CHAPTER II

ELEMENTARY COMPOSITION OF THE HUMAN BODY

(BIOCHEMISTRY)

The elementary composition of the human body is as follows: oxygen —65%; carbon—18%; hydrogen—10%; nitrogen—3%; calcium—1·25%; phosphorus—1%; potassium—0·35%; sulphur—0·25%; sodium—0·15%; chlorine—0·15%; magnesium—0·05%; iron—0·004%; iodine 0·00004%. In addition to these, manganese, copper, zinc, fluorine, silicon, cobalt, molybdenum and certain other elements are also present in traces.

THE PROXIMATE PRINCIPLES

Proteins, lipids, carbohydrates, mineral salts, vitamins and water are collectively known as the proximate principles or the proximate constituents of the body. A brief summary about the chemistry and properties of each one of the proximate constituents is given below.

PROTEINS

Proteins are essential constituents of protoplasm. They differ from carbohydrates and lipids by always containing nitrogen and sometimes phosphorus and sulphur. The elementary composition of protein is as follows: **carbon**—54%; **hydrogen**—7%; **nitrogen**—16%; **oxygen**—22%. Some may contain, **sulphur**—1%; while others, **phosphorus**—0·6%.

The protein molecule is built up by the union of a large number of *amino acids*. The amino acids should be considered as the units with which the protein molecule is composed. It is to be specially noted that the term *protein* is applied only to the complex protein molecule responding to the characteristic tests of protein, e.g., copper-protein (*biuret*) test, etc., due to the presence of two or more peptide linkages whereas amino acids do not respond to the characteristic tests of protein; hence they are called one of the *non-protein nitrogenous* (NPN) constituents.

Classification of Proteins

Proteins are classified in various ways. The following classification is advocated by the English School of Physiologists: (1) **simple proteins,** (2) **conjugated proteins,** (3) **derived proteins.**

I. Simple Proteins

These are pure proteins, uncombined with anything else. The important members of this group are as follows:

1. **Protamines**—found in the sperm of certain fishes; molecular wt. low, about 2,000; strongly basic in character, not coagulated by heat and soluble in ammonium hydroxide.

2. **Histones**—occur in thymus gland and also in haemoglobin (the globin part). Coagulates on boiling in presence of salts. Soluble in water and dilute mineral acids. Precipitated from watery solution by adding ammonia but soluble in excess of the reagent. They are basic in reaction and the most characteristic feature is the high proportion of *diamino acids* in the molecule.

3. **Albumins**—amphoteric reaction, soluble in distilled water as well as in salt solutions; coagulated by heat, acids and alkalies. It is precipitated

by complete saturation by ammonium sulphate or zinc sulphate. It includes the following:

(*a*) *Egg albumin*—forms 10–12% of egg-white. It is heat-coagulable and precipitated by adding a drop of dilute acetic acid, in excess of ether. It is laevorotatory, rotation being—35·5.

(*b*) *Serum albumin*—occurs in serum (4–5%) lymph, tissue fluid, etc. Coagulates at 75°C., differs from egg albumin by its greater rotation (—56) and also by the fact that it is not precipitated by ether and acetic acid.

(*c*) *Lactalbumin*—in milk.

(*d*) *Myoalbumin*—in muscle.

(*e*) *Leucosin*—is a vegetable protein found in wheat.

Some of the albumins are now known to contain carbohydrate radicals, and so truly belong to the conjugated protein group.

4. **Globulins**—insoluble in distilled water but soluble in dilute salt solutions. Coagulated by heat and acids, precipitated by full saturation with magnesium sulphate, NaCl and by half saturation with ammonium sulphate. The important members of this class are the following:

(*a*) *Serum globulin*—occurs in plasma, serum, etc.

(*b*) *Ovoglobulin*—in egg-yolk.

(*c*) *Crystallin*—found in the crystalline lens of the eye.

(*d*) *Fibrinogen*—present in plasma and is converted into fibrin during coagulation.

(*e*) *Vegetable proteins*—for instance, *edestin* from hemp, cotton, etc., *legumin* from peas.

(*f*) *Myosin*—from muscle.

5. **Prolamines**—found in maize, barley, wheat, etc., soluble in 70 to 80% dilute alcohol and insoluble in absolute alcohol and water. Example—*zein* (maize) and *gliadin* (wheat).

6. **Glutelins**—obtained from cereals, soluble in weak acids and alkalies, but insoluble in neutral solution. They are coagulated by heat.

7. **Scleroproteins** (Albuminoids)—They are least soluble of all the proteins.

(*a*) *Keratin*—found in hoofs, horns, nails, hairs, etc. Rich in sulphur.

(*b*) *Elastin*—from elastic tissue, cartilage and ligament.

(*c*) *Collagen*—from white fibrous tissue, bone and cartilage.

(*d*) *Ossein*—in bone and teeth.

II. Conjugated Proteins

In these compounds the protein molecule remains conjugated with another non-protein prosthetic group. This class includes the following:

1. **Chromoproteins.** Here the non-protein substance (prosthetic group) is a colouring matter. The followings are the important chromoproteins:

(*a*) *Haemoglobin*—here the simple protein—globin, remains united with an iron-containing pigment, haem.

(*b*) *Visual purple* (Rhodopsin)—in the retina, prosthetic group is a carotenoid pigment.

(*c*) *Cytochrome*—conjugated protein containing haem.

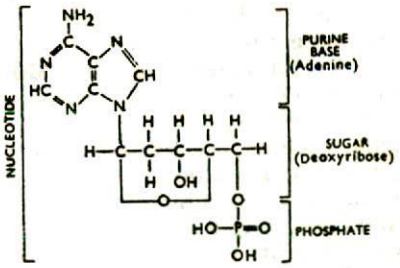
(*d*) *Flavoprotein*—Riboflavin as the prosthetic group.

(*e*) *Haemocyanin*—the blood pigment of Crustacea. The pigment part contains copper.

(*f*) *Catalase* and *Peroxidase*—two separate enzymes, the prosthetic group in both is haem.

2. **Phosphoproteins.** *Caseinogen* of milk, *vitellin* of egg-yolk, etc. Here the protein molecule remains combined with phosphoric acid. They are markedly acid in character, insoluble in water but soluble in alkalies. Their solution in alkali is not coagulated by heat.

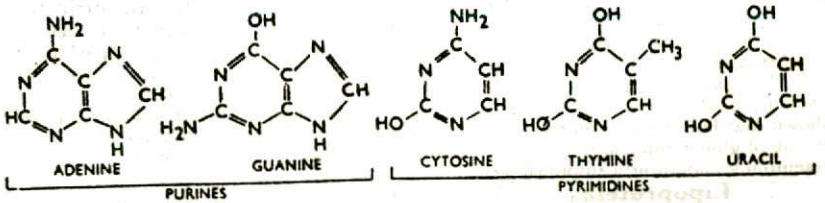
3. **Nucleoproteins.** This protein contains phosphoric acid and also other prosthetic group. Hence it should be placed in a special class. Nucleoproteins are formed by the union of *nucleic acid with two molecules of proteins.* The nucleic acid is made up of four *mononucleotides.* Each mononucleotide is again composed of three different compounds, e.g.,



(*a*) *Phosphoric acid.*

(*b*) *Carbohydrate.* The carbohydrate present in nucleic acid is a *pentose.* In deoxyribonucleic acid (DNA) present in cell nucleus, the pentose is *deoxyribose*, whereas *ribose* is present in ribonucleic acid (RNA) occurring in cytoplasm of cell and also in nucleolus.

(*c*) *Nitrogenous bases.* The bases are of two types: (*i*) *Purine bases*—*adenine* (6-aminopurine) and *guanine* (2-amino-6-oxypurine) both are present in DNA and RNA. (*ii*) *Pyrimidine bases*—the pyrimidine bases



are *cytosine* (2-oxy-4-aminopyrimidine), *uracil* (2,4-dioxypyrimidine) and *thymine* (5-methyl-2,4-dioxypyrimidine). Cytosine is present in both DNA and RNA. Thus, RNA contains adenine, guanine, cytosine and uracil, whereas DNA contains adenine, guanine, cytosine and thymine.

The nucleic acids are necessary for synthesis of protein in the body. There are four types of nucleic acids such as (*i*) DNA, (*ii*) mRNA, (*iii*) tRNA or soluble RNA (sRNA) and (*iv*) ribosomal RNA (rRNA).

DNA or deoxyribonucleic acid containing D-2-deoxyribose is mainly present in cell nucleus, specially in the chromosomes, but other portions of the cell, like mitochondria, also contain DNA. Being present in the chromosomes it is associated with storage and expression of genetic information of heredity. Structure of a DNA molecule shows the two nucleic acid chains to twist in a double helix (FIG. 13). Messenger RNA (mRNA) is formed on the strands of DNA and is responsible for carrying information from the nucleus to the area where protein is synthesised. **mRNA** leaves the nucleus and is stretched itself over the ribosomal surface so as to direct the synthesis of protein (FIG. 14). **RNA** is

ribonucleic acid containing D-ribose and like mRNA there is also tRNA and rRNA. **Transfer RNA (tRNA)** is responsible for transferring specific amino acids to the site of protein synthesis where the mRNA has already stretched over the ribosomal surface and thus expresses the genetic information. The amino acid sequences are determined by the tRNA because it recognises the code for the amino acids that are carrying at the site of protein synthesis where the mRNA has already been attached. The molecular weight of tRNA is 20,000–30,000 and is present in the cytoplasm. **Ribosomal RNA (rRNA)** is found as minute granules in the ribosome,—the protein-synthesising centres of the cells and has got molecular weight of about 2,000,000.

4. **Glycoproteins** and **Mucoproteins.** In these compounds, the protein molecule remains combined with a carbohydrate radicle, containing nitrogen, viz., *hexosamines: glucosamine* or *galactosamine*. Glucosamine and galactosamine are also known as *amino sugars*. Distinction between glycoprotein and mucoprotein is due to percentage of carbohydrate present. Compound containing less than 4 per cent. carbohydrate is glycoprotein and containing more than 4 per cent. carbohydrate is mucoprotein. The carbohydrate part is a *tetrasaccharide*, being made up of two molecules of *acetyl hexosamine*, which are united through two molecules of glucuronic acid. Glycoproteins may be divided into two groups—the *mucins* and the *mucoids*. Mucins are present in the mucous secretions of glands and of mucous membranes. They are not coagulable by heat, precipitated by acids and the precipitate redissolves in alkali. The *hexosamine* derived from mucin is a glucosamine, called *mucoitin*. On being boiled with acids the molecule gives acid metaprotein and another compound,—mucoitin sulphuric acid.

The *mucoids* include a number of compounds which may be extracted from tendons, bones, cartilages, eggs, etc., by dilute alkali. The *ovomucoid* is derived from egg-white, the *chondromucoid* is found in the cartilages and bones. The hexosamine present in such compounds is galactosamine and is called *chondroitin*. On hydrolysis the chondromucoid yields protein and both mucoitin sulphuric acid and chondroitin sulphuric acid. It has been shown that both serum albumin and serum globulin contain a carbohydrate radicle, consisting of glucosamine and mannose. *Heparin* is probably a derivative of some compound resembling chondroitin sulphuric acid.

5. **Lipoproteins.** In these compounds the proteins remain united with lipid. The latter generally belong to the class of phospholipids. It has been derived from plasma, blood corpuscles, eggs, milk, etc.

6. **Metalloproteins.** Protein-containing metallic elements, like Fe, Cu, Mn, Mg, Co, etc.,—found in a large variety of enzymes.

III. Derived Proteins

These proteins are not present in nature as such. They are found as products of hydrolysis of the native protein molecule. The gradual stages are as follows: protein → protean → metaprotein → proteose → peptone → peptides → amino acids.

1. **Proteans.** They are formed especially from certain globulins but differ from them in being insoluble in dilute salt solutions. *Edestan* from edestin and *myosan* from myosin are typical examples of this class.

2. **Metaproteins.** Metaproteins are coagulated by heat, soluble in excess of acid and alkali and are precipitated at isoelectric point or pH. Metaproteins are of two varieties—acid and alkali. (*a*) The *acid metaprotein* is formed by the action of dilute acids on proteins. When the solution is neutralised with dilute alkali the acid metaprotein is precipitated. (*b*) The

alkali metaprotein is formed by the action of alkali and is precipitated on being neutralised by acids.

3. **Proteoses** (such as *albumose* from albumin, *gelatose* from gelatin, *caseose* from casein, etc.). Proteoses are soluble in water, not coagulated by heat, give *violet biuret* reaction. They are also soluble in weak salt solutions, acids and alkalies. Proteoses are of two classes: (*a*) *Primary proteoses*—precipitated by half saturation with ammonium sulphate and full saturation with magnesium or sodium sulphate. (*b*) *Secondary proteoses*—precipitated by full saturation with ammonium sulphate. The proteoses are precipitated by *Esbach*'s solution. The precipitate dissolves on heating and reappears on cooling.

4. **Peptones.** Peptones give a *red biuret* reaction. They are not coagulated by heat, not precipitated by usual precipitating reagents, soluble in water and generally soluble in alcohol. They may be precipitated by tannic acid, phosphotungstic acid, phosphomolybdic acid, etc. Their molecules are of small size and hence do not behave like colloids. Like crystalloids they are easily diffusible through animal membranes.

5. **Polypeptides.** Polypeptides do neither give biuret reaction, nor coagulation reaction nor precipitation reaction. On further hydrolysis the polypeptides yield **lower peptides** which are finally broken down into **amino acids.**

Properties of Proteins

Proteins are colloidal in nature but many of them can be crystallised. They are generally soluble in water, weak salt solutions, dilute acids and alkalies. Each protein has got a **particular isoelectric point** at which it is precipitated. These precipitates again dissolve when the reaction of the medium is shifted from isoelectric point or pH. *During* **precipitation** *proteins do not undergo any intramolecular change. They simply separate out because the medium is not favourable for solution.* Most of the proteins undergo **coagulation** by heat or acid. *Coagulation involves intramolecular change.* There is another kind of change which the proteins undergo, called **denaturation.** This may be done by many kinds of chemical or physical treatment, such as shaking, change of temperature, change of reaction, addition of neutral salts, etc. The exact nature of change undergone during denaturation is not known. It is probable that the molecular arrangement alters. All proteins show the characteristic properties of colloids.

Proteins not only differ from one another in the number and variety of amino acids they contain but also in chemical structure, physical and physiological properties. There are infinite varieties of proteins, each one differing from the rest in certain characteristics. The proteins of a particular tissue of an animal are quite different from those of the same tissue of another species. Moreover, in the same species, each type of tissue contains proteins which are distinct from those found in the other tissues. No two proteins are found to be exactly the same in their physiological properties. This is another characteristic of proteins as a class, which are not found with the carbohydrates or fats. Vegetable proteins differ from animal proteins in the fact that, the former is generally poorer in essential amino acids.

Proteins can be identified by *three* groups of tests: (1) *Colour reaction* (2) *Coagulation reaction,* (3) *Precipitation reaction.*

1. **Colour Reaction.** It depends upon the characteristics radicles in the molecule.

(*a*) **Biuret test** (Piotrowski's or Rose's). To 2 to 3 ml of the protein solution in a test-tube, when an equal volume of concentrated caustic soda solution and one drop or two drops of 1% copper sulphate solution are added, a distinct violet colour develops. This reaction is due to the presence of **peptide linkage** in the protein molecule.

(*b*) **Xanthoproteic reaction.** To 2 to 3 ml of the protein solution when few drops of concentrated nitric acid are added, a white precipitate forms. It is boiled for a minute the precipitate turns yellow and partly dissolves to furnish a yellow solution. It is then cooled and concentrated caustic soda is added till the reaction is alkaline. The yellow colour changes to an orange one. This reaction is due to the presence of the **phenyl group** in the protein molecule.

(*c*) **Millon's reaction.** To 5 ml of the protein solution when few drops of Millon's reagent are added—a white precipitate forms which on heating changes to a brick-red coagulum. This is due to the presence of the **tyrosine** in the protein molecule.

(*d*) **Molisch's reaction.** To 3 or 4 ml of protein solution, three or four drops of an alcoholic solution of *thymol* are added. The solution is well mixed and to it few drops of concentrated sulphuric acid are added when a distinct purple or violet ring develops at the junction of the two liquids. The reaction is due to the presence of **carbohydrate radicles** in the protein molecule.

(*e*) **Adamkiewicz's reaction.** To 3 or 4 ml of protein solution excess of glacial acetic acid is added and heated. It is cooled and few ml of concentrated sulphuric acid are allowed to flow down the side of the inclined test-tube—a purple colour develops at the junction of two liquids within a short time. The reaction is due to presence of *tryptophan* in protein molecule.

(*f*) **Nitroprusside reaction.** To a solution of protein, add sodium nitroprusside in ammoniacal solution. A reddish colour is produced. This is due to the presence of **sulphydryl group** in the protein molecule. (Disulphide groups are to be reduced first to sulphydryls by suitable reducing agent to give this reaction.)

2. **Coagulation reaction.** Albumin and globulin solution when heated—fine flocculi appear. They are denatured.

3. **Precipitation reaction.** In a protein solution the proteins may be precipitated by (*a*) acids, e.g., tannic acid, picric acid, trichloro-acetic acid and phosphotungstic acid. (*b*) Salts of heavy metals. (*c*) Alcohol.

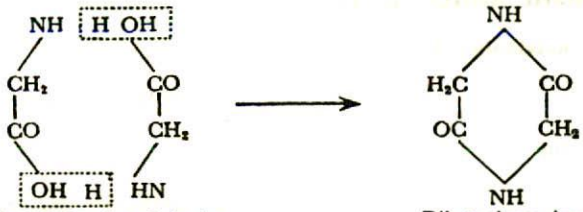
Formation of the Protein Molecule. The synthesis of protein molecule takes place by the union of the $-NH_2$ group of one amino acid with the $-COOH$ group of another, for instance—

$$\underset{\text{Alanine}}{CH_3.CH.NH_2-CO} \boxed{OH+H} \underset{\text{Glycine}}{NH.CH_2.COOH} =$$

$$= \underset{\text{Alanylglycine}}{CH_3.CH.NH_2.CO-NH.CH_2.COOH} + H_2O.$$

In this way infinite number of amino acids may join up in straight chains. This junction, **CO—NH,** through which the amino acids become joined together, is called the **peptide linkage.**

It is obvious that in such a simple chain there will be one free $-NH_2$ group at one end and another free $-COOH$ group at the other. It sometimes so happens that such a straight chain bends upon itself and the basic radicle at one end combines with the acid radicle on the other by forming the same peptide linkage. In this way a ring structure is produced. These compounds are called *diketopiperazines*. The following scheme shows the



Two molecules of glycine — Diketopiperazine

formation of diketopiperazine by the condensation of two molecules of *glycine*.

AMINO ACIDS

The amino acid is an organic acid in which one or more hydrogen atoms are replaced by $-NH_2$ group. Thus it contains at least a free *amino group* ($-NH_2$) and a *carboxyl group* ($-COOH$). The empirical formula is $R-CH.NH_2.COOH$. Amino acids are amphoteric in reaction and form salts with both acids and bases. The amino acids in the body are almost all α-amino acids. They should be regarded as derivatives of saturated fatty acids in which the amino group is attached to that carbon atom which is situated in the α-position, i.e., next to the COOH group. Amino acids are colourless, crystalline substances; soluble in water, easily diffusible and except glycine all are optically active.

Classification of Amino acids. (Those marked with * are essential amino acids in respect to rat growth effects. Arginine can be synthesised by rat but not sufficiently.)

I. Aliphatic Amino Acids

A. Monoamino-monocarboxylic Acids (Neutral Amino Acids)

1. Glycine (Amino acetic acid)—NH_2CH_2COOH.
2. d-alanine (α-aminopropionic acid)—$CH_3.CH(NH_2)COOH$.
3. l-serine (β-hydroxyalanine)—$CH_2(OH).CH(NH_2).COOH$.

*4. **d-threonine** (α-amino-β-hydroxybutyric acid)—$CH_3CH(OH).CH(NH_2).COOH$.

*5. **d-valine** (β-β-dimethylalanine)—$(CH_3)_2CH.CH(NH_2).COOH$.

*6. **l-leucine** (β-isopropylalanine)—$(CH_3)_2CH.CH_2.CH(NH_2).COOH$.

*7. **d-isoleucine** (β-methyl-β-ethylalanine)—$\begin{matrix}CH_3\\CH_3CH_2\end{matrix}\!>\!CH.CH(NH_2).COOH$.

B. Monoamino-dicarboxylic Acids (Acidic Amino Acids)

1. l-aspartic acid (aminosuccinic acid)—$COOH.CH_2.CH(NH_2).COOH$.
2. d-glutamic acid (α-aminoglutaric acid)—$COOH.CH_2CH_2.CH(NH_2).COOH$.

C. Diamino-monocarboxylic Acid (Basic Amino Acids)

*1. **d-arginine** (α-amino-δ-guanidinovaleric acid)—

$$NH=C\begin{matrix}NH_2\\|\\-NH\end{matrix}(CH_2)_3.CH(NH_2).COOH.$$

*2. **d-lysine** (α-α-diaminocaproic acid)—$NH_2(CH_2)_4.CH(NH_2).COOH$.

3. Hydroxylysine (diaminohydroxy caproic acid)—$NH_2.CH_2CH(OH)(CH_2)_2CH(NH_2).COOH$.

D. SULPHUR-CONTAINING AMINO ACIDS

1. l-cysteine (β-thioalanine)—$HS.CH_2.CH(NH_2).COOH$.
2. l-cystine (dicysteine)—$S.CH_2CH(NH_2).COOH$
   |
   $S.CH_2CH(NH_2).COOH$.
*3. **l-methionine** (α-amino- γ-methylthiobutyric acid)—
$CH_3.S.(CH_2)_2.CH(NH_2).COOH$.

II. Aromatic Amino Acids

*1. **l-phenylalanine** (β-phenyl-α-aminopropionic acid)

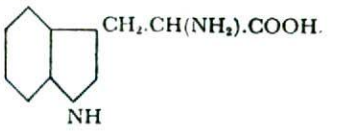


2. l-tyrosine (p-hydroxyphenyl alanine)

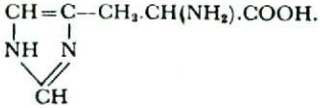


III. Heterocyclic Amino Acids

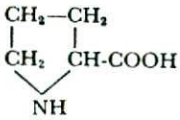
*1. **l-tryptophan** (β-indole-alanine)



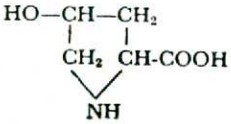
*2. **l-histidine** (β-iminazolalamine)



3. l-proline (α-pyrrolidine-carboxylic acid)



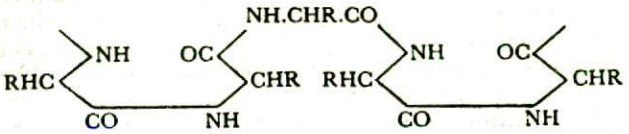
4. l-hydroxyproline



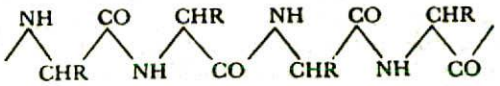
Structure of the Protein Molecule

Some evidence have accumulated to suggest that the amino acids in the protein molecule are arranged in a definite pattern. This is more indicated by the fact that many proteins form well-defined crystals. From X-ray and other observations the existence of two types of proteins is revealed,—the *fibrous form* with elongated molecules and another *rounded form with globular* molecule. *Keratin* is an example of the fibrous type. Keratin may exist in two forms—α and β. By stretching α-keratin, β-keratin is obtained. When the stretch is removed, β-keratin becomes reconverted into α-keratin. If however the stretched hair is subjected to steam, it loses its power of recovery and becomes set permanently as β-keratin. In this way permanent waving of hair is done. X-ray photographs suggest that the keratin

molecule consists of thin bundles of polypeptide chains joined together in a zigzag manner. The two types of keratins have the following structure:



α-keratin (in ordinary hair)



β-keratin (in stretched hair)

Wrinch suggests that the globular type of proteins are also formed in similar lines but vary in detail. She holds that in a folded polypeptide chain a linkage occurs between the NH of one peptide link with the CO of the neighbouring one. Thus a polypeptide chain of this folded type may be closed into hexagonal loops. Six amino acids in a closed polypeptide chain would give a pattern in which the centre is a hexagon. Such a molecule is called **cyclol.** A series of cyclols with 18, 30, 42, 54, etc., amino acids could be formed resulting in a sheet like molecule with repeating pattern. Extension of the sheet may occur in any plain and in this way globular types of protein molecules are formed.

Molecular Weights of Proteins. Protein molecules are large and have varying molecular weights. Their molecular weights cannot be determined by ordinary methods. The most important method for their determination is based upon the measurement of sedimentation rates in the **ultracentrifuge.** They can also be determined by osmotic pressure measurement, light scattering, etc. Molecular weights of some of the typical proteins from different sources are as follows: salmine (salmon sperm)—5,600; cytochrome c (heart)—156,000; lactalbumin (milk)—17,400; gliadin (wheat)—27,400; pepsin (stomach)—35,500; insulin (pancreas)—40,900; haemoglobin (human)—63,000; myogen (muscle)—150,000; thyroglobulin (thyroid)—628,000; myosin (muscle)—1,000,000; virus (tomato)—7,600,000; mosaic virus (tobacco)—60,000,000.

It has been shown by Svedberg that by a change in the protein concentration or in the pH or in the salt concentration of the medium, proteins in solution, may easily dissociate into smaller component units. If the original condition is resorted the fragments may reassociate and form the original molecule. This shows that proteins of higher molecular weights are built up by conglomeration of smaller units.

Virus Proteins. These are nucleoproteins of special interest. Several varieties of virus proteins with very high molecular weights have been isolated in crystalline form, from the juice of plants suffering from virus diseases. The virus protein of *tobacco mosaic disease* has a molecular weight of about 60,000,000, an isoelectric point or pH 3·49, and contains about 5% nucleic acid. It is a highly interesting fact that even minute amount of pure, recrystallised virus proteins induces the virus disease when injected into healthy plants. One-millionth of a milligram of tobacco mosaic protein

invariably infects a healthy plant and from such infected plants, large quantities of the same protein can be recovered. This astonishing property of the virus proteins, in directing the metabolism of the plant cells so that they synthesise more of the same protein, is unique in a chemical compound and is suggestive of the reproduction of a living organism rather than the elaboration of a non-living chemical molecule. The evidence accumulated so far indicates that these giant molecules are purely of chemical nature although we have no absolute proof that they are dead.

Isoelectric pH. Each of the amino acids has at least one carboxyl group ($-COOH$) and one amino group ($-NH_2$). For this reason they are called *ampholytes*. This characteristic of amino acid is also present in intact protein molecule due to the presence of two terminal free amino acids. However there may be more than one acidic or basic group depending on whether they are dicarboxylic or diamino acids. Due to amphoteric nature, in acid solution protein molecule is positively charged and in alkaline one negatively. At certain pH the number of positive charge is equal to the number of negative charge and protein remains as **zwitterion form.** *The isoelectric pH* or *point of a protein is the pH at which the protein does not migrate in an electric field.* At this particular pH the protein molecule does not move either to the positive or negative pole, if placed in an electric field. All proteins, at its isoelectric pH, have got least osmotic pressure, swelling capacity, viscosity, solubility and mobility or migrating power. The proteins are thus precipitated at its isoelectric pH.

Electrophoresis. The property of migration of protein molecules in an electric field has been utilised by Tiselius for electrophoretic method for the separation of protein molecules. Protein solution in an alkaline buffer has maximum number of negative charges, and so the protein molecules move towards the positive pole. Different protein molecules, due to the

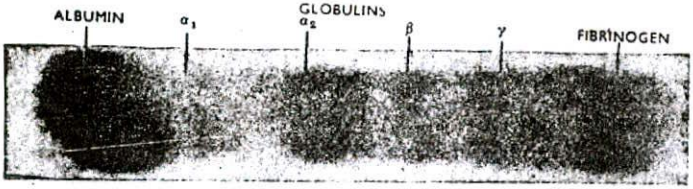


FIG. 79. Paper electrophoresis of normal human plasma showing albumin, globulins (α_1, α_2, β, γ) and fibrinogen.
By courtesy of S. Chatterjee, M.Sc., D.Phil., Department of Physiology, Calcutta University.

presence of charged particles in different amounts, move towards the pole at different rates which can be optically measured. This complicated procedure has been simplified by application on paper. Here a strip of filter paper is moistened with suitable buffer and placed in between two glass plates. The free ends of the paper dip into two small reservoirs of buffer solution, and either positive or negative electrode is placed in each of them. On passing electric current, the protein molecules migrate towards the anode. After definite time interval, the paper is taken out and washed, and a suitable stain is applied to localise the presence of difference in protein molecules (FIG. 79).

Chromatography. It was originally introduced by Tswett in 1906, a Polish Botanist for separation of different colour pigments present in the plant extract.

Amino acids can also be separated from one another by **partition chromatography.** Chromatography might be of different types, such as (1) *paper*, (2) *thin-layer*, (3) *column* and (4) *gas-liquid.* The amino

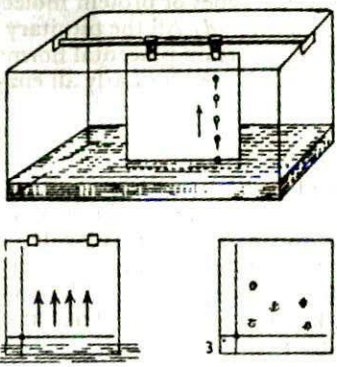


FIG. 80. Separation of amino acid mixture by paper chromatography. 1, placed in a suitable solvent the substances migrate upwards. 2 & 3, separation achieved by turning the paper 90° and by using a different solvent.

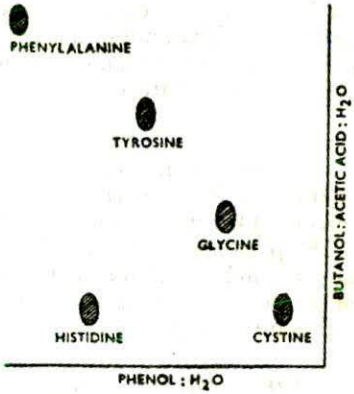


FIG. 81. Two-dimensional paper chromatography of some amino acids.

acids are separated between a stationary and a mobile phase. In **paper chromatography** (FIG. 80), a drop of amino acid mixture is placed on a filter paper and allowed to dry. The paper is then kept in contact with a suitable solvent which is allowed to flow over the dried drop slowly either by capillary action alone **(ascending chromatography)** or in combination with gravitational force **(descending chromatography).** As the solvent moves, it carries along with it the amino acids. Suitable tests are then applied to localise the individual amino acids, which have been found to be carried away to a characteristic distance from the original place of application. *The ratio of the distance travelled by the compound to the distance covered by the solvent on paper is called* $\mathbf{R_F}$ **value** *of the compound.* To have a clearer separation, an improved method, the **two-dimensional chromatography** (FIG. 81) has been developed. Here after suitable chromatographic procedure, the paper is allowed to rechromatograph at a right angle to the first one. In **thin-layer chromatography** (TLC), a suitable adsorbent like alumina, cellulose powder, etc., is spread on glass plates which are then used as paper in chromatographic separation. This technique has some special advantages in separation. In **column chromatography** or **ion-exchange chromatography,**

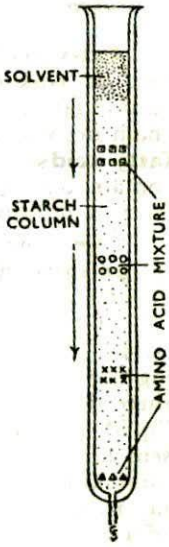


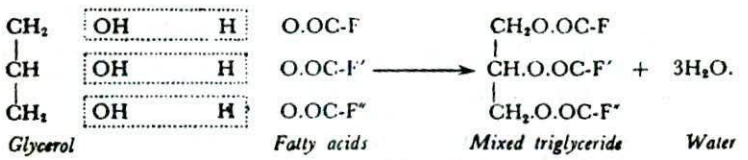
FIG. 82. Column chromatographic separation of amino acid mixture.

various solid adsorbents like starch, cellulose or an ion-exchange resin are placed as columns in glass tubes (FIG. 82). Different amino acids (or other substances) are adsorbed on the column are then eluted with suitable solvents.

Functional importance of proteins. Protein has got multiple functions of which certain physiological importances are described below: (*a*) Protein acts as a growth material for the organism. (*b*) Structures of living materials are composed of different types of protein molecules. (*c*) It also acts as a part of fuel of the organism. (*d*) All the pituitary hormones, hypothalamic-releasing factors (R.F.), certain placental hormones, pancreatic hormones, etc., are proteins in nature. (*e*) Similarly all enzymes are proteins in nature.

LIPIDS AND FATS

Lipids are defined to be the **esters** of higher aliphatic acids with a characteristic property of insolubility in water and solubility in fat solvents like chloroform, ether, benzene and carbon tetrachloride. Due to the presence of replaceable OH group, alcohols behave like alkalies and as such, combine with acids, forming salts and H_2O. These salts—of alcohols with acids—are technically known as esters. They may be represented thus (HOOC–F represents fatty acids).



Fatty acid esters may be formed with alcohols other than glycerol, such as **waxes.** The commonest types of **fatty acids** occurring in natural fats *usually* contain *even* number of carbon atoms and are straight-chain derivatives. They may be saturated or unsaturated. **Saturated fatty acids** do not contain double bonds, but **unsaturated fatty acids** contain one or more double bonds or ethylenic groups. The general formula of fatty acids is $C_nH_{2n+1}COOH$.

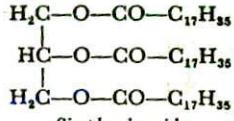
The common *saturated fatty acids* are: (*a*) **butyric acid** (C_3H_7COOH), (*b*) **palmitic acid** ($CH_3(CH_2)_{14}COOH$), (*c*) **stearic acid** ($CH_3(CH_2)_{16}COOH$). The *unsaturated fatty acids* are: (*a*) **oleic acid** ($CH_3(CH_2)_7CH=CH(CH_2)_7COOH$) with one double bond, (*b*) **crotonic acid** ($CH_3–CH=CH–COOH$), (*c*) **linoleic acid** ($C_{17}H_{31}COOH$) containing two double bonds, and (*d*) **linolenic acid** ($C_{17}H_{29}COOH$) containing three double bonds. Consequently the oleic acid is unsaturated, and is liquid at ordinary temperature. Certain other fatty acids may be present in small amounts. Formerly it was believed that natural fat contains fatty acids with an even number of carbon atoms. Lately, thin-layer and **gas chromatographic** techniques have revealed the presence of a good number of fatty acids containing *odd* carbon atoms also. The **simple triglycerides** are those in which glycerol remains united with three molecules of the *same type* (*identical*) *of fatty acids.* The naturally occurring fats are generally all of *mixed type*, in which glycerol remains combined with molecules of different types of fatty acids.

Classification of Lipids

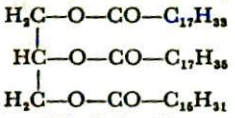
Lipids are classified in (1) **simple lipids,** (2) **compound lipids,** (3) **sterols** or **steroids,** (4) **hydrocarbons.**

I. Simple Lipids

1. **True Fats.** These are esters of fatty acids with glycerol (CH_2.OH-CHOH-CH_2OH) and are generally called **triglycerides.** On hydrolysis with water at high temperature and pressure in an autoclave in the presence of acids, triglyceride may yield *three* molecules of fatty acids and

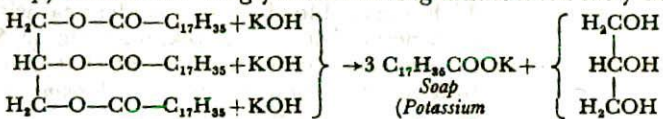


Simple glycerides



Mixed glycerides

one molecule of glycerol. Furthermore, if the triglycerides are boiled with alkali, such as NaOH or KOH then glycerol and Na or K salt of fatty acids (soap) are formed. Triglycerides having unsaturated fatty acids may



Stearin — *Soap (Potassium stearate)* — *Glycerol*

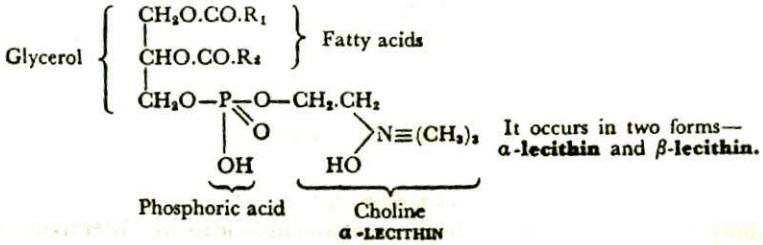
be hydrogenated at their double bonds in the presence of nickel catalyst. Commercial cooking fats are mostly hydrogenated fats and become hard due to hydrogenation.

2. **Waxes.** These are esters of fatty acids with alcohol other than glycerol. In the human body the commonest waxes are the cholesterol esters. They are most abundant in the blood, suprarenal glands, the gonads and the sebaceous glands of the skin. Like true fats, the natural waxes are also complex mixtures. They are solid at ordinary temperature and are not so readily hydrolysed as fats. The three common waxes are as follows: (*a*) *Beeswax.* It is myricyl ($C_{30}H_{61}OH$) palmitate. (*b*) *Lanoline.* It is a mixture of cholesterol palmitate, stearate and oleate. (*c*) *Spermaceti* (in the skull of sperm whale). It is cetyl palmitate.

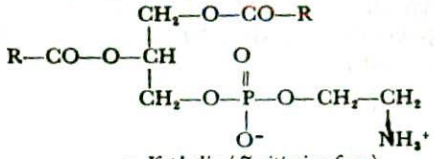
II. Compound Lipids

Fats combined with other non-fatty prosthetic groups.

1. **Phospholipids or Phosphatids.** (Fats containing phosphoric acid and a nitrogenous base in the molecule)—So far three types of phosphatids have been identified. (*a*) **Lecithin.** It is composed of 1 molecule of

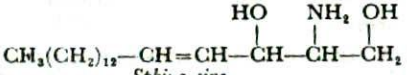


glycerol, 2 molecules of fatty acids and 1 molecule of phosphoric acid. Another nitrogenous base (choline) remains attached to the phosphoric acid. When placed in water it swells up and readily forms an emulsion. Due to presence of acid and basic groups, it combines both with bases and acids. It is a pale-yellow solid which readily absorbs water and oxygen when exposed to air and light, and is thus transformed into a dark-plastic mass. (*b*) **Kephalin** (Cephalin). It is similar to lecithin except that instead



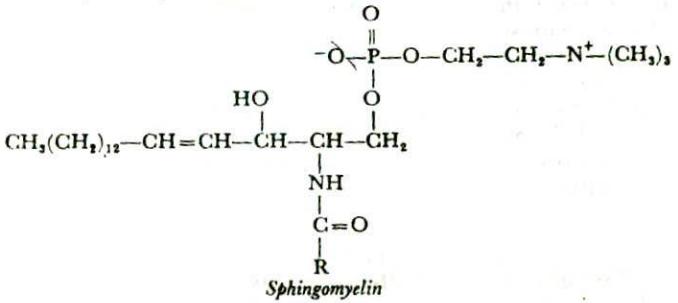
α-Kephalin (Zwitterion form)

of choline the nitrogenous base here, is *ethanolamine*. It occurs in two forms, α-kephalin and β-kephalin. Kephalin and lecithin occur together in the tissue and their properties are generally similar. Howell claims that the substance *thrombokinase* which *initiates* blood clotting is identical with kephalin. It is found specially in nerve tissues, egg-yolk, milk, etc. (*c*) **Sphingomyelin.** Its constituents are same as lecithin except that glycerol is replaced by another unsaturated nitrogenous alcohol called *sphingosine*. It is



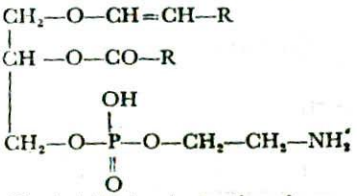
Sphingosine

stable in light and air, and is a white crystalline solid. It is also found in the animal tissues specially in nerve tissues, egg-yolk, milk, etc. Due to the



Sphingomyelin

presence of two asymmetric carbon atoms, 4 stereo-isomers are possible. The natural forms are dextrorotatory. (*d*) **Plasmalogen.** It is another type

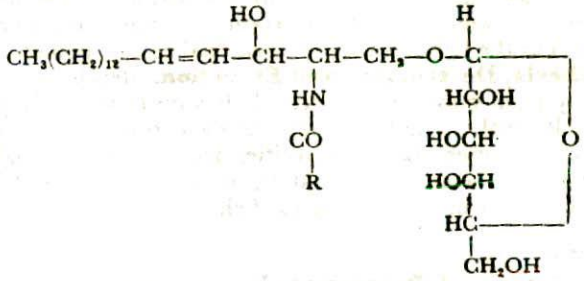


Phosphatidal ethanolamine plasmalogen

phospholipid present in the brain, liver and muscle tissue. After hydrolysis

it yields cholamine esters of α- and β-glycerophosphoric acid along with a mixture of higher aldehyde. Both **palmitic** and **stearic aldehydes** have been found to be the constituents of plasmalogen.

2. **Glycolipids (Cerebrosides).** In general properties they resemble the sphingomyelins. It is also named as **glycosphingosides.** They are specially abundant in the brain. They are characterised by the presence of galactose in the molecule. For this reason they are also called **galactolipids.** Three cerebrosides have been identified, e.g., **phrenosin, kerasin** and **nervon.** The existence of another member of this class **oxynervon,** has also been suggested. They all contain fatty acids, galactose and the nitrogenous base—sphingosine. The difference between cerebrosides is due to



Glycolipid or *Glycosphingoside* or *Cerebroside*

the difference in fatty acids. The function of cerebrosides is not known as yet. Largest amount of them have been found in brain white matter and myelin sheath. In Gaucher's disease a large amount of cerebrosides have been found to occur in liver and spleen.

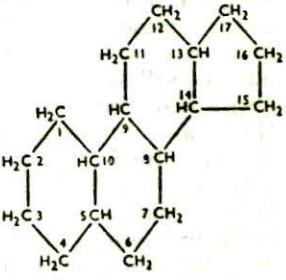
3. **Gangliosides.** They contain sphingosine, fatty acid, hexose, hexose-amine and sialic acid. Gangliosides have been found in nerve cells and also in spleen and red blood cells. In Niemann-Pick disease a large amount of them are present in brain.

4. **Lipoproteins.** Protein molecules conjugated with neutral fats (triglycerides), cholesterol or phospholipids, e.g., α- and β-lipoprotein in plasma. Changes in one or other fraction have been noted in coronary disease, in acidosis accompanying diabetes, in essential hyperlipaemia, etc. Lipoprotein is also present in cell membrane, milk, egg-yolk, etc.

5. **Sulpholipids.** They are sulphuric ester of sphingosine cerebronic acid and galactose (cerebron). Largest amount has been observed in white matter of brain and to a lesser extent in liver, kidney, salivary gland, testis, etc.

III. Steroids or Sterols

Chemistry. Steroids or sterols are not true fats. **Steroids** are derivatives of phenanthrene and have the *parent nucleus* (**perhydrocyclopentanophenanthrene**) with 17 carbon atoms consisting in 3 hexagon and 1 pentagon rings and hydrogen atoms are added until the valency reaches 4. When the natural steroids have a methyl group at C–13 and generally at C–10 and possess one or more alcoholic groups, then



Perhydrocyclopentanophenanthrene

these secondary alcohols are **sterols.** Steroids are found free or in ester bonds with fatty acids (*cholesterides*) and can be extracted by organic solvents along with fats. Cholesterol, coprosterol, ergosterol, male and female sex hormones, adrenocortical hormones and vitamin D are major steroids.

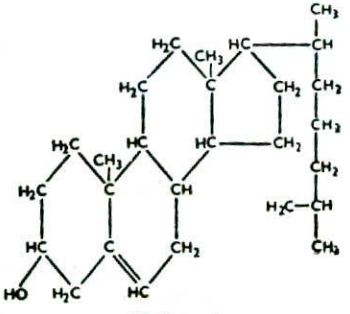
Sterols cannot be hydrolysed by NaOH. Hence, they are not saponifiable. Otherwise, their physical and chemical properties are very similar to those of fats. With fatty acids they form waxes. The various members of this group can be separated by fractional distillation.

Distribution. Sterols are widely distributed in both animal and vegetable kingdoms. In the plants they exist chiefly as *phytosterols.* In the animals they are present in all cells, both in the cell membrane as well as in the cytoplasm. *They always remain along with phospholipids.* They are found both as free sterols as well as sterides. Certain tissues are very rich in this substance, such as the nervous tissue, suprarenal cortex, gonads, etc.

Synthesis, Destruction and Excretion. Sterols can be synthesised in the body from non-sterol sources. It has been definitely proved in the case of cholesterol. The body can destroy sterols to some extent but the power is very limited. Large quantities are excreted through the skin in *sebum* formed by the sebaceous glands, in the stool as *coprosterol* and in the urine as the sex hormones and traces of cholesterol.

Cholesterol

Chemistry and Properties. It is a complex monohydric secondary alcohol being a very important member of the sterol class. With fatty acids it forms waxes. It is a stable white crystalline substance, insoluble in water but readily soluble in chloroform, ether, alcohol and other fat solvents. The crystals have a rhombic or rectangular shape, with one corner broken off. Cholesterol when mixed with fats or oils helps them to absorb a large volume of water due to formation of water in oil emulsion.



Cholesterol

This sterol is present in high amount in nervous tissue and being a poor conductor of electricity, serves the purpose of an insulating cover over the structures through which nerve impulses, which are electrical in nature, are conveyed.

Distribution. The broad facts about the distribution of cholesterol are given below: (1) It is present in all cells—both in the cell membrane and cytoplasm. (2) All body fluids contain cholesterol excepting cerebrospinal fluid in which the amount is negligible. (3) It may exist in the free state as well as in the form of esters, but these two forms are not equally distributed everywhere. In bile it is present only in the free form. (4) Brain (17%) and suprarenals have the richest supply. In the former it is found mainly in the free form. (5) **Blood cholesterol.** Normal blood cholesterol varies between 0·15% to 0·2%. The total quantity is equally distributed between plasma and corpuscles. But in the corpuscles it is present chiefly in the free form, while in the plasma the major part remains as esters. (6) The free cholesterol content of a tissue is characteristic and normally remains very constant but the cholesterol esters may vary in amount. (7) The

distribution of cholesterol in different types of muscles in directly proportional to their degree of activity. The cardiac and smooth muscles have a richer cholesterol content than the voluntary muscles. Moreover, the amount of cholesterol in any tissue is roughly proportional to their degree of activity. (8) Cholesterol and phospholipids always remain together. Moreover, there appears to be a definite ratio between phospholipids and cholesterol for each tissue. So that they not only remain together but also in a definite proportion.

Interrelation with other Sterols. Cholesterol is very closely related to many other sterols of immense physiological importance, for instance (*a*) the **active principles of adrenal cortex,** (*b*) the **male sex hormones,** (*c*) the **female sex hormones,** (*d*) **vitamin D** and **ergosterol,** (*e*) **cholic acid,** etc. Excepting in the case of cholic acid, it has not been proved that cholesterol acts as the mother substance for the other sterols. But since, in the laboratory the male and female sex hormones have been synthesised from cholesterol, it is possible that it may serve as the precursor from which most, if not all, of the physiologically important sterol compounds are derived. For further details *vide* 'Cholesterol Metabolism'.

IV. Hydrocarbons

Hydrocarbons are the substances which have got no structural relationship to fatty acids but yet they are grouped as lipids only because of their similar solubility properties. These are squalene, carotenes, vitamins A, E and K.

Properties of Lipids

(*a*) Physical:

1. **Solubility.** They are insoluble in water but soluble in ether, chloroform, benzene, petroleum-ether, carbon tetrachloride, hot alcohol, etc. (True fats are colourless, odourless and tasteless.)
2. **Consistency.** At ordinary temperature, some fats remain solid whereas others in liquid form. Unsaturated fats are generally liquid. Those fats which remain liquid at ordinary temperature are called **oils.**
3. **Melting Point.** Different fats have different melting points. For instance, pure tristearin melts at 71·5°C.; beef fat melts at 49·5°C.; human fat at 17°C. Glycerides of unsaturated fatty acids have lower melting points than those of saturated fatty acids. It is interesting to note that fats from a particular species of animal have got somewhat characteristic melting point.
4. **Specific Gravity.** It is less than 1·0, i.e., lighter than water. Solid fats are lighter than the liquid fats.
5. **Spreading and Surface Tension.** When liquid fat is poured on water, it spreads uniformly over the surface in a unimolecular layer and reduces the surface tension of water.

(*b*) Chemical:

1. **Reaction.** True fat is neutral in reaction and colourless, but after exposure to air it becomes acid in reaction and yellow in colour owing to partial hydrolysis of fats and oxidation of the unsaturated fatty acids. When thus altered, the fat is said to become *rancid.* 'Antioxidants' present in natural fats prevent this rancidity to some extent.
2. **Hydrolysis.** By boiling with acids or alkalies or by applying superheated steam, fats can be completely hydrolysed into fatty acids and glycerol.

3. **Saponification number (value).** When fats are boiled with alkali, soaps are formed and the process is termed *saponification*. In this way Na, K, Ca, Mg, Li soaps are formed by boiling fats with corresponding hydroxides. By the term *saponification number* (saponification value) of a fat is meant the amount of potassium hydroxide (KOH) in mg required to saponify completely 1 gm of fat. This value gives the idea about the average molecular size of the fatty acids present in the glycerides of the fat and varies inversely with the molecular weight of the fatty acids.

4. **Addition Reactions.** Fats containing unsaturated fatty acids can take up other elements and thus become saturated. Two such addition reactions are of physiological interest. (*a*) **Iodine number**—Under suitable conditions double bonds of unsaturated fatty acids may be made to take up halogens, bromine or iodine. By the term iodine number (regardless of halogens used) of a fat, is meant the number of grams of iodine, taken up by 100 grams of that particular fat. The *degree of unsaturation of a particular fat can be easily assessed by determining its iodine number.* Fats derived from a particular source have generally got a characteristic iodine number. (*b*) **Hydrogenation**—Just like iodine, an unsaturated fat may be made to take up hydrogen atoms in the presence of a catalyst, especially nickel. For instance, addition of two H atoms to oleic acid would give the corresponding saturated acid,—the stearic acid. In this way edible fats can be made from cheaper unsaturated oils. The so-called *vegetable ghee* is manufactured by this process.

5. **Oxidation.** Unsaturated fatty acids when exposed to air, are easily oxidised to form various aldehydes and ketones, which may react further and form complex resinous compounds. For this reason, linseed oil when spread in a thin layer, is rapidly converted into a tough waterproof film which adheres closely upon the surface. Due to this property such oils are called **drying oils.** This property is made use of in the manufacture of paints, varnishes, linoleum, etc.

6. **Acetyl number.** It is defined as the 'mgs of KOH required to combine with the acetic acid liberated by the saponification of 1 gm of acetylated fat'.

7. **Rancidity.** It is a chemical change that may result unpleasant odour and taste within the natural fat due to keeping for a long time. This is due to hydrolysis of fat into free fatty acids and glycerol or mono- and diglycerides. **Acid number** determines the rancidity or free fatty acids present in the fat. It is defined as mgs of KOH is required to neutralise the free fatty acids present in 1 gm of fat. Light hastens and either natural 'antioxidants' or added reducing agents retard the process.

8. **Reichert-Meissl number or Volatile acid value.** It is ml of 0·1 *normal* (*N*) KOH required to neutralise volatile fatty acids obtained during distillation of 5 gm of hydrolised fat after it has been saponified with KOH and then made acid with H_3PO_4 or H_2SO_4. This value actually determines the presence of total fatty acids of low molecular weight.

Functions of lipids. (*a*) *Food material:* Lipid provides a food with a high calorific value (1 gm produces about 9·3 kilocalories of heat). (*b*) *Food reserve:* Lipid acts as reserved food materials, because it is readily stored in the body on account of its insolubility in aqueous solutions. (*c*) *Heat insulations:* Being deposited in subcutaneous tissue lipid acts as an insulator. Subcutaneous fat contents are higher in aquatic animal

(i.e., whale) and in animals living in cold climates. (*d*) *Solvents:* Lipid acts as vehicle of natural fat-soluble vitamins—A, D and E. (*e*) *Structural constituent:* Lipid is a structural component of the cell membrane. (*f*) *Fat transport:* Phospholipids play an important role in absorption and transportation of fatty acids. (*g*) *Hormone synthesis:* Adrenocorticoids, sex hormones, vitamin D and cholic acids are synthesised from cholesterol.

CARBOHYDRATES

A **carbohydrate** *is generally defined as a neutral compound made up of carbon, hydrogen and oxygen, the last two elements remaining in the same proportion as in water.* The general formula is $C_n(H_2O)_n$. But there are many exceptions. For instance, rhamnose ($C_6H_{12}O_5$) is a carbohydrate in which H and O remain in a different proportion. Also there are certain other compounds, such as formaldehyde (HCHO), acetic acid (CH_3COOH), lactic acid ($CH_3CHOHCOOH$), etc., which have got the same empirical formula but are not carbohydrates. Thus chemically, carbohydrates can be defined as the *aldehyde and ketone derivatives of higher polyhydric alcohol* (having more than one 'OH' group).

Classification of Carbohydrates

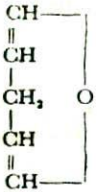
Carbohydrates are classified according to the number of *simple sugar units* present in the molecules, viz., (1) **simple carbohydrates,** (2) **compound carbohydrates.**

1. Simple Carbohydrates or Monosaccharides or Simple sugars

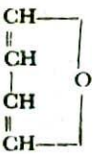
They contain only one unit of simple sugar. For instance, glucose, fructose, galactose, etc. Monosaccharides are further subdivided in two ways: (1) *According to the number of carbon atoms present in the molecule,* viz., monose, diose, triose, tetrose, pentose, hexose, heptose, etc., containing 1, 2, 3, 4, 5, 6 and 7 carbon atoms respectively. (2) *According to the nature of the reducing group they contain,* viz.—(*a*) **Aldoses,** e.g., glucose. Here, the reducing group is an *aldehyde* (—CHO) radicle. (*b*) **Ketoses**—these contain *ketone* (C=O) group, viz., fructose. The general formula of monosaccharides is $C_n(H_2O)_n$.

General properties. (1) They are **colourless crystalline compounds** having a **sweet taste.** (2) Chemically they are **derivatives of polyhydric alcohols.** The aldoses are derived from primary alcohols and the ketoses from secondary alcohols. (3) **Formation of esters.** By virtue of the alcohol group they easily form esters with acids, e.g., acetates, benzoates, etc. Such esters, having great physiological importance, are those of phosphoric acid, viz., hexose phosphates, which play an important role in carbohydrate metabolism; pentose phosphates, present in the nucleic acid and such others. (4) **Reducing power.** By virtue of the aldehyde (—CHO) or the ketone (C=O) groups, the simple sugars are powerful reducing agents. They easily reduce alkaline copper, bismuth or silver solutions. (5) **Isomerism.** Due to the presence of asymmetric carbon atom in the molecule, monosaccharides may remain in different isomeric forms. (6) **Optical rotation.** Simple sugars rotate the plane of polarised light and therefore may exist in either dextro or laevo forms. (7) **Condensation.** Simple sugars condense and form bigger carbohydrate molecules, viz., polysaccharides. (8) **Formation of glycosides.** When replaceable hydrogen atom of a hydroxyl group from sugar is substituted

with other radicles it is called a glycoside; that formed from glucose is a glucoside, from galactose a galactoside and the like. A good number of glycosides occur in leaves, roots, etc., of plants and are bitter solids. Phlorizin (glucose+phloretin), digitonin (galactose+xylose+digitogenin), indican (glucose+indoxyl) are a few examples of them. (9) **Sugar acids.** Either carbon atom one or six in a hexose molecule may be oxidised to carboxyl group. That formed from oxidation of number one is named hexonic acid (glucose→gluconic acid), and from six called uronic acid (glucose→glucuronic acid). Many drugs and a number of hormones combine with glucuronic acid in the body and are excreted as glucuronides. (10) **Formation of hexosamine.** Replacement of hydroxyl group in hexose sugar with amino one produces amino sugar or hexosamine, that formed from glucose is glucosamine. They occur in some complex polysaccharides. (11) **Osazone formation.** All reducing sugars condense with phenylhydrazine and produce osazone compounds. The crystalline forms of these osazone compounds are so characteristic that they can be used for the identification of the particular sugar. However glucose, glucosamine and fructose form similar osazones. (12) **Ring structures of the sugars.** Sugars not only remain as straight chain compounds but may also remain in the form of rings. This ring may include six members (five carbon atoms and one oxygen atom) or five members (4 carbon atoms and 1 oxygen atom). Haworth has suggested all sugars having six-membered rings to be called *pyranoses* (from their relation to pyran) and those forming five-membered rings, *furanoses* (from furan) (*vide* below). (13) **Fermentation.** Sugars, in general, undergo fermentation by yeast and other micro-organisms.



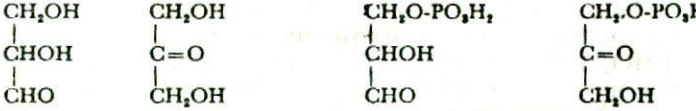
Pyran



Furan

Physiologically Important Monosaccharides

1. **Trioses (three-carbon sugars).** Two trioses are of great physiological importance, *glyceric aldehyde* and *dihydroxyacetone*. The corresponding phosphoric acid esters—*phosphoglyceraldehyde* and *dihydroxyacetone phosphate* —are of great physiological value.

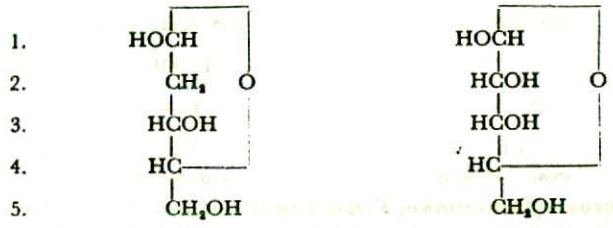


Glyceric aldehyde | Dihydroxyacetone | Phosphoglyceraldehyde | Dihydroxyacetone phosphate

Formation of these triosephosphates is an essential step in the metabolism of sugar.

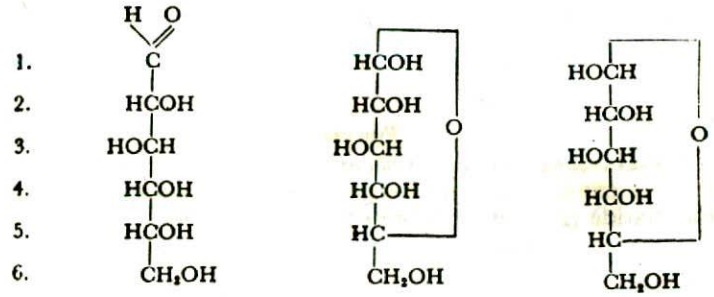
2. **Pentoses (five-carbon sugars).** (*a*) Two different pentoses are present in two nucleic acids, RNA and DNA. Sugar ribose is present in

the RNA, where as that present in DNA is deoxyribose. In the free state both the sugars exist in the pyranose form. In combination with nucleic acids they exist as *deoxyribofuranose* and *ribofuranose*. (*Vide* **nucleoproteins,** page 73.) (*b*) **Arabinose**—It is also a pentose commonly found in gum arabic—chiefly as *l-arabopyranose*.



Deoxyribofuranose Ribofuranose

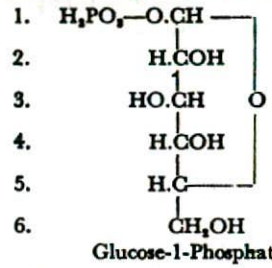
3. **Hexoses (six-carbon sugars).** The following hexoses are of physiological importance. (*a*) **Glucose** (dextrose, grape sugar). It is found in grapes, dextrorotatory and is an aldose. Glucose is found in nature in free form, in the form of disaccharides (maltose, sucrose, etc.), in the form of polysaccharides (starch, glycogen, etc.) and in combination with proteins (glycoproteins). It may remain both as a straight chain compound and as well as, in various ring forms. α- and β-glucose are the isomeric forms.



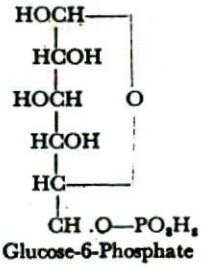
Glucose α-Glucose β-Glucose

The optical reaction of glucose is peculiar. As soon as dextrose is dissolved in water it gives a specific rotation of +112°. On standing the rotation gradually diminishes and finally remains constant at +52·5°. This phenomenon is called *mutarotation*. If however, dextrose be first recrystallised from boiling pyridine, its solution in water at first gives a rotation of +19° and not +112°. This solution on standing also shows mutarotation in which the specific rotation gradually increases and finally becomes constant at the same value, +52·5°. This shows that when in solution one particular variety changes into the other, until an equilibrium mixture of the two forms is produced with a constant rotation of +52·5°. Due to asymmetrical nature of carbon atom 1 in ring form of glucose, it can exist in two forms. The α-glucose has a rotation of +112° and the β form, +19°. A solution of glucose is an equilibrium mixture between the straight chain and

both α- and β-ring forms, the percentage of β being more than α. Glucose forms esters with phosphoric acid.

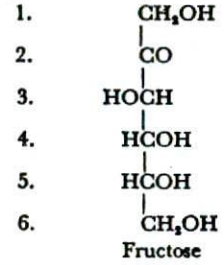


Glucose-1-Phosphate

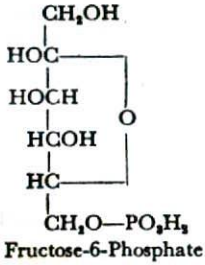


Glucose-6-Phosphate

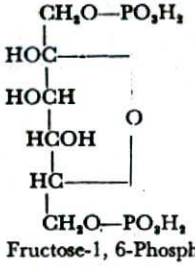
(*b*) **Fructose (Laevulose, Fruit sugar).** It is laevorotatory and is a ketone. Free fructose is present in honey and nearly in all sweet fruits. In combination it is found in cane sugar and inulin. In the human body normally it does not occur in free state and rapidly converted into glucose in the liver and intestine. In contrast with other sugars, fructose is soluble in hot absolute alcohol. Like glucose it also forms phosphoric acid esters which plays an important role in carbohydrate metabolism.



Fructose



Fructose-6-Phosphate



Fructose-1, 6-Phosphate

(*c*) **Galactose.** It does not occur free in nature. In the body it is found as a constituent of lactose (milk sugar) and cerebrosides (galactolipids). As polysaccharide (*galactan*) it is present in lichens, mosses, sea-weeds, etc.



Galactose



Mannose

(*d*) **Mannose** does not occur free in nature. It is found as a constituent of certain animal proteins and converted to glucose in the body. It is also found as a polysaccharide known as *mannan* specially found in the ivory nuts.

4. **Heptoses (seven-carbon sugars).** In the metabolic pathway of *pentose phosphate* by hexose monophosphate shunt, a seven-carbon keto sugar, **sedoheptulose** is formed as an ester with *phosphoric acid* (*vide* Metabolism).

II. Compound carbohydrates

These are made up of from two to one thousand or more than thousand monosaccharide units either with or without non-carbohydrate units. Thus on hydrolysis these may give rise to monosaccharide and non-carbohydrate units. These are principally of two types: (*a*) simpler compound and (*b*) more complex compound. The **simpler compound** carbohydrates contains only a few monosaccharide units and are crystalline, water-soluble and give sweet taste. These simpler compounds are generally called **oligosaccharides.** Oligosaccharides on hydrolysis give rise to at least two to ten monosaccharide units. Thus oligosaccharides are composed of **disaccharides** (two monosaccharide units), **trisaccharides** (three monosaccharide units) and **tetrasaccharides** (four monosaccharide units). **More complex compounds**—These carbohydrates include glycogen (glucan), cellulose, starch and also dextrins which are composed of ten or more monosaccharide units. These are mostly tasteless (dextrins are slightly sweet) and are amorphous solid substance. As these compounds are composed of many monosaccharide units, these are called **polysaccharides** (glycans) which are composed of ten or more monosaccharide units, held together by glycosidic linkages. Those polysaccharides are composed of monosaccharides are called **homoglycans** (e.g., glycogen (glucan), starch, cellulose, etc.) whereas those are made up of two or more different types of monosaccharides are named as **heteroglycans** (e.g., mucopolysaccharides).

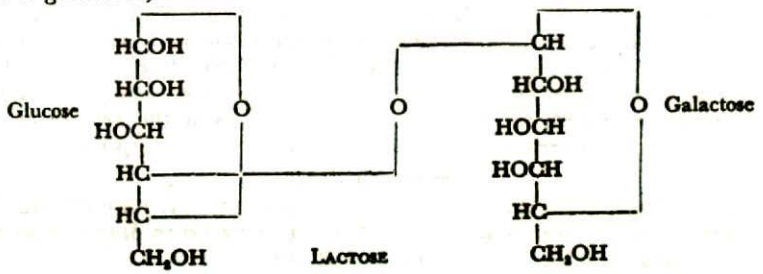
A. Simpler compound carbohydrates

Oligosaccharides include (*a*) disaccharides, (*b*) trisaccharides and (*c*) tetrasaccharides.

Disaccharides

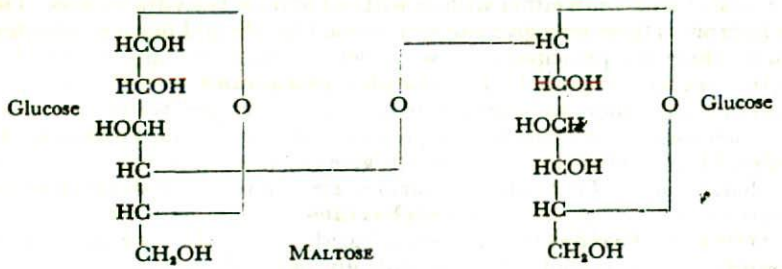
These can be regarded as condensation products of two monosaccharide units with the elimination of one molecule of water. Their general formula is $C_n(H_2O)_{n-1}$. During union the active groups (aldehyde or ketone) become engaged. In lactose only one aldehyde group becomes engaged and one aldehyde group (reducing group) remains free. Similar is the case with maltose. But in cane sugar both the active radicles (—CHO of glucose and —CO of fructose) become engaged. For this reason *lactose and maltose can reduce alkaline copper (although much less than monosaccharides), form characteristic osazone crystals and exhibit mutarotation. Cane sugar,* on the other hand, *in which both the active radicles are engaged, shows no reducing power, does not form any osazone nor shows any mutarotation.* In nature about 16 disaccharides are present. Of these only the following three are of physiological importance.

(*a*) **Lactose (Milk sugar)**—composed of one molecule of glucose and one of galactose, found in the milk of mammals.



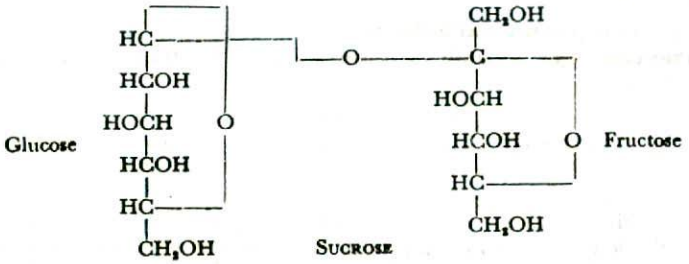
Lactose

(*b*) **Maltose (Malt sugar)**—composed of two glucose units. It is an intermediate product in the digestion of starch.



MALTOSE

(*c*) **Sucrose (Cane sugar)**—On hydrolysis it gives rise to one molecule of glucose and one of fructose. It is the chief form of sugar taken in diet. It is widely distributed in many plants' juices, such as sugar cane, sugar maple, pine apple and also in sugar beets. Sucrose is dextrorotatory but on hydrolysis the resulting mixture becomes laevorotatory owing to the liberation of fructose. The laevo rotation of fructose is greater than the dextro rotation of glucose. For this reason the mixture is known as **invert sugar** and the enzyme (sucrase) which hydrolyses sucrose is also called *invertase*.



SUCROSE

(*d*) **Trehalose** (composed of two units of glucose). It is present in the haemolymph of insects as the principal sugar, also occurs in yeast and fungi.

TRISACCHARIDES

These types of oligosaccharides on hydrolysis give rise to *three* monosaccharide units. These trisaccharides are: (1) **Mannotriose** giving rise to two molecules of galactose and one molecule of glucose on hydrolysis. (2) **Robinose** on hydrolysis gives rise to galactose and two molecules of rhamnose. (3) **Rhamninose** on hydrolysis gives rise to galactose and two molecules of rhamnose. (4) **Raffinose** on hydrolysis gives rise to fructose, glucose and galactose. (5) **Gentianose** on hydrolysis gives rise to fructose and two molecules of glucose. (6) **Melibiose** (melezitose) on hydrolysis gives rise to fructose and two molecules of glucose. Among six trisaccharides described, the first three are *reducing* and the rest three are *non-reducing*. The general formula of trisaccharides is $C_n(H_2O)_{n-2}$.

TETRASACCHARIDES

Only two tetrasaccharides are known. These are (*i*) *stachyose* which is composed of D-glucose, -galactose and -fructose found in *Stachys tuberifera* and (*ii*) *scorodose* in the bulbs of garlic and onion.

B. More complex compound carbohydrates

Polysaccharides

Polysaccharides are made up of a large number of monosaccharide units. During condensation all the active radicles become engaged, so that they do not show any reducing power, do not produce any osazone and are generally not sweet to taste. They are soluble in water excepting cellulose. The molecules are large and hence, show colloidal properties. The empirical formula of polysaccharides is $(C_6H_{10}O_5)_n$.

Polysaccharides are classified according to the nature of the constituent units. For instance, those made up of pentose units are called **pentosans,** those of hexose units **hexosans,** etc. More specifically they are described after the name of the particular units of which they are composed. For instance, **glucosans** (dextrans) are made of glucose, **galactans** of galactose, **fructosans** (laevans) of fructose and such others. Starch, glycogen and cellulose are glucosans. *Inulin is a fructosan. Agar agar is a galactosan* (*galactan*). Gums are mixtures of both pentosans and hexosans.

(*a*) **Starch.** This is the chief form of carbohydrate taken in diet. It is manufactured by the plants and plays the same role in them as glycogen does in animals, i.e., an easily available sugar store. It is the main constituent of food grains. Starch from different sources can be identified from their microscopic peculiarities. It is insoluble in cold water due to the presence of an outer cellulose layer around the granule. On boiling the insoluble cellulose covering ruptures and starch enters into a colloidal solution. It is a glucosan and on hydrolysis breaks down into glucose. It gives blue colour with iodine. It has got no reducing power and is tasteless. Starch is a mixture of two substances having similar structure—*amylose* (mol. wt. 60,000) and *amylopectin* (mol. wt. 300,000) both being composed of chains of 24 to 30 glucopyranose units.

(*b*) **Glycogen.** It is called *animal starch;* because it is in this form that glucose remains stored in the liver and muscles of animal body. Glycogen is also found in those plants which do not possess any chlorophyll, such as yeast, fungi, etc., but not in green plants. *There is probably more than one type of glycogen.* When rabbit is fed with glucose, it synthesises a glycogen containing 12 glucose units, but if fed with galactose it forms a glycogen with 18 glucose units. But curiously enough, mixtures of glycogens with 12 and 18 units are not found. Glycogen is soluble in water, makes an opalescent solution and gives reddish colour with iodine. The glycogen molecule contains many glucose units. The units are joined by linkage between carbon 1 of one unit to carbon 4 of the next one; branches involving 1,6 linkages are frequently present.

(*c*) **Dextrins.** They do not occur naturally, but are the split products of starch resulting from hydrolysis. For this reason they ought to have been described as *derived carbohydrates.* The term dextrin is a group name including several varieties of dextrins. The earlier products of hydrolysis of starch are all called dextrins. The name of the different dextrins are given according to their colour reaction with iodine. The first dextrin formed is *amylodextrin* giving blue colour with iodine, the next product is called *erythrodextrin* giving red colour with iodine and the next product gives no colour with iodine and is called *achroodextrin.* As these products become smaller and smaller, they develop more and more the characteristic properties of monosaccharide, such as the reducing power, a sweet taste and the others.

(*d*) **Cellulose.** It is a stable, insoluble compound, found in the plants and never present in the animal body. Cellulose is taken with vegetable food. It cannot be digested by the human beings. Herbivorous animals can digest cellulose with the help of bacteria. Although indigestible, yet cellulose is of considerable importance in human dietetics, because it adds 'bulk' to the intestinal contents, stimulate peristalsis and thereby help in the formation and evacuation of faeces. Filter papers, commonly used in the laboratories, are almost pure cellulose.

(*e*) **Inulin.** It is a polysaccharide composed of D-fructose units. It is a white crystalline powder and is readily soluble in hot water. It does not give any such characteristic colour with iodine.

Other polysaccharides (heterosaccharides) containing carbohydrate and non-carbohydrate units are: (1) **Heteropentosans** (mucilages, gums, peptic substances, etc.). (2) **Heterohexosans** (hignocellulose, pectocellulose, etc.). (3) **Mucopolysaccharides** (mucin and mucoids) are composed of two to six different monosaccharide units including amino sugar and uronic acid. Many important biological compounds are included in this group, they are hyaluronic acid, chondroitin sulphate, heparin, blood group substances, etc. The first three are called **acid mucopolysaccharides** due to their acidic character. The mucopolysaccharides in tissues are usually present in combination with protein and are called **glycoproteins (mucoproteins, mucoids)**.

Functional importance of carbohydrates. (*a*) It is the readily available fuel of the body. (*b*) It also constitutes the structural material of the organism. (*c*) It also acts as important storage of food material of the organism. (*d*) Carbohydrate also plays as a key role in the metabolism of amino acids and fatty acids.

ENZYMES

Definition. *Enzymes are soluble organic* **catalysts,** *manufactured by living cells but their activities do not depend upon the life of the cells.* It means that enzymes are naturally synthesised by the living cells only, but once they are synthesised, the presence of the living cells is no more necessary for their action.

Nature of enzymes. All enzymes are protein in nature. It is believed that an enzyme consists of two parts which remain united together. Some of the enzymes have characteristic prosthetic groups combined with protein, others have coenzymes which can be easily split off. The nature of the active radicle varies with different enzymes, for instance, the activity of lipase, phosphatase, rennin, etc., depends upon the **—SH** group; that of pepsin depends upon the **—OH** group and so on.

Stability of enzymes. Stability of the enzymes depends upon the pH and the temperature, because they are subjected to denaturation if the pH is changed and the temperature is increased. Besides this, the enzyme is more stable in concentrated solution.

Division of enzymes. Chemically the enzymes can be divided into two groups: (*a*) *simple-protein enzymes* consisting of simple protein and (*b*) *complex-protein enzymes* or *conjugated enzymes* consisting of a protein part—**apoenzyme** and a prosthetic group—**coenzyme.** Combination of apoenzyme with coenzyme constitutes the **holoenzyme.** Thus the holoenzyme is the conjugated enzyme.

How enzymes act. The following are the broad facts about the mode of enzyme action: (1) During enzyme action there is probably some sorts of chemical or physical union between the enzyme and the substrate. The name *substrate* means *the main chemical compound which undergoes alteration in a chemical reaction, upon which the enzyme exerts its influence.* When the substrate is insoluble or less soluble (colloidal), the enzyme is usually **adsorbed** upon it. (2) The active radicle of the enzyme probably attacks some characteristic groupings in the substrate, for instance, the proteolytic enzymes attack the peptide linkages in the protein molecules. (3) The exact nature of chemical action on which enzymes work, varies in different cases. It may be hydrolysis, oxidation, reduction, etc. (4) Enzymes very often cannot work alone. They work with the help of other substances, such as coenzymes, activators, etc. (5) Enzymes may cause both synthetic as well as the breakdown processes. The same enzymes may cause these two types of changes under different conditions. (6) Favourable conditions of the medium, such as optimum pH, temperature, etc., are essential (described below).*

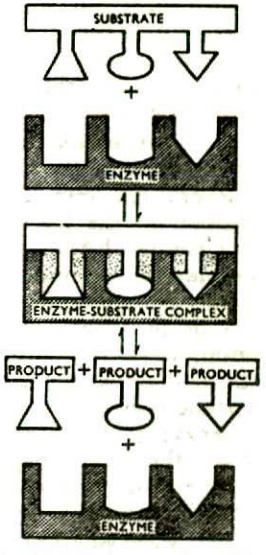


FIG. 83. Demonstration of the mechanism of enzyme action and also the *'lock-key'* hypothesis of enzyme specificity.

Mechanism of enzyme action is mostly based on the theory of Michaelis and Menten —the enzyme-substrate complex (FIG.83). This theory is explained on the following reactions:

(*i*) enzyme + substrate $\rightleftharpoons$ enzyme-substrate complex;

(*ii*) enzyme-substrate complex $\rightleftharpoons$ enzyme + products of enzyme action.

Coenzyme. Certain enzymes require the presence of some other specific non-protein organic molecule for efficient activity. These non-protein organic molecules are called **coenzymes.** They are heat-stable, non-colloidal and are dialysable. In some cases the coenzyme is not a single compound. It is a mixture of many compounds. In such cases it is known as

* The most astonishing fact about the enzymes is the ease with which they can carry out the intricate chemical processes inside the body, without doing the least injury to the living cells. The chemical reactions inside the body must fulfil certain conditions, for instance, (1) there must be an adequate speed of action, (2) there must be a definite direction of reaction—without any harmful side effects, (3) the action must be selective, (4) the pH and the temperature of the medium should be such as not to cause any harm to the tissue cells. All the conditions are wonderfully satisfied by the enzymes.

Some of the enzyme actions can be imitated in the laboratory, such as the hydrolysis of starch, protein and lipids, by boiling them with strong acids. But no cell could remain alive under such conditions. The same actions are effected by the digestive enzymes in the alimentary canal, under such pH and temperature which cause no damage to the living cells.

From these facts, the enzymes should be regarded as the *tools* with which the cells carry out the various chemical reactions upon which their life depends. It should be noted that, it is for the maintenance of their own life, that the cells have developed the chemical skill of synthesising their own enzymes. That is probably the reason, as to why in nature, enzymes are found to be manufactured only by the living cells. Dead things have no need of enzymes, hence, no enzyme is present in the dead world.

coenzyme system. In the muscle one such system is present (consisting of cozymase, adenosine triphosphate and Mg) which helps the enzyme that converts glycogen into lactic acid. Coenzyme remains adherent to the enzyme as a prosthetic group. In some cases the combination is loose, so that the coenzyme can easily be separated by dialysis, while in other instance, the coenzyme remains firmly attached and is not easily separable. Many of the coenzymes are the derivatives of adenosine monophosphate (AMP). The compounds that take part as prosthetic groups or coenzymes are described below:

1. **Vitamins.** (*a*) Vitamin B_1 in the form of thiamine pyrophosphate acts as coenzyme of decarboxylase. (*b*) Vitamin B_2 acts as coenzymes for several enzymes. These coenzymes act in the form of two principal compounds—the flavin mononucleotide (FMN) and the flavin adenine dinucleotide (FAD). (*c*) Nicotinic acid acts as coenzyme in the form of nicotinamide adenine dinucleotide—NAD* and nicotinamide adenine dinucleotide phosphate—NADP** for several enzymes. (*d*) B_6 in the form of pyridoxal phosphate acts as coenzyme of decarboxylase and transaminase. (*e*) Coenzyme A (CoA) contains the pantothenic acid. (*f*) Folic acid acts as coenzyme in the metabolism of one-carbon units. (*g*) Biotin acts as coenzyme of the different carboxylating enzymes. (*h*) B_{12} also acts as coenzyme. The coenzyme forms of B_{12} are **cobamide** and methyl derivative—the methyl-B_{12} coenzyme. (*i*) Ascorbic acid also acts as coenzyme for the enzyme responsible for conversion of *p*-hydroxyphenyl pyruvic acid to 2,5-dihydroxyphenyl pyruvic acid.

2. **Compounds other than vitamins.** (*a*) Lipoic acid or thioctic acid acts as coenzyme for oxidative decarboxylation of α-keto acids between thiamine pyrophosphate and coenzyme A. (*b*) Haem also acts as coenzyme of several enzymes, viz., peroxidase, catalase and cytochrome oxidase. (*c*) UDPG (uridine diphosphate glucose) acts as coenzyme of uridine diphosphogalactose 4-epimerase for the interconversion of glucose and galactose. (*d*) Glutathione also takes part as coenzyme in different enzyme reactions, such as for formaldehyde dehydrogenase in the conversion of formaldehyde to formate. (*e*) S-adenosyl methionine (active methionine) acts as coenzyme of transmethylating enzyme that involves the transfer of methyl group. (*f*) Adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP) act as coenzyme in specific enzyme systems for transphosphorylation or in active transport. (*g*) 2,3-diphosphoglyceric acid acts as coenzyme of enzyme—phosphoglyceromutase that converts 2-phosphoglyceric acid into 3-phosphoglyceric acid. (*h*) CDPC (cytidine diphosphate choline) also acts as coenzyme in the synthesis of lecithins. (*i*) Glucose-1,6-diphosphate acts as coenzyme in the conversion of glucose-1-phosphate to glucose-6-phosphate. (*j*) Active sulphate (adenosine-3′-phosphate 5′-phosphosulphate) acts as coenzyme of enzyme sulphatases for transfer of sulphate or sulphation of phenols and of hexosamine derivatives. (*k*) Ubiquinone—coenzyme Q_6, Q_7, Q_8, Q_9, Q_{10}, takes part in electron transfer system. (*l*) Tetrahydrobiopterin acts as coenzyme for the hydroxylation of phenyl alanine by liver oxidase. (*m*) Certain metals

* NAD—previously known as **coenzyme I** and until recently **diphosphopyridine nucleotide (DPN).**

** NADP—previously known as **coenzyme II** and until recently **triphosphopyridine nucleotide (TPN).**

also act as coenzyme for specific enzyme activities. These are: (*i*) Zn for carbonic anhydrase, (*ii*) Cu for tyrosinase, phenolase and ascorbic acid oxidase, (*iii*) Fe for cytochrome enzyme, catalase, peroxidase, etc., (*iv*) Ca for lecithinase A and C, ATP-*ase* and lipase, (*v*) Co for peptidase, (*vi*) Mn for arginase, phosphoglucomutase, etc., (*vii*) Mg for phosphatases, hexokinases, etc.

Characteristics of Enzyme actions. Enzyme action shows the following characteristics:

1. **Specificity.** One enzyme can act only on a particular type of substrate just as a *key fits a particular lock* (FIG. 83). Thus amylase acts on starch, lipase on lipids, etc. This *law of specificity* strictly holds when the different groups of substrates are concerned. For instance, a fat-splitting or lipolytic enzyme will never act on proteins or carbohydrates. But within the same group, specificity does not strictly hold. Thus a lipase will not only split fats but may also act upon other esters. On the other hand, a group enzyme may not attack all members of the same group, viz., a given phosphatase will not act on all phosphoric acid esters. Often this specificity is so extreme that the enzyme acts strictly on one substance only(*arginase* on arginine; *urease* on urea, etc.) or even on the dextro form of a substance but not on the laevo form. [Liver esterase will act on esters of *d*- but not of *l*-mandelic acid.] Probably, specificity depends upon the interaction between enzyme and special chemical grouping in the substrate.
2. **It is a catalytic agent** and is not destroyed in the process.
3. **It cannot initiate a chemical action.** It only stimulates or retards the intermediate steps in the chemical process.
4. **Optimum temperature.** Every enzyme shows its maximum activity at a particular temperature. Generally, this optimum temperature lies round about the internal body temperature (37°C.). *Boiling destroys all enzymes* (due to alteration of the protein part) but freezing does not destroy them, only suspends their activity.
5. **Optimum pH.** Every enzyme acts best at a limited range of pH which is usually the pH of the medium on which it is acting. Most of the enzymes which are present inside the cell have the highest activity around pH 7·0. Optimum pH for pepsin, which acts in the acid medium of stomach, is 2·0.
6. **Enzyme action is generally reversible.** The direction of reaction is determined by the relative concentration of the substrate on either side. [Probably it is related to the principles of mass action.]
7. **A small amount of enzyme may act upon unlimited quantity of substrate,** provided enough time is allowed, favourable conditions are maintained and the end products of enzyme action are removed.

When viewed in this light, it will be seen that, during digestion of foodstuffs, the digestive process can go on to completion only because the end products are removed by absorption. If adequate absorption fails to take place, the end products will accumulate and will set up a reversible process, so that digestion will come to a standstill. This shows that, although proper digestion is essential for absorption, yet at the same time, absorption is also essential for complete digestion. These two processes are interdependent.

Speed of Enzyme action. Provided all other favourable conditions are maintained the speed of enzyme action depends on the following factors:

1. **Enzyme concentration.** The speed of enzyme action is directly proportional to the enzyme concentration.

2. **Substrate concentration.** Other conditions remaining same, if the concentration of substrate is increased, the speed of enzyme activity also increases, but up to a saturation point of the enzymes with the substrate.

3. **Concentration of reaction products.** If the end products of enzyme reaction are allowed to accumulate, these will inhibit the activity of enzymes. This inhibition is nothing but *negative feedback*. For this retarding effect on the rate of enzyme reaction, some of these end products may be structurally identical to the substrate and therefore they will combine with the enzyme. It has been postulated that these end products as well as other agents combine with the enzyme at a site (allosteric) other than active site and bring about a conformational change in the enzyme protein. This effect is termed as *allosteric effect* that may inhibit the enzyme activity (feedback inhibition).

4. **Temperature.** Within limitations, the enzyme activity increases as the temperature increases.

5. **pH.** Activity of the enzyme changes with alteration in pH level.

6. **Oxidation.** SH group of a number of enzymes mainly dehydrogenases are labile to oxidation forming (S–S) linkages, thus loosing activity.

7. **Radiation.** High energy radiation affects enzyme activity.

8. **Effects of time.** Gortner (1938) has described that there cannot be an optimal hydrogen-ion concentration or an optimal temperature independent of time. He claimed that time factor is important in consideration of other factors that affect the rate of enzyme actions.

9. **Hormones, amino acids** and other biochemical substances can alter enzymatic activity.

10. **Light** may inhibit or accelerate the rate of enzymatic activities.

Activation of enzymes. Some extracellular enzymes are secreted in active form, viz., *ptyalin* of saliva. This is called **zymase secretion.** While many enzymes are quite inactive when they are freshly secreted. Such secretion is known as **zymogen secretion.** They require to be activated by other substances. When these substances are *inorganic* in nature they are called **activators.** For instance, HCl activates pepsinogen into pepsin; amylase requires –Cl or –Br ions; phosphatase requires Mg ions and so on. On the other hand, when these substances are *organic* in nature they are called **kinases.** For instance enterokinase (also called *enteropeptidase*) activates trypsinogen into trypsin.

Inhibition of enzyme action. Enzymes may be inhibited, inactivated or even destroyed by certain substances, in very low concentrations. Salts of heavy metals (Hg, Pb, Au, Ag, etc.), iodine, hydrocyanic acid (HCN), fluorides are very potent in this respect. Certain preservatives, such as, chloroform, thymol, toluene, etc., also inhibit the action of some enzymes, and in this way exert their preservative action. Some highly active substances, such as certain aldehydes, destroy the enzymes altogether.

Competitive inhibition. The competitive inhibitors have close similarity to the substrate of a particular enzyme, and compete for the *active site.* Increasing concentration of the substrate, this inhibition can be prevented. An example of this class is inhibition of succinic dehydrogenase by malonate.

Localisation of enzymes in the cell. In order to determine in which portion of the cell, an enzyme is located the following procedures

may be applied: (*a*) By **differential centrifugation** the sites of maximum enzyme activity in the cell fractions have been determined. Thus the cytochrome oxidase has been found exclusively in the mitochondria along with the enzymes of citric acid cycle. Synthesis of ATP and oxidative phosphorylation occur in the mitochondria also. The microsomes contain the esterases, and the cell sap, enzymes of glycolytic pathway. Most hydrolytic enzymes are present in the lysosomes. (*b*) By **histo-chemical method** an appropriate tissue section is incubated with the substrate forming a coloured reaction product at particular location of the cell. This can be viewed under microscope.

Isozymes. Certain enzymes like lactic dehydrogenase of plasma are present in more than one form distinguishable by chemical or immunological method, but have same enzymatic activity. They are named as isozymes. For example, lactic dehydrogenase, which acts as enzyme for the conversion of lactate to pyruvate in presence of NAD as coenzyme, on electrophoretic separation gives rise to 5 isozymes—LD_1, LD_2, LD_3, LD_4 and LD_5 having equal molecular wt.(130,000). LD_1 and LD_2 are present abundantly in cardiac muscle, and LD_4 and LD_5 are present in liver.

Antienzymes. An agent or particle that retards, inhibits, destroys or neutralises the activity of an enzyme. Extracts of the intestinal parasite *Ascaris* possess pepsin and trypsin inhibitors. This parasite escapes digestion in the intestine. This class of protein inhibitors also occurs in pancreas, raw egg-white and soyabeans.

Allosteric enzyme and feedback inhibition. The term *allosteric site* has been introduced by Monod and Jacob to designate an enzymic site, different from the active site, non-competitively binds molecules other than the substrate and may influence the activity of the enzyme. **Allosteric enzymes** are those which are made up of several subunits with at least two stereospecifically different, non-overlapping receptor sites. Of the two sites, one is the active site which binds the substrate and the other site, the **allosteric site** that binds the compound other than the substrate. The compound, that the allosteric site binds, is known as **allosteric effector.** Allosteric enzyme is also known as *regulatory enzyme* because through **feedback (end product) inhibition** it adjusts the rate of synthesis of metabolic products according to the demands of the cells. In feedback inhibition, the enzyme activity is inhibited by the high concentration of reaction products. If the reaction products are low due to increased metabolic utilisation, then the inhibition will be released. On the contrary, in low metabolic utilisation the enzyme activity is slowed down. In the formation of cytidine triphosphate (CTP) from aspartate and carbamylphosphate, ATC-*ase* (aspartate transcarbamylase) acts as pacemaker because this enzyme catalyses the reaction. When the CTP is produced sufficiently then it inhibits ATC-*ase*, but when the products are sufficiently lowered then the inhibition is released.

Gerhart has described that the binding site of the enzyme ATC-*ase* with the inhibitory end product, the CTP (allosteric effector) is different or distinct from the active site of the enzyme which is sensitive to feedback control. The site at which the allosteric effector binds, is known as *inhibitory site* or *regulatory site* or *allosteric site*. All regulatory or allosteric enzymes have got at least two or more subunits of proteins. Pure ATC-*ase* can be separated into two protein units of which one is the bearer of

the active site having all the catalytic activities of ATC-*ase* and the other is the carrier of the allosteric site having the specific properties of binding CTP (allosteric effector).

Classification of Enzymes

According to the Commission on Enzymes of the International Union of Biochemistry (I.U.B.), 1965, main features for classification and terminology of enzymes are: (*a*) reactions (and enzymes catalysing them) are divided in 6 major classes of which each has 4 to 13 subclasses. (*b*) Enzymes has two parts: (*i*) substrate or substrates, (*ii*) commonly the suffix *-ase* appended to the name of substrate in naming enzymes, which denotes type of reaction catalysed. (*c*) For additional clarification the nature of reaction may be written in parenthesis. (*d*) Each enzyme has a systematic code number (2.1.3.7). First (initial) figure represents main division or group of enzyme. Second figure depicts the sub-class. Third figure expresses the sub-sub-class. The fourth one simply represents a serial number of the enzyme in the sub-sub-class.

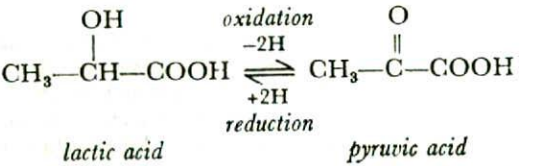
The major classifications of enzymes as proposed by the above Commission are presented with examples:

1. **Oxidoreductases** which catalyse the oxidation-reduction reactions—
   1.1.1.1 Alcohol: NAD oxidoreductase (alcohol dehydrogenase) catalyses the reaction, Alcohol + NAD^+ = Aldehyde or ketone + NADH + H^+.
2. **Transferases** that catalyse the transfer of group, such as
   2.3.1.6 Acetyl-CoA: Choline O-acetyltransferase (choline acyltransferase). It catalyses the reaction, Acetyl-CoA + choline = CoA + O-acetylcholine.
3. **Hydrolases** that catalyse the hydrolysis of ester, ether, peptide bonds, etc. As for example
   3.1.1.8 Acylcholine acyl-hydrolase (pseudocholine esterase) catalyses the reaction, An acylcholine + H_2O = choline + an acid.
4. **Lyases** which catalyse the removal of group from substrates by the process other than hydrolysis. As for example:
   4.2.1.2 Malate: L-Malate hydro-lyase (fumarase) catalyses the formation of fumarate from malate, L-Malate = fumarate + H_2O.
5. **Isomerases.** This group catalyses the interconversion of different isomers (optical, geometric and positional isomers), such as
   5.1.1.1 Alanine racemase catalyses the following reaction, L-Alanine = D-alanine.
6. **Ligases** (or **synthetases**) are capable of binding or linking together of two compounds. Such enzymes are:
   6.2.1.4 Succinate: CoA ligase (GDP) (succinic thiokinase) catalysing the reaction, GTP + succinate + CoA = GDP + Pi + succinyl-CoA.

Of about 840 enzymes, nearly 215 are listed as oxidoreductases, 225 as transferases, 205 a hydrolases, 105 as lyases, 45 as isomerases and 45 as synthetases or ligases.

Although the I.U.B. system is complex as well as precise, descriptive and informative, yet many authors prefer to classify enzymes on the basis of substrate attacked because of better understanding of the beginners. Furthermore, a good number of enzymes discovered earlier retain their trivial names, e.g., pepsin, renin, rennin, trypsin, etc.

I. **Oxidation-Reduction enzymes.** These enzymes form a large group of enzymes concerned with biological oxidations and reductions, i.e., removal or addition of hydrogen atoms. Lactic dehydrogenase is an important example.



Coenzymes are characteristically required for these enzymes.

Therefore it is prosthetic group which donates or accepts hydrogen atoms. NAD and NADP are these coenzymes.

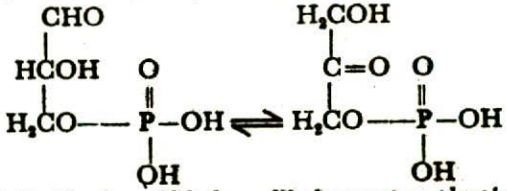
II. **Transferases.** These enzymes effect the transfer of a radical (a group of atoms other than hydrogen) from one compound to another, e.g., (*a*) creatine phosphoryl transferase in muscle. This enzyme transfers 'energy-rich' phosphate group from creatine phosphate to ADP to form ATP. (*b*) *Transaminases*, e.g., glutamic-aspartic transaminase, glutamic-alanine transaminase. (*c*) *Transacylases*, e.g., choline acetylase, glucosamine acetylase. (*d*) *Transmethylases*, e.g., guanido-acetic transmethylase, nicotinamide transmethylase.

III. **Hydrolases (Hydrolysing enzymes).** These enzymes bring about the addition of H_2O to the substrate and thereby initiate splitting of the molecule (a hydrolytic cleavage). Acetylcholinesterase catalyses the cleavage of acetylcholine.

$$\underset{\textit{acetylcholine}}{CH_3CO-O-CH_2-CH_2-N^+-(CH_3)_3} \overset{+H_2O}{\underset{-H_2O}{\rightleftharpoons}} \underset{\textit{acetic acid}}{CH_3COOH} + \underset{\textit{choline}}{HO-CH_2-CH_2-N^+-(CH_3)_3}$$

Many of these enzymes catalyse the breakdown and utilisation of protein, lipid and carbohydrate. (*a*) *Protein-hydrolysing enzymes* (peptide bonds)—These enzymes split the peptide bonds of proteins and peptides. They are further subdivided into proteinases (endopeptidases) and peptidases (exopeptidases). Pepsin, rennin, trypsin, chymotrypsin and cathepsins are *animal proteinases;* and *plant proteinases* are papain, chymopapain, ficin, bromelin. Iminodipeptidase and leucylpeptidase are specific peptidases. Exopeptidases include carboxypeptidases, aminopeptidase, dipeptidase, tripeptidase, aminopolypeptidases. (*b*) *Carbohydrate-hydrolysing enzymes* (*glycosidases*)—Cellulases, α- and β-amylases, sucrase, maltase, emulsin, β-glucuronidase, lactase are main examples. (*c*) *Lipid-hydrolysing enzymes* are cholesterase, esterases, lipases, lecithinases (phospholipases). (*d*) *Amidases* or *Desamidases* (Enzyme-hydrolysing non-peptide C–N linkages)—Arginase, asparaginase, aspartase, glutaminase, hippuricase, purine amidases, adenase, guanase, urease are in this group. (*e*) Other *ester-hydrolysing enzymes* are phosphatases (e.g., phosphomonoesterases, phosphodiesterases, phosphorylase, pyrophosphatase, phytase, nucleoside phosphorylase, nucleinases, nucleotidases), choline-esterase, chlorophyllase, pectinesterase and sulphatases.

IV. **Isomerases.** These enzymes, as described earlier, catalyse the different processes of isomerisation. In the anaerobic breakdown of carbohydrate to lactic acid, triose phosphate isomerase converts 3-phosphoglyceraldehyde to dihydroxyacetone phosphate.



3-phosphoglyceraldehyde *dihydroxyacetone phosphate*

Another examples are retinene isomerase converts *all*-trans-retinene to *cis*-trans-retinene.

V. **Decarboxylases.** Decarboxylase or *carboxylase* is the enzyme which catalyses the decarboxylation of keto acids. For its activity it requires coenzyme. As for example: Pyruvic acid→Acetyl CoA, where enzyme is decarboxylase and coenzyme is thiamine pyrophosphate.

VI. **Miscellaneous enzymes.** Besides these, there are miscellaneous enzymes which are as follows: *Carbonic anhydrase*—present in red blood corpuscles, renal tubule cells, parietal cells of the gastric mucosa, etc. This enzyme accelerates the reaction $H_2O+CO_2 \rightleftharpoons H_2CO_3$. Catalase, hyaluronidase or mucinase, lysozyme, thiaminase, renin, transpeptidase, phosphoamidase. Furthermore there are other enzymes which are transketolase, transaldolase (*vide* Pentose-phosphate metabolism).