals
Arabinose	8-10 mins	Chalk powder shaped crystals
Xylose	6-7 mins	Flower shaped crystals
Sucrose	3-7 mins	Needle shaped crystals form after hydrolysis

Quantitative test for carbohydrates (glucose)

Aim

To estimate the amount of glucose present in the given test solution by Benedict's quantitative method.

Benedict's method

This method is of value in clinic analysis of glucose in blood and urine.

Principle

The cupric ions present in the Benedict quantitative reagent is reduced by the aldehyde group of glucose and form colourless white precipitate on heating.At a fixed volume of Benedicts reagent, the volume of glucose solution consumed is proportional to the concentration of glucose present in it . The end point is the complete disappearence of blue colour

Reagents

(a) Benedict quantitative solution: Dissolve 200 gm of sodium citrate, 75 gm of anhydrous sodium carbonate and 125 gm of potassium thiocyanate in about 600 ml of water with gentle heating. Filter, cool and add 18 gm of copper sulphate dissolved in about 100 ml of water. Mix both the solutions with stirring continuously. Add 5.0 ml of 5% potassium ferrocyanide solution and make up to a litre with distilled water. If the solution is not clear, filter.

(b) Sodium carbonate (anhydrous)

(c) Standard glucose solution: 200 mg of glucose is accurately weighed and dissolved in a 100 ml conical flask with about 20 ml of water. When the substance is completely dissolved, the volume is made up to the mark with water. Concentration of glucose in the standard solution is 2.0 mg /ml

Method

Step 1: Prepare the standard glucose standard glucose solution in 100 ml conical flask as described earlier.

Step 2: Take unknown glucose solution (test) in a standard flask and add distilled water to make up to the mark.

Step 3: Measure accurately 10 ml of the Benedict quantitative reagent in to a 100 ml conical flask and approximately add 1 gm of sodium carbonate. Heat the mixture to boiling. Take the standard sugar solution in a burette and slowly run this solution in to the boiling reagent. A bulky white precipitate formed first, which is cuprous thiocyanide. At this stage, add the sugar solution slowly till the last trace of blue colour has disappeared. Note the volume of the sugar solution required. Repeat the titration till concordant values are obtained.

Step 4: Now, remove the standard sugar solution from the burette. Wash the burette with distilled water and rinse with the given sugar solution (test solution). Conduct the titration as mentioned in Step 3 and repeat the titration with Benedict quantitative reagent till concordant values are obtained.

Step 5: The amount of sugar present in the unknown solution can be calculated by knowing the titre value of both standard and test.

Titration 1 :Titration of standard sugar solution against Benedicts quantitative reagent

Table-1

Volume of Benedict's Reagent (ml)	Burette reading (ml)		Volume of sugar solution consumed (ml)
	Initial	Final	
10 .0	0	8.5	X_1 (8.5)
10.0	8.5	17.0	

Titration 2: Titration of unknown sugar solution against Benedict's reagent

Table- 2

Volume of Benedict's Reagent (ml)	Burette reading (ml)		Volume of sugar solution consumed (ml)
	Initial	Final	
10 .0	0	10.0	X_2 (10.0)
10.0	10.0	20.0	

Calculation

- For model calculation the titre values are taken as mentioned in the table.
- The volume of glucose solution(standard) consumed to reduce 10 ml of Benidict's reagent = 8.5 ml(titration 1)
- The concentration of glucose in the standard solution = 2mg/ml, therefore, the amount of glucose present in 8.5 ml of standard glucose solution= 8.5 x 2 =17.0 mg of glucose
- Therefore 17.0 mg of glucose is required for the complete reduction of 10.0 ml of Benedict's reagent.
- The volume of unknown glucose solution consumed to reduce 10.0 ml of Benedict's reagent=10.0 ml
- Therefore, 8.5 ml of standard glucose solution is equivalent to 10 ml of unknown glucose solution.
- Therefore 10.0 ml of unknown sugar solution must have contained 17.0 mg of glucose.
- Therefore 100 ml of the given test sugar solution must have contained 10 x 17=170 mg of glucose.

Result: The amount of glucose present in the whole of the given solution = 170 mg.

Qualitative tests for amino acids

There are a number of qualitative tests to detect the presence of amino acids and these tests are dependant on the nature of the R groups present in the amino acids and hence specific. The following tests can be carried out with a number of amino acids for which 2 % solutions can be prepared.

(1) Common test for amino acids

Ninhydrin test: Principle- When a solution of aminoacid is boiled with ninhydrin, it is deaminated to hydrindantin and further reacts with excess of ninhydrin and forms a blue coloured compound.

Reagent – Dissolve 200 mg of ninhydrin in 100 ml of ethanol.

Test – Add 1.0 ml of the given solution to 2.0 ml of ninhydrin reagent and keep it in a boiling water bath for 10 minutes. Formation of a bluish violet colour shows the presence of amino acids. Hydroxy proline and proline give orange colour.

(2) Tests for tyrosine The tests for tyrosine are based on the presence of phenolic hydroxyl group present in the amino acid.

a. Xanthoproteic test

Principle – The phenolic hydroxyl group of tyrosine reacts with strong acids such as hydrochloric acid and sulphuric acid and produce reddish orange colour with the addition of sodium hydroxide

Reagents – (i) concentrated nitric acid or conc.H_2SO_4 (ii) sodium hydroxide

Test – Add a few drops of concentrated nitric acid to 1.0 ml of the given amino acid solution. Subsequently, add a few drops of dilute sodium hydroxide. Formation of orange colour shows the presence of tyrosine.

b. Millon's test

Reagents – (i) 15 % solution of mercuric sulphate in 15 % sulpuric acid, (ii) 1 % sodium nitrite solution.

Test - Add a few drops of mercuric sulphate reagent to about 1.0 ml of the given test solution and heat for 10 minutes at 100°C. After cooling add a few drops of sodium nitrite solution. Formation of red colour indicates the presence of tyrosine.

c. Morner's test

Reagent- Morner's reagent- This reagent is a mixture of 1.0 ml of formaline, 45 ml of distilled water and 55 ml of concentrated sulphuric acid .

Test: Add about 3.0 ml of Morner's reagent to about 0.5 ml of the test solution and gently boil the contents. Formation of green colour indicates the presence of tyrosine.

(3) Tests for tryptophan - The tests are mainly based on the imidazole group of tryptophan.

Hopkins Cole test:

Reagent s – (i) glyoxylic acid (ii) concentrated sulphuric acid.

Test – Add 2.0 ml of the given test solution to 2.0 ml of glyoxylic acid. Mix well and then carefully add 2.0 ml of concentrated sulphuric acid through the sides of the tube. Formation of violet ring at the junction indicates the presence of tryptophan.

Ehrlich's test: This test is based on the reaction between aromatic aldehyde and the imidazole group of tryptophan.

Reagent – 10% para dimethyl amino benzaldehyde in 10 % HCl (Ehrlich reagent).

Test - Add 1.0 ml of the given test solution to 1.0 ml of the Ehrlich reagent. Formation of red colour shows the presence of tryptophan.

(4) Test for arginine

Sakaguchi test: This reaction is specific for guanidine group of arginine

Reagents-(1) 40% NaOH.(2) 1 percent α naphthol in alcohol. (3) Dilute bromine water

Test - Mix 3.0 ml of the test solution with 1.0 ml of 40 % NaOH. Add two drops of α naphthol solution to the mixture. Add few drops of bromine water slowly . Formation of red colour indicates the presence of arginine.

(5) Test for sulphur containing amino acids

Test for cysteine:

a. Sullivan's test: This test is based on the sulfhydryl group present in the amino acid cysteine.

Reagents: 1.Sodium sulphite reagent – 15 % solution in 0.5N sodium hydroxide; 2. Sodium bisulphite reagent – 2 % solution in 0.5N sodium hydroxide; 3. 5N sodium hydroxide; 4. Sodium cyanide solution – 1% solution in 0.8N sodium hydroxide.

Test: To 5 ml of the test solution, add 1 ml of sodium cyanide reagent followed by 5 ml of sodium sulphite reagent. Mix the content and let stand for 30 minutes. A reddish brown colour appears. Then add 1 ml of sodium bisulphite reagent. The reddish brown colour will be converted to dark red colour. This shows the presence of cysteine.

b. Lead sulphide test

Reagents: 1. Lead acetate – 10% aqueous solution, 2. 40 % Na OH or KOH.

Test: Add a few drops of lead acetate solution to 1 ml of the test solution. Then add 2 ml of NaOH or KOH . Boil for a few minutes. The solution becomes brown and a black precipitate of lead sulphide appears. This shows the presence of cysteine.

c. Sodium nitroprusside test:

Reagent: 1 % solution of sodium nitroprusside, 2. 5% sodium hydroxide.

Test: Add 1 ml of the test solution with 1 ml of sodium nitroprusside reagent followed by 2 drops of NaOH. Formation of a deep purple colour which fades after a few minutes indicates the presence of cysteine.

d. Ferric chloride test:

Reagents: 1% solution of ferric chloride, 2. 1% solution of copper sulphate.

Test: Add 1 ml of test solution to 0.5 ml of ferric chloride reagent. An indigo blue colour appears and disappears almost immediately. Then add 0.5 ml of copper sulphate solution. Appearance of a transitory violet colour indicates the presence of cystine.

e. Test for cystine

Reagents:

Test: To 5 ml of the test solution add 1 ml of freshly prepared 5% aqueous solution of sodium cyanide. Mix and let it stand for 10 minutes. Then add 1 ml of a freshly prepared 0.5% solution of 1, 2-naphthoquinone – 4 sodium sulpfonate and sodium sulphite. Appearance of a deep red colour in the solution indicates the presence of cysteine.

f. Test for methionine

g. Sodium nitroprusside test: Reagents: (1) NaOH- 5% aqueous solution (2) Glycine- 1.5% aqueous solution (3) Sodium nitroprusside –10 % aqueous solution(to be prepared freshly) 4. Hydrochloric acid- 6 N

T est: To 5.0 ml of the test solution add the following reagents in order, mixing after each addition: 1.5 ml of 5N sodium hydroxide , 1.5 ml of glycine, 0.3 ml of sodium nitroprusside. Place the tubes in the water bath at 37- 45 °C for 15 min , and add 3.0 ml of hydrochloric acid. Shake for 1 min . Formation of a reddish purple colour indicates the presence of methionine.

Quantitative estimation of glycine

The quantity of glycine can be estimated by the Sorenson's formal titration method

Aim: To estimate the amount of glycine present in the whole of the given test solution

Principle: When glycine is treated with formaldehyde , the amino group of glycine reacts with the aldehyde and forms dimethylol compound. Now the carboxyl group is left free and can be titrated with sodium hydroxide and the volume of sodium hydroxide consumed is proportional to the amount of glycine in the given test solution. If the amino group of glycine is not protected with formaldehyde, it will interfere with the end point of the titration with sodium hydroxide.

Reagents:

1. standard oxalic acid solution: 630 mg of oxalic acid is accurately weighed and dissolved with 20 ml of distilled water in a 100 ml standard flask. When the substance is completely dissolved , the volume is made up to the mark. The normality of this solution is 0.1N.

3. Sodium hydroxide 0.1N

4. Formaldehyde- Commercial

5. Phenolphthalein – 0.1 % solution as indicator

Method

Step : 1 The test solution of glycine in the standard flask is made up to the mark accurately

Step : 2 Standardisation of sodium hydroxide

Fill the burette with sodium hydroxide after proper rinsing with the same. Pipette out 10.0 ml of standard oxalic acid (0.1N) in a clean conical flask . Add a drop of of indicator solution . Titrate the oxalic acid solution against sodium hydroxide. Observe the sharp end point which is the appearance of permanent pale pink colour .Note down the volume of sodium hydroxide run down from the burette . Repeat the titration for concordant values.

Step 3 : Titration of standardised used NaOH against glycine (test) solution

Fill the burette with standardised sodium hydroxide. Take 10 ml of the test glycine solution in a clean conical flask and mix well with 10 ml of formaldehyde. The contents are shaken well for the reaction to take place . Now add a drop of of indicator. Titrate the mixture against sodium hydroxide in the burette. Note down the volume of sodium hydroxide run down from the burette at the end point of appearance of pale pink colour. Repeat the titration for concordant values. Find out the blank value by titrating 10 ml of water+ 10 ml of formaldehyde against sodium hydroxide.The difference between the "test" and the "blank" titre values is the volume of alkali consumed for the neutralization of glycine present in the test solution

The normality of "test" glycine solution can be calculated by knowing the normality of sodium hydroxide. From this the amount of glycine present in the test solution can be determined.

Titration :1

Standard oxalic acid against sodium hydroxide

Sl. No.	Volume of Oxalic acid (V_1ml)	Burette reading (ml)		Volume of Sodium Hydroxide (V_2ml)	Indicator
		Initial	Final		
1	10	0	10.2	X_1 (10.2)	Phenolp-hthalein
2	10	10.2	20.4		

Normality of oxalic acid $N_1 = 0.1N$

Volume of oxalic acid used for titration = $V_1 = 10ml$

Normality of sodium hydroxide $N_2 = X$

Volume of sodium hydroxide consumed for titration = $V_2 = 10.2ml$ (for model calculation if the volume of sodium hydroxide consumed is taken as 10.2ml). According to the normality equation.

$$V_1 \times N_1 = V_2 \times N_2$$

$$10 \times 0.1N = 10.2 \times X$$

$$\text{Therefore, } N_2 = V_1 \times N_1 \;/\; V_2$$

$$= 10 \times 0.1\,N \;/\; 10.2$$

$$N_2 = 0.098$$

The normality of sodium hydroxide used for the titration is found to be 0.098 N.

Titration :2

Standard sodium hydroxide (0.098 N) against glycine (Test)

Sl. No.	Volume of NaoH (ml)	Burette reading (ml)		Volume of Sodium Hydroxide (ml)	Indicator
		Initial	Final		
1	10	0	12.0	X_2 (12.0)	Phenolp-hthalein
2	10	12.0	24.0		

Titration :3

Standard sodium hydroxide against blank solution (water + formaldehyde)

Sl. No.	Volume of NaOH (ml)	Burette reading (ml)		Volume of Sodium Hydroxide (ml)	Indicator
		Initial	Final		
1	10	0	1.3	X_3 (1.3)	Phenolp-hthalein
2	10	1.3	2.6		

Calculation

Volume of sodium hydroxide solution consumed for the neutralisation of "test" glycine solution = test titer value - blank titer value.

In the above mentioned experiment the volume of sodium hydroxide consumed = 10.2 - 1.3 = 8.9 = V_1.

Normality of sodium hydroxide N_1 = 0.098

Volume of glycine used for titration V_2 = 10 ml

Normality of glycine = N_2 = X

$V_1N_1 = V_2N_2$

8.9 x 0.098 = 10 x X

X = 8.9 x 0.098 / 10

= 0.087

Therefore nomality of glycine (test) = 0.087 N

The weight of the substance and the normality are related by the equation wt/litre = equivalent weight X normality. The normality of glycine is 75.

Therefore, The amount of glycine present in

1 litre of the given solution = 0.087 x 75

= 6.652 gms

Therefore, the amount of glycine present in 100 ml of the given solution = 6.652 / 10 = 0.665 gms or 665 mg

For model calculation the titre values are taken as mentioned in the table (brackets).

Result

The amount of glycine present in the whole of the given solution = 0.665 gm

Excercise

1. Maintain observation notebook for the entry of results obtained from qualitative and quantitative analysis.
2. For qualitative analysis of carbohydrates and amino acids draw three columns in your observation note books for test, observation and inferences. Write the confirmation test and give your results.
3. For qualitative analysis the tabular column drawn should contain the following details.

Sl.No.	Test	Observation	Inference
1.			
2.			

Result The given unknown solution is found to contain ----------

4. For quantitative analysis draw tabular columns and enter your titer values then perform the calculation.

Result : The amount of glycine / glucose present in the whole of the given solution = ----------