PREFACE

In a bid to standardise higher education in the country, the University Grants Commission (UGC) has introduced Choice Based Credit System (CBCS) based on five types of courses viz. *core, discipline specific, generic elective, ability* and *skill enhancement* for graduate students of all programmes at Honours level. This brings in the semester pattern, which finds efficacy in sync with credit system, credit transfer, comprehensive continuous assessments and a graded pattern of evaluation. The objective is to offer learners ample flexibility to choose from a wide gamut of courses, as also to provide them lateral mobility between various educational institutions in the country where they can carry acquired credits. I am happy to note that the University has been accredited by NAAC with grade 'A'.

UGC (Open and Distance Learning Programmes and Online Learning Programmes) Regulations, 2020 have mandated compliance with CBCS for U.G. programmes for all the HEIs in this mode. Welcoming this paradigm shift in higher education, Netaji Subhas Open University (NSOU) has resolved to adopt CBCS from the academic session 2021-22 at the Under Graduate Degree Programme level. The present syllabus, framed in the spirit of syllabi recommended by UGC, lays due stress on all aspects envisaged in the curricular framework of the apex body on higher education. It will be imparted to learners over the *six* semesters of the Programme.

Self Learning Materials (SLMs) are the mainstay of Student Support Services (SSS) of an Open University. From a logistic point of view, NSOU has embarked upon CBCS presently with SLMs in English / Bengali. Eventually, the English version SLMs will be translated into Bengali too, for the benefit of learners. As always, all of our teaching faculties contributed in this process. In addition to this we have also requisitioned the services of best academics in each domain in preparation of the new SLMs. I am sure they will be of commendable academic support. We look forward to proactive feedback from all stakeholders who will participate in the teaching-learning based on these study materials. It has been a very challenging task well executed, and I congratulate all concerned in the preparation of these SLMs.

I wish the venture a grand success.

Professor (Dr.) Subha Sankar Sarkar

Vice-Chancellor

Netaji Subhas Open University

Undergraduate Degree Programme

Choice Based Credit System (CBCS)

Subject : Honours in Zoology (HZO)

Course : Biochemistry and Metabolic Processes

Course Code : CC - ZO - 08

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Netaji Subhas Open University

UG : Zoology
(HZO)

Course : Biochemistry and Metabolic Processes
Course Code : CC - ZO - 08

Unit-1 □ Biological Macromolecules

Structure

1.0 Objectives

After studying this Unit-I, one will be able to:

- Understand the meaning of 'biological macromolecules'.
- Define and classify carbohydrates.
- Explain isomerism in monosaccharides.
- Understand mutarotation shown by glucose.
- Have an idea of the chemical properties of carbohydrates.
- Understand the physiological importance of carbohydrates.
- Define and classify proteins.
- Understand various orders of protein structure.
- Understand the important properties of proteins.
- Understand the physiological importance of proteins.
- Define and classify lipids.
- Define and classify fatty acids.
- Have an idea of the chemical properties of lipids.
- Understand the physiological importance of lipids.
- Understand Watson and Crick's model of DNA structure.
- Understand the characteristics of different forms of DNA.
- Understand the function of DNA.
- Have an idea of different types of RNA and their function.

1.1 Introduction to biological macromolecules

Macromolecules are giant molecules in which at least a thousand atoms are linked together by covalent bonds. Moreover, macromolecules are long repetitive sequences of an elementary chemical structure called the monomer. Cells and tissues of living organisms are made up of four kinds of biological macromolecules, viz. carbohydrates, proteins, lipids and nucleic acids; the latter are again of two types, viz.

DNA or deoxyribonucleic acid and RNA or ribonucleic acid. The monomer units of carbohydrates are called mono saccharides; those of proteins are called amino acids; those of lipids include fatty acids and alcohol; those of nucleic acids are called nucleotides.

A macromolecule is called a homopolymer, if it is formed by joining of monomers having the same chemical structure, e.g. the carbohydrate called starch is formed by joining of numerous glucose units. On the other hand,a macromolecule is called a heteropolymer, if it is formed by joining of monomers having different chemical structure, e.g.Hyaluronic acid of connective tissue consists of alternating units of glucuronic acid and acetyl glucosamine.

Biological macromolecules are important for not only constituting cells and tissues, but also for serving different physiological functions in the body of living beings. In this chapter, learners will know about the structure, properties and functions of different biological macromolecules.

1.2 CARBOHYDRATES :

1.2.1 Introduction to carbohydrates :

Carbohydrates are widely distributed in both plant and animal tissues. Carbohydrates have profound biological importance: they serve as major source of energy for vital activities in both plants and animals; many carbohydrates form structural components of cells and tissues; some carbohydrates are essential components of other biological macromolecules of cells and tissues; some carbohydrates serve specific physiological functions in animal body.

Definition of carbohydrates : Carbohydrates are derivatives of polyhydroxy alcohols, bearing one or more, free or bound, aldehyde $\left(-C\overset{O}{\underset{H}{\diagdown}}\right)$ or ketone (> C = 0) group. Generally, carbohydrates are composed of carbon, hydrogen and oxygen and have a 2 : 1 ratio of hydrogen and oxygen atoms, as in case of water. However, there are some exceptions.

All compounds that are composed of C, H and O and that have a 2 : 1 ratio of hydrogen and oxygen may not be carbohydrates:

It should be noted that :

(i) All substances that consists of C, H and O and that have a 2 : 1 ratio of hydrogen and oxygen atoms, may not necessarily be carbohydrates,e.g. Formaldehyde (HCHO or CH_2O) and Acetic acid (CH_3COOH or $C_2H_4O_2$).

(ii) There are carbohydrates where the ratio of hydrogen and oxygen atoms is not 2 : 1, e.g. Fucose ($C_6H_{12}O_5$).

(iii) Some carbohydrates contain substances other than C, H and O, e.g. Glucosamine ($C_6H_{11}O_5NH_2$) contains nitrogen in addition to C, H and O.

1.2.2 Classification of carbohydrates :

Carbohydrates may be classified into the following classes :

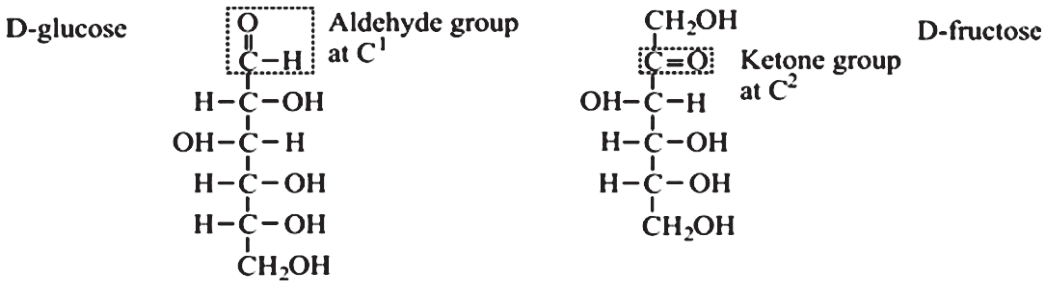
(A) Monosaccharide : Monosaccharides are the simplest carbohydrates, which cannot be hydrolyzed any further and which usually have the empirical formula $C_n(H_2O)_n$. According to the number of carbon atoms, monosaccharides can be subdivided into the following types :

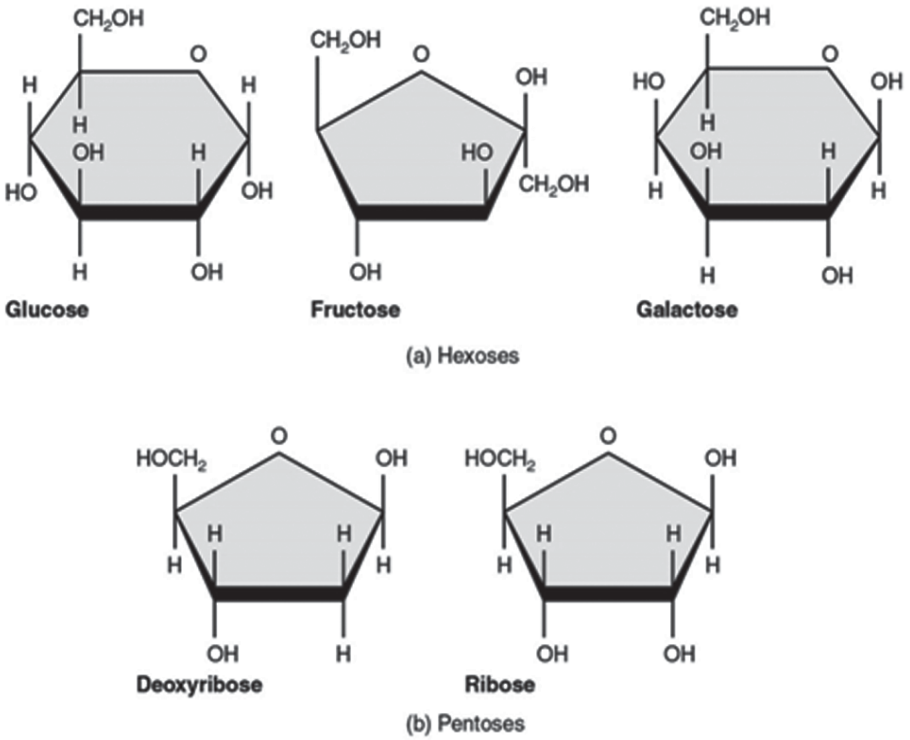
Type	No. of carbon atoms	Empirical formula	Example	Occurrence
Triose	3	$C_3H_6O_3$	Glyceraldehyde	EMP pathway
Tetrose	4	$C_4H_8O_4$	Erythrose	HMP pathway
Pentose	5	$C_5H_{10}O_5$	Ribose	Ribonucleic acid
Hexose	6	$C_6H_{12}O_6$	Glucose	Blood sugar
Heptose	7	$C_7H_{14}O_7$	Sedoheptulose	HMP pathway
Octose	8	$C_8H_{16}O_8$	Glucooctose	Metabolic pathway in plants
Nonose	9	$C_9H_{18}O_9$	Gluconononose	Metabolic pathway in plants
Decose	10	$C_{10}H_{20}O_{10}$	Glucodecose	Metabolic pathway in plants

Again, monosacharides may be subdivided into 2 groups according to the nature of the sugar-group present in the molecules:

(i) Aldose: Monosaccharides having an aldehyde group $\left(-C\overset{O}{\underset{H}{<}}\right)$ at the first carbon (C^1) of their carbon skeleton, e.g. Glucose, Galactose.

(ii) Ketose: Monosaccharides having a ketone group (>C = 0) at a position other than the first carbon in their carbon skeleton, e.g. Fructose.





Ring Structures of five important monosaccharide

[Source : Wikimedia Commons (CC 3.0)]

(B) Disaccharide : Disaccharides are carbohydrates which are composed of two similar or dissimilar monosaccharide units, joined by glycosidic linkage, with the loss of one molecule of water. Upon hydrolysis, disaccharides yield the two constituent monos accharide residues. The general formula of disaccharide is $C_{2n}(H_2O)_{2n-1}$.

Examples	Constituent monosaccharide units	Occurrence
Maltose	Glucose + Glucose (linked by α-1,4 glycosidic bond)	Malt and germinating cereals
Lactose	Galactose + Glucose (linked by β-1,4 glycosidic bond)	Milk
Sucrose	Gluctose + Fructose (linked β-1,2 glycosidic bond)	Sugarcane and Beet
Isomaltose	Glucose + Glucose (linked by α-1,6 glycosidic bond)	Sugarcane and Beet

(C) OIigosaccharide : Oligosaccharides are carbohydrates composed of more than two but less than ten monosaccharide residues, joined together by glycosidic linkages :

Type and Examples	Constituent monosaccharide residues	Occurrence
Trisaccharide	Raffinose = Galactose + glucose + fructose	Sugar-beet
Tetrasaccharide	Stachyose = 2 Galactose + 1 glucose + 1 fructose	Some plant tubers
Pentasaccharide	Verbascose = 3 Galactose + 1 glucose + 1 fructose	Some plant roots

(D) Polysaccharide: Polysaccharides are carbohydrates composed of ten or more monosaccharide units,joined by glycosidic linkages. While mono-, di- and oligosaccharides are soluble in water and sweet in taste, most polysaccharides are insoluble in water and devoid of sweet taste. The empirical formula is $[C_n(H_2O)_{n1}]_x$ where *x* is 10 or larger. Polysaccharides are subdivided into:

(i) Homopolysaccharide or Homoglycan: When the constituent monosaccharides are all similar, e.g. Starch of plant tissues and glycogen of animal bodies are composed of innumerable glucose units only.

(ii) Heteropolysaccharide or Heteroglycan: When a polysaccharide consists of more than one type of monosaccharide units, e.g. Hyaluronic acid of connective tissue consists of alternating units of glucuronic acid and acetyl glucosamine.

Besides, polysaccharides are sometimes, divided into (i) Structural polysaccharides and (ii) Storage polysaccharides. Structural polysaccharides serve as components of different structural parts of organisms,e.g. Hyaluronic acid is a component of connective tissues of animals. Cellulose is a part of cell-wall in plants. Storage polysaccharides are stored in animal or plant bodies for obtaining energy during necessity,e.g. Starch in plants and glycogen in animals.

1.2.3 Some additional information

1.2.3.1 Lowest number of carbon atoms in a carbohydrate :

By definition, a carbohydrate is derivative of polyhydroxy alcohol, having one or more, free or bound CHO or CO group. Hence, a compound must have (i) at least two OH groups and (ii) at least one CHO or CO group. In other words, a carbohydrate molecule cannot have less than 3 carbon atoms.

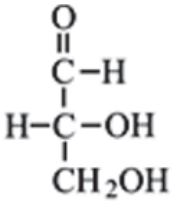
1.2.3.2 Distinction between a triose and a trisaccharide :

Ans. (i) A triose is a monosaccharide having 3 carbon atoms in its carbon-skeleton. E.g. Glyceraldehyde. On the other hand, a trisaccharide is a bigger carbohydrate molecule, composed of 3 monosaccharide units which are joined with one another by glycosidic linkage. E.g. Raffinose – it is a trisaccharide formed by joining of 1 galactose, 1 glucose and 1 fructose residues.

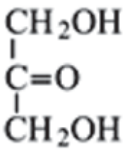
(ii) A triose being a monosaccharide cannot be hydrolysed any further. But, a trisaccharide, when hydrolysed, is broken into 3 monosaccharide residues.

1.2.3.3 The smallest carbohydrates :

Smallest carbohydrates are called as 'triose' which means monosaccharide with 3 carbon atoms,e.g. (i) Glyceraldehyde is an 'aldose' type of triose and (ii) Dihydroxyacetone is a 'ketose' type of triose :



Glyceraldehyde



Dihydroxyacetone

1.2.3.4 Distinction between two polysaccharides – starch and glycogen :

Starch	Glycogen
1. It is a storage polysaccharide of plants.	1. It is a storage polysaccharide of animals.
2. It consists of many glucose units joined by α-1,4-glycosidic linkage. Moreover, a starch molecule has a branched structure; branching occurs in every 24-30 glucose units; one branch remains joined to another branch by α-1,6-glycosidic linkage.	2. It is also composed of many glucose units joined by α-1,4-glycosidic linkage. A glycogen molecule has a highly branched structure ; branching occurs in every 10-15 glucose units; one branch remains joined to another branch by α-1,6-glycosidic linkage.
3. It forms a blue adsorption complex with iodine.	3. It forms a reddish adsorption complex with iodine.

1.2.4 Isomerism in monosaccharides :

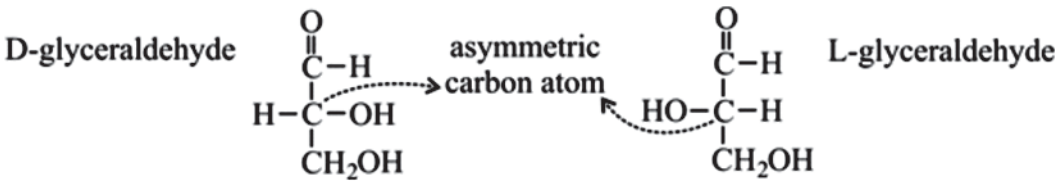
Isomerism : The existence of a compound in different forms that retain the same molecular formula but differ in physical and chemical properties is called isomerism while such different forms are called isomers. Monosaccharides show different types of isomerism:

1.2.4.1 Stereoisomerism :

It refers to the existence of a compound in such different forms that have the same empirical and structural formula but that differ in their spatial configuration (arrangement of constituent atoms in space).

Monosaccharides show stereoisomerism due to the presence of asymmetric carbon atoms in their molecules. An asymmetric carbon atom is such a carbon atom which has four different groups or atoms satisfying its four valencies. Monosaccharides are considered as 'chiral molecules', i.e. the spatial arrangement of different groups around the asymmetric carbon atoms may be variable, which forms the basis of stereoisomerism of different kinds.

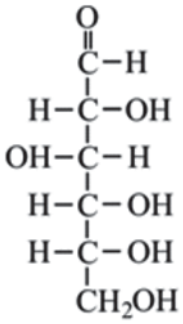
(i) D- and L-isomerism : The most commonly described stereoisomers are D- and L- isomers, which appear as exact mirror-image of each other. The designation of an isomer as D- or L-isomer depends on its spatial relationship to the parental compound of carbohydrate family, the 3-carbon sugar glyceraldehyde. The structure

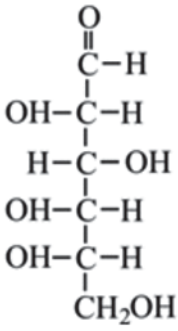


of D-glyceraldehyde and its mirror-image, the L-glyceraldehyde are as follows :

When the highest asymmetric carbon atom i.e. the penultimate carbon atom lying before the terminal primary alcohol carbon has an OH group at its right and a H at its left as in D-glyceraldehyde, the sugar is called D-sugar or D-isomer. But, when the penultimate carbon has OH at its left and H at its right as in L-glyceraldehyde, the sugar is called L-sugar or L-isomer. For instance, glucose is a hexose with the 5^{th} carbon as its penultimate carbon; the structures of D- and L-glucose are as follows :

D-glucose



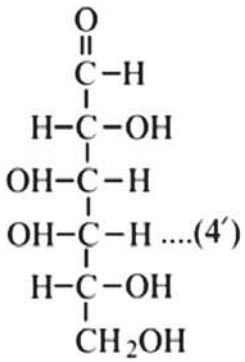


L-glucose

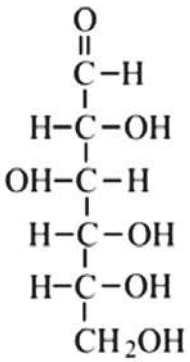
The D- and L-isomers being mirror-images of each other cannot be superimposed on each other. The 2 isomers together are called an enantiomeric pair, and each isomer is said to be an enantiomer to the other.

It may be further added here that most sugars occur as D-isomers in living beings, e.g. D-glucose, D-galactose etc. as aldoses and D-fructose, D-ribulose etc. as ketoses. However, some sugars occur predominantly as L-isomers, e.g. the aldose L-fucose and the ketose L-rhamnose.

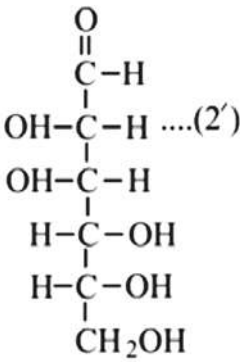
(ii) Epimerization: Stereoisomerism with respect to variation in the arrangement of -OH and -H attached to any single asymmetric carbon atom in molecules that have more than one asymmetric carbon atoms is called epimerization. For instance, D-glucose and D-galactose are epimers differing in configuration around only the 4th carbon. Again, D-glucose and D-mannose are epimers differing in configuration around only the 2nd carbon. Their structures are as follows:



D-galactose



D-glucose



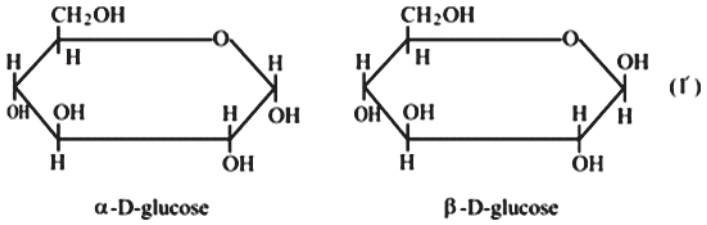
D-mannose

(iii) Anomerism: Stereoisomerism with respect to change in configuration of groups or atoms around the anomeric carbon atom is called anomerism.

In solution, the sugars occur almost exclusively in the form of a ring instead of a chain-structure. In case of an aldose, the ring is a hemiacetal ring, i.e. the ring is formed due to hemiacetal formation or by condensation of the -OH group of C^5 (carbon 5) with the aldehyde group of C^1 (carbon 1). In case of a ketose, the ring is a hemiketal ring, i.e. the ring is formed due to hemiketal formation or by condensation of -OH group of C^5 with the ketone group of C^2. The hemiacetal carbon or C^1 in an aldose and the hemiketal carbon or C^2 in a ketose are called anomeric carbons. During the formation of the ring structure of a sugar, 2 stereoisomers can be formed: the a- and b-anomers, which differ in configuration of groups or atoms around the anomeric carbon.

For instance, in case of D-glucose, in the a-anomer, -H lies above and -OH lies below the C^1 while -H lies below and O -H lies above the C^1 in the â-anomer. The structures are shown below :

The a- and b- anomers of glucose differ in many of their properties, e.g. (i) a-glucose has a melting point of 146^0C while b-glucose melts at 150^0C, (ii) a-glucose



has a solubility of 82.5 g/100 ml of water while b-glucose dissolves at a rate of 178 g/100 ml of water and (iii) the a-anomer of D-glucose shows an initial dextrorotation of $+112.2^0$ which gradually decreases to $+52.7^0$ on standing and the b-anomer shows an initial dextrorotation of $+18.7^0$ which increases to $+52.7^0$ on standing. This type of gradual change in optical activity of a sugar until a stability is reached, is called as mutarotation. Mutarotation occurs due to partial inter-conversion between the a- and b-anomers. The optical activity becomes stable when an equilibrium mixture of the 2 anomers is produced, e.g. In case of D-glucose, the 'equilibrium mixture' contains 38% a-anomer and 62% b-anomer and this mixture shows a stable dextrorotation of $+52.7^0$ only.

1.2.4.2 Optical Isomerism:

Monosaccharides possess optical activity, i.e. they can rotate the plane of polarized light either to the right or to the left, when polarized light is passed through

their solutions. The optical activity is due to the presence of asymmetric carbon atoms in their molecules. The electromagnetic fields of atoms or groups around the asymmetric carbons act on polarized light to cause rotation of its plane.

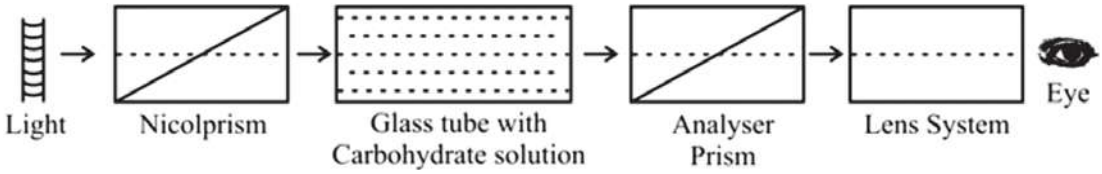
A monosaccharide can exist as two optical isomers: (i) dextrorotatory or d-isomer or (+) isomer which rotates the plane of polarized light to the right and (ii) levorotatory or l-isomer or (-) isomer which rotates the plane of polarized light to the left, e.g. Two optical isomers of glucose are d-glucose or (+) glucose and 1-glucose or (-) glucose respectively.

The rotation of polarized light by an isomer is measured by an instrument called Polarimeter. In this instrument, ordinary monochromatic light is passed through a Nicol prism at a specified angle. The prism gives out polarized light that oscillates in one plane only. The polarized light is passed through the glass-tube containing the sugar solution which rotates the plane of the light. The rotated beam coming out of the solution is passed through an 'analyzer Nicol prism' and a 'lens system' to fall on the eyes or a screen. The 'specific optical rotation' is then calculated as follows:

$$r = \frac{obs \square 100}{1 \square c}$$

(Where, r = specific optical rotation at a specific temperature and a specific wave-length; obs = observed rotation (in degree); 1 = length of the tube containing sugar solution (in decimeter); c = g of sugar/100 ml of solution.)

In case of most sugars, the d-and l-isomers correspond to the D- and L-stereoisomers, respectively, e.g. D-glucose is dextrorotatory and L-glucose is levorotatory and therefore, the 2 isomers can be written as D (+) glucose and L(-) glucose. However, in some sugars, the 2 optical isomers do not correspond to the two stereoisomers, e.g. D-fructose is levorotatory and should be written as D (-) fructose.



Working principle of Polarimeter

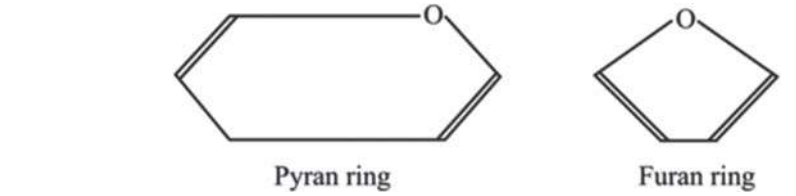
Finally, an equimolar mixture of the d- and l-isomers of a sugar is optically inactive and does not rotate the plane of polarized light at all. Such a mixture is called **racemic mixture.** The two components of a racemic mixture can be separated by specific techniques only.

1.2.4.3. Structural Isomerism :

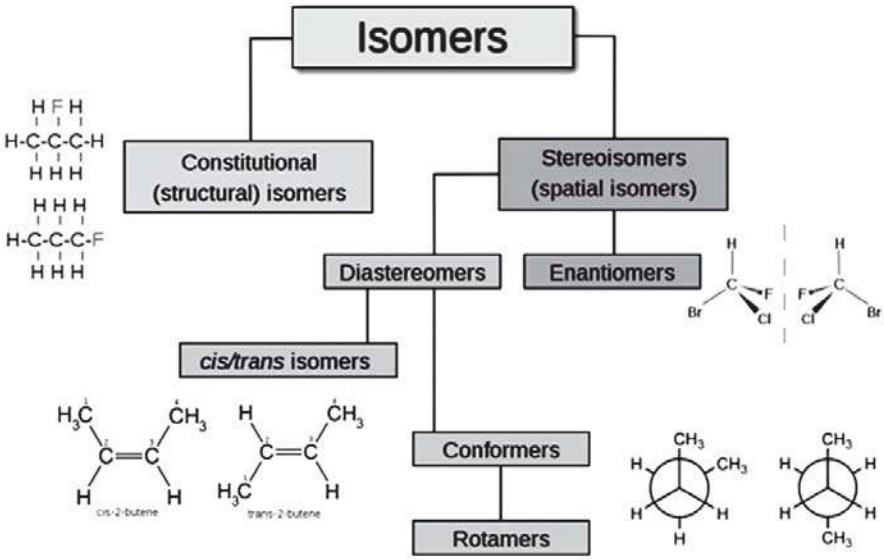
Compounds that have the same molecular formula but differ in molecular structure are called structural isomers and the phenomenon is called structural isomerism, Monosaccharides show structural isomerism as described below:

(i) Aldose-ketose isomerism: The aldohexose glucose and the ketohexose fructose have the same empirical formula of $C_6H_{12}O_6$, but they differ in their molecular structures. Glucose has an aldehyde group at the 1st carbon while fructose has a ketone group at the 2nd carbon; glucose has no ketone group and fructose has no aldehyde group. Thus, glucose and fructose are 2 structural isomers. Their structures have been shown in classification of carbohydrates (Section 1.2.2).

(ii) Pyranose-furanose isomerism: In solution, the sugars exist as ring-structure instead of chain-structure, when the ring is a 6-membered ring having 5 carbons and 1 oxygen like a pyran ring, the sugar is said to be in pyranose form and when the ring is a 5-membered ring with 4 carbons and 1 oxygen like a furan ring, the sugar is said to be in furanose form. The basic structures of pyran and furan rings are as follows :



Pyran ring Furan ring



Schematic representation of different types of isomers

[Source : Wikimedia Commons (CC 3.0)]

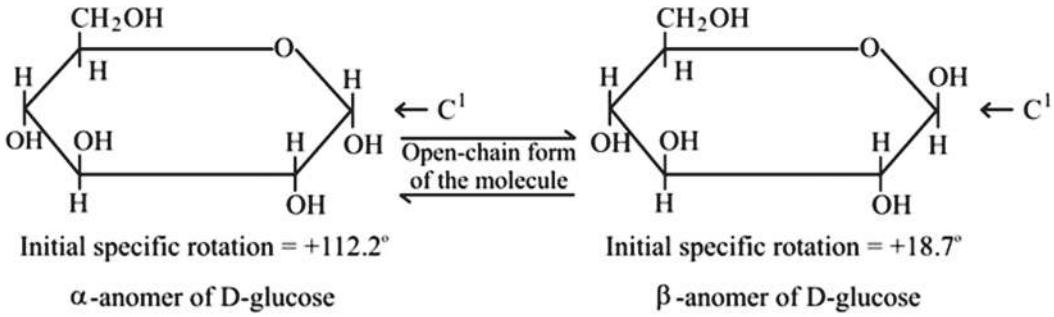
1.2.5 Mutarotation shown by glucose

Definition : The optical activity of a freshly prepared solution of a monosaccharide, with the exception of a few ketoses, shows a gradual change until it becomes stable on standing. This is known as mutarotation.

Mutarotation in glucose : Mutarotation has been studied best in case of glucose. If D-glucose is crystallized from water or dilute alcohol at room temperature, an isomer separates, which in solution shows an initial specific rotation of +112.2º. However, the specific rotation gradually decreases to +52.7º on standing. This isomer is called as a-anomer of glucose.

On the other hand, if D-glucose is crystallized from water or pyridine at 98^0C or higher temperatures, an isomer separates, which in solution shows an initial specific rotation of +18.7º. The specific rotation gradually increases to +52.7º on standing. This isomer is called b-anomer of glucose.

Mechanism of mutarotation: In solution, a sugar molecule mainly exists in a ring form which, in turn, may exist in 2 stereoisomeric forms called as a-anomer and and b-anomer. The structure of the 2 anomers of D-glucose are shown below; the a-anomer has a 'H' above and a 'OH' below the plane of the ring at C^1, while the arrangement of 'H' and 'OH' at C^1 is just the opposite in case of the β-anomer :



Mutarotation in case of glucose

Mutarotation occurs due to partial inter-conversion between the alpha- and beta-anomers through an intermediate open-chain form of the molecule. So long as this inter-conversion goes on, mutarotation or change in optical activity of the sugar solution continues. Finally, an equilibrium mixture of the 2 anomers is established and the optical activity also becomes stable. An equilibrium mixture of D-glucose contains nearly 38% of the a-anomer and 62% of the β-anomer.

1.2.6 Some chemical properties of carbohydrates:

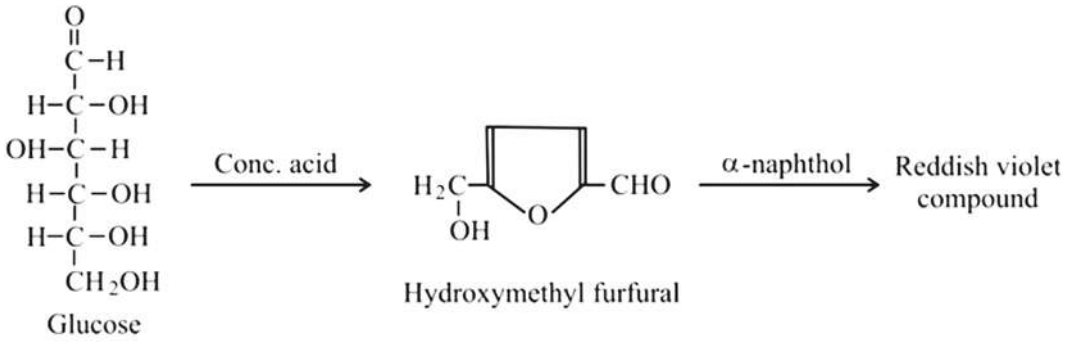
1.2.6.1 Molisch test :

Carbohydrates, in general, respond positively to a colour reaction known as Molisch test. The test is based on the following principle:

On treating with a strong mineral acid, a sugar loses water and forms furfural or furfural-derivative, e.g. when the sugar is a pentose, furtural is formed and when the sugar is a hexose, hydroxymethyl furfural is formed. The furfural or furfural-derivative then condenses with phenolic compounds like a-napthol, thymol etc. to yield coloured complexes.

When Molisch test is carried out, a few drops of an alcoholic solution of a-naphthol is added to the sugar solution. To this mixture, concentrated H_2SO_4 is added very slowly so that the heavy acid forms a separate layer below the previous mixture. A reddish-violet ring appears at the junction of the two layers.

Carbohydrates consisting of more than one or many monosaccharide units also give a positive response to Molisch test, because the treatment with acid hydrolyzes them into monosaccharides.



Reactions in Molisch test

1.2.6.2 Reducing sugars and reduction test :

Sugars possessing a free -CHO or >CO group changes into an enediol in presence of alkali and the enediol, in turn, reduces a metallic ion like Cu^{++} (Cupric) ion or Fe^{+++} (Ferric) ion into a Cu^{+} (Cuprous) ion or Fe^{++} (Ferrous) ion. Due to such reducing action on metallic ion, a sugar with a free -CHO or >CO group is called a reducing sugar. **All monosaccharides are reducing sugars,** as they possess a free -CHO or >CO group. **Among disaccharides, lactose and maltose are reducing sugars,** as they possess a free -CHO group.

A reduction test is a chemical test to identify a reducing sugar. Benedict's test is a common reduction test. Benedict's qualitative reagent contains (i) $CuSO_4$

(Cupric sulphate as a source of Cu^{++} ions), (ii) Na_2CO_3 (an alkali) and (iii) Sodium citrate (which promotes ionization of $CuSO_4$). Upon heating with Benedict's reagent, a reducing sugar reacts with the alkali and changes into an enediol. The enediol reduces Cu^{++} ions into Cu^{+} ions, which combine with OH^{-} ions of water to give yellow CuOH (Cuprous hydroxide). Due to heat, CuOH changes into a **brick-red precipitate of Cu_2O or Cuprous oxide** ($2CuOH = Cu_2O + H_2O$).

Lactose and maltose are reducing sugars but sucrose in a non-reducing sugar. For showing a reducing action, a sugar must possess a free -CHO or >CO group. Among disaccharides, lactose and maltose have a free -CHO group and they are reducing sugars, but sucrose has no free -CHO or >CO group and sucrose is a non-reducing sugar.

In case of maltose, the -CHO group at C^1 of one glucose residue is linked with the C^4 of the second glucose residue. So, the -CHO group at C^1 of the 2nd glucose remains free to make maltose a reducing sugar.

In case of lactose, the -CHO group at C^1 of the galactose residue is linked with the

C^4 of the glucose residue. So, the -CHO group at C^1 of the glucose remains free to make lactose a reducing sugar.

In case of sucrose, the CHO group at C^1 of thee glucose residue is linked with the >CO group at C^2 of the fructose residue. So, there is no free -CHO or >CO group in a sucrose molecule and sucrose cannot have any reducing action.

1.2.6.3 Osazone test :

When reducing sugars are treated with phenylhydrazine in acetic acid at 100°C, the sugar group, i.e. the free -CHO or free >CO group of the sugar reacts with penylhydrazine and sugar-phenylhydrazone is produced. Sugar-phenylhydrazone then reacts further with the excess phenylhydrazine to produce sugar-phenylosazone that precipitates as yellowish or orange crystals with characteristic shape. All monsaccharides and reducing disaccharides like lactose and maltose give a positive Osazone test. The crystals are commonly called as Osazone crystals.

Sugar + Phenylhydrazine Sugar-phenylhydrazone

Sugar-phenylhydrazone + Phenylhydrazine $\longrightarrow$ Sugar-phenylosazone

Glucose and fructose give elongated needle-like crystals. Lactose gives cotton-ball shaped crystals and maltose gives sunflower-shaped crystals.

Glucose/Fructose Lactose Maltose

Osazone crystals of different sugars

1.2.7 Physiological importance of carbohydrates

Monosaccharides : (i) Blood glucose enters into the cells of animal body and is oxidized inside cells for production of energy. (ii) Pentose sugars like ribose and deoxyribose are structural components of RNA and DNA, respectively.

Disaccharides : Milk-sugar or lactose is an essential nutrient for the new-born babies of all mammals. Lactose is hydrolyzed into glucose and galactose by intestinal lactase. Then, glucose and galactose are absorbed into blood and these sugars are oxidized inside cells for production of energy.

Homopolysaccharides : (i) Starch is a storage homopolysaccharide in plant body while glycogen is a storage homopolysaccharide in liver and muscle of animals. Both starch and glycogen are homopolymers of numerous glucose units joined by α-1,4-glycosidic linkage. Both starch and glycogen are broken into glucose whenever required for production of energy in cells. (ii) Cellulose is an insoluble homopolymer of numerous glucose units joined by β-1,4-glycosidic linkage. Cellulose is a structural homopolysaccharide; it is an essential structural component of cell-wall of plant cells. (iii) Chitin is an insoluble homopolymer of numerous N-acetyl-glucosamine units joined by β-1,4-glycosidic linkage. Chitin is a structural homopolysaccharide; it is an essential structural component of exoskeleton of arthropods and wings of insects.

Heteropolysaccharides: (i) Heparin is an acid mucopolysaccharide consisting of alternating units of 2-O-sulphated iduronic acid and glucosamine sulfate. Heparin is secreted from basophils of blood and mast cells of connective tissue. Heparin acts an anticoagulant which prevents clotting of blood in blood vessels. (ii) Hyaluronic acid is a structural heteropolysaccharide cum acid mucopolysaccharide consisting of alternating units of Glucuronic acid and N-acetyl glucosamine. Hyaluronic acid is an essential component of the intercellular matrix of loose connective tissues, synovial fluid and vitreous humor. (iii) Chondroitin sulfate is a structural heteropolysaccharide cum acid mucopolysaccharide consisting of alternating units of Glucuronic acid and N-acetyl galactosamine sulfate. Chondroitin sulfate is an essential component of the intercellular matrix of bone, cartilage, tendon and lung.

1.3 Proteins

1.3.1 Introduction to proteins

Proteins are polymers of the monomers called as amino acids. Proteins are major structural components of cellular membranes, various cell organelles, chromosomes, cytoskeletal structures, extracellular matrix and connective tissue fibers. Besides, proteins in the form of different enzymes, hormones, respiratory proteins and immunoglobulins perform various important physiological functions in the body of living beings.

1.3.2 Classification of proteins

(A) On the basis of molecular shape : On the basis of molecular configuration or shape, proteins are classified into globular and fibrous proteins. **(i) Globular proteins** have their peptide chains compactly folded, exposing polar amino acid side-chains on the surface and hiding non-polar amino acid side-chains within the folds. This makes them soluble in polar solvents like water. The axial ratio (length: diameter ratio) of these molecules ranges from 1 to 4, e.g. Albumin, Globulin. **(ii) Fibrous proteins** have elongated molecules with axial ratios above 10. Non-polar amino acid side-chains lie on their surface, which makes them less soluble in water, e.g. Keratin, Collagen.

(B) On the basis of physiological function : On the basis of physiological role, proteins may be classified into:**(i) Structural proteins** (e.g. Keratin, Collagen, Elastin), **(ii) Contractile proteins** (e.g. Actin, Myosin), **(iii) Carrier proteins** (e.g. Transferrin, Ceruloplasmin), **(iv) Informational proteins** (e.g. Neurotransmitter peptides, Protein hormones and Peptide hormones), **(v) Storage proteins** (e.g. Ferritin, Calsequestrin), **(vi) Enzymes** (e.g. Pepsin, Trypsin), **(vii) Respiratory proteins** (e.g. Cytochromes, Haemoglobin, Haemocyanin), **(viii) Body defense proteins** (Immunoglobulins, Complements). The same protein may sometimes belong to more than one class.

(C) On the basis of solubility: An age-old classification of proteins is based on their solubility in various solvents:

1. Simple proteins: They are composed of amino acids only and have no non-protein part in their molecules. Simple proteins are further classified into :

(a) Protamines: (i) These are the smallest proteins having globular shape. (ii) They are soluble in water, dilute acid or alkali and dilute ammonia. (iii) Protamines are not coagulated by heat. (iv) Protamines do not contain cysteine, cystine, tyrosine and tryptophan, but have a large number of the

cationic, polar amino acid arginine. So, their isoelectric pH is above the normal blood pH 7.4. Consequently, they exist as cationic or basic proteins in the body and combine with nucleic acids, e.g. Protamines like Salmine, Sardinine and Iridine combine with DNA to form Nucleoprotein of fish sperms.

(b) Histones : (i) Histones have globular and larger molecules than protamines. (ii) Their solubility is lower than that of protamines. They dissolve in water, dilute acid or dilute alkali, but not in dilute ammonia. (iii) They are not coagulated by heat. (iv) They are rich in cationic, polar amino acids like arginine and histidine. So, their isoelectric pH is above the normal blood pH 7.4. Consequently, they exist as cationic or basic proteins in the body and combine with nucleic acids and porphyrins. E.g. Histones combine with DNA to form nucleohistone of eukaryotic chromosomes and a histone called globin combines with the porphyrin haem to form haemoglobin of vertebrate RBC.

(c) Albumins : (i) They are large globular proteins. (ii) They dissolve in water, dilute acid or alkali. From solutions, they are precipitated by full saturation with $(NH_4)_2SO_4$, but not by half-saturation. (iii) They are easily thermo-coagulated. (iv) They are deficient in glycine and they have low isoelectric pH, compared to blood pH. Consequently, they are anionic or acidic proteins, e.g. Ovalbumin (egg-white) and lactalbumin (milk albumin) are glycoproteins, but plasma albumin carries no attached oligosaccharides.

(d) Globulins : (i) They have globular and still larger molecules, as compared to albumins. (ii) They have quite low solubility. They are insoluble in water, acid or alkali, but they dissolve in dilute neutral salt solutions (salting in). Globulins are precipitated by half-saturation with $(NH_4)_2SO_4$ (salting out) (iii) They are easily thermo-coagulated. (iv) Many globulins bind with lipids to form lipoproteins (e.g. plasma VLDL or Very Low-Density Lipoproteins), with metals to form metalloproteins (e.g. Transferrin and ceruloplasmin of plasma), and with oligosaccharides to form glycoproteins (e.g. Immunoglobulins of plasma).

(e) Glutelins: These are plant proteins of large size, insoluble in water or salt solution, but soluble in dilute acid or alkali. Having sufficiently big molecules, they are coagulated by heat, e.g. Oryzenin of rice and glutenin of wheat.

(f) Prolamines: They are plant proteins of large size. They are insoluble in water, salt solution and absolute alcohol, but they dissolve in dilute alcohol and dilute acid or alkali. They are coagulated by heat.They are very rich in proline, but poor in lysine, e.g. Gliadin of wheat and hordein of barley).

(g) Scleroproteins: These are fibrous animal proteins with very lowsolubility : (i) Keratins are very insoluble and indigestible. Keratins occur in skin, hair, nail and wool. Keratins are rich in cystine which accounts for interchain S-S linkages, but are poor in proline and hydroxyproline.

(ii) Collagens occur in cartilage, bone, tendon and white fibres of areolar tissue. They dissolve in strong acids and changed to sticky gelatin by heating in water. They are rich in glycine, proline and hydroxyproline, but deficient in tryptophan, tyrosine, cysteine, cystine and methionine.

(iii) Elastins are present in ligaments, tendons and yellow elastic fibres of areolar tissue. They are not gelatinized on heating with water. They are rich in alanine, leucine, valine and proline, but deficient in cysteine, cystine, methionine, hydroxylysine and histidine.

2. Conjugated proteins : These are complexes of simple proteins with non-proteins. The protein part is called apoprotein; the non-protein part is called prosthetic group while the entire molecule is known as a holoprotein. Conjugated proteins are classified according to the prosthetic group:

(a) Chromoproteins: Their prosthetic groups are pigments like porphyrins, carotenoids, melanins and flavins, e.g. Hemoproteins like haemoglobin and cytochromes contain the iron-porphyrin haem. Rhodopsin and iodopsin of retinal rods and cones contain the carotenoid pigment retinol.

(b) Metalloproteins : These are metal-protein complexes, e.g. Carbonic anhydrase of RBC contains Zn^{2+}, ceruloplasmin of plasma contains Cu^{2+} and ferritin of intestinal mucosa contains Fe^{3+}.

(c) Nucleoproteins : Deoxyribonucleoproteins occur in chromosomes, chloroplast stroma and mitochondrial matrix. They have DNA as the prosthetic group while their apoprotein part consists of histones (basic proteins) and non-histones (acidic proteins). Ribonucleoproteins occur in nucleoli and ribosomes. They have RNA as the prosthetic group.

(d) Phosphoproteins : Their prosthetic groups contain phosphates, e.g. Casein of milk and ovovitellin of egg-yolk.

(e) Lipoproteins: Their prosthetic groups are formed of triglycerides, phospholipids, sphingolipids, fatty acids and cholesterol. They are abundant in cellular membranes, plasma and milk. Plasma lipoproteins are classified into VLDL, LDL, HDL etc. according to their densities. The density varies inversely with the percentage of lipids in the molecule.

(f) Glycoproteins: Oligosaccharide chains form the prosthetic groups in these proteins. Oligosaccharide chains are bound either by O-glycosidic linkages with the sidechain-OH of the apoprotein or by N-glycosidic linkages with the

sidechain-NH (of asparagine residues) of the apoprotein. Two major classes are glycoproteins and proteoglycans. Glycoproteins carry hexoses (e.g. galactose, mannose, fucose), N-acetylhexosamines and sialic acid but no hexuronic acid in their oligosaccharide chains. Glycoproteins include mucin, immunoglobulins and some hormones like TSH, FSH and LH. Oligosaccharides of proteoglycan are made of mainly hexuronic acids and N-acetylhexosamines. Proteoglycans occur in extracellular matrix of bones and cartilages.

3. Derived proteins: These are produced from native proteins by various physical and chemical factors:

(a) Denatured proteins or Primary derived proteins: Heat, radiation, acid and alkali can change native proteins non-hydrolytically into denatured proteins. They possess same molecular weight as the original protein, but differ from the latter in solubility, precipitation and crystallization. E.g. Heat, X-ray, UV ray, alcohol or urea change the soluble globular protein ovalbumin into fibrous, insoluble, coagulated protein. This coagulation or clumping of protein occurs due to denaturation or breaking of intra-chain hydrogen bonds in the polypeptide chains.

(b) Secondary derived proteins: Progressive hydrolysis of peptide bonds breaks a protein into smaller molecules: Protein à Peptone à Peptide. They are generally soluble in water and not coagulated by heat.

1.3.3 Protein structure:

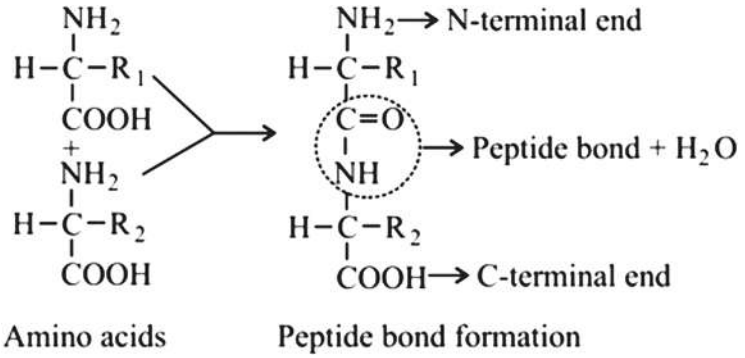
Proteins are made up of one or more polypeptide chains, which in turn are formed by polymerization of many amino acid residues. However, many intra-and interchain connections are formed so that protein molecules assume different higher orders of structure, rather than occurring as simple and discrete polypeptide chains. The formation of higher-order structure fulfils two important purposes: (a) large protein molecules can be accommodated within a limited space in and out of cells and (b) proteins assume the necessary configurations so that they can interact with or act upon other biomolecules. Different orders of proteins structure are described below:

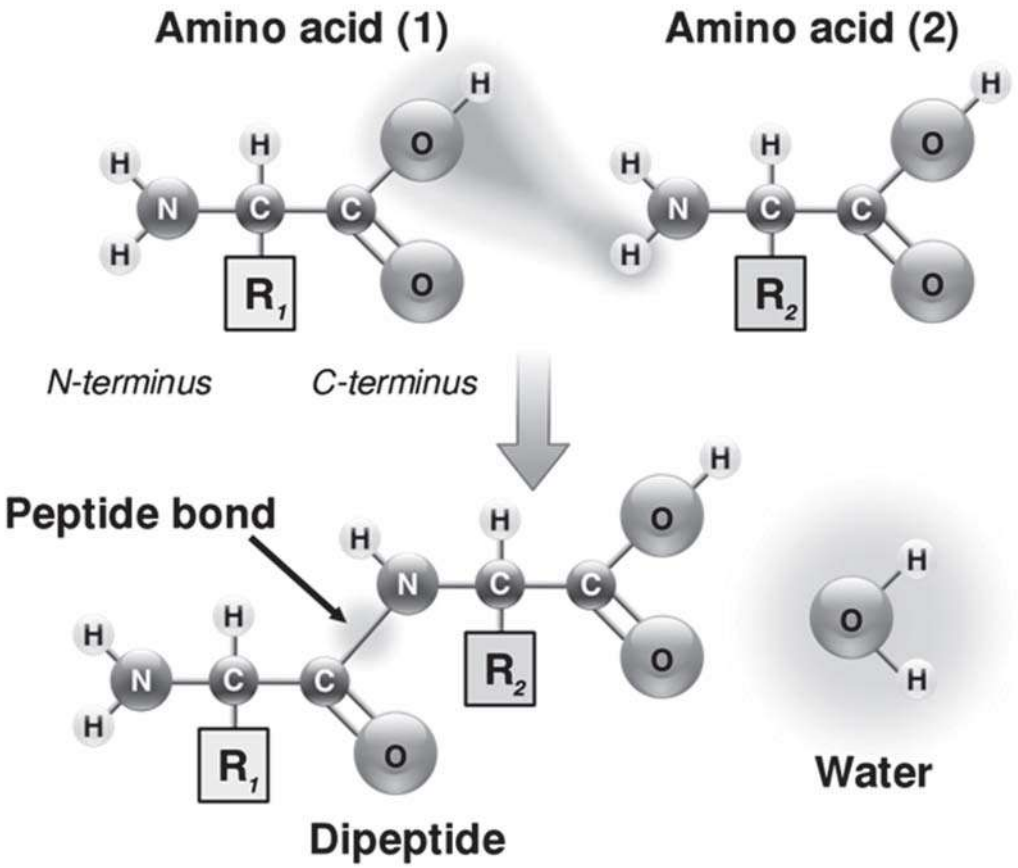
1.3.3.1 Primary structure:

(1) The primary structure of a protein denotes the amino acid sequence of its constituent polypeptide chain or chains.

(2) The primary structure of each polypeptide chain is mainly formed by the formation of covalent bonds called peptide bonds or CONH bonds in between the successive amino acid residues.

(3) A peptide bond links the a-carboxyl carbon of one amino acid with the α-amino nitrogen of the next amino acid while one molecule of water is lost during the formation of a peptide bond.





Peptide bond formation [Source : Wikimedia Commons]

(4) The amino acid residue at one terminal end of a polypeptide chain retains a free α-NH_2 group. This amino acid is called as the first amino acid of a polypeptide chain and this end of the chain is called as the N-terminal end. The amino acid residue at the opposite terminal end of a polypeptide chain retains a free α-COOH group. This amino acid is called as the last amino acid of the chain and this end of the chain is called as the C-terminal end.

(5) The amino acid residues in a polypeptide chain are given serial numbers and shown by respective symbols, starting from the N-terminal end and finishing at the C terminal end, e.g.

$$\text{N terminal end} \quad \overset{1}{\text{Ala}} \quad \overset{2}{\text{Gly}} \quad \overset{3}{\text{Lys}} \quad \overset{4}{\text{Asp}} \quad \overset{5}{\text{Glu}} \quad \overset{6}{\text{Val}} \quad \text{C terminal end}$$

(6) The backbone of each polypeptide chain is constituted by the a-carbon (C_a), the a-carbonyl carbon (C_o) and the a-amide N of the amino acid residues of the chain while the side-chains (R groups) and the H of the amino acids project outwards from the backbone.

Comment: Primary structure of proteins is extremely important for functioning of proteins. Some evidences in favour of this statement are as follows:

(i) Bovine pancreatic ribonuclear is a monomeric protein having a single polypeptide chain with four intrachain disulphide links. If it is treated with 8M urea and â-mercaptoethanol, the hydrogen bonds are disrupted by urea and the disulphide links are disrupted by b-mercaptoethanol. Thus, the secondary and tertiary structure of the protein, which in turn was dependent on the primary structure, is impaired and the enzymatic protein becomes inactive. However, the inactive protein gets reactivated when the denaturing agents are removed and the SH groups are reoxidized with atmospheric O_2.

(ii) In a genetic disease called sickle cell anaemia, the 6^{th} amino acid glutamic acid in each of the two b-chains of haemoglobin is replaced by valine. This abnormal Hb is called HbS and that of normal subjects is called HbA. HbS with nonpolar valine has low solubility; it crystallizes, resulting in sickling of RBCs. The sickle-shaped RBCs rupture easily during their passage through the splenic red pulp.

1.3.3.2 Secondary Structure of Proteins:

The folding or coiling of the polypeptide chain or chains of a protein, due to the formation of intrachain or interchain hydrogen-bonds is called as secondary structure of a protein.

Hydrogen bonding as the cause of secondary structure formation:

(i) The secondary structure of a protein is formed due to formation of intrachain or interchain hydrogen bonds.

(ii) Hydrogen bonds are weak electrostatic bonds formed by sharing of a H^+ ion between 2 electron donor groups lying at a distance of about 2.4 to 3.7 Å. Hydrogen bonds require only 4.5 Kcal of energy per mole to get disrupted.

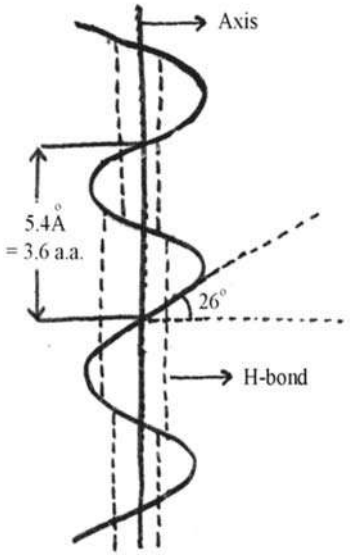
(iii) The secondary structure of a protein is formed by hydrogen bonds between the carbonyl (=CO) group of one peptide linkage and the amide (–NH) group of another peptide linkage (or between the =CO group of one peptide linkage and the OH or NH_2 group in the side-chain of an amino acid residue.

1.3.3.3 Different types of secondary structure :

(a) α-helix :

(i) This type of secondary structure is common in some fibrous proteins like -keratin of hair, wool, nail and skin. It is also found in some regions of some globular proteins like haemoglobin and myoglobin.

(ii) The polypeptide chain forms a helix around a long axis. It is generally a right-handed helix (since the amino acids in body-proteins are L-stereoisomers only). Each complete turn of the helix has a pitch of 5.4 Å). Each turn contains about 3.6 amino acid residues and the angle of the helix is about 26^0).



α-helix

(iii) The helical shape of the chain is maintained by intra-chain H-bonds.

(iv) H-bond runs between the NH group of one peptide bond in one turn of the coil and the =CO group of another peptide bond in the next turn of the coil.

(v) The H-bonds lie parallel to the long axis of the helix.

(vi) The side-chains of amino acid residues project outward from the helix.

(vii) All peptide linkages are involved in H-bond formation.

(b) Triple helix :

(i) This type of secondary structure is found in case of the fibrous protein collagen which consists of 3 polypeptide chains.

(ii) Each of the 3 polypeptide chains occurs as a left-handed helix around a long axis common to all the 3 chains. Thus, the 3 chains together form a triple helix.

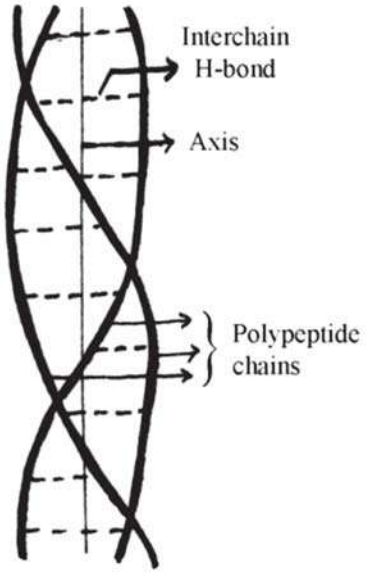
(iii) The triple helical organization is maintained by inter-chain H-bonds.

(iv) H-bonds are established between the -NH and =CO groups of peptide linkages located on the different polypeptide chains.

(v) The H-bonds lie perpendicularly with the long axis of the triple helix.

(vi) The side-chains of amino acid residues project outward from the polypeptide chains.

(vii) Unlike the a-helix, all peptide linkages are not involved in H-bond formation.



Triple-helix

(c) β-pleated sheet:

(i) This type of secondary structure is found in certain fibrous proteins like -keratin of silk and spider's web, which consists of several polypeptide chains.

(ii) The polypeptide chains occur in the form of zigzag chains lying side by side. Two adjacent chains may lie parallel to each other, i.e. their NH_2-terminals lie at the same end and the COOH-terminals lie on the other end. Two adjacent chains may lie in anti-parallel manner also, i.e. the NH_2- and COOH-terminals of 2 adjacent chains lie in the same side.

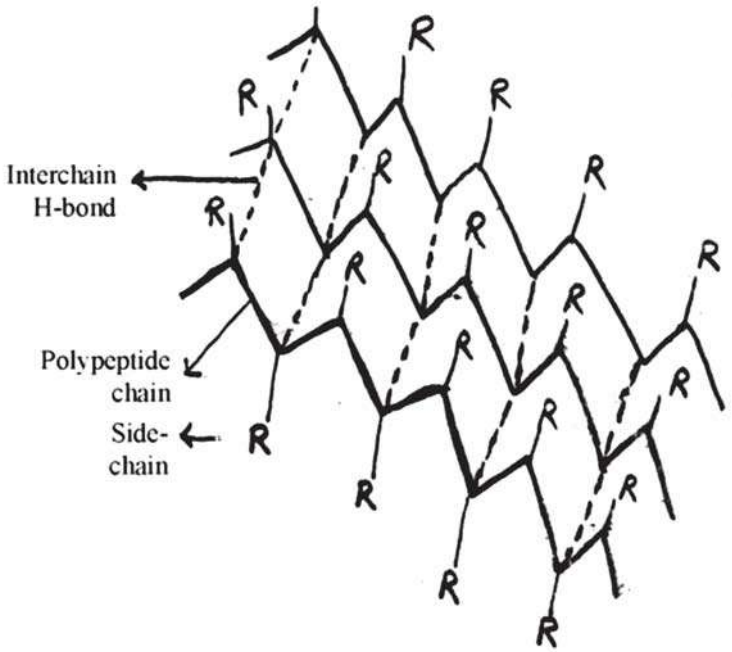
(iii) The zigzag chains are held together by means of inter-chain H-bonds.

(iv) H-bonds run between -NH and =CO groups of peptide linkages lying on adjacent chains.

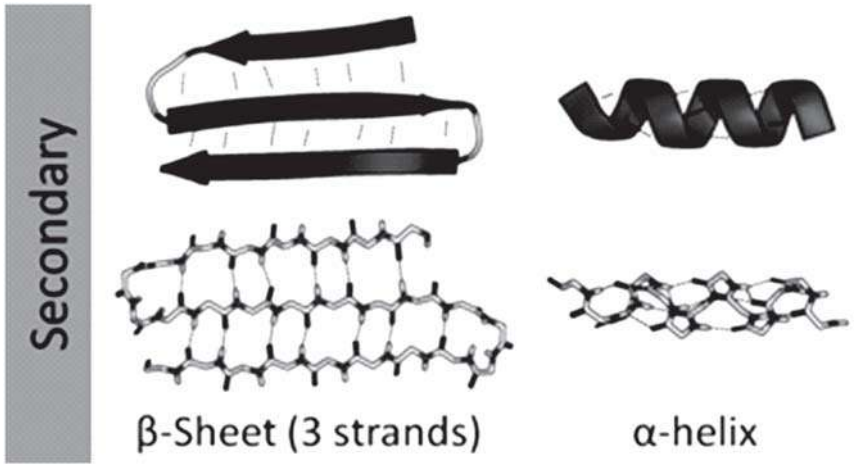
(v) The H-bonds lie perpendicular to the long axis of each chain.

(vi) The side-chains of amino acid residues project either upward or downward from the individual chains.

(vii) Like the alpha-helix and unlike the triple helix, all peptide linkages are involved in H-bond formation.



β-pleated sheet



Secondary Structure of Protein [Source : Wikimedia Commons (CC 4.0)]

1.3.3.4 Tertiary structure of proteins:

In case of globular proteins, the polypeptide chains with the coiled secondary structures again become folded or twisted about themselves due to different types of intra-molecular bonds and forces. Such folding of the secondary structure itself is called tertiary structure of a protein. This is the highest order of structure of a monomeric protein (= a protein having a single polypeptide chain).

The following intra-molecular forces and bonds are responsible for tertiary structure formation:

(i) van der Walls forces: These are very weak attractive forces between atoms, resulting from fluctuations in the distributions of electrons. Such weak forces operating between the side-chains of neutral amino acids help in formation of tertiary structure.

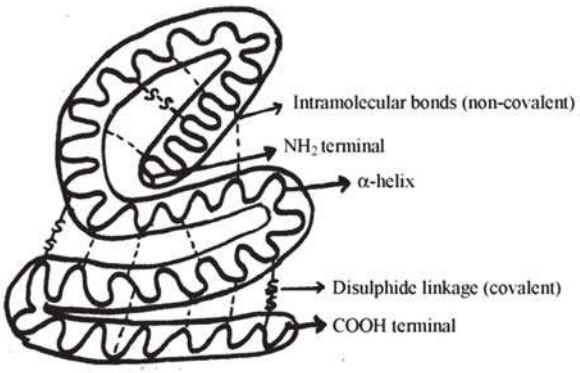
(ii) Hydrogen bonds: These are weak electrostatic bonds formed by sharing of a H^+ ion between 2 electron-donor groups. The formation of the tertiary structure of proteins may be helped by the formation of H-bonds between the CO group of the peptide linkage or the COOH group of the side-chains and the OH and NH_2 groups of the side-chains.

(iii) Ionic bonds: These are strong electrostatic bonds between a +vely charged group and a -vely charged group of the side-chains of amino acids.

(iv) Hydropbobic interaction: This is an attractive interaction between the non-polar side-chains of different amino acids.

(v) Covalent disulphide linkage: Disulphide linkage (-S-S-) is a very strong covalent linkage which can be formed between 2 cysteine residues of a

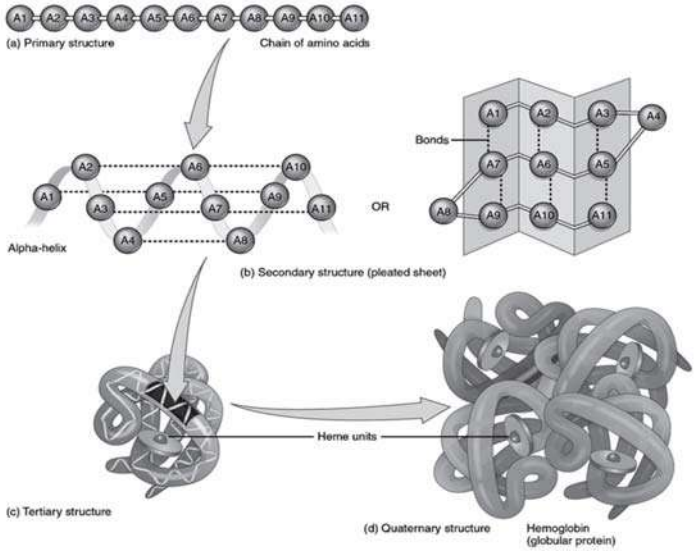
polypeptide chain. It is formed by sharing of electron pairs between 2 sulphur atoms of 2 cysteine residues of a polypeptide chain.



Tertiary structure of a globular protein

1.3.3.5 Quaternary structure :

Many proteins are oligomeric or possess 2 or more identical or non-idendical poly peptide chains called as subunits. These chains remain held together by non-covalent bonds. This assembly is called as quaternary structure of proteins, e.g. Haemoglobin is a tetrameric protein with two α and two β chains. These chains or subunits remain held together by mainly H-bonds and some ionic bonds between side-chains of polar amino acids of the different polypeptide chains. Besides, SH (Sulphydril) groups of cysteine residues of different polypeptide chains may be linked by disulphide (-S-S-) linkage.

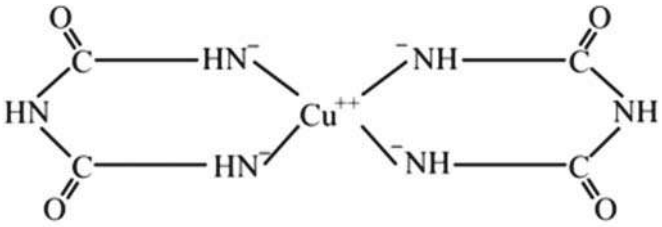


Primary to Quaternary Protein Structure Formation [Source : Wikimedia Commons]

1.3.4 Some important properties of proteins:

1.3.4.1 Biuret test for proteins:

Biuret test is a colour reaction to detect any compound which bears at least two peptide linkages (-CONH) in its molecule. Hence, proteins, peptones and peptides give a positive response to this test. When an alkaline solution of protein, peptone or peptide is added with very dilute copper sulphate solution, a violet or pinkish colour develops due to formation of a complex between the cupric ion (Cu^{++}), peptide nitrogen and oxygen of water.

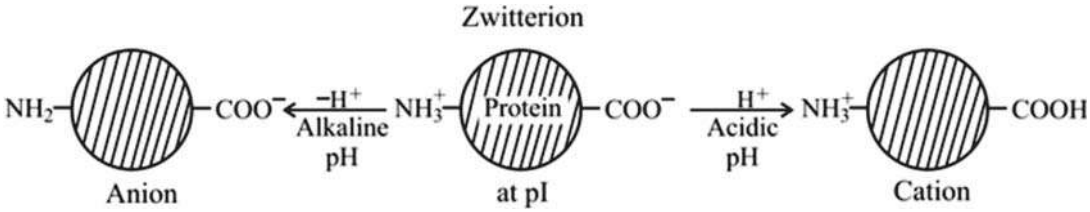


Reaction between Cu^{++} ions with proteins

The colour depends on the complexity of size of the reactant molecules, e.g. Proteins give a purplish violet colour. Peptones have a smaller size than proteins and give a pink colour. Peptides have a further smaller size and give a light pink colour. It may be added here that 'Biuret' which is formed by union of 2 molecules of urea on heating also gives a +ve response (pink colour formation) to biruet test, because 'Biuret' also contains two -CONH linkages in its molecule.

1.3.4.2 pH and protein structure and function :

At a particular pH, called as the **isoelectric pH** or **pI,** a protein exists as a 'zwitterion' or carries equal numbers of +vely and –vely charged groups. In other words, at its pI, a protein bears a net charge of '0'. Hence, at its pI, a protein remains least soluble and also, does not show any migration towards either the cathode or the anode in an electric field, e.g. pI of egg albumin is 4.6 and pI of haemoglobin is 6.8. On the acidic side of the pI, a protein accepts H^+ ions from the medium and converts into a cation. On the alkaline side of the pI, a protein donates H^+ ions to the medium and converts into an anion. In this way, a significant change of pH of the medium may influence the ionic status of proteins, which in turn may influence the solubility of proteins. Moreover, the 3-dimensional structure and activity of proteins may be affected by changes of pH, as the intra- and inter-chain bond formation may be affected by changes of pH.



Effect of pH change on proteins

1.3.4.3 Salting in:

The solubility of several proteins like globulins increases in presence of traces of electrolyte ions. In a dilute solution of a mineral salt, one particular type of electrolyte ion is adsorbed on the protein particles, in preference to the other type of ion from that electrolyte or salt. This increases the like-charges on the protein particles, which then repel one another and remain dispersed in the aqueous medium. However, a concentrated mineral salt solution giving a higher concentration of its ions may precipitate a protein from its solution.

1.3.4.4 Salting out :

Concentrated solutions of neutral mineral salts such as $(NH_4)_2SO_4$, $MgSO_4$ and Na_2SO_4 can precipitate a protein from its solution. This 'salting out' of protein requires a higher concentration of the mineral slat than what is needed for 'salting in'. Firstly, the higher concentration of mineral ions osmotically removes water from the solvation layer around protein particles. Secondly, at high concentrations, both cations and anions from the mineral salt bind with the respective counter-ionic groups on the protein particles to reduce the surface charges of the latter. Both these phenomena enhance the aggregation and precipitation of protein particles. Salting out is most effective near the isoelectric pH of the relevant protein. (Moreover, if a protein solution containing a low concentration of mineral salt is frozen, the protein is salted out from it, because the formation of ice crystals raises the concentration of the mineral ions in the remaining solution and thus precipitates the protein).

1.3.5 Physiological importance of proteins :

(a) Proteins are major structural components of cellular membranes, organelles, cytoskeletal structures, extracellular matrix and connective tissue fibres.

(b) Some structural proteins such as actin and myosin are contractile proteins; they cause contractions of muscle fibres.

(c) Some cytoplasmic or membrane proteins function as molecular receptors. They bind with specific molecules, reaching or entering the cell, so as to mediate in the cellular actions of those molecules.

(d) Some proteins like histones and protamines combine with DNA and RNA to form nucleoproteins of respectively chromosomes and ribosomes.

(e) Carrier proteins bind with specific substances and transport them either across the membranes or in the body fluids, e.g. 'Sodium pump for transmembrane Na+ transport, and transferring for transporting Fe^{3+} in plasma.

(f) Specific proteins may bind with and store specific substances in the cell or in the extracellular fluid, e.g. Ferritin stores iron in spleen and liver cells.

(g) Most enzymes are proteins. Enzymes catalyze various reactions in the body of living beings.

(h) Immunoglobulins and complements are plasma proteins which help in body immunity.

(i) Some proteins are constituents of respiratory pigments like haemoglobin, myoglobin and cytochromes.

(f) When necessary, proteins may be catabolized to produce energy.

(k) Proteins influence and control the osmotic pressure of body fluids.

(l) Proteins influence and maintain ionic and fluid distributions on two sides of membranes.

(m) Some of the hormones are either peptides (e.g. hypothalamic and gastrointestinal hormones) or proteins (e.g. pituitary, pancreatic and parathyroid hormones).

1.4 Lipids

1.4.1 Introduction to lipids

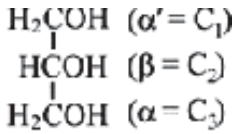
Lipids are esters of aliphatic mono-carboxylic organic acids called fatty acids with different alcohols. They are non-polar organic compounds which are insoluble in water, but soluble in non-poalar organic solvents like chloroform and benzene. They are compounds of mainly C, H and O, but may also contain P, N and S. The ratio of H and O in lipids is quite greater than 2 : 1, which is found in water and many carbohydrates. Lipids may serve as energy in living beings; lipids are structural components of cellular membranes; lipids may also serve different physiological functions.

1.4.2 Classification of lipids

Lipids are classified into 3 broad categories:

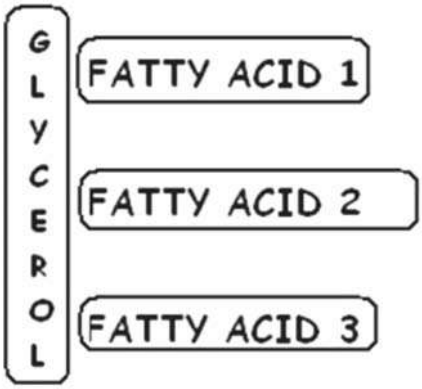
1. Simple lipids: These are esters of fatty acids with different alcohols and do not contain any other substances. Simple lipids are divisible into 2 types:

(A) Glycerides or acylglycerols: (i) These are esters of fatty acids with a trihydroxy alcohol called glycerol.



Glycerol

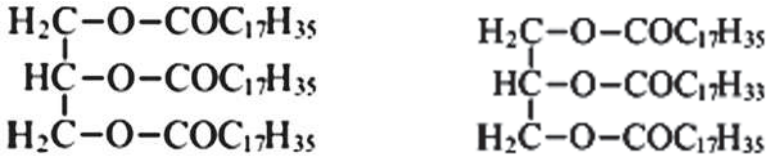
(ii) Glycerides are further divisible into mono-, di- and triglycerides (or mono, di- and triacylglycerols), depending on whether they contain 1, 2 or 3 fatty acid residues, respectively.



Schematic Representation of Tri-glyceride Structure

[Source: Wikimedia Commons (CC 3.0)]

(iii) Triglycerides are again divisible into simple and mixed triglycerides depending on whether the three OH-groups of the glycerol moiety are esterified with only one type of fatty acid or more than one type of fatty acid, respectively.



Simple (left) and mixed (right) triglycerides

(iv) Triglycerides are commonly called as fats; fats remaining solid at room temperature are called solid fats (e.g. Lard and butter of animal origin and 'Vanaspati' of plant origin) and fats remaining liquid at room temperature are called oils (e.g. Mustard oil, Soya bean oil etc. plant oils and Cod liver oil of Cod fish).

Solid fats are triglycerides containing saturated fatty acids while oils are triglycerides containing unsaturated fatty acids whose melting points are lower than those of saturated fatty acids due to the presence of double-bonds in their carbon-chains.

(B) Waxes :

(i) Waxes are solid esters of long-chain fatty acids (C_{16}-C_{34}) with long-chain monohydroxy alcohols (C_{16}-C_{34}).

(ii) Waxes in which the alcohol is an aliphatic alcohol are called aliphatic waxes, e.g. Bee-wax is an ester of palmitic acid ($C_{15}H_{31}COOH$) with myricyl alcohol ($C_{30}H_{61}OH$) or bee-wax is myricyl palmitate ($C_{15}H_{31}COOC_{30}H_{61}$).

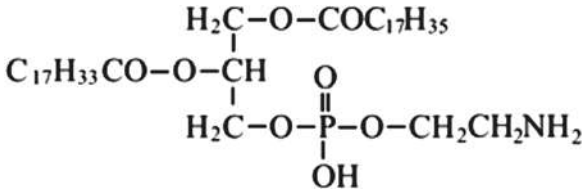
(iii) Waxes in which the alcohol is an alicyclic alcohol are called alicyclic waxes, e.g. Lanolin secreted by the skin of sheep is an ester of fatty acids with alicyclic alcohol commonly called as 'sterol'.

2. Compound lipids: These are also esters of fatty acids with different alcohols, but their molecules contain additional substances like phosphate, nitrogenous base, carbohydrate, protein etc. They are of 3 types:

(A) Phospholipids: These are not only esters of fatty acids with alcohols, but also contain a phosphate and a nitrogenous or non-nitrogenous molecule. Phospholipids may be divided into 2 types:

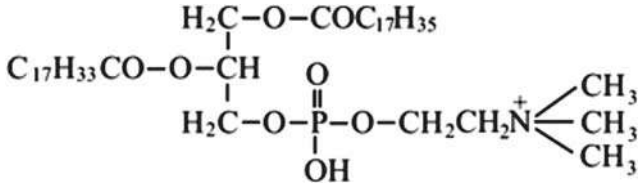
(a) Phosphoglycerides: Phospholipids in which the alcohol part is the trihydroxy alcohol glycerol are called phosphoglycerides. Phospglycerides are of different types:

(i) Cephalin or phosphatidylethanolamine : Here, the α-OH of glycerol is esterified with a phosphoric acid which in turn remains bound with a nitrogenous base called ethanolamine; the β-OH of glycerol is esterified with an unsaturated fatty acid; the $\alpha^{/}$-OH of glycerol is esterified with a saturated fatty acid. Cephalin is found in brain and liver. [Remember, $\alpha = C_{3,}$ $\beta = C_{2,}$ $\alpha^{//} = C_1$].



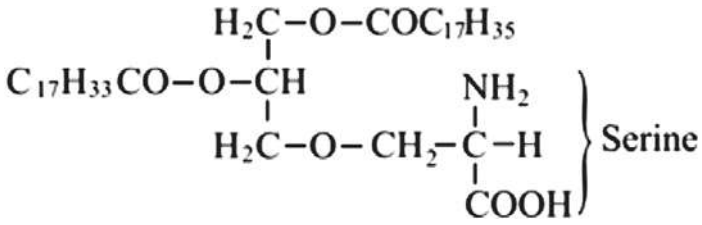
Cephalin [$NH_2CH_2CH_2OH$ = Ethanolamine]

(ii) Lecithin or phosphatidylcholine: Its structure is comparable with that of cephalin, but it contains a nitrogenous base called choline instead of ethanolamine. Lecithin occurs in brain, liver and cardiac muscle.



Lecithin (Choline = trimethylethanolamine with N^+)

(iii) Lipoamino acids: Their structure is comparable with that lecithin, but they contain a hydroxy amino acid like serine or threonine in place of the nitrogenous base choline. Accordingly, they are called as phosphatidylserine or phosphatidylthreonine. Lipo-amino acids occur in brain and RBC.

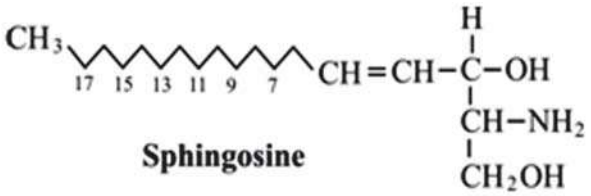


Phosphatidylserine

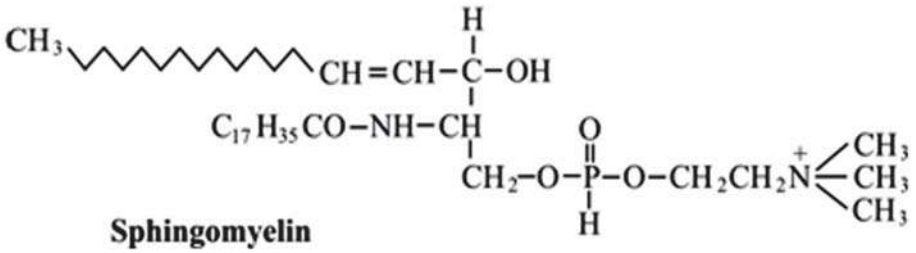
(iv) Plasmalogens: Here, the a-OH of glycerol is esterified with a phosphoric acid which in turn remains bound with either choline or ethanolamine; the b-OH of glycerol is esterified with an unsaturated fatty acid; the a/-C of glycerol is linked by an ether linkage with an unsaturated alcohol. Plasmalogens occur in brain, RBC and cardiac muscles.

(v) Nitrogen-free phosphoglycerides: Their structure is comparable to that of lecithin, but they contain a non-nitrogenous substance like either an inositol (a vitamin) or a glycerol or a phosphatidic acid in the place of choline. They are named as phospha tidylinositol, phosphatidylglycerol and cardiolipin, respectively. These phospholipids are found in brain, cardiac muscle and liver. [Phosphatidic acid = 1 glycerol + 2 fatty acids + 1 H_3PO_4].

(b) Sphingomyelins: Phospholipids in which the alcohol part is a monoamino, dihydroxy, unsaturated, C_{18} alcohol sphingosine are called as sphingomyelins. The NH_2-group of sphingosine remains combined with a fatty acid while the CH_2OH part of sphingosine remains esterified with a phosphoric acid which in turn remains is linked with a choline. Sphingomyelins occur in brain, cardiac muscle and liver.



Sphingosine



Sphingomyelin

(B) Glycolipids: They are not only esters of fatty acids with the nitrogenous alcohol sphingosine, but also contain a carbohydrate moiety. They are of different types :

(a) Carebrosides: Here, the NH_2-group of sphingosine remains combined with a fatty acid while the CH_2OH part of sphingosine remains linked with a galactose or glucose. Accordingly, they are called as galactocerebrosides and glucocerebrosides, respectively and they occur in nervous and non-nervous tissues, respectively.

(b) Sulfatides: Here, the NH_2-group of sphingosine remains combined with a fatty acid while the CH_2OH part of sphingosine remains linked with a galactose which in turn is sulphated. Sulfatides occur in liver, kidney and salivary glands.

(c) Gangliosides: Here, the NH_2 group of sphingosine remains combined with a fatty acid and the CH_2OH part of sphingosine remains linked with an oligosaccharide chain containing hexose, acetylhexosamine and sialic acid. Gangliosides occur in the membranes of neurons and RBC.

(C) Lipoproteins :

These are lipid-protein complexes that assume the shape of small to large rounded particles. Lipids found in lipoprotein particles include triglycerides, phospholipids, cholesterol and free fatty acids. Lipoprotein particles of various size and density occur in blood plasma and help in transport of fats, fatty acids, cholesterol between the liver and the extrahepatic tissues.

3. Derived lipids: Substances derived from simple or compound lipids by their hydrolysis are called as derived lipids. Derived lipids are as follows:

(A) Fatty acids :

Fatty acids are aliphatic mono-carboxylic organic acids that occur in lipid molecules of animal or plant body. Fatty acids are classified as follows:

(a) Straight-chain fatty acids : In these fatty acids, carbon atoms are arranged linearly. These fatty acids are divided into 2 types:

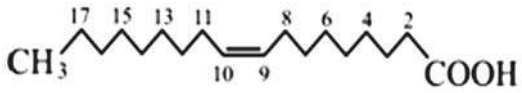
(i) Saturated fatty acids : They have no double-bond between the constituent carbon atoms. All valencies of their carbons, except the carboxyl-C, are saturated with hydrogens. They have a general fomula of $CnH_{(2n+1)}COOH$ and a structure of $\mathbf{CH_3(CH_2)_xCOOH}$. They are again subdivided into 2 groups:

Even-C fatty acids: Saturated fatty acids with an even number of carbons are very common in animal lipids. Even-C fatty acids with 10 or fewer carbons are called as lower fatty acids. E.g. **Butyric acid** [C_4 : C_3H_7COOH or $\mathbf{CH_3(CH_2)_2COOH}$]. Even-C fatty acids with more than 10 carbons are called as higher fatty acids. E.g. **Palmitic acid** [C_{16} : $C_{15}H_{31}COOH$ or $\mathbf{CH_3(CH_2)_{14}COOH}$] and **Stearicacid** [$C_{18}$: $C_{17}H_{35}COOH$ or $\mathbf{CH_3(CH_2)_{16}COOH}$].

Odd-C fatty acids: Saturated fatty acids with an odd number of carbons are more common in plant lipids than in animal lipids. E.g. **Valeric acid** [C_5 : C_4H_9COOH or $\mathbf{CH_3(CH_2)_3COOH}$].

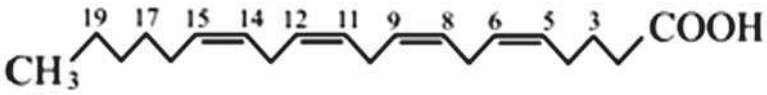
(ii) Unsaturated fatty acids: Straight-chain fatty acids containing double-bonds in the carbon-chain are unsaturated fatty acids. They are generally even-C fatty acids. They are subdivided into:

Monoenoic fatty acids: They have a single double-bond in the carbon-chain. E.g. Oleic acid found ($C_{17}H_{33}COOH$) in both animal and plant lipids is a D^9-cis-monoenoic fatty acid, i.e. it has a double-bond after C_9 and the double-bond is in cis-configuration.



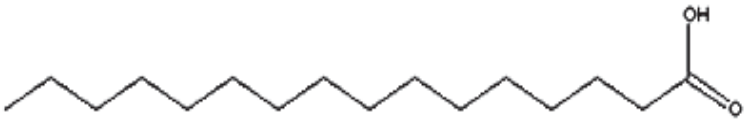
Oleic acid (D^9-cis-monoenoic)

Polyenoic fatty acids: Fatty acids with more than one double-bond in the carbon-chain are called polyenoic fatty acids or polyunsaturated fatty acids (PUFA). E.g. Linoleic acid ($C_{17}H_{31}COOH$) of Saffola and Soya bean oils is a $D^{9, 12}$-cis-dienoic acid having two double-bonds in cis-configuration after C_9 and C_{12}, respectively. Arachidonic acid ($C_{19}H_{31}COOH$) of Peanut and Groundnut oils is a $D^{5, 8, 11, 14}$-cis-tetraenoic acid with four double-bonds in cis-configuration after C_5, C_8, C_{11} and C_{14}, respectively.



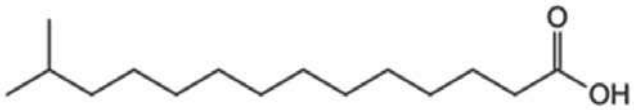
Arachidonic acid ($D^{5, 8, 11, 14}$-cis-tetraenoic)

(b) Branched-chain fatty acids: Some fatty acids have a branched carbon-chain; these are not found in fats but may be present in waxes. Examples of such fatty acids are Isobutyric acid and Isovaleric acid.

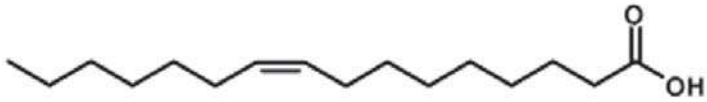


Palmitic Acid – Straight Chain Fatty Acid

13-methyltetradecanoic acid (iso-pentadecanoic acid) – Branched Chain Fatty Acid

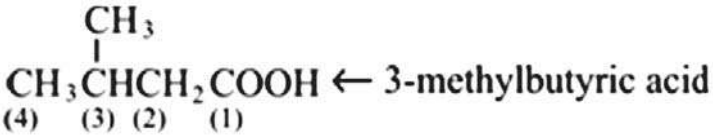


Palmitoic Acid – Unsaturated Fatty Acid



[Source: Wikimedia Commons]

(c) Substitued fatty acids: These are saturated or unsaturated fatty acids in which one or more H-atoms of the hydrocarbon chain are replaced by hydroxyl (OH) or methyl (CH_3) or halide groups, e.g. 3-Methylbutyric acid found in brain.

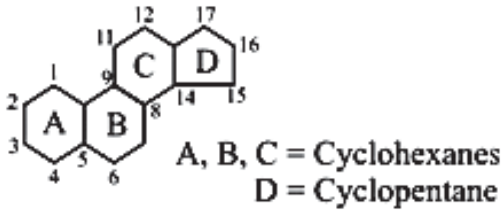


(d) Cyclic fatty acids: These are fatty acids with cyclic ring in their carbon-chain, e.g. Prostaglandins found in different animal tissues are polyunsaturated fatty acids with a cyclopentane ring.

(B) Aliphatic alcohols : The aliphatic alcohol, Glycerol is derived from hydrolysis of glycerides and phosphoglycerides. Another aliphatic alcohol, sphingosine is derived from hydrolysis of sphingomyelins and glycolipids. Some others like myricyl alcohol and cetyl alcohol are derived from hydrolysis of aliphatic waxes.

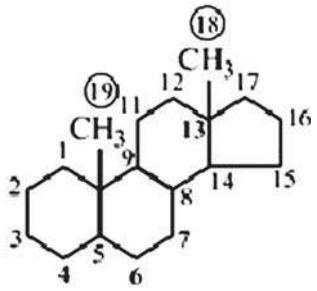
(C) Steroids and sterols: Steroids and sterols are present in association with fat within animal body and can be separated as an 'unsaponifiable residue' after saponification of fat. Moreover, sterols may be derived from hydrolysis or some waxes.

All steroids contain a characteristic 17-carbon, fused, tetracyclic ring-system designated as 'cyclopentanoperhydrophenanthrene nucleus'.



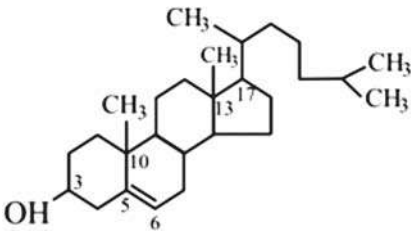
Cyclopentanoperhydrophenanthrene nucleus

The first three rings of this nucleus are cyclohexanes or 6-carbon rings and called as A, B and C, respectively while the 4th ring called as D is a cyclopentane or 5-carbon ring. Fusion of A, B, C and D produces a 17-carbon ring system or nucleus. Besides, 2 methyl (CH_3) groups remain attached with C_{13} and C_{10} of the nucleus and those methyl carbons are designated as C_{18} and C_{19}, respectively. Moreover, other groups may remain attached with different carbons of the nucleus in different steroids.



Basic structure of a steroid

Those steroids which not only carry CH_3-groups at C_{13} and C_{10} of the cyclopentanoperhydrophenanthrene nucleus, but also carry a hydroxyl (OH) group at C_3 and a 8-10 carbon-long side-chain at C_{17}, but no carboxyl or ketonyl oxygen at any carbon, are called sterols,e.g. Cholesterol and lanosterol are two important sterols of animal body. Cholesterol is the precursor of gonadal and adrenocortical steroid hormones; cholesterol carries a 3'-OH, a 8-carbon side-chain at C_{17} and a double-bond between C_5 and C_6.

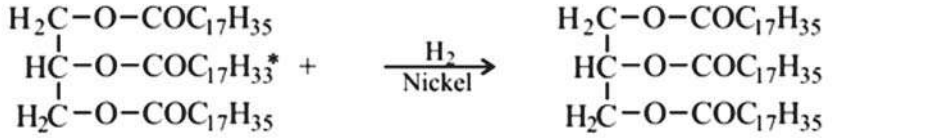


Cholesterol

1.4.3 Properties of fats :

1. Melting point: Fats containing saturated fatty acids have high melting point and remain solid at room temperature (e.g. Lard and butter) while fats containing unsaturated fatty acids have low melting point and remain liquid at room temperature (e.g. Mustard oil, soya bean oil). The former are called solid fats and the latter are called oils. The low melting point of oils is due to their possession of unsaturated fatty acids having cis double-bonds in the carbon-chain; the cis double-bonds prevent the close-packing of the carbon-chains and also reduce the hydrophobic interaction in between those chains.

2. Hydrogenation: When oils, i.e. fats with unsaturated fatty acids are exposed to hydrogen at 155°-220°C in presence of nickel or platinum catalyst, hydrogen is added to the double-bonds in the unsaturated fatty acid residues. One atom of hydrogen is added to each side of a double-bond. As a result, the oil gets reduced into a solid, saturated fat,e.g. Cottonseed oil is changed into margarine and sunflower oil is changed into 'Vanaspati' or 'Dalda' by means of hydrogenation:



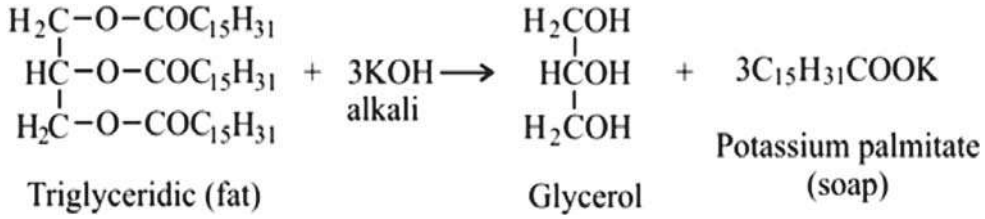
Oil with unsaturated fatty acid (*) Hydrogenated (saturated) solid fat

Hydrogenation of oil

3. Rancidity: An unpleasant odour and taste is often developed by fats left out for a long period in contact with air and light. This is called rancidity of fats. It is due to two causes: (a) Hydrolytic action of lipases from contaminating microbes, resulting in release of glycerol and free fatty acids with characteristic odour; this is called 'hydrolytic rancidification'. (b) Oxidation of unsaturated fatty acids at their double-bonds by atmospheric oxygen, resulting in formation of peroxides which then decompose to form aldehydes with unpleasant odour and taste; this is called 'oxidative rancidification'.

4. Hydrolysis: Fats get hydrolysed into glycerol and constituent fatty acids, when acted upon by the enzyme lipase or when boiled with acidified water under high temperature and pressure.

5. Saponification and saponification number: (i) When boiled with a solution of strong metallic alkali like KOH or NaOH, fats quickly decompose into glycerol and fatty acids. The released fatty acids soon react with the alkali to form metallic salts and water. Those metallic salts of fatty acids are called 'soaps' and the overall process of soap formation is called 'saponification', e.g. If a triglyceride containing glycerol and palmitic acid is boiled with KOH, a soap called 'potassium palmitate' and glycerol are produced. The reaction is facilitated upon addition of an alcohol to the mixture of fat and alkali, as fats are quickly dissolved in alcohol.



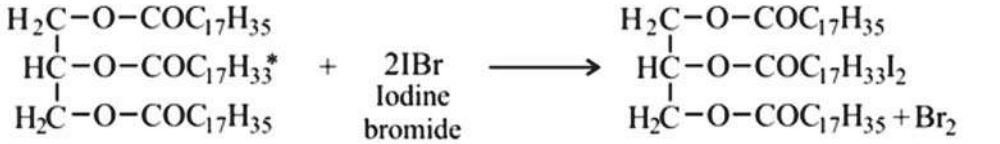
Saponification of fat

(ii) In addition to fats, the waxes, phospholipids and glycolipids can also undergo saponification upon boiling with an alkali. However, in these instances, the alcohol being produced is often different from glycerol. All these lipids are called saponifiable lipids. On the other hand, Steroids and sterols are unsaponifiable lipids.

(iii) **The number of milligrams of KOH required to saponify 1 g of a fat is called as the 'saponification number' of that fat,** e.g. Saponification number of butter is 210-230 and that of castor oil is 175-180.

(iv) **Significance of saponification number:** (a) The higher is the saponification number of a fat, the lower are the chain-length and molecular weight of the fatty acids in that fat and also, the lower is the molecular weight of the fat itself, e.g. Butter is rich in shorter fatty acids and has a lower molecular weight while castor oil is rich in longer fatty acids and has a higher molecular weight. (b) Since 3 molecules of KOH (MW = 56000 mg) are required to saponify 3 fatty acid residues of a triglyceride, saponification number = $\frac{3\times 56000}{M}$ mg of KOH (M = average molecular weight of the triglyceride) or M = $\frac{168000}{\text{saponification number}}$.

6. Iodination and iodine number: (i) When fats with unsaturated fatty acids are treated with halides like IBr (iodine bromide) or ICI (iodine chloride), iodine is added to the double-bonds of the unsaturated fatty acid residues of a fat molecule. One atom of iodine is added to each side of a double-bond resulting in the formation of a saturated halogenated fat.



Iodination of fat

(ii) **The number of grams of iodine absorbed by 100 g of a fat is called as the iodine number of that fat,** e.g. Iodine number of coconut oil is 6-10 and butter is 25-28 but that of saffola oil is as high as 140-155.

(iii) **Significance of iodine number :** A high iodine number of a fat indicates that the fat is rich in unsaturated fatty acids. In other words, iodine number is an index of the degree of unsaturation of fat, e.g. Saffola oil has a much higher degree of unsaturation than coconut oil and butter.

7. Reichert-Meissl number: Reichert-Meissl number or Volatile fatty acid number is a measure of the amount of steam-volatile, lower fatty acids (C_2-C_{10}) present in a fat. A fat sample is first saponified and then treated with a mineral acid to liberate free fatty acids from the soaps. Thereafter, steam is passed through this mixture to let the lower fatty acids get volatilized and carried away with the steam to condense in a receptacle. **The number of milliliters of N/10 KOH required to neutralize the volatile fatty acids liberated from 5 g of a fat is called as Reichert-Meissl number of that fat**,e.g. Reichert-Meissl number of butter is 26-33, but that of olive oil is only 0.5-1.5, indicating that butter contains a much larger amount of volatile fatty acids than olive oil.

1.4.4 Physiological importance of lipids:

(i) Fats stored in adipose tissues may act as a source of energy whenever required by the body.

(ii) Fats deposited under the skin and around the vital organs act as thermo-insulator and shock-absorbing cushion.

(iii) Phospholipids, glycolipids, lipoproteins and sterols form different cellular structures like plasma membrane, nuclear membrane, organelle membranes and myelin sheath.

(iv) Lipoprotein particles of blood plasma help in transport of fats, fatty acids, cholesterol and other non-polar substances between the liver and the extrahepatic tissues.

(v) Sterols are used in the biosynthesis of steroid hormones and vitamin D.

(vi) Phospholipids help in intestinal absorption of fat-soluble vitamins and excretion of cholesterol in bile.

(vii) Prostaglandins, which are modified fatty acids, act as local hormones that influence the functioning of different tissues or organs of the body (contraction and relaxation of smooth muscle, the dilation and constriction of blood vessels, control of blood pressure).

1.5 Nucleic Acids

1.5.1 Introduction to nucleic acids

Nucleic acids are non-protein, nitrogenous, acidic substances in living cells. These are polymers of numerous small units or monomers called as nucleotides. There are two kinds of nucleic acids: DNA or deoxyribonucleic acid and RNA or ribonucleic acid. DNA molecules transmit all the hereditary characters of an organism from one generation to another while RNA molecules carry out protein synthesis in living cells.

DNA or deoxyribonucleic acid occurs in chromosomes and mitochondria of all eukaryotic cells, chloroplasts of plant cells, bacterial chromosome and plasmids. Moreover, DNA is found in the genetic material of DNA viruses. The second type of nucleic acids includes the RNAs or ribonucleic acids which occur in both nucleus and cytoplasm of all eukaryotic cells. In prokaryotes, RNAs are associated with DNA of chromosome and also, in the cytoplasm. Moreover, RNA occurs in the genetic material of RNA viruses.

1.5.2 Watson and Crick's model of DNA structure

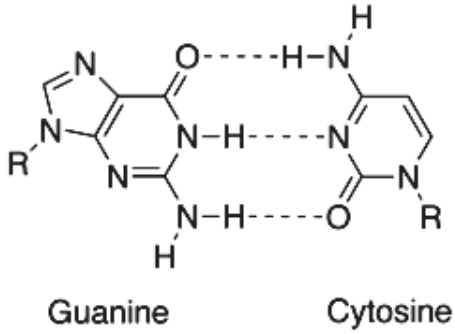
A DNA molecule has a double-helical structure and the double-helix model of DNA structure was proposed by James Watson and Francis Crick in 1953; the model was proposed on the basis of X-ray diffraction studies. The basic postulates of the model are as follows:

1. A DNA molecule consists of two long strands called polynucleotide strands. The two strands remain helically coiled around each other.
2. Each polynucleotide strand is a polymer of numerous small units called nucleotides. Each nucleotide is made up of a nucleoside and a phosphoric acid residue; thus, each nucleotide is a nucleoside phosphate. Again, a

nucleoside consists of a nitrogenous base and a pentose sugar called deoxyribose which lacks oxygen at its C^2. Since the pentose sugar in DNA is deoxyribose, the nucleosides of DNA are more precisely called as deoxyribonucleosides and the nucleotides of DNA are more precisely called as deoxyribonucleotides.

3. The two polynucleotide strands bear two kinds of nitrogenous bases: purines and pyrimidines. Purines possess a purine ring with different substituent groups while pyrimidines possess a pyrimidine ring with different substituent groups. DNA contains two types of purines, viz. adenine and guanine (commonly abbreviated as A and G), and two types of pyrimidines, viz. cytosine and thymine (commonly abbreviated as C and T). Accordingly, the deoxyribonucleosides containing purines (A and G) are called deoxy-adenosine and deoxyguanosine, respectively while the deoxyribo-nucleosides containing pyrimidines (C and T) are called deoxycytidine and deoxy-thymidine, respectively. Moreover, the deoxyribonu cleotides containing purines (A and G) are called deoxyadenosine monophosphate (dAMP) or deoxyadenylic acid and deoxyguanosine monophosphate (dGMP) or deoxyguanylic acid, respectively while the deoxyribonucleotides containing pyrimidines (C and T) are called deoxycytidine monophosphate (dCMP) or deoxycytidylic acid and deoxythymidine monophosphate (dTMP) or deoxyt hymidylic acid, respectively
4. The deoxyribonucleotides of each polynucleotide strand are connected to each other by covalent 3',5'-phosphodiester bond in between the 3'-OH of the deoxyribose of one deoxyribonucleotide and the 5'-OH of the deoxyribose of the next deoxyribonucleotide. Consequently, the two poly-nucleotide strands lie anti-parallel to each other: in one strand, the 3'-OH of the terminal deoxyribonucleotide remains free and at the same end, the other polynucleotide strand has a free 5'-OH of the terminal deoxyribonucleotide.
5. It can be added here that a deoxyribose sugar remains covalently linked by a glycosidic bond with a nitrogenous base within each nucleoside. In case of a purine nucleoside, the glycosidic bond runs in between the N^9 of a purine base (A or G) and the anomeric OH group on C^1 of deoxyribose. In case of a pyrimidine nucleoside, the glycosidic bond runs in between the N^1 of a pyrimidine base (C or T) and the anomeric OH group on C^1 of deoxyribose.
6. Further, the two polynucleotide strands are not free from each other, but instead, remain connected with each other by means of hydrogen bonds that run in between the nitrogenous bases of the opposite polynucleotide strands. The base pairing occurs in a complimentary and very specific manner: two

hydrogen bonds lie in between A and T of the opposite strands and three hydrogen bonds lie in between G and C of the opposite strands. Consequently, the sum of A and G residues in a double-helical DNA molecule equals the sum of T and C residues, or (A + G) : (T + C) = 1 : 1. This is called as Chargaff's rule.



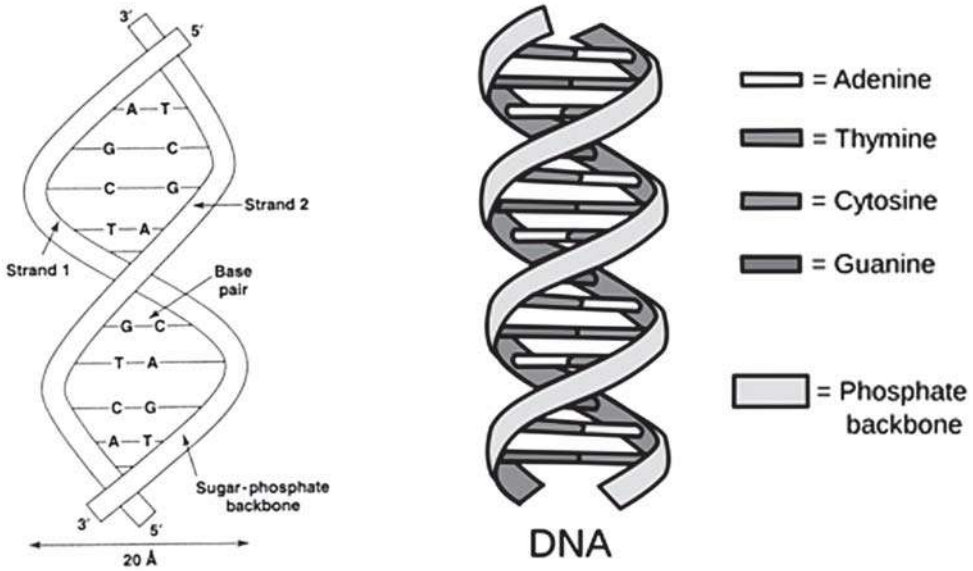
Triple hydrogen bonding between complementary nucleotides Guanine and Cytosine [Source: Wikimedia Commons]

1.5.3 Types of DNA :

In course of time, different forms of DNA have been discovered. The most commonly described forms are B-DNA, Z-DNA and A-DNA. Their characteristics are as follows:

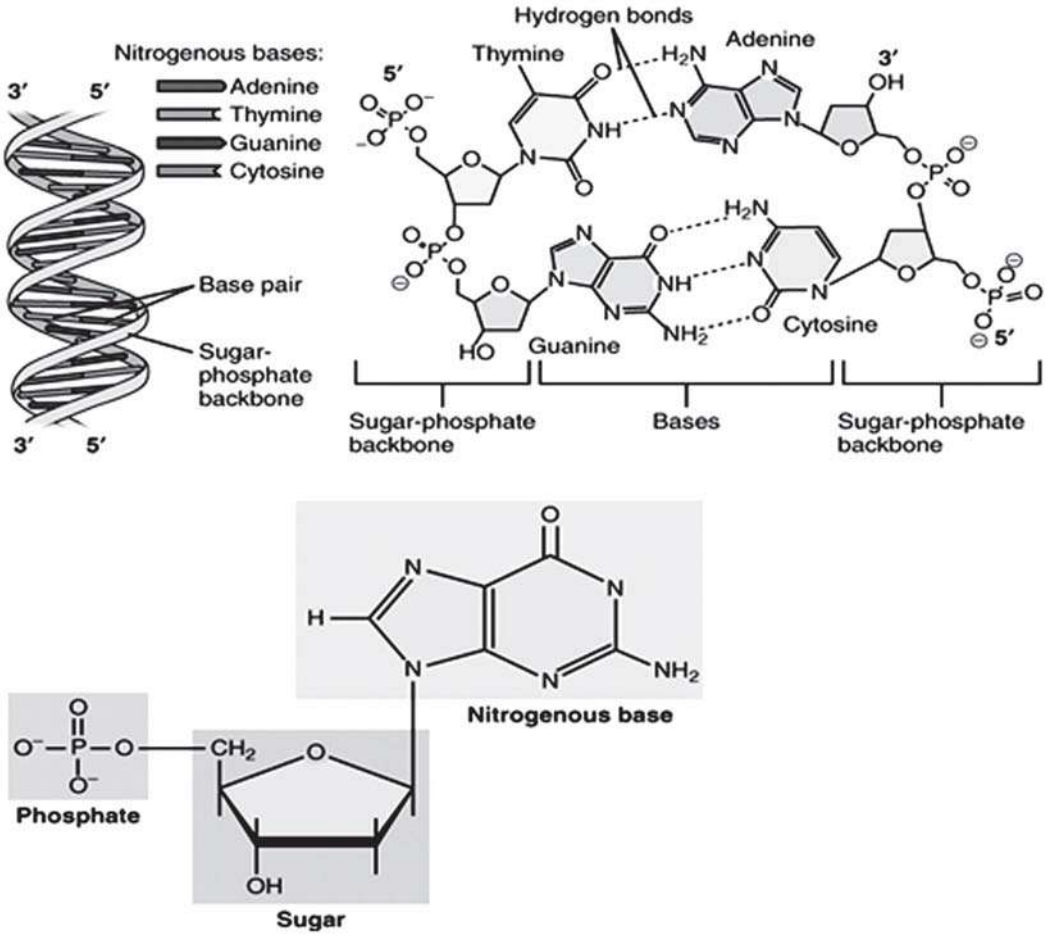
Feature	B-DNA	Z-DNA	A-DNA
Occurrence	B-DNA was vividly described by James Watson and Francis Crick in 1953. It is the prevalent type of DNA found in all living cells.	Z-DNA was discovered by Andrew Wang and Alexander Rich in 1979. It is found in all living cells, but in a much less quantity, as compared to B-DNA.	A-DNA was discovered by Rosalind Franklin in 1953. It is derived from B-DNA under dehydrating conditions. In nature, it is found in bacterial spores.
Helix nature	The two strands of B-DNA form right-handed helix around each other.	The two strands of Z-DNA form left-handed helix around each other.	The two strands of A-DNA form right-handed helix around each other.

Diameter	Diameter of DNA double helix is 20 Angstrom units.	Diameter of DNA double helix is 18 Angstrom units.	Diameter of DNA double helix is 26 Angstrom units.
Pitch	The length of each turn of DNA double-helix is 34 Angstrom units.	The length of each turn of DNA double-helix is 45 Angstrom units.	The length of each turn of DNA double-helix is 28.6 Angstrom units.
Base-pairs per turn	10	12	11
Length of nucleotide	3.4 Angstrom units.	3.75 Angstrom units.	2.6 Angstrom units.



Sketch diagram of B-DNA double-helix **Simple DNA double-helix structure**

(Source: Wikimedia Commons)



Detailed Structure of DNA with its structural components

[Source : Wikimedia Commons (CC 4.0)]

1.5.4 Function of DNA :

Functionally, DNA carries the genetic information (= information for carrying out protein synthesis) of a cell. The genetic information is engraved within the base sequence in the polynucleotide strands of DNA. The genetic information passes from DNA to messenger RNA (mRNA) when the latter is synthesized from the DNA strands by complementary base-pairing. Thereafter, mRNA participates in the synthesis of a particular type of protein.

Moreover, DNA serves as the hereditary material of cells, i.e. DNA transmits genetic information from one generation of a cell to the next. In fact, DNA is a self-duplicating molecule or a DNA produces two identical copies of itself before cell

division. During duplication of DNA, the two strands of a DNA separate from each other and along each of the two separated strands a new strand is synthesized by complementary base-pairing. In this way, from one parental DNA molecule are produced two daughter DNA molecules which are structurally identical to each other as well as with the parental DNA molecule. In other words, identical genetic information is transmitted from one parental DNA molecules to the two daughter DNA molecules.

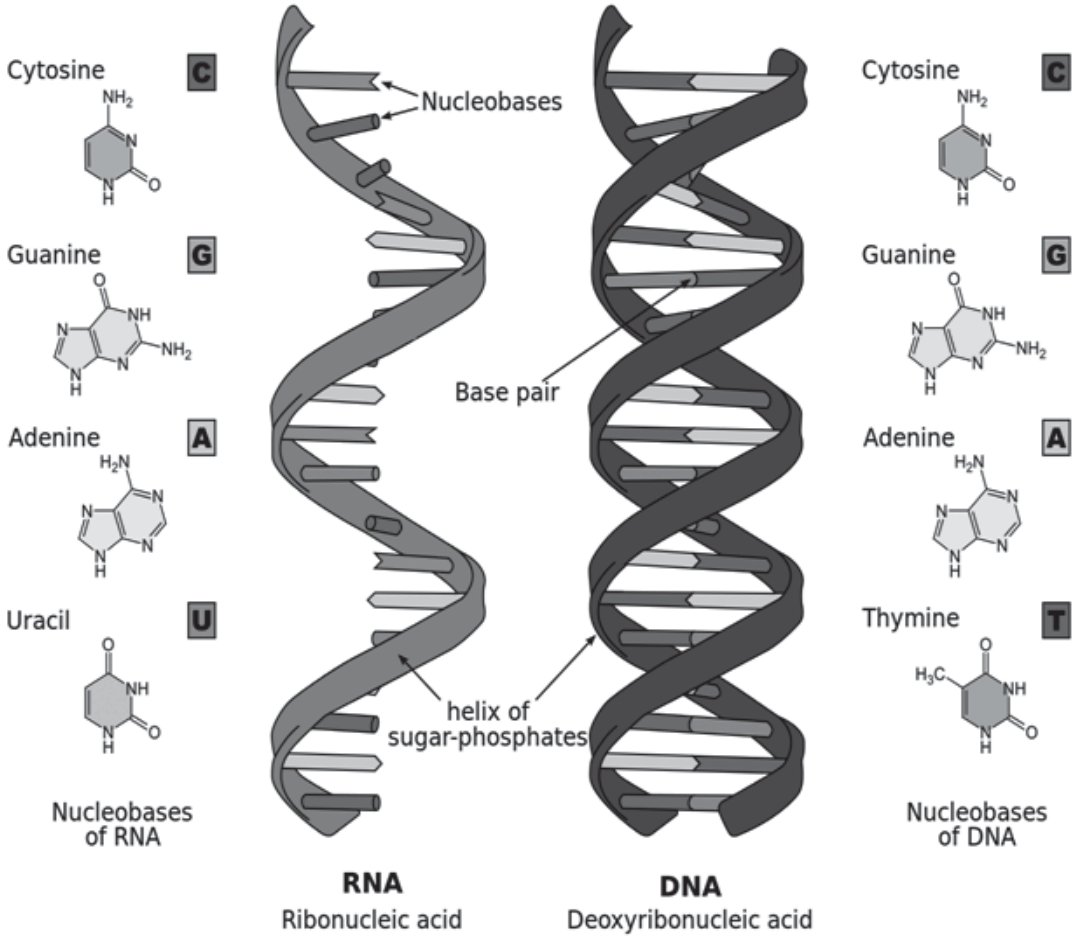
1.5.5 RNA – structure and function:

The second type of nucleic acids includes the RNAs or ribonucleic acids which occur in both nucleus and cytoplasm of all eukaryotic cells. In nucleus, RNA molecules remain associated with DNA of chromosomes and also, in the nucleolus. In prokaryotes, RNAs are associated with DNA of chromosome and also, in the cytoplasm. Moreover, RNA occurs in the genetic material of RNA viruses.

While DNA is double-stranded RNAs are made up of single polynucleotide strands. In DNA, the pentose sugar is deoxyribose but that in RNA is ribose. Besides, the pyrimidine bases in DNA are cytosine and thymine while those in RNA are cytosine and uracil. Purines are similar in both DNA and RNA. However, it may be added here that certain parts of two types of RNA molecules (viz. tRNA and rRNA) are double-stranded due to complementary base pairing in between A and U in one hand and G and C on the other.

RNAs are divisible into three main types: ribosomal RNA or rRNA, messenger RNA or mRNA and transfer RNA or tRNA. About 60-80% of cellular RNAs are rRNA. These RNA molecules remain associated with proteins, forming small cytoplasmic particles called ribosomes which act as the site of protein synthesis inside cells. About 10-20% of cellular RNAs are tRNA. These RNA molecules bind amino acids and carry them to the site of protein synthesis (ribosomes) during protein synthesis. Another 10-20% of cellular RNAs include the mRNA. These RNA molecules accept the message necessary for the cellular synthesis of different protein from DNA and then bind to the ribosomes so that the tRNA molecules can bring the appropriate amino acids for protein synthesis to the ribosomes.

Comparison between general structure of DNA and RNA



[(Source: Wikimedia Commons (CC 4.0)]

1.6 Questions (with hints to answers)

1. Classify monosaccharides (see Section 1.2.2).
2. What are polysaccharides? Classify polysaccharides (see Section 1.2.2).
3. Distinguish between a triose and a trisaccharide (see Section 1.2.3.2).
4. Distiguish between starch and glycogen (see Section 1.2.3.4).
5. Give an account of D- and L-isomerism in sugars (see Section 1.2.4.1).
6. Give an account of optical isomerism in sugars (see Section 1.2.4.2).
7. Explain the phenomenon of mutarotation shown by glucose (see Section 1.2.5).

8. What are reducing sugars and reduction tests? Lactose and maltose are reducing sugars but sucrose is not – explain (see Section 1.2.3.4).
9. What do you mean by osazone test? (see Section 1.2.6.3.).
10. State the physiological importance of carbohydrates (see Section 1.2.7).
11. Classify simple proteins (see Section 1.3.2).
12. Classify conjugated proteins (see Section 1.3.2).
13. Describe á-helix and triple helix types of protein structure (see Section 1.3.3.3).
14. What do you mean by â-pleated sheet of protein structure? (see Section 1.3.3.3).
15. What is biuet test? (see Section 1.3.4.1).
16. What is isoelectric pH of proteins? How does change of pH influence the ionic status of proteins? (see Section 1.3.4.2).
17. What do you mean by salting in and salting out of proteins? (see Sections 1..3.4.3 and 1.3.4.4).
18. Classify phosphoglycerides (see Section 1.4.2).
19. Write notes on lecithin and cephalin (see Section 1.4.2).
20. Define and classify fatty acids (see Section 1.4.2).
21. Write notes on saponification number and Reichert-Meissl number of fat(see Section 1.4.3).
22. Distinguish between fat and oil. Add a note on hydrogenation of fat (see Section 1.4.3).
23. State the physiological importance of lipids (see Section 1.4.4)
24. Describe Watson and Crick's model of DNA structure(see Section 1.5.2).
25. Tabulate the salient features of B-DNA, Z-DNA and A-DNA (see Section 1.5.3).
26. Write a note on structure and function of RNA(see Section 1.5.5).

1.7 References

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Unit-2 □ Bioenergetics

Structure

2.0 Objectives

Upon going through this Unit II, one will be able to :

- Understand the meaning of thermodynamics.
- Understand the features of different types of systems.
- Understand the different laws of thermodynamics.
- Explain the meaning of enthalpy and enthalpy change.
- Understand the meaning and significance of entropy.
- Understand the role of high-energy compounds in living organisms.

2.1 Introduction

Thermodynamics is the science that deals with (i) conservation of energy, (ii) convertibility of different forms of energy into heat energy and (iii) the relationship between energy content and properties of molecular systems.

In thermodynamics, the term‘system’ is used to denote an assemblage of a vast number of one or more kinds of molecules and a specific amount of internal energy, separated from its surroundings by either a real and well-defined boundary or a reasonable imaginary boundary.

The internal energy of any system is the sum of the kinetic energy of all its molecules. The internal energy changes with a change of the thermodynamic state of a system. Thermodynamic state of a system is a collective term that refers to the chemical constituents, pressure, volume and temperature of the system.

Systems can be classified into 3 types :

(a) Isolated system: An isolated system is such a system that is separated from its surrounding by a well-defined adiabatic (insulating) boundary across which neither matter nor energy can pass between the system and its surrounding.

(b) Closed system: A closed system is such a system that is separated from its surrounding by a diathermal boundary across which only energy (e.g. heat or light) but no matter can pass between the system and its surrounding.

(c) Open system: An open system is such a system that is separated from its surrounding by a real or imaginary boundary which allows exchange of both matter and energy between the system and its surrounding.

Living beings represent an 'open, steady-state, non-equilibrium system'.A living being is an 'open system', because, exchange of matter (e.g. intake of food, water and O_2; discharge of faeces, urine and CO_2) and exchange of energy (e.g. gain and loss of heat) can occur between its body and its surroundings.A living being is an 'open, steady-state system', because it tries to maintain a balance between (i) matter and energy received from the surroundings and (ii) matter and energy lost to the surrounding. A living being is, however, a non-equilibrium system, because it tends to maintain an organized state or low-entropy state (less disorderliness) by acquiring as much energy as possible from the surrounding.

In a living body, reversible chemical reactions seldom attain a stable equilibrium. In a living body, reversible chemical reactions seldom attain a stable equilibrium, because (i) the products are continuously removed by other reactions *in vivo, w*ith a constant fall in the rate of a backward reaction, (ii) the reactants are continuously added to maintain a steady forward reaction and (iii) reactions seldom occur under standard conditions inside the body of a living being.

A spaceship together with an astronaut is an 'Isolated system', but the astronaut himself is not. A spaceship with an astronaut inside itself can be considered as an 'isolated system', because the spaceship is well-insulated against exchange of both matter and energy between the spaceship and the surrounding space (outside the spaceship). On the other hand, the astronaut himself should be considered as an 'open system', because considerable exchange of matter as well as energy can occur between his body and the space inside the spaceship.

2.2 First law of thermodynamics

2.2.1 Basic postulate of the law :

The change in internal energy (DE) of a system equals the algebraic sum of (i) heat gained by the system from its surrounding and (ii) heat lost from the system to its surrounding and heat used by the system in doing a work. Thus, the law is virtually the law of conservation of energy – the total energy of a system and its surrounding remains unchanged.

2.2.2 Mathematical presentation of the law :

The first law of thermodynamics can be represented by the equation : $\Delta E = \Delta Q - \Delta W$

(ΔE = change in internal energy ; ΔQ = heat absorbed by a system or released from the system; ΔW = work done by a system or work done on a system). ΔW is positive when a work is done by the system (e.g. expansion in volume) and ΔW is negative,

when a work is done on the system (e.g. contraction in volume). ΔQ is positive when heat is absorbed by a system and it is negative when heat is released by the system. A positive value of ΔE indicates a rise in internal energy and a negative value of ΔE indicates a fall in internal energy of a system.

2.2.3 Enthalpy :

It is the heat content (H) of a system and it amounts to the sum of its internal energy

(E) and the product of its pressure and volume (P and V, respectively) : $H = E + PV$

Any physical or chemical change of a system causes change of its enthalpy. Therefore, the enthalpy change (ΔH) calories or Joules per mole) is the change in the amount of energy of the system in the form of heat absorbed or heat released during a physical or chemical change of the system.

2.2.4 Enthalpy change (ΔH) :

Enthalpy change may be expressed by the following equation:

$\Delta H = \Delta E + \Delta PV = \Delta E + \Delta W$ [because, PV= work done by a system (e.g. expansion in volume) or work done on a system (e.g. contraction in volume) during a physical or chemical change of the system)].

Now, the equation may be rewritten as $\Delta H = \Delta Q$ (total amount of heat absorbed by a system or released from the system during a physical or chemical change), because the first law of thermodynamics tells us the DE = DQ – DW. Moreover, the value of DQ is positive, when heat is absorbed by the system and negative, when heat is released by the system and DW is positive, when a work is done by the system and DW is negative, when a work is done on the system.

2.2.5 Citation of a situation when ΔH = ΔE (or ΔW = 0) :

We know, $\Delta H = \Delta E + \Delta W$. When ΔW or work done by a system or done on a system is '0', we can say that $\Delta H = \Delta E$ in such a situation, e.g. Metabolic reactions take place in a living body at an almost constant pressure and in aqueous solutions. These reactions involve negligible changes in P (Pressure) and V (Volume), or $\Delta PV = 0 = W$ for such reactions. In other words, $\Delta H = \Delta E + 0 = \Delta E$ (apparent heat absorbed or released) for metabolic reactions occurring *in vivo*.

2.2.6. Example of numerical problem :

The latent heat of evaporation of water is 536 cal/g. Calculate DH in converting 1 mole of water at 100°C into steam at the same temperature, assuming water to behave as an ideal gas :

ΔH or heat absorbed by 1 mole (18 g) of water = 536 × 18 cal/mole = 9648 cal/mole.

Now, the work done in converting 1 mole of water at 100°C into steam (gas) at the same temperature = PV = RT = 2 × 373 = 746 cal. [because, R or molar gas constant = 2 and T or absolute temperature = (100+ 273) Kelvin; PV = RT (ideal gas equation)].

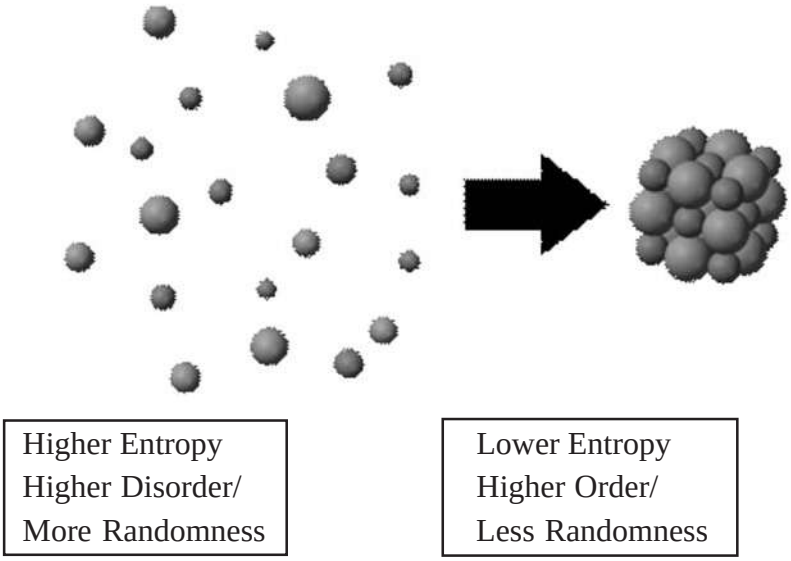
Now, ΔH = ΔE + ΔPV, or 9648 cal = ΔE + 746 cal.

∴ ΔE = 9648 – 746 = 8902 cal.

2.3 Second law of thermodynamics

2.3.1 Second law and its explanation :

The second law of thermodynamics states that each system, given the freedom, always changes spontaneously to increase the randomness or disorder in it; the magnitude of this disorder is called as'entropy'. In other words, the energy of a closed system has always a persistent tendency to get progressively diluted or randomized, thus tending constantly to enhance its entropy**[entropy is that proportion of the total energy of a system which is unavailable for work].**



Conceptual representation of Entropy and System Order
[Source: Wikimedia Commons]

It appears from the second law of thermodynamics that if orderliness is created in any part of a system, a greater or equivalent degree of disorder must simultaneously

appear in another part of that system or in its surrounding so that the overall entropy either rises or at least remains undiminished. Further, the second law asserts that a process can take place spontaneously, only if the total entropy of a system and its surrounding increases during that process.

As a system spontaneously attains equilibrium, its entropy reaches a maximum level for its existing state so that the system can change no further unless some energy (heat) is supplied to it from its surroundings. However, even when energy is supplied to a system, the total amount of the supplied energy fails to be changed to an equivalent amount of work.A part of the supplied energy remains unavailable to work and adds up to entropy.

The second law, therefore, envisages the tendency for an increase in entropy as the driving force for all physicochemical changes in an isolated system. In essence, the following equation may be held to be true for a system at equilibrium at the absolute temperature T :

2.3.2 Entropy :

Entropy (S) refers to the magnitude of randomness or disorder in a system and is expressed in either joules or calories per degree Kelvin per mole. Entropy increases with the increase in randomness or disorderliness in a system. Therefore, entropy can be held to represent that part of the total energy of the system which cannot be transformed into work and is instead, spent in creating disorder in the system.

Entropy is a function of the state of a system, viz., its temperature, chemical constitution, pressure and volume. Whenever the state of a system gets altered, it suffers from an entropy change (ΔS).

Entropy does not change instantly, but tends to increase progressively. So, a system left to itself changes spontaneously towards rising entropy, until its entropy reaches a maximum level or equilibrium for the existing state. After attaining equilibrium with maximum disorder, the system then stays as such, unless it absorbs some further energy from its surrounding.

The stability of a system depends on its entropy.The lower its entropy, the greater is its tendency to change towards the maximum entropy and so. The higher is its tendency for spontaneous changes.

It follows from the second law of thermodynamics that addition of energy or heat (DQ) raises the entropy of a system because a part of the supplied energy must go to enhance the disorder instead of being available for work. At equilibrium,

$$\Delta Q = T\Delta S; \text{ or, } \Delta S = \frac{\Delta Q}{T}$$

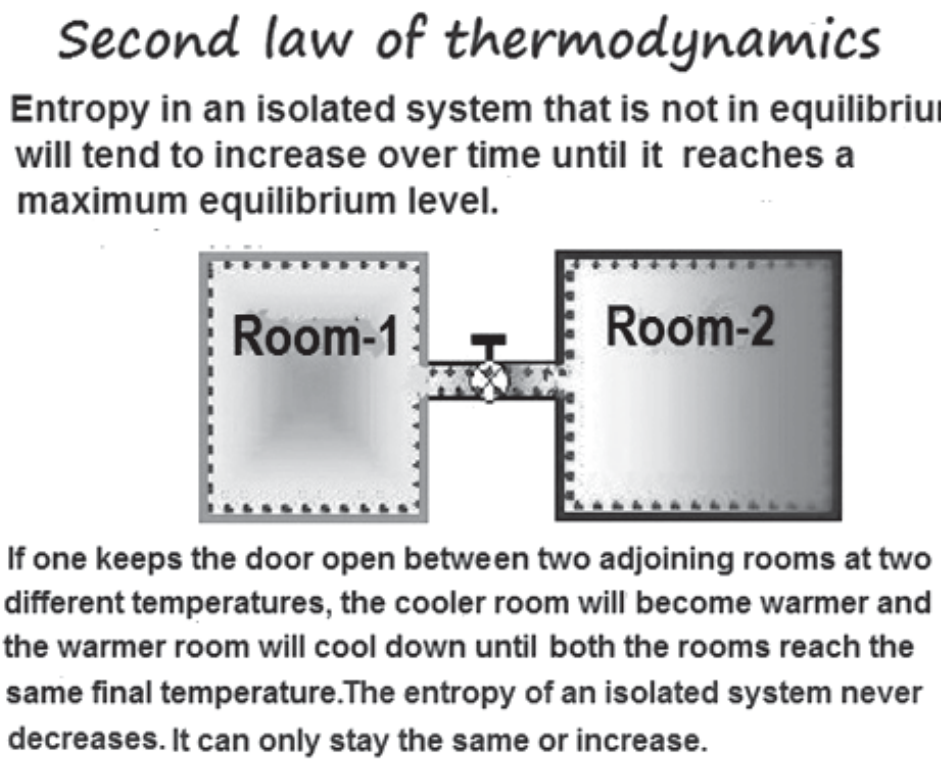
But so long as the system has not attained equilibrium and is undergoing a thermodynamically irreversible process, the following condition prevails:

$$T\Delta S > \Delta Q$$

Again, as ΔQ equals the enthalpy change (ΔH) of the system, it can be rewritten that :

$$T\Delta S > \Delta Q, \text{ or, } T\Delta S > \Delta H; \text{ or, } \Delta H = T\Delta S - \Delta W$$

Ordinarily, a reaction proceeds spontaneously towards the goal of maximum entropy. Only a supply of energy from the surroundings may drive the reaction in the opposite direction against entropy.Entropy is involved in the maintenance of the physiological steady state in biological organisms.



Conceptual representation of the 2nd Law of Thermodynamics

2.4 Third law of thermodynamics

The third law of thermodynamics holds that the entropy of perfect crystals of a compound rises with the increase of temperature from a minimum or zero value at Kelvin scale, i.e. –273°C [–273°C= –273° + 273 = 0° Kelvin].

2.5 Relevance of law of thermodynamics to biological systems

The laws of thermodynamics have considerable relevance to biological systems. From the first law of thermodynamics, we know, DH = DE+DW. When DW or work done by a system or done on a system is '0', we can say that DH = DE in such a situation.This situation is quite evident during metabolic reactions taking place in living organisms. Metabolic reactions take place in a living body at an almost constant pressure and in aqueous solutions. These reactions involve negligible changes in P (Pressure) and V (Volume), or $\Delta PV = 0 = \Delta W$ for such reactions. In other words, $\Delta H = \Delta E+0 = DE$ (apparent heat absorbed or released) for metabolic reactions occurring *in vivo*.

Again, the oxidation of one mole (gram molecule) of glucose yields 686 kcal of energy but the oxidation of one mole of lactic acid yields 326 kcal of energy. Now, the enthalpy as well as oxidation pathways of glucose and lactic acid are different. Therefore, the difference of enthalpy between the two (glucose and lactic acid) when two molecules of lactic acid are produced from one molecule of glucose during glycolysis may be expressed as follows :

$\Delta H_{glycolysis} = (\Delta H_{glucose} - \Delta H_{lactic\ acid}) = (686 - 2 \times 326)$ kcal = 686 – 652 kcal = 34 kcal. In fact, the oxidation of one mole of glucose practically yields only 34 kcal of energy which is utilized for production of high-energy ATP molecules from ADP and P_i in cells. In this way, as per the first law of thermodynamics, it is found that $\Delta H = \Delta E$ during glycolysis in cells.

Again, the second law of thermodynamics indicates that a system tends to progressively increase its entropy or state of disorderliness. But, it is practically found that the cells or tissues in living organisms tend to maintain a steady though non-equilibrium state. How this contradictory situation is made possible? It becomes possible as because the living organisms tend to maintain a balance between (i) matter and energy received from the surroundings and (ii) matter and energy lost to the surrounding.

2.6 High-energy bonds and high-energy compounds

2.6.1 Definition of high-energy compounds:

High-energy compounds aresuch compounds that possess one or more high-energy covalent bonds that yield 7 kcal of energy during hydrolysis of each mole of the compound.On the other hand, low-energy compounds possess only ordinary

covalent bonds; an ordinary covalent bond yields 3.4 kcal of energy during hydrolysis of each mole of a low-energy compound.

2.6.2 Examples of high-energy compounds:

High-energy compounds having high-energy bonds include:

(i) Phosphoric anhydrides like some purine/pyrimidine nucleotides (e.g. ATP or Adenosine triphosphate, ADP or Adenosine diphosphate, GTP or Guanosine triphosphate, GDP or Guanosine diphosphate, CTP or Cytidine triphosphate and UTP or Uridine triphosphate).The tri- and di-phosphates of purine and pyrumidine nucleosides carry two and one high-energy phosphate bonds, respectively in their molecules. The 3rdor g and the 2nd orbphosphate bonds of a nucleoside triphosphate are high-energy bonds while the 2nd orbphosphate bondof a nucleoside diphosphate is high-energy bonds. In both of these tri- and diphosphates, the 1st or aphosphate bond connected directly to the ribose sugar is only a low-energy phosphate bond. Again, adenosine monophosphate (AMP) has a single low-energy aphosphate bond ($\Delta G^{o'}$ –3.4 kcal). Similarly, the lone a phosphate bond of GMP, CMP and UMP molecules is a low-energy phosphate bond.

(ii) Enol phosphates like phosphoenolpyruvate.

(iii) Acyl phosphates like 1, 3-bisphosphogiycerate.

(iv) Phosphoguanidines like creatine phosphate.

(v) Thiol esters like succinyl-CoA.

(vi) Sulfonium compounds like S-adenosylmethionine.

(vii) Cyclized nucleotides like cyclic AMP. The high negative $\Delta G^{o'}$ values of those compounds result largely from:

2.6.3 Energy transfer (utilization) from high-energy compounds in cells :

(a) ATP or Adenosine triphosphate: The high-energy bonds of ATP are hydrolyzed to provide energy for driving a large number of cellular processes. **Hydrolysis of 1 mole of ATP into ADP releases 30 kilo joules or 7 kilo caloriesof energy.** ATP is, therefore, called as the ‘energy currency’ of living cells. A few roles of ATP are cited below:

(i) Active transport of Na^+ and K^+:The enzyme Na^+-K^+-ATPase of plasma membrane catalyzes the hydrolysis of one ATP to ADP and Pi in one hand, and the carrier-mediated active transports of three Na^+ ions outwardand two K^+ ions inward across the plasma membrane against their respective con centration gradients. This role of ATP is essential for maintaining the

polarized state of the membrane, higher extracellular concentration of Na^+ and higher intracellular concentration of K^+.

(ii) Phosphorylation of substrates: In many metabolic pathways, substrates or intermediates are phosphorylated into low-energy phosphate compounds such as glucose 6-phosphate, fructose 1,6-bisphosphate and glycerol 3-phosphate by respective phosphotransferase enzymes utilizing the high-energy phosphate bond of ATP.

(iii) Cellular protein synthesis:The energy released from cleavage of ATP is utilized by amino acyl-tRNA synthetase enzyme for the formation of amino acyl-tRNA, which represents the first step of biosynthesis of proteins in cells.

(iv) Other physiological functions:The cleavage ofATP also provides the energy necessary for contraction of muscles, movement of cilia and flagella, exocytosis and endocytosis.

(b) Cyclic AMP or adenosine 3', 5'-monophosphate: It is a high-energy phosphate formed by cyclization of ATP by adenylate cyclase.It mediates signal transduction across the cell membrane as a second messenger for the actions of many signaling molecules like peptide hormones and adrenaline.

(c) High-energy ribonucleotides and deoxyribonucleotides: The high-energy g-phosphate bond of ATP, GTP, CTP and UTP are cleaved during RNA synthesis and the energy released there from is utilized in forming phosphodiester bonds between successive nucleotides of RNA. Similarly, the high-energy g-phosphate bond of dATP, dGTP, dCTP and dTTP are cleaved during DNA synthesis and the energy released there from is utilized in forming phosphodiester bonds between successive nucleotides of DNA.

(d) Phosphagens: In vertebrate muscles, the enzyme creatine phosphokinase (CPK) cleaves the high-energy phosphate bond of creatine phosphate and utilizes the energy and inorganic phosphate released there from to phosphorylate ADP to ATP. In invertebrate muscles, arginine phosphokinase similarly causes cleavage of high energy phosphate bond of arginine phosphate and utilizes the energy and inorganic phosphate released there from to phosphorylate ADP to ATP.

2.6.4 Energy capture (storage) in high-energy compounds in cells :

The most important high-energy compound of all living cells is ATP or adenosine triphosphate. ATP is produced during aerobic oxidation of glucose and fatty acids inside the mitochondria of cells. During aerobic oxidation, NADH and $FADH_2$ are produced in cells. NADH and $FADH_2$ enter into the mitochondria

wherein electrons from these substances are transported through the 'electron transport chain' (ETC). The transported electrons are combined with H^+ ions (protons) and molecular oxygen to produce water while the energy liberated from the transport of electrons is used to produce ATP from ADP and P_i (inorganic phosphate) by the help of ATP synthetase. The overall process of ATP production is called 'oxidative phosphorylation', because the substrates (viz. NADH and $FADH_2$) give out electrons for transport through the ETC and thus, get oxidized while ADP gets phosphorylated or added with phosphate for formation of ATP.

Again, cAMP or cyclic AMP which is another important high-energy phosphate in living cells is formed by cyclization of ATP by adenylate cyclase. On the other hand, the high-energy phosphagen of vertebrate muscles, known as CP or creatine phosphate is produced from creatine by the catalytic action of creatine phosphokinase in presence of ATP which breaks into ADP and P_i. The high-energy phosphagen of invertebrate muscles, known as AP or arginine phosphate is produced from arginine by the catalytic action of arginine phosphokinase in presence of ATP which breaks into ADP and P_i.

2.7 Questions (with hints to answers)

1. What are systems? Define 'isolated', 'closed'and 'open' systems? [see Section 2.1].
2. Living beings represent an 'open, steady-state, non-equilibrium system' – explain [see Section 2.1].
3. In a living body, reversible chemical reactions seldom attain a stable equilibrium – explain [see Section 2.1].
4. A spaceship together with an astronaut is an 'Isolated system', but the astronaut himself is not – explain [see Section 2.1].
5. State first law of thermodynamics and give a mathematical presentation of the law [see Sections 2.2.1 and 2.2.2].
6. What do you mean by enthalpy and enthalpy change (DH)? [see Sections 2.2.3 and 2.2.4].
7. Cite of a situation when DH becomes equal toDE[see Section 2.2.5].

   2.2.6. The latent heat of evaporation of water is 536 cal/g. Calculate DH in converting 1 mole of water at 100^{o}C into steam at the same temperature, assuming water to behave as an ideal gas [see Section 2.2.6].
8. State and explain the second law of thermodynamics [see Section 2.3.1].
9. What is entropy? State its significance [see Section 2.3.2].

10. Explain the relevance of laws of thermodynamics to biological systems [see Section 2.5].
11. What are high-energy compounds? Give at least 3 examples. Explain the role of ATP in living organisms. [see Sections 2.6.1, 2.6.2 and 2.6.3].
12. What is oxidative phosphorylation? [see Section 2.6.4].

2.8 References

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Unit-3 □ Enzymes

Structure

3.0 Objectives :

Upon going through this Unit-II, students will be able to:

- Define enzymes and distinguish between enzymes and inorganic catalysts.
- Classify enzymes.
- Define code number, turnover number and international number of enzymes.
- Distinguish between coenzymes and cofactors.
- Learn about the 'lock-and-key' and 'induced-fit' models of enzyme-substrate complex formation.

- Gain knowledge on isozymes, ribozymes and abzymes.
- Understand the transition-state theory of enzyme action.
- Understand the meaning of 'enzyme kinetics'and derive of Michaelis-Menten equation.
- Develop thorough concept of K_m and V_{max}.
- Understand the meaning and significance of Lineweaver-Burk plot.
- Understand the mechanisms of multisubstrate reactions.
- Gain thorough knowledge on the factors affecting rate of enzyme-catalyzed reactions.
- Understand the details of competitive, non-competitive and uncompetitive inhibitions of enzymes.
- Develop thorough concept of allosteric enzymes and their kinetics.
- Differentiate between 'K' and 'M' enzymes.
- Learn about reversible and irreversible covalent regulations of enzyme action.

3.1 Introduction

Enzymes are colloidal, thermo-labile, biological catalysts, generally protein in nature and synthesized in living cells. Enzymes catalyze chemical changes of specific substances or substrates either inside or outside cells within the body of living organisms.

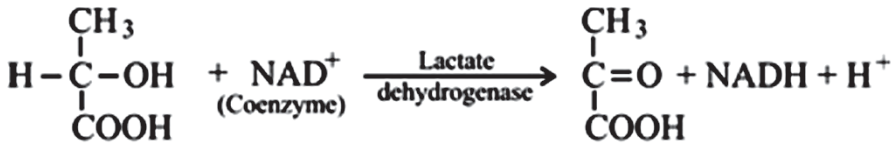
Both inorganic catalysts and enzymes bind to their substrate molecules to form transient binary complexes and both remain unchanged after their catalytic action and both catalyze changes of only covalent bonds in their substrates. However, enzymes are macromolecular entities while inorganic catalysts have small molecular size. Again, an inorganic catalyst can bind to several substrates but an enzyme has a specific three-dimensional substrate-binding site which can bind to a single or a very few specific substrates. Besides, enzymes do not exist outside the body of living beings and their production is a gene-regulated phenomenon. On the other hand, inorganic catalysts are available outside the body of living beings and their production is not any cellular phenomenon.

3.2 Nomenclature and classification

3.2.1 Different classes of enzymes:

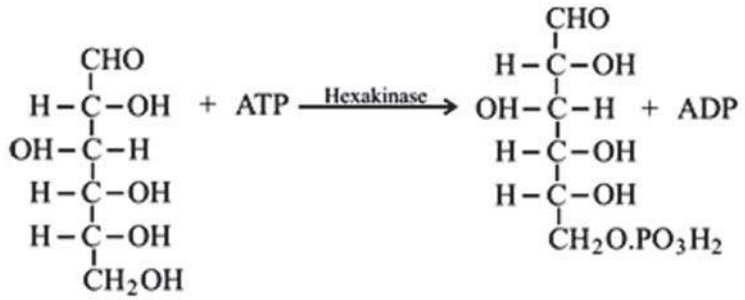
Enzymes are broadly classified into 6 classes. The classification as well as the nomenclature of each class is done according to the types of reaction catalyzed by the enzymes :

Class 1. Oxidoreductases : Enzymes of this class catalyze oxidation or reduction of their substrates. Oxidation is carried out by the removal of electron (often along with H^+) from a specific group or by addition of oxygen to specific group. Reduction is just the opposite change, e.g. Lactate dehydrogenase remove two electrons (along with two H^+) from CH(OH) group of lactic acid and converts it into pyruvic acid :



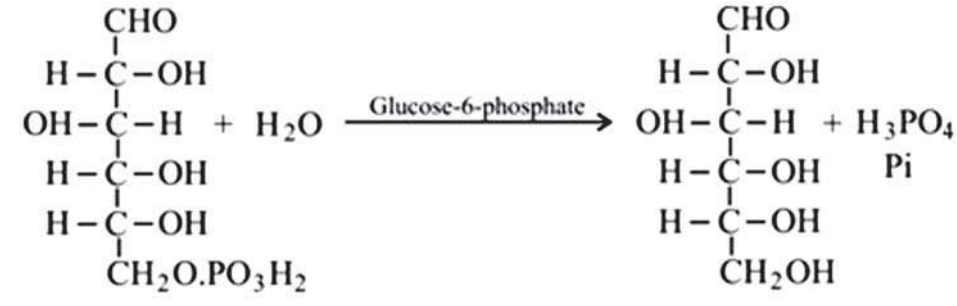
Lactic acid Pyruvic acid

Class 2. Transferses: Enzymes of this class catalyze the transfer of a particular group from one substrate to another, e.g. Hexokinase transfers a phosphate from ATP to glucose, resulting in production of glucose-6-phosphate and ADP:



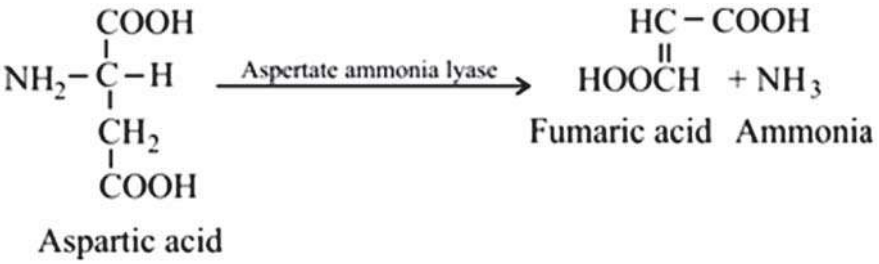
Glucose Glucose-6-phosphate

Class 3. Hydrolases: They catalyze hydrolysis of their respective substrates,e.g. Acetylcholinesterase hydrolyses acetylcholine; Glucose-6-phosphatase hydrolyses glucose-6-phosphate:

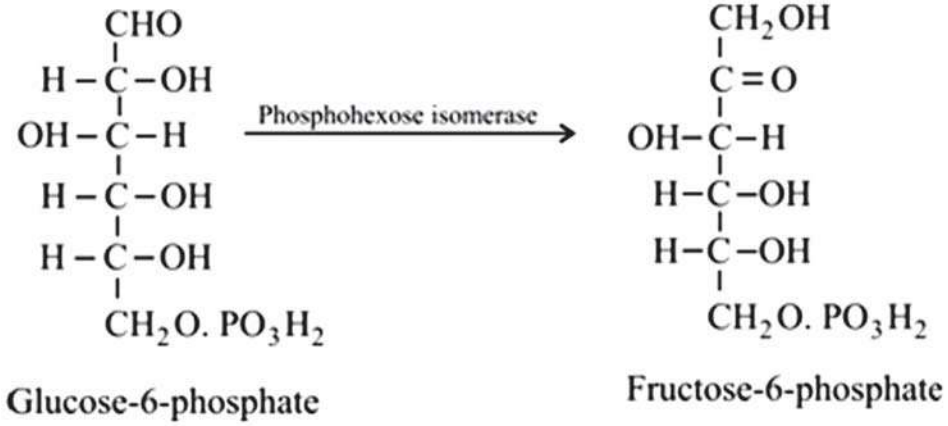


Glucose-6-phosphate Glucose

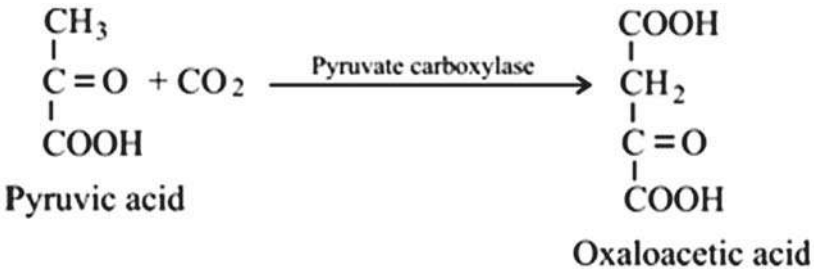
Class 4. Lyases: An enzyme of this class cleaves a covalent bond in the substrate non-hydrolytically and converts it to more than one product. Moreover, the enzyme action also produces a double bond in one of the products, e.g. Aspartate ammonia lyase cleaves a C-N bond in L-aspartic acid to produce fumaric acid along with ammonia; this is an example of non-oxidative deamination of amino acid :



Class 5. Isomerases: An enzyme of this class catalyzes the change of the substrate into another isomeric form by way of intra-molecular rearrangement, e.g. Phosphohexose isomers is an aldoseketose isomerase and isomerizes glucose-6-phosphate into fructose-5-phosphate:



Class 6. Ligases: These enzymes catalyze the formation of a covalent bond between two substrates, which thereby get joined together. Such reaction is endergonic; the necessary energy is derived from a simultaneous cleavage of ATP. Pyruvate carboxylase cause a C-C bond formation between pyruvic acid and CO_2, resulting in formation of oxaloacetic acid :



Pyruvic acid

Oxaloacetic acid

Remarks: However, each class of enzyme is subdivided into some subclasses and each subclass is again divided into sub-subclasses, depending on particulars of their catalytic action. For instance, Glucose-6-phosphatase of the class 'hydrolases' belongs to the subclass 'esterases', as it hydrolyses an ester bond, but pepsin of the same class belongs to another subclass called 'peptide hydrolases', as it hydrolyses a peptide bond.

3.2.2 Some relevant terms :

(a) Enzyme Code Number: Every enzyme has been given an EC No. or Enzyme Code Number in 4 digits by 'The 1972 Recommendations of the Commission on Enzyme Nomenclature'. The first digit indicates its class; the second digit indicates its subclass; the third digit indicates its sub-subclass and the fourth digit indicates its serial number in sub-subclass, e.g. The EC No. of the gastric enzyme pepsin is 3.4.4.1. So, it is an enzyme with a serial number 1 in the sub-classes 4 (Animal endopeptidases) under the subclass 4 (Peptide hydrolases) under the class 3 (Hydroclases).

(b) Enzyme Unit (E): The Commission on Enzymes of the International Union of Biochemists holds that a unit of an enzyme is that amount of the enzyme which catalyzes the transformation of one micromole of substrate into product per minute at 25^{o}C under optimal conditions.

(c) Turnover number: The turnover number of an enzyme refers to the number of substrate molecules transformed into product per minute by a single enzyme molecule under optimal conditions, e.g. Turnover number of b-amylase is as high as 1,100,000.

3.3 Coenzymes and cofactors

3.3.1 Coenzymes :

(i) In many enzymes, a dialyzable, thermolabile, **non-protein but organic compound** remains bound to the main protein part of the enzymes. The whole of such an enzyme molecule is called a holoenzyme; its protein part is

called an apoenzyme and **the non-protein but organic compound attached to the apoenzyme is called as a coenzyme.** A coenzyme helps in the enzyme catalyzed reaction.

(ii) Coenzymes are found in several oxidoreductases, transferases and isomerases, but not in all classes of enzymes.

(iii) When a coenzyme remains non-covalently linked with the apoenzyme, it gets detached from the latter after the enzyme-catalyzed reaction is over, e.g. NAD^+ (Nicotinamide adenine dinucleotide) is such a detachable coenzyme of different anaerobic dehydrogenases like lactate dehydrogenase and pyruvate dehydrogenase. When a coenzyme remains covalently linked with the apoenzyme, it does not dissociate from the latter after the enzyme-catalyzed reaction is over. Such coenzymes are called as prosthetic groups (Lehinger, 1975), e.g. PLP (pyridoxal phosphate) is a prosthetic group of transaminases and FAD (Flavin adenine dinucleotide) is the prosthetic group of D-amino acid oxidase).

(iv) Coenzymes including prosthetic groups are mostly derivatives of vitamins, for instance, NAD^+ is a derivative of pyridoxine or vitamin B_6; FAD is derivative of riboflavin or vitamin B_2. However, a few enzymes like peroxidase and catalase have heme, a metalloporphyrin as the prosthetic group.

(v) Coenzymes help in the action of enzymes in different ways:

(a) In general, the association between coenzyme and apoenzyme helps in the binding of the substrate to the active site of the enzyme, as the binding of the coenzyme provides the proper three-dimensional conformation to the active site of the enzyme.

(b) A coenzyme may accept a particular atom or functional group or electron removed from the substrate by the action of an enzyme. Thus, the coenzyme gets chemically changed but its original form is reproduced later by other cellular reactions, e.g. NAD^+ gets reduced to NADH while it acts as a coenzyme of some anaerobic dehydrogenases. NADH is later re-oxidized to NAD^+ by mitochondrial NADH dehydrogenase :

$$NAD \quad A.H_2 \xrightarrow{Anaerobic dehydrogenase} NADH \quad H \quad A$$

(c) A conenzyme may donate a particular group or atom to be added to the substrate by an enzyme, e.g. Tetrahydrobiopterin donates H_2 when tryptophan hydroxylase causes oxidation of tryptophan into 5-hydroxy-tryptophan and it itself changes into dihydrobiopterin. Dihydrobiopterin is later reduced back to tetrahydrobiopterin by dihydrobiopterin reductase.

(d) A coenzyme may form an intermediate complex with the substrate during an enzyme-catalysed reaction, e.g. PLP, the coenzyme of a transaminase form a Schiff base intermediate with a substrate amino acid. Later, the Schiff base undergoes various chemical changes with the conversion of the amino acid into its corresponding keto acid and reproduction of PLP.

3.3.2 Cofactors :

Sometimes, **a metal ion instead of non-protein organic compound** remains attached with the holoenzyme. **Such a metal ion is called a cofactor**. Enzymes to which the metal ions remain loosely bound are called metal-activated enzymes and enzymes to which the metal ions remain tightly bound are called metalloenzymes. Metal-activated enzymes contain alkali metals like Na^+ and K^+ or alkaline earth metals like Ca^{++} and Mg^{++}, but metalloenzymes contain transitional metals like Cu^{++}, Cu^+, Zn^{++} etc. Metal ions help in the action of enzymes in different ways:

(i) Metal ions may provide the proper three-dimensional conformation to the active site of an enzyme for binding to a substrate, e.g. pyruvate kinase requires Mg^{++} ions while it transfers the phosphate group from phosphoenolpyruvate to ADP for production of ATP.

(ii) A metal may help in substrate-binding by binding to the enzyme in one hand and to the substrate on the other, e.g. Enolase requires the help of Mg^{++} ions when it binds to 2-phosphoglycerate to produce phosphoenolpyruvate.

(iii) A metal ion may act as a donor or acceptor of electron to and from a substrate during an enzyme-catalyzed reaction, e.g. Cu^{++} ions of plasma ceruloplasmin (an enzyme) can oxidize Fe^{++} ions entering the plasma into Fe^{+++} ions.

3.4 Specificity of enzyme action

Enzymes bind to specific substrate or substrates only. Biochemists have proposed two different models for explaining the binding of an enzyme with its specific substrate:

3.4.1 Template model or lock-and-key model :

According to Emil Fischer (1894), the active site of an enzyme always remains in the proper conformation even in absence of its substrate. Thereby, the active site provides a pre-shaped permanent template which fits with the size, shape and groups of the substrate. The binding of the substrate with the active site is like a lock-and-key interaction. The conformation of the active site is not changed after the catalytic action of the enzyme is accomplished.

Merit :

(i) This model fits with the simple substrate-saturation kinetics (hyperbolic substrate-saturation curve)of enzymes.

(ii) This model can explain how certain enzymes can act on a particular stereoisomer, but not on another stereoisomer of the respective substrate.

(iii) The model can also explain the sequential binding of a coenzyme and a substrate to an enzyme.

Demerit : A major demerit of this model is that it cannot explain the change in enzyme activity in presence of allosteric modulators.

3.4.2 Induced-fit model :

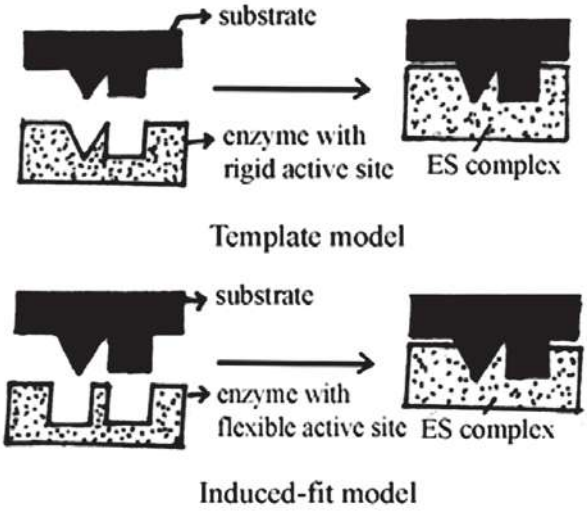
A second model upheld by D. E. Koshland (1958) states that the active site of an enzyme is not a rigid structure and instead, it has a flexible three-dimensional structure. The active site does not bear its fully and complementary conformation in absence of the substrate. Either on approach of the substrate or following an initial loose binding with the substrate, the flexible active site changes its conformation to attain its fully functional form, which binds the substrate more tightly and starts catalytic action. After the catalytic activity is over, the active site returns to its original flexible form.

Merits :

(i) The induced-fit model has received support from X-ray crystallographic studies on the conformation of native enzymes and ES complexes.

(ii) It can explain changes in enzyme activity in presence of an allosteric modulator which binds to an enzyme at a site other than the active site.

(iii) This model can explain complex substrate-saturation kinetics of allosteric enzymes in presence of allosteric modulators.



3.5 Isozymes, Ribozymes and Abzymes

3.5.1 Isozymes :

Some enzymes exist in more than one molecular form in the same species. In other words, these different forms are different proteins catalyzing an identical reaction involving the same substrate. Such different molecular forms of the same enzyme are called isozymes or isoenzymes.

Isozymes of an enzyme differ from each other in their physicochemical properties like (i) primary structure or amino acid sequence, (ii) isoelectric pH, (iii) electrophoretic mobility, (iv) thermolability, (v) sensitivity to different denaturing agents and (vi) sedimentation coefficient. They also differ from each other in their biological properties like (i) optimum pH and temperature for activity, (ii) allosteric property and (iii) immunological reactions. Isozymes can be separated precisely from one another by isoelectric focusing.

Different isozymes of an enzyme may occur in different tissues of an organism, e.g. Lactate dehydrogenase which acts on lactate and converts it into pyruvate, occurs as five different isozyme in rats: LDH-I_1 occurs in heart; LDH-I_2 in brain, kidney and RBC; LDH-I_3 in lungs; LDH-I_4 in WBC, and LDH-I_5 in liver and muscle. Different isozymes may occur in a cell also, e.g. Two distinct isozymes of transaminase occur in cytosol and mitochondria of hepatic cells.

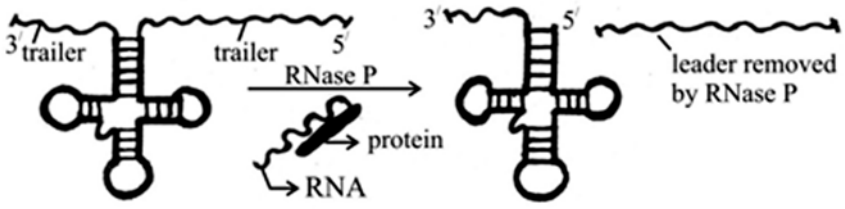
3.5.2 Ribozymes :

Almost all enzymes are basically protein in nature. However, a few RNA with the ability of catalyzing the cleavage of specific RNA molecules have been

discovered in both prokaryotes and eukaryotes in the last two decades. Such RNA molecules acting as biological catalysts are called as 'ribozymes'. A ribozyme may have a protein moiety associated with itself, but the attached protein moiety is not a must for the catalytic action of the ribozyme. The catalytic site lies in the RNA part of the ribozyme and not in the associated protein part.

e.g.(i) The precursor or primary transcript of t-RNA of *E. coli* contains an extra RNA sequence (leader sequence) at the 5' end. During the processing of the precursor of t-RNA, the extra RNA sequence at the 5' end is cut off by the catalytic action (endonuclease action) of a ribozyme called as RNAse P. This ribozyme consists of a 377 nucleotide-long RNA molecule called M1 RNA and a closely attached protein molecule with a molecular weight of 17.5 kD (Nelson and Cox, 2005). The removal of the attached 17.5 kD protein cannot prevent the endonuclease action of RNase P, which proves that the active site of RNase P lies in the RNA part, but not in the attached 17.5 kD protein part. The attached 17.5 kD protein may however, somehow promote the enzymatic activity of RNAse P. It may be added here that the precursor of t-RNA contains as extra RNA sequence (trailer sequence) at the 3' end also. However, during the processing of the precursor molecule, the 3'extra sequence is cut off by an exonuclease called RNase D, which is protein in nature like an ordinary enzyme.

(ii) Spliceosome is a eukaryotic ribozyme. It consists of five snRNA (small nuclear RNA) molecules and some associated proteins. Spliceosome removes the introns or intervening sequences and splices or joins the exons or expressed sequences of primary transcripts of mRNA molecules.



Action of RNase P on precursor of *E.coli* tRNA

3.5.3 Abzymes:

Lerner and Tramontano (1988) discovered that some monoclonal antibodies (a monoclonal antibody is the purest form of an antibody agains a particular antigen) can act as biological catalysts. Such monoclonal antibodies are called abzymes, e.g. Lerner and Tramontano (1988) produced a hapten-carrier complex in which the

hapten was a partially hydrolysed ester (hapten is a small molecule; when it combines with a carrier the hapten-carrier complex becomes an antigen). Then, using the hapten-carrier complex, they produced an 'antihapten monoclonal antibody'. When this monoclonal antibody was incubated in presence of the free and unhydrolysed ester, it catalysed rapid hydrolysis of the ester. So, this monoclonal antibody was an abzyme.Research work is going on to produce abzymes that will be able to (i) dissolve blood clots and (ii) dissolve viral glycoproteins and destroy the infectivity of viruses.

3.6 Mechanism of enzyme action

3.6.1 Basic principle :

An enzyme (E) at first binds with the substrate (S) to form a transient enzyme-substrate (ES) complex. The binding occurs between the active site of the enzyme and a specific group or component of the substrate. ES is an intermediate complex and it soon dissociates into the free, unaltered enzyme (E) and the changed substrate or product (P):

$$E + S \Leftrightarrow ES \Leftrightarrow E + P$$

3.6.2 Some evidence of ES complex formation :

(1) ES complex of glyceraldehyde-3-phosphate dehydrogenase is quite stable and has been chemically isolated.

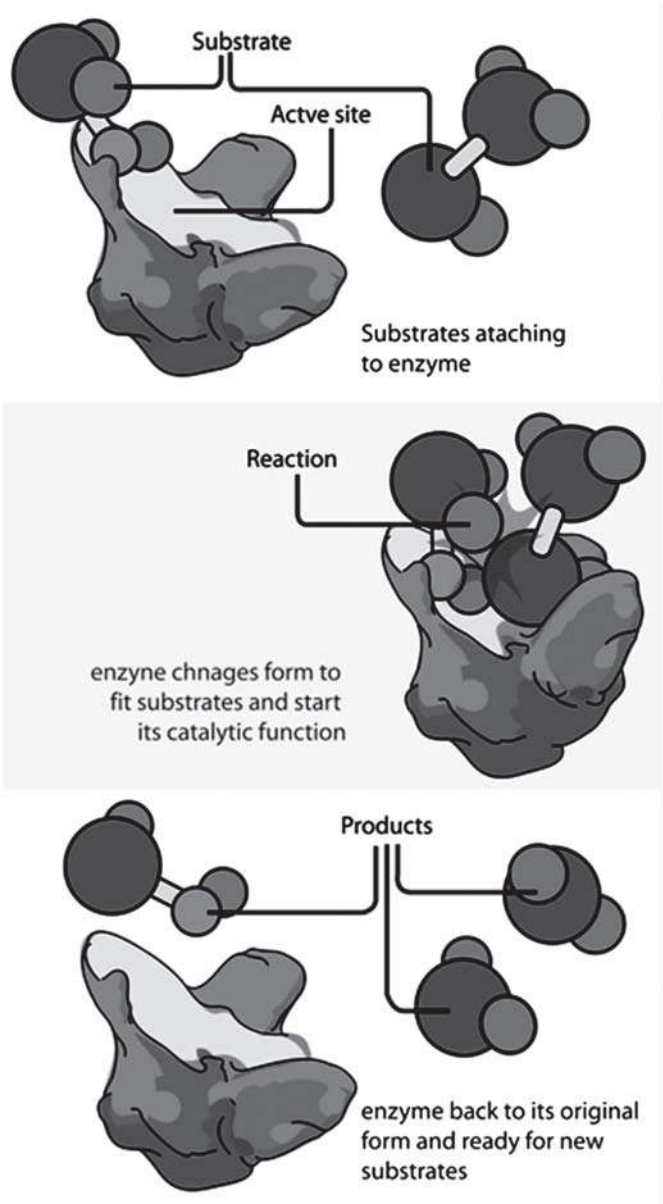
(2) ES complexes of catalase and peroxidase can be detected by spectrophotometry.

(3) ES complex of RNA polymerase can be directly visualized with the help of electron microscopy.

3.6.3 Bonds in ES complex formation :

The active site of an enzyme consists of several amino acid residues, located at some distance from each other in the polypeptide chain but brought close together by the folding of secondary and tertiary structures of the enzyme. Thus, the active site assumes a cleft-like three-dimensional structure. Inside this cleft, side-chains of some specific amino acids constitute a substrate-binding site and side-chains of some other specific amino acids constitute a catalytic site. **[Therefore, Active site = Substrate-binding site + Catalytic-site].** ES complex is formed mainly by non-covalent bonds like (a) hydrogen bonds, (b) electrostatic bonds, (c) Van der Waals forces and (d) hydrophobic bonds between specific amino acid side-chain of the active site of the enzyme and particular groups or residues of the substrate. However, in case of some

enzymes, ES complex is formed by covalent bonding, e.g. A covalent thioester bond is formed between the sulfhydril group of a cysteine of the active site of glyceraldehyde-3-phosphate dehydrogenase and the C1 aldehyde group of the substrate, glyceraldehyde-3-phosphate.



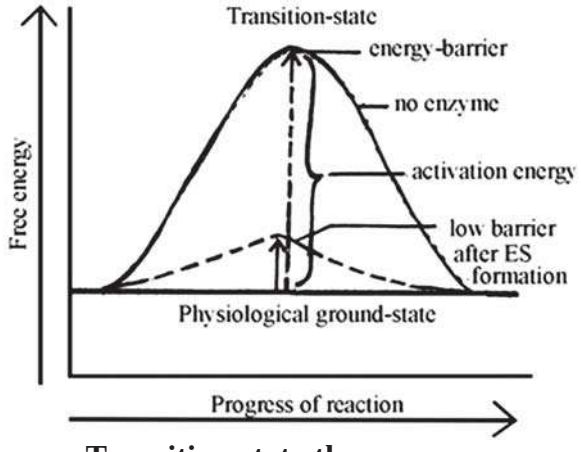
Enzyme-substrate reaction as per induced-fit model
[Source: Wikimedia Commons]

3.6.4 Transition-state theory of enzyme action :

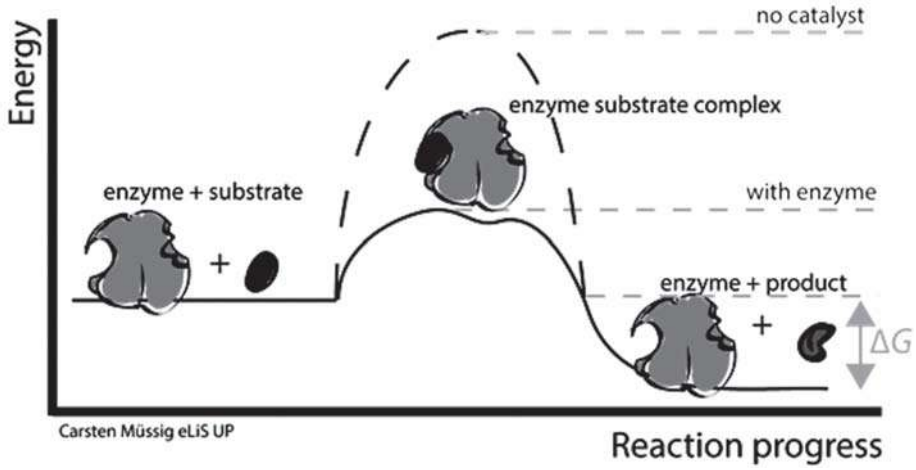
At physiological temperature, the substrate molecules cannot participate in any chemical reaction or formation/breakage of bonds, as they possess insufficient kinetic energy and as such, they fail to collide with or approach each other with sufficient energy, which is needed for formation or breakage of bonds. In other words, the substrate molecules fail to cross the 'energy barrier' for a chemical reaction. Reactant molecules occurring at physiological temperature ('physiological ground state') can be made to participate in a chemical reaction in two ways:

1. One way is to add some amount of energy to the reactant molecules and to shift them to a state of high kinetic energy, when they can collide with each other with sufficient energy and can cause formation or breakage of bonds. This reactive and high-energy state of reactant molecules is called as the 'transition-state'. The amount of energy required to shift the reactant molecules from their 'physiological ground-state' to the 'transition-state' is called as the 'activation energy'. The target energy-level which must be crossed by the reactant molecules for their escalation to the 'transition state' is called as the 'energy barrier' for a chemical reaction. At par with the kinetic theory of chemical reaction, the addition of 'activation energy' to the reactant molecules may be done by increasing the temperature of the molecules. It is obvious that enzymes cannot do so, because enzymes are not any source of heat.

2. There exists an alternative way to enable the reactant molecules to participate in a chemical reaction. This is exactly what the enzymes do with their substrate molecules. Enzymes bind their substrate molecules to their active sites. The formation of enzyme-substrate complex lowers the 'energy barrier' for a reaction to a bare minimum level. As such, a meager amount of 'activation energy' has to be added to the enzyme-bound substrate molecules to make them cross the altered 'energy barrier' and reach the 'transition-state'. This small amount of energy may be quite well provided by the physiological temperature of living beings. Consequently, the enzyme-bound substrates undergo chemical change rapidly, yielding the product.



Transition-state theory



Mechanism of enzymes action in terms of energy (ΔG = change of free energy)
[Source: Wikimedia Commons]

3.7 Enzyme Kinetics

3.7.1 Meaning of 'enzyme kinetics'and derivation of Michaelis-Menten equation :

When the substrate concentration or [S] is very low compared to the enzyme concentration of [E], many enzyme molecules remain free or not bound with substrates, and this makes the velocity (v) of enzyme activity very low. If [S] is gradually increased without disturbing pH, temperature and [E] of the medium, more and more enzyme molecules bind with substrate molecules and 'v' also increases progressively. However, a stage soon comes when all enzyme molecules get linked with substrate molecules and 'v' reaches its maximum level of V_{max}. A further rise in [S] fails to increase the velocity of enzyme activity beyond V_{max}, since the free enzyme molecules are no more available in the medium for binding any more substrate molecules.

The study of the quantitative relationship between the velocity of enzyme action and the substrate concentration is called as the study of enzyme kinetics. The pioneer workers on this issue were L. Michaelis and M. L. Menten. They stated in 1913 that an enzyme-catalysed reaction involves two essential steps. At first, an enzyme (E) reversibly binds to the substrate (S), resulting in formation of a transient enzyme-substrate complex (ES). Then, the ES dissociates into the enzyme and the product (P). The overall changes may be shown by the following equation :

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \underset{K^4}{\overset{K_3}{\rightleftharpoons}} E + P$$

Here, K_1, K_2, K_3 and K_4 are the rate constants for the reactions; K_1 and K_3 for the forward reactions while K_2 and K_4 are for the backward reactions. Now, at a steady state of the enzyme activity, the situation becomes :

Rate of formation of [ES] = Rate of dissociation of [ES]

So, from the law of mass action, it can be represented as follows :

$$K_1[E][S] + K_4[E][P] = K_2[ES] + K_3[ES]$$

$$\text{or, } [E](K_1[S] + K_4[P]) = [ES](K_2 + K_3)$$

$$\text{or, } \frac{[E]}{[ES]} = \frac{K_2 + K_3}{K_1[S] + K_4[P]}$$

Now, at an early stage of the reaction, [P] will be very small and the backward reaction $ES \xleftarrow[K_4]{} E + P$ will also be very slow. As such, we can ignore K_4[P] from the denominator and can write that :

$$\frac{[E]}{[ES]} \quad \frac{K_2 \quad K_3}{K_1[S]}$$

$$\text{or, } \frac{[E]}{[ES]} = \frac{K_m}{[S]}$$

(When the 3 constants $K_1 = K_2$ and K_3 are condensed into a single constant Km, by the relationship, $\frac{K_2 + K_3}{K_1} = K_m$)

The lumped constant Km of the above equation is called as Michaelis constant.

Now, we suppose that the total concentration of enzyme molecules is $[E]_t$. So, the concentration of enzyme molecules bound to the substrate will be equal to the concentration of ES molecules, i.e. [ES]. Therefore, the concentration of free enzyme molecules will be :

Hence, we can further write as follows :

or,

or,

or,

or,

Now, the maximum velocity of enzyme action or V_{max} will be reached when all the enzyme molecules or $[E]_t$ will bind to the substrate. In other words, V_{max} $[E]_t$. Again, the velocity (v) of enzyme action at any given moment is directly proportional to the concentration of enzyme molecules bound to the substrate at that particular moment. In other words, .

Therefore, we can write that $\frac{V_{max}}{v} \alpha \frac{[E]_t}{[ES]}$

Again by substituting $\frac{[E]_t}{[ES]}$ for $\frac{V_{max}}{v}$, we can write as follows :

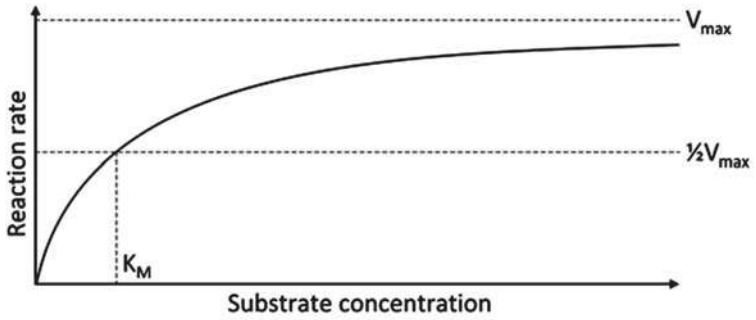
$$\frac{[E]_t}{ES} = \frac{K_m}{[S]} + 1$$

$$\text{or,} \quad \frac{V_{max}}{v} = \frac{K_m}{[S]} + 1 = \frac{K_m + [S]}{[S]}$$

$$\text{or,} \quad \frac{v}{V_{max}} = \frac{[S]}{K_m + [S]}$$

$$\text{or,} \quad v = \frac{V_{max}[S]}{K_m + [S]}$$

The above equation is called as Michaelis-Menten equation for the velocity of enzyme action.



Michaelis-Menten saturation curve showing relation between substrate concentration and reaction rate

[Source: Wikimedia Commons]

3.7.2 Concept of K_m and V_{max} :

Michaelis-Menten equation depicts the velocity of enzyme action at any given moment. From Michaelis-Menten equation, the value of K_m for a given enzyme can be easily calculated. K_m or Michaelis constant or Michaelis-Menten constant is an important and fundamental characteristic of every enzyme. K_m can be defined as a measure of affinity of an enzyme for a particular substrate and it also represents the substrate concentration at which the velocity of enzyme activity reaches or half of the maximum velocity. K_m is independent of enzyme concentration and the higher is the value of K_m, the lower will be affinity of the enzyme for the substrate.

Michaelis-Menten equation states : $v \quad \frac{V_{max}[S]}{K_m \quad [S]}$

If we consider that , then we can represent the situation as follows:

$$\frac{V_{max}}{2} \quad \frac{V_{max}[S]}{K_m \quad [S]}$$

or,

or,

or,

Thus, K_m is equal to the substrate concentration at which the velocity of enzyme action is half of the maximum velocity. Different enzymes have different K_m, e.g. K_m for catalase acting on H_2O_2 is 25 mM/l and K_m for hexokinase acting on glucose is 0.15 mM/l. The K_m of an enzyme may vary with change of pH and temperature of the medium. Again, K_m of an enzyme is increased in case of competitive inhibition, lowered in case of uncompetitive inhibition and remains unaffected in case of non-competitive inhibition of enzyme activity. Besides, if an enzyme acts on more than one substrate, then the enzyme will have different K_m values for different substrates, e.g. Hexokinase acting on glucose has a K_m value 0.15 mM/l, but the same enzyme while acting on fructose has a K_m value of 1.5 mM/L.

From Michaelis-Menten equation, one can easily understand the changes in velocity of enzyme activity along with changes in substrate concentrations :

The equation states : $v = \frac{V_{max}[S]}{K_m + [S]}$

If [S] is very low compound to K_m, then we can modify the equation into :

$v = \frac{V_{max}[S]}{K_m}$ ([S], being very small, is ignored in the denominator)

or, v = a very small quantity (since, [S] in the numerator is very small compared to K_m in the denominator), or, velocity of enzyme action is very low.

When [S] is much bigger than K_m, we can modify the equation as follows :

$v = \frac{V_{max}[S]}{[S]}$ (K_m, being very small, is ignored in the denominator)

or, $v \approx V_{max}$

or, velocity of enzyme action is approaching towards V_{max} ,

or, velocity is very high.

Finally, when 'v' is equal to V_{max} we can represent the situation as follows :

$v = \frac{V_{max}[S]}{[S]}$ (here also [S] is much higher than K_m and K_m can be ignored)

or, $v = V_{max}$

Thus, v is equal to irrespective of any value of [S]. In other words, once V_{max} is reached, the velocity does not increase any further and remains steady even if [S] is increased. In this way, the graph representing velocity (v) of enzyme action in the ordinate and substrate concentration or [S] in the abscissa becomes a hyperbolic curve.

3.8 Lineweaver-Burk Plot

The value of K_m can be easily determined in the laboratory from 'double-reciprocal plot' or 'Lineweaver-Burk plot' devised by Lineweaver and Burk in 1934. Thus plot is derived from Michaelis-Menten equation. Michaelis-Menten equation states :

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

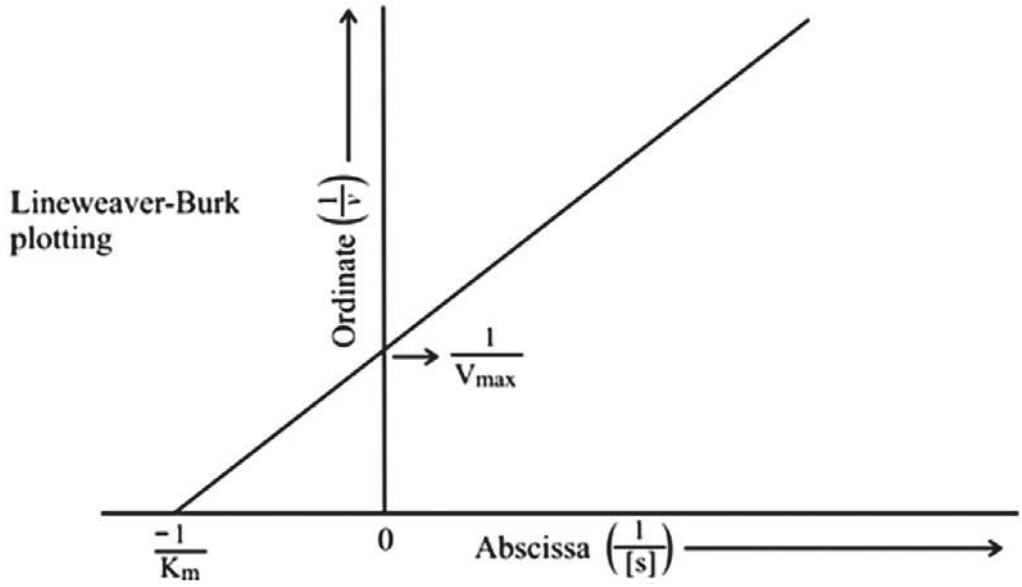
By inverting we get : $$\frac{1}{v} = \frac{K_m + [S]}{V_{max}[S]}$$

$$\text{or,} \quad \frac{1}{v} = \frac{K_m + [S]}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

$$\text{or,} \quad \frac{1}{v} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

The above represents an equation for a straight line, as it may be considered as : $y = ax + b$ (if we consider that $\frac{1}{v} = y, \frac{K_m}{V_{max}} = a, \frac{1}{[S]} = x$ and $\frac{1}{V_{max}} = b$).

Hence, a straight-line graph will be obtained when $\frac{1}{v}$ and $\frac{1}{[S]}$ are plotted on the ordinate and the abscissa respectively. The Y-intercept of the graph represents 'b' or $\frac{1}{V_{max}}$, because the value of 'x' at this point is 0 ; consequently, $y = ax + b$, or, $y = a \times 0 + b = b$. Again, the –ve X-intercept of the graph represents $-\frac{1}{K_m}$, because the value of 'y' at this point is 0; consequently, $y = ax$ or, $0 = ax + b$, or, $x = \frac{-b}{x} = \frac{-1}{V_{max}} + \frac{K_m}{V_{max}} = \frac{-1}{V_{max}} \times \frac{V_{max}}{K_m} = \frac{-1}{K_m}$. From the value of $-\frac{1}{K_m}$, the value of K_m can be easily determined.



3.9 Multisubstrate reactions

Some enzymes act on only one particular substrate, e.g. Isomerases. Here, ES complex generally has a short span of existence and the rate of product formation is proportional to the concentration of ES complex. However, many enzymes act on more than one substrate. Such enzyme actions occur as follows:

(a) Single-displacement reaction: Here, two substrates S_1 and S_2 combine with the enzyme one after another, either in specific sequence or in a random sequence. After both substrates bind to the enzyme, a ternary complex 'ES_1S_2' is formed. Later, ES_1S_2 dissociates into the enzyme and two products P_1 and P_2 which are released in either a specific or a random sequence:

$$E + S_1 \Leftrightarrow ES_1$$
$$ES_1 + S_2 \Leftrightarrow ES_1S_2$$
$$ES_1S_2 \Leftrightarrow EP_1P_2 \Leftrightarrow EP_2 + P_1$$
$$EP_2 \Leftrightarrow E + P_2$$

e.g. Hexokinase can phosphorylate not only D-glucose but also other D-hexoses. The hydrolase, pepsin can hydrolyse casein of milk as well as collagen of connective tissues.

(b) Ping-pong reaction or double-displacement reaction: Here, each of the substrates binds singly with the enzyme in an ordered sequence, each forming only a binary ES complex. No ternary complex or ES_1S_2 is formed, since two substrates never remain simultaneously combined with the enzyme. The first ES complex or ES_1 dissociates to release the first product (P_1) and a modified form of the enzyme (E'). Then, E' binds to the second substrate or S_2 to form a second ES complex or $E'S_2$ which subsequently dissociates into the second product or P_2 and the original form of the enzyme or E :

$$E + S_1 \Leftrightarrow ES_1 \Leftrightarrow E' + P_1$$
$$E' + S_2 \Leftrightarrow E'S_2 \Leftrightarrow E + P_2$$

e.g. Transaminase acts on an amino acid and its corresponding keto acid, respectively, through ping-pong mode of enzyme action.

3.10 Factors affecting rate of enzyme-catalyzed reactions

3.10.1. Enzyme concentration :

An increase in enzyme concentration causes a proportionate rise in the initial velocity of the enzyme action. A linear graph is obtained by plotting the velocity of enzyme action against enzyme concentration.

Generally, in an enzyme-catalyzed reaction, the number of substrate molecules far exceeds the number of enzyme molecules. So, many substrate molecules remain free even after all enzyme molecules have bound to substrate molecules. Therefore, if we increase the concentration of enzyme molecules, the formation of enzyme-substrate complex and the velocity of enzyme action will also increase. However, if the number of enzyme molecules is so much increased that the number of substrate molecules becomes less than the number of enzyme molecules, the velocity of enzyme action will not increase anymore and the linear graph will become hyperbolic in shape.

3.10.2. Effect of reaction products :

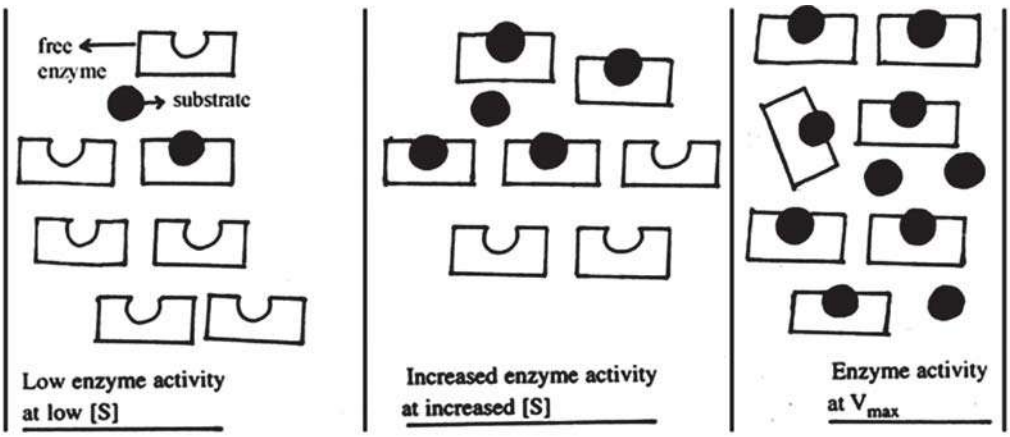
If the reaction products continue to accumulate, the rate of enzyme action is, to some extent, depressed. This happens so because an accumulation of products stimulates the backward reaction :

Enzyme-substrate ← Enzyme (free) + Product

Besides, the products may mask (cover) the active sites of enzymes, which also depress the rate of enzyme action. However, within the body, enzyme actions are generally not prevented by accumulation of products, because different reactions occur in a series in the body. One reaction leads to the formation of one product, but very soon, a second enzyme starts to act on that product to produce a second product. Thus, products can hardly accumulate to depress enzyme actions.

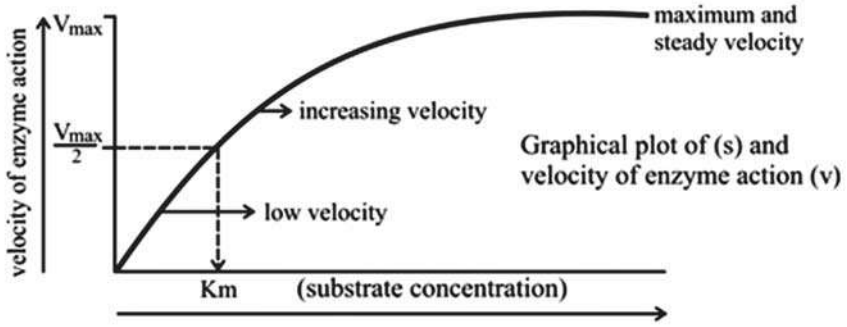
3.10.3 Effect of substrate concentration:

When the substrate concentration or [S] is very low compared to the enzyme concentration of [E], many enzyme molecules remain free or not bound with substrates, and this makes the velocity (v) of enzyme activity very low. If [S] is gradually increased without disturbing pH, temperature and [E] of the medium, more and more enzyme molecules bind with substrate molecules and ‘v’ also increases progressively. However, a stage soon comes when all enzyme molecules get linked with substrate molecules and ‘v’ reaches its maximum level of V_{max}. A further rise in [S] fail to increase the velocity of enzyme activity beyond V_{max}, since free enzyme molecules are no more available in the medium for binding any more substrate molecules.



Effect of substrate concentration on velocity of enzyme action

If the gradually increasing substrate concentrations and the corresponding velocities of enzymes activity are plotted in graph, a hyperbolic curve is obtained:



Hyperbolic curve showing enzyme action

3.10.4. Effect of pH :

Each enzyme acts best at a particular pH, which is called as the optimum pH for that enzyme. The velocity of enzyme action progressively declines, both when the pH of the medium is increased above the optimum pH and when the pH of the medium is lowered below the optimum pH. Thus, a bell-shaped curve will be obtained by plotting the velocity of enzyme action against pH of the medium.

Variations of pH above and below the optimum pH, may affect ionization of different groups in the substrate and also, in the active site of an enzyme. These in turn affect enzyme-substrate complex formation.

Increase or decrease of pH of the medium above and below the optimal pH, may also affect the 3-dimensional structure of the enzyme, which in turn may affect enzyme-substrate complex formation.

Variations of pH may cause dissociation of apoenzyme and coenzyme parts of an enzyme, which in turn may inhibit the activity of an enzyme.

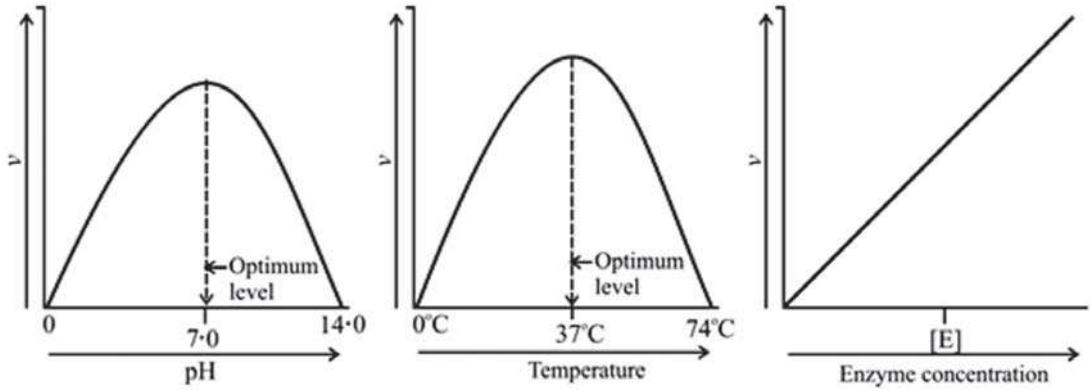
Some enzyme like pepsin may act on more than one substrate. In such cases, the optimum pH of the enzyme varies with substrates, e.g. Pepsin has an optimum pH of 2.2 when it digests haemoglobin, but it has on optimum pH of 1.5 when it acts on egg albumin.

3.10.5. Effect of temperature :

For each enzyme, there is a particular temperature, called optimum temperature, at which the enzyme activity becomes maximum. Above and below the optimum temperature, the activity of an enzyme gets depressed. Thus, a bell-shaped graph is obtained by plotting the velocity of enzyme action against temperature of the medium.

When the temperature is too low, the rate of enzyme action remains slow due to low kinetic energy of both enzyme and substrate molecules. A slow rise in temperature increases the kinetic energy of both enzyme and substrate molecules, which increases their interaction and also, the overall rate of enzyme action. But, when the temperature is raised above an optimum level, the secondary and tertiary structure of the enzyme molecule may be affected. This is turn may affect enzyme-substrate binding and enzyme activity.

The increase in the rate of an enzyme's activity for every 10^{o}C rise of temperature, starting from an initial temperature of 0^{o}C, is called as Q_{10} (temperature coefficient) of an enzyme. For most of the enzymes, the value of Q_{10} is 2. For most of the enzymes found in mammals, the optimum temperature is about 37^{o}C. Most enzymes get completely denatured and lose their activity at 55^{o}C to 60^{o}C. However, the enzymes of thermophilic bacteria (hot-spring bacteria) remain active at even 85^{o}C.



Effect of pH, temperature and enzyme concentration [E] on velocity of enzyme action

3.11 Enzyme inhibition :

3.11.1 Competitive inhibition:

Definition : Sometimes, the activity of an enzyme is inhibited in presence of such an inhibitor, which bears a close similarity with the original substrate of the enzyme and which competes with the substrate for occupying the binding site of the enzyme.

Mechanism : The inhibitor (I) binds with the enzyme by competing with the substrate to form an enzyme-inhibitor complex (EI). Consequently, the enzyme is abstained from binding with the substrate and forming enzyme-substrate complex (ES). Naturally, enzyme activity of lowered.

Characteristics :

(1) Effect of [I] : If the [I] or concentration of inhibitor is progressively increased, the formation of EI is also increased while the formation of ES and the enzyme activity are progressively decreased.

(2) Effect of K_m : A progressive rise in [I] causes a progressive lowering of the affinity of the enzyme for its substrate. Consequently, the K_m value of the enzyme increases progressively.

(3) Reversibility : Competitive inhibition is reversible. If the [S] is much increased relative to the [I], the substrate succeeds to force the inhibitor out from EI complex and to bind with the enzyme to form ES complex and to produce the same maximum velocity as in absence of the inhibitor.

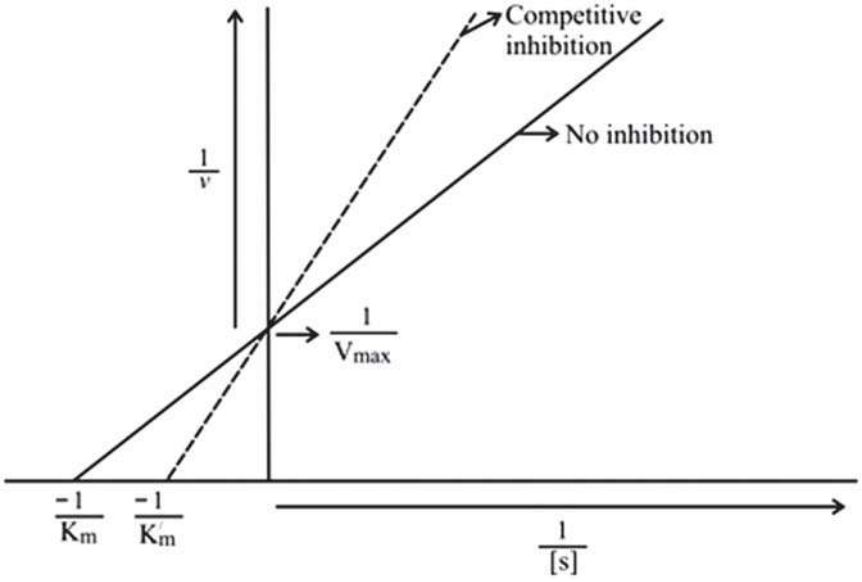
(4) Effects on Lineweaver-Burk plot :

(a) Slope of the curve: The slope of the graph becomes steeper in presence of the inhibitor than in absence of the inhibitor, which indicates a rise in the value of $\frac{1}{v}$ or a decrease in the velocity of enzyme action in presence of the inhibitor.

(b) X-intercept : Along with the increase in [I], the X-intercept becomes reduced in size or moves closer to the Y-axis of the graph, which indicates a decrease in the absolute value of $\frac{1}{K_m}$ or an increase in the value of K_m.

(c) Y-intercept : A rise in [I] does not affect the Y-intercept of the graph. In other words, the value of either $\frac{1}{V_{max}}$ or V_{max} does not change. It means that the inhibitor does not affect the rate of breakdown of whatever number of ES complex is present, into E and P.

Examples: Malonate competes with succinate and inhibits the activity of succinate dehydrogenase; fluorocitrate competes with citrate and inhibits the activity of aconitase.



Lineweaver-Burk plot in competitive inhibition

3.11.2 Non-competitive inhibition:

Definition: Sometimes, the activity of an enzyme is depressed in presence of such an inhibitor, which has no structural similarity with the substrate and does not compete with the substrate for occupying the binding site of the enzyme, but which combines with some other site of the enzyme and inactivates the latter. This is called non-competitive inhibition.

Mechanism: The inhibitor binds to a site other than the substrate-binding site of the enzyme. The binding may occur when the enzyme is free or after the formation of enzyme-substrate complex. Thus, an enzyme-inhibitor (EI) or an enzyme-substrate-inhibitor (ESI) complex is formed. The binding of the inhibitor deforms the three-dimensional conformation of the enzyme, which thus loses its catalytic power. As a result, the enzyme subsequently fails to convert the substrate into the product.

Characteristics :

(1) Effect of [I] : The higher is the concentration of the inhibitor, the higher is the formation of EI or ESI complexes and the lower becomes the velocity of enzyme action.

(2) Effect on K_m : Since the inhibitor does not compete with the substrate for occupying the binding site of the enzyme, it does not affect the substrate-affinity of the enzyme. In other words, the K_m value for the enzyme remains unaffected.

(3) Irreversibility : As the inhibitor binds to the enzyme at a site other than the substrate-binding site, an increase in [I] cannot dislodge the inhibitor from EI or ESI complexes. Thus, non-competitive inhibition is not reversed by an increase of [S].

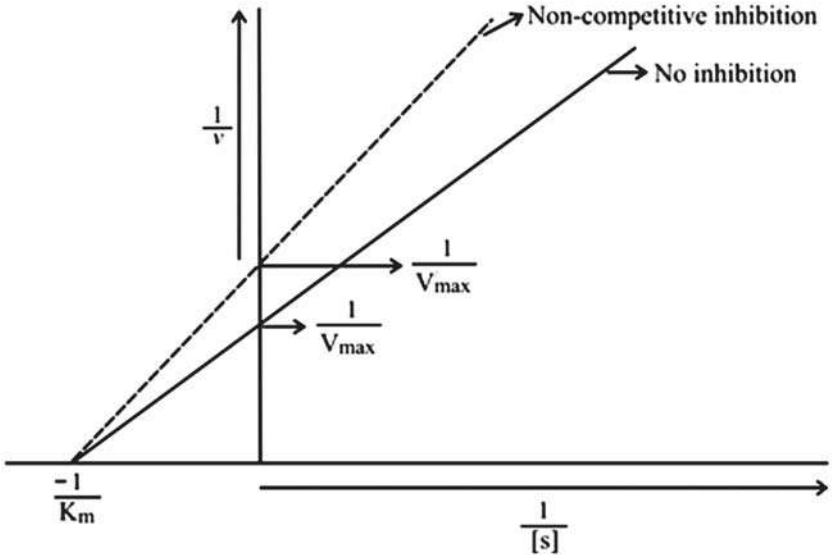
(4) Effects on Lineweaver-Burk plot :

(a) Slope of the curve: The slope of the graph becomes higher than in absence of the inhibitor, indicating a rise in the value of $\frac{1}{v}$ or a decrease in the value of 'v' or velocity of the enzyme activity.

(b) X-intercept: The X-intercept of the graph remains unchanged, indicating no change in the value of $-\frac{1}{K_m}$ as well as K_m.

(c) Y-intercept: The Y-intercept of the graph is increased. In other words, the value of $\frac{1}{V_{max}}$ is increased and the value of V_{max} is decreased. Thus, the maximum velocity of enzyme action is decreased in presence of the inhibitor.

Examples : EDTA (Ethylene diamine tetra-acetate) may bind with the Mg^{++} ions of Mg-dependent enzymes like enclose and inhibit them. Again, heavy metals like Ag^{+} and Hg^{++} may bind to SH groups of sulfhydril enzymes like glyceraldehydes-3-phosphate dehydrogenase and inhibit them.



Lineweaver-Burk plot in non-competitive inhibition

3.11.3 Uncompetitive inhibition:

Definition: In case of certain bi- or multi-substrate enzymes and in a few single-substrate enzymes, enzyme activity is inhibited in presence of such an inhibitor which has no structural similarity with the substrate and does not compete with the substrate for occupying the binding site of the enzyme, but which binds with the enzyme-substrate complex and inhibits the formation and release of the product. This is called uncompetitive inhibition.

Mechanism: The inhibitor does not affect enzyme-substrate complex formation and it binds to enzyme-substrate complex only, forming an enzyme-substrate-inhibitor (ESI) complex. This deforms the conformation of the enzyme in such a way that its catalytic power is suppressed, which prevents the formation and release of the product.

Characteristics:

(1) Effect of [I] : An increase in the concentration of the inhibitor increase the formation of ESI complex and thus, progressively lowers the formation of the product.

(2) Effect of K_m : The inhibitor causes an apparent increase in the substrate-affinity of the enzyme, which in turn, decreases the K_m value of the enzyme [Apparent increase of substrate-affinity = Accumulation of ESI without breakdown into E and P; as if, the substrate affinity of the enzyme is increased].

(3) Irresversibility : The higher is the [S], the higher are the formation of ES as well as ESI, and the inhibition cannot be reversed by increasing the [S].

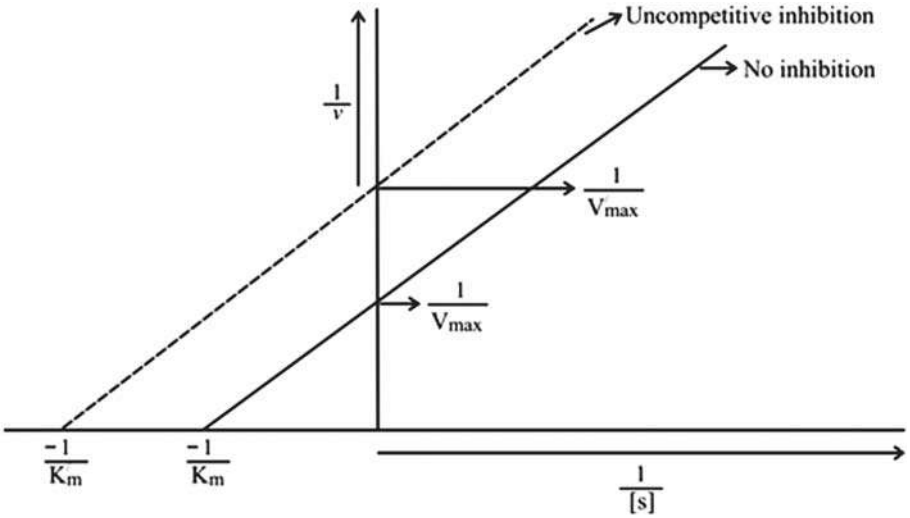
(4) Effect on Lineweaver-Burk plot:

(a) Slope of the curve: The slope of the graph remains unchanged.

(b) X-intercept: The X-intercept becomes larger in size or moves away from the Y-axis of the graph than in absence of the inhibitor, which indicates an increase of the absolute value of $\frac{1}{K_m}$ or a decrease in the value of K_m in presence of the inhibitor.

(c) Y-intecept : The Y-intercept of the graph is increased, indicating an increase in the value of $\frac{1}{V_{max}}$ or a decrease in the value of V_{max} in presence of the inhibitor. Thus, the maximum velocity of the enzyme decreases with a rise in the concentration of the inhibitor.

Example: Uncompetitive inhibition may be noted in cases of bi- and multi-substrate enzymes.



Lineweaver-Burk plot in uncompetitive inhibition

3.12 Allosteric enzymes and their kinetics

The activity of enzymes vary depending on (a) physiochemical conditions of the surrounding medium, e.g. temperature and pH, (b) reaction conditions, e.g. substrate concentration, accumulation of product and (c) presence of inhibitor substances, e.g. different non-cellular chemicals applied from outside and a few cellular substances acting as substrate analogues. Besides, the activity of certain enzymes may be modified (increased or decreased) by the presence of specific cellular substances. The process is called regulation of enzymes and those enzymes are called regulatory enzymes. Regulation of enzymes by cellular substances is divisible into two types:

3.12.1 Non-covalent or Allosteric modulation (regulation) of enzymes:

Sometimes, the activity of an enzyme may be either enhanced or inhibited by the non-covalent binding of some specific low-molecular weight ligands to specific sites of the enzyme, other than the isoteric site or the active site. Such modification or enzymes activity is called as allosteric modulation; the concerned regulatory substances or ligands are called allosteric modulators or effectors; the binding site of an allosteric modulator on the enzyme is called allosteric site; the enzyme thus being regulated is called an allosteric enzyme.

3.12.2 Characteristics of allosteric modulation:

1. An allosteric site is different from the active site of the enzyme. An allosteric stie possesses specificity for binding with a particular allosteric modulator only.
2. An allosteric enzyme may possess only one allosteric site for either a positive modulator or a negative modulator. Or, an allosteric enzyme may possess separate allosteric sites for the binding of a positive and a negative modulator, respectively.
3. Allosteric enzymes are generally oligaomeric enzymes and each enzyme contains more than one substrate binding sites [one on each subunit or polypeptide chain].

3.12.3 Positive and negative allosteric modulations :

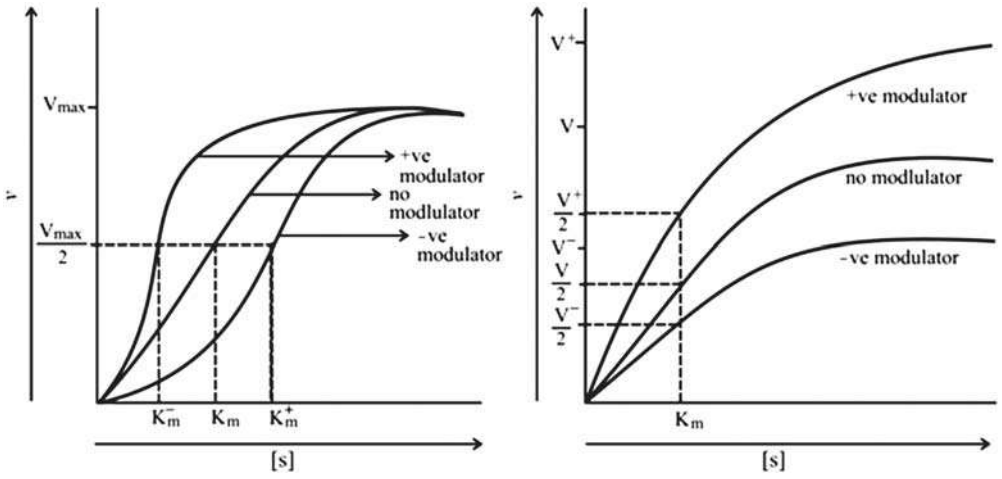
When an enzyme activity is enhanced by allosteric modulation, it is called as positive allosteric modulation and the modulator is called as a positive modulator (or activator). The reverse situation is called as negative allosteric modulation and the modulator is than called as a negative modulator (or inhibitor), e.g. (a) Citrate positively modulates or enhances the activity of acetyl-CoA carboxylase, which

produces malonyl-CoA from acetyl-CoA and (b) Palmityl-CoA negatively modulates or suppresses the activity of acetyl-CoA carboxylase.

The same modulating substance may act as a positive modulator for one enzyme but as a negative modulator for another enzyme, e.g. ATP is an allosteric activator for fructose diphosphatase which hydrolyzes fructose diphosphate into fructose 6-phosphate during glucogenesis, but ATP is an allosteric inhibitor of phosphofructokinase that converts fructose 6-phosphate to fructose diphosphate during glycolysis.

3.12.4 'K'and 'M' types of allosteric enzymes :

Some allosteric enzymes are called 'K' enzymes. In presence of a positive modulator, their K_m values are decreased and in presence of a negative modulator, their K_m values are increased than in absence of any modulator. The V_{max} remains unchanged in both conditions,e.g. Phosphofructokinase. Some allosteric enzymes are called 'M' enzymes. No change in their K_m values occurs in presence of either a positive or a negative modulator. However, V_{max} of an 'M' enzyme rises and falls in presence of a positive and a negative modulator, respectively, e.g. Acetyl-CoA carboxylase.



K_m and V_{max} of 'K' and 'M' enzyme

3.12.5 Molecular mechanism of allosteric modulation:

1. 'M' enzymes: 'M' enzymes are very few in number (Lehninger, 1975). In case of 'M' enzymes, the binding of an allosteric modulator to the allosteric site of the enzyme causes a conformational change of only the catalytic site of the enzyme, which results in a change of velocity of the enzyme action, but the substrate affinity of the enzyme remains unaltered.

2. 'K' enzymes: The mechanism of allosteric modulation of 'K' enzymes is complex. A model, called as 'Sequential model' and proposed by D. E. Koshland in 1973, best explains the mechanism:

Allosteric 'K' enzymes have multiple substrate-binding sites. In absence of any allosteric modulator, those sites remain in a 'partially accessible' conformation where the substrate molecules cannot bind firmly. As a +ve allosteric modulator binds to the allosteric site of the enzyme, a sequential or gradual change of conformation occurs in the enzyme so that its 'partially accessible' substrate-binding sites become 'fully accessible' one after another. As a result, the binding of the first substrate molecule to one substrate-binding site is followed by the binding of another substrate molecule to another substrate-binding site and so on. Apparently, the binding of the first substrate molecule to the enzyme seems to stimulate the binding of the subsequently substrate molecules to the enzyme; the phenomenon is called +ve cooperativity. The substrate-saturation curve of the enzyme becomes sigmoid and shifts to the left of the original hyperbolic curve. Again, when a –ve allosteric modulator binds to the allosteric site of the enzyme, a sequential change of conformation occurs in the enzyme so that its 'partially-accessible' substrate-binding sites become 'too less accessible' one after another. As a result, the binding of the first substrate molecule to one substrate-binding site is followed by negligible binding of the subsequent substrate molecules at other substrate-binding sites; the phenomenon is called –ve cooperativity. The substrate-saturation curve of the enzyme remains sigmoid, but the slope is so lowered that the curve shifts to the right of the original hyperbolic curve.

3.12.6 Non-competitive inhibition vs. allosteric inhibition:

(a) A non-competitive inhibitor generally binds to the cofactor associated with the apoenzyme, but an allosteric inhibitor binds to a specific allosteric site on the apoenzyme itself [exception : a non-competitive inhibitor like Hg^{++} or Ag^{+} ion binds to SH on the apoenzyme of glyceraldehyde-3-phosphate dehydrogenase].

(b) A non-competitive inhibitor is an external agent applied from outside the body of living organisms, but an allosteric inhibitor is a cellular substance or metabolite.

(c) A non-competitive inhibitor does not change the K_m value of the enzyme. An allosteric inhibitor causes an increase of the K_m value of the enzyme.

(d) An enzyme undergoing non-competitive inhibition has a single substrate-binding site, but an enzyme undergoing allosteric inhibition has multiple substrate binding sites.

(e) No cooperativity is found when the substrate binds to an enzyme undergoing non-competitive inhibition, but a –ve cooperativity is found when substrate molecules bind to an enzyme undergoing allosteric modulation.

(f) The substrate-saturation curve is hyperbolic for the first type of enzyme, but becomes sigmoid for the second or allosteric type of enzyme, when an inhibitor effect goes on.

3.13 Covalent regulation of enzyme action

Two types of covalent regulation are found :

3.13.1 Irreversible covalent activation :

Some enzymes are secreted in an inactive precursor form or 'zymogen' form. These are then converted into an active form by an enzyme-catalysed hydrolytic process, which is a type of cleavage of covalent bond, e.g. Trypsinogen is a zymgogen secreted from pancreatic acinar cells. When it passes into the intestine, an intestinal enzyme called enterokinase cleaves a hexapeptide from its N-terminal and produces the active proteolytic enzyme trypsin. Again, pepsinogen is a zymogen secreted by stomach cells. It is converted into an active form called pepsin in the stomach by the action of already-formed pepsin at a low pH, which removes 42 amino acids as a few short peptides from the N-terminal end of pepsinogen.

3.13.2 Reversible covalent modification :

Some enzymes may be reversibly activated or inactivated by change of covalent bond (e.g. addition or removal of phosphate or adenyl groups) by some enzymatic process, e.g. Phosphorylase phosphatase removes four phosphate groups from the active glycogen phosphorylase 'a' and produces two molecules of inactive glycogen phosporylase 'b'; phosphorylase kinase uses four ATP molecules to phosphorylate two molecules of glycogen phosporylase 'b' to produce one molecule of active glycogen phosphorylase 'a'.

3.14 Questions (with hints to answers)

1. What are enzymes? How do they differ from inorganic catalysts? [see Section 3.1].
2. Classify enzymes and explain the functioning of each class with example [see Section3.2.1].
3. Explain the terms: Enzyme code number; turnover number of enzymes; international unit of enzymes [see Section 3.2.2].

4. What are coenzymes? How do they act? [see Section 3.3.1].
4. What are cofactors? How do they act? [see Section 3.3.2].
5. Explain 'lock-and-key model' and 'induced-fit model' of enzyme-substrate binding. State the merit and demerit of each model [see Sections 3.4.1 and 3.4.2 I].
6. Write a short note on isozymes [see Section 3.5.1].
7. What are ribozymes? Cite examples from both pro- and eukaryotes [see Section 3.5.2].
8. What do you mean by abzymes? [see Section 3.5.3].
9. Explain the transition-state theory of enzyme action [[see Section 3.6.4].
10. What is enzyme kinetics? How will you derive Michaelis-Menten equation? [see Section 3.7.1].
11. What is K_m of an enzyme? Prove that K_m = [S], when $v = V_{max}/2$ [see Section 3.7.2].
12. Explain Lineweaver-Burk plot and its significance [see Section 3.8].
13. Explain the principles of single-displacement and double-displacement enzyme actions[see Section 3.9].
14. Explain the effect of substrate concentration on enzyme action [see Section 3.10.3].
15. Explain the effect of temperature on enzyme action [see Section 3.10.5].
16. Give an account of competitive inhibition [see Section 3.11.1].
17. Give an account of non-competitive inhibition [see Section 3.11.2].
18. What isallosteric modulation of enzymes? Define and exemplify positive and negative allosteric modulations.Distinguish between 'K'and 'M' types of allosteric enzymes. Explain the molecular mechanism of allosteric modulation [see Sections 3.12.1, 3.12.3, 3.12.4 and 3.12.5].
19. Write notes on irreversible and reversible covalent modifications of enzymes [see Sections 3.13.1 and 3.13.2].

3.15 References

1. Biochemistry – A. H. Lehninger (1975), Worth Publishers, New York.
2. Outlines of Biochemistry – Conn and Stumpf (1972), John Wiley, New York.
3. Principles of Biochemistry – Nelson and Cox (2005), Freeman & Co., New York.
4. Textbook of Biochemistry – West and Todd (1966), Macmillan, New York.

Unit-4 □ Overview of Metabolism

Structure

4.0 Objectives

Upon going through this Unit IV, learners will be able to :

- Compare between anabolism and catabolism.
- Understand the stages of catabolism.

- Understand the meaning and significance of compartmentalization of metabolic pathways.
- Cite various examples of compartmentalization of metabolic pathways.
- Understand the functioning of glycerophosphate shuttle.
- Understand the functioning ofmalate-aspartate shuttle.
- Understand why ATP is considered to be the 'energy currency' of cells.
- Know about the use of reducing equivalents in cells.
- Know about the use of cofactors in enzyme function.
- Grow knowledge of intermediary metabolism in cells.
- Grow knowledge of mechanisms regulating metabolic pathways in living beings.

4.1 Introduction

Metabolism denotes all the biochemical processes that allow an organism to live, grow, reproduce, heal and adapt to its environment. Metabolism has two components: anabolism and catabolism. Anabolism refers to the process which builds molecules the body needs; it usually requires energy for completion. Catabolism refers to the process that breaks down complex molecules into smaller molecules; it usually releases energy for the organism to use. In this unit, various aspects of metabolism will be discussed.

4.2 Anabolism vs. catabolism

1. Anabolism refers to biochemical processes which build large and complex molecules needed by the body from simple molecules. Catabolism refers to biochemical processes which break down large molecules of the body into simple molecules.
2. Anabolic processes generally require energy for their completion. Catabolic processes generally release energy for utilization by the organism.
3. Anabolic processes are controlled by hormones like growth hormone, insulin, testosterone and estrogen. Catabolicprocesses are controlled by hormones like adrenaline, cortisol and glucagon.
4. Examples of anabolism include biosynthesis of polypeptides from many molecules of amino acids and glycogenesis or biosynthesis of glycogen from many molecules of glucose. Examples of catabolism include glycolysis or breakdown of a glucose molecule into two pyruvic acid molecules and glycogenolysis or breakdown of glycogen into many glucose molecules.

4.3 Stages of catabolism

Catabolism can be divided into 3 main stages. The three stages are as explained as follows :

Stage 1–Stage of Digestion: The large organic molecules like polysaccharides, proteins andfats are digested into their smaller components (monomer units) outside cells. This stage acts mainly on starch, cellulose or proteins or fats that cannot be directly absorbed by the cells. After digestion, starch and cellulose are broken down into glucose; proteins are broken down into different amino acids and fats are broken down into fatty acids and glycerol.

Stage 2 – Release of energy: Once the macromolecules are broken down into monomer units, the latter molecules are taken up by cells. Within the cells, those monomer units are converted into yet smaller molecules through different chemical pathways, along with the production of some amounts of NADH and $FADH_2$ molecules.

Stage 3 – Energy Stored: Finally, NADH and $FADH_2$ molecules enter into the mitochondria of cells. Inside the mitochondria, electrons from NADH and $FADH_2$ are transported through the 'electron transport chain' (ETC). The transported electrons are combined with H^+ ions (protons) and molecular oxygen to produce water while the energy liberated from the transport of electrons is used to produce high-energy ATP molecules from ADP and P_i (inorganic phosphate). ATP acts as the 'energy currency' of cells and provides energy required for various physiological functions carried out by living cells.

4.4 Compartmentalisation of metabolic pathways

4.4.1 Meaning and significance of compartmentalization :

Different metabolic reactions occurring in cells take place in different spaces or compartments, which are separated from other compartments by means of semi permeablemembranes. Such compartments help to separate the microenvironments (pH, ionic environment etc.) and optimise the course of different metabolic reactions. Different enzymes act under different optimal conditions; therefore, compartmentalization provides proper optimal conditions for the activities of the enzymes required for different metabolic reactions. Moreover, compartmentalization allows proper distribution of the machinery required for different metabolic reactions and thereby prevents chaos in running different metabolic reactions, for instance, the electron transport chain being located on the mitochondrial inner membrane

facilitates a systematic flow of electrons and production of ATP by means of oxidative phosphorylation.

4.4.2 Examples of compartmentalization of metabolic pathways :

Different cellular compartments for the occurrence of different metabolic reactions are as follows:

(a) Cytosol (cytoplasm without organelles) :

1) Metabolism of carbohydrates: glycolysis, part of gluconeogenesis, glycogenolysis, glycogenesis, pentose phosphate pathway.
2) Metabolism of fatty acids: fatty acid synthesis.
3) Metabolism of amino acids: synthesis of nonessential amino acids, some of the transamination reactions.
4) Other pathways: parts of heme and urea synthesis, metabolism of purines and pyrimidines.

(b) Mitochondria :

1) **Metabolism of carbohydrates :** part of gluconeogenesis (conversion of pyruvate to oxaloacetic acid).
2) **Metabolism of fatty acids :** beta-oxidation of fatty acids, synthesis and degradation of ketone bodies.
3) **Metabolism of amino acids :** oxidative deamination, some of the transamination reactions.
4) **Other pathways:** Krebs' cycle, electron transport chain and oxidative phosphorylation, parts of heme and urea synthesis.

(c) Rough endoplasmic reticulum :

1) Protein synthesis (translation of mRNA).
2) Posttranslational modifications of proteins.

(d) Smooth endoplasmic reticulum :

1) Triacylglycerol and phospholipid synthesis.
2) Fatty acid elongation (to a maximal length of 24 carbon atoms).
3) Part of steroid synthesis.
4) Biotransformation of xenobiotics.

(e) Golgi complex :

1) Glycosylation of proteins and lipids.

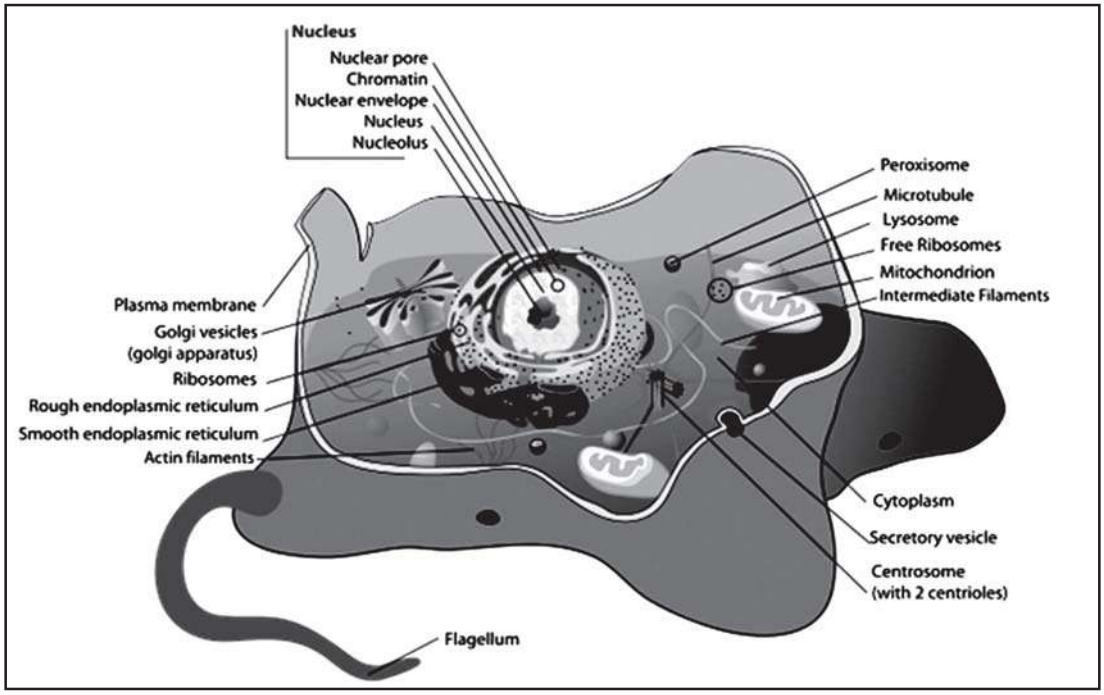
2) Protein sorting and formation of secretory vesicles.

(f) Lysosomes :

1) Hydrolysis of carbohydrates, proteins,lipids and nucleic acids.

(g) Peroxisomes :

1) Degradation of long-chain fatty acids (> 20 carbon atoms).



Schematic diagram of compartmentalized cell interior
(Taken from Wikimedia Commons — https://commons.wikimedia.org/ wiki/ File : Animal_cell_structure_en.svg)

4.5 Shuttle systems and membrane transporters

4.5.1 Definitions :

'Shuttle system'denotes cellular 'membrane transporter systems' whichtransfer the reducing equivalents (electron and H^+ ion) from NADH (which is produced during glycolysis in the cytosol or ground cytoplasm) into the mitochondria across the outer and inner mitochondrial membranes. The two main shuttle systems are (i) α-glycero phosphates huttle and (ii) malate-aspartate shuttle. The presence of shuttle systems is extremely important for the accomplishment of mitochondrial electron transport and ATP production - the inner mitochondrial membrane is impermeable to

NADH. Therefore, electrons and protons from NADH must enter into the inner mitochondrial chamber by the help of shuttle systems, or else electron transport and ATP production will stop.

4.5.2 Glycerophosphate shuttle :

(i) This shuttle system consists of two enzymes, glycerol 3-phosphate dehydrogenase of cytoplasm and flavoprotein dehydrogenase of the inner mitochondrial membrane. The first enzyme transfers H^+ and electron to dihydroxyacetone phosphate which thus gets reduced into glycerol 3-phosphate. (ii) Glycerol 3-phosphate diffuses through the outer mitochondrial membrane and reaches the outer surface of the inner mitochondrial membrane where it gives out H^+ and electron to the FAD part of flavin dehydrogenase; the FAD part thus gets reduced into $FADH_2$ while glycerol 3-phosphate is re-oxidized into dihydroxyacetone phosphate. (iii) Then, $FADH_2$ donates H^+ and electron to CoQ of mitochondrial electron transport chain.

4.5.3 Malate-aspartate shuttle :

(i) Cytoplasmic malate dehydrogenasetransfers H^+ and electron from NADH to oxaloacetate which thus gets reduced into malate. (ii) Then, malate á-ketoglutarate transporter of the inner mitochondrial membrane transports the malate from cytoplasm into the mitochondrion in exchange of mitochondrial á-ketoglutarate. (iii) Thereafter, mitochondrial malate dehydrogenase transfers H^+ and electron from the malate to NAD^+; malate is thus re-oxidized into oxaloacetate and NAD^+ gets reduced into NADH. (iv) Finally, NADH transfers H^+ and electron from itself to the FMN part of NADH dehydrogenase of mitochondrial electron transport chain.

4.6 ATP as ‘energy currency’ of cells

ATP or Adenosine triphosphate is a high-energy purine nucleotide found in all living cells. ATPpossesses two high-energy phosphate bonds in their molecules: the 3rdor g and the 2nd orbphosphate bonds of ATPare high-energy bonds while the 1st or aphosphate bond connected directly to the ribose sugar is a low-energy phosphate bond.The high-energy bonds of ATPor Adenosine triphosphateare hydrolyzed to provide energy for driving a large number of cellular processes.**Hydrolysis of 1 mole of ATP into ADP releases 30 kilo joules or 7 kilo caloriesof energy.**ATP is, therefore, called as the ‘energy currency’ of living cells. A few roles of ATP are cited below :

(i) Active transport of Na^+ and K^+:The enzyme Na^+-K^+-ATPase of plasma membrane catalyzes the hydrolysis of one ATP to ADP and Pi in one hand,

and the carrier-mediated active transports of three Na^+ ions outwardand two K^+ ions inward across the plasma membrane against their respective concentration gradients. This role of ATP is essential for maintaining the polarized state of the membrane, higher extracellular concentration of Na^+ and higher intracellular concentration of K^+.

(ii) Phosphorylation of substrates: In many metabolic pathways, substrates or intermediates are phosphorylated into low-energy phosphate compounds such as glucose 6-phosphate, fructose 1,6-bisphosphate and glycerol 3-phosphate by respective phosphotransferase enzymes utilizing the high-energy phosphate bond of ATP.

(iii) Cellular protein synthesis:The energy released from cleavage of ATP isutilized by amino acyl-tRNAsynthetase enzyme for the formation of amino acyl-tRNA, which represents the first step of biosynthesis of proteins in cells.

(iv) Other physiological functions:The cleavage ofATP also provides the energy necessary for contraction of muscles, movement of cilia and flagella, exocytosis and endocytosis.

4.7 Use of reducing equivalents and cofactors :

4.7.1 Use of reducing equivalents :

A reducing equivalent serves as the electron donor in a redox reaction (= oxidation-reduction reactionbetween two reactants)and becomes oxidized (loses electrons) when it donates an electron to an electron acceptor. A reducing equivalent can donate an electron in multiple forms: a lone electron, hydrogen atom, a hydride, or bond formation with an oxygen atom. Each form of reducing equivalent is characterized by the donation of at least one electron:

(i) Lone electrons : In redox reactions involving metal ions, lone electrons can be transferred from the electron donor to the electron acceptor. That is, no other atoms or protons are transferred along with the electron in the redox reaction. The following reaction between iron and copper is a typical example:

$$\underset{\text{Iron (electron donor)}}{Fe^{2+}} + \underset{\text{Copper (electron acceptor)}}{Cu^{2+}} \longrightarrow Fe^{3+} + Cu^{+}$$

Redox reaction with iron and copper ions

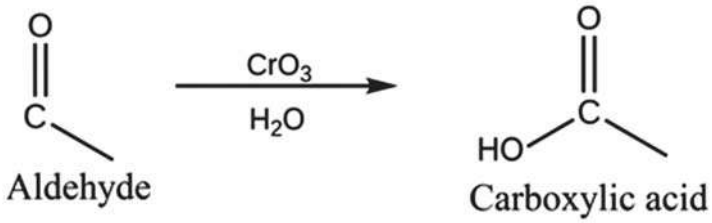
Iron ion serves as the reducing equivalent since it donates an electron to the copper ion.The iron and copper ion each begin the reaction with a double

positive charge ($^{2+}$). At the end of the reaction, the charge on copper decreases to a single positive charge ($^{1+}$) while the charge on iron increased to a triple positive charge ($^{3+}$). The alteration of charges is due to the transfer of a single electron from the iron atom to the copper atom. As a result, iron is oxidized because it loses one of its electrons resulting in a greater positive charge.

(ii) Hydrogen atom : A neutral hydrogen atom consists of one electron and one proton. Hydrogen's electronegativity is less than that of the atoms that hydrogen is commonly bound to, such as oxygen, nitrogen, carbon, or fluorine. When a hydrogen atom forms a covalent bond with a more electronegative atom, the latter will have a greater affinity for the electrons and pull the electrons away from hydrogen. When a highly electronegative atom binds to hydrogen atom, it is reduced because it gains the electrons involved in the covalent bond. Conversely, when an atom loses a hydrogen atom, it is oxidized because it loses electrons. For example, in the reaction involving Flavin Adenine Dinucleotide (FAD) and succinate, FAD is reduced to $FADH_2$ because it accepts two hydrogen atoms from succinate. Succinate serves as the reducing equivalent because it donates electrons to FAD in the form of hydrogen atoms and is itself oxidized.

(iii) Hydride : A hydride is a hydrogen anion that carries two electrons and one proton. The chemical species that accepts a hydride ion will be reduced because it gains the electrons from the hydride ion. The reduced form of Nicotinamide Adenine Dinucleotide (NADH) is a reducing equivalent that donates a hydride ion to an electron acceptor in complex I of the mitochondrial electron transport chain.

(iv) Bond formation with oxygen atom : A chemical species with a lower electronegativity than oxygen can serve as a reducing equivalent when it covalently binds to an oxygen atom. Oxygen is highly electronegative and will have a greater affinity for electrons in a covalent bond, resulting in the reduction of the oxygen atom. When an atom of lower electronegativity forms a bond with oxygen, it is oxidized because the electrons are pulled closer to oxygen and away from that atom. For instance, during the formation of carboxylic acid from the oxidation of an aldehyde, a carbon is oxidized through the formation of a covalent bond with oxygen :



Formation of carboxylic acid from oxidation of aldehyde

(v) Reducing equivalents in the mitochondrial electron transport chain : NADH and $FADH_2$ serve as reducing equivalents and donate electronsto the components of the mitochondrial electron transport chain.During aerobic oxidation, NADH and $FADH_2$ are produced in cells. NADH and $FADH_2$ enter into the mitochondria wherein electrons from these substances are transported through the 'electron transport chain' (ETC). The transported electrons are combined with H^+ ions (protons) and molecular oxygen to produce water while the energy liberated from the transport of electrons is used to produce ATP from ADP and P_i (inorganic phosphate) by the help of ATP synthetase. The overall process of ATP production is called 'oxidative phosphorylation', because the substrates (viz. NADH and $FADH_2$) give out electrons for transport through the ETC and thus, get oxidized while ADP gets phosphorylated or added with phosphate for formation of ATP.

4.7.2 Use of cofactors :

Sometimes, a metal ion instead of a coenzyme (non-protein organic compound) remains attached with the holoenzyme. Such a metal ion is called a cofactor. Enzymes to which the metal ions remain loosely bound are called metal-activated enzymes and enzymes to which the metal ions remain tightly bound are called metalloenzymes. Metal-activated enzymes contain alkali metals like Na^+ and K^+ or alkaline earth metals like Ca^{++} and Mg^{++}, but metalloenzymes contain transitional metals like Cu^{++}, Cu^+, Zn^{++} etc. Metal ions help in the action of enzymes in different ways :

(i) Metal ions may provide the proper three-dimensional conformation to the active site of an enzyme for binding to a substrate, e.g. pyruvate kinase requires Mg^{++} ions while it transfers the phosphate group from phosphoenolpyruvate to ADP for production of ATP.

(ii) A metal may help in substrate-binding by binding to the enzyme in one hand and to the substrate on the other, e.g. Enolase requires the help of Mg^{++} ions when it binds to 2-phosphoglycerate to produce phosphoenolpyruvate.

(iii) A metal ion may act as a donor or acceptor of electron to and from a substrate during an enzyme-catalyzed reaction, e.g. Cu^{++} ions of plasma ceruloplasmin (an enzyme) can oxidize Fe^{++} ions entering the plasma into Fe^{+++} ions.

4.8 Intermediary metabolism and regulatory mechanisms

4.8.1 Intermediary metabolism:

Intermediary metabolism refers to such metabolic reactions that form link between two different metabolic pathways. Some examples are cited below:

(i) Intermediary metabolism involving carbohydrate : Ordinarily glucose is catabolized in cells producing pyruvate (in aerobic condition) or lactate (in anaerobic condition). However, a part of glucose is converted to glycogen for stor-age, particularly, in liver and skeletal muscle. Again, the pentose phos-phate pathways arising from interme-diates of glycolysis is a source of re-ducing equivalents (2H) for biosyn-thesis of fatty acids and cholesteroland it is also a source of ribose which is important for nucleic acid biosynthesis. Moreover, glyceraldehydes-3-phosphate of glycolytic pathway is a precursor of glycerol of fat. Pyruvate of glycolytic pathway and different intermediates of Krebs' TCA cycle form various amino acids.

(ii) Intermediary metabolism involving fat : Oxidation of fatty acids results in the formation of variable numbers of acetyl-CoA molecules which combine with oxaloacetate and enter into TCA cycle for complete oxidation and production of energy.

(iii) Intermediary metabolism involving protein : Metabolism of different amino acids may yield pyruvate, acetyl-CoA or different intermediates of TCA cycle. For instance, serine converts into pyruvate by non-oxidative deamination; Ketogenic amino acids like leucine and tyrosine, after catabolism of their carbon-skeleton, produce acetoacetate which, in turn, converts into acetyl-CoA; Valine, isoleucine, methionine and threonine can change to succinyl-CoA due to metabolism of their carbon-skeleton. All such products may then undergo the final oxidation through TCA cycle along with production of energy. Again, metabolism of either the carbon-skeleton or the nitrogenous part of many amino acids produces gluconeogenic intermediates which are finally converted into glucose in the animal body.

4.8.2 Regulatory mechanisms:

The body of living beings possesses distinct regulatory mechanisms that regulate the rate of different metabolic pathways. A few examples are being cited here:

(a) Regulation of glycolysis :

(1) The rate-limiting step of glycolysis is production of fructose 1,6-diphosphate from fructose-6-phosphate by the catalytic action of phosphofructokinase. It has been found that physiological concentrations of ATP in cells tend to allosterically inhibit the activity of phosphofructokinase while physiological concentrations of AMP try to resist the inhibitory allosteric activity of ATP. So, a rise in ATP/AMP ratio in cells decreases while a rise in AMP/ATP ratio in cells increases the activity of phosphofructokinase and the rate of glycolysis. A 20% fall in ATP concentration raises the rate of glycolysis about tenfold.

(2) Glycogen phosphorylase, which initiates glycolysis in muscles by converting glycogen into glucose-1-phosphate, exists in 2 forms : (a) inactive phosphorylase B, which has no phosphates on their serine residues and (b) active phosphorylase A, which has phosphorylated serine residues. Phosphorylase B is changed to the active phosphorylase a by phosphorylase kinase in presence of ATP.

(3) Adrenaline enhances muscle glycolysis in 2 ways :

(a) In skeletal and cardiac muscles, adrenaline activates adenylatecyclase which converts ATP into PPi and cAMP. The cAMP activates a protein kinase called cAMP-dependent protein kinase, which then uses ATP to phosphorylate and change phosphorylase kinase B into an active phosphorylase kinase A. Phosphorylase kinase a uses ATP to phosphorylate and change glycogen phosphorylase B into an active glycogen phosphorylase A, which initiates glycolysis.

(b) In smooth muscles, adrenaline increases cytosolic Ca^{++} concentration. Ca^{++} ions bind to calmodulin subunits of phosphorylase kinase b, which thus gets activated and changes into an active phosphorylase kinase a without any need of phosphorylation. The kinase A phosphorylates glycogen phosphorylase B to generate an active glycogen phosphorylase A, which initiates glycolysis.

(4) Insulin secretion from the islets of Langerhans of pancreas increases when blood-glucose level rises. Insulin promotes glycolysis in 2 ways :

(a) Insulin enhances biosynthesis of phosphofructokinase and pyruvate kinase, which enhances the rate of glycolysis.

(b) Insulin also induces biosynthesis of hexokinase in kidney, brain and erythrocytes. Hexokinase catalyzes the first step of glycolysis.

(5) In skeletal muscles, AMP concentration rises during heavy exercise. AMP then allosterically activates glycogen phosphorylase B, which can then catalyze conversion of glycogen into glucose-1-phosphate in spite of its so-called inactive form.

(6) A rise in concentration of molecular O_2 depresses glycolysis in all aerobic cells. This is called Pasteur Effect. Aerobic metabolism through TCA cycle produces large amounts of ATP and citrate, which allosterically inhibit phosphofructokinase and reduces the rate of glycolysis.

(ii) Regulation of TCA cycle :

Three enzymes, viz., citrate synthase, isocitrate dehydrogenase and á-ketoglutarate dehydrogenase are the rate limiting enzymes of TCA cycle :

(a) Citrate synthase is activated by the rise of concentrations of its substrates like acetyl-CoA and oxaloacetate. On the other hand, the enzyme is competitively inhibited by its product citrate. Moreover, the enzyme is inhibited by ATP and NADH.

(b) Isocytrate dehydrogenase is activated by the rise of concentration of its substrate isocitrate. It is allosterically activated by ADP. Moreover, it is activated by the rise of intracellular concentration of Ca^{++} ions. On the other hand, the enzyme is inhibited by ATP and NADH.

(c) α-ketoglutarate dehydrogenase is activated by Ca^{++} ions, ADP and AMP. On the other hand, the enzyme is inhibited by ATP and NADH.

4.9 Questions (with hints to answers)

1. Define metabolism. Comapare anabolism and catabolism (see Sections 4.1 and 4.2).
2. Explain the stages of catabolism (see Section 4.3).
3. Give an account of the meaning and significance of compartmentalization of metabolic pathways (see Section 4.4.1).

4. Cite any four examples of compartmentalization of metabolic pathways (see Section 4.4.2).
5. What are shuttle systems and membrane transporters? Describe the functioning ofglycerophosphate shuttle and malate-aspartate shuttle(see Sections 4.5.1, 4.5.2 and 4.5.3).
6. Why is ATP considered to be the 'energy currency' of cells? (see Section 4.6).
7. What are reducing equivalents? Explain their different forms (see Section 4.7.1).
8. What are cofactors ? State their functional significance (see Section 4.7.2).
9. Define and exemplify intermediary metabolism (see Section 4.8.1).
10. Give a brief account of regulation of glycolysis (see Section 4.8.2).

4.10 References

1. Biochemistry – A. H. Lehninger (1975), Worth Publishers, New York.
2. Biochemistry – Debajyoti Das (2005), Academic Publishers, Kolkata.
3. Outlines of Biochemistry – Conn and Stumpf (1972), John Wiley, New York.
4. Principles of Biochemistry – Nelson and Cox (2005), Freeman & Co., New York.
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Unit-5 □ Carbohydrate Metabolism

Structure

5.0 Objectives

After studying the topics included in this chapter, you will be able to :

- Define carbohydrate metabolism.
- Have knowledge of glycolysis and its regulation.
- Develop gross knowledge of TCA cycle.
- Have knowledge of integration of carbohydrate, fat and protein metabolism through TCA cycle.
- Have knowledge of regulation of TCA cycle in aerobic cells.

- Have thorough knowledge of pentose phosphate pathway and its significance.
- Develop thorough knowledge of gluconeogenesis from a wide variety of substrates.
- Develop thorough concept of glycogenolysis with special reference to rate limiting steps.
- Have thorough knowledge of Glycogenesis.

5.1 Introduction

Carbohydrates consumed with food are hydrolyzed into monosaccharides in the small intestine and the monosaccharides are absorbed from the intestinal mucosa. The principal monosaccharide obtained from different carbohydrates is glucose. Another monosaccharide, fructose obtained from digestion of certain dietary carbohydrates is largely converted into glucose in the liver. Glucose is transported to different tissues of the body by means of blood circulation. Glucose undergoes chemical transformations in different tissues for either producing energy or for production of storage carbohydrate (glycogen) and various useful substances (glycoprotein, glycolipid and nucleic acid etc.) in the body. All kinds of chemical transformations of glucose in the body are called as carbohydrate metabolism.

The synthesis of glycogen from glucose in liver and muscles is called glycogenesis. On the other hand, the breakdown of glycogen in liver into glucose is called glycogenolysis. Glucose is utilized for energy production in all tissues of the body. When muscles work for very brief periods (less than 2 minutes), glucose is anaerobically converted into lactic acid with the production of only two molecules of ATP per molecule of glucose ; the process is called as anaerobic glycolysis. On the other hand, in all tissues of the body as well as in muscles working for a considerable period, glucose undergoes glycolysis in presence of oxygen and converts into pyruvic acid which enters into Krebs' TCA cycle for complete oxidation ; aerobic glycolysis followed by TCA cycle produces as many as 38 molecules of ATP per molecule of glucose. Those ATP molecules are used for various activities of different kinds of cells.

5.2 Glycolysis and its regulation

5.2.1 Process of glycolysis :

The anaerobic degradation of the 6-carbon sugar, glucose in the cytosol of living cells into two 3-carbon pyruvic acid or lactic acid alongwith production some amount of ATP molecules is called as glycolysis. The series of reactions in glycolysis is called Embden-Meyerho f-Parnas pathway or EMP pathway after the name of the discoverers.

Starting-points and end-points of EMP pathway : In liver, cardiac and skeletal muscles, glycolysis starts from glycogen as such, but in kidney, brain and

RBCs, it starts from glucose, as these tissues store little glycogen.

In strictly anaerobic condition, glycolysis ends in formation of lactic acid, but in aerobic cells, glycolysis ends in formation of pyruvic acid which then enters into TCA cycle for complete oxidation.

Embden-Meyerhof-Parnas pathway of glycolysis :

(1) Formation of glucose-6-phosphate : In liver, cardiac and skeletal muscles, glycogen reacts with inorganic phosphate under the catalytic action of glycogen phosphorylase. Glucose-1-phosphate is produced and it converts into glucose-6-phosphate by the catalytic action of phosphoglucomutase.

In brain, kidneys and RBCs, glucose is phosphorylated in presence of ATP with the help of hexokinase and glucose-6-phosphate is produced.

(2) Formation of fructose-6-phosphate : Then, glucose-6-phosphate is isomerised into fructose-6-phosphate by the catalytic action of phosphoglucose isomerase.

(3) Formation fructose-1, 6-diphosphate : Fructose-6-phosphate is then phosphorylated to fructose-1, 6-diphosphate in presence of ATP by the help of phosphofructokinase (PFK). Muscle PFK is a distinct isozyme, compared to that found in liver and RBCs and kidneys.

(4) Formation of glycerol dehyde-3-phosphate : Fructose-1, 6-diphosphate is then cleaved by the enzyme aldolase into two triose phosphates, glycerol dehyde-3-phosphate and dihydroxyacetone phosphate. Each has 3 carbons while a hexose has 6 carbons. Aldolase occurs as 3 isozymes in mammalian body ; aldolase A occurs in the muscles, aldolase B in liver and kidneys while aldolase C occurs in brain.

(5) Interconversion of dihydroxyacetone phosphate : Soon, dihydroxyacetone phosphate interconverts into glycerol dehyde-3-phosphate by the enzyme triose phosphate isomerase. Thus, in the long run, 2 glyceraldehyde-3-phosphates are produced from one fructose-1, 6-diphosphate.

(6) Formation of 1, 3-diphosphoglyceric acid : Each glycerol dehyde-3-phosphate is oxidized and phosphorylated in presence of NAD^+, H_2O and inorganic phosphate by the help of glyceraldehydes-3-phosphate dehydrogenase. The products are 1, 3-diphosphoglyceric acid, NADH and H^+ ion.

(7) Formation of 3-phosphoglyceric acid : An enzyme, phosphoglycerate kinase transfers the phosphate group of 1^{st} carbon of diphosphoglyceric acid to an ADP. Thus, 3-phosphoglyceric acid and an ATP are produced.

(8) Formation of 2-phosphoglyceric acid : 3-phosphoglyceric acid is then isomerised into 2-phosphoglyceric acid by the enzyme phospho-glyceromutase.

(9) Formation of phosphoenolpyruvic acid :2-phosphoglyceric acid is then dehydrated into phosphoenolpyruvic acid by the action of enolase.

(10) Formation of pyruvic acid : Then, pyruvic kinase transfers the phosphate group from phosphoenolpyruvic acid to an ADP. Thus, enolpyruvic acid and an ATP are produced. Enolpyruvic acid spontaneously changes into keto-pyruvic acid. Thus, from one initial glucose molecule, 2 pyruvic acid molecules, each with 3 carbons, are formed.

(11) Formation of lactic acid : Under strictly anaerobic conditions, each pyruvic acid is reduced in presence of NADH and H^+ by the help of lactate dehydrogenase. In consequence, lactic acid and NAD^+ are produced.

Comments :

(1) The enzyme hexokinase, phosphor fructokinase, phosphoglycerate kinase and enolase require Mg^{++} ions for their activities.

(2) Pancreatic insulin stimulates glycolysis by inducing the synthesis of hexokinase, phosphofructokinase and pyruvate kinase.

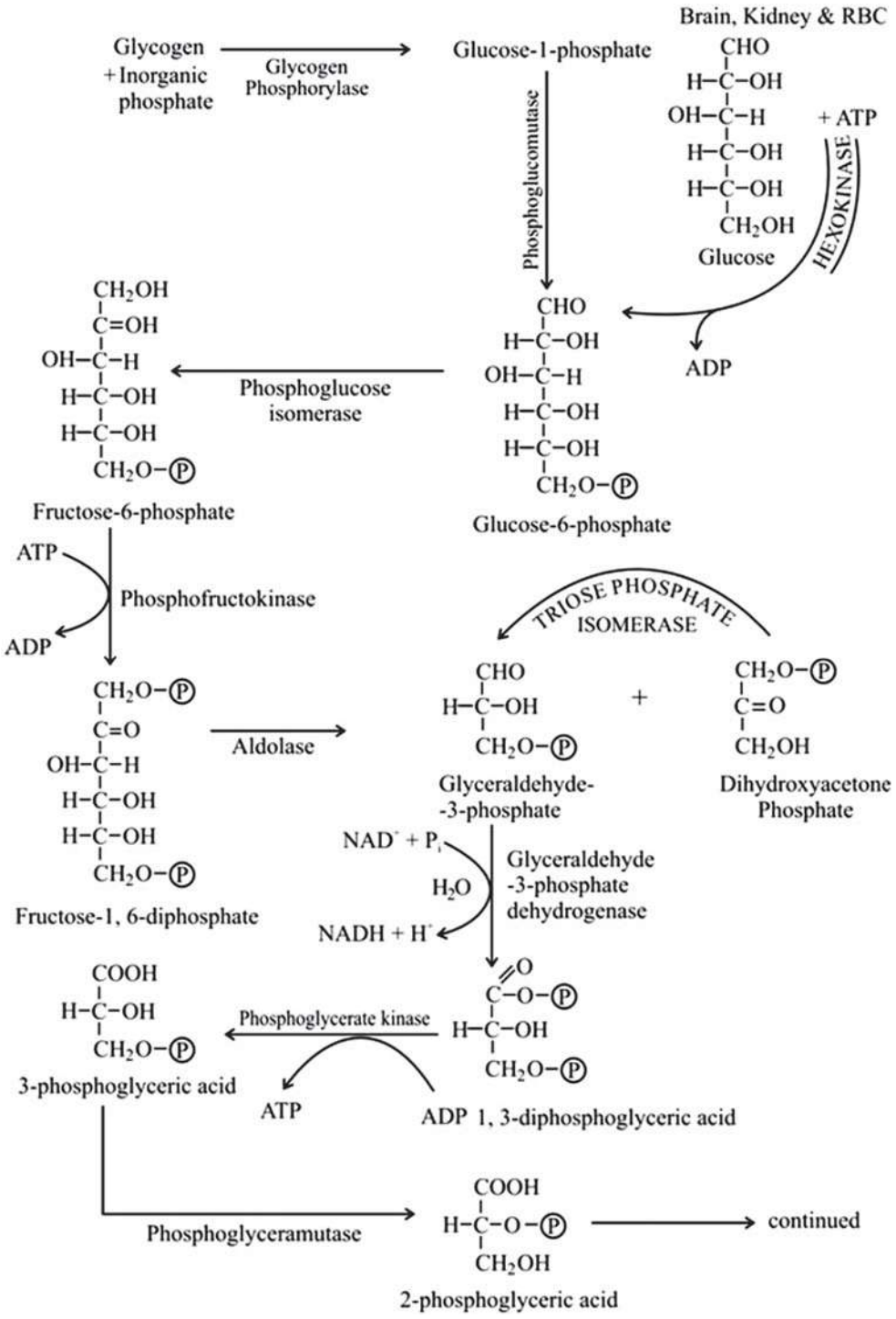
(3) Adrenal medullary adrenalin promotes glycolysis by activating muscle glycogen phosphorylase.

(4) Glycolysis and lactate formation are depressed in presence of high amount of oxygen ; this is called as Pateur effect.

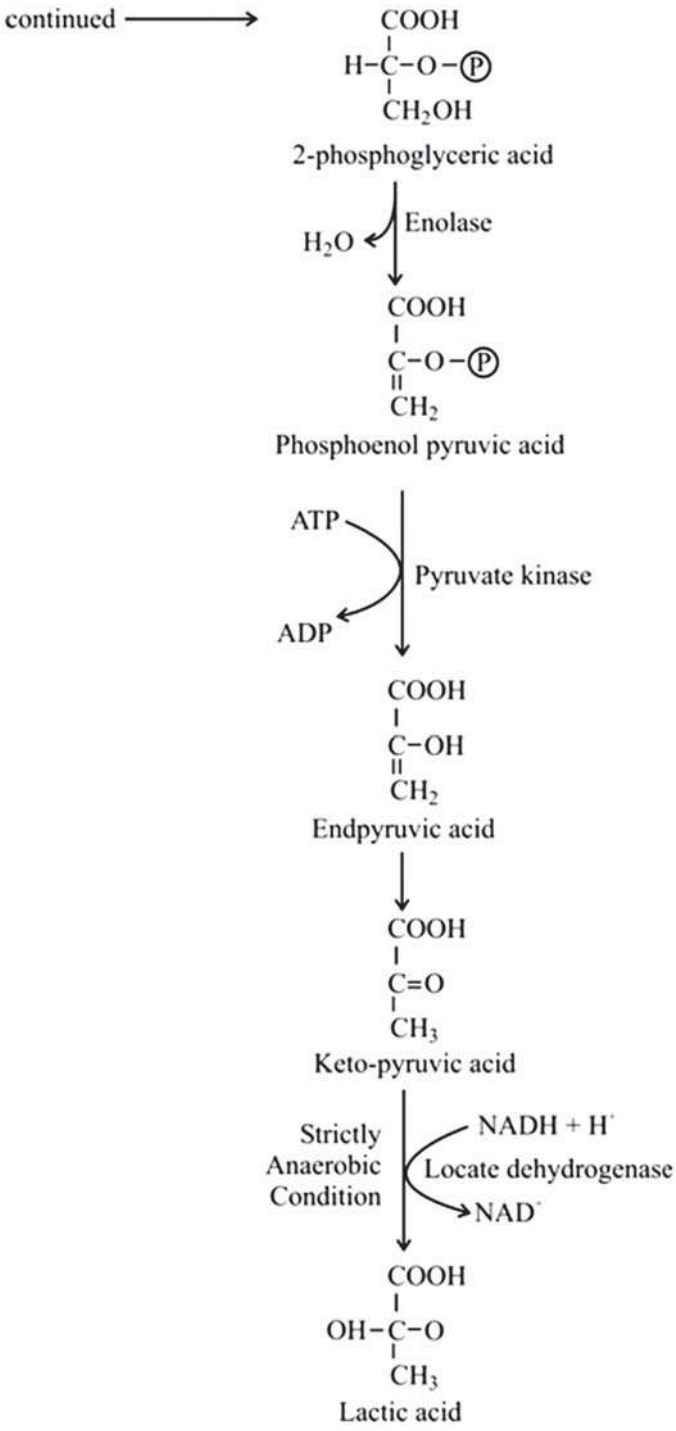
(5) Genetic deficiency of glycogen phosphorylase in muscles lead to type V glycogenosis and that of phosphofructokinase in muscles lead to type VII glycogenosis ; both are characterized by reduced glycolysis and high glycogen in muscles.

Production of high-energy phosphate bonds or ATP in glycolysis :

Reaction	**Enzyme**	**ATP produced**	
		In presence of O_2	**In absence of O_2**
1. 1, 3-diphosphoglyceric acid→ 3- phosphoglyceric acid	Phosphoglycerate kinase	1×2 = 2	1×2 = 2
2. Phosphoenol pyruvic acid→ enolpyruvic acid	Pyruvate kinase	1×2 = 2	1×2 = 2
3. NADH → NAD^+	Mitochondrial respiratory-chain enzymes	3×2 = 6 Total = 10	0 Total = 4
Utilisation of ATP: (1) Glucose → Glucose-6- phosphate = (2) Fructose-6-phosphate→Fructose-1, 6-diphosphate =		 = 1 = 1	 = 1 = 1
Net Gain of ATP :		= 8	= 2



Process of glycolysis (contd. to next page)



Process of glycolysis (contd. from previous page)

5.2.2 Regulation of glycolysis:

(1) The rate-limiting step of glycolysis is production of fructose 1,6-diphosphate from fructose-6-phosphate by the catalytic action of phosphofructokinase. It has been found that physiological concentrations of ATP in cells tend to allosterically inhibit the activity of phosphofructokinase while physiological concentrations of AMP try to resist the inhibitory allosteric activity of ATP. So, a rise in ATP/AMP ratio in cells decreases while a rise in AMP/ATP ratio in cells increases the activity of phosphofructokinase and the rate of glycolysis. A 20% fall in ATP concentration raises the rate of glycolysis about tenfold.

(2) Glycogen phosphorylase, which initiates glycolysis in muscles by converting glycogen into glucose-1-phosphate, exists in 2 forms : (a) inactive phosphorylase b, which has no phosphates on their serine residues and (b) active phosphorylase a, which has phosphorylated serine residues. Phosphorylase b is changed to the active phosphorylase a by phosphorylase kinase in presence of ATP.

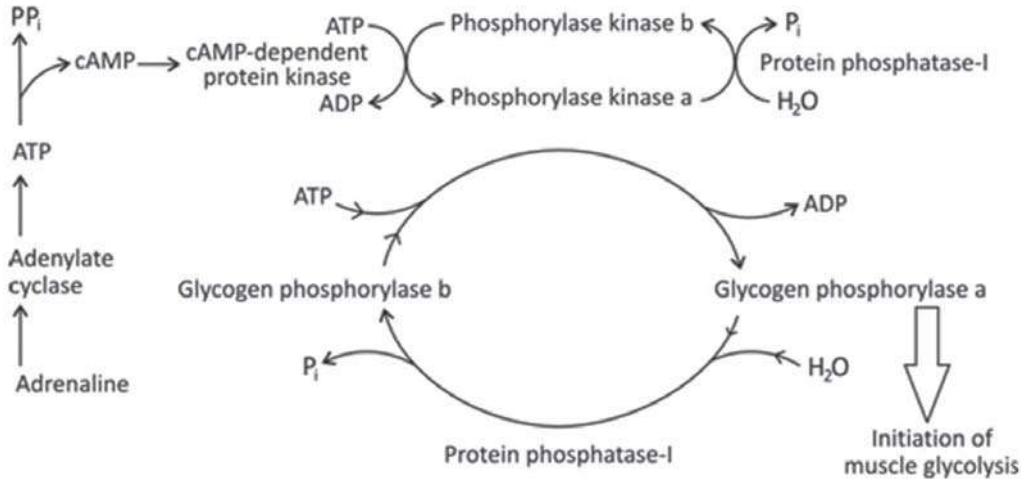
The enzyme phosphorylase kinase itself occurs in an inactive form called as phosphorylase kinase b and an active form called as phosphorylase kinase a. The inactive kinase b changes into the active kinase a when serine residues of the former get phosphorylated by cAMP-dependent protein kinase. The active phosphorylase kinase a then phosphorylates glycogen phosphorylase b, producing the active glycogen phosphorylase a. This enhances the rate of glycolysis in muscles [Fig. 7(iv)].

On the other hand, an enzyme, protein phosphatase-I can cleave phosphate groups from phosphoserine and can thus change glycogen phosphorylase a into the inactive glycogen phosphorylase b and can also change phosphorylase kinase a into the inactive phosphorylase kinase b.

(3) Adrenaline enhances muscle glycolysis in 2 ways :

(a) In skeletal and cardiac muscles, adrenaline activates adenylate cyclase which converts ATP into PPi and cAMP. The cAMP activates a protein kinase called cAMP-dependent protein kinase, which then uses ATP to phosphorylate and change phosphorylase kinase b into an active phosphorylase kinase a. Phosphorylase kinase a uses ATP to phosphorylate and change glycogen phosphorylase b into an active glycogen phosphorylase a, which initiates glycolysis.

(b) In smooth muscles, adrenaline increases cytosolic Ca^{++} concentration. Ca^{++} ions bind to calmodulin subunits of phosphorylase kinase b, which thus gets activated and changes into an active phosphorylase kinase a without any need of phosphorylation. The kinase a phosphorylates glycogen phosphorylase b to generate an active glycogen phosphorylase a, which initiates glycolysis.



Regulation of muscle glycogen phosphorylase

(4) Insulin secretion from the islets of Langerhans of pancreas increases when blood-glucose level rises. Insulin promotes glycolysis in 2 ways :

(a) Insulin enhances biosynthesis of phosphofructokinase and pyruvate kinase, which enhances the rate of glycolysis.

(b) Insulin also induces biosynthesis of hexokinase in kidney, brain and erythrocytes. Hexokinase catalyzes the first step of glycolysis.

(5) In skeletal muscles, AMP concentration rises during heavy exercise. AMP then allosterically activates glycogen phosphorylase b, which can then catalyze conversion of glycogen into glucose-1-phosphate in spite of its so-called inactive form.

(6) A rise in concentration of molecular O_2 depresses glycolysis in all aerobic cells. This is called Pasteur Effect. Aerobic metabolism through TCA cycle produces large amounts of ATP and citrate, which allosterically inhibit phosphofructokinase and reduces the rate of glycolysis. Aerobic metabolism also uses up ADP and Pi for ATP production; depletion of ADP and Pi lowers the activity of phosphoglycerate kinase and pyruvate kinase.

(7) Certain chemicals may inhibit glycolysis by inhibiting the activity of some particular enzymes, e.g. Fluoride inhibits the activity of enolase while iodoacetate inhibits the activity of glyceraldehyde-3-phosphate dehydrogenase.

5.3 Citric Acid Cycle :

5.3.1 Description of TCA Cycle :

Pyruvic acid produced in the cytosol of cells from glycolytic breakdown of glucose undergoes a final oxidation through the TCA cycle or Tricarboxytic acid cycle inside the mitochondria. TCA cycle is a cyclic series of reactions, occurring in

presence of oxygen, inside the mitochondrial matrix of living, aerobic cells. The cycle was originally described by Sir Hans Krebs in 1937 and hence, it is also called as Krebs' cycle or Krebs' TCA cycle. The cycle is described below [Fig.7(i)] :

(i) Pyruvic acid passes from cytosol to mitochondrial matrix where it undergoes oxidative decarboxylation in presence of NAD^+ and also reacts with coenzyme A (CoA.SH) with the help of a multienzyme complex, called pyruvate dehydrogenase system, resulting in the formation of acetyl-CoA, CO_2, NADH and H^+.

(ii) Now, acetyl-CoA enters into TCA cycle. It combines with oxaloacetate of the cycle and water to form citrate by the help of citrate synthase enzyme.

(iii) Citrate is then isomerised into isocitrate by the help of aconitase.

(iv) Isocitrate gets dehydrogenated and decarboxylated in presence of NAD^+ with the help of isocitrate dehydrogenase to yield a-ketoglutarate, CO_2, NADH and H^+.

(v) a-ketoglutarate then undergoes dehydrogenation and decarboxylation in presence of NAD^+ and CoA.SH by the help of a-ketoglutarate dehydrogenase system. Succinyl-CoA, CO_2, NADH and H^+ are produced.

(vi) Succinyl-CoA is then hydrolyzed in presence of GDP, P_i and water by the help of succinate thiokinase. Succinate, CoA.SH and GTP are produced.

(vii) Succinate is then dehydrogenated in presence of FAD by the help of succinate dehydrogenase. Fumarate and $FADH_2$ are produced.

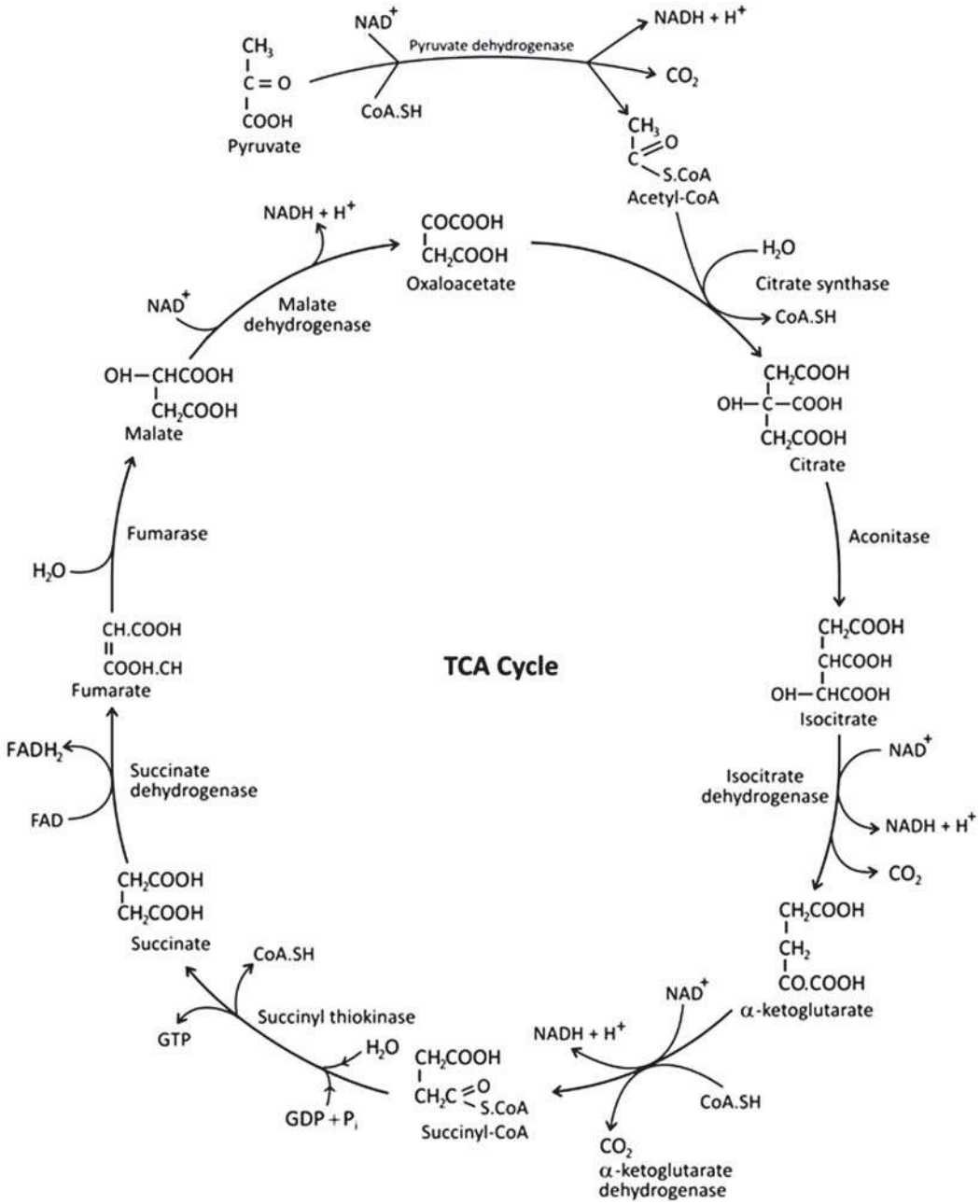
(viii) Fumarate then combines with water by the help of fumarase and malate is produced.

(ix) Finally, malate is dehydrogenated in presence of NAD^+ by the help of malate dehydrogenase. Oxaloacetate, NADH and H^+ are produced. Oxaloacetate is recycled for further continuation of TCA cycle.

Energy output : The complete oxidation of each molecule of pyruvic acid is accompanied by production of 15 molecules of high-energy phosphates (ATP). Of these 15 molecules, 3 are formed when the pyruvate gives rise to acetyl CoA and the other 12 are produced when acetyl CoA is oxidized through the Krebs cycle.

Now, 2 molecules of pyruvic acid are produced in glycolytic breakdown of 1 molecule of glucose. So, 30 (or 15×2) molecules of high-energy phosphate (ATP) are produced during the complete oxidation of those 2 pyruvates.

Further, 8 molecules of high-energy phosphates (ATP) are produced during glycolytic breakdown of glucose (production of 2 pyruvates from a glucose molecule). So, the net gain of high-energy phosphates during the complete oxidation of a glucose molecule (starting from glycolysis upto the end of Krebs' cycle) is 8+30 = 38 molecules of high-energy phosphates or ATP.



Krebs' Tricarboxylic Acid Cycle

5.3.2 Anabolic role of the cycle :

Different intermediates produced in TCA cycle are used in many synthetic reactions of various cells. These synthetic reactions indicate an anabolic role of TCA cycle :

(a) Fatty acid biosynthesis : In hepatocytes, adipocytes and lactating mammary gland cells, acetyl-CoA is utilized in *de novo* synthesis of fatty acids in the cytosol. Mainly palmitic acid is synthesized from acetyl-CoA.

(b) Cholesterol synthesis : Acetyl-CoA is also used for extra-mitochondrial synthesis of cholesterol.

(c) Biosynthesis of heme : Succinyl-CoA is used in erythroid cells and hepatocytes for the biosynthesis of heme. A mitochondrial enzyme called ALA-synthase condenses succinyl-CoA with glycine to produce á-amino-â-ketoadipic acid which is soon decarboxylated into ?-aminolevulinic acid (ALA). Finally, ALA is used for synthesis of heme.

(d) Gluconeogenesis : Malate and oxaloacetate may be utilized for gluconeogenesis.

(e) Amino acid biosynthesis : á-ketoglutarate and oxaloacetate are transaminated by specific transaminase enzymes into glutamic acid and aspartic acid, respectively.

5.3.3 Regulation of TCA cycle :

Three enzymes, viz., citrate synthase, isocitrate dehydrogenase and á-ketoglutarate dehydrogenase are the rate limiting enzymes of TCA cycle :

(a) Citrate synthase is activated by the rise of concentrations of its substrates like acetyl-CoA and oxaloacetate. On the other hand, the enzyme is competitively inhibited by its product citrate. Moreover, the enzyme is inhibited by ATP and NADH.

(b) Isocytrate dehydrogenase is activated by the rise of concentration of its substrate isocitrate. It is allosterically activated by ADP. Moreover, it is activated by the rise of intracellular concentration of Ca^{++} ions. On the other hand, the enzyme is inhibited by ATP and NADH.

(c) α-ketoglutarate dehydrogenase is activated by Ca^{++} ions, ADP and AMP. On the other hand, the enzyme is inhibited by ATP and NADH.

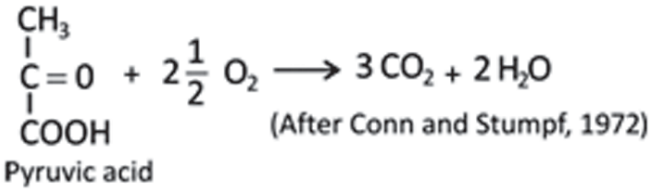
5.3.4 integration of carbohydrate, protein and lipid metabolism through tca cycle :

TCA cycle or 'Tricarboxylic acid cycle' is a cyclic series of reactions, occurring in presence of oxygen inside the mitochondrial matrix of living cells. The cycle was originally described by Sir Hans Krebs in 1937 to account for the oxidation of pyruvic acid into CO_2 and water.

In fact, TCA cycle serves as a common pathway for the final oxidation of all food-stuffs like carbohydrates, proteins and fats. Pyruvic acid produced from glycolytic degradation of glucose, acetyl CoA produced from oxidation of fatty acids and various products of catabolism of different amino acids are finally oxidized through TCA cycle. Thus, TCA cycle is a 'metabolic pool' through which the metabolism of all kinds of food-stuffs is integrated [Fig. 7(ii)].

Link between carbohydrate metabolism and TCA cycle :

The final oxidation of pyruvic acid produced in the cytosol from glycolytic degradation of glucose occurs through TCA cycle, resulting in yield of CO_2 and water (E. E. Conn and P. K. Stumpf, Outlines of Biochemistry, 3rd edition, 1972, John Wiley, New jersey] :



The steps are as follows :

(i) Pyruvic acid passes from cytosol to mitochondrial matrix and undergoes oxidative decarboxylation in presence of NAD^+ and also reacts with coenzyme A with the help of a multienzyme complex, called pyruvate dehydrogenase system, resulting in the formation of acetyl-CoA, CO_2, NADH and H^+.

(ii) Acetyl-CoA enters into TCA cycle. It combines with oxaloacetate of the cycle and water to form citrate by the help of citrate synthase.

(iii) Citrate is isomerised into isocitrate by the help of aconitase.

(iv) Isocitrate gets dehydrogenated and decarboxylated in presence of NAD^+ with the help of isocitrate dehydrogenase to yield a-ketoglutarate, CO_2, NADH and H^+.

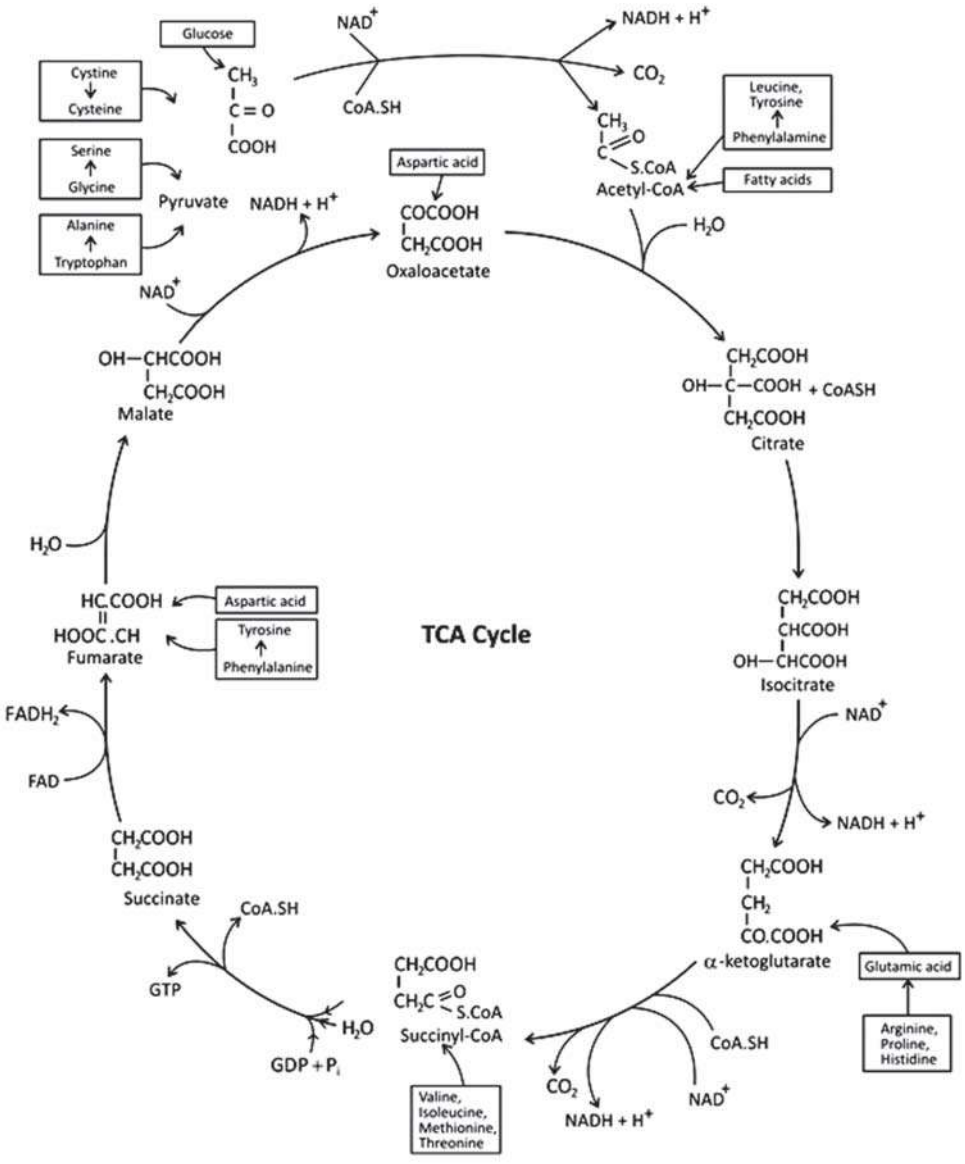
(v) α-ketoglutarate undergoes dehydrogenation and decarboxylation in presence of NAD^+ and CoA.SH by the help of α-ketoglutarate dehydrogenase system. Succinyl-CoA, CO_2, NADH and H^+ are produced.

(vi) Succinyl-CoA is hydrolyzed in presence of GDP, P_i and water by the help of succinate thiokinase. Succinate, CoA.SH and GTP are produced.

(vii) Succinate is dehydrogenated in presence of FAD by the help of succinate dehydrogenase. Fumarate and $FADH_2$ are produced.

(viii) Fumarate combines with water by the help of fumarase and malate is produced.

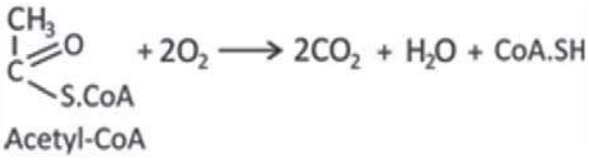
(ix) Finally, malate is dehydrogenated in presence of NAD^+ by the help of malate dehydrogenase. Oxaloacetate, NADH and H^+ are produced. Oxaloacetate is recycled for further continuation of TCA cycle.



Integration of carbohydrate, protein and lipid metabolism through TCA cycle

Link between fat metabolism and TCA cycle :

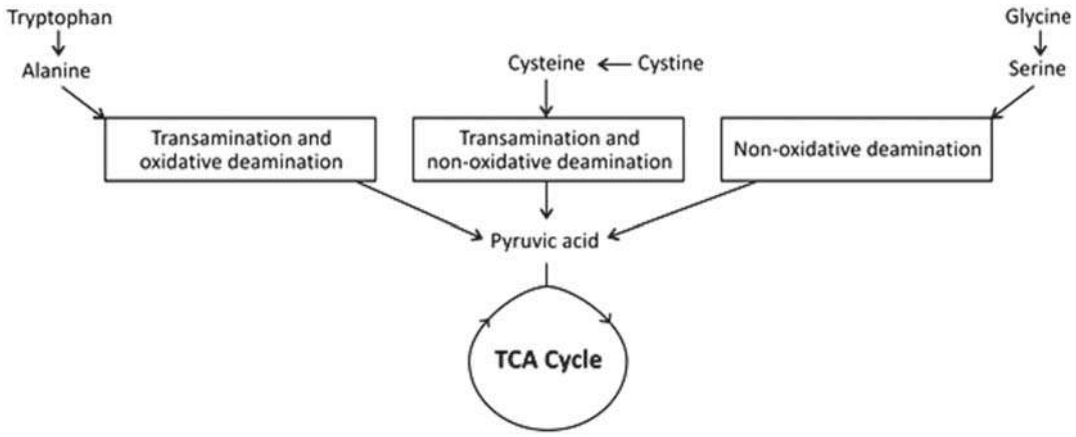
Oxidation of fatty acids results in the formation of variable numbers of acetyl-CoA molecules which combine with oxaloacetate and enter into TCA cycle for complete oxidation as shown below :



Link between protein metabolism and TCA cycle :

Metabolism of different amino acids may yield pyruvate, acetyl-CoA or different intermediates of TCA cycle. All such products may then undergo the final oxidation through TCA cycle :

(1) Formation of pyruvate from amino acids : Cystine breaks into two cysteines which then give rise to pyruvates by both transamination and oxidative deamination. Serine converts into pyruvate by non-oxidative deamination. Tryptophan and glycine may convert into alanine and serine, respectively and the latter two amino acids then convert into pyruvate by non-oxidative deamination. Finally, pyruvate is oxidized through TCA cycle [Fig. 7(iii)].



Formation of pyruvate from amino acids

(2) Formation of acetyl-CoA from amino acids : Ketogenic amino acids like leucine and tyrosine, after catabolism of their carbon-skeleton, produce acetoacetate which, in turn, converts into acetyl-CoA. Phenylalanine can change to tyrosine which later produces acetyl-CoA. Finally, acetyl-CoA is oxidized through TCA cycle.

(3) Formation of α-ketoglutarate from amino acids : Glutamic acid changes to ?-ketoglutarate by transamination. Again, histidine, arginine, proline and ornithine may give rise to glutamic acid by catabolism of their carbon-skeleton and thereafter, glutamic acid produces α-ketoglutarate which finally, enters into TCA cycle for oxidation.

(4) Formation of succinyl-CoA from amino acids : Valine, isoleucine, methionine and threonine can change to succinyl-CoA due to metabolism of their carbon-skeleton. Then, succinyl-CoA is oxidized through TCA cycle inside the mitochondria.

(5) Formation of fumarate from amino acids : Metabolism of the carbon-skeleton of tyrosine can produce fumarate; phenylanine may change to tyrosine which in turn can produce fumarate. Asparatic acid may give rise to fumarate during urea synthesis and purine synthesis. Fumarate is then oxidized through TCA cycle.

(6) Formation of oxaloacetate from amino acids : Transamination of aspartic acid produces oxaloacetate which then may enter into TCA cycle for continuation of the cycle.

Comments :

(i) Thus, TCA cycle serves as a 'common final pathway' through which the final oxidation of all kinds of foodstuffs, viz., carbohydrates, proteins and fats, takes place.

(ii) The number of high-energy phosphate produced depends on which substrate gets oxidized through TCA cycle. When pyruvate is oxidized, 15 high-energy phosphates are produced per pyruvate. If an acetyl-CoA is oxidized, 12 high-energy phosphates are produced. Again, 9, 6 and 3 high engery phosphates are produced from oxidation of α-ketoglutarate, succinyl-CoA and fumarate, respectively, through TCA cycle.

(iii) Besides, TCA cycle not only acts as a catabolic pathway. Different anabolic pathways may start from the intermediates of TCA cycle. For instance, citrate may be used for synthesis of fatty acids and steroids; ?-ketoglutarate and oxaloacetate may be used for synthesis of different amino acids; again, ?-ketoglutarate, succinyl-CoA, fumarate and oxaloacetate may be used for gluconeogenesis.

5.4 Pentose Phosphate Pathway

The principal route of carbohydrate metabolism in most animal tissues involves glycolytic pathway coupled with oxidation in the TCA cycle. However, the 'Pentose

phosphate pathway' or 'Hexose monophosphate shunt' or 'Warburg-Dickens-Lipman pathway' or 'Phosphogluconate pathway' provides an alternative route of carbohydrate metabolism in certain tissues.

Occurrence : Pentose phosphate pathway occurs in the adrenal cortex, testis, liver, adipose tissue, lactating mammary gland, leucocytes and to some extent, in the RBCs. The reactions occur in the cytosol of cells.

5.4.1 Detailed account of the pathway :

(A) Oxidative phase : Formation of pentose phosphates from glucose-6-phosphate :

Step I : At first, glucose-6-phosphate is oxidized by dehydrogenation to 6-phosphogluconolactone by glucose-6-phosphate dehydrogenase in presence of Mg^{++} ions and $NADP^{+}$. After the reaction, $NADP^{+}$ is reduced to NADPH.

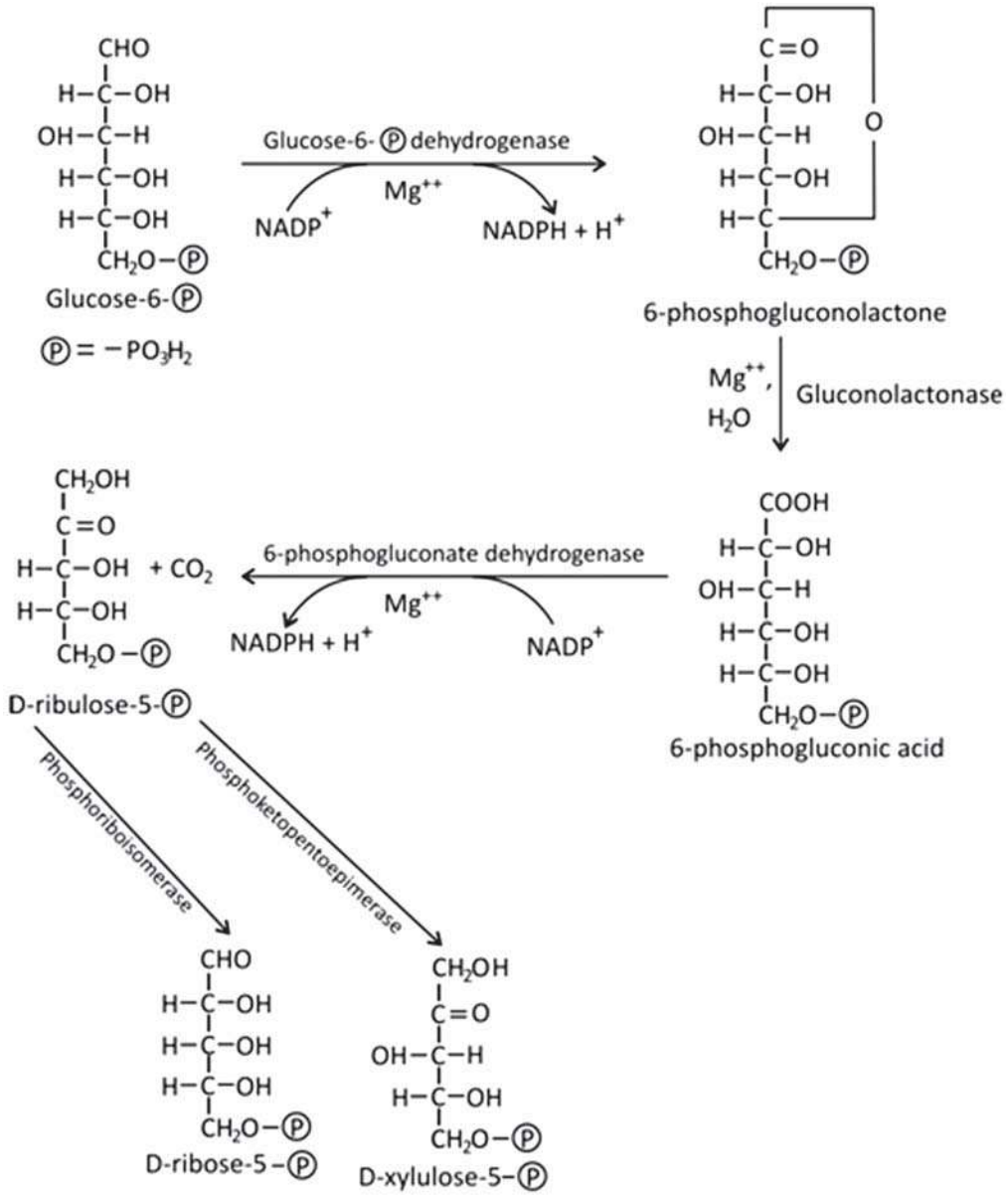
Step II : Now, 6-phosphogluconolactone is hydrolysed into 6-phosphogluconic acid by the action of gluconolactonase in presence of Mg^{++} ions and water.

Step III : Then, 6-phosphogluconic acid is oxidized by dehydrogenation and also decarboxylated to form a pentose phosphate called D-ribulose-5-phosphate by the help of 6-phosphogluconate dehydrogenase in presence of Mg^{++} ions and $NADP^{+}$. After the reaction, CO_2 and NADPH are also produced.

Step IV : (a) In one hand, D-ribulose-5-phosphate is isomerised to D-ribose-5-phosphate by the enzyme phosphoriboisomerase.

(b) On the other hand, D-ribulose-5-phosphate is isomerised into D-xylulose-5-phosphate by the enzyme phosphoketopentoepimerase.

Comment : In this way, starting from 3 molecules of glucose-6-phosphate are initially produced 3 molecules of D-ribulose-5-phosphate and 3 molecules of CO_2. Then, one D-ribulose-5-phosphate converts into D-ribose-5-phosphate while the rest two convert into two D-xylulose-5-phosphates.



Oxidative phase of pentose phosphate pathway : Formation of pentose phosphate

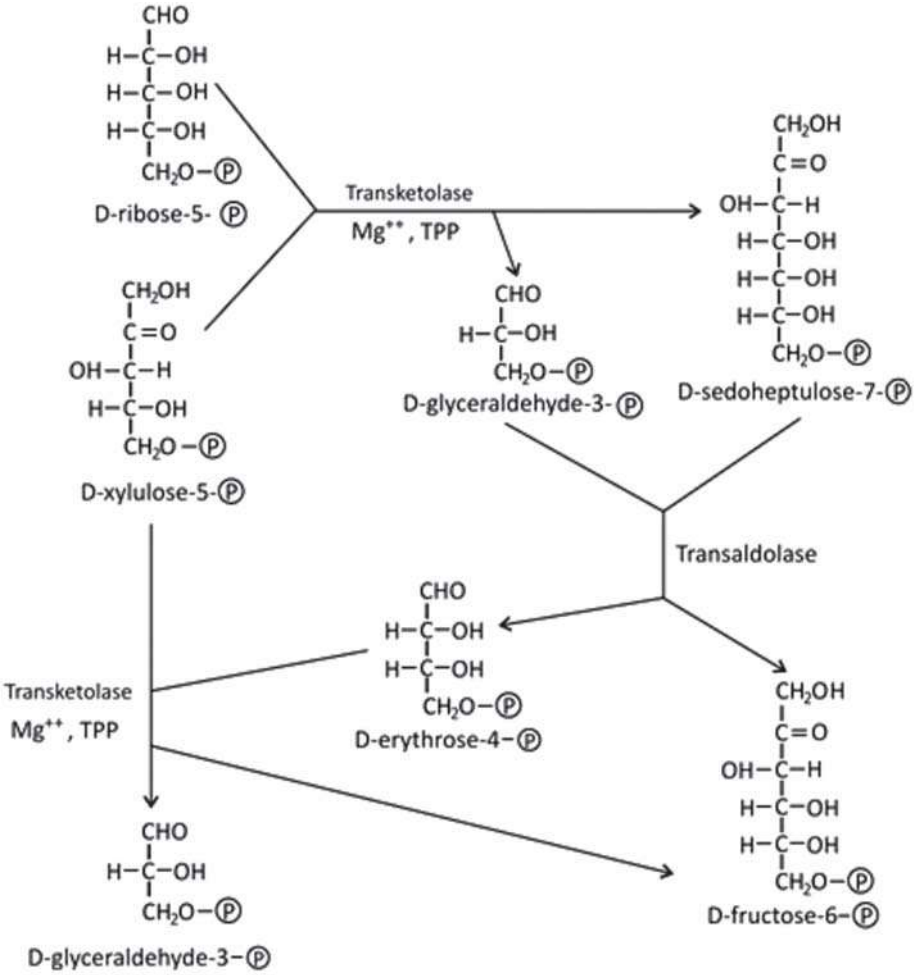
(B) Non-oxidative phase : Conversion of pentose phosphates into hexose phosphates:

Step I : (a) In presence of Mg^{++} ions and TPP (thiamine pyrophosphate), a transketolase enzyme transfers the ketol group ($CO\text{-}CH_2OH$) from D-xylulose-5-phosphate to the 1st carbon of D-ribose-5-phosphate. As a result, D-sedoheptulose-

7-phosphate (with 7 carbons) and D-glyceraldehyde-3-phosphate (with 3 carbons) are produced.

(b) D-sedoheptulose -7-phosphate and D-glyceraldehyde-3-phosphate immediately react with each other under the catalysis of transaldolase to form D-fructose-6-phosphate (with 6 carbons) and D-erythrose-4-phosphate (with 4 carbons).

Step II : Now, the remaining D-xylulose-5-phosphate reacts with D-erythrose-4-phsphate under the catalysis of transketolase in presence of Mg^{++} ions and TPP. The ketol group ($CO\text{-}CH_2OH$) of the former is transferred to the 1st carbon of the latter. As a result, a D-fructose-6-phosphate and a D-glyceraldehyde-3-phosphate are formed.



Non-oxidative phase of pentose phosphate pathway :
Conversion of pentose phosphates into hexose phosphates

Net products : Thus, originally starting from 3 molecules of glucose-6-phosphate, the following are obtained : (i) 2 molecules of D-fructose-6-phosphate, (ii) 1 molecule of D-glyceraldehyde-3-phosphate, (iii) 3 molecules of CO_2 and (iv) 6 molecules of NADPH. The first two [(i) and (ii)] are produced in the non-oxidative phase and the last two [(iii) and (iv)] in the oxidative phase of the pentose phosphate pathway.

Comment : Of the above products, fructose-6-phosphate may be isomerised into glucose-6-phosphate by phosphoglucose isomerase and glucose-6-phosphate may be then recycled for further continuation of the pentose phosphate pathway.

5.4.2 Significance of pentose phosphate pathway :

(i) This pathway provides an alternative route of carbohydrate metabolism, which is independent of glycolysis and TCA cycle.

(ii) This pathway helps in production of pentose phosphates and their inter-conversion with hexose phosphates.

(iii) Ribose-5-phosphate produced by this pathway may be used for synthesis of nucleic acids.

(iv) NADPH obtained from the pathway may be used as a reducing agent during fatty acid and cholesterol biosynthesis.

(v) NADPH is used in RBCs for reduction of 'oxidized glutathione' into 'reduced glutathione' by the enzyme glutathione reductase. Reduced glutathione is then used by glutathione peroxidase for removal of H_2O_2, which tends to oxidize haemoglobin into methemoglobin and reduce the life-span of RBCs.

(vi) NADPH obtained from this pathway may be oxidized into NADP in mitochondria by electon-transport chain. During the oxidation of each NADPH, 3 ATP molecules are produced. When one glucose-6-phosphate enters into pentose phosphate pathway, only one of its carbons is oxidized and 2 NADPH are produced. A glucose-6-phosphate shall enter into the pathway six times for oxidation of all its six carbons and this will produce 12 NADPH. Mitochondrial oxidation of 12 NADPH will be accompanied by production of $12 \times 3 = 36$ ATP molecules, which is comparable to that obtained from glycolysis-TCA cycle. However, NADPH is rarely oxidized in the mitochondria.

5.5 Gluconeogenesis

The formation of carbohydrates in animal body from non-carbohydrates like glycerol, lactate, pyruvate and amino acids is called gluconeogenesis. During gluconeogenesis, some of the steps of glycolysis or TCA cycle may simply be reversed by the same enzymes catalyzing forward reactions or by separate enzymes.

5.5.1 Gluconeogenesis from glycerol :

Occurrence : Gluconeogenesis from glycerol is accomplished through a chain of reactions in the cellular cytosol in liver and kidneys.

Process :

(i) Glycerol is phosphorylated in presence of ATP to L-a-glycerophosphate with the help of glycerokinase.

(ii) L-a-glycerophosphate is then oxidized in presence of NAD^+ to either dihydroxyacetone phosphate or glyceraldehyde-3-phosphate with the help of a-glycerophosphate dehydrogenase.

(iii) Dihydroxyacetone phosphate and glyceraldehydes-3-phosphate combine with the help of aldolase B to form fructose-1, 6-diphosphate.

(iv) Fructose-1, 6-diphosphate is then hydrolysed by Fructose-1, 6-diphosphatase to yield fructose-6-phosphate and inorganic phosphate.

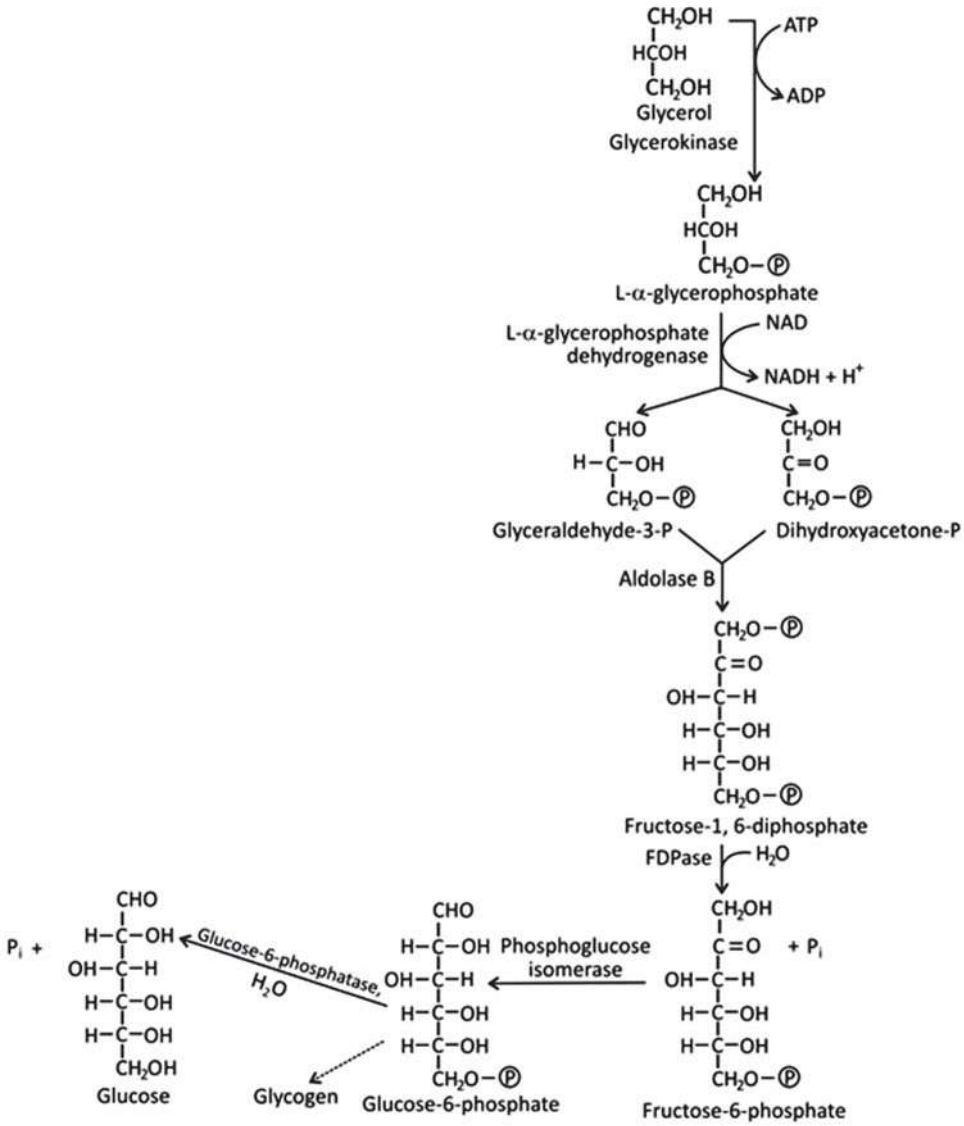
(v) Fructose-6-phosphate is isomerised into glucose-6-phosphate by phosphoglucose isomerase.

(vi) Glucose-6-phosphate then changes into either glucose by glucose-6-phosphatase (not hexokinase) or glycogen by the process of glycogenesis.

Comments :

(i) Adrenal glucocorticoids and adrenaline, and pancreatic glucagon stimulate gluconeogenesis from glycerol by inducing the synthesis of fructose-1, 6-diphosphatase.

(ii) Pancreatic insulin depresses the process by acting as a repressor of fructose-1, 6-diphosphatase.



Gluconeogenesis from glycerol

5.5.2 Gluconeogenesis from lactate :

Occurrence : Gluconeogenesis occurs from lactate in the liver and kidney; some of the reactions occur within cytosol but some others take place inside the mitochondria.

Process :

(i) Lactic acid produced in glycolysis is oxidized back to pyruvic acid in presence of NAD^+ with the help of lactate dehydrogenase in cytosol.

(ii) Pyruvic acid is then converted to phosphoenol pyruvic acid in a complex way; pyruvate kinase that changes phosphoenol pyruvic acid into pyruvic acid during glycolysis cannot catalyse the reverse reaction :

(a) Pyruvic acid enters the mitochondria where ATP-dependent pyruvate carboxylase condenses pyruvic acid with CO_2 to form oxaloacetic acid.

(b) Oxaloacetic acid is reduced by mitochondrial malate dehydrogenase in presence of NADH and H^+. Malic acid and NAD^+ are produced.

(c) Malic acid is transported to cytosol by dicarboxylate transporter of mitochondrial membrane. It is now oxidized back to oxaloacetic acid in presence of NAD^+ by malate dehydrogenase of cytosol.

(d) Thereafter, oxaloacetic acid is both decarboxylated and phosphorylated in presence of GTP by phosphoenolpyruvate carboxykinase (PEP carboxykinase) of cytosol to yield phosphoenolpyruvic acid, GDP and CO_2.

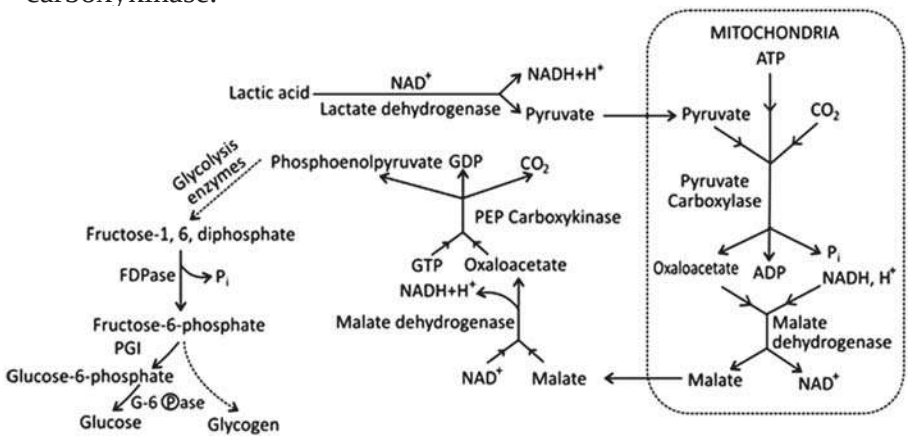
(iii) Phosphoenolpyruvic acid is next changed to fructose-1, 6-diphosphate by reversal of glycolysis steps with the same enzymes that catalyse the forward reactions.

(iv) Fructose-1, 6-diphosphate is hydrolysed by fructose-1, 6-diphosphatase to yield fructose-6-phoshate which subsequently changes into either (i) glucose-6-phosphate by phosphoglucose isomerase ; glucose-6-phosphate is changed to glucose by glucose-6-phosphatase, or (ii) glycogen through the process of glycogenesis.

Comments :

(1) Adrenal glucocorticoids promote gluconeogenesis from lactate by inducing synthesis of PEP carboxykinase.

(2) Pancreatic insulin suppresses the process by suppressing synthesis of PEP carboxykinase.



Gluconeogenesis from lactate

5.5.3 Gluconeogenesis from propionate :

Occurrence : In ruminant mammals, dietary cellulose is converted into lower fatty acids like acetic, propionic and butyric acids due to fermentation caused by microflora in rumen and reticulum of the stomach. These fatty acids, especially propionic acid, after absorption from intestine, convert into glucose by gluconeogenesis in liver and kidneys. The reactions occur partly within the mitochondria and partly in the cytosol of cells.

Process :

(1) Inside the mitochondria, propionic acid is converted into propionyl-CoA in presence of ATP and Mg^{++} ions by the help of acetate thiokinase.

(2) Propionyl-CoA is carboxylated in presence of CO_2, ATP, Mg^{++} ions and biotin by the help of propionyl-CoA carboxylase ; biotin acts as the coenzyme for this enzyme. The product is D-methylmalonyl-CoA.

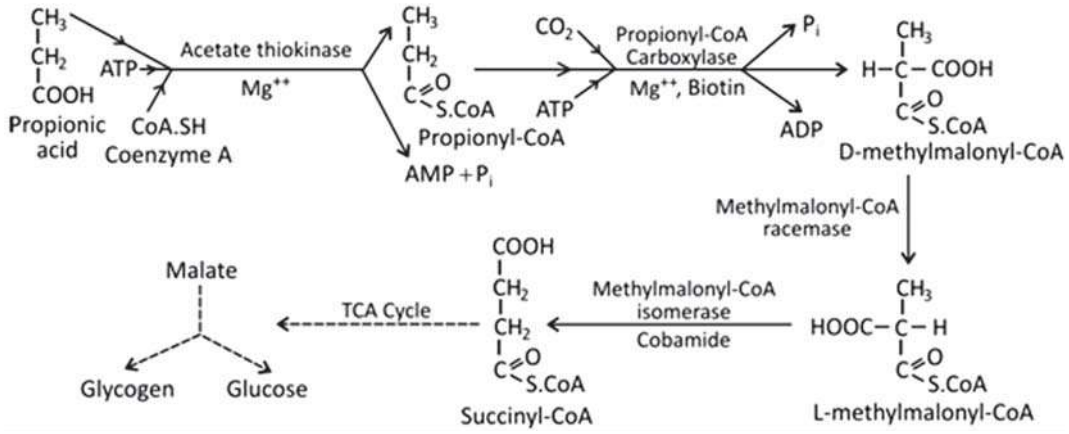
(3) D-methylmalonyl-CoA is now changed by mitochondrial methylmabnyl-CoA recemase to its L-isomer, L-methylmalonyl-CoA.

(4) L-methylmalonyl-CoA is converted to succinyl-CoA by the help of methylmalonyl-CoA isomerase containing cobamide as its coenzyme.

(5) Succinyl-CoA is then converted to malate through TCA cycle inside the mitochondria.

(6) Malate is then transferred to the cytosol where it changes into either glucose or glycogen as it happens in case of gluconeogenesis from lactic acid.

Comment : Deficiency of methylmalonyl-CoA isomerase owing to gene mutation may lead to the disease methylmalonic aciduria characterized by rise in urinary methylmalonate, acidosis and fall in blood pH.



Gluconeogenesis from propionate

5.5.4 Gluconeogenesis from amino acids :

Metabolism of either the carbon-skeleton or the nitrogenous part of many amino acids produces gluconeogenic intermediates which are finally converted into glucose in the animal body. Normally, a small amount of glucose is produced from amino acids but during starvation, amino acids serve as the main source of glucose.

Occurrence : The process occurs mainly in liver and to some extent, in the kidneys.

Process :

(1) Cysteine and alanine give rise to pyruvate through either transamination or deamination; serine produces pyruvate through deamination. Again, tryptophan and glycine may change to alanine and serine, respectively and the latter two amino acids in turn produce pyruvate. Normally, alanine is the largest source of glucose among amino acids.

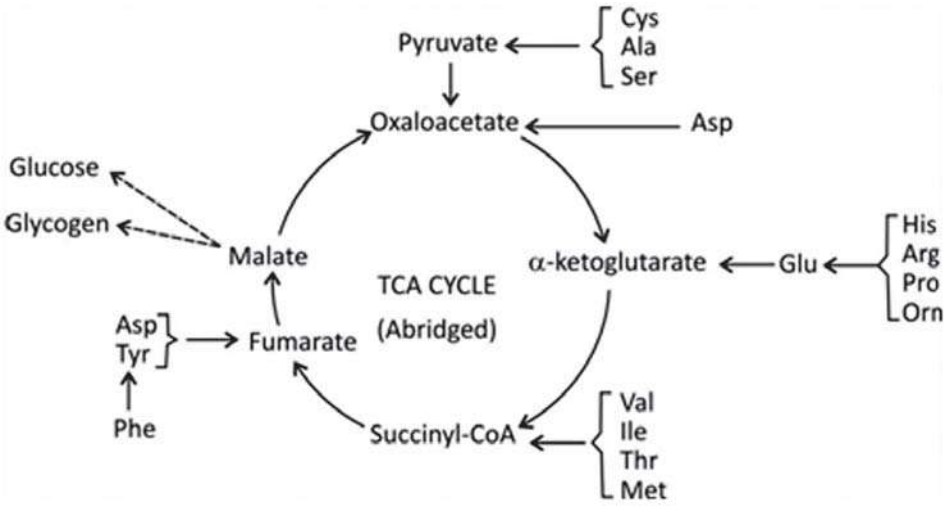
Inside the mitochondria, pyruvate changes to oxaloacetate by the action of pyruvate carboxylase. Some amount of oxaloacetate is produced from aspartic acid through transamination. Oxaloacetate is then changed to malate through TCA cycle.

(2) Glutamic acid can produce a-ketoglutarate by transamination. Again, histidine, arginine, proline and ornithine give rise to glutamic acid due to metabolism of their carbon-skeleton and thereafter, glutamic acid produce a-ketoglutarate by deamination, which in turn changes to malate through TCA cycle in mitochondria.

(3) Valine, isoleucine, methionine and threonine can change to succinyl-CoA due to metabolism of their carbon-skeleton. Then, succinyl-CoA changes to malate through TCA cycle in mitochondria.

(4) The metabolism of the carbon-skeleton of tyrosine and aspartic acid produces fumarate; phenylalanine may change to tyrosine which in turn changes to fumarate. Fumarate is then changed to malate through TCA cycle in mitochondria.

(5) Finally, malate passess out to the cytosol and gradually convert into either glucose or glycogen.



Gluconeogenesis from amino acids

Comments : (i) In normal condition, gluconeogenesis occurs mainly from lactate or pyruvate. During starvation, gluconeogenesis occurs mainly from amino acids (alanine is the largest source of glucose among amino acids). (ii) In both normal and starving conditions, the rate of gluconeogenesis is about ten times higher in the liver than in kidneys.

5.6 Glycogenolysis

The breakdown of the animal storage polysaccharide, glycogen into glucose is called as 'glycogenolysis'. An account follows :

Occurrence : Glycogenolysis occurs mainly in the liver and to some extent, in the kidneys. Although glycogenesis occurs in muscles, no glycogenolysis occurs in muscles, since muscles lack glucose-6-phosphatase enzyme which is essential for glycogenolysis. Brain too does not have this enzyme.

Structure of glycogen : Glycogen has a highly branched molecular structure. 10 to 15 α-D-glucose units are joined by 1,4-glycosidic bonds to form short straight-chains. Many such chains are joined with one another by 1,6-glycosidic bonds while the glucose residues in the chains remain connected by 1,4-glycosidic bonds.

Events in glycogenolysis :

Phase I : Liberation of glucose-1-phosphates and traces of glucose :

(i) A reaction between glycogen and inorganic phosphate is catalysed by glycogen phosphorylase. Initially, the enzyme occurs as inactive phosphorylase b. Pancreatic glucagon and adrenal medullary adrenalin raises

the level of cyclic-AMP in hepatic cells. The increased cAMP activates dephosphophosphorylase kinase which in turn adds two phosphates from two ATPs to phosphorylase b and converts it into active phosphorylase a.

Phosphorylase a phosphorolytically splits the 1, 4-glycosidic bonds between the glucose residues in a branched chain of glycogen, one at a time, starting from the free end of the chain. As a result, glucose-1-phosphates are liberated, one at a time. The enzyme acts until only 4 glucose residues remain attached to the branch-point and is again gets inactivated by phosphophosphorylase phosphatase.

(ii) Now, a second enzyme, oligo (α-1,4 $\rightarrow$ α-1,4)-glucan transferase hydrolytically splits the 3rd 1,4-glycosidic bond from the free end of the shortened branch and transfers the cut out trisaccharide to some other branch of the glycogen molecule. Thus, only one glucose residue remains attached by 1,6-glycosidic bond to the branch-point.

(iii) Then, a third enzyme, the debranching enzyme or amylo-1,6-glucosidase hydrolytically splits the 1,6-glycosidic bond at the branch-point a free glucose is liberated.

Phase-II : Formation of glucose-6-phosphates :

Glucose-1-phosphate produced by the action of glycogen phosphorylase a is isomerised into glucose-6-phosphate by phosphoglucomutase in presence of Mg^{++} ions.

Phase-III : Liberation of free glucose :

Finally, glucose-6-phosphate is hydrolysed into free glucose and inorganic phosphate. The reaction is catalysed by glucose-6-phosphatase in presence of Mg^{++} ions.

Rate limiting steps in glycogenolysis :

The conversion of glycogen into glucose-1-phosphate by glycogen phosphorylase is the rate limiting step in glycogenolysis. The activity of glycogen phosphorylase is increased by adrenaline and glucagon. Glycogen phosphorylase can exist in two forms : An inactive form called glycogen phosphorylase b and an active form called glycogen phosphorylase a. The inactive form of the enzyme is converted into the active form by adrenalin and glucagon in the following way :

Adrenaline and glucagon activate adenylate cyclase of cell membrane, which converts ATP into PPi and cAMP. The increased cAMP activates dephosphophosphorylase kinase which in turn adds two phosphates from two ATPs to phosphorylase b and converts it into active phosphorylase a, which initiates glycogenolysis.

Moreover, adrenaline increases the release of Ca^{++} ions from endoplasmic reticulum into the cytosol of hepatocytes. The binding of Ca^{++} ions with a cytoplasmic protein calmodulin causes activation of calmodulin-sensitive phosphorylase kinase which in turn phosphorylates the inactive glycogen phosphorylase b and to convert it into an active glycogen phosphorylase a, which initiates glycogenolysis.

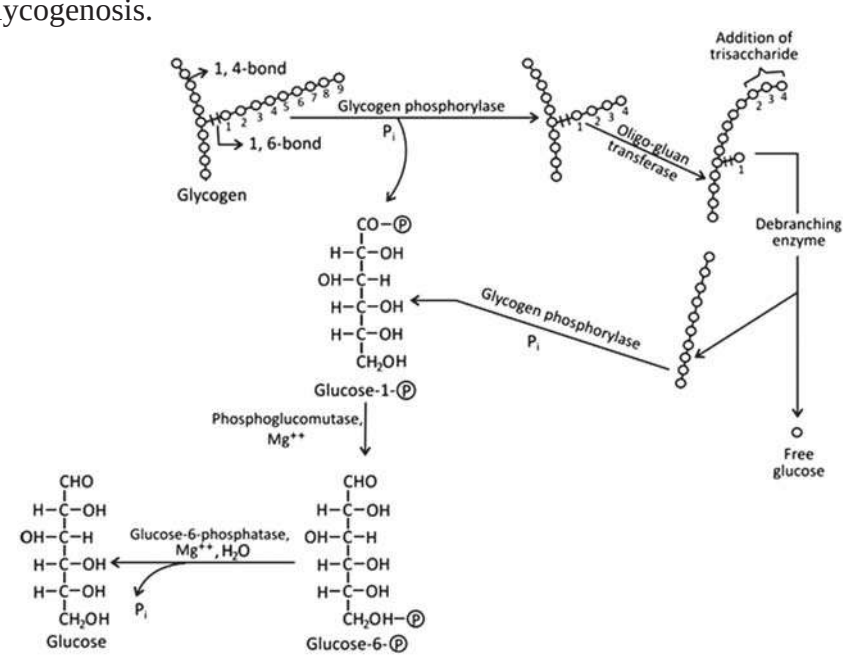
Significance : Glycogenolysis represents an essential part of carbohydrate metabolism in the animal body. The liberated glucose passes to blood for transport to different tissues for its oxidation and production of energy.

Conclusion :

(i) Hormones like pancreatic glucagon and adrenal medullary adrenaline promote glycogenolysis by activating glycogen phosphorylase.

(ii) Hormones like glucocorticoids of adrenal cortex and thyroxine of thyroid also promote glycogenolysis by stimulating glucose-6-phosphatase activity.

(iii) Gene mutations may lead to inborn failure of glycogenolysis, leading to high hepatic glycogen concentration and hypoglycemia. The condition is called as 'glycogenosis'. Deficiency of glycogen phosphorylase is known as type VI glycogenosis ; deficiency of debranching enzyme is called as type III or partial glycogenosis ; deficiency of glucose-6-phosphatase is known as type I glycogenosis.



Reactions in Glycogenolysis

5.7 Glycogenesis

The synthesis of glycogen, which is the storage polysaccharide of animals, from hexose sugars is called glycogenesis. Glycogenesis occurs mainly from glucose.

Occurrence : Glycogenesis occurs mainly in liver and muscles. Some amount of glycogenesis also takes place in spleen, testis and intestine, but no glycogenesis occurs in brain and kidney.

Process :

(i) Formation of glucose-6-phosphate : At first, glucose gets phosphorylated at its C_6 in presence of ATP and Mg^{++} ions by the help of either hexokinase or glucokinase. Glucokinase predominates over hexokinase in liver while hexokinase predominates over glucokinase in muscles and extrahepatic tissues. Interestingly, glucokinase can catalyze glycogenesis when blood sugar level is above 100 mg/100 ml but hexokinase can do so, even when the blood sugar level is much lower. It so happens, because glucokinase has a much higher K_m (5 mM of glucose) than that of hexokinase (50 µm of glucose), which means that glucokinase has a much lower affinity for glucose, compared to hexokinase.

(ii) Formation of glucose-1-phosphate : Then, phosphoglucomutase acts on glucose-6-phosphate in presence of Mg^{++} ions. At first, glucose-6-phosphate accepts a phosphate from a phosphoserine residue at the active site of the enzyme itself, resulting in formation of glucose-1, 6-biphosphate and dephosphorylated enzyme. Then, the 2 products react further with each other so that the phosphate from C_6 of glucose-1, 6-biphosphate is returned to the enzyme. Consequently, glucose-1-phosphate and the original enzyme are produced.

(iii) Formation of UDP-glucose : Then, glucose-1-phosphate reacts with UTP or uridine triphosphate under the catalytic action of UDP-glucose pyrophosphorylase. Two terminal phosphates of UTP are released as PPi or inorganic pyrophosphate while glucose-1-phosphate occupies their place to form UDP-glucose or uridine diphospho-glucose. A pyrophosphatase enzyme soon hydrolyzes the PPi into 2 inorganic phosphates (Pi).

(iv) Elongation and branching of glycogen chain : This step is complex :

(a) At first, a primer protein called glycogenin and having some enzymatic power of itself catalyzes the binding of a glucose residue of a UDP-glucose to a tyrosine residue of itself. The C_1 of glucose binds to the OH group of the tyrosine.

(b) Then, glycogenin catalyzes the binding of 7 more glucose residues from 7 more UDP-glucose molecules. Each time, the C_1 of a glucose residue binds to the C_4 of a glycogenin-bound glucose. In this way, a total of 8 glucose residues get linked to glycogenin.

(c) Then, the catalytic activity of glycogenin stops and glycogen synthase begins to add 12-14 more glucose residues (from UDP-glucose molecues) to the glycogenin-bound oligosaccharide. Each time, a 1,4-glycosidic linkage is formed, i.e. the C_1 of a new glucose residue is linked with the C_4 of the terminal glucose of the glycogenin-bound oligosaccharide.

(d) Thereafter, a branching enzyme (Amylo-1,4 $\rightarrow$ 1,6-transglycosylase) cuts out an oligosaccharide (6-8 glucose unit-long) from the terminus of a glycogenin-bound glucose chain and joins the cut-out oligosaccharide to any other point of the chain by means of a 1,6-glycosidic linkage. In this way, repeated actions of glycogen synthase and branching enzyme produce a highly branched glycogen molecule.

Significance :

(i) Glycogenesis allows storage of excess carbohydrates of the body in the liver in the form glycogen which may be broken into glucose whenever the body needs a large amount of energy.

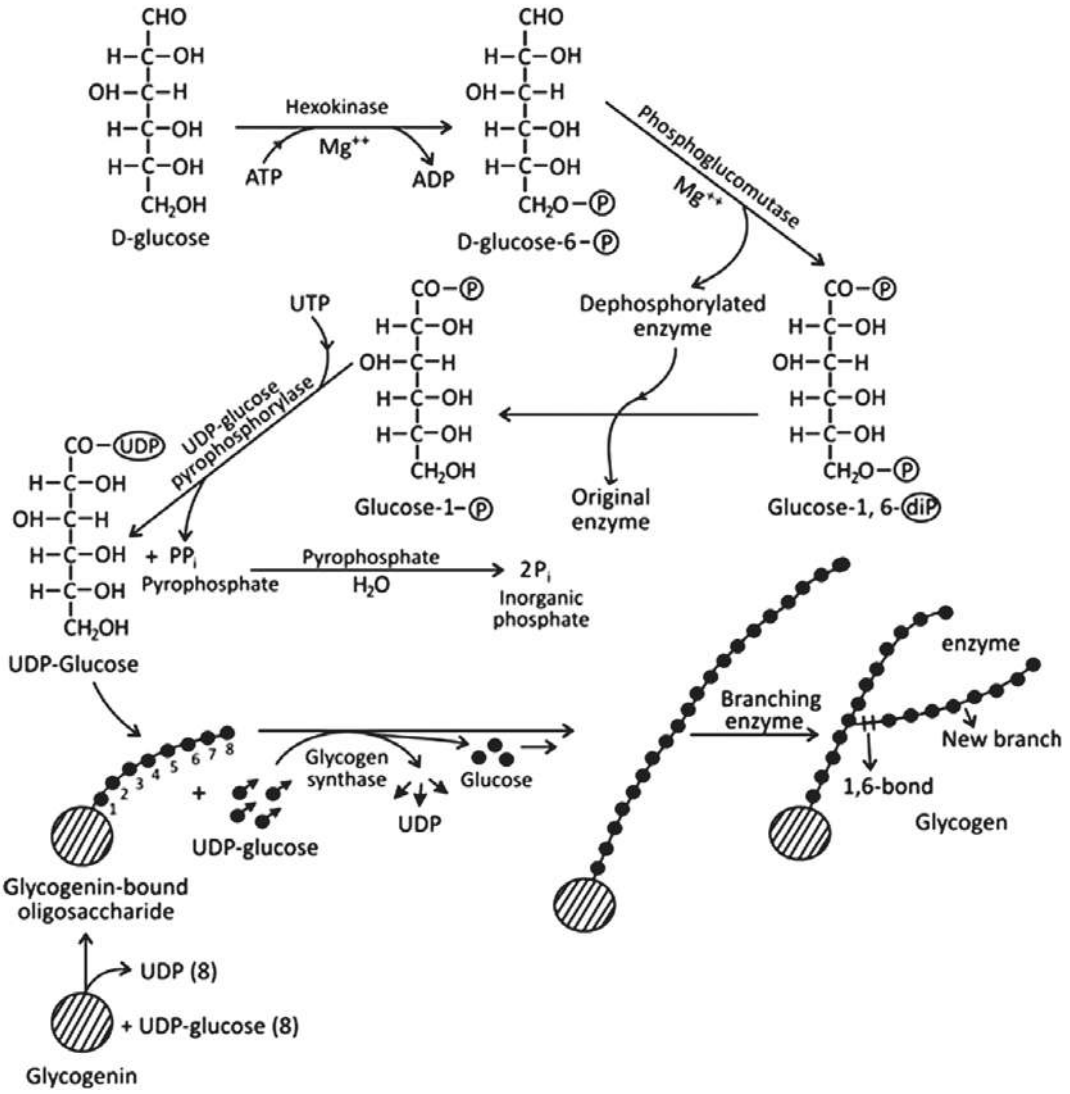
(ii) Glycogenesis prevents any undesirable rise of blood glucose level.

Regulation :

(i) Reversible phosphorylation and dephosphorylation modulates the activity glycogen synthase. The active enzyme called 'glycogen synthesis a' exists in dephosphorylated from. It is changed into an inactive 'glycogen synthase b' due to phosphorylation of its 7 serine residues in presence of ATP by the help of cAMP-dependent protein kinase, Ca^{++}-calmodulin-dependent protein kinase and glycogen synthase kinase. On the other hand, protein phosphatase-I removes the phosphates from phosphoserines and converts the inactive 'glycogen synthase b' into the active 'glycogen synthease a'.

(ii) Adrenaline activates cAMP-dependent as well as Ca^{++}-calmodulin-dependent protein kinase, which in turn phosphorylates the active 'glycogen synthase b'. This retards glycogenesis.

(iii) Insulin stimulates the biosynthesis of glucokinase and glycogen synthase. This in turn promotes glycogenesis in liver.



Glycogenesis

5.8 Questions (with hints to answers)

A. Short-answer type questions :

1. How many ATP molecules are produced from anaerobic and aerobic oxidations of one molecule of glucose ? (see Section 5.3.1).
2. Name the rate limiting enzyme and rate limiting step of glycolysis. (see Section 5.2.2).

3. Name the inactive and active forms of glycogen phosphorylase. (see Section 5.7).
4. Name the rate limiting enzymes of TCA cycle. (see Section 5.3.3).
5. Name two indermediate products of TCA cycle that are used in gluconeogenesis. (see Section 5.5.2).
6. Name two amino acids used in gluconeogenesis. (see Section 5.5.4).
7. Name the substrate used for gluconeogenesis during starvation. (see Section 5.5.4).
8. Name the substrates used for gluconeogenesis in ruminating mammals. (see Section 5.5.3).
9. What is the significance of pentose phosphate pathway in red blood cells ? (see Section 5.4.2).
10. What is the difference between glycogenolysis and glycogenesis ? (see Section 5.6).
11. What is the function of glycogen synthase? (see Section 5.7).
12. What is glycogenin ? (see Section 5.7).
13. State the function of phosphoglucomutase. (see Section 5.6).
14. State the function of branching enzyme ? (see Section 5.7).
15. What is the function of debranching enzyme ? (see Section 5.7).
16. Name two hormones that promote glycogenolysis. (see Section 5.6).
17. Why does glycogenolysis not occur in muscles ? (see Section 5.6).
18. Name the rate limiting enzyme of glycogenolysis. (see Section 5.6).
19. What do you mean by glycogenosis ? (see Section 5.6).
20. What is type I glycogenosis ? (see Section 5.6).

B. Long-answer type questions :

1. Precisely describe the reactions occurring in EMP pathway. (see Section 5.2.1).
2. Give a concise account of Krebs' TCA cycle. (see Section 5.3.1).
3. Give an account of the anabolic role of Krebs' TCA cycle. (see Section 5.3.2).
4. How will link between protein metabolism and Krebs' TCA cycle? (see Section 5.3.4).
5. Give an account of regulation of glycolysis. (see Section 5.2.2).
6. Write a note on regulation of the rate limiting enzymes of Krebs' TCA cycle. (see Section 5.3.3).

7. Describe gluconeogenesis from glycerol. (see Section 5.5.1).
8. Give an account of gluconeogenesis from lactate. (see Section 5.5.2).
9. List the end-products of pentose phosphate pathway. (see Section 5.4).
10. Give an account of glycogenesis. (see Section 5.7).
11. Give an account of glycogenolysis. (see Section 5.6).
12. Give an account of regulation of the rate limiting enzyme of glycogenolysis. (see Section 5.6).

5.9 References

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Unit-6 □ Lipid Metabolism

Structure

6.0 Objectives

After studying the topics included in this chapter, you will be able to :

- Define lipid metabolism ;
- Gain thorough knowledge of β-oxidation of saturated fatty acids ;
- Grow clear concept of ATP production in β-oxidation of saturated fatty acids (both even-carbon and odd-carbon fatty acids) ;
- Gain knowledge of omega-oxidation of saturated fatty acids ;
- Have clear concept of 'fatty acid synthetase system' ;
- Have a detailed idea on biosynthesis of palmitic acid ;
- Gain knowledge on ketogenesis in animal body.

6.1 Introduction

Lipid metabolism refers to all kinds of chemical transformations of lipids and their components in the body of living beings. Such chemical transformations in the animal body include the *de novo* biosynthesis of fatty acids, chain elongation of fatty acids, biosynthesis of fat and different conjugated lipids, breakdown of stored fat, biosynthesis of different steroid hormones (e.g. androgens, oestrogens and adrenal hormones) as well as metabolism of fatty acids obtained from digestion of dietary fats in the alimentary canal and breakdown of fats stored in adipose tissues of the body. It may be added in this connection that the metabolism of fatty acids is accompanied by a significant yield of energy in the form of ATP molecules.

6.2. β-oxidation and omega-oxidation of saturated fatty acids with even and odd number of carbon atoms

6.2.1 Some essential terms :

β = C_2 starting from COOH group. β = C_3 starting from COOH group. Δ = Double-bond. Cis, trans = H atoms attached to same side (cis)/ opposite sides (trans) of two carbon atoms on two sides of a double-bond.	Acyl = Adjective of saturated fatty acid. Enoyl = Adjective of unsaturated fatty acid. L(+) = OH lies on top of a carbon atom. L(–) = OH lies at bottom of a carbon atom. Saturated fatty acid = $CH_3(CH_2)_nCOOH$

6.2.2 Site of β-oxidation of fatty acids :

Most of the saturated fatty acids and all unsaturated fatty acids are metabolized by the process of â-oxidation inside the mitochondria of cells of the liver, kidney, adipose tissue and muscles. The basic mechanism of â-oxidation can be easily explained with reference to the saturated fatty acids ; the pathway was first described by F. Knoop in 1905. In case of unsaturated fatty acids, â-oxidation involves some additional and special steps to change the double-bonds in their molecules so that the process can reach its completion.

6.2.3 β-oxidation of saturated fatty acids (even and odd carbon):

β-oxidation of saturated fatty acids involves the following steps:

1. The fatty is first combined with coenzyme A (CoA.SH) at the outer

mitochondrial membrane-surface by the catalytic action of acyl-CoA synthetase, resulting in the formation of acyl-CoA. This is called as activation of fatty acids. The overall process of activation is simple, but it actually occurs in the following sequence:

(i) $\text{Fatty acid} + \text{ATP} \xrightarrow{\text{Acyl CoA Synthetase}} \underset{\text{(Acyl adenylate)}}{\text{Acyl AMP}} \quad \underset{\text{(Pyrophosphate)}}{\text{PPi}}$

(ii) $\text{Acyl AMP} + \text{CoA.SH} \xrightarrow{\text{Acyl CoA Synthetase}} \text{Acyl CoA} + \text{AMP}$

(iii) $\text{PPi} + \text{H}_2\text{O} \xrightarrow{\text{Pyrophosphates}} 2\ \text{Pi(Inorganic phosphate)}$

$$\text{Fatty acid} + \text{CoA.SH} + \text{ATP} + \text{H}_2\text{O} \rightarrow \text{Acyl CoA} + \text{AMP} + 2\text{Pi}$$

Moreover, there are 3 kinds of acyl-CoA synthetase which activate different fatty acids, depending on the chain-length of the latter, viz. (a) Acetyl-CoA synthetase activates C_2 or C_3 fatty acids like acetic acid (CH_3COOH) and propionic acid (CH_3CH_2COOH) ; (b) Medium-chain acyl-CoA synthetase activates C_4 to C_{12} fatty acids like valeric (C_5) acid [$CH_3(CH_2)_3COOH$] ; (c) Long-chain acyl-CoA synthetase activates fatty acids with more than 12 carbons, such as, palmitic (C_{16}) acid [$CH_3(CH_2)_{14}COOH$ and Stearic (C_{18}) acid [$CH_3(CH_2)_{16}COOH$].

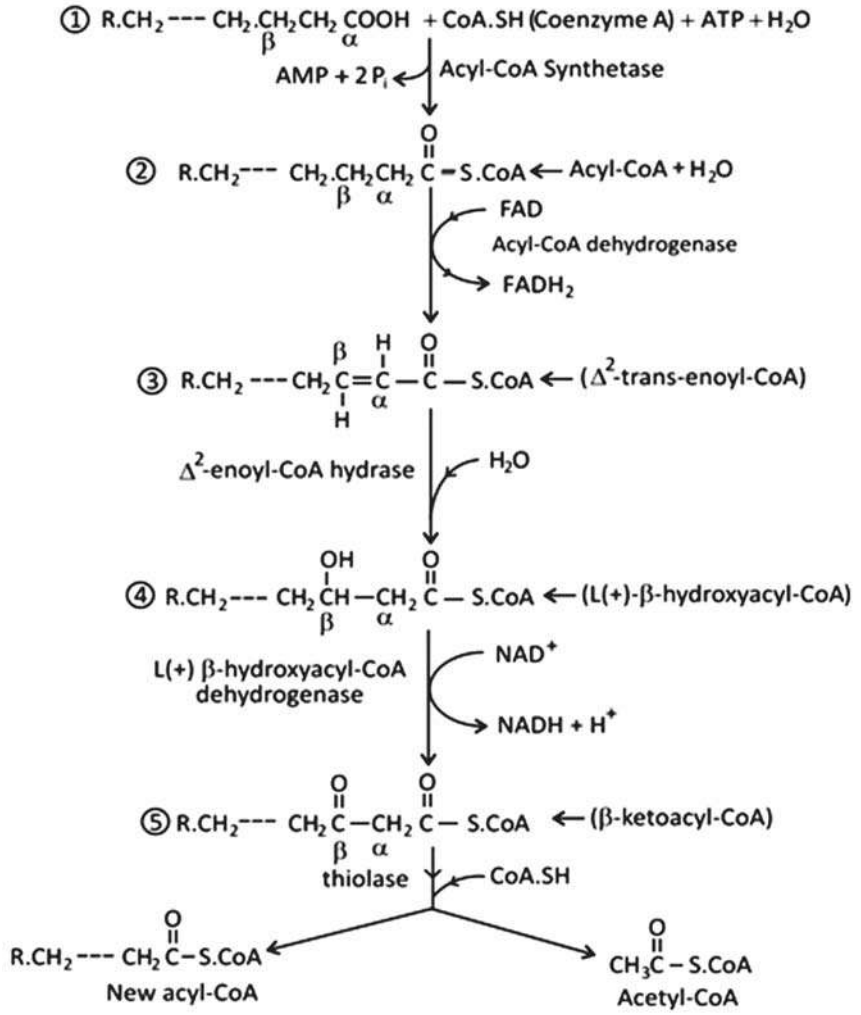
2. Acyl-CoA is then passed to the mitochondrial matrix by the help of a mobile carrier substance called as carnitine. Inside the matrix, acyl-CoA is oxidized due to dehydrogenation [= loss of H_2] under the catalytic action of acyl-CoA dehydrogenase. Acyl-CoA is converted into Δ^2-trans-enoyl-CoA while FAD which is a prosthetic group of the enzyme, acts as an acceptor of H_2 and gets reduced into $FADH_2$.

3. Then Δ^2-trans-enoyl-CoA combines with water under the catalytic action of Δ^2-enoyl-CoA hydrase. As a result, L(+)-β-hydroxyacyl-CoA is formed.

4. Then, L(+)-β-hydroxyacyl-CoA is oxidized due to dehydrogenation at its â-carbon by the catalytic action of L(+)-β-hydroxy-acyl-CoA dehydrogenese. As a result, L(+)-β-hydroxyacyl-CoA is converted into β-ketoacyl-CoA while NAD^+, which is a prosthetic group of the enzyme, acts as an acceptor of H_2 and gets reduced to yield NADH and H^+.

5. Then, in presence of another CoA.SH, thiolase cleaves β-ketoacyl-CoA in between its α and β-carbons (= 2nd and 3rd carbons). Consequently, one molecule of acetyl-CoA (C_2) and a new acyl-CoA, which is shorter than the original acyl-CoA by two carbons, are produced.

6. Thereafter, the steps (2) to (5) are repeated with the new acyl-CoA. Finally, in case of an even-carbon fatty acid, n/2 number of acetyl-CoA is produced, if the total number of carbon atoms in the original fatty acid is 'n'. On the other hand, in case of an odd-carbon fatty acid, one molecule of propionyl-CoA (C_3) and several molecules of acetyl-CoA (C_2) are produced.



β-oxidation of saturated fatty acid

6.2.4 ATP production in β-oxidation of saturated fatty acids :

6.2.4.1 Even-carbon fatty acids :

(i) Each cycle of β-oxidation produces one acetyl-CoA (C_2) and the total number of acetyl-CoA produced from the complete β-oxidation of an even-carbon

fatty acid is $\frac{n}{2}$ (n = number of carbons in the acid). Each acetyl-CoA enters Krebs cycle for oxidation along with production of 12 ATPs, So, $\left(\frac{n}{2}-1\right)$ ATPs will be obtained in this way.

(ii) Now, the last acetyl-CoA is not obtained from â-oxidation, but represents the acetyl-CoA left-out at the end of β-oxidation. Hence, $\left(\frac{n}{2}-1\right)$cycles are necessary for the complete β-oxidation of an even-carbon fatty acid. During each of these cycles, a $FADH_2$ and a NADH get oxidized by the mitochondrial electron transport system along with the production of 2 and 3 ATPs, respectively. So, $\left\{\left(\frac{n}{2}-1\right)\times 5\right\}$ ATPs obtained in this way.

(iii) Now, 2 high-energy phosphates are spent during the initial activation of the original fatty acid.

(iv) So, the net gain of ATP molecules from â-oxidation of an even-carbon fatty acid $=\left\{\left(\frac{n}{2}\times 12\right)+\left(\frac{n}{2}-1\right)\times 5-2\right\}$ molecules of ATP. eg. In case of palmitic acid $[CH_3(CH_2)_{14}COOH]$ with C_{16}, the net gain of ATP will be ;

$=\left\{\left(\frac{16}{2}\times 12\right)+\left(\frac{16}{2}-1\right)\times 5-2\right\}=(96+35-2)=$ 129 ATPs.

Comment: However, according to modern concept (D. Nelson and M. Cox, Principles of Biochemistry, 4th edition, 2005, Freeman and Company, New York), the net gain of ATP from â-oxidation of an even-carbon fatty acid will be $\left\{\left(\frac{n}{2}\times 10\right)+\left(\frac{n}{2}-1\right)\times 4-2\right\}$. The number of ATPs obtained from oxidation of acetyl-CoA, $FADH_2$ and NADH are 10, 1.5 and 2.5, respectively, eg. Palmitic acid : (8×10+7×4 – 2) = 106 ATPs.

6.2.4.2 Odd-carbon fatty acids:

(i) Each cycle of β-oxidation produces an acetyl-CoA (C_2) and the last acyl-CoA is left out as a propionyl-CoA (C_3). So, one will get one propionyl-CoA and $\left(\frac{n-3}{2}\right)$ acetyl-CoA from β-oxidation of an odd-carbon fatty acid (n = number of carbons in the acid). The propionyl-CoA changes into

succinyl-CoA which enters Krebs cycle for oxidation along with production of 6 ATPs while each acetyl-CoA enters Krebs cycle for oxidation along with production of 12 ATPs. So, $\left\{6+\left(\frac{n-3}{2}\right)\times 12\right\}$ ATPs will be obtained in this way.

(ii) Now, the propionyl-CoA is not obtained from β-oxidation, but represents the acyl-CoA left-out at the end of β-oxidation. So, $\left(\frac{n-3}{2}\right)$ cycles are necessary for the complete β-oxidation of an odd-carbon fatty acid. During each of these cycles, a $FADH_2$ and and NADH are oxidized by the mitochondrial electron transport system along with the production of 2 and 3 ATPs, respectively. So, $\left\{\left(\frac{n-3}{2}\right)\times 5\right\}$ ATPs are obtained in this way.

(iii) Now, 2 high-energy phosphates are spent during the initial activation of the original fatty acid.

(iv) So, the net gain of ATP molecules from β-oxidation of an odd-carbon fatty acid $\left\{6+\left(\frac{n-3}{2}\right)\times 12+\left(\frac{n-3}{2}\right)\times 5-2\right\}$ molecules of ATP. In case of valeric acid $[CH_3(CH_2)_3COOH]$ with C_5, the net gain of ATP = $\left\{6+\left(\frac{5-3}{2}\right)\times 12+\left(\frac{5-3}{2}\right)\times 5-2\right\}$ = 21 ATPs.

Comment : According to modern concept (Nelson and Cox, 2005), the net gain of ATP from β-oxidation of an odd-carbon fatty acid will be $\left\{5+\left(\frac{n-3}{2}\right)\times 10+\left(\frac{n-3}{2}\right)\times 4-2\right\}$. The number of ATPs obtained from oxidation of succinyl-CoA, acetyl-CoA, $FADH_2$ and NADH are 5, 10, 1.5 and 2.5, respectively, eg. Valeric acid : $\left\{5+\left(\frac{5-3}{2}\right)\times 10+\left(\frac{5-3}{2}\right)\times 4-2\right\}$

6.2.5 Omega-oxidation of fatty acids :

Omega-oxidation (*ù-oxidation*) is a process of *fatty acid metabolism*in some species of animals. It is an alternative *pathway*to beta *oxidation*that, instead of involving the â-carbon (3rd carbon from the COOH-end of the fatty acid), involves the *oxidation* of the ù-carbon (the carbon most distant from the COOH-end of the *fatty acid*). Omega-oxidation occurs in smooth ER of liver and kidney cells and

involves only some medium-chain fatty acids (C_6 to C_{12}) fatty acids, e.g. Lauric acid. At first, a mxed-function oxidase converts the fatty acid into an omega-hydroxy fatty acid in which the methyl group at the last carbon of the fatty acid is converted by the enzyme into hydroxyl-methyl group. The enzyme utilizes NADPH and molecular O_2 for its function. At the end of its function, NADPH changes into NADP while O_2 changes into H_2O.

Thereafter, an alcohol dehydrogenase and an aldehyde dehydrogenase acts one after another on the omega-hydroxy fatty acid and the latter converts into a alpha,omega-dicarboxylic fatty acid (α, ω-dicarboxylic fatty acid).

Finally, the alpha,omega-dicarboxylic fatty acid undergoes â-oxidation in the mitochondria.

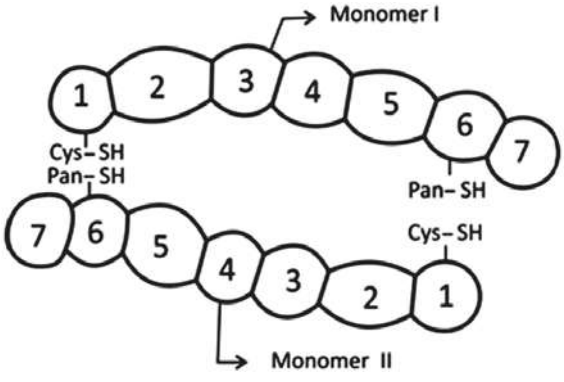
6.3 Biosynthesis of palmitic acid

A multienzyme system called 'fatty acid synthetase' system catalyzes the *de novo* synthesis of palmitic acid (a common, saturated fatty acid of animal body) from acetyl-CoA and malonyl-CoA in the cytosol of the cells of liver, brain, lung, renal cortex, adipose tissue and lactating mammary gland of mammals. An account follows.

6.3.1 'Fatty acid synthetase' system :

This cytoplasmic multienzyme system is an ellipsoid dimer of two identical polypeptide monomers (chains), associated with each other in a head-to-tail fashion. Six enzymes and an acyl-carrier protein (ACP) are covalently linked in each monomer in the following order :

(1) β-ketoacyl synthase, (2) Transacylase, (3) Enoyl reductase, (4) â-hydroxyacyl dehydratase, (5) β-ketoacyl reductase, (6) ACP and (7) Thioesterase.



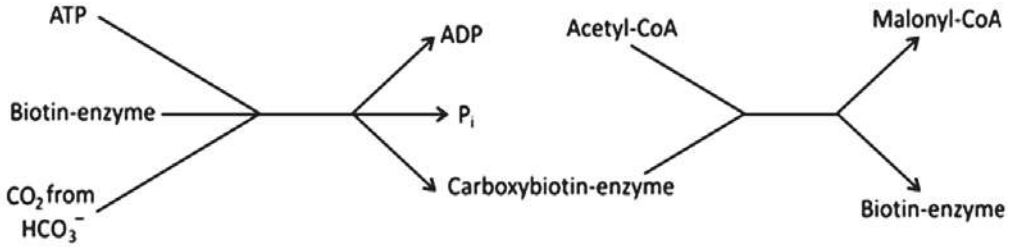
Composition of 'fatty acid synthetase' system

The first member of this complex, viz., β-ketoacyl synthase carries a cysteine residue, while the 6th member of ACP carries a phosphopantetheine residue. Thus, the Cys–SH of one monomer lies close to the Pan–SH of the other monomer.

The individual monomers are inactive when isolated from each other. But, in the dimer form, the two monomers jointly synthesize two palmitates at the two ends of the dimer. The production of each palmitate involves coordinated action of the first two members of one monomer and the last five members of the other.

6.3.2 Carbon-sources for palmitic acid biosynthesis:

The carbon-sources are acetyl-CoA and malonyl-CoA. Acetyl-CoA arises within the mitochondria either by oxidative decarboxylation of pyruvate or by β-oxidation of fatty acids. It is then transferred in other chemical forms through the mitochondrial membranes to the cytosol. Acetyl–CoA is regenerated in the cytosol.



Pathway of malonyl CoA synthesis

Within the cytosol, a large amount of acetyl–CoA is carboxylated to form malonyl-CoA with the help of acetyl-CoA carboxylase, biotin, HCO_3^- and ATP. At first, a biotin-enzyme complex is formed; it is converted into an intermediary compound, carboxybiotin-enzyme which causes carboxylation of acetyl-CoA into malonyl-CoA :

6.3.3 Overall reaction of palmitic acid biosynthesis :

Usually, fatty acid synthetase system produces palmitic acid (C_{16}) as the end-product. It is actually produced by means of 7 cycles of reactions. In the 1st cycle, a C_2 unit is added from a malonyl group to an acetyl group (C_2), resulting in formation of a C_4 acetoacetyl group. Each subsequent cycle adds a new C_2 unit from a fresh malonyl group and finally, palmitoyl group is produced. The overall reaction is thus, as follows :

Acetyl CoA 7malonyl CoA 14NADPH 14H $CH_3(CH_2)_{14}COOH$

6.3.4 Sequence of reactions in palmitic acid biosynthesis :

The synthesis of palmitic acid by the help of 'fatty acid synthetase' system involves the following steps :

(i) Formation of acetyl-malonyl-enzyme : Transcylase of the multienzyme complex transfers the acetyl group from an acetyl–CoA to the Cys–SH of one monomer and the malonyl group from a malonyl–CoA to the adjacent Pan–SH of the other monomer. Thus, an acetyl-malonyl-enzyme complex is formed.

(ii) Condensation reaction : Under the catalytic action of β-ketoacyl synthase, the acetyl group linked to Cys–SH is transferred to the 2^{nd} carbon of the malonyl group linked to Pan–SH, with the release of the free carboxyl group of the malonyl residue as CO_2. The malonyl group is thus, changed into an acetoacetyl group.

(iii) First reduction step : The acetoacetyl group is now reduced into β-hydroxybutyryl group by β-ketoacyl reductase, in presence of NADPH and H^+ ion.

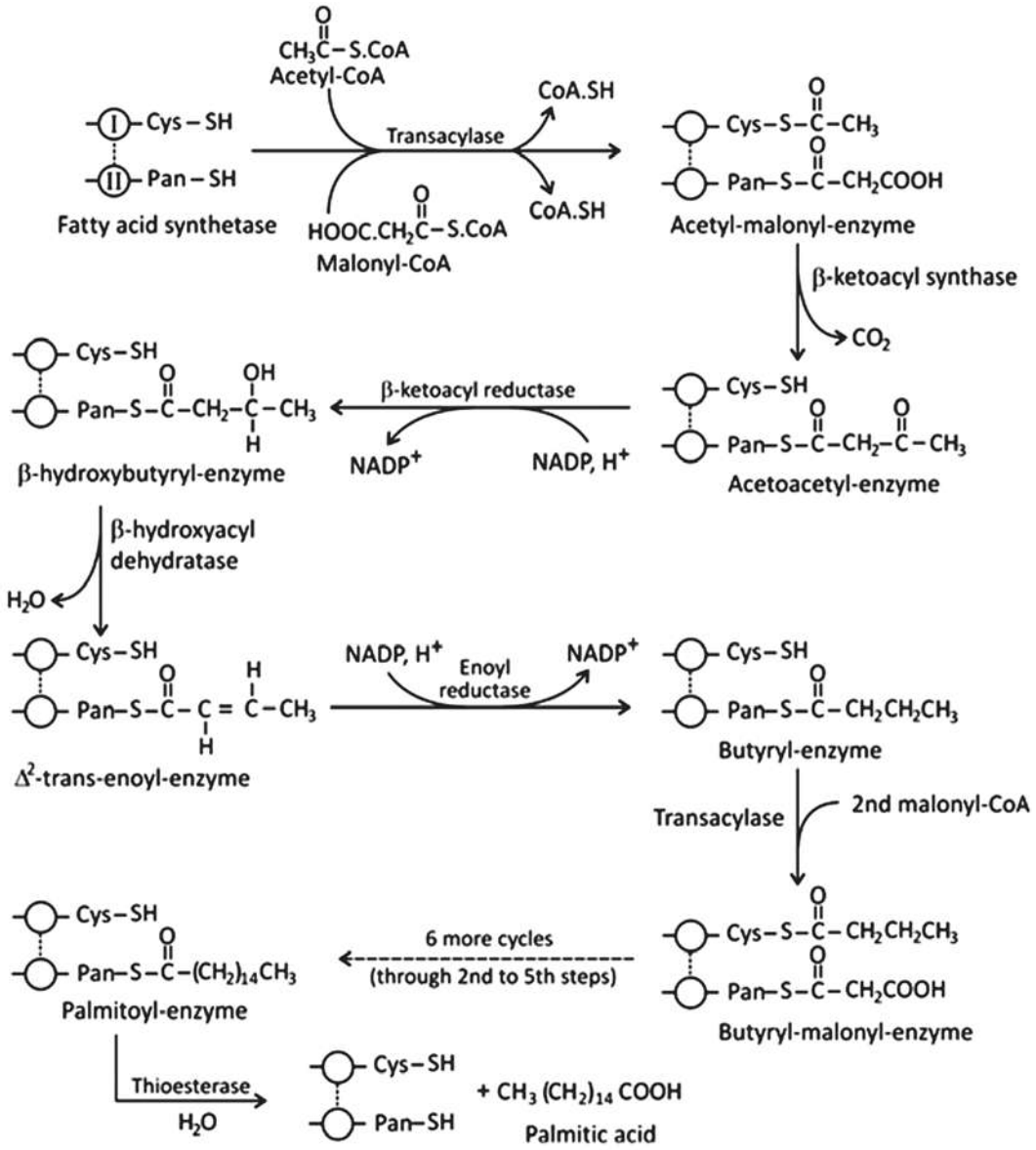
(iv) Dehydration step : β-hydroxyacyl dehydratase now removes water from the β-hydroxybutyryl group which thus, changes into Δ^2-trans-enoyl group.

(v) Second reduction step : The Δ^2-trans-enoyl group is now reduced into butyryl group by enoyl reductase, in presence of NADPH and H^+ ion. This completes the first cycle of reactions.

(vi) 2nd to 7th cycles of reactions : Now, transacylase transfers the butyryl group from Pan–SH of ACP to the adjacent Cys–SH of the other monomer and also transfers the malonyl group from a second malonyl–CoA to the free Pan–SH of ACP. Now, the steps (2) to (5) or 'Condensation reaction' to 'Second reduction step' are repeated. At the end of the each cycle, a new acyl group which is longer than the previous one by 2 carbon atoms is formed. Finally, at the end of 7 cycles, palmitoyl group is formed. The palmitoyl group is released as free palmitic acid ($CH_3(CH_2)_{14}COOH$) by the hydrolytic action of thioesterase of the multienzyme complex.

Comments :

(i) In the liver and most of the other tissues, all the 7 cycles are completed and the end-product is palmitic acid (16 carbon).



Biosynthesis of palmitic acid by 'fatty acid synthetase' system

(ii) In mammary gland, some 10 to 14-carbon fatty acids are synthesized. In such cases, all the 7 cycles of reactions are not completed.

(iii) Sometimes, fatty acids with an odd number of carbons are formed. In such cases, propionyl–CoA (3 carbon) is utilized in the first step, instead of an acetyl–CoA (2 carbon).

6.4 Ketogenesis

Three substances, viz., acetoacetate, acetone and β-hydroxybutyrate are knowns as ketone bodies and their production in the body is called ketogenesis. Ketone bodies are produced from acetyl-CoA in the *mitochondria* of *liver* cells. Further, ketogenesis occurs in response to an unavailability of blood glucose, such as (i) during *starvation*, (ii) prolonged intake of high-fat but low-carbohydrate diet and (iii) in case of diabetes mellitus, a disease in which glucose accumulates in blood but cannot enter into cells for undergoing oxidation. Ketogenesis occurs as follows:

(1) Acetyl-CoA molecules formed from β-oxidation of different types of fatty acids are condensed in pairs by thiolase enzyme, resulting in formation of acetoacetate (acetoacetyl-CoA).

(2) Small amounts of acetoacetate are spontaneously decarboxylated into acetone or decarboxylated into acetone by the help of the enzyme *acetoacetate decarboxylase*. Acetone is eliminated by way of the lungs.

(3) Large amounts of acetoacetate are reduced into â-hydroxybutyrate by the hepatic mitochondrial enzyme D-â-hydroxy-butyrate dehydrogenase which uses NADH as a hydrogen donor during its reducing action. Therefore, â-hydroxybutyrate is the predominant ketone body to be found in blood during starvation and diabetes.

β-Hydroxybutyrate and acetoacetate can pass through cell membranes easily, and are therefore a source of energy for the brain, which cannot directly metabolize fatty acids. The brain derives 60-70% of its required energy from these two ketone bodies when blood glucose levels are low.

However, when the incidence of ketogenesis in the body becomes considerably high, the excess or unused β-hydroxybutyrate from the blood is excreted through the urine; this is called as ketonuria. Moreover, in severe diabetic patients, the incidence of ketogenesis in the body may become excessively high, resulting in ketoacidosis in which the pH of blood becomes lower than normal due to accumulation of β-Hydroxybutyrate and acetoacetate (both are acidic in nature), nausea, vomiting, abdominal pain, deep breathing and weakness.

6.5 Questions (with hints to answers)

A. Short-answer type questions :

1. What do the terms ‘acyl’ and ‘enoyl’ mean ? (See Section 6.2.1).
2. Who discovered the pathway of β-oxidation of fatty acid ? (See Section 6.2.2).

3. Name three kinds of acyl-CoA synthetase ? (See Section 6.2.3).
4. Name a C_{16} fatty acid and a C_{18} fatty acid. (See Section 6.2.3).
5. What is the function of thiolase ? (See Section 6.2.3).
6. How many ATP molecules are produced from β-oxidation of palmitic acid ? (See Section 6.2.4).
7. Name the enzyme system for *de novo* fatty acid synthesis. (See Section 6.3.1).
8. Name the components of each polypeptide chain of fatty acid synthetase. (See Section 6.3.1).
9. What is ACP ? (See Section 6.3.1).
10. Name the carbon sources of *de novo* fatty acid synthesis. (See Section 6.3.2).
11. Name the three ketone bodies. (See Section 6.4).
12. What is ketoacidosis ? (See Section 6.4).

B. Long-answer type questions :

1. Discuss the mitochondrial pathway of β-oxidation of saturated fatty acids. (See Section 6.2.3).
2. Describe the organization of fatty acid synthetase. (See Section 6.3.1).
3. Discuss the sequence of steps in palmiticacid biosynthesis. (See Section 6.3.4).
4. Write a note on activation of fatty acids. (See Section 6.2.3).
5. Give an account of ATP production from β-oxidation of palmitic acid. (See Section 6.2.4).
6. Give an account of ketogenesis. (See Section 6.4).

6.6 References

1. Biochemistry – A. H. Lehninger (1975), Worth Publishers, New York.
2. Biochemistry – Debajyoti Das (2005), Academic Publishers, Kolkata.
3. Principles of Biochemistry – D. L. Nelson and M. M. Cox (2005), Freeman & Co., New York.
4. Textbook of Biochemistry – E. S. West and W. R. Todd (1966), Macmillan, New York.

Unit-7 □ Protein Metabolism

Structure

7.0 Objectives

After studying the topics included in this chapter, you will be able to :

- Define and explain the term protein metabolism ;
- Have a detailed idea on transamination ;
- Have a detailed idea on deamination ;
- Explain the terms ammonotelism, ureotelism and uricotelism ;
- Have a detailed idea on biosynthesis of urea ;
- Have an idea on the fate of C-skeleton of different types of amino acids.

7.1 Introduction

Various proteins consumed with diet are hydrolyzed into different amino acids in the stomach and intestine by the help of protease enzymes secreted from stomach, pancreas and small intestine. Those amino acids are absorbed through the intestinal mucosa into blood and transported to various organs and tissues of the body. However, amino acids cannot be stored for a long time in any tissue or organ of the body. Therefore, all amino acids entering into different tissues are chemically converted into either various substances that are useful for the body or nitrogenous wastes that are eliminated from the body through urine. The chemical transformation

of amino acids in tissues is called as amino acid metabolism or grossly, as protein metabolism.

Most of the amino acids entering into different tissues are utilized for biosynthesis of new proteins including various enzymes in cells by the help of tRNA, mRNA and ribosome. Some of the amino acids entering into some particular tissues or organs are utilized for the biosynthesis of specific useful substances, e.g. thyroxine hormone is produced in the thyroid gland ; catecholamine hormones are produced in the adrenal gland ; melatonin hormone is produced in the pineal gland ; histamine (a vasodilator) is produced in basophil cells of blood and mast cells of connective tissue. Some amino acids like cysteine and alanine as well as the products of metabolism of certain amino acids are utilized for gluconeogenesis in the liver. Some products of amino acid metabolism enter into Krebs' TCA cycle for further oxidation along with production of energy.

Finally, the unused amino acids from various tissues are passed to the liver for undergoing chemical transformation into nitrogenous wastes like ammonia, urea and uric acid which are harmful for the body and consequently, eliminated from the animal body through the gills (in teleost fishes) or the kidneys (in elasmobranch fishes, amphibians, reptiles, birds and mammals).

7.2 Catabolism of amino acids

Catabolism of various amino acids is accomplished mainly through transamination and deamination, as described below :

7.2.1 Transamination :

Transamination is an important method of nitrogen catabolism of amino acids.

Basic principle : Transamination is a reversible process in which the á-amino group of an amino acid is transferred to a keto acid so that the latter changes to a new amino acid while the original amino acid converts into a new keto acid. Thus, transamination involves deamination and amination side by side, but without the liberation of any free ammonia [Fig. 6(x)] :

Net result of transamination

Occurrence : Transamination takes places mainly in the liver and to some extent, in other tissues like the kidney, brain, heart and testis.

Concerned enzymes : Transamination is catalyzed by transaminases or aminotransferases : (i) These enzymes act on the L-amino acids but not on the D-isomers, (ii) They require pyridoxial phosphate as the cofactor, (iii) They occur in both mitochondria and cytosol as separate isozymes and (iv) There are many transaminases ; each acts on a particular pair of amino acid and keto acid.

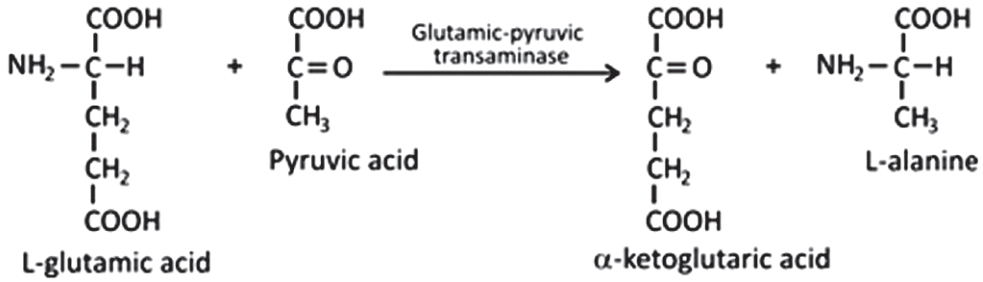
Participant amino acids and keto acids :

(i) Almost all naturally occurring amino acids undergo transamination. Exceptions include the basic amino acid lysine, the hydroxy amino acids like serine and threonine, and also, the heterocyclic amino acids like proline and hydroxyproline.

(ii) Keto acids like pyruvic acid, oxaloacetic acid and á-ketoglutaric acid are commonly involved in transamination. However, glyoxylic acid may also act as an amino-acceptor in transamination.

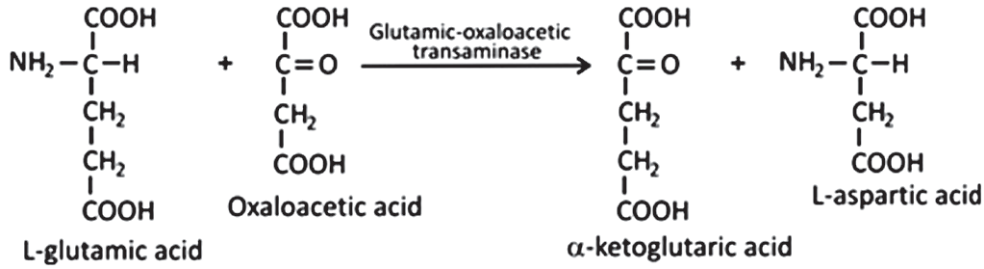
Examples of transamination : Some examples of transamination of amino acids using different amino-acceptors and catalysed by different transaminases are shown below :

(i) Using pyruvate as amino-acceptor :



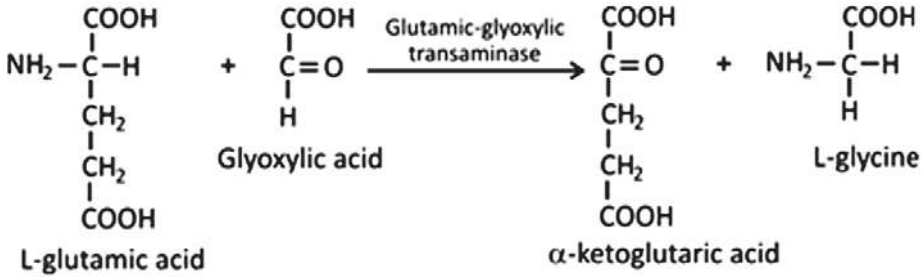
Transamination using pyruvate as amino-acceptor

(ii) Using oxaloacetate as amino-acceptor :



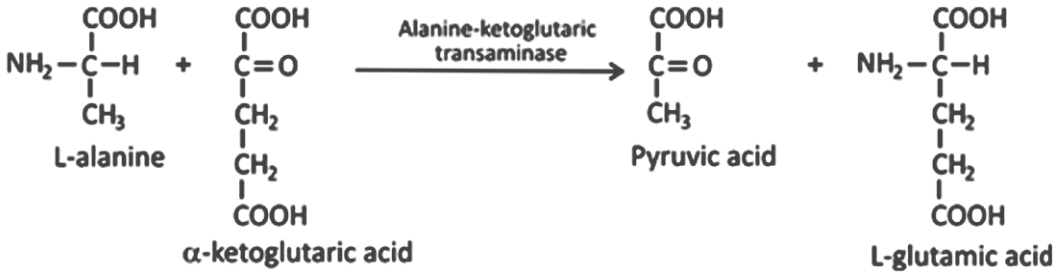
Transamination using oxaloacetate as amino-acceptor

(iii) Using glyoxylate as amino-acceptor :



Transamination using glyoxylate as amino-acceptor

(iv) Using α-ketoglutarate as amino-acceptor :



Transamination using α-ketoglutarate as amino-acceptor

Significance of transamination : Transamination is an important method of nitrogen catabolism of amino acids. It helps in the synthesis of new amino acids from keto acids. It also serves to produce pyruvic acid and oxaloacetic acid, which in turn are used for gluconeogenesis in the liver. Serum levels of glutamic-oxaloacetate transaminase and glutamic-pyruvate transaminase are found to be significantly increased in certain diseases like myocardial infarction of heart, hepatitis and toxic hepatic jaundice.

7.2.2 Deamination :

Deamination is an important method of nitrogen catabolism of amino acids. In this process, the α-amino group (NH_2 group attached to the central C-atom) of an amino acid is converted into ammonia while the amino acid itself converts into its corresponding keto acid. Deamination may be of three kinds :

(A) Oxidative deamination : Here, the presence of oxygen is essential for deamination to occur :

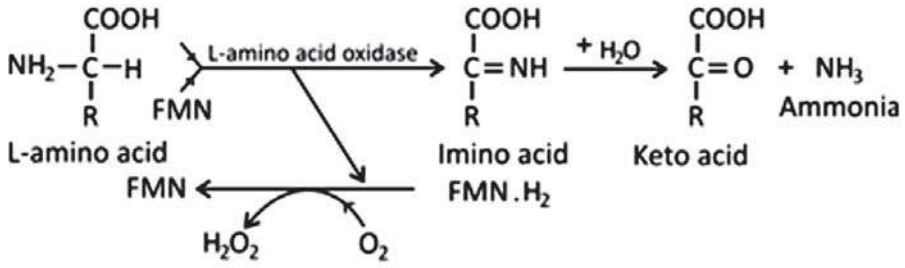
(i) By the help of L-amino acid oxidase : L-amino acids are oxidatively deaminated by L-amino acid oxidase present in mitochondria, ER (endoplasmic reticulum) and peroxisomes of mammalian kidneys. The enzyme cannot act on glycine

(which exists in the body only as L-isomer) and the L-isomers of sulphur-containing, hydroxy, dicarboxylic and basic amino acids. The enzyme contains FMN as the prosthetic group.

During deamination, the enzyme at first catalyzes dehydrogenation of the amino acid which gets oxidized to an imino acid while FMN of the enzyme gets reduced to $FMN.H_2$ by accepting hydrogen. Then, the imino acid spontaneously reacts with water to give the keto acid and ammonia while $FMN.H_2$ is reoxidized by molecular O_2 to produce H_2O_2:

Deamination by L-amino acid oxidase

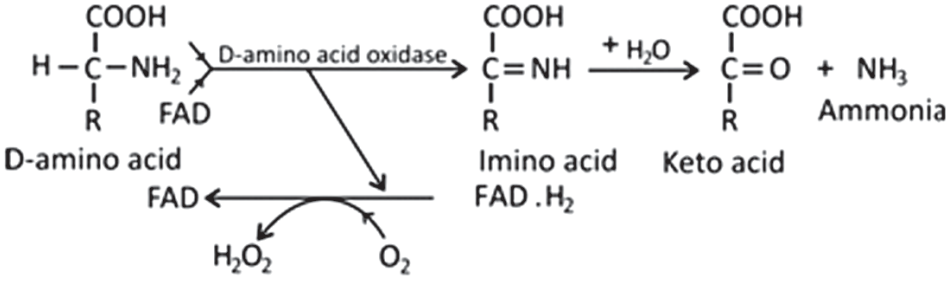
(ii) By D-amino acid oxidase : D-amino acids are oxidatively deaminated by D-



amino acid oxidase present in peroxisomes of mammalian liver and kidney. The enzyme cannot act on the D-isomers of glutamic acid, asparagine, dicarboxylic and basic amino acids. The enzyme contains FAD as prosthetic group. Its mode of action is comparable to that of L-amino acid oxidase:

Deamination by D-amino acid oxidase

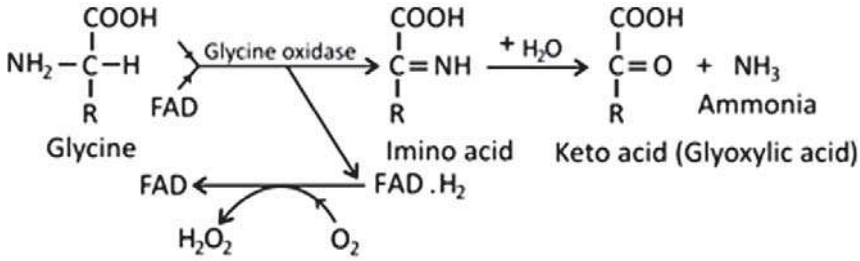
(iii) By Glycine oxidase : Glycine is oxidatively deaminated by a hepatic enzyme,



glycine oxidase instead of either L-or D-amino acid oxidase. Like D-amino acid oxidase, glycine oxidase possesses FAD as the prosthetic group:

Deamination by glycine oxidase

(B) Non-oxidative deamination : In this type of deamination, molecular oxygen (O_2)

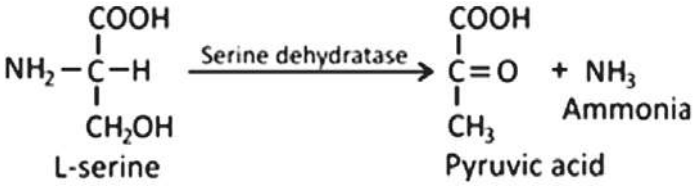


is not directly required for deamination to occur. It occurs mainly in liver and by the help of different enzymes :

(i) By amino acid dehydratase : Amino acid de-hydratases catalyze dehydration, followed by deamination, of hydroxy amino acids like serine and threonine :

Deamination of L-serine

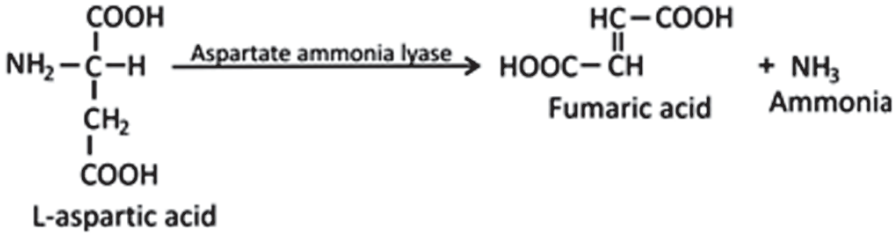
(ii) By amino acid lyase :L-histidine and L-aspartic acid are non-oxidatively



deaminated by C-N amino acid lyases:

Deamination of L-aspartic acid

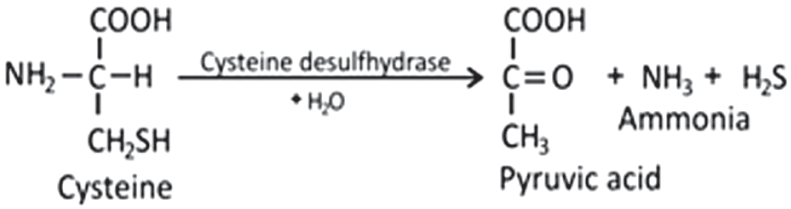
(iii) By amino acid desulphydrase : The sulphur-containing amino acid cysteine is



non-oxidatively deaminated by amino acid desulphydrase in presence of water :

Deamination of L-cysteine

(4) By trans-sulfurase : Trans-sulfurase catalyzes partial deamination of the sulphur-

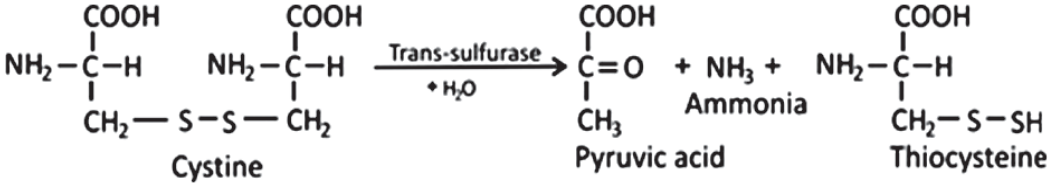


containing amino acid cystine (formed by joining of 2 cysteines) in presence of water, to

yield pyruvic acid, ammonia and thiocysteine which still retains both the sulphur atoms of cystine :

Deamination of cystine

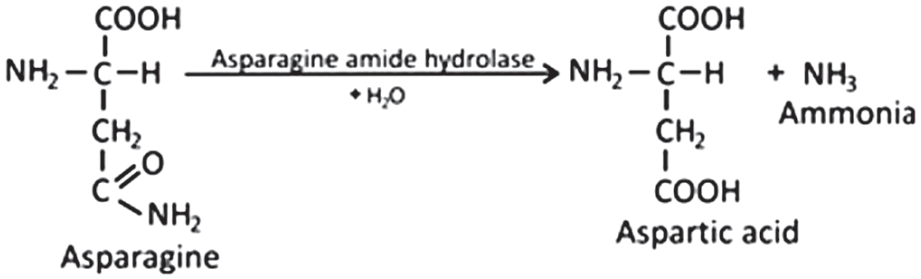
(5) By amino acid amide hydrolase :L-Asparagine may be hydrolytically



deaminated by asparagine amide hydrolase in presence of water :

Deamination of L-asparagine

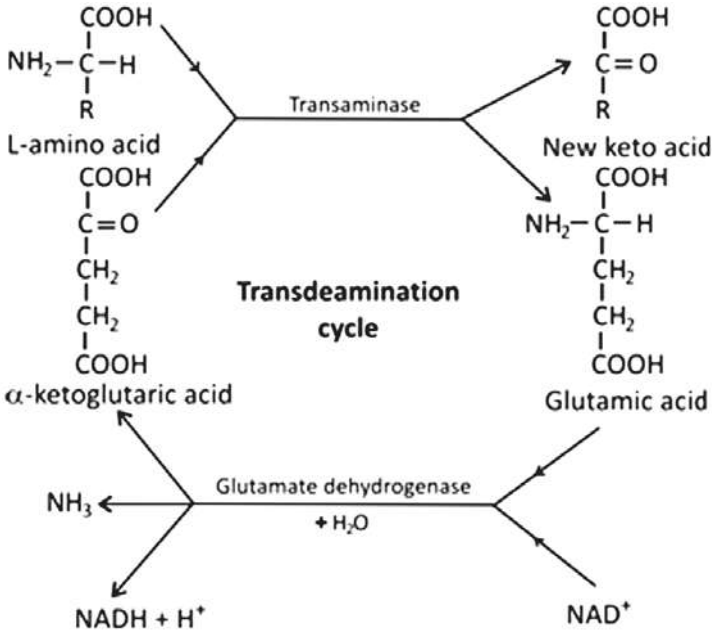
(C) Transdeamination : It is a cyclical process in which (a) at first, transamination



catalyzed by transaminase of mitochondria and cytosol of hepatic cells causes transfer of the α-amino group of a L-amino acid to α-ketoglutaric acid, resulting in the formation of glutamic acid, and (b) thereafter, oxidative deamination of glutamic acid is catalysed by mitochondrial glutamate dehydrogenase which utilizes NAD^+ as hydrogen-acceptor, in presence of water, resulting in reproduction of α-ketoglutatic acid which may be recycled for transamination of another amino acid :

Transdeamination cycle

Significance of deamination: The surplus amino acids of the animal body are catabolized by means of deamination, resulting in the formation of the nitrogenous



waste ammonia. Ammonia may be transformed into urea or uric acid in higher vertebrates. All these nitrogenous wastes are finally excreted through urine. Besides, pyruvic acid produced from deamination of different amino acids may be used for gluconeogenesis in the liver.

7.3 Urea Cycle

7.3.1 Ammonotelism, ureotelism and uricotelism :

Dietary proteins are broken down into amino acids by the activity of proteolytic enzymes in stomach and small intestine. These amino acids are absorbed into blood through the intestinal mucosa. A large part of the absorbed amino acids is utilized for building proteins in cells. The unutilized amino acids are passed from blood to the liver. However, amino acids cannot be stored indefinitely in liver and instead, these are catabolized in the liver, resulting in the formation of nitrogenous excretory substances (Ammonia is produced first and it may be transformed into urea or uric acid in different animals). Finally, the nitrogenous wastes are eliminated from the animal body through the gills (in teleost fishes) or the kidneys (in elasmobranch fishes, amphibians, reptiles, birds and mammals).

Depending on the nature of the end-product of catabolism of amino acids, animals have been classified into three groups, viz., ammonotelic, ureotelic and uricotelic animals. In teleost fishes, the predominant end-product of amino acid catabolism is ammonia. Hence,

teleosts are called ammonotelic animals. In elasmobranch fishes, amphibians and mammals, the predominant end-product of amino acid catabolism is urea. Hence, these groups of animals are called ureotelic animals. On the other hand, in reptiles and birds, the predominant end-product of amino acid catabolism is uric acid. Hence, reptiles and birds are called uricotelic animals.

7.3.2 Description of urea cycle :

Urea is the end-product of amino acid catabolism in elasmobranch fishes, amphibians and mammals. Chemically, urea is known as 'carbamide' or 'diamide of carbonic acid'. Deamination of amino acids produces large amounts of NH_3 which is toxic for the animal body. Different animals have acquired different mechanisms to eliminate this NH_3. Teleost fishes are ammonotelic and directly excrete NH_3 through the gills ; reptiles and birds convert NH_3 into uric acid for excretion through the kidneys and they are, thus, uricotelic ; mammals, amphibians and elasmobranch fishes convert NH_3 into urea for renal excretion and they are called as ureotelic.

In ureotelic animals, urea synthesis occurs through a cyclical pathway [Fig. 6(xv)] called as 'urea cycle' or 'arginine-urea pathway' or 'Krebs-Henseleit ornithine cycle', after the name of the discoverers. Urea is formed mainly in the liver and to a very small extent, in the kidneys and brain.

Details of urea cycle :

(i) Formation of carbamoyl phosphate : NH_3, CO_2 and one phosphate of an ATP are condensed by the mitochondrial enzyme, carbamoyl phosphate synthetase I, in presence of Mg^{++} ions and N-acetylglutamate. The result is the formation of carbamoyl phosphate. Another ATP is broken down into ADP and inorganic phosphate during this process and the liberated energy is utilized for the reaction.

(ii) Formation of citrulline : Now, the carbamoyl group of carbamoyl phosphate is transferred to the amino acid, L-ornithine by the mitochondrial enzyme L-ornithine transcarbamoylase. The product of the reaction is L-citrulline.

(iii) Formation of arginosuccinic acid : From the mitochondria, L-citrulline passes into the cytosol where the enzyme arginosuccinate synthetase causes condensation of L-citrulline with L-aspartic acid, in presence of Mg^{++} ions and ATP so that L-arginosuccinic acid and water are produced ; the ATP breaks into AMP and pyrophosphate to provide energy for the reaction.

(iv) **Formation of arginine :** Another cytosol-enzyme arginosuccinase then cleaves L-arginosuccinic acid into L-arginine and fumaric acid.

(v) **Formation of urea :** Finally, the cytosol-enzyme arginase irreversibly cleaves L-arginine into L-ornithine and urea, in presence of water. Thus, L-ornithine is regenerated and it is recycled for further continuation of the urea cycle.

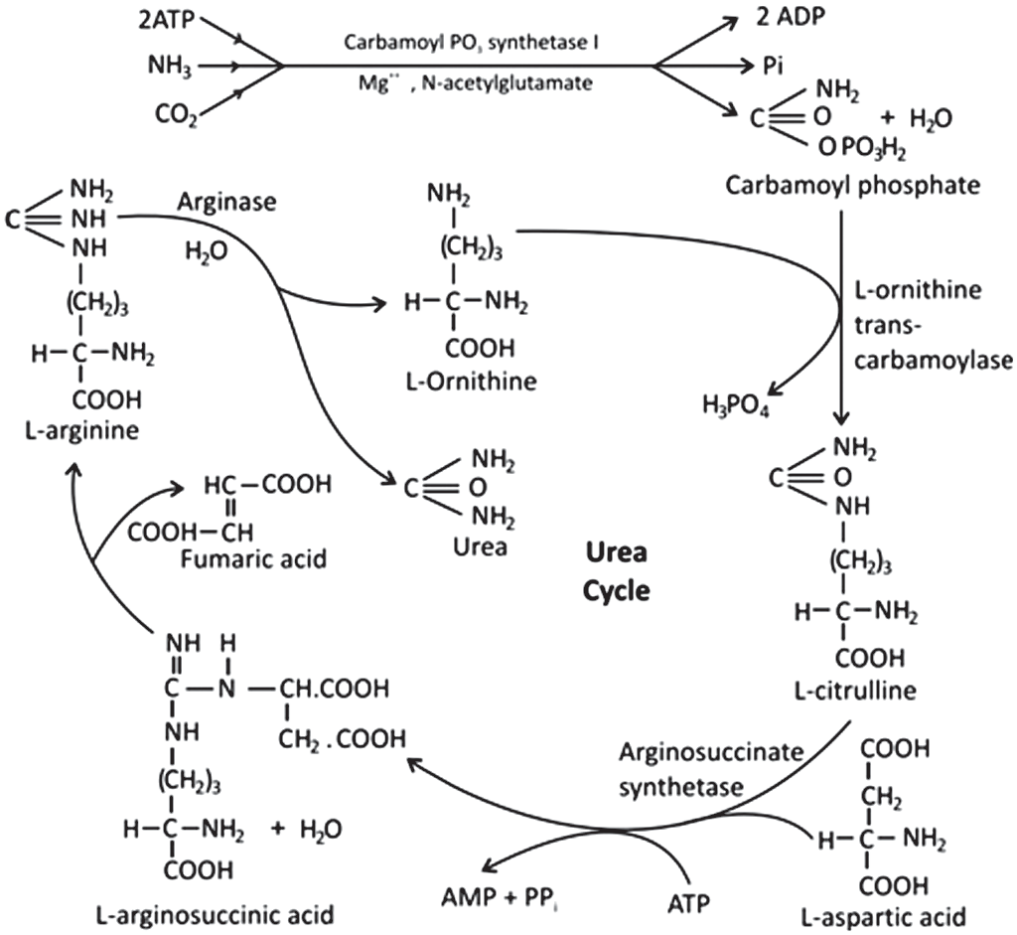
Comments : Liver is the major site of urea synthesis, as it possesses all the enzymes necessary for the urea cycle. The mitochondrial enzymes, carbamoyl phosphate synthetase I and ornithine transcarbamoylase are absent in kidneys and brain. Hence, citrulline can't be formed in these organs and only a little urea can be formed from citrulline reaching these organs from the liver via blood.

In man, hereditary deficiency of carbamoyl synthetase I may lead to hyperammonemia showing high blood ammonia levels, nausea, tremor, blurring of vision and mental retardation. Again, hereditary deficiency of arginase may lead to argininemia characterized by accumulation of arginine in blood, nausea, lethargy and mental retardation.

Urea cycle

7.4 Fate of C-skeleton of glucogenic and ketogenic amino acids

We now turn to the fates of the carbon skeletons of amino acids after the removal of the α-amino group. The fate is to transform the carbon skeletons into (1) metabolic intermediates that can be converted into glucose or (2) metabolic intermediates that



can be oxidized by the Krebs' citric acid cycle. The carbon skeletons of the 20 fundamental amino acids are funneled into only seven metabolic intermediates : Acetyl-CoA, Acetoacetyl-CoA, Pyruvate, Á-Ketoglutarate, Succinyl-CoA, Fumarate and Oxaloacetate.

Amino acids that are degraded to acetyl-CoA or acetoacetyl-CoA are termed ketogenic amino acids because they can give rise to ketone bodies. Amino acids that are degraded to pyruvate, á-ketoglutarate, succinyl-CoA, fumarate, or oxaloacetate are termed glucogenic amino acids. The net synthesis of glucose from these amino acids is feasible, because pyruvate, á-ketoglutarate, succinyl-CoA, fumarate, or oxaloacetate can be converted into phosphoenolpyruvate and then into glucose. It may be mentioned here that mammals lack a pathway for biosynthesis of glucose from acetyl-CoA or acetoacetyl-CoA

Of the 20 fundsmental amino acids, only leucine and lysine are solely ketogenic. Isoleucine, phenylalanine, tryptophan and tyrosine are both ketogenic and glucogenic - their carbon skeletons change partly to acetyl-CoA or acetoacetyl CoA, and partly to the precursors of glucose. The other 14 amino acids are solely glucogenic in nature.

7.5 Questions (with hints to answers)

A. Short-answer type questions :

1. State the function of transaminase. (See Section 6.2.1).
2. How does transamination help in gluconeogenesis? (See Section 6.2.1).
3. Name at least two keto acids that participate in transamination. (See Section 6.2.1).
4. Name the prosthetic groups of L-amino acid oxidase and D-amino acid oxidase, respectively. (See Section 6.2.2).
5. Name the keto acids produced from deamination of glycine, serine, aspartic acid and cysteine, respectively. (See Section 6.2.2)
6. What is the source of ammonia liberated from deamination ? (See Section 6.2.2)
7. Cite one example each of ammonotelic, ureotelic and uricotelic animals.
8. Give the chemical structure of urea.
9. Why is urea not produced in the kidney ?

B. Long-answer type questions :

1. Cite any two examples of transamination. (See Section 6.2.1).
2. Give an account of deamination of glycine. (See Section 6.2.2)
3. Distinguish between the prosthetic group and modes of action of L-amino acid oxidase and D-amino acid oxidase. (See Section 6.2.2)
4. Cite any three examples of non-oxidative deamination. (See Section 6.2.2)
5. Write a note on transdeamination. (See Section 6.2.2)
6. Give an account of urea cycle. (See Section 6.3.2)
7. Discuss the fate of C-skeleton of glucogenic and ketogenic amino acids. (See Section 6.4)

7.6 References

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NOTES