Biochemistry

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1

Introduction

1.1 Biochemistry is a term introduced to distinguish the body of knowledge from the term *Organic Chemistry*. The name organic chemistry was originally introduced to the branch of chemistry dealing with "organic" or "organized" systems (i.e. living organisms), as distinct from the non-living, unorganized inorganic chemistry. Organic chemistry has grown beyond the scope of chemicals occurring only in living systems. Today it encompasses all compounds of carbon, including many obtained only in the laboratory, through synthetic chemical reactions. It became necessary to distinguish the chemicals of living systems from those obtained purely synthetically, even if they resembled in many respects, the *biochemicals*. The term *physiological chemistry* was actually introduced, but has since been superseded by the term **biochemistry** (*bios* means life). From a branch of chemistry, biochemistry has developed into a full and comprehensive branch of science itself, with close relationship to biology, chemistry, medicine, microbiology etc.

Biochemistry deals with the study of chemical substances and processes involved in any living system, as also in simulated systems with biochemicals, even in the absence of living cells. Biochemical processes are similar to chemical processes (reactions) but take place under mild conditions obtainable in living organisms. All the physico-chemical principles that influence chemical reactions also influence the biochemical reactions, more or less in a similar manner. Biochemical reactions in living cells cause the formation of new chemicals and bring change in the energy status in such cells. These in turn are responsible for the various physiological phenomena in living cells and tissues. In fact, it can be said that the very nature of life is the result of biochemical processes. All biochemicals are active in low concentrations. Most biochemical processes are reversible and consist of several steps of reactions.

For example, the conversion of glucose to carbondioxide and water can be done in the laboratory by the addition of strong acid. Lot of heat energy is generated in the process. In a living cell or tissue glucose can

be converted to carbondioxide and water by a series of biochemical reactions, all taking place under mild conditions of pH, temperature etc., yielding energy needed for living organisms. Such a process is called *metabolism* and the distinct series of steps a *pathway*. In different organisms the metabolic pathways differ to a minor extent or to a large extent. For example the end product of glucose metabolism in some organisms may be ethyl alcohol or in others acetic acid etc. Thus there may be different metabolic pathways operating in different organisms or sometimes in the same organism under different conditions. Small changes in pH, temperature, redox potential, ions and their concentration, water etc. can cause profound changes in the course and extent of biochemical reaction. Normal working of a living cell is thus dependent on a delicate balance of biochemical processes taking place inside it at any given time. Any disturbance in the normal biochemical processes will result in an undesirable change in the function of a cell or tissue. It can be said that "health" is a state in which normal biochemical processes are taking place in a living organism and that "disease" is a state in which some biochemical process in a living organism has been disturbed or are abnormal. Disturbances may be because a metabolic pathway is slowed down or runs too fast, or diverted through an alternate pathway leading to an abnormal end product, which may result in an abnormal physiology (pathology), or may altogether be blocked. These are all responsible for "disease" conditions. A knowledge of both "normal" and "abnormal" biochemical processes is necessary for a better understanding of living organisms. Biochemistry therefore deals with both *health* and *disease* conditions.

1.2 Brief Survey of Organic Chemistry

In order to understand biochemistry clearly, a sound knowledge of organic chemistry is necessary. For the purpose of this book, only a brief survey of organic chemistry will be included here, that is relevant to the topics dealt with in this book.

Carbon, with its four valencies, is considered as having a tetrahedron structure, the four atoms attached to carbon, occupying the four corners (apex) of the tetrahedron. This structure is the result of sp^3 hybridization of the four valency electrons of carbon, because all four bonds are equal in all respects and indistinguishable from each other. When all four corners are occupied by other atoms it gives rise to a saturated carbon. The bond angles are equal to 109.5°. The atomic distances (bond lengths) are different for different bonds. In this concept, the bond formed is by sharing the two electrons, one donated by carbon and the other by the atom forming the bond. This is a covalent bond.

With di- and tri-valent atoms like oxygen, sulphur, nitrogen, phosphorous (which are commonly encountered in biochemicals) and of course carbon, there will also be double and triple bonds in many compounds. Some of these atoms have non-bonding electrons (unshared pair of electrons) in the outer shell, (oxygen, sulphur, nitrogen etc). Such electrons often enter into co-ordinated covalent bonds, particularly with metals, allowing metals to become part of biochemicals. Such elements also form hydrogen bonds, sharing a hydrogen between two such atoms. This is of great significance in biopolymers.

TABLE 1.1 **Hydrocarbon Residues (R)**

Open Chain		*Cyclic*
Saturated (alkyl) :		**Cycloalkyl (saturated) :**
$C_nH_{(2n+1)}$		$C_nH_{(2n)}$
Straight chain	Branched chain	
CH_3- methyl	$(CH_3)_2$-CH- isopropyl	C_5H_{10}- cyclopentyl
C_2H_5- ethyl	C_2H_5-CH(CH_3)- sec. butyl	C_6H_{12}- cyclohexyl
C_3H_7- propyl	$(CH_3)_3$-CH- tert. butyl	C_7H_{14}- cycloheptyl
C_4H_9- butyl	C_2H_5-CH(CH_3)-CH_2- amyl	
$C_{15}H_{31}$- pentadecyl	$(CH_3)_2$-CH-CH_2-CH_2- isopentyl	
$C_{18}H_{37}$- octadecyl		**Aryl :**
		C_6H_5- phenyl
		Aralkyl :
		C_6H_5.CH_2- benzyl
		C_6H_5.CH_2.CH_2- phenethyl
Unsaturated : (alkenyl)		
CH_2=CH- vinyl;	CH_2=CH-CH_2- allyl	

When a carbon bond carries carbon-hydrogen residues (organic hydrocarbon residues) they are simply designated as R group, which may be alkyl (meaning an open-chain hydrocarbon residue) or aryl (meaning aromatic hydrocarbon system) or arakyl or cycloalkyl. The smallest of these groups is CH_3 or methyl. As the size of the 'R' group increases, the 'R' group becomes less and less polar until it becomes completely non-polar. The more polar groups have a greater affinity for water (*hydrophilic*) and the less polar groups have greater affinity for lipid type of systems and repel water (*lipophilic or hydrophobic*). By varying the size of the 'R' groups, the solubility of a compound in aqueous and lipid systems can be controlled. Most biochemicals are characterized by these properties, facilitating their transport and storage in the body.

As in organic compounds functional groups are quite common in biochemicals. The more frequently found functional groups are: hydroxyl (-OH), oxo (C=O and -CHO), carboxyl (-COOH), amino ($-NH_2$), thiol (-SH). Less frequently groups like nitro ($-NO_2$), cyano ($-C\equiv N$) are found. Other functional groups, particularly derivatives of the above (like $-OCH_3$, $-NCH_3$, $-SCH_3$, $-CONH_2$ etc.) are often encountered in biochemicals.

All these functional groups exhibit their usual properties. For example the hydroxyl functions are easily esterified by both organic acids and inorganic (mineral) acids like phosphoric and sulphuric acids. Similarly acylated amino-groups are found in many biochemicals. Several organic acids can be converted to their anhydrides in course of some processes. Inorganic phosphoric acid anhydride (pyrophosphoric acid) and its organic acid mixed anhydride are very active in many biochemical molecules.

TABLE 1.2 **Functional Groups**

Hydroxy Compounds

Alcohols : $R–CH_2OH$ (primary)

$R^1–CHOH–R^2$ (secondary)

$R^1–C(R^2)(R^3)–OH$ (tertiary)

Polyhydroxy compounds

$R^1–CHOH–CHOH–CHOH–CHOH–R^2$

$R^1–CHOH–CHOH–CHOH–CH_2OH$

Glycols : $CH_2OH–CH_2OH$; $R–CHOH–CH_2OH$

Phenols

$C_6H_5–OH$ (Ar–OH)

(Ar = aryl)

Oxo Compounds

Aldehydes : $R–CHO$, $C_6H_5–CHO$, $Ar–CHO$

Ketones : $R^1–CO–R^2$, $Ar–CO–R^1$, $Ar–CO–Ar$

Carboxyl Compounds

	Monocarboxylic	Dicarboxylic		Tricarboxylic
Fatty acids :	$R–COOH$	$COOH–COOH$	:	$COOH–CH(COOH)–(CH_2)_n–COOH$
Aromatic acids :	$Ar–COOH$, $C_6H_5–COOH$			

...continued

TABLE 1.2 **Functional Groups** *(continued)*

Amino Compounds

Open chain	Cyclic (saturated)*	Cyclic (unsaturated)*
$R–NH_2$ (primary)	$C_4H_8–NH$	$C_4H_4–NH$
$R^1–NH–R^2$ (secondary)	$C_4H_8–N–R^1$	C_5H_5N
$R^1–N(R^3)–R^2$ (tertiary)	$C_5H_{10}–NH$	
	$C_5H_{10}–N–R^2$	

*These are heterocyclics

Sulphur Compounds

Thiols (mercaptans) : R–SH; Sulphides : $R^1–S–R^2$; $R^1–S–S–R^2$

Esters

$R^1–CH_2–O–CO–R^2$ (primary alcohol & monocarboxylic acid); $R^1–CH_2–S–CO–R^2$ (thio-ester)

R^1, R^2 $CH–O–CO–R^3$ (secondary alcohol & monocarboxylic acid)

$C_6H_5–O–SO_2–OH$ (phenol sulphuric acid ester)

$R^1–CH_2–O–PO–(OH)_2$ (phosphoric acid ester)

$C_6H_5–O–CO–R^1$ (phenolic ester)

Anhydrides

$R^1–CH(R^2)–CO$ O (intramolecular) : $R^2–CH–CO$

$R^1–CO–O–CO–R^2$ (mixed organic)

$R^1–CO–O–PO–(OH)_2$ (mixed organic-phosphoric)

Compounds having amino-groups are basic. Similarly compounds with carboxyl groups are acidic. Many biochemicals are responsible for maintaining acidic or basic reaction in a medium (pH) by virtue of such functional groups in their molecules. Similarly polarity is regulated by the presence or absence of polar functional groups like -OH and -COOH or by protonation of amino-groups to yield ions like NH_3^+. The hydroxy compounds undergo dehydration to yield unsaturated molecules. This and its reverse reaction (hydration of double bonds) are quite frequent in biochemical reactions. Oxidation, dehydrogenation and hydrolysis reactions are also well known among biochemical processes. Most such reactions are like the ones encountered in organic chemistry, with similar mechanisms of addition and substitution. Table 1.3 summarises some of the typical reactions occuring in biochemical systems.

TABLE 1.3 **Reaction Types**

Ionization (Dissociation)

$R^1–COOH + H_2O \longrightarrow R^1–COO^- + H_3O^+$ (carboxylic acid)

$R^1–NH_2 + H_2O \longrightarrow R^1–NH_3^+ + OH^-$ (protonation)

$R–O–PO–(OH)_2 + H_2O \longrightarrow R–O–P(OH)_2–O^- + H_3O^+$ (phosphoryl comp.)

Dehydration and Hydration

$R^1–CH_2–CHOH–R^2 \longrightarrow R^1–CH{=}CH–R^2 + H_2O$

$R^1–CH{=}CH–R^2 \xrightarrow{H_2O} R^1–CHOH–CH_2–R^2$

Oxidation

$R^1–CH_2OH \longrightarrow R^1–CHO$ (dehydrogenation)

$R^1–CHO \longrightarrow R^1–COOH$ (oxygenation)

$R^1–CHOH–R^2 \longrightarrow R^1–CO–R^2$ (dehydrogenation)

$R^1–CH{=}CH–R^2 \longrightarrow R^1–CHO + R^2–CHO$ (oxidative cleavage)

Reduction

$R^1–CHO \longrightarrow R^1–CH_2OH$ (hydrogenation)

$R^1–CO–R^2 \longrightarrow R^1–CHOH–R^2$ (hydrogenation)

Hydrolysis

$R^1–CO–O–R^2$ (ester) $\xrightarrow{H_2O} R^1–COOH + R^2–OH$ (hydration)

Many organic compounds exhibit different types of isomerism : position isomers, optical isomers, geometric isomers are all encountered among biochemicals.

TABLE 1.4 **Different Types of Isomers**

Position Isomers

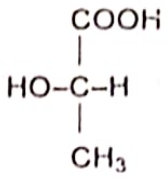
$CH_2–O–CO–R^1$ | CHOH | CH_2OH	$CH_2–OH$ | $CH–O–CO–R^1$ | CH_2OH
Glycerol-1-ester	Glycerol-2-ester

... continued

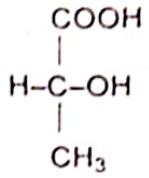
TABLE 1.4 **Different Types of Isomers** (*continued*)

Stereoisomers

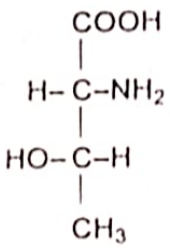
Optical isomers (enantiomers)



L-Lactic acid

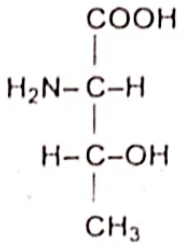


D-Lactic acid



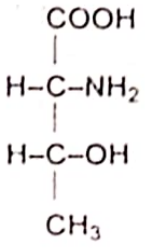
(I)

D(+)Threonine



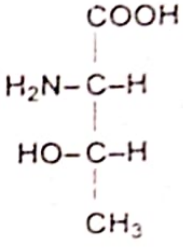
(II)

L(−)Threonine



(III)

D-*allo*-Threonine

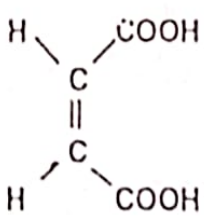


(IV)

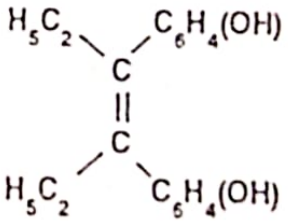
L-*allo*-Threonine

I & II and III & IV are enantiomer pairs. However, I & III, II & III, I & IV and II & IV are *diastereomers* (not mirror images).

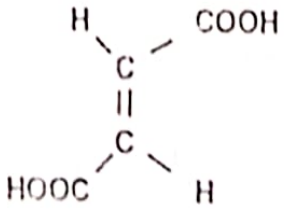
Geometrical Isomers



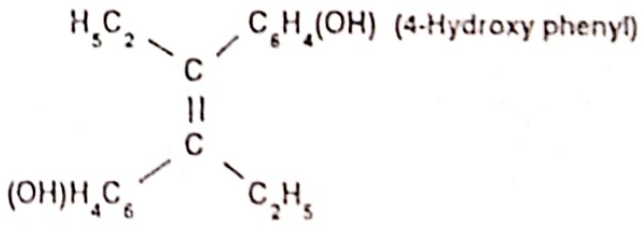
Maleic acid (*cis*)



cis-Diethylstilboestrol



Fumaric acid (*trans*)



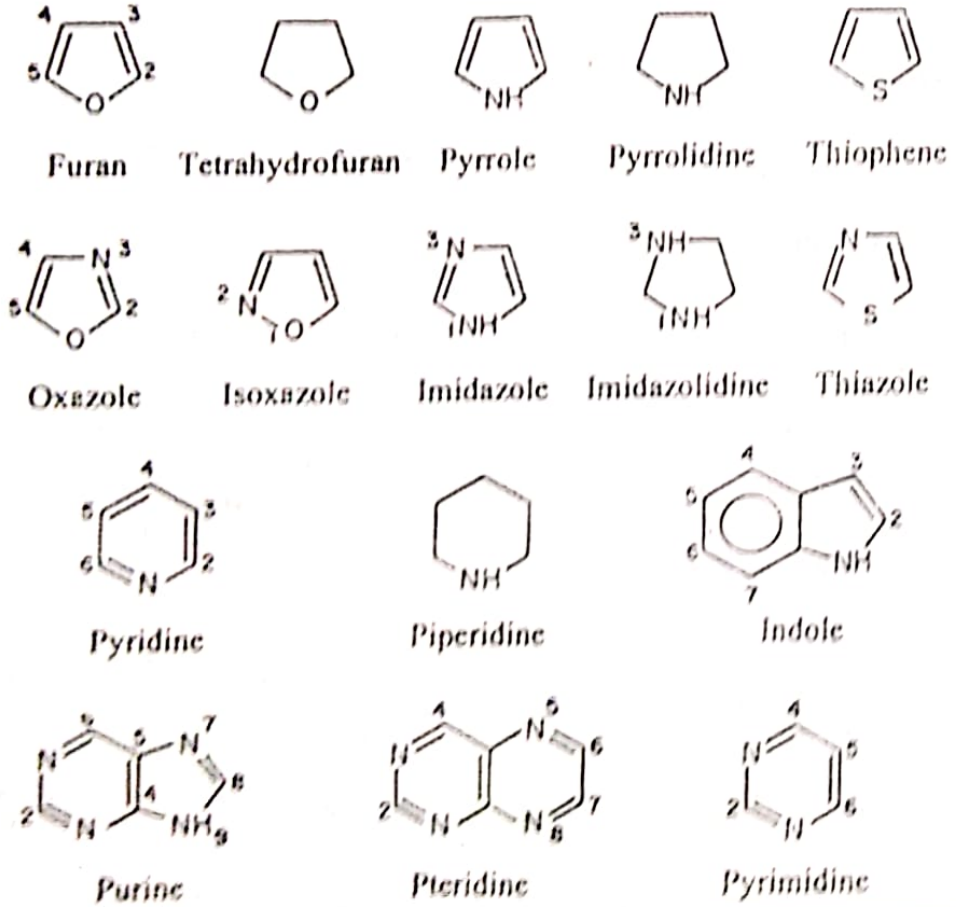
trans-Diethylstilboestrol

These play a very important role in living systems, because they distinguish the isomers very sharply in processing. This imparts a high degree of specificity for the biochemical reactions unlike in some organic reactions. An interesting and important aspect is that a specific isomer

alone is bio-synthesized in living systems, a situation that is very difficult to achieve in the laboratory.

Organic compounds with ring systems are very well known. These are the aromatic (benzenoid and polynuclear aromatics), alicylic (cyclohexane and related fused systems e.g. steroids) and heterocyclic systems, with oxygen and nitrogen hetero atoms more frequently and sulphur hetero atoms occasionally. There are a wide variety of such systems found in living organisms. Some of the systems are prominent among proteins, carbohydrates and hormones. Numerous and varied heterocyclic systems have been discovered among secondary metabolites of plants, like alkaloids, flavonoids, saponins etc. and of micro-organisms of plants, like antibiotics. Most of these are biosynthesized by appropriate cyclisation reactions.

CHART 1.1 **Hetrocyclic Systems**



1.3 Brief Survey of Physico-Chemical Principles

Biochemical processes are very well regulated by achieving physico-chemical conditions, within cells, tissues, fluids, membranes etc. Before attempting to

understand and appreciate biochemistry, it will be worthwhile to consult comprehensive books on physical chemistry for a thorough understanding. Here, only a very brief outline of some of these will be given, with a view to drawing attention to them.

1.3.1 Ionization

When an electron of an outer-shell (valency shell) of an atom is transferred to another atom forming a compound, an electro-valent bond results. In solution, the two atoms separate as electrically charged particles, one as a *cation*, with loss of electron and the other as *anion* with gain of electron. This phenomenon called *ionization* or *dissociation* operates in most biological systems also. Such compounds are known as electrolytes. When dissociation is complete or 100% (i.e. every molecule is dissociated), the compound is termed a strong electrolyte. Most inorganic salts, acids and bases of organic compounds dissociate only partially and are called weak electrolytes.

$NaCl \longrightarrow Na^+ + Cl^-$ (strong electrolyte, 100% dissociation)

$NH_4OH \rightleftharpoons NH_4^+ + OH^-$ (weak electrolyte, partial dissociation)

$H_2PO_4^- \rightleftharpoons HPO_4^{--} + H^+$

$CH_3COOH \rightleftharpoons CH_3COO^- + H^+$

Water, which is the medium for these dissociation phenomena, itself is a weak electrolyte. In 1 litre of pure water, which is approximately equal to 55.5 moles, only 1.0×10^{-7} moles of hydrogen ion and an equal amount of hydroxyl ion are found. The ion product of water is equal to 1×10^{-14} (i.e. the product of the ionic concentrations expressed in moles per litre). This dissociation is a reversible reaction.

$$H_2O \rightleftharpoons H^+ + OH^-$$

$$H^+ + H_2O \rightleftharpoons H_3O^+$$

Therefore it reaches equilibrium, when the value is 1.8×10^{-16}. The equilibrium can be easily disturbed by increase in either of the ions, H^+ or OH^-. When strong acid is dissolved in water, it contributes hydrogen ions, which force reversal of ionization of water. Usually the H^+ associates with water giving rise to hydronium ion, H_3O^+. Similarly the bases which contribute OH^- ions force reversal and dissociation of water is suppressed. However, weak electrolytes will dissociate to a limited extent and the final reaction of the medium is dependent on the H^+ or OH^- concentration. If the H^+ concentration is expressed as negative logarithm, then the term pH is applied. Pure water has a pH of 7.0. Weak acids will

contribute H^+ reducing the pH. The lower the pH, the stronger the acid, Similarly the higher the pH the stronger the base. Compounds which dissolve in water, but do not contribute H^+ or OH^- ions, are termed neutral. Most sugars, alcohols, aldehydes, ketones are neutral compounds. Acids are considered as proton donors or acceptors of a pair of electrons.

1.3.2 Acids and Bases

Most organic acids are carboxylic acids. If they have only 'R' groups (alkyl, aryl etc.) their dissociation is usually low and becomes less and less as the 'R' group size increases (i.e. becomes less polar). Many organic acids found in living systems, are substituted, i.e. have other functional groups. If the functional groups are polar (like -OH) or electron withdrawing (like C=O, $-NO_2$, -Cl etc.), the acids are usually stronger, with a lower pKa value. Thus citric acid, tartaric acid, malic acid, pyruvic acid are all stronger than acetic acid. Many biochemicals carry inorganic polybasic acids like phosphoric acid in their molecules. For example glycero-phosphoric acid is a strong acid compared to other organic acids.

Most organic bases contain amino-groups and their substituted derivatives. Nitrogenous compounds, including heterocyclics, are basic, unless compensated by the presence of acidic functions or electron-withdrawing groups in their close vicinity within the molecule. Bases are proton acceptors or donors of electron pair. Accordingly many organic bases may become ionizable on accepting a proton (protonation). In this manner, they can be easily dissovled and transported.

1.3.3 Buffers

Since water is a weak electrolyte, its dissociation is influenced by other electrolytes, weak and strong. Actually water is participating in the dissociation reaction of the electrolyte. This becomes prominent, when a salt of weak acid and a strong base (or salt of weak base and strong acid) is dissolved in water. The salt dissociates fully thus : (e.g. sodium acetate in aqueous medium)

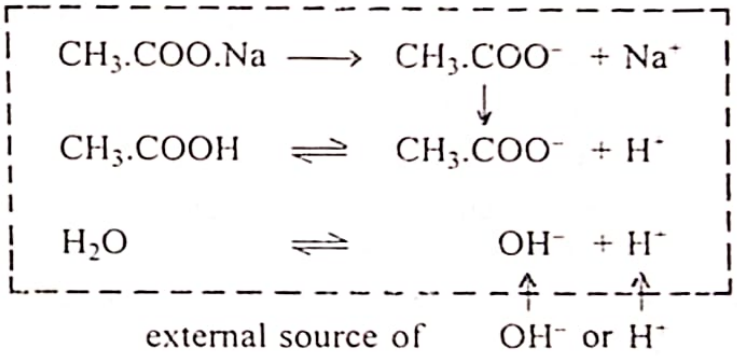
$$CH_3COONa \longrightarrow CH_3COO^- + Na^+$$

$$H_2O \rightleftharpoons H^+ + OH^-$$

Since acetic acid is a weak acid and has a dissociation constant, the two ions of H^+ and CH_3COO^- will immediately combine to yield undissociated acetic acid :

$$H^+ + CH_3COO^- \rightleftharpoons CH_3COOH$$

This results in lowering of H^+ and CH_3COO^- ions in the system leaving a slight excess of OH^- or a basic pH (above 7.0). Actually water has brought about a decomposition of sodium acetate to yield acetic acid and sodium hydroxide to a small extent. Therefore hydrolysis has taken place. In general, salts of this type (formed from weak and strong partners) undergo hydrolysis easily, changing the pH of the medium, depending on concentration of salt and undissociated weak partner (acid or base) and the pH of the weak acid or weak base. To this system, if a small amount of H^+ or OH^- are added in the form of another acid or base, immediately the system tries to fix the H^+ or OH^-, as the case may be. In the process, the concentration of the original salt/acid or salt/base is not changed as also the dissociation constant. The result is therefore practically no change in pH.



Within narrow limits, change in pH is resisted by this system, which is termed a *buffer*. Such buffer systems play a very important role in biochemical processes. Besides the numerous simple organic acids and bases found in cells and tissues, many proteins and peptides also act as buffer systems in the body.

Organic acids found in the body are found both in undissociated form as well as salts. More often, they are accompanied by cations (like Na^+, K^+ etc.). Thus they may be considered as salts of corresponding strong bases. For this reason, in biochemistry, acids are always designated as salts (e.g. *acetate*, *citrate*, *malate*, *pyruvate* etc. rather than as acetic acid, citric acid, malic acid, pyruvic acid etc.). To a limited extent these also form buffers in their own systems.

1.3.4 Oxidation Reduction

Many biochemical processes are described as oxidation-reduction reactions. These reactions are essential to living systems, because ultimately all energy is generated by oxidative process. Oxidation consists of introduction of oxygen in addition to whatever was already present (oxygenation).

$R.CHO \longrightarrow R.COOH$

$R.CH_2.CH_2.COOH \longrightarrow R.CO.CH_2.COOH$

$R.CH_2.CH_2.COOH \longrightarrow R.CHOH.CH_2.COOH$

Oxidation also consists of removal of hydrogens (dehydrogenation)

$R.CH_2.CH_2.OH \longrightarrow R.CH_2.CHO$

$R.CH_2.CH_2.COOH \longrightarrow R.CH{=}CH.COOH$

Ascorbic acid $\longrightarrow$ Dehydroascorbic acid

$NADH + H^+ \longrightarrow NAD^+ + H_2$

$FAD.H_2 \longrightarrow FAD + H_2$

Oxidation also occurs when an electron is withdrawn from an atom or ion.

$Fe^{++} \longrightarrow Fe^{+++}$

ferrous ferric

Reduction is the reverse process. Oxidation and reduction always go hand in hand. Oxidation is effected by an oxidising agent. In the process this agent itself gets reduced. The reduction product may not be mentioned, as in the above examples, but it is always there. For example ascorbic acid is oxidised to dehydro-ascorbic acid by say iodine. Here iodine is reduced to iodide. This may be indicated thus :

Ascorbic acid + $I_2^0 \longrightarrow$ Dehydroascorbic acid + $2I^-$

In the above process elementary iodine has gained an electron to be reduced to iodide. Thus ascorbic acid acts as a reducing agent, by donating electons. Similarly ferric is reduced to ferrous by gaining an electron.

Reduction also occurs when oxygen is eliminated from a compound or hydrogen is added to compounds. Following example, illustrate these :

$R.CHOH.CH_2.COOH \longrightarrow R.CH{=}CH.COOH$

$R.CH{=}CH.COOH \longrightarrow R.CH_2.CH_2.COOH$

$R.NO_2 \longrightarrow R.NO$

Flavine adenine dinucleotide (FAD) + $H_2 \longrightarrow FADH_2$

$NAD^+ + H_2 \longrightarrow NADH + H^+$

$NADP^+ + H_2 \longrightarrow NADPH + H^+$

Cytochrome-$(Fe^{+++}) \longrightarrow$ Cytochrome-(Fe^{++})

In the above examples also, the reducing agent and its corresponding oxidised product are not shown. The following example illustrates a fuller description of oxidation reduction process :

$$CH_3.CH_2.OH + NAD^+ \xrightarrow[dehydrogenase]{alcohol} CH_3.CHO + NADH + H^+$$

In the biochemical process numerous enzymes and few co-enzymes are involved in oxidation reduction reactions. A whole chain of reactions have been discovered which end in utilising atmospheric oxygen (inhaled in respiration) as the final oxidising agent.

2

Proteins and Amino Acids

2.1 Introduction

The name protein is derived from the Greek word *proteios*, meaning the first (and foremost). Proteins are a class of chemicals, which occupy the foremost position among biological chemicals. It is also believed that origin of life was made possible because of amino-acids, which are the building blocks of all proteins.

Proteins have two major functions in all living cells or tissues or organs : (1) structural and (2) dynamic. Structural proteins have rigid, usually fibrous form and support the physical framework of living beings, e.g. connective tissue, cell membrane, muscle fibre etc. Dynamic proteins are responsible for all active life processes like biochemical reactions (metabolism), control of body functions, protection of cells and tissues against foreign organisms, transport of other chemicals, maintenance of favourable physico-chemical conditions like osmotic pressure, viscosity etc. Examples of proteins involved in these dynamic functions include enzymes, hormones, antibodies, hemoproteins, serum proteins etc. Earliest studies on proteins were conducted on egg white, blood fibrin and silk fibre. Based upon these studies, proteins were classified as (*a*) *albumins*, which are soluble in water and salt solutions; e.g. egg white; (*b*) *globulins*, which are sparingly soluble in water but soluble in salt solutions; e.g. serum proteins; (*c*) *prolamines*, which are insoluble in water (found in wheat, barley etc; (*d*) *glutelins*, which dissolve in acids or alkalis (wheat and rice) (*e*) *scleroproteins* (also known as *albuminoids*), which are insoluble and are structural proteins (e.g. hair, nails). *Protamins* and *histones* are two other classes, which are soluble in water but are not coagulated.

Proteins have a high molecular weight. Most proteins are amorphous solids, but a few have been crystallized. On breakdown (usually hydrolysis) they yield a mixture of amino-acids. A protein usually yields about a dozen or less of amino-acids, which by repeating themselves in some order, build up a protein molecule. Thus *proteins are biological polymers*

(bio-polymers) of high molecular weight formed from amino-acid units. Proteins, chemically linked with units other than amino-acids are called conjugated (or complex) proteins. Examples of conjugated proteins : *glycoproteins* (containing also sugar residues), *lipoproteins* (containing lipids) and *nucleoproteins* (containing nucleic acids). There are thousands of proteins in living organisms, each chemically different from another depending on the source and function. For example albumin obtained from egg differs from serum albumin. But all proteins are made up of not more than two dozen amino-acids. A study of amino-acids is therefore necessary to understand the chemistry and properties of proteins.

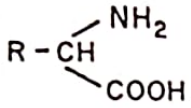
Table 2.1 **Classification of Proteins**

Class	*Characteristics*	*Examples*
Simple Proteins : Made up of only α-amino-acids		
(a) Albumins	Soluble in water & dilute electrolyte solutions	egg albumin serum albumin
(b) Globulins	Sparingly soluble in water but soluble in dilute electrolyte solutions	serum globulins
(c) Prolamines	Insoluble in water; soluble in 70% alcohol	seed proteins
(d) Glutelins	Soluble only in acidic or alkaline media	wheat gluten
(e) Scleroproteins (Albuminoids)	Insoluble in aqueous media (structural proteins)	hair, nails
(f) Protamines	Highly basic	sperm proteins
(g) Histones	Less basic than protamines	thymus gland proteins
Complex or Conjugated Proteins	Contain also other compounds and/or metals	
(a) Glycoproteins	Sugar residues are present	blood group proteins
(b) Lipoproteins	Lipids are present	egg yolk
(c) Nucleoproteins	Nucleic acids are present	virus proteins
(d) Metalloproteins	Metal ions are present	cytochromes, myoglobin
(e) Chromoproteins	Coloured organic compounds are associated	hemoglobin

2.2 Amino Acids

Chemistry and classification : All amino-acids contain at least one amino group and at least one carboxyl group in the molecule. Both the

amino group and carboxyl group are attached to the same carbon atom, to which another organic residue is attached. The general structure is :



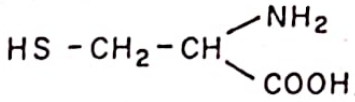
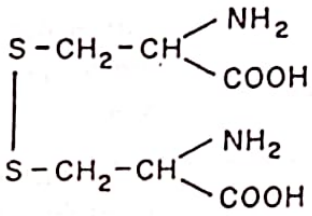
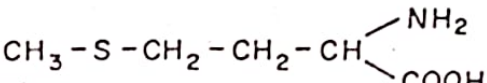
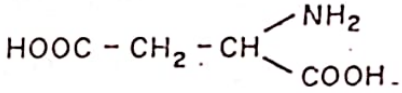
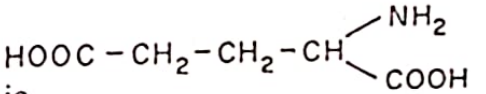
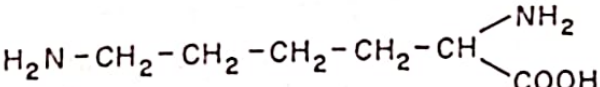
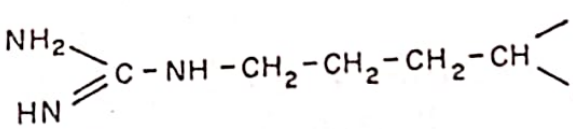
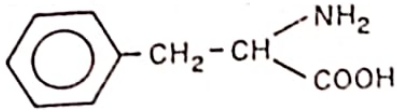
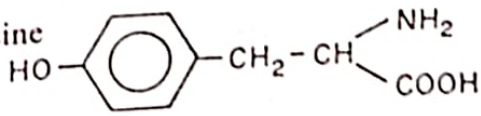
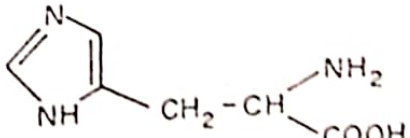
As the amino group is attached to the carbon *alpha* to the carboxyl group, these are also known as *alpha*-amino-acids. Organic acids may have an amino group anywhere in the molecule and called amino acids (e.g. Paraaminobenzoic acid, PABA can be called an amino acid or gamma-amino butyric acid, GABA, can also be called an amino-acid). But *proteins are built up entirely from α-amino acids,* without exception.

Different amino-acids differ from each other only in the nature of R group (organic residue) in the above general structure. All the known α-amino acids are shown in the table below, with additional information about them.

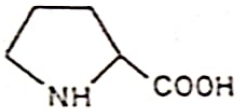
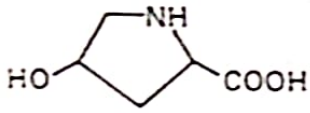
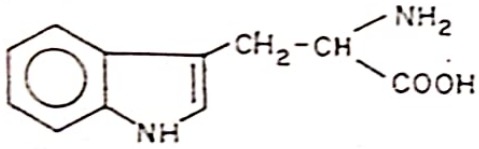
TABLE 2.2 **Alpha-Amino Acids**

No. Name	*Structure*	*Chemical Name*	*Abbreviated Name*
1. Glycine	NH_2-CH_2-COOH	2-Amino-acetic acid	Gly
2. L-Alanine	$CH_3-CH(NH_2)-COOH$	L-2-Amino-propionic acid	Ala
3. L-Valine	$(CH_3)_2CH-CH(NH_2)-COOH$	L-2-Amino-iso-valeric acid	Val
4. L-Leucine	$(CH_3)_2CH-CH_2-CH(NH_2)-COOH$	L-2-Amino-4-methyl-valeric acid	Leu
5. L-Isoleucine	$C_2H_5(CH_3)CH-CH(NH_2)-COOH$	L-2-Amino-3-methyl-valeric acid	Ileu
6. L-Serine	$HO-CH_2-CH(NH_2)-COOH$	L-2-Amino-3-hydroxy-propionic acid	Ser
7. L-Threonine	$HO-CH(CH_3)-CH(NH_2)-COOH$	L-2-Amino-3-hydroxy-butyric acid	Thr

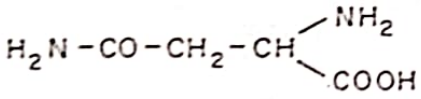
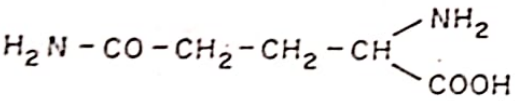
...continued

No. Name	Structure	Chemical Name	Abbreviated Name
8. L-Cysteine		L-2-Amino-mercapto-propionic acid	Cys
9. L-Cystine			Cys-S-S-Cys
10. L-Methionine		L-2-Amino-4-methyl-thiobutyric acid	Met
11. L-Aspartic acid		L-2-Amino-succinic acid	Asp
12. L-Glutamic acid		L-2-Amino-glutaric acid	Glu
13. L-Lysine		L-2,6-Diamino-caproic acid	Lys
14. L-Arginine		L-2-Amino-5-guanidino-valeric acid	Arg
15. L-Phenyl-alanine		L-2-Amino-3-phenyl-propionic acid	Phe
16. L-Tyrosine		L-2-Amino-3-(4-hydro-xyphenyl)-propionic acid	Tyr
17. L-Histidine		L-2-Amino-3-(5-imida-) zolyl)-propionic acid	His

...continued

No. Name	*Structure*	*Chemical Name*	*Abbreviated Name*
18. L-Proline		L-Pyrrolidine-2-carboxylic acid	Pro
19. L-Hydroxy proline		L-Pyrrolidine-4-hydroxy-2-carboxylic acid	Hypro
20. L-Tryptophan phan		L-2-Amino-3-(3'-indolyl)-propionic acid	Trp

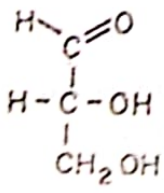
Two of the above amino-acids also occur in their amide forms in many proteins, where the extra carboxylic group is converted to carboxamide thus :

21. L-Asparagine		Asn
22. L-Glutamine		Gln

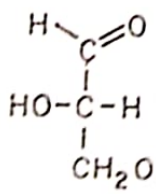
In addition to the above, hydroxylysine has been obtained from gelatin. From some marine animals and corals, di-iodotyrosine and even dibromotyrosine have been obtained. From the above table a few facts can be observed :

(1) Glycine is the simplest of α-amino-acids having no organic residue (*i.e.* R = H). This is also the only α-amino-acid which is optically inactive.

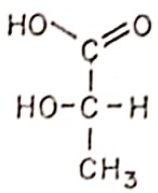
(2) All other amino-acids correspond to L-series, i.e they are stereochemically related to L-glyceraldehyde or L-lactic acid.



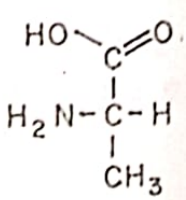
D-Glyceraldehyde



L-Glyceraldehyde



L-Lactic acid



L-Alanine

However, it must be remembered that the optical rotation actually measured may be *dextro* or *levo* depending on other factors in the mol-

ecule. For example L-alanine is dextro-rotatory whereas L-phenylalanine is levo-rotatory.

(3) The first 14 amino-acids in the table (and their derivatives 20 and 21) can be classified as aliphatic α-amino-acids. Phe and Tyr may be classified as aromatic α-amino-acids. His, Pro and Trp may be described as heterocyclic α-amino acids.

(4) The amino-acids Cys and Met can be described as sulphur containing amino-acids.

(5) Ser, Thr and Tyr may be described as hydroxylated amino-acids.

(6) Most amino-acids are described as mono-amino-monocarboxylic acids. Lysine is a diamino-monocarboxylic acid, making it more basic. Arg, His and Trp also are more basic because of more basic groups in their molecules. Asp and Glu are dicarboxylic monoamino-acids and are more acidic. Asn and Gln are neutral because of conversion of the extra carboxyl group to its neutral amide form.

(7) Proline is unique in that its α-amino-group is part of a heterocylic ring.

(8) Cystine is a dimer of cysteine (*note the spelling!*). It is usually formed by oxidation of cysteine molecules.

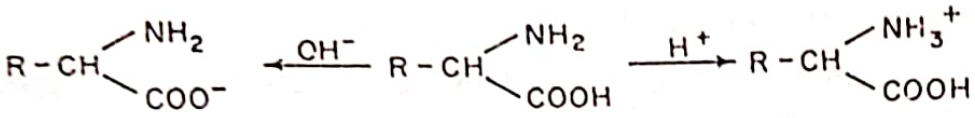
TABLE 2.3 **Classification of Amino-Acids**

Class	*Examples*
Aliphatic Amino Acids (no ring system)	
Mono-amino-monocarboxylic acids	glycine, alanine, valine
(no other functional groups)	leucine, isoleucine
Hydroxy-mono-amino-monocarboxylic acids	serine, threonine
Sulphur containing amino-acids	cysteine, (cystine)*
Mono-amino dicarboxylic acids (acidic)	aspartic, glutamic
Basic amino-acids	lysine, arginine
Amido amino-acids (neutral)	asparagine*, glutamine*
Aromatic & Heterocyclic Amino Acids	
Aromatic amino-acids	phenylalanine, tyrosine
Heterocyclic amino-acids	proline, hydroxyproline histidine, tryptophan

*These are derived amino-acids

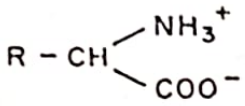
2.3 Properties of Amino Acids

(1) Most a-amino acids are fairly to sparingly soluble in pure water, undergoing feeble ionisation. In dilute acids or alkalis they dissolve with greater ionisation :



Amino acid dissociation

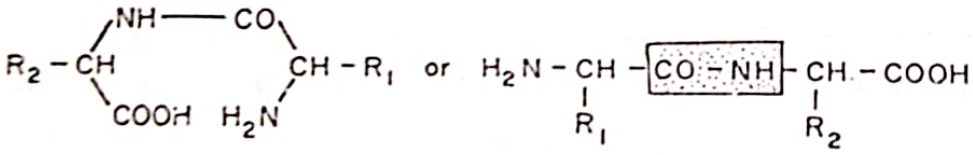
In neutral solutions α-amino-acids exist as amphoteric species, known as *zwitterion.*



Zwitterion

As seen above, in basic medium the protonation of the NH_2 group is suppressed and in acidic medium ionisation of the COOH group is suppressed. If the pH is carefully adjusted, ionisation and protonation can both be suppressed and neutral amino-acid will result. The solubility of the unionised unprotonated amino-acid is least and it may get precipitated. This is called *iso-electric pH.* As proteins are made up of α-amino-acid units, they also behave similarly in this respect (i.e. protonation, ionisation and precipitation at iso-electric pH).

(2) All a-amino acids form amide bonds, in which the α-amino group of one reacts with the a-carboxyl group of another amino-acid.



Peptide bond

This amide bond is responsible for building up of chains of amino-acids which are present in proteins and peptides. The bond is known as the *peptide bond.* Note that in the above new compound (called a *dipeptide*) formed from two amino-acids, one amino and one carboxyl group are still available for further reactions, just as in a single amino-acid. Peptide molecules are known as dipeptides, tripeptides, tetrapeptides, polypeptides etc. depending on the number of amino-acids in them and

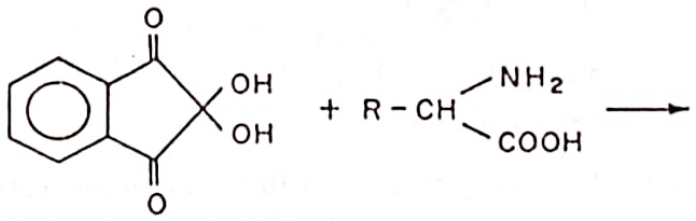
the relative molecular weight. High molecular weight polypeptides with over 50 amino-acids are termed proteins.

(3) The free carboxyl groups of amino-acids and peptides behave like any other carboxyl group in their chemical reactions. Thus esters of amino-acids can be obtained as also amides of amino compounds including amino-acids. These reactions are useful in the study of chemistry of proteins and peptides.

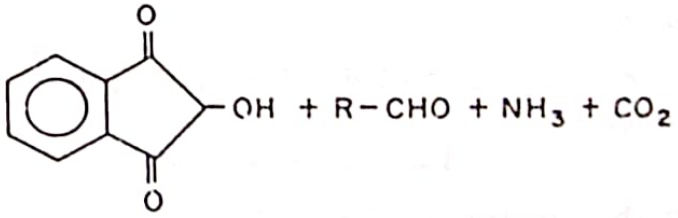
(4) The free amino groups of amino-acids and peptides can be acylated with other carboxylic acids. They can be substituted with alkyl or aryl groups. The primary amino groups also react with nitrous acid, liberating nitrogen quantitatively. These reactions form the basis for qualitative and quantitative analysis of amino-acids, peptides and proteins.

(5) Functional groups present in the 'R' residues of amino-acids and peptides (like -OH, -SH, -NH etc.) also yield characteristic reactions of such groups.

(6) An important reaction of amino-acids is their reaction with *ninhydrin* (triketo-hydrindene hydrate or indane trione hydrate). Most amino-acids react with ninhydrin giving rise to a mixture of carbon dioxide, ammonia and an aldehyde, all derived from the amino-acid. Ninhydrin itself is reduced to hydrindantin.

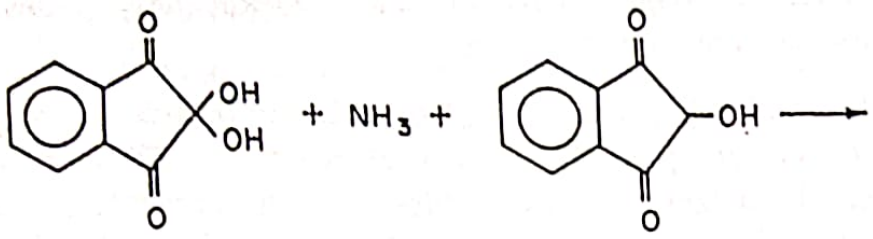


Ninhydrin Amino acid

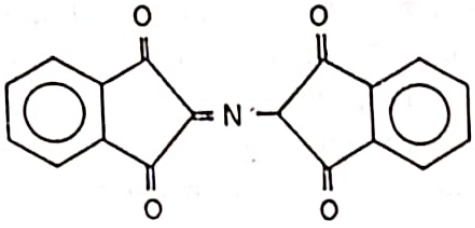


Hydrindantin

The liberated ammonia reacts with more ninhydrin to yield a coloured compound. The colour ranges from light pink to deep violet for different amino-acids. This reaction has been used both for qualitative detection of the amino-acids, especially in paper chromotography and thin layer chromatography and for quantitative estimation of amino-acids.



Ninhydrin Ammonia Hydrindantin



Coloured complex

2.4 Proteins and polypeptides

Polypeptides usually are made up of less than 50 amino-acids. The smallest peptides are di-and tri-peptides. *Glutathione* is a tripeptide occurring in living systems. Its chemistry is represented thus :

Glutamic Cysteinyl Glycine

Glutathione

It can be named γ-glutamyl-cysteinyl-glycine or in the short hand form as γ-glu-cys-gly. (*Note*—It is the *gamma* carboxyl of glutamic acid that has formed the peptide bond with cysteine instead of the usual α-carboxyl group). The short hand form is useful for writing structures of poly-peptides.

A pentapeptide called enkephalin occurring in a variety of tissues has the structure :

Tyr-Gly-Gly-Phe-Met (Met-enkephalin)

In this molecule Tyr and Met are end amino-acids of the chain. Conventionally the end amino-acid with the free amino-group is written at the beginning of the chain. Occasionally, to make this fact clear, the peptide may be written thus :

H_2N-Tyr-Gly-Gly-Phe-Leu-COOH (Leu-enkephalin)

1 2 3 4 5

which also numbers amino-acids in the chain in their correct sequence. For example, it is easy to explain that "5-methionine is replaced by 5-leucine in Leu enkephalin".

A long polypeptide chain is physically not rigid and will undergo distortion or deformation in biological media. As some of the functions of polypeptides and proteins are dependent on the shape of the molecule, rigid shape may be essential. Such rigidity is achieved through hydrogen bonding between -NH groups and -C=O groups of peptide bonds. In long chain peptides this is achieved by : (*a*) twisting the chain to form a *helix* so that the hydrogen bond occurs between an amino acid and its fourth successor or predecessor in the chain (called the α-*helical structure*) or (*b*) having parallel chains or helices so that the hydrogen bonds are formed between chains or helices (called the *β structure*). In long fibrous proteins (as in keratin of hair and fibroin of silk etc.) the β structure is a *pleated structure*. The three types are shown below.

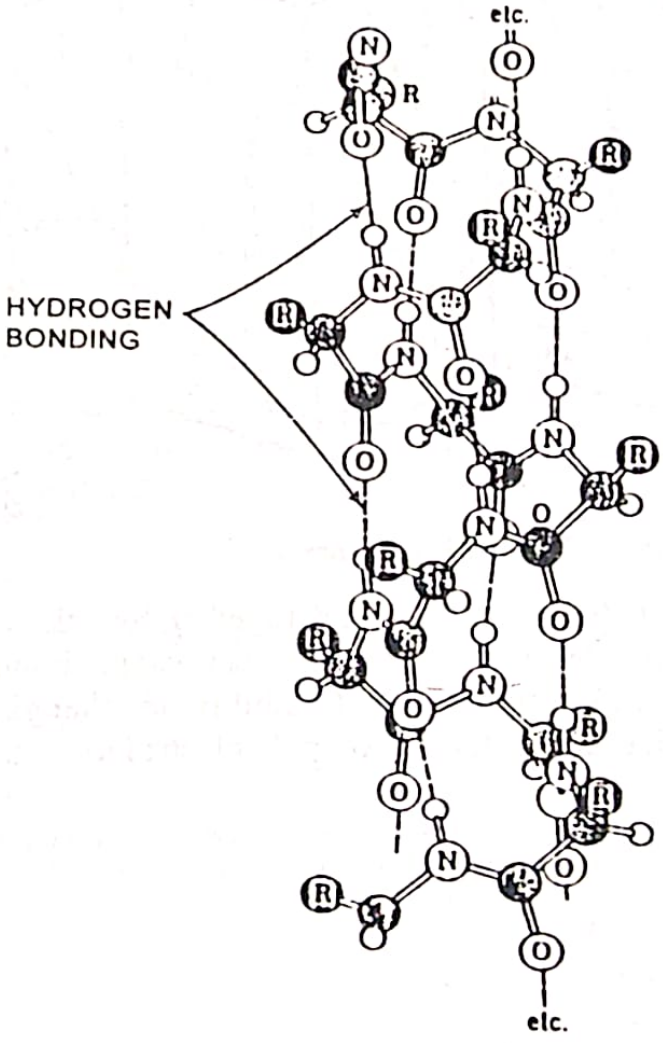


Fig.2.1 α-Helix

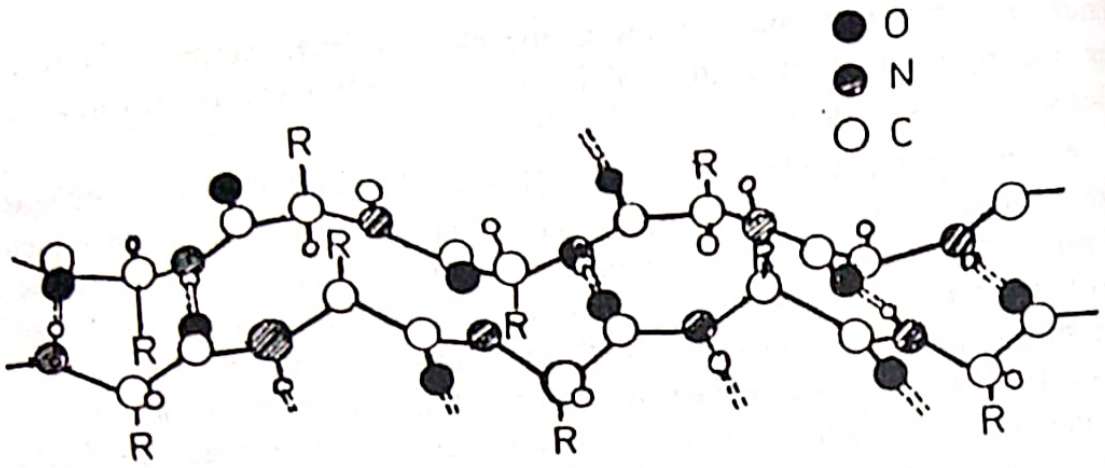


Fig.2.2 β-Structure

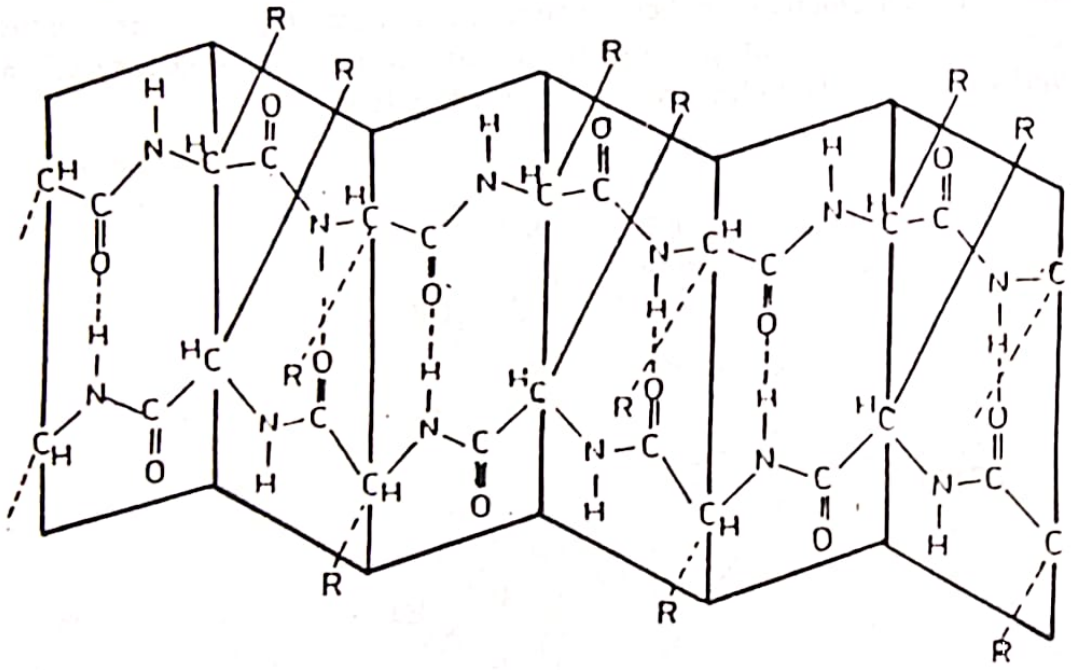


Fig.2.3 β-Pleated Structure

Polypeptide chains and helices are also held together by other forces like hydrophobic bonds, van der Waal's forces, electrostatic bonds etc. However, all these bonds and forces allow flexibility in changing the shape of the molecule under the influence of pH, electrolytes etc. (*see* Fig. 2.4).

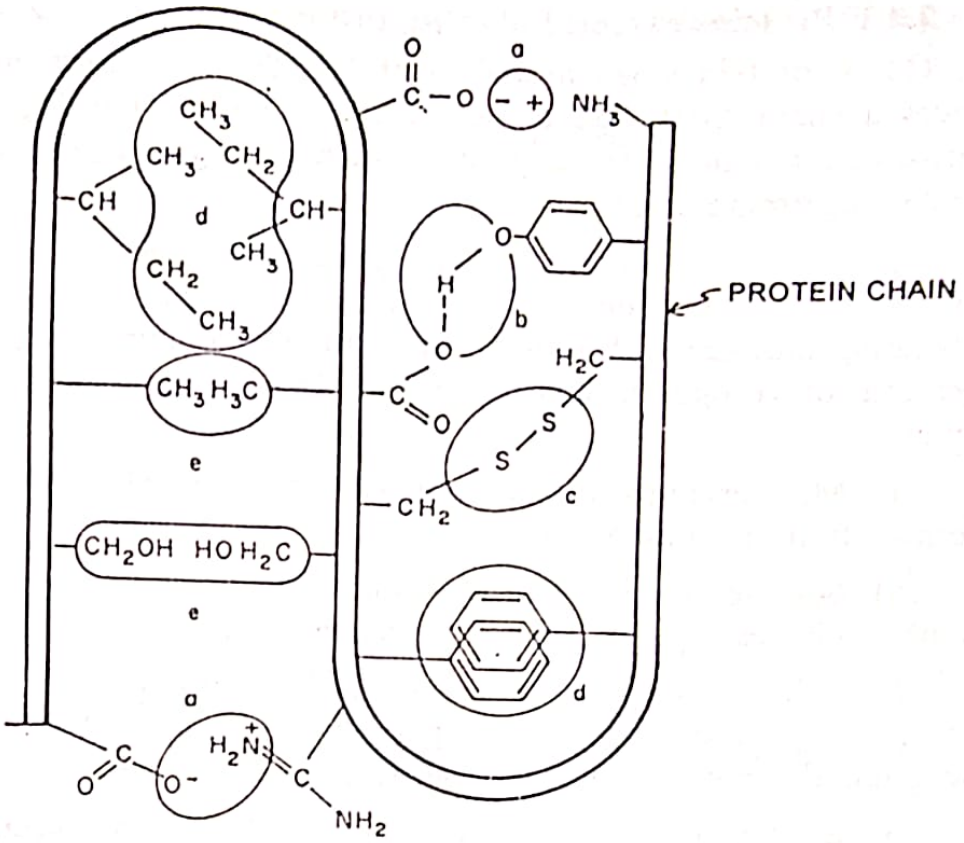


Fig. 2.4 Various bonds in proteins
(a) Electrostatic interaction; (b) Hydrogen bonding; (c) Disulfide linkage; (d) Nonpolar side chains; (e) van der Waals interactions

Some polypeptide molecules may have more than one chain. The two chains are held together generally through sulphide bridges formed between a cystein of one chain with the cystein of another chain. Insulin, a polypeptide hormone is an excellent example of this arrangement, as shown in the Fig 2.5 :

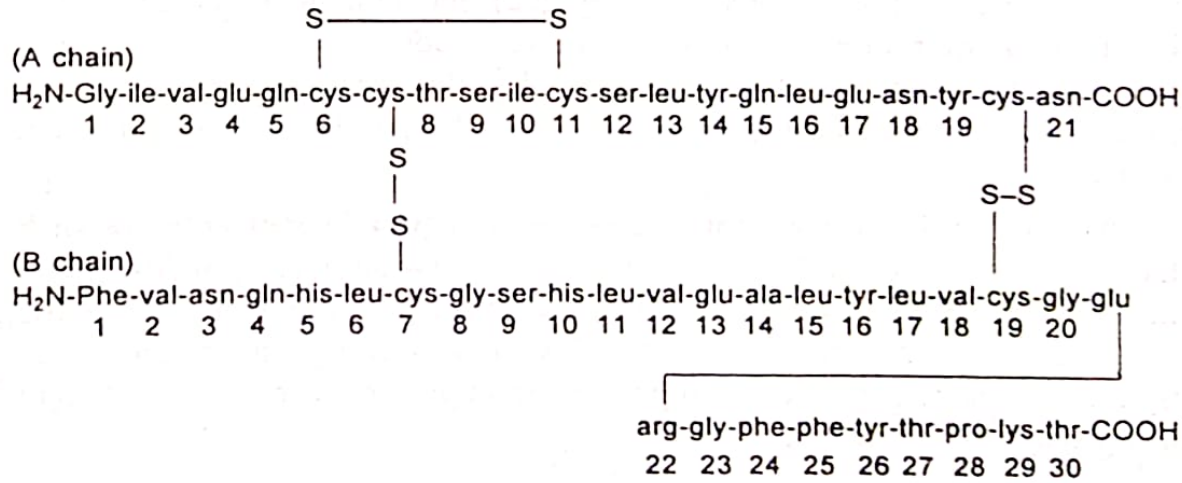


Fig. 2.5 Human Insulin

2.4.1 Proteins exhibit following properties :

(1) A protein when heated in dry condition or burnt in flame, produces a characteristic obnoxious smell, reminding burning hair or skin. The smell is caused by sulphides and mercaptans formed from sulphur containing amino-acids.

(2) Presence of nitrogen in proteins and polypeptides can be detected by the usual Lassaigne's test for elements (sodium fusion test) and by liberating nitrogen with nitrous acid. Proteins contain between 12 and 19 per cent of nitrogen in their molecules, 16% being assumed popularly as an average.

(3) Most proteins on warming with strong alkalis liberate ammonia, formed from the amino groups.

(4) Soluble proteins, like globulins, precipitate on warming (coagulation). This destroys the biological properties of proteins (*denaturation*).

(5) Soluble proteins are precipitated by strong mineral or organic acids like trichloro-acetic acid, phosphomolybdic acid, phosphotungstic acid and also picric acid. These also cause denaturation.

(6) Strong electrolyte concentrations (salts like magnesium sulphate, ammonium sulphate etc.) precipitate proteins, but do not cause denaturation. Removal of the electrolyte by dialysis, restores the protein properties. Sufficient concentration of ethyl alcohol also produces similar effects on proteins.

(7) Proteins are least soluble at their iso-electric point (i.e. pH at which ionisation is least). On ionisation in buffered systems, they can be subjected to migration under influence of electrical field. The process is known as *electrophoresis*. Migration is dependent on molecular size, nature of net charge (positive or negative) and amount of charge. The direction of migration (towards cathode or anode) and the rate of migration are characteristic of a protein. The electrophoretic behaviour of proteins has been utilised in identification and separation of proteins from each other.

(8) The molecular weight and shape of a protein molecule are such that these can be considered as colloidal or sub-colloidal particles. With the help of an ultra centrifuge, these particles can be made to "settle down" from solutions. The rate of *sedimentation* of a protein in an ultracentrifuge is useful for computing the approximate molecular weight of a protein molecule.

(9) Because of their high molecular weight, many of them are antigenic (i.e. they induce the body to produce antibodies) when injected. A

reaction between an antigen and its antibody can produce occasionally anaphylactic shock or *hypersensitivity* or allergy.

TABLE 2.4 **Properties of Some Proteins**

Protein/Polypeptide	*Approx. Mol. Wt*	*Iso-electric Point*
Insulin	6000	4.3-5.3
Egg Albumin	45000	4.6
Serum Albumin	69000	4.7
Hemoglobin	66000	6.7

2.4.2 Colour tests for proteins

(1) Proteins give blue to violet colour with *ninhydrin*, like amino-acids, and peptides.

(2) All proteins and peptides give reddish to violet colour with a drop of dilute copper sulphate solution added to a warm alkaline solution of the protein or peptide. The reaction is characteristic of peptide bonds. As the test was first discovered with biuret (H_2N-CO-NH-CO-NH_2), it is known as *biuret test*.

(3) **Xanthoproteic test** is given by proteins and peptides which have phenylalanine or tyrosine or tryptophan (aromatic amino-acids) in their molecules. On treatment with concentrated nitric acid, these amino-acids undergo nitration in the aromatic ring. These nitro compounds (particularly nitro-phenols) are yellow in colour. This is the same reaction which occurs when our skin comes in contact with concentrated nitric acid in the lab.

(4) **Millon's test** : When proteins or peptides are treated with Millon's reagent (a solution of mercuric and mercurous nitrates in nitric acid) a white precipitate is formed. When this is heated, a red colour or precipitate is formed. This test is given only by proteins containing tyrosine in their molecules, forming red mercury complexes with the nitrophenol formed. The test is also given by other phenolic compounds and is therefore *not specific* to proteins.

(5) **Sodium nitroprusside** reagent gives a red colour with peptides and proteins which yield cysteine or cystine on hydrolysis. As many proteins contain these, the test is answered by most proteins.

(6) **Sakaguchi** reaction is specific for the presence of arginine in proteins. Protein hydrolysates will give red colour with α-naphthol-sodium hypochlorite reagent if arginine is present. The reaction is due to the guanidine group.

(7) **Hopkins-Cole** reaction is carried out by adding glacial acetic acid followed by concentrated sulphuric acid to a solution of protein. A violet colour is produced if tryptophan is present in the protein. The reaction is due to the glyoxalic acid formed on acid hydrolysis of typtophan.

2.4.3 Structure of Proteins

Primary Structure : The exact sequence of amino-acids in each chain of a single protein molecule is termed the primary structure. This is similar to the structural formula of organic compounds. The full structure of glutathione and the full structure of insulin in the short hand notation, given earlier represent primary structures. The determination of the primary structure of protein involves the following stages :

(1) **Purification** to a homogeneous substance (free from closely related proteins). This may be achieved by : (*a*) precipitation with salt or alcohol or at isoelectric point etc.; (*b*) ion-exchange chromatography; (*c*) gel filtration; (*d*) electrophoresis; (*e*) ultracentrifuge etc. Care should be taken to avoid denaturation. More than one method may be needed.

(2) **Hydrolysis** to yield all the amino-acids without destruction. This may be achieved by acid, alkaline and enzymic hydrolysis. Tryptophan will not be found in acid hydrolysed proteins, due to destruction. Hence more than one method may be required.

(3) **Separation, isolation and identification** of the amino-acids. The mixture of amino-acids can be separated by paper chromatography or TLC (if necessary by two dimensional technique) and detected by spraying with ninhydrin reagent. The mixture of amino-acids (hydrolysate) can also be treated with reagents which react with the amino-acids to yield characteristic derivatives. Dinitro-fluoro-benzene (DNFB) is one such reagent which reacts with the free amino-groups to yield coloured dinitrophenyl derivatives (DNP). Similarly phenyl-isothiocyanate on reaction yields phenylthiocarbamyl (PTC) derivative which on acid treatment gives a phenylthiohydantoin (PTH) derivative. Such derivatives can be separated chromatographically on paper or thin-layer and identified by their characteristic Rf values and colours.

(4) **Quantitative estimation** of the amino-acids will be necessary to determine their relative proportions. For example beef insulin has a total of 51 amino-acids made up of only one mole each of ileu, thr, pro, lys and arg, two moles of his, three moles each of ala, ser, asn, gln and phe, four moles each of gly, glu and tyr, five moles of val, and six moles each of cys and leu.

(5) **Sequence** of amino-acids starting from the amino-acid (N-terminal amino-acid) to the carboxyl end of the chain (C-terminal amino-acid).

This is usually achieved by step by step cleavage of the peptide chain by enzymes like *amino-polypeptidase*, which starts cleavage from N-terminal amino-acid or by *carboxy-polypeptidase*, which starts cleavage from the C-terminal amino-acid. The sequence can also be determined by the Edman's PTH method using phenylisothiocyanate. The degradation can be repeated step by step, as each time only the N-terminal amino-acid is cleaved. These methods are also known as *end group analysis*.

(6) **Determining the number of chains in the molecule** : This is easily determined by finding out the number of N-terminal amino-acids (and/or C-terminal amino-acids). Each such amino-acid represents one chain, because each chain must begin with a free amino group and end with a free carboxyl group. For example, insulin has two N-terminal and two C-terminal amino-acids, hence has two chains.

(7) **Determining the nature and position of bridges** holding the chains together. Usually sulphide bridges are found between the cystein units of different chains. During sequence determination and end group analysis, these positions are revealed.

Secondary Structure : The secondary structure refers to the weak bonds and forces that hold together the peptide chain. The hydrogen bond is the most important of these. Within a single molecule of a protein, the exact positions of hydrogen bonds are determined to reveal the secondary structure. The α-helix form or the β-structure are the usually occurring secondary structures. This is still dealing with bonds and forces that are holding together the primary structure, but not the spatial distribution of the protein or peptide molecule.

Tertiary structure : The protein molecule which may have an α-helix or a β-structure, twists itself at different parts within the molecule, to form a layered or three dimensional mass. The tertiary structure can be determined by X-ray studies, somewhat similar to crystallography. The distances between different atoms are mapped and the molecule reconstructed using the co-ordinates and distances to determine the tertiary structure. The tertiary structure is easily altered sometimes even under mild conditions (like simple acid medium, or warming or chemical reagents etc.). Denaturation disturbs the tertiary structure irreversibly.

Quaternary Structure : Most porteins are not single molecules but an arrangement of more than one molecule held rigidly together in association. They behave as though it is a single molecule in some physico-chemical experiments. They may be considered as loosely held oligomers. The number of molecules forming such an associated structure and the forces holding them together are described in the quaternary structure. A metal

ion or an organic complex structure may hold the protein molecules. Hemoglobin is made up of four sub-units. The enzyme *phosphorylase* is active only when it has the four unit quaternary structure, the individual monomeric form being inactive. In this enzyme all four sub-units are identical (homogeneous) but in some others like in hemoglobin, the tertiary and secondary structures of the subunits may be dissimilar to a small extent (heterogeneous).

2.5 Role of Proteins in Diet

As explained earlier proteins have a variety of functions in the body and the body has a variety of proteins to carry out these functions. All the proteins found in the body are produced in the body (synthesized) by a complex mechanism using amino-acid units (protein biosynthesis). In this process the DNA, RNA and ribosomes are involved in addition to certain enzymes and the amino acids themselves. The body has also a mechanism of synthesizing amino-acids from other molecules like acids, ammonia, sugars and other amino-acids. Of the twenty amino-acids that are found in body proteins, eight cannot be synthesized in the body. They usually have an 'R' group that the body is unable to synthesize. Hence we are dependent on our dietary source for these amino-acids. Without these, many proteins in the body cannot be produced by the body, thus affecting many functions. These amino-acids are therefore termed "*essential or indispensable amino-acids*". These are — *Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan and Valine* (remember as **ILL, MP, TTV** in alphabetical ascending order).

Arginine and histidine have been given the semi-essential status, as they are needed during growth years. Later the body is able to synthesize these. Methionine is recycled in the body, hence becomes partially essential.

Proteins, when ingested as foods, are broken down to amino-acids with the help of enzymes, both in the stomach and intestines, although at different pH. Only aminoacids are absorbed and circulated in the body. From this pool, amino-acids are utilized for the biosynthesis of proteins. Therefore the food proteins must contain all the indispensable amino-acids in sufficient amounts needed by the body. The usefulness of a protein is measured by the extent it is capable of supplying the essential amino-acid and is expressed as *bioloigcal value* (BV) or *net protein utilization* (NPU). It is not practical to estimate the essential amino-acid content of nutritional proteins. Hence, the nitrogen content of such proteins is determined. After controlled experiments, the amount of nitrogen that is estimated from the body after administering the protein is also determined. The

difference gives nitrogen retained in the body. The end product of protein metabolism is urea, which is exclusively eliminated through the kidney. Estimating urea nitrogen in the urine gives the amount of nitrogen not retained in the body or which has undergone metabolism. NPU is calculated thus :

$$N\ P\ U = \frac{\text{N retained}}{\text{N intake}} \times 100 \quad or \quad \frac{\text{(N intake} - \text{N excreted)}}{\text{N intake}} \times 100$$

The biological value (BV) is a corrected value taking into account nitrogen content of food *not absorbed.* This is determined by estimating the nitrogen in the faeces during the period of the experiment. BV is calculated thus :

$$B\ V = \frac{\text{N retained}}{\text{N absorbed}} \times 100 \quad or \quad \frac{\text{(N intake} - \text{N in faeces)} - \text{N in urine}}{\text{(N intake} - \text{N in faeces)}}$$

Both the values are difficult to determine, because of experimental limitations and tediousness of determinations. Moreover, these are not absolute but are useful as only approximate relative values to compare different dietary proteins. These methods assume that all essential amino-acids are retained in the body and do not contribute to the urinary nitrogen. The NPU values of some proteins are listed below (from WHO technical report) :

TABLE 2.5

Protein Source	*NPU (in children)*
Human milk	95
Whole hen egg	87
Cow's milk	81
Polished rice	63
Ground nut	57
Soya been flour	54
Whole wheat	49
Maize	36

As explained above the body retains substantial amount of nitrogen from the proteins (and other nitrogen containing compounds) of foods ingested. The difference between nitrogen intake and nitrogen excretion (i.e. nitrogen retention) gives not only the value of the food, but also a good idea of the condition of the body efficiency in nitrogen turn-over or

protein metabolism. As proteins are also part of the structural tissues, it is obvious that the nitrogen retention is high in growing children and relatively low in elderly persons. In the youth and active adults the nitrogen excretion is carefully regulated, depending on the amount of intake. This phenomenon is known as "*nitrogen balance*" or "*nitrogen equilibrium*". In the normal course of life in the growth period, nitrogen balance is "positive", i.e. nitrogen retention in the body is high. During adulthood it is marginally fluctuating between positive and negative. In elderly persons it may be on the negative side, i.e. more nitrogen is excreted than the intake. The additional nitrogen is from the degradation of structural tissues and cells. Negative nitrogen balance may also be seen in persons who are on diet or fasting or starving (malnutrition) but also in overeating (obesity), and in certain disease conditions, which have caused impaired protein metabolism. Positive nitrogen balance is also seen in pregnant females. Thus determining nitrogen retention in the body can give valuable information on the condition of the body.

2.6 Protein and Amino-Acid Deficiency Diseases :

These are caused by :

(a) *Malnutrition* : lack of required amount of proteins and essential amino-acids in the diet.

(b) *Malabsorption* : lack of ability to digest and/or absorb the protein and amino-acid nutrients from ingested food.

(c) *Inability to ingest food through oral route* (e.g. due to injury to the buccal cavity of the oesophagus).

(d) *Surgical removal of organs* involved in digestion, absorption, metabolism etc. (stomach, intestine, liver, pancreas, etc.).

(e) *Metabolic disorders*, due to genetic or other reasons body is unable to metabolise some amino-acids.

Kwashiorkor is a dietary protein deficiency disease seen in Asian children, who live mostly on starchy foods like corn, plantain, tapioca etc. **Hypoproteinemia** and **anorexia nervosa** are conditions related to malnutrition. They are treated by administering proteins of predigested proteins of high biological value. Malabsorption states are treated with predigested proteins containing all the needed amino-acids. If oral administration is not possible, then parenteral route may be employed using sterilised preparations. Liver diseases like **cirrhosis**, also cause severe deficiency of amino-acids. Total parenteral nutrition (TPN) may be needed to treat the patient. TPN products are formulated according to individual patient's needs and include amino-acid mixtures besides minerals, glucose, vitamins and fats.

Protein intolerance (e.g. cow's milk intolerance), gluten sensitivity (gluten is wheat protein) also known as **coeliac disease** require specially formulated protein digests (protein hydrolysates) for treatment.

Phenylketonuria is a metabolic disorder, in which the body is unable to convert phenylalanine to tyrosine. This results in accumulation and excretion of phenylpyruvic acid, a keto-acid. Both these situations are undesirable. Tyrosine, needed for the bio-synthesis of several proteins and hormones, is not available in adequate amounts. The condition is treated with special amino-acid mixture preparation to provide just adequate amounts of both phenylalanine and tyrosine.

Many products containing proteins of high biological value are available in the market, as food supplements and for malnutrition. Protein hydrolysates are also available for persons suffering from malabsorption states. Sterile preparations containing protein hydrolysates (*Protein hydrolysate injection USP* is the only official product of this type) are formulated from pure amino-acids for parenteral administration. These are not commercially available. These have to be made in a hospital pharmacy. Some marketed protein supplement products also contain other nutrients like carbohydrates, minerals, fats and vitamins.

There are a number of proteins and polypeptides which are used for purposes other than nutrition. Enzymes and hormones are some such, which will be discussed at appropriate places. Gelatin is a derived protein, obtained by partial hydrolysis of collagen (structural protein found in skin, connective tissue, bones etc.). It is official in the Indian Pharmacopoeia (IP) and is used as a pharmaceutical aid (for making empty capsules, as suspending agent and binding and coating agent for tablets). It is obtained as colourless or yellow flakes or as coarse powder. It has very little nutritional value. Absorbable gelatin sponge or foam was earlier available for use as local hemostatic during surgery.

2.7 Blood Protein Products in I.P.

Human normal serum albumin (Human albumin) is obtained as a clear liquid, the colour ranging from amber to deep orange-brown depending on protein content. It is prepared from pooled blood or plasma or serum, by removing most of the other proteins and organic components of blood by addition of alcohol or salts and adjusting pH to precipitate these. The final pharmacopoeial product contains between 5 and 25% w/v of protein. It is intended for use as blood volume supporter by intravenous injection.

Human plasma is a product obtained from pooled unclotted human blood from healthy donors, by removing the blood cells by sedimentation

or centrifugation. It contains 4.5% w/v of protein. It is used as a plasma volume restorer.

Dried human plasma is obtained by freeze drying human plasma preparations as explained above. It is a pale to deep cream coloured powder. It should be reconstituted with water for injection to yield a solution containing 4.5% w/v of protein.

Human plasma protein fraction is also used as blood volume supporter and is administered by intravenous infusion. It is a transparent, colourless or slightly brownish liquid. It is obtained from pooled human blood or plasma or serum and contains both normal globulins and albumins but no fibrinogen and antibodies (immunoglobulins). A combination of solvents, ionic strength, pH and temperature are employed to eliminate unwanted proteins. The final product contains about 5.2% w/v of proteins.

Human normal immunoglobulin (also known as immune human serum globulin or Human gamma globulin) is an important passive immunising agent, useful in prevention of measles in children, rubella in pregnant women and infective hepatitis in general. It is prepared from pooled human blood or plasma or serum from individuals who have high content of the required antibodies in their blood. The gamma globulins are precipitated selectively using a combination of alcohol strength, ionic strength, and pH. The separated gamma globulins are dissolved in aqueous vehicle and may contain added preservative or stabilising agent. The final product is a transparent, colourless or slightly brownish liquid containing between 10 to 18% w/v of protein. Dried human normal immunoglobulin (dried human gamma globulin) is a white or slightly yellowish powder obtained by freeze-drying the human normal immunoglobulin preparation, as explained above. It is reconstituted with water for injection, before administration.

Dried human anti-hemophilic fraction is another protein product made by fractionation of human serum, suitably to enrich it with the specific anti-hemophilic fraction. It still contains about 80% fibrinogen, but should have at least 0.1 unit of anti-hemolytic fraction (clotting factor VIII) in 1 mg of the product. It is obtained by freeze drying the fractionated protein solution. It is stored in sealed ampules under an atmosphere of nitrogen (no oxygen should be present).

Since the advent of biotechnological techniques (like recombinant DNA cloning, gene splicing etc.), several proteins and polypeptides are being produced by such methods, instead of from animals, plants or microorganisms. These are relatively very pure, single chemical sub-

stances (as compared to the mixture of isolated proteins from tissues) with low antigenic properties. Moreover, they can be produced in large amounts. Today *insulin, interferon, growth hormone, erythropoietin, hepatitis B vaccine and tissue plasmonigen activator* are already being produced by biotechnological processes and marketed. More products are expected in future. Drug industry is likely to be heavily biotechnology oriented in future.

3

Enzymes

3.1 Introduction

Enzymes are a class of proteins, that are extensively distributed in all living systems. They exhibit all the characteristic properties of proteins. Their function in the body is to bring about chemical changes in biochemical molecules, under the conditions prevailing in the body. They are also called *biocatalysts*, as their activity is similar to chemical catalysts. In the chemical reaction they catalyse, they are not destroyed or rendered useless. They are regenerated to enable re-use of the enzyme. Hence they are needed in small quantities. Since all biochemical reactions (also known as *biotransformations*) take place only with the help of enzymes, they are essential to all life processes. They are produced in specialised cells and released when needed. Some enzymes are produced in special organs, released, transported to distant organs, stored in such organs and secreted through ducts to the site of action. Many digestive enzymes are of this type.

The term enzyme was introduced by Kuhne in 1878 and means "in yeast", as yeast was found to be rich in enzymes. However, the activities of some of the enzymes (earlier known as *ferments*) had been recognised. *Diastase* of malt, *emulsin* of bitter almonds, *pepsin* of stomach juice, *trypsin* of the pancreatic juice and *invertase* of yeast were the earliest of enzymes described, although not obtained in pure form. The substance which is biotransformed by an enzyme is known as *substrate*. An extraordinary fact was that the enzymes were active not only inside living cells (*in vivo*), but also in cell-free laboratory experiments (*in vitro*, meaning in glass ware) on the same substrate. The discovery has helped greatly in advancing knowledge of enzymes.

3.2 Nomenclature and Classification

Early researchers named the enzymes on the basis of source or the type of reaction catalysed or the substrate acted upon by the enzyme. Table 3.1 lists a few of the enzymes.

TABLE 3.1 **Some Enzymes**

Name	*Basis of Name*	*Biotransformation Catalysed*
Pepsin	occurs in stomach	peptide bonds cleaved
Papain	occurs in papaya	peptide bonds cleaved
Pancreatin	occurs in pancreatic juice	digestion of foods
Amylase	substrate amylum (starch)	starch degraded
Lipase	substrate : lipids	hydrolysis of fats and oils
Urease	substrate : urea	urea degraded
Phosphorylase	causes phosphoryl-ation of substrate	(e.g) Sugars phosphorylated
Dehydrogenase	causes dehydrogen-ation of substrate	(e.g) Alcohol dehydrogenated
Peroxidase	breaks down peroxi-de substrate	(e.g) hydrogen peroxide decom-posed
Invertase	causes inversion of optical activity	(e.g) sucrose hydrolysed to mixture of glucose and fructose

While many of the classical names of enzymes are retained today, the new names of most enzymes are based on the recommendation of International Union of Biochemistry (IUB). According to this the full name consists of the (a) substrate; (b) cofactor if any; and (c) reaction type (biotransformation), in that order. The name ends in the suffix-*ase*. The IUB has also recommended a numbering system. The enzyme *alcohol dehydrogenase* is correctly designated according to IUB as *alcohol : NAD oxido-reductase*. Often the names are simplified to include only the substrate and type of reaction.

TABLE 3.2 **IUB Classification of Enzymes**

1. Oxido-reductases :	Dehydrogenase, oxidases, reductases, per-oxidases, catalase, oxygenases and hydroxylases
2. Transferases :	Kinases, phosphomutases, transaldolase, acyl-, glucosyl, phosphoryl-, methyl-, transferases etc.
3. Hydrolases :	Esterases, glycosidases, peptidases, amidases, phosphatases, deaminases, lipases, ribonucleases etc.
4. Lyases :	Decarboxylases, aldolases, hydratases, lyases, synthases, dehydratases etc.
5. Isomerases :	Racemases, epimerases, mutases, isomerases etc.
6. Ligases :	Synthetases, carboxylases etc.

The IUB has divided all enzymes into six major classes according to the biotransformation involved. Each of these classes are sub-divided into

sub-classes, to group the substrate types. The sub-classes contain the list of specific enzymes acting upon the specific substrate. The major classes and some of the sub-classes are shown in Table 3.2 above.

3.3 Properties

All enzymes are high molecular weight proteins, soluble in aqueous media in the body. Many enzymes have been obtained in pure crystalline form. They are precipitated by strong electrolyte concentrations (salts), organic solvents like ethanol and at their iso-electric point. They behave like other proteins when subjected to electrophoresis, ultracentrifugation, ion-exchange chromatography etc. They are irreversibly denatured by heat (usually above 56°C), strong acids (protein precipitants) and heavy metal ions like lead, mercuric etc. (protein poisons). Many of them exhibit their full activity in their simple protein forms. Some are not active in the form in which they are stored and secreted. They require the help of another enzyme to release the active protein enzyme by removing a protective unit. Such inactive enzymes are called *pro-enzymes* or *zymogens* : e.g trypsinogen and chymotrypsinogen which are converted to the active trypsin and chymotrypsin respectively.

Some enzymes require the presence of other non-peptide organic or metal ion co-factors for their full activity. The protein enzyme part in such cases is known as *apoenzyme*. The co-factors like *co-enzymes* or metal ions are usually present only during the actual reaction.

Some apoenzymes have non-peptide organic *prosthetic groups*, which are always attached to the apoenzyme and necessary for the activity. The prosthetic groups can be removed or restored under certain conditions. The apoenzyme together with the co-factors and/or prosthetic groups, are then termed *holoenzyme* with full activity. With proper conditions, such a complete system can function *in vitro* also. Certain small parts of the large enzyme molecule are designated as *sites*. Some are *active sites* involved in the particular enzyme action, allowing the substrate to be attached to that site. Some sites accommodate co-factors and other molecules which are required for activation. Enzymes can be antigenic like other proteins. Hence parenteral administration of enzymes should be avoided.

3.4.1 Enzyme Reactions

(1) Enzymic reactions are specific, either acting on a single substrate or on a group of closely related substrates and bring about usually a single step specific biotransformation. *Urease* is a single substrate enzyme. *Pepsin* is a multi-substrate enzyme, i.e. many peptides and proteins can be its substrates. But it breaks the peptide bonds between tyrosine

(contributing the amino-group to the peptide bond) and other amino-acids, formed in the interior of protein molecules.

(2) Enzymic reactions are reversible. Like reversible chemical reactions, the physico-chemical conditions of the medium (concentrations, pH, solubility, ionisation, complexation etc.) decide whether an enzymic reaction proceeds in the forward or reverse direction. Enzymic reactions can therefore be controlled by a variety of conditions.

(3) Enzymes (E) form a complex with the substrate (binding to the active site). The complex usually changes in shape or charge etc. forcing the substrate (S) to change its chemistry. Now a new complex of enzyme and products has resulted. This then undergoes dissociation, releasing the products and the enzyme which quickly rearranges to the original active form of the enzyme. This sequence of steps in the enzymic reactions can be represented thus :

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

In some cases, there may be two successive reactions taking place quite fast, appearing like a single reaction. For example *glutamate* substrate is bound to the enzyme and converted to *keto-glutarate* and *ammonia*. If in the medium *oxalo-acetate* is also present, it binds on to the enzyme and accepts ammonia to form *aspartate*. This second reaction is simply a reverse of the first reaction, excepting that a second α-keto-acid has taken the place of the product of the first reaction. The net result of this reaction would appear as though the amino-group of glutamate has been transferred to yield aspartate from oxalo-acetate. The enzyme is called *glutamate-oxalo-acetate-transaminase* (GOT) or *aspartate transaminase* (AST).

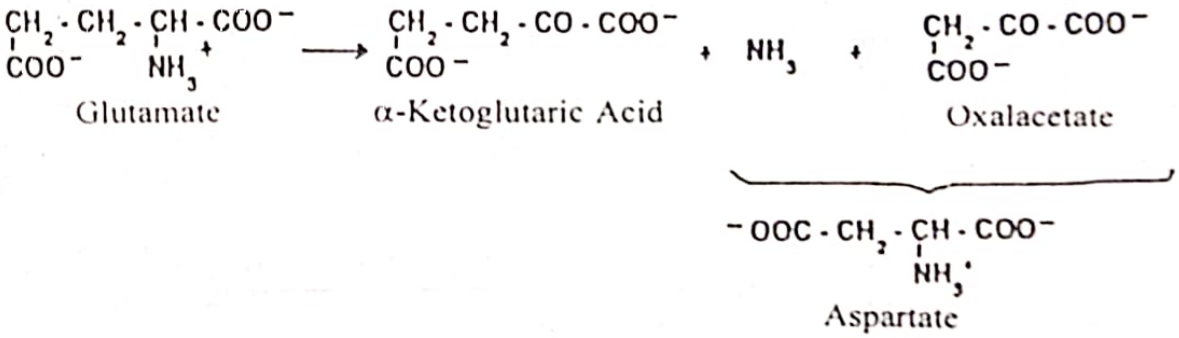


Chart 3.1

Similarly formation of alanine from pyruvate and glutamate is catalysed by the enzyme *glutamate-pyruvate-transaminase* (GPT), which is also known as *alanine transaminase* (ALT). Estimation of serum levels of GOT (SGOT) and GPT (SGPT) are common clinical investigations used to diagnose liver and heart conditions.

(4) An enzyme alters the rate of a reaction. It does not effect the energy status of a reaction, *i.e.* the amount of energy released or utilised in a chemical reaction remains same whether an enzyme catalyses the reaction or is achieved by some other means. Since all enzymic reactions are reversible the reaction velocities can be indicated as below :

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + P$$

If we assume that the product formed is entirely removed, then k_4 can be ignored as only k_3 will be operative. Now the formation of ES is entirely dependent on concentrations of E and S. It has been shown that if the availability of the substrate is large, then k_1 doubles when enzyme concentration is doubled. On the other hand, in cells the enzyme concentration is low and constant. In such cases the rate of reaction is dependent on the substrate concentration. As S increases the velocity rapidly increases, until all the available enzyme has entered the reaction. As the ES complex formation increases, the k_2 starts rising thus reducing the k_1. Finally k_1 becomes equal to k_2 and an equilibrium is reached. As the substrate cocentration is increased beyond saturation levels, no further changes in velocity will be observed and a maxiumum velocity V_{max} is reached. The phenomenon is indicated in the accompanying diagram :

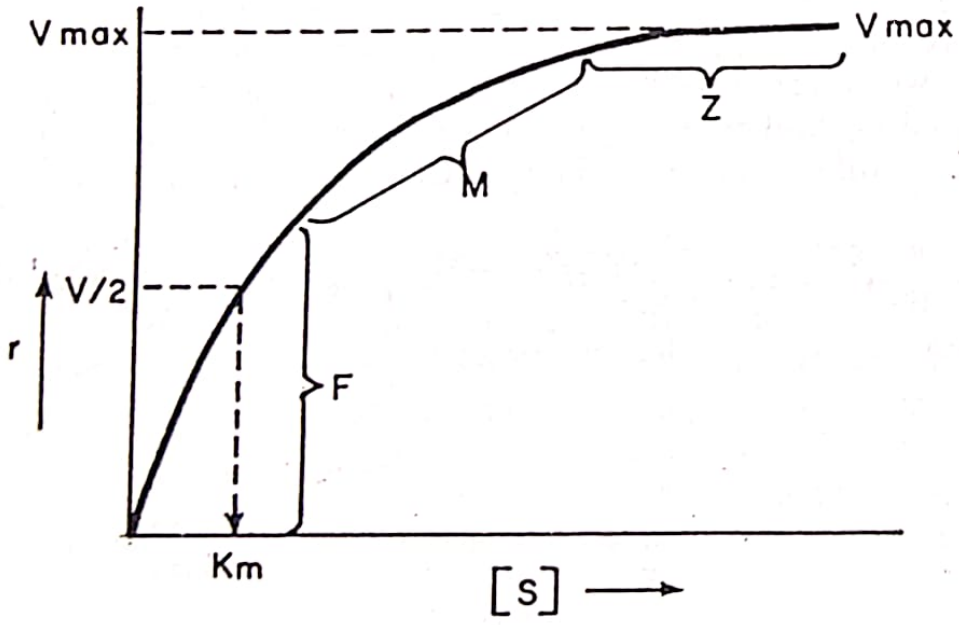


Figure 3.1 Enzyme Kinetics

Initially first orders kinetics (F) will be observed, followed by a mixed order kinetics (M) and finally by zero order kinetics (Z). When the velocity of reaction is equal to half of V_{max} (designated as V/2), the concentration of substrate corresponds to what is known as K_m or Michaelis-

Menten constant. This has been derived both algebraically and graphically (as shown in the diagram). K_m is a constant for a given enzyme-substrate pair. This helps in deciding which is the true substrate (or enzyme), as enzymes act upon multiple substrates. In general, K_m corresponds to the substrate concentration obtainable in living cells. A high K_m value indicates low affinity between E and S, whereas a low K_m value indicates a high affinity between the E and S.

(5) It has been shown that the enzyme lowers the activation energy of the reacting system. This enables a biotransformation to take place with ease at the mild conditions existing in living systems.

(6) In order that the enzyme reaction takes place efficiently there are several factors (or conditions) which should be at optimum levels. These are : (a) *Temperature* : In general, body temperature of 37°C is optimum. But the reaction rate increases upto about 45-50°C, then slowly and finally but abruptly, the reaction comes to a stand still usually at 55°C or near about. Actually the enzyme is denatured. (b) *Hydrogen ion concentration or pH* : Enzymes are most active at narrow pH ranges characteristic of the enzymes. For example pepsin is active at pH 1.5 to 3.0 whereas trypsin is active at pH 7.0 to 10.0. However, optimum pH for pepsin is close to 2.0 and for trypsin it is 8.0. Some enzymes may be irreversibly denatured at pH far removed from their optimal pH values. The pH of a system is well regulated in the body by biological buffer systems, which include ions like bicarbonate, phosphate, acetate as also proteins and peptides.

(7) Some enzymes are activated by the presence of some co-enzymes. Most co-enzymes are derived from vitamins and will be discussed in more detail in that chapter, e.g NAD is a co-enzyme that is associated with a number of *dehydrogenases*. Some enzymes also need the presence of metal ions for their activity, e.g. copper for *cytochrome oxidases* and *superoxide dismutase;* zinc for *RNA polymerase;* manganese for *pyruvate decarboxylase* etc.

3.4.2 Enzyme Inhibitors

An important aspect of enzyme reactions is inhibitors of enzyme activity. Specific substrates are accommodated on specific sites on an enzyme molecule. The stereochemistry (3-dimensional distribution of atoms and bonds) and physical chemistry (ionisation, lipid solubility etc.) of a site is designed to accommodate only the normal substrate. However, other very closely related chemicals having similar stereochemical and physicochemical properties fit into the site. This prevents the availability of the enzyme for the usual reaction. Hence the enzyme is

inhibited and the abnormal substrate is the *inhibitor*. If an inhibitor molecule competes for the binding site of the enzyme and blocks it from the true normal substrate molecule, it is called *a competitive inhibitor*. By increasing normal substrate availability the process can be reversed and the enzyme becomes available, due to successful competition from the normal substrate molecules with their inhibitor molecules. For example *malonate* can be competitive substrate for the *succinate dehydrogenase* enzyme. A number of chemotherapeutic drugs can be described as *competitive inhibitors* of some important enzyme. For example sulphonamides compete and inhibit the enzyme *dihydropteroate synthetase*. A few other enzyme inhibitors used as drugs are : methotrexate binds *dihydrofolate reductase*; alpha-methyl DOPA inhibits *L-DOPA-decarboxylase*; alloupurinol inhibits *xanthine oxidase*; isocarboxazide inhibits *mono-amino-oxidase* (MAO) etc.

There are also non-competitive inhibitors. These chemicals do not bind the active site of enzyme but are bound to other functional sites on the enzyme molecule. This leads to a more firm attachment and alters the spatial arrangement of the enzyme molecule, making it unsuitable for enzymic activity. In the normal body conditions, such inhibitors produce *irreversible inhibition*. However, through dialysis the inhibitor can be removed and the active enzyme regenerated. Fluorouracil used as an anticancer drug, is an irreversible inhibitor of *thymidylate synthetase*. A number of enzyme inhibitors have been developed as drugs by imitating the chemistry of normal substrates, which are formed in the body as key or *essential metabolites*. For example methotrexate and trimethoprim are *anti-metabolite* type inhibitors used as drugs.

3.5 Application of Enzymes

Enzymes find several applications depending on their specific activity. For these applications, enzymes need not be purified to the extent of crystalline nature. A reasonably pure or high activity preparation is usually adequate. *Papain*, a proteolytic enzyme is obtained by simply air and sun drying of the juice of unripe papaya fruit. *Diastase* (or *alpha-amylase*) is obtained from filtrate of culture medium of *Aspergillus oryzae* fungus and partially purified by removing all non-peptide and some peptide impurities. However, it will still be a mixture of partially purified *amylases*. Such enzyme preparations are kept in dry condition. In recent years enzymes are being prepared by newer biotechnological methods (e.g. *tissue plasminogen activator* by recombinant DNA technology). Such preparations are usually pure and less antigenic. Their activity is estimated in terms of *units of micromoles* (μ mol) representing the quantity of the substrate converted to product in one minute. The *standard unit* is

usually equal to the enzyme of one μ mol/minute activity. The IUB has proposed that enzyme activity should be expressed as mol/second conversion of the specific substrate. The relative activity of different enzymes are better expressed as *turnover number* (or *catalytic constant*). This is *equal to the number of units of activity per mol of enzyme i.e. μmol/min/mol of enzyme.* The turn over number is dependent on number of active sites on an enzyme molecule and the rate at which the enzyme is regenerated (recycled) for further activity. In case of *alpha amylase I.P.*, the activity is expressed as number of grams of starch completely digested in 1 hour by 1 gram of the sample. Other types of units are also expressed for certain enzymes (e.g. turbidity units for *hyaluronidase*).

TABLE 3.3 **Applications of Enzymes**

Enzyme	*Source*	*Application*	*Action*
Alpha-amylase (I.P.)	Fungal (Aspergillus oryzae) Bacterial (Bacillus subtilis)	Digestive enzyme	Starch converted to dextrin and maltose (800 μ/g)
Hyaluronidase (I.P.)	Testes & serum of mammals	Spreading factor	Depolymerise muco-polysaccharide (300 μ/mg)
Pancreatin (I.P.)	Mammalina pancreas	Digestive enzyme	Proteolytic, amylolytic and lipolytic activities
Papin (I.P.)	Unripe papaya fruit juice	Proteolytic enzyme	Endopeptidase
Pepsin (I.P.)	Gastric mucosa of hog or cattle	Proteolytic enzyme	Endopeptidase
Trypsin	Ox pancreas	Proteolytic enzyme	Endopeptidase
Chymotrypsin	Mammalian pancreas	In cataract surgery	Proteolytic
Alteplase	Recombinant DNA technology	In myocardial infarctions	Tissue plasminogen activator
Streptokinase	Cultural filtrates of Streptococcus haemolyticus	Thromboembolic disorders and myocardial infarction	Plasminogen activator
Penicillinase (Beta lactamase)	Culture filtrates of bacilli (B. subtilis, B. cereus etc.)	Penicillin inhibitor in diagnosis & tests	Hydrolysis of beta lactam antibiotics

Important applications of enzyme preparations is medicine are as:

(1) Therapeutic agents and/or aids in surgery.

(2) Some are used in diagnostic tests.

(3) Some find application in quality control laboratories.

(4) A major use of enzyme preparation is in industry for differen transformations.

Examples of all these are listed in the Table 3.3. In addition to thes direct applications of enzymes or their preparations, knowledge of the rol of enzymes in certain cells or tissues or in very specific biotransformation is exploited in (5) Clinical diagnosis by quantitative estimations of som enzymes and in (6) Research in biochemical pharmacology, which ca lead to rational design of new drug molecules.

4

Carbohydrates

4.1 Introduction

"Carbohydrate" means hydrated carbon, which indicates the relative proportion of carbon, hydrogen and oxygen atoms in a carbohydrate. For example glucose has a molecular formula $C_6H_{12}O_6$ i.e. $C_6(H_2O)_6$. It also indicates that these are the only three elements in this group of natural compounds. However, like the complex (conjugated) proteins, some carbohydrates also may have amino-groups. Carbohydrates also have two major functions in living organisms : (1) they provide energy needed for life processes and (2) some are part of structural units of cells and tissues, especially in plants and many lower animals.

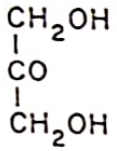
Energy is released from carbohydrates by metabolism, consisting of a series of biotransformations. Structural carbohydrates are usually fibrous like *cellulose* or hard layers like *chitin* of insects. Well known simple carbohydrates are *glucose, fructose,* (both occur in fruits), *lactose* (occurs in milk), and *sucrose* (cane sugar). Starch (rice, potato, maize, wheat etc.) is a high molecular weight carbohydrate. As the simple sugars are all sweet, they are also popularly known as sugars. Plant foods are rich in starch and related carbohydrates, which yield energy after metabolism.

4.2 Classification

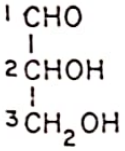
Simple carbohydrates have the proportion $C_n(H_2O)_n$ and are known as *monosaccharides.* Two molecules of these can condense with elimination of a molecule of water, yielding a *disaccharide*, e.g. sucrose with the formula $C_{12}H_{22}O_{11}$ (i.e. $C_6H_{12}O_6 + C_6H_{12}O_6 - H_2O$). This process can repeat yielding *oligosaccharides* (generally upto 6 units of mono-saccharides). These three classes are water soluble and possess sweet taste. Higher polymers are known as polysaccharides, which are less soluble in water and become completely insoluble in water as the molecular weight increases. Hydrolysis of polysaccharides yields oligosaccharides, disaccharides and monosaccharides.

Monosaccharides are further classified based on the number of carbon atoms in the molecule : *pentoses* and *hexoses* (widely distributed), *trioses,*

tetroses and *heptoses* (less common). All these are characterised by the presence of one hydroxyl group (-OH) for each carbon atom, one of them having been oxidised to an aldehyde or ketone group. On the basis of this, *a monosaccharide is defined as a polyhydric aldehyde or polyhydric ketone with the proportion of one H_2O for each carbon atom.* The two simplest sugars that fit the definition are shown below :

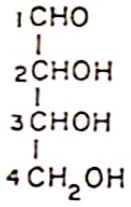


Dihydroxyacetone

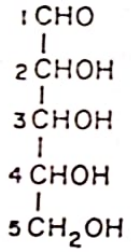


Glyceraldehyde

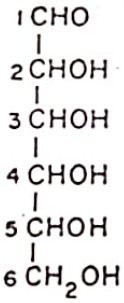
Both these are trioses, which are found in tissues where metabolism is actively going on. Following are other examples of monosaccharides :



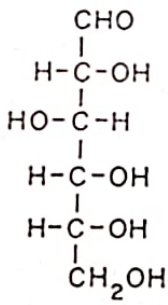
Erythrose (tetrose)



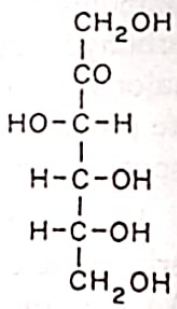
Ribose (pentose)



Allose (hexose)

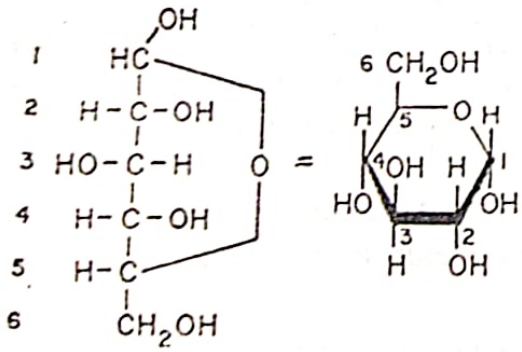


Glucose (hexose)



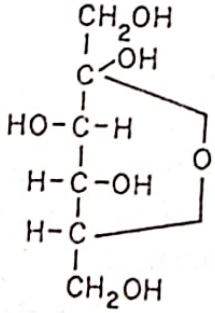
Fructose (hexose)

Since there are only two functional groups in the monosaccharides, they are also designated on the basis of these : those containing a ketone group are called *ketoses* and those containing aldehyde groups are called *aldoses*. These terms are combined with the system based on number of carbons thus : *aldotetrose* (e.g. erythrose), *aldopentose* (e.g. ribose), *ketohexose* (e.g. fructose) etc. The pentoses and hexoses exist as cyclic structure to a greater extent than the open chain structures shown above. For example :

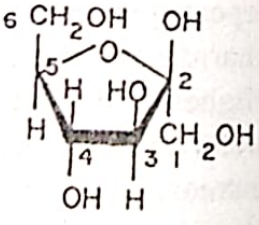


Glucose

Glucose

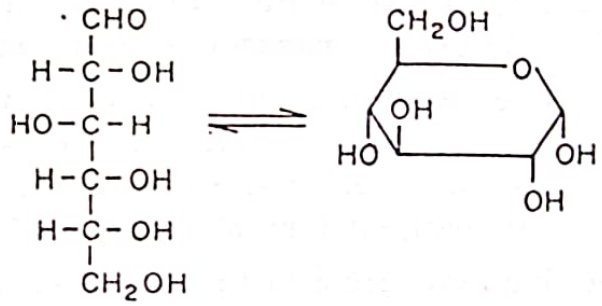


Fructose



Fructose

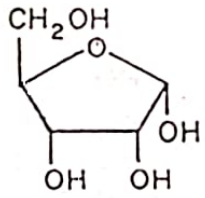
The cyclic structures give rise to (1) a six membered ring with oxygen hetero-atom called a pyran ring; or (2) a five membered ring with oxygen hetero-atom called a furan ring. The ring is formed bacause of intra-molecular condensation of the oxo group (-CHO or -C=O) with the alcoholic group. This is called a hemiacetal or hemi-ketal structure, with a new hydroxyl group on the carbon with the oxo group. The pyran containing sugars are called *pyranoses* (e.g. glucopyranose) and the furan sugars are called *furanoses* (e.g. fructofuranose). Note that these cannot be called aldoses and ketoses any longer, having lost these groups in hemi-acetal formation. It has been experimentally shown that the open chain and ring structures exist in equilibrium.



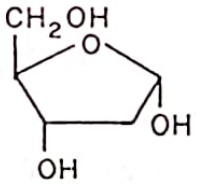
Open Chain Cyclic

D (+) Glucose

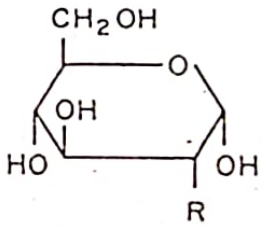
As explained earlier, each carbon in a carbohydrate carries an oxygen. However, in nature there are a few derived carbohydrates, which also participate in biochemical processes . If one or two carbons do not carry oxygen, such sugar molecules are called *desoxy sugars* (also *deoxy*) e.g. *desoxyribose*, a widely distributed desoxy monosaccharide :



Ribose



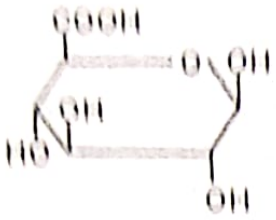
2-Deoxyribose



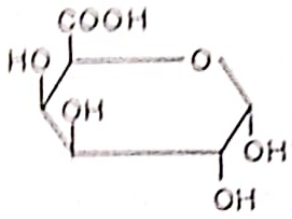
2-Glucosamine (R = NH_2)

2-N-Acetylglucosamine (R = $NH.CO.CH_3$)

Hydroxyl functions are also replaced by amino-groups in some mono-saccharides yielding *amino-sugars* (amino-monosaccharides), e.g. glucosamine. Chitin, the hard shell of insects is a polymer of *N*-acetyl-glucosamine. Another derived monosaccharide contains a carboxyl group instead of the primary alcohol in the molecule, i.e. it contains more oxygen proportion-ately, e.g. glucuronic acid (found in hyaluronic acid) and galacturonic acid (found in pectins) :



Glucuronic Acid



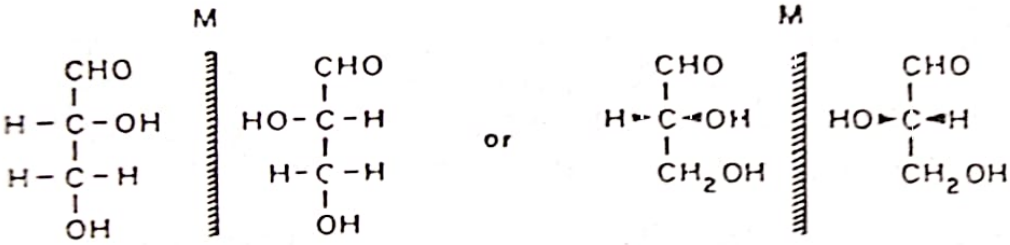
Galacturonic Acid

Summary

1. Carbohydrates are classified as mono-, oligo-, and poly saccharides, depending on number of repeating units in the molecule, the unit being a mono-saccharide.
2. Monosaccharides are known as trioses or tetroses or pentoses or hexoses, depending on the number of carbon atoms in their molecules.
3. Monosaccharides exist both in an open chain and closed ring structures. In the open chain form, they are known as aldoses or ketoses (aldopentose, ketohexose etc.) based on the presence of aldehyde or keto group in their molecules.
4. The closed ring forms are called pyranoses or furanoses, depending on the six or five membered heterocycle in the molecule.
5. Derived sugars are designated as desoxy sugars or amino-sugars or uronic acids based on the chemical change.

4.3 Stereochemistry

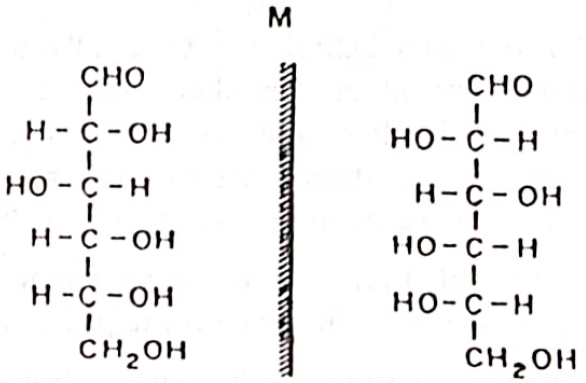
The carbohydrates exhibit a variety of stereo-chemical variations. Only simple monosaccharides will be considered here to explain this phenomenon. The simplest aldotriose has one asymmetric centre (chiral centre)



Aldo-triose (Glyceraldehyde)

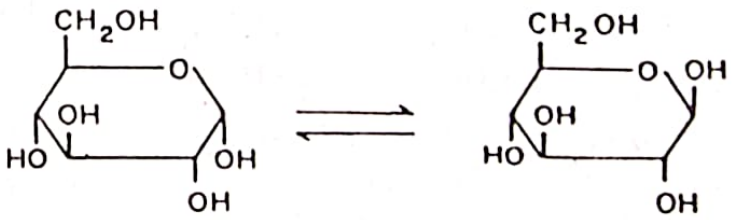
and therefore can exist in two optically isomeric forms (mirror images) known as *enantiomers*, each of them known as *enantiomorph* or *epimer*. As represented on the paper, the penultimate carbon (next to the CH_2OH or the last carbon in the chain) is taken as reference. On this carbon, if the hydroxyl group (or other substituent) is written projecting to the right, the

enantiomorph is referred as D and the opposite as L. The measured (actual) molar optical rotation for these enantiomorphs happen to be *dextro* and *levo* rotatory respectively. However, for other monosaccharides these may not correspond. For example glucose is dextro-rotatory (hence also known as *dextrose*). But its enantiomers mannose (2-epimer) and galactose (4-epimer) are also dextrorotatory. All these belong to the D-series. But D-fructose is levorotatory (hence known as *levulose*) as also D-ribose which is levorotatory. To avoid confusion and still provide information about the series as also the actual rotation, the correct designations for the above examples will be as follows : D(+)Glyceraldehyde; L(–)Glycerose; D(+)Glucose; D(+)Mannose; D(+)Galactose; D(–)Fructose; D(–)Ribose respectively. A series is derived by keeping the last and penultimate carbons constant and adding the carbons of the chain between the aldehyde or keto group and the penultimate carbon. These enantiomorphs are not epimers, because at all centres there is inversion, as depicted by the mirror images. Other stereo-isomers of D(+)Glucose are not exact mirror images and hence known as *diastereomers*. Mannose and galactose are diastereomers of glucose.



D(+)Glucose L(–)Glucose

Note that in glucose (which always means D(+)Glucose or dextrose), there are four chiral centres, the carbon atoms 2, 3, 4 and 5 in its open chain form. In the ring structure carbon 1 also is chiral. This gives rise to two new isomers called *anomers* and are designated as α and ß



α-D-Glucopyranose (+112°) β-D-Glucopyranose (+19°)

depending on the position occupied by the new 1-OH group with reference to the rest of the molecule. If a sample of α-D-glucose is dissolved in water, slowly the measured dextrorotation decreases until it reaches the value of +53° and remains steady (equilibrium). The solution contains a mixture of the α and β forms. This type of change is known as *mutarotation.*

In di-saccharides, oligosaccharides and polysaccharides the bonds between mono-saccharide units may be α or β, thus leading to two series of carbohydrates.

4.4 Tests and Properties

(1) Monosaccharides and disaccharides are usually crystalline, sweet tasting, hygroscopic, highly water soluble solids.

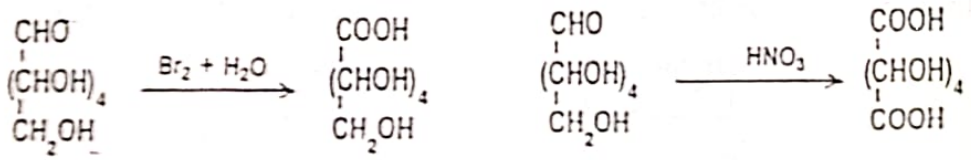
(2) When heated dry or with strong sulphuric acid, they undergo charring with dehydration, with a characteristic burning sugar smell (caramelisation).

(3) Carbohydrates yield characteristic reactions of hydroxyl functions like esterification, etherfication, oxidation, dehydration, substitution with halogens etc.

(4) Monosaccharides also exhibit reactions of aldehydes and ketones. They reduce Fehling's reagent or Benedict's reagent, the alkaline cupric ion (blue colour) present in these reagents being precipitated as reddish brown cuprous oxide. Some disaccharides also reduce these reagents. Sucrose does not reduce these reagents.

(5) Reducing sugars also react with Tollen's reagent (silver ammonia ion solution) leaving a fine silver mirror on the glass wall of the tube.

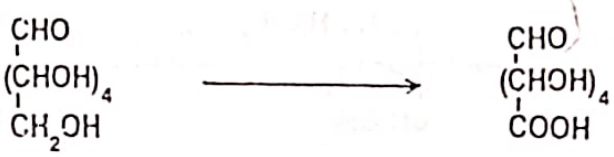
(6) Bromine water is decolourised by aldoses but not ketoses. Oxidation of monosaccharides results in formation of different products, depending on the type of oxidising agent used. Bromine water oxidises selectively the aldehyde group to carboxyl group, giving rise to the general class of compounds called *aldonic acids*.



Glucose — Gluconic Acid — Glucose — Glucaric Acid (Saccharic Acid)

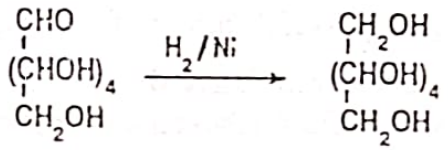
(7) Concentrated nitric acid oxidises both the aldehyde and primary alcohol groups to the dicarboxylic acids known by the general name *aldaric acids*.

(8) All the above oxidation products can also be obtained by enzyme oxidation. In nature another oxidation product known by the general name *uronic acids* are also found. The uronic acids are often found as conjugating groups in biotransformations. The uronic acids retain the reducing aldehyde group, the carboxyl group being formed by oxidation of the primary alcohol function (last carbon). Glucuronic acid, galacturonic acid and mannuronic acid are examples for such oxidised monosaccharides.



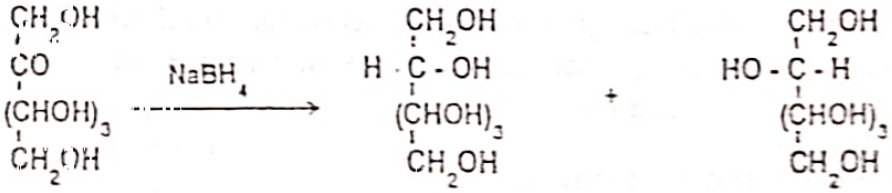
Glucose Glucuronic Acid

(9) The aldehyde or keto groups of monosaccharides can also be reduced to the corresponding alcohols (primary or secondary respectively), yielding polyhydric alcohols.



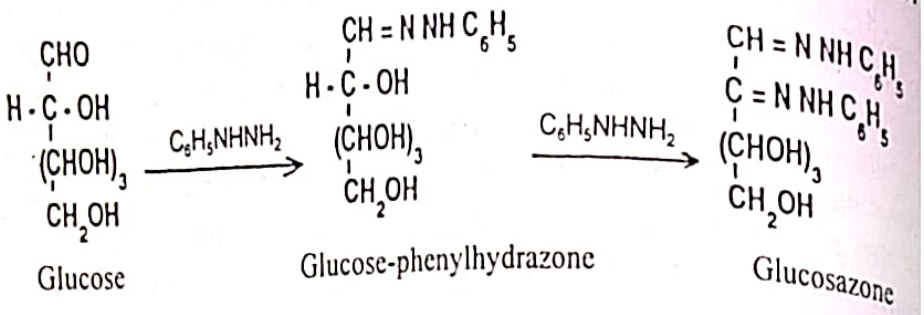
Glucose Glucitol (Sorbitol)

Similarly mannitol, erythritol and ribitol are known. Note that ketose on reduction gives secondary alcohol, which is a new chiral centre. Accordingly ketoses on reduction yield a mixture of polyhydric alcohols (usually epimers).

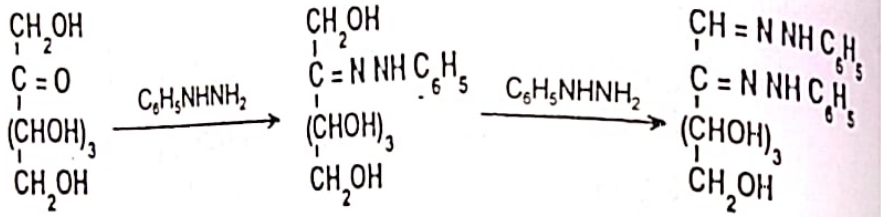


Fructose Sorbitol Mannitol

(10) All reducing sugars (i.e. having an oxo function) react with phenyl hydrazine forming a phenylhydrazone. This is similar to any other aldehyde or ketone. However, the sugar phenylhydrazone reacts with more phenylhydrazine to yield a compound known as *osazone*. Glucose and fructose are identical in respect of carbon atoms 3, 4, 5 and 6 (superimposable). They differ only in the chemistry of carbon atoms 1 and 2. In the osazone formation, only these two carbons are involved,



Glucose — Glucose-phenylhydrazone — Glucosazone



Fructose — Fructose-phenylhydrazone — Fructosazone

forming exactly identical products. Hence glucose and fructose yield the same osazone. Most osazones are yellow crystalline substances. The crystal nature, the melting point and the rate at which they are formed, under similar experimental conditions, are characteristic of a particular sugar. Thus the osazone test is useful in identifying sugars.

(11) Aldohexoses and aldopentoses are dehydrated and cylised to yield hydroxymethyl furfural and furfural respectively, by treatment with strong acid and heat :

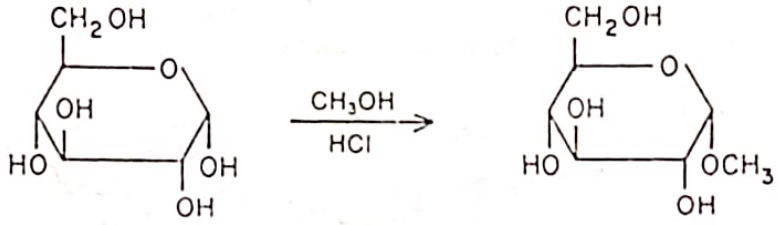
Hydroxymethyl-furfural — Furfural

These aldehydes react with α-naphthol (as also with other phenols, aromatic amines etc.) to give coloured products. Molisch test employs sulphuric acid and α-naphthol, to obtain a violet colour from a monosaccharide (aldohexose usually).

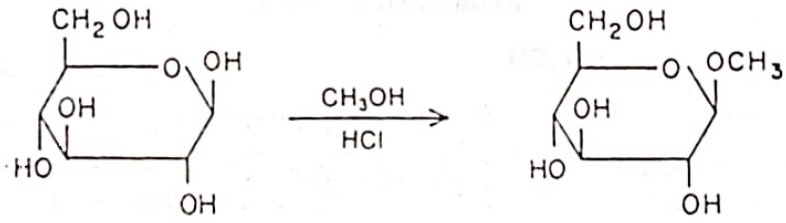
4.5 Glycosides and Disaccharides

Cyclic monosaccharides are considered as hemi-acetals resulting by the interaction of an oxo group (i.e. -CHO or -C=O) with an alcohol, within the molecule (intra-molecular). The full acetal, where two alcohol functions react with one oxo-group, is not possible within the molecule. It also implies that one more molecule with an alcohol group can react with the cyclic mono-saccharide. The open chain structure should theoretically react with two molecules of say methanol, to give an acetal. But D(+)glucose reacts with only one mole equivalent of methanol and gives a mono-methyl *glucoside*. This reaction actually proves that there is a

block in the molecule caused by an intramolecular hemi-acetal or cyclic hemi-acetal. Therefore the external alcohol reacts with the anomeric hydroxyl group. As this hydroxyl may be α or β, the methyl glucoside formed may be α or β or a mixture as the case may be.



α-D(+)-Glucopyranose α-D(+)-1-Methyl-glucoside

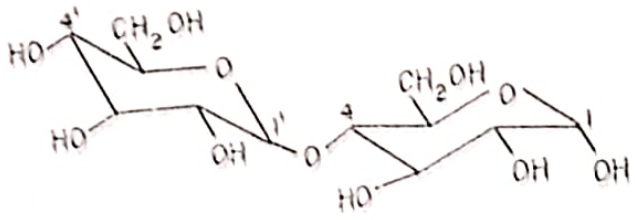


β-D(+)-Glucose β-D(+)-1-Methyl-glucoside

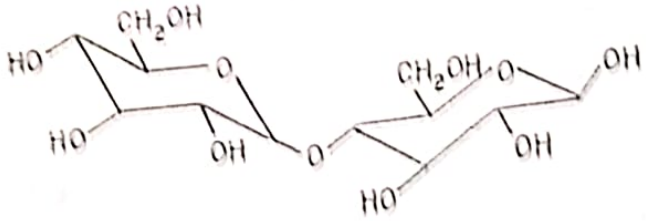
The anomeric hydroxyl group is partially reacting as an oxo-group. Further in solution the reducing group (-CHO or -C=O) is available for reactions. Hence the anomeric carbon is also often called the potential aldehyde (or keto) group. The anomeric hydroxyl function can react with any other hydroxyl compound (including phenols) giving rise to acetals. These are known as glycosides. Such glycosides derived from a reducing sugar and an alcohol or a phenol (with a wide variety of carbon skeleton residues) are extensively distributed in nature. (e.g. flavonoid glycosides, steroidal glycosides, anthraquinone glycosides, cyanogenetic glycosides, amino-glycosides etc).

An interesting situation is when one molecule of a reducing sugar acts as the hemi-acetal sugar and another molecule of the same or another sugar acts as the source of the alcohol function to form glycosyl-glycoside. Anyone of the available alcohol groups of the second molecule may participate in this glycosidic linkage.

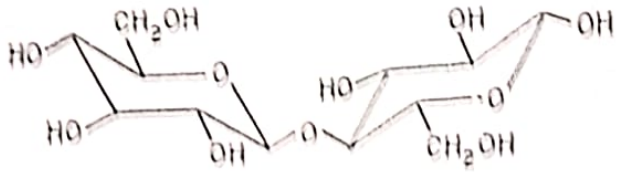
Maltose (α-form) is formed by interaction between the anomeric α-OH function of one glucose molecule and the 4-hydroxyl group of the second glucose molecule. The anomeric hydroxyl group of the second glucose is free to further react as a reducing group or to form further glycosidic linkage. The resulting compound (maltose) is termed a disaccharide because it has two sugars. The linkage here is 4:1α meaning : the fourth carbon carrying the alcohol -OH has reacted with the first carbon carrying the anomeric hydroxyl, which is having the α-configuration.



Maltose (α–anomer)



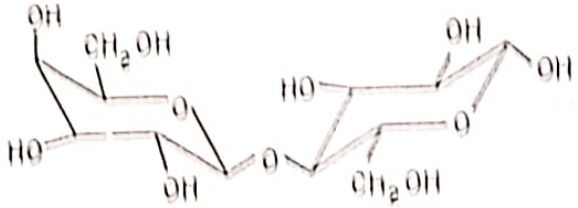
Maltose (β-anomer)



(glucose) (glucose)

Cellobiose (β-anomer)

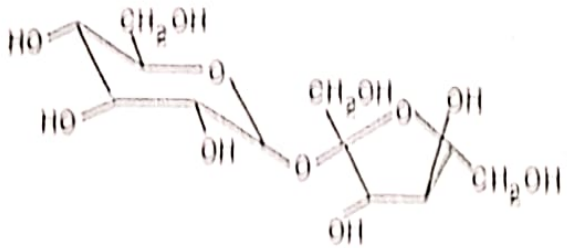
4:1-β-D-Glucosidyl-glucoside



(galactose) (glucose)

Lactose (β-anomer)

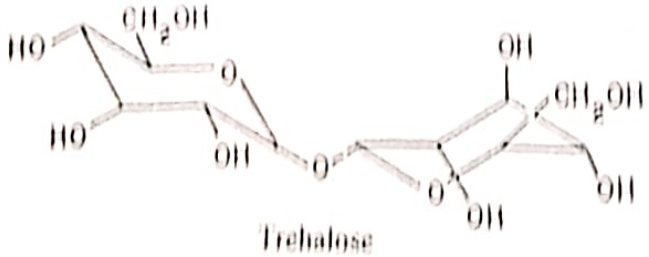
β-4-(β-1'-D-Galactopyranosyl)-D-glucopyranose



(glucose) (fructose)

Sucrose

1'-α-D-Glucopyranosyl-2-β-D-fructofuranoside



Trehalose

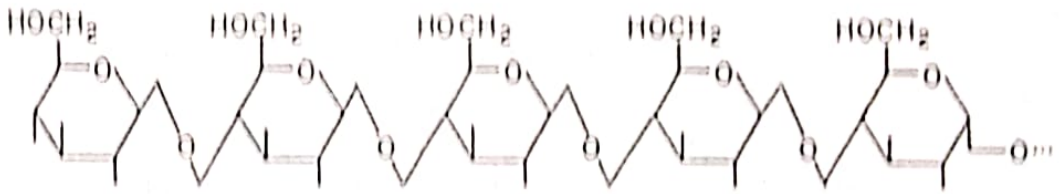
Maltose does not occur free in nature, but is obtained in the β-form, although in solution it may mutarotate to give a mixture of the α and β forms. In maltose there is still a reducing group available. But in trehalose as shown above, the linkage is 1α : 1α thus fixing the potential aldehyde group in a glycosidic linkage. Trehalose is a non-reducing sugar. Thus all disaccharides resulting through 1:1 linkages are non-reducing. Sucrose is another such example. Some more important disaccharides are shown below :

The disaccharides and monosaccharides can be tested for their properties. They are identified by the osazone test. Mixtures are separated by paper or thin-layer chromatography and detected with reagents like Tollen's or phenylhydrazine etc.

4.6 Polysaccharides

Oligo-saccharides and polysaccharides are formed by continuing the glycosidic condensation between mono-saccharides and disaccharides. A long chain of repeating units of ß-glucose 1:4 linked, will produce a linear fibrous polymer like cellulose with molecular weights greater than 100000. Cellulose, on enzymic hydrolysis yields the disaccharide cellobiose. Similarly 1:4 linked α-glucose units give rise to polymers found in starches.

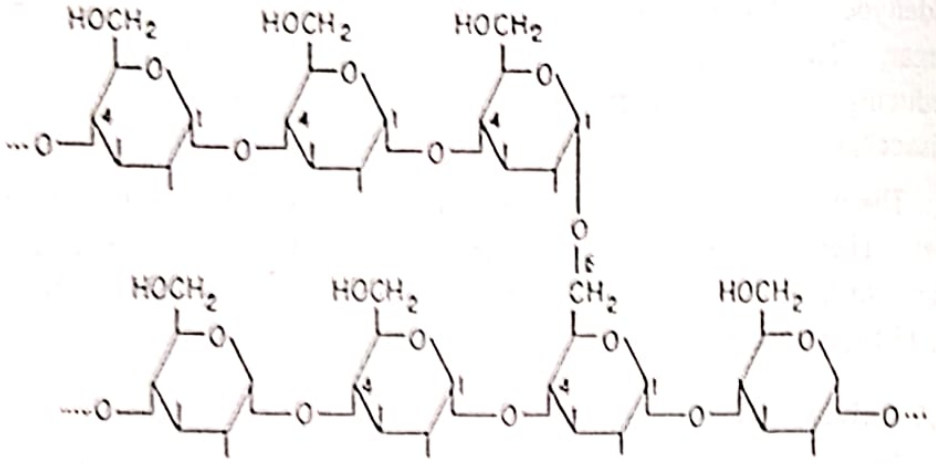
Simple starch (potato) molecules may have a molecular weight of as low as 4000, whereas some others may have molecular weights upto 500000 (*amylopectin* of rice starch). *Amyloses* which accompany amylopectin, have molecular weights of about 35000. Yet none of the starches have a linear fibre structure. This is because in starches besides 1:4 linkages there are also 1:6 linkages on some glucose units. This gives rise to branching of the chain. Profuse branching keeps the molecule compact and less linear. Starches occur in plants. In animals a polysaccharide called *glycogen* is present. Its structure resembles amylopectin very closely. However, the molecular weight of glycogen is considerably higher, (approaching 4000000). Starches and glycogen on hydrolysis yield maltose and glucose. Polysaccharides obtained by polymerisation of pentoses are known as *pentosans* and those of hexoses as *hexosans*.



Cellulose (partial structure)

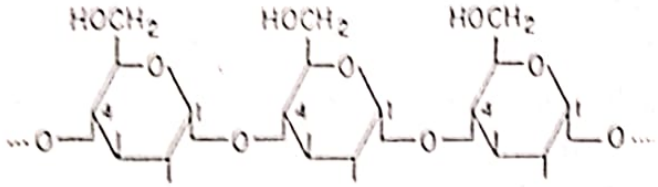
If a single sugar like xylose is involved, the polysaccharide is known as xylan. Similarly glucan, galactan, mannan etc. are known.

Polysaccharides derived from reducing sugars, do not themselves show reducing properties. They are insoluble in water, but absorb water and swell, depending on temperature and duration of contact. Starches give a deep blue colour with iodine solutions (due to amylose). Partially hydrolysed



Schematic Representation Partial Structure

Amylopectin (Glycogen)



Amylose Partial Structure

starches are known as *dextrins*. They give a reddish colour with iodine solutions (hence sometimes known as *erythrodextrins*). Cellulose, amylose, amylopectin, glycogen are polysaccharides derived from only one monosaccharide unit i.e. glucose. Other polysaccharides obtained by co-polymerisation of two different mono-saccharides or their derivatives are also known. e.g. : Hyaluronic acid, heparin, pectins etc.

Both cellulose and starch are abundant in nature. They have a number of primary alcohol groups in their molecules, which can be converted to their esters by acids, yielding products like cellulose acetate, cellulose acetate-phthalate etc. They can also be oxidised to carboxyl groups, which may be further esterified or converted to salts. Carboxy cellulose, carboxy methyl cellulose are such examples. The secondary alcoholic functions in these molecules can also be partly oxidised yielding products like oxycellulose, oxystarch etc. All these have special applications in pharmacy.

Inulin is a small molecular weight oligo-saccharide (mole mass 5200). It occurs in dahlia bulbs, artichokes and tap roots of some other plants, as

a storage carbohydrate, like starch in most foods. It consists of a single chain of about 30 fructo-pyranose units, linked β-2→1. At the ends of the chain D-glucose is linked 1→2. Thus there are no reducing groups available. Because of small size of the molecule, inulin is soluble in water. Besides its nutritional value, it has an important application. It is used to estimate glomerular filtration rate (GFR) in diagnosis of kidney function, as it gives reliable and reproducible results.

Complex polysaccharides are polysaccharides which are associated with non-carbohydrate biological molecules. *Glycolipids* and *glycoproteins* belong to these. Glycolipids will be explained in the chapter on lipids. Glycoproteins are found on the surface of many bacterial membranes and erythrocytes. Because of the presence of proteins, these show antigenic properties (induce formation of antibodies). When they come in contact with their specific antibodies the cells aggregate together (called agglutination). The antibodies are also known as agglutinins. Besides the bacterial glycoproteins and their agglutinins, the human erythrocyte glycoproteins (blood group antigens) and their agglutinins in the serum of other individuals, are very important. They help in classifying blood groups (for genetic relationship purposes) and also in ensuring safe blood transfusion.

4.7 Carbohydrates and Nutrition

Polysaccharides may be hydrolysed using acids, higher temperature, pressure etc. The products formed are usually lower molecular weight polysaccharides, oligosaccharides, disaccharides and monosaccharides. Due to the drastic conditions of reaction some destruction also takes place. The yield of monosaccharides (e.g. glucose) may be low. To achieve better yields and purer product, enzymic hydrolysis is preferred. Both amylose and amylopectin of starch are hydrolysed by *amylase*. The human pancreas produces α-*amylase*. Therefore only starches and glycogen, which have α-linked glucose units are hydrolysed. The end product of this is maltose. Maltose is further hydrolysed to glucose units by the enzyme *maltase* (α-glucosidase). The α-amylase cannot hydrolyse cellulose and other polysaccharides having β-linkages. Hence cellulose is not digested by human beings. Malt (germinating barley) is rich in α-amylase. Some fungi, like *Aspergillus oryzae* are also rich in α-amylase and are good source of the enzyme on a commercial scale. Many soil bacteria and *actinomycetes* are able to degrade cellulase by producing *cellulase* enzymes. This is a natural process of bio-degradation of large quantities of cellulose, which accumulates from plant materials.

As food materials human beings can make use of starches, sucrose, lactose, glucose and fructose. Most vegetarian foods derived from grains,

tubers and pulses, are rich in starches. The monosaccharide lactose (from milk) is first hydrolysed by the enzyme *lactase* (β-*galactosidase*) to a mixture of galactose and glucose, when absorption takes place. This enzyme is in high proportion in infants and children, whose main nutrition is milk. In later years the enzyme content diminishes and lactose digestion may not be complete. Sucrose is digested to a mixture of glucose and fructose by *sucrase* (*sucrose* α-*glucosidase*) and absorbed. If the human digestive system is deficient in these enzymes or α-amylase, nutritional deficiency can occur. In many patients, digestive process can be facilitated by administering fungal or malt amylase. In acute nutritional deficiency, intravenous administration of glucose (dextrose) is given in the form of glucose-saline. Excessive consumption of leguminous seeds (pulses and beans), causes **flatulence** or gas formation in the large intestine. The pulses and beans contain an oligo-saccharide called *raffinose*, which cannot be degraded by our body enzymes. Raffinose is thus not absorbed and reaches the large intestine, where bacteria degrade it and produce gases causing flatulence and discomfort.

Most of the energy needed for the activities of life comes from the breakdown of carbohydrate foods. Our daily intake must therefore be rich in digestive carbohydrates. An optimum amount is about 60% of the food consumed. However, in India the average diet is richer in carbohydrates, usually approaching 90%, and deficient in proteins, minerals, vitamins and fats. Carbohydrate deficiency may be observed only in starvation conditions.

In many children conditions known as **lactose intolerance** and **galactose intolerance**, also known as **galactosemia** may be found. As these are due to enzyme deficiencies, the best way to treat these conditions is to administer to the patient, diets free of lactose and galactose. Occasionally **fructose intolerance** may also be found in infants, due to genetic reasons. Such infants should not receive fructose, sorbitol and sucrose. *Glycogen* **storage disease** is a carbohydrate metabolite disorder. It causes accumulation of glycogen in body tissues, but is not degraded to release glucose. This is also due to lack of the degrading enzymes. Some types of the disease may respond to special diets.

4.8 Glucose Estimation

I. In Blood Samples : In our blood there is a narrow range of glucose (90 to 120 mg/100 ml at normal periods. In diabetic conditions, starvation etc. these values change. Hence an accurate estimation of glucose in the blood (actually in serum) is a useful diagnostic test. It is therefore estimated by following steps :

(a) first blood is clotted to obtain serum.

(b) Proteins of serum are separated by acid coagulation (usually trichloroacteic acid).

(c) The clear aqueous filtrate is then treated with a reagent which produces a colour reaction.

(d) The colour is then estimated by colourimetry or spectrophotometry.

The step (c) is the critical one. There are many reagents and reactions used by different laboratories and scientists. The more well known are :

(1) *Folin-Wu*, which uses alkaline cupric ion reduction, forming red cuprous oxide; this is dissolved in phosphomolybdic acid to give a blue colour.

(2) *Nelson-Somogyi* method uses iodine to react with the cuprous ion formed as above and the excess iodine is titrated with thiosulphate.

(3) *Hagedorn-Jensen* method uses ferricyanide as the oxidising agent instead of cupric reagents.

II. In Urine Samples : Here *Benedict's reagent* is taken in a china dish, which is kept at boiling temperature. From the burette a diluted urine sample is added. The discharge of blue colour of the cupric reagent with the formation of white precipitate of copper thiocyanate indicates the end point of titration.

III. In Pharmaceutical Products : In dextrose injection and glucose-saline preparations, the Indian Pharmacopoeia recommends a simple method of direct measurement of optical rotation of the sample. The concentration is calculated directly from the mentioned value of optical rotation using a standard factor.

4.9 Pharmacopœial Products

Although a number of carbohydrates are included in pharmacopœias, only some are used as biochemicals, i.e. for nutritional or replacement therapy. By virtue of strong hydrophilic properties, some of the official carbohydrates are used as pharmaceutical aids. Only a brief account of these will be given here.

Dextrose (I.P.) : Both anhydrous glucose (mole mass 180.16) and the monohydrate (mole mas 198.17) are official. It is a white crystalline or granular powder obtained usually by hydrolysis of starch. It should be free from dextrins and oligosaccharides. Its purity is controlled by optical rotation, which should be between +52.5° and +53°. It is a nutrient, used directly orally or it may be used to prepare injectable products of different

strengths, usually 5, 10, 25 and 50% w/v. Pharmacopœia also includes dextrose saline solutions for intravenous infusion purposes.

Malt extract (I.P.) is another carbohydrate nutrient included in the Pharmacopœia. It is obtained by digesting malted cereals like barley, wheat or sorghum. The germinating cereals produce amylase, which hydrolyses starch, causing accumulation of maltose. The soluble solids include dextrins besides maltose, which is the major constituent of malt extract. Negligible amount of proteins may be present. It has an agreeable taste, making it useful as a masking agent for bitter drugs and for cod-liver or shark liver oils. It is used for these purposes.

Lactose (I.P.) is a disaccharide, used as a pharmaceutical aid, as a dry diluent for potent drugs, in making tablets or capsules. **Sucrose** (I.P.) is also a disaccharide, used as pharmaceutical aid, as a sweetening agent in syrups, elixirs, lozenges etc. **Starch** (I.P.) is a polysaccharide, obtained from different plant sources (potato, wheat, rice etc.), used as a pharmaceuticals aid, as filler, binder and disintegrant in tablet formulation. It is also useful as a dusting powder during manufacture. **Microcrystalline Cellulose** (I.P.) is used for suspending and diluting purposes, in pharmaceutical formulations. **Pectin** (I.P.) is a polygalacturonic acid, used as a suspending agent, but also as a protectant in gastric ulcers. Polysaccharide gums like those of **Acacia, Tragacanth and Guar (all I.P.) gums** are extensively used as suspending and binding agents.

Heparin sodium (I.P.) is a preparation obtained from animal tissues, particularly lungs and intestinal mucosa of oxen, pigs or sheep. It is the natural anti-coagulant of our body. It is a polysaccharide formed from the disaccharide unit called mucoitin (N-acetyl-glucosamine and glucuronic acid, which resemble hyaluronic acid), partly esterified by sulphuric acid, On account of the acidic group present, it is easy to prepare the sodium salt rendering it soluble in water. It should be free from proteins (to prevent allergic reactions) and has a potency of 110 to 130 units in 1 mg. It is used as an anti-coagulant.

Dextran (I.P.) is another official polysaccharide obtained from fermenting sucrose with *Leuconostoc mesenteroides*. It is a polysucrose. It is made available in two grades : (1) average molecular weight of 40000; and (2) average molecular weight of 100000. Both are dissolved in 5% dextrose or normal saline (0.9% w/v of sodium chloride) and should be free of proteins. It is used as plasma substitute by intravenous infusion, in severe loss of blood due to hemorrhage.

Sorbitol (I.P.) is the polyhydric alcohol obtained by the reduction of glucose or fructose. Also known as glucitol, this is used as a pharmaceu-

tical aid, as a sweetening agent and tablet excipient. It is a crystalline or granular powder.

Mannitol (I.P.) is also obtained by reduction of fructose or mannose, and is a polyhydric alcohol like sorbitol. It is also a crystalline or granular powder. It finds application as a diuretic (increasing urine output) by increasing osmolality of glomerular filtrate. It is also used as a diagnostic agent in renal function test. (See chapter on excretion).

24. _____

25. False.

27.

28.

5

Lipids

5.1 Introduction

Lipids are a large group of naturally occurring compounds, characterised only by their water repellant property : hence called *hydrophobic*. Their solubility in organic solvents is variable. Some are soluble in polar organic solvents like alcohol and others in non-polar solvents like hexane, benzene etc. Although many proteins and polysaccharides are also not soluble in water, they do not repel water. They are easily wetted by water, may absorb and swell with water. Hence they are *hydrophilic*. Unlike the proteins or carbohydrates, the lipids have no specific functional group or linkage characterising all of them, although a majority belong to the group of oils and fats. The lipids are widely distributed in nature and are produced on large scale, mainly from plant sources for various purposes. In cold parts of the world, animal and fish oils are produced for the same purpose as vegetable oils in tropical and temperate regions. Lipids, like carbohydrates, provide energy for work and life processes. They also form part of some structures in the cell membranes, walls of tissues and organs. They are stored in animal tissues and plant materials (especially seeds) as food sources. The lipids also provide a non-aqueous medium in the body, which helps in regulation of transport of many chemicals, due to their partition between lipid and aqueous layers. Thus lipids play an important role in living organisms. As most of the lipids are bio-synthesized in our body, low fat consumption does not lead to deficiency. As energy source, the lipids are secondary to carbohydrates. Lipids also occur in conjugation with proteins and are termed *lipo-proteins*.

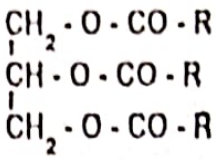
5.2 Classification and Chemistry

They may be classified into (a) *Simple lipids* (b) *Phospholipids*; and (c) *Complex lipids*. Simple lipids can be further sub-divided into *(1) Glycerides; (2) Waxes; (3) Sterols; and (4) Ethers*

5.2.1 Simple Lipids

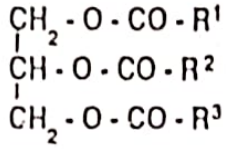
5.2.1.1 Glycerides : The glycerides are esters of mono-carboxylic

acids and the trihydric alcohol glycerol (also known as glycerin). Their general structure is represented thus :



Glycerides (general structure)

Here R represents the carboxylic acid portion or no substitution (i.e. R = H). If only one carboxylic acid reacts with glycerol, then we obtain *mono-glyceride* (mono-ester). Similarly *di-glycerides* and *tri-glycerides* are obtained by progressive esterification. Most oils and fats are tri-glycerides. Hence the structure can be re-written thus :

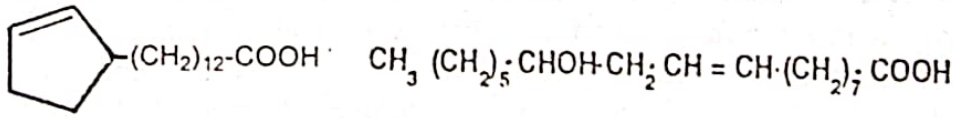


Glycerol Esters

Although the simplest carboxylic open chain compounds like acetic acid and propionic acid are well known, these are not found in oils and fats. However, most of the higher homologues of this series with C_4 to C_{24} are found in oils and fats. As most of the higher acids were first obtained from fats, the series is known as fatty acids. Most of the naturally occurring fatty acids are straight chain acids. Both the saturated and unsaturated acids are well known. All the acids have even number of carbon atoms. A few exceptions are known, with odd number of carbon atoms and also branched chain acids. There are also some unusual cyclic acids and acids with a substituent group. The more well known acids are indicated in the Table 5.1.

The unsaturated fatty acids linoleic and linolenic acids are considered as *essential*, because our body is not able to synthesize adequate amounts of these. At least one must be present in the diet, to enable the body to synthesize the others from it.

A few uncommon but interesting acids are indicated below :



Chaulmoogric Acid
(from oil of chaulmoogra)

Ricinoleic Acid
(from castor oil)

TABLE 5.1 Fatty Acids Commonly Found in Fats

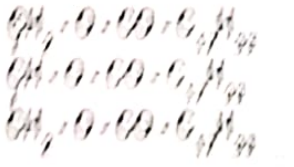
Common Name	Systemic Name	Structure	Commonly Written as	Source
Saturated Acids				
Butyric	Butanoic	$CH_3(CH_2)_2COOH$	C_3H_7COOH	Butter
Caproic	Hexanoic	$CH_3(CH_2)_4COOH$	$C_5H_{11}COOH$	Palm oils
Caprylic	Octanoic	$CH_3(CH_2)_6COOH$	$C_7H_{15}COOH$	Coconut oil
Capric	Decanoic	$CH_3(CH_2)_8COOH$	$C_9H_{19}COOH$	Coconut oil
Lauric	Dodecanoic	$CH_3(CH_2)_{10}COOH$	$C_{11}H_{23}COOH$	Laurel oil
Myristic	Tetradecanoic	$CH_3(CH_2)_{12}COOH$	$C_{13}H_{27}COOH$	Nutmeg fat
Palmitic	Hexadecanoic	$CH_3(CH_2)_{14}COOH$	$C_{15}H_{31}COOH$	Widely found
Stearic	Octadecanoic	$CH_3(CH_2)_{16}COOH$	$C_{17}H_{35}COOH$	Widely found
Arachidic	Eicosanoic	$CH_3(CH_2)_{18}COOH$	$C_{19}H_{39}COOH$	Groundnut oil
Unsaturated Acids				
Palmitoleic	Hexadec-9-enoic*		$C_{15}H_{29}COOH$	Widely found
Oleic	Octadec-9-enoic*		$C_{17}H_{33}COOH$	Widely found
Linoleic	Octadeca-9,12-dienoic*		$C_{17}H_{31}COOH$	Linseed oil
Linolenic	Octadeca-9,12,15-trienoic*		$C_{17}H_{29}COOH$	Linseed oil
Arachidonic	Eicosa-5,8,11,14-tetraenoic*		$C_{19}H_{31}COOH$	Animal fats

* All cis isomers

The triglycerides of fatty acids may contain the same acid as R^1, R^2 and R^3, as shown below.

Tripalmitin

Tristearin



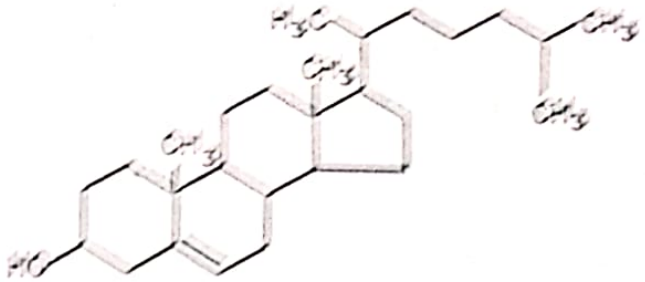
Triolein

The fatty acids R^1, R^2, R^3 may be different and may be any combination of the naturally occurring fatty acids.

5.2.1.2 *Waxes* : Waxes are also esters of fatty acids. The alcohol is usually a monohydric alcohol. Many waxes also contain free alcohols and/or free acids. Both the alcohols and acids are of higher molecular weights, usually between C_{16} and C_{30}. Straight chain alcohols like myricyl alcohol ($C_{30}H_{61}OH$), cetyl alcohol ($C_{16}H_{33}OH$), unsaturated alcohols like lanosterol ($C_{30}H_{49}OH$) are some of the examples of alcohols

found in some waxes and related substances. Beeswax, carnauba wax, wool fat are examples for this class of lipids.

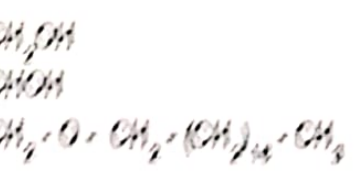
5.2.1.3 Sterols : These are of particular interest to human biochemistry. Structure of cholesterol is shown below :



Cholesterol

This occurs both free and as esters in the body as part of cell membranes, in circulation and in nerve cells (brain tissue). It also serves as the source for biotransformation to steroidal hormones (e.g. hydrocortisone, cortisone, progesterone, testosterone, oestradiol etc.) and bile acids (e.g. cholic acid). A small amount may be converted to vitamin D_3. In plants other sterols like stigmasterol, ergosterol, sitosterol etc occur.

5.2.1.4 Glycerol mono-ethers of higher alcohols formed by reduction of corresponding acids or glycerides are known to occur in marine organisms like sharks, squids, starfish etc. For example batyl alcohol is a monoether of glycerol and stearyl alcohol with the structure :



Glycerol Mono-ethers

5.2.1.5 Tests and properties of simple lipids

(1) These are not water soluble. They are immiscible with water (do not mix but remain separated). Their specific gravity is less than water. Hence they float on aqueous media.

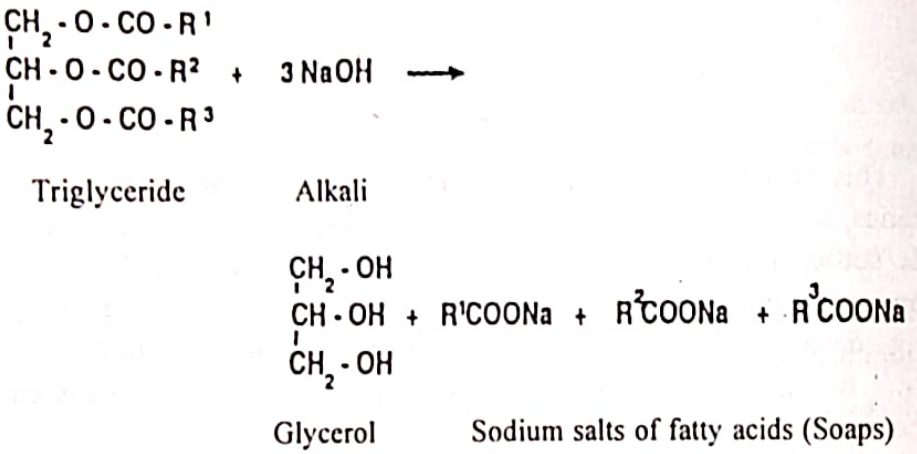
(2) The oils are liquid at room temperature. Fats differ from oils only in their being solids at room temperature. For example coconut oil becomes a fat at cool temperatures and may be correctly called coconut fat. Waxes and sterols are solids at room temperature.

(3) They dissolve in many organic solvents, particularly in ether, chloroform, benzene, hexane etc.

(4) The fats and waxes do not have sharp melting points, but only melting ranges. On cooling, oils solidify (congeal), forming fine granular or thin flake like structures. The sterols have sharp melting points and are obtained in fine crystalline forms.

(5) On heating, the lipids do not boil, but decompose, with charring.

(6) As the lipids are esters or free alcohols, they are neutral (not acidic or basic). The oils and waxes can be hydrolysed by strong alkaline solutions.

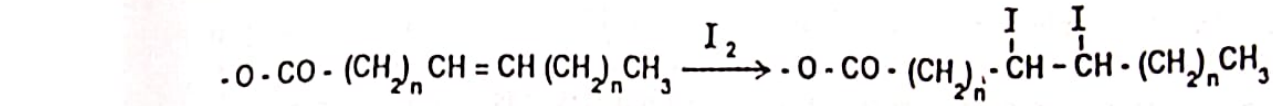


Glycerol and the sodium soaps are water soluble. Hence a clear solution will be obtained. With waxes, it depends on the higher alcohol, which is generally not soluble in water. However, if some alcohol has been used in the reaction, it also may dissolve. In this reaction soap is formed. Hence the reaction is known as *saponification.*

This reaction is the basis for quantitative characterisation of an oil (or fat) or wax. *The number of milligrams of potassium hydroxide (KOH) required to neutralise (i.e. form salts) the fatty acids liberated on complete hydrolysis of one gram of an oil, fat or wax is known as saponification value.* It may also be known as *sap value or sap equivalent or saponification number.* Because each oil or fat contains different fatty acids as triglycerides, the sap value of different oils will be slightly different. However, this is constant within a narrow range for a given oil and provides a good quantitative characteristic for that oil. This reaction is not given by sterols, ethers and alcohols found in lipids. Therefore after saponification, they may be extracted with ether, dried and weighed to determine *unsaponifiable matter* of the oil. The soaps do not dissolve in ether and are not extracted.

(7) Many oils and fats (but rarely waxes) contain unsaturated fatty acids like oleic, linoleic, linolenic acids. Therefore such oils will slowly

decolourise bromine or iodine, when kept in contact, in an organic solvent. This is because they add to the double bond in the acid causing saturation. This reaction can be represented thus :



Part of Glyceride Iodine addition to double bond

This reaction also forms the basis for another important quantitative characterisation method for oils and fats. *The number of grams of iodine absorbed by 100 grams of the oil or fat is designated as iodine value (or iodine number).* In actual experiment other reagents like *iodine monochloride* or *iodine monobromide* or *pyridine bromide* etc. may be employed in place of iodine itself. These reagents are more reliable and give quantitative reactions. However, the calculation must be made to give the iodine value. Oils with high unsaturation, like olive oil and linseed oil have high iodine value, whereas palm oils with more of saturated acids have low iodine value. The iodine value is also a characteristic of a given oil and forms a good criterion for the evaluation of an oil or fat.

(8) The unsaturation in oil molecules renders them susceptible to oxidation. Atmospheric oxidation causes degradation of the unsaturated acids, resulting in the formation of acids and aldehydes of shorter chain with characteristic disagreeable odour. This is called *rancidity*. Moisture and light hasten this process. Many vegetable oils contain *tocopherols* (vitamin E), which are naturally occurring oil soluble anti-oxidants, preventing rancidity. Animal fats (like butter fat) become rancid quickly due to liberation of butyric acid.

(9) Sterols, which are part of lipids can be tested by the following reactions:

(a) *Liebermann-Burchard* reaction : A sterol sample in chloroform solution is treated with a few drops of cold acetic anhydride followed by a drop of concentrated sulphuric acid. A *green colour* in the chloroform layer will be seen if cholesterol (and some related sterols) is present.

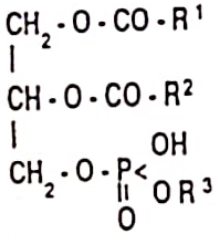
(b) *Salkowski* reaction is carried out without addition of acetic anhydride. Green colour will be seen both in choloform and acid layers.

(c) *Rosenheim* reaction is carried out also with a chloroform solution of a sterol. But trichloroacetic acid is added. *Pink to red* colour is produced by ergosterol (and a few other sterols).

5.2.2 Phospholipids

As the name indicates these contain phosphorous in their molecule in the form of phosphoric acid esters of the hydroxyl groups. Like glycerides, they may also be called *phosphatides*. As phosphoric acid is polybasic, the phosphatide is still a strong acid (like monosodium dihydrogen phosphate). Hence the name *phosphatidic acid* is also often employed. There are two major subgroups of phosphatides based on the hydroxyl part of the molecule : *(1) Glyceryl phosphatides; and (2) Sphingosyl phosphatides.*

5.2.2.1 Glyceryl phosphatides (also known as *phosphoglycerides*) contain glycerol, phosphoric acid, fatty acids and a basic hydroxy compound, which reacts with the phosphatidic acid to give another ester. Their general structures are indicated below :



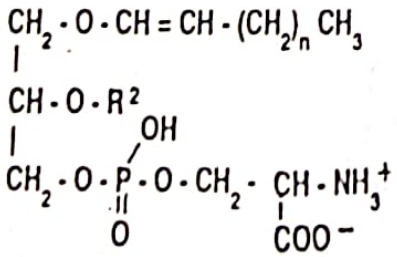
Phospholipids

R^1 and R^2 are usually palmitic or oleic acids.

Lecithins have choline as the basic alcohol at R^3, i.e. R^3 = $-CH_2CH_2-N^+(CH_3)_3$. Choline is $HO-CH_2CH_2-N^+(CH_3)_3.OH^-$. As the first carbon in glycerol is involved in phosphatide link, lecithins are also α-lecithins. They may also be called phosphatidyl cholines. They may also be considered as derivatives of α-glycerophosphoric acid. Lecithins occur extensively in brain tissue, liver, egg yolk, soya bean, wheat germ, yeast etc.

Cephalins yield the basic alcohol, ethanolamine (2-amino-ethanol, $HO-CH_2CH_2-NH_2$) at R^3 or the α-amino-acid serine at R^3. These occur extensively in cell membranes together with lecithins and other phospholipids.

Plasmalogens are also phosphoglycerides containing glycerol, fatty acid, phosphoric acid and ethanolamine or serine (as in cephalins). But at

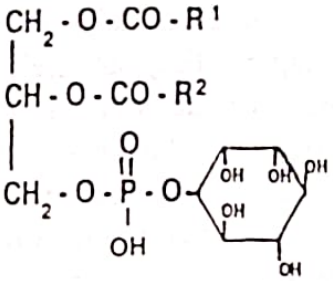


Serine Plasmalogen

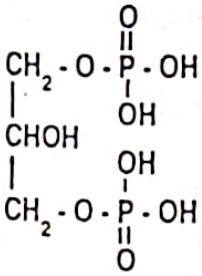
carbon 1 of glycerol, instead of an ester there is an ether linkage with a long chain unsaturated alcohol.

The plasmalogens are abundant in nerve and heart tissues.

Diphosphatidyl glycerol (or cardiolipin), which occurs in mitochondria and many bacterial membranes, differs from the phosphoglycerides in lacking a fatty acid and basic alcohols.



Phosphatidyl Inositol

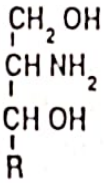


Cardiolipin

Phosphatidyl inositol is another phosphoglyceride which lacks an amino-alcohol, but has the hexahydric inositol in its place.

The above two phospholipids do not have nitrogen in their molecules.

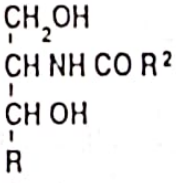
5.2.2.2 Sphingolipids are characterised by the presence of amino-group in the glyceryl part of the molecule.



R = -CH=CH-$(CH_2)_{12}$-CH_3 in Sphingosine

R = -$(CH_2)_{14}$-CH_3 in Dihydrosphingosine

The 'R' group here is a saturated or unsaturated long chain alkyl group with 15 carbon atoms. The amine of the sphingosine is converted into an amide with a fatty acid, giving *ceramide*. Thus:



Ceramide (R^2 may be palmityl or oleiyl)

This ceramide has still a primary and a secondary alcohol function. The primary alcohol is linked to choline through phosphoric acid in *sphingomyelins*, found as the major sphingolipid in mammalian tissues.

5.2.3 Complex lipids

In brain and nerve tissues, the ceramide mentioned earlier is found in glycoside linkage with sugars, involving the primary alcoholic group of the ceramide and usually the anomeric hydroxyl function of cyclic monosaccharides like glucose, galactose but also oligo saccharides, amino-sugars etc. These are called *cerebrosides*. The cerebrosides are *glycolipids (glycosphingolipids)* and are complex lipids.

As the lipids are hydrophobic and have to exist side by side with hydrophilic compounds like proteins and sugars, they are held together through physical (solubility, particle size) as well as weak forces to the proteins, particularly in blood and most tissues. The association with protein is particularly high with phospholipids and sphingolipids. Cholesterol esters are also extensively associated with membrane proteins and plasma proteins. Such complex lipids are known as *lipoproteins*. Egg yolk, brain tissue, myelin sheath of nerves, photoreceptive structures of the retina (rods and cones), mitochondria etc. are rich in lipoproteins.

5.3 Role of Lipids

(1) Lipids, particularly the glycerides, are exceptionally high energy sources, if they can be fully degraded. Unfortunately this is not achieved. A considerable amount of carbohydrate is also necessary for efficient oxidation of fats. However, body tries to conserve fats and stores them in almost any tissue. Excessive fatty diet tends to produce **hyperlipidemia** leading to **obesity** (over weight). Only vigorous exercise can mobilise better utilisation of fats. Food must also contain adequate amounts of the highly unsaturated fatty acids linoleic and linolenic acids.

The human body is unable to introduce double bonds into the long alkyl chain, far away from the carboxyl group. Foods with high content of saturated fatty acids are not encouraged, because they cause heavy burden on the liver metabolic activity. The vegetable oils are susceptible to oxidation leading to rancidity. To prevent this and prolong keeping capacity of cooking oils, they are partially hydrogenated to reduce unsaturation. But this leads to increased workload to the body. The hydrogenated oils are not suitable to persons suffering from any type of liver or cardiovascular disorders.

(2) Complex lipids like glycolipids, lipoproteins and sphingolipids are important parts of living cell membranes, nerve cells and fibres. The lipid

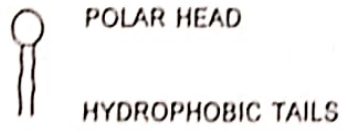
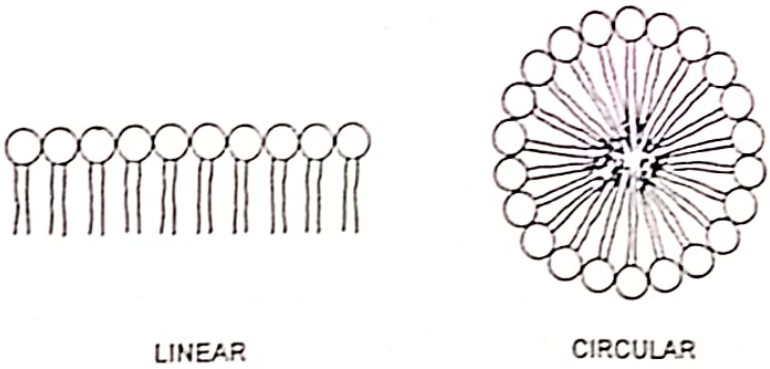


Fig 5.1

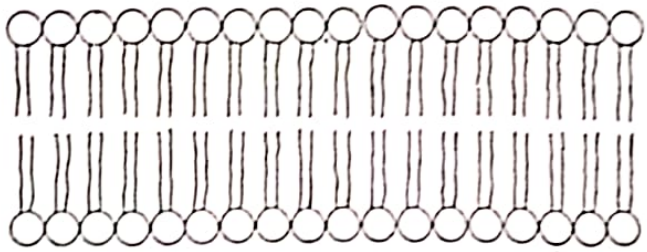
molecules are characterised by a polar (hydrophilic) group on one end of the long molecule and the long non-polar hydrocarbon chain (hydrophobic).

Groups of lipid molecules can arrange parallel to each other, to form a membrane with hydrophilic groups on one side and a hydrophobic (or lipophilic) surface on the other side. Circular arrangement can enclose a small lipophilic volume trapping lipophilic substances inside.

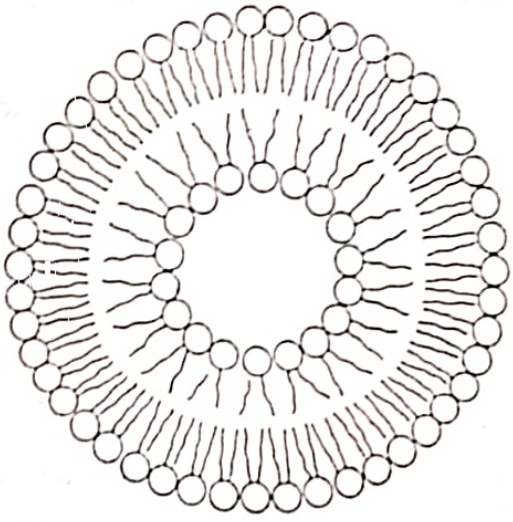


LINEAR CIRCULAR

Monolayers



LINEAR



CIRCULAR MICELLS

Bilayers

Fig. 5.2

Lipids are thus able to transport lipophilic substances by trapping them inside small micelles which circulate in the blood and body fluids. Layered and bi-layered lipids form semi-permeable membranes, which regulate migration of molecules across the membranes.

5.4 Pharmacopœial Products

A number of lipids are official in the Pharmacopœia. Most of these find application as aids in pharmaceutical formulations and manufacture. **Arachis oil** (I.P.) or groundnut oil is used as oily vehicle for oil soluble drugs. **Castor oil** (I.P.) is useful as a plasticizer (softening agent), but also finds application externally as an emollient (soothing). It is also administered internally as a cathartic.

Oleic acid (I.P.) is obtained by the hydrolysis of suitable oils containing mainly oleic esters of glycerol. However, small amounts of stearic and palmitic acids may be present. It is a pale yellow liquid. It is used as an emulsifier along with other such agents, in pharmaceutical formulations.

Stearic acid (I.P.) is also similarly obtained by hydrolysis of suitable oils or fats. It is usually a mixture of stearic and palmitic acids. It is a solid at room temperature. It is used as an adjunct to emulsification, besides as a lubricant in tablet manufacture.

Ethyl oleate (I.P.) is a synthetically prepared ethyl ester of oleic acid. This is obtained in a much purer form than the natural products. Hence it is preferred as a vehicle for oily injections (steroidal hormones and some antibiotics).

Ceto-stearyl alcohol (I.P.) is obtained by the reduction of stearic acid, but consists of a mixture of stearyl alcohol ($C_{17}H_{35}$-CH_2OH) and cetyl alcohol (or palmityl alcohol, $C_{15}H_{31}$-CH_2OH). It is also a solid at room temperature. It is used as a base for ointments, usually along with other similar agents.

Glyceryl mono-stearate (I.P.), also popularly known as monostearin or GMS, is also a mixture of glycerylmonostearate and glyceryl monopalmitate. It may also contain small amounts of di- and tri glycerides. This is because of the method of preparation, usually by partial hydrolysis of triglycerides. If obtained by controlled esterification of glycerol by stearic acid, the proportion of the di- and tri glycerides will be low. It is a waxy mass, used as an emulsifying agent in ointments (for external use only).

Glycerol (I.P.) itself is used as a solvent and humectant (retaining water), in many formulations.

Beeswax (I.P.) is the wax obtained by purifying the wax of the honeycomb and is usually yellow. It can be bleached to yield white beeswax. Both are official in the I.P. It consists mainly of myricyl palmitate and myricyl alcohol, besides higher fatty acids (e.g. cerotic acid). These are used as pharmaceutical aids, as stiffening agents in pastes and ointments and as polishing agents in tablet manufacture.

Wool fat (I.P.), also known as lanolin or wool wax, is obtained by recovering the waxy material adhering to the raw wool of the sheep. This is a byeproduct. When partially purified, it contains about 25 to 30% water emulsified in it. This is called hydrous wool fat. On melting, it separates into two layers of water at the bottom and wax on top. If water is driven off, anhydrous lanolin can be recovered. Chemically it consists of a mixture of cholesterol and lanosterol in free form and esterified by higher fatty acids. It is a soft oily mass (like soft paraffin) and finds extensive use in emulsification and formulation of ointment bases.

6

Nucleic Acids

6.1 Introduction

These are so called because they were first encountered in the study of nucleus of cells and were found to be strongly acidic. It is now known that they are present both inside the nucleus and outside in the cytoplasm. The nucleic acids are generally found in conjugation with proteins, i.e. as nucleoproteins, inside the cells. Like proteins and carbohydrates, the nucleic acids are also high molecular weight polymers of repeating units, called *nucleotides*. They may also be called therefore, *polynucleotides*. There are two types of nucleic acids. (a) *ribonucleic acids* (RNA); and (b) *deoxyribonuleic acids* (DNA).

6.2 Functions

(1) Nucleic acids carry genetic information (or code), which is transmitted to all cells and subsequent generations.

(2) Nucleic acids regulate the synthesis of all proteins.

(3) The nucleotides are part of high energy bonded compounds in the body (e.g. ATP) which store and release energy needed for critical biotransformations.

(4) Some of the nucleotides and their derivatives are part of some coenzyme molecules. (e.g. NAD, FAD, Coenzyme A).

(5) Some nucleotides or their derivatives are known to be physiological mediators and/or regulators in some metabolic processes. (e.g. cAMP, cGMP, ADP).

(6) They may also be needed for activation (or priming) of some biochemical intermediates.(e.g. UDP, GDP, CDP etc).

(7) The nucleotides also supply the units for the biosynthesis of polynucleotides. (i.e. nucleic acids).

Although the nucleic acids and their units (nucleotides) are essential for all life processes, fortunately they are all biosynthesized from other

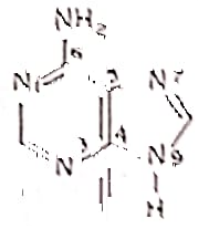
food products, mainly amino-acids, glucose and inorganic phosphate. Once biosynthesized these are recycled many times before being metabolised.

6.3 Chemistry and Inter-Relationship

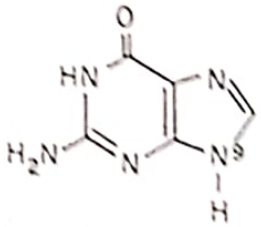
Nucleotides are units of nucleic acids (like amino-acids are units of proteins or monosaccharides of polysaccharides). *A nucleotide is an ester of phosphoric acid and a nucleoside.* In its turn *a nucleoside is made up of a base and a pentose.* There are two pentoses which are found in nucleosides : (a) *ribose*; and (b) *deoxyribose*. These pentoses are involved in a direct linkage of carbon-1 of the pentose and a ring nitrogen of the base (somewhat similar to a glycosidic linkage).

There are two types of heterocyclic bases found in nucleosides : (a) *Purine* type; and (b) *Pyrimidine* type. The purine bases commonly found are *guanine* and *adenine* and to a lesser extent *hypoxanthine* and *xanthine*. The pyrimidine bases commonly encountered in the nucleosides are *uracil, cytosine* and *thymine*. Cytosine and ribose are mostly present in ribonucleic acids. Thymine and deoxyribose are present likewise only in deoxyribonucleic acids. The other bases are found both in RNA and DNA. The structure and names of the more common bases, nucleosides and nucleotides are shown below :

6.3.1 Purine bases



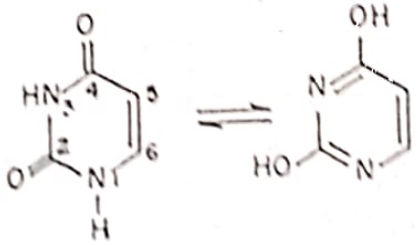
Adenine
(6-Amino-purine)



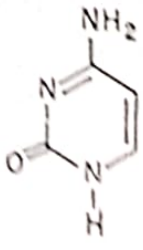
Guanine
(2-Amino-6-oxo-purine)

The N^9 is involved in linkage with C-1 of pentose. Note that guanine can exist in a tautomeric form (lactam-lactim).

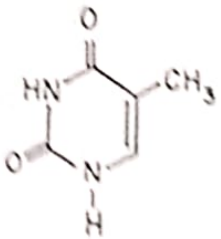
6.3.2 Pyrimidine bases



Uracil
(2,4-Dihydroxy-pyrimidine)



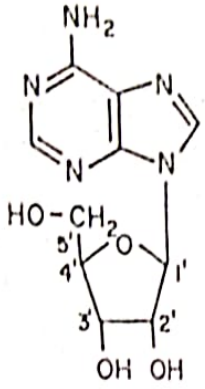
Cytosine
(2-Oxo-4-amino-pyrimidine)



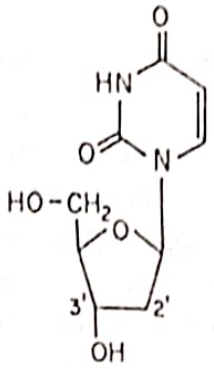
Thymine
(5-Methyl-uracil)

The N^1 of pyrimidines is involved in linkage with C-1 of pentose.

6.3.3 Nucleosides



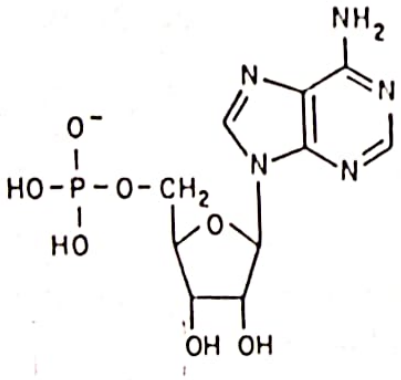
Adenosine
(Adenine Riboside)



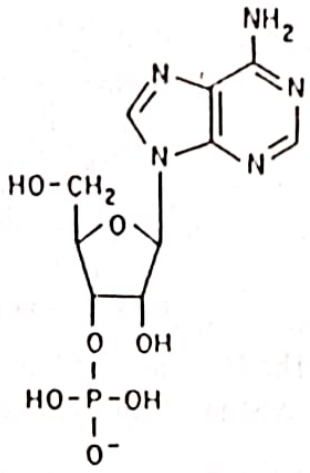
Thymidine
(Thymine Deoxyriboside)

Similarly, *Guanosine, uridine* and *cytidine* are obtainable with the corresponding base and ribose. *Deoxyadenosine, deoxyguanosine, deoxyuridine* are the corresponding *deoxyribosides*.

6.3.4 Nucleotides : When one of the hydroxyl functions of the pentose in nucleosides is esterified with phosphoric acid, a mono-nucleotide is obtained. Because of the polybasic phosphoric acid, these mono-nucleotides are also acidic and have trivial names. Structures of some of the more common nucleotides are as follows :

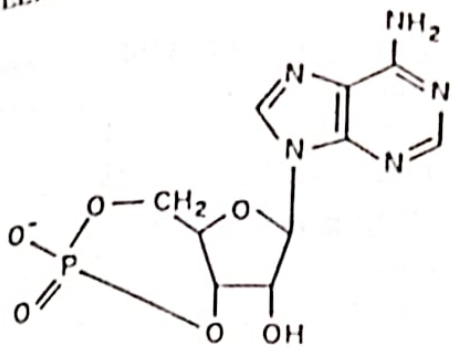


Adenosine-5'-monophosphate (AMP)
(5'-Adenylic Acid)

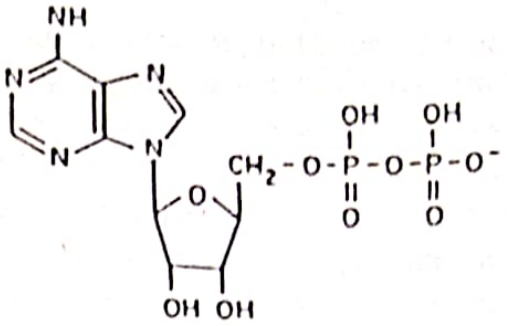


Adenosine-3'-mono-phosphate
(3'-Adenylic Acid)

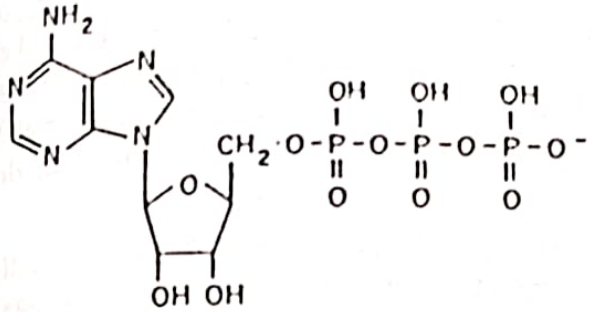
Similarly other structures for the following may be written : Guanosine-2'-phosphate, Guanosine-3'-phosphate, Guanosine-5'-phosphate, Guanosine-5'-diphosphate (GDP), GTP, UDP, UTP, CMP, CDP, CTP, TMP, TDP, TTP etc for the ribosides. With 2'-deoxy-ribose also one can write other nucleotides similarly.



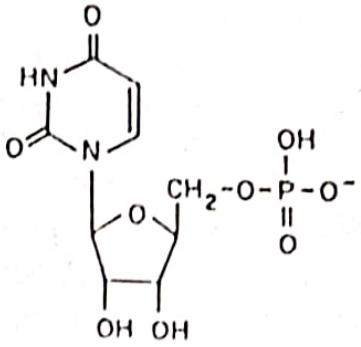
Cyclic AMP



Adenosine-5'-diphosphate

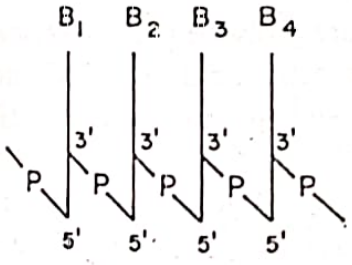


Adenosine-5'-triphosphate
(ATP)

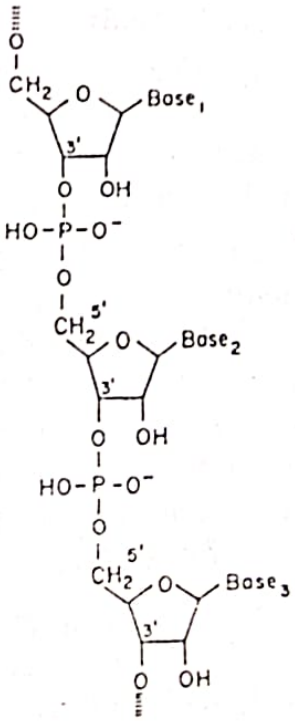


Uridine-5'-mono-phosphate
(UMP)

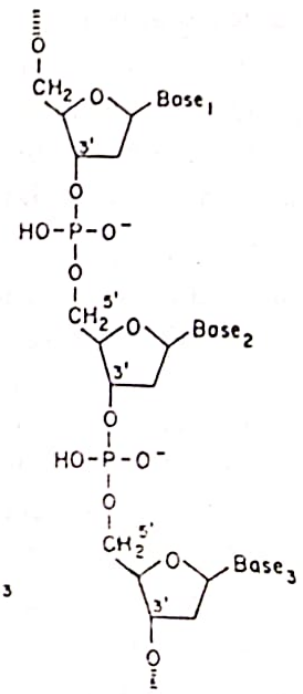
6.3.5 Nucleic acids : As seen earlier, nucleosides may be phosphorylated at positions 3' or 5' or both in ribose and deoxyribose and also at 2'



Shorthand form for
Partial Structure of
Nucleic Acids



RNA Chain



DNA Chain

in ribose. This provides an opportunity for one nucleotide to condense with another nucleotide via the 3' phosphate (usually), giving a dinucleotide. Continuing such a condensation leads to a polymer known as the nucleic acid. The general structure of these is indicated on above.

It would appear that the pentose and phosphate provide the back bone for the nucleic acids. The bases may be any of the ones mentioned earlier (with the exceptions). Each chain may be very long and strand like. This is the primary structure of nucleic acids. In the cells two strands of the DNA (probably also RNA) twist around each other to form a double helix (similar to α-helical structure of some proteins). However, the spacing of the twists and the close neighbours of bases are very specific and makes the DNA structure very unique. The double helix is stabilised by hydrogen bonds between the two neighbouring bases, one from each strand. Adenine in one strand is always found as a neighbour to thymine of the other strand.

Similarly cytosine and guanine are always paired. This is usually simplified as : A=T and C≡G indicating that there are two hydrogen bonds stabilising the A=T pair and three hydrogen bonds stabilisng the C≡G pair. This type of structure is considered as secondary structure of nucleic acid, analogous to proteins. Single stranded DNA and RNA structures are also known. In addition to linear helices, circular double stranded and super helical (coiled coils) DNA are also known.

6.4 Biosynthesis of Nucleic Acids

(1) Ribose is biosynthesized from glucose by the pentose phosphate pathway. Deoxyribose is formed from ribose by reduction, after its incorporation into the nucleotide. The ribose-5'-phosphate is converted to the active form 5-phospho-ribose-pyrophosphate (PRPP) by ATP.

(2) Pyrimidine bases of the nucleotides are obtained by a series of transformations, involving CO_2, NH_3 and aspartic acid to yield orotic acid. Condensing orotic acid with 5-phospho-ribose-1-pyrophosphate (PRPP), yields the uridine-5'-mono-phosphate (UMP). *This is the key step*, as UMP is easily converted to UTP through the mediation of ATP. Amination of UTP results in CTP. UMP is also converted to deoxy-UMP by the mediation of cyanocobalamin (vitamin B_{12}), which is then converted to TMP involving tetrahydrofolate (THF).

(3) Purine bases are obtained in a complex manner, by building up the heterocylic base on the PRPP molecule. Glutamine, glycine, aspartic acid, together with CO_2 and formate, contribute to the skeleton of the purine base. ATP and folic acid are needed for some of the steps. The *key*

purine formed is inosine-5'-monophosphate (IMP). With the involvement of aspartate and GTP, adenosine-5'-monophosphate is obtained from IMP. The IMP can also be converted to xanthine-5'-phosphate by mediation of NAD^+ and by amination to guanosine-5'-phosphate (GMP). Both AMP and GMP so formed can be reduced to the corresponding deoxy-AMP and deoxy-GMP, involving vitamin B_{12}.

(4) The polymerisation of the mononucleotides, in the form of triphosphates (*e.g.* ATP, UTP, CTP, GTP etc.) is catalysed by the enzymes *RNA-polymerase* and *DNA-polymerase*. This process takes place only inside the nucleus of the cell. Further it requires the presence of DNA primer (already formed). It has been shown that the exact sequence of RNA is dependent on the DNA primer present. The information of sequence (code) is present in the DNA primer. Similarly a new molecule of DNA is formed only when the DNA primer is present along with the DNA polymerase and a supply of needed mononucleotides. A very important fact discovered in this process was that the newly formed DNA molecule is a replica of the DNA primer.

Overview of Nucleic Acid Biosynthesis

1. Glucose $\longrightarrow$ Ribose $\xrightarrow{(ATP)}$ PRPP

2. CO_2, NH_3, Aspartic acid $\longrightarrow$ Orotic acid + PRPP $\longrightarrow$ UMP

3. UMP $\xrightarrow{(ATP)}$ UTP $\xrightarrow{(amination)}$ CTP

   UMP $\xrightarrow{(B_{12})}$ deoxy-UMP $\xrightarrow{(THF)}$ TMP

4. Glutamine, glycine, aspartic, formate, CO_2 + PRPP $\longrightarrow$ IMP

5. IMP $\xrightarrow{Asp + GTP}$ ATP

   IMP $\longrightarrow$ Xanthine-5'-phosphate $\xrightarrow[NAD^+]{(amination)}$ GMP $\xrightarrow{B_{12}}$ deoxy-GMP

   Xanthine-5'-phosphate $\longrightarrow$ AMP $\xrightarrow{B_{12}}$ deoxy-AMP

6. ATP, UTP, GTP, CTP —RNA polymerase→ Ribonucleic acids

7. d-ATP, d-UTP, d-GTP, TTP —DNA polymerase→ Desoxyribouncleic acids

This brief account of the nucleic acids is like the tip of the iceberg. Enormous amount of knowledge about the nucleic acids has accumulated in recent years. This has led to a better understanding of many diseases, which are called hereditary or congenital or genetic defects etc. Some of the defects can be corrected today by newer techniques which tend to repair the gene or the nucleic acid. Another important outcome of this vast knowledge is the development of biotechnology. With these techniques it is possible to produce any desired protein in the laboratory in adequate quantities, so that they are available for therapeutic applications. Yet another application of the knowledge of biosynthesis of nucleic acids, is the possibility of developing drugs which can interfere at specific stages of the biosynthesis of abnormal nucleic acids (as in cancer).

7

Vitamins and Coenzymes

7.1 Introduction

The term *vitamines* was coined by Funk in 1912 to a group of substances present in foods which cured diseases like *beri beri, pellagra, scurvy, rickets* etc. He believed they were all basic amino-compounds. Later it was found such substances may be neutral and some are also acids. Hence the term is now changed to *vitamin*, dropping the terminal 'e'. Other diseases called *xerophthalmia* and *night blindness* were cured by factors which were found in milk fat and other oils. This was called 'fat soluble A factor', to distinguish from the water soluble B factors occurring in the other foods. Thus subsequently discovered factors were designated by alphabet C, D, E, K etc.

A vitamin is defined as an essential nutritional factor needed in small quantities for the normal physiological and biochemical activities of the body. Diet with adequate proteins, carbohydrates and fats are unable to satisfy normal growth without these factors. Vitamins are not synthesized in our body. We are entirely dependent for these factors, on external dietary sources.

The capacity to biosynthesize such essential molecules is different in different species of living organisms. Hence a vitamin for humans may not necessarily be a vitamin for other organisms. For example many micro-organisms can synthesize some of the human vitamins. These are therefore not vitamins for them. On the other hand some other essential factors may be vitamins for them, e.g. PABA is needed by some bacteria, but humans cannot utilise this as a vitamin.

7.2 Classification

The early classification based on solubility is satisfactory and is accepted even today. These are: (a) Fat soluble and (b) Water soluble vitamins. Another possible method of classification of vitamins is based upon their chemical nature : (a) *acidic* (b) *basic* and (c) *neutral vitamins*. It is convenient to follow the first classification.

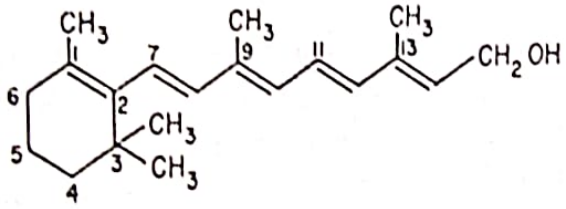
The fat soluble vitamins are A, D, E and K vitamins.

The water soluble vitamins are B complex group and C vitamins.

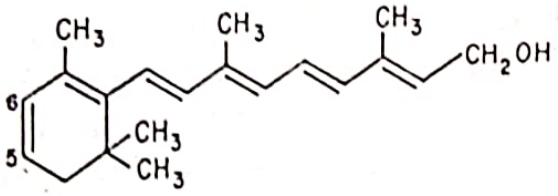
7.3 Chemistry and Role of Vitamins

7.3.1 Fat Soluble Vitamins

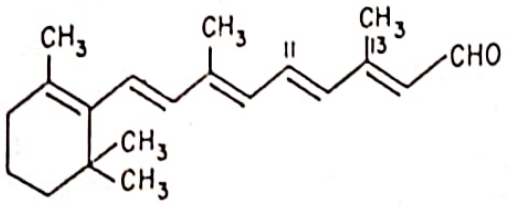
7.3.1.1 Vitamin A : Chemically it is known as *axerophthol* or *retinol*. It occurs only in animals as the active form, particularly in fish oils and milk fat. In plants like carrots, tomatoes etc. the colouring matter *carotene* (a tetraterpenoid) occurs, which can be converted to vitamin A. Hence carotene is a *precursor* of vitamin A or *provitamin A*. Carotenes have been investigated well. It has been shown that *β-carotene* yields two equivalents of vitamin A per mole. Therefore the chemistry of vitamin A is closely related to half the molecule of beta-carotene. The structures of vitamin A group of closely related molecules are shown below :



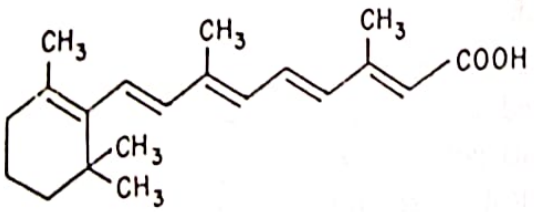
All-*trans*-Retinol
(A_1 alcohol; Axerophthol)



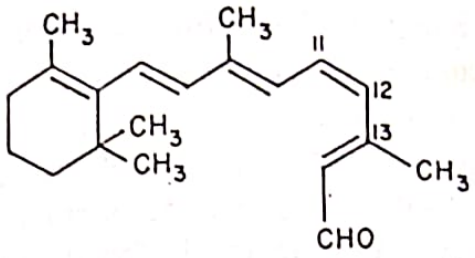
Vitamin A_2
(all-*trans*-5,6-Dehydro-retinol)



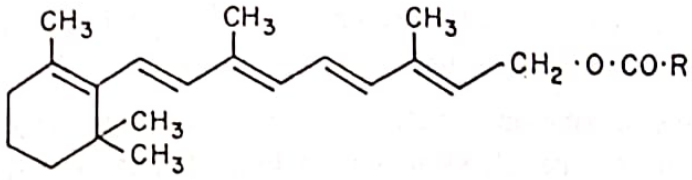
All-*trans*-Retinal



All-*trans*-Retinoic Acid



11-*cis*-Retinal



All-*trans*-Retinol Esters

Vitamin A_1 occurs in marine fish whereas A_2 occurs in fresh water fish. Vitamin A_2 possesses approximately 40% activity of vitamin A_1. All *trans*-retinal and retinoic acid possess activity equal to retinol. However, 9-*cis*, 11-*cis*, 13-*cis*, 11,13-*di-cis* and 9,13-*di-cis* isomers are also known but are less active than the all *trans*-isomer. Vitamin A is stored in the liver as its palmitate ester. To facilitate its transport, it is rendered more polar as its phosphate. Vitamin A group is susceptible to oxidation by oxygen. Therefore anti-oxidants are added to vitamin A preparations. The sources of vitamin A are shown in Table 7.1, at end of this chapter.

(1) The most important function of vitamin A is in vision mechanism, as it is present on the retina. A protein called opsin is also involved in the process.

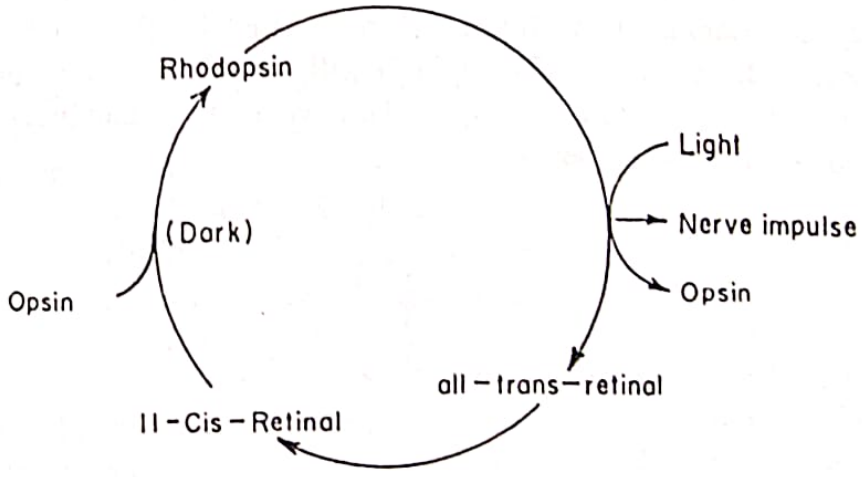


Fig. 7.1 Rhodopsin Cycle

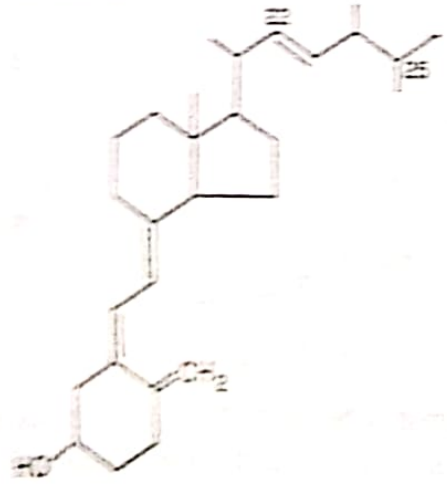
Besides this, vitamin A has been shown to be important for : (2) synthesis of some glycoproteins and mucopolysaccharides; (3) regulating growth

and differentiation, (4) mobilisation of iron from the liver to form hemoglobin, (5) protect cells from infection and cancer etc.

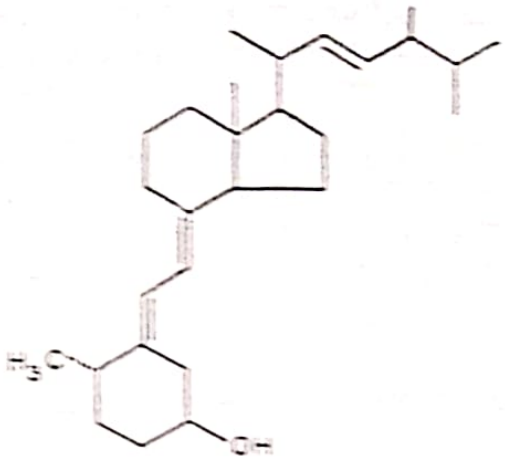
The unit of vitamin A activity is found in 0.344 micrograms of all-*trans*-retinol-acetate or 0.3 microgram of all-*trans*-retinol. The daily requirement for vitamin A is estimated between 500 and 750 micrograms for healthy adults. Deficiency of vitamin A is commonly found in undernourished children and in adults whose food habits are defective (i.e lacking all normal sources of vitamin and provitamin). The provitamins like carotenes are oxidised to vitamin A by bacteria in our large intestines, which form the source of vitamin A for us.

Symptoms of vitamin A deficiency are : (a) xerophthalmia, (b) night blindness and (c) rough skin due to hyperkeratosis (d) anemia, even when iron is available in the body. Excessive intake of vitamin is known to cause toxic reactions. Symptoms of hypervitaminosis A are : bone pain, dermatitis, enlargement of liver and spleen, nausea, diarrhoea etc. The Indian Pharmacopoeia includes two preparations containing Vitamin A, concentrated solution of vitamin A and concentrated solution of vitamin A and D. Fish liver oils with high content of vitamin A are produced on commercial scale. It is also produced synthetically from citral found in lemon grass oil.

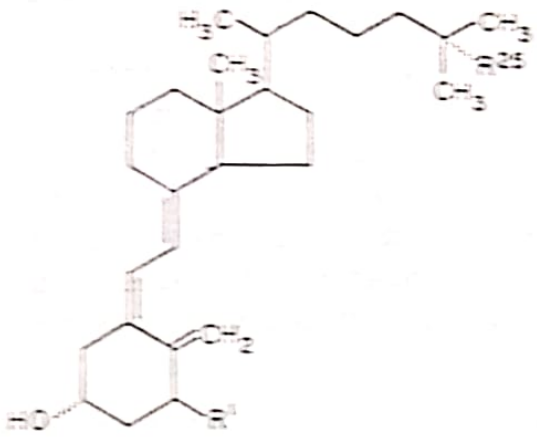
7.3.1.2 **Vitamin D** : Chemically it is known as *calciferol*. The vitamin D also is represented by a group of closely related chemicals. The vitamin D group also occur in animal fats like fish oils, milk fat, liver fat, eggs etc. Plants produce sterols like *ergosterol and stigmasterol* which can form *precursors* of vitamin D, in the laboratory. Small amounts of *cholesterol* are converted to vitamin D by exposure to sunlight. Deficiency of vitamin D in children is recognised by the condition called rickets. Administration of vitamin D will prevent and even cure rickets. Hence it is also known as *anti-rachitic* vitamin. Vitamin D group of compounds are shown below :



Ergocalciferol (Vitamin D_2)



Dihydrotachysterol



Cholecalciferol (Vitamin D_3) : $R^1 = R^{25} = H$
Calcifediol : $R^1 = H : R^{25} = OH$
Alfacalcidol : $R^1 = OH : R^{25} = H$
Calcitriol : $R^1 = R^{25} = OH$

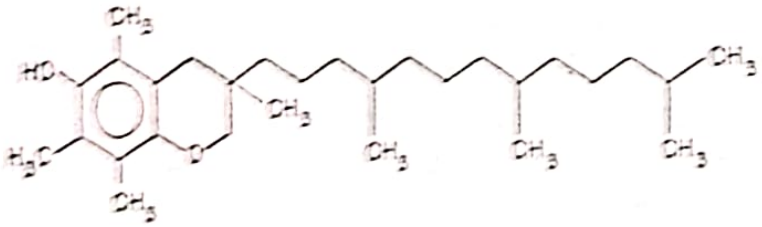
Ergocalciferol is obtained when ergosterol (from yeast) is irradiated with ultra-violet rays. It is called vitamin D_2. (Vitamin D_1 was a mixture of active and inactive compounds. The name is now not used). When cholesterol is similarly irradiated, *cholecalciferol* (vitamin D_3) can be obtained. 7-Dehydro-cholesterol gives better yields of vitamin D_3 when irradiated. There is evidence of occurrence in the body of *alphacalcidol, calcifediol and calcitriol*. All these are formed from the calciferol consumed through foods. Therefore calciferols (ergo- or chole-) are the true vitamins. Stigmasterol, an abundant plant sterol can similarly be converted to *stigma-calciferol* (vitamin D_4). The reduction of the double bond at C-22 in the side chain of ergo-calciferol yields *dihydroergocalciferol* (D_5). *Dihydrotachysterol* is obtained by reduction of methylene group of ring A in tachysterol, which is obtained as bye-product of ergosterol

irradiation. Calciferol is found in circulation, whereas calcitriol is generally found in renal tubules. The natural sources of calciferols are usually the fish oils and milk fat.

The most important function of the calciferols is absorption of calcium from foods, its transport and deposition in bones (*calcification* or *bone mineralization*). They are in concert with *parathyroid hormone*. The demineralization of bones (*bone resorption*) and excretion of calcium are also regulated by the calciferols. In this form, they may be considered as hormones (like steroid hormones) rather than as vitamins. Each milligram of calciferol contains approximately 40000 units of vitamin (antirachitic) activity.

The daily requirement of vitamin D is about 200 units for healthy adults. Deficiency is rare, except in malnourished children and those not exposed to sunlight. Vitamin D deficiency may cause rickets (bow or knock legs in children, **hypocalcaemia**, **hypophosphataemia**, soft bones, **bone pain** (**osteomalacia**) and related conditions. Vitamin D preparations are used in all the above deficiency conditions and also in **hypoparathyroidism**. Excessive administration of vitamin D leads to **hypercalcaemia** and related conditions. Concentrated solution of vitamin D and A is official in Indian Pharmacopoeia. Hydrogenated oils and milk foods are required to contain minimum recommended quantities of both vitamins A and D.

7.3.1.3 **Vitamin E** : This is also known as *α-tocopherol*. It occurs in vegetable oils, particularly wheat germ oil, sunflower oil and cotton seed oil. It also occurs in most cereals and egg yolk. Its structure is shown below :

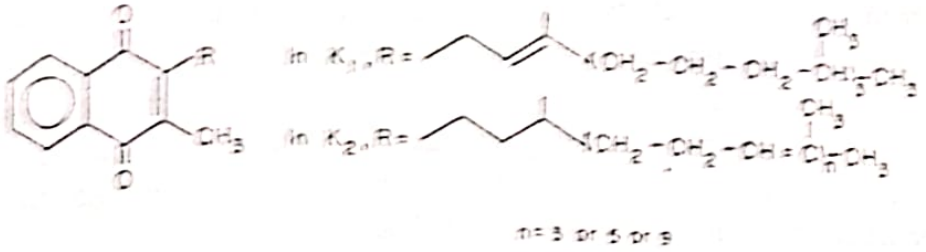


dl-α-Tocopherol

Vitamin E is a powerful anti-oxidant, which is soluble in oils. It is slightly yellow in colour, imparting this colour to the vegetable oils in which it occurs. It is not destroyed by cooking. The racemic mixture (*dl*) of the various possible optical isomers is generally employed. Its role in human biochemistry is not fully understood. In experimental animals, induced deficiency causes **protein deficiency, neurological disorders, sterility and muscular dystrophy**. However its benefit in humans with

similar conditions, has not been well established. It is believed to have a role in maintaining a suitable redox state in muscles and metabolic reactions. In spite of incomplete evidence it is used in conditions like **haemolytic anemia, sickle cell anemia, intermittent claucidation, sterility, muscular dystrophy, neurological disorders** etc. There is no daily requirement, as most food materials contain adequate amounts of it. Its requirement increases with diets containing high proportion of unsaturated oils and fats. One unit of activity is contained in 1 mg of α-tocopherol. Vitamin E is also used as an anti-oxidant in some oily pharmaceutical preparations.

Vitamin K : This is also known as *phytonadione* or *phytomenadione* or *phylloquinone* or vitamin K_1. These occur in plants. In some animal fats and bacteria closely related vitamin K_2 or *menaquinones* occur. Their chemistry is shown below :

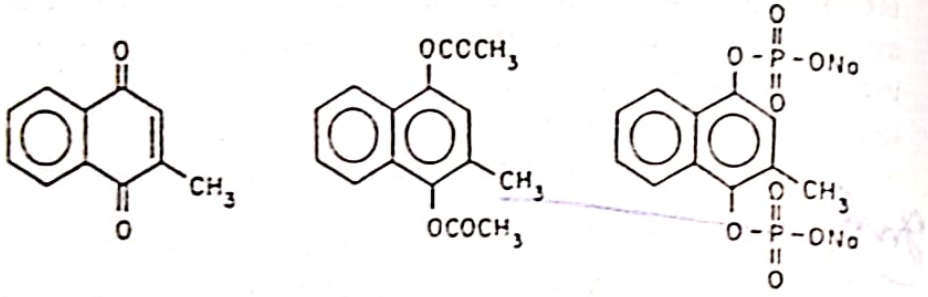


Vitamin K

Vitamin K_1 is well distributed in plants. Cabbage, spinach and cereals are rich in K_1. Animal foods like liver, milk and egg yolk also contain adequate amounts of K_1. In the large intestines, bacteria produce menaquinones, which are absorbed easily and satisfy vitamin K requirements. It is usually stored in the liver. The daily requirement is estimated as about 2 micrograms per kg body weight.

Vitamin K is essential for the biosynthesis of prothrombin and other blood clotting factors (factors VII, IX and X) in the liver. It is specifically involved in causing activation of pre-prothrombin to prothrombin. Vitamin K deficiency is rare. Dietary vitamin K is absorbed from the intestines with the help of bile acids. *Malabsorption* of fats and lack of ability to absorb K_2 from large intestines are the only reasons for vitamin K deficiency. In infants, where the intestinal flora are not yet established (therefore poor availability of K_2), there is considerable danger of deficiency. Vitamin K deficiency causes increased coagulation time (i.e. **delayed coagulation**) with the risk of **hemorrhage** and **bleeding**. This risk is also great in prolonged therapy with broad spectrum antibiotics, which destroy most of the intestinal flora.

Synthetic analogs of vitamin K have been found to possess equal activity. These are used in place of natural vitamin K, for corrective therapy.



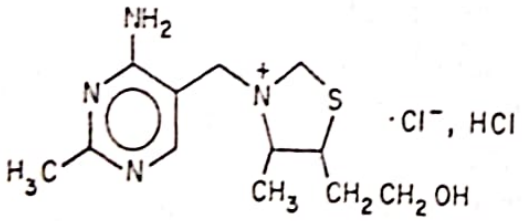
2-Methyl-naphthaquinone (Menadione) | Acetomenaphthone | Menadiol Sodium Phosphate

Menadione and acetomenaphthone are fat soluble, like the natural vitamins, and are dependent on fat absorption. Menadiol-sodium phosphate is highly soluble in water. It can be absorbed from the intestine even when fat absorption is not satisfactory. Moreover, it can be made into aqueous injections for parenteral administration. Menadione and acetomenaphthone are official in Indian Pharmacopœia.

7.3.2 Water Soluble Vitamins

Vitamins B group

7.3.2.1 Vitamin B_1 : This is also known as *thiamine* or *aneurine*. This was first obtained from rice polishings. It is basic, forms salts, soluble in water and insoluble in oils. Its structure is shown below :



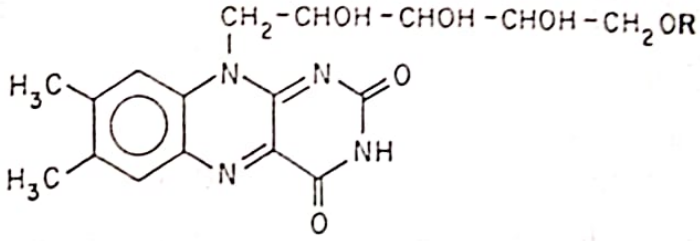
Thiamine chloride hydrochloride

It is sensitive to light and heat, partially destroyed by cooking. It is fairly distributed in plants. Cereals, nuts, beans, peas and yeast are rich sources of vitamin B_1. Other satisfactory food sources are : green vegetables, fruits, milk, fish, eggs and meats. Its requirement is about 1 to 1.5 mg per day for an adult. The ingestion of high carbohydrate diet may enhance the daily requirement of thiamine. Thiamine deficiency is caused by improper diet, leading to a disease called **beri-beri** or **peripheral neuritis**. **Bradycardia, muscle weakness** and **paralysis** may also be

noticed with thiamine deficiency. Severe conditions like **cardiac failure** and **demyelination** of the nerve fibres may also appear in severe deficiency. For the treatment of thiamine deficiency, both single vitamin preparations and multivitamin preparations are available. Supplementary foods also contain added thiamine. The vitamin is not stored in the body. Excess consumption does not cause any severe toxicity, but the excess will be excreted from the body in urine.

The most important function of thiamine in the body, is its participation in metabolic transformations. In the body thiamine is converted to its pyro-phosphate (TPP), which is the co-enzyme of *α-keto-acid decarboxylases*, particularly of pyruvate and α-keto-glutarate, both important steps in intermediary metabolism. It is also involved as a conenzyme of *transketolases* and *phosphoketolases*. Deficiency of thiamine leads to blockade of these metabolic paths and the substrates accumulate, causing the various symptoms.

7.3.2.2 Vitamin B_2 : This is also known as *riboflavine* or *lactoflavine*. This was first obtained from milk, later from egg yolk, rice polishings etc. It is coloured yellow (hence the name flavine). It is sparingly soluble in water, almost neutral and stable to heat treatment (not destroyed during cooking). Its structure is as follows :



R = H = Riboflavine

R = −P(=O)(−OH)−O = Phosphoriboflavine

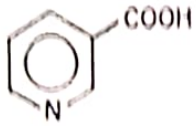
Riboflavine and Riboflavine phosphate

It is found in some green vegetables, yeast and many animal products like milk, cheese, yeast, egg yolk, liver and kidney. Its daily requirement for healthy adults is about 1.5 mg. It is well absorbed from foods, but not stored in the body. Excess intake will result in excretion through kidney, urine being coloured yellow. Riboflavine deficiency causes **ariboflavinosis**, characterised by **dermatitis**, **alopecia**, **cheilosis**, **glossitis**, **stomatitis**, particularly of the nose and ano-genital regions. **Burning eyes**, **photophobia**

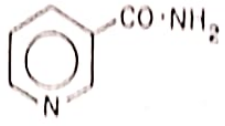
and **corneal vascularisation** are also noticeable. These conditions usually occur along with other vitamin deficiency diseases like pellagra and other dermatitis states, as the same food sources contain those vitamins also. Deficiency often occurs in chronic alcoholics. Deficiency is treated by administering riboflavine sodium phosphate along with other vitamins. Hence 'B' complex preparations are often marketed.

Riboflavine is converted in the body to riboflavine-5'-phosphate or FMP (*Flavine-mono-phosphate*) also known as *flavine mononucleotide*. FMN is further converted to *flavine-adenine-dinucleotide* (FAD). Both these are co-enzymes involved with many dehydrogenases, reductases and oxidases (e.g. *succinic dehydrogenase, nitrate reductase, xanthine oxidase*) essential for a number of oxidation-reduction biotransformations and cellular respiration, which yield energy. The flavine co-enzymes are strongly bound to their apoenzymes. Such proteins are called *flavoproteins* (e.g. D-amino acid oxidase enzyme).

7.3.2.3 Niotinic acid : Both *nicotinic acid* and *nicotinamide* belong to the B group although no number is given to these. They are also known as PPF (*Pellagra preventive factor*). The structures of these compounds are :



Nicotinic Acid
(Niacin)



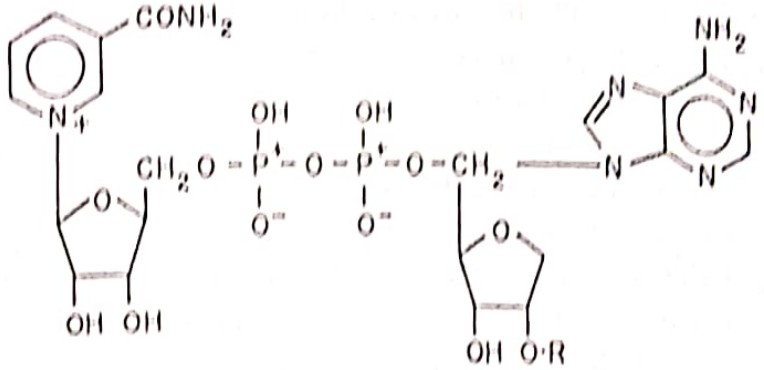
Nicotinamide
(Niacinamide)

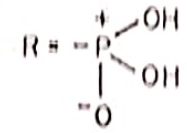
Niacin is acidic whereas nicotinamide is neutral. The acid is soluble in water and the amide is freely soluble. Dilute alkaline media dissolve niacin very easily. There is no appreciable loss of niacin during cooking. Niacin and *niacinamide* occur in many foods : green vegetables, potatoes, yeast, fish, meat etc. A small quantity may also be formed in the body through degradation of the essential amino-acid tryptophan. The human daily requirement is recommended to be about 15 to 20 mg of niacin.

Deficiency may be rare, but may be caused in conditions which cause deficiency of other 'B' vitamins, i.e. imbalanced diet, starvation, malnutrition etc. Niacin is well absorbed from foods and well distributed in the body. Excess intake will result in excretion in urine. In deficiency conditions, it is administered orally along with other vitamins of the B group.

Niacin is essential for many oxidation reduction biotransformations in the body like the flavine co-enzymes. Niacin and niacinamide are part of

co-enzymes and are associated with a number of dehydrogenases and reductases (e.g. *alchol dehydrogenase, lactic dehydrogenase, quinone reductase* etc). There are two co-enzymes derived from the niacinamide : (1) *nicotinamide-adenine-dinucleotide* (NAD'). This was earlier known as *coenzyme I* and DPN (*Diphosphopyridine-nucleotide*). These names are not used any longer. (2) *Nicotinamide-adenine-dinucleotide-phosphate* (NADP'). This was earlier known as *coenzyme II* or TPN (*Triphosphopyridine-nucleotide*). These names are also redundant. Their structures are shown below :



R = H in NAD^+ ; R =  in $NADP^+$

NAD' and NADP'

NAD' may be remembered as nicotinamide-ribose-phosphate-phosphate-ribose-adenine. Both NAD' and NADP' are capable of accepting hydrogen to give the uncharged and reduced forms written as NADH and NADPH. In a few biotransformations like reduction of glutathione, nitrate and cytochrome C, both the NADP and FAD coenzymes are involved successively. The following example illustrates this :

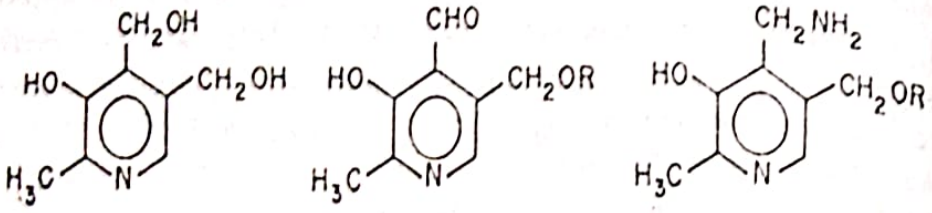
$$\text{Malate} + \text{NAD}^+ \longrightarrow \text{Oxalacetate} + \text{NADH} + \text{H}^+$$

$$\text{NADH} + \text{H}^+ + \text{FAD} \longrightarrow \text{NAD}^+ + \text{FAD.H}_2$$

$$\text{FAD.H}_2 + \text{Cytochrome.(Fe}^{+++}\text{)} \longrightarrow \text{FAD} + \text{Cytochrome.(Fe}^{++}\text{)}$$

Thus many oxidation-reduction reactions, especially in the respiratory chain, are dependent on the adequate supply of the vitamins. As mentioned earlier, deficiency of niacin vitamin causes **pellagra** with symptoms like **skin lesions, hyperpigmentation, hyperkeratinisation** (together called **dermatitis**). For treatment nicotinamide is preferred, because nicotinic acid also produces **vasodilatation**.

7.3.2.4 Vitamin B_6 : This is also known as *pyridoxine*. Three closely related structures are equated as vitamins B_6.



Pyridoxine Pyridoxal (R = H) Pyridoxamine (R = NH_2)

It is basic, forms salts like hydrochloride and soluble in water. It is not appreciably destroyed in cooking. It is widely distributed in foods like vegetables, fruits, cereals, eggs, fish, liver, meat etc. The daily requirement is about 2 mg for healthy adults. It is also well absorbed from foods. Deficiency of pyridoxine is rare in human. It may occur in conditions which may lead to deficiency of other B group vitamins. Pyridoxine deficiency may be induced by drugs as in iso-niazid therapy and some other drugs. The deficiency causes **peripheral neuritis, metabolic disorders** and **dermatoses** different from those due to deficiency of other B group vitamins. In such conditions pyridoxine hydrochloride alone or along with other B group vitamins, is administered. Excessive administration of pyridoxine causes severe **neuropathies**.

Pyridoxine participates in intermediary metabolism as a coenzyme, in the form of phosphate of pyridoxal or pyridoxamine (R = $-PO_3H_2$ in structure given above), together with numerous enzymes. It is most active in :

(1) amino-acid metabolism in *transmination* reactions (interconversion of amino-acids), including glutamate-oxalacetate-transmination (GOT), glutamate-pyruvate-transmination (GPT);

(2) amino-acid *decarboxylation* reactions like tryptophan, tyrosine, histidine decarboxylations;

(3) *energy release* from amino-acids;

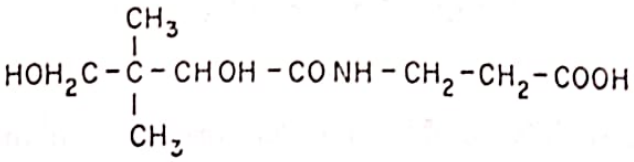
(4) in the *biosynthesis of heme*; and

(5) *conversion of glycogen to glucose-1-phosphate* through *glycogen phosphorylase* and many other biotransformations.

7.3.2.5 Pantothenic acid : This is extremely wide spread in nature, in all living organisms. Hence it must be considered as a very essential factor in life. At the same time, because of its extensive availability, no deficiency of the vitamin occurs in human beings. Only in excessive antibiotic therapy this may be needed. It is absorbed well from food sources and does not cause any toxic symptoms. Yeast, legumes, cereals,

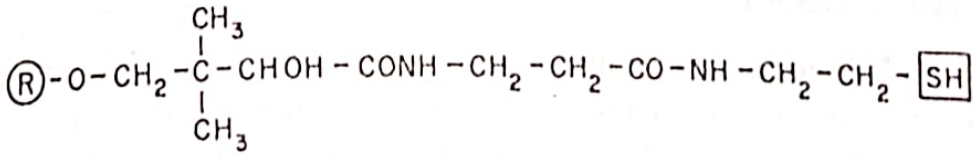
fruits, eggs, milk etc. are rich sources. In rare cases of pantothenic acid deficiency, no well defined symptoms are known. But the conditions may resemble those of deficiency of other B group vitamins. No daily requirement is recommended, but about 5 mg per day is easily available from normal foods.

Pantothenic acid is acidic, soluble in water and forms salts. For convenience *calcium pantothenate* is employed as a source of pantothenic acid. Its structure is as follows :



Pantothenic Acid

The amide of the vitamin and its reduction product *panthenol* are also active. Pantothenic acid is converted in the body to *coenzyme A (acylation coenzyme)*, by linking to 2'-phospho-adenosine diphosphate (R in the structure below) at the primary alcohol and to β-alanyl-cysteamine at the carboxyl group. The relevant part of the coenzyme A molecule is shown below :



Coenzyme A

(R = 2'-phospho-adenosine-diphosphate)

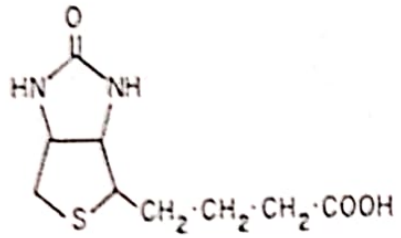
In the thiol form it is usually written as CoA-SH. the thiol group links to acyl groups thus :

Co-S-CO-R

Acyl thioester of coenzyme A

In this manner coenzyme A is the only acyl group transferring factor in the body. More than 70 enzymes active as acyl group transferases, utilise coenzyme A as co-factor. Fatty acid, carbohydrate and protein metabolisms, tri-carboxylic acid members etc. are all biotransported with the participation of coenzyme A. Note that pantothenic acid is only a small part of the large molecule of coenzyme A.

7.3.2.6 Biotin : This is a factor which is extensively distributed in normal foods. Groundnut, egg yolk, liver, yeast are excellent sources for this vitamin. Its deficiency occurs very rarely, as in prolonged antibiotic therapy. Biotin structure is shown below :

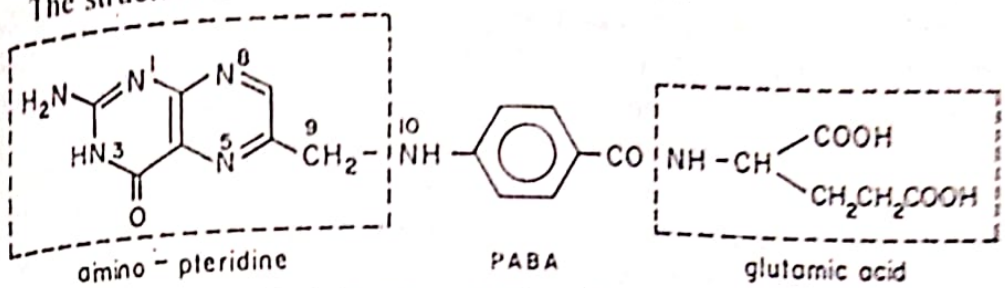


Biotin

It is acidic, slightly soluble in water, insoluble in oils and organic solvents. It normally occurs in conjugation with proteins, to which it is strongly bound. In very rare cases of its deficiency, symptoms like **dermatitis, alopecia, muscular pain** etc. may be noticed. No daily requirement for human beings has been recommended, since it is adequately available from natural foods. Its major function in the body is as a coenzyme in carboxylation reactions (i.e. CO_2 added to certain molecules to convert them to carboxylic acids). *Pyruvate carboxylase* and *acetyl-CoA-carboxylase* are two enzymes with which biotin coenzyme is definitely associated.

7.3.2.7 Folic acid : It is also known as *pteroyl-glutamic acid* (PGA). It is well distributed in nature. Leafy green vegetables (from which its name is derived) like spinach, nuts, yeast and animal foods like liver and kidney contain considerable amounts of readily absorbable folic acid. In the large intestines bacteria biosynthesize closely related pteroyl-polyglutamic acids, from which PGA is formed by deconjugation prior to absorption. Under normal conditions therefore folic acid deficiency does not occur. However, folic acid deficiency is well known due to (a) inadequate dietary intake (b) malnutrition (c) malabsortpion (d) greater demand during pregnacy and hemolytic anemia and (e) excessive administration of drugs which are folate antagonists. From food it is well absorbed except in patients suffering from malabsorption. It is stored in liver upto about 10 mg. It is usually recycled and only small amounts are excreted in urine. Its daily requirement is recommended as about 400 micrograms for an adult. Folic acid is destroyed during cooking and also deteriorates on exposure to air. Deficiency of folic acid causes **megaloblastic anemia**. Folic acid administration in this condition must be carefully balanced with concurrent administration of vitamin B_{12}.

The structure of folic acid is as shown below :



Folic Acid (Pteroyl Glutamic Acid; PGA)

It is acidic, insoluble in water, but soluble both in alkaline and acidic media. It is sensitive to heat, ultraviolet light and atmospheric oxygen. The complex molecule consists of amino-pteridine linked to para-amino-benzoic acid (PABA), which is linked to glutamic acid. PGA is converted in the body to 5, 6, 7, 8-*tetra-hydrofolic acid* (THF or FH_4), through initial formation of 7, 8-dihydrofolic acid (FH_2). These reactions are catalysed by enzymes as shown below :

$$\text{Folic acid} \xrightarrow{\textit{folate reductase}} \text{Dihydrofolic acid } (FH_2)$$

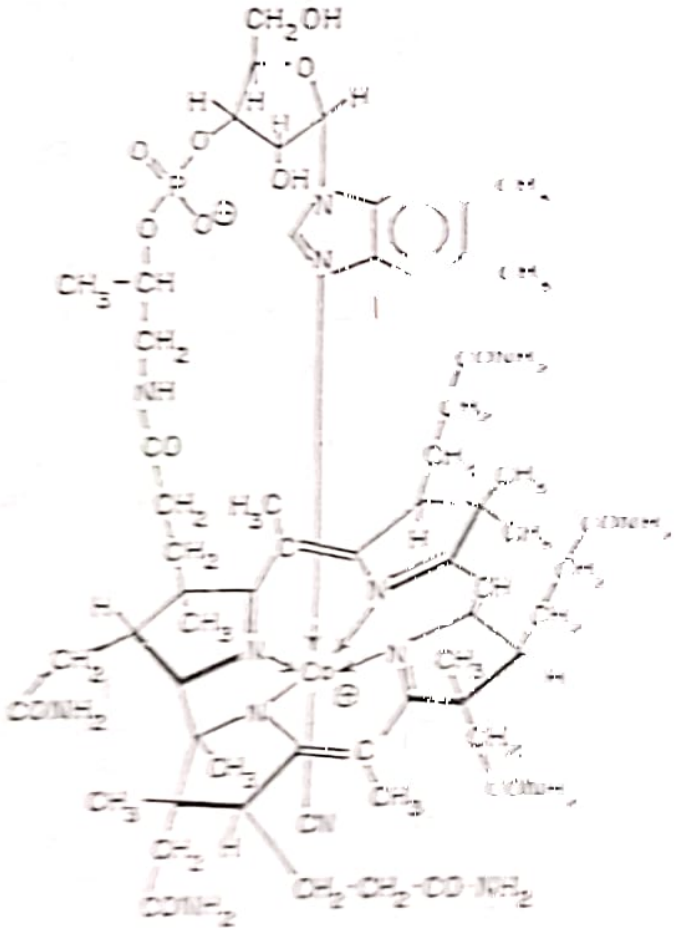
$$\text{Dihydrofolic acid} \xrightarrow{\textit{dihydrofolate reductase}} \text{Tetrahydrofolic acid } (FH_4)$$

In both cases NADPH is involved. The FH_4 or THF is the actual coenzyme for effecting 1-C transfers (one carbon units) from formic acid. The biosynthesis of purines and pyrimidines, which are part of DNA, requires the THF coenzyme. Formation of serine, glycine, choline and methionine is also regulated by THF. These may indirectly influence porphyrin biosynthesis. Megaloblastic anemia is the result of lack of the proper DNA in folic acid deficiency.

Many drugs have been developed which interfere with the formation of THF from folic acid. These inhibit either of the enzymes indicated above. Such folate antagonists are used as anti-cancer drugs (e.g. *methotrexate* and *aminopterin*), diuretics (*triamterene*) etc.

7.3.2.8 Vitamin B_{12} : This is also known as *cyanocobalamin* or *cobamin*. The name B_{12} also includes closely related compounds like *hydroxocobalamin*. This is the only vitamin containing a metal atom, cobalt, in its molecule. Moreover, it is a large molecule, with the molecular formula $C_{63}H_{88}CoN_{14}O_{14}P$ and mole mass 1355. The structure given alongside reveals the complexity of the molecule. It is a red coloured crystalline substance, soluble in water, insoluble in oils and organic solvents. It is not affected by heat, but is affected by light.

Cobamin is found only in animal foods like meats, fish, liver, kidney, eggs and milk. It is not found in plants. Some micro-organisms are capable of producing this vitamin. Daily requirement of cobamin is about 2 to 3 micrograms for an adult. Cobamin is stored in liver and recycled. A well stocked liver may supply cobamin for almost six years. Partly it may be excreted in urine rapidly after administration. Deficiency occurs in persons, who are exclusively on vegetarian diet (i.e. without even milk and egg products). Absorption of B_{12} from intestines requires an *intrinsic factor*. Individuals may suffer from malabsorption of B_{12} because of lack of intrinsic factor. A number of drugs also affect absorption of B_{12}. Surgical removal of parts of gastrointestinal tract (e.g. *gastrectomy*) also leads to deficiency of B_{12}. In all such cases administration of cobamin becomes a necessity. For pharmaceutical purposes vitamin B_{12} is obtained by fermentation method from some bacteria or *streptomyces* species.

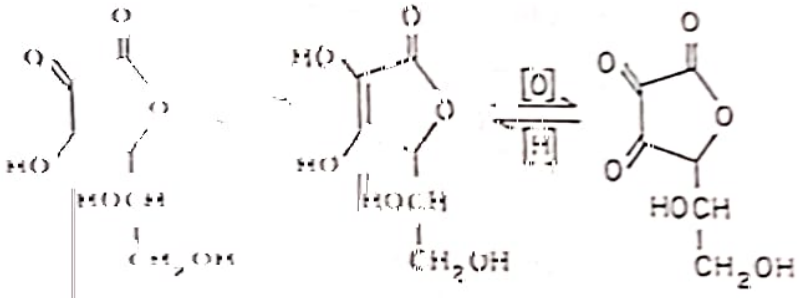


Cyanocobalamin (Vitamin B_{12})

Deficiency of B_{12} causes megaloblastic anemia and neurological disorders, similar to the deficiency of folic acid. When intrinsic factor is missing, a specific condition called pernicious anemia is caused. Deficiency is treated with administration of B_{12} with or without folic acid and

in doses regulated by conditions of the deficiency symptoms. Hydroxocobamin (which has -OH group in place of -CN group of cyanocobalamin) also occurs in liver. In the body other related products like methylcobalamin and adenosylcoabalamin also occur. These two appear to act as coenzymes in the biosynthesis of DNA. Just like folic acid, cobamin is also involved in 1-C transfer reactions particularly in conversion of homocysteine to methionine and methyl-malonyl-coenzyme A to succinyl-coenzyme A. Cobamin is also needed to regenerate tetrahydrofolic acid from N5-methyl-THF.

7.3.3 Vitamin C This is also known as *ascorbic acid.* It has the chemical structure as shown below :



Ascorbic Acid Dehydroascorbic Acid

Chemically ascorbic acid is known as the enolic form of 3-keto-L-gulo furano lactone. This is highly water soluble crystalline substance, with an acidic taste. In dry condition it is stable, but must be protected from oxygen of atmosphere, moisture and light. Solutions deteriorate rapidly. Cooking destroys the vitamin considerably. Sodium ascorbate is also used beside the acid itself in pharmaceutical products like tablets and capsules.

Ascorbic acid is not biosynthesized in human body, but some animals and many plants have the ability to do so and are not in need of this vitamin from outside sources. Daily adult human requirement is estimated to be about 30 to 50 mg. This quantity is available from most fresh fruits (particularly citrus) and vegetables. Milk and animal foods contain small amounts. Gooseberry (or *amla*) and chillies are rich sources of ascorbic acid. It is readily absorbed and well distributed in the body. Deficiency is rare, but may occur in individuals, who may be consuming no fresh vegetables and fruits. It is also recommended in large doses (mega doses) of 1 to 4 gm per day in certain conditions like colds and flu. Deficiency causes a disease called scurvy. The name ascorbic is derived from anti scorbutic meaning preventing scurvy. Its symptoms are characterized by bleeding, small hemorrhages, slow wound healing, osteoporosis and anemia. In all such conditions oral administration of

ascorbic acid is highly beneficial. Excess of ascorbic acid is excreted in urine, unchanged or as oxalate.

It has two major functions in the body : (1) *biosynthesis of collagen and intercellular material*; and (2) *maintaining a suitable redox potential* in cells and tissues as needed. Faulty or inefficient formation of collagen affects capillary walls (leading to *capillary fragility*), organic matrix in bones (leading to *osteoporosis*) and improper maintenance of connective tissue (leading to *slow wound healing*). Thus all the symptoms of scurvy are a result of the improper collagen formation. Ascorbic acid is a powerful reducing agent, itself getting oxidised to dehydro-ascorbic acid, which can be reversibly reduced to ascorbic acid. A suitable mixture of these two, maintains the needed redox potential in a tissue to allow the action of other substances to go on smoothly. Some of the examples are :

Reduction of ferric to ferrous state in the stomach before absorption; aiding conversion of folate to tetrahydrofolate; preventing oxidation of vitamins A, E and some of B group; as anti-oxidant in the adrenal gland, etc.

For pharmaceutical purposes, ascorbic acid is manufactured in large quantities from glucose. Ascorbic acid is estimated by using standard solution of iodine or a standard solution of 2,6-dichlorophenol indophenol.

7.3.4 Minor Vitamins

The possible necessity of some other food factors have been reported. Some such "vitamins" are ; *inositol* and *choline* grouped under the B group and *vitamin P*, which also is water soluble. Vitamin P is a name given to closely related *bio-flavonoids*, particularly *rutin* and *hesperidin* occurring in citrus fruits. Their role is implicated in **capillary resistance** and **permeability**. However, their essential role has not been established *Inositol* is implicated in glucose metabolism as essential. However, it is widely distributed and deficiency symptoms have not been demonstrated. Choline is precursor of acetylcholine, which is an important **neurotransmitter**. Choline donates methyl groups in some 1-C transfer reactions. However, its deficiency has not been demonstrated.

7.3.5 Multivitamin preparations

A very large number of multi-vitamin preparations are marketed. There are several important aspects to be considered in connection with multi-vitamin products. These are discussed below :

(1) **Solubility** : All liquid preparations have to be made by very special techniques because the fat soluble vitamins cannot be simulta-

neously present with water-soluble vitamins. One technique is to emulsify the oil part containing the fat soluble vitamins in the aqueous medium containing B group vitamins. Another method is to chemically convert the vitamins into water soluble derivatives.

(2) **Incompatibility** : As already noted some B vitamins are bases and some other are acids. These may neutralize each other in preparations. At pH above 7.0 (alkaline) the basic vitamins may be precipitated. Hence a buffering medium may be needed. Similarly, reducing and oxidising properties of the vitamins may affect each other.

Thus liquid orals and injections suffer from technical problems of preparation. In addition they also suffer from deterioration of some of the vitamins.

(3) **Overages** : To keep the potency for the life time of product, usually higher amounts of vitamins are actually added than declared on the label. This excess (overage) is expected to compensate for the loss that may occur. However, it adds to the cost of the preparation. The water soluble vitamins are excreted, if present in excess of requirement. But the fat soluble vitamins are stored in the body and cause toxicity, particularly vitamins A and D. Excess of vitamins may also be due to intake of vitamin rich foods while undergoing multivitamin therapy.

(4) **Stability** : Multi vitamin preparations usually require addition of stabilizing agents. They are also to be protected from light and moisture (if dry products like tablets or capsules). Amber coloured containers are to be preferred. Tablets and capsules in air-tight containers and with added desiccant (like silica gel, in cloth bags) keep well. These do not keep well in strips, which are generally not air and moisture tight.

(5) **Requirement** : The deficiency of one vitamin cannot be cured by other vitamins. Thus in certain anemias only one or two vitamins may be needed. Administering multi vitamins is not only a waste and expensive, but may also cause undesirable side reactions in some patients.

TABLE 7.1 **Vitamins : Summary**

Vitamin	*Name(Daily Adult Requirement)*	*Solubility*	*Food Sources*	*Deficiency Diseases or Symptoms*	*Pharmacopœal Products (IP' 85)*	*Potency/ Usual Strength**
A	Axerophthol/Retinol (500-750 mcg)	Fats	Fish liver oils, milk fat; carrots & tomatoes (Provitamin A)	Xerophthalmia, night blindness, hyperkeratosis, anemia	1. Vitamin A (oil or solid)	30000 u/gm
					2. Concentrated Vit. A. soln	5000 u/gm
					3. Shark liver oil	6000 u/gm
					4. Dilute shark liver oil	1000 u of A and 100 u/gm of D
					5. Shark liver oil with vit. D.	6000 u/of A and 1000 u of D/gm
					6. Conc. vit A and D solution	50000 u of A and 5000 u of D/gm
					7. Vit. A and D capsules	As per label
D	Calciferol (200 u)	Fats	Fish liver oils, milk fat, eggs; Yeast (Provitamin)	Rickets, hypocalcemia, hypophosphatemia osteomalacia	1. Cholecalciferol	40000 u/mgm
					2. Ergocalciferol	40000 u/mgm
					3. Conc. Vit. D. solution	10000 u/gm
					4. Dilute shark liver oil	100 u of D and 1000 u of A/gm
					5. Shark liver oil with Vit. D.	1000 u of D and 6000 u of A/gm
					6. Conc. Vit. A and D solution	5000 u of D and 50000 u of A/gm
					7. Vit. A and D caps.	As per label

(...continued)

Vitamin	*Name(Daily Adult Requirement)*	*Solubility*	*Food Sources*	*Deficiency Diseases or Symptoms*	*Pharmacopœal Products (IP' 85)*	*Potency/ Usual Strength**
E	α-Tocopherol (not established)	Fats	Vegetable oils, cereals, egg yolk	Protein deficiency, neurelegical disorders, muscular distrophy	1. Tocopheryl Acetate	1 u/mgm
K	Phytonadione (2 mcg/Kg body wt.)	Fats	Leafy vegetables, cereals, egg yolk, milk	Delayed coagulation time (risk of hemorrhage and bleeding)	1. Menadione 2. Acetomenaphthone 3. Acetomenaphthone tablets	Pure synthetic product Pure synthetic product
B_1	Thiamine/ Aneurine Hydrochloride (1-1.5 mg)	Water (Freely)	Cereals, nuts, beans, yeast, fruits, milk, fish	Polyneuritis (beri-beri), bradycardia, muscle-weakness	1. Thiamine hydrochloride 2. Thiamine hydrochloride injection 3. Thiamine hydrochloride tablets 4. Thiamine mononitrate	Pure synthetic product 100 mg/ml 5 or 10 or 50 mg Pure synthetic product
B_2	Riboflavine/ Lactoflavin (1.5 mg)	Water (slightly)	Green vegetables, yeast, milk, egg yolk, liver, kidney	Aribeflavinosis, dermatitis, alopecia, glossitis, stomatitis	1. Riboflavine 2. Riboflavine tablets 3. Riboflavine phosphate sod.	Pure synthetic product 2 mg or 5 mg 1.4 mg = 1.0 mg riboflavine.
Niacin	Nicotinic acid/ Nicotinamide (15-20 mg)	Water (sparingly/ freely)	Green vegetables, potatoes, yeast, fish, meat	Pellagra, dermatitis	1. Nicotinamide 2. Nicotinamide tablets 3. Nicotinic acid	Pure synthetic product 50 mg Pure synthetic product

(...continued)

Vitamin	Name(Daily Adult Requirement)	Solubility	Food Sources	Deficiency Diseases or Symptoms	Pharmacopeial Products (IP '85)	Potency/ Usual Strength*
B_6	Pyridoxine hydrochloride (2 mg)	Water (freely)	Vegetables, fruits, cereals, eggs, fish, liver, meat	Neuritis, dermatoses, metabolic disorders	1. Pyridoxine hydrochloride	Pure synthetic product
					2. Pyridoxine tablets	5 mg
Panthenol	Pantothenyl alcohol (not established)	Water (completely)	Yeast, legumes, cereals, fruits, eggs, milk	Metabolic disorders; (dermatitis)	1. D-Panthenol	Pure synthetic product
					2. Calcium Pantothenate	Pure synthetic product
Folic acid	Pteroyl glutamic acid (PGA) (400 mcg)	Water (very slightly)	Green leafy vegetables, nuts, yeast, liver, kidney	Megaloblastic anemia, metabolic disorders	1. Folic acid	Pure synthetic product
					2. Folic acid tablets	5 mg
B_{12}	Cyanocobalamin (2-3 mcg)	Water (sparingly)	Liver, kidney, fish, meat, eggs, milk	Anemia, metabolic disorders	1. Cyanocobalamin	Pure Product
					2. Cyanocobalamin injection	100 or 500 or 1000 mcg/ml
					3. Hydroxocobalamin	Pure Product
					4. Hydroxocobalamin injection	500 or 1000 mcg/ml
B complex	Protein + B complex (not established)	Not soluble in water	Dried yeast	As for individual vitamins	Dried yeast	45% protein; 0.3 mg niacin, 0.04 mg riboflavine, 0.1 mg thiamine
C	Ascorbic acid (30-50 mg)	Water freely	Citrus fruits, Amla, green chillies, vegetables	Scurvy, capillary fragility, slow wound healing, osteoporosis, anemia,	1. Ascorbic acid	Pure synetic product
					2. Ascorbic acid inj.	0.5g in 2 ml
					3. Ascorbic acid tabs.	50 or 100 or 500 mg
					4. Sodium ascorbate	Pure synthetic product

8

Digestion and Absorption

8.1 Introduction

Food materials are consumed in order to supply the body with (a) energy; (b) chemicals needed for body building; and for (c) growth regulation. However, these cannot be utilised directly by the body. The carbohydrates, proteins and fats of the food materials have to be first broken down to their simpler units. Thereafter they can be absorbed. Inside the body, the simpler molecules are processed as needed by metabolic steps. For this purpose the gastro-intestinal tract is considered outside the interior organs and systems. Food is consumed through the oral cavity, which is open to the outside. The breakdown of food materials is called *digestion*, which takes place in the gastro-intestinal tract, followed by absorption. The digestion of proteins, lipids and carbohydrates and the absorption of the products of digestion will be outlined briefly.

8.2 Proteins

Human diet consists of proteins in the form of animal and plant proteins. Normally less than 100 g of proteins are consumed per day by most adults. The protein digestion is efficient enough to render about 90% of this absorbable. There is no digestion in the mouth or oesophagus.

(A) Gastric digestion takes place in a highly acidic medium, the pH is in the range of 2. The acid medium is essential and is well regulated by several factors including *acetylcholine, histamine, gastrin* etc. The proteolytic enzyme *pepsinogen* is secreted into the stomach from special cells of the gastric mucosa. A polypeptide gastrin stimulates secretion of pepsinogen. This enzyme is inactive and is converted to the active *pepsin* by the low pH of the stomach or by the activity of a small amount of pepsin already present. In the activation, a part of the peptide chain of pepsinogen is removed. Pepsin is called an *endopeptidase*, meaning that it cleaves peptide bonds in the inside of the long chain of the protein

polymer. It breaks the peptide bonds involving aromatic amino-acids (like phe and tyr) and also leucine. If these acids are present at the ends of the peptide chains, free amino-acids will be formed. Normally the proteins are cleaved to oligo-peptides. The partially digested acidic *chyme* passes on to the intestines.

(B) Some of the free amino-acids, peptides and the acidic pH stimulate the duodenal lumen cells, to liberate *cholecystokinin* (or *pancreozymin*). This peptide, in its turn stimulates the secretion of pancreatic enzymes into the small intestine. Another later part of the small intestine epithelium also has cells which secrete *secretin*, when the pH is around 5 caused by the partially neutralised acidic chyme. The polypeptide stimulates the secretion of pancreatic juices with high bicarbonate, to enable neutralisation of the acid and render the medium neutral or slightly alkaline. The pancreatic peptidases are : *trypsinogen, chymotrypsinogen, proclastase, pro-carboxy-polypeptidases* A and B. All these are inactive at time of secretion. The trypsinogen is activated by an intestinal enzyme *enteropeptidase*, which removes a hexapeptide from the N-terminal end of trypsinogen moleculce to form trypsin. This is a critical step. All other pancreatic peptidases are activated by trypsin. The active enzyme formed are : *chymotrypsin, elastase* and *carboxypolypeptidase* A and B.

(1) *Trypsin* is an endopeptidase like pepsin, but cleaves the peptide bonds formed between the -COOH of arginine or lysine (both basic amino-acids) and any other amino-acid.

(2) *Chymotrypsin* also is an endopeptidase, acting on peptide bonds involving the -COOH of tyrosine or tryptophan or phenylanine or leucine or methionine. In this respect it is almost like pepsin, but acting in a neutral medium in the intestines.

(3) *Elastase* is another endopeptidase which cleaves the peptide bonds formed by the -COOH of alanine, glycine or serine.

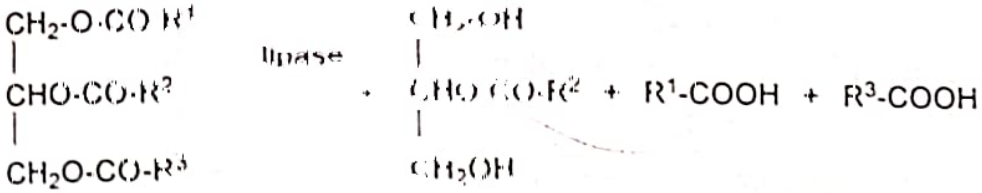
(4) The *carboxypolypeptidases* A and B require the zinc ions for their activity. They cleave the peptide bonds at the carboxyl end of a protein chain (hence called *exopeptidases*) formed between the amino-group of the terminal amino-acid and -COOH of any other amino-acid. However the terminal amino-acids are preferably valine, leucine, isoleucine, alanine, arginine or lysine. In practice, the smaller peptides formed by the action of other peptide enzymes are degraded step by step from the carboxyl end by removing the end amino-acid, which liberates the -COOH of the next amino-acid, for the next step. The pancreatic enzymes cause the liberation of a number of free amino-acids and oligo-peptides with 2 to 8 amino-acid residues.

The small intestine wall epithelial cells secrete a number of minor peptidases, most of which are *amino-polypeptidase* and *dipeptidases*. The amino-poly-peptidase cleaves a peptide chain from the $-NH_2$ terminal amino-acid, without specificity. The di-and tri-peptides are cleaved by the dipeptidases.

(C) Absorption of free aminoacids from the small intestines is accomplished with ease. There are more than half a dozen different transport mechanisms, depending on the type of amino-acids (e.g neutral, acidic, basic, branched, aromatic etc). Some of these are Na^+ mediated. Others may attach to the epithelial cells and pass through. A few di- and tri-peptides are cleaved on the surface of the intestinal lumen and are thus transported to the other side of the lumen. Undigested proteins are not normally absorbed, except in new born babies. However, a process called *pinocytosis* may operate marginally to transport some proteins.

8.3 Lipids

Human diet consists of a high proportion of fats. A small quantity of lipids (less than 10% of total fats) other than fats are also ingested. All these materials are water repellant and insoluble in water. The body has developed a unique process of digestion and absorption of fats. Enzymes like pancreatic *lipase*, hydrolyse the fats partially to fatty acids and glycerol monoesters (mono acyl glycerols).



Triglyceride (fat)

Generally the liberated fatty acids are of lower chain length, whereas the longer chain fatty acid remains attached to glycerol. The mono-acyl glycerol is now more hydrophilic (because of the two hydroxyl groups) than the triglyceride. However, the free fatty acid molecules and the mono-acylglycerol form a *micelle* which is emulsified by bile acids present in the lumen. Transportation of micelles into the cells across the intestinal wall now takes place easily. Inside the cells the mono-acylglycerol is re-esterified by some of the free fatty acids, to triglycerides. These are then usually transported to sites in the body for storing. Some of the mono-acylglycerol is converted to phosphatides and later to phospholipids, by esterification with phosphoric acid.

8.4 Carbohydrates

The food carbohydrates that can be digested and absorbed are : starch (made up of amylopectin and amylose), sucrose, lactose, glucose and fructose. Amylopectin (and glycogen found in animal tissues) is highly branched having both α 1:4 linkages (linear) and α-1:6 linkages (branched). Amylose has mainly α-1:4 linkages (linear). Both these are digested partially by α-amylase of the saliva, giving rise to oligo-saccharides. The reaction is insignificant as contact time is too low, the enzyme being inactivated in the acidic gastric medium. Carbohydrate digestion takes place mainly in the intestines.

(1) For efficient digestion of amylopectin, glycogen and amylose, hydration is necessary. Hence starch foods are digested well when cooked. The pancreatic α-*amylase* is an *endosaccharidase* (like endopeptidases), which attacks the α-1:4 linkages. From amylose a small amount of glucose (from the end of the chain) and large amount of the disaccharide maltose are formed. Because of branching, amylopectin and glycogen are broken down to a small amount of glucose (from end of chain), considerable amount of maltose (from the linear part of the molecule), α-limit dextrin (an oligo-saccharide from the branched part of the molecule) and some maltotriose (a trisaccharide closest to the branched part of the molecule). α-Amylase is the abundant enzyme of the pancreatic juice. Amylose is also degraded to glucose by intestinal enzyme *glucoamylase*.

$$\text{Amylose} \xrightarrow{\alpha\text{-amylase}} \text{Glucose} + \text{Maltose}$$

$$\text{Amylopectin} \xrightarrow{\alpha\text{-amylase}} \text{Glucose} + \text{Maltose} + \text{Maltotriose} + \alpha\text{-Limit dextrin} + \text{isomaltose (1:6)}$$

$$\text{Amylose} \xrightarrow[(\alpha\text{-1:4-glucosidase})]{\text{glucoamylase}} \text{Glucose}$$

Further degradation of the carbohydrates takes place in the intestines with the help of locally secreted enzymes. These are also called surface enzymes, secreted by the epithelial cells of the small intestine. The following changes are more pronounced.

$$\alpha\text{-Limit dextrin \& Isomaltose} \xrightarrow[(\alpha\text{-1:6-glucosidase})]{\text{isomaltase}} \text{Glucose}$$

$$\text{Maltose and Maltotriose} \xrightarrow[(\alpha\text{-}1{:}4\text{-}glucosidase)]{maltase} \text{Glucose}$$

Thus starches and glycogen are completely degraded to glucose, which is then absorbed without further degradation.

(2) Lactose is degraded to glucose and galactose by the enzyme *lactase* (or *ß-galactosidase*) found in small intestines. In infants and children, who consume milk and milk products this enzyme is essential for digestion. In most adults this enzyme may not be prominent.

$$\text{Lactose} \xrightarrow{lactase} \text{Glucose} + \text{Galactose}$$

(3) Sucrose is another disaccharide which is consumed in considerable amounts and is digested by the enzyme *sucrase* or *sucrose-α-glucosidase* to yield one mole each of glucose and fructose.

$$\text{Sucrose} \xrightarrow{sucrase} \text{Glucose} + \text{Fructose}$$

All other complex carbohydrates (i.e di-, oligo- and poly-saccharides) are not digestible and are eliminated from the small intestines to large intestines. They include trisaccharides like raffinose, disaccharides like trehalose and polysaccharides like cellulose. In the large intestines these may be degraded by bacteria, producing acids and gases (fermentation).

The absorption of the three monosaccharides (end products of digested carbohydrates) occurs both by passive diffusion (fructose) and by active transport with participation of Na^+ (glucose and galactose).

9

Metabolism

9.1 Introduction

The term metabolism is derived from a Greek word meaning *change*. In biochemistry the term is applied to any chemical change that takes place in a living system (i.e. under the conditions of life) and with the participation of constituents of living matter (e.g. enzymes, coenzymes etc). Note that it excludes non-living systems But it also includes chemical changes to a foreign chemical (e.g. synthetic drug) inside a living system. Such changes are called *biotransformations*. In a living system the biotransformations are usually in successive stages (called a path or pathway) and a whole chain of reactions can be recognised. *In vitro*, changes are almost always limited to a single step Therefore even if we can conduct a biotransformation in a non-living (cell-free) system with the help of an enzyme, it is not a metabolism.

Metabolism includes (1) degradation of normal biochemicals (e.g. foods), occasionally called *catabolism*; (2) synthesis of biochemicals (e.g. hormones, proteins, glycogen, phospholipids, nucleic acids, lipoproteins etc), also called *anabolism*; and (3) transfer of energy or *bioenergetics*. The aim of catabolism is to breakdown complex molecules of nature to simple units, (e.g. glucose from starch or glycogen) which can be used to build up other complex molecules. Similarly the purpose of anabolism is to build up complex molecules from the simple units available (e.g. proteins from amino-acids). Bio-energetics include such reactions which yield energy or store liberated energy. All these biotransformations are between the finished complex molecules (e.g. stored foods and structural parts) and the simple molecules that are excreted (e.g. carbondioxide, urea etc). The term *intermediary metabolism* is often used to denote these various steps. The intermediary metabolism of carbohydrates, proteins and lipids are exclusive only to a limited extent. Several pathways of metabolism of these three major biochemicals become common and mingle considerably. Such areas may be known as *integrated metabolism*. This aspect is important because the living systems show a flexibility of conversion of one to the other as and when needed.

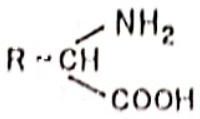
In order to understand the complex metabolic pathways it is advantageous to study them separately and then try to appreciate the integration. The process of digestion of foods in the gastrointestinal tract is not part of the intermediary metabolism. The aim of digestion is simply to breakdown a food molecule into its simple units like amino-acids or monosaccharides to enable the living system to absorb these molecules. Thereafter begins the intermediary metabolism. Digestion and absorption have been dealt with in the previous chapter.

9.2 Protein Metabolism

This consists of (1) amino-acid metabolism and (2) protein biosynthesis.

9.2.1 Amino-acid metabolism

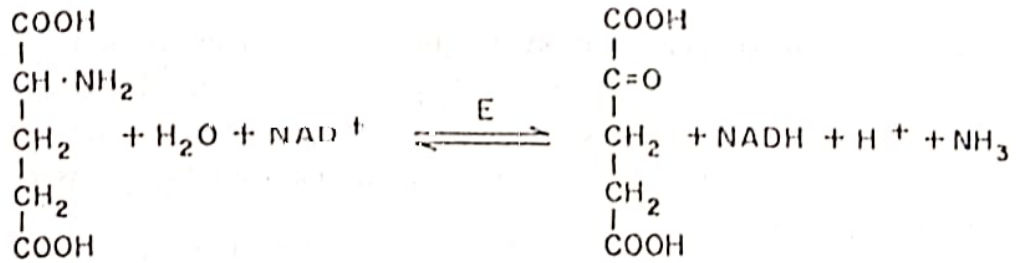
The amino-acid is represented by the general structure



Amino Acid

There are two functional groups, the *amino-* and the *carboxyl*. Most of the biotransformations of amino-acids involve either or both of these groups. In a few amino-acids which have additional functional groups (e.g. -OH, $-NH_2$, $-COOH$, $-SH$, $-SCH_3$, $-S-S-$, etc.), biochemical reactions are known involving these groups also.

9.2.1.1 Deamination reactions : Deamination of an amino-acid releases ammonia and a deaminated acid. If the reaction involves introduction of oxygen into the deaminated acid, then it is known as oxidative deamination :

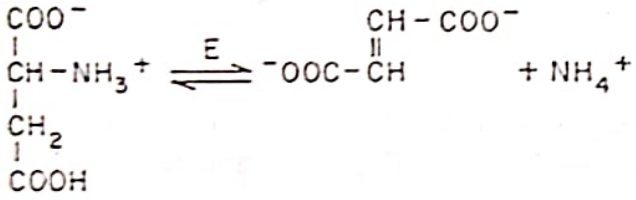


Glutamic Acid α-Keto-glutaric Acid

E = *glutamic dehydrogenase* enzyme

Oxidative deamination reactions are catalysed by both *dehydrogenases* (as above) and by *amino-oxidases*, but by slightly different mechanisms. Again the coenzyme involved may be either the *pyridine nucleotide* (NAD^+) or the *flavine nucleotide* (FAD).

In certain reactions oxygen is not introduced. These are non-oxidative deamination reactions : e.g.

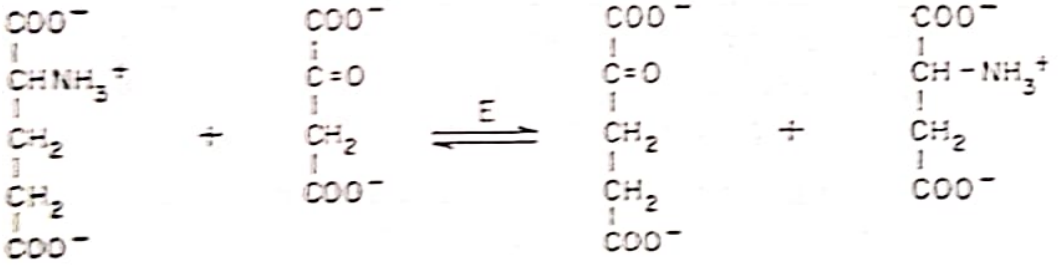


Aspartate Fumarate

E = *aspartase* enzyme

In both the above reactions ammonia is formed, which may be eliminated in the form of urea or may be recycled to yield a different amino-acid. (This is the reverse reaction of the oxidative deamination, the keto-acid being a different one). The keto-acid or the unsaturated acid formed in the above reaction is further integrated with other metabolic pathways.

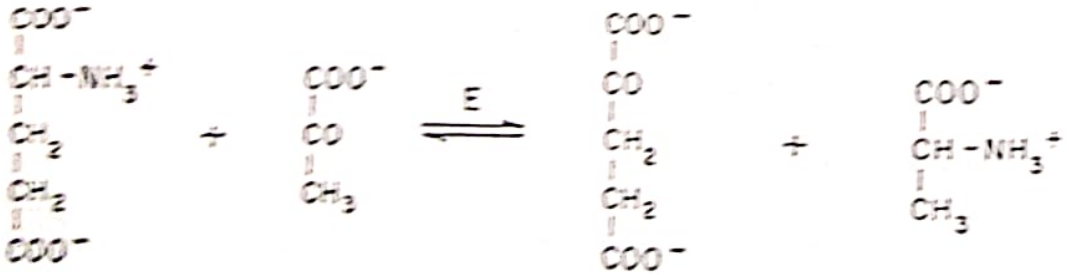
9.2.1.2 Transamination reactions : In the above deamination process, if a keto-acid is readily available, the ammonia formed may undergo immediate incorporation into the second keto-acid to yield a new amino-acid.



Glutamate Oxalacetate α-Keto-glutarate Aspartate

E = *transaminase*

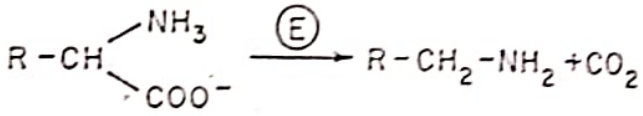
The succession of deamination of glutamate and amination of oxaloacetate take place so rapidly as to appear to be simultaneous. The enzyme involved in the above reaction is *glutamic-oxalacetic-transaminase* (GOT) found in serum, heart and liver. Similarly another transamination reaction is well known.



Glutamate Pyruvate α-Keto-glutarate Alanine

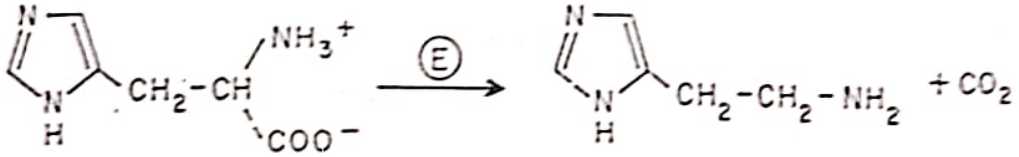
The enzyme *glutamic-pyruvic-transaminase* (GPT) is involved. All the transamination reactions need the coenzyme *pyridoxal pyro-phosphate*. The coenzyme accepts the amino group and is converted to *pyridoxamine pyro-phosphate*, which in turn donates the amino-group to an α-keto-acid. The transamination reactions are very useful for biosynthesizing α-amino acids from either ammonia or other amino-acids and an α-keto-acid. The α-keto-acids are available from carbohydrate and fatty acid metabolism.

9.2.1.3 Decarboxylation : The removal of a carboxyl group of α-amino-acids can be achieved by *amino-acid decarboxylases.*



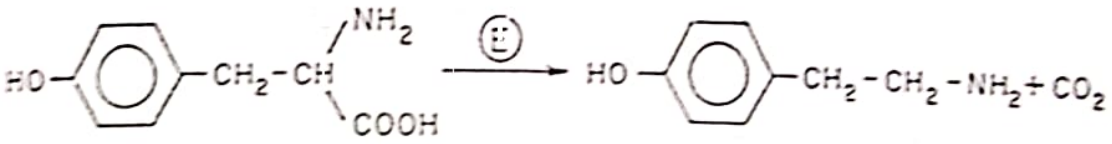
Decarboxylation

The carbondioxide may be transported as gas and eliminated or may be converted to biocarbonate by another enzyme *carbonic-anhydrase*, and enter circulation. The amines formed thus are usually specific and important metabolites in the body. Some examples are shown below :



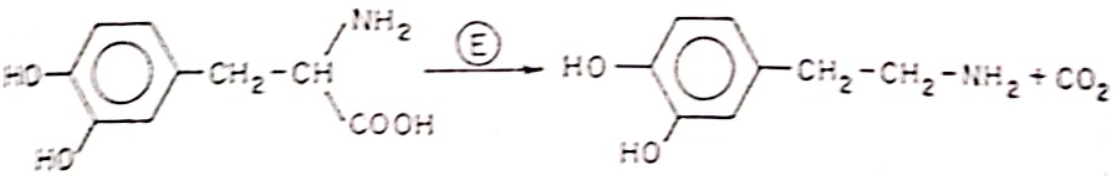
Histidine Histamine

E = *Histidine decarboxylase*



Tyrosine Tyramine

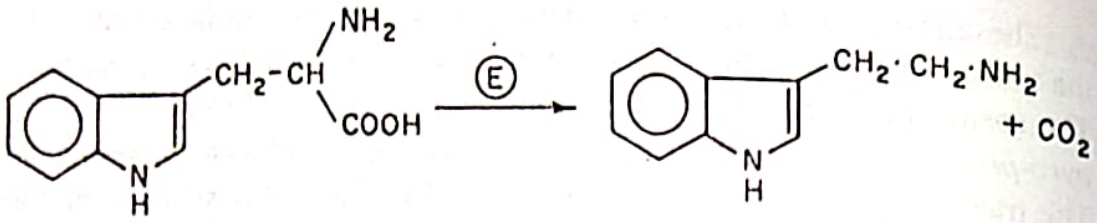
E = *Tyrosine decarboxylase*



Dihydroxyphenylalanine (DOPA) Dopamine

E = *DOPA decarboxylase*

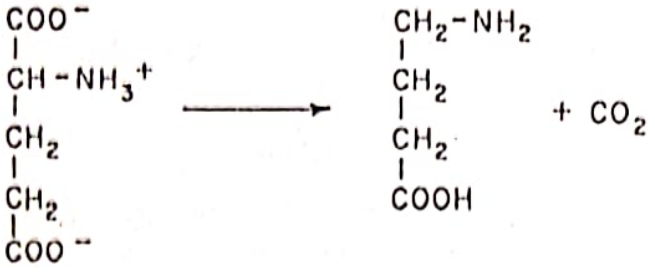
(Both *tyramine* and *dopamine* are biotransformed to *nor-adrenaline* and *adrenaline*)



Tryptophan Tryptamine

E = *Tryptophan decarboxylase*

(*Tryptamine* is biotransformed to 5-hydroxytryptamine, 5-HT, or *serotonin*).



Glutamate γ-Amino-butyric Acid (GABA)

E = *Glutamic decarboxylase*

All these amines have important physiological roles in the body and are known as biogenic amines. The decarboxylation reactions also require pyridoxal pyrophosphate coenzyme.

9.2.1.4 Other reactions involving α-amino-acids are known and are equally important. Some of these are : Formation of porphyrin involving glycine; conjugation of unusual carboxylic acids (like benzoic acid, aromatic and heterocylic acids) with glycine, ornithine, glutamine and cysteine in various detoxication processes, etc.

9.2.2 Protein Biosynthesis

It is an important part of amino-acid metabolism (*anabolism*). In the process, the peptide bond is generated, linking two α-amino-acids yielding a dipeptide. The dipeptide in its turn can be further converted to oligopeptides and finally to proteins. However, this simple scheme does not work in reality. Protein biosynthesis is a very complicated affair involving the DNA, different types of RNA, ribosomes, ATP and several enzymes, besides the amino-acids. This is because, the exact sequence of amino-acids in a chain and the cross linking of chains requires a carefully controlled system, generally known as the genetic code. The process therefore is not simply an amino-acid metabolic process, but integrates with the nucleic acids in a complex manner.

9.3 Lipid Metabolism

Lipid metabolism consists of (1) fatty acid degradation; (2) fat biosynthesis; and (3) other lipids metabolism.

9.3.1 Fat Degradation

9.3.1.1 Beta oxidation of saturated fatty acids : The fatty acids have long chain of hydrocarbon residue with a carboxyl group at one end. Due to lack of functional groups in the long chain biotransformation is not common in this part of the molecule. On the other hand the *beta* (β) carbon from the carboxyl group is activated and oxidised, giving rise to β-keto-fatty acid. This is then broken (cleaved) between the α and β carbons, with the formation of acetic acid and a long chain fatty acid with 2 carbon atoms less. The process requires the involvement of *coenzyme A*, ATP, FAD and NAD besides the respective enzymes. Five distinct steps have been identified in this scheme as shown :

(1) $R.\overset{\beta}{C}H_2\overset{\alpha}{C}H_2CH_2CH_2.COOH + ATP + CoA.SH \xrightarrow{\text{thiokinase}} R.CH_2CH_2CH_2CH_2.CO.S.CoA + AMP + Pyrophosphate$

(2) $R.CH_2CH_2CH_2CH_2.CO.S.CoA + FAD \xrightarrow{\textit{acyl dehydrogenase}} R.\overset{\beta}{C}H_2\overset{\alpha}{C}H_2.CH{=}CH.CO.S.CoA + FAD.H_2$

(3) $R.CH_2CH_2.CH{=}CH.CO.S.CoA + H_2O \xrightarrow{\textit{enoyl hydrase}} R.CH_2CH_2.\overset{\beta}{C}HOH.CH_2CO.S.CoA$
(β-hydroxyacyl coenzyme A)

(4) $R.CH_2CH_2.CHOH.CH_2.CO.S.CoA + NAD^+ \xrightarrow{\beta\textit{-hydroxyacyl dehydrogenase}} R.CH_2CH_2.\overset{\beta}{C}O.CH_2CO.S.CoA + NADH + H^+$

(5) $R.CH_2CH_2.CO.CH_2CO.S.CoA + CoA.SH \xrightarrow{\beta\textit{-ketoacyl thiolase}} R.CH_2CH_2.CO.S.CoA + CH_3CO.S.CoA$
(Product fatty acid 2) (acetyl CoA)

This scheme is repeated now with the product fatty acid 2, which at the end of the above set of reactions, yields another acetyl coenzyme A

molecule and a third product fatty acid. The cycle can continue until the entire long hydrocarbon chain is converted to acetic acid (2 C units). The activation of the fatty acid in the first reaction requires the energy rich ATP. But it is not needed for the second cycle or subsequent cycles as the product fatty acid is already an activated thiol ester of the coenzyme A. Some of these reactions require the presence of magnesium or manganese ions. All the reactions take place inside a mitochondrion. The hydrolysis of the thioester provides high energy.

9.3.1.2 Unsaturated fatty acids : As was shown above in reaction 3, a double bond can be hydrated prior to oxidation and cleavage. Unsaturated fatty acids, having double bonds far from the carboxyl group, do undergo this type of reaction to a limited extent. Prior to oxidation to the keto acid, the hydroxy acid undergoes isomerisation. Unsaturated fatty acids also undergo β-oxidation scheme until the double bond is encountered. However, the body conserves the unsaturated fatty acids, as they are needed for specific biotransformations.

9.3.1.3 Other Pathways of Degradation

(1) A minor pathway is α-oxidation. In this case the hydroxyl group is directly introduced on the α-carbon atom, which is then oxidised to the α-keto-acid and finally a mole of carbondioxide and a new acid with 1 carbon atom less, are formed.

$$R.CH_2CH_2.COOH \longrightarrow R.CH_2.CHOH.COOH \longrightarrow$$
$$R.CH_2.CO.COOH \longrightarrow R.CH_2.COOH + CO_2$$

(2) Another minor pathway of fatty acid degradation is the ω-oxidation (*omega*). In this, the last carbon atom farthest from the carboxyl group (i.e. the $-CH_3$) is oxidised to the primary alcohol and then to the carboxyl, yielding a long chain dicarboxylic acid. This is then degraded by the β-oxidation scheme from both ends, hastening the oxidation process.

$$CH_3\text{-}(CH_2)_n\text{-}COOH \longrightarrow \underset{\displaystyle OH}{\underset{|}{CH_2}}\text{-}(CH_2)_n\text{-}COOH \longrightarrow \underset{\displaystyle COOH}{\underset{|}{(CH_2)_n}}\text{-}COOH$$

9.3.2 Fat Biosynthesis

All acylation processes in the body proceed with involvement of coenzyme A. As thiol esters, the acyl groups become activated and are transferable. Thus a mono-acylglycerol absorbed after digestion and undergoing circulation can be acylated with any of the numerous acyl coenzyme A molecules present in mitochondria. This is the simplest and most common pathway of fat biosynthesis at the site of storage of fats.

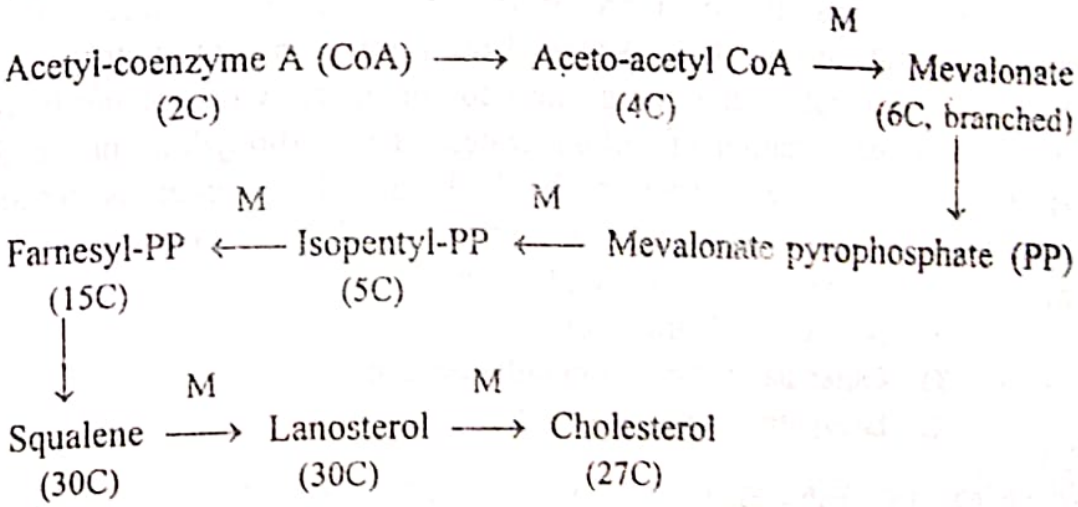
However, the other major pathway of synthesis involves the reverse of the β-oxidation scheme. Two acetyl coenzyme A molecules interact to give a 4C acid. This may further react with another acetyl conzyme A to give a 6C acid or with another 4C acyl coenzyme A to give an 8C acid. Thus many alternatives are available for this type of synthesis. It should be noted that all fatty acids resulting from this scheme will have even no. of carbon atoms only. This also explains why all naturally occurring fatty acids have even no. of carbon atoms.

To a very limited extent unsaturated fatty acids are formed by direct unsaturation of specific carbon atoms in the long chain of a saturated fatty acid. For example formation of oleic acid from stearic acid takes place with the help of *desaturase* enzyme, NADPH, cytochrome b_5 and a *reductase* in presence of oxygen.

9.3.3 Metabolism of Other Lipids

Cholesterol metabolism : Cholesterol is an important lipid found in the body as part of cell membranes. It is also the precursor of bile acids and steroidal hormones. Both the cholesterol biosynthesis and its regulation in the body are quite important. An impaired cholesterol metabolism can lead to fatal diseases of the heart and circulatory systems. A brief understanding of these processes is essential.

Cholesterol in the body comes from two major sources : (1) absorption from food sources; and (2) biosynthesis from acetate units, from fatty acid and carbohydrate metabolic pathways. In either case the circulatory system transports it to various tissues, particularly to liver, where it undergoes degradation or elimination. Its biosynthesis is a very complicated phenomenon, involving a number of steps, enzymes, cofactors etc. The following outline of cholesterol biosynthesis indicates important steps only (in a simplified manner) :



M : Multiples Steps

The cholesterol formed in the liver is transported by lipoproteins called LDL (*low density lipoprotein*) and VLDL (*very low density lipoprotein*) to the blood. In the blood, part of the LDL attaches to the vascular vessels causing thickening. Free cholesterol may remain circulating or gets attached to HDL (*high density lipoproteins*) and transported back to the liver. This process is essential to dispose of excess cholesterol. The liver oxidises cholesterol to bile acids, which are secreted along with free cholesterol through the bile into intestines. Although some of the free cholesterol is reabsorbed and goes through the whole cycle again, a good part is excreted in faeces. There is thus a critically balanced cholesterol metabolism. The HDL is beneficial in reducing incidence of **ischaemic heart disease** and **atherosclerosis**. The HDL contains over 40% proteins, nearly 50% total lipids of which 20-30% are phospholipids and only about 12% cholesterol in esterified form. On the other hand LDL and VLDL have a low protein (20-25%) but high lipids (75 to 90%) of which phospholipids form less than 20% and a total of over 35–45% of cholesterol in LDL.

Atherosclerosis is a disease of the blood vessels caused by defective cholesterol metabolism as explained above. The deposit on the vessel walls appear as yellow plaques containing the LDL lipids besides cholesterol. The heart has to work more to pump blood through thickened vessels. **Hyperlipidemia**, **obesity** and **diabetes** are some of the conditions leading to atherosclerosis. Diets with low cholesterol and low lipids in general, help in reducing the incidence of this disease. Cholesterol biosynthesis can also be controlled by inhibing any of the enzymes involved in its long biosynthetic route. Clofibrate is one such drug. There are other methods also to control cholesterol levels in the blood.

9.4 Carbohydrate Metabolism

Although fats liberate more energy (mole for mole) than carbohydrates, the fats are not fully degraded but a large amount is stored in the body. Thus most of the energy needed for all activities of the body is obtained by degradation of carbohydrates. The carbohydrate metabolism is therefore more extensive in the body and has numerous reactions compared to the protein or the lipid metabolism. The carbohydrate metabolism can also be studied under subheadings.

A. Anaerobic degradation;
B. Other pathways of degradation and
C. Biosynthesis of carbohydrates.

9.4.1 Anaerobic Degradation

This takes place extensively with glucose. In the series of reactions (biotransformations) of this pathway, no elementary oxygen is utilised.

Therefore it is called anaerobic degradation or *glycolysis*. Although many scientists have contributed to the discovery of this pathway, it is popularly known as the *Embden-Meyerhof scheme*. A better understanding of the glycolysis can be had by considering it in different ways.

In the body, prior to glycolysis, glycogen has to be converted (degraded) to glucose. This process is known as *glycogenolysis*. Glycogen has a structure similar to amylopectin. However, in glycogenolysis, glycogen is transformed to glycogen with one glucose unit less, the end glucose unit being converted to glucose-1-phosphate by the enzyme *glycogen phosphorylase* and inorganic phosphate. In glycogen, only the end glucose has the 1-hydroxyl group free (anomeric hydroxyl), all others being in $1 \rightarrow 4$ chain linkages. The enzyme being specific, only the end unit is liberated from the chain, but immediately exposes another glucose end unit for further phosphorylation. Glucose-1-phosphate is then converted to glucose-6-phosphate by the enzyme *phospho-gluco-mutase*. In the muscle, glucose-6-phosphate enters the glycolysis pathway. In the liver (from liver glycogen), it may be dephosphorylated by *phosphatase* enzyme to inorganic phosphate and glucose. The free glucose is available for circulation. The glycogenolysis can be schematically represented thus

$$(\text{Glucose})_n \xrightarrow[\text{glycogen phosphorylase}]{\text{inorganic phosphate}} (\text{Glucose})_{n-1} + \text{Glucose-1-phosphate}$$

(glycogen)

$$\text{Glucose-1-phosphate} \longrightarrow \text{Glucose-6-phosphate} \quad (\text{liver})$$

$$\text{Glucose-6-phosphate} \longrightarrow \text{Pyruvate} \quad (\text{glycolysis})$$

$$\text{Glucose-6-phosphate} \longrightarrow \text{Glucose} + \text{i.Phosphate}$$

There are three major stages in glycolysis (1) activation or priming (2) cleavage or splitting; and (3) oxidation to end products. In the following summarised schemes, all the reactions are reversible, like all enzyme catalysed reactions. But the equilibrium constants are such that most of these proceed in the direction of the arrow most of the time.

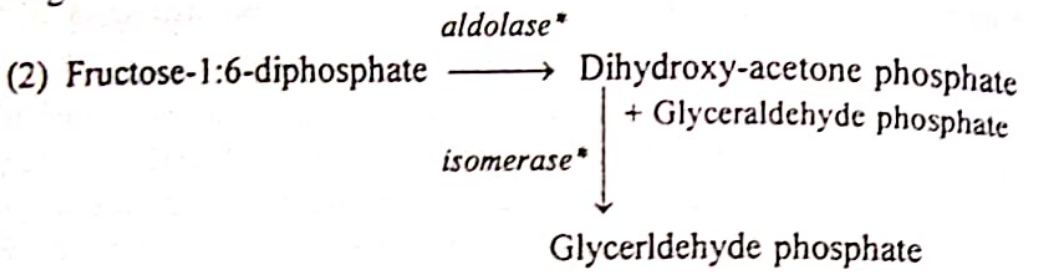
$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{++}]{\textit{hexokinase}^*} \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} \xrightarrow{\textit{isomerase}^*} \text{Fructose-6-phosphate}$$

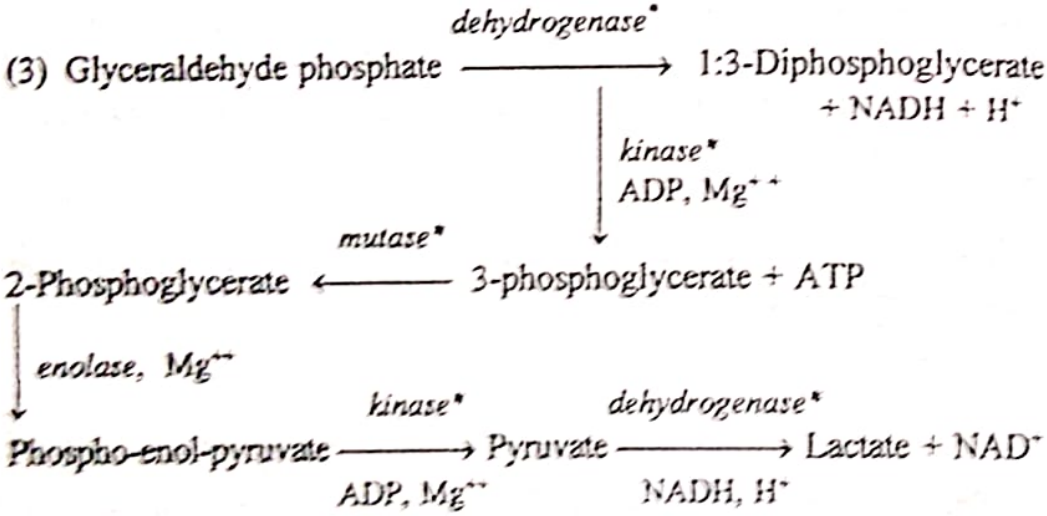
$$\text{Fructose-6-phosphate} + \text{ATP} \xrightarrow[Mg^{++}]{\textit{fructokinase}^*} \text{Fructose-1:6-diphosphate} + \text{ADP}$$

In this process glucose has been activated to glucose-6-phosphate by using a high energy phosphate bond of ATP. The doubly activated

fructose 1:6 diphosphate formed in the first stage, is ready to undergo cleavage.



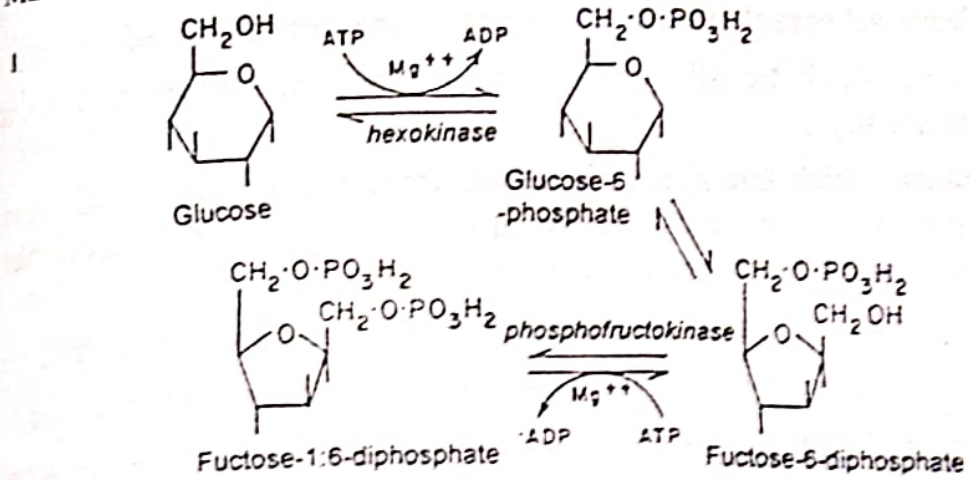
In this stage, the doubly activated fructose-diphosphate is cleaved (split) in the middle yielding two trioses, each carrying an active phosphate. The *aldolase* catalysed reaction is freely reversible, thus facilitating re-synthesis of hexoses from trioses. The further degradation from this stage is possible only through glyceraldehyde phosphate. Thus dihydroxyacetone phosphate has to be converted to glycerldehyde phosphate as and when it is degraded.



*For the sake of simplicity, full names of the enzymes have not been given.

There are very important aspects to this stage. Oxidation and reduction reactions are first seen at this stage of glycolysis. The two ATP molecules utilised in the first stage have been regenerated and are ready for recycling. The NAD^+ also is recycled in this stage itself. Inorganic phosphate is incorporated into the triose molecule generating a high energy bond. Pyruvate produced in this stage is a key intermediate, which can be integrated into amino-acid metabolism (alanine to pyruvate and reverse has been mentioned earlier) and fat metabolism.

The glycolytic path is explained below with structure, stressing on the intricate chemical change in the molecules at each step. The enzymes are mentioned in full and the co-factors have been indicated.



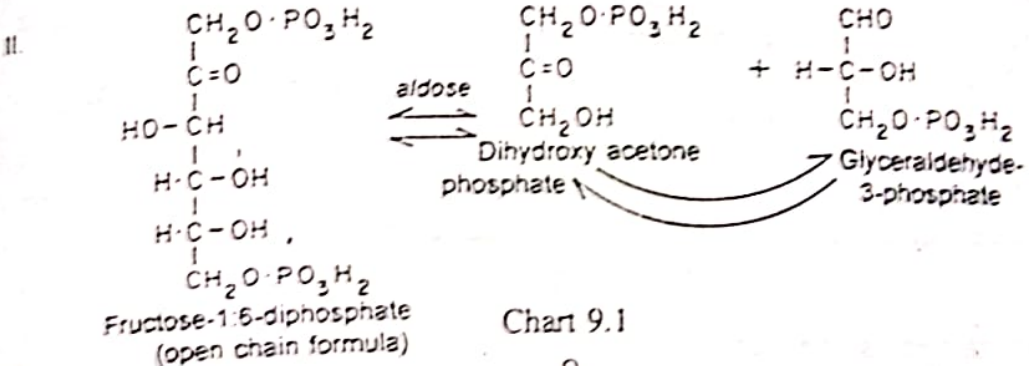


Chart 9.1

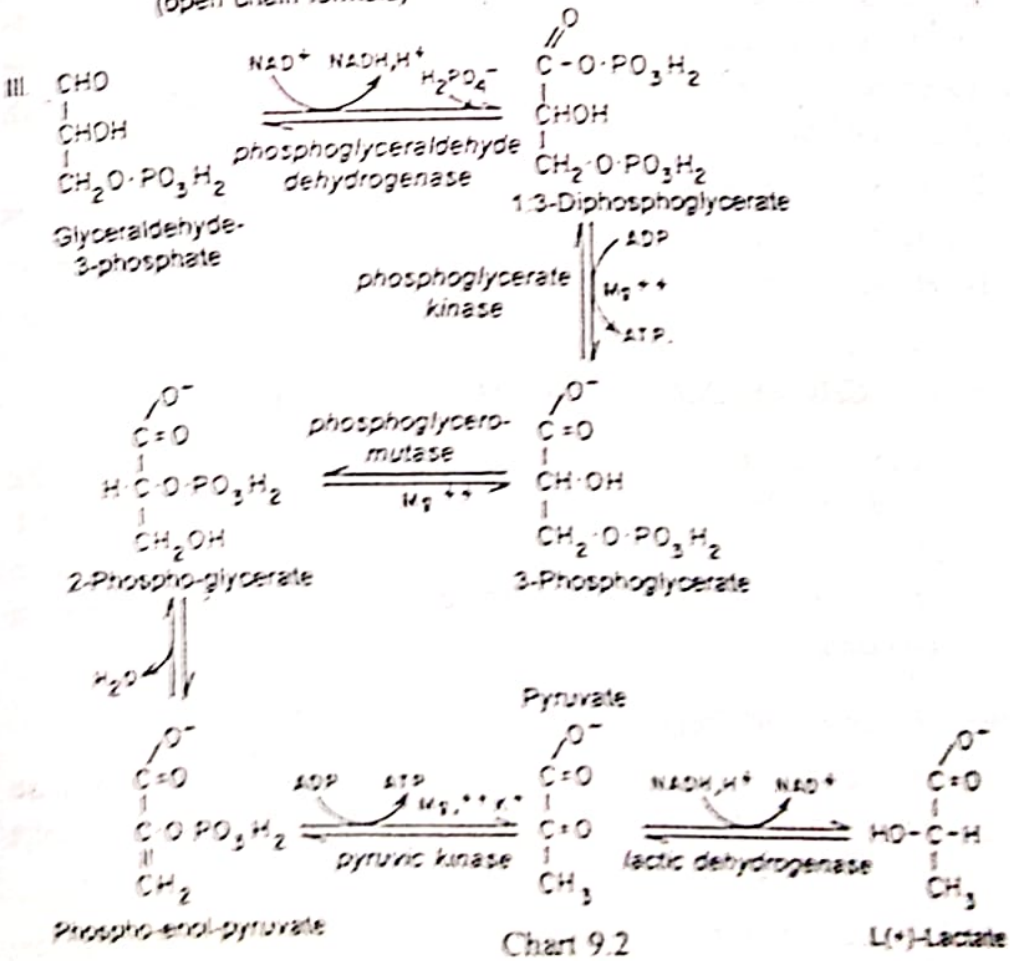


Chart 9.2

Note : The reverse of pyruvate to phospho-enol-pyruvate proceeds through a complex mechanism involving malate. (see later).

In the above scheme there are four types of enzymes involved :

(a) *kinases*, which transfer a high energy phosphate from ATP to a substrate sugar;

(b) *mutases*, which transfer a phosphate from one position to another position within the molecule, without involving energy. Both the kinases and mutases need megnesium ions;

(c) *isomerase*, which convert aldoses to ketoses and *vice-versa*; and

(d) *dehydrogenases*, which bring about oxidation reduction reactions.

In yeasts and some other organisms the glycolytic path is identical upto the formation of pyruvate, which then undergoes the following changes :

$$\text{Pyruvate} \xrightarrow[\text{thiamine pyrophosphate, } Mg^{++}]{\textit{pyruvic decarboxylase}} \text{Acetaldehyde} + CO_2$$

$$\text{Acetaldehyde} \xrightarrow[NADH,\ H^+]{\textit{alcohol dehydrogenase}} \text{Ethanol} + NAD^+$$

Also in yeasts the starting material is usually sucrose, which is converted to a mixture of glucose and fructose by means of the enzyme *invertase* (sucrase). The corresponding phosphates are formed which enter the glycolytic path.

In muscles, during exercise and some other conditions, lactic acid is formed by anaerobic oxidation of glucose. The pyruvate is hydrogenated. by the enzyme *lactic dehydrogenase.*

$$CH_3COCOO^- \xrightarrow{H_2} CH_3CHOHCOO^-$$

When muscle is exhausted of its stored glycogen by this anaerobic glycolysis, the muscle becomes fatigued and cannot do anymore work. As soon as sufficient oxygen becomes available. the reverse glycosis takes place, lactic acid disappearing rapidly. On restoring muscle glycogen, the muscle is reactivated.

9.4.2 Other Pathways of Degradation

Although glycolysis is the major pathway of obtaining energy from glucose by forming ATP and other high energy bonded compounds, there are several other pathways in living organisms for degrading hexoses.

9.4.2.1 Galactose which is obtained from lactose digestion, is converted to galactose-1-phosphate and then to glucose-1-phosphate by an

epimerase enzyme. The glucose-1-phosphate can then be utilised for biosynthesis of glycogen or converted to glucose-6-phosphate, to enter the glycolytic pathway.

Galactosemia : This is a rare inborn metabolic disorder, caused due to the absence of enzymes that are required to metabolise galactose. In early childhood and infancy, milk is the major food, which contains lactose. Lactose on hydrolysis yields glucose and galactose. Galactose is phosphorylated by a *kinase* to galactose-1-phosphate. The galactose-1-phosphate is transferred to uridine-diphospho-glucose, to form UDP-galactose. Only through this intermediate galactose is epimerised to glucose-1-phosphate, to be fully utilised as an energy source. In infants, who lack the ability to transfer galactose-1-phosphate to UDP-glucose, this is very serious and fatal, if not recognised early. A lactose and galactose free diet may help prolong life. If the *galactosemia* is due to inability to phosphorylate galactose (i.e. lack of *galactokinase*), the disease is not serious and can be controlled. This generally leads to **cataract** formation. In later years body develops alternative pathway to form UDP-galactose directly from galactose. Therefore proper care taken in early years helps a patient to survive and live with galactose diets in later life.

9.4.2.2 Fructose is abundant in foods, ranging from 30 to 60% of carbohydrates consumed. Fructose is absorbed passively from the intestines, once it is liberated from its sources (e.g. sucrose). Free fructose carried to the liver, undergoes phosphorylation by *hexokinase* and ATP, to yield fructose-6-phosphate, just like glucose. This then enters glycolytic pathway at the appropriate stage. However, it also undergoes phosphorylation to fructose-1-phosphate by a *special kinase*. Fructose-1-phosphate so formed does not get isomerised to fructose-6-phosphate or further phosphorylated to fructose-1:6-diphosphate. Thus it does not enter glycolysis in the usual manner. The liver also has another enzyme, *fructose-1-phosphate aldolase*, which directly cleaves this to trioses, glyceraldehyde and dihydroxyacetone-1-phosphate. Thus fructose can be directly utilised. In some persons the specific kinase may be deficient, causing an accumulation of fructose in the blood (**fructosemia**), which may further lead to **fructosuria**. Another closely related disease is **fructose intolerance**. This is an inborn error, characterised by inability to metabolise fructose, due to lack of the enzyme *fructose-1-phosphate aldolase*. Persons who are unable to metabolise this sugar, will show symptoms like **hypoglycemia**, caused due to depletion of inorganic phosphate in forming the fructose-1-phosphate. Fructosemia and fructosuria (fructose in urine) may also be noticed. Such persons should be provided a fructose-free diet, also free of sucrose and sorbitol.

9.4.2.3 It will be noted that ordinary food materials are not the main sources of pentoses like ribose and deoxyribose. Both these pentoses are found in all the nucleic acids, ATP, coenzyme A, NAD, FAD etc. The body has mechanism of converting glucose to ribose by a route known as *pentose phosphate pathway* or *hexose monophosphate shunt*. The pathway is characterised by the conversion of glucose to ribose-5'-phosphate in one sequence of reactions. Briefly these are shown in Chart 9.3.

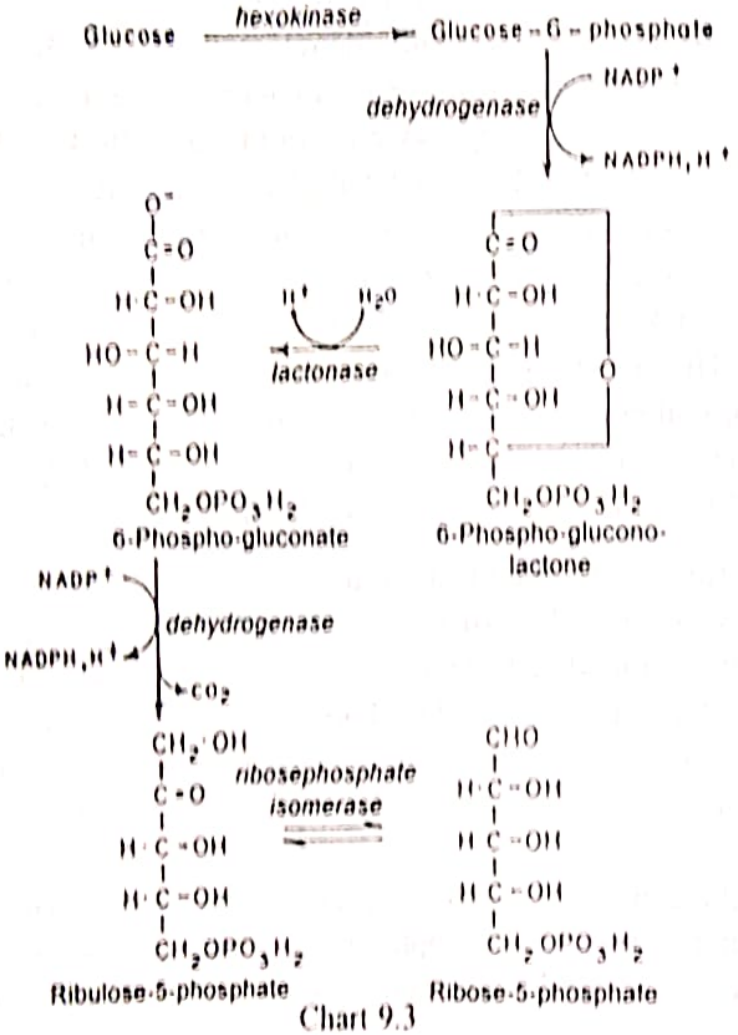


Chart 9.3

In another pathway, the ribulose-5-phosphate formed is converted by series of biotransformations either to (a) fructose-6-phosphate, which then enters the glycolytic pathway or to (b) glucose-6-phosphate which then re-enters the pentose phosphate pathway. In either case hexose monophosphate is regenerated. Hence the scheme is known as hexose monophosphate shunt. Note that one CO_2 is produced for each cycle of the pentose phosphate pathway, but 2 NADPH are produced at the same time. If the cycle is continued for six times, the net result will be :

$$6\ \text{Glucose-6-phosphate} + 12\ NADP^+ \xrightarrow{H_2O}$$
$$5\ \text{Glucose-6-phosphate} + 6CO_2 + 12NADPH + 12H^+ + H_2PO_4^-$$

Thus one molecule of glucose is completely oxidised to carbon dioxide. A large amount of reduced form of NADP is made available.

9.4.3 Biosynthesis of Carbohydrates

Carbohydrates are the major source of energy in the body. Even so all the consumed and absorbed monosaccharides are not at once degraded to yield energy and end products. A reasonable amount of glucose is circulating in the blood and also present in most tissues and cells for immediate needs. As soon as this normal concentration is exceeded, the glucose is converted to glycogen and stored. Gycogen is found in muscles and liver to a greater extent than in other tissues and organs. The process of formation of glycogen is known as *glycogenesis*. This is the reverse of glycogenolysis.

9.4.3.1 Glycogenesis : The formation of glycogen from circulating glucose or glucose-6-phosphate obtained by reverse glycolysis, involves several steps as against the simple glycogenolysis. This is summarized below :

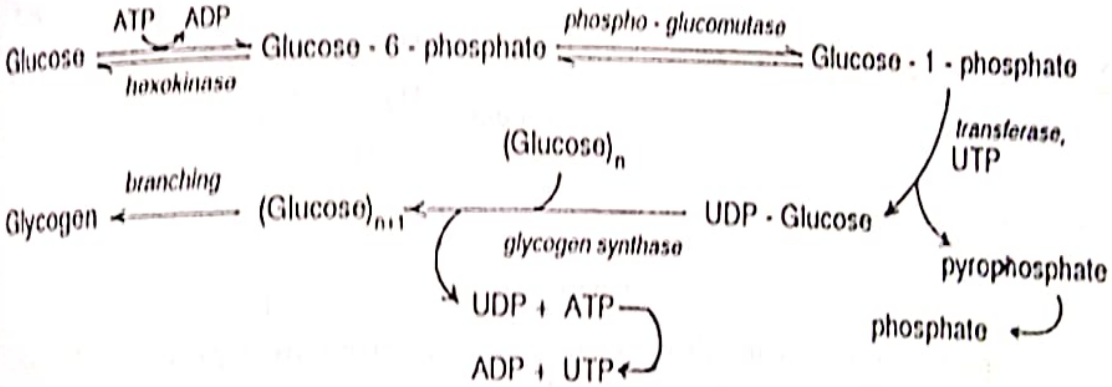


Chart 9.4 (Glycogenesis)

The unique features of this pathway are : (a) Uridyl-5'-triphosphate (UTP) is involved in carrying the glucose. (b) Glucose gets attached only as the end unit of already existing activated (primed) glucose polymer (glucan). (c) *Glycogen synthase* is specific and produces only a linear 1:4 linked oligosaccharide by repeated addition of end glucose units; and (d) Branching of this amylase structure to amylopectin structure (1:6 linkage) is accomplished by a branching enzyme to the final glycogen structure.

Glycogen storage disease : This is an inherited disease and is characterised by accumulation of glycogen in body tissues. The glycogen metabolism, particularly its breakdown, is affected, because of lack of one or more enzymes needed for the process. Several diseases (e.g. **Von Gierke's disease, Pompe's disease, Cori's disease** etc.) have been recognised as

related glycogen storage diseases. The liver, muscle and heart are affected. Some of these can be controlled by special diets, but most are incurable and may result in early death due to **cardiomegaly** and related conditions.

9.4.3.2 In the muscle, glycogen is degraded to lactic acid. During vigorous activity muscle glycogen is depleted by the glycogenolysis and glycolysis processes, with accumulation of lactic acid. Part of the lactic acid is converted to glycogen by a *reverse glycolysis*. However, formation of phospho-enol-pyruvate from pyruvate follows a complex route as shown :

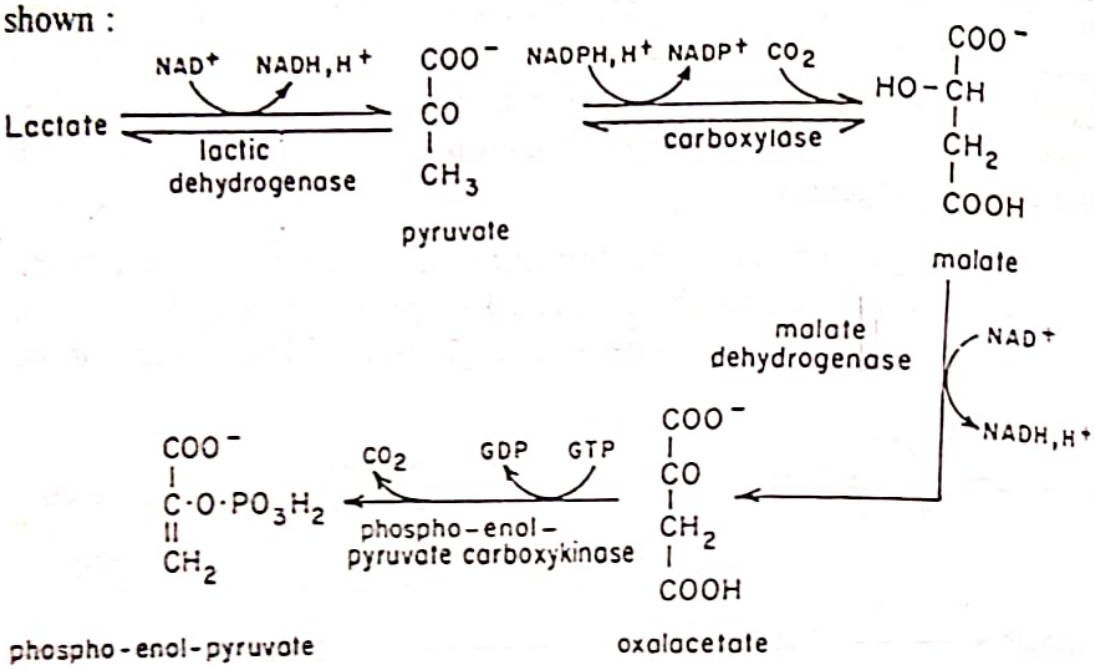


Chart 9.5

Phospho-enol-pyruvate thus formed is converted to 2-phosphoglycerate and backwards in the glycolytic scheme to glucose-6-phosphate.

9.4.3.3 Biosyntheis of carbohydrates also takes place from products of protein or fat metabolism. Such a process of conversion on non-carbohydrate metabolites to carbohydrates is known as *gluconeogenesis*. (a) From amino-acid metabolism, pyruvate, oxalacetate and propionate are formed. Both pyruvate and propionate are converted to oxalacetate which enters the reverse glycolytic path via phospho-enol-pyruvate. Amino-acids, which are metabolised to pyruvate or oxalacetate or propionate can all be converted to glucose as indicated. These are called glucogenic amino acids. Only leucine and lysine are not glucogenic. (b) Fatty acid oxidation yields acetate. This two carbon metabolite cannot be directly converted to glucose. In rare cases branched chain fatty acids or odd number carbon fatty acids may yield propionate. Cholesterol oxidation to bile acids also yields propionate. Thus propionate can be converted to oxalacetate before it is used in gluconeogenesis. Any fatty acid metabolite which can enter

the tri-carboxylic acid cycle (TCA or Krebs cycle) can eventually be led to gluconeogenesis. (c) On the other hand glycerol which is formed in fat hydrolysis is readily convertible to glycerol-3-phosphate and to dihydroxy acetone phosphate, which is a major product of hexose diphosphate cleavage in the glycolytic pathway. Thus glycerol contributes to gluconeogenesis, by reversing the glycolysis.

9.5 Tricarboxylic Acid Cycle (TCA)

This was proposed by Hans Krebs in 1937, whose contribution in this area is monumental. In his honour this is also popularly known as *Krebs cycle*. Several steps in the cycle are characterized by acids with three carboxyl groups, which justifies the name TCA. It is also known as *citric acid cycle*, the major tricarboxylic acid in the cycle being citric acid.

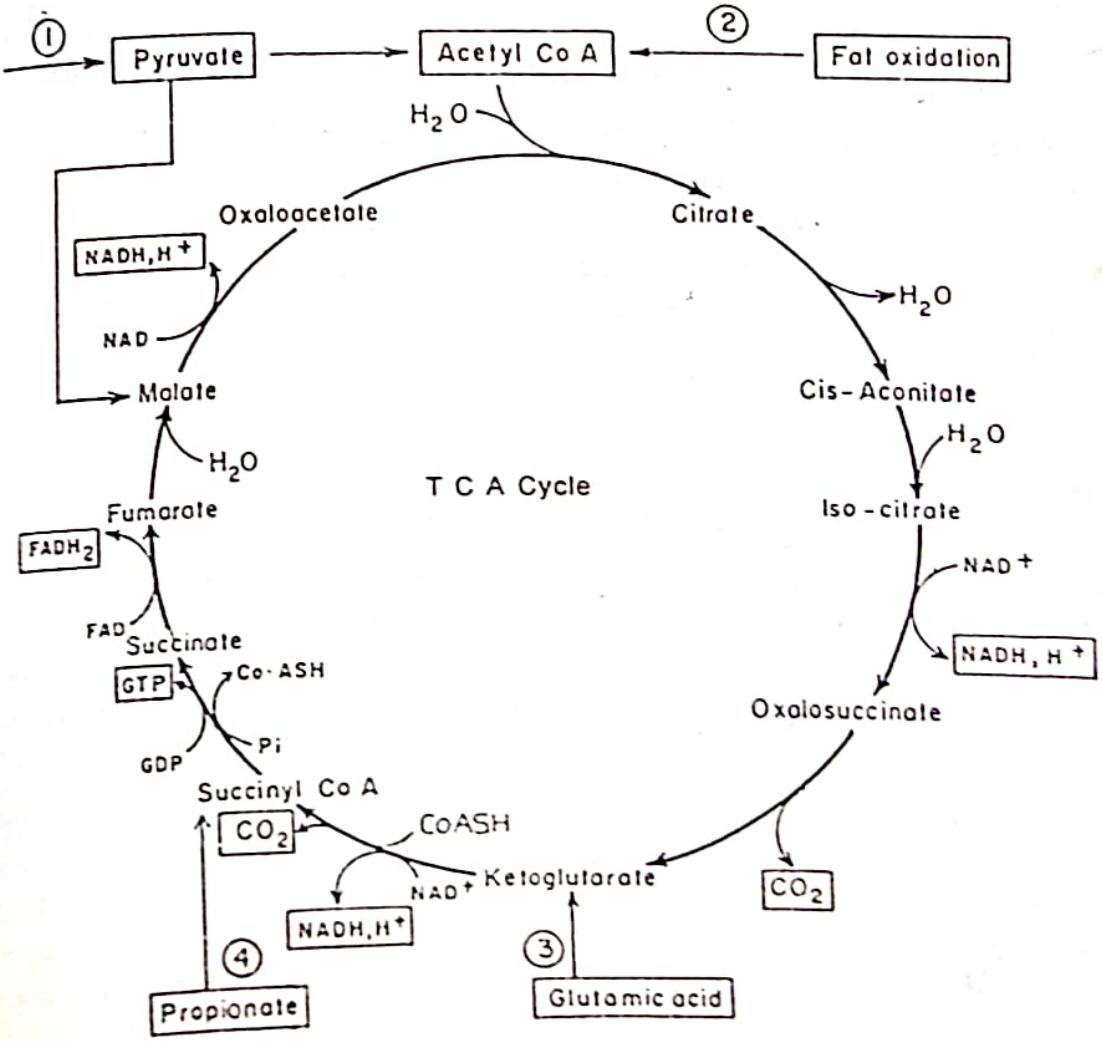


Chart 9.6 (TCA Cycle)

It is the major pathway of degradation of the products of protein, carbohydrate and fat metabolism, particularly to obtain energy and fully oxidise these to end products, to be eliminated from the body. The

scheme can be compared to a mill which receives materials for grinding, but itself is not ground. Following scheme is a simplified version of the TCA, highlighting the members of the cycle and changes that take place. The point of entry into the cycle by the products of carbohydrate, fat and protein metabolism is indicated.

In the scheme shown above, entry point (1) represents pyruvate from carbohydrate metabolism (i.e. glycolysis). Pyruvate may be converted tomalate, which then enters the TCA cycle. Pyruvate may also be converted to acetate by decarboxylation. The acetate is then incorporated into TCA cycle via citrate. The entry point (2) is more important to fatty acid metabolism, in which acetate accumulates. Entry point (3) represents products of protein metabolism, particularly aspartate as oxalacetate and glutamate as a-ketoglutarate. Branched chain amino-acids and odd number carbon fatty acids accumulate propionate, which enter TCA cycle via succinyl coenzyme A at point (4). There are many other possibilities for the metabolites to enter the TCA cycle.

Note that acetate, once it enters into the TCA cycle is fully oxidised to $2CO_2$, yielding energy which is stored in the form of GTP and in the reduced forms of NADH and $FADH_2$. Coenzyme A is utilized in the TCA cycle for the transport of acyl groups. The TCA cycle takes place inside the mitochondrion, which is also the site of oxidation of fatty acids. The TCA cycle is the major scheme integrating the carbohydrate, fat and protein metabolism.

9.6 Bioenergetics

Every life activity, like physical (motion) or physiological (e.g. sensory, transport across membranes, nerve conduction, excretion etc.) or biochemcial (e.g. biotransformations) or hormonal, etc. requires energy. In the body energy is carefully regulated. The three stages of its regulation are : (a) production; (b) storage or conservation; and (c) efficient utilisation.

All energy in the body is produced by oxidative processes. The final oxidising agent is elementary (atmospheric) oxygen, entering the cells through respiration. The food sources like glucose and fatty acids are converted to simpler molecules by metabolic pathways, which do not involve elementary oxygen. Finally the path must lead to a stage where elementary oxygen is utilised. The entire mechanism is highly intricate involving many steps and alternative pathways.

In the first stage : (i) Pyruvate is produced by glycolysis, in case of carbohydrates, providing only a small gain in energy. (ii) Acetyl coenzyme A (or simply acetate) is formed from fatty acid metabolism, again

providing the system with little or no gain in energy. (iii) Keto-acids (e.g. pyruvate, oxalacetate, ketoglutarate etc.) and propionate are formed from amino-acid metabolism, again with little gain in energy.

In the second stage these products are fed into the TCA cycle, which produces considerable amount of energy. Pyruvate may enter the TCA by different paths. The net result is that each glucose can be fully oxidised to CO_2 by entering the TCA cycle successively several times, yielding its full complement of energy. This is the major energy producing mechanism. Similarly, a fatty acid molecule, converted to acetate is theoretically fully oxidised in the TCA cycle. In practice, the acetate is partly diverted for the biosynthesis of sterols, fatty acids, ketone bodies etc. Approximately slightly less than half of the acetate from fats, yields energy through the TCA cycle. Similarly, protein degradation products yield small amount of energy through the TCA cycle, as it involves only a few amino-acids and to a small degree. Part of the energy produced in the TCA cycle is stored in the form of high energy compounds.

In the third stage, part of the energy utilised in the TCA cycle for the reactions, is regenerated through a series of reactions, which finally utilise elementary oxygen (respiratory oxygen). A simplified form of this is given below :

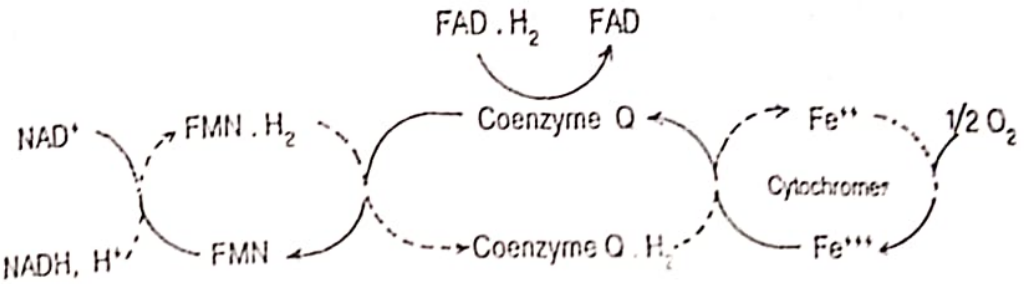


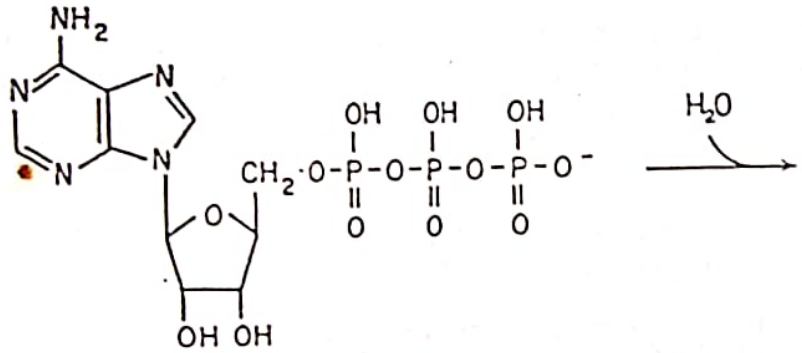
Chart 9.7 (Electron Transfer Scheme/Sequence)

The above scheme taking place in a mitochondrion, involves electron transfer throughout. Cytochromes, which contain iron, act as the donor to oxygen. It may be remembered that NADH and $FADH_2$ are formed during TCA cycle and other metabolic pathways. They are oxidised by transferring their electrons as shown in the scheme above.

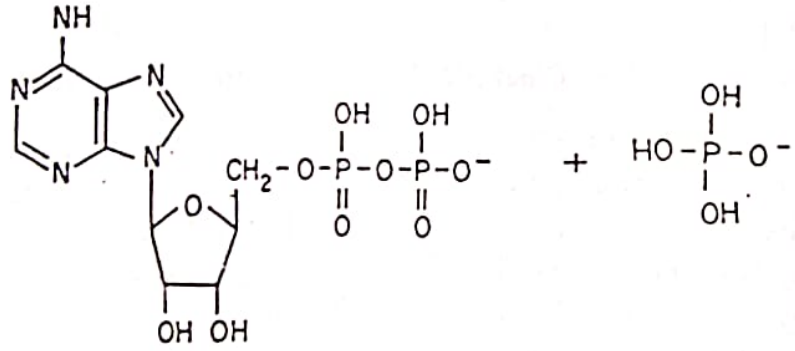
Energy is usually expressed as calories or kilocarlories (kcal) per gram or per mol. If completely oxidised to yield maximum energy, the relative values for the foods are : carbohydrates and proteins 3 to 4 Kcal/gm, whereas for fats it is about 9 Kcal/gm. Weight for weight fats yield nearly three times the energy obtainable from carbohydrates. In practice, the body uses a maximum of about 45% of fats for energy production and has a tendency to store them as much as possible. Even in case of carbohydrates a maximum of about 85% undergoes oxidation to produce energy.

Every compound can be considered as having free energy (like potential energy of matter by virtue of physical status and position). During a chemical reaction a chemical is changed into one or more chemicals, each of which have a free energy. If the total free energy of the products is less than the free energy of starting compound, then excess energy is liberated and becomes available. Such reactions are termed *exergonic*. If on the other hand energy is consumed to bring about a chemical change, the reaction is termed *endergonic*. In reversible reactions, the tendency is to favour the exergonic direction of the reaction. However, in enzyme catalysed biochemical reactions, compounds with higher free energy are formed with ease, when energy is available, as in oxidation processes of TCA cycle or glycolysis or fatty acid oxidation etc.

Adenosine-5'-triphosphate (ATP) is the most common energy rich compound in the body. On hydrolysis of ATP to ADP, by any method, about 8000 cal/mol of energy is liberated.



ATP



ADP Inorganic Phosphate (Pi)

Similarly :

$$ADP \xrightarrow{H_2O} AMP + H_3PO_4 + 6500 \text{ cal/mol}$$

$$AMP \xrightarrow{H_2O} Adenosine + H_3PO_4 + 2200 \text{ cal/mol}$$

$$\text{cyclic AMP} \xrightarrow{H_2O} \text{Adenosine} + H_3PO_4 + 11900 \text{ cal/mol}$$

On account of this behaviour, the pyrophosphate bonds are termed *high energy bonds*. Formation of high energy bonds (or energy rich compounds) during metabolic activity is a method of storing excess energy. The energy rich compounds found in the body and the energy liberated on hydrolysis are given below (Table 9.1) :

Table 9.1 **Energy Rich Compounds**

Energy Rich Compd.	*Product*	*Energy Release (kcal/mol)*
ATP	ADP	7.3
ADP	AMP	6.5
c-AMP	Adenosine	11.9
Acetyl phosphate	Acetate	10.3
1:3-Diphospho-glycerate	3-Phosphoglycerate	10.1
Creatine phosphate	Creatine	10.3
Phospho-enol-pyruvate	Pyruvate	14.8
Acetyl coenzyme A (thiol ester)	Acetate	7.7

Note that in glycolysis the energy rich compounds diphosphoglycerate and phospho-enol-pyruvate, transfer the excess energy to ADP → ATP while undergoing further transformation.

It has been estimated that full oxidation of one mole of glucose by glycolysis followed by several cycles of TCA scheme, produces energy equivalent to 38 ATP molecules. Similar calculation shows that a molecule of palmitic acid (C_{16}) completely oxidised by way of TCA cycle would theoretically produce energy equivalent to about 108 ATP molecules. In practice, less than 50 ATP molecules may be formed.

9.7 Regulation of Metabolic Processes

The body has a precise and efficient control over the numerous metabolic steps taking place. There are several regulatory events.

(1) *Site dependent* regulation is achieved by locating the enzymes and cofactors at a particular site. The substrate has to be transported to the site for transformation. This is particularly evident between mitochondria and cytoplasm outside it, the membrane acting as a barrier.

(2) All enzymic reactions obey *laws of chemical kinetics*. Thus the rate and direction of the reversible enzymic reactions are automatically

regulated by the accumulation of products. If there are several steps involved in a pathway, a product of a later stage specifically inhibits the release of the enzyme of first stage. This is called *feed back control*. Stimulation of a pathway or induction is often effected by initial activation or priming. The primer is usually a small amount of the product itself.

(3) In addition to the above obvious mechanisms, the metabolic activities are well regulated by *hormones*. Some hormones are exclusive and specific whereas some others have more than one regulatory effects. These are discussed briefly below.

9.7.1 Lipotropin from anterior pituitary has been shown to mobilise fats from adipose tissue and to promote oxidation of fatty acids. Thyroid hormones, *thyroxine* (T_4) and *tri-iodothyronine* (T_3) are known to increase basal metabolic rate (BMR) by promoting oxygen consumption and catabolism of carbohydrates, proteins and lipids. They facilitate increased absorption of glucose from intestines leading to increased blood glucose levels. However this is countered by increased utilisation of glucose. Thyroid hormones promote protein anabolism, i.e. formation of proteins (tissue proteins in particular) from amino-acids. In lipid metabolism they facilitate better utilisation of fats and cholesterol, thus reducing fat and cholesterol accumulation and deposition.

9.7.2 Insulin, the pancreatic hormone from β-cells of islets of Langerhans, is liberated in response to increased blood glucose levels, encountered after absorption of glucose from intestines. In carbohydrate metabolism, its primary function is to promote glycogenesis (i.e. formation of glycogen from circulating glucose). Both liver and muscle glycogen formation is promoted. Insulin alters the permeability in the muscle cells, facilitating transport of glucose to inside the cell. This action also promotes transport of amino-acids into the cell, which in its turn leads to protein synthesis. The fat cells are also similarly affected, whereby mono-acylglycerol and fatty acids tend to be transported into the cells, which are then converted to triacylglycerols (fats). Fatty acid synthesis is also triggered by insulin. In the muscle it triggers glycolysis and TCA cycle, to provide adequate energy for the above important anabolic activities.

For therapeutic purposes large amounts of insulin are prepared. The Indian Pharmacopoeia includes as many as seven different preparations of insulin. These are briefly described here :

Insulin injection (I.P.) is a soluble form and is generally prepared from pancreas of oxen, pigs or sheep. Porcine insulin is closest to human insulin in its chemistry. It has at 30 B alanine whereas the human insulin

has at 30 B threonine (*see* Fig 2.5). Thus pig insulin causes least reactions (hypersensitivity) when administered to patients. All other insulins differ also in two amino-acids in A chain. In recent years, human insulin has become available, by modification of pig insulin through removal of 30 B alanine and introducing threonine in its place by chemical manipulation. Alternatively human insulin is also being produced by biotechnological methods (recombinant DNA technology). Proinsulin is also produced by such methods and insulin generated in the body of the patient. Insulin injection is supplied in potencies of 20, 40 and 80 units per ml. It contains a very low amount of zinc, less than 40 µgm per 100 units of insulin. *Globin zinc insulin* (I.P.) is also a soluble form, but contains the protein globin and added zinc chloride, not less that 0.25 mg of zinc ion per 100 units of insulin. All other products of insulin are suspensions.

Insulin zinc suspension (I.P.) is an *amorphous* form of insulin, suspended with very fine particles. This is intended for prompt action, by quick release of insulin. Another suspension of the same material differs only in having larger size of *crystalline* insulin, instead of amorphous particles. This releases insulin slowly over a longer period of time, hence used for extended activity.

Isophane insulin (I.P.) and *Protamine zinc insulin* (I.P.) are both suspensions containing added protamine protein (obtained from some fish). No zinc is added to the isophane insulin, whereas the other preparation contains added zinc chloride. All the insulin preparations have different advantages and are used to suit individual patients.

9.7.2.1 Diabetes : The disease is known as **diabetes mellitus**, indicating a sweet urine, recognised quite long time age. Today it is known that sugar appears in urine (**glycosuria**) after the disease is well advanced. Prior to this situation, there is a high and persistent level of glucose in blood, called **hyperglycemia**. Thus it is possible to diagnose diabetes much early by blood glucose estimation. As indicated earlier, the blood sugar levels is the result of a number of controlling mechanisms. Any one of them can go wrong, leading to this disease. Normally the blood glucose is either utilised by energy liberation through glycolysis or is stored by glycogenesis. These processes require the mediation by the hormone insulin and the enzyme hexokinase. Many factors may lead to poor control of glucose metabolism. However, insulin defective diabetes (Type I) can be controlled by administration of insulin to the patient on a regular basis throughout life. Another form of diabetes (Type II) is usually the result of over-eating and high carbohydrate diet, and occurs in elderly persons with a tendency to become obese. In such individuals diet control and symptomatic treatment with oral anti-diabetic drugs may be

adequate. In both types a patient is prone to infections, slow healing of wounds, **polyuria**, **tiredness** etc. In type I, the urine and even exhaled breath may contain *ketone bodies*, which result from incomplete and abnormal breakdown of fats. In urine they appear as keto-acids (**keto-acidosis**). Detection of ketone bodies in breath by its sweetish smell or in urine by chemical tests, is an early indication of severe hyperglycemia.

9.7.3 Glucagon, another pancreatic hormone (from α-cells of islets of Langerhans), is more or less oppose to the actions of insulin. It activates the *phosphorylase* system of liver, which triggers glycogenolysis (breakdown of glycogen) to glucose. This enters circulation, causing increased levels of blood glucose (*hyperglycemia*). Glucagon also stimulates *gluconeogenesis* in the liver (i.e. formation of glucose from non-carbohydrate metabolites) by increasing the availability of glucose precursors in the liver. In the fat cells, glucagon promotes fat catabolism, leading to formation of fatty acids and glycerol.

9.7.4 Adrenaline or epinephrine is an important hormone with many functions. This hormone promotes *glycogenolysis* in the liver, just like glucose-6-phosphate formed from glycogen. Glucose so formed enters the blood and is transported to tissues for local oxidation. In skeletal muscle also adrenaline causes liberation of glucose-1-phosphate from muscle glycogen. Due to lack of *phosphatase*, the glucosephosphate is allowed to undergo glycolysis in the muscles, providing ready energy. Epinephrine also is known to stimulate lipolysis in adipose tissue.

9.7.5 Corticosteroids (adrenal cortical hormones) are active in glucose metabolism. Particularly cortisol (hydrocortisone), cortisone and corticosterone are prominent among these. The effects of these hormones on the carbohydrate metabolism are termed gluco-corticoid. These and some other corticosteroids also possess mineralo-corticoid activity (which will be discussed elsewhere). Glucocorticoid activities are somewhat opposite to the effects of insulin on the carbohydrate metabolism. Cortisol promotes *gluconeogenesis* in the liver, leading to increased levels of blood glucose. This also promotes deposition of liver glycogen. In the muscle cells this action is not seen. In fact muscle glycogen is not formed due to non-availability of glucose (and amino-acids). Membrane transport is affected. In this respect it is *anti-anabolic*. In the muscle, protein catabolism (breakdown) is promoted by corticosteroids. However, in the liver, protein anabolism actually takes place.

There are several other hormones and hormone-like substances and other biochemicals that in some minor way regulate the metabolism of the major food chemicals.

10

Excretion and Drug Metabolism

10.1 Introduction

The human body has a very efficient mechanism of eliminating unwanted materials from the body. Primarily these are end products of metabolism, formed from foods, after producing energy from them. As explained in an earlier chapter, many of the metabolites are usually retained in the body and recycled. However, energy production necessarily yields end products, which are of no more use to the body. Carbon dioxide, water, ammonia, urea etc. belong to this category. Carbon dioxide is oxidation end product whereas water is formed by reduction of oxygen. These are processed by specific paths and excreted. Ammonia is the major end product of amino-acid (i.e. protein) metabolism. Respiratory elimination of carbon dioxide is also of great importance. Lot of excess water escapes from the body not only through the kidney but also in exhaled gases and perspiration. Undigested food materials are eliminated as excreta (faeces) along with some end products, which may be secreted into the intestines. In this chapter only the urinary excretion of urea and related materials will be discussed.

Human beings consume a number of non-food materials for various reasons. The most important of these are the 'drugs' or more accurately *therapeutic agents*. The body has developed general processes which help in the elimination of such substances through the kidneys. This may be termed *drug metabolism* or *drug biotransformation* or *detoxication*. A brief review of this important biochemical activity in the body will also be presented in this chapter. No student of pharmacy can ignore the importance of this aspect of human biochemistry.

10.2.1 Urea Cycle

Protein metabolism leads to nitrogen containing end products. Small amounts of amino-acids are excreted in conjugation with other substances. But the major end product of amino-acid metabolism is ammonia. Ammonia is a toxic material and should not accumulate in body (**ammonemia**).

As and when ammonia is formed it is converted in the body into harmless urea and excreted through the kidney. Ammonia is combined with carbon dioxide, the other major end product of carbohydrate and fat metabolism (through the tricarboxylic acid cycle). The result is *urea*. Hans Krebs was also the discoverer of the process of urea formation, which also is a cyclic process. Hence the process is known as *urea cycle* or *Krebs-Henseleit cycle* or *ornithine cycle*. The cycle is fully described today with many details. The following is a simplified version of the urea cycle.

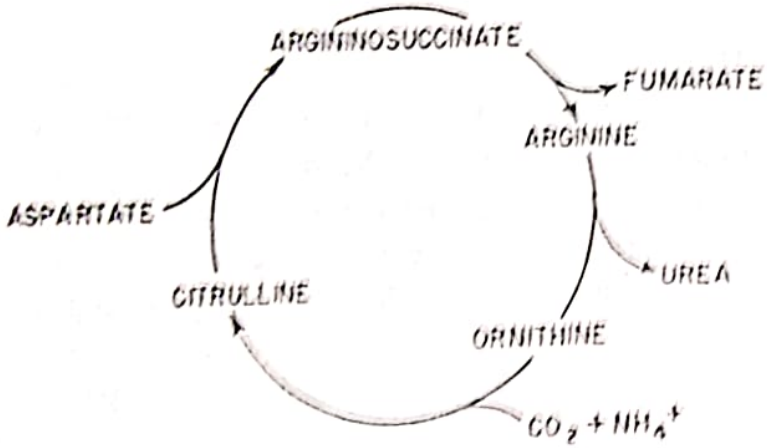
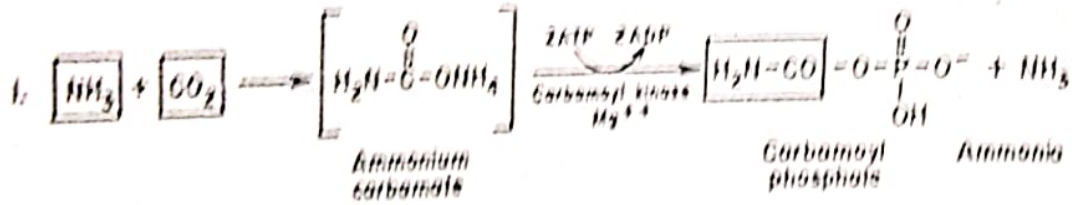


Chart 10.1 Urea Cycle

The details of these reactions are as follows :



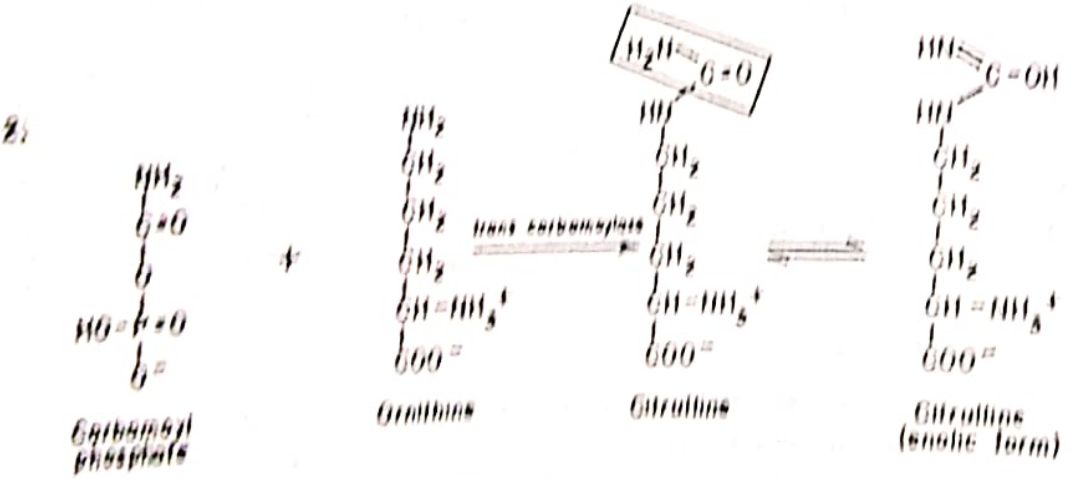


Chart 10.2

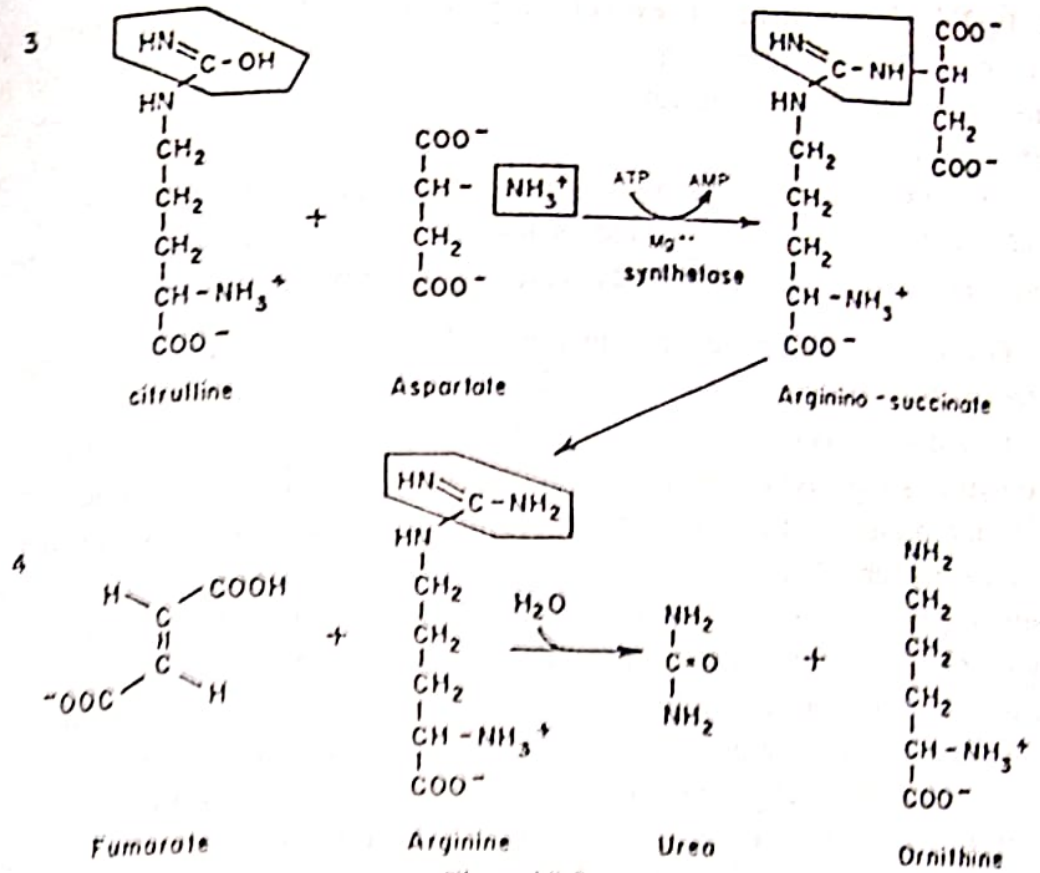


Chart 10.3

The following important points may be noted :

(a) One mole of ammonia and one mole of carbondioxide combine with the amino-group of aspartate to form urea. Hence aspartate is essential for the urea formation.

(b) Two moles of ATP are needed to supply energy for the successful formation of urea.

(c) Fumarate may enter the TCA for further metabolism, while ornithine is recycled to form more and more urea.

(d) Aspartate needed for recycling comes from amination of oxalacetate (GOT) by glutamate. This is also nitrogen from an amino-acid.

While urea is an excretory product, without any value to the body, it finds application as a diuretic. As it is the end product of metabolism, it is naturally not metabolised when consumed. It is directly excreted through the kidney. Its high concentration in the glomerular filtrate increases the osmolality. To overcome this, body adds more water to the urine, diluting to a considerable extent. Thus more fluid is excreted from the body, with increased urine output (diuresis). Urea acts as a water carrier. *Urea* (I P) finds application as a diuretic. For this purpose it is obtained synthetically. It may be remembered that urea was the first synthetic organic compound prepared by Wöhler.

10.2.2 Other nitrogen excretory products : A small amount of *uric acid* may also be formed from amino-acid metabolism. Uric acid is a purine, whose skeleton is formed from glutamine, glycine, aspartate, carbon dioxide and formate. Thus uric acid is also a product of amino-acid metabolism. Uric acid is also formed, from purine metabolism (nucleic acid) in the liver. As uric acid is insoluble, it is not very well excreted and may accumulate in the body, causing discomfiture.

Gout : It was pointed out in purine biosynthesis that IMP is the key intermediate normally leading to formation of both adenylic and guanylic acids and then to nucleic acids. However, part of IMP also is diverted to the synthesis of hypoxanthine to xanthine and finally to uric acid. This is a minor pathway and only small quantities of uric acid are formed. In certain persons, due to genetic defect or in rare cases due to secondary causes (some drugs or in some other diseases or even consumption of foods rich in purines etc.), the pathway may become prominent and uric acid may appear in higher concentrations in blood (**hyperuricaemia**), urine and related organs. Both uric acid and urates are sparingly sobluble and get crystallised. These crystals may be found in some polymorphonuclear cells (due to phagocytosis) and in the synovial fluid of joints (toes, limb joints). The latter condition causes acute pain to the patient. Uric acid and urates may also appear as **urinary calculi** (kidney stones).

The *xanthine oxidase* responsible for conversion of xanthine to uric acid can be inhibited by certain drugs like *allopurinol*, thus reducing urate formation. There are also drugs which promote excretion of uric acid, called *uricosuric agents* (e.g. *probenecid or sulfinpyrazone*). These are useful in long term treatment. For acute attacks of gout only symptomatic treatment may be useful.

10.2.3 An indirect way of elimination of protein nitrogen is via *creatinine*. All the creatinine formed in the body is excreted in the urine. Creatinine is formed when creatine phosphate (or phosphocreatine) is utilised in the muscle for release of energy during activity. The phosphocreatine in its turn is formed from glycine, arginine and methionine. However, the nitrogen of creatinine is from arginine and glycine. The muscle mass and its capacity for activity is often related to creatinine content of urine. The guanidino group is transferred to glycine to provide guanidine-acetate, by arginine, which itself is converted to arnithine. Methionine provides methylation of guanidino-acetate forming creatine. Part of creatine is cyclised to creatinine. It may be first activated to phosphocreatine before cyclisation. The creatinine formation is summarised below.

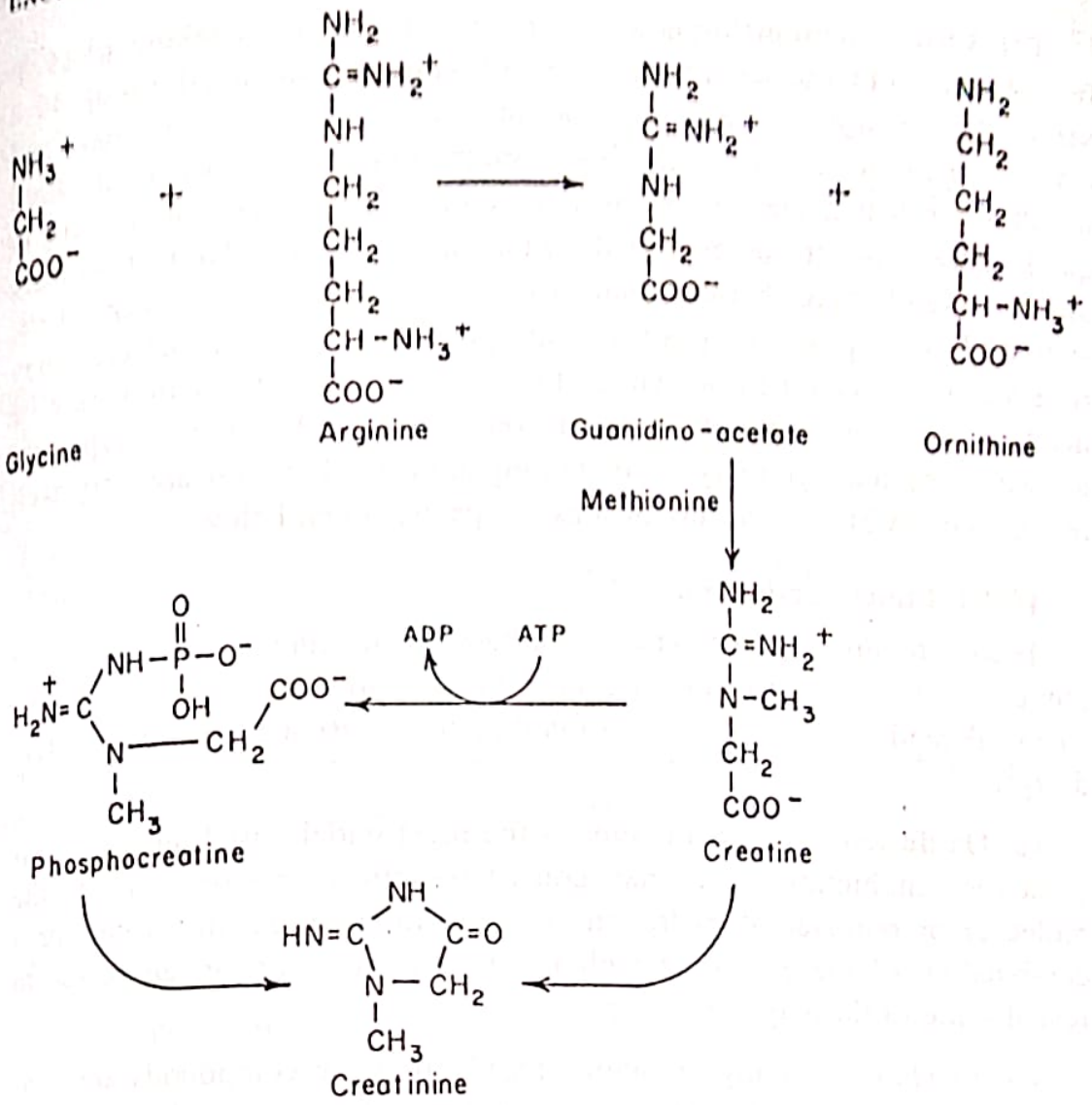


Chart 10.4

10.3 Drug Metabolism

The changes that are brought about in the body on drug molecules and others 'foreign' to the body are numerous and have been known for a long time. In most cases the 'toxicity' (or activity) of a drug molecule is reduced after the biotransformation. Hence the process was known as *detoxication* or *detoxification*. However, several biotransformations result in more active molecules and sometimes with therapeutically more desirable properties. There are also a number of *prodrugs*, which are themselves inactive, but are rendered active in the body, through normal biotransformation processes. Any chemical which is not normally found in the human body (i.e foreign) or is not a nutritional substance, is termed a *xenobiotic*. Most drugs like antibiotics, alkaloids etc. are xenobotics. Substances like hormones, vitamins, enzymes etc. even if employed as therapeutic agents, are not xenobiotics. The human body makes every effort to biotransform drugs and xenobiotics and eliminate them from the body.

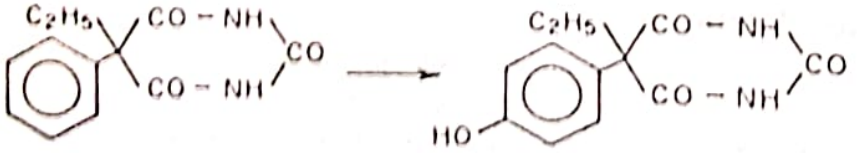
The various biotransformations can be considered as taking place in two stages : (1) Introducing into or releasing a functional group in a xenobiotic or drug. This may be also called *functionalisation*; and (2) *Conjugation* with specific body metabolites to render elimination feasible. In actual fact, often the two stages may be rapid and occur in quick succession. In some cases the first stage may be redundant, as there may be already enough functional groups present in the drug. In others, simple physical processes (like osmotic pressure, diffusion, pH etc.) may be adequate to eliminate a xenobiotic and no biotransformation may be needed. The functionalisation step is very broad and numerous types are known. The number of types of conjugation are few and are very well recognised. A brief overview of these steps are given below.

10.3.1 Functionalisation

There are three major types of either introducing a new functional group or releasing a functional group already present, but blocked by a chemical method. These are (a) Oxidation; (b) Reduction; and (c) Hydrolysis.

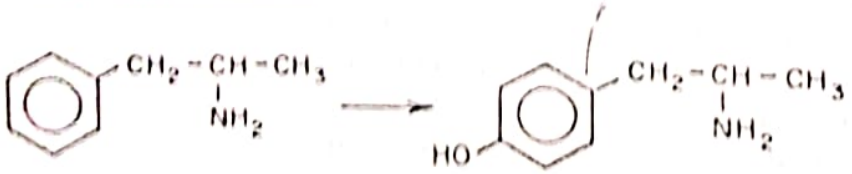
(a) **Oxidation** : By far this type is the most widely used biotransformation for xenobiotics. This may consist of introducing oxygen into the molecule or removal of hydrogens or removal of reducing groups or a combination of these. Some well known examples are given below to reveal some of these type reactions.

Hydroxylation : Many aromatic and heterocyclic compounds are converted to their hydroxy derivatives, thus rendering them more polar (solubility in water increased).



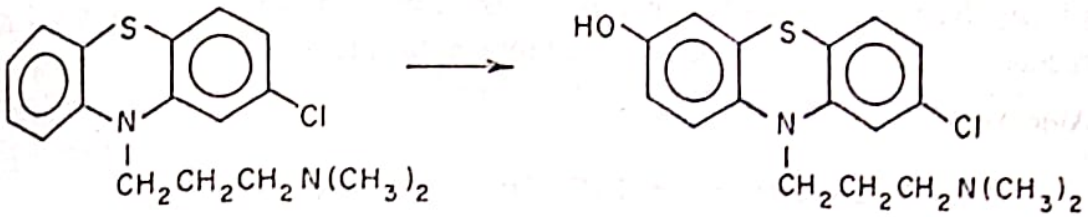
Phenobarbitone
(*Hypnotic & Anticonvulsant*)

(4-Hydroxy)-phenobarbitone



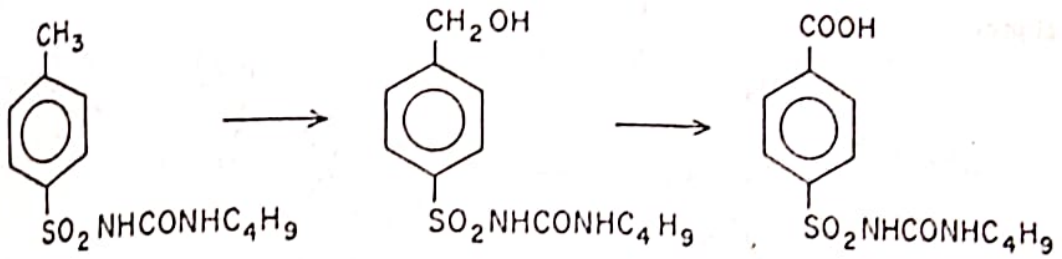
Amphetamine
(*CNS Stimulant*)

(4-Hydroxy)-amphetamine



Chlorpromazine
(*Tranquillizer*)

(7-Hydroxy)-chlorpromazine

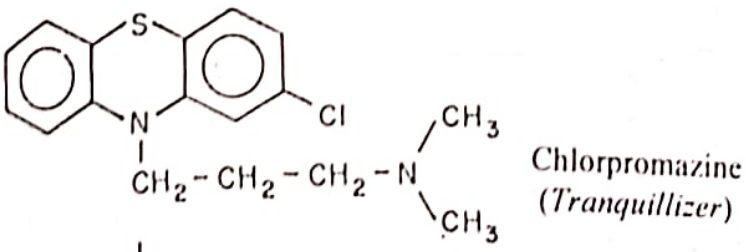


Tolbutamide
(*Anti-diabetic*)

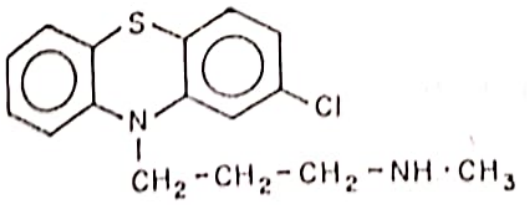
Hydroxyl Derivative
(side chain)

Carboxyl Derivative

Oxidative dealkylation and *oxidative deamination* are common among drugs containing amino-groups, as illustrated by a few examples.



Chlorpromazine
(*Tranquillizer*)



Desmethyl-chlorpromazine + Formaldehyde

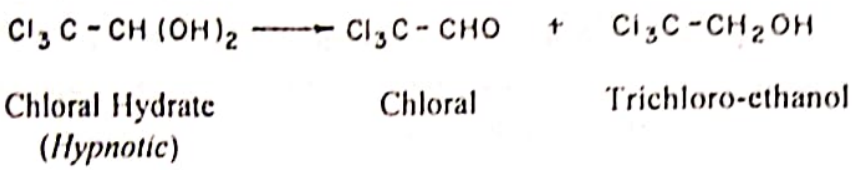
$$F_3C\text{–}CHClBr \longrightarrow F_3C\text{–}COOH + HBr + HCl$$

Halothane
(*Anaesthetic*)

Trifluoro-acetic
Acid

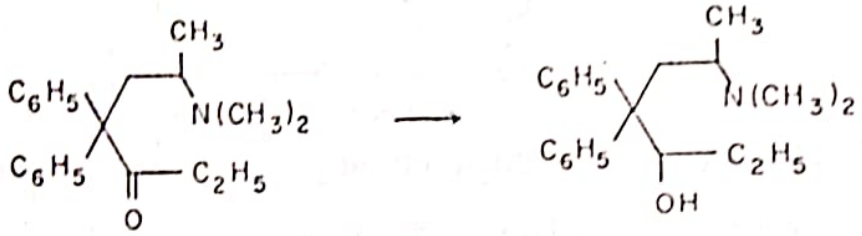
(b) **Reduction** : Although this is less common, several examples of reduction of drugs to less active and more polar metabolites are known.

Aldehydes :



Chloral Hydrate (*Hypnotic*) — Chloral — Trichloro-ethanol

Ketones:

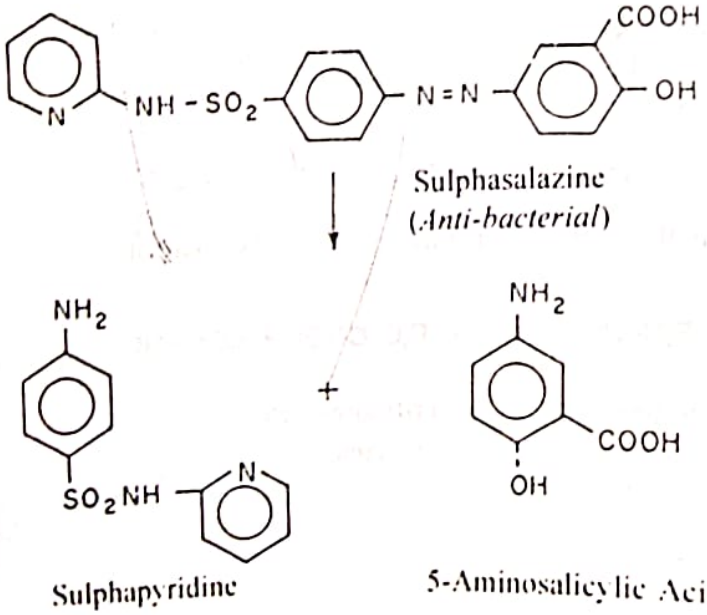


Methadone (*Analgesic*) → Methadol

Nitro Groups:

Metronidazole (*Anti-protozoal*)

Azo Groups:

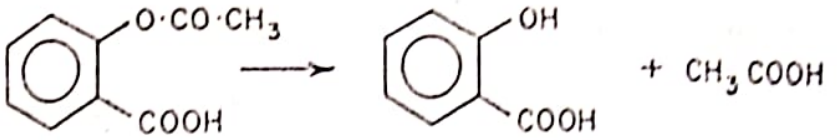


Sulphasalazine (*Anti-bacterial*)

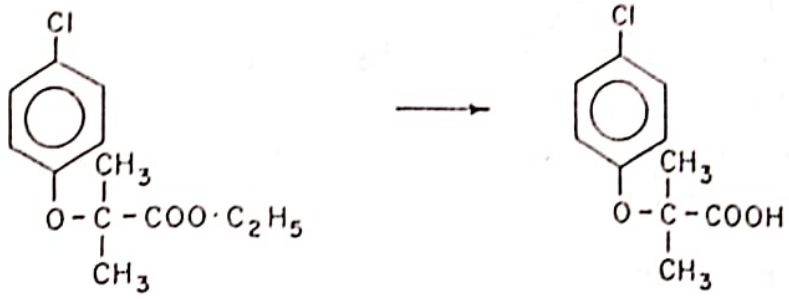
Sulphapyridine + 5-Aminosalicylic Acid

(c) **Hydrolysis** : Drugs and xenobiotics having ester, amide, and ether linkages, usually undergo hydrolysis liberating a functional group, which can then be biotransformed to facilitate elimination. Some examples are given below.

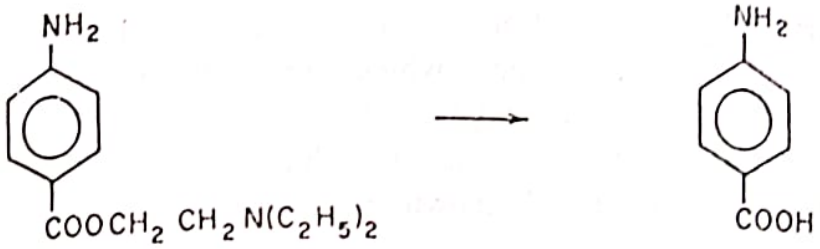
Esters:



Aspirin (*Analgesic*) — Salicylic Acid — Acetic Acid

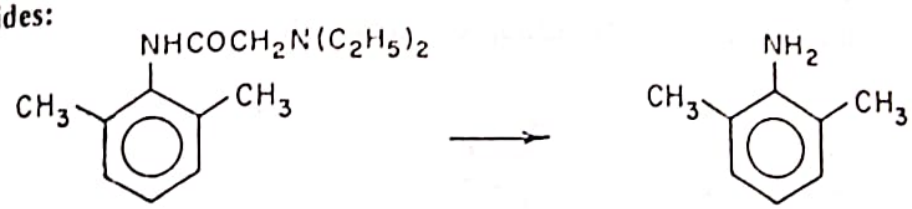


Clofibrate (*Anti-cholesterolemic*)

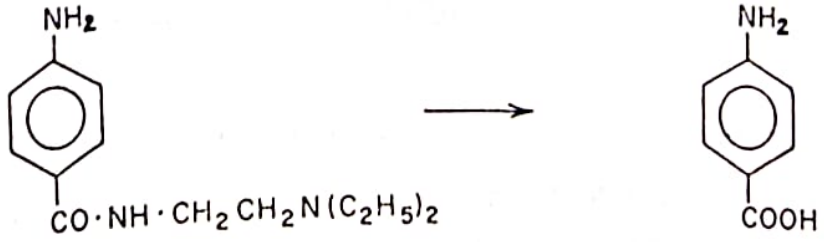


Procaine (*Local anaesthetic*) — PABA

Amides:

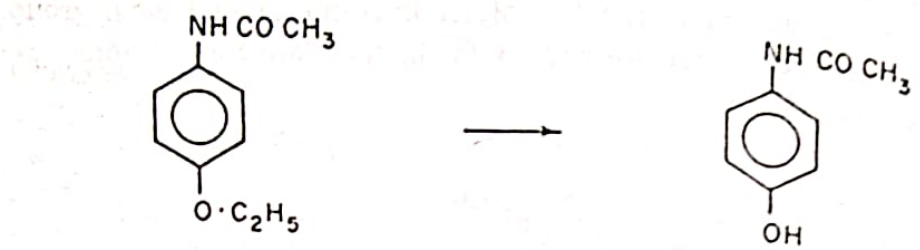


Lignocaine (*Local anaesthetic*) — Xylidine

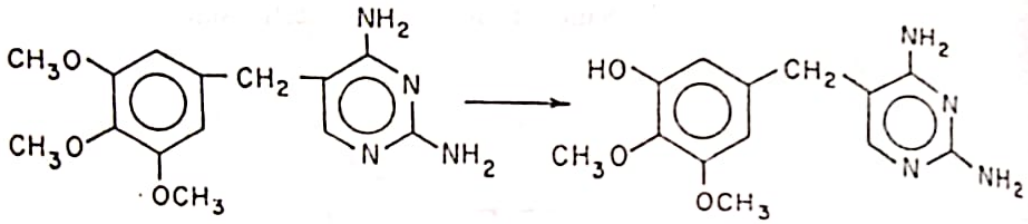


Procainamide (*Anti-arrhythmic*) — PABA

Ethers:



Phenacetin (*Analgesic*) Paracetamol

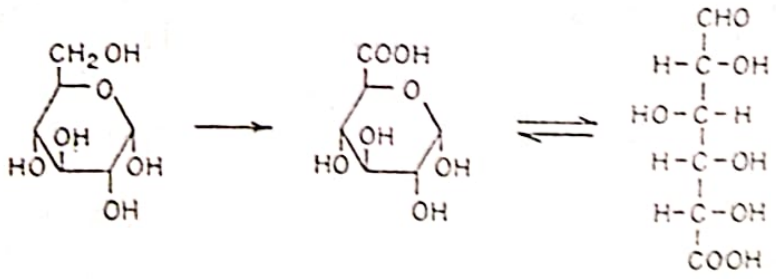


Trimethoprim (*Anti-bacterial*) Desmethyl Derivative

10.3.2 Conjugation :

Conjugation is the step in attaching a transferable moiety to a functional group of a drug or xenobiotic. These are usually easily available in the tissues that are metabolically active (e.g. liver, kidney, brain, intestines etc). The functional groups most often involved are the hydroxyl groups, amino-groups and carboxyl groups, which are easily formed during functionalisation reactions. The conjugating groups are : (a) glucuronic acid; (b) sulphuric acid; (c) amino-acids; (d) glutathione; (e) acetic acid; (f) methyl radicals etc. Some well known examples for these conjugation reactions are given below.

10.3.2.1 Glucuronides : Glucuronic acid is formed from glucose on oxidation of the primary alcohol group of glucose (C-6) :



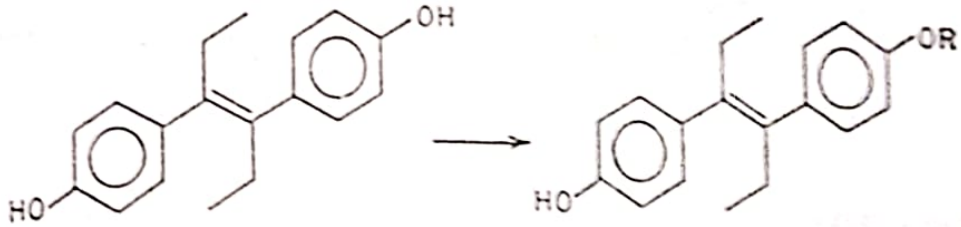
Glucose Glucuronic Acid

This is a unique metabolite in the body, with three different functional groups : the hydroxyl, the carboxyl, and the potential aldehydic group. It is highly polar and easily enters into reactions with a variety of functional

groups, providing highly soluble conjugates, which are excreted through urine. These conjugates are known as *glucuronides*. The glucuronidation involves the formation of uridine-diphospho-glucuronic acid (UDPGA) from glucose-1-phosphate and the transfer of the glucuronyl group to the drug or xenobiotic, mediated by a transferase enzyme. Glucuronidation of phenolic, alcoholic, enolic, carboxylic and N-hydroxylic groups are very well documented. Both aromatic and aliphatic amines, amides and thiols are also conjugated by glucuronic acid. Glucuronide conjugation is a normal pathway for several normal constituents of the body also (e.g. steroidal hormones, bilirubin etc).

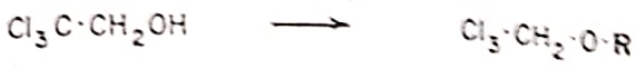
Phenolic Groups:

Paracetamol (*Analgesic*) — Glucuronide of Paracetamol

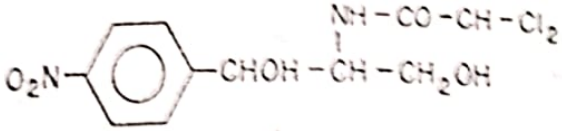


Diethyl-stilboestrol (*Oestrogen*) — Glucuronide Conjugate R = $C_6H_9O_6$

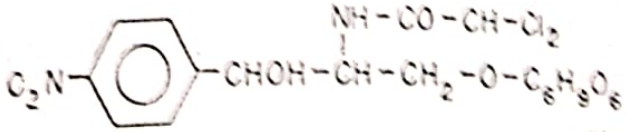
Alcoholic Groups:



Trichloro-ethanol (*Sedative*) — Glucuronide Conjugate

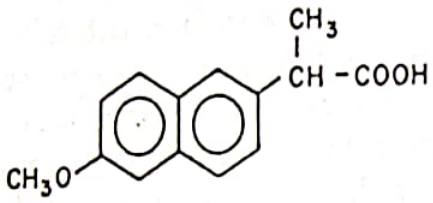


Chloramphenicol (*Antibiotic*)

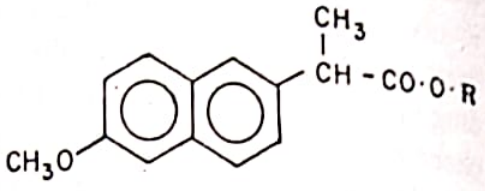


Glucuronide Conjugate

Carboxyl groups:

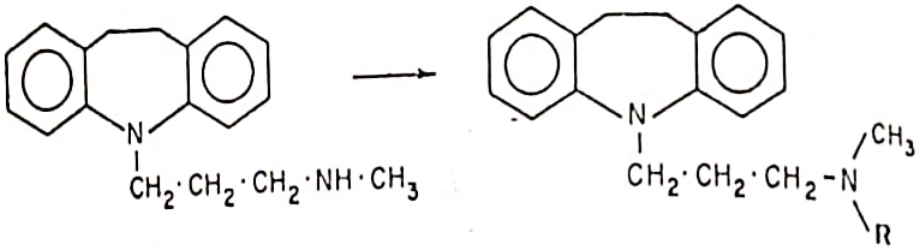


Naproxen
(*Anti-inflammatory Analgesic*)



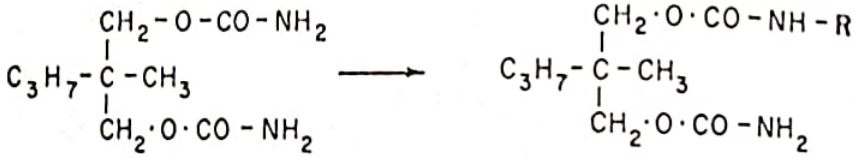
Glucuronide Conjugate

Amino and Amido Groups:



Desipramine
(*Anti-depressant*)

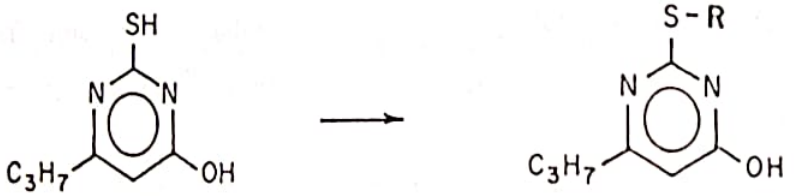
Glucuronide Conjugate



Meprobamate
(*Anxiolytic*)

Glucuronide Conjugate

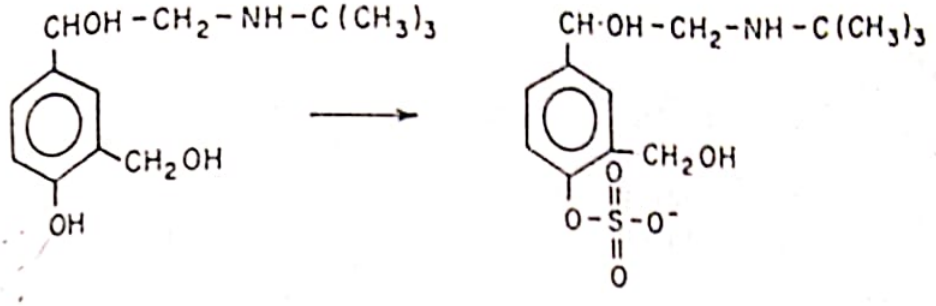
Thiol Groups:



Propyl-thio-uracil
(*Anti-thyroid*)

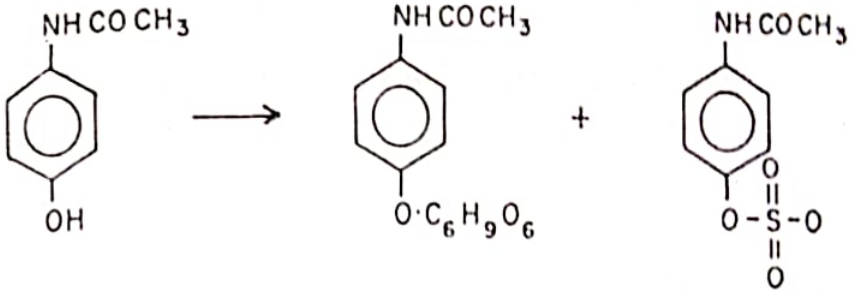
Glucuronide Conjugate

10.3.2.2 Sulphate conjugation : Sulphuric acid is formed by oxidation of the amino-acids cysteine and methionine by different routes to inorganic sulphate. However, both organic sulphate and inorganic sulphate are activated by enzyme and ATP which is then transformed to substrate drugs or xenobiotics. Phenols are the main functional groups undergoing sulphate conjugation. Even then it may be a minor path, glucuronidation being the major route.



Salbutamol
(*Bronchodilator*)

Sulphate Conjugate

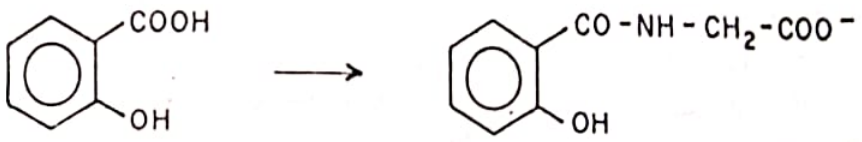


Paracetamol

Glucuronide
(major product)

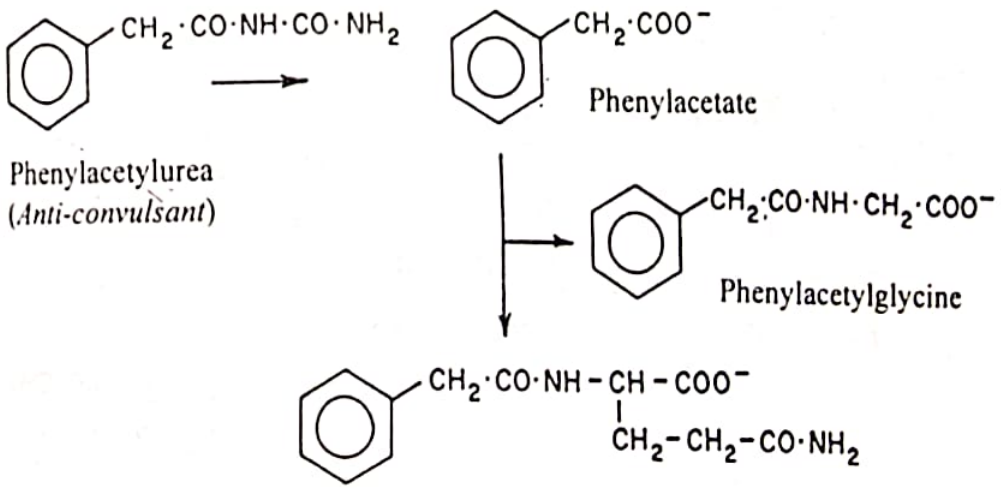
Sulphate
(minor product)

10.3.2.3 Amino-acid conjugates : The amino-acid glycine and glutamine are often directly involved in conjugation of carboxylic acid groups in drugs. Cysteine is indirectly introduced for conjugation. Other amino-acids may also enter into conjugation reactions in a minor way.



Salicylic Acid
(Glycine Conjugate)

Salicyluric Acid

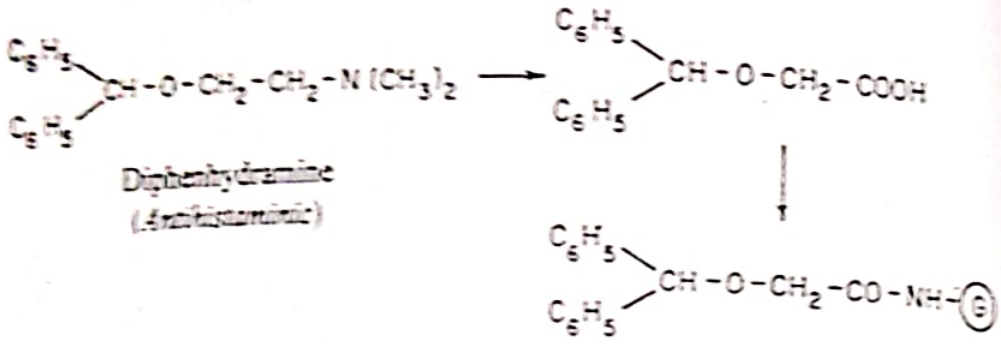


Phenylacetylurea
(*Anti-convulsant*)

Phenylacetate

Phenylacetylglycine

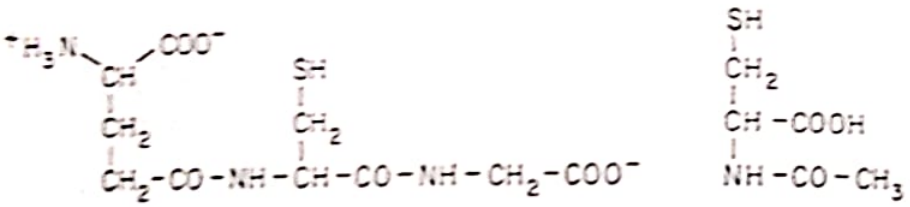
Glutamine Conjugate



Diphenhydramine (Antihistaminic)

Glutamine Conjugate

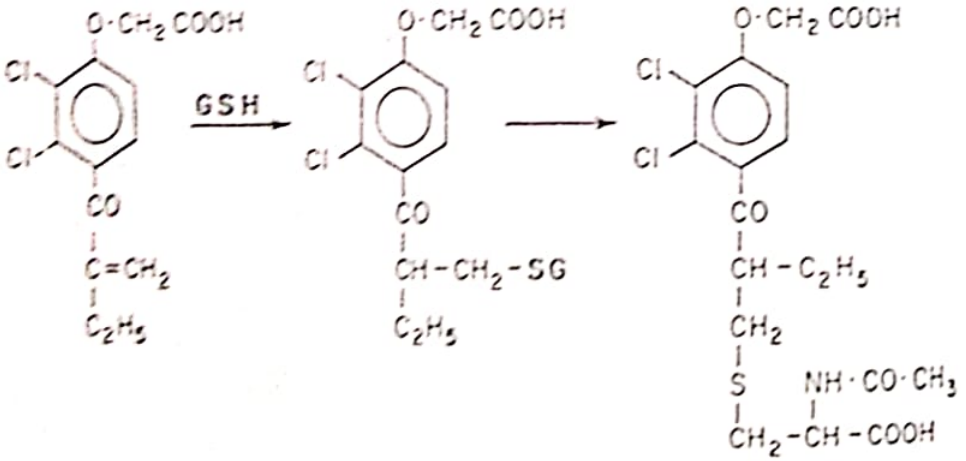
10.2.3.4 Glutathione conjugation : Glutathione (GSH) is an important tripeptide in the body, with several functions. One of these is to conjugate drugs and xenobiotics and convert them to mercapturic acid derivatives.



Glutathione (GSH)

N-Acetyl Cysteine

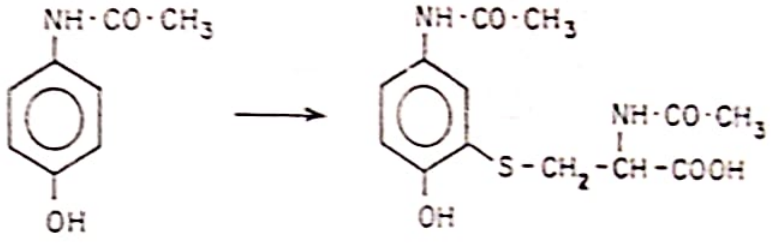
Glutathione in its reduced form acts as a nucleophilic agent and reacts with electron deficient carbon in drugs and xenobiotics. Compounds with electron withdrawing groups like halogens, oxygen, aromatic and heterocyclic ring carbons, are susceptible to glutathione conjugation. After glutathione adds to the molecule, via the sulphur atom, glutamine and glycine are delinked leaving behind cysteine attached to the drug, which is then acetylated at cysteine amino-group by acetyl coenzyme A. This final product is called mercapturic acid.



Ethacrynic Acid (*Diuretic*)

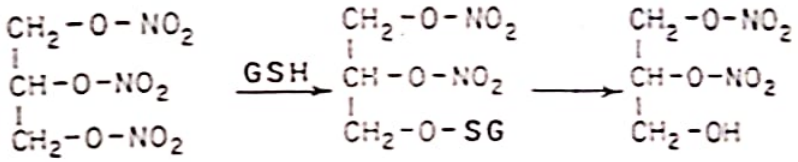
Glutathione Adduct

Mercapturic Acid Derivative



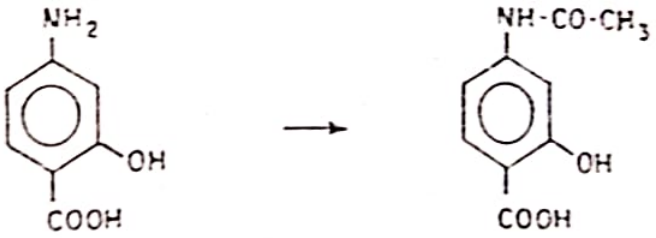
Paracetamol — Mercapturic Acid Derivative

Glutathione conjugation also leads to simple hydrolysis in case of organic nitrates, like nitroglycerin and isosorbide dinitrate.



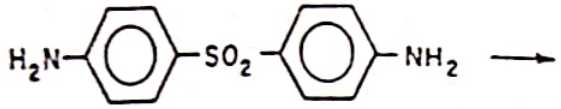
Nitroglycerine (*Anti-angina Drug*) — Glutathione Adduct — 1,2-Dinitroglycerine

10.3.2.5 Acetylation : This is a fairly common route of conjugation, particularly for amino-groups of drugs. Other related groups like amides, hydrazines, hydrazides also undergo acetylation reactions. Most sulphonamides, sulphones containing amino-groups are frequently acetylated. Isonicotinic acid hydrazide (INH) is acetylated extensively.

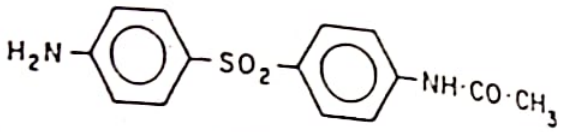


Para-amino-salicylic Acid (*PAS ; Anti-tubercular*) — *N*-Acetyl-PAS

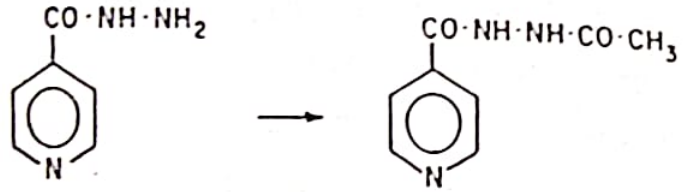
Sulphonamides (*Anti-bacterials*) — N^4-Acetyl-sulphonamides



Dapsone
(*Anti-Leprotic*)



Acedapsone

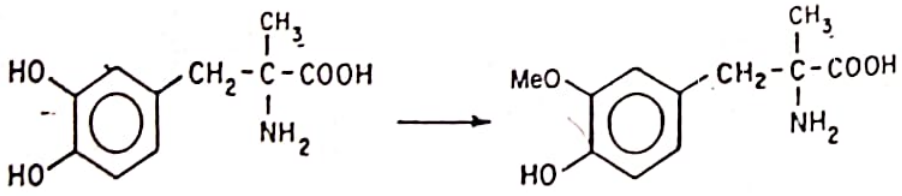


Isoniazid : INH
(*Anti-tubercular*)

Acetyl-INH

Although acetylation generally renders the drug less toxic (detoxication), the acetyl derivatives are usually less soluble than the parent drugs. Acetyl coenzyme A is the main acetylating agent in all these reactions.

10.3.2.6 Methylation : Methylation is a normal process in the body, whereby important compounds in the body are formed like acetylcholine and adrenaline. Also inactivation of adrenaline, nor-adrenaline, dopamine (all called catecholamines) takes place routinely by *O*-methylation of the phenolic hydroxyl groups. *N*-methylation and *S*-methylation also take place in the body to yield products like adrenaline, trigonelline etc. Methylation is not a major conjugating (detoxication) process in the body. Following examples are illustrative of the type reaction. Methionine is usually the immedIate donor of methyl groups.



Alpha-methyl DOPA

(3-O-Methyl)-α-methyl-DOPA

Nor-ephedrine

Ephedrine

Nicotinic Acid Trigonelline

Propylthio-uracil *S*-Methyl Derivative

10.3.3 Conclusion : It should be noted that the same drug or xenobiotic may be metabolised by several different pathways. The choice of the pathways depends on many factors like species differences, genetic differences, age, food habits etc. It should be also noted that rarely a single step is used. More often several steps are involved in conjugation before elimination from the body. Further there are numerous instances of conjugation leading to more active (toxic ?) compounds. In fact several prodrugs have been prepared which ensure release of bio-active molecules in the body after undergoing biotransformations.

11

Minerals and Water

11.1 Introduction

The human body is rich in fluid and minerals. The role of organic compounds like proteins, carbohydrates, fats, nucleic acids, vitamins, hormones and enzymes, has been outlined earlier. All these compounds contain carbon, hydrogen and oxygen in abundance and nitrogen, sulphur and phosphorous to a considerable extent. Moreover, these are generally inter-convertible, i.e. the body can synthesize one from the other via the intricate and interwoven metabolic pathways. On the other hand minerals are inorganic and are not inter-convertible. Hence the human body is dependent on dietary mineral sources. Not all the elements of the periodic table are needed for normal living processes. Only a few are needed for some important processes, some in quantities that are considered large enough to be called *macro-minerals* and others needed in very small quantities and designated as *micro-minerals*. (*Note* : These terms should not be confused with macro and micro nutrients, which include other nutritional substances, also mentioned earlier).

Macrominerals include calcium (1.4% w/w of human body), phosphorous (0.8%), potassium (0.27%), sodium (0.26%), chloride (0.25%), sulphur (0.2%), and magnesium (0.1%). The microminerals include iron, zinc, cobalt, copper, iodine, manganese, molybdenum, selenium and chromium. Water plays a very important role in the body in transport of these minerals besides providing conditions suitable for biochemical reactions. The water content of the body is fairly well regulated. Electrolytes and water are also discussed in detail in books on Inorganic Pharmaceutical Chemistry. (e.g. by the same author and same publisher). Readers are advised to go through the relevant chapters there for more useful and related information.

11.2 Macrominerals

11.2.1 Sodium : Sodium ion is widely distributed in the body. It exists as sodium ion, paired usually by chloride ion in most extracellular

fluids, but also by bicarbonate or phosphate anions in some parts of the body. Its main functions are to maintain : electrolyte concentration, regulate osmotic pressure, membrane potential and acid-base regulation (pH). An adult needs about 2 to 5 g per day. Usual dietary intake provides several times this amount. Hence most of excess of sodium and chloride is excreted through urine and/or perspiration. Sodium deficiency is very rare. It may occur due to loss of body fluids as in accidents or **diarrhoea** and when drugs which promote sodium excretion are frequently used.

11.2.2 Potassium : Potassium ion in the body, is largely found inside the cells (intracellular). This ion is also paired with chloride or bicarbonate or phosphate. Its functions are similar to sodium in maintaining acid-base and membrane potential. Potassium ions are also needed in some enzyme catalysed reactions (e.g. *pyruvic kinase*). Potassium deficiency occurs rarely, as most diets contain adequate potassium salts. Moreover, potassium ion is extensively recycled in the body and only little is excreted. However, when drugs are used, excretion of potassium may occur leading to **hypokalemia**. The condition can be corrected by administering electrolyte fluids containing potassium. On the other hand potassium sparing drugs and sodium excreting drugs can cause **hyperkalemia**. Sodium competes with potassium and hydrogen ions and can easily disturb the balance, leading to **alkalosis** and **hypokalemia** or **acidosis** and **hyperkalemia**, as the case may be.

11.2.3 Chloride is never found to be deficient either in the body or in diets. It is always associated with sodium or potassium. Its functions are closely related to these two cations. It is also the ion accompanying hydrogen ion in the stomach.

11.2.4 Calcium : Calcium is an essential and most abundant macromineral in the body. It is the major constituent of bone, but is found in micro levels in blood and other body tissues. It has thus two important roles. (1) structural, providing strength to the bone; and (2) dynamic, needed for both physiological and biochemical processes. It is essential in the blood coagulation mechanism, mediating the binding (activiating) of prothrombin II and other factors to the phospholipid membranes. Muscle contraction and neuro-muscular irritability are regulated by calcium ions, which maintain suitable potential. The calcium level of blood is regulated within a narrow range of 2.25 to 2.6 mmol/litre. Any increase beyond the maximum is termed **hypercalcemia** and below the minimum is called **hypocalcemia**. Both these conditions may lead to severe consequences. Hence maintenance of calcium levels in the blood is necessary.

Calcium level in plasma is affected by (1) absorption from food sources; and (2) transport of calcium to and from bone. Absorption of food calcium is dependent on availability. About 500 mg/day is considered as adequate for an adult. For growing children this is not adequate and is in the range of 800 mg to 1 g per day. This amount is also recommended for pregnant and lactating women. There is also evidence indicating the higher requirement of about 1 g per day in elderly persons and post-menopausal women. Milk is rich source of calcium. Absorption of calcium from foods is affected adversely by some plant acids (e.g. phytic acid). Its absorption is increased by calcitriol (1,25-dihydroxycholecalciferol), a hormonal form of vitamin D. Plasma calcium is regulated by hormones and other electrolytes like magnesium and phosphate. Nearly a half of plasma calcium is bound to plasma protein and the rest is available for physiological activity in the form of inorganic cation.

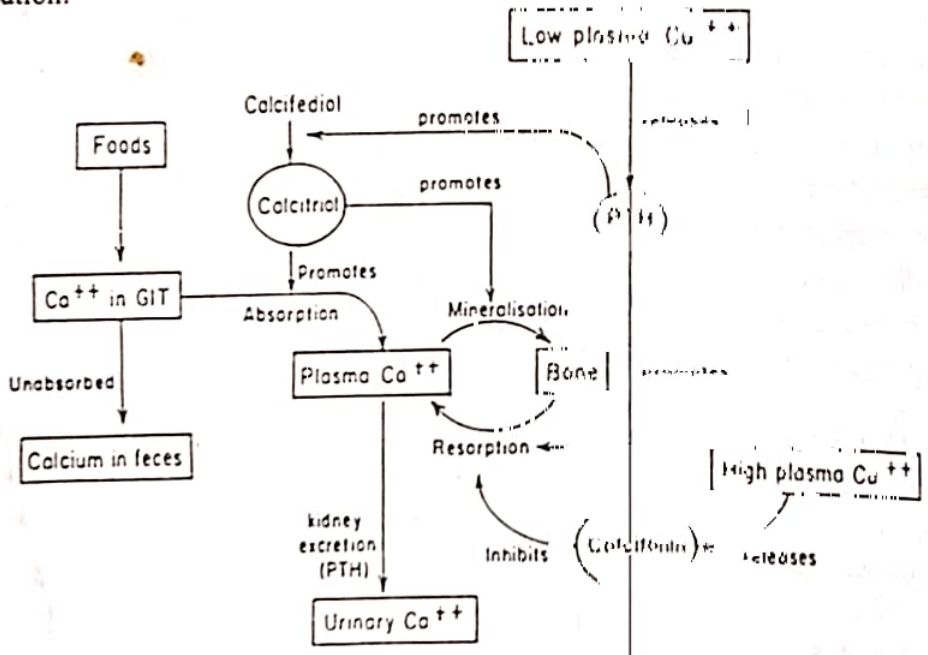


Chart 11.1 Calcium Regulation

Increased calcium ion level in plasma, after absorption from intestines, will be brought under control by (a) excretion through the kidney; and (b) deposition in bones. Proteins promote excretion of calcium (along with phosphate). Hence persons used to high protein (animal) diet may need high calcium intake, to compensate higher excretion in urine. *Parathyroid hormone* (PTH) also indirectly causes overall calcium excretion as a result of phosphate excretion followed by increased plasma calcium levels. *Calcitonin* is responsible for the reduction of plasma levels of calcium due to inhibition of calcium resorption from bones (i.e. release of Ca^{++} by dissolution of hydroxyapatite of the bone). On the other hand

parathormon (PTH) induces production of *calcitriol* in the kidneys, which in its turn promotes absorption of Ca^{++} from the GIT, resulting in higher plasma levels. PTH also promotes bone resorption (opposite effect to that of calcitonin), again contributing to plasma Ca^{++} levels. The activities of calcitonin and PTH themselves are triggered by high or low plasma Ca^{++} levels respectively. Thus it is a closely self regulated system. Above chart summarises plasma Ca^{++} regulation.

The deficiency of vitamin D indirectly results in poor mineralisation (calcification) of bones causing **osteomalacia** in adults and **rickets** in children. This is partially due to poor absorption of Ca^{++} from GIT, causing hypocalcemia. This condition may also be caused by poor absorption from GIT due to dietary defects. Accordingly both vitamin D and adequate amounts of calcium should be supplied. In elderly persons, excretion of Ca^{++} through kidney increases, causing bone resorption, which in its turn results in **osteoporosity**. Increased intake of calcium and calcitonin may help reduce rate of resorption (decalcification), but recalcification is not very significant. Calcium preparations available for these purposes include : clacium chloride, calcium phosphates, calcium gluconate, calcium lactate, calcium levulinate (all these are official in I.P.). (For more details consult Inorganic Pharmaceutical Chemistry by same author).

11.2.5 Phosphate : Phosphorous is always found as phosphates in all living organisms. Hence foods from natural sources (animals or vegetables) always contain adequate amounts of phosphate. Nutritional deficiency of phosphates is unknown. However, plasma levels of phosphate may deviate from normal ranges, due to failure of regulatory mechanisms. High calcium levels in plasma tend to reduce phosphate levels. PTH, as already mentioned, promotes urinary excretion of inorganic phosphate. Bone resorption results in plasma phosphate levels. Inorganic phosphate is converted in the body to organic phosphates. Innumerable examples of bio-organic phosphates are known, which are involved in biochemical pathways.

11.2.6 Magnesium : This cation is on the borderline of macro and micro-minerals, being about 15 mmol/l in intracellular fluids and about 1 mmol/l in plasma. Magnesium is well distributed in foods. Its deficiency is therefore rare. However, **hypomagnesemia** is a condition, which can be easily corrected by administering magnesium enriched electrolyte fluids. Magnesium is involved in neuromuscular transmission and many enzyme activities like phosphatases, kinases, enolase, pyruvic carboxylase etc.

11.3 Micro Minerals

11.3.1 Iron : The metal iron is unique in the human biochemistry and physiology. It is relatively less toxic, though it is a heavy metal. Its salts ionise in water to form two series of ions, ferrous (Fe^{++}) and ferric (Fe^{+++}) with one electron difference. The inter-conversion of these two ions takes place easily at physiological conditons of the body, providing a very useful redox system. Thus in oxidation reduction processes the Fe^{++}/Fe^{+++} system could participate effecting electron transfer. An indication of this was made while discussing bio-energetics. Its major participation is in the oxygen and carbondioxide transportation by hemoglobin of the erythrocytes. Hemoglobin and myoglobin are two important porphyrin-iron-protein complexes involved in this. Iron is also a component of cytochromes and a few other enzymes. It amounts to about 4 g, less than 0.01% of a normal adult person. Most of this (about 68%)) is part of tissue *ferritin*. Another protein, *transferrin* and enzymes together account to less than 0.8% of the total body iron.

Unlike in the case of calcium, the body does not carefully regulate iron levels either in the plasma or in tissues. In fact, the body has a large capacity to retain iron. It is excreted to a very small extent through the kidney. A daily requirement of 10-15 mg for a normal adult may be adequate. In pregnancy the requirement is much higher. However, its absorption from foods is somewhat regulated, being low if body is already loaded with iron and moderate if there is iron deficiency in the body. The protein *apoferritin* is involved in this regulation. Foods like meat, dried legumes, fruits and milk contain absorbable forms of iron. In many foods iron is bound strongly to some organic acids, such that ionic form of iron cannot be released in the stomach to enable absorption. The gastric acid reduces Fe^{+++} forms to Fe^{++} which is generally absorbed from duodenum and near intestine. In more alkaline pH iron hydroxides may be precipitated reducing absorption.

Fortunately low absorption does not matter much, if the body is already storing adequate amount of iron. Iron is recycled in the body very efficiently. When hemoglobin is degraded after the normal life of an erythrocyte, the protein and porphyrins find their way to elimination. Protein is degraded to amino-acids, entering into the usual channels of amino-acid metabolism. The porphyrin is converted to biliverdin and then to *bilirubin*, which may be excreted. But the iron is almost entirely recycled, with the help of transferrin to the liver. In the liver, ferritin is formed. If iron content is high, ferritin associates with particles of iron protein complexes, forming *hemosiderin*. One atom of ferrous iron forms four co-ordinated linkages with four nitrogen atoms of tetrapyrrole ring of protoporphyrin, forming the pigment *heme*.

The central ferrous atom has still capacity for two more co-ordinated bonds. One of these is utilised by the globin polypeptide to bind to iron through a histidine nitrogen. The sixth bond is utilised by oxygen for attachement and transportation. This bond may also be blocked competitively by other groups like methyl, cyano etc. irreversibly thus reducing the oxygen carrying capacity of hemoglobin.

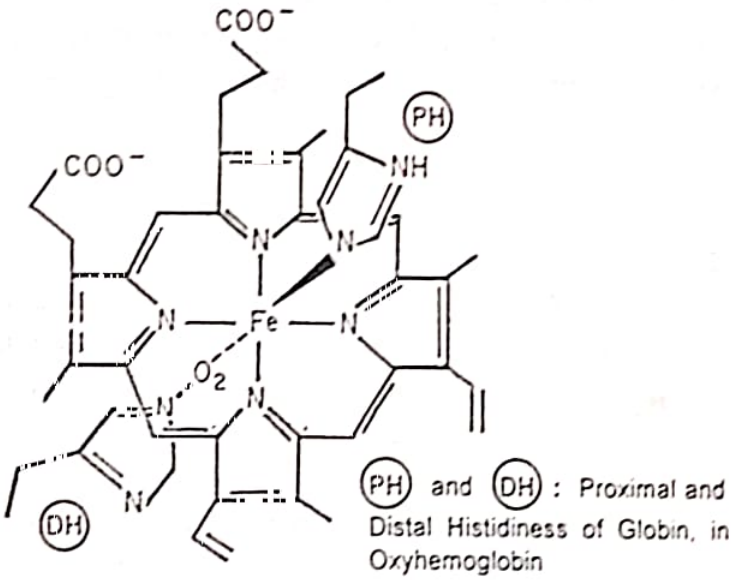


Fig.11.1 Oxyhemoglobin

Iron deficiency leads to specifically **microcytic hypochromic anemia**, i.e. small size erythrocytes containing lesser amounts of hemoglobin. This condition is easily corrected by administering oral iron preparations, if absorption is not impaired. A large number of products with well absorbable forms of iron, are available in the market. These include IP products like ferrous sulphate, ferrous fumarate, ferrous gluconate, ferric ammonium citrate for oral administration, and iron dextran injection for parenteral use. A number of other iron salts like carbonate, phosphate, iodide, chloride, lactate, tartrate etc. are also often marketed. (For more details consult Inorganic Pharmaceutical Chemistry by the same author.)

11.3.2 Zinc : The presence of zinc in the body and some of its functions have been well established. However, its content in the body is difficult to estimate, but is probably less than 0.005%. Further it is distributed throughout the body, with no regulated levels in serum or other tissues. It is also not regularly excreted through the kidney. It is widely distributed in foods, particularly meat, cereals and milk. Absorption of zinc from the GIT is poor, although deficiency is rare. In rare cases of zinc deficiency (e.g. in general malnutrition), growth and sexual development in children may be affected, as also lack of taste perception.

Most of the body zinc seems to be recycled. It is relatively non-toxic and can accumulate in the body without causing any pathological conditions. On an average, an adult absorbs about 10 to 15 mg daily from foods.

Zinc is part of the active form of insulin molecule. It is also part of several enzymes including *carbonic anhydrase, alcohol dehydrogenase, alkaline phosphatases, RNA and DNA polymerases* etc. Zinc ions also promote wound healing. Several zinc compounds are used as astringents for topical application and for adding to mineral supplements. The I.P. includes zinc chloride and zinc sulphate for this purpose.

11.3.3 Copper : This is a trace metal and its content in the body or its level in any tissue is difficult to determine. Its requirement may be about 1 mg/day for a normal adult. Normally foods (particularly if cooked in copper or bronze or brass vessels) contain more than adequate amount of copper. Its deficiency is rare, except in general malnutrition cases, causing symptoms like **anemia, bone dimineralisation** etc. On the other hand copper may accumulate in the body and cause **Wilson's disease**, characterised by liver and brain disorders. Copper removal by dialysis and/or chelation is indicated in such conditions. Copper is part of some enzymes like *ferroxidase* (responsible for conversion of Fe^{++} to Fe^{+++} after absorption, to enable binding to transferrin), *cytochrome C oxidase, dopamine ß-hydroxylase, superoxide dismutase* etc.

Hepato-lenticular Degeneration : This is also known as **Wilson's disease**. This is generally considered a rare disease, because it is caused by accumulation of copper in several tissues and organs in the body, including brain, kidneys, liver etc. This is probably an inborn disease and may lead to **mental impairment**. It affects the liver leading to **jaundice, cirrhosis** etc. If recognised early, it may be controllled by avoiding copper-rich diet.

11.3.4 Other micro-minerals : In the body extremely low quantities of a few other metals are detected. Some of these are undoubtedly essential. But nutritional deficiencies of these minerals are not known.

Manganese is part of some enzymes, e.g. *pyruvic carboxylase*. It is involved in fatty acid synthesis and ATP synthesis.

Selenium is known to be associated with *glulathione peroxidase*.

Molybdenum is a component of *xanthine oxidase*.

Chromium is known to be associated with glucose tolerance factor (GTF).

Cobalt is part of the vitamin cyanocobalamin (and hydroxocobalamin). However, none of these ions are considered as essential nutrients. Probably these are absorbed directly in the form in which they are active and recycled in the body. For example cobalt salts cannot promote formation of cyanocobalamin, which itself has to be present in the food as an essential nutrient.

11.3.5 Iodide : The only mineral anion which belongs to the micro mineral category is iodide. It occurs as trace element in the body. Almost all the body iodine is found as part of the thyroid hormone molecules, *thyroxine* (T_4) and *tri-iodo-thyronine* (T_3). Iodide from food is very well absorbed and transported rapidly to the thyroid gland. The inorganic iodide is converted to organic iodinated di-iodo-tyrosine, mediated by *thyroid stimulating hormone* (TSH). The role of iodine in the body is essentially the role of thyroid hormones. Thus the basal metabolic rate (BMR), growth and development of the body is influenced by iodide. Iodide is also recycled and partly excreted. Marine foods are good sources of iodides. Even salt prepared from sea usually contains adequate iodide. However, people living far removed from the sources of sea food and salt (as in mountains), are likely to experience iodide deficiency.

Prominent among the symptoms of iodide deficiency is **goitre**, an enlargement of the thyroid gland. Goitre may be wide spread in areas, where sub-soil drinking water is deficient in iodides and the population is unable to obtain marine salt and food. This may assume the proportion of an epidemy (*endemic goitre*) and may need large scale health measure. In India it is estimated that over 60 million people may be affected by goitre. To avoid such severe situations, it is now a general practice to add a very small amount of iodide (about 1 in 25000), to common table salt (iodised salt). This effectively controls goitre in endemic areas and prevents iodide deficiency. All forms of inorganic iodine (iodine itself, iodides or iodates) may be used for this purpose. The I.P. includes iodine, potassium iodide and sodium iodide in its monographs.

11.3.6 Fluoride : Fluoride is a trace material in our body. It has not been definitely established that it is essential. It is generally obtained from food and water in adequate amounts. In some regions, drinking water is totally devoid of fluoride. In such places, people suffer from **dental caries**. This may assume alarming proportions, similar to endemic goitre due to iodine deficiency. In such areas, *fluoridation* of municipal water supply is generally undertaken as a health measure (compare iodination of common salt). The role of fluoride in human nutrition and metabolism has not been demonstrated. Dental caries is caused by corro-

sion of teeth dentine by acids, produced by micro-organisms growing in the crevices and cavities of teeth. Fluoride seems to be incorporated into dentine material, making it hard and resistant to acid. This type of mineralisation may also occur in bones, making them hard and increasing their density. This is believed to be beneficial in **osteoporosis**.

About 1 mg of fluoride per day is considered to be adequate and 2 mg may be upper limit. About 1 part per million (ppm) in drinking water amounts to 1 mg per day, if about 1 litre of water is consumed. Tea is a very rich source of fluoride. When fluoride ingestion exceeds the acceptable limits, it may cause **mottling** of teeth, **muscular weakness, gastric disturbances, convulsions** and even **cardiac failure**. For prevention of dental caries, tooth pastes and dentifrices with added fluoride are adequate, which provide fluoride in very small quantities locally. For internal use sodium fluoride (I.P.) and sodium monofluoro-phosphate may be employed.

11.4 Water

Water is the largest chemical constituent of the body, amounting to about 75% of the body weight. Within a range of 2%, the body is able to maintain this amount under normal conditons in a healthy adult. Water content is necessary to be regulated because most body chemicals are maintained at regular and acceptable levels. This is called *homeostasis*. This is possible only by free transport of such chemicals between cells and tissues, facilitated by water. Water has some unique properties, which makes it a very suitable medium of life processes. These are :

(*i*) It is a polar solvent dissolving a number of chemicals.

(*ii*) It has a high dielectric constant, facilitating ionisation and maintenance of potential across membranes.

(*iii*) It has high specific heat and thermal conductivity, both of which help in quick dissemination of heat energy within the body, maintaining a constant body temperature.

(*iv*) It has a high heat of vaporization and moderate vapour pressure at ambient temperatures. This helps in preventing loss of fluid at body temperature due to evaporation.

(*v*) It is a weak electrolyte and provides suitable pH for the body reactions, with the help of small quantities of dissolved chemicals (regulation of acid base equilibrium).

(*vi*) It participates in and is a product of many biochemical reactions.

(*vii*) Its small molecular weight, high surface tension and moderate viscosity are well suited for its transport and retention in tissues.

Water is supplied to the body through foods and direct ingestion. Part of the orally ingested water is eliminated in faeces. Rest of it is absorbed. Body excretes large amount of water in the form of urine, about 1.5 litre per day for an average adult, which carries waste products from metabolism, like urea, creatinine, sodium, chloride, bilirubin etc. Some amount of water escapes from the body in the form of vapour through the exhaled air. This quantity is dependent on vapour pressure of water at body temperature and vapour content in the atmosphere. In warm climate and after exertion, water also escapes as sweat, which also carries salts and other chemicals.

Other forms of escaping water are milk secretion, tear secretion, spittle etc. all of which are of minor significance. Large quantities of water from the body may be lost in conditions like vomiting, diarrhoea, hemorrhage, excessive urination etc. The human body is sensitive to losses of water. Small losses cause thirst (due to increased electrolyte concentration followed by increased intracellular osmotic pressure). This stimulation prompts us to consume water and set right the water balance. In large losses as in vomiting, diarrhoea, hemorrhage, excessive urination, water alone will not satisfy the body, because besides water large losses of electrolytes have also occurred. Hence consumption of mineralised waters (electrolyte solutions or rehydration salt solutions) is to be preferred. (see also Inorganic Pharmaceutical Chemistry, by the same author).

Within the body the electrolyte and water equilibrium is regulated by physico-chemical processes like osmotic pressure, membrane potential, pH, solubility, interwoven with that of electrolyte regulation within and outside the cells. However, the elimination of water (and electrolytes) from the body, mainly in urine is regulated by the action of hormones. Two major hormonal controls of water in urine, are well known.

(1) The posterior pituitary secretes a hormone called *Anti-Diuretic Hormone* (ADH) or *Vasopressin* or *Argipressin* or *Beta-hypophamine*. This hormone directly influences the amount of water secreted as urine, causing water retention. In its absence or deficiency, there is excess of dilute urine eliminated by the kidney. These conditions may be called **polyuria, diabetes insipidus etc.** While considerable amount of water is eliminated the proportionate amount of salts, urea etc. are not found in the urine. Hence the name *diabetes insipidus*. In the normal body ADH regulates retention of water in the body.

(2) *Corticosteroid hormones*, particularly *aldosterone* (and to lesser extent *deoxycorticosterone* and others) cause re-absorption of sodium from the filtered primary urine (at glomerulus) at the distant tubules, before

elimination. At the same time, potassium excretion is increased (i.e its reabsorption is decreased). There is also inhibition of water re-absorption at the distant tubules, increasing total urine output, which has low sodium, but high potassium. The conditon may also be caused by inhibition of ADH by these hormones. The corticosteroid secretion is regulated by the *anterior pituitary hormone* ACTH. Thus ACTH indirectly interferes in the regulation of water, sodium and potassium excretion.

11.4.1 Pharmaceutical Products

Vasopressin (I.P.) is the anti-diuretic hormone (ADH) of the posterior pituitary gland. It is obtained by collecting the posterior pituitary glands of cattle or sheep (from slaughter houses) and extraction and purification. It may also be produced by synthesis, as it is oligopeptide with only nine amino-acids. Although it has mild vasopressor activity, it is used only as an anti-diuretic, in treating *diabetes insipidus*. As it may be accompanied by *oxytocin*, a closely related posterior pituitary hormone, particularly if obtained from collected glands of slaughtered animals, the Pharmacopœia restricts the oxytocic activity of vasopressin preparations.

Deoxycortone acetate (I.P.), also known as desoxycorticosterone acetate or DOCA, is the acetate ester of the corticosteroid hormone of the adrenal cortex. It is now obtained by partial synthetic methods, along with other steroidal hormones. Its main application is for regulating salt excretion and retention in the body. Although *aldosterone* is the more potent naturally occurring hormone of the cortex, it is more difficult to obtain. Hence DOCA and other synthetic analogs are used in place of aldosterone.

Many other factors like adrenaline, histamine, drug consumption etc. also influence water equilibrium in the body, besides pathology of kidney itself.

12

Pathology of Blood and Urine

12.1 Introduction

As pointed out from time to time in earlier chapters, the body has mechanisms of *homeostasis* (steady state) for a number of vital components. In normal healthy adults, the concentration of many such substances, lies within a narrow range in most body fluids. The blood is a fluid tissue and is circulating in the body. This confers on it the role of a transport vehicle. Therefore the blood has also to maintain specific levels of most constituents, to ensure availability of these to tissues and organs whenever needed or to carry excess or unwanted chemicals. Thus a careful analysis of blood constituents gives useful information on the state of the body. Whenever a disease condition exists (pathological condition), some component of the blood is affected, which is directly related to the tissue or organ which may have been behaving abnormally. For example, the normal level of cholesterol in serum is within a range of 120-220 mg/100 ml. An increase in cholesterol level indicates a pathologic condition called **hypercholesterolemia**, which may lead to **atherosclerosis**. Blood is a fluid and is easy to withdraw from the body of a patient in small quantities. It can be measured easily and quantitative estimations with it carried out rapidly.

Similarly, urine is a normal excretory product which is also a liquid. It can be collected easily, measured and analysed quantitatively. Normal urine contains only excretory or waste products. Any constituent other than these, would indicate some pathologic condition in the body. For example, glucose is not a normal constituent of urine. If in the urine of a person glucose is detected, one can suspect several possible causes, all of which are pathologic. Hence abnormal constituents of urine or abnormal concentrations of normal constituents are found only in pathologic urine.

Therefore an examination of blood and/or urine can be very useful in diagnosis of disease status of a patient. Although good correlation has been established between presence and/or concentration of many constituents of blood and several of urine, few of these tests or estimations are

adequate to diagnose number of common diseases. The following sections are restricted to a few selected pathological examinations of blood and urine.

12.2 Pathology of Blood

The blood can be examined in several ways : (a) physical (physicochemical); (b) physiological; (c) microscopical; (d) biochemical; (e) microbiological; and (f) immunological (serological).

(a) The quantity of blood, rate of flow of blood, viscosity (rheology), specific gravity etc. are all useful parameters to evaluate pathologic conditions.

(b) Clotting time, bleeding time, hemoglobin content, cell volume, cell count etc. are physiological parameters and their determination furnishes valuable information on the pathology of the associated diseases.

(c) Microscopic examination may reveal abnormal blood cell structures (morphology), sizes, presence of bacteria and other foreign bodies, some of which are associated with specific diseases (e.g. sickle cell anemia). For cell counts, microscopic examination also becomes necessary.

(d) The blood cells are carried in the fluid known as plasma. If blood or plasma is coagulated, a clear liquid known as serum is obtained. Most of the dissolved components are found in the serum, including enzymes, electrolytes, hormones, vitamins, foods, drugs, metabolic intermediates and end products etc. Most quantitative estimations on blood are usually with serum, in which various biochemicals are within narrow ranges in normal persons and deviate both quantitatively and qualitatively in pathological situations.

(e) For confirmation of infectious diseases, microbiological examination may be resorted to by culturing the blood in suitable media.

(f) As an improvement over the microbiologic examination, infectious diseases (including sometimes of past infections) can be well established by using serologic testing of blood samples.

Many of the above tests are beyond the scope of this book.

In a normal adult of about 70 Kg body weight, about 5.6 litres of blood will be present. The plasma accounts for 55% v/v of the blood, the cells settling down to about 45% v/v. Most of the blood cells are produced by the bone marrow and directly dumped into circulating blood. However, the cells are all of one type called *multi-potent uncommitted stem cells*, because any one of them can be developed into any type of

blood cell. In the finished form there are three types of blood cells : (1) the *erythrocytes (RBC)*; (2) the *leucocytes*; and (3) the *platelets*.

12.2.1 Red Blood Cells (RBC)

The blood cells are distinguished on the basis of size, shape of nucleus if present, granules or cytoplasm and staining characters. The erythrocytes are biconcave disks or sacs without nuclei. They are about 7.5 μm in diameter and 2 μm in thickness. They enclose *hemoglobin*, the red pigment made up of iron-porphyrin complex *heme* and a protein called globulin. Because of the red colour of hemoglobin these cells are called erythrocytes (or red blood cells, RBC).

Each red blood cell contains about 29 picograms (10^{-12}gm) of hemoglobin. Normal adult blood has 5.4×10^6 (i.e. 5.4 million) RBC in each μl (microlitre) of blood (i.e 5.4×10^9 in each ml or 5.4×10^{12} in each liter) in males and 4.8×10^6 per μl of female blood. This is about 3×10^{13} cells in the total blood containing about 900 g of hemoglobin, which is about 15 g per 100 ml. Therefore normal values for erythrocytes can be deduced by count per μl, size, shape, hemoglobin content, hematocrit and mean cell volume.

A lower content of hemoglobin indicates **anemia**. In anemic condition, the blood transports less amount of oxygen from the lungs and to the tissues, which in turn impairs oxidation processes responsible for energy production. Lack of hemoglobin also reduces capacity of blood to carry carbondioxide to be eliminated, resulting in accumulation of CO_2 (and biocarbonate) in tissues. This may result in **respiratory acidosis**. Anemia may be caused by (a) deficiency of vitamin B_{12} and/or folic acid. This condition is recognised by enlarged size of the RBC with low hemoglobin. Here porphyrin synthesis is affected. (b) Deficiency of iron, in which the cells are small (microcytic) and contain less amount of iron, with low overall hemoglobin content. (c) Faulty hemoglobin, in which globulin protein differs chemically slightly (usually due to genetic mistake), leading to hemoglobin S. This is less soluble than the usual hemoglobin in low oxygen blood. This causes the RBC containing hemoglobin S to curve and form a sickle shaped cell (hence **sickle cell anemia**). These cells are deformed and do not freely flow, particularly in capillaries, causing blocks and **anoxia** in tissues. The sickle shaped cells can be recognised under microscopic examination. These cells also undergo rupture leading to **haemolysis**. Another related disease is **betathalassemia**, which is also due to faulty hemoglobin formation. In this case, the formation and maturing of RBC is affected and immature RBC (nucleated and pigmented with hemoglobin) may be recognised.

If the blood sample contains a general reduction in the count of RBC, leucocytes and platelets, it indicates a serious condition of **bone marrow degeneration**. The condition is called **aplastic anemia**. Anemias are therefore a major pathologic condition of the blood, the different types caused by different deficiences and aberrations. All the types can be diagnosed by (*a*) count of cells; (*b*) size and shape of cells; and (*c*) hemoglobin content. **Polycythemia** is a disease associated with bone marrow *hyperactivity* or *proliferation*. This results in high blood cell count, particularly of erythrocytes. Although the exact causes are not known, low oxygen availability (as in high altitudes), burns, drug induced and hormonal imbalances are some of the reasons.

The condition of erythrocytes can be quickly judged by the rate of settling down in blood samples treated with an anti-coagulant. The test is known as *erythrocyte sedimentation rate* (ESR). It ranges between 0 to 9 mm/hr in blood of normal men and maybe upto 20 mm/hr in normal women. In some infectious conditions and inflammatory diseases it may give an early indication of pathology. However, it is a non specific test and needs to be followed up by other more definitive tests.

The blood coagulation is an important process to prevent loss of blood from injured blood vessels. It is usually prompt in normal individuals. Formerly, whole blood *clotting time* was determined, as a measure of an individual's capacity to coagulate blood promptly. There are a number of improved tests to diagnose the defects in coagulation mechanism. The more popular ones are : *activated partial thromboplastin time* (APTT) and *prothrombin time* (PTT). These tests are useful not only in determining the state of coagulation mechanism in a patient's blood, but also are useful in following up of patients, under treatment with anti-coagulants.

12.2.1.1 Blood Products : Transfusion of blood is very common in hospitals. During severe hemorrhage and in certain surgical procedures, blood may be lost to such an extent that immediate transfusion of human blood may become essential to save the life of the patient. For this purpose, blood banks have been established, where blood is collected, stored, processed, matched with recipient's blood and supplied. There are many aspects of blood transfusion, which are beyond the scope of this book. Here only mention will be made of two blood products included in the pharmacopoeia. A brief review of blood protein products, derived from plasma or serum (without the cells) has already been given in the chapter on Proteins.

Whole Human Blood (I.P.) is a blood replenisher. It is mainly intended to supply hemoglobin, so that respiratory oxygen is carried to tissues and cells. Therefore it is expected to contain not less than

9.7% w/v of hemoglobin. On keeping, it usually separates into lower layer of red blood cells and upper yellow layer of plasma. This should not be pink, which is an indication of hemolysis. A grey layer of leucocytes and platelets will also be found between the two layers. On the surface there is usually a thin layer of emulsified lipids. On gentle shaking, the cells are redistributed in the whole blood. To prevent coagulation of blood, anti-coagulant (usually citrate) is added. The blood is to be stored between 1 and 6°C. All precautions are taken to maintain sterility and prevent infection or other adverse conditions caused due to the blood donor's history of health and disease.

Concentrated Human Red Blood Corpuscles (I.P.) is the packed red blood cells, separated from the plasma of the whole human blood. This is to reduce the fluid, protein and added electrolytes (as anti-coagulants), from being administered to patients, when only hemoglobin is needed. The RBC may be obtained by simply decanting the supernatant plasma after sedimentation is complete or by centrifugation. In either case, sufficient plasma should be left over, to keep cells covered, moist and in good condition. For this purpose the whole blood used should not be older than three weeks. The product contains not less than 15.5% w/v of hemoglobin.

12.2.2 White Blood Cells (WBC or Leucocytes)

White blood cells or leucocytes is a name given to all large cells, usually nucleated, which do not have any pigment (i.e. not coloured). All are involved in body defence mechanisms. Three major types are recognisable : (1) *Lymphocytes*; (2) *Monocytes*; and (3) *Polymorphonuclear* cells. The lymphocytes and monocytes have a clear cytoplasm without any recognisable granules. They are also therefore known as *agranulocytes*. The polymorphonuclear cells are known as *granulocytes*, because of granular cytoplasm which can be recognised by staining techniques.

12.2.2.1 Lymphocytes : Some of the bone marrow uncommitted cells are developed into large nucleated agranulocytes called lymphocytes and are directly released into the lymphatic system. But many lymphocytes are also developed in lymph nodes, thymus gland and spleen, although the immature cells originate in the bone marrow. From the lymph a number of lymphocytes enter blood stream via the thoracic duct. There are about 2750 (range 1500 to 4000) of these per µl of blood (about 20 to 40% of WBC). Although morphologically (i.e. microscopically) indistinguishable, there are two types of lymphocytes, the B and T lymphocytes. These differ in their functions in the immune system.

12.2.2.2 Monocytes : These cells are formed in the bone marrow and transported through blood to various tissues to form *tissue macrophage* cells. The different tissues transform the cells suitably and retain them in these tissues. (e.g. *Kupffer cells* in the liver, *osteoclasts, alveolar macrophages* etc.) to form the tissue macrophage system (also sometimes called *reticulo endothelial system*). Only a small number of circulating monocytes are found in blood, about 300 to 600 (average 540) per µl. These are the largest nucleated cells in the blood with an irregular shape and kidney shaped nucleus.

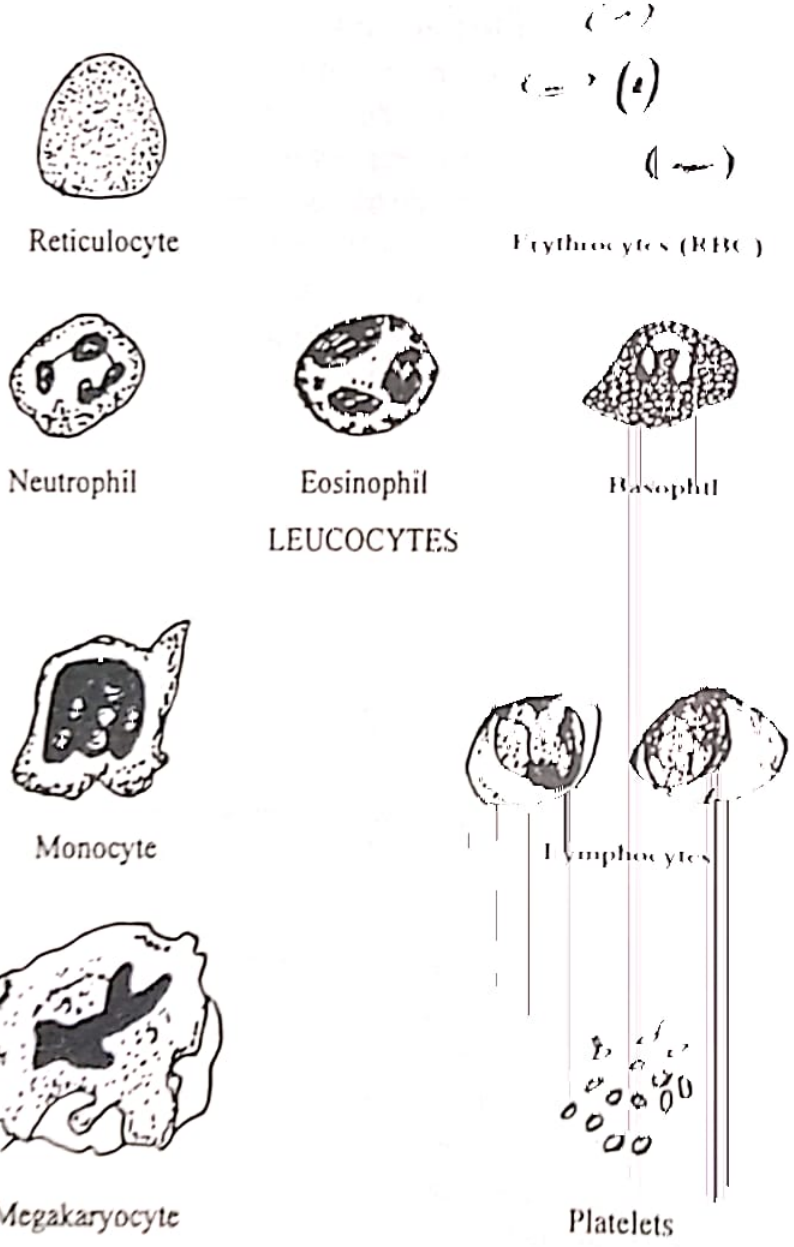


Fig. 12.1 Blood Cells

12.2.2.3 Polymorphonuclear cells : A large proportion of the stem cells of the bone marrow evolve into three closely related and resembling

nucleated cells. The nuclei in these cells differ in segmentation. The cytoplasm also differ in extent of granulation. However, these are distinguished microscopically by their differential staining. If the granules are readily stained by acid dyes like eosin (a phthalein dye), the cells are called *eosinophils*, which have segmented (lobed) large nuclei and equal amount of cytoplasm granules. Some cell granules are stained by basic dyes (para rosaniline dyes) and are designated *basophils*, which have large nucleus and small amount of cytoplasmic granules. Most of the leucocytes have granules that are stained by neutral dyes and are called *neutrophils*. These have polysegmented (3 to 5 lobes) small nucleii and large amount of cytoplasm. Since all the three differ in the shape of nucleus, together they are also called *polymorpho-nuclear cells*.

These are the microphage cells, providing defense to the body by engulfing bacteria, viral cells and other foreign bodies, which may gain access to the blood. At the site of an injury with infection, large numbers of these cells appear and after death of the cells in the process of protection, are reduced to pus cells. All of them put together (Total Count) may be about 4000 to 11000 (average 9000) per ml of blood. When counted by differential staining techniques (DC), over 60% are shown to be neutrophils, 1-4% eosinophils and less than 0.5% basophils, among all the nucleated cells. Lymphocytes amount to about 30% and monocytes 7-8% in the blood. These relative counts are carried out by blood smear and differential staining techniques, and by using hemocytometer. The neutrophils have a short life (half life is about 6 hrs) and are produced in large numbers (over a 100 billion a day).

In pathological conditions the total and differential counts are altered and the values may indicate specific disease conditions. If the total count of granulocytes is very much below the normal count, a disease called **agranulocytosis** is noticed. This results in low resistance to infections. This is also a bone marrow disease. It may also result from use of some drugs or due to genetic causes. On the other hand, there may be a phenomenal increase in the total number of leucocytes in the blood. If the number is slightly high, an infection may be the reason. But too high a count is termed **leukemia**. This may be because of too many and too early influx of leucocytes from the bone marrow into the blood. This condition can be recognised by the presence of a large number of immature leucocytes, characterised by unsegmented large nucleii, with little granular cytoplasm and large size. Leukemias may also be caused by non-removal of mature cells (low turn over) from the blood stream. Besides genetic reasons, exposure to toxic chemicals, drug toxicity, radiation etc. also contribute to leukemias. If a high count of eosinophils

above 10% as against normal 1-4% is observed (**eosinophilia**), there is a strong indication of body reaction to certain parasitic infections.

12.2.3 Platelets or Thrombocytes

These are also formed from the uncommitted stem cells of bone marrow. Initially large nucleated cells called *megakaryocytes* are developed, from which cytoplasm fragments into small units, which become enclosed in a cell membrane, giving rise to platelets. The megakaryocyte is about 40 µm in diameter, whereas platelets are 2 to 4 µm. The platelets also have a short life (half-life of about 4 days). There are on an average 300000 platelets in one ml (range 150000 to 400000) or 300 x 10^9/l of blood. The platelets are involved in the coagulation mechanism. In normal blood, the platelets may adhere to each other forming small clusters, but are easily separated and continue to flow smoothly along with blood. When a blood vessel is injured (cuts, bruises etc.), the rough surface breaks the platelet cell membrane releasing a substance which promotes aggregation of platelets and adhesion to the collagen of the vessel wall. This forms thus a barrier, which further seals and constricts the opening, facilitating not only blocking blood from escaping from the injured vessel but also sealing and healing of the ruptured vessel. They also trigger the coagulation mechanism by reacting with coagulating proteins. Occasionally a higher quantity of platelets may be found in circulating blood, leading to frequent small clots. If such clots occur in large vessels, these will be flushed and in due course dissolved by *fibrinolysin*

On the other hand when such clots occur in capillaries of heart muscle, a condition called **myocardial infarction** will be precipitated. The clot blocks the capillary and cuts off nutrition and oxygen supply to the heart muscle. A weak heart cannot cope up and death results. To reduce the risk of such clots the platelet count must be kept within normal limits. Small doses of aspirin have proved beneficial in some patients, by reducing platelet count and aggregation. On the other hand there may be low platelet count, called **thrombocytopenia**. This may be due to low production of thrombocytes as in **aplastic anemia** or in **megaloblastic leukemia** (diversion of platelet forming megaloblasts) or due to bone marrow diseases. Platelet deficiency may also be caused due to excessive destruction of platelets due to various causes. In either case there will be frequent hemorrhage and can be noticed as **purpura** (reddening of tissues).

12.2.4 Blood Serum

As explained earlier blood serum is an excellent representative body fluid, whose qualitative and quantitative analysis for some specific constituents, reveals the pathologic state of the body. The more important

and common estimations are briefly discussed below (without practical details).

12.2.4.1 Glucose : Glucose concentration in the blood varies widely during the day, depending on food consumption and physical activity. Immediately after absorption of glucose from digested food, the blood glucose may be very high (200 mg%). This condition remains for only a brief period, most excess glucose getting deposited as glycogen. The normal resting value of glucose is about 70 to 90 mg%. Persistent higher values above 90 mg% are described as **hyperglycemia**. The blood can manage to retain upto about 180 mg% in circulation as a normal level for some individuals. This is a diabetic condition. When the sugar (i.e. glucose) content exceeds this value, glucose which is filtered in the glomerulus is not reabsorbed from the distant tubules, because it has crossed the threshold value. Appearance of reducing sugars (i.e generally glucose) in urine is called **glycosuria** or **diabetes mellitus**. Since hyperglycemia exists long before a patient's urine begins to show presence of glucose, its estimation in blood (i.e serum) can help detect diabetic tendency at quite an early stage (*see* chapter 4.8).

Glucose estimation may be carried out early in the morning on an empty stomach (*fasting level*) which reveals the lower values, establishing hyperglycemia if present. Sometimes hyperglycemia may be due to faulty hormonal control (by insulin), in which peripheral utilization of glucose and/or storage of excess glucose as glycogen are affected. Another test called *glucose tolerance test* (GTT) is helpful in revealing these possibilities. In this test, the blood glucose is estimated over a two hour period immediately after administering a challenge dose of glucose orally. A return to normal level within 2 hrs indicates no pathologic condition, whereas in diabetic individuals, the level remains much higher than even the initial normal levels for such individuals.

12.2.4.2 Urea : Urea is the end product of protein metabolism. Urea formed in various organs (notably in liver) is transported by the blood and when passing through the kidney, much of this is filtered and eliminated by urine. However a small but constant amount of urea is found in circulating blood, ranging between 8 to 25 mg/litre. The blood carries many other nitrogenous chemicals. Hence estimation of nitrogen in serum filtrates (free from serum proteins) may include all the others and does not provide a useful parameter. Specific methods of estimating only urea nitrogen in blood samples are available (usually making use of enzymatic degradation of urea) and are known as blood urea nitrogen (BUN) determination. An excess of BUN may indicate a syndrome known as **uremia**. High BUN may be caused due to impaired kidney. In uremia other toxic

substances also accumulate in the blood causing severe symptoms. Hence removal of urea by using an artificial kidney (dialysing machine) may be necessary.

12.2.4.3 Cholesterol : The plasma has a normal range of 120 to 200 mg/100 ml of total cholesterol, of which 60 to 70% is found as cholesterol esters of fatty acids. There is facile interconversion of free cholesterol and its esters. Both these are sparingly soluble and are transported by the low density lipids (LDL). An increase in cholesterol in the blood (plasma or serum is generally used in estimations) indicates either excess formation or poor exchange from blood to tissues or poor breakdown in the liver to bile acids. In any case such **hypercholesterolemia** can lead to deposition of cholesterol on the walls of the blood vessels, resulting in hardening of the vessels (**atherosclerosis**). Gall stones, rich in cholesterol, may also be formed. Hence cholesterol (free and total) estimation is a routine test applied to plasma of individuals for better diagnosis (*see* chapter 9.3.3).

12.2.4.4 Alkaline phosphatase : This is an enzyme which is widely distributed in the body and has a narrow range, 13 to 39 units per liter of blood (serum) in normal adults. Its estimation is also routinely carried out. A high alkaline phosphatase level in serum indicates impaired gall bladder functioning. There may be obstruction in the bile ducts (**cholestasis**). A moderate increase may be also found in liver cell damage, in **osteomalacia** (bone resorption) and some types of **cancer**. In the third trimester of pregnancy also a higher level of alkaline phosphatase in serum becomes evident.

12.2.4.5 Transaminases : The transaminases are enzymes required for the biosynthesis of amino-acids from corresponding keto acids. The serum transaminases are found in narrow ranges and are very specific. Their estimation has diagnostic value. Two serum transaminases are commonly estimated : (1) *alanine transaminase* (ALT, glutamic-pyruvic transaminase or SGPT) and (2) *aspartate transaminase* (AST, glutamic-oxalacetic transaminase or SGOT). Their range is between 5 and 30 international units/liter. High levels of SGPT or SGOT are noticed in **hepatocellular damage** and moderately high levels in **cirrhosis** and **cholestasis**. In cardiac and circulatory impairement and **hypoxia** also, a raised SGPT or SGOT level may become noticeable. SGOT levels are increased in **cardiac infarction**. Therefore their estimation, separately, contributes to confirmation of the diagnosed pathological situation.

12.2.4.6 Amylase and/or lipase : Both these enzymes are in circulation and can be detected and estimated in blood (serum). A value

exceeding 120 units for *amylase* (also known as *diastase*) or 24 units for *lipase* per litre of serum indicates **pancreatitis**. It may also become noticeable in kidney failure.

12.2.4.7 Calcium : Importance of calcium in the body has already been discussed. Its level in blood is carefully regulated within a narrow range of 8.5 to 10.5 mg/100 ml or 4.3 to 5.3 meq/litre. Diseases associated with bone mineralization and resorption, with blood coagulation, muscle sensitivity etc are all dependent on calcium content. Hence its estimation in blood provides valuable diagnostic information. Calcium estimation is carried out routinely. Calcium in serum is estimated by direct precipitation of calcium as calcium oxalate, by addition of ammonium or sodium oxalate to diluted and measured quantity of serum. Calcium oxalate is separated by centrifugation, washed free of adhering fluids, then dissolved in sulphuric acid to liberate oxalic acid quantitatively. This is then titrated with standard potassium permanganate solution. There are also other methods available utilising sodium edetate (EDTA) as titrant (complexometry)

12.2.4.8 Bilirubin The red blood cells have a life of about 12 days. When they degenerate by rupture, hemoglobin is released in the tissues. Hemoglobin is broken down into its protein part and heme part. The heme is further degraded to liberate *biliverdin*, a linear porphyrin molecule (instead of the cyclic one present in heme), or *bilirubin* and iron. The iron is recycled in the body But the coloured bilirubin adheres to serum albumin and enters circulation In the liver, most of the bilirubin is quickly conjugated to give bilirubin glucuronide. This conjugate is normally secreted into the intestine along with the bile. From there it passes to large intestine, where bacterial flora convert this conjugate to several reduced forms called *stercobilin or urobilinogen* etc. Most of these are excreted in faeces. A small amount may be absorbed and after circulation may appear in urine In blood serum there is therefore albumin-bound free bilirubin and also conjugated bilirubin from hepatic circulation. In normal adults about 1 mg/100 ml may be found.

Methods have been developed to estimate both free and conjugated or total bilirubin in the serum. A fairly constant amount may also be found in urine. However, serum bilirubin estimation provides useful diagnostic information. If the liver clearance of bilirubin (i.e. conjugation and secretion into the intestine) is affected, the bilirubin content of blood rises. Free bilirubin increase suggests liver damage (**hepatitis**). Increase in conjugated bilirubin indicates normal liver function, but inability to secrete through the bile. This may be due to obstruction in bile ducts. As the conjugated bilirubin accumulates in the liver, it enters circulation

(*regurgitation*) again. A higher concentration of conjugated bilirubin in blood is a strong indication of obstruction. When total bilirubin in blood reaches a concentration beyond 8 mg/100 ml, tissues start extracting the yellow pigment (which is more lipid soluble than in blood serum) and get coloured yellow, a condition known as **jaundice**. For the same reason of poor solubility in blood, compared to lipid-rich tissues, bilirubin removal takes quite some days, even after controlling bilirubin concentration in blood by treatment or body's natural adjustment. Thus the yellow jaundice condition remains for sometime.

(*Note* : Related estimations are : bilirubin and urobilinogen in fresh urine.)

12.3 Pathology of Urine

Urine is a major excretory product of the body, carrying most of the soluble waste (and unwanted) material formed from different activities in the body. It is put together in the kidney after a series of processes. The blood passing through the kidney is filtered in the glomerulus, the rate of filtration depending on blood pressure and efficiency of the glomerulus. *Glomerular filtration rate* (GFR) is a useful test to determine the efficiency of the kidney. The filtrate goes through a long tube, before entering a collecting duct, which empties the final urine into the urinary bladder. The tubule has two functions : one of re-absorbing substances which are needed by the body (threshold substances) because the glomerular filtrate contains many soluble substances. The second function is to further add to the tubular fluid by secretion of other unwanted substances. The final urine is therefore the result of *glomerular filtration*, *tubular re-absorption* and *tubular secretion*. These processes are affected not only by the kidney cells and membranes, but also by hormones and the constituents of the glomerular filtrate.

12.3.1 Physical examination of urine

A normal person excretes about 1.0 l of urine a day. Changes in this volume may be noticed if a person consumes large amount of fluid or does not drink enough fluid, or consumes beverages that promote *diuresis* (e.g. tea) etc. Normal urine does not contain any insoluble substances, therefore is clear and does not yield sediment on keeping. However, a microscopic examination should be carried out if a sample of urine appears to be cloudy or turbid. A sample of urine can be subjected to centrifugation and the sediment examined. Presence of bacterial cells indicates kidney infection. Blood cells, particularly RBC, are rarely seen but can be suspected if urine is tending towards red colour, as a result of hemolysis. These conditions indicate damaged tubules or collecting ducts

or bladder or urethra etc. Similarly presence of leucoytes may be explained. The sediment may also contain very fine crystals, which may be due to crystallising uric acid or calcium oxalate etc. all of which may indicate impaired metabolism.

12.3.2 Physico-chemical Examination of Urine

The specific gravity of normal urine is in the range of 1.015 to 1.020. However, this measurement is not of sufficient diagnostic value in several pathological conditions. It is therefore recommended to measure the *osmolality* of urine, which is a better measure of urine concentration. Normal urine may have values between 300 and 500 mosm/litre of urine. A higher osmolality indicates high concentration due to high re-absorption of water (anti-diuretic hormone influenced !) and/or poor re-absorption of electrolytes. In *diabetes insipidus*, urine output is high, but urine osmolality can be lower than 100 mosm/l. On the other hand ADH (or vasopressin) induced concentration may reach 1400 mosm/l. Osmolality can be determined by freezing point determination method.

The pH of urine has a wide range, but is usually slightly acidic at about 5.5. Normally hydrogen ion is excreted along with phosphate and creatinine or ammonia. An alkaline pH is indicative of pathological conditions. It suggests **metabolic acidosis** (i.e. retention of hydrogen ion in the body) and **hypokalemia** etc. However, further investigations are necessary.

12.3.3 Chemical Examination of Urine

12.3.3.1 Urea As explained earlier this end product of protein metabolism is found in blood also. The BUN (blood urea nitrogen) is actually a measure of kidney efficiency. All the blood urea is found in the glomerular filtrate, but reabsorption also takes place in the tubules. As a result only about half of the filtered urea is excreted. Excretion is dependent on diet, being as low as about 2 g in protein-poor diet and as high as 13 g in high protein diet. Therefore urea estimation of urine does not give clear indication of kidney function. The BUN determination is preferred. This has already been discussed.

12.3.3.2 Creatinine : Creatinine is the cyclic derivative of creatine. As explained in another chapter, muscle activity consumes the energy stored in the form of creatine phosphate, releasing creatine. Although a small amount of creatine is present in urine, most of it is converted to creatinine. This is not only excreted completely through the kidney but is practically not re-absorbed and not secreted in the tubules. Moreover in normal conditions, creatinine excretion is fairly constant, being about 150 mg in 100 ml, generally not influenced by diet, metabolic rate or urine

output. This creatinine clearance by the kidney over 24 hr period is a good diagnostic tool for measuring kidney function and is also used to calculate GFR. Better correlations are obtained by determining ratio of BUN to urine creatinine concentration. Normal creatinine clearance is about 120 ml/min in males and 96 ml/min in females. The BUN/ creatinine ratio is approximately 10. The kidney function in all its aspects and high protein diets in kidney impaired persons, are clearly indicated by these tests.

Note : Clearance is defined as "*concentration of the substance in urine multiplied by flow of urine per minute divided by plasma level of the substance*". This can be expressed thus :

$$\text{Clearance of compound D} = D_{ur} \times V_{ur} / D_{pl}$$

where D_{ur} is concentration of D in urine, V_{ur} is volume of urine flow in 1 minute and D_{pl} is concentration of D in plasma. If the compound being studied is a drug, then the drug clearance by the kidney can be determined by this method. This is quite useful in pharmacokinetic studies.

12.3.3.3 Glucose : Glucose is essential for the body as it provides energy. It is not a waste substance. But it gets filtered in the glomerulus like many other soluble substances of the blood. However, it is almost completely re-absorbed from the urinary tubules and no glucose normally appears in the excreted urine. As explained earlier, if the plasma concentration of glucose exceeds 180 mg%, for sufficiently long period of time (due to poor peripheral utilization or inability to store excess glucose, **hyperglycemia** results. A high concentration in blood adversely affects renal reabsorption. Thus glucose (commonly called sugar) starts appearing in the urine.

The detection of glucose in urine (**glycosuria**) is a clear indication of a severe pathological condition. Sugar being food, urine containing it can promote bacterial growth in case of kidney or bladder infections. Although its appearance in urine occurs after advanced hyperglycemia, its detection and estimation is very useful. Qualitatively it is detected by a reducing agent, like Fehling's solution. Other reducing agents also may be employed. Its estimation in urine is carried out by using urine as a titrant against Benedict's reagent. It can also be estimated by colourimetric methods, as in case of blood glucose estimation. Only small amounts of blood are available for estimations, necessitating micro-chemical methods like colourimetric procedures, whereas large amounts of urine are easily collected and are available for simpler inexpensive titrimetric methods. There is no standard value for glucose in urine, as it is an abnormal constituent and is dependent on the severity of disease in the patient. The greater its amount in the urine the more severe the disease.

REVISION QUESTIONS

CHAPTER 2 : Proteins and Amino Acids

1. Enumerate functions of proteins in living organisms.
2. Classify proteins, indicating the property on which the classification is based. Give suitable examples.
3. Classify alpha-amino-acids on the basis of 'R' groups with examples.
4. What is zwitterion? What is its importance in protein chemistry?
5. With structures, write the reaction between an α-amino-acid and ninhydrin. What is the importance of this reaction?
6. Write the structure of (a) glutathione in full, indicating the peptide bonds and (b) a pentapeptide (hint: enkephalin) in shorthand notation indicating the amino- and carboxyl- end amino-acids.
7. Which properties of proteins are useful for separation and isolation of purified proteins? Explain suitably.
8. What is denaturation? How does it occur and what are its effects on proteins?
9. Describe briefly colour tests for proteins and polypeptides.
10. Briefly explain the different steps needed for the determination of primary structure of proteins.
11. What is the significance of the four structures (primary, secondary, tertiary and quaternary) to the biological activities of proteins?
12. Which are the essential amino-acids and why are they essential?
13. Explain the terms biological value and net protein utilization. How are they calculated?
14. What is understood by nitrogen balance and its significance in human life?
15. Briefly describe the well-known diseases associated with deficiency of amino-acids and proteins.
16. Enumerate different types of protein products available in the market and their applications.
17. Describe briefly pharmacopoeial blood protein products and their applications.
18. Which protein products, produced by biotechnological methods, are becoming available for medicinal uses?

CHAPTER 3 : Enzymes

1. Define and explain the concept of enzymes. How is the term biocatalyst for an enzyme justified?
2. Where do the following enzymes occur and what are their main activities in living systems?

   (a) amylase (b) pepsin (c) lipase (d) invertase (e) phosphorylase (f) trypsin.
3. Enumerate the major classes of enzymes as per IUB, with suitable examples.
4. Explain the following terms : (a) zymogen (b) apoenzyme (c) co-enzyme (d) prosthetic group (e) holoenzyme (f) active site.
5. Briefly explain the mechanism of an enzymic reaction.
6. How is the rate of an enzymic reaction affected by : a) concentration of enzyme and (b) concentration of substrate?
7. What is K_m and what is its importance?
8. How are enzyme reactions affected by : (a) temperature (b) pH and (c) product concentration ?
9. Explain the concept of enzyme inhibition and its importance in medicine and drug development. Give suitable examples.
10. How is the activity of an enzyme quantitatively expressed?
11. Enumerate some enzyme preparations used in medicine and pharmacy, giving their sources and specific applications.
12. List the official (I.P.) enzyme preparations, their sources, uses and activity wherever known.

CHAPTER 4 : Carbohydrates

1. Classify carbohydrates : (a) on the basis of functional groups, (b) on the basis of structural types and (c) on the basis of molecular size. Give examples for each class.
2. Give examples of dervied sugars and show their relationship to normal sugars.
3. Oxidation of glucose can yield three carboxylic acid compounds, without loss of carbon. Write their structures, name them and indicate their importance.
4. Explain the α and ß-linkages in sugars with suitable examples and structures. What is the importance of these types of linkages?

5. Explain why some sugars are reducing and some are not.
6. Describe briefly the following tests , including the chemical basis involved :

   (a) Molisch's test (d) Fehling's test (c) Tollen's test (d) Osazone test
7. Explain briefly the chemistry of and differences between : starch and cellulose.
8. Which are the enzymes involved in the breakdown of starch in the body to yield glucose? Indicate their specific function.
9. Why do the following abnormal conditions occur and how can they be treated? (a) galactosemia (b) lactose intolerance (c) fructose intolerance (d) glycogen storage disease.
10. Write briefly on different methods of glucose estimation, for various purposes.

CHAPTER 5 : **Lipids**

1. What are the functions (role) of lipids in the body?
2. Classify lipids, giving examples for each class.
3. Which are considered as essential fatty acids and why?
4. How do the waxes differ from fats? What is the difference between oils and fats?
5. Define saponification equivalent and explain its usefulness.
6. What is iodine number? Which different reagents may be employed for determining this value? What is the significance of this estimation?
7. Explain the term rancidity. Why does it occur and how can it be reduced?
8. Describe two tests for sterols.
9. Classify phospholipids, give suitable examples and the role of different phospholipids in the body.
10. What are lipoproteins and glycolipids? Explain with examples, how they participate in cell membranes and micelles.

CHAPTER 6 : **Nucleic Acids**

1. What are the functions of nucleic acids in the body?
2. Explain the terms : nucleoside, nucleotide, nucleic acid and their relationship.

3. What is understood by double helix and how is it rendered stable?
4. What do the notations "A=T and C≡G" signify? Explain.
5. In the biosynthesis of nucleic acids, which are the key intermediates related to (a) ribose (b) pyrimidine bases and (c) purine bases? Which vitamins are needed for some of these processes?
6. How is a study of nucleic acid biochemistry useful to medicine and pharmaceutical industry?

CHAPTER 7 : Vitamins and Coenzymes

1. Define vitamins and explain why they are essential in our diet.
2. What are provitamins? Give examples and explain how they become useful.
3. Mention different compounds possessing vitamin A activity.
4. Enumerate the most important functions (role) of vitamin A in the body.
5. Enumerate the symptoms of vitamin A deficiency.
6. Which are the different forms of vitamin D that are used and what are their sources?
7. What is the role of vitamin D and its derivatives in the body?
8. Enumerate the symptoms of vitamin D deficiency.
9. What are tocopherols? What is considered to be their role in the body?
10. Which are the common sources for vitamin K? What is its role in human body?
11. Which are the different forms of vitamin K that are available for medicinal purposes?
12. Enumerate the symptoms of thiamine deficiency. How are they overcome?
13. What is the role of thiamine in human biochemistry?
14. What are the symptoms of ariboflavinosis?
15. Explain the role of riboflavine in human biochemistry.
16. Indicate the nutritional sources for (a) niacin (b) pyridoxine (c) folic acid and (d) vitamin B_{12}.
17. Which forms of vitamin B_{12} are official in the Indian Pharmacopoeia and what is their strength?

18. What symptoms are associated with deficiency of (a) folic acid and (b) vitamin B_{12}? How are they treated?
19. What is the role of folic acid in the body biochemistry?
20. Discuss briefly the chemistry, properties and role of vitamin C in human biochemistry.
21. Which are the coenzymes related to niacin? Name them and indicate their role in human biochemistry.
22. Which are the different forms of vitamin B_6 that are active?
23. How does pyridoxine participate in metabolic reactions? Explain briefly.
24. What is coenzyme A and what is its role in intermediary metabolism?
25. Write a brief note on multi-vitamin preparations.

CHAPTER 8 : Digestion and Absorption

1. Outline briefly gastric digestion and its regulation.
2. How are proteins digested and absorbed? Explain different steps, organelles, enzymes and processes involved.
3. What are proenzymes (or zymogens) and what is their role? How are they activated?
4. Explain briefly absorption and absorption of fats.
5. Which are the different enzymes involved in digestion of carbohydrates and how exactly they achieve this?

CHAPTER 9 : Metabolism

1. With an example describe briefly the transamination reactions and their importance in intermediary metabolism.
2. Explain amino-acid decarboxylation reactions and show how some important biogenic amines are formed.
3. Explain with the help of formulae, the beta-oxidation scheme of fatty acid degradation.
4. Besides the beta oxidation method, which other routes of fatty acid oxidation are known?
5. What is the importance of LDL and HDL in lipid metabolism? How is atherosclerosis caused? How can it be controlled?
6. Explain the key reactions involved in glycogenesis and glycogenolysis.

7. Which are the four important enzymatic type reactions involved in glycolysis and what do they achieve?
8. Briefly indicate conditions that may lead to diabetes mellitus type I. What are its symptoms? How is it generally treated?
9. What is the pathway of galactose utilisation in the body? What is galactosemia and what are its symptoms?
10. How is ribose formed in the body?
11. What is understood by gluconeogenesis and what is its importance?
12. Outline briefly the major steps in the tricarboxylic acid cycle.
13. At what stages of the TCA the carbohydrate, fat and protein metabolic pathways get integrated (or enter)?
14. Explain briefly what is understood by high energy bond. Mention a few energy rich compounds available in the body and how they release energy on hydrolysis.
15. What are the general principles of regulation of metabolic activities in the body?
16. Explain briefly the role of the following in regulating metabolism:

    (a) insulin (b) glycogen (c) thyroxine (d) adrenaline (e) corticosteroids.

CHAPTER 10 : **Excretion and Drug Metabolism**

1. Outline briefly the urea cycle and show how the two end products of metabolism of proteins, carbohydrates and fats are incorporated into the urea molecule.
2. How is uric acid formed in the body?
3. How does uric acid get accumulated in the body? What disease symptoms does it cause? How is this condition treated?
4. Outline briefly importance of functionalisation reactions in the metabolism of xenobiotics.
5. Which are the prominent conjugating agents employed by the body to metabolise drugs and xenobiotics? Explain their role with suitable examples.
6. How is glutathione (GSH) involved in 'detoxication' of some drugs?
7. Show how a drug may be metabolised in a variety of ways (e.g. paracetamol).

CHAPTER 11 : Minerals and Water

1. Explain the role of calcium in the body. How is its level in serum regulated?
2. What are the symptoms (or diseases) caused by deficiency of (a) calcium and (b) magnesium?
3. Discuss the absorption, transport, storage and biochemical role of iron in the body.
4. How do the following minerals participate in human biochemistry? (a) Zinc (b) Copper (c) Manganese (d) Molybdenum (e) Chromium.
5. What are the symptoms/diseases of deficiency of (a) zinc (b) copper?
6. Explain the role of iodide in the body.
7. Describe briefly regulation of water content in the human body and its importance.
8. What is diabetes insipidus and why is it caused? How can it be controlled? How does it differ from diabetes mellitus ?

CHAPTER 12 : Pathology of Blood and Urine

1. What are the symptoms/diseases associated with abnormal erythrocytes (and their count)? Why are they caused? Which diagnostic methods are used to detect these conditions?
2. How are the following conditions recognised? (a) agranulocytosis (b) leukemia (c) eosinophilia
3. What is the normal count and role of thrombocytes in the body? How is their count affected and what symptoms/diseases may it produce?
4. Outline briefly estimation of glucose in (a) blood and (b) urine.
5. What is the diagnostic importance of estimation of serum (a) cholesterol (b) alkaline phosphatase (c) transaminases (d) calcium?
6. How is bilirubin formed and excreted? What is the diagnostic utility of estimation of serum bilirubin?
7. Which qualitative and quantitative tests are commonly performed in urine analysis? What is the significance of each of these tests?
8. What is understood by 'clearance'? How can clearance of a drug be determined? What is the importance of such a determination?

MEDICAL TERMS INDEX

GENERAL INDEX