AS PER PCI REGULATIONS
FIRST YEAR B. PHARM. | SEMESTER-II

BIOCHEMISTRY

Dr. Mrs. PADMAJA H. AGARKAR
Dr. YOGESH KULKARNI
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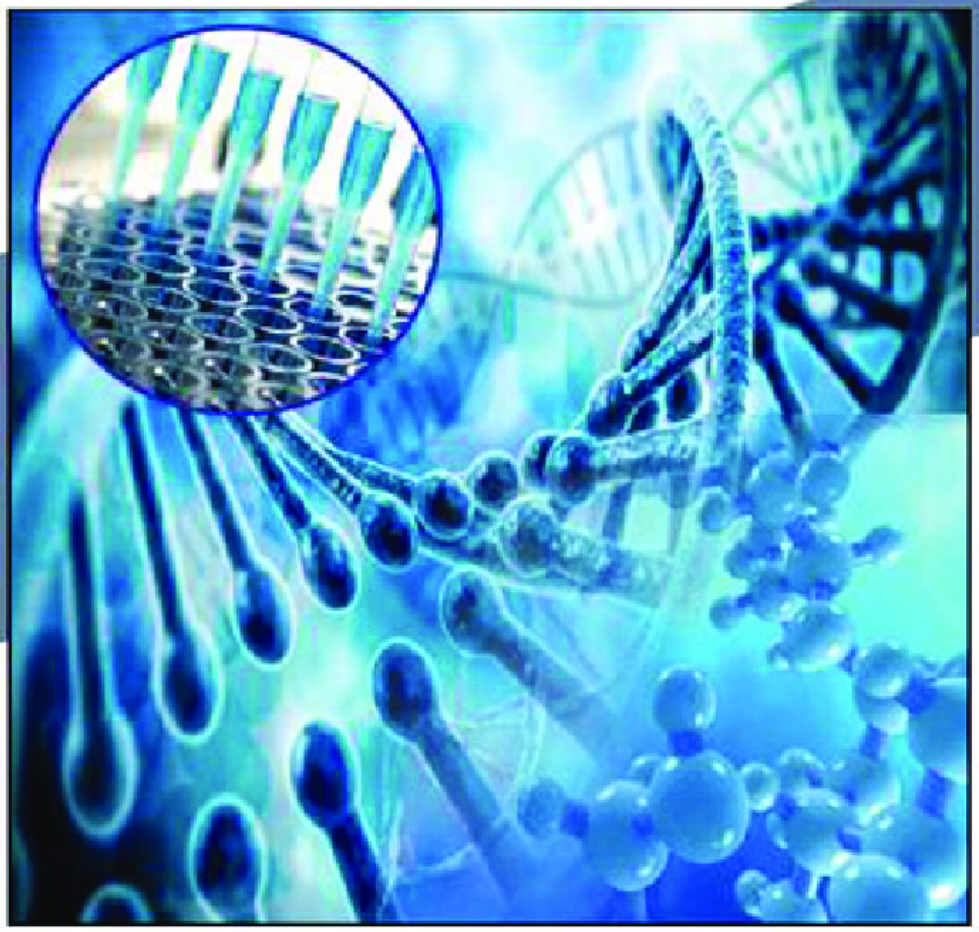

NIRALI PRAKASHAN
ADVANCEMENT OF KNOWLEDGE

A Text Book Of

BIOCHEMISTRY

As Per PCI Regulations

First Year B. Pharm.
Semester II

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This book is dedicated to Dr. Hemant S. Agarkar.

Earnestly blissful for his constant encouragement and incessant guidance.

Authors

Preface

I feel a deep sense of gratitude to find overwhelming response to all seven editions of my book*-, "Biochemistry: Basic and Applied". This new, book entitled "Biochemistry" is orchestrated according to latest PCI (Pharmaceutical Council of India) Syllabus. The book provides systematic coverage of basic, clinical, applied and molecular aspects of biochemistry which can be viewed as standard textbook as well as a review before examination.

Sound knowledge of Biochemistry is imperative for students to understand the significance of molecular aspects of life processes in biological science. One has to accept the fact that biology is fast becoming a molecular science. A student can easily keep pace with latest advances in the subject by learning biochemistry thoroughly which deserves a special place in pharmaceutical and medical science.

New topics included in this edition are:

(i) Diabetes and Insulin

(ii) Metabolism of cholesterol into (a) Bile acid, (b) Vitamin D, (c) Steroid hormones

(iii) Substrate level phosphorylation

(iv) Glucose-6-phosphate dehydrogenase deficiency

(v) Decarboxylation of amino acids

(vi) Catabolism of heme (bilrubin production), hyperbilirubinemia, Jaundice

(viii) Catabolism of nucleotides, hyperurecemia and gout.

(IX) Role of Cyclic AMP

(X) Energy coupling reactions

I do hope in addition to pharmacy, medical, dental, veterinary, microbiology and biotechnology students will find this book a worthy reference.

I am grateful beyond measure to my guru, reverend professor S.B. Gokhale for his blessings and guidance in the venture of of the book.

Thank you to wonderful team of Nirali Prakashan specially Mrs. Manasi Pingle, Mr. Akbar Shaikh, Mrs. Prachi Sawant and Mrs. Roshan Khan for their earnest efforts behind making every edition beautiful and error free.

And most of all, I would like to express my sincere gratitude towards respected Mr. Jigneshbhai Furia of Nirali Prakashan for his guidance and support which is beyond reckoning. I cannot complete preface without thanking profusely my valued coauthors Dr.Rammohan Rao and Dr.Yogesh Kulkarni for their timely help.

I do hope that students will find this book "Biochemistry" equally helpful like its previous book "Biochemsitry : Basic and Applied".

Dr. Padmaja Hemant Agarkar

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Previous Editions

Following List Displays Publication Years of all previous Editions of the Book, 'Biochemistry: Basic and Applied'.

Edition No.	Year
Ist Edition	May 2004
IInd Edition	June 2005
IIIrd Edition	October 2006
IVth Edition	August 2009
Vth Edition	September 2011
VIth Edition	October 2013
VIIth Edition	July 2015

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Syllabus

Unit I **[08 Hours]**

- **Biomolecules:** Introduction, Classification, Chemical Nature and Biological Role of Carbohydrate, Lipids, Nucleic Acids, Amino Acids and Proteins.
- **Bioenergetics:** Concept of Free Energy, Endergonic and Exergonic Reaction, Relationship between Free Energy, Enthalpy and Entropy; Redox Potential.
  Energy Rich Compounds; Classification; Biological Significances of ATP and Cyclic AMP.

Unit II **[10 Hours]**

- **Carbohydrate Metabolism:** Glycolysis - Pathway, Energetics and Significance.
  Citric Acid Cycle - Pathway, Energetics and Significance.
  HMP Shunt and its Significance; Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency.
  Glycogen Metabolism Pathways and Glycogen Storage Diseases (GSD).
  Gluconeogenesis - Pathway and its Significance.
  Hormonal Regulation of Blood Glucose Level and Diabetes Mellitus.
- **Biological Oxidation:** Electron Transport Chain (ETC) and its Mechanism.
  Oxidative Phosphorylation and its Mechanism and Substrate Level Phosphorylation.
  Inhibitors ETC and Oxidative Phosphorylation/Uncouplers.

UNIT III **[10 Hours]**

- **Lipid Metabolism:** β-Oxidation of Saturated Fatty Acid (Palmitic Acid).
  Formation and Utilization of Ketone Bodies; Ketoacidosis.
  De Novo Synthesis of Fatty Acids (Palmitic Acid).
  Biological Significance of Cholesterol and Conversion of Cholesterol into Bile Acids, Steroid Hormone and Vitamin D.
  Disorders of Lipid Metabolism: Hypercholesterolemia, Atherosclerosis, Fatty Liver and Obesity.
- **Amino Acid Metabolism:** General Reactions of Amino Acid Metabolism: Transamination, Deamination and Decarboxylation, Urea Cycle and its Disorders.
  Catabolism of Phenylalanine and Tyrosine and their Metabolic Disorders (Phenyketonuria, Albinism, Alkeptonuria, Tyrosinemia).
  Synthesis and Significance of Biological Substances; 5-HT, Melatonin, Dopamine, Noradrenaline, Adrenaline.
  Catabolism of Heme; Hyperbilirubinemia and Jaundice.

UNIT IV **[10 Hours]**

- **Nucleic Acid Metabolism and Genetic Information Transfer:** Biosynthesis of Purine and Pyrimidine Nucleotides.
  Catabolism of Purine Nucleotides and Hyperuricemia and Gout Disease.
  Organization of Mammalian Genome.
  Structure of DNA and RNA and their Functions.
  DNA Replication (Semi Conservative Model).
  Transcription or RNA Synthesis.
  Genetic Code, Translation or Protein Synthesis and Inhibitors.

UNIT V **[07 Hours]**

- **Enzymes:** Introduction, Properties, Nomenclature and IUB Classification of Enzymes.
  Enzyme Kinetics (Michaelis Plot, Line Weaver Burke Plot).
  Enzyme Inhibitors with Examples.
  Regulation of Enzymes: Enzyme Induction and Repression, Allosteric Enzymes Regulation.
  Therapeutic and Diagnostic Applications of Enzymes and Isoenzymes.
  Coenzymes - Structure and Biochemical Functions.

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UNIT I

Chapter ... 1

CHEMISTRY OF CARBOHYDRATES

♦ LEARNING OBJECTIVES ♦

- *Understand Chemical Structures, Types of Bonding and Isomerisms in different Carbohydrate. Molecules like Monosaccharides, Disaccharides and Polysacharides.*
- *Recognize of Intricacies of Structures of all Carbohydrates.*
- *Know Chemical Reactions of Monosaccharides.*
- *Appreciate the Concepts of Invert Sugar and Inversion.*
- *Acknowledge Biochemical properties of different Carbohydrate Molecules.*

1.1 INTRODUCTION

Carbohydrates may be defined as 'polyhydroxy aldehydes or ketones, or compounds which yield one of these compounds on hydrolysis'.

Many carbohydrates have empirical formula $(CH_2O)_n$, where n is three or larger. Basically, these compounds are made up of C, H and O. On earth, there are probably more carbohydrates than any other organic compounds. The very meaning of term carbohydrate is *hydrates of carbon*. In the course of time, many other compounds have been discovered, that have general properties of carbohydrates, but contain nitrogen, phosphorus and sulfur in addition to carbon, hydrogen and oxygen. There are several non-carbohydrate compounds like acetic acid [CH_3COOH], lactic acid [$C_3H_6O_3$], which can also be called as hydrates of carbon. Moreover, most abundant cellular sugar, deoxyribose, has the molecular formula $C_5H_{10}O_4$, rather than $C_5H_{10}O_5$. Therefore, all carbohydrates cannot be called as hydrates of carbon.

1.2 BIOCHEMICAL ROLE OF CARBOHYDRATES

1. Carbohydrates are important constituents of the cell structure in the form of glycolipid, glycoproteins, heparin, cellulose, starch etc.
2. Carbohydrates serve as an important source as well as a store of energy.
3. Carbohydrates are important starting material for many organic compounds like amino acids, nucleic acid and lipids.
4. Carbohydrates are the first storage form of energy, in the form of glycogen, to compensate for immediate energy demands of body.
5. Carbohydrates are an important raw material for the production of products like glucose, maltose, enzymes, alcohol, acids etc.

1.3 CARBOHYDRATE CLASSIFICATION

There are four major groups of carbohydrates:

1. Monosaccharides, 2. Disaccharides, 3. Oligosaccharides, 4. Polysaccharides.

1.3.1 Monosaccharides: (Synonym – Simple Sugars)

They have the general formula $C_n(H_2O)_n$. They cannot be further hydrolysed into simpler forms.

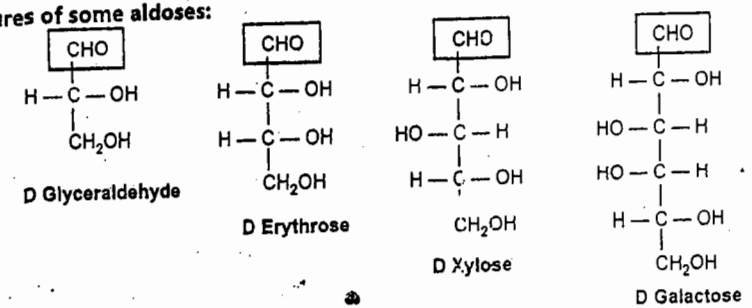
Monosaccharides are further subdivided into different classes depending on the number of carbon atoms or functional group.

(A) Classification of Monosaccharides based on Functional Group

Aldoses: Aldoses are those monosaccharides having the (– C = O) aldehyde as one of their functional groups. e.g. glucose, galactose, glyceraldehyde.

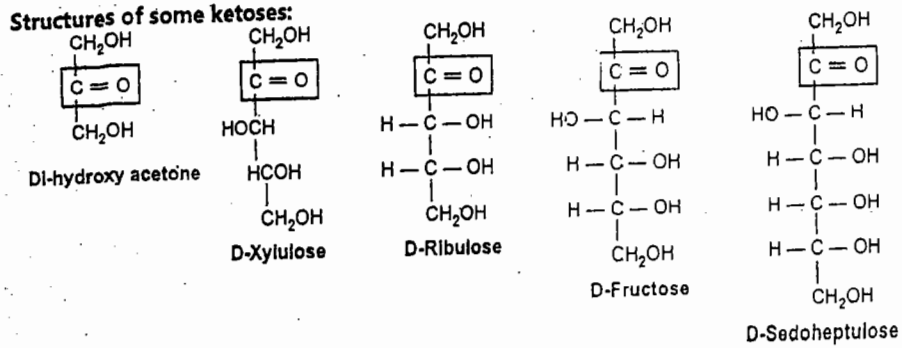
Ketoses: Ketoses are those monosaccharides having the (– C –) keto group, as one of their functional groups e.g. fructose, dihydroxy acetone, ribulose.

Structures of some aldoses:



Examples of aldoses of biochemical importance

Structures of some ketoses:



Examples of ketoses of biochemical importance

(B) Classification of Monosaccharides based on the Number of Carbon Atoms

Number of Atoms	Name	Example
3	Triose	Dihydroxy acetone, Glyceraldehyde
4	Tetrose	Erythrose
5	Pentose	Ribulose, Xylulose, Ribose
6	Hexose	Glucose, Fructose, Mannose, Galactose
7	Heptose	Glucoheptose, Galactoheptose, Sedoheptose

Classification of monosaccharides can also be done according to the number of carbon atoms present in their structure.

Trioses

Glyceraldehyde and dihydroxy acetone are examples of trioses. These two monosaccharides are formed in the glycolytic pathway. 3-Phosphoglyceraldehyde is another triose and is found in the hexose monophosphate (HMP) shunt pathway.

Tetrose

An example of tetrose, erythrose 4-phosphate is formed by HMP shunt pathway.

Pentoses

Ribose and deoxyribose are examples of pentoses. These two pentoses are important constituents of nucleic acids. Xylulose and ribulose are ketopentoses.

Hexoses

Glucose, galactose, fructose, mannose etc. are examples of hexoses of which glucose is the fuel of life. Galactose is a constituent of lactose (milk sugar). Fructose is found in fruits, honey and as a constituent of sucrose, and mannose is a constituent of plant polysaccharides.

Heptoses

Sedoheptulose is a ketoheptose existing in the plant kingdom. Sedoheptulose-7-phosphate is one of the intermediates of the HMP shunt. The same compound is one of the products of photosynthesis.

1.4 ISOMERISM IN MONOSACCHARIDES

1.4.1 Stereoisomers or Geometric Isomers

Stereoisomers: Stereoisomers are compounds which have same structural formula but differ in their spatial configuration.

The presence of asymmetric carbon atoms in the compounds allows the formation of such isomers.

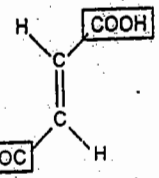
1.4.1.1 Asymmetric Carbon Atom

The carbon atom to which four different atoms or groups are attached is called as asymmetric carbon atom.

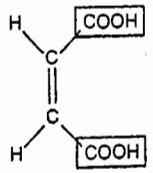
Thus, it is the number (n) of asymmetric carbon atoms in the compound which determines the number of possible isomers of the compound. Thus, galactose with 4 asymmetric carbon atoms has 2^n i.e. $2^4 = 16$ isomers.

1.4.2 Cis and Trans Isomerism

Compounds with double bonds show this type of isomerism. Rigidity of double bond, resulting in restriction in rotation about the carbon-carbon double bond is responsible for geometrical isomerism. ***Cis*** isomer is one in which two similar groups are on the same side of the double bond.



Fumaric acid (Trans-structure)



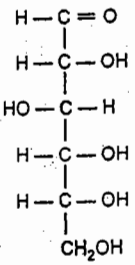
Maleic acid (Cis-structure)

Trans isomer is one in which two similar groups are on the opposite side of the double bond.

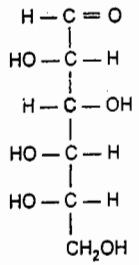
1.4.3 D and L Isomerism

The D and L isomers are mirror images of each other. All the naturally occurring monosaccharides of mammalian tissues are mostly of D-configuration. All the cellular enzymes are specific for D series of monosaccharides.

The spatial orientation of –H and –OH groups on the carbon atom that is adjacent to terminal primary alcohol carbon (e.g. C_5 of glucose) determines whether the sugar is D or L series.



D-Glucose



L-Glucose

1.4.3.1 Optical Activity of Sugars

When a beam of polarised light is passed through a solution of optical isomers, it will be rotated either to the right or left. The term dextro rotatory (+) is used for compounds that rotate the plane of polarised light to the right i.e. clockwise and levo rotatory (–) is used for compounds that rotate the plane of polarised light to left i.e. anti-clockwise.

Optical isomers: Optical isomers are a type of stereoisomers. The unique feature of optical isomers is that they have the ability to rotate plane polarised light. This property is referred to as optical activity.

1.4.3.2 D-L mixture (Racemic mixture)

The mixture that contains equal amounts of dextro rotatory and levo rotatory isomers is called as D-L mixture or racemic mixture.

Such a mixture has no optical activity as the optical activity of one isomer is cancelled by the other isomer. Synthetically produced compounds are generally racemic.

1.4.3.3 Resolution

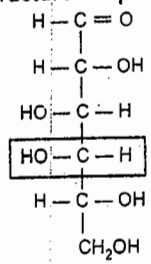
Synthesis of an optically active compound produces a mixture of both (+) and (–) isomers in equal amounts. Such a mixture is called racemic mixture or racemate.

The separation of optically active isomers from the racemic mixture is called as resolution. The mixture is resolved into optically active counterparts or compounds (+ and – isomers). Resolution is of two types – (1) Chemical resolution, (2) Enzymatic resolution.

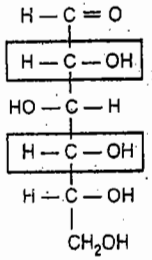
1.4.4 Epimers

Glucose, galactose and mannose are called as the epimers of each other. They are formed by interchange of the –OH and –H, on the carbon atom 2 or 4 of glucose. Enzyme epimerase is responsible for the interconversions of epimers.

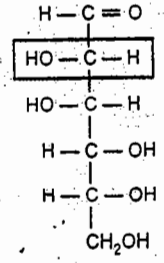
Structure of Epimers:



D-Galactose



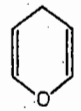
D-Glucose



D-Mannose

(glucose and mannose are epimers at C_2 while glucose and galactose are epimers at C_4)

1.4.5 Pyranose and Furanose Ring Structures

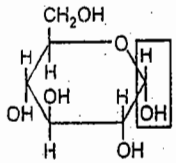


Pyran

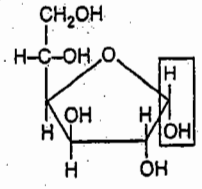
Furan

Ring structures of sugars were proposed by Haworth in 1929 in which all sugars forming six membered rings are called pyranose from their relation to pyran and name furanose was given to those sugars, forming a five membered ring. Pyranose and furanose forms of the glucose are given below.

Structure of glucose-pyranose and -furanose forms.



α-D-Glucopyranose

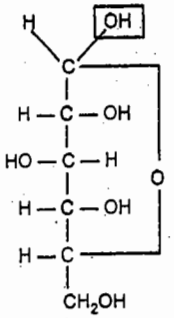


α-D-Glucofuranose

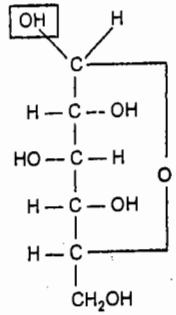
1.4.6 α and β Anomers of D-glucose

The cyclic structure of glucose is retained in the solution. But isomerism takes place around the carbon atom 1. This occurs along with the optical rotation by which the positions of H and –OH groups are changed around carbon number 1. α and β cyclic forms of glucose are called as anomers. They differ from each other in configuration only at C_1 known as anomeric carbon atom. The physical and chemical properties of anomers are different.

Mutarotation: Intraconversion of α and β glucose in solution along with the change in their optical activity is called as *mutarotation*. It can be defined as 'the change in the optical rotation due to interconversion of α and β forms of D-glucose to form an equilibrium mixture'.



α-D-Glucose

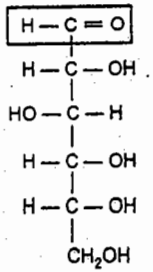


β-D-Glucose

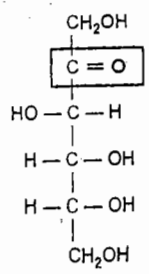
1.4.7 The Aldoses and Ketoses

Glucose has the same molecular formula as that of the fructose. But both of them differ from each other with respect to their structural formula. Glucose is an aldose sugar due to the presence of aldehyde group and fructose is a ketose sugar due to the presence of the keto group in its structure.

Chemical structures of D-glucose (aldohexose) and D-fructose (ketohexose) are given below:



D-Glucose
(Aldohexose)



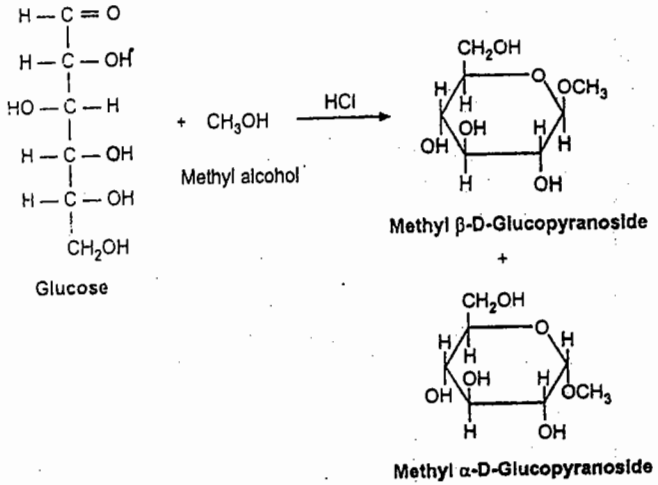
D-Fructose
(Ketohexose)

1.5 GLYCOSIDES

A condensation reaction between sugar and hydroxyl group of other compounds, also called as a aglycone, forms a compound known as a glycoside. The aglycone may or may not be another sugar. If the carbohydrate portion is glucose, the resulting compound is undergoes condensation *glucoside*, if galactose the compound formed is called as *galactoside*. One of the important properties of monosaccharide is its ability to form glycosides.

Condensation of the reducing sugar with dry methyl alcohol in the presence of catalyst like dry HCl results in glycoside formation.

Glycoside Formation:



Glucose + CH_3OH (Methyl alcohol) $\xrightarrow{HCl}$ Methyl β-D-Glucopyranoside + Methyl α-D-Glucopyranoside

Some examples of aglycones are as follows:

methyl alcohol, glycerol, other sugars and sterol.

Glycosides are found in many species, in animal tissues and in many drugs. An antibiotic like streptomycin is a glycoside. Some cardiac glycosides are very important in medicine because of their action on the heart. All the cardiac glycosides contain steroid as the aglycone component. These glycosides are obtained from digitalis e.g. digoxin and strophanthus e.g. ouabain, are inhibitors of Na^+–K^+ ATPase of the cell membrane.

1.6 AMINO SUGARS (HEXOSAMINES)

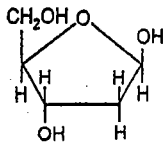
These sugars are components of glycoproteins, gangliosides and glycosaminoglycans.

Sugars containing amino groups are called as the amino sugars. Examples of amino sugars are D-glucosamines, D-galactosamine and D-mannosamine.

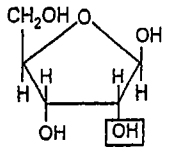
Several antibiotics contain amino sugars e.g. erythromycin and carbomycin. *Erythromycin contains dimethyl amino sugars. Carbomycin contains 3 amino-sugars* e.g. 3 amino D-ribose. The antibiotic activity of the drug is due to the presence of amino sugars. Glucosamine is also a constituent of hyaluronic acid.

1.7 DEOXY SUGARS

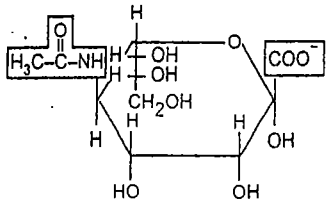
Hydroxyl group attached to the ring structure of these sugars has been replaced by hydrogen atom. e.g. deoxyribose sugar is found in the DNA (nucleic acid). Deoxy sugars cannot form osazones. These sugars are very unstable and form resins.



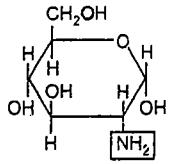
D-2-Deoxyribose (β-form)



D-ribose (β-form)



N acetyl neuraminic acid



αD-Glucosamine

Structures of few monosaccharides and monosaccharide derivatives

1.8 MONOSACCHARIDE GLUCOSE

Glucose: Glucose is the most important monosaccharide in the body. It is the most widely distributed sugar in the body. The molecular formula of glucose is $C_6H_{12}O_6$. The structural formula of glucose was given earlier in the chapter.

1.8.1 Structure of Glucose

The molecule of glucose contains five –OH groups.

The presence of five hydroxyl groups is confirmed due to the formation of a pentacetyl derivative on acetylation. It gives the following set of reactions:

$$\text{Glucose} \xrightarrow{\text{Reduction}} \text{Sorbitol} \quad \ldots (1.1)$$

$$\text{Glucose} \xrightarrow{\text{Oxidation}} \text{Gluconic acid} \quad \ldots (1.2)$$

$$\text{Glucose} \xrightarrow[\text{Killiani Reaction}]{\text{HCN}} \text{Heptylic acid} \quad \ldots (1.3)$$

All three reactions of glucose indicate the presence of $\left(\begin{matrix}-C-H\\ \|\\ O\end{matrix}\right)$ group.

Killiani reaction involving reaction with HCN, hydrolysis of cyanohydrin and reduction with HI gives normal heptylic acid, which is a straight chain compound. This confirms that glucose is also a straight chain compound.

However, straight chain structure of glucose is unable to explain the following properties:

1. Mutarotation of glucose.
2. No compound formation with $NaHSO_3$.
3. No restoration of colour with Schiff's reagent.
4. Formation of two isomeric glucosides with methyl alcohol and dry HCl gas.

In order to explain the above properties of glucose, Fischer suggested that glucose molecule must be existing in the ring structural form. Both six membered ring and five membered ring are possible and their existence has also been proved experimentally. Six membered ring i.e. a pyranose form is more stable. Further, glucose exists in α and β forms too.

1.9 CHEMICAL REACTIONS OF MONOSACCHARIDES

1.9.1 Reduction

Monosaccharides are reduced to their corresponding alcohols by reducing agents such as sodium amalgam.

$$\text{Glucose} \xrightarrow[\text{Na/Hg}]{\text{Reduction}} \text{Sorbitol (gluatol)}$$

$$\text{Galactose} \xrightarrow[\text{Na/Hg}]{\text{Reduction}} \text{Dulcitol (galactitol)}$$

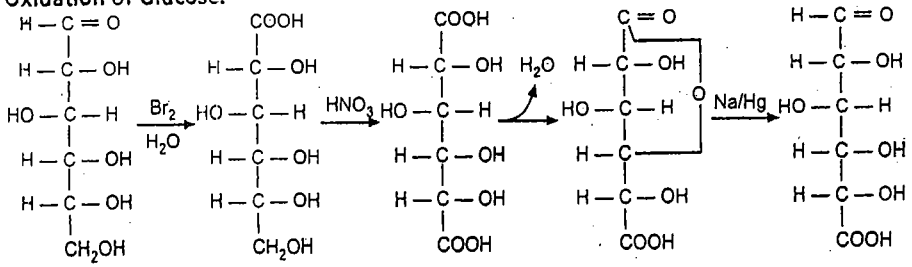
$$\text{Mannose} \xrightarrow[\text{Na/Hg}]{\text{Reduction}} \text{Mannitol}$$

$$\text{Fructose} \xrightarrow[\text{Na/Hg}]{\text{Reduction}} \text{Mannitol + Sorbitol}$$

1.9.2 Oxidation

Oxidation of the aldehyde group of the glucose forms aldonic acids. If the aldehyde group does not participate in the reaction and if the primary alcoholic group is oxidised then uronic acids are formed. The oxidation of glucose gives glucuronic acid under the following sets of conditions.

Oxidation of Glucose:



D-Glucose — Gluconic acid — Saccharic acid — Saccharolactone — Glucuronic acid

1.9.3 Heat

Glucuronic acid on heating produces lactones. These are cyclic structures which resemble pyranoses and furanoses.

1.9.4 Alkali

Monosaccharides react in various ways with alkali:

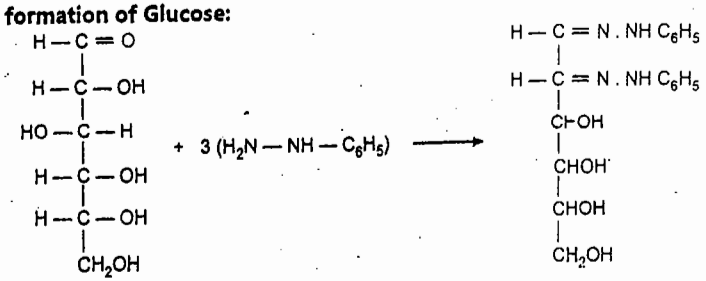
(a) In dilute alkali, sugar will change to the cyclic α- and β- structures with equilibrium between two isomeric forms.
On standing the rearrangement will occur which produces an equilibrium mixture of glucose, fructose and mannose through enediol form.

(b) Sugar produces a series of decomposition products in concentrated alkali. Yellow and brown pigments are developed and salts may be formed. Many double bonds between carbon atoms are formed, C-C bonds (carbon to carbon) may also rupture.

1.9.5 Osazone Formation

On addition of a mixture of phenylhydrazine hydrochloride and sodium acetate to sugar solution and heating in the boiling water bath, crystals are formed. The reaction takes place between carbonyl group (aldehyde or ketone) and next adjacent carbon with the N of phenylhydrazine. The reaction with the aldose is shown below. The hydrazone so formed reacts with the two additional molecules of phenylhydrazine to form osazones. The ketones also show a similar reaction.

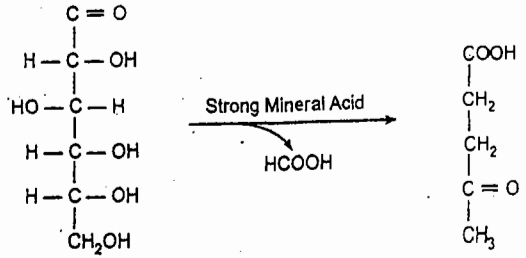
Osazone formation of Glucose:



D-Glucose Phenylhydrazine Osazone

1.9.6 Strong Mineral Acids

Glucose on treatment with strong mineral acids gives levulinic acid.



D-Glucose Levulinic acid

1.9.7 Iodo Compounds

Aldose sugar on heating with concentrated hydroiodic acid (HI) loses all its oxygen and is converted into an iodo compound.

$$C_6H_{12}O_6 + HI \longrightarrow C_6H_{13}I$$

Glucose Iodohexane

1.9.8 Ester Formation

The presence of hydroxyl group in sugars allows the reaction of esterification. All free – OH groups are esterifiable.

1.10 DISACCHARIDES

Disaccharides are composed of two monosaccharide units joined by a glycosidic bond. Some commonly occurring disaccharides are:

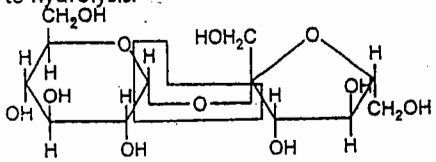
(1) Sucrose = 1 molecule of glucose + 1 molecule of fructose.
(2) Lactose = 1 molecule of glucose + 1 molecule of galactose.
(3) Maltose = 1 molecule of glucose + 1 molecule of glucose.

(A) Sucrose:

It is present in sugarcane, carrot, honey, pineapple, beetroot etc. It does not exist in the human body. The enzyme sucrase also known as invertase hydrolyses sucrose to glucose and fructose. The molecule of sucrose is formed by glycosidic linkage between aldehyde group of glucose and keto group of the fructose. There is no free aldehyde or ketone group available in the sucrose molecule. This is the reason why sucrose is a non-reducing sugar. It does not exhibit mutarotation and does not exist in α and β forms. It is unable to form osazone with phenylhydrazine. It is also unable to reduce Benedict's reagent, Fehling's solution or Barfoed's reagent.

It is also known as *invert sugar* due to the following reasons:

The specific rotation of the sucrose solution is + 66.5°. This rotation changes to −19.5° during hydrolysis. This is because levo rotation of fructose (a hydrolysis product) is greater than dextro rotation of glucose. Sucrose is known as invert sugar because of the inversion in the specific rotation due to hydrolysis.



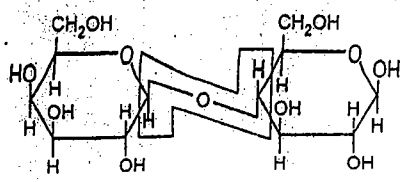
Glucose Fructose

Sucrose

(α-D-glucosyl (1→2) β-D-fructose)

(B) Lactose:

Lactose is also known as milk sugar. It is made up of a molecule of glucose and a molecule of galactose. It is formed in lactating mammary gland. It is present in milk. The enzyme lactase hydrolyses it to glucose and galactose. Lactose has free aldehyde group of glucose. The free aldehyde group of its constituent galactose is engaged in glycosidic linkage with the 4th carbon of glucose. It is a reducing sugar. It shows mutarotation. It can also exist in α and β forms. It can reduce Benedict's solution, Fehling's solution, Barfoed's reagent. It can also form osazone with the phenylhydrazine.



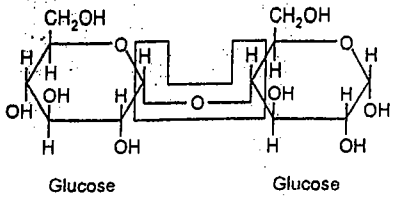
Lactose

(β-D-galactosyl (1→4) β-D-glucose)

(C) Maltose:

Maltose is found in germinating seeds, cereals and malt. It is made up of two molecules of glucose. Maltose is formed during hydrolysis of starch by the enzyme diastase. Chemically, maltose is glucose α-glucoside. Enzyme maltase hydrolyses maltose to glucose. It has free aldehyde groups and hence shows mutarotation. It can exist in α or β forms.

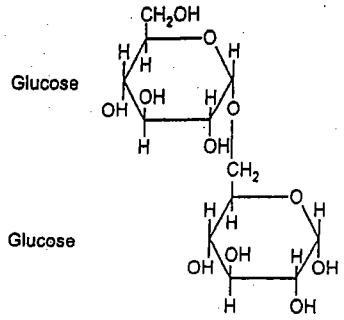
Maltose can reduce Fehling and Benedict's solutions being a reducing sugar. It forms an osazone with phenylhydrazine.



Maltose

(α-D-glucosyl (1→4) α-D-glucose)

(D) Isomaltose: Another disaccharide obtained during the hydrolysis of certain polysaccharides like glycogen, amylopectin and dextran. It is similar to maltose except it has an α (1 → 6) linkage.



Isomaltose

(α-D-glucosyl (1→6) α-D-glucose)

1.11 POLYSACCHARIDES

Long chains of monosaccharides joined together are collectively called polysaccharides. Polysaccharides are of two types:

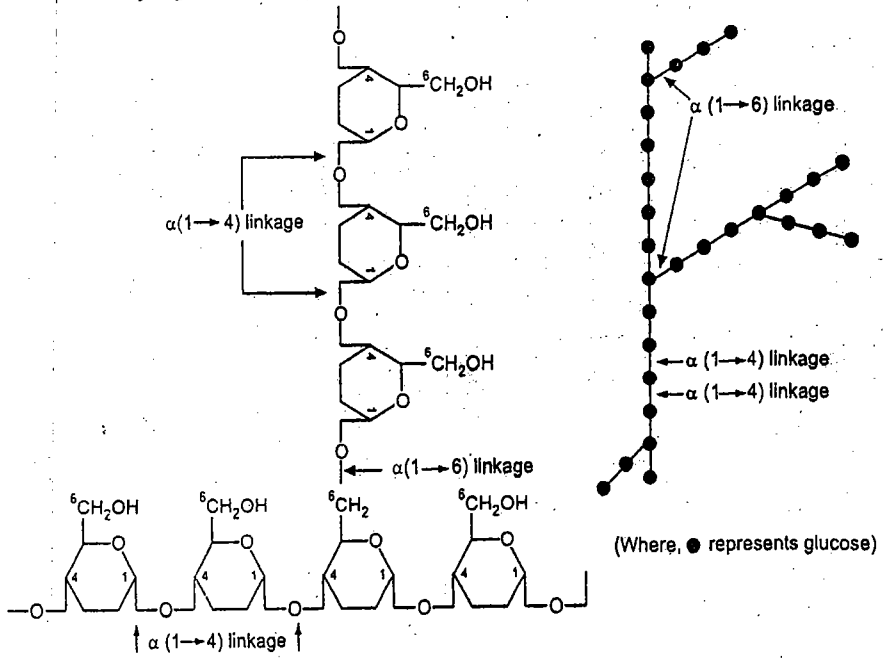
1. **Homopolysaccharides:** These are very large molecules which contain only single type of monosaccharide component. e.g. starch, glycogen, dextran, cellulose etc.
2. **Heteropolysaccharides:** These molecules are formed from one or more monosaccharide component as their basic units e.g. heparin, chondroitin, sulphates, hyaluronic acid, murein etc.

The major storage polysaccharides are glycogen (in animals), starch (in plants) and dextran (in yeast and bacteria). Glycosaminoglycans is a mucopolysaccharide. Cellulose is a structural polysaccharide found in plant cell walls.

(A) Glycogen (Animal Starch):

Glycogen is a storage polysaccharide and is found in animal tissues. It is a branched chain polysaccharides made up of glucose as a basic unit. Glucose molecules are linked by α (1 → 4) bonds with α (1 → 6) branch points. The branching in glycogen makes it more accessible to glycogen phosphorylase during its degradation. It is also referred to as the animal starch.

Structure of Glycogen:

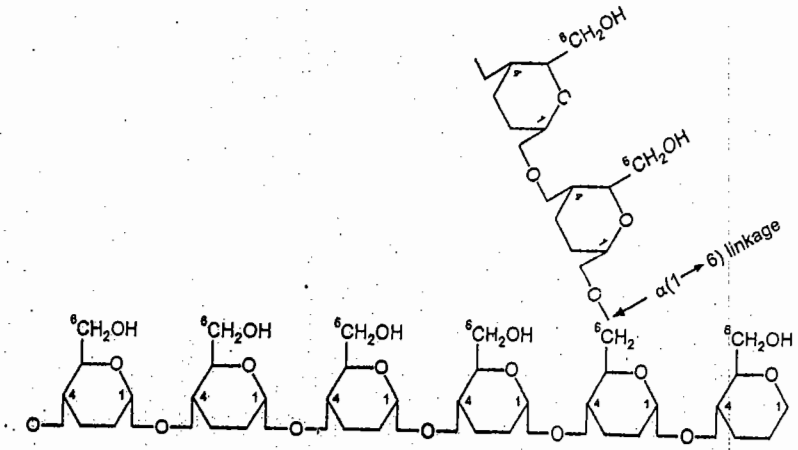


(B) Starch:

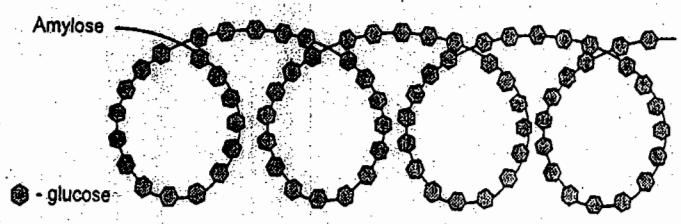
Starch is formed of an α glycoside chain. Such a compound, yielding only glucose on hydrolysis, is a homopolymer called glucan. It is the most important food source of carbohydrate and is found in cereals, potatoes, legumes and other vegetables. The two chief constituents of starch are amylose (15-20%) (amylose is a non-branching helical structure) and amylopectin which contains branched chain composed of 24-30 glucose residues united by (1 → 4) linkage in the chains and by (1 → 6) linkage at the branch points.

Structure of Starch:

(Amylose and Amylopectin)



(Amylopectin) (Branched chains composed of glucose units joined together by α (1→4) linkage in chains and α (1→6) linkage at the branch points.



(Amylose) (non-branching helical structure)

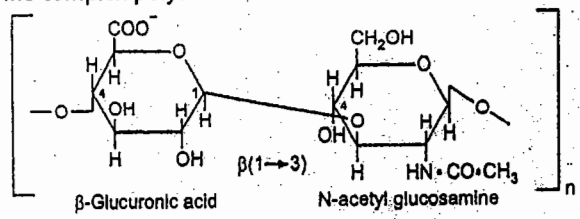
(C) Cellulose:

Cellulose is the chief constituent framework of plants. It is insoluble and consists of β-D-glucopyranose units linked by β (1 → 4) bonds to form long straight chains strengthened by cross-linked hydrogen bonds. It is also known as structural polysaccharide. Cellulose cannot be digested by many mammals including human, because of the absence of a hydrolase that attacks the β linkage. Thus, cellulose is an important source of fibre in the diet. Cellulase, an enzyme that degrades cellulose is absent in mammals, but it is produced by some bacteria, fungi, protozoa and herbivorous animals.

1.12 HETEROPOLYSACCHARIDES (GLYCOSAMINOGLYCANS OR MUCOPOLYSACCHARIDES)

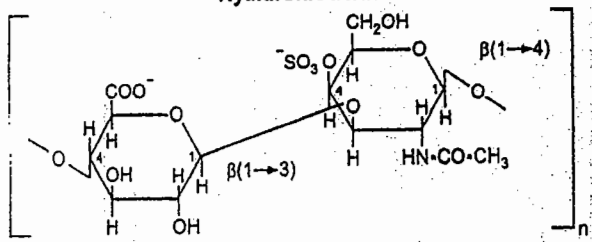
These are composed of derivatives of sugars such as amino sugars and uronic acid. Hence, the name glucosaminoglycans. They consist of repeated disaccharide units in which uronic acid is linked glycosidically to 3-position of an acylated amino sugar. They are acidic and occur in association with peptide group. e.g. hyaluronic acid, chondroitin, sulphate, heparin etc. Mucopolysaccharides are gelatinous substances of high molecular weight (upto 5×10^6) that both lubricate and serve as a sticky cement.

Structures of some complex polysaccharides:



β-Glucuronic acid N-acetyl glucosamine

Hyaluronic acid



β-Glucuronic acid N-acetyl galactosamine 4 sulphate

Chondroitin 4 sulphate

(A) Chitin:

It is an important polysaccharide of invertebrates. It is found in exoskeleton of crustaceans and insects. Structurally, chitin consists of N-acetyl D-glucosamine units joined by β (1 → 4) glycosidic linkage.

(B) Hyaluronic Acid:

It is a heteropolysaccharide composed of alternating units of D-glucuronic acid and N-acetyl D-glucosamine. The two different monosaccharides are linked by a β (1 → 3) bond to form a disaccharide and a heteropolysaccharide. Hyaluronic acid contains repeating units of the above mentioned disaccharide, that is linked by β (1 → 4) to the next.

Hyaluronic acid is water soluble, but it forms a viscous solution. It is found in the viterous humor of the eye and umbilical cord. It is also present in the synovial fluid of the joints where it acts as a lubricant.

(C) Chondroitin Sulphate:

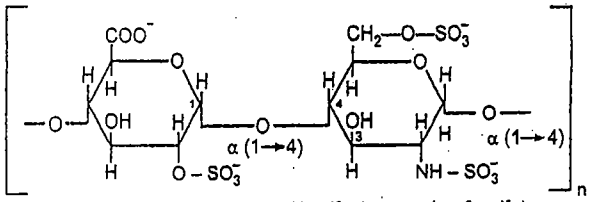
This heteropolysaccharide is made up of repeating units of disaccharide made up of D-glucuronic acid and N-acetyl D-galactosamine sulphates linked by a β (1 → 3) bond. Each disaccharide molecule is linked by β (1 → 4) to the next.

Chondroitin sulphate is very abundant in cartilage tissue and skin.

(D) Heparin:

Heparin is composed of alternating repeating units of N-sulfo-D-glucosamine-6-sulfate and glucuronate-2-sulphate linked by α (1 → 4), α (1 → 4) linkage. It may contain ioduronate in place of glucuronate.

Heparin is a natural anticoagulant secreted by mast cells in the lungs, intestinal mucosa and is found in the blood, spleen, kidney, liver etc. Another function of heparin is release of enzyme lipoprotein lipase. The enzyme is helpful in clearing the turbidity of lipemic plasma. It prevents the blood clotting by inhibiting conversion of prothrombin to thrombin. Heparin is also given intravenously to bone fracture patients to avoid the danger of clot formation prior to surgery.



D-glucuronate-2-sulphate N-sulfoglucosamine-6-sulfate

Heparin

1.13 GLYCOPROTEINS

These are proteins containing the carbohydrates in varying amounts. The carbohydrates are attached as short, long, branched or unbranched chains. The carbohydrates are covalently bonded to proteins. Glycoproteins occur in tissues, nucleus, secretions of respiratory tract, vagina, uterus etc. The other glycoproteins include plasma proteins, blood group substances etc.

QUESTIONS

1. Explain the term carbohydrate.
2. Give detailed classification of carbohydrates.
3. Write short notes on the following:
   (a) Non-reducing sugar (sucrose)
   (b) Lactose
   (c) Glucosaminoglycans
   (d) Structure of starch and glycogen.
4. Explain the term isomerism. Give the salient features of stereoisomerism in carbohydrates.
5. Define - mutarotation.
6. Why is sucrose known as invert sugar?
7. Define epimer. Give the examples of the same.
8. What is the difference between disaccharide and polysaccharide?

9. Which test will you perform to detect the presence of reducing sugar? Explain the principle of the same test.
10. Give an account of biological importance of glucose.
11. Describe structure and function of mucopolysaccharides.
12. Discuss the structure and function of homopolysaccharides.
13. Outline the salient features of the importance of glycosaminoglycans.
14. Define a glycoside. Explain the glycoside formation with a neat labelled diagram.
15. What is the biological significance of glycoproteins?
16. Explain the action of phenylhydrazine on the glucose molecule.

Chapter ... 2

CHEMISTRY OF AMINO ACIDS AND PROTEINS

♦ LEARNING OBJECTIVES ♦

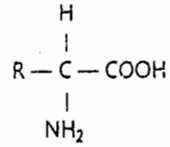
- *Explain what Amino Acids are and their Role in Protein Formation*
- *Understand Structural Classification of Amino Acid*
- *Recognize various ways of Classification of Amino Acids and Proteins*
- *Appreciate Biochemical importance of Proteins*
- *Understand various Levels of Structural Organization of Proteins and its Significance*

2.1 INTRODUCTION

Amino Acids

Amino acids are a group of organic compounds containing two functional groups, carboxyl (–COOH) and amino ($–NH_2$).

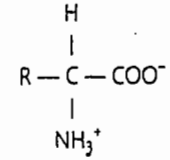
In α amino acids, both groups are attached to the same carbon atoms.



Representation of Amino Acid Structure

Amino acids play central roles both as building blocks of proteins and as intermediates in metabolism. There are about 300 amino acids occur in nature. Only twenty amino acids are found in proteins. The exact sequence and content of amino acids in proteins is determined by the sequence of bases in the gene that encodes the protein.

The side chain R is different for all 20 amino acids found in proteins. The amino acids mostly exist in ionised form in the biological system.



2.2 STEREO FORM OF AMINO ACIDS IN BIOLOGICAL SYSTEM (L & D FORM OF AMINO ACID)

With the exception of glycine, all four groups linked to α carbon atom of amino acids are different.

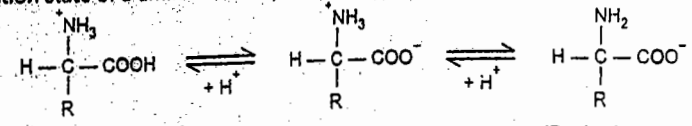
All proteins contain L amino acids as their building blocks, whereas D amino acids are constituents of antibiotics.

Zwitter ions: Amino acids in solution at a physiological pH (7.4) are predominantly dipolar ions (or zwitter ions) rather than unionized molecules. In the dipolar form of an amino acid molecule, the amino group is ionized ($-NH_3^+$) and carboxyl group is dissociated ($-COO^-$).

The ionization state of an amino acid changes with the pH. In an acid solution, the carboxyl group is unionized and the amino group is ionized ($-NH_3^+$) (e.g. at pH =1).

In an alkaline solution (e.g. pH = 10), the carboxyl group is ionized ($-COO^-$) and the amino group is unionized ($-NH_2$).

Ionization state of a amino acid depends on the pH.



(Predominant form at pH – 1) (Predominant form at pH – 7) (Predominant form at pH – 10)

2.3 CLASSIFICATION OF AMINO ACIDS

Amino acids can be classified in different ways as follows:

(A) Classification based on structure.
(B) Classification based on chemical nature.
(C) Classification based on nutritional requirement.
(D) Classification based on metabolic fate.

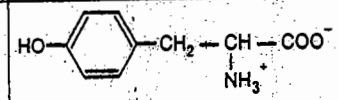
2.3.1 Amino Acid Classification Based on Structure

Each amino acid is assigned 3 letters or 1 letter symbol. There are 7 distinct groups of 20 amino acids.

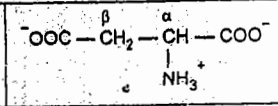
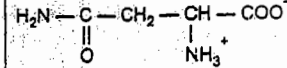
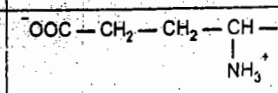
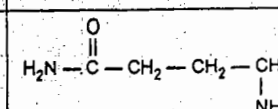
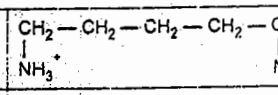
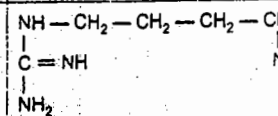
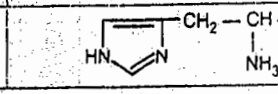
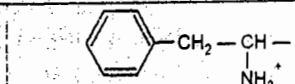
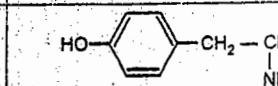
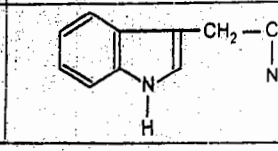
Table 2.1: Classification of amino acids

Name	Symbol		Structure	Group unique to amino acid
	1 letter	3 letter		
I. Amino acids containing aliphatic side chains				
1. Glycine	G	Gly	$H-CH(NH_3^+)-COO^-$	
2. Alanine	A	Ala	$CH_3-CH(NH_3^+)-COO^-$	

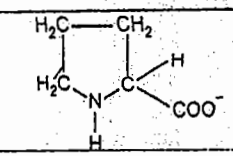
contd. ...

3. Valine	V	Val	$(H_3C)_2CH-CH(NH_3^+)-COO^-$	Branched chain
4. Leucine	L	Leu	$(CH_3)_2CH-CH_2-CH(NH_3^+)-COO^-$	Branched chain
5. Isoleucine	I	Ile	$CH_3-CH_2-CH(CH_3)-CH(NH_3^+)-COO^-$	Branched chain
II. Amino acids containing hydroxyl group				
6. Serine	S	Ser	$CH_2(OH)-CH(NH_3^+)-COO^-$	Hydroxyl
7. Threonine	T	Thr	$H_3C-CH(OH)-CH(NH_3^+)-COO^-$	Hydroxyl
Tyrosine (This amino acid is also a member of aromatic amino acids).	Y	Tyr		Phenol
III. Sulfur containing amino-acids				
8. Cysteine	C	Cys	$CH_2(SH)-CH(NH_3^+)-COO^-$	Sulfhydral
9. Cystine			$CH_2(S-S-CH_2)-CH(NH_3^+)-COO^-$ (×2)	Disulfide
10. Methionine	M	Met	$CH_2(S-CH_3)-CH_2-CH(NH_3^+)-COO^-$	Thioether

contd. ...

IV. Acidic amino acids and their amides				
11. Aspartic acid	D	Asp		β carboxyl
12. Asparagine	N	Asn		Amide
13. Glutamic acid	E	Glu		γ carboxyl
14. Glutamine	Q	Gln		Amide
V. Basic amino acids				
15. Lysine	K	Lys		ε Amino
16. Arginine	R	Arg		Guanidino
17. Histidine	H	His		Imidazole
VI. Aromatic amino acids				
18. Phenyl alanine	F	Phe		Benzene or phenyl
19. Tyrosine	Y	Tyr		Phenol
20. Tryptophan	W	Trp		Indole

contd. ...

VII. Imino acids				
21. Proline	P	Pro		Pyrrolidine

1. **Amino acids with aliphatic side chains:** Structurally simplest amino acids, glycine, alanine, valine, leucine, isoleucine belong to this class. They contain monoamino, monocarboxylic groups. The valine, leucine, isoleucine have branched chains in their structure.
2. **Hydroxyl group containing amino acids:** Serine, threonine and tyrosine are hydroxyl group containing amino acids of which tyrosine is also classified under aromatic amino acids.
3. **Sulfur containing amino acids:** Cysteine, cystine and methionine are members of this class. They are characterised by the presence of sulfhydryl, disulfide and thioether group respectively. Amino acid cystine is formed by condensation of two molecules of cysteine.
4. **Acidic amino acids and their amides:** Aspartic acid and glutamic acid are dicarboxylic monoamino acids. While asparagine and glutamine are respective amines of these dicarboxylic acids. They are acidic amino acids.
5. **Basic amino acids:** Lysine, arginine, histidine are dibasic, monocarboxylic amino acids. Presence of one more amino group makes them basic in nature. Histidine contains imidazole ring.
6. **Aromatic amino acids:** Amino acids phenylalanine, tyrosine and tryptophan are characterised by the presence of aromatic ring. The phenylalanine and tyrosine contain benzene ring and tryptophan contain indole ring. Histidine can be grouped in here. But owing to its basic nature, it is also considered under basic amino acids.
7. **Imino acids:** Presence of secondary amino group in proline makes it imino acid. Hydroxylation of proline converts it into hydroxyproline. Hydroxyproline is the active component of cross linking of collagen.

2.3.2 Classification of Amino Acids based on Polarity (Chemical Nature)

The nature of R group attached to amino acids determines the polar nature of amino acids. The amino acids may be divided into two broad groups on the basis of polar nature of R groups:

1. **Non-polar amino acids:** They are also known as *hydrophobic* or water hating amino acids. The absence of charge on the R groups of these amino acids makes them non-polar amino acids. The amino acids – alanine, leucine, isoleucine, valine, methionine, phenylalanine, tryptophan and proline are members of this group.

2. ***Polar amino acids:*** They are also referred to as *hydrophilic* amino acids. The amino acids like arginine, aspargine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, lysine, serine, threonine are in this class of amino acids. These amino acids may contain no-charge on their R groups e.g. glycine, threonine etc. or they may contain positive side chains of R groups e.g. lysine, arginine and histidine. Amino acids like aspartic acid and glutamic acids are characterised by negatively charged R side chains.

2.3.3 Classification Based on the Nutritional Requirement

In this way of classification, all 20 amino acids are divided into two groups viz.

1. Essential (or indispensable), 2. Non-essential (dispensable) amino acids.

Our body contains sufficient biochemical machinery in terms of enzymes, co-enzymes and favourable cellular sites to support the complete synthesis of non-essential amino acids. Thus, non-essential amino acids are not a necessary component of diet.

The number of essential amino acids changes from animal to animal. Humans can produce 10 of the 20 amino acids and others must be supplied in the food.

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

*These amino acids are considered as essential, although they are synthesised by humans, because of the following reasons:

Arginine : The rate of synthesis is insufficient to meet the growth needs of the body.

Methionine : It is required to produce cysteine if the latter is not adequately available in the diet.

Phenylalanine : It is required to produce tyrosine if the latter is not adequately available in the diet.

If our body fails to produce even one of the ten essential amino acids then it results in the degradation of muscles. This way, the body tries to obtain that one amino acid. It is important to note that unlike fats and carbohydrates, human body does not store excess amino acids. Hence, amino acids i.e. proteins must be present in the food everyday.

Most micro-organisms and plants are able to synthesize all amino acids on their own.

The enzymes meant for metabolism of essential amino acids are specific to L amino acids only.

2.3.4 Based on the Nature of Metabolic End Products, Amino Acids are Classified into Two Groups

1. **Glucogenic (glycogenic) amino acids:** A group of amino acids whose carbon skeleton is finally catabolised to pyruvate or one of the intermediates of TCA cycle like oxaloacetate, fumarate, succinyl CoA and α-ketoglutarate. These intermediates are substrates for gluconeogenesis leading to formation of glucose or glycogen.
2. **Ketogenic amino acids:** Amino acids whose carbon skeleton is metabolised (catabolised) to acetoacetate or acetyl CoA which can be converted to fat (e.g. fatty acids or ketone bodies).

Some of the amino acids are both glycogenic as well as ketogenic as they can serve as precursors for both, glucose or fat.

The details of catabolism of amino acids to amphibolic intermediates are given below:

1. Acetyl CoA and acetoacetate – Tyrosine, Phenylalanine, Tryptophan, Isoleucine, Leucine, Lysine.
2. Oxaloacetate – Aspartate and Aspargine
3. Fumarate – Phenylalanine, Tyrosine
4. Succinyl CoA – Methionine, Isoleucine, Threonine, Valine.
5. α–ketoglutarate – Glutamate, Glutamine, Arginine, Histidine, Proline.
6. Pyruvate – Alanine, Cysteine, Glycine, Hydroxyproline, Serine and Threonine.

Table 2.2: Classification of amino acids on the fate of carbon skeleton

Glycogenic (glucogenic)	Glycogenic and Ketogenic	Ketogenic
Alanine	Phenylalanine	Lysine
Arginine	Isoleucine	Leucine
Aspartate	Tyrosine	
Cysteine	Tryptophan	
Glutamine		
Glutamate		
Glycine		
Histidine		
Hydroxyproline		
Methionine		
Proline		
Serine		
Threonine		
Valine		

2.4 PROTEINS

(a) Peptides and Proteins: 20 amino acids are commonly found in proteins. These twenty amino acids are linked together through peptide bond forming peptides and proteins. The chains containing less than 50 amino acids are called peptides while those containing greater than 50 amino acids are called proteins.

(b) Proteins can be defined as 'high molecular weight mixed polymer of α amino acids joined together with peptide linkage (– CONH –)'.

Biological Role of Proteins:

Proteins contain carbon, hydrogen, oxygen, nitrogen and sulphur as major constituents. Some proteins also contain phosphorus as the chief constituent. Proteins are present as the main constituent of all living matter. Proteins are among the central molecules of biology. In fact, along with nucleic acids, they are the most essential as virtually all the chemical functions of the living cell are performed by proteic enzymes (there are a minority of RNA-based enzymes also). In addition, most of the scaffoldings that hold cells, chromosomes and other molecules together are made up of proteins. Proteins transmit and commute signals from the external environment to cell interior, duplicate genetic information, transform the energy in light, carry out chemical reactions with tremendous efficiency, transport molecules between cell compartments etc. In a way, proteins constitute the most complex, precise and minute example of nanotechnology known by humans. Proteins are the most perfect nanomachines.

Proteins make up over 50% of the solid components in the cell and range from small simple peptides e.g. neurotransmitters, to large complex structures, e.g. haemoglobin.

2.4.1 Biochemical Importance of Proteins

1. Proteins are basic constituents of cytoplasm of the cell.
2. Proteins are fundamental constituent of the structural and functional organisation of the cell.
3. Chemically enzymes are proteins.
4. Many of the hormones are proteins.
5. Proteins play a major part in the transport of the O_2 and CO_2 by haemoglobin and special enzymes in red blood cells.
6. Proteins like thrombin, fibrinogen etc. participate in the blood clotting as clotting factors.
7. Antibodies are proteins by nature, which act as defence against infections.
8. Some proteins like actin and myosin carry out mechanical work in the muscle.
9. A protein rhodopsin of retina carry out the function of sensing the light.
10. Plasma proteins function in the homeostatic control of the volume of the circulating blood and that of the interstitial fluid.

2.5 CLASSIFICATION OF PROTEINS

Proteins can be classified by two ways:

1. On the basis of their solubility.
2. According to increasing complexity of their structure.

2.5.1 On the Basis of Solubility

(a) **Fibrous proteins:** These proteins are fibrous in nature. Basically, fibrous proteins are long linear chains of proteins held together by intermolecular hydrogen bonds. Hydrolytic proteolytic enzymes are not able to digest fibrous proteins. Proteins of skin–collagen, hair–keratin, silk–fibron are examples of fibrous proteins.

(b) **Globular proteins:** These are highly branched condensation products of basic and acidic amino acids. Polypeptide chains are held together by cross-linked groups. Globular proteins are soluble in water and in dilute acids, alkalies and salts. Globular proteins include some hormones, enzymes and O_2 carrying proteins.

2.5.2 According to Increasing Complexity of Their Structure

1. **Simple proteins:** These proteins yield only amino acids on their hydrolysis.

(a) **Albumins:** These are soluble in water. They are coagulated by heat. They can be precipitated by high salt concentration.

(b) **Globulins:** These are insoluble in water. They are coagulated by heat. They are precipitated by half saturated salt solutions. Examples are plasma globulins, serum globulins, vitelin, tuberin, legumin, myosinogen etc.

(c) **Glutelins:** These are insoluble in water but are soluble in acids and bases. They are coagulated by heat. e.g. oryzenin (rice), glutenin (wheat).

(d) **Prolamines:** These are insoluble in water, but soluble in ethanol. These are not coagulated by heat. e.g. gliadin of wheat, zenin (maize) etc.

(e) **Protamines:** These are soluble in water. They are not coagulated by heat. e.g. salmine from salmon sperm.

(f) **Sclero proteins (albuminoids):** These are water insoluble proteins with a characteristic. e.g. a keratin (protein of hair, horn, nail) and collagen (protein of skin, bone and tendons).

2. **Conjugated proteins:** These proteins are characterised by presence of non-protein group united with the protein molecule. The non-protein part is also called as *prosthetic group*. Few of the examples of conjugated proteins are:

(a) Nucleoproteins
(b) Lipoproteins
(c) Chromoprotein

(d) *Phosphoprotein*
(e) Metalloprotein
(f) Glycoproteins
(g) Flavoproteins

2.6 STRUCTURE OF PROTEINS

Proteins are made up of many amino acids, which are covalently bonded to each other. This covalent backbone of proteins is actually made up of hundreds of individual bonds. The covalent peptide bonds are rigid in nature. The free rotation, or even the fraction of rotation of each bond is not possible. This is the reason why there are limited number of three dimensional structures of protein.

Different Levels of Structural Organisations of Proteins

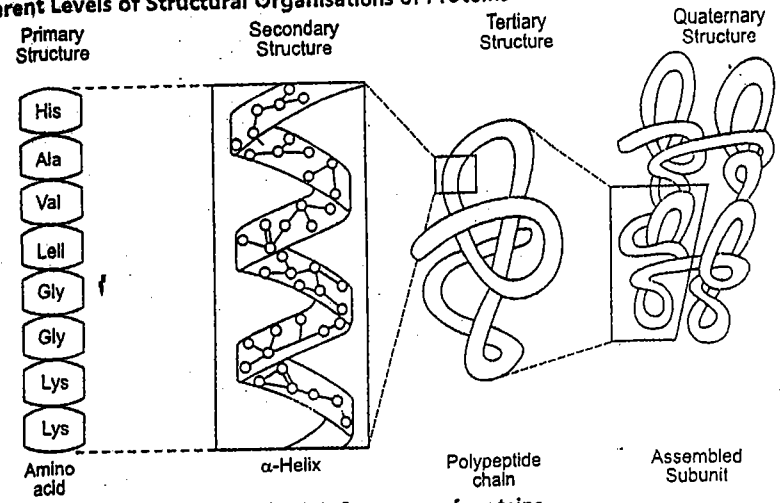


Fig. 2.1: Structures of proteins

Each protein has specific chemical or structural function and its own unique three dimensional structure.

The spatial arrangement of atoms in a protein is called conformation.

The proteins in their functional conformation are called native proteins.

To understand the function of proteins, the protein structure can be considered at four levels:

(a) **The primary structure:** It defines the sequence of amino acids and sites of disulphide bonds.

(b) **The secondary structure:** Refers to regular repeating arrangements in the space of adjacent amino acid residues in the polypeptide chain.

(c) **A supersecondary structure:** A stable gathering of several elements of secondary structure is sometimes referred to as supersecondary structure.

Rao and Rossmann (1973) were the first to observe that some structural components comprising of few alpha-helices or beta strands were frequently repeated within the structure, called "supersecondary structure" (intermediate to secondary and tertiary structure). "Green Key" is another secondary structure. Levitt and Chothia (1976) were first to classify proteins on the basis of structural comparisons:

- **All α:** The tertiary structure of these molecules is composed of only alpha helix bundles. For example, myoglobin.
- **All β:** In these protein molecules, all strands are beta sheets. Beta sheets can be parallel or anti-parallel. For example, immunoglobulin.
- **α + β:** These proteins contain a random mixture of alpha and beta structures.
- **α/β:** Generally, these proteins are made up of a large number of beta-alpha-beta units. Beta strands are parallel and alpha and beta strands occur consecutively. Many enzymes are alpha beta proteins.

(d) **Tertiary structure:** It refers to spatial relationship among all amino acids in polypeptide. It can also be called as the complete three-dimensional structure of polypeptide.

The boundary between the secondary and tertiary structure is not always clear. Many secondary structures are found to be present within the three dimensional structure of large proteins.

(e) **Quaternary structure:** Proteins containing more than one polypeptide chain exhibit a fourth level of protein structure called quaternary structure. This level of structure represents the spatial arrangement of polypeptide subunit and nature of the interacting forces between them.

(f) **Domain:** This refers to a compact region including perhaps 40 to 400 amino acids, that is a separate structural unit within a larger polypeptide chain.

Peptide bond: Linear sequences of amino acids in proteins are linked together by peptide bonds. Peptide bond is a chemical covalent bond formed between α-amino group of one amino acid and α-carboxyl group of another.

The peptide bond shows partial double bond character due to the closeness of the carbonyl carbon-oxygen double bond, allowing resonance structure.

A peptide unit made up of CO–NH atoms is thus relatively rigid and planar. Although free rotation can take place around C–N and C–C bonds permitting adjacent peptide units to be at different angles giving rise to different levels of structures of protein.

2.6.1 Primary Structure of Protein

The primary level of structure in a protein is the linear sequence of amino acids joined together by peptide bonds. The sequence of amino acids in the primary structure is

determined by the sequence of nucleotide bases in the gene encoding protein. Primary structure also reveals the location of any other covalent bond. There are primary disulfide bonds between cysteine residues that are near to each other in space but away from each other in linear amino acid sequence. These disulfide covalent cross links can take place between two separate polypeptide chains or between different parts of the same chain. They are formed by oxidation of SH groups on cysteine residues that are exposed to each other in space. The disulfide so formed is called as the cysteine residues.

e.g. Primary structure of Insulin – as discovered by Sanger is as follows:

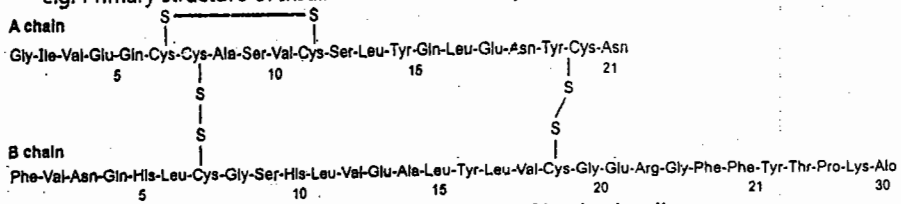
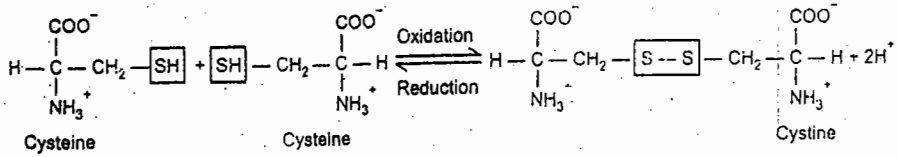


Fig. 2.2: Amino acid sequence of bovine insulin

The figure below represents the formation of disulfide bond between two cysteine residues with formation of cystine.



The determination of the complete primary structure can be carried out by the following stages viz.

(i) Purification

(ii) Determination of amino acid composition by means of acid, alkali or enzyme peptidase to its constituent amino acids, which are then separated by ion exchange chromatography followed by reaction with ninhydrin.

(iii) End-group determination i.e. determination of N terminal or C terminal amino acids of polypeptide chain by various standard methods.

The significance of the primary structure can be sited with the example of the primary structure of haemoglobin.

Polypeptide of haemoglobin S – contains the following amino acids:

His – Val – Leu – Leu – Thr – Pro – **Val** – Glu – Lys – β chain

Polypeptide of haemoglobin A – contains the following sequence of amino acids:

His – Val – Leu – Leu – Thr – Pro – **Glu** – Glu – Lys – β chain

If in β chain of haemoglobin A there is valine amino acid in place of glutamic acid, this gives rise to a different haemoglobin– known as the haemoglobin S and the individual showing such a genetic defect suffers from a genetic disease known as sick cell anaemia, characterised by sickle shaped red blood cells.

From the above example we can understand the importance of the primary structure in determining the property of the proteins.

2.6.2 Secondary Structure of Protein

The regular folding of the regions of the polypeptide chain gives rise to a conformation called as the secondary structure of protein.

Most commonly occurring protein folds in terms of secondary structures are – α helix and β sheet.

Linus Pauling and Robert Corey predicted the existence of these structures (1951) long before the 1st protein structure was elucidated.

2.6.2.1 α-Helix

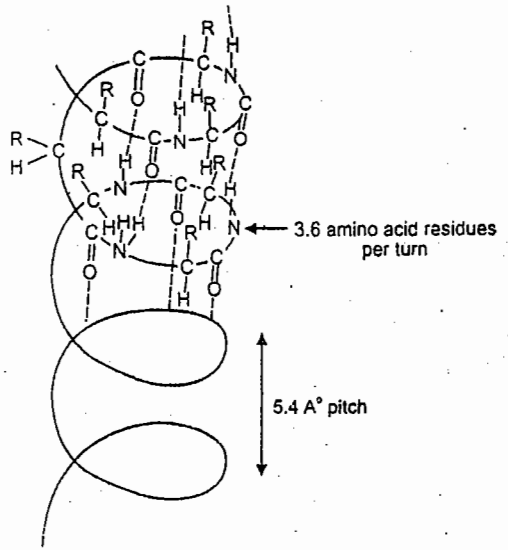


Fig. 2.3: Secondary structure α–helix (H . . . . O) intramolecular hydrogen bonding

Amino acids are found in regular helical conformation. The central strength of the helix occurs in the stubborn, non-rotating peptide bonds. It represents a pack of regular coiled structure of polypeptide which contains amino acid side chains positioned outward, away from the central axis.

The H of amino group forms a hydrogen bond with the carbonyl O, of the every fourth amino acid. The direction of hydrogen bond is parallel to the axis of the helix. Each turn of the helix contains nearly 3.6 amino acids which cover the distance of 0.54 nm. And the spacing of each amino acid is 0.15 nm, along the helix axis.

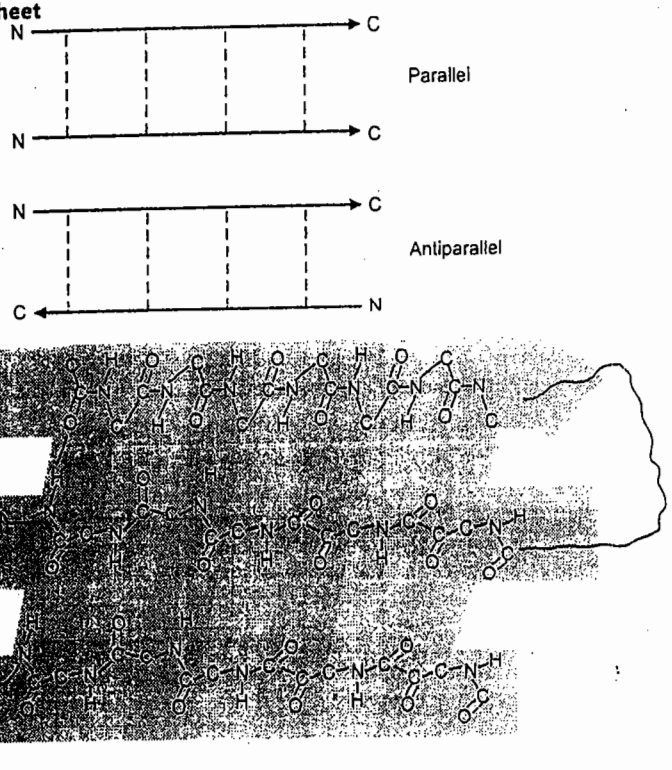
α helix represents a stable conformation with the lowest energy. There are two types of *α helix viz. right handed* helix and left handed helix. The right handed helix is more stable than the left handed helix.

Amino acid proline is generally not found as a constituent of α helix as it is unable to form the correct system of hydrogen bonds owing to the absence of H atom attached to nitrogen atom (the N in proline is engaged in the ring). Actually, proline is found at the end of the helix, which changes the direction of the helix thus terminating the helical structure.

Large number of hydrogen bonds produced by carbonyl (O) and amino (H) (except the last and the first peptide bonds) together give the helix stability.

e.g. 1. A molecule of myoglobin has 8 α helixes.
2. Keratin – the hair protein is an example of α helix conformation.

2.6.2.2 β-pleated sheet



β-pleated sheet. The side chain groups (R) are not shown.

- **A and B are antiparallel: the two strands are in opposite orientations.**
- **B and C are parallel: the two strands are in the same direction**

Fig. 2.4: Polypeptide chains (having polarity) β-pleated sheet form

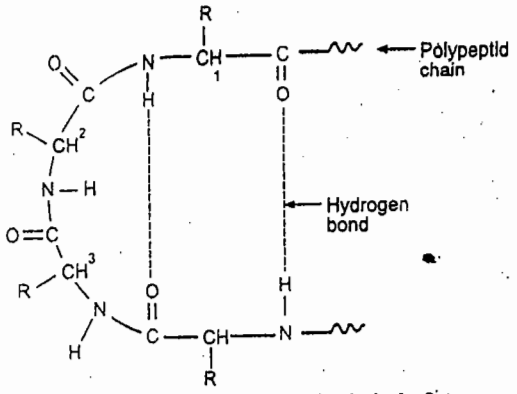


Fig. 2.5: Folding of polypeptide chain in β-turn

This conformation consists of nearly straight extended polypeptide chains held together by hydrogen bonds. The backbone of the polypeptide chain is extended into zigzag rather than a helical structure. The hydrogen bonding takes place between different polypeptide chains or in different sections of the same polypeptide chain. The actual structure of β-pleated sheet represents amino acid residues coming out above or below the sheet.

Adjacent polypeptide chains of β-pleated sheets can be parallel or antiparallel depending on if they run in the same direction or in the opposite directions respectively. The distance between one α atom to next is 0.35 nm. The sheet can close to form a β-barrel shape.

e.g. Protein - silk fibrin consists of almost all stacks (arranged side by side) of antiparallel β-pleated sheet or else, β turn or hairpin loop is observed when polypeptide folds itself in reverse direction as seen in the globular proteins e.g. all peptide linkages of β-keratin participate in intrachain hydrogen bonding.

In β-conformation, the hydrogen bonds can be either intrachain or interchain between peptide linkages of the adjacent polypeptide chain.

Some examples of β-keratin such as the silk fibrin and protein of spider web have very high content of Gly and Ala residues.

Stability of Secondary Structures:

The α-helix and β-conformation are stable because steric repulsion is minimized and hydrogen bonding is maximized.

2.6.3 Tertiary Structure of Proteins

Three dimensional arrangement of protein structure is known as tertiary structure. e.g. The protein myoglobin represents the tertiary structure. The compact structure contains hydrophilic group on the surface of the protein molecule with hydrophobic side chains towards the interior of the molecule.

The types of bonds which are the integral part of the tertiary structure are:

1. Hydrogen bonds
2. Ionic interactions
3. Disulfide bonds
4. Hydrophobic interactions
5. Hydrophilic interactions.

The tertiary structure also imparts stability to the molecule and also represents three dimensional, biologically active conformation (a native conformation).

The enzymes like lysozyme, nuclease, α-chymotrypsin having one, two and three chains respectively are examples of native tertiary structures. Cytochrome C, ribonuclease also exhibit tertiary structure. X rays are used to reveal tertiary structure of protein.

2.6.3.1 Denaturation of Proteins

The three dimensionally folded structure of protein can be opened up or unfolded by, extremes of pH, heat, light, ultraviolet light, or by chemicals such as acids or alkali or organic solvents, synthetic detergents or vigorous mechanical shaking.

This unfolding process is referred to as denaturation. The denaturation can be reversible in some cases or it is irreversible in many other cases.

The protein loses its secondary and tertiary structure during denaturation but primary structure of protein is maintained.

A denatured protein loses its biological activity as well as solubility. But chemical activity of various proteins is increased due to large exposure obtained to the various functional groups in protein.

The boiling of egg denatures the tertiary structure of the protein albumin and is an example of irreversible denaturation. Whereas the treatment of ribonuclease with urea and mercaptoethanol denatures its tertiary structure due to breakdown of the disulfide linkage and uncoiling of the polypeptide chains. The same denatured protein on slow reoxidation with urea is converted into its native, original tertiary structure.

2.6.4 Quaternary Structure of Proteins

Some proteins contain two or more polypeptides chain held together by non-covalent interactions. Such a structure is called as the quaternary structure of protein.

The individual polypeptide chains of a quaternary protein is called as the subunit or monomer.

These monomers are bound to each other to form a quaternary structure. There are different types of forces which can bind these monomers. They are of four types:

1. Ionic bonds
2. Hydrophobic interactions
3. Hydrogen bonds
4. Covalent linkages e.g. disulphide bonds.

The oxygen carrying protein of erythrocytes, haemoglobin is one of the well known example of the multisubunit protein. Haemoglobin exists in quaternary structure in native form. It is made up of four polypeptide chains namely α_2 and β_2 (i.e. two identical α chains and two identical β chains). Each chain binds a haem group. There are very minute differences in twisting between the α-chain and β-chain. These differences are due to differences in primary structure. Four globin chains make up a regular tetrahedral arrangement in the quaternary structure of haemoglobin.

Another example is enzyme phosphorylase. It contains four identical subunits. Many enzymes are active when present in their quaternary structure form. These enzymes lose their catalytic activity in absence of their quaternary structure. Many multisubunit proteins serve regulatory functions. Their activities are altered by the binding of certain small molecules.

Enzymes like lactate dehydrogenase, aspartate transcarbomylase also exist in quaternary conformations.

The larger and more complex multisubunit protein is the enzyme RNA polymerase of *E. coli*. It is responsible for the initiation and synthesis of RNA chain.

So we can conclude that arrangements of proteins and protein subunits in three dimensional complex constitute a quaternary structure.

X ray analysis is necessary for revealing the complete structure of some quaternary proteins.

2.7 PROTEIN FAMILIES

Evolutionary related proteins are grouped together into families. Amino acid residue similarities between proteins of the same family is 30% greater. In some cases although amino acid residue similarity does not exist, still there is close similarity between protein function. For example, globins form a family but some members have sequence identities of only 15%.

2.8 PROTEIN SUPERFAMILIES

Some proteins have very low amino acid similarity but their functional and structural properties suggest a evolutionary connection and such proteins are placed together in superfamilies. Criteria used for grouping proteins into a superfamily are not clear. Some of the criteria are extreme structural similarity, a similar mechanism or a similar binding site. Many times, proteins grouped in a superfamily may show only 5 - 10% amino acid sequence similarity.

QUESTIONS

1. Describe the classification of amino acids along with their structures.
2. Give an account of the classification of proteins with suitable examples.
3. Give a classification of amino acids on the basis of nutrition.

4. Write a detailed note on the various levels of organisation of protein structures with *suitable examples.*
5. Explain the classification of proteins with suitable examples.
6. Write notes on:
   (a) Zwitter ion.
   (b) Isoelectric pH of amino acids.
   (c) α-helix.
   (d) Tertiary structure of protein.
7. What is the importance of primary structure of protein?
8. Explain in detail the structure of β-pleated sheet.
9. What is meant by secondary structure of protein? Add a note on the α-helical structure.
10. Why do we call some amino acids as glucogenic and some as ketogenic? Enlist the members of glucogenic and ketogenic amino acids.

Chapter ... 3

CHEMISTRY OF LIPIDS

♦ LEARNING OBJECTIVES ♦

- *Understand Unique Nature, Function and Structure of Lipid Molecules.*
- *Appreciate Biological Role of Lipids and their Structural Diversities.*
- *Recognize Parameters to which can Qualify various Lipid Molecules.*
- *Role of Glycerol and Other Alcohols in the Structure of Lipids.*
- *Know Importance of Lipid from different Sources.*

3.1 INTRODUCTION

Lipids can be defined as 'a chemically diverse group of organic compounds, insoluble in water, soluble in organic solvents (like alcohol, ether) and performing diverse biological functions'.

The name lipid is derived from the *Lipos* (in greek - Lipos means fat). Lipids represent most important storage form of energy. They also play major role in the cellular infrastructure and many other biochemical functions.

One important distinguishing characteristic of lipids is: they never exist in the polymer form like proteins and polysaccharides. On the contrary they are present in the form of smaller compounds.

In our body, fats are much more efficient forms of energy storage compared to carbohydrates. This is because, fats are highly reduced compared to carbohydrates. This is obvious because of preponderance of C–H bonds where electrons "belong" to C compared to C–O bonds where electrons belong to O. Hence, during catabolism there will be more electrons transferred to O_2 per gram of fat than per gram of carbohydrate, so more energy is released (9 kcal/gm fat compared to 4 kcal/gm of carbohydrates).

Also fats are highly insoluble in water, so there is little (if any) water of hydration associated with stored fats compared to glycogen. This is the reason, a gram of hydrated fat yields more than six times as much energy compared to a gram of hydrated carbohydrate.

For example, in a typical 70 kg man with about 11% body fat stored in the abdominal cavity, will have about 1,00,000 kcal of energy stored as fat as compared to 600 kcal in glycogen.

3.2 BIOLOGICAL ROLE OF LIPIDS

1. Fats and oils, being highly reduced compounds, are used as the storage form of energy in living organisms.

2. Lipids function as the medium and source of fat soluble vitamins.
3. Phospholipids and sterols make up about half the mass of the biological membrane and regulate membrane permeability.
4. Other lipids, although in small amounts, play an important role as enzyme cofactors, light absorbing pigment, electron centres, emulsifying agent and hydrophobic anchors.
5. Lipids play an important role of cellular messenger (phosphatidyl inositol) as well as that of cellular metabolite regulator.
6. Being a component of inner mitochondrial membrane, lipids participate in the electron transport chain.
7. Lipids function as the insulators of internal organs of the body.

3.3 CLASSIFICATION OF LIPIDS

Lipids can be broadly classified into the following classes:

I. Simple Lipids
II. Compound or Complex Lipids
III. Derived Lipids.
IV. Miscellaneous Lipids.
V. Neutral Lipids.

I. Simple Lipids:

This class is made up of esters of fatty acid with alcohol. This class is further divided into two subclasses:

1. **Neutral fats and oils (Triacyl glycerol):** The esters of fatty acids with glycerol make this class of lipids. The term fat refers to those lipids which are solid at room temperature. Whereas oils refer to the lipids which are in liquid state at room temperature.
2. **Waxes:** Waxes are made up of esters of fatty acids (having long carbon chain) with the alcohol other than glycerol e.g. acetyl alcohol.

II. Compound or Complex Lipids:

These are esters of fatty acids with alcohol containing additional group such as carbohydrate, protein, nitrogen base or phosphate.

This class can be further subdivided into:

(a) **Phospholipid:** Lipids containing fatty acid and alcohol esters along with the phosphoric acid and many times a nitrogen base or amino acid or some other species like inositol.
   (i) **Glycerophospholipid:** These phospholipids contain glycerol as the alcohol. The examples are lecithin and cephalin.
   (ii) **Sphingophospholipid:** It is characterised by the presence of alcohol sphingosin e.g. sphingomyelin.

(b) **Glycolipid:** These lipids contain a fatty acid, carbohydrate and a nitrogen base. The alcohol is sphingosine. Hence, they are also called as the glycosphingolipids. Glycerol and phosphate are absent in this class of lipids. e.g. gangliosides, cerebrosides.

(c) **Lipoproteins:** They represent the large molecular complexes of lipids with proteins.

(d) **Other complex lipids:** Examples: aminolipids, lipopolysaccharides and sulfolipids are members of this class.

III. Derived Lipids:

These represent the derivatives obtained from the hydrolysis of group I and group II lipids, having the properties of lipids. The examples are fatty acids, glycerol, mono and diacyl glycerol, lipid soluble vitamins, steroid hormones, ketone bodies.

IV. Miscellaneous Lipids:

Large number of compounds show characteristics of lipids e.g. squaline, carotenoids, hydrocarbons such as pentacesane, terpenes etc.

V. Neutral Lipids:

Uncharged lipids are called as neutral lipids. They are monoacyl glycerol, diacyl glycerol, triacyl glycerol, cholesterol and cholesteryl esters. Neutral lipids are commonly found in cells as storage fats and oils. The reason they are called as neutral lipids is because at cellular pH they have no charged groups. Usually all neutral lipids are a combination of fatty acids with glycerol, as alcohol.

3.4 FATTY ACIDS

Fatty acids are carboxylic acids with hydrocarbon side chains (the side chain may contain 4 to 36 carbons).

The principal component associated with most lipids is monocarboxylic acids that have even number of carbon atoms in a straight chain.

As fatty acids are toxic, they occur to a only limited extent in their free form. Generally, these acids are found as oxygen esters in the complex lipids e.g. triacyl glycerol, glycollpids and phospholipids.

Different types of lipids mentioned above may contain fatty acids in the esterified form. Most of the fatty acids occurring in common lipids are aliphatic monocarboxylic acid with even number of carbon atoms ranging from C_4 to C_{24}. The fatty acids can also be branched and dicarboxylic but these are not found so often. A few fatty acids contain three carbon rings or hydroxyl groups.

Simplest nomenclature of fatty acid indicates the chain length number and number of double bonds separated by a colon (:).

The 18 carbon saturated stearic acid is abbreviated as 18: 0. The 18 carbon oleic acid with one double bond is abbreviated as 18: 1. Number indicating total number of double bonds is then followed by the sign of semicolon (:). After which the numbers indicating the position of double bonds are written which are separated by comma (,) from one another.

The counting of number of double bond should be from carboxyl end of fatty acid.

So saturated fatty acid; Stearic acid should be written as 18 : 0, Palmitic acid is 16 : 0 and unsaturated fatty acid; Arachidonic acid can be written as **20 : 4; 5, 8, 11, 14**. It indicates that there are four double bonds in the 20 carbon fatty acid. The positions of the double bonds are between carbon 5 and 6, 8 and 9, 11 and 12, 14 and 15 respectively.

The table 3.1 gives the list of some naturally occurring fatty acids. The even number of carbon atoms of fatty acids results from the mode of synthesis of these compounds involving the condensation of 2 carbon unit acetates.

The fatty acids are broadly classified as follows:

(a) Saturated fatty acids

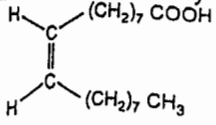
(b) Unsaturated fatty acids.

Classification of Fatty Acids

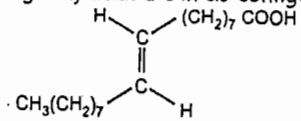
The saturated fatty acids do not contain double bonds whereas unsaturated fatty acids contain one or more double bonds. Both saturated and unsaturated fatty acids occur in nature. The fatty acids with one double bond are called as monounsaturated fatty acids and those with two or more double bonds are called as polyunsaturated fatty acids (PUFA).

Cis Trans-Isomerism in Fatty Acids

The double bonds present in unsaturated fatty acid are always separated by methylene groups. The double bonds in naturally occurring fatty acids are in cis-configuration.



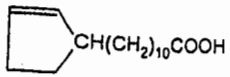
Oleic acid
(cis form)



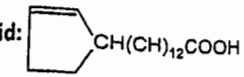
Elaidic acid
(trans form)

Cyclic Fatty Acids: The fatty acids containing ring in their structure are called as cyclic fatty acids.

e.g. 1. Hydnocapric acid:



2. Chaulmoogric acid:



The Hydnocapric acid and chaulmoogric acid are cyclic fatty acids present in the chaulmoogra oil. The chaulmoogra oil was used in the treatment of leprosy.

Table 3.1: List of some naturally occurring important fatty acids

Common name	Systematic name	Abbreviation	Structural formula
1. Saturated Fatty acids:			
Acetic acid	Ethanoic acid	2: 0	CH_3COOH
Propionic acid	n-Propanoic acid	3: 0	$CH_3\ CH_2\ COOH$
Butyric acid	n-Butanoic acid	4: 0	$CH_3\ CH_2\ CH_2\ COOH$
Valeric acid	n-Pentanoic acid	5: 0	$CH_3 - CH_2 - CH_2 - CH_2 - COOH$
Caproic acid	n-Hexanoic acid	6: 0	$CH_3 — CH_2 — CH_2 — CH_2 — CH_2 — COOH$
Caprylic acid	n-Octanoic acid	8: 0	$CH_3 — CH_2 — CH_2 — (CH_2)_3 — CH_2 — COOH$
Capric acid	n-Decanoic acid	10: 0	$CH_3 — (CH_2)_8 — COOH$
Lauric acid	n-Dedecanoic acid	12: 0	$CH_3 — (CH_2)_{10} — COOH$
Myristic acid	n-Tetradecanoic acid	14: 0	$CH_3\ (CH_2)_{12} - COOH$
Palmitic acid	n-Hexadecanoic acid	16: 0	$CH_3\ (CH_2)_{14}\ COOH$
Stearic acid	n-Octadecanoic acid	18: 0	$CH_3\ (CH_2)_{16}\ COOH$
Arachidonic acid	n-Eicosanoic acid	20: 0	$CH_3\ (CH_2)_{18}\ COOH$
Behenic acid	n-Docosanoic acid	22: 0	$CH_3\ (CH_2)_{20}\ COOH$
Lignoseric acid	n-Tetracosanoic acid	24: 0	$CH_3\ (CH_2)_{22}\ COOH$

3.5 ESSENTIAL FATTY ACIDS (EFA)

The fatty acids that cannot be synthesized by the body and therefore should be supplied in the diet are known as essential fatty acids (EFA).

They belong to the group of polyunsaturated fatty acids (PUFA). They are:

Linoleic acid **18 : 2; 9, 12**

$CH_3(CH_2)_4\ CH = CH\ CH_2\ CH = CH\ (CH_2)_7\ COOH$

Linolenic acid **18 : 3; 9, 12, 15**

$CH_3CH_2CH = CH\ CH_2\ CH = CH\ CH_2\ CH = CH(CH_2)_7\ COOH$

Arachidonic acid **20 : 4; 5, 8, 11, 14**

$CH_3(CH_2)_4\ CH = CH\ CH_2\ CH = CH\ CH_2\ CH = CH\ CH_2\ CH = CH(CH_2)_3\ COOH$

The arachidonic acid becomes essential if its precursor linoleic acid is not provided. In most of the fatty acids, the double bonds have cis-conformation.

The structures of the above three fatty acids contain the double bonds beyond carbon 9. Humans lack an enzyme that can introduce double bond beyond C_9 to C_{10}. This makes these three fatty acids as essential fatty acids to human being. However, this specific desaturase enzyme is widespread in plant tissues. High concentration of essential fatty acid is required for reproductive functions.

3.6 BIOCHEMICAL FUNCTIONS OF ESSENTIAL FATTY ACIDS

The Essential Fatty Acids (EFA) are required for the integrity of membrane, both structurally and functionally.

EFA are also essential for prevention of fatty liver. They are also needed for transport of cholesterol and formation of lipoproteins.

Constant supply of EFA is a must for the synthesis of a group of substances known as eicosanoids.

EFA have a positive influence on the prolongation of the clotting time and increase the fibrinolytic activity. The addition of this fatty acid in the diet of the babies cure eczema. EFA are also called as skin tonics.

Daily intake of essential fatty acids in the diet reduces the risk of atherosclerosis.

3.6.1 ω-3 Poly Unsaturated Fatty Acids (ω-3 PUFA)

Eicosapentaenoic acid [EPA, **20:5;5,8,11,14,17**] and Docosa Hexarnoic acid [DHA, 20 : 6, $\Delta^{4, 7, 10, 13, 16, 19}$] are two ω-3 polyunsaturated fatty acids present in the fish oils. They are also present in eggs, milk and other fats but at low concentration.

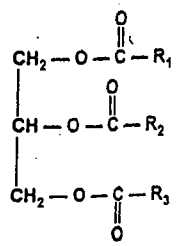
However, because of their beneficial effects, designer eggs containing adequate quantities of these ω-3 PUFA are being produced in several advanced countries. This is achieved by feeding chickens a diet (fish oils) rich in ω-3 fatty acids. They are not essential fatty acids. However, EPA and DHA are required for development of brain and for photoreceptors present in the brain. In the body, DHA is synthesised from linolenic acid, an essential ω-3 PUFA.

3.6.2 Significance of ω-3 PUFA

Regular consumption of ω-3 fatty acids reduce the incidence of cardiovascular diseases and atherosclerosis. They reduce, incidence of inflammatory and autoimmune diseases. They protect the body, from developing cancer of lung, colon, pancreas and prostrate.

3.7 TRIACYL GLYCEROLS

The ester of one glycerol molecule with three different fatty acid molecules is called as triacyl glycerol.



(R_1, R_2, R_3, represent carbon chains)

A general structure of triacyl glycerol

Simple triacyl glycerol: These contain the same fatty acid in all three positions. They are named after the fatty acid they contain. e.g. tristearin, tripalmitin containing stearic acid and palmitic acid respectively.

Mixed triacyl glycerol: They contain two or more different fatty acids. Triacyl glycerols are nonpolar hydrophobic molecules, essentially insoluble in water.

Biochemical Significance of Triacyl Glycerol

1. Adipocytes or fat cells of vertebrates store a large amounts of triacyl glycerol as the fat droplets which occupy maximum space in the cell.
2. Triacyl glycerols are stored in the seeds of many plants, providing energy and biosynthetic precursors for seed germination.
3. In animals, triacyl glycerol is stored under the skin and serves as an insulation against very cold temperatures along with its normal use as energy store.
4. The low density of triacyl glycerols stored in the sperm whales allow the animals to match the buoyancy of their bodies to that of their surrounding during deep dives in cold water.
5. Vegetable oils like corn, olive oil are composed of triacyl glycerol with the unsaturated fatty acids.
6. Most of the naturally occurring fats such as those in vegetable oils, dairy products, animal fats are complex mixtures of simple and mixed triacyl glycerol.
7. Triglycerols are never a component of membrane lipid.

Biochemical Properties of Triacyl glycerols

1. **Saponification:**

   The ester linkage of triacyl glycerol is hydrolysed by an alkali like NaOH or KOH to produce glycerol and Na or K salt of fatty acid and this process is called as the saponification.

   $$\text{Triglycerol} + 3\ \text{KOH} \xrightarrow{\text{Saponification}} \text{Glycerol} + \underset{\text{Soap}}{3\ \text{R}-\text{COOK}^+}$$

   The most important property of the soap is its ability to solubilise water insoluble material (dirt) by forming microscopic aggregates.

2. **Hydrolysis:**

   Intestinal lipases carry out stepwise hydrolysis of triglycerols to free fatty acids and glycerol. The hydrolysis of triglycerol is necessary for digestion, fat mobilisation and also in germinating seed.

3. **Rancidity:**

   It can be defined as 'the complete or incomplete oxidation or hydrolysis of fats and oil exposed to air, light, moisture or by bacterial action resulting in unpleasant taste and odour'.

The fats containing unsaturated fatty acids are more susceptible to rancidity. As oxygen is added across the double bonds, this results in the formation of unpleasant products such as aldehydes, ketones, dicarboxylic acids which have offensive odour.

Oxygen brings about the formation of peroxides. The rancid fats are unsuitable for consumption.

Antioxidants: The group of compounds which can prevent the formation of rancidity are called as antioxidants.

The Vitamin E (tocopherol) is a natural antioxidant, whereas gallic acid, α-naphthol, hydroquinones, O- and P-diphenols are synthetic antioxidants. They are added to commercial preparations of fat.

Antioxidants are also added to food preparations to increase their shelf life. They are BHT (butylated hydroxy toluene), BHA (butylated hydroxy anisole) etc.

Tests to Determine Purity of a given Fat Sample

Set of tests described below are useful in detecting the age or freshness of the sample and presence of adulterants.

1. **Acid number:** It represents the number of milligrams of KOH required to completely neutralize the free fatty acids present in one gram of fat or oil.

   The refined oils are free from any free fatty acids. But the oils on standing for longer periods, decompose due to bacterial lipase or chemical contamination. This gives rise to free fatty acids.

   This decreases the shelf life of fats and increases their acid number which makes them unsuitable for human consumption.

2. **Saponification number:** It is defined as 'the milligrams of KOH required to saponify or hydrolyse one gram fat or oil'.

   Saponification number gives the idea of average molecular size of the fatty acids present in the given sample. Fats containing more number of short chain fatty acids show higher sap value or saponification number.

   Sap value of some fat samples:

   Butter: 230 – 240

   Coconut oil: 250 – 260

3. **Iodine number:** It represents the grams of iodine absorbed by 100 g of fat or oil.

   Iodine number is useful to know the relative degree of unsaturation in fats and oil. The number increases directly with the content of unsaturated fatty acids. Higher iodine number indicates the higher degree of unsaturation of fats and oils.

Table 3.2: Iodine numbers of some fixed oils

Lipid Sample	Iodine Number
Linseed oil	175 – 200
Sunflower oil	125 – 135
Cottonseed oil	100 – 110
Groundnut oil	85 – 100
Olive oil	80 – 85
Butter	25 – 28
Coconut oil	7 – 10

4. **Reichert-Meissl (RM) Number:** It is defined as 'volume (ml) of 0.1 N KOH required to completely neutralize soluble, volatile fatty acids distilled from 5 g fat'.

   RM number is helpful in testing the purity of the butter as it contains significant amount of volatile fatty acids.

   The RM number of butter is in the range of 25–30. But RM numbers of other edible oils are less than 1.

   One can easily find out any adulteration in the given butter sample with the help of RM number.

Analytical Methods for Separation of Fats and Fatty Acids

1. **Paper Chromatography:** It is used for analytical separation of fatty acids in chloroform (99%) + butanol (1%) using a filter paper soaked in a grease. The water is used as a mobile phase (reversed phase chromatography).
2. **Column Chromatography:** The column of silica gel or Hyflow-Supercel is used for separation of fatty acids.
3. **Thin Layer Chromatography (TLC):** It uses glass plates coated with the silica gel G, or alumina or kieselguhr for separation of fatty acids.
4. **Gas Liquid Chromatography (GLC):** It can be used to separate different fatty acids in the form of their methyl esters, especially of volatile lipid derivatives.
5. **High Performance Liquid Chromatography (HPLC):** It is a recent technique employed for the separation of fatty acids.
6. **Specific Hydrolysis:** Using acid or alkali and enzyme treatment or combination of specific hydrolysis with characterisation of products by TLC or GLC often allows the determination of the structure of lipids.
7. **Mass Spectral Analysis:** Mass spectral analysis of lipids or their volatile derivatives is very important to determine the length of hydrocarbon chain or position of double bonds.

3.8 PHOSPHOLIPIDS

Phospholipids are complex compound lipids. They contain phosphoric acid in addition to alcohol, nitrogen base and fatty acids.

They are further classified into two classes glycero-phospholipids and sphingo-phospholipids:

I. Glycero-phospholipids:

They are also known as phosphoglycerides. They contain glycerol as the alcohol. These are major components of the cell membrane.

All the glycero-phospholipids are derivatives of the phosphotidic acid. They are named after their highly polar head groups.

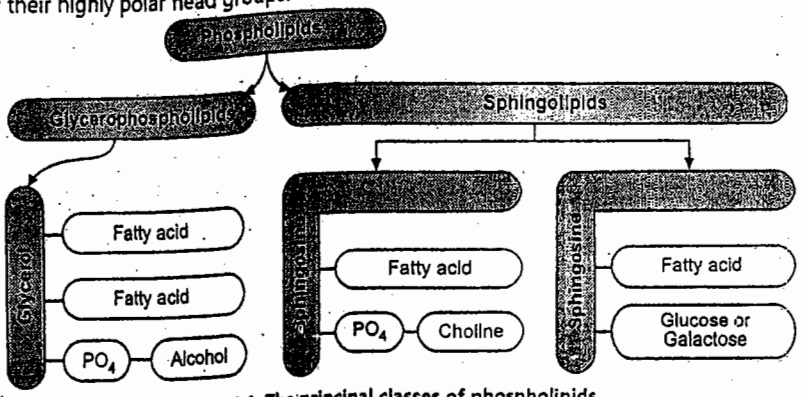
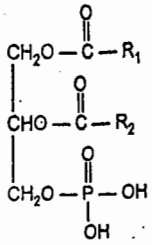
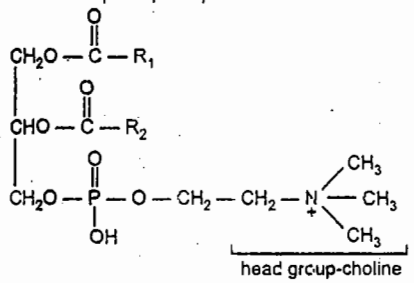


Fig. 3.1: The principal classes of phospholipids

1. **Phosphotidic acid:** It is the simplest of all phospholipids. It is also an intermediate product in the synthesis of triglycerols and phospholipids.



Phosphotidic acid



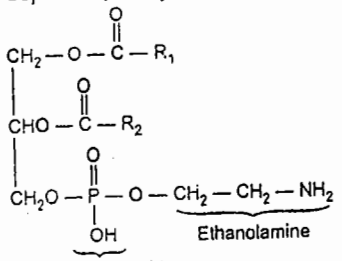
Lecithin (Phosphotidyl choline)

2. **Lecithin (Phosphotidyl choline):** It is a major constituent of the cell membrane. It is present in egg yolk, brain tissue and a wide variety of animal fats and plants. Its chemical structure contains phosphotidic acid attached to the nitrogen base choline. Choline in the lecithin participates in the transmission of nerve impulse and in various methylation reactions.

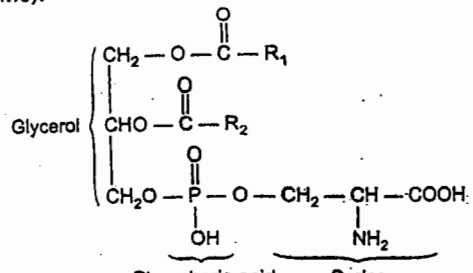
R.D.S. (Respiratory Distress Syndrome)

Dipalmitoyl lecithin is an important phosphotidyl choline, secreted by lungs. It functions as a very good surfactant. It prevents the adherence of inner surface of the lungs due to its surface tension reducing action. The dipalmitoyl lecithin is not secreted in some newborns. This causes death of the newborn infants by a disease called as respiratory distress syndrome.

3. **Cephalin (Phosphotidyl ethanolamine):**



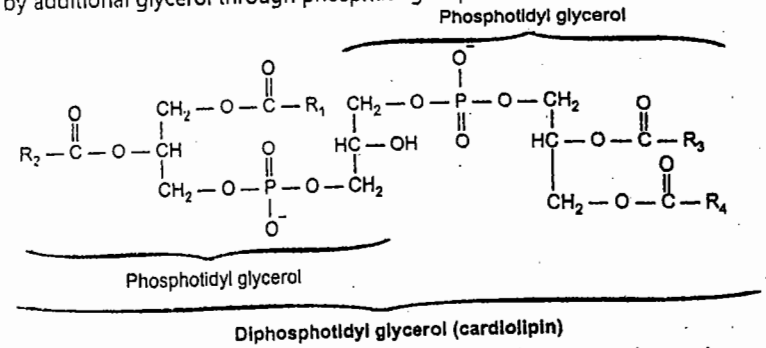
Phosphotidyl ethanolamine



Phosphotidyl serine

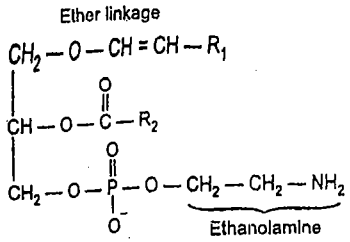
Cephalins are part of the thromboplastin enzyme.

4. **Cardiolipin:** Cardiolipins were first isolated from the lipid extracts of the heart tissue. Cardiolipin is used as an antigen for the VDRL test meant for the detection of syphilis. Structural formula consists of two phosphotidic acid molecules sandwitched by additional glycerol through phosphate groups.



Diphosphotidyl glycerol (cardiolipin)

5. **Phosphotidyl inositol:** This fraction of phospholipid is present in soyabeans and the brain. It also serves as a membrane constituent like other phospholipids. Its biochemical function deserves a special mention, for its role as a mediator of the hormones vasopressin and oxytocin.
6. **Plasmalogen:** When C_1 of glycerol in glycerophospholipid contains attached fatty acid in ether linkage, the compound so formed is plasmalogen. Generally, C_1 contains an unsaturated fatty acid. The nitrogen base choline can be replaced by serine or phosphotidyl ethanolamine.

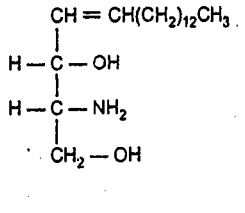


Phosphotidal ethanolamine

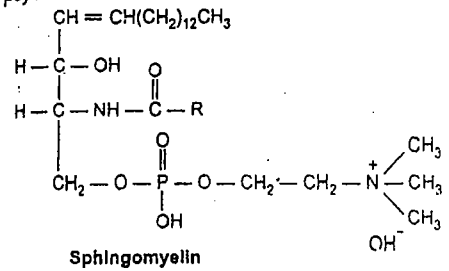
Thus, plasmalogens are glycerol ether phospholipids. Broadly there are two types - alkyl ether and alkenyl ether. Plasmalogens are divided into three classes: choline, ethanolamine and serine plasmalogens. Choline plasmalogen is found in cardiac tissues whereas, ethanolamine plasmalogen is found in myelin.

II. Sphingo-phospholipid:

These lipids contain sphingosine, a complex amino alcohol, along with a fatty acid, a phosphoric acid and choline. The glycerol is not present in these lipids. In sphingomyelin, there is an amide (–CONH–) linkage between sphingosine (an amino alcohol) and a fatty acid. These lipids are present in high concentration in myelin sheath of neurons. A sphingosine is formed from a complex series of reactions involving palmitoyl-CoA and serine. Cardiolipid, sphingomyeline and psychosine are its derivatives.



Sphingosine



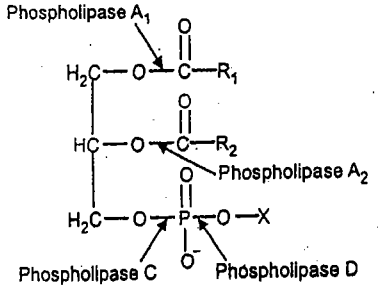
Sphingomyelin

Phospholipases:

Phospholipids are degraded by phospholipases. These enzymes are found in the mammalian tissues, snake venom and pancreatic juice. They cleave the phosphodiester linkage.

There are various phospholipases which exhibit substrate specificities for different positions in phospholipids. The products of these phospholipases are called as lysophospholipids.

Phospholipase A_2 is an important enzyme. This enzyme is responsible for the release of arachidonic acid from the membrane lipids. Arachidonic acid is a substrate for the synthesis of prostaglandins and leukotrienes.



Sites of action of the phospholipases A_1, A_2, C and D

Glycolipids: (Glyco-sphingolipids)

They represent a class of conjugated lipids. They are present in brain and spinal chord. They are important constituents of the cell membrane. Cerebrosides are one of the examples of simplest glycolipids.

Glycolipids contain three different molecules, namely, amino alcohol, fatty acid and a carbohydrate. The amino alcohol is attached with an amide linkage to a fatty acid and it is attached to a carbohydrate with the glycosidic linkage. Glycolipids do not contain phosphate.

Glycolipids can be further classified into: (a) Cerebrosides, (b) Gangliosides.

(a) **Cerebroside:** It contains a galactose, a sphingosine and a high molecular weight fatty acid. Presence of sphingosine in the compound, makes them a member of sphingolipids too.

Cerebrosides are chief constituent of the myelin sheath. There are different types of cerebrosides due to the presence of different types of fatty acid in the molecule. e.g. –

(i) **Nervon:** containing an unsaturated homologue of lignoceric acid called as nervonic acid.

$[CH_3—(CH_2)_7—CH = CH—(CH_2)_{13}—COOH]$

(ii) **Cerebron:** containing hydroxylignoseric acid (cerebroinic acid).

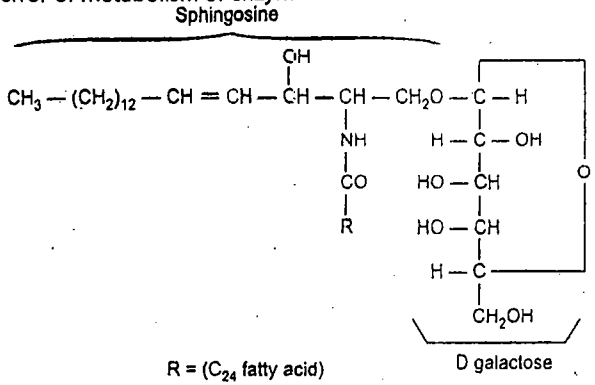
$[CH_3—(CH_2)_{21}—CH(OH)—COOH]$

(b) **Gangliosides:** These represent most complex form of the glycosphingolipids. They are found in the ganglions of brain.

Structurally, gangliosides are made up of a ceramide, glucose, galactose, N-acetyl galactosamine and N-acetylneuraminic acid. They are also known as GM_1, GM_2, GM_3. Ganglioside GM_2 accumulates in brain in Tay Sachs disease.

GM_2: Ceramide — Glucose — Galactose < **N-acetyl galactosamine**, **N-acetyl neuraminic acid**

It causes mental retardation and muscular weakness. The disease occurs due to inborn error of metabolism of enzyme — Hexosaminidase.



Structural formula for cerebroside

3.9 LIPOPROTEINS

Molecular complexes of lipids and proteins are called as lipoproteins. The protein part of the lipoprotein is known as apoprotein. This combination of lipid with the protein is helpful in the transport of the otherwise insoluble lipids in the blood. The major functions of lipoproteins are transport and delivery of the lipids (triacyl glycerol, cholesterol) to their different tissue destinations in the body. In addition, lipoproteins occur as a component of the membranes. They are present in the membranes of mitochondria, nuclei, endoplasmic reticulum and bacteria.

Lipoprotein Structure

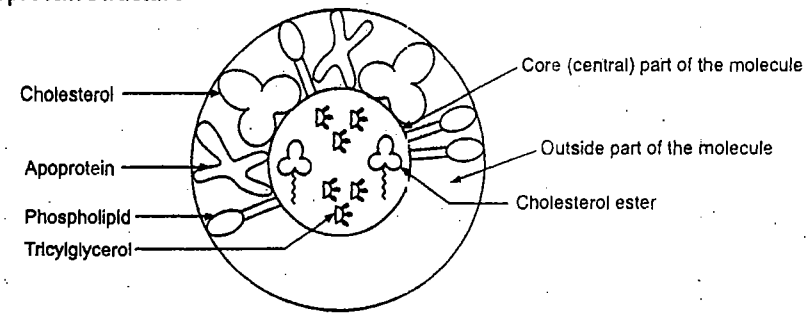


Fig. 3.2: Lipoprotein complex

Lamellar lipoprotein systems occur in the myelin sheath of nerves and chloroplasts. Structurally, lipoprotein molecule consists of a lipid core (made up of cholesteroyl esters and triglycerol) surrounded by the cap of apoprotein, phospholipid and cholesterol. The polar parts of the cholesterol and phospholipids are towards the outside surface of lipoprotein. This makes the lipoprotein molecule soluble in the aqueous solution e.g. blood.

Classification of Lipoproteins

Various combinations of lipids and proteins in the lipoprotein molecules, produce the lipoprotein particles of different densities.

The five major classes of lipoproteins exist in the human plasma and are based on their separation by electrophoresis or ultracentrifugation.

1. **Chylomicrons:** These lipoproteins are synthesized in the intestine. They contain about 99% lipids and 1% concentration of protein. Chylomicrons fraction contains the highest concentration of lipids and lowest concentration of proteins. They are characterized by lowest density and highest size amongst all lipoproteins.
2. **Very low density lipoproteins (VLDL):** The site of production of VLDL is liver and intestine. They are responsible for the transport of only those triglycerols which are synthesized endogenously in the body.
3. **Low density lipoproteins (LDL):** They are formed from the VLDL in the blood circulation. LDL carries out the function of transport of cholesterol from liver to other tissues.
4. **High density lipoproteins (HDL):** HDL are synthesized in the liver. They take part in the transport of cholesterol from peripheral organs to the liver.
5. **Free fatty acid-albumin:** Circulation of free fatty acid in the blood occurs in the form of free fatty acid albumin complex. One molecule of albumin can bind with 20-30 molecules of free fatty acids. This lipoprotein is not separated by the electrophoresis.

Electrophoresis of Serum Lipoproteins

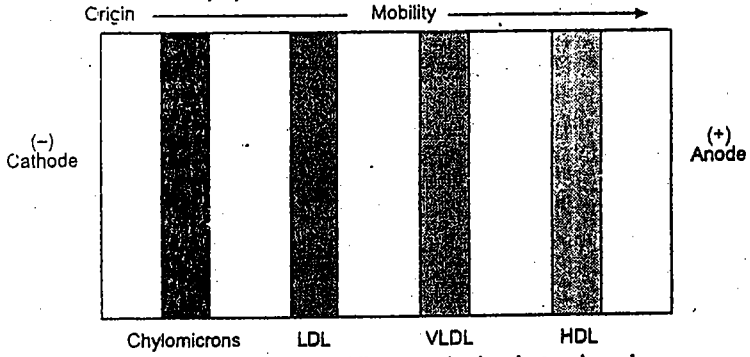


Fig. 3.3: Bands of separated lipo proteins by electrophoresis

Steroids

Compounds containing cyclopentanoperhydrophenanthrene nucleus in their structure are called as steroids. Steroids are many times found in association with the fat. Fig. 3.4 displays the numbering and structure of steroid nucleus. Most of the steroids contain side chains at the position 17. Presence of one or more hydroxyl groups on the steroid nucleus change them into sterols. The compound gains the suffix – (ols).

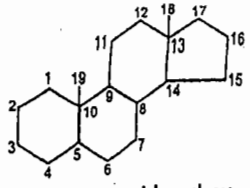


Fig. 3.4: Steroid nucleus

Steroid Nucleus (Cyclopentanoperhydrophenanthrene)

There are many steroids found in biochemical environment.

They can be grouped in the following manner:

Cholesterol, Ergosterol, – Sterols.

Testosterone, Estradiol – Sex hormones.

(1, 25, DHCC) or 1, 25 dihydroxycholecalciferol, – Vitamin D.

Corticosterone – Adrenocortical hormone.

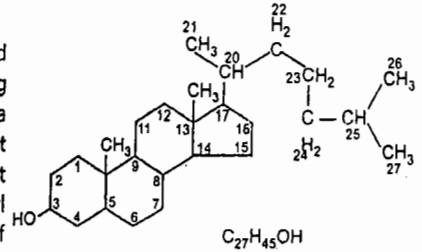
Glycocholic acid, Taurocholic acid – Bile acids.

3.10 CHOLESTEROL

Cholesterol is found only in the animal kingdom. It is absent in plant kingdom. All the animal tissues contain cholesterol mainly as a part of cell membrane and also as one of the constituent of lipoproteins. The egg yolk, adrenal cortex, brain and nervous tissue are rich sources of cholesterol. Cholesterol is also found in liver, kidney, small intestine and skin in the moderate concentrations.

Structure

A molecule of cholesterol contains steroid (cyclopentanoperhydrophenanthrene) ring made-up of 19 carbon atoms and a hydrocarbon side chain consisting of eight carbon atoms. It contains double bond at carbon number 5 and 6. The one hydroxyl group is situated at C_3 whereas C_{10} and C_{13} of cholesterol carries two methyl groups which are numbered C_{19} and C_{18} respectively.



$C_{27}H_{45}OH$

Cholesterol

Being an important part of the plasma membrane as well as the membrane of cell organelles, cholesterol plays a major role in the membrane permeability properties. Cholesterol undergoes esterification of –OH group at C_3 with the fatty acid.

Biochemical Properties

Cholesterol was first isolated from bile. It is a yellow coloured crystalline solid. The crystals of cholesterol have typical notches when observed under the microscope. It is insoluble in water and soluble in organic solvents like benzene, ether and chloroform.

Cholesterol can be qualitatively identified with the help of many biochemical reactions like:

1. Liebermann Burchard reaction.
2. Zak's Test.
3. Salkowski Test.

A quantitative estimation of cholesterol can also be done using well established biochemical assays.

Biochemical Role of Cholesterol

1. Being a component of nervous tissue, it functions as an insulator cover of the nervous tissue.
2. It is a major constituent of the membrane lipid and hence directly influences membrane permeability.
3. It serves as the important precursor of vitamin D_3, corticosteroid hormone, sex hormone etc.

Plant Sterol (Ergosterol)

Ergosterol occurs exclusively in the plant kingdom. It is an important precursor of vitamin D_2. Ergosterol is converted into ergocalciferol, a compound possessing vitamin D activity.

Some Other Animal Sterols

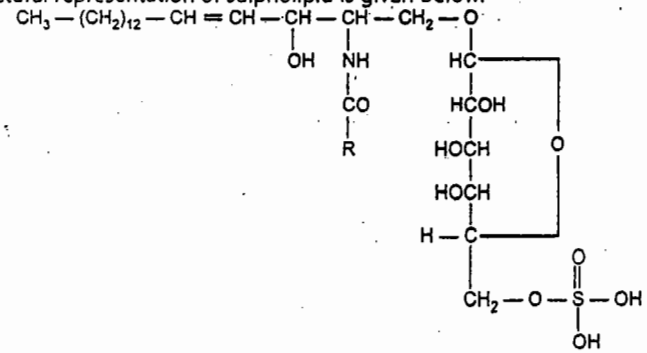
7 Dehydrocholesterol and coprosterol or fecal sterol.

7 Dehydrocholesterol is formed from cholesterol in the skin fat. The same compound happens to be a precursor of vitamin D_3 or cholecalciferol. Cholesterol entering the intestines is reduced by intestinal bacteria into coprosterol.

3.11 SULPHOLIPIDS (SULPHATIDES)

Sulphur containing lipids are found in brain, liver, kidney. The sulpholipids are made up of four compounds namely, sphingosine, fatty acid, sulphuric acid, galactose.

The structural representation of sulpholipid is given below:



Sulphatide: Structural formula

Sulphatides are sulfuric acid esters of galactocerebrosides. Excess accumulation of sulphatides is observed in sulfatide lipidosis (metachromatic leukodystrophy).

QUESTIONS

1. Give a detailed account of classification of lipids.
2. Write short notes on the following topics:
   (a) Simple lipids
   (b) Compound or complex lipids
   (c) Derived lipids
   (d) Neutral lipids
   (e) Rancidity and antioxidants
   (f) Phospholipids
   (g) Lipoproteins.
3. Define fatty acids. Explain the nomenclature of fatty acids.
4. Explain the term essential fatty acids. Why are they called so? What is the biochemical role of essential fatty acids?
5. Explain the terms saponification, hydrolysis, rancidity with reference to triglycerol.
6. What is the use of antioxidants? Name some natural and synthetic antioxidants.
7. How will you determine the purity of a given fat sample?
8. Define the following terms and state the importance of the same:
   (a) Iodine number
   (b) Saponification number
   (c) Acid number
   (d) Reichert Meissl number.
9. Which analytical methods are used to separate fats and fatty acids?
10. Define glycolipid. Write a note on the different types of phospholipids.
11. What is meant by lipoproteins? What is their biological significance?
12. Give the structure of cholesterol. What is the role of cholesterol in an eukaryotic cell?

Chapter ... 4

BIOENERGETICS

♦ LEARNING OBJECTIVES ♦

- *Understand Concept of Free Energy, Coupling reaction, Reduction Potential, Energy Rich Compounds.*
- *Recognize Role of Cyclic AMP and ATP in Metabolism.*
- *Appreciate Relationship among Free Energy, Enthalpy and Entropy.*
- *Acknowledge the Enerdeergonic and Exergonic Reactions.*

4.1 DEFINITION

Bioenergetics is 'the study of energy changes in biological reactions'. These reactions take place in the b ological system and are isothermic making use of chemical energy for the living activities.

4.2 CONCEPT OF FREE ENERGY

This is a thermodynamic concept and deals with free energy (G) contents of any substance When substance A is converted to product B, the difference in the free energy content of substance (G_A) and product (G_B) determines change in free energy (ΔG); to positive or negative quantity.

$$A \rightleftharpoons B \qquad \ldots (4.1)$$

$$\Delta G = G_B - G_A \qquad \text{OR} \qquad -\Delta G = G_B - G_A \qquad \ldots (4.2)$$

If the free energy change (ΔG) in the reaction is positive, the reaction involves an increase in free energy, while if free energy change is negative then the reaction occurs with decrease in free energy and occurs spontaneously. Later reaction will occur only if the energy is provided to catalyse the reaction. The reactions which have negative ΔG are called as exergonic reactions and those that have positive ΔG are called as endergonic reactions.

Many reactions taking place in our body are endergonic e.g. synthesis, transport of substances against the concentration gradient or electrical potential gradient. The free energy change in the following reactions can be studied using thermodynamic properties of reactant A and product B and can be expressed as:

$$\text{Reactant (A)} \rightleftharpoons \text{Product (B)}$$

$$\Delta G = \Delta H - T\Delta S \qquad \ldots (4.3)$$

where,

ΔG = Free energy change

ΔH = Enthalpy change (change in heat content)

T = Absolute temperature at which reaction takes place

ΔS = Entropy change (change in degree randomness or disorder in a system)

In the standard state, when pH is 7, temperature is 25°C and the concentration of solute is 1 molar at 1 atmospheric pressure, the actual free energy change in the reaction can be calculated by the following equation

$$\Delta G = \Delta G^\circ + RT \ln \frac{[Product]}{[Reactant]} \quad \ldots (4.4)$$

where, ΔG = Free energy change

ΔG° = Standard free energy change (with 1 M reactants and products, at pH 7)

R = Gas constant (1.987×10^{-3} kcal $\times$ deg^{-1} $\times$ mol^{-1})

T = Temperature (298 °K)

ln = Natural logarithm (can be converted to $\log_{10}$ multiplying by 2.303)

Enthalpy ΔH (change in heat content) for the reaction can be measured using calorimeter at constant pressure. Studying the measurement of entropy change (ΔS) does not come under the purview of this book. But, it is understood that if the entropy of product B becomes larger as compared to reactant A, the term $T\Delta S$ becomes positive and reaction becomes exergonic i.e. ΔG becomes more negative.

4.3 DETERMINATION OF FREE ENERGY FROM EQUILIBRIUM CONSTANT

When the rate of forward reaction is equal to the rate of backward reaction, the reaction is said to be at equilibrium. In the state of equilibrium, there is no change in free energy, i.e. $\Delta G = 0$ and the ratio of product [B] to reactant [A] is fixed and denoted by K_{eq}. Hence, the equation (4.4) becomes

$$\Delta G^\circ + RT \ln (K_{eq}) = 0 \quad \ldots (4.5)$$

$$\Delta G^\circ = -RT \ln (K_{eq}) \quad \ldots (4.6)$$

When we put values in equation (4.5), the equation becomes

$$\Delta G^\circ = 1.987 \times 298 \times 2.303 \log_{10} K_{eq} \quad \ldots (4.7)$$

$$\Delta G^\circ = -1363 \log_{10} K_{eq} \quad \ldots (4.8)$$

uation shows the relationship between standard free energy change (ΔG°) and stant K_{eq}. Hence, we can calculate ΔG° for any specific reaction. Now, at ntration of reactant and product can be measured. We get K_{eq} and ee energy change (ΔG°) can be calculated using equation (4.8). If K_{eq} or very small, then this method of determining ΔG° is not useful, o determine very small concentration of reactants and products at

If we consider the K_{eq} values varying from (very small) 0.001 to (very large) 1000, the standard free energy change will vary from + 4089 to – 4089 respectively. If K_{eq} value is considered as 1 then standard free energy change will be 0. In this case, the standard free energy change ΔG° will be equal to ΔG. In another words, at standard state, when $K_{eq} = 1$, is equal to free energy change (ΔG). If K_{eq} is more than 1, the reaction proceeds with decrease in free energy. In this case, tendency of the reaction to proceed in the almost complete formation of product B. If K_{eq} is less than 1, very less product is formed because equilibrium will not be attained unless energy is provided to form the product.

In the biological system, any reaction, occurring at any pH rather than 7, the standard free energy change is corrected for difference in pH, because equation assumes that the hydrogen ion produced or reacted will be taken at 1 M or pH = 0.

Alternatively, the equilibrium of the reaction can be measured at any one pH other than 0, and the resulting free energy change is denoted by ΔG. If hydrogen ion is neither produced nor utilized then ΔG equals to ΔG°.

Example:

Glucose-1-phosphate is converted into glucose-6-phosphate in the presence of *phosphoglucomutase*.

$$\text{Glucose-1-phosphate (0.020 mole, at 25°C)} \xrightarrow{\text{phosphoglucomutase}} \text{Glucose-6-phosphate} \quad \ldots (4.9)$$

As the reaction proceeds, the concentration of glucose-1-phosphate decreases to 0.001 M and concentration of product i.e. glucose-6-phosphate increases to 0.019.

$$\text{Hence, } K_{eq} = \frac{[Product]}{[Reactant]} = \frac{0.019}{0.001} = 19 \quad \ldots (4.10)$$

The standard free energy change (ΔG°) may be calculated by equation (4.6).

$$\Delta G^\circ = -RT \ln K_{eq}$$

$$= 1.987 \times 298 \times 2.303 \log_{10} 19$$

$$= -1363 \log_{10} 19$$

$$= -1363 \times 1.28$$

$$= -1745 \text{ calories.}$$

The reaction is independent of pH, because acid is not produced or utilized. Hence, no need to correct the standard free energy change (ΔG°).

Actually, in biological systems, glucose-6-phosphate is continuously formed and utilized to achieve a steady state condition and thermodynamic principles need to be applied on steady state rather than equilibrium condition.

4.4 RELATIONSHIP FREE ENERGY, ENTHALPY AND ENTROPY

Energy

The understanding of free energy change (ΔG) is important in analysis at any biological system. The concept of free energy was articulated by Josiah Gibbs. It is also called as Gibbs free energy which is related to entropy and enthalpy as follows;

$$\Delta G = \Delta H_1 - TS$$

where, H – Enthalpy

ΔG – Free energy

S – Entropy

T – Temperature

The free energy change (ΔG) is the measure of the spontaneity of a process under conditions of constant temperature and pressure. If the free energy change has negative value, it indicates spontaneous process.

This indicates that there are two possible driving forces,

(i) Decrease in enthalpy (negative values of ΔH)

(ii) Increase in entropy (positive values of ΔS)

A process having negative value of ΔG is spontaneous which is called as exergonic process. A process with positive value of ΔG is non-spontaneous, which is referred as endergonic. The free energy becomes zero at equilibrium. This indicates that entropy is at a maximum.

In biological systems, many chemicals reactions occur spontaneously. Entropic measurements are not sufficient to measure the spontaneity of reaction. If we take into consideration, the heat released by the reaction, the overall entropic change is positive.

All processes try to approach equilibrium. The pressure and volume changes in biological systems are very minor or negligible. The free energy change or ΔG represents maximum possible work occurring at the completion of reaction. This shows that ΔG is the maximum possible amount of useful energy that can be obtained from a reaction.

ΔG reflects only the direction in which the process takes place and does not include the time required for the process to occur. ΔG can be called as measure of spontaneity, but it has no concern of the rate of the reaction. Some reactions have negative ΔG (very small) and it may takes place in fraction of a time. Whereas some reactions might have very large value of negative ΔG and still require the millions of years to occur (in biological system). This way ΔG is a measure of spontaneity, but is unrelated to rate.

Enthalpy

Enthalpy is measurement of the energy in a thermodynamic system. Enthalpy is thermodynamic quantity which is equivalent to total heat content of a system. It is equal to internal energy of the system plus product of pressure and volume.

Enthalpy (H) is concept closely related to total energy E.

$$H = E + PV$$

where, P and V are pressure and volume respectively.

In biological system measurements, enthalpy describes system energy changes. The enthalpy simplifies the description of energy transfer. The total enthalpy H of a system cannot be measured directly. At constant pressure enthalpy change is equal to energy transfer from environment through heating work (other than expansion).

Entropy

Entropy is translated as the measure of disorder or uncertainty in a system. Entropy is one of the consequences of the second law of thermodynamics. The higher the disorder, higher the entropy of the system. Reversible processes do not increase the entropy of the universe.

Addition of polar molecules to water molecule forms micells around this foreign polar molecule. Being highly ordered molecules' micells formation, the entropy of the system decreases.

Small molecules bind to a surface (like protein surface) in many biochemical reactions. There is increase in the entropy in this case as binding of small molecules is associated with release of water molecules from protein surface. So the phenomenon of binding of water molecules can be called as entropically favoured.

4.5 COUPLING OF REACTION

The thermodynamically unfavourable reaction i.e. endergonic reaction (ΔG is positive) can be driven by coupling of reaction to favourable reaction.

Example:

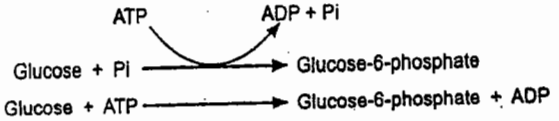
$$\text{Glucose} + \underset{\text{(Inorganic phosphate)}}{\overset{\text{Pi}}{}} \longrightarrow \text{Glucose-6-phosphate} + \text{water} \qquad \text{... (4.11)}$$

This reaction is thermodynamically an unfavourable reaction with a ΔG° of +3.3 kcal/mol.

When this reaction is coupled to hydrolysis of ATP to ADP and inorganic phosphate which is exergonic reaction with ΔG° of –7.3 kcal/mol provides energy for glucose phosphorylation.

$$\text{ATP} \longrightarrow \text{ADP} + \text{Pi} \qquad \text{... (4.12)}$$

The coupled reaction is



Finally, $\text{Glucose} + \text{ATP} \longrightarrow \text{Glucose-6-phosphate} + \text{ADP}$ ... (4.13)

With a net $\Delta G^\circ = 3.3 + (-7.3) = -4$ kcal/mol.

4.6 REDUCTION POTENTIAL

Free energy change (ΔG) of a biochemical reaction can be conveniently related to change in oxidation-reduction potential (ΔE_0). Reducing agent is a substance that donates electrons and gets oxidized.

$$F^{++} \longrightarrow \underset{\text{1 electron}}{F^{+++}} \longrightarrow Fe^{++} \quad \text{... (4.14)}$$

The reactions in which electrons are shown as either consumed or formed but the donor or acceptor is not shown are called as half reactions. The property of these donor/acceptor (moiety) to accept or donate electron is due to the specific potential of that compound. H_2 is considered as a standard form and comparing the potential of such moiety whose reduction potential E_0 is considered as 0.00 V at pH 0, for the following half σ reaction.

$$H^+ + 1\,e^- \longrightarrow \tfrac{1}{2} H_2 \quad \text{... (4.15)}$$

In this reaction, the proton is utilized and the potential of this half reaction varies with pH. In the standard state i.e. at pH 7, the reduction potential is – 0.420 V.

By taking this as a standard, one can determine the reduction potential of any compound able to get oxidized or reduced with reference to hydrogen. Following are the potentials for few reactions written as reduction.

Table 4.1: Reduction potentials of half reactions

Half reaction (Written as reduction)	E_0 at pH 7 in V
1. $\frac{1}{2} O_2 + 2H_2 + 2e^- \longrightarrow H_2O$	0.82
2. $Fe^{+++} + 1\,e^- \longrightarrow Fe^{++}$	0.77
3. Cytochrome a – $Fe^{+++} + 1e^- \longrightarrow$ Cytochrome a – Fe^{++}	0.29
4. Fumarate + $2H^+ + 2e^- \longrightarrow$ Succinate	0.03
5. Oxaloacetate + $2H^+ + 2e^- \longrightarrow$ Mallate	– 0.10
6. Acetaldehyde + $2H^+ + 2e^- \longrightarrow$ Ethanol	– 0.16
7. 1, 3 Diphosphoglyceric acid + $2H^+ + 2e^- \longrightarrow$ Glyceraldehyde-3 phosphate + Pi	– 0.29
8. $NAD^+ + 2H^+ + 2e^- \longrightarrow NADH + H^+$	– 0.32
9. Acetate + $2H^+ + 2e^- \longrightarrow$ Acetaldehyde + H_2O	– 0.047

If the reaction is going on as oxidation rather than reduction, the E_0 value will be changed from positive to negative or negative to positive. When any two reactions are coupled, there is a change in reductive potential of overall reaction. The reaction with more positive reduction potential will be a reduction, and other half reaction having less positive reduction potential is oxidation. It has been observed that substances with more positive reduction potential are oxidizing agents and those with negative reduction potential are reducing agents.

We can corelate the change in reduction potential (ΔE_0) with free energy change (ΔG) by using the following equation,

$$\Delta G = -nF\Delta E_0 \quad \text{... (4.16)}$$

where, ΔG = Free energy change

n = number of electrons transferred in redox reaction

F = Faraday's constant (23063 cal)

ΔE_0 = Difference in reduction potential of half reaction containing oxidizing and reducing agents at pH 7.0

Consider the following two reactions in which acetaldehyde is converted to ethanol (E_0 = – 0.16 V) and NADH is converted to NAD^+ (E_0 = – 0.32 V)

$$\text{Acetaldehyde} + 2H^+ + 2e^- \longrightarrow CH_3CH_2OH \text{ (Reduction)} \quad \text{... (4.17)}$$

$$NADH + H^+ + 2e^- \longrightarrow NAD^+ + 2e^- + 2e^- \text{ (Oxidation)} \quad \text{... (4.18)}$$

When these two reactions are coupled, there will be change in reduction potential.

$$\text{Acetaldehyde} + NADH + H^+ \longrightarrow NAD + CH_3CH_2OH \quad \text{... (4.19)}$$

ΔE_0 = [E_0 of reduction] – [E_0 of oxidation]

= [– 0.16] – [– 0.32]

= 0.16 V

Now, the free energy change is calculated using the equation

$\Delta G = nF\,\Delta E_0$

= (– 2) (23063) (0.16)

= – 7400 cal.

As ΔG is a larger negative quantity, the reaction is thermodynamically feasible.

Oxidation of NADH by molecular oxygen is a general reaction in biological system. The ΔG can be calculated for this reaction as described below:

$$NADH + H^+ \longrightarrow NAD^+ + 2H^+ + 2e^- \text{ (Oxidation)} \quad \text{... (4.20)}$$

$$\tfrac{1}{2} O_2 + 2H^+ + 2e^- \longrightarrow H_2O \text{ (Reduction)} \quad \text{... (4.21)}$$

Overall coupling reaction is

$$NADH + H^+ + \frac{1}{2}O_2 \longrightarrow NAD^+ + H_2O \quad \text{... (4.22)}$$

$$\Delta E_0 = 0.82 - [-0.32]$$
$$= 1.14\ V$$

The free energy change is calculated as

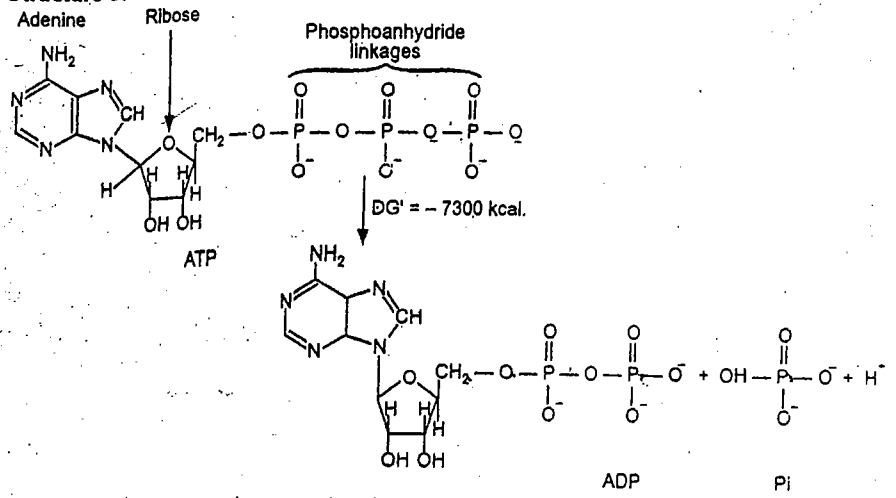
$$\Delta G = (-2)(23063)(1.14)$$
$$= -52{,}600\ kcal$$

In this coupled reaction, though ΔG is a larger negative quantity, there is no guarantee that NADH will be rapidly oxidized, because NADH is stable in presence of oxygen and oxidized only in presence of suitable enzyme.

4.7 ENERGY RICH COMPOUNDS (HIGH ENERGY COMPOUNDS)

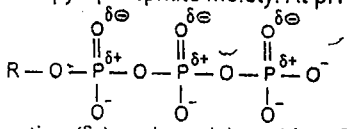
Adenosine triphosphate is a compound which is involved in linking endergonic reaction to others that are exergonic. ATP exhibits decrease in free energy when it undergoes hydrolytic reaction. Hence, they are called 'energy - rich' or 'high - energy' compounds. Such compounds are unstable in acidic and alkaline conditions and are susceptible to heat.

Structure of ATP and ADP



This reaction is highly exergonic. The products, adenosine diphosphate and inorganic phosphate are more stable because they exhibit more resonance stabilization as compared to ATP and the intramolecular electrostatic repulsion is reduced between the negatively charged groups on beta (β) and gamma (γ) phosphates.

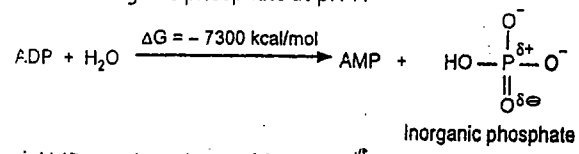
ATP can act as energy carrier between catabolic and anabolic pathways. The structure of importance in the ATP molecule is pyrophosphate moiety. At pH 7 it is fully ionized.



There exists a partial negative (δ–) and partial positive (δ+) charge on oxygen and phosphorus atom which results in polarization of P=O bond.

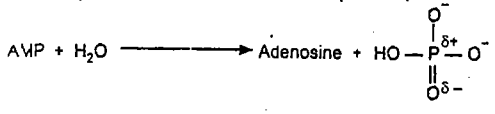
The presence of residual positive charges on adjacent phosphorus atom in ATP and ADP indicates that they must contain enough energy to overcome electrostatic repulsion between adjacent like charges.

When pyrophosphate moiety is cleaved upon hydrolysis, this energy will get released and decrease the ΔG of the reaction (*orthophosphate cleavage*). Similarly, when ADP is converted to AMP and inorganic phosphate at pH 7.



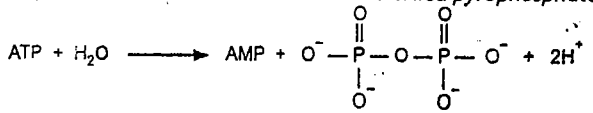
... (4.23)

Hydrolysis of AMP to adenosine and inorganic phosphate results in less free energy for reason already mentioned above. (ΔG = – 22000 kcal at pH = 7)



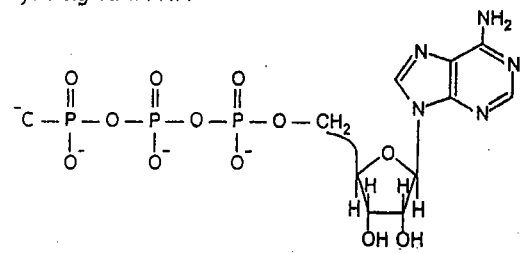
... (4.24)

There are few important reactions in which ATP is converted directly to AMP and inorganic pyrophosphate with ΔG = – 8600 kcal. This is called *pyrophosphate cleavage.*



... (4.25)

Artificial ATP analogs have been designed which are resistant to cleavage of the terminal phosphate by hydrolysis e.g. AMPPNP.



AMPPNP (ATP analog)

Such analogs are generally used to study the dependence of coupled reactions on ATP hydrolysis.

Biological Significance of ATP as Energy Currency

Reactions pursued in biological environment are either endergonic or exergonic. Some times energy liberated in the exergonic reactions is utilized by endergonic reactions, to achieve completion. High energy compounds present in the cell are responsible for driving many reactions. It is observed that, hydrolysis of high energy bonds is coupled with the undergoing reactions.

Maximum number of high energy compounds are made up of phosphorus and oxygen atoms. The hydrolysis of bonds between phosphorus and oxygen is utilized by many endergonic reactions as a energy source.

$$R-O \sim PO_3 + HOH \longrightarrow R-O-H + PO_3^-$$

(~ sign indicates high energy bond. This sign was assigned for the first time by Lipman, to indicate high energy bond.)

If the energy of hydrolysis of the compound is more than 7.3 k cal/mole, it is referred as the high energy compound and if the energy of hydrolysis of compound is less than 7.3 k cal/mol, then it is a low energy compound.

This way ATP bears the central role. ATP is the most common compound involved in the process of energy transfer in the body. ATP can be hydrolysed to ADP, which can be further hydrolysed to AMP.

$$ATP + H_2O \longrightarrow ADP + Pi + 7.3 \text{ kcal/mole}$$

$$ADP + H_2O \longrightarrow AMP + Pi$$

ATP can be hydrolysed to AMP

$$ATP + H_2O \longrightarrow AMP + PPi$$

where Pi – is standard abbreviation of inorganic phosphate

and PPi – is standard abbreviation of pyrophosphate (inorganic)

The examples of inorganic pyrophosphates are $P_2O_7^{-4}$, $H_2PO_7^{-2}$ or $H_3PO_7^-$ or $HP_2O_7^{-3}$

ATP acts as a energy currency in the cell. It traps the energy released in the exergonic reactions or in the hydrolysis of high energy compounds. ATP can also supply energy to the endergonic reaction by hydrolysis of its γ phosphate bond to form ADP + Pi or by hydrolysis of its β phosphate bond to form AMP + PPi.

Structure of ATP:

The molecule of ATP is made up of purine base odenine and a ribose sugar which together makes a nucleoside adenosine. The adenosine is bonded with one phosphate ester bond and two phosphate anhydride bonds through its ribose sugar. The ATP molecular structure also contains a glycoside bond between N atom of adenine and C of the ribose sugar.

ATP molecule is found in all forms to life; animals and plants.

ATP owes its high energy to phosphates within its structure. Phosphates are high energy molecules. However the energy released during the ATP hydrolysis (i.e. when phosphate groups are removed) is due to two reasons :

(i) Removal of phosphate groups from the molecule.

(ii) Total interactions of all the atoms within ATP molecules.

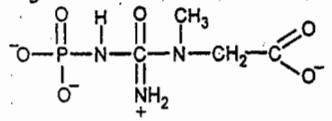
ATP and Energy Transfer :

A 70 kg resting man also shows turnover of 40 kg worth of ATP. The high energy bond in ATP is fairly easy to break, making ATP, an ideal universal energy carrier. The phosphor-anhydrate bond in ATP is fairly unstable may be due to lot of negative charged compounds around triphosphates.

The biological reactions in the cell requires almost equivalent amount of energy released by the ATP hydrolysis. This is the reason why very less amount of energy is wasted.

Phosphocreatine

Phosphocreatine is also called as *creatine phosphate*. Like ATP, it is also a compound with a "high energy" phosphate linkage. It is used in the muscle cells for storage of ~P bonds.



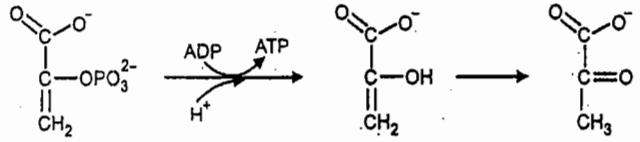
Phosphocreatine (creatine phosphate)

Creatine kinase catalyzes the following reaction:

$$\text{Phosphocreatine} + ADP \rightleftharpoons ATP + \text{creatine}$$

During extensive use of muscles (exercise), phosphate is transferred from phosphocreatine to ADP, thus replinishing ATP. Phosphocreatine is also used to transport "high energy" phosphate between different compartments of cell.

Phosphoenolpyruvate



Phosphoenolpyruvate Enolpyruvate Pyruvate

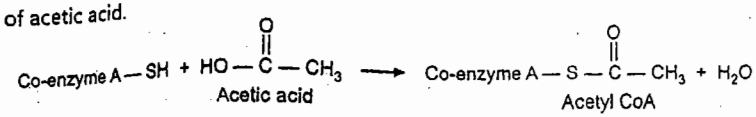
Phosphoenolpyruvate is involved in the production of ATP during hydrolysis. It has a larger negative ΔG of phosphate hydrolysis than ATP. In this aspect it is quite unique as other phosphate esters have a lower negative ΔG of phosphate hydrolysis.

Other "high energy" bonds

Thioesters

Thioesters are also "high energy" bonds. Thioesters are esters of carboxylic acid and a thiol group (SH). Compared to their phosphate counterparts, thioesters have a large negative ΔG of hydrolysis.

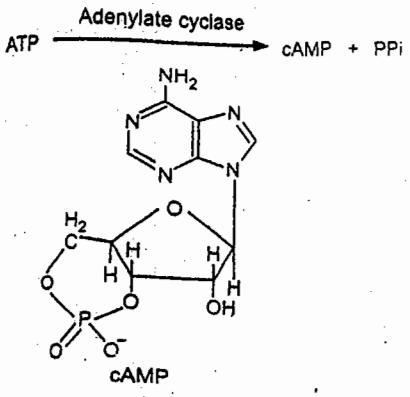
For example, acetyl-CoA is formed when thiol of co-enzyme A reacts with carboxyl group of acetic acid.



Cyclic AMP

Cyclic AMP (cAMP) is a abbreviation of the cyclic adenosine monophosphate. cAMP is a derivative of adenosine triphosphate (ATP) and it work as a second messenger for many biological reactions and processes. It works for intracellular transduction in many different organisms, wherein it plays role in cAMP dependent pathway. The major role of cAMP is observed in the control of the enzyme catalyzed process in living cells.

Formula: $C_{10}H_{12}N_5C_6P$

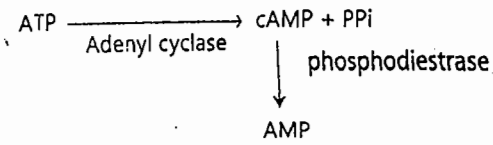


Cyclic AMP

Ease of hydrolysis makes cyclic AMP an excellent signal transduction molecule.

cAMP is involved as a secondary messenger in the mechanism of action of hormones like epinephrine and many other hormones.

Synthesise of cAMP occurs from the ATP with the help of adenyl cyclase enzyme which is located on the inner side of the plasma membrane as well as it is anchored at different locations in the interior of the cells. The cAMP so formed is deactivated once its role as a secondary messenger is over with the help of enzyme phosphodiesterase.



Synthesis and deactivation of cyclic AMP

Enzyme adenylate cyclase is activated by a range of singnalling molecules through the activation of a adenylate cyclase stimulatory G(Gs) – protein coupled receptors. The inhibition of the adenylate cyclase occurs through agonists of adenylate cyclase inhibiting G(Gi) – protein coupled receptors. It is observed that, adenylate cyclase found in the liver responds or shows its action to hormone glucagon and muscle adenylate cyclase shows strong response to hormone adrenaline.

Major Biochemical Functions:

(i) cAMP works as a secondary messenger, which is used for intracellular signal transduction which involves the transferring into cells the effects of hormones like adrenaline and glucagon. The hormones are unable to pass through plasma membrane. This is the reason why they require secondary messenger like cAMP.

(ii) cAMP binds to ion channels like HCN and regulates the passage of specific ion through it across the membrane.

(iii) cAMP plays a role in the activation of the protein kinase enzymes which participate in biochemical regulation of glycogen, carbohydrate and lipid metabolism (These reactions are called as pKA or protein kinase A dependent functions of cAMP).

(iv) Activation of a Ca-channel, which involves activation of growth hormone. Releasing hormone to release the growth hormone occurs through the action of cAMP. However, this function of cAMP does not involve any mediation or participation of PKA or protein kinase A.

QUESTIONS

1. Explain the concept of free energy.
2. Determine free energy from equilibrium constant.
3. What are energy rich compounds. Give their importance.

4. Explain with structures:
   (a) *Phosphocreatine*
   (b) Phosphoenolpyruvate
   (c) Thioesters
   (d) 3' 4' - Cyclic 4 MP (cAMP)
5. Describe major biochemical function of cyclic AMP.

UNIT II

Chapter ... 5

CARBOHYDRATE METABOLISM

♦ LEARNING OBJECTIVES ♦

- *Recognize Central Role of Glucose in Metabolism of Carbohydrates.*
- *Understand Significance of Metabolic pathways of Glycolysis, TCA Cycle, Pentose Phosphate Pathway, Gluconeogenesis, Glycogenolysis and Glycogenesis.*
- *Know energetics and Hormonal Control of Pathways of Carbohydrate Metabolism.*
- *Recognize consequences of Glucose-6-Phosphate Dehydrogenase Deficiency.*

5.1 INTRODUCTION

The major pathways of carbohydrate metabolism will be discussed here. They are *glycolysis, citric acid cycle, glycogenolysis, glycogenesis, gluconeogenesis, hexose monophosphate shunt.*

Most important energy source of the body is carbohydrates. Almost all cells of the body utilize glucose very easily. A minimum amount of glucose is always required for normal functioning of cells. If the amount of carbohydrate is not sufficient for this purpose, body tries to produce glucose from non-carbohydrate sources. The normal fasting plasma glucose level is 80 – 120 mg / dL.

5.2 METABOLISM OF GLUCOSE

The preferred source of energy for most of the body tissues is glucose. There are many pathways for the metabolism of glucose, primarily for the production of energy as well as for the conversion of glucose into its storage form i.e. glycogen.

Embden - Meyerhof Pathway of Glycolysis:

Glycolysis is defined as 'the catabolic pathway converting glucose or glycogen to pyruvate or lactate under aerobic or anaerobic conditions along with production of ATP'.

Significance of Glycolysis:

All the reactions of this pathway occur in the cytoplasm. Glucose is converted to pyruvate or lactate with concomittant production of energy. This pathway can operate in both conditions i.e. aerobic or anaerobic. The only pathway that occurs in each cell of the body is glycolysis and it is the only source of energy in erythrocytes. In strenuous exercise, muscle tissues do not have enough oxygen, here anaerobic glycolysis is the major source of energy. Under aerobic conditions, the pyruvate is the end product which then gets oxidized to acetyl CoA and enters into citric acid cycle, which is operating in mitochondria and where large quantity of energy is trapped as ATP. This pathway also provides carbon skeletons for the synthesis of certain non-essential amino acids as well as glycerol part of fat. Many reactions of this pathway are reversible and are also used for gluconeogenesis.

Glucose from the extracellular fluid gets transported into the cell with the help of *translocase* enzyme. This enzyme is present on the membrane of all the cells except liver cells. The activity of this enzyme is under the control of insulin. Hence, in diabetes mellitus, entry of glucose into the cells is hampered.

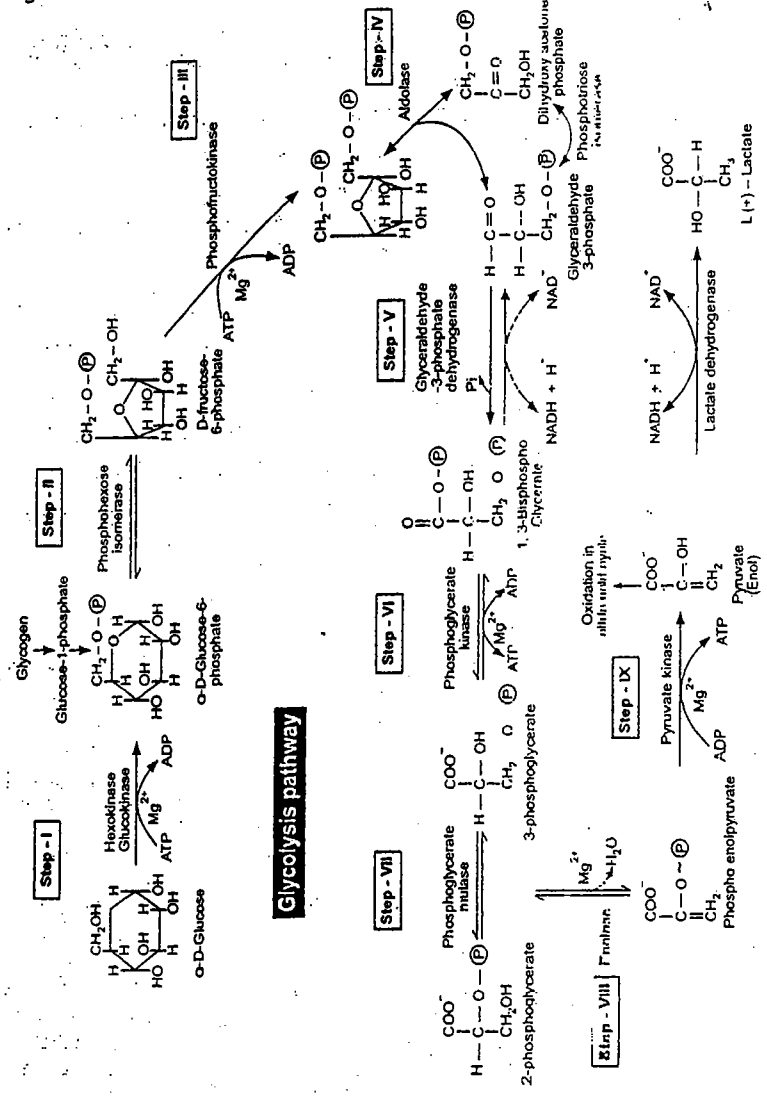


Fig. 5.1: Glycolysis pathway

5.3 STEPS INVOLVED IN GLYCOLYSIS

Step I: Initial reaction of glycolysis is catalysed by hexokinase. Hexokinase phosphorylates glucose at 6 carbon in presence of Mg^{2+} and ATP. Phosphate is donated by ATP. Formation of Mg^{2+}.ATP complex is essential. Hexokinase is an allosteric enzyme. It phosphorylates other hexoses like galactose and fructose. The reaction catalysed by this enzyme is irreversible under normal physiological conditions. One high energy phosphate bond is used in this reaction to generate glucose-6-phosphate. Hexokinase is widely distributed.

Liver contains glucokinase which phosphorylates only glucose. Glucokinase is an inducible enzyme. Its K_m for glucose is high compared to K_m of hexokinase. Hence, it phosphorylates glucose when blood glucose concentration is high, whereas hexokinase phosphorylates glucose at lower concentrations.

Step II: Conversion of glucose-6-phosphate to fructose-6-phosphate: This is the reversible reaction where glucose-6-phosphate isomerizes to fructose-6-phosphate with the help of enzyme *isomerase* (aldose-ketose isomerisation).

Step III: Conversion of fructose-6-phosphate to fructose-1, 6-biphosphate: This is an irreversible reaction where fructose-6-phosphate is further phosphorylated to form fructose-1,6-biphosphate. This reaction is governed by *phospho fructokinase* enzyme in the presence of ATP.

Step IV: This is a reversible reaction in which fructose-1, 6-biphosphate is cleaved into one molecule of glyceraldehyde-3-phosphate and one molecule of dihydroxyacetone phosphate. Since the backward reaction is an aldol condensation, name of the enzyme involved is *aldolase*.

Dihydroxyacetone phosphate formed from the cleavage of fructose-1, 6-diphosphate is isomerised to glyceraldehyde-3-phosphate with the help of enzyme *phosphotriose isomerase*. Thus, from one molecule of glucose, two molecules of glyceraldehyde-3-phosphate are formed.

Neutral fat is hydrolysed to fatty acid and glycerol. The glycerol is phosphorylated to give glycerol-3-phosphate, which after dehydrogenation in the presence of NAD^+ produces dihydroxyacetone phosphate. Thus, glycerol portion of neutral fats can enter in gluconeogenesis and glycolysis pathways.

Step V: This is a reversible reaction in which glyceraldehyde-3-phosphate is dehydrogenated and simultaneously phosphorylated to form 1, 3-biphosphoglycerate with the help of *glyceraldehyde-3-phosphate dehydrogenase* enzyme in the presence of NAD^+.

Step VI: This step is an example of substrate level phosphorylation where energy of 1, 3-biphosphoglycerate is trapped to synthesize one ATP molecule with the help of *kinase* enzyme.

This reaction is reversible where 1,3-biphosphoglycerate is converted to 3-phosphoglycerate with the help of enzyme *1, 3-biphosphoglycerate kinase.*

Step VII: This is a reversible reaction. In this reaction, the phospho group on the third carbon of 3-phosphoglycerate is shifted to the second position resulting in the formation of 2-phosphoglycerate. This reaction is governed by the enzyme *phosphoglyceromutase*.

Step VIII: In this reaction, water molecule is removed from 2-phosphoglycerate forming phosphoenol pyruvate. The enzyme responsible for this reaction is *enolase* and it requires Mg^{++} ion. The fluoride ion blocks the activity of this enzyme.

Step IX: This is an irreversible reaction. The phosphoenol pyruvate is converted to pyruvate with the help of *pyruvate kinase*. This reaction is also an example of substrate level phosphorylation. (Because there is formation of one ATP).

***The pyruvate kinase* is a key glycolytic enzyme.**

Step X: The main metabolic fate of pyruvate is dehydrogenation, producing acetyl CoA which enters into citric acid cycle. Citric acid cycle operates only in the presence of excess oxygen. In shortage of oxygen, the major pathway of pyruvate is blocked. Since in 5th step of glycolysis, two molecules of NAD^+ are reduced to NADH. The availability of this co-enzyme inside the cell is limited and for smooth operation of this pathway, NADH has to be reconverted to NAD^+. This reconversion can be done in two ways i.e. one by oxidative phosphorylation in the mitochondria, but in the absence of oxygen this reconversion is blocked. The 2nd way is that pyruvate is reduced to lactate which generates NAD^+ and is reutilized for 5th step of the pathway. This reduction is governed by the enzyme *lactate dehydrogenase*.

Thus, in anaerobic glycolysis, end product is lactate which enters in Cori's cycle, whereas in aerobic condition the end product is pyruvate which enters in citric acid cycle for complete oxidation.

5.4 REGULATION OF GLYCOLYSIS

Generally, metabolic activities are regulated by altering the activities of some enzymes of the pathway. Glycolysis is under hormonal and allosteric control.

Insulin activates glycolysis by stimulating key glycolytic enzymes, whereas glucogen activate gluconeogenesis by stimulating the key enzymes and have inhibitory effect on glycolysis. Phosphofructokinase is the most important rate limiting enzyme, whereas hexokinase and pyruvate kinase also have regulatory impact on the glycolysis. Phosphofructokinase is an allosterically regulated enzyme. The most important allosteric inhibitor of phosphofructokinase is ATP; another inhibitor is citrate. When the citrate levels are high, it indicates that energy charge of the cell is high and therefore glucose degradation and thus ATP synthesis is inhibited. Thus, citrate acts by potentiating the inhibitory effect of ATP. AMP acts as an allosteric activator of phosphofructo kinase. AMP also reverses the inhibitory effect of ATP. Fructose-6-phosphate also increases the activity of phosphofructokinase. pH also has an effect on phosphofructokinase enzyme, low pH inhibits the phosphofructokinase activity.

In 1980, Henri-Gery Hers and Emile van Schaftingen discovered that the efficient regulator of phosphofructokinase is fructose-2,6-bisphosphate, which increases the activity of this enzyme.

Another regulation of glycolysis is at the level of hexokinase, which is inhibited by glucose-6-phosphate. When phosphofructokinase is inhibited, the accumulation of fructose-6-phosphate and glucose-6-phosphate occurs. Due to this, inhibition of hexokinase occurs. In liver, phosphorylation of glucose continues because glucokinase has a higher K_m and therefore excess glucose is converted to glycogen and stored. Since glucose-6-phosphate is not only used for glycolysis but is also used for glycogen synthesis hence hexokinase cannot be the only enzyme for the regulation.

Pyruvate kinase also has a regulatory effect on glycolysis. It is inhibited by ATP and phosphoenol pyruvate. Insulin activates the activity of pyruvate kinase, whereas glucagon inhibits the activity.

Important Physiological Controls of Glycolysis:

Controller	Inhibition	Activation
Carbohydrate	Glucose-6-phosphate	Glucose Fructose-6-phosphate Fructose-1,6-biphosphate Fructose-2,6-biphosphate
Nucleotide	ATP	AMP
Miscellaneous	Citrate phosphorylation of enzymes	

5.5 ENERGY YIELD FROM GLYCOLYSIS

In anaerobic glycolysis, when one molecule of glucose is degraded to two molecules of lactate, it results in the net yield of two molecules of ATP. As such four molecules of ATP are produced by the two substrate level phosphorylations i.e. at steps 6 and 9, but two molecules of ATP are consumed in step 1 and 3. Hence, the net yield is two ATP. The complete reaction can be summarized as;

$$\text{Glucose} + 2Pi + 2ADP \longrightarrow 2 \text{ lactate} + 2ATP$$

This occurs in anaerobic condition but in aerobic condition the two NADH molecules which are generated in step 5 enter into mitochondria via electron transport chain. Each NADH produces 3 ATPs. Since 2 NADH molecules are produced from one molecule of glucose, 6 ATPs are produced. Thus, in aerobic condition, 8 ATPs are produced while in anaerobic condition, 2 ATPs are produced.

Table 5.1: Energy yield per molecule of glucose in glycolysis under anaerobic condition

Step	Enzyme	Source	No. of ATPs gained per glucose molecule
1.	Hexokinase	–	– 1
2.	Phosphofructokinase	–	– 1
3.	1, 3-bisphosphoglycerate kinase	ATP	1 × 2 = 2
4.	Pyruvate kinase	ATP	1 × 2 = 2
			∴ Total = 4 – 2 = 2 ATP

Table 5.2: Energy yield per molecule of glucose in glycolysis under aerobic conditions

Step	Enzyme	Source	No. of ATPs gained per glucose molecule
1.	Hexokinase	–	– 1
2.	Phosphofructokinase	–	– 1
3.	Glyceraldehyde-3-phosphate dehydrogenase	NADH	3 × 2 = 6
4.	1, 3-bisphosphoglycerate kinase	ATP	1 × 2 = 2
5.	Pyruvate kinase	ATP	1 × 2 = 2
			Total = 10 – 2 = 8 ATPs

5.6 METABOLIC FATE OF PYRUVATE

Under aerobic condition, pyruvate is converted to acetyl CoA by oxidative decarboxylation. Acetyl CoA which is formed enters in TCA cycle to give CO_2, H_2O and ATP. Acetyl CoA is also used for the synthesis of fatty acids, cholesterol and ketone bodies. This conversion i.e. pyruvate to acetyl CoA is catalysed by enzyme complex called pyruvate-dehydrogenase complex (PDH), which is a mitochondrial enzyme. This reaction requires thiamine pyrophosphate, lipoamide and FAD as cofactors, whereas CoA-SH and NAD^+ are the co-enzymes. The pyruvate dehydrogenase complex is made up of pyruvate decarboxylase, dihydrolipoyl transacetylase and dihydrolipoyl dehydrogenase.

$$\text{Pyruvate} + NAD^+ + \text{CoA} \xrightarrow{\text{PDH}} \text{Acetyl CoA} + NADH + H^+ + CO_2$$

The oxidative decarboxylation of pyruvate is catalysed by pyruvate decarboxylase of the PDH. This reaction occurs in 2 steps. First pyruvate combines with thiamine pyrophosphate to form hydroxymethylthiaminepyrophosphate liberating CO_2 and second hydroxyethyl thiamine pyrophosphate is oxidised to form acetyl group, which is then transferred to lipoamide to form acetyl lipoamide. Oxidation of hydroxy ethyl group leads to the acetate group and lipoamide is reduced to dihydrolipoamide. The acetyl group is transferred to co-enzyme A with the help of dihydrolipoyl transacetylase. Finally, oxidation of dihydrolipoamide by dihydrolipoyl dehydrogenase occurs. The hydride ion which is formed is first transferred to FAD to form $FADH_2$ and then to NAD^+. After completion of reaction, cofactors i.e. thiamine pyrophosphate, lipoamide and FAD are regenerated. This reaction is completely irreversible.

5.7 CITRIC ACID CYCLE

In 1937, Sir Hans A. Krebs described the complete cycle and for this work he was awarded the Nobel prize in 1953. The cycle is named after him i.e. Krebs cycle. He studied the condensation of oxaloacetate with acetyl CoA to form one molecule of tricarboxylic acid. In 1948, Ogsten and in 1949, Polter showed that the tricarboxylic acid formed in this condensation was citric acid and hence gave it the name citric acid cycle.

5.8 REACTIONS INVOLVED IN CITRIC ACID CYCLE

All the enzymes involved in this pathway are located inside the mitochondria. Pyruvate, which is the end product of glycolysis, is oxidatively decarboxylated to form acetyl CoA with the help of pyruvate dehydrogenase enzyme. This reaction is a link between the TCA cycle and glycolysis. This conversion occurs in mitochondria and pyruvate enters into the mitochondria with the help of a carrier. Acetyl CoA is also derived from β-oxidation and this reaction occurs inside the mitochondria.

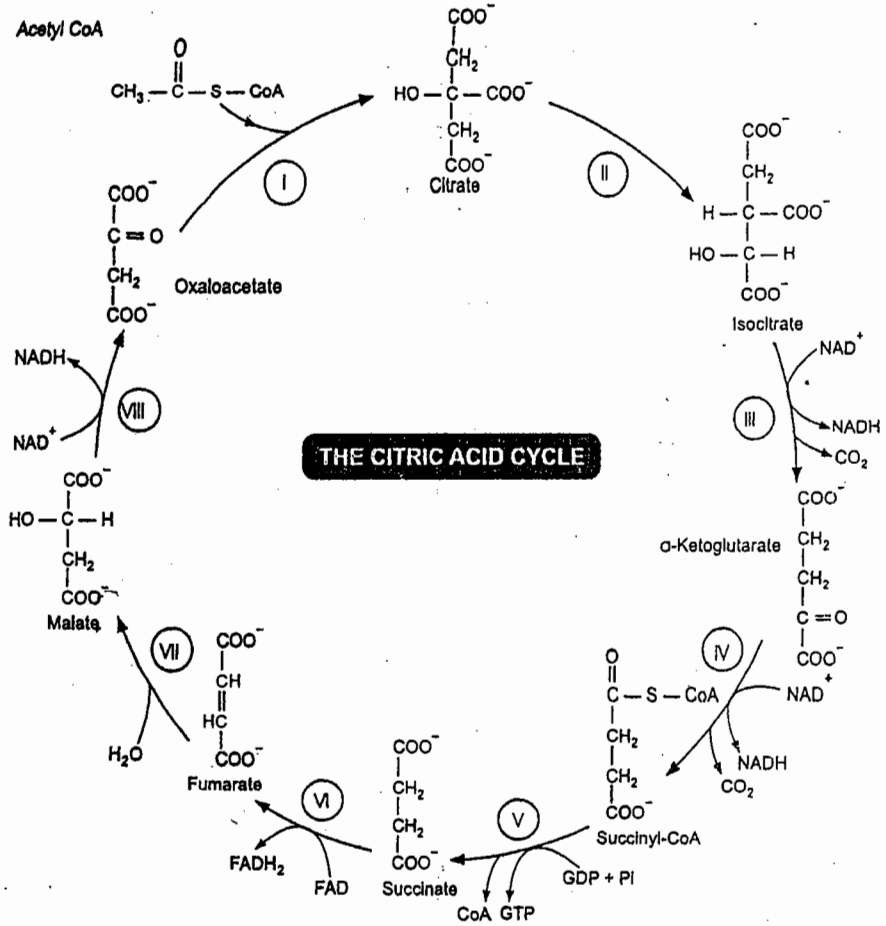
Step I: The first step of this cycle is the condensation of 4 carbon oxaloacetate with 2 carbon acetyl CoA forming 6 carbon compound citrate with the help of citrate synthase. This reaction involves an aldol condensation forming citroyl CoA which upon hydrolysis yields citrate. It is a rate limiting reaction and irreversible. One water molecule is consumed in this reaction.

Step II: In this step, citrate is isomerised to form isocitrate via intermediate formation of cis-aconitate. This reaction is catalysed by aconitase enzyme, which is an iron-sulphur protein. Citrate loses one water molecule forming cis-aconitate. Then water molecule is added forming isocitrate. The position of the hydroxyl group is shifted in the product.

Step III: In this step, isocitrate undergoes dehydrogenation to form unstable β-keto acid oxalosuccinate, which undergoes spontaneous decarboxylation to yield α-ketoglutarate. This reaction is catalysed by isocitrate dehydrogenase. The mitochondrial enzyme utilizes NAD^+, whereas cytoplasmic enzyme is $NADP^+$ dependent. This reaction has a regulatory role. The NADH formed in this step is oxidised in the electron transport chain.

Step IV: In this step, α-ketoglutarate is oxidatively decarboxylated to form succinyl CoA. This reaction is catalysed by α-ketoglutarate dehydrogenase, which is a multi-enzyme complex having 3 enzyme proteins and 5 co-enzymes. The NADH which is formed in this reaction enters into the electron transport chain to form 3 ATPs.

Step V: Succinyl CoA is converted to succinate with the help of succinic thiokinase. This reaction is an example of substrate level phosphorylation. A molecule of GDP is phosphorylated to GTP, which can be converted to ATP by reacting with the ADP molecule.



(The Roman numbers indicate the number of individual steps of the cycle)

Fig. 5.2: Reaction chart of Citric Acid Cycle in mitochondria from pyruvate

Step VI: Succinate is dehydrogenated to form fumarate, an unsaturated dicarboxylic acid, with the help of succinate dehydrogenase. The liberated hydrogen atoms are accepted by FAD forming $FADH_2$, which enters the electron transport chain forming 2 ATPs. The succinate dehydrogenase is inhibited by malonate and this inhibition is competitive. The enzyme is flavoprotein in nature with FAD covalently linked to a histidine residue of the enzyme.

Step VII: Malate is converted to form fumarate with the help of fumarase. In this reaction, water molecule is added and the H^+ and OH^- ions are added in a stereo specific manner, hence only L-malate is formed as the product.

Step VIII: Malate is dehydrogenated to form oxaloacetate with the help of malate dehydrogenase in the presence of co-enzyme NAD^+. The oxaloacetate which is generated, condenses with another molecule of acetyl CoA and continues the cycle.

Stoichiometry of the TCA Cycle:

Acetyl CoA → $2\ CO_2$ + SH–CoA
Oxaloacetate → Oxaloacetate
FAD → $FADH_2$
3 NAD^+ → 3 NADH
GDP + Pi → GTP

Table 5.3: Energetics of TCA Cycle

Step No.	Enzyme	Coenzyme	ATPs generated
3.	Isocitrate dehydrogenase	NADH	3
4.	α-ketoglutarate dehydrogenase	NADH	3
5.	Succinic thiokinase	GTP	1
6.	Succinate dehydrogenase	$FADH_2$	2
7.	Malate dehydrogenase	NADH	3
		Total ATPs generated	**12**

5.9 FUNCTIONS OF TCA CYCLE

1. It is the final common oxidative pathway which oxidises acetyl CoA to give CO_2.
2. This pathway is the source of a significant fraction of reduced co-enzymes that drive the respiratory chain.
3. It is a link between catabolic and anabolic pathways.
4. The pathway also provides precursors for the synthesis of proteins and nucleic acids.
5. Components of the cycle have a direct or indirect controlling effect on key enzymes of other pathways.

5.10 SIGNIFICANCE OF TCA CYCLE

1. TCA cycle is the final common oxidative pathway for all the major ingredients of food stuffs. Carbohydrates enter via pyruvate and acetyl CoA. Fatty acids are broken down into acetyl CoA. Amino acids, after transamination enter in this cycle.
2. Without carbohydrates, fat cannot be metabolised, because for complete oxidation of fat, oxaloacetate is required, which is formed from pyruvate. Oxaloacetate acts as a true catalyst, as it enters in the cycle but is regenerated at the end.
3. Excess carbohydrate is converted to neutral fats which are stored in the body. The pathway for the formation of neutral fat is: Glucose → Pyruvate → Acetyl CoA → Fatty acid. The conversion of pyruvate to acetyl CoA is catalysed by pyruvate dehydrogenase, which is an irreversible reaction. Hence, fat cannot be converted to glucose.
4. Most amino acids enter in TCA cycle after deamination. For example, glutamic acid enters at α-ketoglutarate whereas aspartate enters at oxaloacetate. These amino acids which are converted as members of the cycle can also enter in gluconeogenesis via oxaloacetate. Such amino acids are called gluconeogenic amino acids.

5. The amino acids like leucine, are metabolised to acetyl CoA. Acetyl CoA either enters TCA cycle and gets completely oxidised or is channeled to ketone body formation. Such amino acids are called as ketogenic.
6. All other pathways are either catabolic or anabolic, but TCA cycle is purely amphibolic i.e. catabolic + anabolic. TCA cycle acts as a source for the precursors of biosynthetic pathways, e.g. heme is synthesized from succinyl CoA whereas aspartate forms oxaloacetate. This is also called as anapleorotic role of TCA cycle.

5.11 REGULATION OF THE TCA CYCLE

The regulation of the TCA cycle is mainly by the cellular need for ATP. When the energy charge of the cell is high, the cycle operates slowly and vice-versa. ATP is an allosteric inhibitor of citrate synthetase which catalyses the conversion of oxaloacetate to citrate. Another regulation of cycle is through through isocitrate dehydrogenase. NADH is an inhibitor of this enzyme which displaces NAD^+ from its binding sites. NADH along with succinyl can also inhibit α-ketoglutarate dehydrogenase.

As Pi is required for the substrate level phosphorylation, its availability in the medium has a regulatory effect on the cycle. Oxygen also has a regulatory effect since in hypoxia, electron transport chain will be inhibited.

5.12 INHIBITORS OF TCA CYCLE

Fluroacetate non-competitively inhibits aconitase, which catalysis the conversion of citrate to aconitate.

$$\text{Citrate} \xrightarrow[\text{non-competitive inhibitor}]{\text{Fluroacetate}} \text{Aconitate}$$

Arsenite non-competitively inhibits α-ketoglutarate dehydrogenase. This enzyme catalyses the conversion of α-ketoglutarate to succinyl CoA.

$$\alpha\text{-Ketoglutarate} \xrightarrow[\text{non-competitive inhibitor}]{\text{Arsenite}} \text{Succinyl CoA}$$

Malonate competitively inhibits succinate dehycrogenase, which converts succinate to fumarate.

$$\text{Succinate} \xrightarrow[\text{competitive inhibitor}]{\text{Malonate}} \text{Fumarate}$$

5.13 GLUCONEOGENESIS

Gluconeogenesis is a process whereby new glucose molecule is synthesizec from non-carbohydrate source like lactate and glycogenic amino acids. The other sources of gluconeogenesis are glycerol part of fat and propioryl CoA from odd chain fatty acids. All the gluconeogeneic precursors are converted tc either pyruvate or to TCA cycle intermediates or to triose phosphate.

The primary organ responsible for this pathway is liver followed by muscle. Renal cortex has the ability but only $^1/_{10}^{th}$ of liver. This pathway occurs partly in mitochondria and partly in cytoplasm. It employs several enzymes of glycolysis but the enzyme systems are different for irreversible reactions of glycolysis. There are four key enzymes of the pathway, namely pyruvate carboxylase, phosphoenol pyruvate carboxy kinase, fructose-1,6-bisphosphatase and glucose-6-phosphatase.

5.14 STEPS INVOLVED IN GLUCONEOGENESIS

Carboxylation of pyruvate to oxaloacetate is the first reaction of this pathway. This reaction is catalysed by mitochondrial enzyme pyruvate carboxylase, which is a biotin containing enzyme. The biotin is attached to lysine residue of enzyme and acts as a carrier for CO_2. Hydrolysis of ATP is required to drive the reaction. This reaction also requires Mg^{2+} and Mn^{2+} as cofactors. This enzyme is a regulatory enzyme which is activated by acetyl CoA. The gene of this enzyme is located on chromosome 11.

Another reaction occurs in cystol which is catalysed by a cytosolic enzyme, phosphoenol pyruvate carboxykinase. Thus, oxaloacetate which is formed in mitochondria is transported to cystol. This is achieved by malate shuttle. In malate shuttle, oxaloacetate is converted to malate. This occurs in mitochondria. The malate which is formed diffuses into cytosol where it gets converted to oxaloacetate. These two reactions are catalysed by malate dehydrogenase.

The oxaloacetate is then converted to phosphoenol pyruvate with the help of phosphoenol pyruvate carboxykinase. This conversion occurs by removing CO_2 molecule and adding high energy bond. The phosphoenolpyruvate which is formed, undergoes further reactions to form fructose-1,6-bisphosphate. The enzymes responsible for these reactions are glycolytic enzymes.

The fructose-1,6-bisphosphate is converted to fructose-6-phosphate with the help of fructose-1,6-bisphosphatase. Fructose-6-phosphate gets isomerised to glucose-6-phosphate, which is then hydrolysed to form glucose. The enzymes responsible for these reactions are hexosephosphate isomerase and glucose-6-phosphatase, respectively. Glucose-6-phosphatase is active in liver, kidney and intestinal mucosa. The free glucose which is formed is transported to cells via blood.

Important Physiologic controllers of gluconeogenic enzymes are:

Enzyme	Controller	Effect on reaction	Mechanism
Pyruvate carboxylase	Acetyl CoA	Positive	Allosteric
Phosphoenol pyruvate carboxykinase	AMP	Negative	Allosteric
Fructose-1,6-biphosphate	Fructose-1,6-biphosphate	Negative	Competitive inhibition

5.15 (A) SUBSTRATES FOR GLUCONEOGENESIS

The most important substrate for gluconeogenesis is lactate, which is formed in the muscle or RBC and is transported to the liver, where it is converted to pyruvate with the help

of cytoplasmic lactate dehydrogenase. In mitochondria, pyruvate formed from lactate is converted to glucose. The glucogenic amino acids are transaminated to TCA cycle intermediates which form oxaloacetate or pyruvate. Alanine is the major substrate for gluconeogenesis which is released from muscles. Alanine is transported to liver where it is transaminated to form pyruvate, which is then converted to glucose. Glucose can enter in glycolysis forming pyruvate, which can be transaminated to form alanine. This cycle i.e. **glucose-alanine** is of primary importance in starvation.

The odd chain fatty acids and carbon skeleton of some amino acids form propionyl ion, which is then converted to succinyl ion with the help of biotin dependent carboxylation.

The glycerol part of fat is phosphorylated to form glycerol-3-phosphate which in turn forms glucose via dihydroxy acetone phosphate. This conversion i.e. glycerol to glycerol-3-phosphate and glycerol-3-phosphate to dihydroxyacetone phosphate is carried out by glycerol kinase which requires ATP and glycerol-3-phosphate dehydrogenase which requires NAD^+ respectively.

5.5 (D) THE CORI CYCLE

Under anaerobic conditions, the lactate formed from the oxidation of glucose is one of the important precursors of gluconeogenesis.

However, the lactate is the metabolic deadend, meaning it cannot be metabolised further until it is converted back to pyruvate.

Owing to the absence of enzymes of gluconeogenesis in muscle, namely:

1. Glucose-6-phosphatase.
2. Fructose 1, 6 biphosphatase, lactate or pyruvate produced in muscle cannot be utilized for glucose synthesis.

Lactate diffuses out of the muscle membrane and is carried via blood to the liver. Inside the liver it is converted back to pyruvate. The pyruvate is converted to glucose by gluconeogenesis. The glucose can be transported to skeletal muscle.

Thus, overall picture of the cycle shows that there occurs a reformation of glucose in the liver from skeletal muscle lactate. The glucose again becomes available to muscle tissue via circulation. This process is known as the *Cori cycle* or *Lactic acid cycle*.

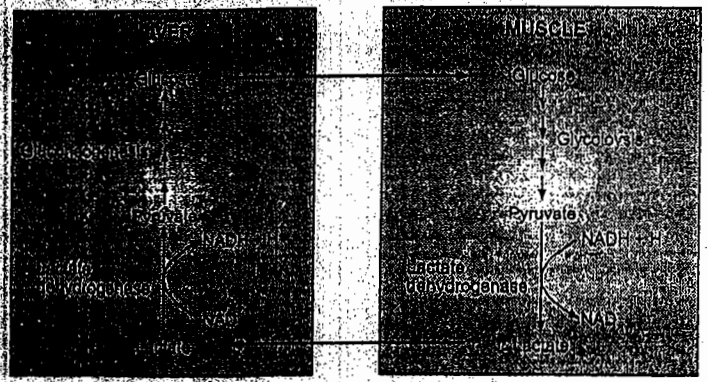


Fig. 5.3 (a): The Cori cycle (Lactic acid cycle)

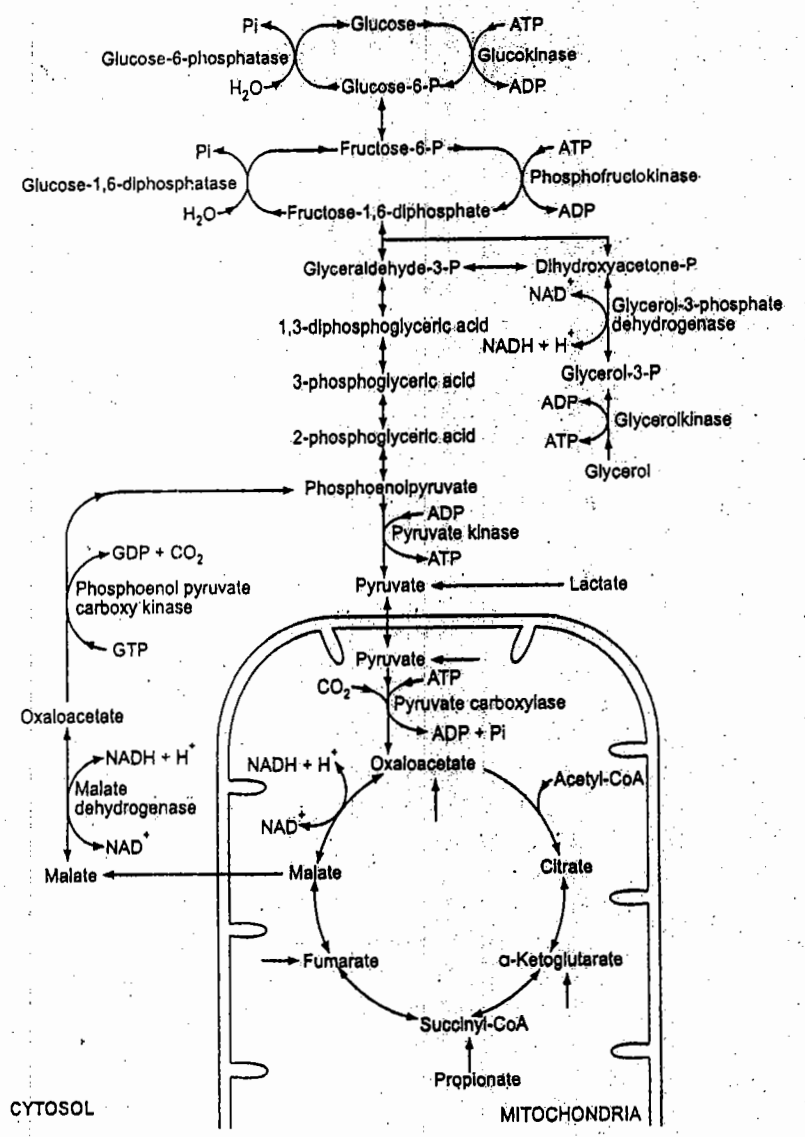


Fig. 5.3 (b): Reaction steps in Gluconeogenesis

5.16 REGULATION OF GLUCONEOGENESIS

Enzymes of gluconeogenesis are subjected to allosteric regulation and hormonal regulation. Pyruvate carboxylase and fructose 1, 6 biphosphatase regulate gluconeogenesis, allosterically. Whereas, all the key enzymes of gluconeogenesis are under hormonal control.

Hormonal regulation: Insulin decreases the synthesis of key enzymes of gluconeogenesis, thus inhibiting gluconeogenesis, whereas glucagon and glucocorticoid favour gluconeogenesis.

Allosteric regulation: Pyruvate carboxylase is an allosteric enzyme, acetyl CoA is its activator. Supply of glucose and fatty acid oxidation generates acetyl CoA, this in turn activates the gluconeogenesis. Fructose 1,6 biphosphatase is another allosteric enzyme. AMP is its allosteric inhibitor. So when there is an energy crisis, gluconeogenesis is inhibited due to binding of AMP to F-1-6-biphosphatase.

5.17 SIGNIFICANCE OF GLUCONEOGENESIS

The major metabolic significance of gluconeogenesis is the maintenance of blood glucose levels, especially in starvation. The glycogen storage gets depleted in 12-18 hours of prolonged starvation. This process enhances lipolysis and protein catabolism so that blood glucose level is maintained to normal.

5.18 PENTOSE PHOSPHATE PATHWAY OR PHOSPHOGLUCONATE OXIDATIVE PATHWAY OR HEXOSE MONOPHOSPHATE (HMP) SHUNT

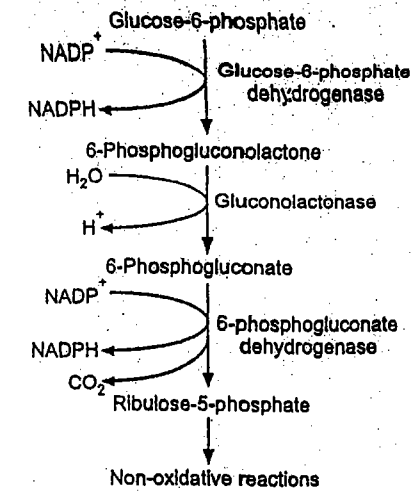
This pathway is an alternative pathway for glucose metabolism. This pathway operates in RBCs, adrenal cortex, testis, liver, etc. In mammals this anabolic pathway occurs in cytoplasm; in plants most steps take place in plastids. The major purpose of this pathway is to produce reduced NADPH and pentose phosphates for biosynthetic reactions and nucleotide synthesis. Although pathway operates in all cells, highest levels of PPP enzymes are found in neutrophils and macrophages. These white blood cells utilize NADPH to generate superoxide radicles from molecular oxygen.

This pathway has two phases viz: oxidative and non-oxidative. Oxidative phase involves generation of NADPH and pentose phosphate, whereas non-oxidative phase involves a series of reactions whereby the pentose phosphate formed is converted to intermediates of glycolysis pathways, which can be converted to glucose-6-phosphate and channeled to pentose phosphate pathway or glycolysis pathway.

5.19 REACTIONS INVOLVED IN THE PENTOSE PHOSPHATE PATHWAY

This pathway starts with the oxidation of glucose-6-phosphate with the help of $NADP^+$ dependent glucose-6-phosphate dehydrogenase. The 6-phosphogluconolactone which is formed from glucose-6-phosphate is hydrolysed to form 6-phosphogluconic acid with the help of hydrolase enzyme.

Oxidative Stage of Pentose Phosphate Pathway



Non-Oxidative Stage of Pentose Phosphate Pathway

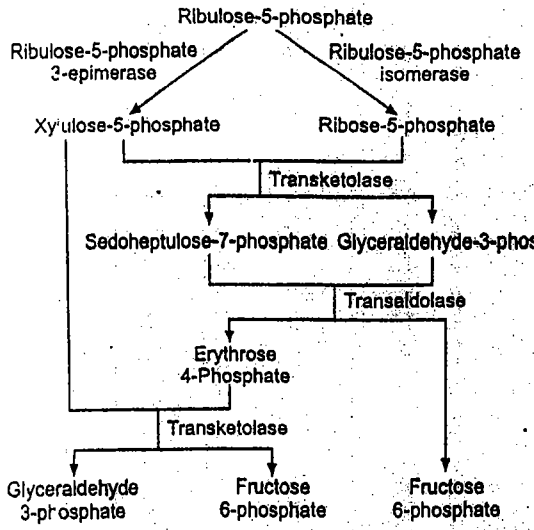


Fig. 5.4: Reactions of the pentose phosphate pathway

This conversion is a rate limiting step and is catalysed by glucose-6-phosphate dehydrogenase, which is a dimer enzyme and its activity is inhibited by NADPH.

6-Phosphogluconic acid is further oxidised and is coupled with decarboxylation. The first carbon of 6-phosphogluconic acid is lost as CO_2, forming ribulose-5-phosphate. The enzyme involved is 6-phospho gluconate dehydrogenase and it requires the presence of $NADP^+$. The ribulose-5-phosphate then isomerises to form ribose-5-phosphate with the help of isomerase enzyme. This ribose-5-phosphate is required for nucleic acid synthesis and also for nucleotide co-enzymes. When the pentose phosphate is synthesized in sufficient amount, then remaining pentose phosphate molecules are converted to intermediates of glycolysis by the 3 group transfer reactions. Glucose-6-phosphate is shunted through this pathway to glycolysis, hence it is also known as shunt pathway. Hexose monophosphate is formed, hence it is also called as HMP shunt. Ribulose-5-phosphate epimerizes to xylulose-5-phosphate with the help of epimerase. The ribulose-5-phosphate and xylulose-5-phosphate serve as reactant for next step.

5.20 TRANSKETOLASE REACTION

Transketolase is a thiamine pyrophosphate dependent enzyme. Transketolase helps to transfer glycol aldehyde moiety from xylulose-5-phosphate to ribulose-5-phosphate which results in the formation of 7 carbon ketose sugar i.e. sedoheptulose-7-phosphate leaving glyceraldehyde-3-phosphate. The activity of this enzyme decreases in thiamine deficiency.

5.21 TRANSALDOLASE REACTION

The transfer of 3 carbon i.e. dihydroxy acetone phosphate from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate forming fructose-6-phosphate is carried out by transaldolase enzyme.

In another transfer reaction, a 2 carbon unit from xylulose-5-phosphate is transferred to erythrose-4-phosphate forming fructose-6-phosphate and glyceraldehyde-3-phosphate. This transfer is catalysed by transketolase enzyme.

Depending upon cellular needs, further metabolism of these compounds occur:

1. When the NADPH demand is high this pathway proceeds to completion i.e. 1 molecule of glucose is completely oxidised to CO_2. The pentose phosphate is recycled, fructose-6-phosphate and glyceraldehyde-3-phosphate are converted to glucose-6-phosphate that re-enters HMP pathway. The reaction is as follows:
   Glucose-6-phosphate + 12 $NADP^+$ + $7H_2O \rightarrow 6\ CO_2$ + 12 NADPH + $12H^+$ + Pi
2. If the balanced amount of ribose-5-phosphate and NADPH is required then the oxidative phase of the pathway operates, forming 1 molecule of ribose-5-phosphate per molecule of glucose-6-phosphate that enters in the pathway.
   Glucose-6-phosphate + 2 $NADP^+$ + $H_2O \rightarrow$
   Ribose-6-phosphate + 2NADPH + $2H^+$ + CO_2
3. When ribose-5-phosphate demand is more than NADPH then fructose-6-phosphate and glyceraldehyde-3-phosphate are converted back to pentose by reversing the transaldolase and transketolase enzymes.

5.22 REGULATION OF PATHWAY

Rate limiting step of this pathway is catalysed by glucose-6-phosphate dehydrogenase enzyme. This enzyme is inhibited by NADPH. Thus, main regulation of this pathway is at the level of $NADP^+$. $NADP^+$ controls the oxidative phase whereas non-oxidative phase is controlled by the requirement of pentose. Insulin stimulates glucose-6-phosphate dehydrogenase.

5.23 GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY (G6PD)

This genetic disorder occurs almost exclusively in males. The condition may affect red blood cells, which function to carry oxygen from lungs to tissues of the body. The people suffering have the glucose-6-phosphate dehydrogenase enzyme molecule in defective form. This causes the red blood cells to break down permanently. This destruction of red blood cells is called hemolysis. G6PD results from mutation in the G6PD gene.

The common medical problem associated with glucose-6-phosphate dehydrogenase deficiency is hemolytic anemia, which occurs when red blood cells die faster than body can replace them.

This type of anemia causes paleness, yellowing of skin and whites of the eyes (jaundice), dark urine, fatigue, rapid heart rate, shortness of breath. In people with this deficiency – hemolytic anemia is triggered by viral or bacterial infection or by certain drugs (like antimalarial drugs or certain antibiotics).

Enzyme G-6-P dehydrogenase converts glu-6-phosphate into 6 phosphogluconate and produces NADPH as byproduct. The NADPH reducing equivalents are utilized to neutralize reactive oxygen species and free radicals produced during gaseous exchange in RBC. Unavailability of NADPH in G6PD causes breakdown of RBC membrane prematurely.

This type of deficiency is significant cause of mild to severe jaundice in new born. Many people with this disorder never experience signs or symptoms and totally unaware that they suffer from this condition.

Hemolytic anemia is also reported in people after eating fava beans or inhaling pollen from fava plants (The reaction is called as favism).

The G6PD gene is on X chromosome. The patients at higher risk of G6PD deficiency are those with African or Mediteranea ancestry.

Most diagnosis of G6PD is made via screening the enzyme activity rather than genotype.

The G6PD dihydrogenase enzyme is involved in the normal processing of carbohydrates. It also protects the red blood cells from the effects of toxic molecules produced like reactive oxygen species, which are by products of oxygen exchange and cellular processes. People having defective G6PD lack this protection of the enzyme and suffer severe RBC breakdown.

5.24 SIGNIFICANCE OF PATHWAY

The major role of this pathway is to supply cytoplasmic NADPH for reductive biosynthesis of fatty acids, steroids, etc., as well as formation of ribose-5-phosphate. NADPH is required for the production of superoxide anion radicals by the macrophages during phagocytosis. NADPH is also used for preserving the transparency of lens. The maximum concentration of NADPH is seen in eye lens.

The main significance of NADPH is to keep glutathione in the reduced state. This is required by RBC i.e. reduced glutathione is required for keeping the membrane integrity of RBC. The enzyme glutathione reductase requires NADPH, it converts oxidised glutathione to the reduced form. The reduced form reacts with the peroxides and the free radicals formed and detoxify them. The reduced form also prevents the formation of disulphide bonds between the membrane SH groups. Erythrose-4-phosphate is used in the synthesis of aromatic amino acids. Aromatic amino acids are precursor of many substances, one of which is lignin in wood.

The NADPH is also required by haemoglobin to keep the ferrous ion in reduced state and prevent the accumulation of methemoglobin. Dietary pentose sugar derived from digestion of nucleic acids may be metabolised through pentose phosphate pathway.

5.25 (A) GLYCOGEN METABOLISM

Glycogen is a polymer of glucose residues linked by α(1 → 4) glycosidic bonds, mainly and α(1 → 6) glycosidic bonds at branch points. Glycogen chains and branches are longer than shown. Glucose is stored as glycogen predominantly in the liver and muscle cells in the biochemical process called as glycogenesis.

Glycogen is broken down to glucose whenever body needs internal supply of glucose, in the biochemical process known as glycogenolysis. Highly branched structure of glycogen make it possible for immediate conversion of glycogen into glucose easily.

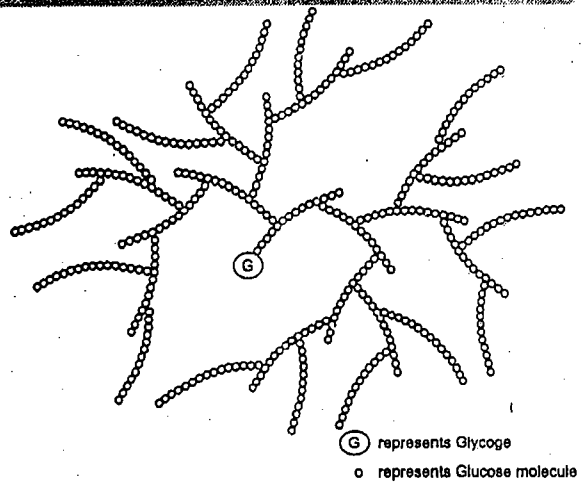


Fig. 5.5: Structural representation of glycogen, showing branching

5.25 (B) GLYCOGENOLYSIS

In glycogenolysis, glycogen undergoes phosphorylatic cleavage with the help of enzyme glycogen phosphorylase forming glucose-1-phosphate.

This enzyme removes glucose unit one at a time from non-reducing end of the glycogen molecule. The α (1 → 4) linkage is cleaved and inorganic phosphate is added. This reaction is reversible. This enzyme contains pyridoxal phosphate as a prosthetic group held in a Schiff's base linkage with the ε-amino group of a lysine residue of the enzyme. This enzyme attacks only at α (1 → 4) and is unable to hydrolyze α (1 → 6) linkages. The debranching enzyme i.e. α (1 → 6) glucosidase, hydrolyses the remaining glucosyl units held in α (1 → 6) linkage at the branch point.

In the liver, glucose-1-phosphate is converted to glucose-6-phosphate with the help of phosphoglucomutase. This conversion involves the formation of enzyme bound intermediate glucose-1, 6-bisphosphate. Glucose-6-phosphate is converted to free glucose, which is released into blood stream. This conversion is brought about by glucose-6-phosphatase.

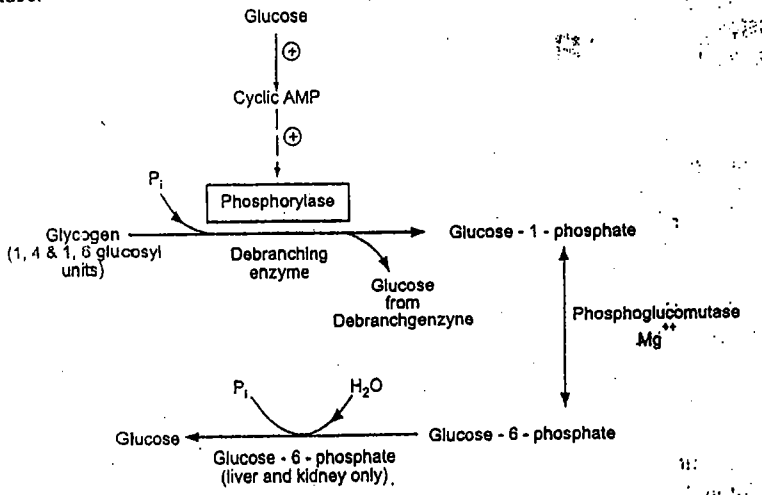


Fig. 5.6 (a): Glycogenolysis

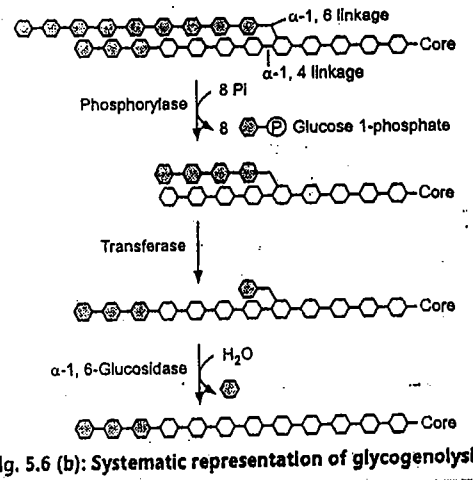


Fig. 5.6 (b): Systematic representation of glycogenolysis

5.26 GLYCOGENESIS

The synthesis of glycogen from glucose or glucose-1-phosphate is called as glycogenesis. The glycogenesis occurs by a different pathway from that of glycogen breakdown. Glucose is first activated to UDP - glucose with the help of UDP-glucose

pyrophosphorylase, which is then added to glycogen primer. This UDP-glucose is formed from glucose-1-phosphate and uridine triphosphate with the help of UDP-glucose pyrophosphorylase. The energy of hydrolysis drives the reaction forward. The glucose is added to glycogen at the non-reducing end forming an α (1 → 4) glycosidic linkage liberating UDP. The primer made up by protein-carbohydrate complex is essential as the acceptor of the glycosyl unit. Since glycogen synthase adds glucose molecules only in α (1 → 4) linkage, a branching enzyme is required to create α (1 → 6) linkages. Branching is done by amylo-[1, 4] → [1, 6] transglycosidase. At new branch point, glucose molecule can be added in α (1 → 4) linkage by glycogen synthetase.

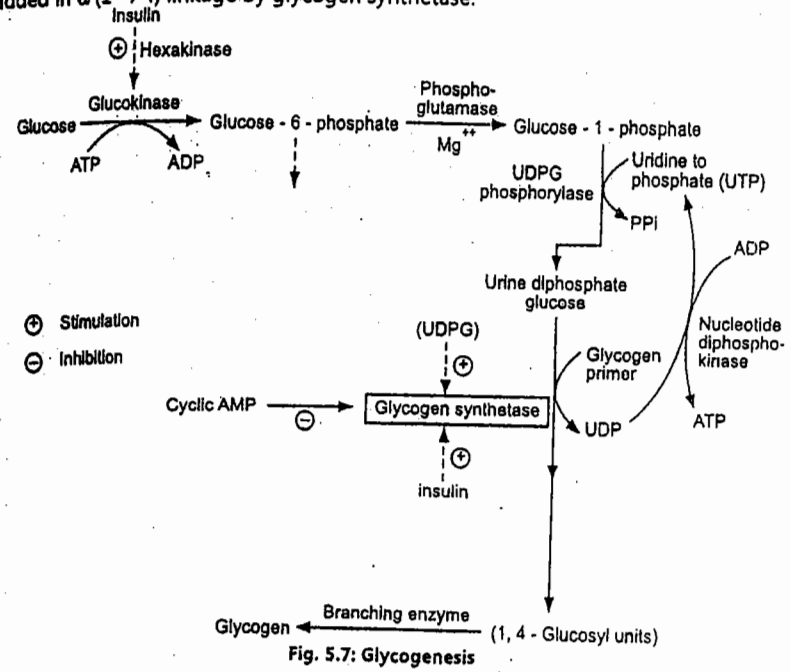


Fig. 5.7: Glycogenesis

QUESTIONS

1. Describe the pathway of aerobic glycolysis.
2. Why is pyruvate kinase considered as key glycolytic enzyme?
3. How is glycolysis pathway regulated?
4. What is the significance of TCA cycle?
5. Explain functions and energetics of TCA cycle.
6. What is the significance of Pentose Phosphate Pathway?
7. Write a note on glycogenesis and glycogenolysis.
8. Define and describe in detail, glyconeogenesis.
9. Describe the importance of transaldolase reaction of pentose phosphate pathway.
10. What are the cellular sites of (i) Glycolysis, (ii) TCA cycle, (iii) Pentose phosphate pathway, (iv) Glyconeogenesis, (v) Glycogenesis, (vi) Glycogenolysis.

Chapter ... 6

DIABETES MELLITUS AND INSULIN

♦ LEARNING OBJECTIVES ♦

- *Understand the Biochemical Perspectives of Type I and Type II Diabetes.*
- *Recognize Worth of Various Tests: In Diagnosis of Diabetes and Terms Hyperglycemia, Hypoglycemia, Glycosurea.*
- *Appreciate the Chemical Structure and Synthesis of Insulin Molecule.*
- *Recognize Structure and Significance of Insulin Receptor.*
- *Compare the Relation between High Blood Sugar Levels and Lipid Profile.*

6.1 INTRODUCTION

Diabetes mellitus is a metabolic disorder i.e. it is a disorder of the way our body uses digested food for growth and energy, especially inability to oxidize carbohydrates. Clinically, it is characterized by increased blood glucose levels. It occurs due to insufficient insulin levels or inefficient insulin actions. This means that insulin is not produced in sufficient quantity or it is inefficient in its action on target tissues. As a result, blood glucose levels are increased along with the presence of glucose in urine.

Diabetes mellitus affects 2-3% of world population and about 5-7% of population in developed countries. India has a dubious distinction of being a country with the largest number of diabetic people.

About 85% of total diabetic patients have Type II or non-insulin dependent diabetes mellitus (NIDDM). The major biochemical feature of diabetes is the inability of the target tissues to take up glucose inspite of its higher concentration in the surrounding. It is equally important to remember that diabetes is the third leading cause of death in the world. The first and second being heart disease and cancer, respectively.

What deserves a special attention in diabetes is its complications. It affects the nervous system, kidneys, eyes and peripheral organs. Untreated diabetes is one of the major causes behind limb amputation, renal failure and heart attacks.

Criteria, which clinically establish an individual as suffering from diabetes mellitus, include:

1. Having a fasting plasma glucose levels in excess of 140 mg/dl. The normal levels being 80-120 mg/dl.

2. Having plasma glucose levels in excess of 200 mg/dl at two time points during a glucose tolerance test (GTT), one of which must be within 2 hr of ingestion of glucose.

Uncontrolled diabetes is characterized by the following chemical pathology:

(i) Hyperglycemia - increased blood sugar levels.
(ii) Glycosuria - excretion of glucose in urine.
(iii) Polyuria – frequent urination.
(iv) Increased triglyceride and cholesterol in the blood.
(v) Increased urea and non-protein nitrogen in the blood.

Diabetic patients may experience all or some of the following symptoms:

- Frequent urination
- Excessive thirst
- Dry and itchy skin
- Slow healing of cuts or wounds
- Skin infections
- Blurry eyesight
- Always being very hungry
- Feeling tired and weak
- Weight loss
- Numbness or tingling in feet

6.1.1 Causes of Diabetes

Two factors play an important role in the development of diabetes.

Heredity: A person is most likely to develop diabetes if he/she has a parent, grandparent, brother, sister, or even a cousin who has diabetes. A person is at 5% risk of developing Type II diabetes if his/her mother, father, or sibling has diabetes. This risk can go as high as 50% if the person is also overweight.

Obesity: Eighty percent of Type II diabetic patients are overweight. Diabetic symptoms may disappear in some of these obese patients when they lose weight.

Various other factors are also known to cause or trigger diabetes, such as:

- **Age:** As people grow older, there may be fewer insulin-producing β-cells.
- **Faulty immune system:** Multiple factors are known to trigger the immune system to destroy β-cells.
- **Physical trauma:** An accident or injury may destroy the pancreas.
- **Drugs:** Drugs used to treat other conditions may unmask diabetes.
- **Stress:** Hormones released during periods of stress may block the effect of insulin.
- **Pregnancy:** Hormones produced during pregnancy may block the effect of insulin.
- **Viruses:** Certain viruses may destroy β-cells in susceptible people.

6.2 TYPES OF DIABETES MELLITUS

Diabetes mellitus is the heterogeneous clinical disorder with numerous causes. Two main classes of diabetes mellitus exist:

1. Idiopathic (primary) diabetes mellitus
2. Secondary diabetes mellitus

The idiopathic diabetes mellitus i.e. primary diabetes mellitus is further divided into two main types:

Type I: Insulin Dependent Diabetes Mellitus (IDDM). This type is generally seen in younger population, which cannot secrete insulin.

Type II: Noninsulin Dependent Diabetes Mellitus (NIDDM). This type is generally seen in the older population; especially obese people who retain the capacity to secrete insulin but insulin actions are inefficient.

Secondary diabetes mellitus is the result of many causes:

(i) Maturity onset type diabetes of the young (MODY). MODY is characterized by onset prior to age 25. These patients show impaired β-cell function and may also exhibit insulin resistance and late β-cell failure. Mutations in ~10-12 genes have been considered to be responsible for the development of MODY.

(ii) Pancreatic disease: Pancreatectomy, cystic fibrosis and pancreatitis can lead to destruction of pancreas.

(iii) Endocrine disease: Various endocrine diseases have been correlated with diabetic symptoms e.g. Cushing syndrome (excess cortisol secretion), acromegaly (excess growth hormone), pheochromocytoma (excess epinephrine) and glucagonomas (excess glucagon).

Gestational diabetes is usually found during pregnancy. Gestational diabetes usually disappears when the pregnancy is over. Women who had gestational diabetes during pregnancy have a 20-50% chance of developing type II diabetes within 5 to 10 years. It is more prevalent in African Americans, American Indians, Hispanic Americans, and among women with a family history of diabetes.

Diabetes insipidus is another disorder characterized by large volumes of urine excretion due to the deficiency of antidiuretic hormone. The major symptom of diabetes insipidus occurs due to the inability of the kidneys to reabsorb water.

Brittle diabetes is a form of diabetes and is very difficult to control. It is characterized by unexplained oscillations between hypoglycemia and acidosis.

IDDM (Type I diabetes)

It is also known as juvenile onset type diabetes mellitus. IDDM is a chronic endocrine disease characterized by severe loss of insulin secretion and hyperglycemia associated with

considerable morbidity, reduced life expectancy and significant health costs. This type is generally seen early in life, before the age of 30 and later on develops much more serious symptoms and has near certain prospect of vascular involvement in later stages. It is the result of autoimmune destruction of β-cells of pancreas. It is defined by the development of ketoacidosis in the absence of insulin therapy. There is a strong association between IDDM and other endocrine autoimmunities e.g. Addison's disease. Additionally, there is an increased prevalence of autoimmune diseases in the family members of IDDM patients.

β-cells destruction may occur due to a variety of reasons e.g. drugs, viruses and autoimmunity. In some cases due to genetic variations, β-cells are recognized as non-self and thus they are destroyed by body's own immune system. The patient of IDDM requires insulin therapy. About 15-20% of total diabetic patients fall into this category.

Symptoms of IDDM usually develop over a short period, although β-cell destruction may begin months, even years, earlier. Symptoms include increased thirst and urination, blurred vision, constant hunger, weight loss, and extreme tiredness. If not diagnosed and treated with insulin, a person can lapse into a life-threatening coma.

NIDDM (Type II diabetes)

NIDDM is also referred to as adult onset type of diabetes mellitus. It occurs in about 85-90% of diabetic population. It is comparatively milder and has more gradual onset than IDDM. It is more prevalent in adult people above 35-40 years of age and is most common in adults over the age of 55. NIDDM most commonly occurs in obese patients; approximately 80% of NIDDM patients are overweight. Type II diabetes is now increasingly being diagnosed in children and adolescents. However, comprehensive data on prevalence of type II diabetes in youth are not available.

NIDDM is characterized by persistent hyperglycemia but ketoacidosis is seen rarely. It can be a result of genetic defect and can cause both insulin resistance and insulin deficiency. In most of the cases, when type II diabetes is diagnosed, the pancreas is functional i.e. it produces enough insulin, but for unknown reasons, the body cannot use the insulin effectively. This condition is called as insulin resistance. After several years, eventually insulin production decreases. The net result is the same as that of type I diabetes i.e. high blood glucose levels. In addition, body cannot make efficient use of glucose.

There are two major forms of Type II diabetes:

1. Late onset associated with obesity.
2. Late onset not associated with obesity.

Obesity acts as a diabetogenic factor in genetically predisposed individuals by increasing the resistance to the insulin actions. Decrease in the number of insulin receptors on insulin responsive targets is also seen in this type of diabetes.

The symptoms of NIDDM develop gradually and are not as noticeable compared to IDDM. Symptoms include feeling ill or tiredness, fatigue or nausea, frequent urination (especially at night), unusual thirst, blurred vision, weight loss, frequent infections, and slow healing of sores/wounds. Some type II diabetic patients show no symptoms.

Comparison of Type I and Type II diabetes is shown in Table 6.1.

Table 6.1: Comparison of Type I and Type II diabetes mellitus

Characteristics	Type I	Type II
Age of onset	Childhood and young adult	Middle and old age
Family history	Not common	Common (95%)
% Of all diabetics	Less than 10%	More than 90%
Appearance of symptoms	Rapid	Slow
Ketosis	Yes	No
Obesity	Not common	Common (95%)
Cause	Autoimmune	Variable
Insulin levels	Decreased	Variable
Sensitivity to insulin	Sensitive	Resistant
Pathology	Reduction or absence of β-cells	β-cell mass normal or slight reduction

Pre-diabetes or other forms of impaired glucose metabolism

Pre-diabetes is a state between "normal" and "diabetes". People with pre-diabetes are at increased risk of developing diabetes, heart attacks and strokes. However, weight loss and increased physical activity can prevent or delay the development of diabetes. There are two forms of pre-diabetes:

Impaired fasting glucose

In this case, impaired fasting glucose (IFG) is observed i.e. fasting plasma glucose is between 100 -125 mg/dl. Thus it is slightly higher than normal but less than the diabetic level.

Impaired glucose tolerance

Impaired glucose tolerance (IGT) means that blood glucose during the oral glucose tolerance test is slightly higher than normal but less than seen in diabetic patients. IGT is diagnosed when the glucose level is 140 to 199 mg/dl 2 hours after the intake of 75 grams of glucose.

Pre-diabetes is more prevalent between ages 40 to 74.

6.3 TESTS FOR DIABETES MELLITUS

One of the most commonly used tests for the diagnosis of diabetes mellitus is oral glucose tolerance test (OGTT).

Oral Glucose Tolerance Test (OGTT)

The body's ability to utilize glucose may be ascertained by measuring its glucose tolerance. Glucose tolerance represents ability to regulate blood glucose concentration after administration of test dose of glucose. There are certain prerequisite conditions before undergoing OGTT and they are as follows:

- It is mandatory for a patient to consume carbohydrate rich diet for at least 3 days prior to test.
- The patient is advised to discontinue all the drugs related to carbohydrate metabolism for at least 4-5 days.
- The patient should avoid any kind of strenuous exercise one day before the test.
- The patient should fast overnight, at least 10 hours, but should be allowed to drink water.
- During the test procedure patient should be in a comfortable sitting position and smoking or exercising should not be allowed.

OGTT Procedure

The patient is given an oral dose of 1.0 gm of glucose per kilogram body weight (or 75 gm of anhydrous glucose dissolved in 300 ml water to be drunk within 5 min) and blood glucose levels are followed for next few hours.

The test is preferably conducted in the morning. A fasting blood and urine samples are collected. The blood and urine samples are then collected every 30 min upto 120 min. All the blood samples are screened for the amount of glucose and urine samples are tested qualitatively for the presence of glucose.

Interpretation of OGTT

Normal people show a modest rise in the blood sugar level ~ 120 to 140 mg/dl in about 1 hour. This is insufficient to exceed the normal renal threshold i.e. the concentration at which glucose begins to appear in urine; 160 to 180 mg/dl. This rise in blood glucose is followed by a rapid decline to normal levels within 2-3 hours period (Fig. 6.1). The rapid decline in blood glucose levels in normal individuals is due to an increased rate of glucose utilization by the tissues. This increased utilization of glucose is evoked by increased secretion of insulin and decreased secretion of glucagon from the pancreas in response to increased blood glucose levels.

Diabetics on the other hand show a much higher increase in their blood glucose concentration, which is initially higher than normal. They also show a more prolonged period of hyperglycemia and a slower decline in blood glucose levels. They may also show a glucosuria after the test dose of glucose (Fig. 6.1).

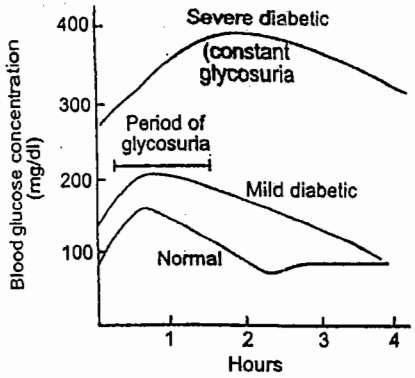


Fig. 6.1: Glucose tolerance test for normal, mildly diabetic and severely diabetic individuals

WHO Criteria for the Diagnosis of Diabetes are as follows (WHO report 1980):

(i) If fasting glucose is abnormal (> 7.8 mmol/l i.e. 140 mg/dl), patient is diabetic.

(ii) If fasting glucose is between 6.5–7.8 mmol/ml, it is considered as borderline diabetic and oral OGTT is recommended.

(iii) 2 hours after OGTT if blood glucose levels are > 11.1 mmol/l i.e. 200 mg/dl, patient is diabetic.

The fasting plasma glucose level is less than 100 mg/dl in normal individuals. Normal person also shows increase in plasma glucose concentration in response to oral glucose load, which reaches a peak value of less than 150 mg/dl in less than one hour and returns to normal within 2 hours. Urine samples of non-diabetic person show absence of glucose.

The OGTT may give normal response or abnormal response.

Normal Response OGTT

In this case, the rise in blood sugar level is normal and it never crosses renal threshold. The elevated blood sugar levels return to normal within 1 to 1.5 hours (Fig. 6.2).

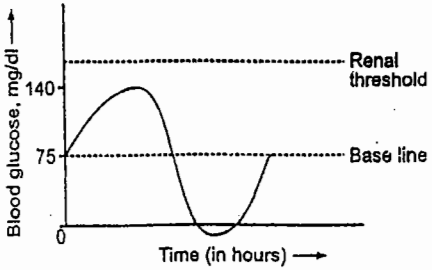


Fig. 6.2: Normal response GTT

Abnormal Response OGTT

I. OGTT may give abnormal responses for a variety of reasons.

(i) Diminished glucose tolerance

In this case, fasting blood glucose is higher than normal and the rise in blood sugar levels is more than normal. In addition, returning of increased levels to normal is delayed. Glycosuria is present. (Fig. 6.3)

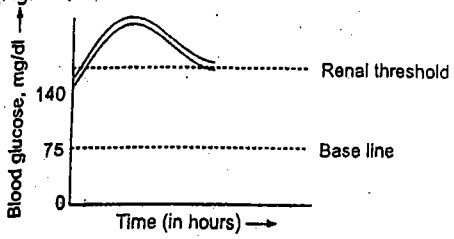


Fig. 6.3 OGTT: Diminished glucose tolerance

(ii) Increased glucose tolerance

In this case, the rise in blood sugar level is not very high and fasting blood glucose level is normal. Glycosuria is absent. This is generally found in dysfunction of the pituitary, thyroid or adrenal glands and in malabsorption. (Fig. 6.4)

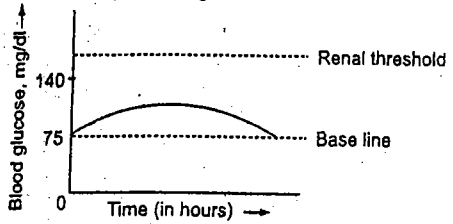


Fig. 6.4 OGTT: Increased glucose tolerance

(iii) Decreased renal threshold

In this case, blood sugar levels are not high but glycosuria is present. This is because of decreased renal threshold. This is generally found in late stage of pregnancy and Wilson's desease. (Fig. 6.5)

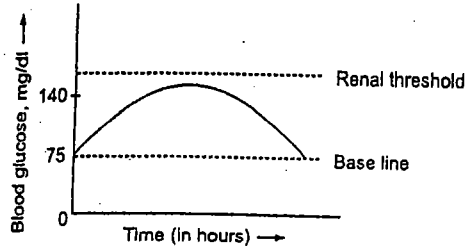


Fig. 6.5: OGTT: Decreased renal threshold

Modification in the OGTT procedure:

Under some conditions few modifications are done in the OGTT procedure. For example,

- An intravenous instead of oral glucose administration is carried out in individuals with suspected malabsorption.
- A corticosteroid stressed OGTT is advised to detect latent diabetes in some individuals.
- In case of children, oral glucose dose is 1.5 – 1.75 gm/kg body weight.
- Many laboratories perform "mini OGTT". Only fasting for 2 hours blood and urine samples are taken.

Other biochemical tests for diabetes mellitus

It is mandatory for diabetic patient, at any stage or type, to undergo periodical check-ups for some of the related biochemical parameters. This helps in understanding and quickly interpreting the efficacy of the treatment. Traditionally, blood glucose and urine glucose detection have been carried out. Now more advanced, reliable and sensitive indices in the form of biochemical tests have been developed.

1. **Glycated haemoglobin:**

   Glucose derived products of normal adult haemoglobin (Hb) is known as glycated haemoglobin. Glycation refers to post-translational and non-enzymatic addition of sugar residues to amino acids. Hb_{1C} is the most abundant form of glycated haemoglobin.

   Diagnostic importance of Hb_{1C}: The rate of synthesis of Hb_{1C} in RBCs is directly related to the exposure of RBCs to glucose over the previous two months. This test reflects the mean glycaemia over the previous two months, two months is the half-life of Hb. Thus, screening of Hb_{1C} in the blood samples gives an idea of mean glucose levels over previous two months. A close correlation has been observed between half-life of RBCs (6-8 weeks) and Hb_{1C} concentration. Normal range of Hb_{1C} concentration is about 3-5% of total haemoglobin. In diabetic patients, the concentration of Hb_{1C} can be upto 10-15%. It is accepted as a good measure of glycaemic control in the clinical environment instead of plasma blood glucose measurements.

2. **Microalbuminuria:**

   Diabetic patients show a small increase in albumin excretion when compared to normal individuals. Normal values of albumin excretion range from 2.5 to 3.0 mg/dl. In diabetic conditions this excretion may go as high as 250-300 mg/dl. This increase in albumin is actually an indicator of defective renal function due to diabetes, especially early reversible renal damage.

3. **Fructosamine:**

   Fructosamine can be used instead of Hb_{1C}. Glycated albumin is the major contributor to serum fructosamine measurements and its half-life is less than that of Hb. Hence, serum fructosamine measurements indicate glycaemic control over the last 3 weeks.

4. **Plasma C-peptide:**
   Pre-pro-insulin is cleaved to produce pro-insulin, which is cleaved further to produce C-peptide and insulin. Equimolar amounts of C-peptide and insulin are produced and hence it can be established as to how much insulin an insulin-treated diabetic is producing (plasma insulin measurements will record both produced and injected insulin).
   NIDDM patients will have some plasma C-peptide while IDDM patients with total pancreatic β-cell failure will have no plasma C-peptide.
   This marker is also helpful in differentiating hypoglycaemia due to over injection of exogenous insulin (high plasma insulin, low plasma C-peptide) and that due to an insulinoma (high plasma levels of both insulin and C-peptide).
5. **Glycosuria - urine dipstix:**
   The renal threshold at which glucose is excreted into urine is when plasma glucose is 10 mmol/l. Hence, this is not a sensitive test.
6. **Serum lipid profile:**
   Determination of serum lipids namely total cholesterol, triglycerides. LDL. HDL, VLDL and chylomicrons is included in the lipid profile test.
   The determination of lipid profile focuses on the overall lipid metabolism and general metabolic control in diabetic patients. Hence, diabetic patients are advised to undergo a lipid profile test, in addition to the above mentioned tests.

6.4 METABOLIC CHANGES IN DIABETES

Diabetes mellitus is associated with many metabolic alterations. Some of these alterations are manifested clinically in the form of hyperglycemia, ketoacidosis and hypertriglyceridemia.

Hyperglycemia: The very first sign of uncontrolled diabetes is elevation of blood glucose levels. Hyperglycemia in diabetes is attributed to reduced glucose uptake by peripheral tissues as well as its increased production by gluconeogenesis and glycogenolysis.

Ketoacidosis: In diabetes, presence of excess ketone bodies in the blood is observed due to increased mobilization of fatty acids from the adipose tissue.

Hypertriglyceridemia: In diabetes, increased levels of triglycerides are observed due to increased conversion of fatty acids to triglycerols and due to higher secretion of VLDL and chylomicrons. The low activity of the enzyme lipoprotein lipase is also responsible for higher plasma levels of chylomicrons, VLDL and triacylglycerol.

Hypercholesterolemia: Diabetes is also associated with hypercholesterolemia.

Besides the above mentioned metabolic changes, diabetes is also associated with the following complications:

- Diabetics are more prone to myocardial infarction.
- Gangrene of feet and toes.

- Defective circulation in legs.
- Hypertension, particularly systolic hypertension is more prevalent.
- Cataract is more prevalent in old age patients suffering from diabetes.
- Poor control of diabetes is correlated with lower resistance to infections e.g. urinary tract infections, pulmonary tuberculosis and carbuncle.

6.5 BIOCHEMICAL BASIS OF DIABETIC COMPLICATIONS

Hyperglycemia, a major clinical sign of diabetes, is responsible for many complications. For example, nephropathy, atherosclerosis, retinopathy and neuropathy. The basic biochemical defects in diabetes are still not completely understood.

A secondary set of symptoms arising in chronic long lasting diabetes includes, degeneration of blood vessel walls, particularly of small capillaries and their basement membranes.

Some of the above changes and complications are believed to occur due to microvascular changes caused by glycation of proteins. These proteins are called as Advanced Glycated End Products (AGE). It is important to note that the glycation of proteins is non-enzymatic. The glycation of proteins leads to many adverse effects e.g. inactivation of enzymes, cross-linking of glycated proteins, decreased proteolysis and increased immunogenecity.

Another mechanism responsible for chronic complications in diabetes is the intracellular accumulation of sorbitol. Sorbitol is a byproduct of glucose metabolism. Elevated levels of sorbitol are found in various tissues involved in diabetic complications e.g. lens epithelium, Schwaan cells of perpheral nerves, kidney, islets of Langerhans and retinal blood vessels.

6.6 MANAGEMENT OF DIABETES

Following four steps are involved in the management of diabetes.

Dietary Management: Dietary modification is fundamental to the successful treatment of diabetic patients. A diabetic patient should consume low calories, high protein and fibre rich diet. Carbohydrates should be in the form of complex carbohydrates e.g. starch and complex sugars. Patient is advised to reduce the fat intake and the fat intake should be just enough to take care of essential fatty acid requirements of the body. High-complex carbohydrates, high-fibre diet (HCF diet) is generally recommended. HCF diet is high in cereal grains, legumes, root vegetables and restricted intake of simple sugars and fat.

Oral Hypoglycemic Drugs: There are two classes of these drugs – sulfonylureas and biguanides.

Sulfonylureas are known as insulin secretogogues. They mimic the action of ATP on potassium channels and induce the release of endogenous insulin and thus help in reducing blood glucose levels.

Examples:

First generation sulfonylureas: Tolbutamide, acetoxamide, chlorpropamide, tolzamide. These are not routinely prescribed in the developed countries.

Second generation sulfonylureas: Glipizide, glimepiride and glyburide.

Sulfonylureas have no significant effects on circulating triglycerides, lipoproteins or cholesterol.

Biguanides e.g. metformin is currently the most widely prescribed insulin-sensitizing drug. Metformin does not increase insulin release but it increases insulin sensitivity by increasing the insulin receptor tyrosine kinase activity.

In addition to these drugs, other drugs are also available e.g. α-glucosidase inhibitors – acarbose and miglitol. These drugs interfere with the action of the α-glucosidases present in the small intestinal brush border. Thus, reducing the digestion and the consequent absorption of glucose into the systemic circulation. Meglitinides – repaglinide and nateglinide, these drugs represent non-sulfonylurea insulin secretogogues. These drugs are comparatively fast acting and have short duration of action. Thiazolidinediones e.g. rosiglitazone, and pioglitazone, are useful in treating the hyperglycemia associated with insulin-resistance in both type II diabetes and non-diabetic conditions. These drugs are agonists of the nuclear receptor peroxisome proliferator-activated receptor-g (PPARg). PPARg is a member of a superfamily of transcription factors. The ultimate effect of these drugs is the potentiation of the actions of insulin in liver, adipose tissue and skeletal muscle, increased peripheral glucose disposal and a decrease in glucose output by the liver.

Diabetes vaccine: Type I diabetes is an autoimmune disease which when triggered, causes body's own killer T cells to kill β-cells of pancreas which produce insulin. Diabetes vaccine attempts to stop T cells from attacking body's own cells. Studies have been able to treat diabetes in mice, but a working vaccine in humans has yet to receive pharmaceutical approval.

6.7 HYPERGLYCEMIA

Normal range of blood sugar concentration is 80-120 mg/dl. Increase in blood sugar is called as hyperglycemia. It is a pathological condition as excess of glucose in blood causes osmotic disturbances and affects the life of RBCs. Hyperglycemia is primarily due to reduced glucose uptake by the tissues and its increased production because of gluconeogenesis and glycogenolysis. When blood glucose levels go beyond renal threshold (> 10 mmol/l), it is excreted in urine (glycosuria).

Renal Threshold: A concentration of a substrate beyond which the substance starts appearing in the urine.

High threshold substances: These are entirely or almost entirely reabsorbed e.g. chloride and glucose.

Low threshold substances: These are reabsorbed in limited quantities e.g. urea and phosphate.

No threshold substances: These are excreted entirely and do not show any reabsorption e.g. creatinine sulfate.

6.8 HYPOGLYCEMIA

A person is said to be hypoglycemic when blood glucose levels fall below 45 mg/dl.

The clinical symptoms of hypoglycemia include headache, anxiety, confusion, sweating, slurred speech, seizures and ultimately coma or death if untreated. All the symptoms mentioned above are because of deprivation of glucose to central nervous system, mainly brain. A mammalian body has a meticulous homeostatic biochemical machinery to maintain blood glucose levels. Hence, generally normal individuals do not suffer from hypoglycemia.

Generally, in clinics physicians encounter hypoglycemia because of one of the following four reasons:

1. **Hypoglycemia due to fasting:** This is not a very common occurrence. It can be observed in patients with pancreatic β-cell tumor or hepatocellular damage.
2. **Post-prandial hypoglycemia:** It is observed in patients with elevated insulin secretion after a meal. It is a transient phase of hypoglycemia and is associated with mild symptoms.
3. **Hypoglycemia due to alcohol intake:** It occurs due to reduction in the rate of gluconeogenesis because of alcohol consumption. It occurs as follows: if a person is engaged in prolonged exercise or is a victim of prolonged starvation, the alcohol consumption will lead to hypoglycemia. This also leads to accumulation of NADH, as enzyme alcohol dehydrogenase diverts the pyruvate and oxaloacetate (both are substrates for gluconeogenesis) to form lactate and malate respectively. The overall effect is reduction of gluconeogenesis because of alcohol consumption.
4. **Hypoglycemia due to overdose of insulin:** An excess of insulin may cause hypoglycemia resulting in convulsions and ultimately death unless glucose is administered. It might be observed in patients who are on intense insulin treatment regimen.

6.9 GLYCOSURIA

Presence of glucose in urine is termed as glycosuria. Traces of sugars, particularly glucose, may appear in normal urine, but are below the detectable limits of commonly used qualitative tests.

In routine urine analysis, the presence of reducing sugars is indicative of diabetes mellitus. It is found when blood sugar level exceeds renal threshold (~ 170 mg/dl or ~ 10 mmol/l) – recall that normal fasting level of blood glucose is usually between 80 to 120 mg/dl.

Besides diabetes, glycosouria may also result from disorders of other endocrine glands e.g. hypophysis, adrenals, thyroid and ovaries, excess carbohydrate intake, excessive glycogenolysis or reduction in renal threshold.

6.10 INSULIN

Insulin is an anabolic hormone. Insulin is synthesized and stored in the granules in β-cell of islet of Langerhans in pancreas.

Insulin was the first hormone identified. It is a polypeptide hormone. It plays a major role in the metabolism of carbohydrates, fats and proteins. Inefficient or insufficient insulin is actually responsible for occurrence of diabetes.

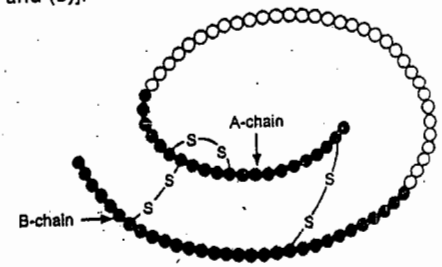
In normal individuals, daily secretion of insulin amounts to 30-40 units, which is about 25% of total pancreatic content. The principle factor that evokes insulin secretion is high blood glucose concentration.

6.10.1 Structure of Insulin

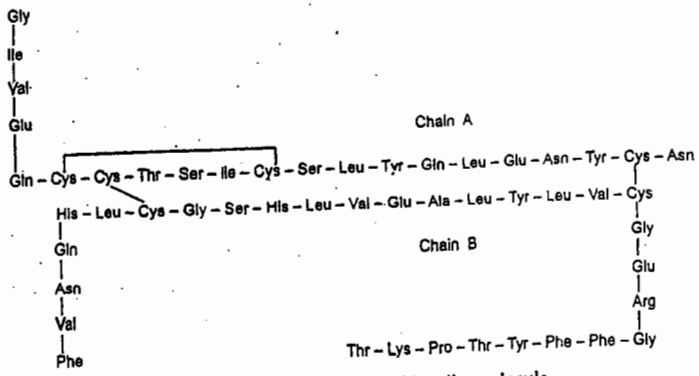
In 1953, Sanger (Nobel Prize winner of 1956) identified the amino acid sequence of insulin. Insulin molecule consists of 51 amino acids and its molecular weight is ~ 6kD. A molecule of insulin is a polypeptide with two peptide chains, namely A and B.

- Chain A contains 21 amino acids.
- Chain B contains 30 amino acids.

Chain A is linked to chain B by disulfide bridges at positions A_7-B_7 and A_{20}-B_{19}. The insulin molecule also shows the presence of intrachain disulfide linkage in chain A between A_6 and A_{11}. [Fig. 6.6 (A) and (B)].



(A) Structure of insulin. Open circles denote C-peptide



(B) Amino acid sequence of insulin molecule

Fig. 6.6: Human Insulin

Structure of insulin shows very little species dependent variations. These variations are most commonly seen at positions 8, 9 and 10 in chain A.

Now-a-days, human insulin is prepared either by enzyme modification or by more advanced techniques such as recombinant DNA technology. Porcine and bovine insulins were standard therapy for diabetes before human insulin became available.

6.10.2 Insulin Biosynthesis

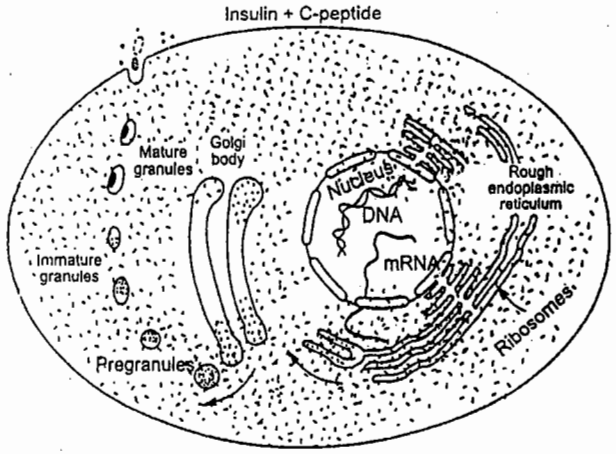


Fig. 6.7: Schematic representation of the biosynthesis of insulin in a β-cell of islet of langerhans

The major insights into the biosynthesis of insulin were because of the discovery of proinsulin by D. F. Steiner in 1967. Insulin production occurs in the pancreatic β-cells of islet of Langerhans. There are approximately 1 million islets of Langerhans and each is composed of around 3000 cells, in the normal pancreas. These constitute ~3% of the total pancreatic mass. These cells also synthesize chromogranin A or an islet amyloid peptide but insulin is the most dominant secretion. Transcription and translation of insulin gene result in the formation of a peptide with 110 amino acids. This peptide is called as preproinsulin. Insulin is synthesized from preproinsulin in two steps. (Fig. 6.8)

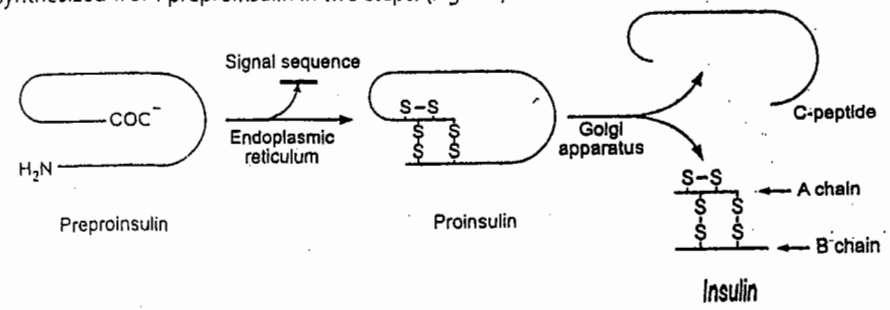


Fig. 6.8: Biosynthesis of insulin

Preproinsulin has a lipophilic sequence of 24 amino acids, which is used only to pass through the membrane of endoplasmic reticulum. The first step involves the removal of its signal peptide (the 24 amino acid sequence) from preproinsulin to form proinsulin in the cisternae of the endoplasmic reticulum and its consequent packaging into the secretory vesicles of Golgi apparatus. Proinsulin molecule has 86 amino acids. The proinsulin molecule is locked in its conformation by the formation of two disulfide bonds. The second step involves a protease activity, which removes the center of the molecule in the form of C-peptide (35 amino acids), forming insulin molecule (51 amino acids). The insulin molecule formed contains B peptide chain bonded to the A peptide chain. Once secreted, insulin has a half-life of 4-5 mins. An enzyme, insulinase (found in liver and kidney) degrades insulin.

6.10.3 Regulation of Insulin Secretion

Insulin secretion is regulated by a variety of factors.

1. **Plasma glucose:** Insulin secretion from β-cells is principally regulated by plasma glucose levels. The increased glucose uptake by the pancreatic β-cells leads to an elevation of ATP/ADP ratio. Increased levels of ATP lead to inhibition of ATP-sensitive potassium channels. The net result is the depolarization cell membrane leading to influx of calcium and exocytosis of insulin i.e. secretion of insulin.
2. **Amino acids:** Leucine and arginine are potent stimulators of insulin secretion. A transient rise in the amino acid concentrations observed after meals also causes stimulation of insulin release.
3. **Hormones:** Gastrointestinal hormones e.g. pancreaozymin, secretin and gastrin elevate insulin secretion by increasing preproinsulin mRNA levels and enzymes involved in the processing of preproinsulin. Chronic increase in other hormones e.g. growth hormone, placental lactogen, estrogen and progestins are also known to increase insulin secretion.

   Hormones like epinephrine are inhibitors of insulin release. Epinephrine suppresses insulin secretion even in the presence of glucose.
4. **Pharmacological agents:** Many drugs stimulate insulin secretion. Drugs such as sulfonylureas and glucosidase inhibitors stimulate insulin secretion (For details, please refer to section 6.7).

6.10.4 Effects of Insulin on the General Metabolism

Insulin is an anabolic hormone with antidiabetic characteristics. Insulin shows profound influence on carbohydrate, lipid and protein metabolism.

(A) Effects of insulin on carbohydrate metabolism

Insulin influences glucose metabolism in three major ways:

(i) By increasing the rate of removal of glucose from the blood.

(ii) By promoting its utilization and storage.

(iii) By inhibiting its endogenous production.

The major effect of the above actions is hypoglycemia i.e. lowering of blood glucose concentration by the following biochemical steps.

Effects of insulin on glucose uptake by tissues: Uptake of glucose by muscles (smooth, skeletal and cardiac), adipose tissue, mammary gland and leukocytes is dependent on insulin.

Many organs e.g. brain, kidney, nerves, erythrocytes, intestinal mucosa and retina do not require the help of insulin for the uptake of glucose.

Liver is a unique organ it does not require insulin for the glucose uptake but insulin stimulates glucose utilization in liver.

Insulin increases glucose uptake by increasing the number of plasma membrane glucose transporters (GLUTs). GLUTs are in a continuous state of turnover. Increase in the plasma membrane content of transporters in response to insulin is because of an increase in the rate of recruitment of new transporters into the plasma membrane. These are derived from a special pool of preformed transporters localized in the cytoplasm. GLUT1 is present in most tissues, GLUT2 is found in liver and pancreatic β-cells, GLUT3 is in the brain and GLUT4 is found in heart, adipose tissue and skeletal muscle.

Effects of insulin on glucose utilization: Insulin increases the rate of glycolysis in muscle and liver. This is achieved by increasing the amounts of enzymes e.g. glucokinase, phosphofructokinase and pyruvate kinase and also by activating these enzymes.

Insulin stimulates glycogen production by increasing the activity of glycogen synthetase.

Insulin stimulates the pentose phosphate pathway by increasing the activity of glucose-6-phosphodehydrogenase.

Effects of insulin on glucose production: Insulin inhibits the enzymes of gluconeogenesis e.g. pyruvate carboxylase, phosphoenol pyruvate carboxy kinase and glucose-6-phosphatase. Insulin also inhibits gluconeogenesis by inactivating the enzyme glycogen phosphorylase.

(B) Effects of insulin on protein metabolism

Insulin plays a vital role in enhancing protein synthesis from amino acids and also decreases the degradation of proteins. It is also responsible for stimulating entry of amino acids into cells. Insulin plays a major role in the stimulation of cell growth and replication.

(C) Effects of insulin on lipid metabolism

Overall effects of insulin on lipid metabolism can be summarized as follows:

(i) Decrease in the release of fatty acids from adipose tissue.

(ii) Decrease in the production of ketone bodies.

Effects of insulin on lipogenesis: Insulin is a potent stimulator of acetyl CoA carboxylase, the key enzyme in fatty acid synthesis.

Effects of insulin on lipolysis: Insulin suppresses the activity of the hormone sensitive lipase. This way insulin inhibits the release of free fatty acids from the stored fat in adipose tissues. It also plays an important role in decreasing the mobilization of fatty acids from the liver. Insulin also increases the formation of VLDL in liver. Thus, insulin plays a central role in maintaining the normal levels of free acids.

Effects of insulin on ketogenesis: Insulin suppresses the activity of HMG CoA synthetase, an enzyme involved in the synthesis of ketone bodies. This way insulin decreases the ketogenesis.

Ketone bodies characteristically accumulate in the blood of diabetic patients. Prolonged exercise by a person with sedentary lifestyle also results in the elevated levels of ketone bodies.

Athletes rarely slow accumulation of ketone bodies as they have elevated levels of enzymes that utilize ketone bodies in the peripheral muscle tissues.

Effects of insulin on lipoprotein metabolism: Insulin secretion is very essential for the proper utilization of VLDL and LDL in the tissues. This is the reason why diabetic patients show higher concentrations of VLDL, LDL and cholesterol in the blood. These high concentrations are responsible for the pathogenesis of heart diseases (atherosclerosis).

6.11.5 Mechanism of Action on Insulin

High glucose concentrations evoke the release of insulin from pancreas, which is then transported via blood to various tissues. Target tissues of insulin such as muscles, adipose tissues etc. possess specific receptors for insulin. These receptors are present on the plasma membrane. Insulin binds to these specific receptors. The interaction of a molecule of insulin and receptor results in a series of events, which ultimately leads to various biochemical effects. There are three different mechanisms of insulin action.

I. **Signal transduction or transmembrane signal induction by insulin:** It involves a cascade of biochemical events that ultimately results in biolog cal effects of insulin. Insulin enables the transport of glucose and cations to cross the cell membrane, thus changing in membrane potential. Also there is activation of various second messengers e.g. G-proteins, diacylglycerol and inositol triphosphate.

II. **Insulin mediated glucose transport:** This involves the binding of insulin molecule to the receptor, which signals translocation of vesicles containing glucose transporters from the intracellular pool. These vesicles fuse with the plasma membrane and thus recruit glucose transporters in the membranes. These glucose transporters are responsible for insulin mediated uptake of glucose by the cell.

III. **Induction of enzyme synthesis by insulin:** Insulin is responsible for promoting the synthesis of enzymes such as glucokinase, phosphofructokinase etc. Insulin stimulates both transcription and translation thus resulting in an increase in the protein synthesis.

6.10.6 Insulin Receptors

Insulin receptor is originally synthesized as a single polypeptide. This polypeptide is cleaved into α and β subunits. The α and β subunits are assembled afterwards. The half-life of insulin receptor is 6-12 hours. Each mammalian cell possesses on an average 20,000 insulin receptors.

At a molecular level, insulin receptor is a tetramer i.e. it is made of four subunits - $\alpha_2\beta_2$. These subunits are in glycosylated form. They are held together by disulfide linkage. The molecular weight of an α-subunit is 13,500 and that of β-subunit is 95,000. α-subunit is extracellular and contains insulin binding site whereas, β-subunits has a transmembrane-protein (Fig. 6.9).

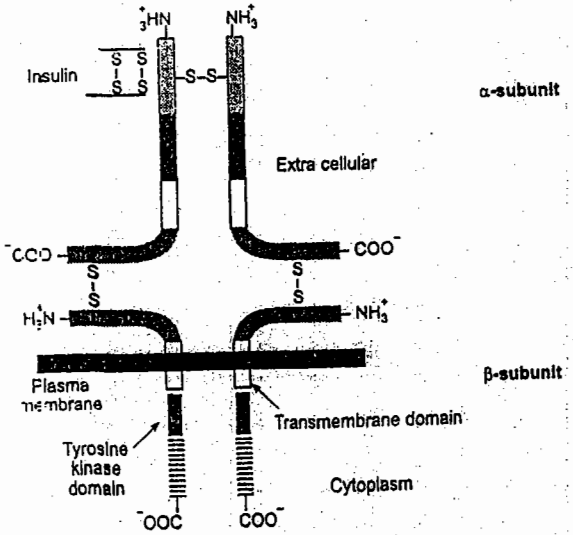


Fig. 6.9: The insulin receptor

α-subunit shows negative co-operativity i.e. binding of one insulin molecule decreases the binding of second molecule. Binding of insulin molecule leads to an activation of tyrosine kinase activity on β-subunits. Then activated protein kinase further transmits the singles by phosphorylating the cytoplasmic proteins.

QUESTIONS

1. Define diabetes. Explain its various types.
2. Write a note on juvenile onset diabetes.
3. What is the rationale behind screening of Hb_{A2c} – in diabetic patients?
4. What is meant by OGTT? What is the prerequisite condition for the same?
5. Explain in detail – metabolic changes occurring in diabetes?
6. Give a brief account of the complication in diabetes. Write the biochemical bases for the same.
7. Explain the different measures necessary to take the different types of diabetes.

Chapter ... 7

BIOLOGICAL OXIDATION

♦ LEARNING OBJECTIVES ♦

- *Recognize the Application of the Concept of Oxidation reduction in Biochemical Science.*
- *Understand Components, Cellular Site and Working of Electron Transport Chain.*
- *Know Site, Process, Mechanism, Inhibition and Energetics of Oxidative Phosphorylation in Mitochondria and detailed Account of ATP Synthesis and ATP Cycle.*
- *Understand Theories of ATP Synthesis and about High Energy Compounds.*
- *Know the Concept of Uncoupling in Brown Adipose Tissues.*

7.1 INTRODUCTION

Mitochondria are the sites of oxidative phosphorylation in eukaryotes. It was discovered in 1948 by Eugene Kennedy and Albert Lehniger. Mitochondrian has appropriately been termed the 'Powerhouse' of the cell, as it is within this organelle that most of the capture of energy derived from respiratory oxidation takes place.

Oxidative phosphorylation (ATP synthesis) driven by electron transfer to oxygen is the most important energy transduction reaction in the biosphere. Oxidative phosphorylation is the end of energy yielding metabolism in aerobic organisms. All the enzymatic steps in oxidative degradation of carbohydrates, fats and amino acids in aerobic cells converge at this final stage of cellular respiration, in which electrons flow from catabolic intermediates to O_2, yielding energy for generation of ATP from ADP and Pi.

7.2 OXIDATION - REDUCTION

In any biochemical reaction,

A compound is said to be oxidised when:

(i) It loses hydrogen or an atom having relatively less attraction for electrons.
(ii) It loses one or more electrons.
(iii) It combines with oxygen or other atoms having a strong attraction for electrons.

A compound is said to be reduced when:

(i) It gains hydrogen or an atom having relatively weak attraction for electrons.
(ii) It gains one or more electrons.
(iii) It loses oxygen or other atoms having a strong attraction for electrons.

Oxidation of the various compounds in the body brings about a free energy change (release). Generally, compounds of high energy levels are converted into compounds of low energy levels after oxidation. A portion of energy released in the form of free energy is usually stored in the body in the form of certain energy rich compounds like adenosine triphosphate (ATP), while the remaining portion of energy is liberated as heat.

Most of the oxidative activity of the cell takes place in the mitochondria. Little oxidative activity is also present in microsomal and cytosol fraction.

The electrons lost in oxidation are accepted by an acceptor which is said to be reduced. Thus, oxidation-reduction is a synchronous process going hand in hand.

7.3 STRUCTURE OF MITOCHONDRIA

Mitochondria like gram-negative bacteria have two membranes: The outer membrane, the inner membrane and the intermembrane space, the cristae and the matrix. (Fig. 7.1)

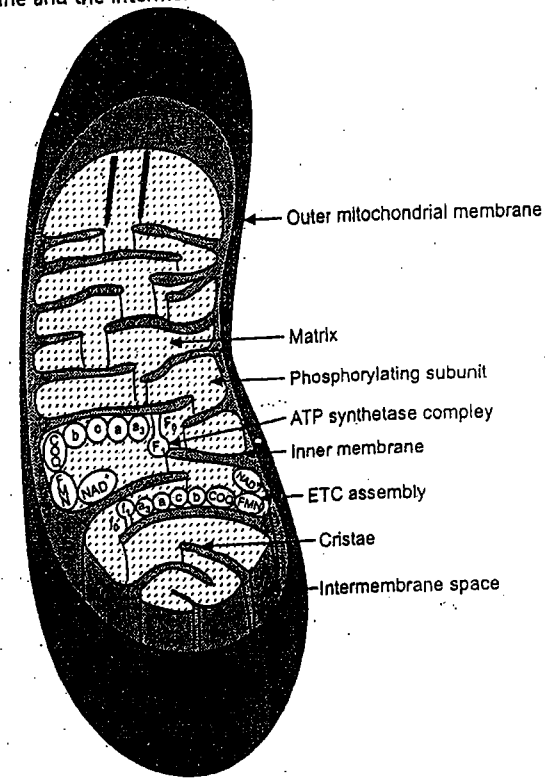


Fig. 7.1: Structure of mitochondria (showing carriers of electron transport)

The outer mitochondrial membrane is readily permeable to small molecules and ions through its transmembrane channels. Whereas, inner mitochondrial membrane is impermeable to most small molecules and ions including protons (H^+). The only species crossing the inner membrane bears the components of the respiratory chain and the surface area of the inner membrane is greatly increased due to folding in the form of *cristae*. The inner surface of the inner mitochondrial membrane possesses typical particles. They look like a stick and a bead on its top. These particles are phosphorylating subunits. The ATP synthesis takes place in these particles. The space enclosed by the inner membrane contains mitochondrial matrix. Matrix contains enzymes of citric acid cycle, fatty acid β-oxidation pathway and pathways of amino acid oxidation, pyruvate dehydrogenase complex. In short, all the pathways of fuel oxidation.

7.4 RESPIRATORY CHAIN OF MITOCHONDRIA (ELECTRON TRANSPORT CHAIN)

The cells of a eukaryote contain, intracellular organelles called mitochondria, which produce ATP. Energy sources such as glucose are initially metabolized in cytoplasm. The products are imported into the mitochondria. Mitochondria continue the process of catabolism using metabolic pathways including kreb cycle, fatty acid oxidation and amino acid oxidation.

The end result of these pathways is the production of two kinds of energy rich electron donors, NADH and $FADH_2$. Electrons from these donors are passed through an ETC to oxygen, which is reduced to water. This is a multi-step redox process that occurs on mitochondrial inner membrane.

The mitochondrial respiratory chain consists of a series of electron carriers as components. All the components of the respiratory chain are located on the inner mitochondrial membrane. The inner mitochondrial membrane can be disrupted into five distinct respiratory enzyme complexes – namely complex I, II, III, IV and V. Complex I to IV are carriers of electrons, while complex V is responsible for ATP synthesis.

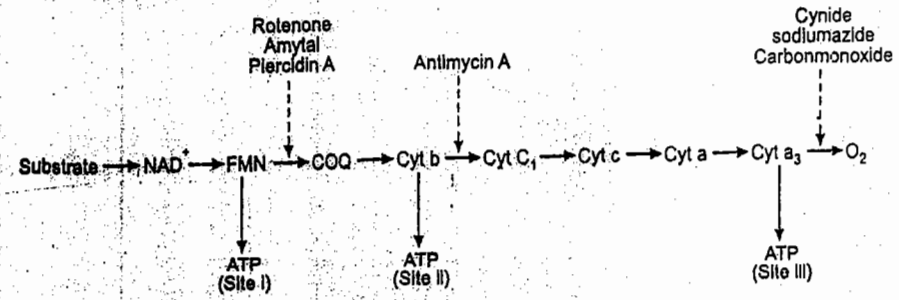
Each component of the chain can accept electron from the preceding one and transfer them to the next in a specific sequence. Some of the reactions in the sequence transfer one electron and others involve transfer of pairs of electrons. Whatever may be the mode of transfer, the term **reducing equivalent** is used to designate a single electron equivalent that is transferred in an oxidation-reduction reaction.

The enzyme complexes (I – IV) and the mobile carriers are collectively involved in the transport of electrons which finally combine with oxygen to produce water.

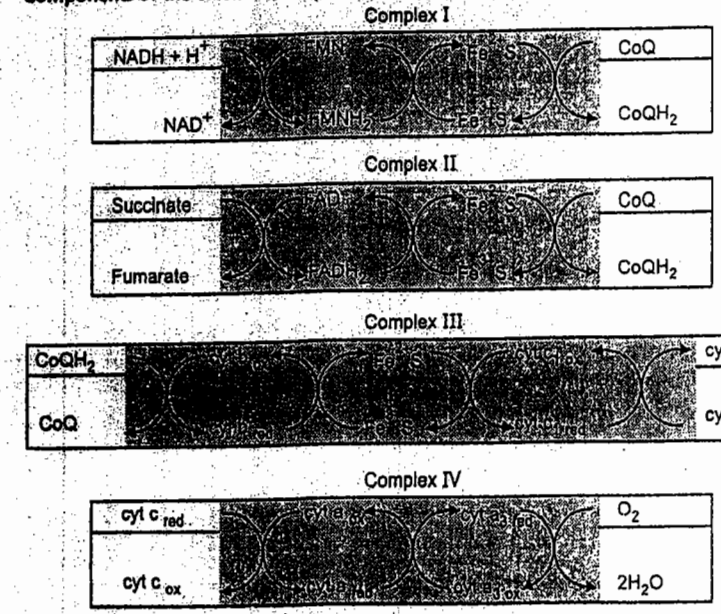
Respiratory chain: The sequence of enzymes and carriers responsible for the transport of reducing equivalents from substrates to molecular oxygen represents the respiratory chain.

Redox Potential: It represents the measure of tendency of the redox pair to lose or gain electrons. In oxidation-reduction reactions, free energy exchange (ΔG^0) is proportional to the redox potential (E). More positive redox potential represents the greater tendency to accept electrons, whereas more negative redox potential represents the greater tendency to lose electrons.

The major compounds of respiratory chain, arranged in the order of increasing redox potential are shown in the following flow sheet:

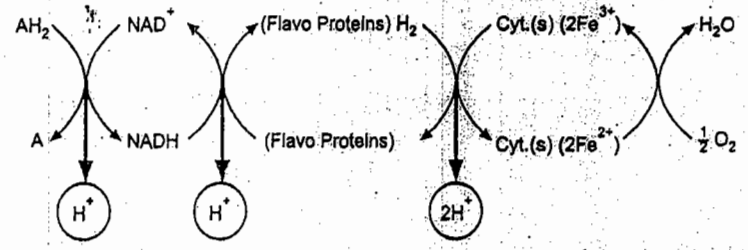


Components of the electron transport chain with inhibitors and 3 sites of ATP synthesis



FMN – Flavin mononucleotide, $Fe^{2+}S$ - reduced iron-sulfur center, $Fe^{3+}S$ - oxidized iron-sulfur center, cyt - cytochrome, CoQ - Coenzyme Q.

7.5 ELECTRON TRANSPORT CHAIN IN DETAIL



(AH_2 – Reduced substrate
Cyt.(s) – Cytochromes)

Fig. 7.2: Actual flowsheet of electron transport chain

The energy rich compounds like carbohydrates, lipids and proteins in the course of metabolism undergo a series of biochemical changes and finally get oxidised to CO_2 and H_2O. The reducing equivalents produced in the above mentioned reactions are transferred to co-enzyme NAD^+ and FAD to produce NADH + H^+ and $FADH_2$. These two reduced co-enzymes travel the path of electron transport chain and reduce O_2 to H_2O at the end of the respiratory chain. There is concurrent loss of free energy occurring during the passage of electrons through the electron transport chain. The standard reduction potential for respiratory chain and related electron carriers is shown in Table 7.1.

The part of free energy so released during electron passage on inner mitochondrial membrane is utilised by the ATP synthesizing enzyme (ATP synthetase) and ADP and Pi present in the granules (phosphorylating subunits) to generate ATP. The remaining free energy is released in the form of heat.

Overview of the Pathway

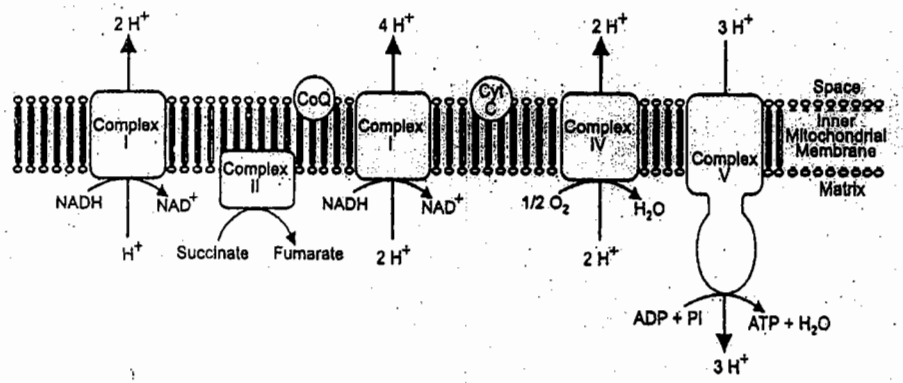


Table 7.1

Standard reduction potential for respiratory chain and related electron carriers Redox Potential (half reaction)	E_0(V)
1. $2H^+ + 2e^- \rightarrow H_2$	– 0.414
2. $NAD^+ + H^+ + 2e^- \rightarrow NADH$	– 0.320
3. $NADP^+ + H^+ + 2e^- \rightarrow NADPH$	– 0.324
4. NADH dehydrogenase (FMN) + $2H^+ + 2e^- \rightarrow$ NADH dehydrogenase ($FMNH_2$)	– 0.30
5. Ubiquinone + $2H^+ + 2e^- \rightarrow$ Ubiquinol	0.045
6. Cytochrome b (Fe^{3+}) + $e^- \rightarrow$ Cytochrome b (Fe^{2+})	0.077
7. Cytochrome C_1 (Fe^{3+}) + $e^- \rightarrow$ Cytochrome c_1 (Fe^{2+})	0.22
8. Cytochrome (Fe^{3+}) + $e^- \rightarrow$ Cytochrome c (Fe^{2+})	0.254
9. Cytochrome a (Fe^{3+}) + $e^- \rightarrow$ Cytochrome a (Fe^{2+})	0.29
10. Cytochrome a_3 (Fe^{3+}) + $e^- \rightarrow$ Cytochrome a_3 (Fe^{2+})	0.55
11. $1/2\ O_2 + 2H^+ + 2e^- \rightarrow H_2O$	0.816

7.6 SUBSTRATE LEVEL PHOSPHORYLATION

It is the metabolic reaction that results in the formation of ATP or GTP by direct transfer of a phosphoryl (PO_3) group to ADP or GDP from another phosphorylated compounds

Substrate level phosphorylation offers quicker, less efficient source of ATP formation. However, most of the ATPs are generated by oxidative phosphorylation in the body.

Human erythrocytes have no mitochondria, and produce ATP through the substrate level phosphorylation. Oxygen depleted muscles and brain receive energy through substrate level phosphorylation.

Glycolysis taking place in cytoplasm of the cells, has two steps wherein ATP production occurs by substrate level phosphorylation.

Substrate Level Phosphrylation in Glycolysis:

Step 1: 1-3-bi Ⓟ glycerate $\xrightarrow[\text{phosphoglycerate kinase}]{}$ 3 - Ⓟ - glycerate + ATP

Step 2: Phosphoenol pyruvate $\xrightarrow[\text{pyruvate kinase}]{}$ Pyruvate + ATP

where Ⓟ – stands for phosphate PO_3^-

Substrate Level Phosphorylation in Kreb Cycle:

Kreb cycle which takes place in the mitochondrial matrix, produces GTP by substrate level phosphorylation.

Succinyl CoA + GDP + Pi $\xrightarrow[\text{succinyl synthetase}]{}$ Succinate + GTP + CoA

The GTP formed reacts with ADP to form ATP, as per the need of cell of ATP.

GTP + ADP ⟶ ATP + GDP

Substrate Level Phosphorylation in Oxygen Depleted Muscles:

Creatine phosphate in the muscles breaks down to donate phosphate group to ADP which produces ATP when body requires energy currency of ATP.

Creatine phosphate + ADP $\xrightarrow[\text{creatine phosphokinase}]{}$ Creatine + ATP

It is also known as ATP-creatine phosphate system.

Substrate level phosphorylation is also observed in fermentation process.

7.7 OXIDATIVE PHOSPHORYLATION

The electron transfer through respiratory chain releases more than enough free energy to form ATP. The process of synthesizing ATP from ADP and Pi coupled with electron transport chain is known as *oxidative phosphorylation*. The complex V of the inner mitochondrial membrane is the site of oxidative phosphorylation. The transport of electrons through electron transport chain is linked with the release of free energy.

7.7.1 Sites of Oxidative Phosphorylation in Electron Transport Chain

1. Between NAD and FAD
2. Between Cyt b and Cyt c_1
3. Between Cyt a_3 and oxygen.

Each one of the above represents a coupling site for ATP production.

7.7.2 P : O Ratio

It is defined as 'the number of inorganic phosphates esterified to form ATP per atom of oxygen utilized in oxidation'. OR, in other words, it represents 'the number of ATP molecules synthesized per pair of electrons carried through respiratory chain'.

P:O ratio is 3 for substrates directly passing their reducing equivalents to NAD. Whereas P:O ratio is 2 for substrates directly passing their reducing equivalents to FAD (e.g. succinate oxidation). Some substrates directly pass their reducing equivalents to cytochromes, bypassing the previous carrier i.e. two sites of ATP formation and thus form only one ATP for a pair of electron removed, their P:O ratio is only 1.

7.7.3 Energetics of Oxidative Phosphorylation

The transport of electrons across the entire respiratory chain may be written as transport of electrons from NAD^+ / NADH (E_0 – 0.32) to finally the redox pair $1/2\ O_2$ / H_2O (E_0 – 0.82). It may be represented in the form of an equation as follows:

$$\frac{1}{2} O_2 + NADH + H^+ \rightarrow H_2O + NAD^+$$

The redox potential difference between these two redox pairs is (1.14 V).

It is equivalent to an energy 52 cal/mol.

The energy of hydrolysis of ATP is 7.3 cal. Since three ATPs are synthesized in the electron transport chain when NADH is oxidised, it is equal to 21.9 cal.

Hence, the efficiency of energy conservation is calculated as:

$$\frac{21.9 \times 100}{52} = 42\%$$

Hence, about 42% of energy is trapped if NADH is oxidised in the form of 3ATP and remaining is lost as heat.

7.8 ELECTRON CARRIERS OF RESPIRATORY CHAIN

7.8.1 Nicotinamide Nucleotides

Source of nicotinamide nucleotide is vitamin niacin. Two biochemically active forms of this vitamin, namely NADH and NADPH are water soluble electron carriers. The NADH is more actively involved in electron transfer chain. The reversible dehydrogenase enzyme reduces NAD^+ to $NADH^+$. Thus, NADH acts as a diffusible carrier which transports electrons derived from catabolic reactions to their point of entry into respiratory chain. NADPH is a diffusible carrier which supplies electrons to anabolic reactions like fatty acid synthesis, cholesterol synthesis, with the help of its specific enzyme, oxido-reductase, commonly known as dehydrogenase. The general reactions are:

$$AH_2 + NAD^+ \rightleftharpoons A + NADH + H^+$$

$$BH_2 + NADP^+ \rightleftharpoons B + NADPH + H^+$$

where, AH_2 – Reduced substrate, BH_2 – Reduced substrate.

Some of the examples of the reduced substrates include – pyruvate, glyceraldehyde-3-phosphate, isocitrate, malate, α-ketoglutarate, lactate for NAD linked dehydrogenase.

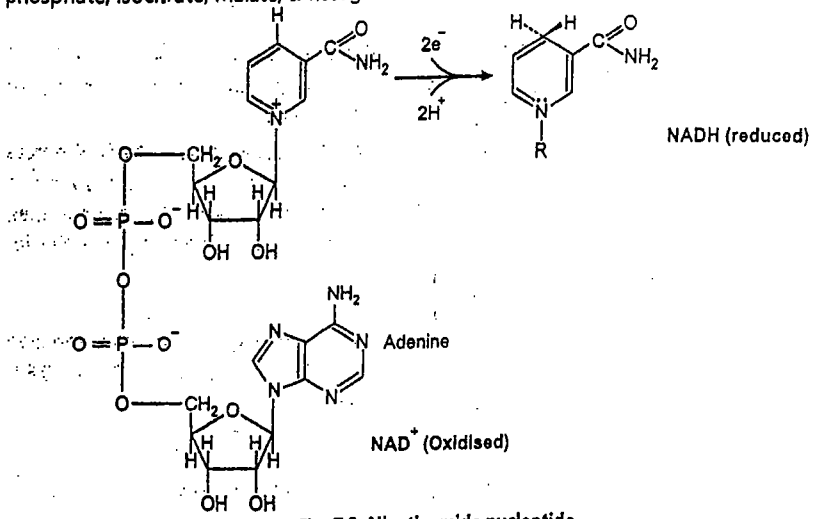


Fig. 7.3: Nicotinamide nucleotide

7.8.2 Flavoproteins

Flavoproteins are enzymes that catalyse oxidation-reduction reactions using either flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as a cofactor. These cofactors are derived from the vitamin riboflavin. The isoalloxazine ring of flavin nucleotide undergoes reversible reduction accepting one or two electrons in the form of hydrogen atom.

Flavoproteins contain very tightly bound, sometimes covalently bound nucleotide, either FMN or FAD e.g. in the enzyme succinic dehydrogenase residing on inner mitochondrial membrane.

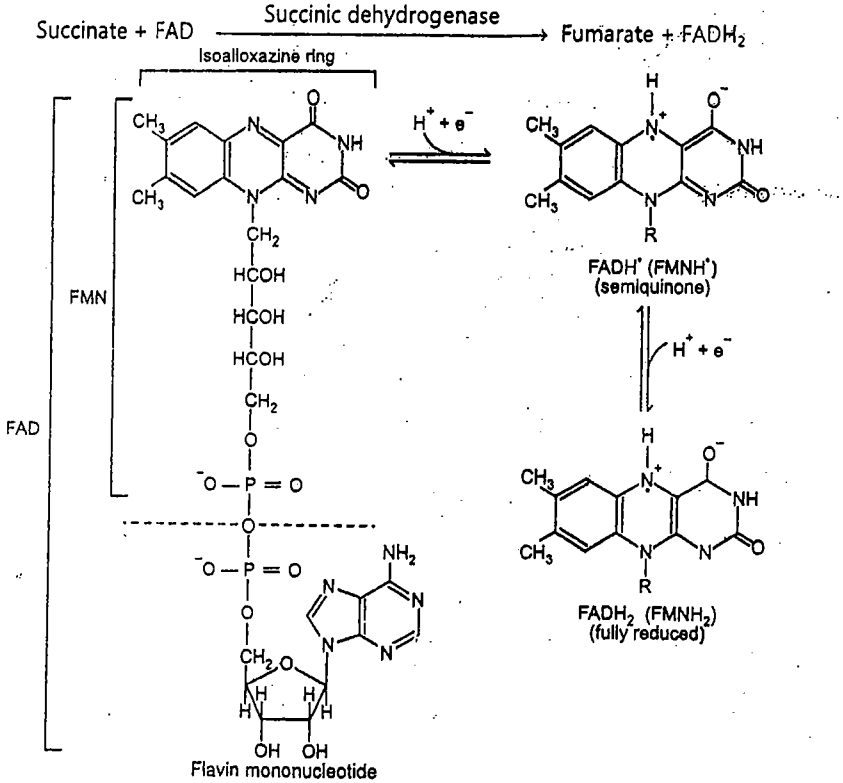


Fig. 7.4: Flavoproteins

Iron Sulfur Proteins

Iron containing electron transfer proteins contain iron in association with inorganic sulfur atoms of Cys residues of protein. About 5 to 6 iron sulfur proteins associated with respiratory chain are known today. These Fe–S centers range from simple structure with single Fe atom to structures with 2 or 4 Fe atoms in it. They exist in oxidised (Fe^{3+}) or reduced (Fe^{2+}) state.

The mechanism of action of iron sulfur proteins is not very clearly understood. Iron sulfur protein associated with cytochrome b and cytochrome c_1 participate in the transport of electrons. Whereas one Fe-S protein is also known to transfer electrons from FMN to co-enzyme Q.

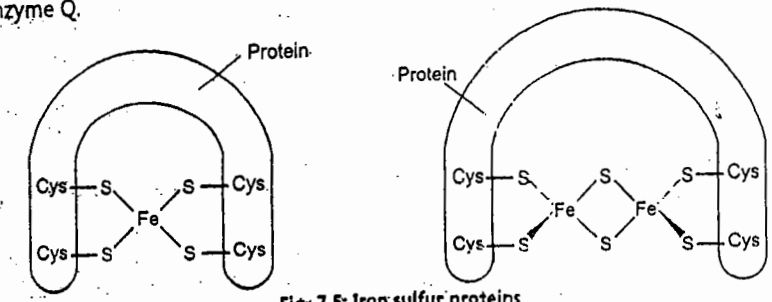


Fig. 7.5: Iron sulfur proteins

Co-enzyme Q:

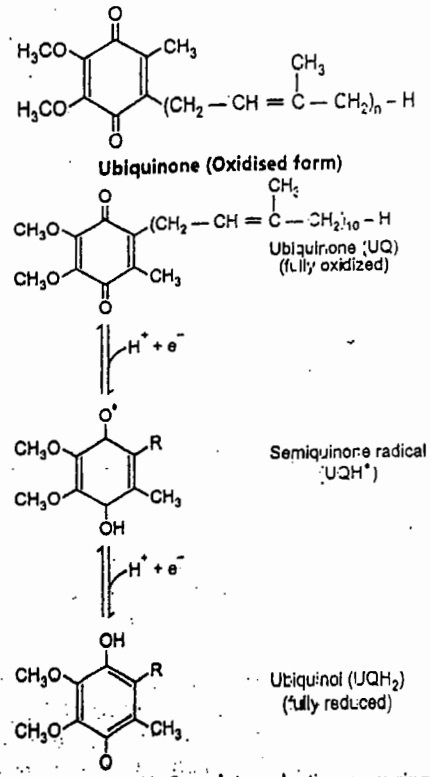


Fig. 7.6: Ubiquinone (or Co-enzyme Q), Complete reduction occurring in two steps, through semiquinone radical intermediate formation

Co-enzyme Q is a fat soluble benzoquinone with a long isoprenoid side chains. It is also known as unbiquinone, since it is ubiquitous (present everywhere) in the living cell. Its structure is closely related to plastoquinones found in chloroplast. This carrier of respiratory chain links the flavoproteins to cytochrome b.

Unbiquinone can accept one electron to become the semiquinone radical UQH or two electrons to form ubiquinol (UQH_2). Hence, like flavoprotein carriers, it can junction between a two electron donor and one electron acceptor.

Quantity of ubiquinone in lipid bilayer of mitochondrial membrane is more than any other carrier of the respiratory chain.

7.8.3 Cytochromes

Cytochromes are heme containing electron transfer proteins of mitochondrial inner membrane. The heme group contains four porphyrin rings with the central core of iron atom.

There are three classes of cytochromes distinguished by differences in their light absorption spectra. They are designated as a, b and c. The iron of heme in cytochromes is reversibly oxidised (Fe^{3+}) and reduced (Fe^{2+}) for the transport of electron in electron transport chain.

Cytochromes are also found on the thylacoid membrane of chloroplasts and plasma membrane of bacteria.

The electron transfer occurs from co-enzyme Q to cytochromes in the following order: i.e. $b \rightarrow c_1 \rightarrow c \rightarrow a \rightarrow a_1$.

Cytochrome C: It is ubiquitous (i.e. present everywhere in nature). It is smaller in size with 104 amino acid residues.

Cytochrome Oxidase: The cytochromes a and a_3 are collectively called as the cytochrome oxidase. It represents the terminal of electron transport chain. The heme iron of cytochrome oxidase directly combines with molecular oxygen. This oxidase also contains copper ions that undergo oxidation-reduction as $Cu^{2+} \rightleftharpoons Cu^{+}$ during transport of electrons.

7.9 THE WORKING OF RESPIRATORY CHAIN IN MITOCHONDRIA

The components of respiratory chain are arranged in the order of increasing redox potential from left to right as shown (earlier) in the flowsheet.

The respiratory chain in mitochondria starts from the NAD linked dehydrogenase system on the one hand and via cytochromes to the molecular oxygen on the other hand.

Initially, the substrate molecule is oxidised by NAD linked dehydrogenase. NAD transfers its hydrogen to FAD requiring dehydrogenases. The hydrogen is then passed on to ubiquinone (co-enzyme Q). Thereafter, hydrogen undergoes ionisation to release two protons (H^+) and two electrons (e^-). Subsequent portion of electron transport chain carries only electrons. Since a molecule of hydrogen releases two electrons and one cytochrome can accept only one electron at a time at its Fe^{3+} and gets converted to Fe^{2+}, hence thereafter, 2 moles of each cytochromes are required for 2 electrons. These electrons are thereafter successively transferred to Cyt b_1c_1, c and Cyt a + a_3 (also known as cytochrome oxidase) and finally come in contact with oxygen and protons forming H_2O molecule.

In the electron transport chain, the system having relatively more positive redox potential is able to oxidise another system having lower redox potential. Hydrogen or electrons pass through different carriers in increasing sequence of positive redox potential releasing packets of energy at each step.

A major portion of the released energy is captured in the form of ATP, provided surrounding medium contains ADP, Pi and ATP synthetase. There are three sites of ATP synthesis across the electron transport chain:

1. Between NAD and FAD.
2. Between Cyt b and Cyt c_1.
3. Between Cyt a_3 and oxygen.

7.10 MECHANISM OF OXIDATIVE PHOSPHORYLATION

The most fundamental point regarding mitochondrial oxidative phosphorylation is – How does the electrons flow through the respiratory chain channel energy into the synthesis of ATP? What is the chemical mechanism by which energy released in one exergonic reaction (electron transport chain) is channeled into second endergonic reaction (i.e. condensation of ADP and Pi). Hence, to understand the process of oxidative phosphorylation, we need to study the physical and chemical changes that result from electron flow and cause ADP phosphorylation.

Several hypothesis have been put forward to explain the process of oxidative phosphorylation. The most important among them are: chemical coupling, conformational coupling, chemiosmotic.

7.10.1 Chemical Coupling Hypothesis

It was put forward by Edward Slater in 1953. It states that, series of high energy intermediates (i.e. phosphorylated high energy intermediates) are initially synthesized during the passage of electron across the respiratory chain. These intermediates are utilized for the synthesis of ATP. The reactions of intermediate formation are similar to substrate level phosphorylation, taking place in glycolysis and TCA cycle.

But owing to lack to experimental evidence, the hypothesis is not yet proved as each and every attempt so far to isolate any one of the high energy intermediates have not been successful.

7.10.2 Conformational Coupling Hypothesis

The hypothesis was put forward by Paul Boyer in 1964. According to this hypothesis there is induction of conformational change in membrane proteins. This according to Paul Boyer is responsible for the synthesis of ATP. The protein attains a high energy conformation due to electron transport. Then same protein reverts back to a random state of low energy which results in the synthesis of ATP from ADP and Pi.

The hypothesis was put forward on the basis of research work stating evidence of conformational changes in membrane proteins of mitochondria.

However, there is no conclusive proof which can corelate such changes with ATP synthesis.

7.10.3 Chemiosmotic Coupling Hypothesis

This revolutionary hypothesis explaining the mechanism of oxidative phosphorylation was put forward by Peter Michell in 1961.

This hypothesis proposes that the energy liberated by electron transport is used to create a proton gradient across the mitochondrial inner membrane and this proton gradient is used to drive ATP synthesis. Thus, function of coupling of electron transport chain and oxidative phosphorylation is done by the proton gradient. The actual ATP synthesis is carried out by the enzyme ATP – synthetase present on the inner mitochondrial membrane.

The electron transport down the respiratory chain from NADH oxidation causes H^+ ions to be pumped out of mitochondrial matrix across the inner mitochondrial membrane into the intermembrane space by the three H^+ pumps, namely NADH dehydrogenase, the cytochrome bc_1 complex and cytochrome oxidase. The pumping of H^+ ions generates a higher concentration of H^+ ions in the intermembrane space and electrical potential. This makes the side of inner mitochondrial membrane facing intermembrane space positive. Thus, there is a building up of overall proton gradient.

The protons flow back into mitochondrial matrix through ATP synthetase and this drives ATP synthesis. The ATP synthetase is driven by proton motive force which is the sum of pH gradient (i.e. chemical gradient of H^+ ions) and the membrane potential.

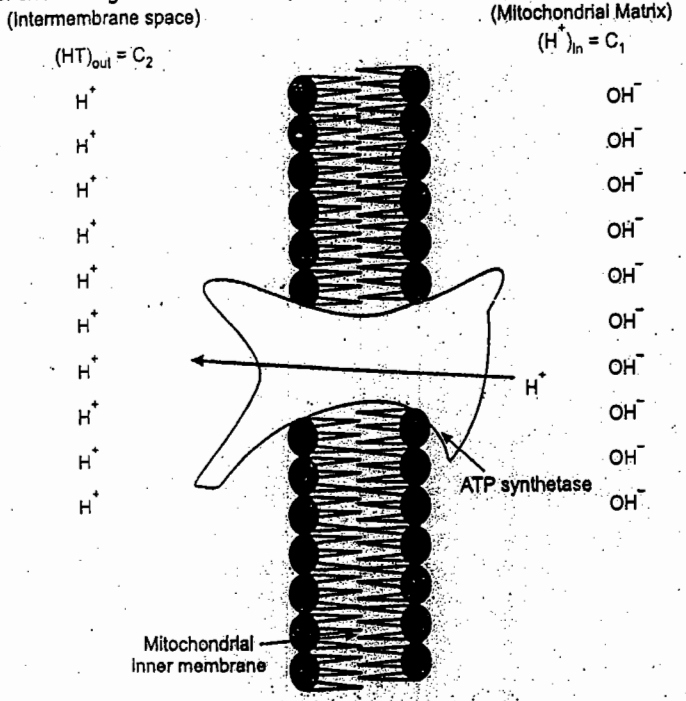


Fig. 7.7: Mechanism of oxidative phosphorylation according to chemiosmotic theory

Fig. 7.8 shows the likely organisation of the sub-unit to form conducting 'F_0' portion and the ATP synthesizing 'F_1' unit of ATP synthetase enzyme. F_1 is made up of three subunits and F_0 is made up of proton channel.

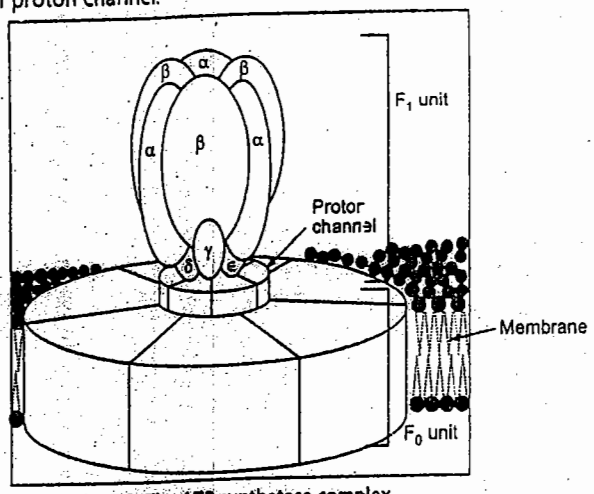


Fig. 7.8: The ATP synthetase complex

7.11 RESPIRATORY CONTROL OF OXIDATIVE PHOSPHORYLATION

There is tight coupling between electron transport chain and oxidative phosphorylation. The electrons do not flow through the electron transport chain towards oxygen unless ADP is simultaneously phosphorylated to ATP and ATP is not synthesized unless electron transport is occurring to provide the proton gradient.

The rate of oxidative phosphorylation is set by the availability of ADP. Addition of ADP to mitochondria increases the rate of oxygen consumption as electrons flow down the chain and concurrently rate of oxygen utilization falls when all the ADP has been phosphorylated to ATP. The overall process represents the respiratory control.

This mechanism ensures that electrons flow down the chain only when ATP synthesis is needed.

7.12 ATP SYNTHESIS

Synthesis of ATP by oxidative phosphorylation is carried out by the membrane bound enzyme – ATP synthetase. The enzyme is present in the complex V. This enzyme has also the capacity to hydrolyze ATP to ADP and Pi reversibly (ATPase activity).

ATP synthetase is a complex enzyme and is made up of two functional subunits F_1 and F_0 (Fig. 7.8)

The enzyme contains the channel for the passage of the protons from intermembrane space into the mitochondrial matrix. This passage of protons into the matrix leads to the synthesis of ATP with the help of ATP synthetase.

7.13 EVIDENCES OF CHEMIOSMOTIC HYPOTHESIS

1. The intact mitochondrial membrane is able to carry out oxidative phosphorylation.
2. The impermeability of inner mitochondrial membrane towards H^+ and other ions like K^+, Cl^- and OH^-.
3. Proof of ATP generation by increasing H^+ ion concentration by adding HCl on the outer side of mitochondria.
4. Compounds like 2–4 dinitrophenol inhibit ATP synthesis as they are found to increase permeability of protons through the inner mitochondrial membrane.

All the above mentioned experimental evidences support the principle of chemiosmotic hypothesis.

7.14 INHIBITION OF OXIDATIVE PHOSPHORYLATION

7.14.1 Uncouplers

Certain chemicals – such as 2, 4, dinitrophenol (DNP) act as uncoupling agents when added to cells. They stop ATP synthesis but electron transport still continues along with oxygen consumption. In this way, DNP uncouples or delinks electron transport chain from oxidative phosphorylation.

The DNP is lipophilic or lipid soluble small molecule which can bind H^+ ions and transport them across the membrane. The electron transport chain builds up the proton gradient across inner mitochondrial membrane. But DNP in the same membrane carries the H^+ ons into mitochondria. This way it prevents the formation of H^+ gradient.

As no proton gradient is formed, no ATP synthesis takes place and the energy derived from electron transport is released as heat. The heat so produced by uncoupling is called thermogenesis.

7.14.1.1 Uncoupling in Brown Adipose tissues

In the new borns, hairless animals, hibernating animals and in the animals of the cold regions, maintenance of body temperature is of prime importance. These animals possess a specialised type of adipose tissue called as brown adipose tissue (or brown fat) in the neck and back portion of the body. The fuel oxidation does not used to produce ATP, but to generate heat to maintain the body temperature. The brown adipose tissue possesses m tochondria which are rich in the electron carriers. These mitochondria carry out oxidation uncoupled from phosphorylation. This liberates heat when fat is oxidised in brown adipose tissue. The heat liberated is helpful in maintaining body temperature of these animals. The uncoupling of electron transport chain from phosphorylation occurs due to presence of the unique membrane bound protein, **thermogenin** is also called as uncoupling protein (UCP). This protein provides the path for protons to return to the matrix without passing through the F_0F_1 complex. As a result, the energy of oxidation is not conserved by ATP formation but is liberated as heat. The heat is helpful in maintaining body temperature.

7.14.2 Ionophores

The group of lipid soluble or lipophilic substances are called as ionophores. They promote the transport of ions across the biological membrane. e.g. antibiotic valinomycin, nigercin act as ionophores for K^+ ions. Both compounds have the capacity to disturb the proton gradient formed across the inner mitochondrial membrane and ultimately inhibit the oxidative phosphorylation.

7.14.3 Atractyloside

This is a plant toxin. It inhibits oxidative phosphorylation by indirect mechanism. A non-stop phosphorylation in mitochondrial matrix can take place only in presence of an adequate quantity of ADP, Pi and transport of ATP into the extramitochondrial system. The passage of ATP and ADP occurs with the help of adenine nucleotide carrier system.

Atractyloside inhibits adenine nucleotide carrier. This way it blocks an adequate supply of ADP. Thus, phosphorylation is inhibited in the absence of ADP.

7.14.4 Oligomycin

Antibiotic oligomycin stops or prevents mitochondrial oxidation and phosphorylation. It blocks the proton H^+ channels by binding with the enzyme ATP synthetase. This way antibiotic prevents the re-entry of protons into the mitochondrial matrix. This gives rise to accumulation of protons into the intermembrane space. This stops the electron transport chain as protons cannot be pumped out against a steep gradient. The inhibition by oligomycin shows linking between two processes; electron transport chain and oxidative phosphorylation.

7.15 ENZYMES PARTICIPATING IN BIOLOGICAL OXIDATION

All enzymes carrying out biological oxidation belong to the class – oxidoreductase. There are five groups as follows:

1. Oxidases
2. Aerobic dehydrogenase
3. Anaerobic dehydrogenase
4. Hydroperoxidase
5. Oxygenase.

1. **Oxidases:** Function of oxidases is the removal of hydrogen from the substrate which is accepted by oxygen to form water or H_2O_2. The examples of oxidases are:
   (i) Cytochrome oxidase (product is H_2O)
   (ii) Ascorbate oxidase (product is H_2O)
   (iii) Amino acid oxidase (product is H_2O_2)

2. **Aerobic dehydrogenase:** These enzymes are flavoproteins containing FAD or FMN as the prosthetic group. The product of aerobic dehydrogenase – catalytic oxidation is H_2O_2, e.g. xanthine oxidase.
3. **Anaerobic dehydrogenase:** The oxidation-reduction reactions carried out by these enzymes are in the absence of oxygen. The reversible transfer of hydrogen from one substrate to another is brought about by these enzymes.
   e.g. (i) FAD dependent dehydrogenase e.g. succinate dehydrogenase.
   (ii) FMN dependent dehydrogenase e.g. NADH dehydrogenase.
   (iii) $NADP^+$ dependent dehydrogenase e.g. HMG CoA reductase.
   (iv) NAD^+ dependent dehydrogenase e.g. glycerol-3-P-dehydrogenase.
   (v) The cytochromes: Namely the cytochromes of electron transport chain except the terminal cytochrome oxidase ($a + a_3$) belong to this group.
4. **Hydroperoxidases:** These enzymes act on hydrogen peroxide and detoxify this harmful compound which is produced by the action of aerobic dehydrogenase (as a product). The examples of hydroperoxidases are:
   (i) Peroxidase
   (ii) Catalase.
5. **Oxygenase:** The direct incorporation of oxygen into a substrate molecule is carried out by catalytic action of oxygenase. e.g. L - tryptophan pyrrolase.

7.16 HIGH ENERGY COMPOUNDS (ENERGY RICH COMPOUNDS)

Biochemical compounds releasing free energy during hydrolysis can be classified into two classes:

(i) High energy compounds
(ii) Low energy compounds.

The high energy in these compounds is restricted to some atoms or bonds and not distributed in the whole molecule.

It was Lipmann who suggested to use the symbol (~) to denote the high energy bonds. The symbol (~) also represents the group of transfer potential since groups or bonds attached to this bond transfer large quantity of free energy to the acceptor molecule or atom.

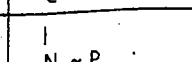
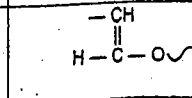
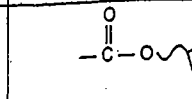
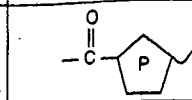
All such compounds are classified into high energy class, if the free energy of hydrolysis is atleast 7.3 kcal/mole or more at pH 7. The 7.3 kcal/mole represents the energy of hydrolysis of ATP. Whereas certain other compounds which liberate less than 7.3 kcal/mole energy of hydrolysis are termed as low energy compounds.

In short, substances capable of ATP formation are high energy compounds.

The high energy compounds like phosphoenol pyruvate, 1,3, biphosphoglycerate, phosphocreatine etc. liberate more energy than that of ATP on hydrolysis. Most of the high energy compounds contain a phosphate group. Hence, they are also called as high energy phosphates. Acetyl CoA is an example of non-phosphate high energy compound. For details please refer to Chapter 4, Page No. 4.6.

Table 7.2 shows some examples of high energy compounds; whereas Table 7.3 gives energy of hydrolysis of some energy rich compounds.

Table 7.2: Examples of high energy compounds

Class	Bond	Example(s)
1. Thiolesters (thioesters)		Acetyl CoA, acyl CoA
2. Guanidino phosphates		Phosphocreatine Phosphoarginine
3. Enol phosphates		Phosphoenol pyruvate
4. Acyl phosphates (Carboxyl phosphates)		Acetyl phosphate, 1, 3 biphosphoglycerate carbamoyl phosphates
5. Pyrophosphates		ATP, pyrophosphate, ADP
6. β keto acid		Acetoacetate (R = CH_3)

where  = Phosphates

Table 7.3: Standard free energy of hydrolysis of compound

Compounds	ΔG° (cal/mol)
High Energy Phosphates	
Carbamoyl phosphate	– 12.3
Phosphoenol pyruvate	– 14.8
Cyclic AMP	– 12.0
1, 3 biphosphoglycerate	– 11.8
Phosphocreatine	– 10.3
Acetyl phosphate	– 10.3
S-Adenosyl methionine	– 10.0
Pyrophosphate	– 8.0
Acetyl CoA	– 7.0
ATP → ADP + Pi	– 7.3
Low Energy Phosphates	
ADP → AMP + P_i	– 6.6
Glucose-1-phosphate	– 5.0
Fructose-6-phosphate	– 3.8
Glucose-6-phosphate	– 3.3
Glycerol-3-phosphate	– 2.2

The amino acid esters of sRNA, diester phosphate linkage in nucleic acid and acyl imidazoles possess additional high energy type of linkage.

ATP is the energy currency of the cell. It is formed during many catabolic energy yielding reactions and utilized during many anabolic energy requiring reactions.

Table 7.4: Low energy compounds

Class	Bond	Example
Peptide		Proteins
Glycoside	H — C — OR	Polysaccharide
Phosphoric ester	R — O — PO_3 H_2	Hexose, pentose, triose phosphates, AMP.

ATP is a unique high energy compound. This compound is made up of adenine, ribose and triphosphate species. ATP is also formed concurrently after the breakdown of other high energy compounds like creatine phosphate. Energy in high energy bonds of ATP is also utilized for the synthesis of many phosphodiester bonds.

1. Creatine ~ Phosphate ⟶ Creatine + Phosphate
   (ADP + Pi ⟶ ATP)
2. ATP + glucose ⟶ *Glucose - 6 - P + ADP + Pi*

7.17 ATP-ADP CYCLE

ATP is the biochemical link between catabolism and anabolism. Animal cells obtain free energy in the chemical form by catabolism of nutrient molecules and use this energy to make ATP from ADP and Pi. ATP then donates some of its chemical energy to endergonic reactions for the synthesis of metabolic intermediates and macromolecules from smaller precursors and also for the transport of substances across membranes against the concentration gradient and for mechanical motions like muscle contraction.

In general, the function of coupling of energy of ATP breakdown to energy consuming substrate transformations is carried out not by ATP hydrolysis, but through transfer of phosphate and adenylate from ATP to a substrate or enzyme molecule.

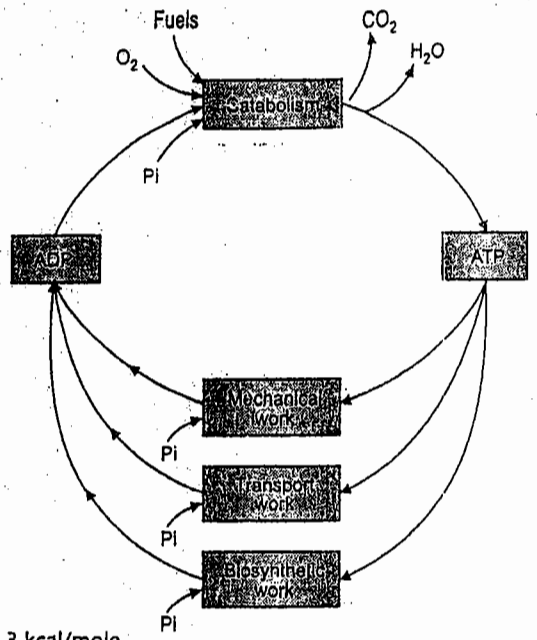


Fig. 7.9: ATP-ADP cycle

The energy of hydrolysis of ATP is 7.3 kcal/mole.

$$ATP + H_2O \longrightarrow ADP + Pi + 7.3 \text{ kcal.}$$

Besides, ADP can accept high energy phosphates from compounds having higher free energy content to form ATP.

In other words, we can call ATP as the immediately available energy currency of the cell which is constantly being utilized and regenerated as per cellular demands.

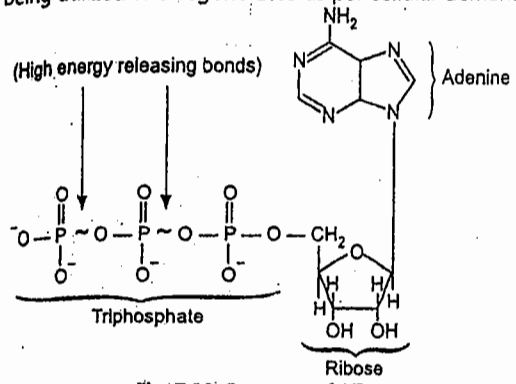


Fig. 7.10: Structure of ATP

The above mentioned reactions are in fact represented in ATP-ADP cycle. These represent the basic energy exchange reactions in the living cell.

A 65 kg resting man consumes about 40 kg of ATP per day. The rate of ATP consumption increases during strenous activities.

The cycle also deserves a special mention of creatine phosphate. The excess of energy in the body is stored in the form of creatine phosphate. It is one of the high energy compound in muscles. Creatine in muscles is phosphorylated whenever there is an excess of cellular energy.

Creatine + ATP $\rightleftharpoons$ Creatine ~ Phosphate.

The creatine phosphate is hydrolysed reversibly whenever cell requires energy.

The core molecule of ATP cycle can be synthesized in two ways:

1. **Oxidative phosphorylation (discussed earlier in the same chapter):** It serves as the major source of energy for aerobic organisms.
2. **Substrate level phosphorylation:** It can be defined as 'the direct synthesis of ATP during breakdown of high energy compounds like phosphoenol pyruvate 1,3, bisphosphoglycerate, succinyl CoA', etc.

   e.g.

1, 3-bisphosphoglycerate $\xrightarrow[Mg^{2+}]{\text{Phosphoglycerate kinase}}$ 3-phosphoglycerate (glycolysis)

ADP + Pi → ATP

Phosphoenol pyruvate $\xrightarrow[]{\text{Pyruvate kinase}}$ Pyruvate (glycolysis)

ADP + Pi → ATP

Thus, vicious cycle of ATP-ADP goes on and on in the living cell to furnish cellular demands.

7.18 INBORN ERRORS OF OXIDATIVE PHOSPHORYLATION

1. Laber's hereditary optic neuropathy (LHON)

All the five enzymes of the respiratory chain are encoded by 37 genes of the circular mitochondrial genome or DNA. The remaining genes of the mitochondria encode for the ribosomal and transfer RNA molecules required for the protein synthesis in mitochondria.

Mutations in the mitochondrial DNA are known to cause the human disease called as Laber's hereditary optic neuropathy (LHON). Although a very rare genetic disease, it affects the central nervous system including the optic nerves. It causes a bilateral loss of vision due to neuroretinal degeneration.

2. Myoclonic Epilepsy and Ragged Red Fiber Disease (MERRF):

This inborn error of metabolism is caused due to the mutation in the mitochondrial gene that encodes a transfer RNA. The clinical signs of the disease are uncontrollable muscular jerking due to defective production of many mitochondrial proteins. The skeletal muscle fibres of individuals with MERRF have abnormally shaped mitochondria i.e. paracrystalline shape.

QUESTIONS

1. Explain the structure of mitochondria. Draw and label the necessary diagram.
2. Write a note on the Electron transport chain.
3. Give only the flow sheet of electron transport chain, with special mention of various inhibitors and their sites.
4. Define oxidative phosphorylation. What is the cellular site of oxidative phosphorylation?
5. Describe in detail chemiosmotic hypothesis of oxidative phosphorylation.
6. Give an account of energetics of oxidative phosphorylation.
7. What is meant by electron carriers of respiratory chain? What is the mode and sequence of electron transport across the respiratory chain?
8. Write short notes on:
   (a) Chemical coupling hypothesis.
   (b) Conformational coupling hypothesis.
   (c) Nicotinamide as an electron carrier.
   (d) Iron sulfur proteins.
   (e) ATP synthesis.
9. Explain the role of flavoproteins in the Electron transport chain.
10. What is meant by cytochromes? How do they participate in the respiratory chain?
11. Explain in detail working of respiratory chain in mitochondria with a special mention of various sites of ATP synthesis.
12. Define oxidative phosphorylation. Write a note on P: O ratio.
13. Explain the role played by co-enzyme Q in the electron transport chain.
14. Describe the respiratory control of oxidative phosphorylation.
15. Explain in detail Inhibition of oxidative phosphorylation.
16. Give an account of enzymes participating in oxidative phosphorylation.
17. Explain in detail 'High Energy Compounds'.
18. Discuss ATP – ADP cycle in the biological system.
19. Give a detailed account of inborn errors of oxidative phosphorylation.

UNIT III

Chapter ... 8

METABOLISM OF LIPIDS

♦ LEARNING OBJECTIVES ♦

- *Appreciate Distinct Nature of Metabolism of each Class of Lipid.*
- *Understand Conversion of Cholesterol into Steroid Hormones, Vitamin D and Bile Acids.*
- *Know Importance of β-oxidation and Energetics related to it.*
- *Appreciate, Significance and Synthesis of Ketone Bodies.*

8.1 INTRODUCTION

Distribution of Dietary Lipids in the Body

Lipids comprise 18-25% of body weight in a lean adult.

Lipids are distributed in three important compartments in the body, they are liver, adipose tissue and blood. The major part of the lipid metabolism takes place in the liver and adipose tissue. The blood works as a medium of transport for lipids. The tissues like cardiac and skeletal muscle require lipids as well as ketone bodies for their regular metabolism.

8.2 MICELLES FORMATION

Pancreatic lipase enzyme brings about degradation of triacyl glycerol to a molecule of glycerol and free fatty acids in the lumen of intestine. The degradation is carried out in the presence of conjugated bile acids (which contain lipid soluble polar parts of taurine/glycine). Bile acids are very good surfactants. The micelle formulation takes place, when bile acids, glycerol and free fatty acids are present together. The non-polar molecules are centrally present in the micelles. Micelles offer a absorption system for fat soluble vitamins and cholesterol.

8.3 METABOLISM OF LIPIDS IN THE INTESTINAL EPITHELIAL CELLS AND CHYLOMICRON FORMATION

The intestinal epithelial cells absorb micelles containing free fatty acids, glycerol etc. The enzymes present in the endoplasmic reticulum of epithelial cell bring about the synthesis of triacyl glycerol. Freshly synthesized triacyl glycerols, phospholipids, and proteins along with the dietary cholesterol are combined together in the endoplasmic reticulum of the epithelial cells and excreted into lacteals as chylomicrons. The chylomicrons are stable particles having a diameter of about 200 nm. Their constitution is as follows:

Table 8.1: Constitution of chylomicrons

Phospholipids	10%
Proteins	0.2 – 0.5%
Cholesterol	2 – 3%
Triglycerols	80 – 90%

These particles travel the route from intestinal lacteals via lymphatic system to thoracic duct. The chylomicrons are discharged into the blood system at the left subclavian, in the form of a milky suspension. It takes 20 minutes to remove chylomicrons from the blood.

Most of the chylomicrons are transported to the adipose tissue for the fat storage. But whenever body is in short supply of energy, chylomicrons are utilized primarily by cardiac muscle, skeletal muscle as well as liver to meet their energy demands.

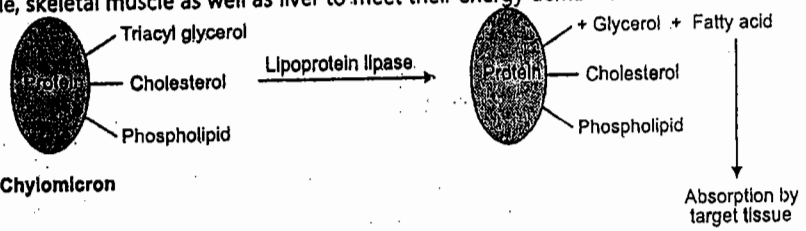


Fig. 8.1: Action of lypoprotein lipase on chylomicrons

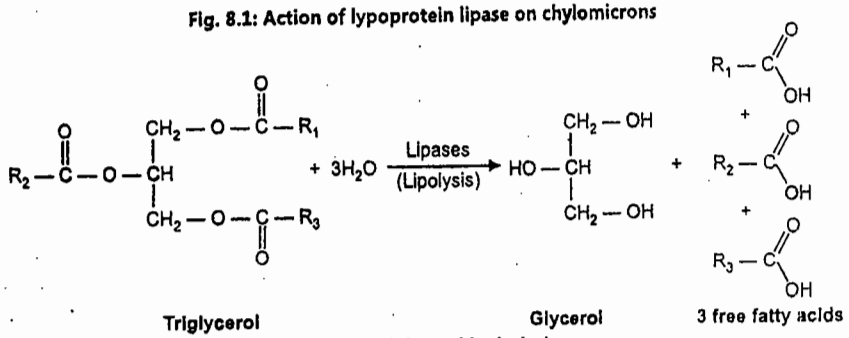


Fig. 8.2: Triglycerol hydrolysis

The enzyme lipoprotein lipase catalyses the cleavage of triacyl glycerol to glycerol and free fatty acids in target tissues like muscle, as well as adipose tissue.

8.4 LIPIDS IN ADIPOSE TISSUE

The adipocytes of adipose tissue contain triglycerol as a stored fat that occupies 99% of the total cell volume, and a very thin envelope of cytoplasm. Fats are never permanently deposited in the adipose tissue. The cycle of constant mobilization as well as deposition of lipids goes on and on in the adipose tissue. The hormone sensitive lipases remove the fatty acids from the stored triglycerol molecule.

Lipolysis: The complete degradation of triglycerol to glycerol and free fatty acids is called as lipolysis.

8.5 HORMONAL REGULATION OF LIPOLYSIS

Hormones namely, epinephrine, norepinephrine, glucagon and adrenocortico-trophine, induce or stimulate lipolysis. The cyclicAMP is a second messenger in the activation of lipolysis in the adipose tissue cells.

Insulin inhibits lipolysis by decreasing the cAMP levels. Lipase is present in the inactive form. Hormones mentioned above, convert it into an active form with the help of cAMP dependent protein kinase. The mechanism of activation of lipase is given in the flowsheet below.

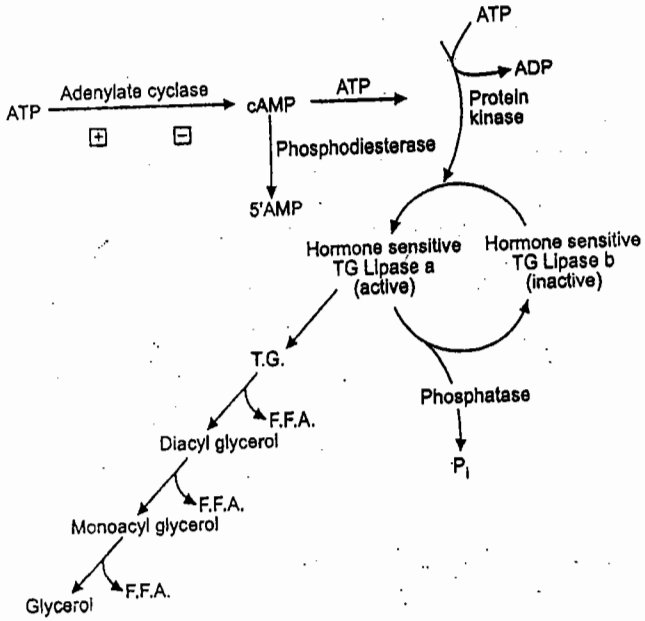


Fig. 8.3: Hormonal regulation of lipolysis

(−) Inhibition of Lipolysis

- **Insulin**
- **Niacin**
- **PGE_1**

(+) Activation of Lipolysis

- **Epinephrine**
- **Norepinephrine**
- **Glucagon, Thyroxin,**
- **Glucocorticoid**

8.6 OXIDATION OF FATTY ACIDS

Fatty acids in the cells are generally oxidised by β-oxidation. Oxidation of the fatty acid at the β-carbon is defined as β-oxidation.

The β-oxidation involves the removal of two carbon fragment of acetyl CoA from the long chain fatty acid molecule.

The site of β-oxidation in eukaryotes is mitochondrial matrix.

German scientist, Franz Knoop in 1904 fed the dogs with straight chain fatty acids in which carbon was joined to phenyl group. Knoop found that urine of those dogs contained a derivative of phenylacetic acid, when they were fed with even carbon fatty acids. The excretory product of the odd carbon fatty acid was benzoic acid. Knoop deduced from the above findings that degradation of fatty acids occur by oxidation at β carbon.

8.6.1 Phases of β-Oxidation

There are three phases of β-oxidation:

1. In cytosol – activation of fatty acid to fatty acyl CoA.
2. Transport of activated fatty acid to mitochondrial matrix across mitochondrial inner membrane.
3. The β-oxidation - pathway in mitochondrial matrix.

8.6.1.1 Fatty Acid Activation

In the cytosol, the molecule of fatty acid is activated by forming an acyl CoA thioester link between the carboxyl group of fatty acid and sulfhydryl group of CoA to form acyl CoA. The enzyme catalysing the activation of fatty acid is fatty acid thiokinase or acyl CoA synthetase. Activation reaction takes place in two steps.

Fatty acid reacts with ATP to form acyladenylate. The acyladenylate combines with the co-enzyme A to produce acyl CoA. The overall reaction requires ATP, co-enzyme A, and Mg^{2+}. Activation reaction utilizes two high energy phosphates. (1^{st} ATP and 2^{nd} PP_i hydrolysis with the help of the enzyme inorganic pyrophosphatase). The quick removal of PP_i makes the activation reaction totally reversible. The overall reaction is as follows:

$$R-CH_2-CH_2-COO^- + ATP + HS-CoA \rightleftharpoons R-CH_2-CH_2-\overset{O}{\overset{\|}{C}}-S-CoA + AMP + PP_i$$

Fatty acid **Acyl CoA**

The actual reaction takes place as follows:

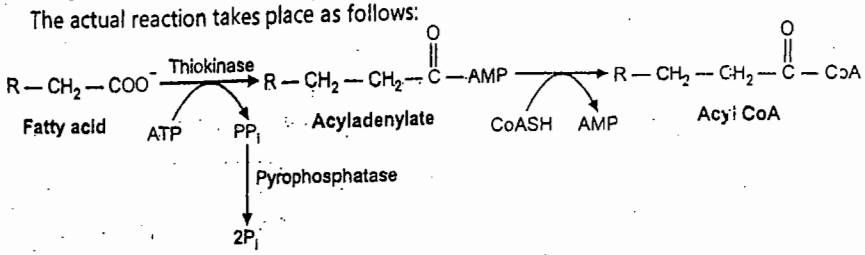


Fig. 8.4: Reaction of acyl co-enzyme formation

8.6.1.2 Transport of Activated Fatty Acid from Cytosol into Mitochondria

Fatty acids (upto 10 carbon atoms) are readily able to cross the inner mitochondrial membrane by diffusion. However, the inner mitochondrial membrane is not permeable to long chain fatty acyl CoA derivatives, and these are transported into mitochondria as carnitine derivatives - a special carnitine carrier system. The actual transport takes place as follows:

Carnitine is actually a protein β-hydroxy γ-trimethyl amino butyrate.

(A) The first step involves the transfer of acyl group of acyl CoA to the polar carnitine molecule with the help of the enzyme *carnitine acyl transferase I*. The enzyme is found on the outer surface of the inner mitochondrial membrane (intermembrane space).
The enzyme removes the CoA group and substitutes it with the carnitine molecule.

(B) The acyl carnitine is then transported across the inner mitochondrial membrane into the mitochondrial matrix by *acyl carnitine translocase*, a membrane bound enzyme. The enzyme actually transfers the fatty acids into the matrix.

(C) The enzyme, carnitine acyl transferase II found on the matrix side of inner mitochondrial membrane transfers acyl group back to CoA, releasing free carnitine.

(D) The carnitine released reaches the intermembrane space for its reuse.

Thus, there are two separate CoA pools in the cell (one in cytosol and another in the mitochondrial matrix). There is consumption of two ATPs, one in cytosol, and another in mitochondria for activation.

8.7 CARNITINE TRANSPORT SYSTEM ACROSS THE INNER MITOCHONDRIAL MEMBRANE

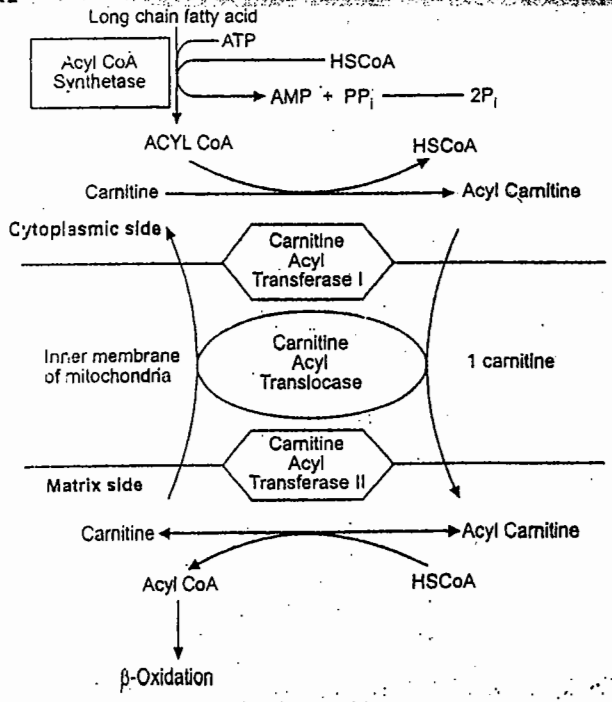


Fig. 8.5: Flow-chart showing carnitine transport system

β-OXIDATION PATHWAY

Flow chart of β-oxidation of fatty acids (Even number of carbon containing saturated fatty acid).

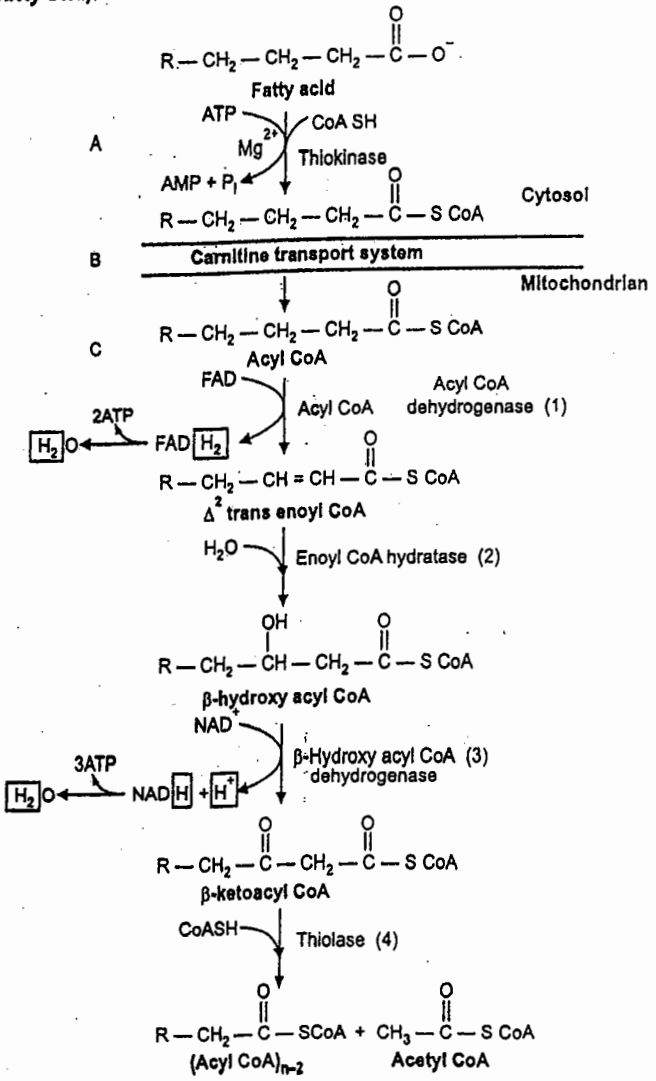


Fig. 8.6: Pathway of β-oxidation of even number of carbon atoms containing fatty acids

β-oxidation of saturated and even number of carbon containing fatty acids occurs most frequently in biological systems. The individual reactions of β oxidation of fatty acid occur in the sequence of four reactions.

1. **Oxidation:** Oxidation of the fatty acyl CoA to enoyl CoA forms a trans Δ^2 – double bond on the fatty acyl chain and produces $FADH_2$. This reaction is catalysed by an FAD dependent flavoenzyme, acyl CoA dehydrogenase. The reaction introduces a double bond between α and β carbons.
2. **Hydration:** Hydration is catalysed by enoyl CoA hydratase. The reaction involves actual hydration of double bond of trans Δ^2 enoyl CoA to form 3-hydroxy acyl CoA.
3. **Oxidation:** The enzyme hydroxyacyl CoA dehydrogenase catalyses the second oxidation of 3-hydroxyacyl CoA to 3 ketoacyl CoA and NADH formation.
4. **Cleavage or thiolysis of 3-ketoacyl CoA to a second CoA molecule:** This reaction involves the liberation of acetyl CoA (2 carbon fragment) and formation of new acyl CoA (shortened by 2 carbon atoms) with the help of enzyme β-ketoacyl thiolase.

The new acyl CoA formed in the fourth step (containing two carbon less than original) now re-enters the β-oxidation cycle. The cycle of β oxidation goes on and on until the fatty acid fragment becomes - 2 carbon fragment in length.

Each cycle of β-oxidation shows the following overall reaction:

$$(Acyl\ CoA)_n + FAD + NAD^+ + H_2O + CoASH \rightarrow (Acyl\ CoA)_{n-2} + Acetyl\ CoA + FADH_2 + NADH + H^+$$

Fatty acid in each cycle of β-oxidation, gives one acetyl CoA molecule and fatty acid chain length is reduced by 2 carbon atoms.

8.8.1 Energetics of β-oxidation

The standard free energy release due to palmitate oxidation is 2,340 cal. The energy released in terms of ATP by palmitate oxidation is 129 ATP.

$$C_{16}H_{32}O_2 + 23\ O_2 \longrightarrow 16\ CO_2 + 16\ H_2O$$

(Palmitic acid) $\Delta G' = -2340$ kcal/mole

(The energy of hydrolysis of 1 mole of ATP = 7.3 cal.)

Hence, energy gained by its oxidation = $129 \times 7.3 = 940$ cal.

Hence, efficiency of energy conservation by fatty acid oxidation = $\frac{940}{2,340} \times 100 = 40\%$

8.8.2 Energetics of Palmitic Acid Oxidation

Palmitic acid is a C_{16} fatty acid. The overall reaction for palmitic acid oxidation can be written as:

$$Palmitoyl\ CoA + 7\ CoASH + 7FAD + 7NAD^+ + 7H_2O \longrightarrow 8\ Acetyl\ CoA + 7FADH_2 + 7NADH + 7H^+$$

Palmitic acid is 16 carbon fatty acid. It undergoes 7 cycles of β-oxidation to produce 8 molecules of acyl CoA (acetyl CoA).

Each cycle (or turn) of β-oxidation produces 5 ATP (of which 2 are obtained by $FADH_2$ oxidized by electron transport chain and 3 by NADH oxidized through electron transport chain).

7 cycles of β-oxidation produce $7 \times 5 = 35$ ATP.

8 Acetyl CoA molecules, after entering into TCA cycle produce $8 \times 12 = 96$ ATP.

(Each cycle of tricarboxylic acid produces 12 ATP). Thus, net energy from one molecule of palmitoyl CoA = 35 + 96 = 131 ATP.

But 2 ATPs are utilized for activation of palmitic acid to palitoyl CoA, 131 – 2 = 129 ATP.

Net yield of complete oxidation of 1 molecule of palmitic acid = 129 ATP.

8.8.3 β-oxidation of Unsaturated Fatty Acid

Complete degradation of unsaturated fatty acid requires some additional processing, prior to its β-oxidation.

Unsaturated fatty acyl CoA with double bonds at odd numbered carbon atoms, are acted upon in such a way that acyl CoA dehydrogenase comes into picture at the cis Δ^3 enoyl CoA at the end of the third round. This occurs when double bond is present between C_9 and C_{10}.

One more enzyme participation: Isomerase is required for oxidation of PUFA (polyunsaturated fatty acid) having the double bond at even number carbon atoms.

8.8.4 Oxidation of Oleic acid – Monounsaturated acid

The molecular formula of oleic acid is $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$. This C_{18} unsaturated fatty acid has one double bond between C_9 and C_{10}. The activation and transportation of oleic acid to oleyl-CoA occurs in the same way as that of saturated fatty acid, across the inner mitochondrial membrane.

Oleyl-CoA then undergoes 3 cycles of β oxidation of saturated fatty acids. Cis-Δ^3 enoyl CoA is the product of the third round of β oxidation. But this compound is not the substrate for the enzyme acyl-CoA-dehydrogenase, which can attack only on the Δ^2 enoyl CoA.

The enzyme enoyl CoA isomerase catalyses the reversible shift of the double bond from Δ^3 cis to Δ^2 trans position. The Δ^2 trans enoyl CoA formed is the normal substrate for the next enzyme of the fatty acid sequence. It yields acetyl CoA in a similar manner as (mentioned above) that of saturated fatty acid.

Flow Chart of β-Oxidation of Unsaturated Fatty Acid (Oleic acid):

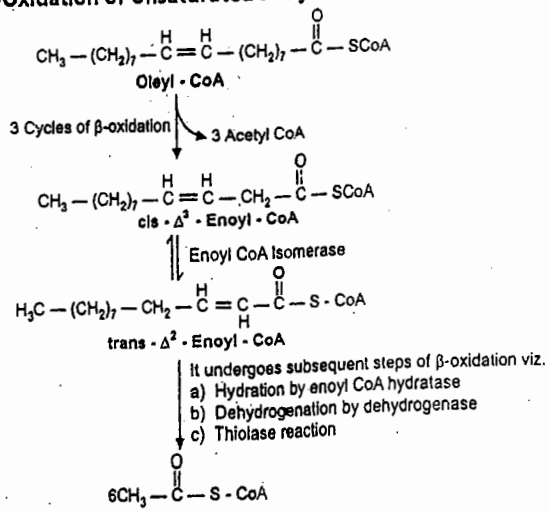


Fig. 8.7: 18 carbon containing oleyl CoA on β-oxidation gives 9 acetyl co-enzymes at the end

18 C-Oleyl CoA on β-oxidation gives 9 acetyl CoA molecules at the end. There is participation of one more enzyme-isomerase.

8.9 α-OXIDATION OF FATTY ACIDS

Only free fatty acids are subjected to α-oxidation. It is helpful in the removal of one carbon unit at a time.

α-oxidation occurs in liver, brain and in leaf tissues of plant. α-oxidation neither involves the binding of co-enzyme A to fatty acid, nor does it liberate energy during oxidation.

8.9.1 Inborn Error of α-Oxidation System

Refsum's disease: Patients with Refsum's disease, a rare inheritable neurological disease have lost their α-oxidation system. Hence, patients accumulate large quantities of phytanic acid. Source of phytanic acid is phytol of chlorophyll, in plant food, milk and animal fats.

Carbon No. 3 of phytanic acid contains a methyl group, owing to which, this fatty acid has to undergo initial α oxidation step for the removal of methyl group. This is followed by β-oxidation.

Patient suffering from Refsum's disease should avoid diet containing chlorophyll, especially leafy and green vegetables.

8.10 ω-OXIDATION OF FATTY ACIDS

The methyl carbon atom at the distal end of the chain is called as ω-carbon. ω-oxidation is observed in bacteria. It is carried out by bacterial mixed function oxygenases in microsomes. These enzymes rapidly degrade terminal hydrocarbons of fatty acids to form water soluble products. The pathway of ω oxidation takes place by initial hydroxylation of terminal methyl group of fatty acid to hydroxyl group and its further oxidation to carboxylic acid.

$$\boxed{CH_3}\text{–}CH_2\text{–}(CH_2)\text{–}COO^-$$

$$\downarrow \quad \omega\text{-carbon atom}$$

$$\boxed{HO - CH_2} - CH_2 - (CH_2)_n - COO^- \rightarrow OOC - CH_2 - CH_2 - (CH_2)_n - COO^-$$

The reactions of ω-oxidation require cytochrome P_{450}, NADPH and O_2 along with specific enzymes.

8.11 OXIDATION OF FATTY ACIDS WITH ODD NUMBER OF CARBON ATOMS

Fatty acids with odd number of carbon atoms are oxidised by the pathway of β oxidation until three carbon propionyl CoA remains. The following set of reactions convert propionyl CoA to succinyl CoA.

1. Carboxylation of propionyl CoA (in presence of ATP, CO_2 and vitamin biotin) to D methyl malonyl CoA.
2. An enzyme methyl malonyl CoA epimerase converts D methyl malonyl CoA to L form.
3. A vitamin B_{12} dependent enzyme, methyl malonyl CoA mutase catalyses the conversion of L-methyl malonyl CoA to succinyl CoA. Succinyl CoA is an intermediate of the TCA and hence enters the TCA cycle.

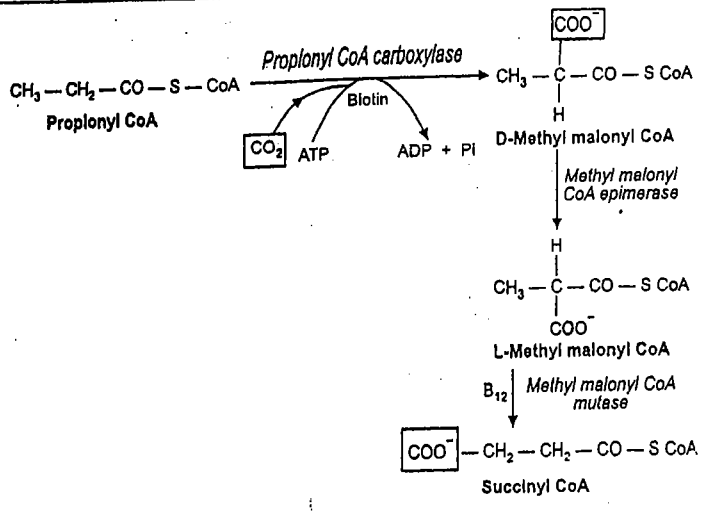


Fig. 8.8: Oxidation of fatty acids with odd number of carbons

8.11.1 Methylmalonic Acidemia

There are two types of methylmalonic acidemias reported:

1. This disease is due to defect in enzyme mutase.
2. A disease occurring due to deficiency of enzyme mutase.

The clinical characteristics of both diseases show accumulation of methylmalonic acid in the body. This gives rise to metabolic acidosis and retards the growth and damages the central nervous system. The victim of this disease dies in the early years of life.

8.12 OXIDATION IN PEROXISOMES

Eukaryotic cells possess organelles, known as peroxisomes. The mode of oxidation of fatty acids in peroxisome is β-oxidation. But unlike mitochondrial β-oxidation, the peroxisomes do not channel reducing equivalents formed (i.e. $FADH_2$) towards ATP formation.

In fact $FADH_2$ produced in peroxisomal oxidation are not passed on to the mitochondrial electron transport chain, but they directly react with O_2. This results in H_2O_2 formation. H_2O_2 is degraded by catalase.

ATP synthesis does not take place in the peroxisomal β-oxidation, but heat is liberated.

Actually the high fat diet contains the long chain fatty acids (C_{20}, C_{22}). The initial oxidation of these long chain fatty acids is carried out by inducible enzymes in the peroxisomes. Administration of hypolipidemic drug, clofibrate, shows the induction of peroxisomal enzyme and similar type of oxidation.

8.12.1 Zellweger's Syndrome

The individuals suffering from Zellweger's syndrome show absence of peroxisomes in almost all tissues.

As a result, long chain fatty acids are not oxidised in these patients. This results in accumulation of long chain fatty acids in the brain, kidney and liver. This is the reason why disorder is also called as cerebrohepatorenal syndrome.

8.13 KETOGENESIS

Ketogenesis

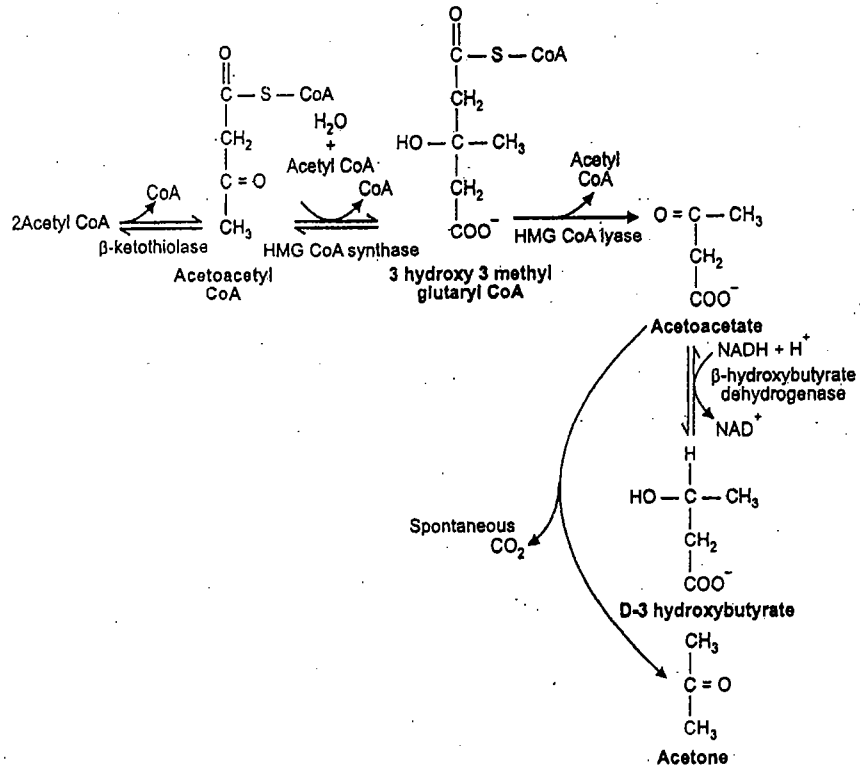


Fig. 8.9: Reactions showing synthesis of ketone bodies from acetyl CoA

Ketone Bodies Synthesis

The compounds namely acetone, acetoacetate and β-hydroxybutyrate are known as ketone bodies.

The ketone bodies are synthesized in the mitochondrial matrix in the liver. The end product of the carbohydrate, fat or some amino acid metabolism is acetyl CoA. Ketone bodies are formed from acetyl CoA.

Normally, the concentration of ketone bodies in the blood is not more than 1 mg/100 ml. A normal healthy adult excretes ketone bodies by way of urine. The urinary concentration of ketone bodies is as low as 1 mg per day.

8.13.1 Synthesis of Ketone Bodies

The process of formation of ketone bodies is called as *ketogenesis*. Ketogenesis occurs in the liver. The enzymes synthesizing ketone bodies are located in the mitochondrial matrix. The utilization of ketone bodies occurs in the extrahepatic tissues.

The end product of β-oxidation is acetyl CoA. Under certain metabolic conditions, the concentration of acetyl CoA increases in excess of that required for entry nto the citric acid cycle, then acetyl CoA is diverted into the formation of acetoacetate and D-3-hydroxy-butyrate, known as ketogenesis.

The first step of ketogenesis involves the joining or condensation of two molecules of acetyl CoA to form a molecule of acetoacetyl CoA. The acetoacetyl CoA reacts with another molecule of acetyl CoA to form 3-hydroxy 3-methyl glutaryl CoA (HMG CoA). The HMG CoA molecule is then cleaved to form acetoacetate and acetyl CoA.

The acetoacetate can be metabolised in two different ways:

1. Acetoacetate is reduced to the D-3 hydroxy butyrate in the mitochondrial matrix.
2. Or it undergoes a slow as well as spontaneous decarboxylation to yield acetone.

The set of reactions involved in the formation of ketone bodies are shown in Fig. 8.9.

Ketolysis: The process of breakdown of ketone bodies occurs in the extrahepatic peripheral tissues and is known as ketolysis.

Ketonemia: Higher than normal concentrations of ketone bodies in the blood is termed as ketonemia.

Ketonuria: The condition in which more than normal excretion of ketone bodies occurs in the urine is called as the ketonuria.

Ketosis: Elevation of ketone bodies concentration in tissues and blood is known as ketosis.

8.13.2 Significance of Ketone Bodies

- Ketone bodies like acetoacetate, D-3 hydroxybutyrate are not just degradation products of lipid metabolism produced in liver. Certain tissues like muscle, heart, kidney, cortex use these ketone bodies as energy source in preference to glucose.
- The cells like RBC, which do not contain mitochondria are unable to utilize ketone bodies.
- The utilization of ketone bodies by the tissues becomes more essential when glucose supply to tissue is absent, especially in the conditions like starvation and diabetes mellitus.

- Brain starts utilizing the ketone bodies as a fuel source during prolonged starvation. One can call this act of brain as the adaptation for survival.
- Non-availability of the carbohydrate to the tissues makes it necessary for the tissues to use the fatty acids for the energy productions. This gives rise to overproduction of acetyl CoA and ultimately ketone bodies.
- The hormone insulin inhibits the synthesis of ketone bodies, whereas the hormone glucagon stimulates ketogenesis.
- Decreased insulin synthesis or decreased insulin availability in diabetes mellitus stimulates the ketone body formation.

8.13.3 Ketogenic and Antiketogenic Substances

Ketogenic substances represent those chemical compounds which when introduced into the body are found to increase the formation of ketone bodies. The examples are phlorizin and ammonia.

Antiketogenic substances: Compounds whose introduction into the body lower the concentrations of blood ketone bodies, are called as the antiketogenic substances. Common examples are oxaloacetic acid, thiamine pyrophosphate, pyruvic acid, α-ketoglutaric acids and carbohydrates.

Ketoacidosis: Ketone bodies are acidic and when produced in excess over a long period, as in diabetes, cause ketoacidosis, which is ultimately fatal.

Detection of ketosis: In a normal individual, the concentration of ketone bodies is very less (in traces). This is why, it is not detected by the usual tests. But in ketosis, the concentration of ketone bodies in urine increases. Hence, it can be detected by Rothera's test.

8.14 LIPID BIOSYNTHESIS (FATTY ACID BIOSYNTHESIS)

Lipids are important constituents of the cell membrane. They also represent major group of stored form of energy containing substances in majority of organisms.

Some specialised variety of lipids like sex hormones, cofactors (vitamin K), pigments (retinal), detergents like bile acids, transporters (dolichols), anchors for membrane proteins (phosphotidyl inositol, covalently attached fatty acids), other hormones (vitamin D derivatives), play a major role in the cellular biochemistry.

Hence, all organisms require sufficient biochemical ability to synthesize different lipids.

Fatty Acid Biosynthesis:

The exact cellular site of fatty acid biosynthesis is cytosol.

Major components namely acetyl CoA and NADPH along with the ATP are essential for fatty acid biosynthesis. Wherein, ATP serves as a source of metabolic energy and NADPH acts as a reductant. Basically, acetyl CoA is produced by oxidation of pyruvate, the reaction is catalysed by pyruvate dehydrogenase complex.

There are still different biochemical routes to produce acetyl CoA. They are:

1. Oxidation of fatty acids in mitochondria.
2. Degradation of carbon skeleton of amino acids.
3. From ketone bodies.

But mitochondrial inner membrane is impermeable to acetyl CoA. Hence, acetyl CoA enters into the cytosol by some indirect biochemical routes as follows.

8.14.1 Journey of Acetyl CoA

The mitochondrial acetyl CoA and oxaloacetate undergo condensation in the presence of citrate synthetase to form citrate. The molecule of citrate is easily transferred across the mitochondria into the cytosol. The enzyme citrate lyase, residing in the cytosol, cleaves the citrate into acetyl CoA and oxaloacetate. Now, cytosol contains acetyl CoA for fatty acid biosynthesis. The oxaloacetate molecule is further converted into malate in the TCA cycle. The malic enzyme in the cytosol acts on malate and brings about its conversion into pyruvate. The above reaction catalysed by malic enzyme also produces CO_2 and NADPH as byproducts, which are also utilized for fatty acid biosynthesis.

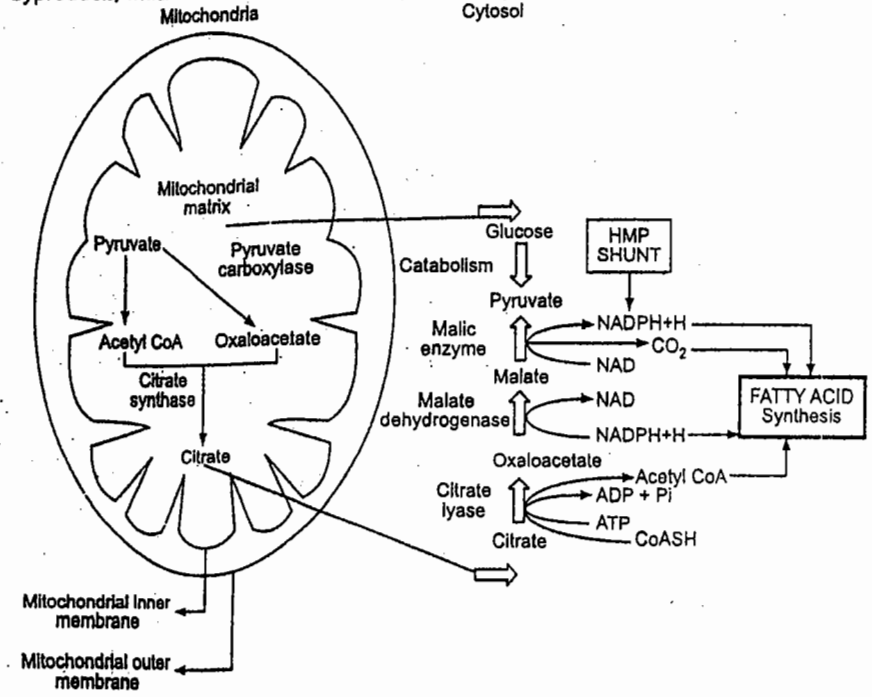


Fig. 8.10: Entry of acetyl co-enzyme into cytoplasm in the form of citrate

In cytosol, citrate is converted into the acetyl CoA for fatty acid biosynthesis. (HMP shunt - Hexose monophosphate shunt).

8.14.2 Co-ordination of Acetyl CoA Transport with NADPH Production

There is a fine co-ordination of acetyl CoA transport from mitochondria into the cytosol along with the production of the NADPH and CO_2 which is beneficial to the cell for the maximum fatty acid production. Fatty acid biosynthesis requires both acetyl CoA and NADPH, which is also supplied by the HMP shunt like pathways.

The biosynthesis of fatty acids takes place in two phases:

(i) Malonyl CoA formation.

(ii) Fatty acid synthetase complex reactions.

(i) Malonyl CoA Formation:

Acetyl CoA reacts with CO_2 in the presence of ATP and biotin to form malonyl CoA. The reaction is carried out with the help of an enzyme acetyl CoA carboxylase.

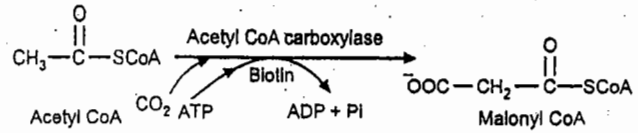


Fig. 8.11

This is the rate limiting (regulatory) step. Acetyl CoA carboxylase is the key enzyme of the fatty acid biosynthesis.

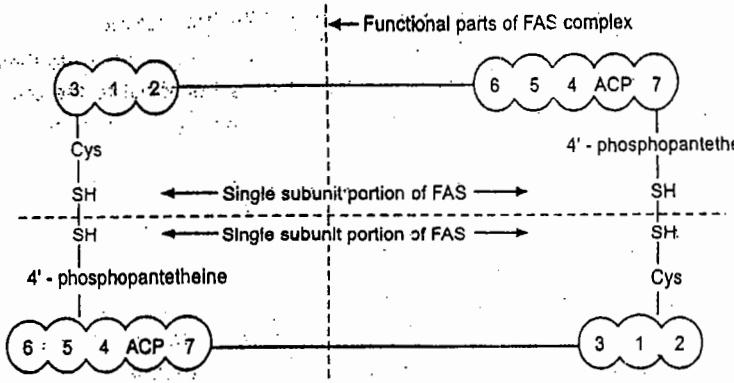
(ii) Fatty Acid Synthetase Complex Reactions:

A multienzyme complex known as fatty acid synthetase (FAS) takes part in the synthesis of fatty acids.

The multienzyme complex is made up of two similar subunits (dimer) in humans and in eukaryotic cells. Each monomer subunit possesses an acyl carrier protein (ACP) attached with its 4'-phosphopantetheine (vitamin panthothenate) group and seven enzymes participating in the complex. The complex is active in its dimer form. The two subunits are antiparallel to each other. The FAS complex model shown in Fig. 8.12 is hypothesised based on the work of Wakil.

The one end of the single unit of dimer contains –SH group of cysteine and another end contains 4' - phosphopantetheinyl group. Seven enzymes present in each monomer are:

1. Acetyl CoA-ACP transacylase.
2. Malonyl CoA-ACP transacylase.
3. β-keto acyl ACP synthetase.
4. β-keto acyl ACP reductase.
5. β-hydroxy acyl ACP dehydratase.
6. Enoyl ACP reductase.
7. Palmitoyl thioesterase.



Multienzyme complex of fatty acid synthetase in cytoplasm containing ACP (acyl carrier protein). Two similar subunits aligned head to feet on the either side.

Fig. 8.12: Structure of fatty acid synthetase complex

The prokaryotic fatty acid synthesis takes place in the multienzyme complex containing a separate acyl carrier protein (ACP), unlike eukaryotic FAS containing inbuilt ACP.

The following set of reactions represent each step in the formation of 16 carbon palmitoyl CoA in cytosol. Since, the FAS can synthesize only 16 carbon length fatty acid i.e. palmitoyl CoA.

1. An enzyme acetyl CoA-ACP transacylase catalyses transfer of two carbon containing acetyl CoA to ACP of fatty acid synthetase complex. The acetyl moiety is further transferred from ACP to the cysteine residue of the enzyme. This makes the ACP - site of the enzyme vacant.
2. The molecule malonate from malonyl CoA is transferred to ACP of FAS complex. The reaction of malonate transfer is catalysed by the enzyme malonyl CoA-ACP transacylase.
3. The enzyme β-keto acyl ACP synthetase catalyses the reaction in which acetyl group attached to cysteine of FAS complex is transferred to the malonyl unit already attached to ACP of the FAS complex. There is concurrent loss of the CO_2 from the malonyl molecule. (Recall that CO_2 was added with the help of enzyme, acetyl CoA carboxylase). This decarboxylation (loss of CO_2) occurs along with the loss of free energy which facilitates the progress of reaction in the forward direction. The β-ketoacyl ACP is the product of the reaction.
4. The next enzyme of the FAS complex, β-keto acyl ACP reductase carries out the reduction of β-keto acyl ACP into β-hydroxy acyl ACP. The reduction occurs with the help of reducing equivalents supplied by NADPH.
5. The β-hydroxy acyl - ACP - dehydratase - brings about the dehydration of β-hydroxy acyl ACP with the elimination of H_2O molecule and introduction of the double bond (between α and β atoms) into a molecule to form trans Δ^2 enoyl ACP.
6. An enzyme enoyl ACP reductase brings about the NADPH - dependent reduction. This produces four carbon acyl - ACP (the acyl in here represents the butyryl group).

The carbon chain so formed is now transferred to cysteine residue of FAS complex. The 6 more cycles of reactions from number 2 to 6 take place repeatedly. Each cycle (2 to 6) increases the chain length of the growing fatty acid by 2 carbon atoms.

Fatty Acid Synthetase Complex Reactions:

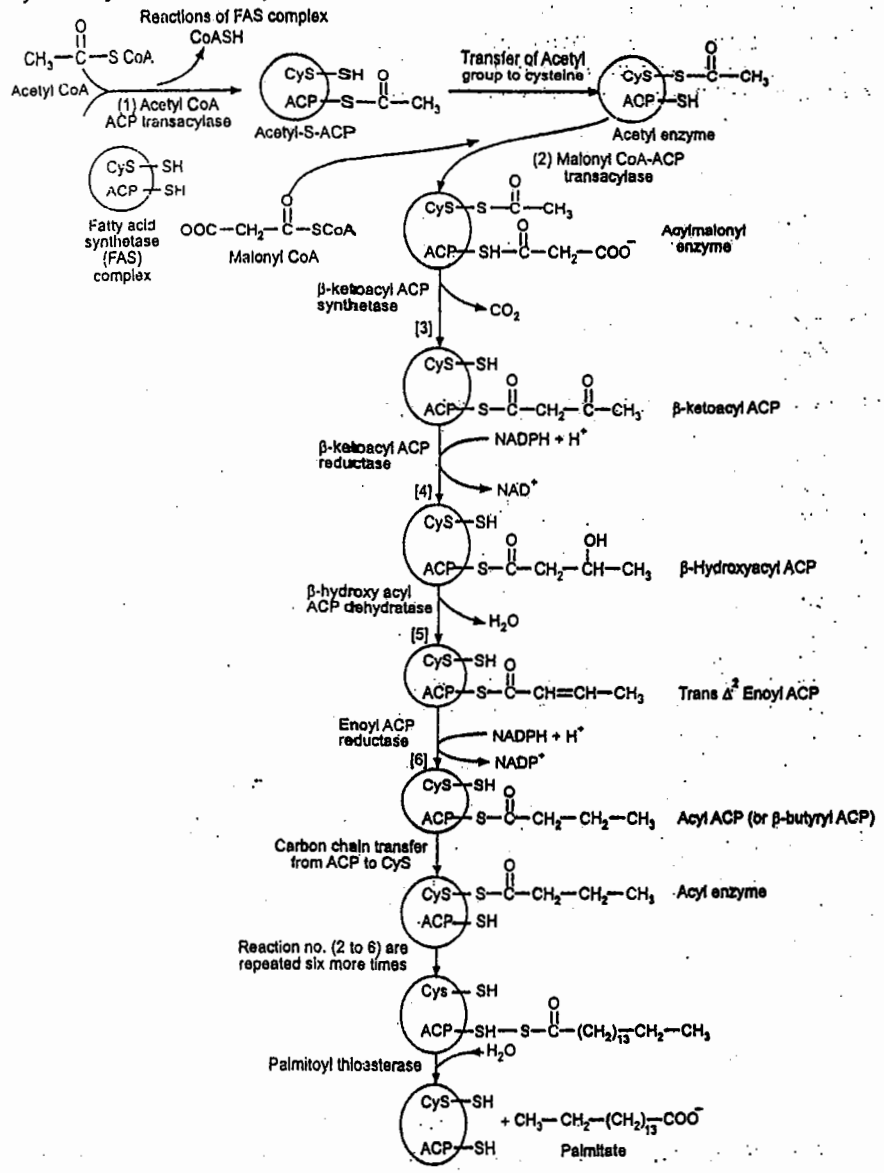


Fig. 8.13: Fatty acid synthetase complex reaction showing biosynthesis of long chain fatty acid-palmitate

A 16 carbon containing saturated molecule of palmitate is formed at the end of 7th cycle. This completes the fatty acid synthesis of FAS complex.

The 7th enzyme of FAS complex, palmitoyl thioesterase separates the newly formed molecule of palmitate from the FAS complex.

8.14.3 The Overall Reaction for Palmitate Synthesis

The overall reaction for synthesis of palmitate from acetyl CoA can be written into two parts:

I. Formation of seven malonyl CoA molecule.

$$7 \text{ acetyl CoA} + 7\ CO_2 + 7ATP \longrightarrow 7 \text{ malonyl CoA} + 7\ ADP + 7Pi$$

II. Then seven cycles of condensation and reduction

$$\text{Acetyl CoA} + 7 \text{ malonyl CoA} + 14\ NADPH + 14\ H^+ \longrightarrow \text{Palmitate} + 7CO_2 + 8\ CoA + 14\ NADP^+ + 6H_2O$$

The overall reaction can be written as:

$$8 \text{ Acetyl CoA} + 7\ ATP + 14\ NADPH + 14H^+ \longrightarrow \text{Palmitate} + 8\ CoA + 6H_2O + 7ADP + 7Pi + 14\ NADP^+$$

8.14.4 Importance of FAS Complex in Lipid Biosynthesis

1. The multienzyme complex of FAS is coded by one gene.
2. Being in one complex, all enzymes are easily available without any biochemical permeability problems and work with greater efficiency.

8.14.5 Regulation of Fatty Acid Biosynthesis

Number of factors including hormones, dietary lipids, other metabolites, enzymes and end products regulate fatty acid biosynthesis.

1. **Allosteric Regulation:** The reaction catalysed by acetyl CoA carboxylase is controlled allosterically. It is the rate limiting step of fatty acid biosynthesis.
   The enzyme exists as an inactive monomer or active polymer. The palmitoyl CoA, malonyl CoA promote monomer formation and thus inhibits fatty acid biosynthesis. Whereas, citrate promotes polymer formation and increases fatty acid synthesis.
2. **Hormonal Regulation:** A mechanism of hormonal regulation of acetyl CoA carboxylase is different from the one mentioned above.

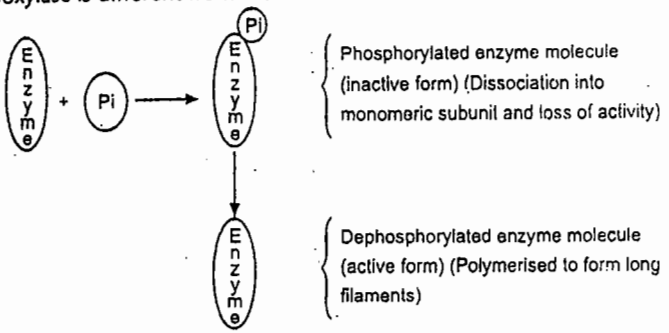


Fig. 8.14: Hormonal regulation

A cAMP dependent phosphorylation inactivates the enzyme. This inactivation is brought about by hormones like epinephrine, norepinephrine, glucagon. Whereas insulin dephosphorylates the enzyme thus activating it.

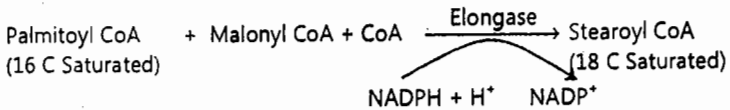
3. **Dietary Lipids:** High fat diet and fasting conditions decrease fatty acid biosynthesis by reducing the synthesis of the two enzymes, namely:
   1. Acetyl CoA carboxylase.
   2. Fatty acid synthetase.
4. **NADPH:** The reactions of fatty acids synthesis require NADPH as reducing equivalents which are obtained either from HMP or from citrate transport.

The decrease in the cellular concentration of NADPH affects the fatty acid synthesis negatively.

8.15 CHAIN ELONGATION OF FATTY ACID (SYNTHESIS OF LONG CHAIN FATTY ACIDS FROM PALMITATE)

Palmitate happens to be the main product of cytosolic fatty acid synthetase system in the eukaryotic cell. It is the precursor of other long chain fatty acids (saturated or unsaturated).

Chain elongation of palmitate molecule can take place in smooth endoplasmic reticulum or in mitochondria by a different mechanism. Group of enzymes known as elongase bring about fatty acid elongation. In the more active chain elongation, there is successive addition of malonyl CoA in the presence of NAPDH.



The reaction mechanism is similar to palmitoyl CoA formation involving:

1. Donation of 2 carbon by malonyl ACP
2. Reduction
3. Dehydration
4. Reduction

8.16 FATTY ACID DESATURATION

Palmitate and stearate are the most common prerequisite precursors of the fatty acid desaturation system.

Desaturation is carried out by the microsomal enzyme fatty acyl CoA desaturase. The reaction requires molecular O_2, NADH and flavin dependent cytochrome 5 reductase. The monounsaturated fatty acids synthesized are as follows:

Stearic acid → Oleic acid

Palmitate → Palmitoleic acid

Essential Fatty Acids

Linoleic acid (18 : 2; 9, 12) and linolenic acid (18 : 3; 9, 12, 15) are essential fatty acids for humans. This is because they lack the enzymes which can introduce double bond beyond carbon atom no. 9 and 10. Hence, these must be obtained from plant material in the diet. However, (20 : 4; 5, 8, 11, 14) arachidonic acid represents a semiessential fatty acid as it can be synthesized from the linoleic acid after the desaturation and chain elongation.

8.7 TRIGLYCEROL BIOSYNTHESIS

Most of the dietary fatty acids and those synthesized by an organism have one of the two fates:

1. Incorporation into triglycerol for the storage of metabolic energy.
2. Incorporation into phospholipid components of the membrane.

Sites of triglycerol synthesis are liver and adipose tissue. The amount of triglycerol synthesis is very less in other tissues.

The total amount of the stored triglycerol in a 70 kg human is about 15 kg. Triacylglycerol is transported around the body in the form of large lipid-protein particles called lipoproteins.

Synthesis of Triacylglycerol

Triacylglycerol is synthesized from fatty acyl CoA and glycerol-3-phosphate.

The actual synthesis begins with the reduction of glycolytic intermediate - dihydroxy acetone phosphate to glycerol-3-phosphate.

The glycerol-3-phosphate is then acylated by glycerol-3-phosphate acyl transferase to form lysophosphotidic acid. This in turn reacts with another acyl CoA molecule to form phosphotidic acid.

The next step is the removal of phosphate group from phosphotidic acid. It gives rise to a compound known as diacylgycerol. The diacylglycerol is then acetylated with the help of the third acyl CoA molecule to form triacylglycerol. (Fig. 8.15).

The triacylglycerol biosynthesis is carried out at the cost of energy released by the cleavage of high energy thioester bond between the acyl moiety of CoA.

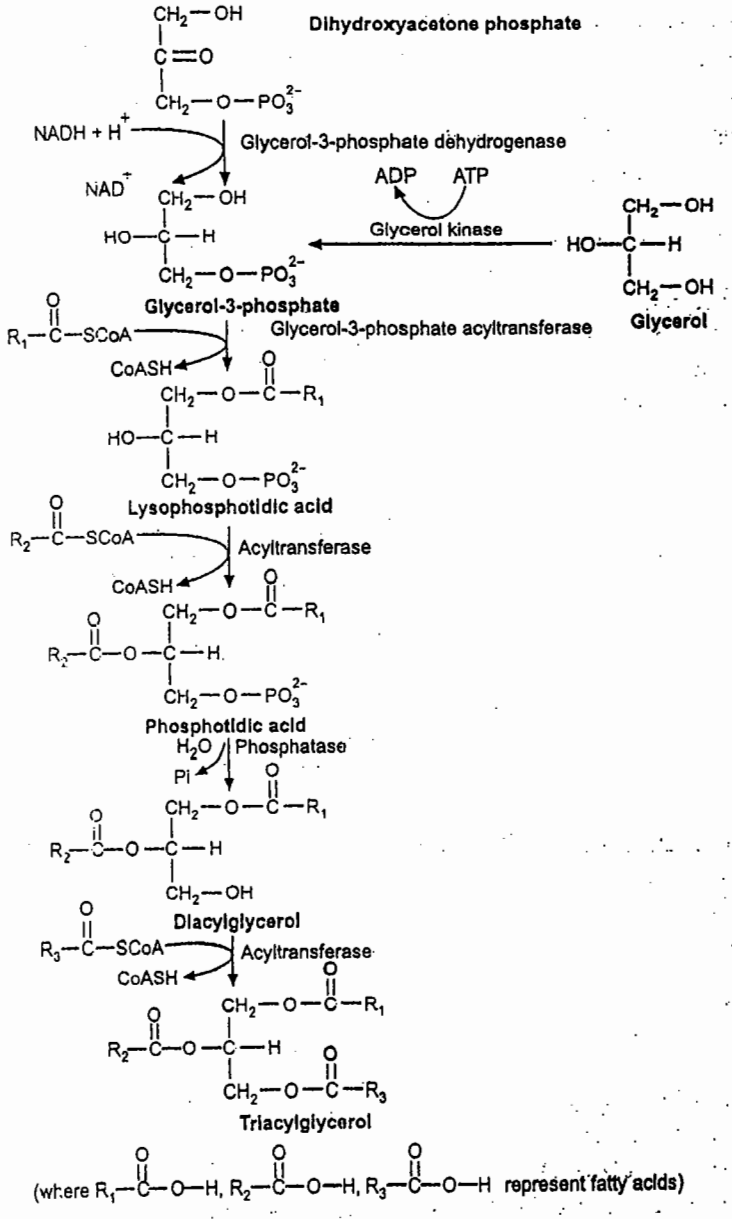


Fig. 8.15: Triacylglycerol biosynthesis

8.17.1 Synthesis of Triacylglycerol in Liver

The synthesis of triacylglycerol in the liver begins with the phosphorylation of the glycerol with the help of enzyme glycerol kinase. This enzyme is unique to the liver.

8.17.2 Regulation of Triacylglycerol Biosynthesis

Insulin stimulates the conversion of dietary carbohydrates and proteins into fat. In diabetic patients (untreated), acetyl CoA obtained from catabolism of carbohydrates and proteins is channeled towards ketone bodies production. This condition occurs due to lack of insulin.

Insulin stimulates the formation of triacylglycerol by decreasing the level of cAMP. cAMP promotes the dephosphorylation and inactivation of hormone sensitive lipase (which breaks down triacylglycerol).

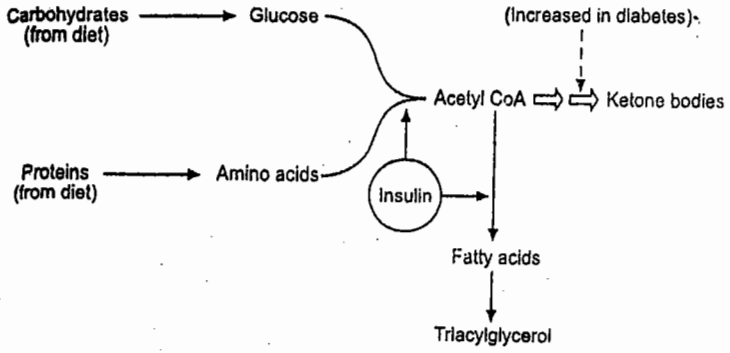


Fig. 8.16: Regulation of triacylglycerol biosynthesis

Insulin plays an important role in the regulation of triacylglycerol biosynthesis.

CHOLESTEROL METABOLISM

Cholesterol is a component of the cell membrane and is also a precursor of steroid hormones and the bile acids.

Structure of cholesterol contains 27 carbons. All of its carbon atoms are provided by acetate.

Cholesterol synthesis thus requires ATP, NADPH and acetyl CoA.

Cholesterol synthesis takes place in almost all tissues of the body. The cellular site is cytosol and microsomes.

Konrad Bloch deserves a special mention for elucidating the origin of the carbon atoms in the entire molecule of cholesterol (1940).

Biosynthesis of Cholesterol:

Series of reactions take place in the *de novo* synthesis of entire molecule of cholesterol. All 27 carbon atoms of cholesterol are derived from the acetyl CoA.

In the beginning, two moles of the acetate combine with each other to form acetoacetyl CoA. To the acetoacetyl CoA molecule, one more molecule of acetyl CoA is added. This gives rise to HMG CoA (3 hydroxy, 3 methyl glutacyl CoA). This cytosolic HMG CoA is utilized for the cholesterol biosynthesis. (Recall that HMG CoA produced in the mitochondria is utilized for the ketone bodies synthesis).

HMG CoA is then reduced to mevalonate by HMG CoA reductase. Mevalonate formation is the committed step in cholesterol biosynthesis and is the key control point. Mevalonate is converted into 3-isopentenyl pyrophosphate, with the help of the three consecutive reactions requiring ATP. CO_2 is released in the last reaction.

The IPP (isopentenyl pyrophosphate) isomerises to dimethyl pyrophosphate (DPP). IPP and DPP are 5-C isoprenoid units.

IPP and DPP condense to produce 10 C geranyl pyrophosphate (GPP). One more molecule of IPP condenses with GPP to form 15 C farnesyl pyrophosphate (FPP). Two units of farnesyl pyrophosphate condense and get reduced to produce a 30 carbon compound squaline.

Squaline is then converted into squaline epoxide in a reaction that uses O_2 and NADPH. The squaline epoxide cyclises to form a lanosterol. Finally, three methyl groups are removed from the lanosterol along with the reduction of one double bond by NADPH and shift of one double bond to form a molecule of cholesterol. (Fig. 8.17).

8.18.1 Regulation of Cholesterol Biosynthesis

Biosynthesis of cholesterol is controlled by the HMG CoA reductase (the rate limiting enzyme of the pathway). Many biochemical metabolic controls participate in the control of HMG CoA reductase.

1. **Feedback regulation of HMG CoA reductase:** Increase in the cellular concentration of cholesterol, the end product of the pathway, inhibits the key enzyme HMG CoA reductase. The synthesis of HMG CoA reductase is actually reduced at the genetic level. Both transcription and translation of the gene (responsible for HMG CoA reductase) are reduced.
2. **Regulation at hormone level:** Enzyme HMG CoA reductase is inactivated by phosphorylation by cAMP activated protein kinase.

   Hormones, namely glucocorticoids and glucagon stimulate the formation of inactive HMG CoA reductase. This way, cholesterol synthesis is decreased. Hormones like thyroxine and insulin enhance the formation of active form of enzyme HMG CoA reductase (dephosphorylated form). This increases the rate of cholesterol synthesis.
3. **Therapeutic inhibition:** Administration of the fungal compounds lovastatin (mevinolin) and compactin which competitively inhibit the enzyme thus ultimately decrease cholesterol biosynthesis.

   Therefore, these compounds are used in the clinical practice for the treatment of the hypercholesterolmia (high levels of blood cholesterol).
4. **Control by increasing rate of degradation:** HMG CoA reductase activity is also controlled by increasing the rate of its degradation.

Reactions of Cholesterol Biosynthesis

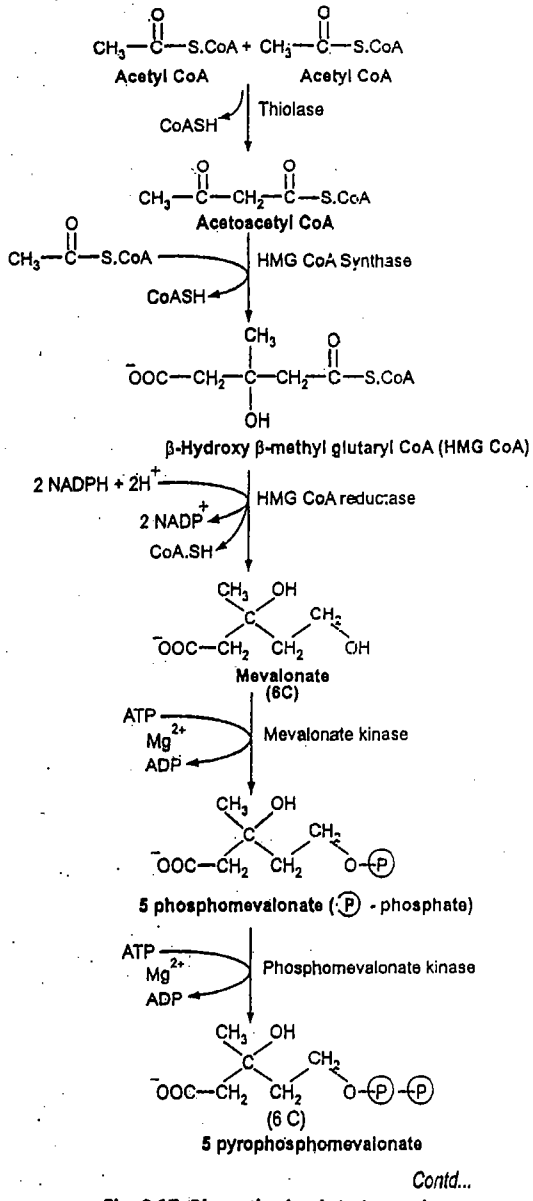


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Fig. 8.17: Biosynthesis of cholesterol

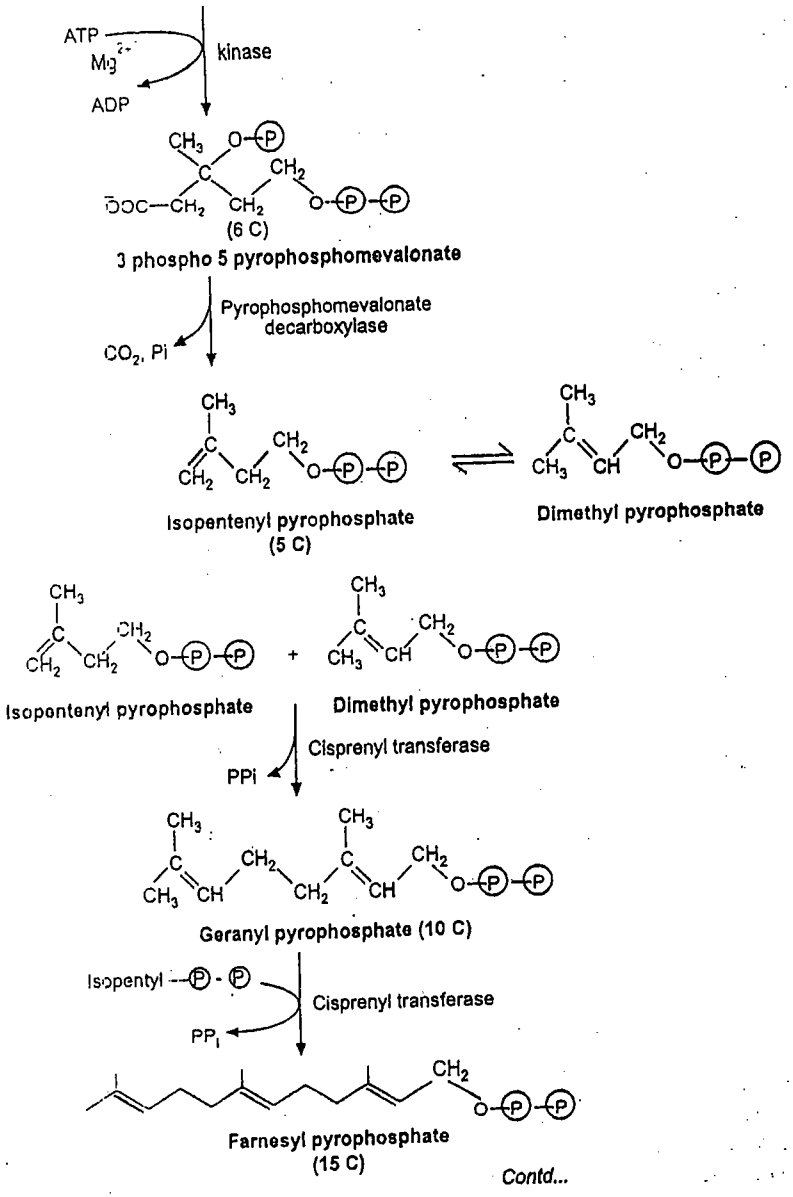

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Fig. 8.17: Biosynthesis of cholesterol

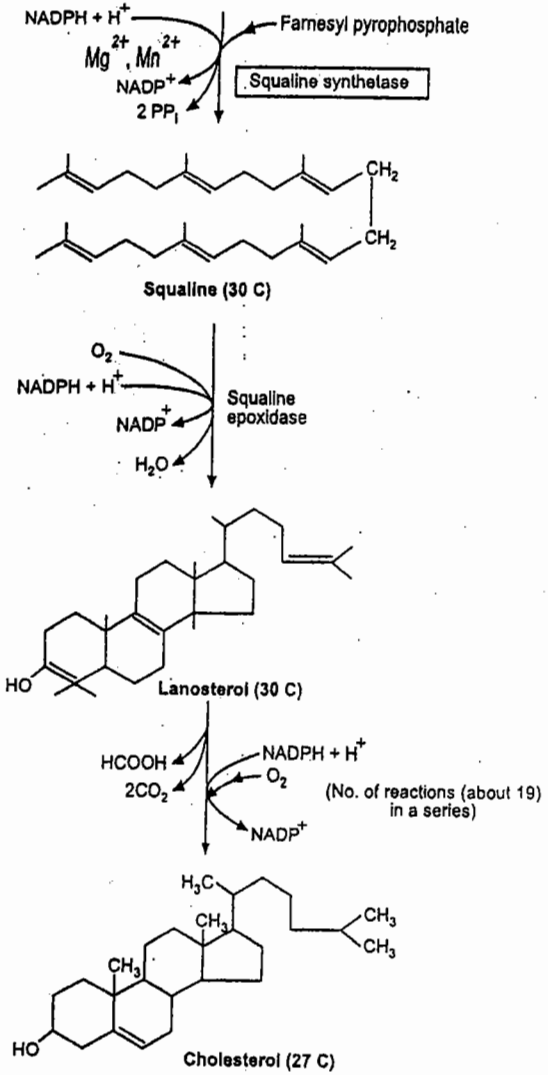


Fig. 8.17: Biosynthesis of cholesterol

8.18.2 Cholesterol as a Precursor

1. Cholesterol is the precursor of five major classes of steroid hormones, namely:
   (i) Glucocorticoids (ii) Progestins (iii) Mineralocorticoids (iv) Androgens (v) Estrogens
2. Vitamin D is synthesized from 7-dehydrocholesterol by the action of the UV component of the sunlight on the skin. UV component brings about the rearrangement of the double bonds of the molecule to form provitamin D_3. This molecule undergoes spontaneous isomerisation to form vitamin D_3 (cholecalciferol). The subsequent two different hydroxylation reactions are accomplished in the liver and kidney to produce calciferol, the active hormone.
3. Bile acids (or bile salts) are polar derivatives of cholesterol and form a major pathway for the excretion of cholesterol in mammals.

8.18.3 Biochemical Importance of Cholesterol

Cholesterol belongs to the class of molecules known as lipids or fats. Cholesterol is essential for normal cell function and hormonal balance. Cholesterol is also required for the absorption of fat soluble vitamins from food. Cholesterol is also required for the production of bite acids, which helps in the digestion of fats. Cholesterol is biosynthetic precursor of bile acids.

In humans and animals, cholesterol is the important constituent of the cell membrane. Cholesterol influences the physical properties of membrane proteins, which in turn modulate functions of membrane protein receptors. Cholesterol is precursor of steroid hormones and bile acids. Intermediate of cholesterol biosynthesis are required to make vitamin D and also for post translational modifications of membrane proteins.

High concentration of plasma cholesterol promotes atherosclerosis.

Steroid hormones like adrogens, estrogens, projestins, calfiferol, glucocortecoids, mineralocorticoids are produced from the cholesterol molecules.

A 70 kg normal healthy adult human contains serum cholesterol in the range of 150-250 mg/dl. In blood, cholesterol is found in different fractions of lipoproteins, namely HDL, VLDL, LDL etc.

Cholesterol levels increase with age. Especially in women, the levels increase after menopause.

Different fractions of cholesterol have clinical importance. Elevation of LDL cholesterol is not beneficial to body. As it may lead to clinical complications like atherosclerosis or other coronary heart diseases (CHD). On the contrary, elevation of the plasma HDL-cholesterol is beneficial to body, since it protects the body from coronary heart diseases including atherosclerosis.

8.18.3.1 Factors Affecting Blood Cholesterol Level

1. **HMG coenzyme reductase:** This enzyme participates in the beginning of the pathway involving synthesis of cholesterol in the liver. There is marked decrease in the activity of the enzyme during fasting which explains reduced synthesis of cholesterol and associated decrease in the blood cholesterol levels.
2. There is a feedback mechanism by which HMG coenzyme reductase in the liver is inhibited by mevalonate as well as cholesterol itself. Mevalonate is the immediate precursor and cholesterol is the final product.
3. **Hypercholesterolemia:** Increase in plasma cholesterol concentration (above 250 mg/dl) is known as hypercholesterolemia. It is a clinical sign of many disorders.
   - **Hypothyroidism:** Hypercholesterolemia in hypothyroidism is generally observed due to decrease in the HDL receptors on hepatocytes. Owing to which, cholesterol is channeled towards the blood.

- **Nephrotic syndrome:** This disorder is characterised by an increase in the plasma globulin fraction. Cholesterol elevation is the consequence of the elevated plasma lipoproteins observed in this disorder.
- **Obstructive jaundice:** In this disorder, elevation in the plasma cholesterol occurs due to obstruction of the cholesterol excretion through bile.
- **Diabetes mellitus:** Since the concentration of the acetyl CoA increases in diabetes, cholesterol synthesis also increases leading to hypercholesterolemia.

4. **Consumption of dietary fibres:** Vegetable fibres in the diet decrease the cholesterol absorption from intestine.
5. **Dietary Poly Unsaturated Fatty Acids (PUFA)** in the diet is found to reduce the plasma cholesterol levels.
   It is hypothesized that PUFA stimulate the transport of cholesterol by LCAT mechanism and its excretion from the body.
   PUFA deserves a special mention in the stimulation of cholesterol excretion into the intestine and stimulation of oxidation of cholesterol into bile acids.
6. **Carbohydrate rich diet,** precisely sucrose rich diet is responsible for the hypercholesterolemia.
7. **Drugs:** Several drugs are known to act at various stages in the biosynthetic pathway of cholesterol.
   - The fungal inhibitors of HMG-CoA reductase e.g. mevastatin and lovostatin, decrease the cholesterol synthesis and thus plasma cholesterol levels.
   - Drugs like clofibrate and gemfibrozi decrease the plasma cholesterol and triglycerol levels by stimulating the activity of the lipoprotein lipase
   - Colestipol and cholestyramine resin bird with bile acids and make it unavailable for intestinal reabsorption. Due to feedback mechanism more and more cholesterol is converted into bile acids, which are ultimately excreted.
   - Nicotinic acid: It acts, in part, via decrease in the release of free fatty acids from adipose tissue, thereby decreasing the influx of free fatty acids into the liver, the hepatic reesterification of free fatty acids and the rate of production of hepatic very low-density lipoprotein (VLDL). The mechanism by which nicotinic acid elevates HDL is unknown.
8. **Surgical treatment:** Hypercholesterolemia may be surgically treated. The procedure causes the block in the reabsorption of bile acids.

8.18.4 Catabolism of Cholesterol

Cholesterol is metabolised in a different manner. About half of the body cholesterol is converted into bile acids. It is secreted in bile and finally excreted in faeces. Remaining amount of cholesterol is utilized for the synthesis of the vitamin D and steroid hormones.

Cholesterol and Bile Acid Synthesis:

Many cell types in the body possess several active enzymes for the bile acid synthesis. However complete biosynthesis of bile acids occurs only in liver. Synthesis of bile acids is one of the predominant mechanisms of the excretion of excess cholesterol. However total amount of cholesterol cannot be excreted through bile in the from of bile acids.

Synthesis of Bile Acids and Catabolism of Cholesterol: Most abundant bile acids in the human bile juice are chenodeoxycholic acid (45%) and cholic acid (31%). These two are primary bile acids. Bile acids before their secretion into canalicular lumen, are conjugated with either amino acid glycine or taurine (the amide bond formation takes place between terminal carboxyl group of bile acid and amino group of the amino acid) to form the glycocholic acid and taurocholic acid respectively. The sodium and potassium salts of these conjugated bile acids are called as bile salts.

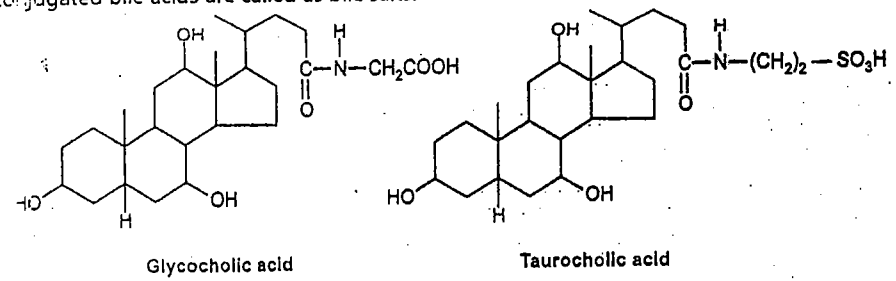


Fig. 8.18: Conjugated bile acids

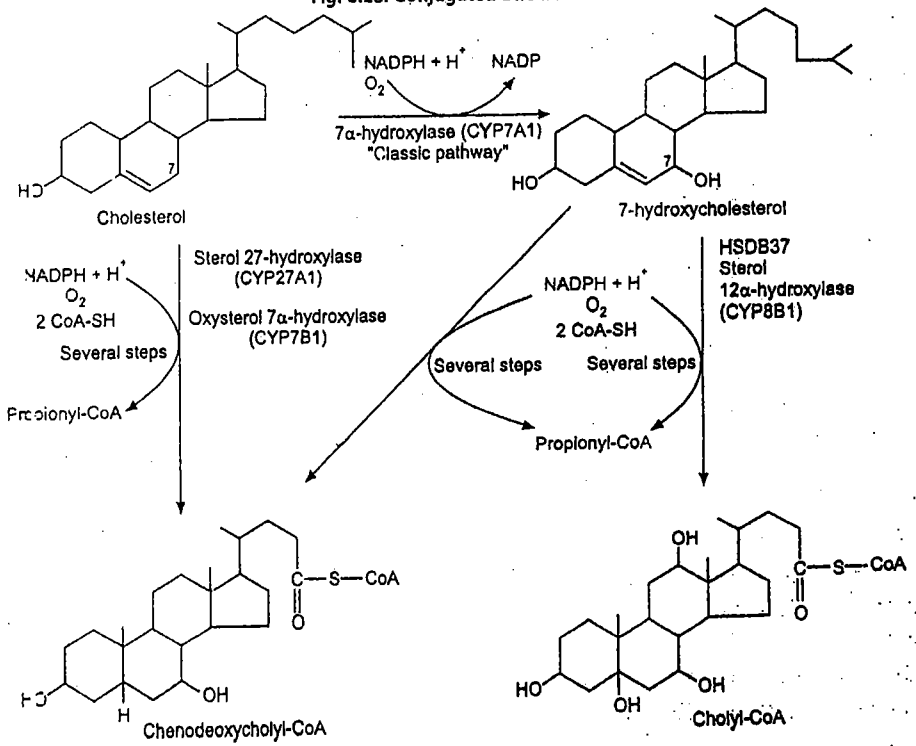


Fig. 8.19: Detailed account of bile acid synthesis and metabolism

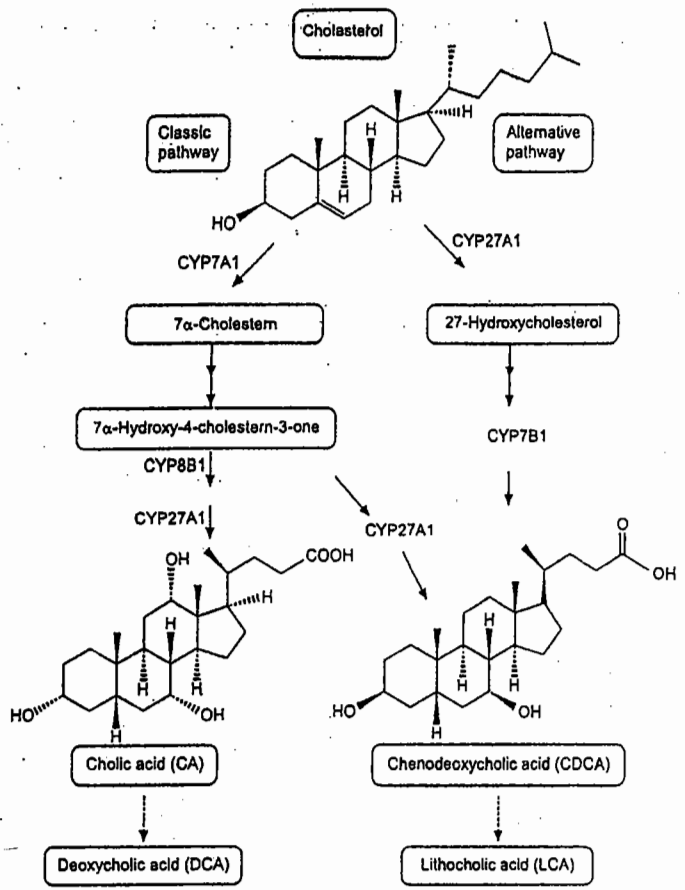


Fig. 8.20: Bile acid synthesis and metabolism

The reaction catalyzed by the 7α hydroxylase is the control point or rate limiting step in bile and synthesis. The enzyme 7α hydroxylase is expressed only in liver. Sterol 12α hydroxylase is rate limiting step for the synthesis of cholic acid and its production is controlled at the level of transcription.

Two major pathways are involved in the synthesis of bile acids. The neutral or classic pathway is controlled by CYP7A1 in the endoplasmic reticulum. The alternative or acidic pathway is initiated by sterol CYP27A1 in mitochondria CYP8B1 is required to synthesize CA. Oxyterol 7 hydroxylase (CYP7B1) is involved in the formation of CDCA in the acidic pathways. The neutral pathway can also from CDCA by CYP27A1. Primary bile acids are metabolized by gut bacteria to form secondary bile acids, DCA and LCA.

8.18.5 Atherosclerosis

The disease is characterised by the hardening of the arteries because of the cholesterol rich arterial thickening (atheroma is the important feature of the disease).

The onset of the disease occurs over the period of a time. There is an intracellular deposition of the lipids. Deposition is specifically observed in the smooth muscle cells of the arterial wall.

Progressively, depositions become fibrous and calcified. The plaques so formed ultimately block the artery. The blocks in the artery deprive the related tissues of oxygen and nutrient's supply.

If the blockage occurs in the coronary arteries i.e. arteries supplying blood to the heart, then it culminates into myocardial infarction or heart attack.

8.18.6 Familial Hypercholesterolemia

It represents an inheredited disorder. The individuals suffering from the familial hypercholesterolemia show extremely elevated levels of cholesterol in their blood. The afflicted individual develops severe atherosclerosis in childhood. Increase in the blood cholesterol eventually gives rise to the formation of atheroma in the blood vessel which can cause death of the person due to the pathological conditions of myocardial infarction.

The lack of functional LDL receptor is the biochemical defect. The tissues are unable to take up the blood cholesterol. This gives rise to high concentration of the blood cholesterol and formation of atherosclerotic plaques.

The individuals suffering from the hypercholesterolemia can be treated with the hypolipidemic drugs like lovastatin, compactin, clofibrate etc. Drugs like cholestipol and cholestyramine bind with the bile acids and reduce their reabsorption. Clofibrate increases the activity of lipoprotein lipase and reduces blood cholesterol and triglyceride.

Hypercholesterolemia is characterised by increase in the plasma cholesterol above 250 mg/dl. It is also observed in the pathological conditions like hypothyroidism, nephrotic syndrome and diabetes mellitus.

In addition to drugs, plasma cholesterol can also be controlled by the factors like:

1. Dietary fibre in the diet.
2. Avoiding high carbohydrate diet.
3. Consuming polyunsaturated fatty acids in the diet.

8.18.7 Hypocholesterolemia

In pathological conditions like pernicious anaemia, malabsorption syndrome, hyperthyroidism, the individuals are found to suffer from the hypocholesterolemia.

Disorders of Lipid Metabolism:

Fatty Liver (Sleatosis)

A condition wherein there is abnormal accumulation of fats (triglycerides) inside liver cells. The liver triglycerol synthesis provides the quick stimulus for the formation and secretion of VLDL. Abnormal VLDL formation or secretion leads to non-mobilization of lipid components from the liver, resulting in fatty liver.

The causes of fatty liver include the imbalance between the rate of triglyceride formation and its export from liver. There are different reasons responsible for accumulation of lipids in the liver. Expansive accumulation is considered as the pathological condition. When the accumulation of the lipids in the liver becomes continuous process (a chronic condition), fibrotic changes occur in the liver that proceed to cirrhosis condition and creates problems in the normal functioning of liver occur.

Fatty liver falls into the main categories:

(A) More synthesis of triglycerides

(B) Defective VLDL synthesis

(A) More synthesis of triglycerides

There is increased synthesis of triglycerides in the liver due to more availability of fatty acids and glycerol. The source of fatty acids to triglyceride synthesis is of the two types:

(i) Fatty acids from diet.

(ii) From acetyl CoA, which is a derivative of carbohydrate metabolism.

Thus, high carbohydrate diet triggers the denovo synthesis of fatty acids by providing excess of acetyl CoA and high fat diet provides increased influx of fatty acids from the diet that can be esterified with glycerol to provide excess triglycerides.

(B) Defective VLDL synthesis

This type of fatty liver occurs due to metabolic block in the production of plasma lipoproteins, causing accumulation of triclycerides in the liver.

The theoretical reasons for the defective VLDL synthesis are:

(i) Block in apolipoprotein synthesis.

(ii) Failure to provide the phospholipids that are part of lipoprotein (VLDL).

(iii) Defective glycosylation of lipoprotein by arotic acid (can be observed in orotic aciduria – a defect in pyrimidine nucleotide biosyntehsis).

(iv) Impaired secretion of VLDL-in oxidative stress causing membrane disruption of lipoproteins.

Obesity:

Obesity can be defined in the context of biochemistry as 'a disorder of body weight regulatory systems, which causes accumulation of excess body fat'.

Obesity is associated with the following pathological conditions:

(i) Hypercholesterolemia	(ii) Diabetes mellitus
(iii) High plasma triglycerides	(iv) Hypertension
(v) Heart diseases	(vi) Cancer
(vii) Gall stones	(viii) Gout
(ix) Arthritis	(x) Mortality

Risk of hypertension, insulin resistance, diabetes, dislipidimia and coronary heart diseases are generally observed in obese people. Due to increase in fat intake, adipocytes increase in size and expand and divide. Fat cells once gained are never lost. Reduction in weight causes reduction in size of adepocyte. This explains, why gaining weight is easier after loosing.

The energy imbalance, where calories intake is not equal to calories utilized is one of the major cause of obesity. The factors like genetics, individual behaviour, social interactions and environmental reasons are also responsible in different cases of obesity.

Hormonal control over obesity is due to three hormones secreted by adipocytes, namely – Leptin, adiponectin and resistin. Adiponectin and resistin cause insulin resistance observed in obesity. The protein hormone Leptin secreted in adipocyte is responsible to keep body weight under control. Leptin causes increase in metabolic rate and decrease appetite in humans.

Metabolic changes in obesity include Dyslipidemia – which can be defined as elevation of plasma cholesterol, triglycerides, or both, or low high-density lipoprotein level that contributes to development of atherosclerosis.

8.18.8 Role of LCAT (Lecithin Cholesterol Acyl Transferase) in Lipid Metabolism

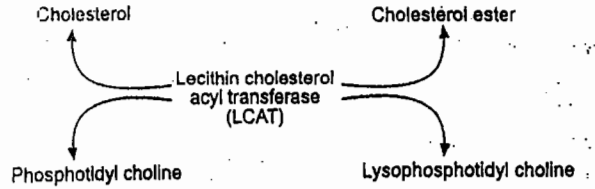


Fig. 8.21

8.18.8.1 Reaction Catalysed by LCAT

Lecithin cholesterol acyltransferase, an enzyme synthesized in the liver is responsible for the transport and elimination of cholesterol from the body. HDL or High density lipoprotein is also responsible for the transport and the elimination of cholesterol from the body.

Plasma enzyme LCAT catalyses the transfer of fatty acid from the second position of phosphotidyl choline (Lecithin) to the hydroxyl group of cholesterol. HDL cholesterol is the actual substrate for the LCAT. The reaction carried out by the LCAT is a reversible reaction. Cholesterol present in the periferal tissues is taken up in HDL with the help of reaction catalysed by the LCAT. Then it reaches to liver for degradation and excretion. The activity of LCAT is engaged in apo-A_1 of HDL.

8.19 PHOSPHOLIPID METABOLISM

The metabolism of phospholipid occurs in all tissues of the body. Efficient biosynthesis of phospholipids occurs in the brain, intestine and liver.

Exact cellular site of phospholipid biosynthesis is the smooth endoplasmic reticulum.

1. **Phosphotidyl Serine:** Ethanolamine head group of the phosphotidyl ethanolamine exchange with a free serine molecule to produce phosphotidyl serine.

   Decarboxylation of the phosphotidyl serine gives rise to phosphotidyl ethanolamine.

2. **Phosphotidyl Inositol (PI):** A phosphotidic acid produces CDP diacyl glycerol. The latter compound can combine with inositol to form phosphotidyl inositol. The arachidonic acid in the phosphotidyl inositol is the source and precursor of prostaglandin synthesis.

3. **Lecithin and Cephalin:** The nitrogen bases, namely choline and ethanolamine are first phosphorylated and afterwards, they combine with CTP to form respectively - CDP - choline and CDP - ethanolamine.

   CDP - ethanolamine + 1, 2, diacyl glycerol ⟶ Phosphotidyl ethanolamine

   CDP - choline + 1, 2, diacyl glycerol ⟶ Phosphotidyl choline

   The Interconversion of Lecithin and Cephalin:

   Phosphotidyl ethanolamines $\xrightarrow{\text{methylation}}$ Phosphotidyl choline

   Cardiolipin: The CDP diacyl glycerol combines with the glycerol-3-phosphate to form phosphotidyl glycerol which in turn condenses with another molecule of CDP diacylglycerol to form cardiolipin.

   Plasmalogen: Dihydroxyacetone phosphate combines with the acyl CoA to form 1-acyl dihydroxy acetone phosphate. This compound undergoes a series of reactions shown in Fig. 8.22 to form 1-alkenyl 2 acylglycerol 3 phosphoethanolamine (plasmalogen).

   Sphingomyelins: Sphingomyelins are made up of phosphoric acid, fatty acid, choline and sphingosine. A sphingosine is a complex amino alcohol.

A molecule of sphingosine is acylated to produce ceramide. Ceramide further combines with the CDP-choline to produce sphingomyelin.

8.20 SPHINGOMYELIN SYNTHESIS

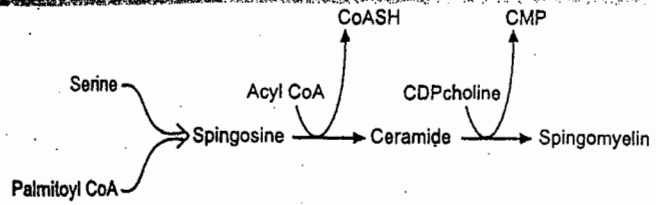


Fig. 8.22: Flowchart of sphingomyelin synthesis

8.21 PLASMALOGEN BIOSYNTHESIS

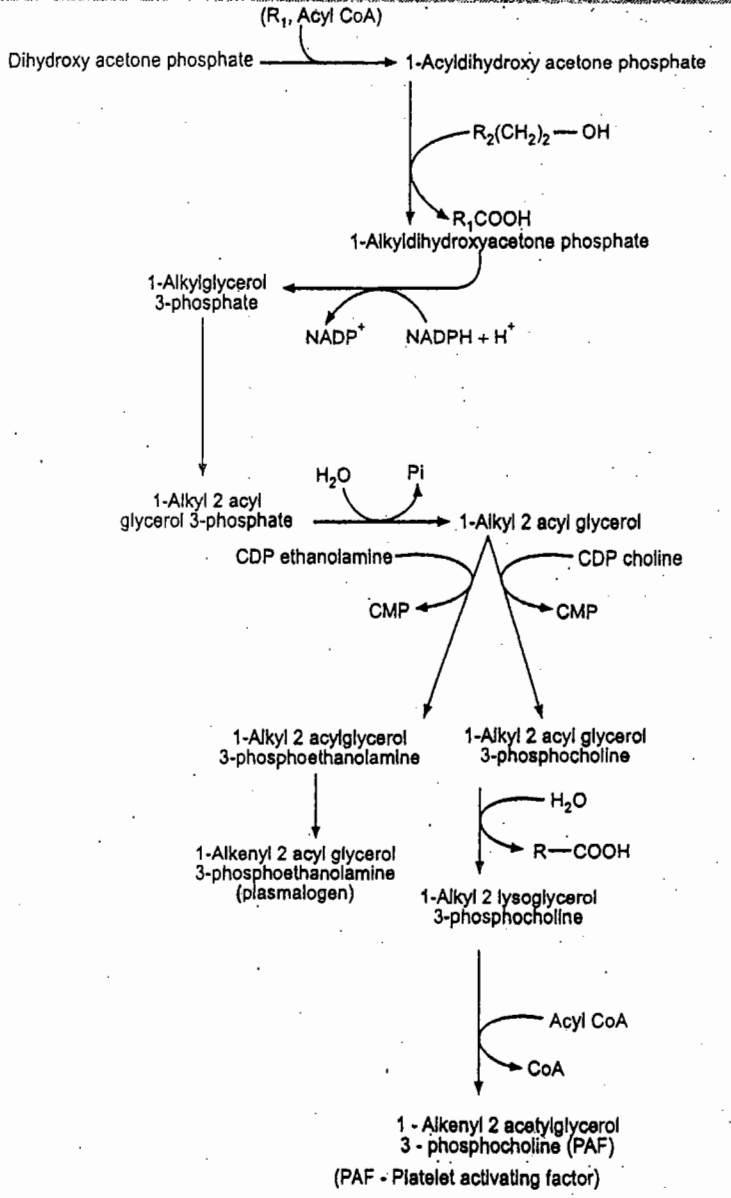
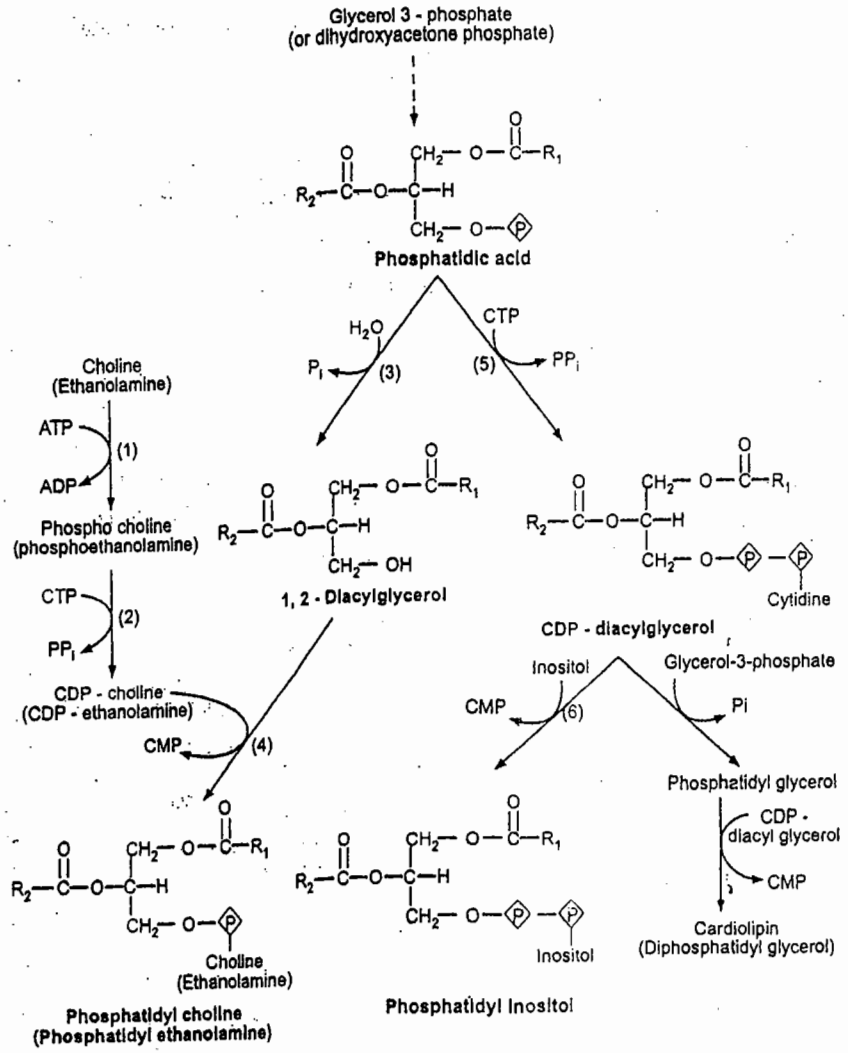


Fig. 8.23: Plasmalogen - biosynthesis

8.22 BIOSYNTHESIS OF PHOSPHOLIPIDS



1. Choline kinase, 2. Phosphocholinecytidyl transferase, 3. Phosphatidase phosphohydrolase, 4. Phosphocholine diacylglycerol transferase, 5. CTP phosphotidate cytidyl transferase, 6. CDP - Diacylglycerol Inositol transferase

Fig. 8.24: Phospholipid biosynthesis

8.23 CATABOLISM OF PHOSPHOLIPIDS

Group of enzymes known as phospholipases degrade the phospholipids. Phospholipases breakdown phosphodiester bond in phospholipids.

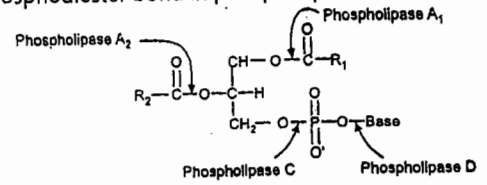


Fig. 8.25: Various sites of action of phospholipases

Various Sites of Action of Phospholipases:

Phospholipase A_1: This enzyme is present in *Penicillium notatum, Aspergillus oryzae* and attacks the phosphodiester linkage at the position 1 of the phospholipid giving a product lysophospholipid. The lysophospholipid is acted upon by lysophospholipase.

Phospholipase A_2: This enzyme brings about the hydrolysis of fatty acid at the C_2 position of the phospholipid. Bee venom and snake venom are rich sources of the phospholipase A_2. Enzyme is also found in many tissues and pancreatic juice.

Phospholipase C: This enzyme is found in the *Clostridium welchi* and in lysosomes of hepatocytes. It specifically splits phosphoryl choline from phospholipids to produce 1, 2, diacylglycero .

Phospholipase D: The enzyme is found in various plant sources like cotton seed and cabbage.

The enzyme catalyses the removal of nitrogenous base from phospholipids.

Various products of the phospholipids enter the metabolic pool and are utilized as and when required.

8.24 CATABOLISM OF SPHINGOMYELINS

Lysosomal enzyme, sphingomyelinase catabolise the sphingomyelins to ceramide and phosphotidyl choline. Degradation of ceramide produces sphingosine and free fatty acids.

Faber's Disease:

A defect in the enzyme ceramidase is responsible for the Faber's disease. Clinical signs of the disorder are subcutaneous nodules, skeletal deformation, dermatitis, mental retardation. It is fatal in early life.

Niemann Pick Disease:

This inherited disorder is due to the defect in the enzyme sphingomyelinase. Disease is characterised by the accumulation of the large pale cells or Niemann-pick cells containing sphingomyelins in liver, kidney, spleen and lung. This causes enlargement of the organs. Victims of Niemann pick disease suffer from mental retardation and death in early childhood. Several clinical features are common with Gauchers disease.

8.25 GLYCOLIPID METABOLISM (GLYCOSPHINGOLIPIDS)

Glycolipids contain ceramide. The ceramide is in turn formed from the fatty acid bound to the sphingosine. Presence of carbohydrate in the glycolipid (generally galactose) is responsible for their nomenclature as glycolipid.

Glycolipids are also known as cerebrosides.

Cerebroside is the common name for a group of glycosphingolipids called monoglycoceramides which are important components in animal muscle and nerve cell membranes.

Synthesis of galactosyl ceramide and glucosylceramide occurs in endoplasmic reticulum and on cytosolic side of the early Golgi membranes respectively.

Glycolipids are part and parcel of the cerebral lipids. Some of the examples of them are:

1. Glucocerebrosides 2. Galactocerebrosides.

Glucocerebrosides are formed during the course of the metabolism of complex glycosphingolipids. The galactocerebroside is a major fraction of the nervous tissue membrane.

8.26 METABOLISM OF GLYCOLIPIDS

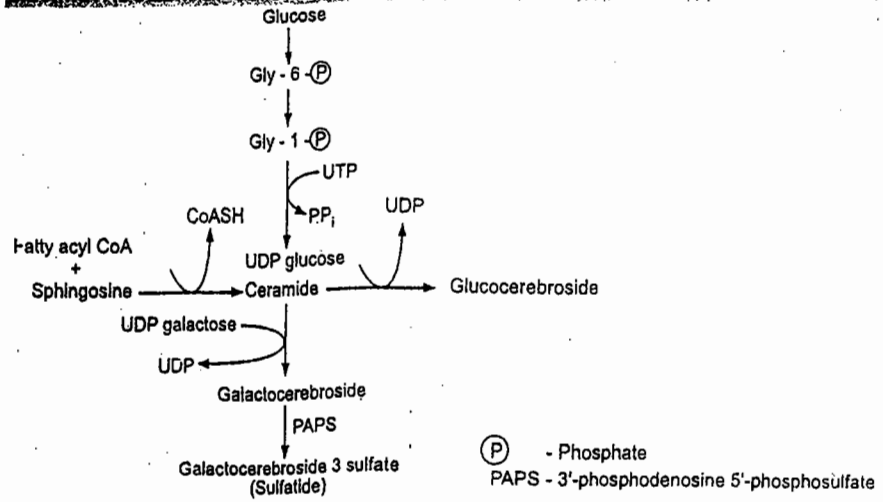


Fig. 8.26: Glycolipid metabolism

8.27 LIPOTROPIC FACTORS

Lipotropic factors represent the group of compounds that promote the transportation and utilization of fats and help to prevent the accumulation of fat in the liver.

The examples of lipotropic factors include - choline, methionine, inositol, betain, folic acid and vitamin B_{12}, essential fatty acids required for VLDL and lipoprotein synthesis

The lipotropic factors are directly or indirectly participating in the transmethylation reactions which are channeled towards the choline synthesis. Protein deficiency disease like Kwashiorkar causes the fatty liver. The root cause of fatty liver in the Kwashiorkar is the deficiency of the methyl group donating amino acid - methionine.

Besides being methyl group donors, choline and inositol are constituents of phospholipids too. This justifies their necessity in the synthesis of the lipoproteins and phospholipids.

Sometimes, glycine and serine also play the role of lipotropic factors. Lipotropic factors are important because they can help liver function better as well as get rid of toxins.

The deficiency of lipotropic factors in the body is correlated with the accumulation of the fat in the liver. Lipotropic factors prevents fatty liver but cannot reverse the condition. Casein, heparin, Vit. E, Selenium, Pantothenic acid play role of lipotropic factors.

Humans derive steroid hormones from cholesterol. Two classes of steroid hormones are synthesized in the cortex of adrenal gland, mineralocorticoids, which control the reabsorption of inorganic ions (Na^+, Cl^- and HCO_3^-) by the kidney and glucocorticoids, which help regulate gluconeogenesis and reduce inflammatory response.

Sex hormones are produced in male and female gonads and the placenta. They include progesterone, which regulates female reproductive cycle and androgens (such as testosterone) and estrogens (such as estradiol), which influence development, secondary sexual characters in males and females respectively.

Steroid hormones are effective at very low concentrations and therefore synthesized in relatively small quantities. Therefore the amount of cholesterol consumed for the synthesis of steroid hormones is comparatively less than that consumed for bile salt production.

8.28 SYNTHESIS OF STEROID HORMONES

Steroid hormones (glucocorticoids, mineralocorticoids and sex hormones) are produced from cholesterol by alteration of side chain and introduction of oxygen atoms into steroid ring system.

Synthesis of steroid hormones requires removal of some or all of carbons in 'side chain' on C-17 of the D ring system of cholesterol (For structure of cholesterol Refer Article 3.10, Page 3.16). Side chain removal takes place in the mitochondria of steroid producing tissues. Removal involves the hydroxylation of two adjacent carbons in the side chain (C-20 and C-22) followed by cleavage of the bond between them.

Synthesis of various other steric hormones, also involves the introduction of oxygen atoms. Enzymes, known as 'mixed function oxidases' are involved in the all hydroxylation and oxygenation reations mentioned above. Mixed function oxidases use NADPH, O_2 and cytochrome P-450.

Cytochrome P-450 acts as a carrier in this mixed function oxidase system that oxidises adjacent carbons. The process also requires electron transferring proteins adrenodoxin and adrenodoxin reductase. This system for cleaving side chains is found in mitochondria of the adrenal cortex, wherein active steroid production occurs.

Pregnenolone is the precursor of all other steroid hormones as shown in the adjacent flowchart.

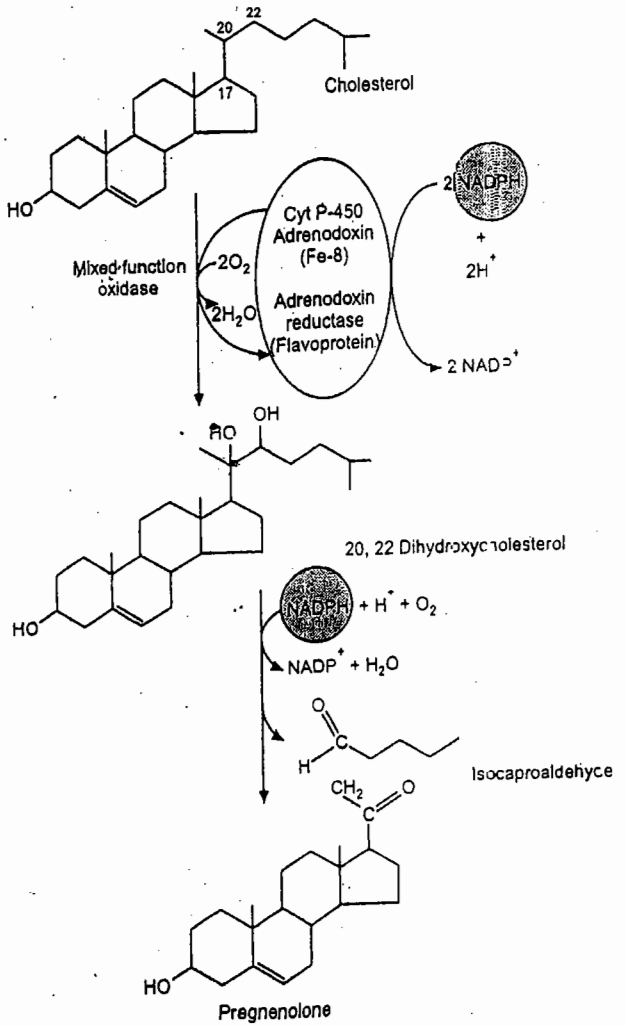


Fig. 8.27: Some steroid hormones derived from cholesterol

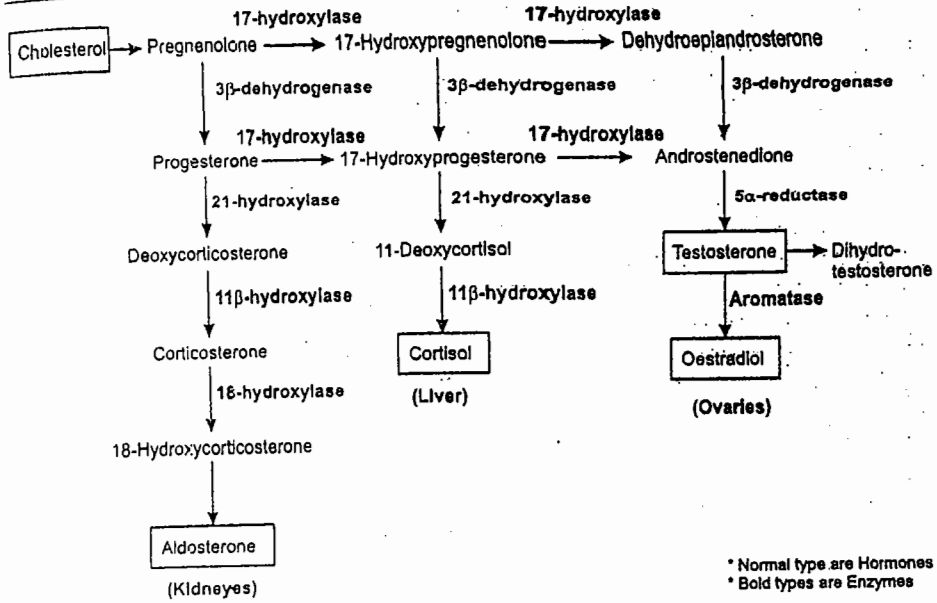


Fig. 8.28: Synthesis of steroid hormones

8.29 SYNTHESIS OF VITAMIN D FROM CHOLESTEROL

Vitamin D is also known as calcitriol, (active form of it). There is a close connection between Vitamin D and cholesterol. One of the biggest sources of Vitamin D for individuals is contact with sunlight, upon exposure to sunlight, body can synthesize its own Vitamin D. Cholesterol is involved in the process of synthesizing vitamin D from sunlight.

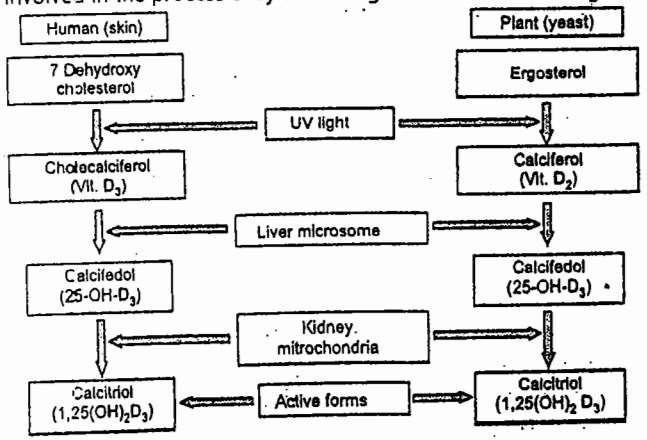


Fig. 8.29: Flowchart of journey of vitamin D formation

Vitamin D cannot be synthesized in absence of cholesterol. In fact, it has been proved that, less cholesterol in the diet resulted in less Vitamin D in blood.

After your skin is exposed to UV rays in the sunlight, molecules of 7 dehydrocholesterol (also known as provitamin D_3) is converted into cholecalciferol (or Vitamin D_3). This reaction takes place in the skin.

Cholecalciferol reaches liver by way of blood circulation, wherein this molecule is hydroxylated at 25 position to form 25 hydroxy cholecalciferol (25 hydroxy vitamin D_3). This hydroxylation in liver is brought about by the specific hydroxylase enzyme.

Next step of hydroxylation takes place in the kidney, with the help of 25 hydroxy cholecalciferol hydroxylase. It produces a molecule of 1, 25 dihydroxy cholecalciferol (active Vitamin D molecule or 1, 25 dihydroxy Vitamin D_3).

The liver and kidney hydroxylation reactions are carried out in the presence of the cyclochrome P_{450}, NADPH and molecular oxygen.

Regulation of Vitamin D Synthesis:

Plasma levels of calcium and phosphates are major factors deciding the synthesis of active Vitamin D molecule (1, 25 dihydroxy cholecalciferol). Plasma calcium and phosphate levels control the hydroxylation reactions at position 1 (occurring in kidney). This reaction is tightly controlled and serves as the major control point in production of active hormone.

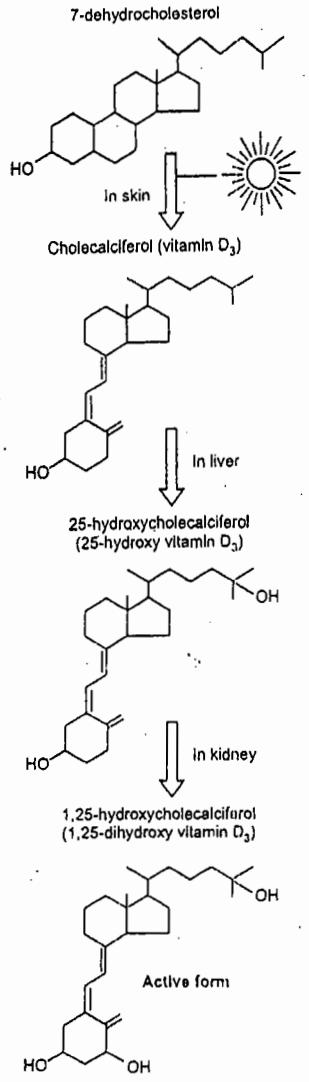


Fig. 8.30: Vitamin D synthesis

QUESTIONS

1. Explain in detail the pathway of fatty acid biosynthesis.
2. Enlight the salient features of the β-oxidation of fatty acid.
3. Give energetics of the β-oxidation of C_{18} fatty acid.

4. How many numbers of ATPs will be produced, if the C_{20} fatty acid is completely oxidised to CO_2 and H_2O?
5. Explain in detail the pathway for the cholesterol biosynthesis.
6. Discuss in detail the metabolism of phospholipids, lecithin and cephalin.
7. What is meant by ketone bodies? What is their biochemical significance?
8. Describe in short synthesis of ketone bodies.
9. Describe the pathway for the production of steroid hormones from cholesterol.
10. Write short notes on: (a) Obesity (b) Lipotropic factors. (c) Atherosclerosis.

Chapter ... 9

METABOLISM OF AMINO ACIDS AND PROTEINS

♦ LEARNING OBJECTIVES ♦

- *Understand Pathways of Synthesis of Biochemically Active Substances like Tryptamine etc.*
- *Understand Catabolic Pathways of Amino acids and Ammonia Disposal in Urea Cycle.*
- *Appreciate General Reactions of Amino Acids and Correlate their Biochemical Significance.*
- *Appreciate the Pathway of Catabolism of Heme and related diseases.*
- *Understand Biochemical Reasons behind Diseases related to Amino Acid Metabolism.*

9.1 INTRODUCTION

There are twenty amino acids in proteins with different structural formulas. Correspondingly, there are twenty different catabolic pathways for amino acid degradation. In humans total amount of energy produced by these pathways, accounts for 10-15% of body's energy production.

The twenty catabolic pathways converge to form seven products, all of which enter the citric acid cycle. From here carbon skeleton can be diverted to gluconeogenesis or ketogenesis or they can be completely oxidised to CO_2 and H_2O.

All or part of twenty amino acids are ultimately broken down to yield acetyl CoA.

When amino acids are present in excess of metabolic needs, their carbon skeletons are catabolized to amphibolic intermediates for use as a source of energy or else as a substrate for carbohydrate and lipid biosynthesis.

Removal of α-nitrogen by transamination is the initial reaction of amino acid catabolism. Subsequent reactions remove any additional nitrogens (if at all present) and restructure the remaining hydrocarbon skeleton for conversion to amphibolic intermediates such as:

1. Oxaloacetate
2. α-Ketoglutarate
3. Pyruvate
4. Acetyl CoA
5. Succinyl CoA
6. Fumarate
7. Acetoacetate.

The above mentioned carbon skeleton can be metabolised in the following manner:

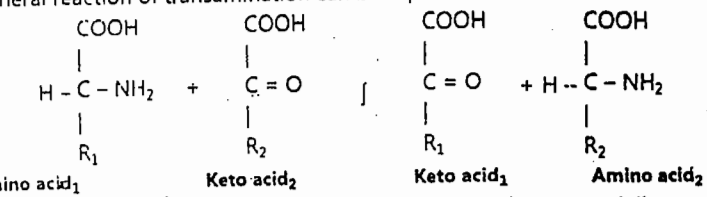
1. Synthesis of glucose.
2. Formation of lipids.
3. Oxidation via TCA cycle to produce energy.
4. Synthesis of non-essential amino acids.

9.2 AMINO ACID METABOLISM

9.2.1 Transamination

The transfer of an amino ($-NH_2$) group from an amino acid to keto acid is known as *transamination*. This reaction involves reversible transfer of an amino group from amino acid to keto acid. The keto $acid_2$ is converted into amino $acid_2$ and amino $acid_1$ to keto $acid_1$. Transamination reaction is catalysed by group of enzymes called transaminases, they are also known as aminotransferases. Transaminases in mammals are found predominantly in liver.

The general reaction of transamination can be represented as follows:



The most important characteristics of transamination reaction are as follows:

All amino acids except lysine, proline, hydroxyproline and threonine can participate in the transamination reaction. The keto acids participating in the transamination reaction are only three, namely α-ketoglutaric acid, oxaloacetic acid and pyruvic acid. All transaminases require pyridoxal phosphate (PLP), a cofactor derived from vitamin B_6. There are specific transaminases for every pair of amino and keto acids. Significant contribution to transamination is given by aspartate transaminase (SGOT or recently known as AST) and alanine transaminase (SGPT, recently known as ALT). There is no free NH_3 liberated in the transamination reaction. It involves only transfer of amino group. Transamination is a reversible reaction. Transamination is very important for the redistribution of amino groups and production of non-essential amino acids in the cell, since all the amino acids are not available in the proportion needed for protein synthesis. Transamination is not restricted to α-amino group, e.g. amino group of the ornithine is also transaminated.

Transamination involves both catabolism and anabolism of amino acids.

Excess of amino acids can be diverted towards energy generation with the help of transamination. All the amino acids undergoing transamination, finally concentrate nitrogen in the form of glutamate. In fact, glutamate is the only amino acid that undergoes oxidative deamination to a significant extent to liberate free NH_3 for urea synthesis in urea cycle.

SGPT and SGOT have a diagnostic importance. Serum levels of SGPT increase in liver diseases and serum levels of SGOT increase in myocardial infarction. Reactions catalysed by transaminases are anergonic as they do not require input of metabolic energy. The reactions are freely reversible. The direction of the reaction is determined by relative cellular demands in terms of concentrations of amino acid and ketoacid pair.

9.2.1.1 Mechanism of Transamination

Transamination reaction takes place in two steps.

1. Transfer of amino group of amino acid to cofactor pyridoxal phosphate to form pyridoxamine phosphate.
2. The amino group of pyridoxamine phosphate is then transferred to keto acid to produce new amino acid along with the regeneration of the enzyme and PLP

PLP a derivative of vitamin B_6, linked with ε amino group of lysine residue, at the active site of enzyme forms a Schiff base (imine linkage). When amino acid (substrate) comes in contact with the enzyme, it displaces lysine at the active site of the enzyme and a new Schiff base linkage is formed with amino acid substrate. The complex of amino acid, PLP-Schiff base tightly binds with the enzyme by multiple non-covalent interactions. The amino acid is then hydrolyzed to form an α-keto acid and pyridoxamine phosphate, α-amino group having been temporarily transferred from the amino acid substrate onto pyridoxal phosphate. Steps discussed so far, constitute half of the transamination reaction. The second half occurs by a reversal of above reaction with second α-keto acid reacting with pyridoxamine phosphate to yield a second amino acid with the regeneration of enzyme pyridoxal phosphate complex.

Flowchart of Transamination Mechanism

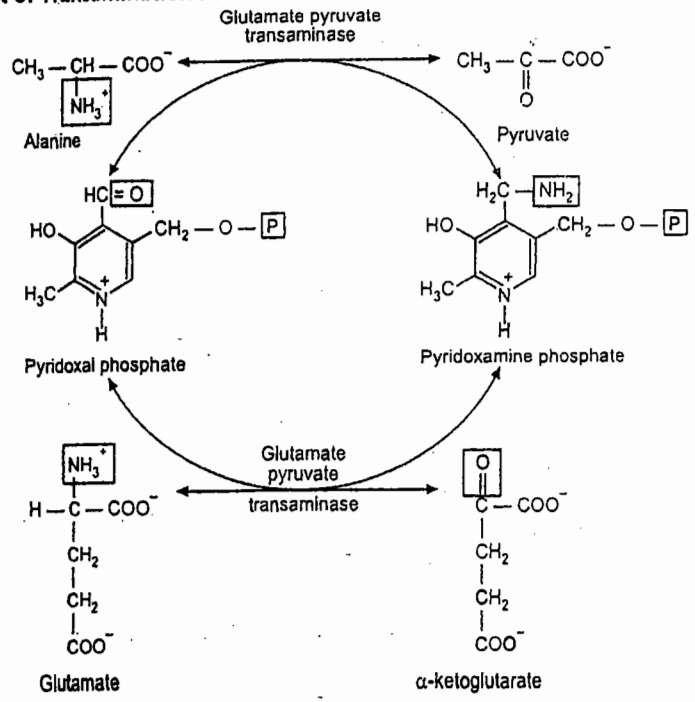


Fig. 9.1 (a): Role of Pyridoxal phosphate in Transamination

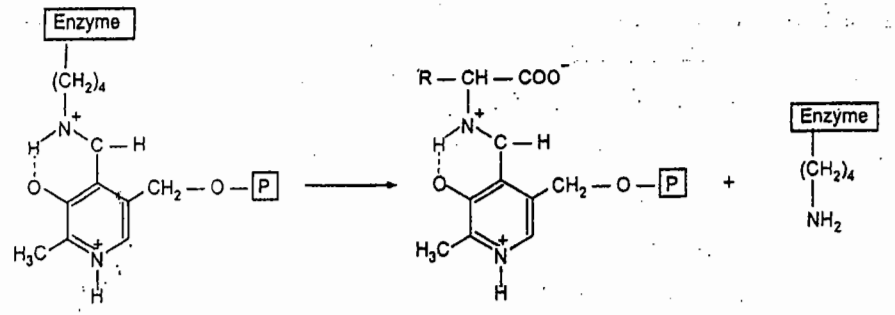


Fig. 9.1 (b): Role of Pyridoxal phosphate in Transamination

9.2.2 Deamination

Deamination involves removal of amino group from amino acid in the form of NH_3. The ammonia liberated is diverted for urea synthesis. The remaining carbon skeleton of amino acid is catabolised to keto acid.

Deamination can be oxidative or non-oxidative. Although transamination and deamination are separately discussed, they occur simultaneously. The glutamate is involved as a central molecule in these two reactions. Hence, many people call these two reactions as transdeamination, while describing the reaction of transamination and deamination particularly involving glutamate.

9.2.2.1 Oxidative Deamination

Liberation of free ammonia from amino group of an amino acid coupled with oxidation takes place in oxidative deamination. The reaction occurs in the liver and kidney.

Glutamate Dehydrogenase Participation:

The elimination of NH_2 of amino acid could be considered to be routed through glutamic acid in the following sets of reactions. In the process of transamination, the amino group of most amino acids are generally transferred to α-keto glutarate to produce glutamate. This glutamate serves as a 'reserve' or store of amino groups in biological systems. An enzyme glutamate dehydrogenase carries out oxidative deamination of glutamate to liberate ammonia. The glutamate dehydrogenase is the only enzyme utilizing either NAD^+ or $NADP^+$ as cofactors in the biological system.

The conversion of glutamate to α-ketoglutarate occurs through the formation of an intermediate, namely α-iminoglutarate. Glutamate dehydrogenase is widely distributed in all the tissues and its activity is very high.

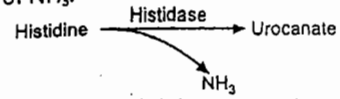
The reaction is very important because it reversibly links glutamate metabolism with TCA cycle via α-ketoglutarate.

Oxidative deamination can also be brought about by L amino acid oxidases which require FMN as coenzymes whereas D amino acid oxidases require FAD as coenzymes. They act on corresponding enzymes to produce α-ketoglutaric acid and NH_3.

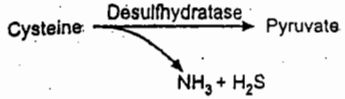
9.2.2.2 Non-Oxidative Deamination

Some of the amino acids can be deaminated to liberate NH_3 without undergoing oxidation.

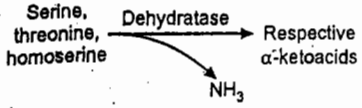
(a) **Deamination of Histidine:** The enzyme histidase converts histidine into urocanate with the liberation of NH_3.



(b) **Amino acid desulfhydratase:** Sulphur containing amino acids namely cysteine and homocysteine, undergo deamination which is coupled with desulfhydration to give ketoacids.



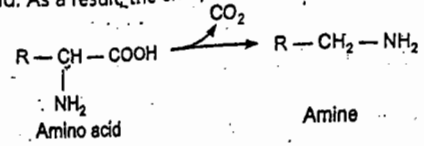
(c) **Amino acid dehydratase:** Hydroxy group containing amino acids, namely, serine, threonine and homocysteine, undergo PLP dependent deamination carried out by dehydratase.

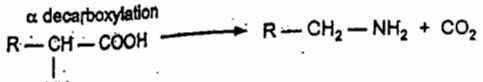


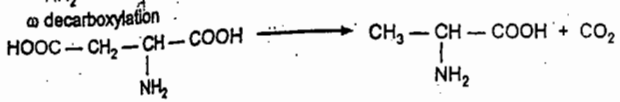
9.2.3 Decarboxylation

Decarboxylation is the chemical reaction that removes carboxyl group from amino acids and releases carbon dioxide.

Decarboxylase enzymes break the bond between the –COOH group to rest of the amino acid. As a result, the end product is basic chemical compound.







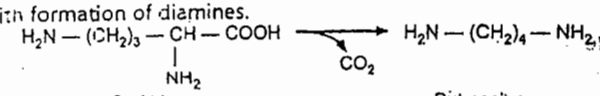
Arginine decarboxylase, ornithine decarboxylase and lysine decarboxylase are examples of few decarboxylase enzymes.

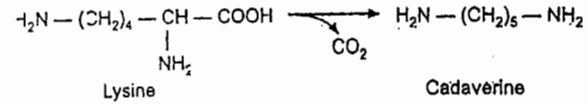
Amino acids undergo α-decarboxylation to form corresponding amines. Usually amines have high physiological activity. Amino acids also show ω decarboxylation reactions as shown.

Enzymes carrying out decarboxylation are called as decarboxylase which require pyridoxal phosphate as the coenzyme. Examples are as follows:

Decarboxylation reactions of amino acids are responsible for the formation of physiologically active compounds like GABA, Tyramine, Histamine.

Enzymes of microorganisms (in colon; in dead organisms) decarboxylate amino acids with formation of diamines.





1. Glutamic acid → Gamma amino butyric acid (GABA) (CO_2)
2. Tyrosine → Tyramine (CO_2)
3. Histadine → Histamine (CO_2)

Following table 9.1 gives examples of other decarboxylation reactions of amino acids:

Table 9.1

Name of an acid	Product obtained after decarboxylation
Tryptophan	Tryptamine
Phenyl alanine	Phenyl ethylamine
Serine	Ethanolamine
Lysine	Cadaverine
Arginine	Agmatine
Ornithine	Putrescine
3-hydroxytryptophan	Serotonin
L DOPA	Dopamine

Oxidative decarboxylation reactions are oxidation reactions in which carboxylate group is removed forming CO_2. Many biological systems show these reactions.

9.3 AMMONIA METABOLISM

Ammonia is constantly liberated in the amino acid metabolism. Ammonia exists as NH_4 ions at the physiological pH.

Formation of ammonia mainly occurs from amino acids (transamination and deamination), catabolism of purines, pyrimidines, biogenic amines, and by the action of intestinal bacteria.

Please refer Ch. 5, Article 5.6, 'Decarboxylation of Pyruvate' under title; Metabolic Fate of Pyruvate.

Our body has efficient mechanism for NH_3 transport and its immediate utilization for urea synthesis. This is the reason why plasma ammonia levels are normally maintained at 10-20 µg/dl.

Ammonia transport occurs in the form of glutamate or alanine and not as free ammonia. Alanine transport from muscle to liver is done by glucose alanine cycle.

Glutamine serves as storage and transport form of NH_3. Its synthesis mostly occurs in liver, muscle and brain. Brain detoxifies ammonia in the form of glutamine.

An enzyme glutamine synthetase, a mitochondrial enzyme is responsible for the synthesis of glutamine from glutamate and ammonia. This reaction is unidirectional and requires Mg^{2+} ions and ATP.

Animals can be classified into different classes on the basis of their mode of ammonia disposal.

(i) **Ureotelic:** Mammals including man convert NH_3 to urea, a non-toxic easily excretable compound.

(ii) **Uricotelic:** In reptiles and birds, ammonia is converted to uric acid, thus excreted.

(iii) **Ammoniotelic:** Aquatic animals can dispose off NH_3 into the surrounding water.

9.3.1 Ammonia Toxicity

Brain is very vulnerable to marginal concentration of ammonia. Elevated blood ammonia levels cause blurring of vision and slurring of speech. If not corrected in time, it may lead to coma or death.

9.3.2 Hyperammonia

Elevation in blood ammonia level may be genetic or acquired. Defect in any one enzyme of urea cycle can give rise to the same condition. Acquired hyperammonia may be due to hepatitis, alcoholism etc.

Mental retardation is the clinical sign of hyperammonia.

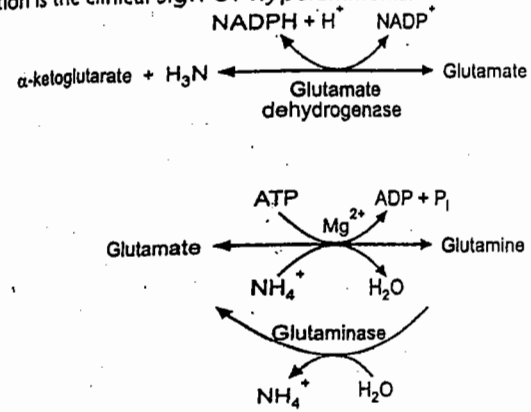


Fig. 9.2: Synthesis of glutamine and its conversion to glutamate are both independent reactions

Toxic effects of ammonia can be explained by the above mentioned reactions, specifically glutamate dehydrogenase. Elevated blood ammonia levels shift the equilibrium

to the right with more glutamate formation. Glutamate formation involves more utilization of α-ketoglutarate, the key intermediate of TCA cycle. The net result is the reduced production of energy (ATP) by brain. Thus, toxic effects of NH_3 on brain are due to reduction in ATP formation.

9.4 UREA CYCLE

Urea is synthesized in the liver by urea cycle. It is then secreted into blood stream and taken up by the kidneys for excretion in the urine. Urea cycle was the first metabolic pathway to be discovered by Hans Krebs and Kurt Henseleit in 1932. Urea cycle takes place partly in mitochondria and partly in cytoplasm.

Urea is the end product of protein metabolism or amino acid metabolism. The ammonia formed from amino acid nitrogen is toxic to the body. It is detoxified and converted to urea in urea cycle.

Urea molecule $H_2N - \underset{\underset{O}{\|}}{C} - NH_2$, contains two amino groups. One of the nitrogen atom of urea comes from ammonia. The other is transferred from amino acid aspartate.

The overall reaction of the urea cycle is as follows:

$$NH_4^+ + HCO_3^- + H_2O + 3\ ATP + Aspartate \rightarrow Urea + 2ADP + AMP + 2P_i + PP_i + Fumarate$$

Urea synthesis is a five step cyclic process, with five distinct enzymes. The first two enzymes are present in the mitochondria and rest are localised in the cytosol.

9.4.1 Carbamoyl Phosphate Synthetase I

Carbamoyl Phosphate Synthetase I (CPS I) catalyses the condensation and activation of ammonia and CO_2 (in the form of bicarbonate, HCO_3^-) to form carbamoyl phosphate in mitochondria. The hydrolysis of 2ATP molecules makes this reaction irreversible and rate limiting. Another enzyme, carbamoyl phosphate synthetase II (CPS II) present in cytosol accepts amino group from glutamate. This reaction takes place in the mitochondria.

9.4.2 Ornithine Transcarbamoylase

Ornithine transcarbamoylase catalyses the transfer of carbamoyl group from carbamoyl phosphate to ornithine. This reaction forms another non-standard amino acid citrulline which then has to be transported out of mitochondria into cytosol where remaining reactions of the cycle take place. In cytosol, ornithine is regenerated and used in the urea cycle. Ornithine and citrulline are basic amino acids which are never found in proteins due to lack of codons.

Citrulline produced in this reaction is transported to cytosol by a transporter system.

9.4.3 Arginosuccinate Synthetase

This enzyme condenses citrulline with aspartate to produce arginosuccinate. The second amino group of urea is incorporated in this reaction. This step requires ATP which is cleaved to AMP and pyrophosphate. Pyrophosphate is immediately broken down to inorganic phosphate.

9.4.4 Arginosuccinase

This enzyme removes the carbon skeleton of aspartate from arginosuccinate in the form of fumarate, leaving the nitrogen atom on the other product, arginine.

Fumarate liberated here provides connecting link with TCA cycle and gluconeogenesis.

9.4.5 Formation of Urea

Urea is formed from arginine by the action of arginase with the regeneration of ornithine. Ornithine is thus regenerated and can be transported into mitochondria to initiate another round of urea cycle.

9.4.6 Regulation of Urea Cycle

The first reaction catalysed by carbamoyl phosphate synthetase I is the rate limiting reaction of urea cycle. CPS I is allosterically controlled by N acetyl glutamate. It is synthesized from glutamate and acetyl CoA by synthetase and degraded by a hydrolase.

Flowchart of Urea Cycle:

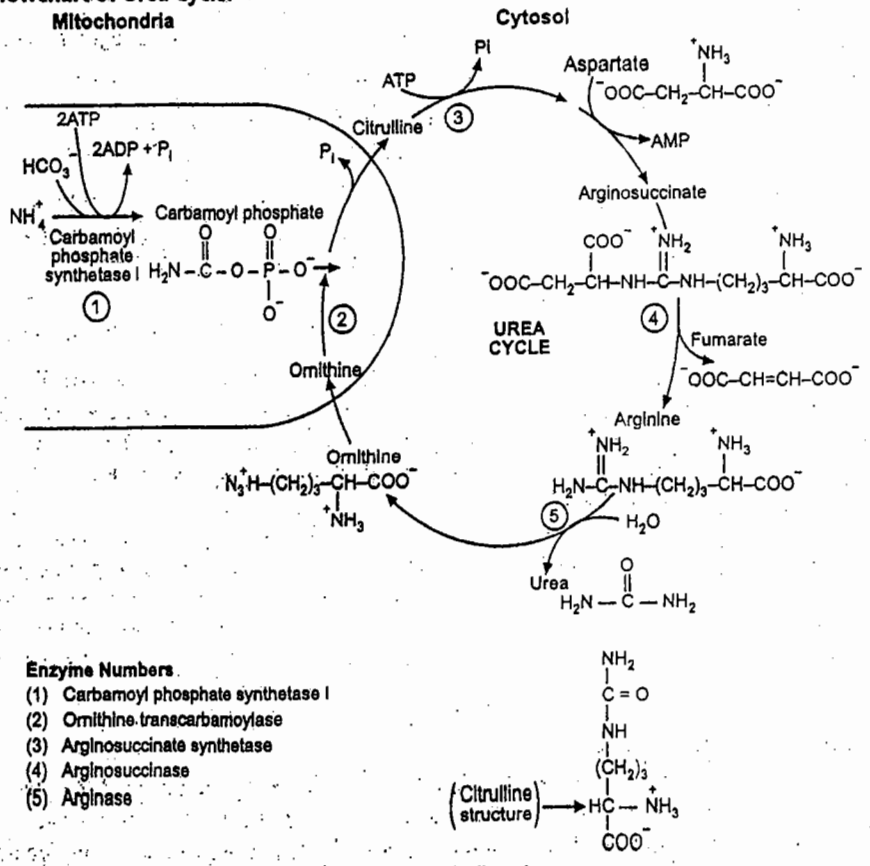


Fig. 9.3: Urea cycle flowchart

9.5 INBORN ERRORS OF METABOLISM (IEM)

It represents a group of metabolic diseases, hereditary in origin, related to lack of an enzyme in a particular metabolic pathway, occurring as a result of mutation in a specific gene responsible for enzyme synthesis.

Mutation of the gene causes synthesis of a protein different from a natural one. These mutations are transmitted from generation to generation and abnormal enzyme so produced becomes hereditary in nature.

Such an altered protein produced may or may not have the harmful effects on metabolism.

However, if such mutation occurs in the part of the genome, which is not transcribed, the mutation reflects no effect on the metabolism. If mutation occurs in the part of the genome, which is transcribed, it gives rise to an altered protein.

If such altered protein is an enzyme it may lose its catalytic ability. This is manifested in the form of accumulation of the substrate of the enzyme. This in turn can result into its feedback effects.

If a gene becomes partly mutant and partly normal, there might occur a deficiency of a certain enzyme. This is reflected in the form of disturbance in the metabolism of certain substances. The excretion of the accumulated substance in urine is a natural compensatory phenomenon.

Research is actively going on in the field of genetic defects. Gene therapy could be one of the solutions for genetic diseases like inborn errors of metabolism.

OR else, to certain extent, such diseases can be controlled as follows:

(a) Reduce the intake of the substance which cannot be metabolised due to genetic error.

(b) In case of complete or partial failure in production of some substance, it could be supplied in calculated amounts externally.

(c) If there is overproduction of some substance (unwanted), it can be selectively reduced by knowledge and use of inhibitors.

(d) The existence of an abnormal metabolic trait of genetic origin could be decreased by marriage counselling.

9.5.1 Metabolic Disorders of Urea Cycle

People with genetic defects in urea cycle enzymes have impaired ability to convert ammonia to urea.

All the disorders of urea cycle ultimately lead to build up in blood ammonia (hyper-ammonemia) leading to toxicity. Metabolic defects associated with each of the five enzymes of urea cycle have been reported.

The clinical symptoms associated with defect in urea cycle enzymes include vomiting, lethargy, irritability, ataxia and mental retardation.

Disorders of Urea Cycle

All enzymes of urea cycle, have witnessed genetic defects in different individuals. Although it is extremely rare to find such defects in population. Defect described in OTC or arnithine trans carbamoylase is x linked. The defect in the enzyme, 1. The carbomoyl phosphate synthase, and 2. *Ornithine-trans caramoylase causes accumulation of ammonia.*

Where as defect in later enzymes causes accumulation of intermediates of urea cycle *and these are autosomal recessive.*

Such children having genetic defect in the enzymes of urea cycle having feeding difficulty, lethargy, irritability, protein induced vomiting, poor intellectual development, cerebral edema, seizures and the patient may suffer from coma and succumb to death.

9.6 METABOLISM OF INDIVIDUAL AMINO ACIDS: (AMINO ACID DEGRADATION)

There is no provision for the storage of excess amino acids in the body. Moreover, every protein in the body has its own half life. There is constant turnover of amino acids and proteins in the body.

The first step of degradation of amino acid include removal of the α-amino group of the amino acid. The remaining carbon skeleton is converted into one of the metabolic intermediates. These intermediates obtained in the course of degradation are used as metabolic fuels.

There are only seven molecules formed at the end of degradation of twenty amino acids. These are pyruvate, acetyl CoA, aceto acetyl CoA, α-ketoglutarate, succinyl CoA, fumarate and oxaloacetate. [amino acids are classified as glucogenic or ketogenic based on the nature of degradation or catabolism product - (discussed in the Chapter 2 - Chemistry of Amino Acids and Proteins)].

The flowsheet below represents the fates of various amino acid catabolism.

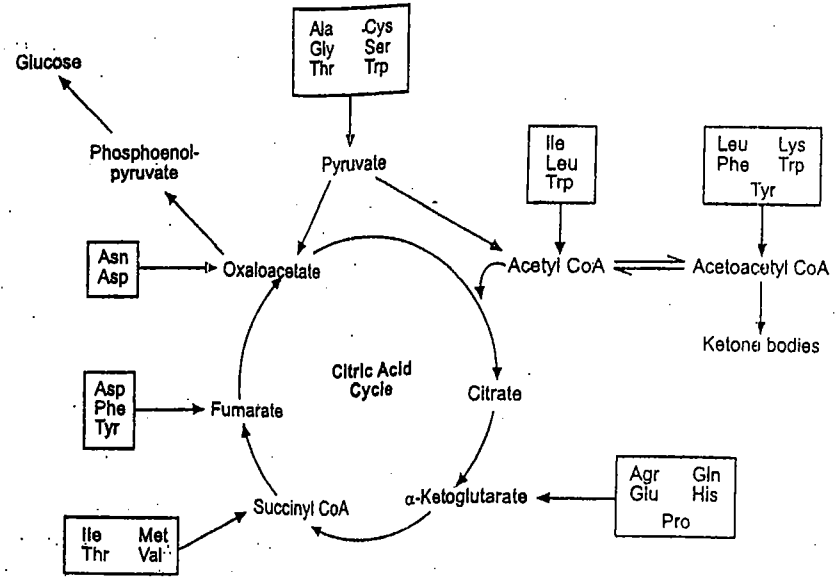


Fig. 9.4: Different fates of the amino acid carbon skeletons

9.6.1 Catabolism of Sulfur Containing Amino Acids

Sulfur containing amino acids are:

Cysteine
Cystine
Methionine

- Methionine is the essential amino acid among above mentioned amino acids.
- Methionine serves as a precursor of cysteine and cystine.
- Cysteine and cystine are interconvertible.
- Cystine is found exclusively in proteins.
- Methionine is required for the initiation of protein biosynthesis.
- Cystine can spare requirement of methionine in the diet.
- Cysteine is a constituent of glutathione.
- Cysteine is a constituent of scleroproteins (e.g. keratin of skin and hair).

9.6.1.1 Metabolism of Methionine

Methionine metabolism could be distributed into three main steps.

1. Transmethylation.
2. Conversion to cysteine and cystine.
3. Cysteine degradation.

1. Transmethylation: This involves the transfer of methyl group from active methionine to an acceptor. A methionine is activated to S-adenosyl methionine (SAM) or active methionine to donate the methyl group.

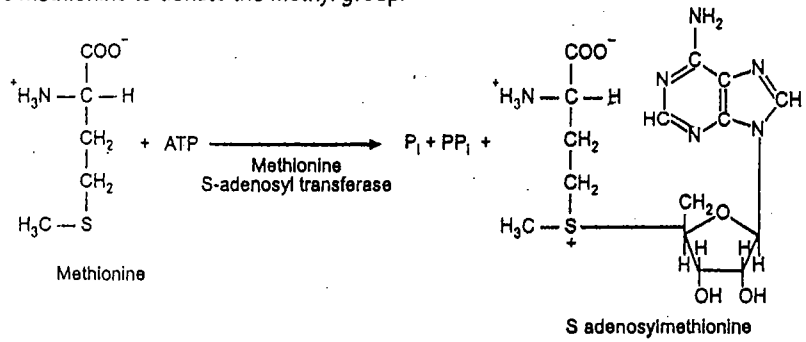


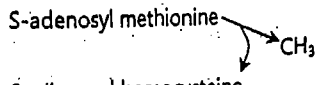
Fig. 9.5: Synthesis of SAM

Synthesis of S-adenosyl methionine occurs by the transfer of adenosyl group from ATP to sulphur atom of methionine. This reaction is catalysed by methionine S-adenosyl transferase. The activation of methionine is unique as the sulphur becomes sulfonium ion. Uniqueness of the reaction lies in the fact that all the three phosphates of ATP are eliminated as pyrophosphates (PP_i) and inorganic phosphates (P_i). Three high energy phosphates (3 ATP) are consumed in the formation of SAM.

S-Adenosyl methionine is highly reactive due to the presence of positive charge. Enzyme transferring methyl groups are collectively called as methyl transferase. S-adenosyl methionine transfers the methyl group to an acceptor and gets itself converted to S-adenosyl homocystine. SAM also participates in the synthesis of polyamines.

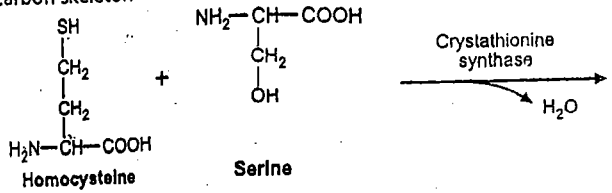
Transmethylation is a very important reaction biologically, since many compounds become functionally active only after transmethylation.

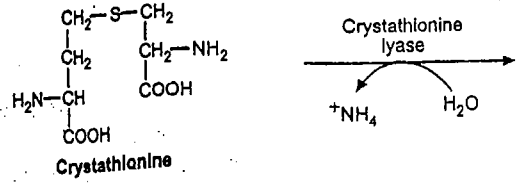
Methylation protects the protein from self-degradation. In plants. S-adenosyl methionine is the precursor for the synthesis of plant hormone, ethylene, which regulates the plant growth and development. It is also involved in the ripening of fruits.

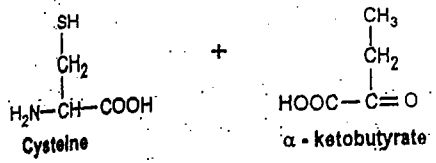


Methyl group acceptor	Methylated product
Norepinephrine	Epinephrine
Epinephrine	Metanephrine
Ethanolamine	Choline
Serine	Choline
t RNA bases	Methylated t RNA bases

2. Synthesis of Cysteine: A PLP dependent enzyme cystathionine synthetase brings about condensation of homocysteine to serine to form dipeptide cystathionine. The next enzyme cystathionase (cystathionine lyase) cleaves the cystathione to cysteine and α-ketobutyrate. Thus, in all, there is a transfer of sulfur group from homocysteine to cysteine. The carbon skeleton of the cysteine is obtained from serine.







Cysteine is used for protein synthesis and other body needs. α-ketobutyrate is decarboxylated and converted to propionyl - CoA.

3. Degradation of Cysteine: Cysteine oxidation by cysteine dioxygenase yields cysteine sulfinate, which on oxidation is converted into cysteic acid. The cysteic acid on decarboxylation produces taurine. Taurine conjugates with bile acids, alternatively, cysteic acid can also be degraced to keto acid pyruvate.

An enzyme disulfhydrase catabolises cysteine into sulfur (in the form of H_2S), ammonia and pyruvate.

9.6.2 Cystine and Cysteine

The major catabolic fate of cystine in mammals is conversion to cysteine. This reaction is catalysed by cystine reductase (Fig. 9.6). Catabolism of cystine then merges with that of cyste ne. Cysteine is catabolised via two catabolic pathways:

(1) The direct oxidative pathway (cysteine sulfinate) (2) The transamination (3-mercaptopyruvate).

1. Conversion of cysteine to cysteine sulfinate is catalysed by cysteine dioxygenase. Cysteine dioxygenase requires Fe^{2+} and NAD(P)H. Further catabolism of cysteine sulfinate probably involves its transamination to β-sulfinylpyruvate. Conversion of β-sulfinylpyruvate to pyruvate and sulfite catalysed by desulfinase, occurs even in the absence of enzymatic catalysis.
2. Cysteine transaminase catalyses reversible transamination of cysteine to mercaptopyruvate, also known as thiopyruvate. Reduction of 3-mercaptopyruvate by lactate dehydrogenase forms 3-mercaptolactate (Fig. 9.7). Later it is present in normal human urine as its mixed disulfide with cysteine and excreted in large quantity by patients with mercaptolactate cysteine disulfiduria.

Catabolism of Cystine

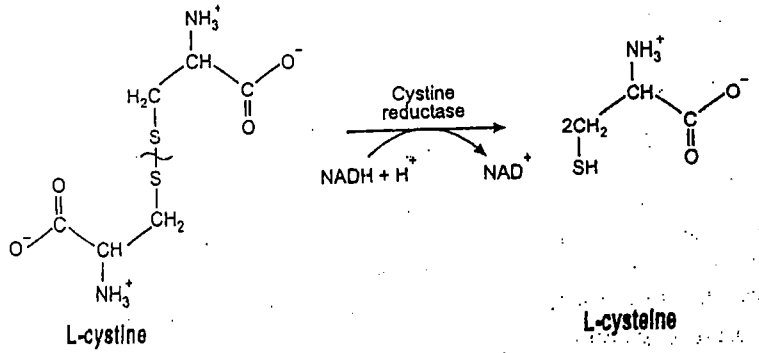


Fig. 9.6: Cystine reductase reaction

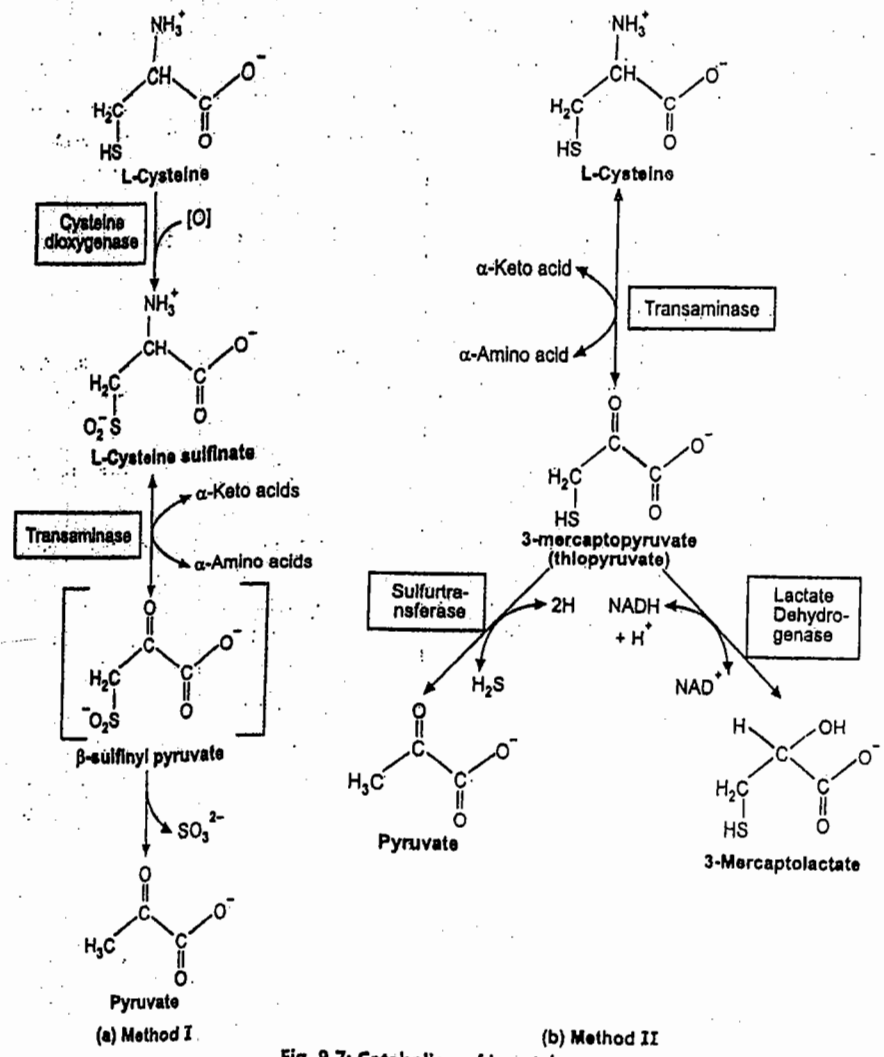


Fig. 9.7: Catabolism of L-cysteine

Cystinosis:

It is also known as cystine storage disease. It is a lysosomal disorder characterised by defective carrier mediated transport of cystine. Cystine crystals are deposited in tissues and organs, particularly the reticuloendothelial system. Cystinosis generally occurs along with amino aciduria. Other renal functions are also seriously affected. The patients die at a young age from acute renal failure.

A-Cystinuria:

This clinical condition is characterised by urinary excretion of cysteine upto 30 times the normal level. Excretion of lysine, arginine and ornithine is also increased, suggesting defect in renal reabsorptive mechanisms of these four amino acids. Relatively insoluble cysteine forms cysteine calculi in the renal tubules of cysteinuric patients. The mixed disulfide of L cysteine and L homocysteine present in the urine of cystinuric patient is more soluble than cystine and reduces the formation of cystine crystals and calculi.

Homocystinuria:

This is a heritable defect of methionine catabolism. Missing or impaired cystathionine synthetase leads to homocystinuria. About 300 mg of homocystine, sometimes together with S-adenosyl methionine is excreted daily in the urine. There is considerable rise in the plasma methionine level. At least four metabolic defects cause homocystinuria. In type I homocystinuria, clinical findings include thrombosis, osteoporosis, dislocated eye lenses and mental retardation.

A diet low in methionine and high in cystine prevents pathological changes if initiated early in life.

Other types of homocystinuria reflect defect in remethylation cycle (II, III, IV).

9.6.3 Tryptophan

Chemically, tryptophan is α-amino β-indole propionic acid. It contains an indole ring in its structure. It belongs to the group of essential amino acids. It also belongs to both ketogenic and glucogenic group of amino acids. Tryptophan is a precursor for the synthesis of important compounds like serotonin, melatonin, NAD^+ and $NADP^+$.

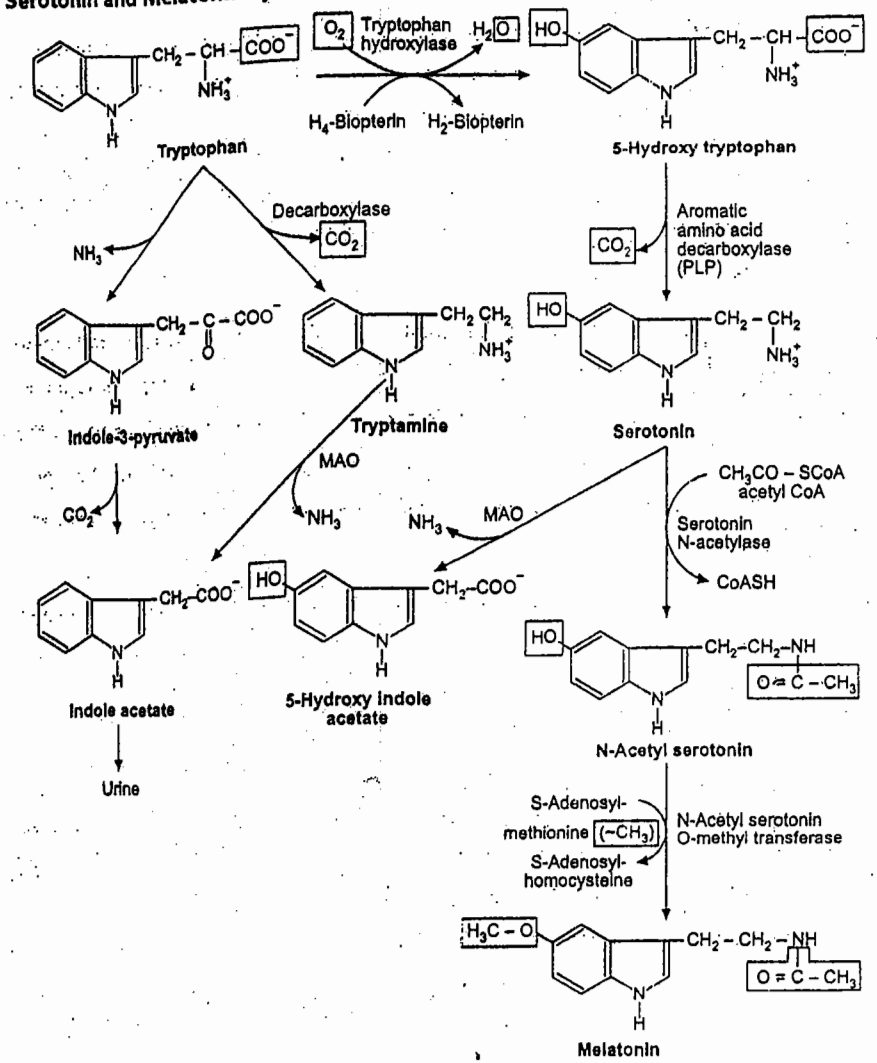
The metabolism of tryptophan is divided into two pathways:

1. Serotonin pathway.
2. Kynurenine pathway.

9.6.3.1 Serotonin Pathway

Serotonin is a neurotransmitter, synthesized from tryptophan. Chemically, serotonin is 5-hydroxytryptamine. The production of serotonin occurs in the target tissues e.g. intestinal cells and neurons.

Serotonin and Melatonin Synthesis:



(MAO – Monoamino Oxidase, PLP – Pyridoxal Phosphate)

Fig. 9.8: Tryptophan Metabolism

Synthesis of Serotonin:

Enzyme tryptophan hydroxylase, hydroxylates tryptophan at the 5th carbon in the 1st step. This enzyme requires tetrahydrobiopterin as a cofactor. 5-hydroxytryptophan is decarboxylated by aromatic amino acid decarboxylase to give serotonin.

9.6.3.2 Biochemical Role of Serotonin

Serotonin is a neurotransmitter and performs a variety of functions as follows.

It is necessary for the motility of gastrointestinal tract, also known as peristalsis. Serotonin stimulates the release of peptide hormone from the gastrointestinal tract. Serotonin controls behavioural patterns, sleep, body temperature and blood pressure. It is involved in the regulation of cerebral activity. Being a vasoconstrictor, serotonin influences smooth muscle contraction in bronchioles and arterioles. Degradation of serotonin occurs with the help of enzyme monoamino oxidase. This enzyme converts serotonin into 5-hydroxy indole acetate, which is excreted in urine.

9.6.3.3 Kynurenine Pathway

Liver is the site of this pathway. Tryptophan is converted to vitamin niacin in the following set of reactions.

Tryptophan pyrrolase, also known as oxygenase, breaks the five membered ring of tryptophan to produce N formyl kynurenine. Tryptophan pyrrolase is a metalloprotein containing iron porphyrin ring. The enzyme is controlled by feedback mechanism. The activity of this inducible enzyme, increases with rise in concentration of the substrate, tryptophan. Tryptophan pyrrolase activity is also elevated by corticosteroids.

The next enzyme formamidase hydrolyses formyl kynurenine and liberates formate and kynurenine. The formate then enters the one carbon pool and kynurenine formed in this reaction can undergo two different reactions as follows:

1. Kynurenine hydroxylase, hydroxylates the kynurenine to 5-hydroxy-kynurenine. The reaction is dependent on NADPH.
2. A PLP dependent enzyme kynurinase breaks off the alanine from the 3-hydroxy kynurenine along with the formation of 3-hydroxy anthranilate.

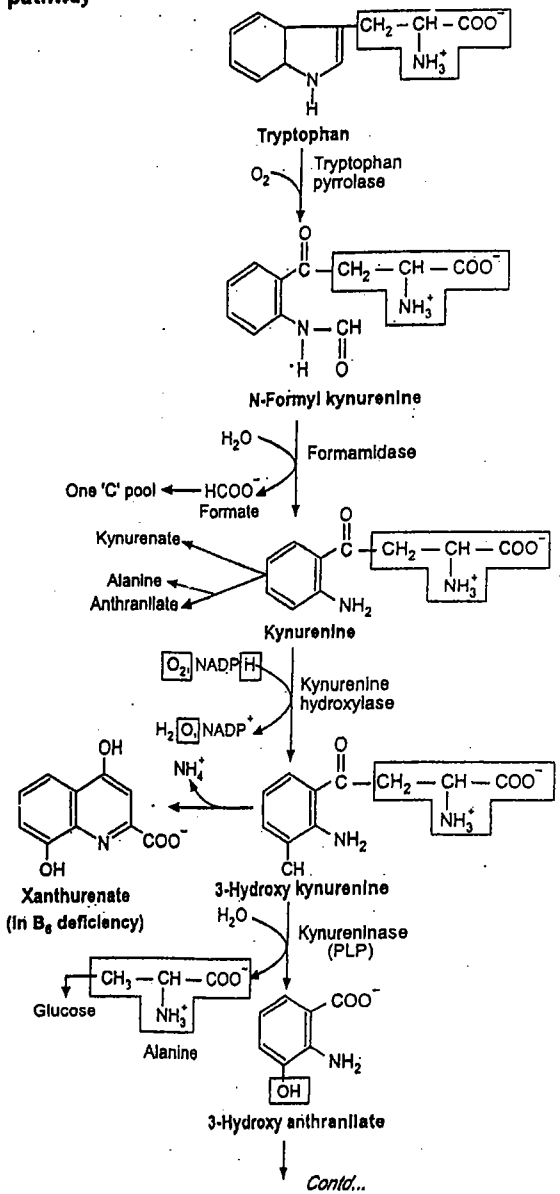
Enzyme kynureninase is very sensitive to vitamin B_6 deficiency. Hence, kynureninase reaction can be blocked due to lack of PLP. The 3-hydroxykynurenine is converted into xanthurenate. Elevated excretion of xanthurenate serves as an indication of B_6 deficiency. Administration of antituberculosis drug isoniazide induces B_6 deficiency and causes reduced synthesis of NAD^+ and $NADP^+$ from tryptophan. Symptoms of pellagra can be explained on this basis.

3-Hydroxyanthranilate is splitted by an oxidase (Fe^{2+}) dependent to 2 amino, 3 carboxy muconate semi-aldehyde. This compound can undergo three different reactions as follows:

1. Picolinate carboxylate, decarboxylates the same compound to picolinate.
2. It can undergo spontaneous cyclization to form quinolinate which is used for NAD^+ synthesis.
3. It can undergo decarboxylation catalysed by amino carboxy semi-aldehyde decarboxylase to produce 2 aminomuconate semi-aldehyde which enters the glutarate pathway. The semi-aldehyde is converted to 2 aminomuconate by a dehydrogenase. The aminomuconate is ultimately converted to glutaryl CoA and finally to acetyl CoA. The acetyl CoA can be completely oxidised via TCA cycle or can be converted to fat (since tryptophan is ketogenic).

Flowchart of Reaction from Tryptophan:

Kynurenine pathway



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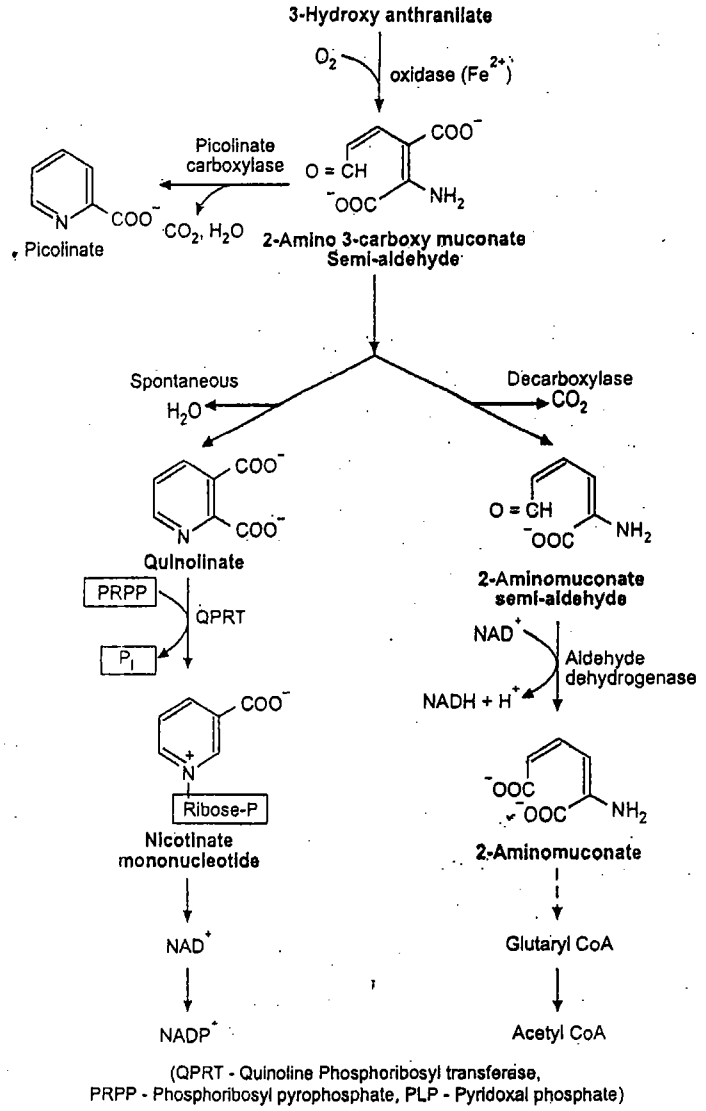


(QPRT - Quinoline Phosphoribosyl transferase,
PRPP - Phosphoribosyl pyrophosphate, PLP - Pyridoxal phosphate)

Fig. 9.9: Kynurenine pathway for metabolism of tryptophan

NAD^+ Pathway: Quinolinate undergoes decarboxylation and is converted to nicotinate mononucleotide by the enzyme quinolinate phosphoribosyl transferase (QPRT). Thus, tryptophan synthesizes NAD^+ and $NADP^+$.

Indole Acetate Formation:

Tryptophan can undergo deamination and decarboxylation to produce indole pyruvate and tryptamine respectively.

Both of these compounds can be converted to indole acetate and excreted in urine.

9.6.3.4 Melatonin

A pineal gland synthesizes hormone melatonin. A tryptophan is convertec to serotonin which is explained in the above mentioned pathway. Serotonin is acted upon by serotonin N acetylase to give N acetyl serotonin. This undergoes methylation, S-adenosyl methionine is a methyl group donor. The synthesis and secretion of melatonin from pineal gland is controlled by light.

Melatonin has some inhibitory effect on ovarian functions. Melatonin also performs neurotransmitter function. Melatonin inhibits the production of melanocyte stimulating hormone (MSH) and adrenocorticotropic hormone (ACTH). It plays an important role in sleep and wake process and hence is important for diurnal rhythms.

9.6.3.5 Hartnup's Disease

It is a hereditary disorder of tryptophan metabolism. The disorder was first reported in the family of Hartnup, hence the name, Hartnup's disease. Hartnup's disease has its root cause in the defective absorption and transport of tryptophan and other neutral amino acids from intestine and kidney tubules.

Clinical Symptoms: Dermatitis, ataxia, mental retardation. Disease is characterised by low plasma levels of tryptophan and other neutral amino acids and their elevated urinary excretion. Increased urinary excretion of indole acetic acid and indole pyruvic acid is also observed in the disease.

9.6.4 Branched Chain Amino Acids

Valine, leucine and isoleucine are branched chain as well as essential amino acids. They all contain branched chain aliphatic group with common metabolic features. All three initially form respective keto acids and then change their route to form respective end products.

These three amino acids undergo common pathways for the initial three steps of reactions.

9.6.4.1 Transamination

The reversible transamination to form their respective keto acids, occurs in the first step.

9.6.4.2 Oxidative Decarboxylation

This reaction is brought about by the α-keto acid dehydrogenase complex present in mitochondria.

The enzyme requires many coenzymes viz. FAD, TPP, coenzyme A, lipoate, NAD^+, for its action. α-keto acid dehydrogenase catalyses oxidative decarboxylation of α-keto acids to the corresponding acyl CoA thio esters. This is an important control point in the catabolism of branched chain amino acids.

9.6.4.3 Dehydrogenation

Dehydrogenation reaction requires FAD as the coenzyme. There is incorporation of double bond in these reactions. Infact there are two enzymes catalysing dehydrogenation reactions.

After initial three common reactions mentioned above, metabolism of individual amino acid takes independent route.

In the series of reactions that follow as given in the flowsheet, valine is converted to propionyl CoA, a precursor of glucose.

Leucine produces acetyl CoA and acetoacetate.

Isoleucine is degraded to propionyl CoA and acetyl CoA. Thus, we can say that –

Valine	–	Glycogenic
Leucine	–	Ketogenic
Isoleucine	–	Glycogenic and ketogenic

Catabolism of Valine, Leucine, Isoleucine

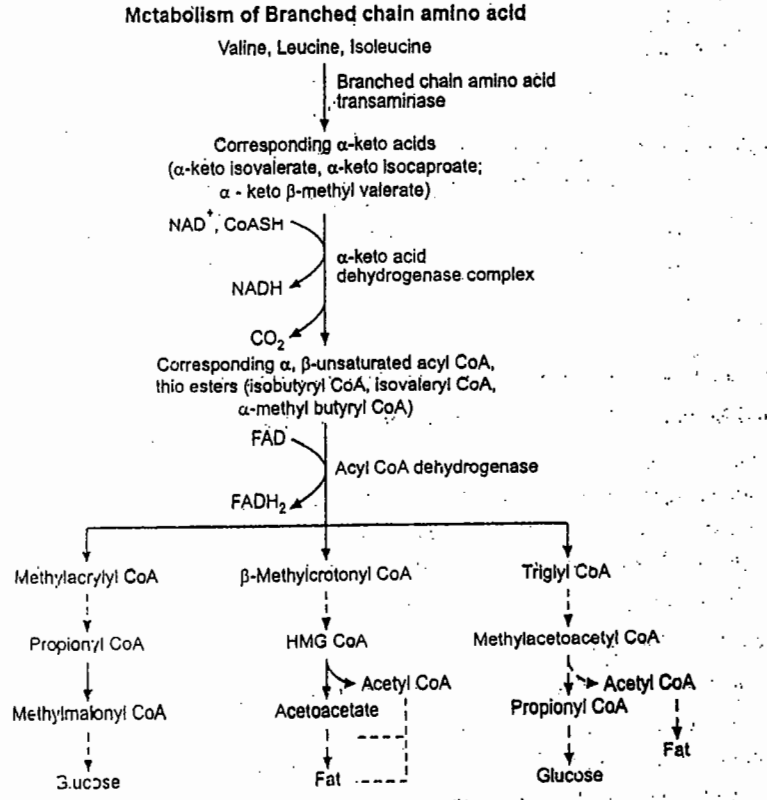


Fig. 9.10: Metabolism of branched chain amino acids

Catabolism of proline begins with its oxidation to pyroline 5-carboxylate which undergoes non-enzymatic conversion to glutamate 5-semi-aldehyde. This compound is then converted to glutamate and ultimately transaminated to α-ketoglutarate.

Metabolic Defect of Proline Metabolism:

Hyperprolinemia type I – It is observed due to defect in the proline dehydrogenase.

Hyperprolinemia type II – It is observed due to defect in hydroxyproline metabolism.

9.6.8 Lysine

Lysine is an indispensable amino acid to human beings and higher organisms. It does not exchange its nitrogen with other amino acids of the pool i.e. it does not participate in transamination reaction. Lysine is a ketogenic amino acid.

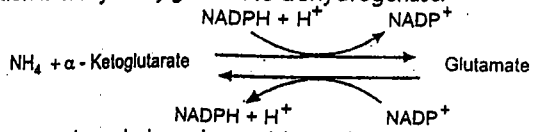
Catabolism of lysine begins with its conversion to saccharopine. Saccharopine is oxidised and cleaved by dehydrogenase to remove the glutamate and produce α-amino adipate ε semi-aldehyde. Second compound is then converted to α-amino adipate by the action of dehydrogenase. The latter then undergoes transamination to form α-ketoadipate. This compound is converted to glutaryl CoA by the enzyme α-ketoacid dehydrogenase. A double bond is introduced into glutaryl CoA in the next reaction. This gives rise to glutaconyl CoA which is decarboxylated to crotonyl CoA. The final product acetoacetyl CoA is obtained after degradation of crotonyl CoA.

9.6.8.1 Methylation of Lysine

S-adenosyl methionine (SAM) methylates some of the lysine residues in proteins. Methyl lysines are obtained after degradation of such proteins. e.g. trimethyl lysine serves as a precursor for the synthesis of carnitine, a protein, participating in fatty acid transport from cytosol to mitochondria. Only lysines present in the protein are methylated and not the free lysines. Hence, free lysine cannot serve as a precursor of carnitine synthesis.

9.6.9 Glutamate

Glutamate is synthesized from α-ketoglutarate by a simple one-step transamination reaction. This reaction is catalyzed by glutamate dehydrogenase.



Glutamate plays a major role in amino acid catabolism. Glutamate is a non-essential amino acid. It is synthesized in the body. It is important in the fixation of dietary ammonia and amination of keto acids.

Glutamic acid is decarboxylated in brain to GABA by glutamate decarboxylase. GABA is the inhibitory neurotransmitter required for proper neuronal function.

Glutamic acid is directly involved in the final transfer of amino group for urea synthesis.

Amino acids, histidine, proline and arginine are converted to glutamate in their metabolism.

Glutamate is also involved in the production of certain important compounds like:

1. γ-Aminobutyric acid (GABA), an inhibitory neurotransmitter.
2. A derivative of glutamate – N acetyl glutamate is an allosteric regulator of enzyme carbamoyl phosphate synthetase I, the enzyme of urea cycle.
3. Glutathione – A tripeptide contains glutamate as a constituent of amino acid.

9.6.9.1 Glutamine

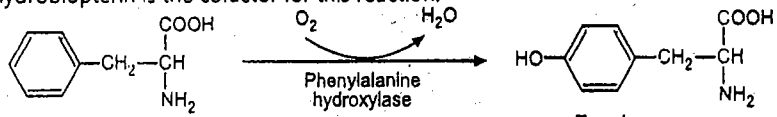
An enzyme glutamine synthetase in an irreversible reaction synthesizes glutamine from glutamic acid, ammonia, ATP and Mg^{2+}. Glutamine, is also known an an amide of glutamic acid. The breakdown of glutamine to glutamic acid is carried out by the enzyme glutaminase.

Glutamine is an important temporary reservoir of the ammonia in the tissues. Glutamine takes part in transamination. Glutamine is freely diffusible and hence easily transported. Glutamine can easily cross blood brain barrier. Glutamate shows active participation in the conjugation reaction. Glutamine donates nitrogen for the purine and pyrimidine synthesis. NH_3 production is elevated in acidosis to maintain acid base balance. The glutamate donates NH_3 to the kidneys.

9.6.10 Phenylalanine and Tyrosine

Phenylalanine is an essential amino acid, however tyrosine is non-essential. Both of them are aromatic amino acids having similarity in their structures. Our body is not able to synthesize phenylalanine. But tyrosine can be synthesized from the phenylalanine.

The exzyme involved is phenylalanine hydroxylase which is a mixed-function oxygenase. One atom of oxygen is incorporated into hydroxyl of tyrosine and other into water. Tetrahydrobiopterin is the cofactor for this reaction.



Phenylalanine Tyrosine

Phenylalanine and tyrosine are part of the infrastructure of protein. They are also required for different metaboiic reactions. They are required for the synthesis of melanin pigments, norepinephrine, epinephrine, catecholamines as well as thyroid hormones. Phenylalanine and tyrosine are both glucogenic as well as ketogenic as they can be converted into the compounds which can serve as the precursor of glucose or can be channeled towards lipogenesis.

9.6.10.1 Catabolism of Tyrosine and Phenylalanine

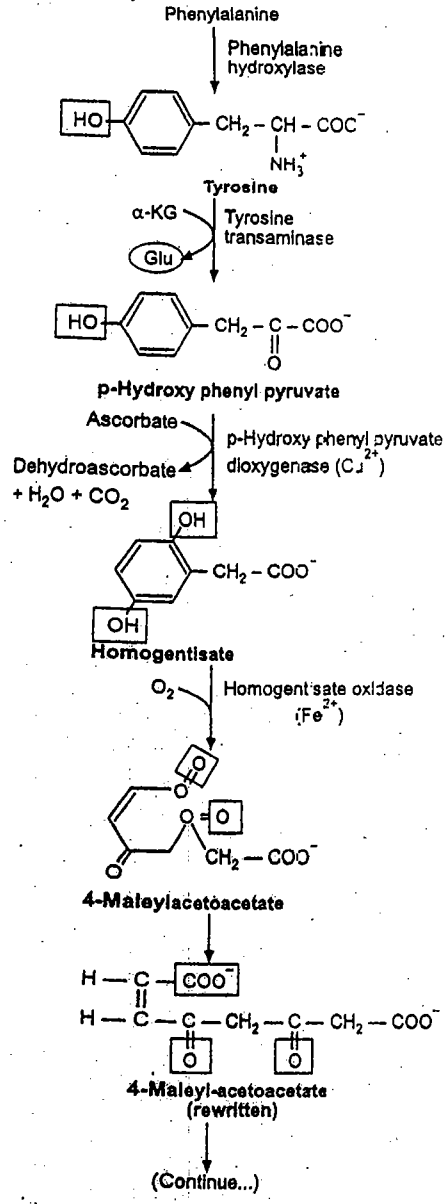
The metabolism of phenylalanine and tyrosine is considered together. As phenyl- alanine is converted to tyrosine in the course of its catabolism, a single pathway is responsible for the catabolism of both of these amino acids.

Tyrosine is catalysed by the tyrosine transaminase into p-hydroxy phenyl pyruvate. The enzyme requires PLP as the cofactor.

p-Hydroxy phenyl pyruvate undergoes vitamin C dependent oxidative decarboxylation along with hydroxylation of phenyl ring to produce homogentisate. This conversion shifts the hydroxyl group from para to meta position as well as there is addition of new hydroxyl group at para position on homogentisate.

A benzene ring of the homogentisate is cleaved by the homogentisate oxidase to form 4-maleylacetoacetate. Oxygen molecule is responsible for the cleavage of the benzene ring.

Metabolism of Tyrosine and Phenylalanine:



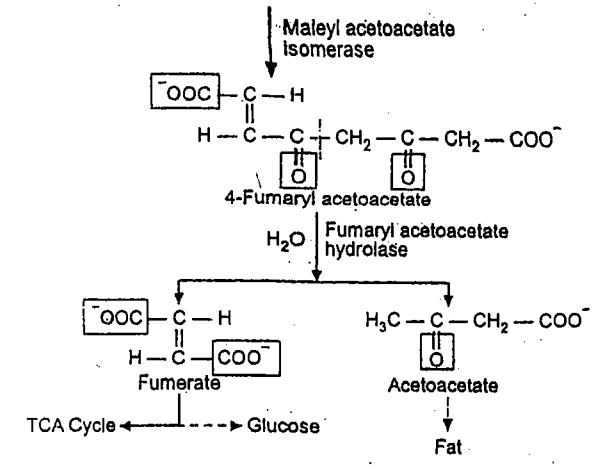


Fig. 9.15: Metabolism of Tyrosine (Glu – Glutamate, α KG – α ketoglutarate)

The next step is the isomerization of maleylacetoacetate to 4-fumaryl acetoacetate catalysed by the enzyme maleylacetoacetate isomerase.

4 Fumaryl acetoacetate is hydrolysed to fumarate and acetoacetate by the enzyme fumaryl acetoacetate hydrolase.

Fumerate is an intermediate of TCA cycle and also one of the precursors of gluconeogenesis. Whereas acetoacetate is one of the ketone bodies from which fats can be synthesized.

9.6.10.2 Thyroid Hormone Synthesis

Thyroxine-tetraiodothyronine (T_4) and tri-iodothyronine (T_3) are thyroid hormones, synthesized from tyrosine of protein thyroglobulin and activated iodine. Iodination of tyrosine ring occurs to produce monoiodotyrosine, diiodotyrosine, T_3 and T_4. The protein thyroglobulin undergoes proteolytic breakdown to release the free hormones - namely T_3 and T_4.

9.6.10.3 Biosynthesis of Catecholamines

Catacholamines, dopamine, norepinephrine (noradrenaline) and epinephrine (adrenaline) are synthesized from the tyrosine. The conversion of tyrosine to catecholamines occurs in adrenal medulla, sympathetic nervous system and CNS.

The dihydroxylated phenyl ring is called as the catechol and amine derivatives of catechol are referred to as catecholamines.

Catecholamines carry out a number of important functions in the body. Epinephrine and norepinephrine stimulate the degradation of triglycerol as well as glycogen. They regulate carbohydrate and lipid metabolism. They cause an increase in blood pressure.

A deficiency of DOPA (L-dopa) or decreased production of dopamine is responsible for Parkinson's disease. It is a neuromuscular disorder affecting older people. Clinical symptoms include – muscular rigidity, tremors, expressionless face, lethargy, involuntary movements etc. The disease is treated by the administration of L-dopa. L-dopa enters into

Catabolism of Branched Chain Amino Acids:

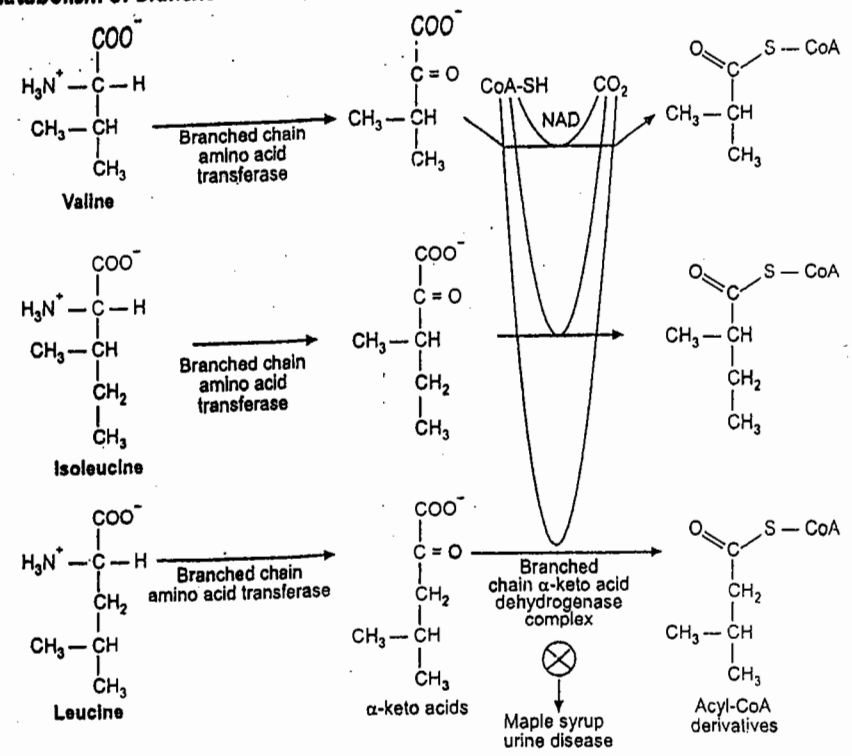


Fig. 9.11: Three branched chain amino acids share the first two enzymes in their catabolic pathway

9.6.4.4 Metabolic Defect of Branched Chain Amino Acids

The most common defect is in the α-keto acid dehydrogenase. Note that there is only one dehydrogenase enzyme for all three amino acids and hence all the three α-keto acids accumulate in the body and are excreted in the urine. This disease is known as *Maple syrup urine disease* because of the characteristic odor of the urine. The urine smells like maple syrup or burnt sugar because of the presence of α-keto acids.

Biochemical Changes:

Branched chain amino acids competitively inhibit glutamate dehydrogenase. The protein biosynthesis is reduced. The disease results in acidosis, lethargy, convulsion, mental retardation, coma and finally death within 1 year after birth. The neurological problems are because of poor formation of myelin in the CNS.

Investigations:

An early diagnosis within 1st week of life is useful.

Estimations of urinary branched chain amino acids and keto acids are helpful in the diagnosis.

Management requires formulated diet intake, low content of branched chain amino acids. Unfortunately, as these are essential amino acids, they cannot be restricted too much. Hence, life of these patients is short and development is abnormal.

9.6.5 Histidine, Proline, Arginine

All the three amino acids, histidine, proline, arginine are converted to glutamate in catabolism. Hence, metabolism of histidine, proline and arginine is considered together.

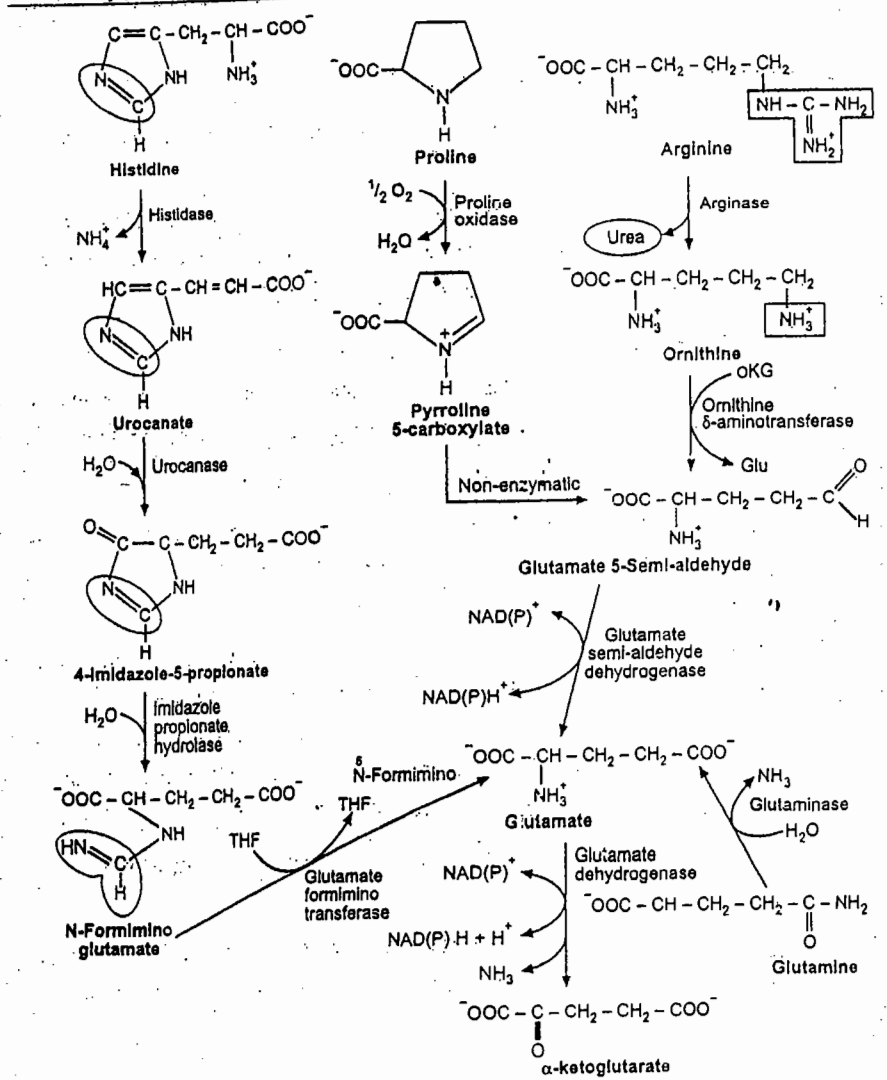
9.6.5.1 Histidine

The conversion of histidine to five carbon glutamate occurs in a multistep pathway. The extra carbon is released in the step as **formamino groups** that employs tetrahydrofolate as a cofactor.

The enzyme histidase acts on histidine to remove ammonia with the formation of uroconate. The uroconate formed in this reaction is acted by urocanase to produce 4 imidazole 5 propionate. Imidazole ring of the histidine molecule is cleaved by hydrolase to give N-formimino glutamate (FIGLU). Tetrahydrofolate (THF) takes the formimino group to form N 5-formimino THF and glutamate is liberated.

Deficiency of folic acid blocks this reaction and causes elevated excretion of FIGLU in urine.

Histamine is produced after decarboxylation of histidine. Histamine regulates HCl secretion by gastric mucosa. Excessive production of histamine causes asthma and allergic reactions.



(Glu – Glutamate, α-KG – α ketoglutarate, THF – Tetrahydrofolate)

Fig. 9.12: Metabolism of histidine, proline, arginine, glutamate, glutamine

9.6.5.1.1 Metabolic Defect of Histidine

Histidinemia: It is a rare disorder of histidine metabolism. It is due to defective enzyme histidase. The disease is characterised by elevated blood histidine level and increased excretion of imidazole, pyruvate and histidine in urine.

Patients of this disease suffer from mental retardation and defect in speech.

There is no cure available for this disease till date.

9.6.6 Arginine

Amino acid arginine is broken down by arginase to liberate urea and produce ornithine. The δ amino group of ornithine so formed undergoes transamination to form glutamate γ-semi-aldehyde which is converted to glutamate. Though arginine is formed in the body during urea cycle, it is considered semidispensable. The biosynthesis of creatinine requires arginine. Hyperargininemia is an inborn error in arginine metabolism due to defect in enzyme arginase and the rate of its synthesis is too slow to provide normal growth and maintenance.

Catabolism of Arginine

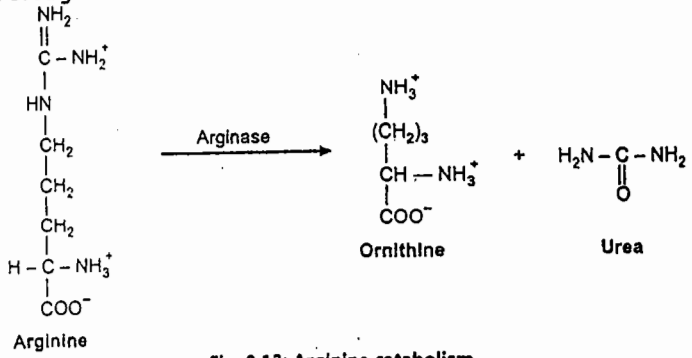


Fig. 9.13: Arginine catabolism

9.6.7 Proline and Hydroxyproline

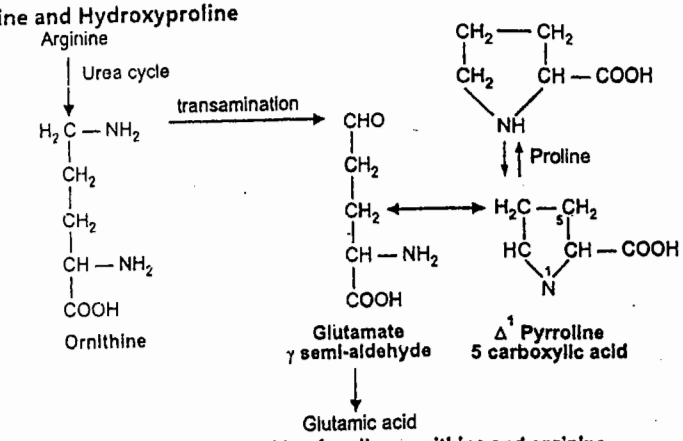


Fig. 9.14: Relationship of proline, ornithine and arginine

Proline is interconvertible with ornithine through the formation of glutamic γ-semi-aldehyde. Hydroxyproline is present in collagen, precisely in cross linking of collagen fibres. It requires vitamin C and oxygen for conversion of proline to cross-linked hydroxyproline in collagen fibres.

the brain where it is decarboxylated to dopamine which alleviates (lessen) the symptoms of the disease. Administration of dopamine is of no use as it cannot cross blood brain barrier.

Biosynthesis of Melanin (Metabolism of Tyrosine):

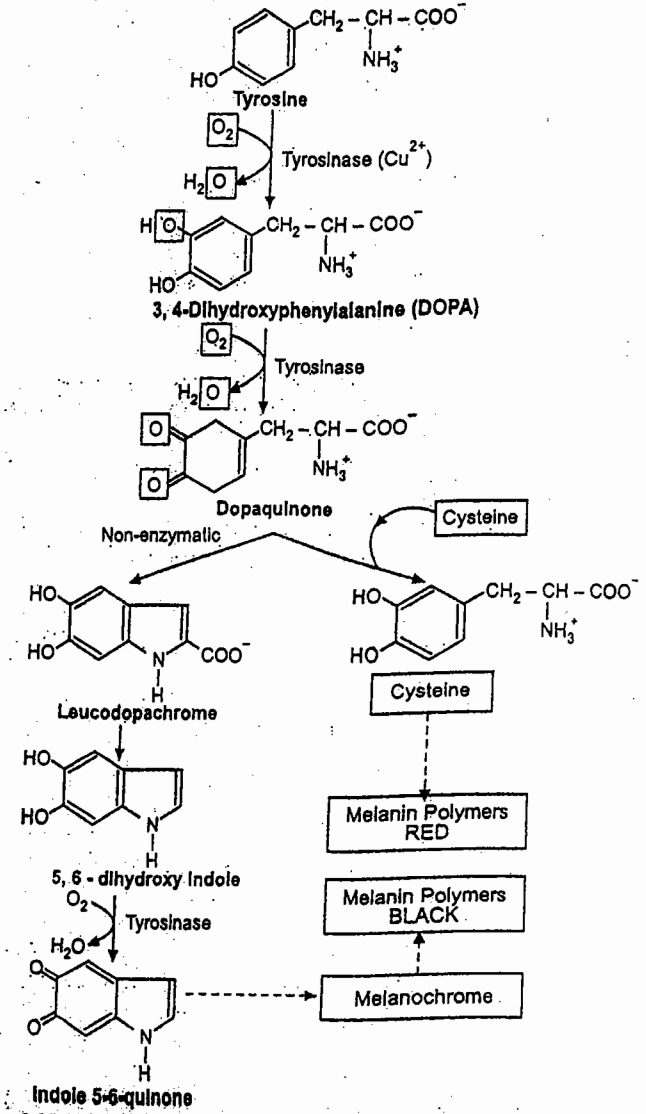


Fig. 9.16: Tyrosine metabolism showing melanin formation. Genetic error in Tyrosinase causes non-production of melanin – a condition called as Albinism

Synthesis of Thyroid Hormones (Tyrosine Metabolism):

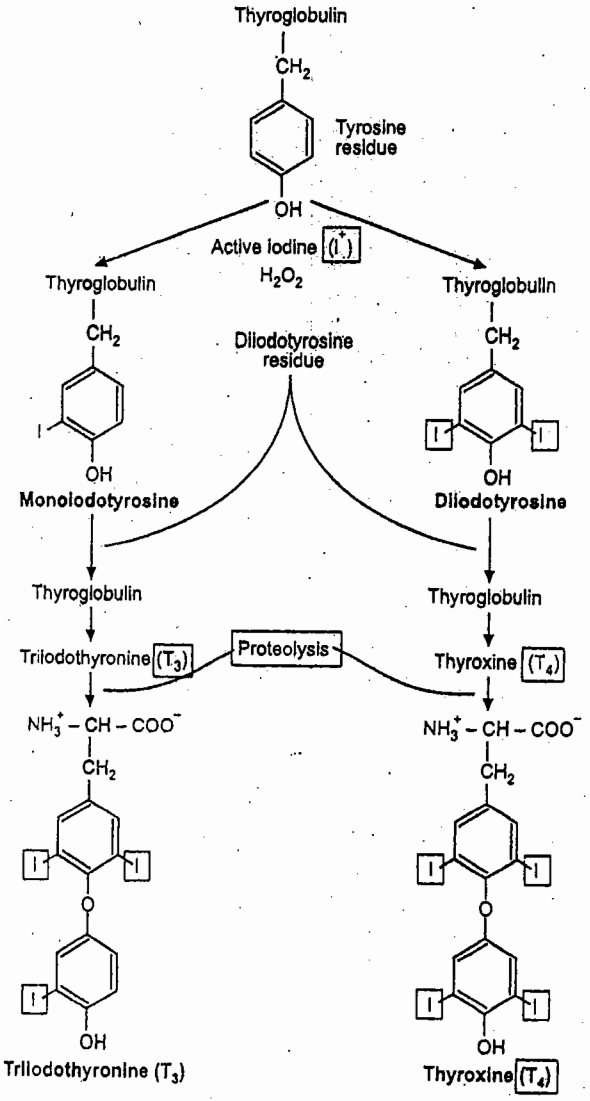
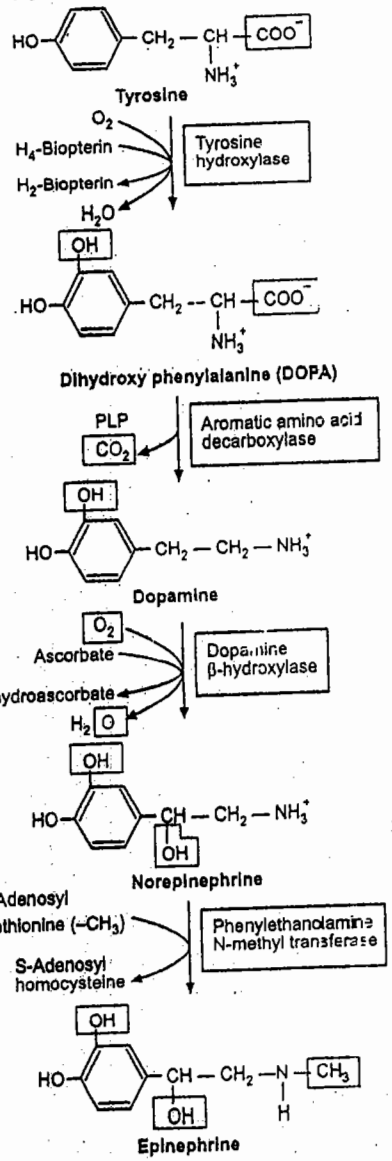


Fig. 9.17: Synthesis of Thyroid hormones

Synthesis of Catecholamines (Tyrosine Metabolism):



(Synthesis of Dopamine, Norepinephrine, Epinephrine)

Fig. 9.18: Catecholamine synthesis

Disorders of Tyrosine and Phenylalanine Metabolism:

1. **Phenyl Ketonuria (PKU)**

A deficiency of hepatic enzyme phenylalanine hydroxylase is responsible for phenyl ketonuria. This enzyme deficiency impairs the synthesis of tetrahydrobiopterin required for the action of phenylalanine hydroxylase. Deficiency of the above mentioned enzyme leaves phenylalanine unmetabolised.

There is accumulation of phenylalanine in the tissues and blood leading to its increased excretion in urine. Urine contains increased concentrations of metabolite phenyl pyruvate ($C_6H_5CH_2 - CO - COO^-$), phenyl acetate and phenyl lactate. Hence, the name Phenyl Ketonuria.

Biochemical Changes in PKU:

1. If untreated, patients show very low I.Q., mental retardation, failure to walk or talk, failure of growth, tremor and seizures.
2. There is defect in myelin formation.
3. An enzyme tyrosinase, synthesizes melanin with the help of amino acid tyrosin. Accumulation of the phenylalanine competitively inhibits tyrosinase and in effect reduces melanin formation.
4. Urine of PKU patients show mousy odour due to accumulation of the above mentioned keto acids.

Investigations of PKU should be done in new born babies for increased plasma levels of phenylalanine (normal 1-2 mg/dl) with the help of Guthrie test.

2. **Neonatal Tyrosinemia**

The disorder is caused due to the absence of enzyme P-hydroxy phenyl pyruvate dioxygenase. The condition responds to ascorbic acid.

3. **Tyrosinemia type II (Richner Hanhart Syndrome)**

The defect in the enzyme tyrosine transaminase is responsible for this disorder. The disorder affects or blocks the normal catabolic pathway of tyrosine.

The disorder is characterised by the accumulation of tyrosine metabolites like N acetyl tyrosine, tyramine, p-hydroxyphenylacetate, p-hydroxyphenyl pyruvate, p-hydroxyphenyl lactate.

Clinical signs of the disease include confusion, disturbed self co-ordination, skin and eye lesions and very rarely mental retardation.

4. **Albinism**

Albinism is an autosomal recessive disorder. It is an inborn error due to lack of the synthesis of pigment melanin. Albinism develops due to the inability to convert DOPA to melanin because of the deficiency of the enzyme tyrosinase in the melanocyte cells.

Many possible causes for albinism are known such as –

1. There is presence of inhibitors of tyrosinase.
2. Due to lack of the enzyme tyrosinase.

3. Defect in melanin polymerization.
4. Lack of protein matrix in melanosomes.
5. Limitation of tyrosine availability i.e. substrate to the enzyme.
6. Number of melanosomes and melanocytes is decreased.

The core or central cause of albinism is defect or deficiency of tyrosinase, the main enzyme synthesizing melanin. In this condition, there is no pigmentation in skin, hair and iris.

Person suffering from albinism is known as albino. Lack of melanin impairs the ability of such patients to tolerate sunlight. As most important function of melanin is protection of body from radiations of sun, albinos are more susceptible to skin cancer. They also suffer from photophobia.

5. Alkaptonuria

Observations of alkaptonuria by Garrod are actually responsible for the discovery of the inborn errors of metabolism. Alkaptonuria is an autosomal recessive disorder.

Alkaptonuria was first described by Lusitanus in 1649.

An enzyme catabolizing tyrosine, homogentisate oxidase is absent in alkaptonuria. This gives rise to accumulation of homogentisate in tissues and blood and its excretion into urine. If the urine sample is left undistributed then on standing for some time, homogentisate is oxidised to the corresponding quinones. Quinones polymerize to give black or brown colour.

Biochemical Changes

The enzyme polyphenol oxidase oxidizes homogentisate to benzoquinone acetate. The latter undergoes polymerization.

6. Tyrosinemia type I

It is also known as tyrosinosis. It occurs due to deficiency of the fumarylacetoacetate hydroxylase and malenylacetoacetate isomerase. This serious but rare disorder causes liver failure, rickets, polyneuropathy, renal tubular dysfunction. Clinical signs of the disease are characterised by the urinary excretion of the tyrosine and its metabolites along with many other amino acids.

In acute tyrosinosis, the infant suffers from diarrhoea, vomiting and cabbage like odour. Death may occur due to the liver failure within 1 year.

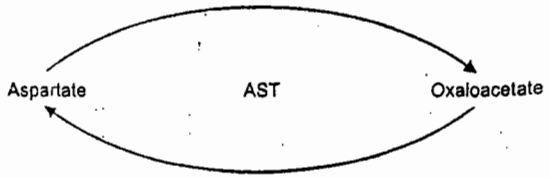
Treatment: Diet formulation low in tyrosine, phenylalanine and methionine.

9.6.11 Aspartate and Aspargine

Aspartate and Aspargine are glycogenic as well as non-essential amino acids. Both are converted to oxaloacetate in the course of catabolism. Aspartate forms oxaloacetate, (an intermediate of TCA) by transamination (AST).

Aspargine is first converted to aspartate due to action of asparginase which on transamination is converted into oxaloacetate.

Since the transamination catalysed by AST plays the most important role in central amphibolic functions, it is incompatible with life. Hence, no known metabolic defect is associated with this catabolic pathway.



Metabolism of Aspartate and Asparagine and Glutamate

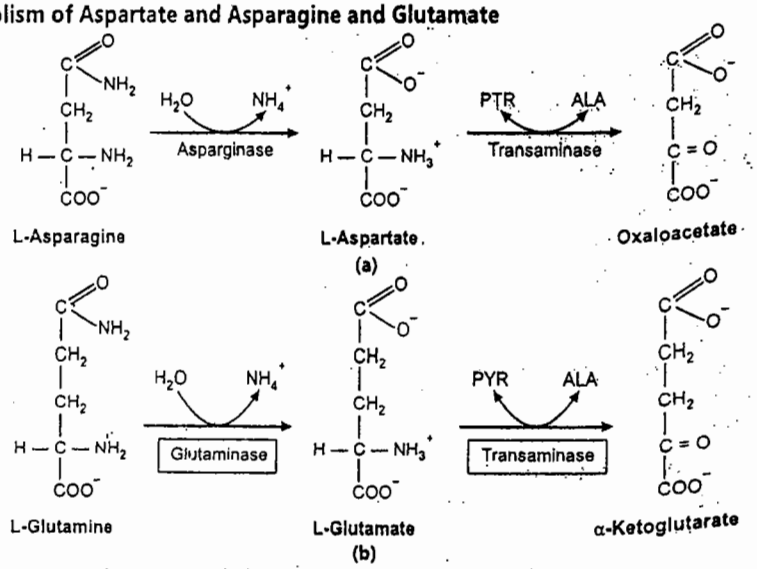


Fig. 9.19: Metabolism of Aspartate, Asparagine and Glutamate

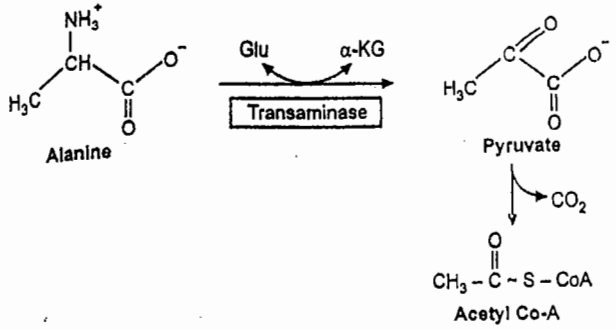
Aspartate is responsible for linking urea cycle and TCA cycle. It is also utilized for the synthesis of purines and pyrimidines, and donates amino group for the urea synthesis. Aspartate along with malate forms malate aspartate shuttle which transfers reducing equivalent from cytosol to mitochondria.

9.6.12 Alanine

Alanine is a non-essential amino acid.

Transamination of alanine forms pyruvate.

Catabolism of Alanine:



$$\text{Alanine} \xrightarrow{\text{ALT}} \text{Pyruvate} \xrightarrow{\text{PDH}} \text{Acetyl CoA}$$

ALT – Alanine transaminase

PDH – Pyruvate dehydrogenase

There is no metabolic defect reported for α-alanine catabolism.

Alanine Pyruvate Shuttle:

Alanine is helpful in detoxification of ammonia along with glutamate and glutamine.

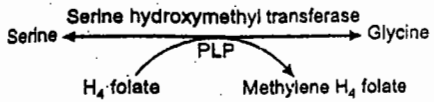
Pyruvate is produced at the end of glycolysis in many tissues like muscle, kidney etc. Pyruvate is converted to alanine by transamination reaction. Alanine is transported to liver. The pyruvate can be regenerated from alanine in the liver. The pyruvate serves as a precursor for glucose, whereas amino group goes for the urea formation.

Thus, alanine pyruvate shuttle carries nitrogen for reutilization or for conversion of the nitrogen into urea.

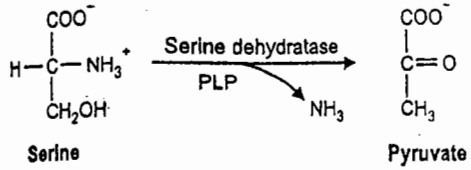
9.6.13 Serine

Serine is a non-essential and glycogenic amino acid.

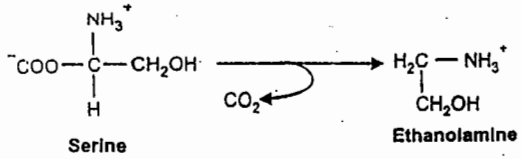
1. Catabolism of serine takes place primarily via glycine in human and many other vertebrates.



2. Serine can undergo deamination to form pyruvate. This reaction takes place in rodent liver.



3. Serine can also undergo PLP dependent decarboxylation to form nitrogen base ethanolamine.



9.6.14 Threonine

Threonine does not take part in transamination reactions. It is an essential as well as glucogenic amino acid. Yeast and E.coli can synthesize threonine from the aspartic acid.

An enzyme threonine dehydratase catabolises threonine to α-ketobutyrate. The later is then converted to propionic acid.

Threonine → α-Ketobutyric acid → Propionic acid → Glucose.

Theonine can be cleaved to glycine and acetaldehyde by serine hydroxymethyl transferase. Sequential dehydrogenation and decarboxylation of threonine results in amino acetone which can be ultimately converted to pyruvate or lactate.

Threonine is also required for incorporation into proteins. Like serine it is a carrier for the phosphate group in phosphoproteins.

9.6.15 Glycine

Glycine is a non-essential, optically inactive and glycogenic amino acid. Glycine actively participates in the synthesis of many specialised products like heme, purine, creatine, etc. It is also incorporated in the proteins. It is also utilized for the synthesis of serine. It actively participates in one carbon metabolism.

An enzyme serine hydroxymethyl transferase synthesizes glycine from serine. Glycine can also be obtained from threonine with the help of the enzyme threonine aldolase.

Oxidative deamination of glycine by glycine synthetase takes place to liberate NH_4^+, CO_2 and one carbon fragment N^5, N^{10} methylene (THF). This provides a major route for breakdown of glycine in mammals.

Glycine synthetase is a multienzyme complex and requires PLP, NAD^+ and THF as coenzymes. The reaction is reversible, hence, it can synthesize glycine too.

Serine hydroxymethyl transferase converts glycine into serine. It requires THF for its activity. Serine is degraded to form glyoxalate which in turn undergoes transamination to give back glycine. Glyoxalate is converted to oxalate (an excretory product) and formate, which enters one carbon pool.

Different Routes of Catabolism of Glycine:

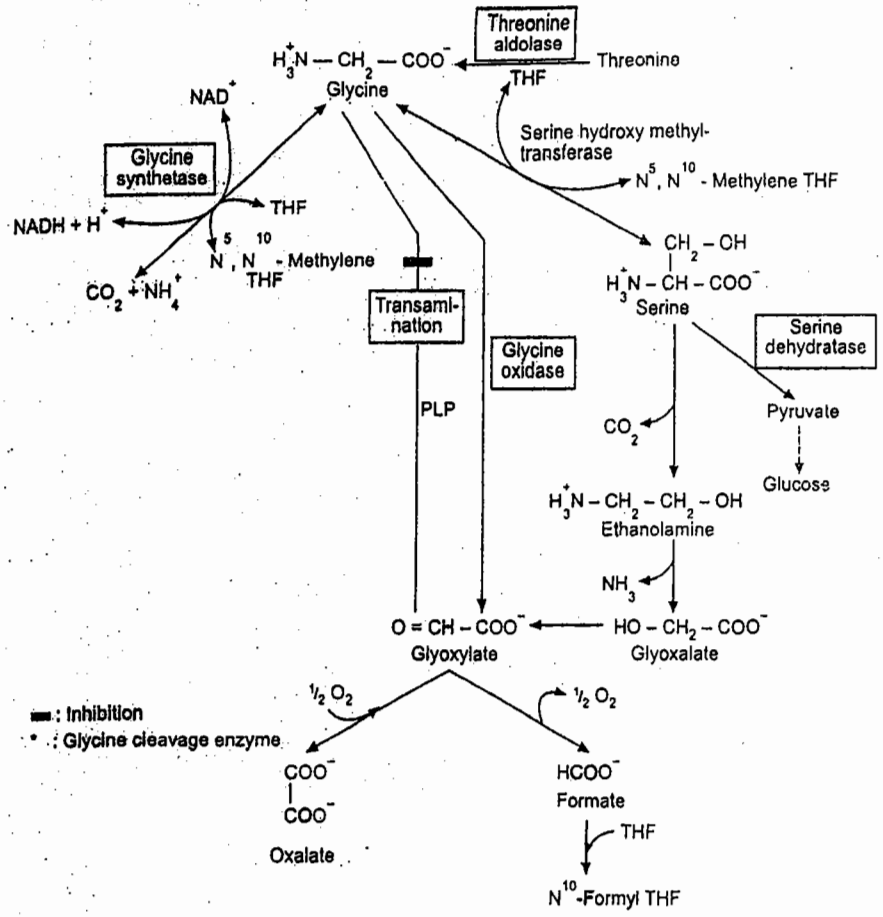


Fig. 9.20: Glycine metabolism

Disorders of Glycine Metabolism:

1. **Primary hyperoxaluria:** An increased urinary excretion of oxalate is the characteristic symptom of this disorder. Patient suffers from oxalate stones. Deposition of oxalates in various tissues is also observed. The disorder is due to increase in glycine transaminase along with the defective glyoxalate oxidation into formate. Dietary consumption of oxalate is not blamed for the urinary excretion, as it is entirely endogenous in origin.

2. **Glycinuria:** This rare disorder is characterised by elevated levels of excretion of glycine in the urine. On the contrary, the serum glycine concentration is within the normal range. The disorder is believed to be due to defective renal reabsorption. Glycinuria patients have increased tendency for the formation of oxalate renal stones but urinary oxalate levels are normal.

9.7 NITROGEN BALANCE

Nitrogen balance represents the difference between total nitrogen intake and nitrogen loss in faeces, urine and perspiration. The maximum amount of nitrogen in the body is present in the proteins. Hence, nitrogen balance is talked about in terms of proteins. Proteins like many other body constituents, are synchronously synthesized and broken down. A synchronous anabolism and catabolism is essential for the proper metabolic functioning of the body.

A positive nitrogen balance represents ingestion of more nitrogen than excreted. Positive nitrogen balance occurs in growth. The group of individuals showing positive nitrogen balance are pregnant women and infants. Growth is characterised by addition of new proteins or new cells to the body. Nitrogen ingested is used for assimilation in growth and very less amount of nitrogen is excreted in growth.

Normal adults are subject to nitrogen equilibrium, their nitrogen intake matches the nitrogen output.

In conditions like surgery, advanced cancer, starvation, wasting diseases like tuberculosis, burns, the tissue proteins are catabolised to a greater extent than they are formed. Consequently, there is loss in body weight, owing to loss of body proteins. Such a condition represents negative nitrogen balance in which nitrogen output exceeds intake.

9.8 AMINO ACID BIOSYNTHESIS

The essential amino acids namely valine, tryptophan, threonine, phenylalanine, methionine, lysine, leucine, isoleucine, histidine are required to be supplied through the diet. There are no biosynthetic pathways for them in animals. But non-essential amino-acids serine, tyrosine, proline, glycine, glutamate, glutamine, arginine, aspartate, aspargine, cystine, alanine are synthesized by diverse metabolic pathways. These biosynthetic pathways change from one animal to another. But one thing that remains common to all these biosynthetic pathways is the origin of carbon skeletons of amino acids. The carbon skeletons of all biosynthetic pathways are obtained from the intermediates of important metabolic pathways like glycolysis, citric acid cycle, pentose phosphate pathway. The primary amino group of amino acid generally comes from the transamination of amino acid glutamate. There are six different metabolic intermediates serving as the carbon skeleton of the non-essential amino acids. Leading to it are six different metabolic routes for amino acid biosynthesis.

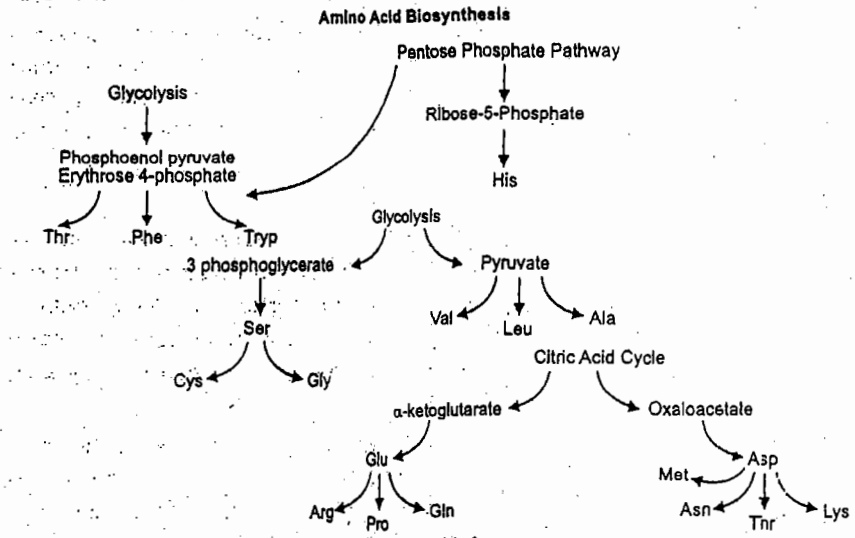


Fig. 9.21: Synthesis of amino acids from common precursors

Flowchart represents six different metabolic routes for synthesis of the non-essential amino acids.

9.8 CATABOLISM OF HEME AND BILIRUBIN PRODUCTION

The end product of heme catabolism are bile pigments, bilirubin and biliverdin.

The cellular site of heme catabolism is macrophages of reticuloendothelial system. In a normal healthy adult body, 6 g of heme breaks down per day from which 250 mg of bilirubin is formed.

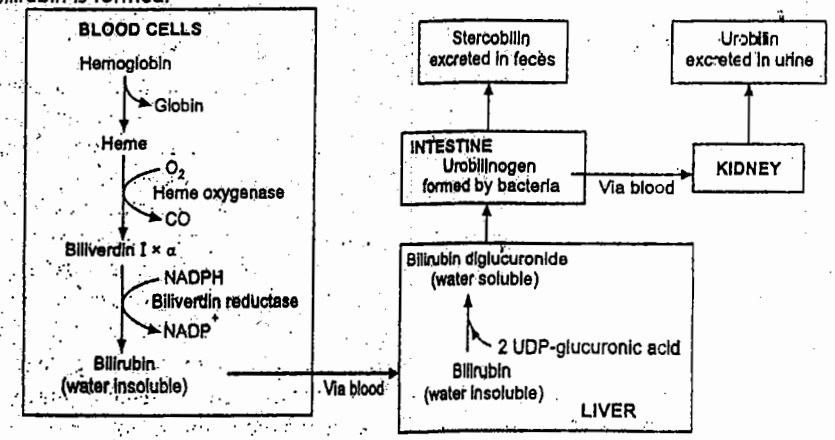


Fig. 9.22: Course of catabolism of hemoglobin

Bilirubin Production at Molecular Level:

A molecule of Hemoglobin looses its protein part a globin in the blood cell. This creates a separate Heme molecule. Enzyme Heme oxygenase removes a molecule of CO in presence of oxygen from Heme. This gives a new compound Biliverdin I x ∝. The same compound is reduced with the help of enzyme biliverdin reductase and NADPH to bilirubin. Bilirubin, a water insoluble compound enters the liver via blood circulation. The liver enzymes attach molecules of glucuronic acid by virtue of which there is a formation of bilirubin diglucuronide, a water soluble compound. This compound enters intestine in the course of blood circulation, wherein it gets converted into urobilinogen by action of intestinal bacteria. Urobilinogen can be excreted in the urine as urobilin, in the course of its passage from intestine to kidney and finally into urine.

Urobilinogen is converted into stercobilin in the large intestine and excreted in feces.

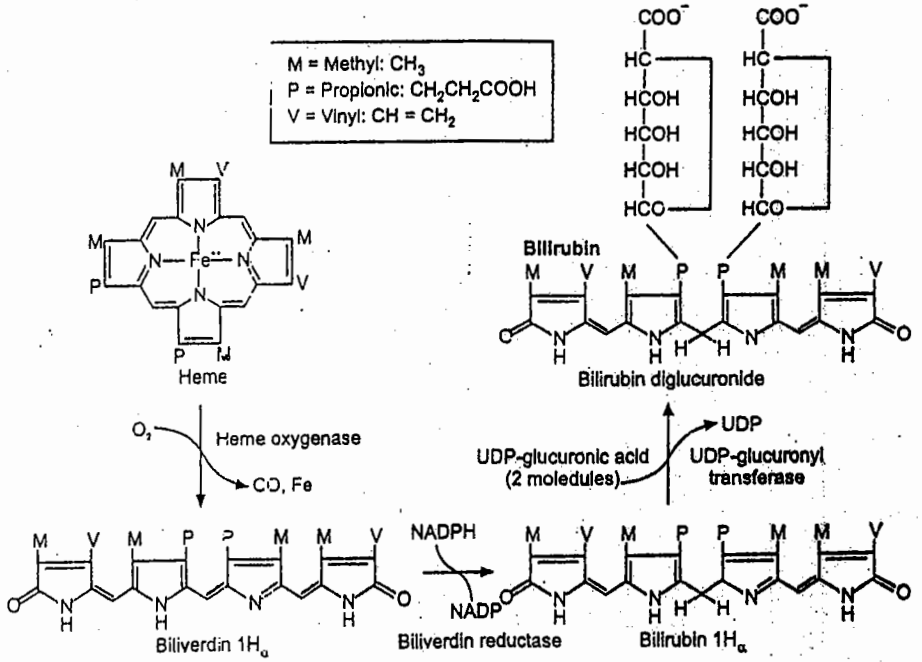


Fig. 9.23: Heme degradation and bilirubin production flowchart

Hyperbilirubinemia (Jaundice):

Excessive hemolysis gives rise to excessive production of bilirubin. This causes excessive level of bilirubin in the blood, giving the skin, urine mucus membrane and a yellow colouration of sclera of eyes, a condition known as jaundice.

Excessive level of bilirubin in the blood is a pathological condition called as hyper-bilirubunemia.

Especially new born babies are not able to get rid of bilirubin and it can build-up in the blood and other tissues and fluids of body.

In pregnancy, bilirubin from babies body is removed through the placenta.

Jaundice is the term used to describe yellowish tinge to the skin and whites of the eye. Body fluids may also be yellow. Jaundice is also known as icterus.

Jaundice most often happens as a result of an underlying disorder that either causes the production of the too much bilirubin or prevents the liver from getting rid of it. Both of these results in bilirubin being deposited in tissues.

Some underlying conditions that may cause jaundice are: 1. Acute inflammation of liver 2. Inflammation of bile duct 3. Obstruction of bile duct 4. Hemolytic anemia 5. Cholestasis 6. Gilbert's syndrome

Symptoms of jaundice include yellow tinge of skin and whites of the eye. Accompanying symptoms of jaundice include high bilirubin level, fatigue, fever, nausea, vomiting, pale stools and dark urine.

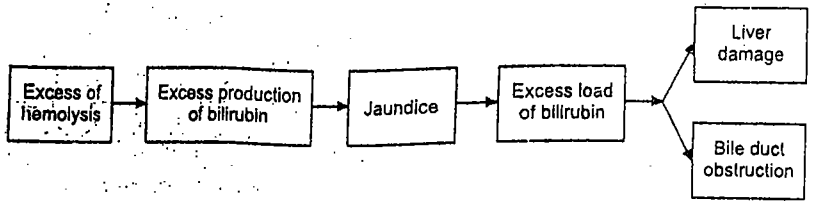


Fig. 9.24: Heme degradation

Hyperbilirubinemia can be caused by:

(i) Increased bilirubin production.

(ii) Decreased uptake of bilirubin in liver cells.

(iii) Impaired conjugation of bilirubin.

(iv) Problems in the secretion of conjugated bilirubin.

Examples of Diseases Showing Hyperbilirubinemia

(i) **Hemolytic jaundice:** Disease results due to increase in production of bilirubin. Here, more than normal amount of bilirubin is conjugated and excreted. However, amount of bilirubin produced is more than the capacity of body to conjugate it. This results in the large amount of unconjugated bilirubin in the blood.

(ii) **Gilbert's disease:** The cause of the disease is inability of the hepatocyte to take up bilirubin from the blood. As a result unconjugated bilirubin is accumulated in the blood.

(iii) **Physiological jaundice:** It is the condition wherein conjugation is impaired. As a result, unconjugated bilirubin retains in the body.

(iv) **Dublin – Johnson syndrome:** The disease results due to inability of hepatocytes to secrete conjugated bilirubin in the bile after its formation. Conjugated bilirubin returns to blood. It is a rare, autosomal recessive benign disorder which causes black liver.

(v) **Biliary obstruction:** Biliary calculi causes backup and reabsorption of conjugated bilirubin. This gives rise to elevated blood levels of conjugated bilirubin.

QUESTIONS

1. Define inborn errors of metabolism. Add a detailed note on the inborn errors of amino acid metabolism.
2. Describe in detail the pathway of catabolism of amino acid tyrosine and phenyl-alanine.
3. Write a brief account of catabolism of sulphur containing amino acids.
4. Explain the fate of carbon skeleton of amino acids.
5. Write notes on the catabolism of the following amino acids:

   (a) Histidine, (b) Proline, (c) Arginine, (d) Valine, (e) Leucine, (f) Isoleucine
6. Explain in detail the pathway for the synthesis of thyroid hormone.
7. Write a note on the pathway of synthesis of catecholamines.
8. Explain the importance of glycine in the synthesis of specialised products in biochemistry.
9. Explain the pathway for the synthesis of urea.
10. Define transamination. Add a note on the significance and mechanism of the same.
11. What is meant by deamination? Write a note on the same.
12. Give account of significance of the one carbon metabolism in biochemistry.
13. Write notes on: (a) Cystinuria (b) Catabolism of serine (c) Nitrogen balance (d) Amino acid biosynthesis

UNIT IV

Chapter ... 10

CHEMISTRY AND METABOLISM OF NUCLEOTIDES

♦ LEARNING OBJECTIVES ♦

- *Recognize Structures of Nucleotides and Sources of Various Atoms in Nucleotides.*
- *Understand Pathways of Purine and Pyrimidine Biosynthesis.*
- *Understand Pathways of Catabolism of Purine Nucleotides.*
- *Learn important Aspects of Hyperurecemia and Gout from Biochemical Perspective.*

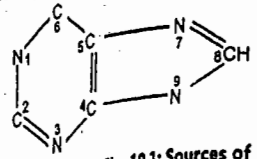
10.1 INTRODUCTION

Nucleotides play a variety of roles in the cells. First, they are precursors of RNA and DNA. Second, ATP and to some extent GTP are important and essential carriers of chemical energy. Third, group of nucleotides are components of cofactors NAD, FAD, S-adenosylmethionine and coenzyme A and activated biosynthetic intermediates like UDP glucose and CDP diacyl glycerol. cAMP and cGMP are also cellular second messengers.

Radiolabelled elements, also known as tracers are used to study the biosynthesis of nucleotides. Tracers enable us to understand the exact source of each and every element in a nucleotide.

10.2 SOURCES OF DIFFERENT ATOMS TO PURINES

Position 5 and 4 of the purine are provided by the glycine. The nitrogen at the 7 position is also obtained from the glycine. Thus, the entire molecule of the glycine is incorporated in the purine nucleus. Amino nitrogen of aspartic acid provides the nitrogen at position 1. Whereas glutamine provides amino nitrogens at position 3 and 9. The respiratory carbon dioxide is the donor of C atom at position 6. One carbon compound FH_4 is the donor of carbon at position 2 and 8. (FH_4 – Tetrahydrofolate).



Amino nitrogen of aspartate – N_1
α-carbon of glycine and FH_4 – C_2, C_8
Amide nitrogen of glutamine – N_3, N_9
Glycine – C_4, C_5, N_7
Respiratory CO_2 – C_6

Fig. 10.1: Sources of different atoms in purine biosynthesis

In the first committed step of the pathway, an amino group donated by glutamine is at C – 1 of PRPP. The resulting 5 phosphoribosyl amine is highly unstable. The purine ring is subsequently built upon this structure. The above reaction is catalysed by the enzyme glutamyl amidotransferase.

10.3 BIOSYNTHESIS OF NUCLEOTIDES

There are two types of pathways leading to nucleotides:

1. De novo pathways 2. Salvage pathways.

De novo synthesis of nucleotides begins with their metabolic precursors, amino acids, ribose 5 phosphate, CO_2 and NH_3.

Whereas salvage pathways recycle the free bases and nucleosides released from nucleic acid breakdown. Both pathways are important in cellular metabolism.

De novo pathways are similar in all living organisms. The free bases are not synthesized first and then attached to the ribose. In fact, purine ring structure is built-up one or a few atoms at a time, attached to the ribose throughout the process.

The pyrimidine ring is synthesized in the form of orotate, attached to ribose and then converted into the common pyrimidine nucleotides used in nucleic acid synthesis.

10.4 DE NOVO SYNTHESIS OF PURINE NUCLEOTIDES

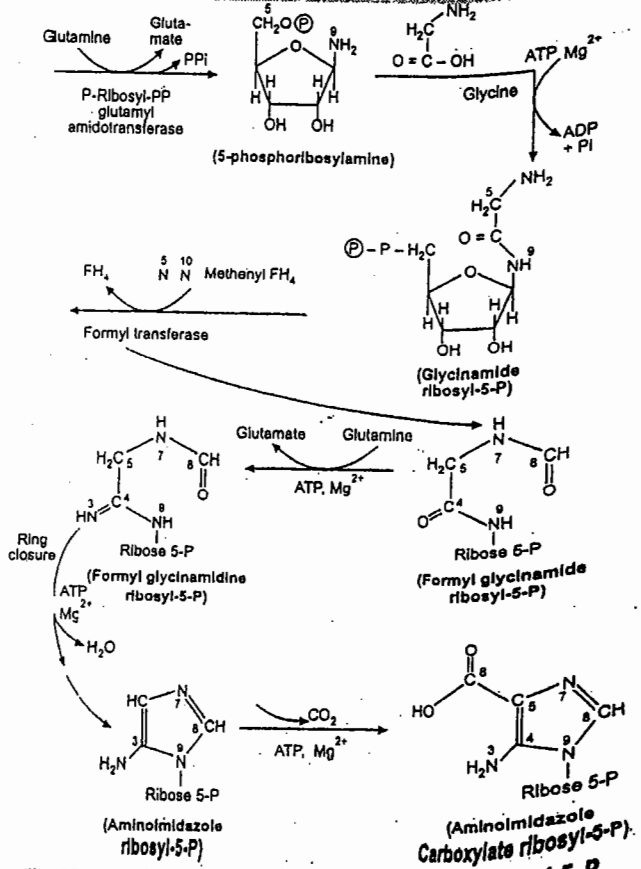


Fig. 10.2: Synthesis of amino imidazole carboxylate ribosyl 5-P

Biosynthesis of purine nucleotides take place in liver and other tissues except brain and erythrocytes.

De novo synthesis of purine nucleotides begins with PRPP (phosphoribosyl pyrophosphate). The first reaction takes place in the liver. It involves the ribose-5-phosphate, ATP, and magnesium ions and gives AMP and 5 phosphoribosyl - 1 - pyrophosphate. The reaction is catalysed by the enzyme, P-Ribosyl-PP synthetase. The ribonucleotides are reduced into deoxyribonucleotides. The reaction is catalysed by nucleotide reductase.

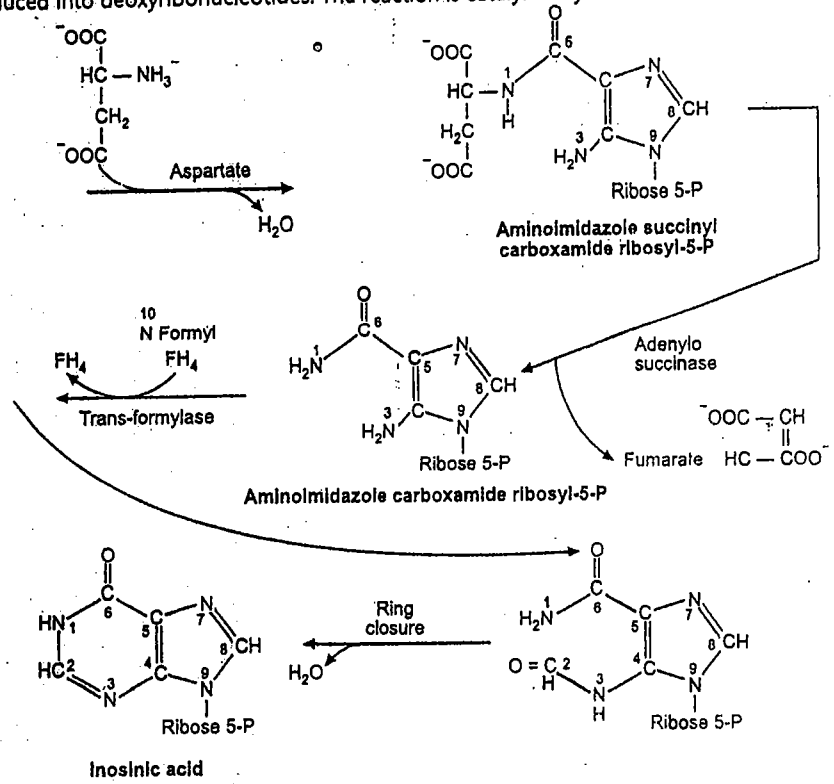


Fig. 10.3: De novo synthesis of purine nucleotides

Further reaction of phosphoribosyl pyrophosphate and glutamine is catalysed by glutamine amido transferase to form 5 phosphoribosylamine, glutamine donates N_9 of the purine ring. 5 phosphoribosyl amine then reacts with glycine to form glycinamide ribosyl - 5 - phosphate.

The activity of the enzyme phosphoribosyl pyrophosphate glutamine amido transferase is regulated by feedback inhibition. Particularly, AMP, GMP and IMP are its feedback modulators.

Formylation of glycinamide ribosyl phosphate occurs in the reaction in which transformulase and tetrahydrofolic acid (N^5 N^{10} methenyl FH_4) participate. Transfer of one carbon moiety from glycine to position 8^{th} of the purine ring occurs in the above reaction. Glutamine, the amino group donor, donates amino group to carbon 4 of the formylated glycinamide. The new coming nitrogen occupying position 3 in the purine nucleus. Next step is the ring closure forming amino imidazole ribosyl 5 (P) followed by carbamoylation. The respiratory carbon dioxide supplies carbon for carbamoylation and aspartic acid is the source of nitrogen in the above reaction. The carboxylation reaction does not require biotin.

Splitting of fumaric acid occurs in the next step. Formylation occurs again with F^{10} FH_4 and the newly added carbon accounts for the carbon - 2 of the purine nucleus. Ring closure occurs again resulting in the synthesis of the first purine nucleotide namely inosinic acid. Amination of hypoxanthine or xanthine nucleotides respectively gives rise to the adenine and guanine nucleotides. Antimetabolites like amethopterin, a folic acid antagonist, block formylation by way of preventing the reduction of dihydrofolate to tetrahydrofolate.

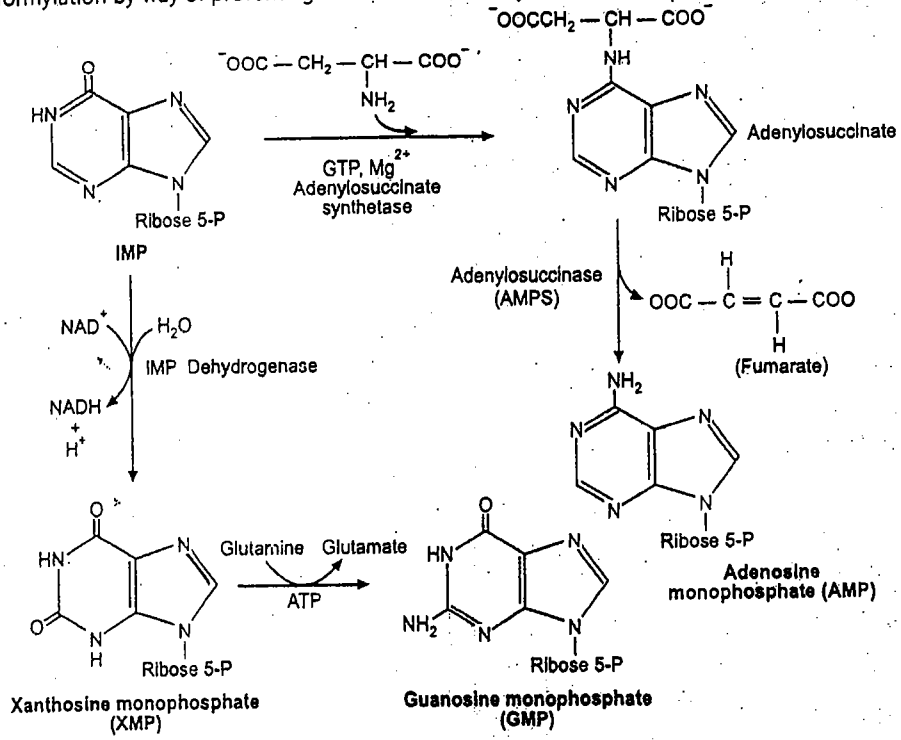


Fig. 10.4: Conversion of IMP into AMP, GMP and XMP

10.5 SALVAGE PATHWAY FOR PURINE BIOSYNTHESIS

Brain, erythrocytes and neutrophils are not able to synthesize purines by de novo synthesis. Liver is the major site for the supply of purines, which are salvaged by the brain, erythrocytes and neutrophils.

The salvage pathway recycles nucleosides produced from nucleic acid degradation. These pathways involve two important routes:

1. Phosphorylation of purine nucleosides at 5' hydroxyl group.
2. Phosphoriboxylation of preformed purines of purine nucleosides by the specific enzymes.

Salvage of purine nucleoside to purine nucleotide is carried out by the enzyme adenosine kinase.

The major salvage pathway is catalysed by the enzymes ike adenine phosphoribosyl transferase (APRTase). Another salvage pathway is catalysed by the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRTase).

Interconversion of IMP and GMP into IDP and GDP, and dIMP and dGMP into dIDP and dGDP is carried out by the purine 5' nucleotidase. These nucleosides are further phosphorylated at the sugar moiety producing their respective sugar phosphates and release the nitrogen bases free. Hypoxanthine and guanine can be phosphorylated in a similar manner.

The following three enzymes are found to compete for the substrate, PRPP. These are:

1. Hypoxanthine, guanine phosphoribosyl transferase.
2. PRPP glutamine amido transferase.
3. Adenine phosphoribosyl transferase.

GMP, IMP, AMP, the products formed, inhibit the enzyme PRPP glutamine amido transferase by the feedback inhibition.

Urinary as well as serum uric acid is also produced by the enzymes APRTase and HGPRTase. This prevents the de novo synthesis of uric acid. Dietary purines cause a shortage of PRPP and thus inhibit de novo synthesis of purines. This is because salvage pathway enzymes, APRTase and HGPRTase consume the substrate PRPP more rapidly than the enzyme PRPP amido transferase.

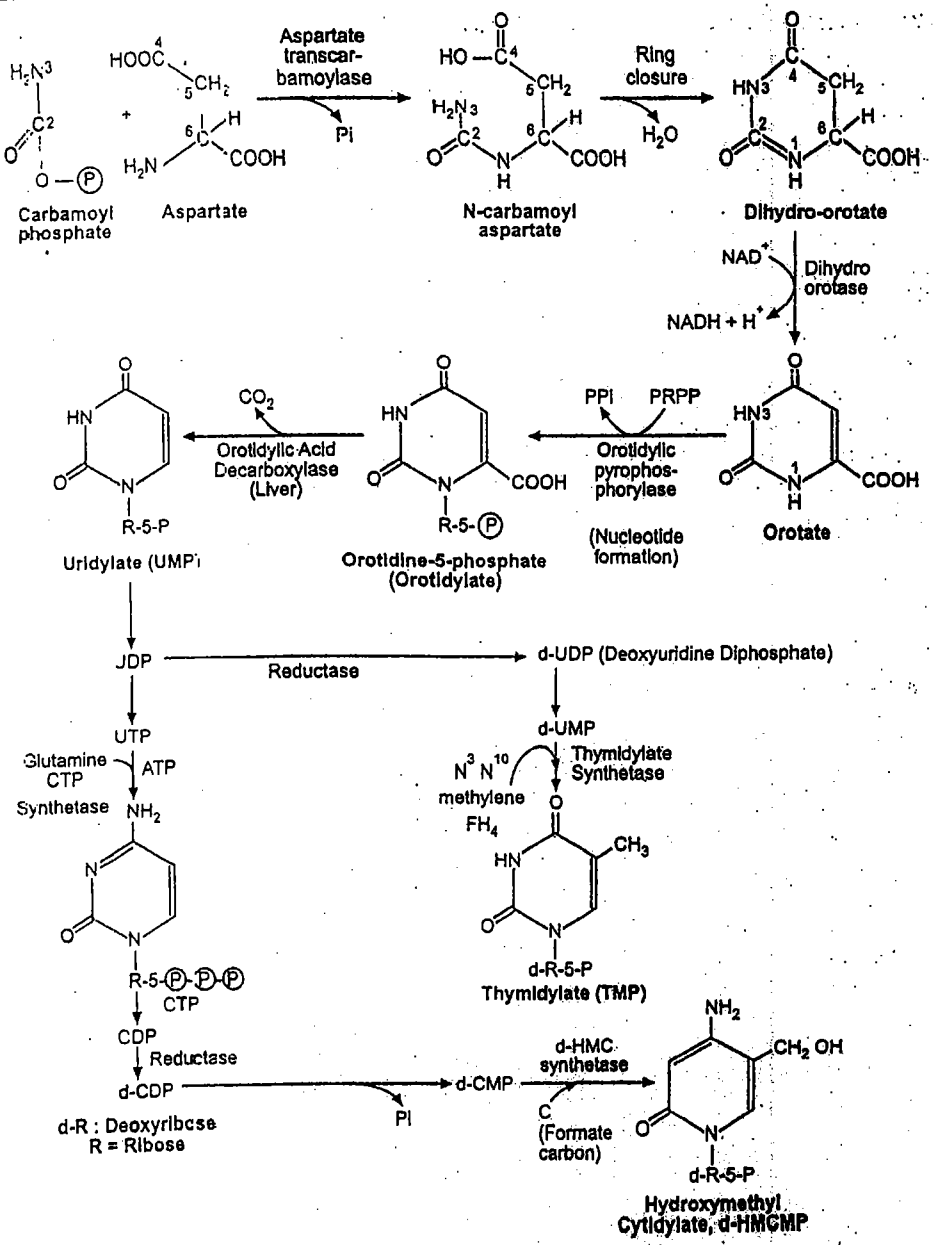


Fig. 10.5: Biosynthesis of pyrimidine nucleotides

10.5 PYRIMIDINE NUCLEOTIDE BIOSYNTHESIS

Pyrimidine nucleotides are made from aspartate and ribose - 5 - phosphate.

Pyrimidine nucleotide biosynthesis proceeds with the initial formation of a six membered pyrimidine ring. The ring is then attached to ribose 5 phosphate. This process requires carbamoyl phosphate, also an intermediate to urea cycle. Carbamoyl phosphate synthetase II, a cytosol enzyme, carries out pyrimidine biosynthesis. (Carbamoyl phosphate synthetase I employed in urea synthesis is present in the mitochondria).

Carbamoyl phosphate reacts with aspartate to yield N-carbamoyl aspartate in the first step of pyrimidine biosynthesis. The enzyme aspartate transcarbamoylase carries out this reaction, by removal of water from N-carbamoyl aspartate. The reaction is catalysed by dihydroorotase. A pyrimidine ring is closed to form L dihydroorotate. This compound is oxidised to yield the pyrimidine derivative orotate, a reaction in which NAD^+ is the ultimate electron acceptor.

Orotate is then attached to yield orotidylate. Orotidylate is then decarboxylated to yield uridylate, which is phosphorylated to UTP. CTP is formed from UTP by the action of cytidylate synthetase. This reaction occurs by way of acyl phosphate intermediate (consuming one ATP), and the nitrogen donor is glutamine in animals or NH_4^+ in some bacteria.

Regulation of pyrimidine nucleotide biosynthesis occurs by feedback mechanism. Bacterial enzyme, aspartate transcarbamoylase (ATCase), catalysing the first reaction is inhibited by CTP. Uridylic acid is also a competitive inhibitor of orodylic acid decarboxylase.

10.7 NUCLEIC ACID BIOSYNTHESIS

DNA and RNA, two different nucleic acids are produced by the process of replication and transcription respectively.

DNA is formed in the cell before cell division by the replication process. DNA flow is comparatively lower in the non-dividing cells.

Whereas RNA synthesis occurs continuously in all the cells. Not only mRNA, but rRNA, tRNA are also synthesized by transcription.

Ribonucleotides are reduced to deoxyribose nucleotides (i.e. reduction of hydroxyl group).

10.8 CATABOLISM OF PURINE NUCLEOTIDES

Nucleotides of the cell undergo regular and continual wear and tear. The old nucleotides are catabolized and new ones are formed. The order of breakdown of nucleotides occurs as follows:

Nucleotide
↓ Nucleotidase
Nucleosides
↓ Nucleoside phosphorylase
FreeNbases + Ribose-1-phosphate

(Some of the bases are reused in the salvage pathway.)

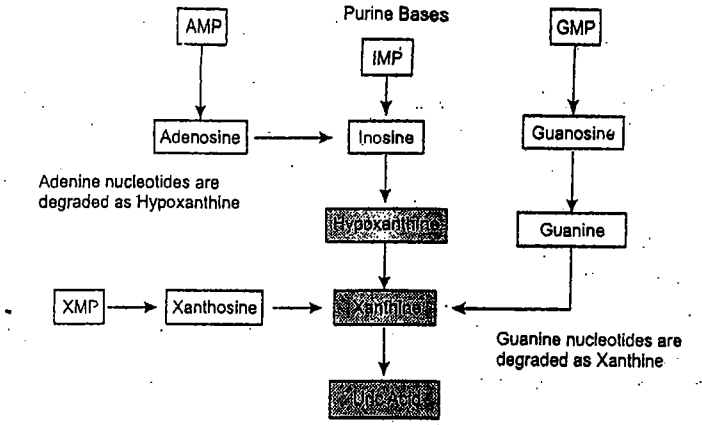


Fig. 10.6: Flowchart showing catabolism of purines

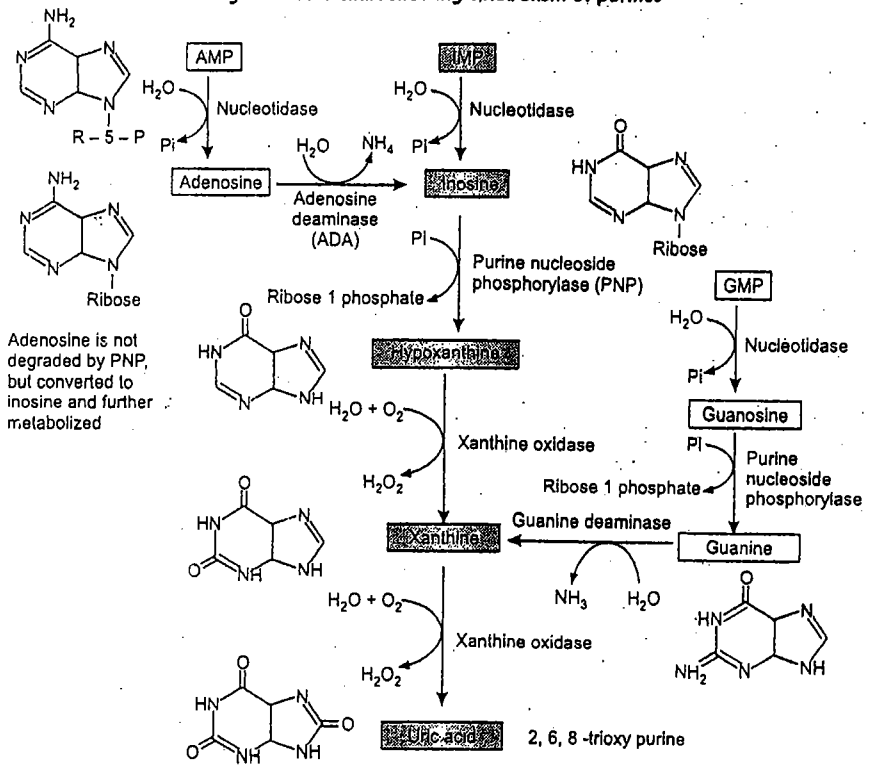


Fig. 10.7: Reactions showing catabolism of purines and production of uric acid

Other bases are degraded to products that are excreted. Uric acid is the end product of purine and amino acid catabolism. Some mammals and lower primates possess enzyme uricase which converts uric acid to allantoin which is more water soluble. Man does not have this enzyme uricase hence uric acid is the end product of purine catabolism. Uric acid is formed primarily in liver.

Normal serum uric acid concentration is 5-6 mg/dL. Uric acid is cleared off the body by glomerular filtration and tubular secretion.

Hyperuricemia and Gout:

It is the condition of increased uric acid level, above 7 mg/dL in men and 6 mg/dL in women.

It results from inherited metabolic disorders, malignanecies, pre-eclampsia or excessive alcohol consumption.

Gout is a metabolic disorder of purine catabolism which results from over production of uric acid. Uric acid is found in minimal soluble form, monosodium urates – which easily precipitate at lower temperature.

Types of Gout

- **Primary gout:** It is found to be a causative factor of 90% of cases of gout. It occurs due to inborn error of metabolism caused by defective enzyme of purine synthesis.
- **Idiopathic gout:** It occurs in 10% of cases of gout. Primary gout occurs due to different reasons like:

1. **Elevation of glutathione reductase enzyme.** This enzyme converts oxidized glutathione to reduced form by using NADPH, from the HMP shunt. Abnormal excessive activity of the glutathione reductase produces large amounts of $NADP^+$. Rise in the $NADP^+$ levels rises Ribose-5-phosphate and PRPP synthesis, which is responsible for the over production of purines.
2. **Glucose-6-phosphatase deficiency** - Van Gierke's disease. Owing to deficiency of the enzyme, excessive levels of Glucose-6-phosphate builds-up, which enter the HMP shunt and produce excess of Ribose-5-phosphate and PRPP, this way causing purine overproduction. Besides. lactic acidosis in Von-Gierke disease interfers with uric and excretion,
3. **A variant form of PRPP synthase enzyme** produces lot of purines. This genetically variant enzyme cannot be controlled by the allosteric mechanism, hence the control over purine production is lost. Excess of purines are responsible for excess of uric acid production.
4. **A one more variant of PRPP** glutamyl amidotransferase enzyme is not subjected to feedback control and product purines ceaselessly.

Secondary Gout:

It occurs due to different diseases causing increased synthesis and decreased excretion of uric acid.

(a) **Overproduction of uric acid occurs due to rise in turnover rate of nucleic acids as occurring in the following conditions:**
1. In psoriasis – due to increased tissue turnover.
2. In rapidly growing malignant tissues in cancers – like leukemia, lymphomas, polycythemia.
3. In the conditions leading increased tissue breakdown as in radiotherapy and chemotherapy, trauma and starvation.

(b) **Reduced excretion of uric acid:**
1. In chronic renal failure due to reduce GFR.
   (i) Increased alcohol consumption – leads to lactic acidosis, and lactic acid is responsible for decreasing tubular excretion of uric acid.
   (ii) Ketoacidosis – decreases the tubular excretion of uric acid.
   (iii) Thiazide diuretics – inhibits tubular secretion of uric acid.

Symptoms and Biochemistry Behind Gout Pathogenesis:

A low solubility of uric acid is the core reason behind the clinical symptoms. Typical gouty arthritis affects great toe or phalangeal joint. It is the classical sign of gout. The serum urate level exceed solubility limit leading to the rise in formation of monosodium urate crystals which get deposited in joints. These deposits are called 'Tophi'. Tophi deposition causes inflammation of joints, painful arthritis which culminates into chronic gouty arthritis. Other complications include urolithiasis and renal damage.

QUESTIONS

1. Explain the sources of different atoms in purine nucleotides.
2. Write a note on the de novo synthesis of purine nucleotides.
3. What is meant by salvage pathway? Add a note on the salvage pathway for purine nucleotide biosynthesis.
4. Explain the metabolic pathway for the biosynthesis of the pyrimidine nucleotides.
5. Discuss the de novo pathway for purine nucleotides biosynthesis.
6. Write short notes on:
   (a) Regulation of pyrimidine nucleotides biosynthesis.
   (b) Regulation of purine nucleotide biosynthesis.
   (c) Metabolic disorders associated with catabolism of purine nucleotides.
   (d) Inhibitors of purine synthesis.
   (e) Role of carbamoyl phosphate synthetase II.
7. Explain in detail the biosynthesis of inosine monophosphate.
8. Describe in detail the role of PRPP in purine and pyrimidine biosynthesis.
9. Explain with flow chart catabolism of purine nucleotides.
10. Describe in detail the terms – *gout and hyperuricemia.*

Chapter ... 11

DNA, REPLICATION AND RECOMBINANT DNA TECHNOLOGY

♦ LEARNING OBJECTIVES ♦

- *Revise Important Aspects of the DNA Structure.*
- *Understand Roles of Different Enzymes in the Process of Replication.*
- *Understand DNA Mutation and Repair.*
- *Understand Recombinant DNA Technology.*
- *Compare Prokaryotic and Eukaryotic Replication Process.*

INTRODUCTION

DNA (deoxyribonucleic acid) is the prime genetic material, which carries hereditary information within chromosomes. A portion (segment or region) of DNA that contains information required for the synthesis of a functional biological product (protein or RNA) is called as a *gene*. Typically, cell has many thousands of genes and thus DNA molecules are very large. *Storage of biological information is the only known function of DNA.*

DNA is a polymer of *deoxy*nucleotides. Each nucleotide is made up of:

1. A nitrogenous base
2. A pentose sugar
3. A phosphate group.

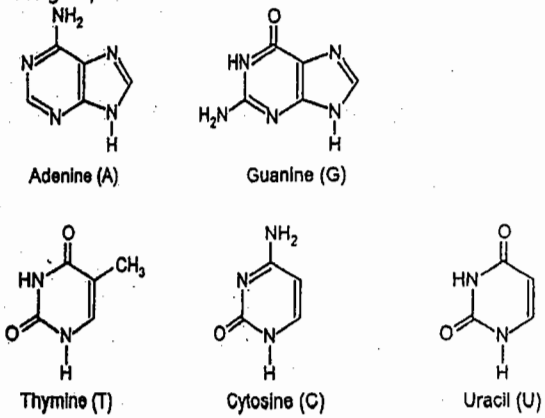


Fig. 11.1 (A): Structures of purines (A, G) and pyrimidines (T, C, U)

The phosphate groups are strongly acidic, thus the name nucleic acid. The nitrogen bases are of two types: purines and pyrimidine. In DNA, pyrimidines are thymine (T) and cytosine (C) and purines are adenine (A) and guanine (G). RNA contains uracil (U) instead of thymine. [Fig. 11.1 (A)]. The structures of the respective dideoxynucleotides are shown in Fig. 11.1 (B).

Pentoses are also of two types: deoxyribose in DNA and ribose in RNA. In deoxyribose there is one less oxygen atom than ribose.

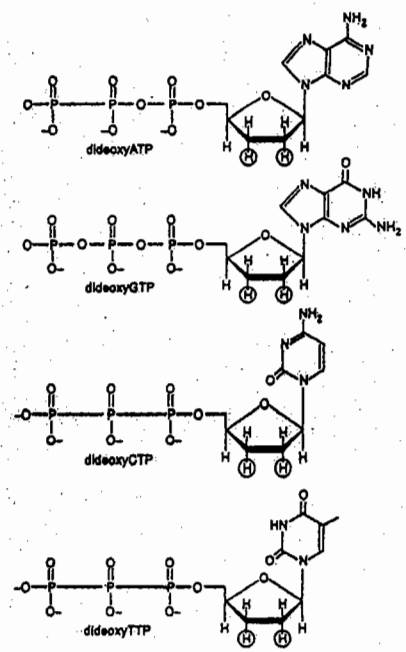


Fig. 11.1 (B): Dideoxynucleotides

The base is joined covalently (at position N–1 of pyrimidines and N–9 of purines) via a N-glycosyl linkage to the 1' carbon of the pentose and the phosphate is esterified to the 5' carbon. The successive nucleo-tides of both DNA and RNA are covalently linked via phosphate group bonds. The 5'–hydroxyl group of one nucleotide unit is joined to 3'-hydroxyl group of next nucleotide by a *phosphodiesterase linkage.*

The backbone of DNA and RNA consist of alternating phosphate and pentose residues and thus is hydrophilic. Both DNA and RNA have distinct 5' and 3' ends: 5' end has a phosphate group but lacks a nucleotide at the 5' position and 3' end has a hydroxyl group but lacks nucleotide at 3' position. (Fig. 11.2)

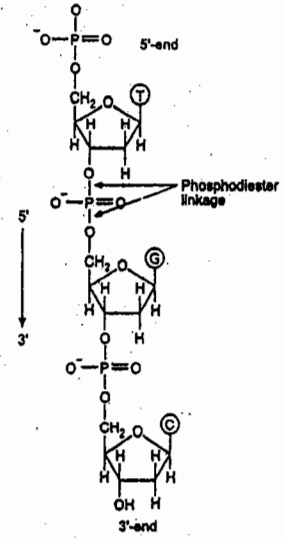


Fig. 11.2: Backbone structure of DNA segment showing phosphodiester linkage

11.2 DNA STRUCTURE

In 1950, Erwin Chargaff at Columbia University showed that no matter which tissue from an animal was looked at, the percentage content of each of the four nucleotides was the same. However, the percentage varied from species to species. Importantly he also showed that in all animals:

$$\%G = \%C$$
$$\%A = \%T$$

These findings were crucial in elucidating the structure of DNA.

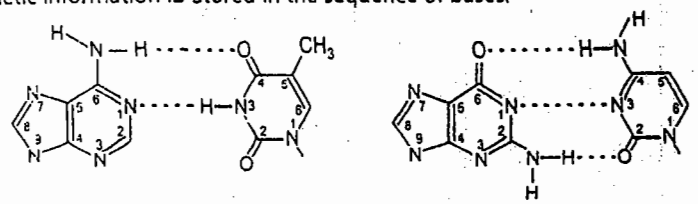
The structure of DNA was proposed by Watson and Crick in 1953 based on the X-ray diffraction data of Wilkins and Franklin. This structure suggests that DNA is composed of two right handed helical polynucleotide chains that form a double helix around the same central axis. These two strands are antiparallel and bases are stacked inside the helix in a plane perpendicular to the helical axis. These two strands are held together by hydrogen bonds between the nitrogenous bases moieties of opposite strands and only certain base pairs fit into the structure. Thus, the only pairs possible are AT and GC. There are two hydrogen bonds between A and T and three between G and C and hence GC pair is more stable than AT pair (Fig. 11.3). Note that hydrogen bonds are formed only between a hydrogen atom on one base and oxygen or nitrogen atom on the other base. This is because a hydrogen bond can only be formed when hydrogen atom comes in close proximity to an oxygen or nitrogen atom of a base pair on the opposite strand. Hence, only two bonds can be formed between AT pair and three between GC. The *two anti-parallel polynucleotide chains of DNA are not identical in base sequence or composition but are complementary to each other.*

The features of Watson-Crick model of DNA can be summarised as given below:

- There are two *complementary* polynucleotide strands held together by hydrogen bonds.
- The hydrogen bonds are formed between the bases A and T and between G and C.
- This base pairing leads to a ladder-like structure and as the bases are hydrophobic, succeeding pairs attract each other producing a twist in the structure creating a right-handed double helix.
- The strands are referred to as complementary, as sequence on one determines what the sequence on the other must be e.g. the sequence ACTTGA on one, pairs only with TGAACT on the other.
- The two strands are *antiparallel*, meaning they run in opposite direction. Hence, 5'- ACTTGA-3' pairs with 3'-TGAACT-5'.

```
5' - A C T T G A - 3'
     : : : : : :
3' - T G A A C T - 5'
```


- Genetic information is stored in the sequence of bases.



Adenine: Thymine **Guanine: Cytosine**

Fig. 11.3: The A.T. and G.C. base pairs of DNA

Most of the DNA is right handed and of the B-DNA form. The B-DNA contains two kinds of grooves, called the *major groove* and the *minor groove*. (Fig. 11.4). The minor groove contains the pyrimidine – O_2 and the purine – N_3 of the base pair. The major groove is slightly deeper than the minor one and is on the opposite side of the base pair.

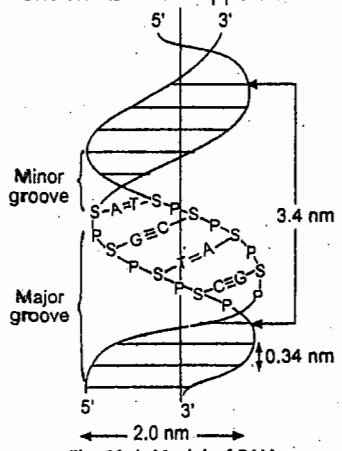


Fig. 11.4: Model of DNA

The dehydrate (relative humidity below 75%) shows a different form of DNA than described above, called A-DNA. This change in the conformation is reversible. A-DNA is wider, flatter and shorter right-handed helix compared to B-DNA. In A-DNA, the planes of the bases are tilted to the helix axis rather than normal like in B-DNA. Thus, A-DNA has a deep major groove and a very shallow (almost non-existent) minor groove.

A-helix form is also seen in double stranded regions of RNA and RNA-DNA hybrids.

A-form DNA	B-form DNA
• Shorter, wider helix than B. • Most RNA and RNA-DNA duplex are in this form. • Deep, narrow major groove and is not easily accessible to proteins.	• Narrower, elongated helix than A. • Most common DNA conformation *in vivo*. • Wide major groove, easily accessible to proteins.

contd....

• Wide, shallow minor groove. • Base pairs tilted to helix axis and displaced from axis. • Favoured conformation at low water concentration.	• Narrow minor groove. • Base pairs are approximately perpendicular to helix axis. • Favoured conformation at high water concentration.

The third type of DNA was identified during the crystal structure determination of d(CGCGCG) by Alexander Rich and co-workers. They revealed that duplex of antiparallel strands was left handed and was named Z-DNA because of zig-zagged nature of phosphate backbone. Z-DNA contains only one deep minor groove and no identifiable major groove.

Most of the DNA in any genome is the classic Watson-Crick B-DNA form. The additional A-DNA and Z-DNA forms show that DNA is flexible and dynamic.

11.3 CHROMOSOME

Chromosomes are long, thin, rod-like structures present in the nucleus. They have the following characteristics:

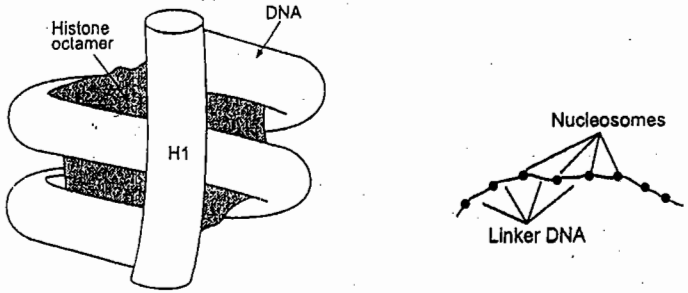
- All cells of an organism and all organisms of the same species have the same number of chromosomes, except sperm cells and egg cells.
- There are two copies of each type of chromosome, such cells (i.e. somatic cells) are called as diploid cells.
- Germ cells i.e. sperm and egg have one copy of each chromosome type. Such cells are called as haploid cells.
- The fertilization of an egg with a sperm cell produces a diploid cell i.e. zygote, which has the same number of chromosomes as that of somatic cells.
- During mitosis, the number of chromosomes double just prior to the cell division.

Eukaryotic chromosomes are larger and have a higher degree of structural organisation than those of prokaryotes. The DNA in eukaryotes is tightly bound to small basic proteins called as *histones*. In eukaryotic chromosomes, half the mass is histones and half being DNA. This nucleoprotein chromosomal material is called as *chromatin*. *Chromatin* is a term which designates a structure in which DNA exists within the cell. The histones and DNA can be separated from each other by treating chromatin with salt or dilute acid. The unique characteristic of histones is their high content of positively charged side chains (~ one in four residues is either lysine or arginine). In all, there are five types of histones – H1, H2A, H2B, H3 and H4 , the mass ranges from 11 kD to 21 kD.

A chromatin is made up of repeating units each consisting of 200 bp of DNA and two of H2A, H2B, H3 and H4. These repeating units are called as *nucleosomes*. The adjacent nucleosomes are attached by *linker* DNA. Thus, a chromatin fibre is a flexibly joined chain of nucleosomes; like beads on a string. (Fig. 11.5 (A)).

Nucleosomes from different organisms and cell types range from about 160 – 240 bp of DNA. Irrespective of the initial DNA content of nucleosome, a *core particle* of 140 bp of DNA is obtained after the treatment of nucleosomes with nuclease. This 140 bp of DNA is not further digested and this nucleosome core is probably nearly the same in all eukaryotes. This

core consists of 140 bp of DNA bound to a histone octamer; two each of H2A, H2B, H3 and H4. (Fig. 11.5 (B)). The other class of DNA binding proteins is a diverse group of proteins called as *non-histone proteins*. Non-histone proteins include transcription factors, polymerases, hormone receptors and many nuclear enzymes. In any given cell generally there are more than 1000 types of non-histone proteins bound to the DNA.



(A) Nucleosome and linker DNA (B) Nucleosome

Fig. 11.5

The chromosomes of many viruses and bacteria are circular molecules of double stranded DNA. In this circular duplex molecule, both strands are covalently joined by DNA *ligase*. DNA ligase catalyzes the formation of a phosphodiester bond between the 3'-OH group at the end of one DNA chain and the 5'-phosphate group at the end of the other. This enzyme cannot link two molecules of single-stranded DNA or circularise single stranded DNA.

Circular duplex DNA molecules sometimes have a peculiar twisted appearance, known as supercoiling or superhelicity. Supercoiled DNA is more compact than "relaxed" molecule with the same number of nucleotides. (Fig. 11.6).

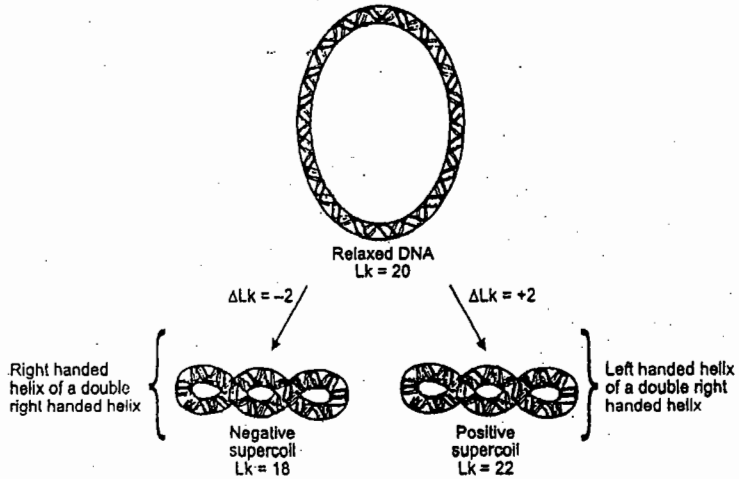


Fig. 11.6: Circular DNA and supercoiling

A geometric property of double helical DNA is that its number of coils cannot be altered without first cleaving atleast one of the strands. Mathematically this can be expressed as:

$$L = T + W$$

L, the linking number, the number of times a strand of DNA winds in the right hand direction around the helix axis. This number cannot be changed as long as both strands remain covalently intact (DNA molecules differing only in linking numbers are topological isomers [*topoisomers*]).

T, the twist, complete revolutions (helical winding) of strands around each other. T is considered to be positive for right-handed duplex. For B-DNA it is normally the number of base pairs divided by 10.4 (observed number of base pairs per turn of B-DNA in aqueous solution).

W, the writing number, measure of DNA supercoiling. Number of turns of duplex axis around the superhelix axis.

11.4 REPLICATION OF DNA

The double helical structure of DNA proposed by Watson and Crick has the following features:

(i) Two polynucleotide chains running in opposite directions coil around a common axis to form a right-handed double helix.

(ii) The purine and pyrimidine are on the inside of the helix, whereas the phosphate and deoxyribose units are on the outside.

(iii) Adenine (A) is paired with thymine (T) and guanine (G) with cytosine (C). AT base pairs are reinforced by two hydrogen bonds and GC by three such bonds.

One DNA strand is the complement of the other. The strict base-pairing rule suggests that the use of one strand as a template will result in another strand with a predictable, complementary sequence.

Cells (both prokaryotic and eukaryotic) do not live forever and hence must pass their genetic information to new cells (offspring). Thus, they should be able to replicate the DNA to be passed to the offspring. The process of accurate, efficient and rapid duplication of the genome is called as *DNA replication.*

DNA replication has a *semi-conservative* meaning. If each DNA strand serves as a template for the synthesis of a new strand, then two daughter DNA molecules will be formed, each having one new strand and one old strand.

The hypothesis of semi-conservative replication was proposed by Watson and Crick and was proved in 1957 by Mathew Meselson and Franklin Stahl.

11.4.1 Meselson – Stahl Experiment

E.coli cells were grown for many generations in a medium which contained $^{15}NH_4Cl$, a heavy isotope of nitrogen. (The DNA isolated from these cells had a density of about 1% greater than the normal [^{14}N] DNA and can be separated by centrifugation in a cesium chloride density gradient). (Fig. 11.7).

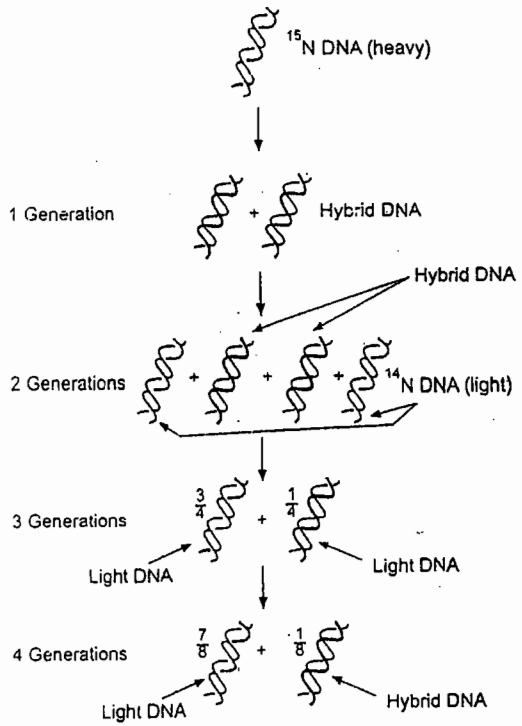


Fig. 11.7: Meselson and Stahl experiment

These cells were then transferred to a normal medium (^{14}N) and were allowed to grow until the population just doubled. The DNA isolated from these first generation cells gave only one band in the cesium chloride gradient suggesting that the DNA from daughter cells were hybrids containing one new 'normal' [^{14}N] and one parental [^{15}N] strand.

This semiconservative nature of replication was further proved when cells were allowed to double in number again in the normal medium. The DNA of this cycle exhibited two different bands on cesium chloride gradient, one with density equal to that of light DNA and the other having the density of hybrid DNA.

11.4.2 Replication of Circular DNA or Bacterial Replication

DNA synthesis begins at a site termed as origin of replication. Each bacterial chromosome has only one origin of replication. Synthesis of DNA proceeds bidirectionally around the bacterial chromosome - eventually meeting at the opposite side of the chromosome where replication ends. Directionality of DNA strands at replication fork.

Duplication of circular DNA does not involve the creation of linear DNA. The parental strands maintain a circular form throughout the replication. The synthesis of daughter DNA proceeds outwardly in both the clockwise and anti-clockwise directions.

11.4.3 Chain Growth Occurs In Both Directions

The two daughter strands being synthesized at each replicating fork run in opposite directions. Hence, direction for chain growth must be 5' → 3' and 3' → 5' for the other strand. But enzymes that add nucleotide precursors to DNA i.e. DNA polymerases, extend chain only in the 5'→ 3' direction. The reason being the reaction catalysed by DNA polymerases allow a nucleotide triphosphate to react only with the free 3'–OH end of a polynucleotide strand. The energy for formation of the phosphodiester bond in 3' to 5' is on the 5' end of the growing chain. That is, if wrong base is put in and removed, then no energy will be left on the resulting monophosphate end for the condensation. But in case of 5' to 3', the energy for condensation is on the triphosphate of the incoming base. Thus, if there is editing, there is still energy for the bond formation and hence because of the need of the polymerase to edit its work, replication occurs in 5' to 3' direction. Inspite of lot of research no replicating enzyme that would add nucleotides onto free 5' – ends have been found. As replication proceeds, one strand is synthesized continuously whereas other newly synthesized DNA strand exists as small fragments, 100 to 1000 bp long, called as *Okazaki fragments*, that later link upto the main daughter strand by DNA ligase. This suggested that DNA chain whose ultimate direction of growth is 3' → 5' might, in fact, be formed by the joining of Okazaki fragments, which grow in the conventional 5' → 3' direction. The strand formed from Okazaki fragments is termed as the *lagging strand*, whereas the one synthesized continuously is termed *leading strand*. Thus, the discontinuous assembly of the lagging strand enables 5' → 3' polymerisation at the nucleotide level to give rise to overall growth in the 3' → 5' direction (Fig. 11.8).

In eukaryotes, chromosomes are linear and are very large. So to complete the replication in a reasonable time, beginning of replication occurs at several origin points (Fig. 11.9). Each replication origin grows in both directions with similar process as described above (leading and lagging strand). But the precise nature or origin of replication in higher eukaryotes is not clear.

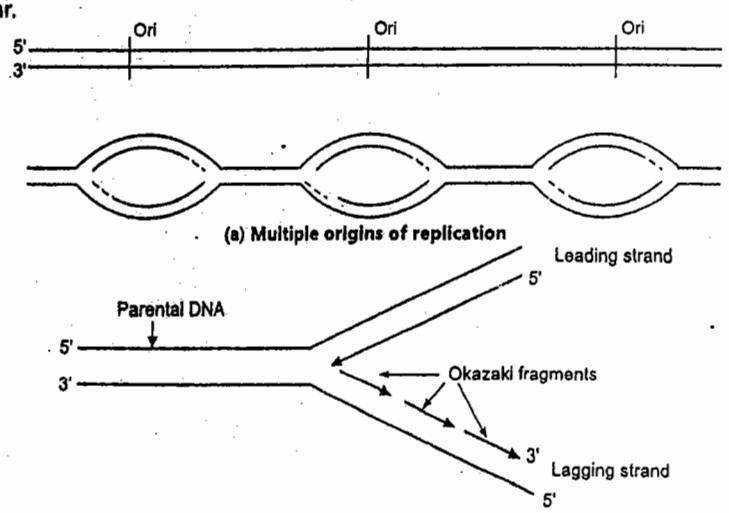
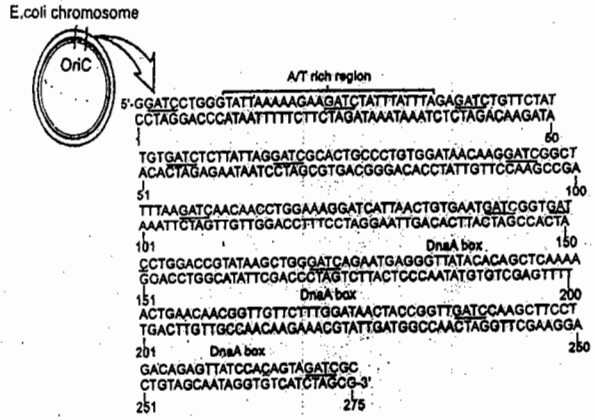
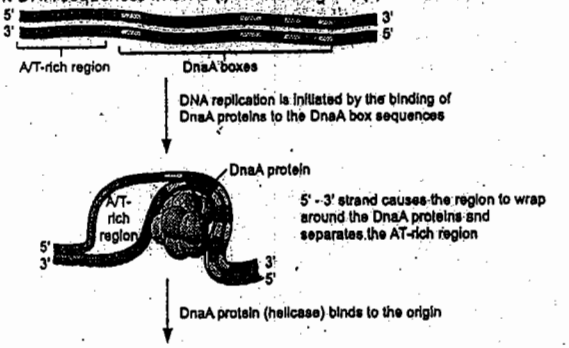


Fig. 11.8



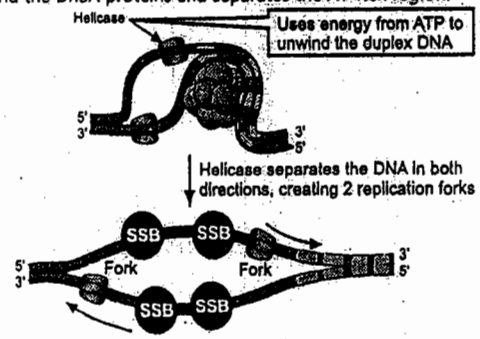
(a)

The origin of replication of *E. coli* is termed as ori C-origin of chromosomal replication. There are two types of important DNA sequences in ori C (i) AT-rich region, (ii) DnaA boxes.



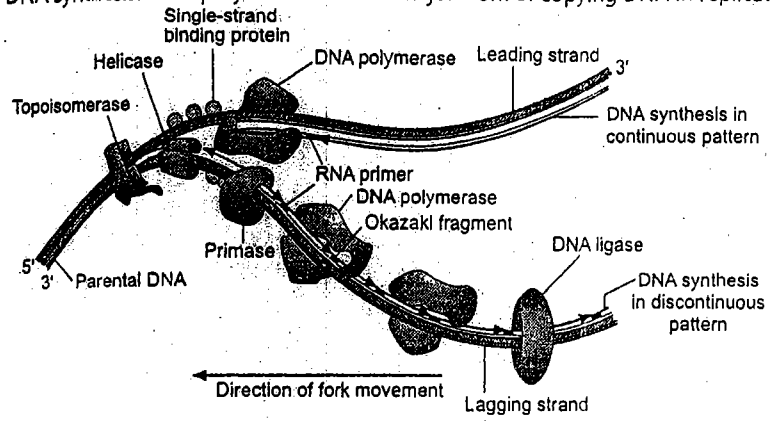
(b)

DNA replication is initiated by binding of DnaA protein to DnaA box sequence. Dna protein causes the region to wrap around the DnaA proteins and separates the AT-rich region.



(c)

DnaB protein helicase binds to origin. Helicase bind to single strand of DNA at the origin. Helicase uses energy from ATP to unwind duplex DNA. Helicase separates the DNA in both directions causing replication fork. Helicase separates the two DNA strands by breaking the hydrogen bonds between them. This generates positive supercoils ahead of fork. DNA gyrase travels ahead of the helicase and alleviates these supercoils. Single stranded binding proteins bind to separated DNA strand to keep them apart. Then short (10-12 nucleotides) RNA primers are synthesized by DNA primase. These short RNA primer strands start or prime DNA synthesis. DNA polymerase III does major work of copying DNA in replication.



(d) Replication fork

Fig. 11.9: Picture of replication fork showing role of different proteins and enzymes

11.4.4 DNA Polymerases

In *E. coli* there are three different DNA polymerases (I, II and III). Although it was thought for long, DNA polymerase I was the major enzyme which joins together deoxyribonucleotides. Now, it is known that multisubunit assembly containing polymerase III synthesies new DNA, whereas polymerase I erases primer and fills the gap. DNA polymerase II is not needed for DNA replication but participate in DNA repair. It has been suggested that DNA polymerase II could be dispensable as mutant cells having no or if any, very few molecules of DNA polymerase II which are known to survive. In *E. coli* it has been shown that besides DNA polymerase I and III, there are more than 20 proteins, which participate in DNA replication.

In eukaryotes, there are five distinct polymerases – α, β, γ, δ and ε (Table 11.1). The eukaryote DNA polymerase which is equivalent to *E.coli* DNA polymerase III is polymerase-α. *E.coli* DNA polymerase I is equivalent to eukaryotic DNA polymerase-β. Eukaryotic DNA polymerase-γ is responsible for the replication of mitochondrial DNA.

Table 11.1: Eukaryotic DNA polymerases

Polymerase	Location	Function
α	Nucleus	Lagging strand replication
β	Nucleus	DNA repair
γ	Mitochondria	Mitochondrial DNA replication
δ	Nucleus	Leading strand replication
ε	Nucleus	Replication

All the three DNA polymerases possess 3' → 5' exonuclease activity, meaning all DNA polymerases have the ability to cut back nascent polynucleotides as well as to extend them. Generally, synthesis is overwhelmingly favoured over degradation. This exonuclease activity preferentially acts on incorrectly paired bases. Thus, high fidelity is achieved in DNA replication because of 3' → 5' exonuclease activity which acts as a proof reading mechanism.

11.4.5 Helicases

To permit the rapid rates of DNA polymerisation observed, the double helix should come apart frequently but this does not occur spontaneously. The facilitation of strand separation is done by specific proteins called as *helicases*. Two different helicases have been identified. Helicase II or III binds to the template for the lagging strand and moves in 5' → 3' direction. Whereas, the other helicase, called *Rep protein*, binds to template for leading strand and moves in the 3' → 5' direction. Helicases are proteins that are bound to DNA and derive the energy from ATP to move progressively along with DNA and increase the rate of strand separation. The unwound portion of DNA is then stabilised by single strand binding protein (SSB). SSB is a tetramer and binds co-operatively to single-stranded DNA. Thus, the single-stranded DNA tightly covered by SSB is rigid, semiextended without bends or knicks. This is essential in order to avoid its tendency to fold back on itself to form imperfect base-paired hairpin loops.

11.4.6 RNA Primers

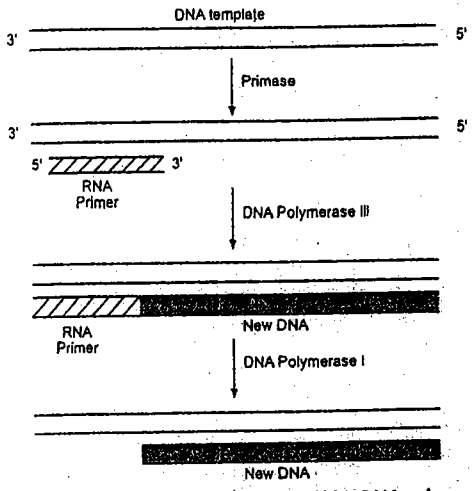


Fig. 11.10: DNA replication is primed by RNA primers

DNA polymerases require a primer, pre-existing polynucleotide chain, with a free 3'-OH group for DNA synthesis. How is this primer formed? It was observed that RNA synthesis is essential for the initiation of DNA synthesis. Thus, starting points for DNA synthesis are recognised by enzymes that transcribe DNA to RNA. These RNA chains thus generated serve

as primers. A specialised RNA polymerase called *primase* joins prepriming complex called as *primosome and prepares primers for Okazaki fragments (Fig. 11.10).* Primosome consists of primase, polypeptide of 60 kD, present in 50 – 100 copies per cell in *E.coli*, and six or seven other polypeptides. The components of primosomes ensure movement along the DNA, the displacement of SSBs, the recognition of appropriate start site and the polymerisation of ribonucleotides into RNA. The RNA primer is removed by RNase H and the gap is filled by DNA polymerase I.

11.4.7 DNA Polymerase III

The active form of DNA polymerase III holoenzyme (an assembly of seven different polypeptides) positions at the replication fork and begins the synthesis of the leading strand using the RNA primers (see above).

One of the seven polypeptides is α, which carries the 5' → 3' exonuclease activity as well as the polymerisation function, while the ε (epsilon) polypeptide possesses the 3' → 5' exonuclease activity. The functions of the remaining polypeptides are not known.

The duplex DNA ahead of DNA polymerase III is unwound by helicase, SSBs keep the unwound strands of DNA extended and accessible. DNA polymerase III continuously synthesizes the leading strand and does not release the template until the replication has been completed.

As seen earlier the lagging strand is synthesized in fragments so that 5' → 3' polymerisation leads ultimately to growth in 3' → 5' direction. (See Section 11.4.3). This is achieved by looping lagging strand template through the one subunit of polymerase so that it passes in the same direction as the leading strand template in the other subunit. DNA polymerase III releases the lagging strand template after addition of about 1000 nucleotides. Then a new loop would be formed synthesizing short stretch of RNA primer for another Okazaki fragment.

11.4.8 Telomerases

There is a problem when replication approaches end of a linear structure of chromosome (telomer). Remember that a primer is needed. It was not understood, how is this last part of 3' end of chromosome repliacted? This mystery was solved by the discovery of an enzyme - *telomerase*. Telomerase is like reverse transciptase, it has a small RNA template of its own. This template is used for the addition of telomer repeat (repeated sequences in telomers) at 3' end. Thus, RNA primer is synthesized and then the last piece of DNA for lagging strand is synthesized.

11.5 DNA MUTATION AND REPAIR

Mutations are inheritable changes in the DNA. Mutations are passed on to next generation when the DNA replicates and are also transcribed into RNA; they may result in changes in the protein produced.

Mutations in DNA can be of various types:

(i) Substitution of one pair for another.

(ii) Deletion of one or more base pairs.

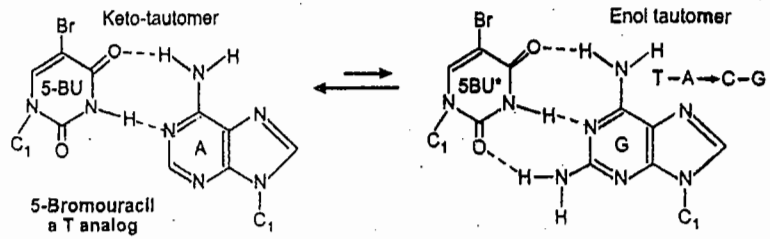
(iii) Insertion of one or more base pairs.

The most common type of DNA mutation is the substitution of one base for another. There are further two types possible, and they are:

(a) Transition: Replacement of one purine by other purine or one pyrimidine by other pyrimidine.

(b) Transversion: Replacement of purine by a pyrimidine or pyrimidine by a purine.

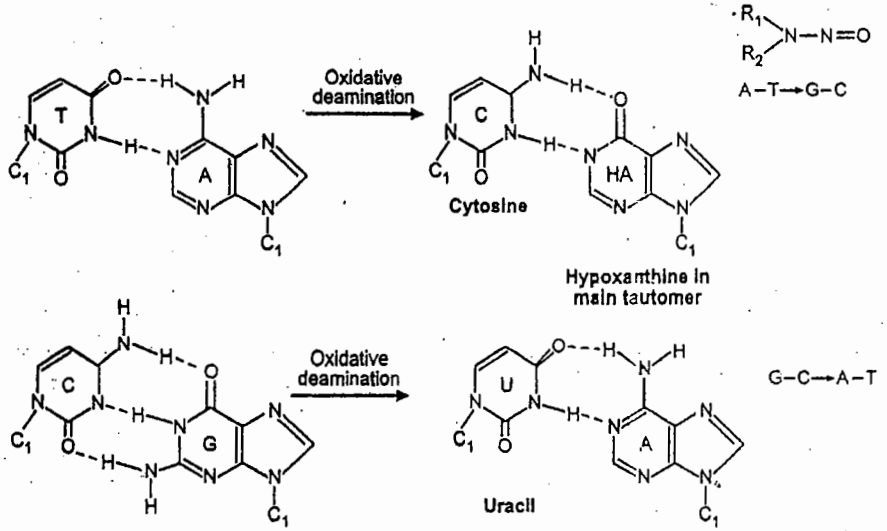
Transition mutations can be generated by base analogs e.g. 5-bromouracil and 2-aminopurine. 5-Bromouracil, a base analogue of thymine normally pairs with adenine, whereas, 2-aminopurine normally pairs with thymine.



Point mutations are single base pair changes.

A *sense mutation* has no effect on protein sequence. For example, if AGU is changed to AGC, the protein would still have the appropriate serine at that position. A *missense mutation* leads to changes in the mRNA code. This change will alter the shape or properties of the protein. For example, if AGU is changed to AGA, then protein will have arginine instead of serine. A *nonsense mutation* creates a stop codon and hence shortens the resulting protein.

Mutations can also be generated by chemical modifications of the bases of DNA e.g. nitrous oxide generally reacts with bases which contain amino groups. Nitrous oxide can oxidatively deaminate adenine to hypoxanthine, cytosine to uracil and guanine to xanthine. Nitrous oxide causes AT↔GC transition i.e. hypoxanthine pairs with cytosine, uracil pairs with adenine rather than guanine and xanthine pairs with cytosine.



Flat aromatic molecules like acridine produce insertion or deletion of base pair(s) leading to alteration of the reading frame in translation. These molecules slip in between adjacent base pairs i.e. they *intercalate* in the DNA.

Not only chemicals but ionising radiation and ultraviolet light can also damage DNA. DNA can be damaged (or lesioned) in a variety of ways e.g. alteration or loss of bases, breaking of phosphodiester bonds and covalent crosslinking of strands. As genetic information is stored in both strands, the information lost by one strand can be retrieved from the other, thus much of the damage can be sustained.

Let us discuss an example. When DNA is exposed to ultraviolet light, pyrimidine dimers are formed i.e. adjacent pyrimidine are covalently linked, thus cannot fit into double helix and hence inhibit replication or gene expression. In *E. coli*, initially an enzyme complex consisting of a protein (which is a product of uvrABC gene) detects the defect because of pyrimidine dimer. Then the uvrABC enzyme cuts the damaged DNA strand at two positions – on 5' side, eight nucleotides away and on 3' – side 4 nucleotides away. This 12-deoxynucleotide portion is then cut by a specific enzyme called *exinuclease*. After this DNA polymerase I enters the gap and carries out repair synthesis.

Pyrimidine dimer can also be repaired photochemically by a photoreactivating enzyme called as *DNA photolyase*. This enzyme binds to the defective region of DNA and absorbs a photon at near UV and blue spectral region of light. The energy created by absorption at photon cleaves the dimer into its original bases.

DNA repair is clinically very important. *Xeroderma pigmentosum* (an autosomal recessive disorder characterised by dry, rough discoloured skin) is a disease in which pyrimidine dimers can not be repaired. This disease may be a result of defect in the exinuclease and/or in other genes necessary for DNA repair.

Most mutations are deleterious and cause many of the genetic diseases. However, mutations can result in beneficial new genes and functions, which will enable an organism to adapt to the changing environment.

11.6 RECOMBINANT DNA TECHNOLOGY

Recombinant DNA technology is also referred to as ***genetic engineering. Recombinant*** DNA technology was introduced in early 1960s. Initially this technology was used mainly by academicians to understand the basic mechanisms of various cellular processes.

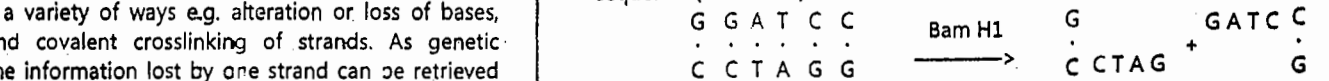
Isolation and manipulation of DNA is the main objective of recombinant DNA technology (rDNA technology). The major components of rDNA technology are as follows:

(i) Restriction endonucleases
(ii) Preparation of chimeric DNA molecule
(iii) Transfer of hybrid DNA into host cell
(iv) Gene expression.

(i) Restriction Endonucleases:

Restriction endonucleases (or restriction enzymes) recognise and cut specific DNA sequence. These enzymes help in protecting host cells from foreign organism. Host DNA is methylated so it is not cut (or cleaved or digested) by these enzymes. The restriction endonucleases are named according to the bacterial source e.g. Bam H1 from *Bacillus amyloliquefaciens, EcoRI* from *E. coli.*

Each enzyme is very specific in recognizing and cutting particular double stranded DNA sequence (Table 11.2).



Till to date more than 700 restriction endonucleases have been isolated.

Table 11.2: Examples of restriction endonucleases (↓ denotes cleavage site)

Name of the restriction endonuclease	Source	Sequence cleaved
EcoRI	*Eschericia coli*	↓ G A A T T C C T T A A G ↑
Hint III	*Haemophilus influenza*	↓ A A G C T T T T C G A A ↑

contd. ...

Pst I	*Providencia stuartii*	↓ C T G C A G G A C G T C ↑
Sma I	*Serratia marcescans*	↓ C C C G G G G G G C C C ↑
Sst I	*Streptomyces stanford*	↓ G A G C T C C T C G A G ↑

(II) Preparation of Chimeric (hybrid) DNA Molecule:

Pieces of DNA cleaved by endonucleases are then joined by DNA ligase. Thus, a new piece (segment) of the DNA can be inserted. The genetic material i.e. DNA segment, is incorporated into the DNA molecule of bacterial plasmid or phages. Bacterial plasmids or phages used for this purpose are called as *cloning vectors. The* only shortcoming is that there is no control over the orientation of the insertion.

Plasmids are small, circular duplex DNA molecules. Phages usually are linear DNA molecule into which foreign DNA can be inserted. Large fragments of DNA are generally cloned in *cosmids.*

The commonly used plasmid vector is pBR322.

(III) Transfer of Hybrid DNA into Host Cell:

The process of transferring plasmid hybrid DNA into the host cell is called as *transformation.* While transfer of phage hybrid DNA is called as *transfection.*

Various techniques are available to transfer the hybrid DNA into bacteria. The basic theme is to make the bacterial membrane permeable to the DNA. $CaCl_2$ is widely used for this purpose.

(iv) Gene Expression:

The main interest of rDNA technology is to ultimately produce proteins for clinical or other applications. The process of protein production from its gene is called as *'expression'* and the DNA component (DNA carrying vector) in this process is called as *'expression vector'*. Remember that the production of protein is occurring in the host cell. The host cell and expression vector are jointly called as an *expression system.*

Above mentioned techniques are summarised in Fig. 11.11.

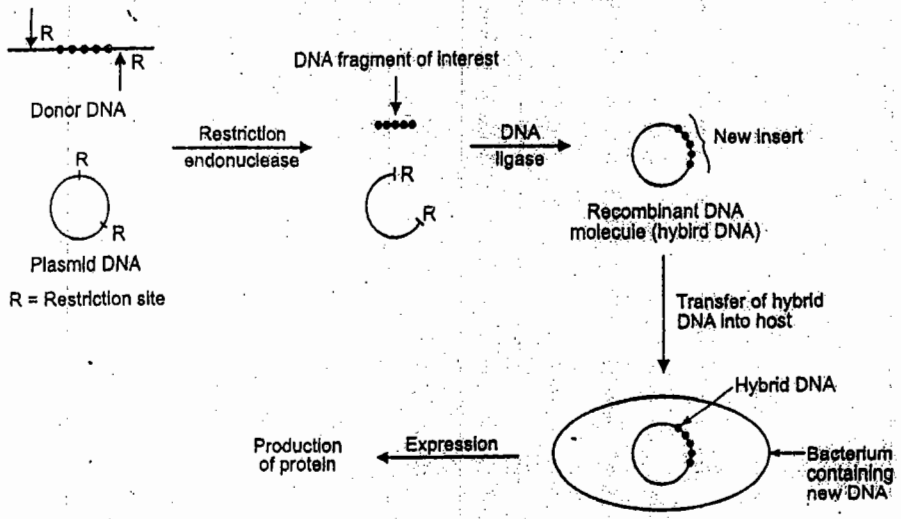


Fig. 11.11: Techniques in recombinant DNA technology

Applications of Recombinant DNA Technology:

Genetic Mapping:

Specific genes on different chromosomes can be localised. This has tremendously helped in mapping the human genome. The genes involved in various hereditary disorders can be located.

Pharmaceutical Application:

The pharmaceutical industry has taken a good advantage of this technology. Proteins which could not be produced on a large scale by conventional production method, can be produced by recombinant DNA technology e.g. human insulin, interferon, urokinase, somatostatin and many vaccines such as hepatis B. Recombinant DNA technology has also been used for diagnostic purposes such as AIDS testing.

11.7 THE POLYMERASE CHAIN REACTION

The Polymerase Chain Reaction (PCR) is a simple, faster, convenient and powerful technique for the amplification of specific DNA segment. This technique was invented by *Kerry Mullis* in 1985 and received the Nobel prize for the same in 1993. PCR contains double-stranded DNA segment of interest, two primers which will hybridize the flanking sequences (end sequences of DNA fragment) on opposing strands, all four deoxyribonucleotides (dNTPs) and a DNA polymerase. For PCR, it is not essential to know

the entire sequence of the DNA segment, only the short sequences at the end of the DNA segment of interest needs to be known. PCR uses heat stable DNA polymerase. Heat stable DNA polymerases are obtained from thermophilic bacteria i.e. bacteria that live in high temperature surroundings e.g. hot water streams. Examples of such DNA polymerases are - Taq DNA polymerase (obtained from *Thermus acquaticus*), *Pfu* DNA polymerase (obtained from *Pyrococcus furiosus*) which are now commercially available.

PCR (also known as *cell free molecular cloning*) consists of the following steps:

1. **Denaturation of DNA:** PCR mixture is heated at 95°C for 15 - 30 sec. The heat separates double stranded DNA into single strands which can act as templates for new DNA synthesis.
2. **Primer annealing:** The reaction mixture is then rapidly cooled. This allows the primer to anneal with their complementary sequence on both the strands of DNA. Setting appropriate annealing temperature is important so as to allow binding of primers only to the desired sequence on DNA.
3. **Elongation/Amplification:** The temperature is again raised (usually to 70°C) for a predetermined time period. This allows synthesis of new DNA strand. Thus, single stranded DNA becomes double stranded.

Above three steps constitute one PCR cycle which is then repeated for a number of times. These steps are summarised in Fig. 11.12.

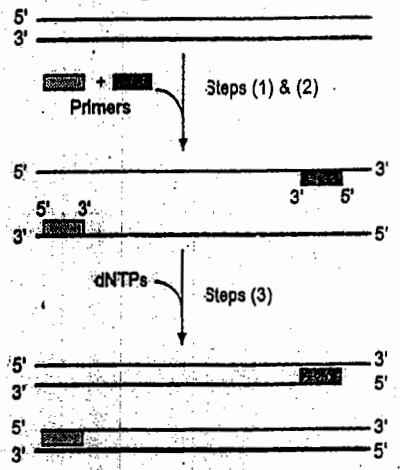


Fig. 11.12: One cycle of PCR

Generally, twenty cycles PCR will increase the amount of target DNA sequence by one million (10^6) fold with high specificity and after 30 cycles by one billion fold (10^9). The amplification factor is 2^n (n = number of amplification cycles). Now-a-days, PCR is routinely performed by using automated *thermocyclers*.

Applications of PCR:

PCR has a wide variety of applications, some of them are as follows:

- Due to its specificity, PCR can be used to amplify single DNA molecule from a complex mixture.
- PCR is commonly used in DNA sequencing.
- PCR is used for creating point, deletion and insertion mutations and hence is used in the study of gene expression and function.
- PCR is used in the diagnosis of diseases such as AIDS and genetic diseases.
- As PCR is extremely sensitive it is used in forensic medicine - to amplify DNA from hair or drop of blood, also in archeology to study evolution from DNA of archeolog cal samples.

11.8 HYBRIDIZATION TECHNIQUES

Hybridization techniques are also called as blotting techniques and are used for identification of DNA, RNA or proteins with specific sequence.

Various steps involved in the hybridization technique for DNA (Southern blotting) are shown in Fig. 11.13.

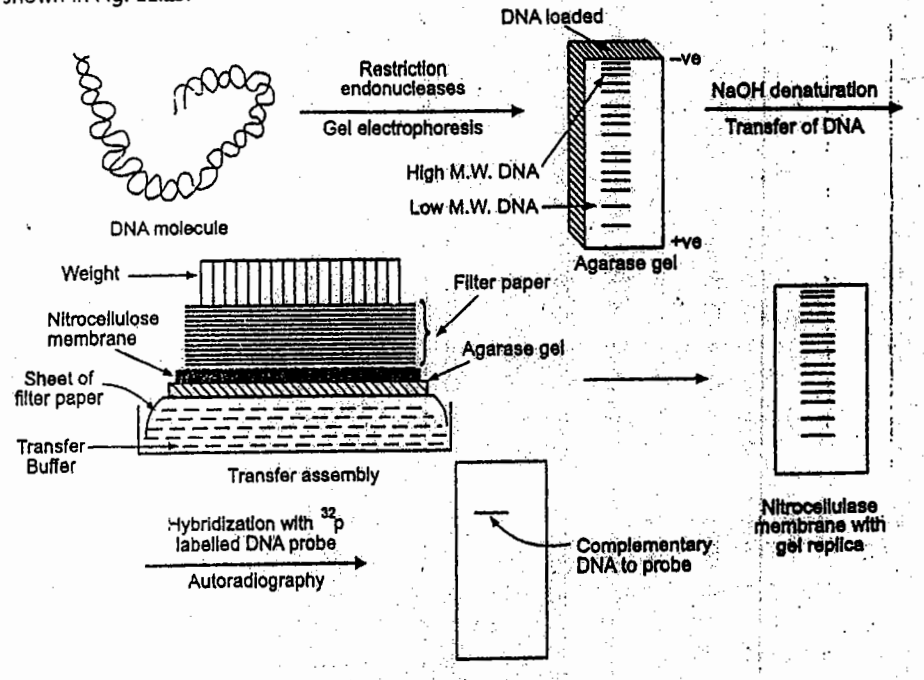


Fig. 11.13: Hybridization technique

First the DNA is digested with restriction endonucleases. The resultant fragments are then separated on agarose gel. Note that electrophoretic mobility of DNA fragment in the gel varies according to molecular weight (i.e. number of base pairs). Now, the gel is soaked in NaOH which converts DNA to single stranded DNA. This gel is then blotted on the nitrocellulose membrane. Nitrocellulose membrane has the property of tightly binding to single stranded DNA; nylon or polyvinylidine difluoride membranes can also be used for this purpose. To permanently fix the DNA on membrane, membrane is vacuum dried at 80°C. Now, the ^{32}P - labelled single stranded DNA (probe) which is complementary in sequence to the DNA of interest is added on the membrane. Radiolabelled DNA (probe) hybridizes with the DNA of interest, unbound radioactive probe is washed away. This is then exposed to X-ray film and position of restriction fragment with a sequence complementary to that of the probe can be identified.

Thus, a particular DNA fragment present among the thousands to million others can be easily identified. This useful technique for DNA was developed by *Edwin M. Southern* and hence called as *Southern transfer technique* or *Southern blotting*. Similarly, using the analogues procedure, RNA molecules with specific sequences can be identified. The analogues technique for RNA for some whimsical reason is called as *Northern blotting* (RNA is opposite to DNA and Northern is opposite to Southern !). This whimsicalness with word continued and analogues technique for identifying particular sequence in protein is called as *Western blotting*. Southern, Northern and Western blots are also called as DNA, RNA and protein blots.

QUESTIONS

1. Explain the replication of DNA with a neat labelled diagram.
2. Give an account of – Roles of various proteins in the replication of DNA.
3. Describe the salient features of DNA replication in eukaryotes.
4. Write a note on the polymerase chain reaction.
5. Describe the terms of replication fork and Okazaki pieces.
6. Write notes on:
   (a) DNA polymerase III.
   (b) Meselson and Stahl experiment.
   (c) Role of helicases in replication.

   (d) Replication fork.
   (e) Recombinant DNA technology.
   (f) Restriction endonucleases.
7. Define mutation. Explain various types of the same. Add a note on the DNA repair.
8. Give the significance of hybridization techniques.
9. Explain in detail the significance of rDNA technology.

Chapter ... 12

RNA, TRANSCRIPTION AND TRANSLATION

♦ LEARNING OBJECTIVES ♦

- *Understand the Structure of RNA and Different Types of RNA.*
- *Understand the Process of Transcription.*
- *Recognize the Role of RNA Polymerase and Different Proteins in the Transcription.*
- *Recognize Significance of Promoter.*
- *Recognize Process of Reverse Transcription.*
- *Understand Concept of Genetic Code and Its Role in Protein Synthesis.*
- *Learn important Steps of Protein Synthesis.*
- *Recognize various ways of Inhibition of Translation.*

12.1 INTRODUCTION

RNA is almost always single-stranded but almost every RNA molecule has many short double-helical regions. This is because two sections of an RNA chain within a hairpin loop are in the correct antiparallel orientation to base-pair.

Each cell contains many RNA molecules with lengths varying from less than 50 nucleotides to tens of thousands of nucleotides.

There are four major types of RNA:

- **mRNA – messenger RNA:** It is a copy of a gene and has a sequence complimentary to one strand of the DNA and identical to the other strand. The mRNA carries the information stored in the DNA in the nucleus to the cytoplasm where ribosomes can use it to make proteins.
- **tRNA – transfer RNA:** It is a small RNA and has a specific secondary and tertiary structure. It binds an amino acid at one end and mRNA at the other end. It is an adapter to carry an amino acid to the appropriate place as coded by the mRNA.
- **rRNA – ribosomal RNA:** It is one of the structural components of the ribosomes. It constitutes about two-thirds of the ribosomal mass.
- **snRNA – small nuclear RNA:** It is involved in processing RNAs as they travel between the nucleus and the cytoplasm.

All types of RNAs are shown to hybridize with complementary sequences on DNA from the same organism. Hence, all RNAs are transcribed from DNA templates. In addition, RNA, like DNA, is a chain of four different nucleotides which indicates that genetic information of DNA is transferred to a complementary sequence of RNA nucleotides.

12.2 RNA POLYMERASE

RNA polymerase is a multisubunit complex and is involved in DNA directed synthesis of RNA (*transcription* of DNA to RNA). This enzyme couples together the ribonucleotide triphosphates, ATP, CTP, GTP and UTP.

RNA polymerase consists of four subunits. The composition of the entire enzyme i.e. holoenzyme is $\alpha_2\ \beta\ \beta'\ \gamma.\ \sigma$ subunit identifies transcription beginning site (promoter site), initiates RNA synthesis and dissociates from the rest of the enzyme leaving behind *core enzyme* ($\alpha_2\ \beta\beta'$). The α subunits bind to regulatory proteins, the β' subunit binds to DNA template and β subunit binds to ribonucleotide triphosphates.

The DNA strand that serves as a template i.e. its sequence is complementary to that of the RNA is called as *antisense* or *non-coding strand.* The other DNA strand having the same sequence as transcribed RNA (except replacement of U with T) is called as *sense* or *coding* strand.

The actual template for RNA synthesis is single-stranded DNA but RNA polymerase normally uses duplex DNA for RNA synthesis. RNA synthesis is an extremely accurate process and there is no evidence for any proof reading. Thus, overall precision is not as perfect as DNA-replication. As RNA is not self-replicating, the mistakes that do occur are not genetically preserved.

12.3 TRANSCRIPTION INITIATION

Transcription starts at promoters on the DNA template (base sequences recognised by σ factor of RNA polymerases). When many promoters are compared, a striking pattern is seen. Two common sequences of six nucleotide occur about 10 and 35 base pairs located on the 5' side (upstream) of the transcription initiation site. These are called as – 35 (5' – TTGACA – 3') and – 10 (5' – TATAAT – 3') sequences; the nucleotide encoding the beginning of the RNA chain is termed as + 1. The – 10 position is sometimes called as ***Pribnow box.*** (Fig. 12.1)

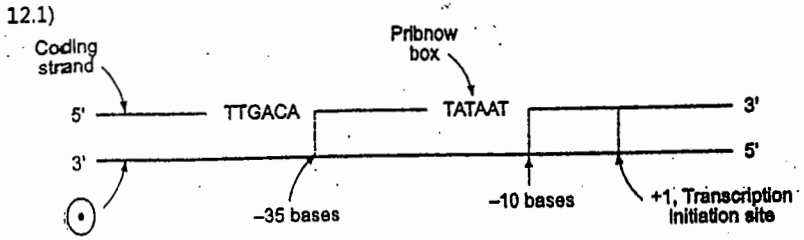


Fig. 12.1: Promoter region

The exact – 10 and – 35 *consensus sequences* are found in only few promoters. Most of the promoters differ from them by only few nucleotides. Additional upstream sequences may also influence RNA polymerase binding to DNA.

Many protein-coding genes, known as structural genes, are individually transcribed in eukaryotes. But in case of prokaryotes, genes are arranged along a single strand so they can

be transcribed together. These genetic units called as *operons*, generally contain genes with related function. Thus, we can have a model (Fig. 12.2) in which there is the regulator gene, operator site and a set of structural genes.

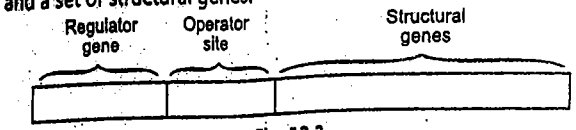


Fig. 12.2

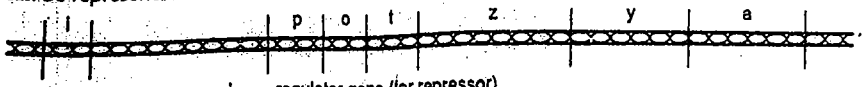
In order to explain the induction or repression of enzyme synthesis, '*Operon model*' was proposed by F. Jacob and J. Monod in 1961. In *operon model*, a unit of transcription called '*operon*' was proposed. Operon consists of:

(a) Operator gene, site for activator or repressor and decides which whether or not transcription should be initiated.

(b) Set of structural genes which may vary in number. Protein products of these structural genes i.e. enzymes, are involved in the different steps of biosynthetic pathway in a co-ordinated manner.

LACTOSE OPERON

Lactose operon, known as *lac operon* is one of the extensively studied operon. *Lac operon* can be represented as:



where,
- i = regulator gene (for repressor)
- p = promoter gene (for transcription initiation)
- o = operon gene
- t = translation initiation site
- z = structural gene for β-galactosidase
- a = structural gene for acetylase
- y = structural gene

Fig. 12.3

β-Galactosidase is synthesized by *E.coli* only when lactose is present. Normally, 'repressor' molecules are present in the cell and these repressors check the activity of the gene (in this case gene for β-galactosidase). In general, the inactive repressor is made active by combining with co-repressor. However, in the case of inducible system, active repressor is made inactive because of an inducer (in this case lactose).

Lac operon is regulated by two proteins:

(i) lac repressor, inactivated by inducer (lactose) - negative control

(ii) catabolic gene activator protein (Cga protein), cAMP activates Cga protein-positive control.

Lac operon regulation is an example of negative control regulation. Meaning, the gene is not allowed to express unless required. But via Cga and cAMP, a positive control is also exercised in lac operon.

There is another operon in *E.coli* called as *arabinose operon*. For this operon, the negative control is exercised by a repressor named C-protein which acts as operator "O".

Thus, regulation of gene expression system have both positive as well as negative control.

The identification of promoter region to which RNA polymerase binds was done by a process called as *foot printing* ('protection' experiment). Briefly, DNA is incubated with a protein to which it binds (e.g. RNA polymerase) and then treated with alkylating agents such as dimethyl sulfate (DMS). Due to alkylation of bases there is cleavage of backbone except for the protein – DNA bound portion which is protected from cleavage. This resulting pattern of protection is called the *protein's footprint* (Fig. 12.4).

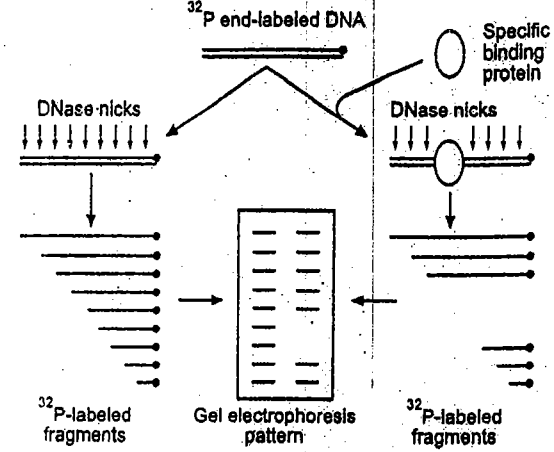


Fig. 12.4: Foot printing technique

The initial recognition by RNA polymerase occurs at – 35 region (mutations in – 35 region impair the binding to the promoter) and a 'closed' complex is formed. Then a 17-bp segment of DNA is unwound (which corresponds to 1.6 turns of B-DNA helix). This resulting 'open complex' is similar to the region of unwound DNA at the replication origin. Notice that in 'closed complex', DNA is double helical whereas in 'open complex', DNA is unwound. Now, all the elements are in place to form the first phosphodiester bond of the new RNA chain.

12.5 RNA CHAIN ELONGATION

Like DNA synthesis, RNA synthesis also proceeds in the 5' → 3' direction. The growing RNA molecule has a 5' – triphosphate group and is quite unique. It is usually pppA or pppG, sometimes pppC and rarely pppU.

The important thing about elongation of RNA chain is the loss of σ-factor, core enzyme thus left behind tightly binds to the DNA template. In fact, the enzyme is bound to the template until a termination signal is reached.

At the point of RNA synthesis, double-stranded DNA is opened up. This allows the antisense strand to be transcribed. The RNA chain thus formed forms a short-length of RNA-DNA hybrid complex. This open initiation complex (the region containing RNA polymerase, DNA and nascent RNA, called as *transcription 'bubble'*, so called because of the presence of unpaired 'bubble' of DNA) presumably travels along the DNA with the RNA polymerase (Fig. 12.5)

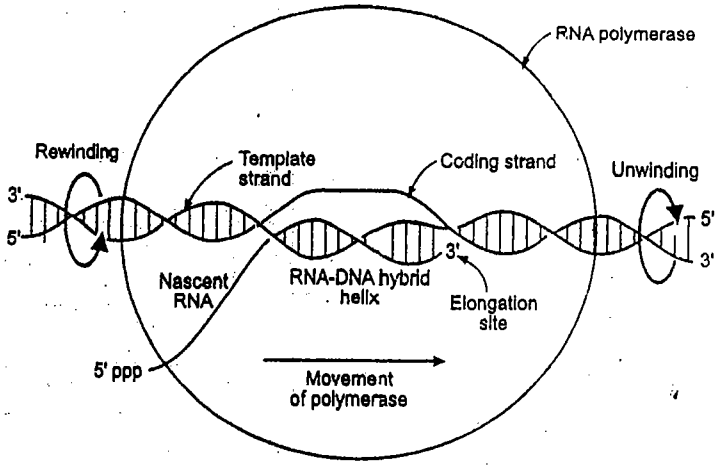


Fig. 12.5: Schematic representation of transcription bubble

Note that the length of the RNA – DNA hybrid and unwound portion of DNA remains somewhat constant. This suggests that DNA is rewound at almost the same rate at the rear as it is unwound at the front of RNA polymerase.

Generally, the *in vivo* rate of transcription is 20 – 50 nucleotides per second at 37°C. The error frequently in RNA synthesis is 10^4 to 10^6 higher than that of DNA synthesis (one wrong base incorporation for every ~ 10^4 transcribed). These errors are tolerated because most genes are repetitively transcribed. Also genetic code contains numerous synonyms and in protein, amino acid substitutions are often functionally not harmful.

12.6 RNA CHAIN TERMINATION

There are signals in DNA called "terminators". They stop RNA synthesis at specific points. At these points, the enzyme stops the polymerisation of nucleotides, releases the RNA chain and leaves the DNA. At most of the sites, core enzyme is capable of carrying out all these steps but at some steps an additional protein, ρ (rho) factor is needed.

The termination sequence (stop signal) shares common features such as palindromic GC-rich region followed by an AT-rich region. The *palindromic sequence* is self-complementary, thus forms a hairpin structure which is terminated by several U residues. (Fig. 12.6). Once the hairpin fold is formed, RNA polymerase pauses. At this stage,

RNA – DNA hybrid fold is formed. At this stage, RNA – DNA hybrid is not very stable because of the rU.dA base pair, which is the weakest among base pairs. Thus, nascent RNA dissociates from the DNA template and from the RNA polymerase. In the bubble region, duplex DNA is again formed and as core enzyme has less affinity for duplex DNA it gets released.

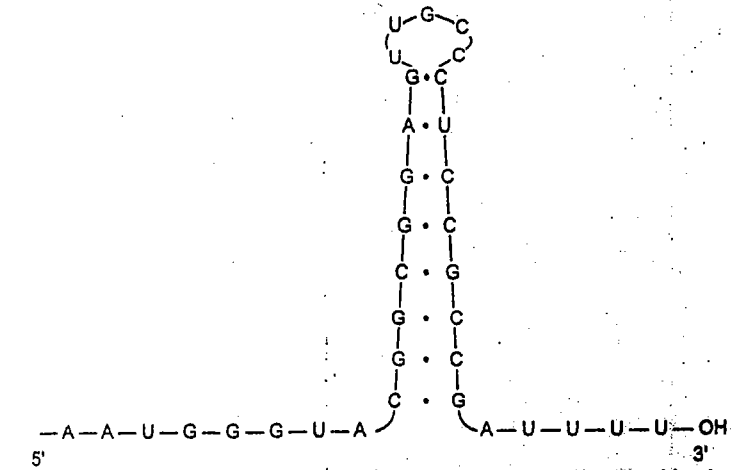


Fig. 12.6: Termination sequence (Palindromic sequence and four U residues)

At some sites, termination of transcription is not spontaneous as described above but requires the participation of additional factor (protein), called as *rho* factor. Rho factor is a hexamer of 419 - residue subunits. It helps in termination of non-spontaneously terminating transcripts and also increases the termination efficiency of spontaneously terminating transcripts.

12.7 TRANSCRIPTION IN EUKARYOTES

In prokaryotes, RNA is synthesized by a single kind of polymerase (as described above), but is accomplished by three enzymes in eukaryotic cells: RNA polymerase I, II and III. The fundamental principles of transcription remain similar in prokaryotes and eukaryotes.

The three RNA polymerases differ in template specificity, localisation and susceptibility to inhibitors. RNA polymerase I is located in nucleoli and synthesizes precursors of most ribosomal RNA (r RNA) viz. 18S, 5.8S and 28S.

RNA polymerase II is located in nucleoplasm and synthesizes precursors of messenger RNA (mRNA).

RNA polymerase III is located in nucleoplasm and synthesizes 5S rRNA and transfer RNA (tRNA).

In a given eukaryotic cell, numerous rRNA genes have essentially identical sequences, thus RNA polymerase I recognises only one promoter. RNA polymerase I requires *core promoter element* (position – 31 to + 6) but for efficient transcription it also requires an *upstream promoter element* (position – 187 and – 107).

A considerable diversity is seen in promoters recognised by RNA polymerase II. The *structural genes (the house keeping genes which are constitutively transcribed)* have the GC rich sequences (GC box). The GC box(es) function similar to prokaryotic promoters. The genes which are selectively expressed have a conserved AT-rich sequence located at – 25 to – 30, called as *TATA box* (similar to – 10 region of a prokaryotic promoter). Many genes also have conserved sequence of CCAAT (CCAAT box) located at – 70 to – 90. Alteration in these regions changes the transcription rate of gene. The sequence upstream of TATA box is the binding site for RNA polymerase II. In addition, there are sequences located hundred or even thousand base pairs upstream which act as *enhancers or silencers*. These enhancers or silencers may alter the conformation of DNA so as to promote or interfere with binding of RNA polymerase II. Transcription regulatory proteins (*transcription factors*) which act as activators or repressors bind to enhancers or silencers thereby influencing the binding of RNA polymerase to the promoter. Note that transcription factors are proteins whereas enhancers or silencers are DNA sequences (also known as *transcription elements*).

The promoters recognised by RNA polymerase III are not in front (upstream) but within the transcribed sequences.

In general, initiation and elongation are similar in prokaryotes and eukaryotes, however, there are several important differences (Table 12.1).

Table 12.1: Differences in Gene Expression between Prokaryotes and Eukaryotes

Prokaryotes	Eukaryotes
1. All RNA species are synthesized by a single RNA polymerase.	1. Three different RNA polymerases are responsible for the different classes of RNA molecule.
2. mRNA is translated during transcription.	2. mRNA is processed before transport to the cytoplasm, where it is translated. Caps and tails are added, and internal portions of the transcript are removed.
3. mRNAs are often polycistronics.	3. Genes are split and are not contiguous, instead the coding sequences are interrupted by intervening sequences (introns).
4. Genes are contiguous segments of DNA that are colinear with the mRNA that is translated in a protein.	4. mRNA are monocistronics (one gene per mRNA).

Regulation of transcription also differs in prokaryotes and eukaryotes and is summarized below:

Regulation of Transcription in Prokaryotes:

- Clusters of genes (operons) are transcribed from a single promoter. They are not found in eukaryotes. Within the promoter region of an operon is a nucleotide sequence called the operator. Some proteins (called as repressors) bind to the operator and prevent RNA polymerase from transcribing the DNA into RNA.
- Repressors are generally present at the operator when the end product of the biosynthetic pathway they are regulating is present. Thus, in the absence of the end product, the repressor is released and transcription starts to make the mRNA that encodes the protein which ultimately synthesizes the end product.
- There are also proteins which activate transcription (activators). Activators make the promoters tightly bind to RNA polymerase and initiate transcription.

Regulation of Transcription in Eukaryotes:

- In eukaryotes, there are three RNA polymerases.
- RNA polymerase is assisted by a large set of proteins called as general transcription factors which assemble at the promoter. These general transcription factors recognize TATA box.
- Initiators and activators influence the initiation of transcription even when they are bound to nucleotides thousands of base pairs away from the promoter.
- Eukaryotic transcription takes into account the packaging of DNA into nucleosomes.

12.8 TRANSFER RNA AND RIBOSOMAL RNA

Ribosomal RNA (rRNA) and transfer RNA (tRNA) molecules are generated by cleavage and modification of nascent RNA.

In eukaryotes, there are several hundred to several thousand tRNA genes. tRNA precursors are converted into mature tRNAs by cleavage of 5' leader sequence, splicing to remove an intron, replacement of the 3' - terminal UU by CCA (the site where amino acid sequence is attached) by enzyme tRNA nucleotidyl transferase.

There are three types of rRNAs in *E.coli*: 5S, 16S and 23S. In eukaryotes, rRNA transcript is 45S RNA which contains 18S, 5.8S and 28S rRNA separated by spacer sequence. Initially, 45S RNA is specifically methylated at ~ 110 sites and subsequently cleaved and trimmed.

12.9 MESSENGER RNA (MRNA)

In prokaryotes, most mRNA transcripts are translated without any modifications. In eukaryotes, mRNA synthesis occurs in nucleus and translation in cytosol. Hence, eukaryotic mRNA may undergo post-transcriptional modification.

Eukaryotic mRNAs have a *cap structure*. A phosphate is released from 5' triphosphate end. The resulting diphosphate 5' - end attacks the α phosphorus atom of GTP to form a unique 5' - 5' triphosphate linkage called a *cap*. The termina. guanine is then methylated to form cap 0. Further, riboses may be methylated to form cap 1 or cap 2. These caps help in enhancing the translation of mRNA in protein synthesizing systems. Also, caps provide the protection to mRNAs at the 5' end against phosphatases and nucleases.

The termination signal in eukaryotes is imprecise. Hence, 3' sequences of RNA transcripts are heterogeneous. However, most eukaryotic mRNAs contain a polyadenylate (poly (A)) tail at their 3' end. The poly (A) tail is enzymatically added (and not encoded by DNA). The poly (A) tail is generated by ATP through the action of *poly (A) polymerase*. The poly (A) tail protects mRNA from nucleases. Although considered to be not essential, absence of poly (A) tail makes mRNA a less effective template for protein synthesis.

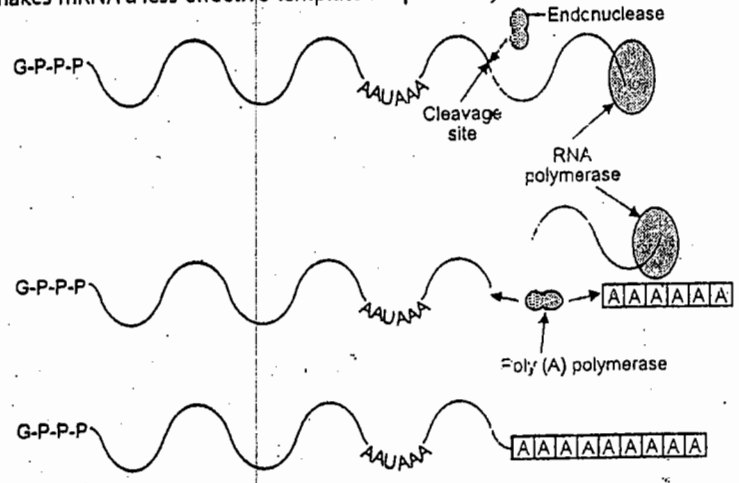


Fig. 12.7

Unlike prokaryotes, eukaryotic genes are interspersed with unexpressed regions. Thus, pre-mRNAs (primary transcript) are much larger than expected size from the protein. The pre-mRNA has non-expressed intervening sequences (introns) and expressed sequences (exons). Thus, pre-mRNAs are processed by the excision of introns, following which the flanking exons are joined or spliced together. Introns are precisely spliced out of mRNA precursors. The base sequence of an intron begins with GU and ends with AG. These 5' and 3' splice sites are important in determining where splicing occurs.

The introns are removed by unusual enzymes composed of protein and RNA. They are called as *small nuclear ribonucleoprotein particles* (snRNPs). At each intron a group of snRNPs assembles onto the RNA, cleaves the RNA, and religates the flanking exons. The spliced intron is released and is eventually degraded in the nucleus. RNA portion of the snRNPs are involved in the recognition of introns by complementary base pairing. The snRNPs align GU (at the 5' end) and an AG (at the 3' end) in a lariat formation to allow precise splicing:

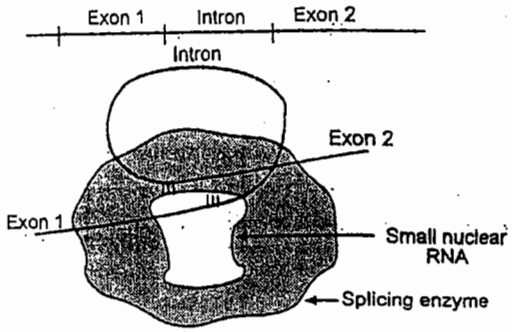


Fig. 12.8

Complex containing the snRNP, mRNA and associated proteins is called as *spliceosomes*.

Introns and splicing of introns are important:

- Splicing of introns allows variations of a gene and hence gene product to be made.
- Splicing allows for evolutionary modifications by making genetic recombination between exons.
- Many viruses have spliced mRNAs and hence understanding this process may lead to new therapeutic approaches.

Some α2-thalassemias (anaemia due to imbalance of α and β haemoglobin subunits) have been attributed to a defect in polyadenylation (aberrant splicing). Specifically, there is a mutation in the cleavage site from AAUAAA → AAUAAG.

12.9.1 The Lifetime of mRNA

The length of time the mRNA remains in the cytosol affects the amount of protein that can be produced. Eventually, all mRNAs are degraded but lifetime of individual mRNA varies considerably. In prokaryotes, such as bacteria, a typical mRNA has a lifetime of 3 minutes. In eukaryotes, lifetime of mRNA varies from 30 minutes to 10 hours. The lifetime of mRNA is partly determined by nucleotide sequences between the 3' end of the coding sequence and the poly A tail.

PROTEIN SYNTHESIS

The flow of genetic information is from DNA to RNA to protein.

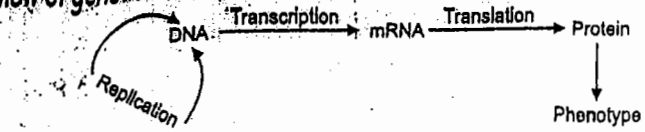


Fig. 12.9

Protein synthesis is the most complex of all the biosynthetic mechanisms. About 300 different macromolecules e.g. ribosomal proteins, enzymes to activate amino acids, auxillary enzymes, protein factors for initiation, elongation and termination of protein synthesis, enzymes for final processing, tRNA and rRNA, are involved in the synthesis of polypeptide or proteins. The base sequence of DNA is *transcribed* into RNA and then *translated* into protein. Protein synthesis is called as translation because four-letter alphabet of nucleic acids (A, T (U), G, C) is translated into the entirely different alphabet of proteins. In other words, expression of genetic information as the amino acid sequence of proteins from mRNA is called *translation*.

THE GENETIC CODE

Recall that DNA is composed of: (i) phosphoric acid, (ii) deoxyribose sugar and (iii) nitrogen bases. So theoretically all three moieties can be used for coding the genetic information. But poly-sugar-phosphate backbone is always the same, so it is not possible that these moieties can carry diverse genetic information. But nitrogen bases vary from DNA to DNA and hence can carry the diverse genetic information. In fact there exists a *colinearity* i.e. sequence of nitrogen bases is identical to the amino acid sequence in protein molecule. Thus, a group of several bases, termed as *codon* is necessary to specify a single amino acid. Remember there are four types of nucleotide residue and twenty types of amino acids. Thus, a doublet codon (2 bases per codon) will not be sufficient as $4^2 = 16$. But a triplet codon (3 bases per codon) will be sufficient as $4^3 = 64$. The genetic code has been deciphered and perfected by the efforts of many biochemists, namely Marshall Warren Nirenberg and Har Gobind Khorana who were awarded by the 1968 Noble Prize along with Robert Holley who was the first scientist to determine the nucleotide sequence of several tRNAs. This triplet codon allows more than one codon to specify single amino acids and is termed as *degenerate* codon. The codon which acts as a stop signal for chain termination is called as 'non-sense' codon (Table 12.2).

Table 12.2: Genetic Code

First Position	Second Position								Third Position
	U		C		A		G		
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cyst	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG		UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	UUA	Met	ACG		AAG		AGG		G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G

Experimental evidence suggests that genetic code has the following characteristics:

(i) the code is triplet

(ii) the code is not overlapping

(iii) the code is commaless i.e. it is continuous
(iv) the code is non-ambiguous
(v) the code is universal.

As discussed before triplet code is the minimum requirement. Due to triplet code we will have 64 codons (4^3 = 64) and as there are twenty amino acids, there is an excess of 44 codons. This suggests that there are more than one codon for the same amino acid i.e. the code is degenerate (Refer Table 12.2). The code is non-overlapping, means that the adjacent codons do not overlap. Codes are comma-free, meaning that there is no signal to indicate the end of one codon and the beginning of the another. Code is non-ambiguous, meaning that there is no ambiguity (doubt) about a codon. A particular codon will always provide the code for a particular amino acid. The genetic code is universal and applies to all organisms. In other words, genetic code is conservative i.e. genetic code was developed in bacteria approximately three billion years ago and has not undergone any change (but there are exceptions).

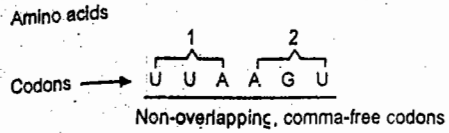


Fig. 12.10

Also, the genetic code is read in a sequential manner starting from a fixed point. Thus, any insertion or deletion will shift the *reading frame*. Hence, insertions or deletions are called as *frame shift mutations*.

12.11.1 Important features of Genetic Code

1. The genetic code is highly degenerate.
   Arg, Leu and Ser are specified by six different codons. Only Met and Trp are coded by a single codon. Codons that specify the same amino acids are termed as *synonyms*.
2. Mostly, codons which are synonyms differ only in their third nucleotide. Changes in the first codon tend to specify similar amino acids. Pyrimidine at second position encodes mostly hydrophobic amino acids whereas polar amino acids are encoded when purines are present at second position.
3. UAG, UAA and UGA are stop codons or non-sense codons and do not encode amino acids. UAG, UAA and UGA are often referred as *amber, ochre* and *opal codons*.
4. The codons AUG and less frequently GUG specify the starting point (initiation) for polypeptide chain synthesis. AUG encodes Met whereas GUG encodes Val.

Variations in Genetic Code

Earlier it was mentioned that genetic code is universal and has not undergone any change during the evolution. But over the last few years, variations of genetic code have

been found in yeast and mammalian mitochondria. For example, in yeast mitochondria UGA instead of 'stop signal' codes for tryptophan, CGG instead of coding arginine codes for methionine in mammalian mitochondria.

Variation of genetic code has also been identified in a protozoa (*Mycoplasma capricolum*). In this case, UAA and UAG code for glutamine instead of 'stop signal'.

12.11.2 Role of tRNA

Nucleic acids do not specifically bind amino acids. So initially an "adapter" molecule was hypothesized to carry specific enzymatically modified amino acid and recognise the corresponding codon. Subsequently this 'adapter' molecule was identified as tRNA. tRNA is involved in the translation of the nucleic acid message into the amino acids of proteins.

All the tRNAs have clover leaf secondary structure having following characteristics: (Fig. 12.11)

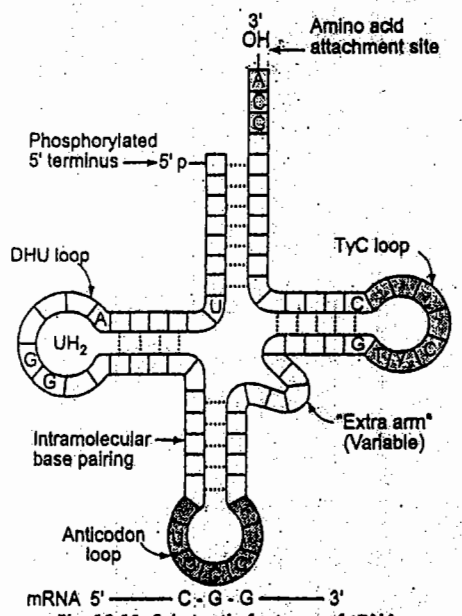


Fig. 12.11: Schematic features of tRNA

- Phosphate group at 5' terminal.
- An *acceptor* or *amino acid stem* (amino acid residue carried by the tRNA is appended at 3'-terminal of acceptor), assembly of 7-base pair stem including 5'-terminal nucleotide. Sometimes this assembly may contain non-Watson-Crick base pairs such as GU.
- 5 to 7-nucleotide loop is attached with 3 or 4 base pair stem containing modified base (d hydro uridine). This structure is collectively called as *D arm*.
- *Anticodon arm* contains anticodon loop and 5 bp stem. Anticodon is a 3 nucleotide sequence and is complementary and antiparallel to the mRNA codon.
- T arm contains loop having pseudouridine (ψ, sequence TψC).

Thus tRNA recognises both the enzymes that attach the correct amino acid and anticodon on mRNA. One of the unique characteristics of tRNA is the presence of unusual bases (~ 25% post transcriptionally modified bases). These modified bases help to promote attachment of amino acid to the acceptor stem or even strengthening codon-anticodon interactions.

The clover leaf structure discussed above does not give the complete picture of tRNA structure. The three dimensional structure of yeast phenylalanine tRNA was resolved by X-ray crystailography in 1974. This three-dimensional structure of tRNA has the following characteristics:

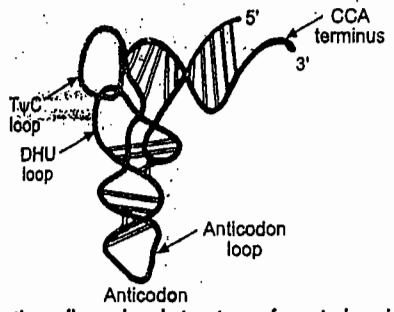


Fig. 12.12: The three-dimensional structure of yeast phenylalanine tRNA

- The tRNA molecule is L shaped.
- Amino acid attachment site (CCA group) and anticodon loop are at the two ends of L.
- Corner of the L is formed by dihydrouracil (D) rich and TΨC loop.
- Hydrogen bonding is also present between non-conventional base pairs e.g. GG.AA and AC.
- The two segments of double helix are like A-DNA.
- Amino acid attachment site and anticodon loop do not interact strongly with the rest of the molecule. These sites may change conformation during amino acid activation and protein synthesis.

3-D shape also plays a role in determining which amino acid will be attached to the amino acid attachment site. Recent research indicates that the anticodon loop, the D loop and the amino acyl stem are all important. The correct attachment of the amino acid to it's tRNA is considered the *2nd genetic code* and a lot of research is being done to understand the details of this code.

12.11.3 Aminoacyl tRNA Synthetase

Two vital steps are involved in translation:

- Aminoacyl tRNA selects the appropriate amino acid for the covalent attachment to tRNA and
- Correct pairing of aminoacyl tRNA with an mRNA codon on ribosome.

The formation of peptide bond between amino group of one amino acid and carboxyl group of another is thermodynamically an unfavourable reaction. Hence, *amino acid esters* (carboxyl group of amino acids is linked to 2' - or the 3' - hydroxyl group of ribose at 3' - end of tRNA); activated intermediates are formed. These amino acid esters are called as *charged tRNA* or an *amino acyl - tRNA*. The overall reaction is as follows:

$$AA + ATP \xrightarrow{\text{Amino acyl-tRNA synthetase}} AA \sim AMP + PPi$$

$$AA \sim AMP + tRNA \xrightarrow{\text{Amino acyl-tRNA synthetase}} AA \sim tRNA + AMP$$

Amino acids cannot recognise the codons on mRNA, hence formation of amino acyl tRNA is important. As tRNA can recognise codons on mRNA, the synthesis of amino acyl tRNA is carried out by activating enzymes called as amino acyl - tRNA synthetases. There are 20 amino acyl tRNA synthetases - each recognizing one amino acid and all the tRNAs to which that amino acid is to be attached.

There are two classes of amino acyl - tRNA synthetases called as class I and class II. Large and more hydrophobic amino acids (Arg, Gys, Glu, Ile, Len, Met, Trp, Try and Val) are activated by class I tRNAs. Whereas, smaller amino acids (Ala, Asn, Asp, Gly, His, Lys, Phe, Ser, Pro and Thr) are activated by class II tRNAs. All class I enzymes produce activated amino acyl tRNAs via amino acid esterification at ribose 2'-OH group whereas, class II enzymes esterify at the 3'-OH group.

The activation of tRNA with the corresponding amino acid is a remarkably accurate process. The synthetases can distinguish different tRNAs because different base sequences are present on tRNA molecules that accept different amino acids. The high degree of accuracy is surprising because some amino acids are structurally very similar e.g. difference between Ile and Val is that Ile contains a methylene group. Error frequency seen *in vivo* is 1 in 3000 only. Thus, the synthetase corrects its own errors e.g. If tRNA specific for Ile has Val then this tRNA hydrolyses the Val - AMP and avoids the wrong incorporation into proteins. Synthetases recognise the corresponding tRNA also on the basis of anticodes e.g. when UAG anticodon of Val-tRNA was replaced with CAU, the anticodon for methionine, the Val-tRNA containing the CAU anticodon was amino acylated by methionyl tRNA synthetase. This was called as *identity swap* experiment.

12.11.4 Codon-Anticodon Interactions

The anticodon on tRNA is the recognition site for the codon on mRNA and is accomplished by the base pairing. The amino acid in amino acyl - tRNA does not play a role in selecting a codon. Also, a tRNA molecule can recognise more than one codon and is called as *Wobble hypothesis*. It is suggested that first two base-pairings are normal Watson - Crick pairings and there could be some *steric freedom or wobble* in the pairing of the third anticodon position. In fact, genetic code suggests that if first two bases are same then those

codons always code for the same amino acid e.g. GCU, GCC and GCA code for alanine. Thus, if codon differs in either of first two bases then they are recognised by different tRNAs e.g. UUA and CUA code for leucine but are recognised by different tRNA.

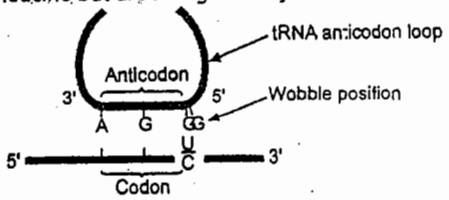


Fig. 12.13: Schematic representations of codon-anticodon pairings allowed by the Wobble Rules

Table 12.3: Codon-Anticodon pairings allowed by the Wobble Rules

5' end of anticodon	3' end of codon
G	U or C
C	G only
A	U only
U	A or G

12.12 RIBOSOMES

The synthesis of proteins is catalyzed by the *ribosome*. Ribosomes are important as they co-ordinate the interaction of tRNA, mRNA and proteins and catalyse the peptide bond formation. Structurally and functionally, prokaryotic and eukaryotic ribosomes resemble each other e.g. eukaryotic 18S and 28S rRNA are similar to prokaryotic 16S and 23S. (Note that S refers to a sedementation value of the structure in a sucrose gradient). Besides the rRNA, the ribosome also contains binding sites for tRNA and mRNA.

Each protein (polypeptide chain) has two ends, at one end amino acid with free carboxyl group (C terminus) and at the other, amino acids bearing a free amino group (N terminus). The direction of polypeptide chain growth is from the amino to carboxyl i.e. *peptidyl transferase* activity attaches an incoming amino acid to an ongoing polypeptide's C terminus. Ribosomes read the mRNA in the 5' → 3' direction. Recall that, mRNA is also synthesized in 5' → 3' direction. In fact, in *E-coli*, both transcription and translation are closely coupled in space and time. But this is not the case in eukaryotes, because transcription occurs in nucleus whereas translation in cytosol.

Single mRNA molecule is translated simultaneously by many ribosomes. The binding of many ribosomes to mRNA looks like beads-on-a-string and this structure is called as *polysome* or *polyribosome*. Each mRNA contains a definite beginning and end signals for the synthesis of polypeptide chain (See Fig. 12.14).

The unique form of Met-tRNA, carrying a formylated methionine residue, initiates translation. The most common initiating codon in mRNA is AUG and rarely GUG. AUG, also codes for internal Met. Thus, besides initiation codon, more information must be needed to specify initiation site. Thus, it was found that a purine rich sequence is present ~ 10 nucleotides upstream (5'-side) of the start codon. This purine rich sequence is called as *Shine - Dalgarno sequence*. The complementary pyrimidine rich sequence is present on the 16S rRNA. Thus, base pairing between *Shine-Dalgarno* sequence of mRNA and 16S rRNA

plays an important role in the selection of an appropriate initiation site. This process also requires *initiation factors* (IF – 1, IF – 2 and IF – 3) but these factors are not permanently associated with the ribosome.

IF – 1: Promotes dissociation of ribosomal subunits.

IF – 2 (•GTP): Required for fMet - $tRNA^{met}$ binding.

IF – 3: Required for mRNA binding, finding the AUG.

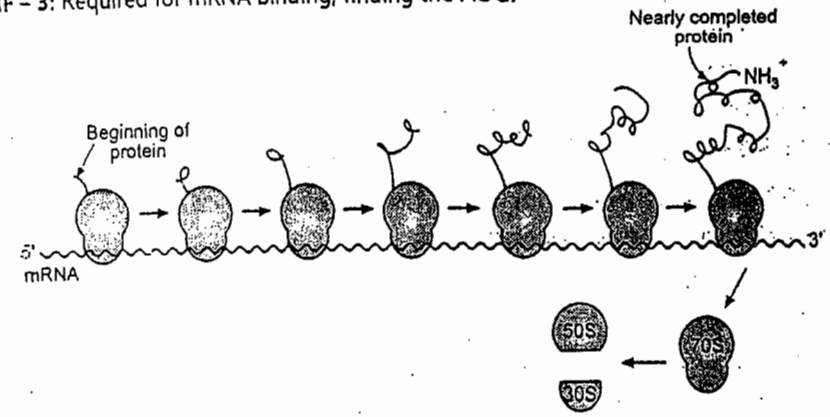


Fig. 12.14: Diagram of polyribosomes

The chain elongation occurs at a rate of ~ 40 residues per second and requires elongation factors (EF – Tu, EF – Ts, EF – G). EF – Tu is bound to GTP and amino acyl - tRNA. GTP is hydrolysed when EF–Tu positions the amino acyl-tRNA to the ribosomal A site. A second elongation factor EF–Ts dissociates GDP, EF–Ts is then displaced by GTP. It is important to note that just like any other amino acyl-tRNA met-tRNA binds to EF–Tu. However, formyl methionyl - tRNA does not interact with EF–Tu. Thus, initiator tRNA is not delivered to the ribosomal A site.

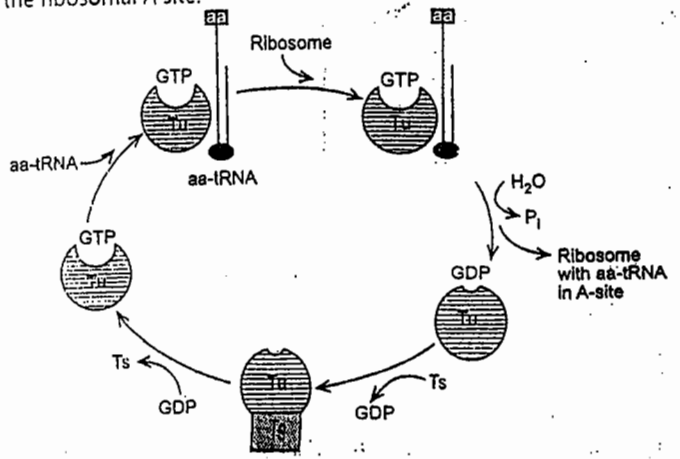


Fig. 12.15: Cycle of elongation factor

To ensure fidelity, the incoming amino acyl-tRNA is carefully scrutinised. Ribosomes take a while before deciding whether bound amino acyl-tRNA is the right one because formation of peptide bond does not take place until EF-Tu is released (see above) and release of EF - Tu depends on hydrolysis of GTP. Thus, hydrolysis of GTP and subsequent release of EF - Tu - GDP gives enough time (several milliseconds) for incorrect amino acyl-tRNA to leave. The hydrolysis of GTP also causes conformational changes in EF-Tu but the correct amino acyl-tRNA interacts strongly with mRNA in both the conformations (Fig. 12.15).

EF-Tu (-GTP): Binds amino acyl - tRNA to the ribosome.

EF-Ts: Regenerates EF-Tu-GTP

EF-G(-GTP): Increases translocation rate.

The second stage of elongation is the formation of peptide bond, catalysed by *peptidyl transferase*, an enzymatic activity of the 50S ribosomal subunit. The 23S rRNA forms the peptide transferase active site. The two tRNAs are arranged on A and P sites, a third binding site *E (exit) site* transiently binds the tRNA once peptidyl group is donated. The deacylated tRNA occupies E site on 50S subunit while staying in the P-site on 30S subunit. The new dipeptidyl-tRNA occupies the P-site on the 50S while staying in the A-site of 30S. This is consistent with the fact that ribosome can bind three tRNAs. During elongation, a residue is added at C-terminus and nascent polypeptide is transferred to A-site, this process is called as *transpeptidation*. The next step in elongation is *translocation*. It is mediated by EF-G (elongation factor G or translocase). In this step, the deacylated tRNA moves out of the P-site on the 30S subunit, the dipeptidyl - tRNA translocates from A-site to P-site on 30S and finally mRNA moves a distance of codon i.e. three nucleotides. Thus, the next codon is positioned for reading by the new incoming amino acyl-tRNA. This translocation is driven by the hydrolysis of GTP of EF-G. Now, EF-G is released and new elongation cycle can begin. Besides conformational changes, EF-G drives translocation by actively displacing the peptidyl-tRNA from A-site. Thus, A-site is empty and a new round of elongation can start. It is important to take a note that A and E-site cannot be simultaneously occupied and filling of A-site induces the release of deacylated tRNA from E-site. Thus, the unidirectional movement of ribosomes through transpeptidation and translocation is accomplished by alternating activities of EF–Tu and EF–G accompanied by GTP hydrolysis (Fig. 12.16).

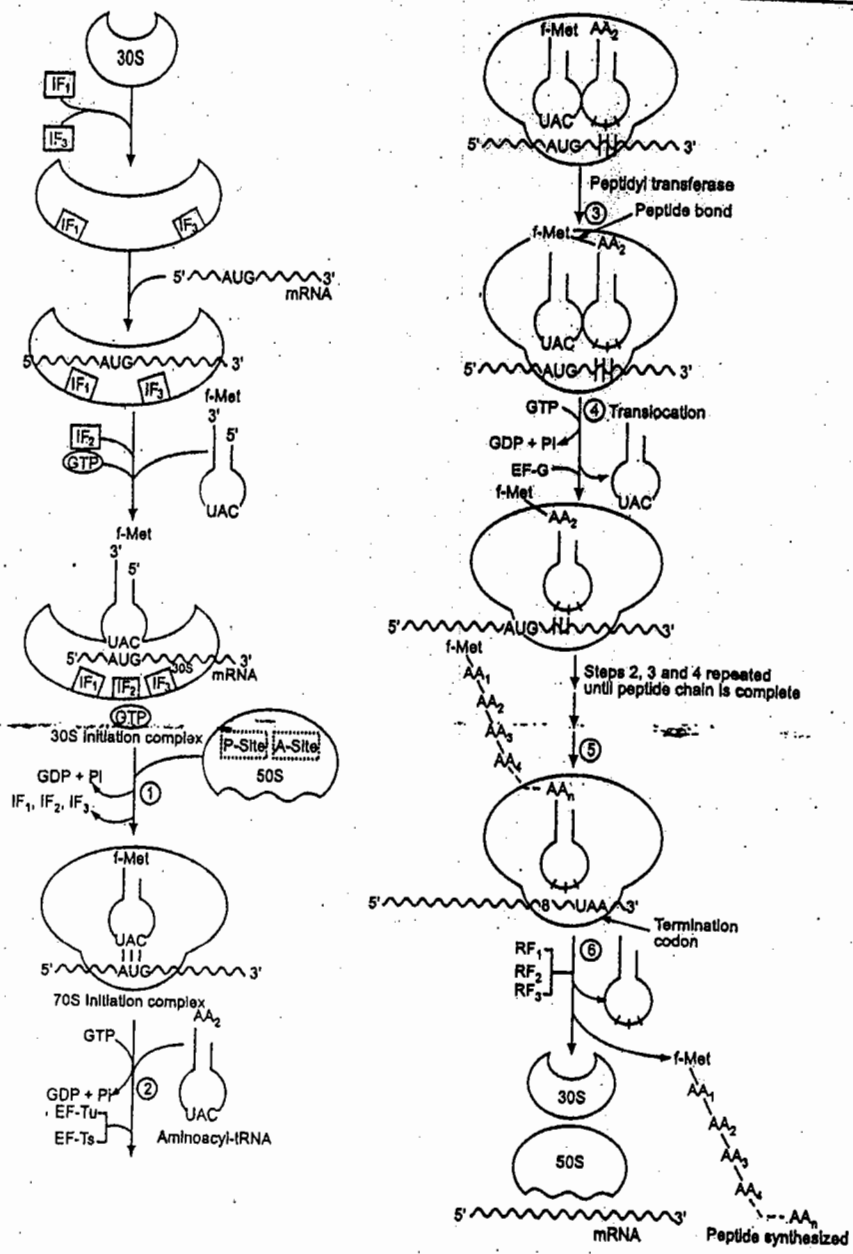


Fig. 12.16: Protein biosynthesis

Normally, cell does not contain a tRNA with anticodon complementary to 'stop signals' or 'non-sense codons'. Thus amino acyl-tRNA does not bind to A-site of ribosomes if the codon is UAA, UGA or UAG. These termination codons are recognised by proteins called as *release factors* in *E. coli*. RF-1 recognises UAA and UAG, whereas RF-2 recognises UAA and UGA, RF-3 (GTP - binding protein) enhances the ribosomal binding of RF-1 and RF-2. But in eukaryotes, all the three termination codons are recognised by a single release factor, *eRF*.

The hydrolysis of bond between the polypeptide and the tRNA in the P site is done by peptidyl transferase, which is activated by binding of release factors to a termination codon in the A-site. Thus, peptidyl transferase transfers the peptidyl group to water rather than amino group. Then free polypeptide and uncharged tRNA are dissociated from ribosome. The dissociation of release factors is accomplished by hydrolysis of GTP. Before starting a new round of protein synthesis, mRNA is released from inactivated ribosomes.

12.12.1 Important Features of Protein Synthesis

Protein synthesis is carried on ribosomes and is summarized in Fig. 12.17. Protein synthesis occurs intracellularly. Some of the features to be remembered are:

(i) DNA molecules never leave chromosomes during protein synthesis.

(ii) Protein synthesis is an accurate process; error rate is about 0.01%.

(iii) Protein synthesis is cyclical.

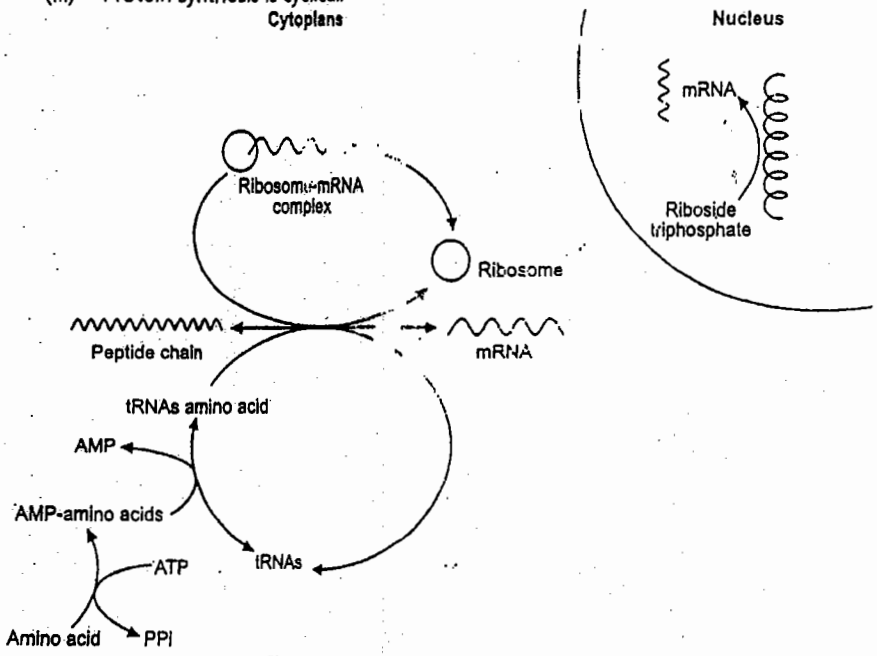
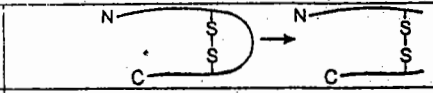
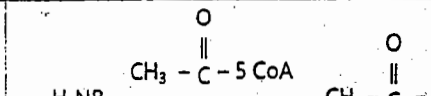
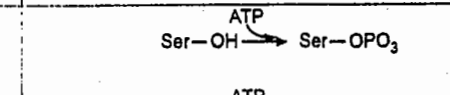
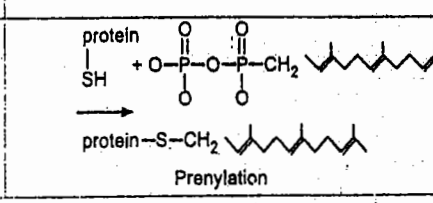


Fig. 12.17: Summary of protein synthesis

In addition many proteins are known to undergo modifications known as *post translation modifications* e.g. removal and/or derivatisation of specific residues. Many proteins are synthesized as precursors - *proproteins*. These are activated by limited proteolysis e.g. serine proteases.

Table 12.4: Post translational modifications

Proteolysis		Proteases
Glycosylation	Asn – NH_2 → Asn – NH_2 – Sugar (N-linked) Ser – OH → Ser – OH – Sugar	Glycosyl transferase
Acylation Acetylation		Acylase Acetylase
Phosphorylation		Kinases
Lipid attachment		Lipid transferases

12.12.2 Inhibition of Translation

Many antibiotics act as inhibitors of protein synthesis. Streptomycin interferes with the binding of formylmethionyl - tRNA to ribosomes and thus inhibits initiation and / or causes misreading of mRNA. Puromycin, an analog of the terminal amino acyl-adenosine portion of amino acyl-tRNA, binds to A-site of ribosome and inhibits the entry of amino acyl-tRNA and thus causes premature chain termination. Chloramphenicol binds to 23S rRNA and blocks protein synthesis by inhibiting peptidyl transferase activity. *Thus, it is important to note that protein synthesis inhibition by most antibiotics is mainly linked to RNA and not protein component of ribosome.*

12.13 REVERSE TRANSCRIPTION

As discussed above the usual flow of information is from DNA to RNA to proteins i.e. the central dogma of molecular biology.

There are exceptions to the central dogma e.g. retroviruses (RNA viruses) like HIV-1. Reverse transcription is a process that makes DNA from an RNA template. This is achieved by an RNA-directed DNA polymerase, called *reverse transcriptase*. Reverse transcriptase turns RNA to DNA in a similar way that DNA polymerase turns DNA to RNA. Thus, there is a reversal to first part of central dogma i.e. RNA to DNA, instead of DNA to RNA, and hence they are called as *retro*viruses. In Latin, *retro* means 'backward'. The process of making DNA from RNA template by reverse transcriptase is called as *reverse transcription*.

A modified schematic diagram of central dogma including reverse transcription can be shown as in Fig. 12.18.

12.13.1 Structure and Functions of Enzyme: Reverse Transcriptase (RT)

HIV-1 RT exists as a heterodimer consisting of tightly associated p^{51} and p^{66} subunits.

This enzyme has three distinct catalytic functions:

1. An RNA dependent DNA polymerase activity.
2. Utilizing viral RNA strand as a template. It synthesizes a complimentary strand of DNA. As this strand is being formed, activity residing RNAase H domain of p^{66} serves to digest RNA template.
3. A DNA-dependent DNA polymerase to complete the synthesis of the double stranded proviral DNA.

Hence, RT is responsible for the synthesis of double stranded viral DNA from proviral RNA for subsequent incorporation into a host cell chromosome.

The function of RT is essential for the replication of HIV. Therefore, this is a suitable target for chemotherapeutic intervention.

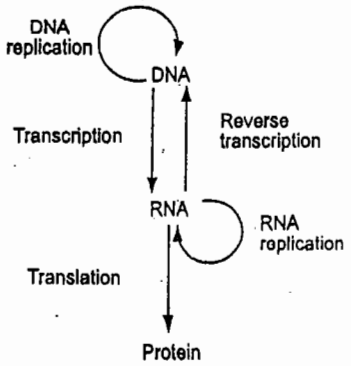


Fig. 12.18: Modified central dogma of molecular biology

David Baltimore in 1975 shared the Nobel Prize in Physiology or Medicine for his discovery of reverse transcriptase.

Retroviruses are important, as some of them are responsible for cancer and AIDS. Reverse transcriptase has been used as the target for the anti-HIV drugs. For example, 3'-Azido-2', 3'-dideoxythymidine (AZT) is an inhibitor of HIV reverse transcriptase.

QUESTIONS

Transcription

1. Define transcription. Add a note on transcription in prokaryotes and eukaryotes.
2. Enlist the proteins participating in transcription. Describe the role of each protein in short.
3. Write short notes on:
   (a) Role of mRNA in transcription.
   (b) RNA chain termination.
   (c) Inhibitors of transcription.
4. Give a schematic representation of transcription bubble.

Translation

1. Define the term genetic code and discuss its characteristic features.
2. Explain in detail – Protein biosynthesis.
3. What is meant by inhibitor? Add a note on the inhibitors of translation.
4. Write short notes on:
   (a) Ribosomes
   (b) Codon-anticodon interactions.
   (c) Inhibition of translation.
5. Explain the role of tRNA in translation. Give a schematic representation of tRNA.
6. What is meant by polyribosomes? Add a note on the role of polyribosomes in protein synthesis.

UNIT V

Chapter ... 13

ENZYMES AND CO-ENZYMES

♦ LEARNING OBJECTIVES ♦

- *Understand about Biological Catalyst and its Tremendous Potential to Pursue various reactions at Body Temperature.*
- *Understand the Role of Coenzymes, Cofactors, Activators, Inhibitors and factors like Temperature, pH, Concentration in Enzyme Catalyzed Reactions.*
- *Knowledge of Concept of Inhibitor and its Application in the Study of Enzyme.*
- *Recognize how Enzymes Regulate Biochemical Reaction within the Cell and Mechanism of Enzyme Action.*
- *Knowledge of Nomenclature and Classification of Enzyme.*
- *Knowledge of Restriction, Oncolytic and Pharmaceutical Importance of Enzymes.*

13.1 INTRODUCTION

Enzymes are remarkable and highly specialised proteins. *Enzymes are catalysts of the biological system* and are often much more efficient as compared to synthetic catalysts.

Enzymology is a branch of science dealing with the study of enzymes and has its origin in the early days of biochemistry. In the 1850's Louis Pasteur suggested that conversion of sugar to alcohol by yeast is catalysed by *'ferments'*, later named *enzymes*. Further, Edward Buchner showed that yeast extract can convert sugar to alcohol. This finding proved that enzymes can work outside the cells. Later, James Sumner in 1926 crystallised urease and John Northrop in 1930's crystallised pepsin and trypsin. These findings established that *enzymes are proteins* (although a small group of RNA molecules also have catalytic properties).

The most striking characteristics of enzymes are their catalytic power and specificity.

Endoenzymes:

Endoenzymes are also called as 'intracellular enzymes'. These enzymes are used in the cells which make them.

Exoenzymes:

Exoenzymes are also called as 'extracellular enzymes'. These enzymes are produced by cells and are secreted to different parts of the body. Enzymes of the digestive tract are the best examples of exoenzymes.

Constitutive enzymes:

Constitutive enzymes are formed at constant rates and in constant amount regardless of the metabolic state of the cell or organism. This is the permanent and basic machinery of the cell. For example, enzymes of glycolytic sequence. In general, constitutive enzymes carry out 'housekeeping functions' of the cell.

Inducible enzymes:

Normally inducible enzymes are present in very small amounts in a cell. When enzyme substrate is present, enzyme concentration can increase thousand fold or more. *β-galactosidase* is a classic example of inducible enzyme. Cyclo-oxygenase-2 (Cox-2) is also an inducible enzyme present in inflammatory cells.

Extracellular enzyme which is secreted in active form is called as *zymase*. Whereas the one which is secreted in inactive form and is activated by an agent (or enzyme) secreted by other cells is called as *zymogen*.

13.2 ENZYME NOMENCLATURE AND CLASSIFICATION

Initially, enzymes were named in an arbitrary manner. For example, names like trypsin, pepsin does not provide information regarding enzyme function or the substrate on which enzyme is acting. Now-a-days, enzymes are commonly named by adding the suffixase to the name of the substrate or to a phrase describing the catalytic action of the enzyme. Thus, lipase acts on lipids, urease catalyses hydrolysis of urea and alcohol dehydrogenase catalyses the oxidation of alcohols. Sometimes enzymes are given names which does not provide clue to their function e.g. catalase. To avoid such confusions, Enzyme Commission was appointed in 1961 by International Union of Biochemistry (IUB). Since 1964, the IUB system of enzyme classification is in use. Enzymes are classified and named according to the nature of the chemical reactions they catalyse (Table 13.1).

Table 13.1: Classification of enzymes

Enzyme Class	Type of Reaction Catalysed	Example
1. Oxidoreductases	Oxidation-reduction reactions (Transfer of electrons)	Alcohol dehydrogenase, cytochrome oxidase.
2. Transferases	Transfer of functional groups	Hexokinase, transaminase, transmethylase, phosphorylase.
3. Hydrolases	Hydrolysis reactions (Transfer of functional groups to water)	Lipase, choline esterase, pepsin, urease, phosphatases.
4. Lyases	Group elimination to form double bonds, addition of groups to double bonds	Aldolase, histidase.
5. Isomerases	Isomerisation	Retinol isomerase, glucose phosphate isomerase.
6. Ligases	Bond formation coupled with ATP hydrolysis.	Glutamine synthetase, succinate thiokinase.

All enzymes are divided in six major classes. Each class has subclasses based on the type of reaction catalysed. Each enzyme is given a four digit classification number (Enzyme Commission, (EC) number) and a systematic name, which identifies the reaction. Consider the following example:

ATP + D-glucose → ADP + D-glucose – 6 – phosphate

Hexokinase is the trivial name of the enzyme which catalyses the above reaction. The systematic name of the enzyme is ATP: glucose phosphotransferase (specifying that it catalyses the transfer of phosphate group from ATP to glucose). The EC number of this enzyme is 2.7.1.1.

- The first digit (2) denotes the name of the class. In this case, it is transferase.
- The second digit (7) denotes the subclass. In this case, it is phosphotransferase.
- The third digit (1) denotes sub-sub class. In this case, it is phosphotransferases with a hydroxyl group as acceptor.
- The fourth digit (1) denotes arbitrarily assigned serial number in it's sub-sub class. In this case, it is D-glucose as the phosphate group acceptor.

Systematic names of the enzymes are long and complicated and hence trivial names are used in day-to-day practice.

13.3 PROPERTIES OF ENZYMES

All enzymes are proteins. Although, recently few RNA molecules have been shown to function as enzymes (Refer Section 13.13). Each enzyme has its own tertiary structure and particular conformation which is necessary for its catalytic activity. The enzyme–catalysed reaction occurs within a pocket of the enzyme called the *active site*. The functional form of the enzyme is called as *holoenzyme*. Holoenzyme is constituted of two parts – *apoenzyme* (the protein part) and a *co-enzyme* (non-protein organic part). The co-enzyme can be separated by dialysis. Sometimes non-protein part is very tightly bound to apoenzyme and cannot be separated by dialysis. Such a group is called as *prosthetic group*.

Enzymes which are made up of a single polypeptide chain are called as *monomeric enzymes* e.g. ribonuclease and trypsin. Whereas, enzymes which contain more than one polypeptide chains are called as *oligomeric enzymes* e.g. lactate dehydrogenase. Certain reactions are catalysed by *multienzyme complex* e.g. pyruvate dehydrogenase. In such cases, only the intact complex is functionally active and not the individual units of the complex.

Enzyme catalysed reactions are approximately 10^6 to 10^{12} times faster compared to uncatalysed reactions. Also, enzyme driven reactions require much milder conditions compared to chemical reactions e.g. nearly neutral pH, atmospheric pressure and temperatures much below 100°C. Compared to chemical reactions, enzyme catalysed reactions rarely produce side products because of much higher substrate specificity. In addition, enzyme catalysed reactions are well regulated. (Refer Section 13.9).

Enzymes show all the general properties of proteins.

13.4 CO-ENZYMES AND COFACTORS

The catalytic activity of many enzymes depends on the presence of small, non-protein molecules called as *cofactors*. Cofactors can be divided into two groups - metallic ions and small organic molecules. The enzyme carbonic anhydrase requires Zn^{++} for its activity - an example of metallic ion cofactor. The enzyme glycogen phosphorylase requires pyridoxal phosphate for its activity - an example of a small organic molecule.

Cofactors that are small organic molecules are called as *coenzymes. Coenzymes can be defined as non-protein, organic, low-molecular weight substances associated with the enzyme activity*. Coenzymes are often derived from vitamins and can be either loosely bound e.g. NAD^+ or tightly bound, e.g. heme prosthetic group of cytochrome C, to the enzyme. The tightly bound coenzymes are called as *prosthetic groups*. Loosely bound coenzymes are like cosubstrates i.e. they bind to and are released from the enzyme just like substrates and products.

The functional enzyme i.e. catalytically active complex of enzyme-cofactor is called as *holoenzyme*. The enzyme without its cofactor is called as *apoenzyme*.

Apoenzyme + Cofactor ⟶ Holoenzyme

Like substrates, coenzymes also undergo alteration during enzyme reaction. However, unlike substrates, coenzymes are regenerated.

Vitamins and Co-enzymes

Many co-enzymes are derived from vitamins (Refer Table 13.2).

Table 13.2: Vitamins and Co-enzymes

Vitamin	Co-enzyme	Reaction
Biotin	Biocytin	Carboxylation
Cobalamine (B_{12})	Cobalamine co-enzymes	Alkylation
Riboflavin (B_2)	Flavin co-enzymes	Oxidative - reduction
Niacin	Nicotinamide co-enzymes	Oxidative - reduction
Pyridoxin (B_6)	Pyridoxal phosphate	Amino group transfer
Folic acid	Tetrahydrofolate	One-carbon transfer
Thiamine (B_1)	Thiamine pyrophosphate	Aldehyde transfer

Most of the coenzyme precursors are water-soluble vitamins but fat-soluble vitamins (such as A, K and D) are also essential.

Vitamin K is required for normal blood clotting and participates in the carboxylation.

Vitamin K is required for the synthesis of γ-carboxy-glutamate (an extra COOH on the glutamate side chain): 10 of these residues are found at the N-terminal of thrombin and are required for Ca^{+2} binding (which anchors it to phospholipid membrane).

Vitamin A is a precursor of retinal, a light sensitive group in rhodopsin and other visual pigments.

Ascorbic acid is essential in reducing the ferric ion of the inactivated form of prolyl hydroxylase and thus regenerating the active form of prolyl hydroxylase. Prolyl hydroxylase is required for the synthesis of hydroxyproline residues of collagen.

But not all co-enzymes are derivatives of vitamins. There are organic molecules which are not vitamins but serve as co-enzymes (Refer Table 13.3).

Table 13.3: Co-enzymes other than vitamins

Co-enzyme	Function
ATP	Donates phosphate
CDP	Essential for the synthesis of phospholipids
UDP	Required for glycogen synthesis
S-adenosylmethionine	Methyl group donor

It is important to note that a particular co-enzyme may participate in different reactions catalysed by different enzymes. In other words, specificity of the enzyme is not determined by co-enzyme. Rather it is decided by apoenzyme e.g. NAD^+ acts as a co-enzyme for both lactate dehydrogenase and alcohol dehydrogenase

13.5 FACTORS AFFECTING CATALYTIC ACTIVITY

Various factors influence the rate of enzyme reactions.

(i) Concentration of enzyme:

Rate (velocity) of reaction and enzyme concentration are directly proportional. Hence, rate of reaction increases proportionally as the concentration of enzyme is increased. Here it is assumed that the substrate concentration is in excess and hence the reaction is independent of substrate concentration. Thus, any change in the amount of product formed over a particular time period will depend on the enzyme concentration. This can be represented graphically as shown below:

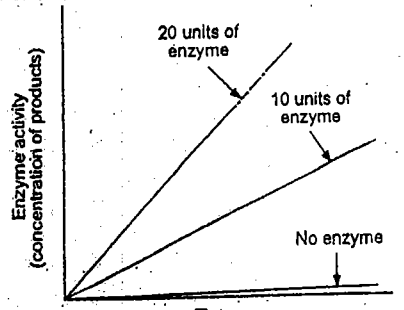


Fig. 13.1: A plot of concentration of enzyme Versus time

(ii) Concentration of substrate:

Till a threshold concentration, increase in rate of reaction is seen with increase in the substrate concentration. At very low substrate concentration rate of reaction is directly proportional to the substrate concentration. Whereas, at very high substrate concentration the rate of reaction is independent of substrate concentration.

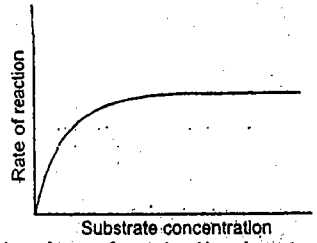


Fig. 13.2: A plot of rate of reaction Vs substrate concentration

(iii) Temperature:

At a particular temperature called as *optimum temperature*, the enzyme activity is maximum. Enzyme reactions are extremely temperature sensitive and change in reaction temperature as small as 1 or 2°C may change the reaction rate by 10 to 20%. At high temperature there is denaturation of proteins and hence inactivation of enzymes.

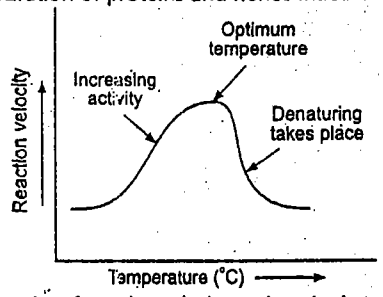


Fig. 13.3: A plot of reaction velocity against rise in temperature

For humans the optimum temperature is ~ 37°C but for other animals it is ~ 40°C.

At low temperature enzyme activity decreases. At 0°C, most enzymes are inactive.

(iv) pH:

Maximum enzyme activity is seen at *optimum pH* and is between pH 5 to 9. A small change in pH will significantly alter the enzyme activity. Extremely high or low pH usually results in complete loss of activity for most enzymes.

Changes in pH can alter the state of ionization of charged amino acids e.g. Asp, Lys and hence catalytic action of an enzyme. The effect of pH on the enzyme activity can be seen as shown below (Fig 13.4). Optimum pH for maximum activity is different for different enzymes. For example, pepsin is active in acidic media, whereas trypsin is active in neutral to slightly alkaline medium. This makes sense because pepsin is an enzyme that is mainly found in stomach where pH is low because of the presence of hydrochloric acid. Trypsin is found in the duodenum and hence its optimum pH is in the range of neutral to slightly alkaline to match the pH of the duodenum.

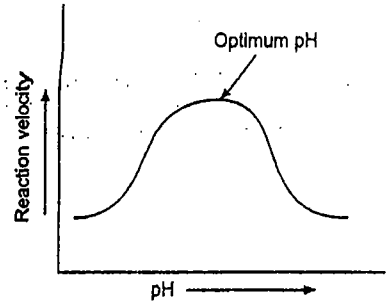


Fig. 13.4: A plot of reaction velocity against change in pH

The optimum pH for maximum activity is different for different enzymes (see below).

Enzyme	Optimum pH
Pepsin	1.5 – 1.6
Invertase	4.5
Maltase	6.1 – 6.8
Catalase	7.0
Trypsin	7.8 – 8.7

13.6 ENZYME KINETICS

For enzyme reactions, rate (velocity) of the reaction (V) varies with the substrate concentration ([S]) as shown in Fig. 13.5.

Basically, the overall enzymatic reaction is composed of two elementary reactions: first, in which enzyme (E) and substrate (S) combine to form an unstable enzyme-substrate complex (ES) and second, in which ES decomposes to form products (P) and (E).

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_3} E + P \qquad \text{... (13.1)}$$

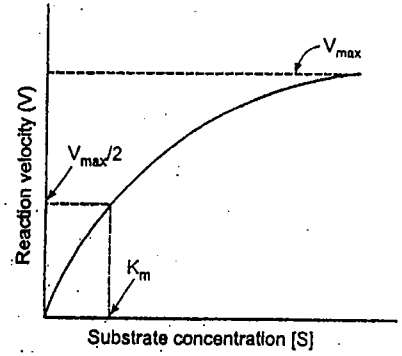


Fig. 13.5: A plot of velocity of enzymatic reaction versus substrate concentration [S]

K_1 is the rate constant for the formation of ES, K_2 is the rate constant for the dissociation of ES back to E and S and K_3 is the rate constant for the formation of product P.

The *Michaelis–Menten constant*, K_m is defined as:

$$K_m = \frac{K_2 + K_3}{K_1} \qquad \text{... (13.2)}$$

With appropriate mathematical manipulations we will get equation (13.3), also known as *Michaelis-Menten equation*, which is the basic equation of enzyme kinetics.

$$V = \frac{V_{max}[S]}{K_m + [S]} \qquad \text{... (13.3)}$$

where, V = Measured velocity
V_{max} = Maximum velocity
S = Substrate concentration
K_m = Michaelis-Menten constant

13.6.1 Significance of Michaelis – Menten Constant

Let us consider that $K_m = [S]$, hence equation (13.3) can be rewritten as follows:

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

i.e. $$V = V_{max}\frac{[S]}{2[S]}$$

hence, $$V = \frac{1}{2} V_{max} \qquad \text{... (13.4)}$$

Thus, K_m is equal to the substrate concentration at which the velocity of the reaction is half its maximum value.

Consider a situation where substrate concentration is very low i.e. [S] much less than K_m. In this case, equation (13.3) can be rewritten as follows:

$$V = \frac{V_{max}[S]}{K_m} \quad \text{as } K_m + [S] \sim K_m \qquad \text{... (13.5)}$$

From equation (13.5) it is apparent that when substrate concentration is very low, velocity of reaction is directly proportional to substrate concentration (as V_{max} and K_m are constants).

Now, consider a situation where substrate concentration is very high i.e. [S] is much higher than K_m. In this case, equation (13.3) will be rewritten as follows:

$$V = \frac{V_{max}[S]}{[S]} \quad \text{as } K_m + [S] \sim [S]$$

i.e. $$V = V_{max} \qquad \text{... (13.6)}$$

From equation (13.6) it is clear that when substrate concentration is very high, velocity of reaction is at its maximum and is independent of substrate concentration.

13.6.2 Lineweaver - Burk Plot

Lineweaver - Burk plot is also called as *double-reciprocal plot*. It was formulated by Hans Lineweaver and Dean Burk for determining values of V_{max} and K_m. This method uses the reciprocal of Michaelis-Menten equation (eq. 13.3).

Thus, $$\frac{1}{V} = \frac{K_m + [S]}{V_{max}[S]}$$

i.e. $$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{[S]}{V_{max}[S]}$$

hence, $$\frac{1}{V} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}} \quad \ldots (13.7)$$

This is a linear equation (similar to y = mx + c). Hence, plot of $\frac{1}{V}$ and $\frac{1}{[S]}$ wil. give a straight line (See Fig. 13.6).

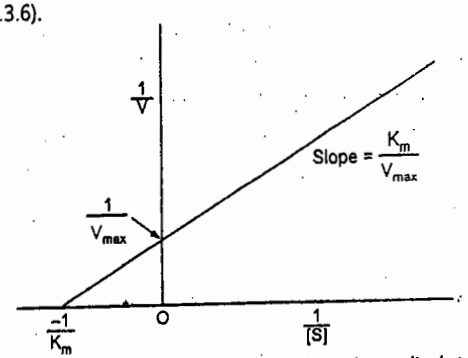


Fig. 13.6: Lineweaver - Burk (double reciprocal) plot

This is called as *Lineweaver - Burk* or *double-reciprocal plot*. The slope of the line is $\frac{K_m}{V_{max}}$, the intercept on y-axis $\left(\frac{1}{V}\right)$ is $\frac{1}{V_{max}}$ and the extrapolated x-axis $\left(\frac{1}{[S]}\right)$ intercept is $-\frac{1}{K_m}$. Thus, calculation of K_m and V_{max} is now much easier.

13.7 ENZYME UNITS

For all practical purposes, enzymes are expressed only as their activities and not their concentration viz. mg or µg etc. Various units which have been used to express enzyme activities are King-Armstrong units, Reitman-Frankel units, Somogyi units, Spectrophotometric units etc.

Katal

To maintain the uniformity, Enzyme Commission of IUB introduced a new unit called as Katal (abbreviated as Kat). *One kat indicates the conversion of one mole substrate per second* (mol/sec.).

International Units

One International Unit (IU) or Standard Unit or System International (SI) unit is defined as a *conversion of one micromole of substrate per minute*.

IU and Katal are interconvertible as shown below:

$$1 \text{ IU} = 60 \text{ µ Kat}$$

or

$$1 \text{ n Kat} = 1.67 \text{ IU}$$

Old units, such as King-Armstrong units or Somogyi units are still in use, especially in clinical laboratories.

13.8 ENZYME INHIBITION

An enzyme *inhibitor* is a substance that binds with the enzyme and decreases the catalytic activity of the enzyme. Inhibition can be defined as 'the process which decreases the catalytic activity of the enzyme'. As enzymes are involved in almost all the cellular processes, enzyme inhibitors are very important pharmaceutical agents. In addition, enzyme inhibitors can provide information about mechanism of enzyme action.

Enzyme inhibition can be:

1. Reversible
2. Irreversible
3. Allosteric

1. Reversible Inhibition:

In this type of inhibition, inhibitors bind non-covalently with enzyme, and the inhibition can be reversed by removal of inhibitor. Sometimes the effect of reversible inhibitor can also be reversed by decreasing the concentration of inhibitor (e.g. by dilution or dialysis). Reversible inhibition can be of the following types:

(i) Competitive inhibition
(ii) Non-competitive inhibition
(iii) Uncompetitive inhibition

(i) Competitive inhibition:

Competitive inhibitor is a substance that competes directly with a normal substrate for the active site of the enzyme. Competitive inhibitor usually resembles the substrate but does not undergo any catalysis. The main feature of competitive inhibition is that it can be overcome by increasing the concentration of substrate. The enzyme reaction can be shown as below:

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

$$+ I \uparrow\downarrow$$

$$EI$$

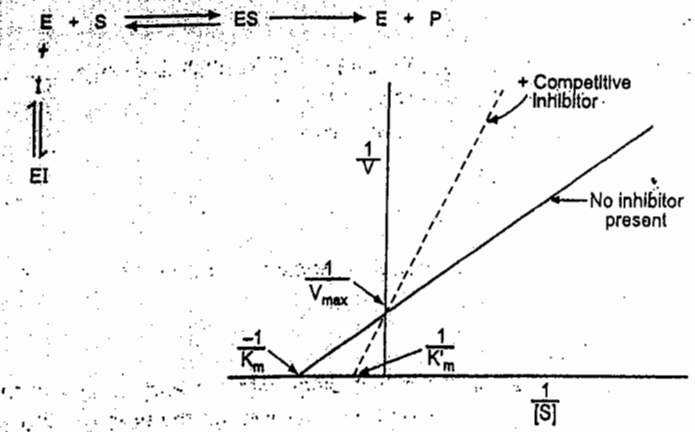
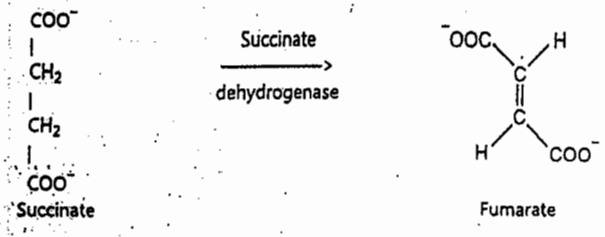
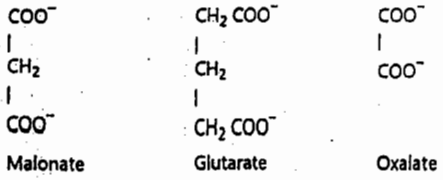


Fig. 13.7: Effect of competitive inhibitor on a Lineweaver - Burk plot (dashed line with inhibitor)

In competitive inhibition, intercept of $\frac{1}{V}$ (y–axis intercept) is the same in presence and absence of inhibitors but slope of the lines differ (See Fig. 13.7). This suggests that V_{max} is not altered by competitive inhibition and K_m value increases. The slope of the line indicates strength of binding of competitive inhibitor.

An example of competitive inhibition is succinate dehydrogenase (a citric acid cycle enzyme) which normally converts succinate to fumarate. This enzyme is competitively inhibited by substances like malonate, glutarate and oxalate, as they have structural similarity with succinate.





More examples of enzymes, their substrate, inhibitor and therapeutic implication are given in Table 13.4.

Table 13.4: Few examples of enzymes with their competitive inhibitors

Enzyme	Natural Substrate	Competitive Inhibitor	Therapeutic Implication
Acetylcholine esterase	Acetylcholine	Succinyl choline	Muscle relaxation
Dihydrofolate reductase (bacterial)	Dihydrofolic acid	Trimethoprim	Antibacterial
Monoamine oxidase - B	Dopamine	Selegiline	Antiparkinsonism
Xanthine oxidase	Hypoxanthine	Allopurinol	Control of gout

(ii) Non-competitive inhibition:

In this case, inhibitor binds to both the enzyme and enzyme-substrate complex. This is also called as *mixed inhibition*. This type of inhibitor has no structural similarity to substrate. Non-competitive inhibitor changes the enzyme conformation and hence can affect both, substrate binding and catalysis. The inhibitor generally binds to a site distinct from the substrate binding site.

The double-reciprocal plot corresponding to non-competitive inhibition is shown in Fig. 13.8.

As can be seen in Fig. 13.8, $\frac{1}{V}$ intercept (y-axis intercept) increases i.e. in non-competitive inhibition, V_{max} decreases.

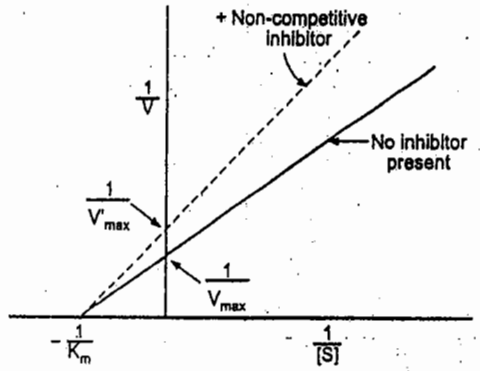
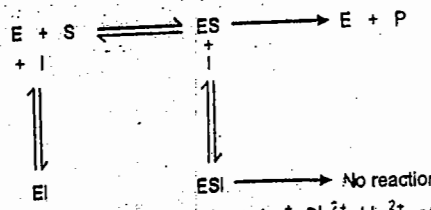


Fig. 13.8: Effect of non-competitive inhibitor on a Lineweaver-Burk Plot (dashed line with inhibitor)

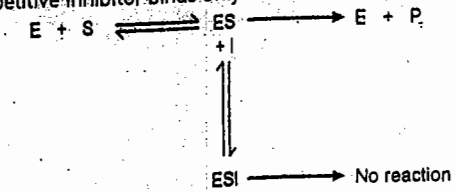
There is often little or no effect on K_m. Non-competitive inhibition cannot be overcome by increasing the substrate concentration. Non-competitive inhibition can be demonstrated as follows:



Inhibition of enzyme by heavy metals such as Ag^+, Pb^{2+}, Hg^{2+}, etc. is an example of non-competitive inhibition.

(iii) Uncompetitive inhibition:

In this case also, inhibitor binds at a site distinct from the substrate binding site. However, an uncompetitive inhibitor binds only to the ES complex.



Uncompetitive inhibitor may not structurally resemble substrate and presumably distorts the active site. Thus, making the enzyme catalytically inactive.

The double-reciprocal plot corresponding to uncompetitive inhibition is shown in Fig. 13.9.

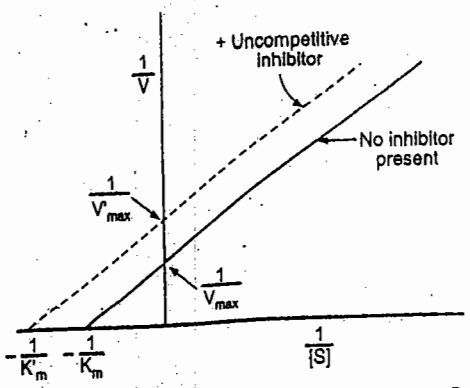


Fig. 13.9: Effect of uncompetitive inhibitor on a Lineweaver-Burk plot (dashed line with inhibitor)

As can be seen from Fig. 13.9, uncompetitive inhibitor decreases both K_m and V_{max}. The inhibition by uncompetitive inhibitor is not reversed by increasing substrate concentration. Uncompetitive inhibition is not very common and is significant only in multisubstrate enzymes.

2. Irreversible Inhibition:

When an inhibitor irreversibly binds to an enzyme, it is called as *inactivator*. Irreversible inhibitors combine with or destroy a functional group on the enzyme that is essential for its activity. The irreversible inhibitor binds covalently with the enzymes.

Irreversible inhibitors decrease the V_{max} without changing K_m. Hence, double reciprocal plot of irreversible inactivation resembles that of non-competitive inhibition. (Refer Fig. 13.8).

Suicide inhibitors (also called as *mechanism-based inactivators*) are a special class of irreversible inhibitors. These type of inhibitors act as substrates, but instead of being transformed into the normal product, are converted to reactive compounds that combine irreversibly with the enzyme.

These inhibitors can be used to identify catalytic site e.g. Diisopropylphosphofluoridate (DIPF) which specifically reacts with only Ser 195 of chymotrypsin, thus, demonstrating that this residue is in the enzyme's active site.

Difluoromethylornithine, a suicide inhibitor of ornithine decarboxylase, is used in the treatment of African sleeping sickness (trypanosomiasis).

Many organophosphate insecticides act as irreversible inhibitor of acetylcholine esterase and thus produce paralysis of nerve conduction.

Penicillins act as irreversible inhibitors of enzymes in bacterial cell wall synthesis.

3. Allosteric Inhibition:

For details please refer to 'Enzyme Regulation' (Section 13.10).

13.8.1 Degree of Inhibition

If an enzymatic reaction occurs in the absence of an inhibitor with rate (velocity) V and in the presence of an inhibitor with rate (velocity) V_i, the *degree of inhibition* is defined as:

$$\text{Degree of Inhibition} = \frac{V - V_i}{V} \quad \ldots (13.8)$$

As degree of inhibition is a ratio of rates, it has no units.

13.9 ISOENZYMES

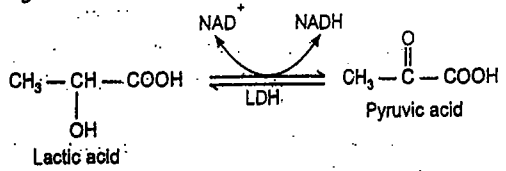
Multiple proteins (enzymes) demonstrating the same enzyme activity (and which are coded by different genes i.e. which are genetically different) are called as *isoenzymes* or *isozymes*. These multiple proteins should be present in the same organ (tissue) of the same organism.

If different enzymes catalysing the same reactions are present in different organs of same animal or same organ of different organisms then they are called as *heterozymes*.

Generally, isoenzymes differ in their physical and chemical properties which include *electrophoretic, immunological, kinetics, regulatory properties,* in the requirement of cofactor or in their subcellular distribution. Isoenzymes generally have similar, but not identical amino acid sequences.

13.9.1 Isoenzymes of Lactate Dehydrogenase

Lactate dehydrogenase (LDH; systematic name is L-lactate-NAD^+-oxidoreductase) catalyses the following reaction:



LDH was one of the first enzyme found to have isoenzymes. LDH occurs as atleast five different isoenzymes which can be separated by electrophoresis.

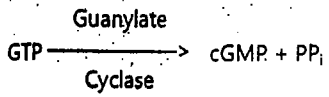
LDH is a tetramer made-up of two kinds of subunits (each ~ 35 KD). M type (also designated as A chain) in skeletal muscle and liver and H type (also designated as B chain) in heart. The M-type isoenzyme appears to mainly function in the reduction of pyruvate to lactate by NADH. But H-type isoenzyme is better adapted to catalyse the reverse reaction. These subunits associate to give five types of tetramers viz. H_4, H_3M_1, H_2M_2, H_1M_3 and M_4 (A_4, A_3B, A_2B_2, AB_3 and B_4). The H_4 isoenzyme (LDH_1) has higher affinity for substrates compared to the M_4 isoenzyme (LDH_5). The different LDH isoenzymes have different values of V_{max} and K_m (especially for pyruvate).

The M_4 isoenzyme reduces very low concentrations of pyruvate to lactate in skeletal muscle. And H_4 isoenzyme appears to favour rapid oxidation of lactate to pyruvate in the heart. Also H_4 isoenzyme is allosterically inhibited by high levels of pyruvate but M_4 is not. The remaining isoenzymes have intermediate properties depending on the ratio of the two kinds of chains.

Isoenzymes of LDH have great importance in the diagnosis of disorders of heart and liver. For example, heart attacks cause the death of heart muscles, which eventually rupture and release H-type LDH into the blood. Thus, a blood test demonstrating the presence of H-type LDH is a diagnostic of heart attack. Whereas, increased activity of M_4 isoenzyme in blood is an indicator of liver disease.

13.9.2 Isoenzymes of Guanylate Cyclase

There are at least two isoenzymes of guanylate cyclase.



One of the isoenzyme is an integral part of the plasma membrane. This isoenzyme is activated by the binding of the hormone atrial natriuretic factor.

A second and distinctly different isoenzyme of guanylate cyclase is a cytosolic protein. This isoenzyme is activated by nitric oxide (NO) and by several nitrovasodilators e.g. nitroglycerin, nitroprusside. These drugs are used in the treatment of heart disease.

13.9.3 Isoenzymes of Alkaline Phosphatase

There are atleast six isoenzymes of alkaline phosphatase (ALP) viz. α_1-ALP, α_2-heat labile ALP, α_2-heat stable ALP, pre-β ALP, γ-ALP, etc.

Increase in α_2-heat labile ALP is an indication of hepatitis whereas, pre β-ALP is an indicator of bone disease.

13.9.4 Isoenzymes of Alcohol Dehydrogenase

There are two isoenzymes of alcohol dehydrogenase: $\alpha\beta_1$ (found in White Americans and Europeans) and $\alpha\beta_2$ (found in Japanese and Chinese). $\alpha\beta_2$ isoenzyme rapidly converts alcohol to acetaldehyde compared to $\alpha\beta_1$. Due to rapid conversion by $\alpha\beta_2$ isoenzyme, there is accumulation of acetaldehyde which produces facial flushing and tachycardia. Hence, Japanese and Chinese show increased sensitivity to alcohol compared to White Americans and Europeans.

13.9.5 Isoenzymes of Hexokinase

There are several isoenzymes of hexokinase. These enzymes catalyse the conversion of glucose to glucose-6-phosphate. The main form of hexokinase isoenzyme in the liver is hexokinase D, also called as *glucokinase*. Glucokinase differs in a couple of respects from the hexokinase in muscle.

First, glucose concentration at which glucokinase is half-saturated (~ 10mM) is higher than the usual blood glucose concentration.

Second, glucokinase is inhibited by fructose-6-phosphate and not by its reaction product glucose-6-phosphate.

13.9.6 Isoenzymes of Creatinine Phosphokinase

Creatinine phosphokinase (CPK) catalyses the following reaction:

CPK consists of two subunits – M (muscle) and/or B (brain) and has three isoenzymes: CPK_1 (BB, brain), CPK_2 (MB, heart) and CPK_3 (MM, skeletal muscle).

After myocardial infarction, CPK_2 increases in the serum to as high as 20% (against 2% normal levels) within 6-18 hours. Thus, CPK_2 is a reliable index of myocardial infarction.

13.10 ENZYME REGULATION

Regulation of enzyme activity is essential to co-ordinate various metabolic processes. Regulation of enzyme activity takes place by one of the following ways:

(i) Allosteric regulation
(ii) Activation of latent enzymes
(iii) Compartmentation
(iv) Enzyme availability
(v) Isoenzymes
(vi) Cofactor availability.

(i) Allosteric Regulation (Allosteric Inhibition):

In allosteric regulation, *the binding of substrate to one active site can affect the properties of other active sites in the same enzyme molecule.* The term allosteric derives from Greek *allos,* 'other', and *stereos,* 'solid' or 'shape'. Allosteric regulation occurs through reversible, non-covalent binding of a regulatory metabolite called a *modulator (effector). Allosteric modulators* can increase (by binding to activator site) or decrease (by binding to inhibitor site) the enzyme activity (See Fig. 13.10). Thus, allosteric activator will shift the curve to the left, whereas allosteric inhibitor will shift the curve toward right.

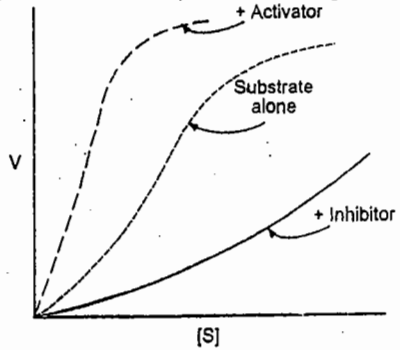


Fig. 13.10: Effect of allosteric activator and allosteric inhibitor

The allosteric theory suggests that an allosteric enzyme possesses two distinct binding sites: the active or *catalytic site* and the regulator or *allosteric site.* The regulator has no structural similarity to the substrate. Also enzyme has extreme specificity toward the regulator molecule and substrate and regulator molecules do not compete for the same site on the enzyme.

Allosteric enzymes (enzymes regulated by allosteric mechanisms) can be grouped under two classes:

(i) **K-class:** In this class of allosteric enzymes, effector molecule changes the K_m but not V_{max}. Thus, Lineweaver-Burk plot will be similar to competitive inhibition e.g. phosphofructokinase. (See Fig. 13.7).

(ii) **V-class:** In this class of allosteric enzymes, effector molecule changes the V_{max} and not the K_m. Thus, Lineweaver - Burk plot will be similar to non-competitive inhibition e.g. acetyl CoA carboxylase. (See Fig. 13.8).

Model of Allosteric Regulation:

Imagine an allosteric enzyme consisting of two identical subunits. The A (tense) form has low affinity and B (relaxed) form has high affinity for substrates. An allosteric inhibitor will shift the conformational equilibrium toward A-state (low affinity for substrate) whereas, allosteric activator will shift the conformational equilibrium toward B-state (high affinity for substrate). Thus, allosteric activator will increase and allosteric inhibitor will decrease the substrate binding.

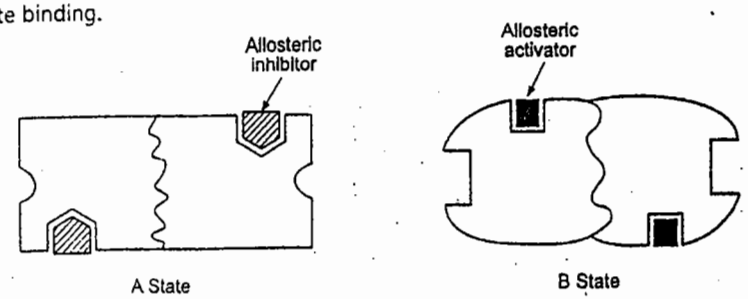


Fig. 13.11

For example, aspartate transcarbamoylase (ATCase, enzyme involved in the biosynthesis of pyrimidines) has two subunits: catalytic (c) and regulator (r) subunit. The activator ATP preferentially binds to active state of ATCase (high substrate affinity). Whereas, CTP binds to inactive state (low substrate affinity) and thus decreases affinity for the substrate without affecting V_{max}. Only the regulatory subunit is responsive to ATP or CTP but the catalytic subunit is unresponsive to ATP or CTP.

Feedback Regulation:

Feedback regulation is the inhibition of first step in a series of reactions in a pathway by the final product. The end product of the pathway binds to an allosteric site on the first enzyme in the pathway and shuts down the entire pathway. Feedback inhibition is also called as *end product inhibition* or *negative feedback inhibition.*

It is the most flexible and biologically widespread mechanism of metabolic control.

ATCase is an example of allosteric enzyme inhibited by feedback mechanism. ATCase converts carbamoyl phosphate to N-carbamoyl aspartate which is further converted to CTP. Thus, when CTP levels are high, it inhibits ATCase and inhibits earlier step in its own biosynthesis.

Another example is feedback inhibition of isoleucine biosynthesis in E. coli, as shown below.

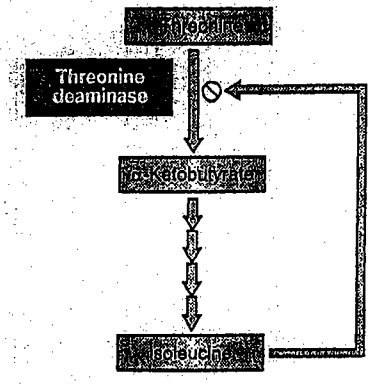


Fig. 13.12

The amino acid isoleucine is synthesized by a series of reactions starting from threonine. The first step in the pathway is catalysed by the enzyme threonine deaminase. This enzyme is inhibited by isoleucine, the end product of the pathway. Thus, once the adequate amount of isoleucine is formed in the cell, the further synthesis is blocked. Thus by regulating the activity of threonine deaminase, the cell synthesises only required amount of isoleucine and avoids wasting energy on the synthesis of more isoleucine than is needed.

(ii) Activation of Latent Enzymes:

The inactive precursors of the enzymes are called as *zymogen* (or *proenzymes*).

Many proteolytic enzymes of the stomach and pancreas are regulated in this manner.

Following are examples of zymogens (Refer Table 13.5).

Pepsinogen is activated by the low pH in the stomach. A protease known as enteropeptidase (or enterokinase) activates trypsinogen, which in turn activates chymotrypsinogen. These pancreatic proteases are also auto-catalytic i.e. they activate their own zymogens.

This approach of enzyme regulation is quite important in case of proteases, especially relatively non-specific ones e.g. proteases in digestion, as they are potentially lethal to the cells that produce them unless their activity is controlled.

Table 13.5: Zymogen and respective active enzyme

Zymogen	Active enzyme
Pepsinogen	Pepsin
Chymotrypsinogen	Chymotrypsin
Trypsinogen	Trypsin
Proelastase	Elastase
Procarboxypeptidase	Carboxypeptidase

Blood coagulation is also mediated by a cascade of proteolytic activations.

A series of serine proteases are activated by each other in turn, finally converting pro-thrombin into thrombin. Thrombin then converts, fibrinogen into fibrin, which spontaneously polymerizes to form the clot, which is subsequently stabilised by covalent cross-linking.

Some protein hormones are synthesized as inactive precursors e.g. insulin is synthesized from proinsulin.

Collagen, a fibrous protein and the major constituent of skin and bone is obtained from procollagen.

Many enzymes are regulated by phosphorylation. In this, cyclic AMP plays a crucial role. *Cyclic AMP (cAMP)* is synthesized by the cyclization of ATP and this conversion is catalysed by *adenylate cyclase.* Adenylate cyclase is stimulated by hormones and thus intracellular effects of hormones are mediated by cAMP.

cAMP mediates its effect via protein kinase A (PKA). cAMP Binds to regulatory subunits and releases catalytic subunit of PKA. Activated PKA then phosphorylates a variety of enzymes and regulates their activity. For example:

- In glycogen metabolism, *glycogen synthase* is inhibited because of the phosphorylation and thus further glycogen synthesis is inhibited.
- *Cystic fibrosis transmembrane regulator* (CFTR), a chloride channel on epithelial cells, is opened because of the phosphorylation of regulatory domain by PKA. This channel is defective in *cystic fibrosis*, a lethal genetic disorder.
- PKA also controls the expression of various genes by phosphorylating a transcriptional factor called as cAMP-response element binding protein.

(iii) Compartmentation:

Different forms of the same enzyme (isozymes) may be located in different parts of the cell e.g. cytosol/mitochondria, or different organs, heart/muscle. As a general rule, anabolic enzymes (synthetic) and catabolic enzymes (breakdown) are active in different cellular organelles. For example, enzymes involved in the synthesis of fatty acids are present in cytosol but enzymes involved in the oxidation of fatty acids are present in mitochondria.

(iv) Enzyme availability:

The amount of enzyme in a cell depends on its rate of synthesis (both transcription and translation) and rate of degradation. These rates are controlled by cells and are subject to change.

Enzyme synthesis can be induced or repressed. Insulin causes the induction of various enzymes involved in glucose metabolism viz. glycogen synthetase, glucokinase, pyruvate kinase etc. Whereas, pyruvate carboxylase (an enzyme involved in the synthesis of glucose) is repressed by glucose.

Each enzyme has a certain half-life and half-lives of enzymes are highly variable. For example, half-life of amylase is 3 to 5 hours but of LDH_4 is 5 to 6 days. It is important to note that many rate limiting enzymes have short half-lives and thus are rapidly degraded.

(v) Isoenzymes:

Different forms of the same enzyme helps in the regulation of activity *For details please refer previous section (13.9).*

(vi) Cofactor availability:

Significant alteration of enzyme activity and hence the metabolic pathway can be achieved if the concentration of the cofactor is modulated. For example, variation in the concentration of carnitine, a cofactor, can regulate fatty acid oxidation without affecting other metabolic processes. Although not a physiological regulation, many vitamin-deficient diseases can be explained on the basis of lack of specific cofactors.

13.10 MECHANISM OF ENZYME ACTION

The enzyme catalysed reactions are essential for life. Many reactions in the biological system will not occur at required rates in the absence of enzymes.

It is important to remember that enzyme is just a catalyst and it does not alter the equilibrium of the reaction. In other words, an enzyme will accelerate both forward and backward reactions by the same factor. Thus, enzymes accelerate the achievement of equilibrium but do not shift the position of equilibrium.

There is a huge *energy barrier* between substrate (S) and product (P). This energy barrier is nothing but the energy required for the alignment of reaction groups, bond rearrangements and various transformational changes. For a reaction to occur, the molecule must overcome this barrier and thus must be raised to a higher energy level i.e. *transition state*. The difference between the energy level of the ground state and transition state is called as *activation energy* (See Fig. 13.13).

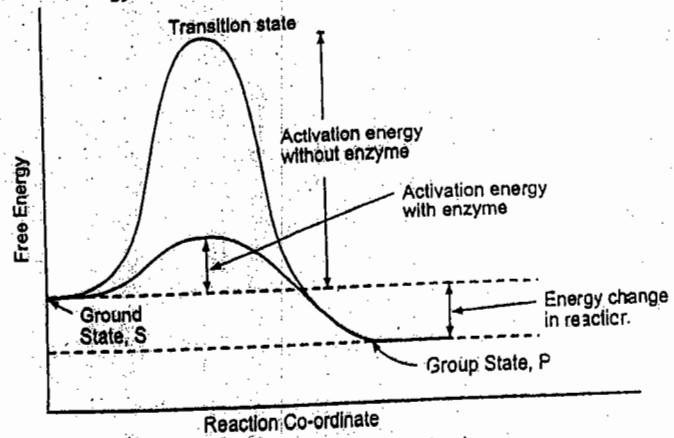


Fig. 13.13: Effect of enzyme on activation energy

Activation energy provides information about rate of reaction: higher the activation energy, slower is the rate. Enzyme (in general sense a catalyst) lowers the activation energy and thus increases the rate of reaction.

Thus, enzymes do not affect the equilibrium constant of the reaction but increase the rate of reaction.

13.11.1 Enzyme-Substrate Complex

Formation of enzyme-substrate complex (ES) is the prequisite for enzyme catalysis. The substrates bind to a specific region of the enzyme known as *active site.*

Following are the theories of ES formation.

Lock and Key Model

This is also known as the *Fischer's template theory* and was proposed in 1809 by Emil Fischer, a German biochemist.

According to Lock and Key model, a substrate must have a matching shape as that of active site of enzyme. Specificity of substrate-enzyme binding depends on the well defined arrangement of atoms in an active site (Fig. 13.14). *It is assumed that the conformation of enzyme is rigid.*

Thus, substrate fits into an active site just the way key fits into a proper lock.

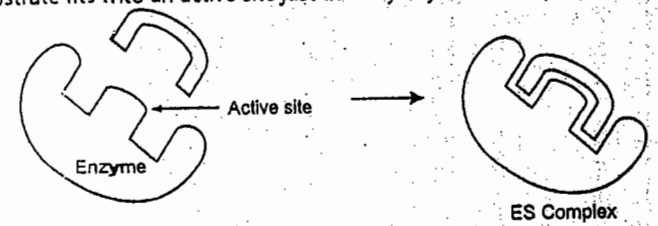


Fig. 13.14: Lock and Key model

Induced Fit Theory

Also known as *Koshland's model*, and was proposed by Daniel E. Koshland, Jr. in 1958.

According to this model, active site of enzymes takes the shape that is complementary to the substrate i.e. *active site is not a rigid structure.* This process of dynamic recognition is called as *induced fit.* (Fig. 13.15). This model suggests that the binding of substrate to an enzyme induces a localised structural change in the active site and creates the correct three dimensional arrangement of amino acids in the active site of the enzyme. Thus, structure of the substrate is not only important for binding but also for generating the *catalytic conformation* of the enzyme.

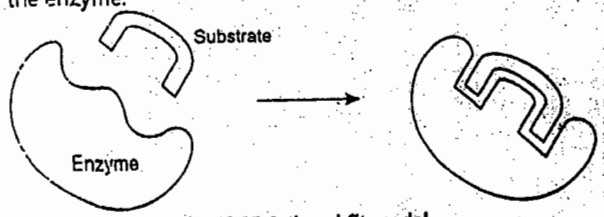


Fig. 13.15: Induced fit model

Induced fit model is a more realistic model of ES complex formation.

13.12 CATALYTIC MECHANISMS

The catalytic mechanisms used by enzymes can be classified as:

(i) Acid-base catalysis
(ii) Covalent catalysis
(iii) Metal ion catalysis
(iv) Electrostatic catalysis
(v) Proximity and orientation effect
(vi) Preferential binding of transition state complex.

(i) Acid-base catalysis:

Acid-base plays an important role in the enzyme catalysis. Acid catalysis can be defined as 'a process of partial proton transfer from an acid which lowers the free energy of the transition state'. A reaction is said to be base catalysed when reaction rate is increased by partial proton abstraction by a base.

Many amino acid residues have pK's near the physiological pH and hence act as acid and/or base catalyst e.g. Asp, Glu, His, Cys, Tyr and Lys. Thus, protonated form of histidine acts as an acid.

RNaseA (a digestive enzyme secreted by pancreas), which hydrolyses RNA to its component nucleotides, is an example of enzymatically mediated acid-base catalysis.

(ii) Covalent catalysis:

In covalent catalysis there is a transient formation of a catalyst-substrate covalent bond. This is also called as *nucleophilic catalysis*, because there is an interaction between nucleophilic group (usually on enzyme) and an electrophilic group (usually on substrate).

Decarboxylation of acetoacetate catalysed by primary amines is an example of covalent catalysis.

Many co-enzymes viz. thiamine pyrophosphate, pyridoxal phosphate also function along with respective apoenzymes as covalent catalysts.

(iii) Metal ion catalysis:

Enzymes which require the presence of metal ions for the catalytic activity can be classified into two groups:

Metalloenzymes: These enzymes contain tightly bound metal ions as cofactors.
e.g. Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} or Mn^{2+}.

Metal-activated enzymes: These enzymes contain loosely bound metal ions.
e.g. Na^{+}, K^{+}, Mg^{2+} or Ca^{2+}.

Metal ions may help catalytic process either by:

(a) binding to substrate for proper orientation or
(b) change its own oxidation state thereby carrying out oxidation-reduction reaction or
(c) electrostatically stabilize the charges.

Carbonic anhydrase contains an essential Zn^{2+} ion and it is considered to play an important role in the enzyme's catalytic mechanism.

(iv) Electrostatic catalysis:

Electrostatic catalysis resembles metal ion catalysis. In many enzymes, charge distribution helps to guide the polar substrate towards their respective binding sites. The charge distribution around the active site also helps in stabilizing the transition state.

(v) Proximity and orientation effect:

Proximity and orientation means that reactants must come together with the proper spatial relationship. Enzymes are known to bind with their substrates in proper orientation. Reaction is catalysed most readily when enzyme and substrates are in proper relative orientation. For example, in case of S_N^2 reaction, the incoming nucleophilic group attacks the target from the direction opposite to that of leaving group.

(vi) Preferential binding of transition state complex:

Many enzymes preferentially bind with the transition complex. This increases the concentration of transition state and thus increases the reaction rate. Hence, more tightly the enzyme binds with transition state, greater is the reaction rate. This is because of the stabilisation of the transition state. In fact, enzymes have a tendency to bind with transition state with higher affinity than substrates.

Transition state analogues, which are stable molecules and resemble transition state geometrically and electronically are inhibitors of the enzyme.

13.13 RIBOZYMES

Ribozymes are also known as *RNA enzymes* or catalytic RNA. Tom Cech (and his student) first observed that protein-free (enzyme-free) preparation still catalyzed the splicing of an intron from rRNA of *Tetrahymena* (a ciliated protozoan). Later on Thomas Czech and Sidney Altman won the Nobel prize for chemistry for their discoveries of catalytic RNA.

Now, it is well recognized that certain RNA molecules can act as enzyme-like catalysts. Ribozymes can carry out RNA splicing by transesterification (splicesome) and peptidyl transfer (in ribosomes). The mechanism of catalysis of the hepatitis delta virus ribozyme, includes general acid-base catalysis.

RNase P and self-splicing group I or intron ribozymes are the best characterized ribozymes. Intron ribozymes catalyse either hydrolysis of nucleotide or phosphate transfer. RNase P is a ribo-protein i.e. it contains both protein and RNA (M1RNA) RNase P functions in the processing of tRNA (formation of mature 5'-end). It has been demonstrated that under appropriate ionic conditions the RNA component alone could carry out the catalytic reaction and the protein component alone has no catalytic activity. Protein portion is needed either to stabilize the RNA or facilitate the function.

So far naturally occurring substrates for ribozymes have all been nucleic acids, however, synthetic substrates have been developed.

Ribozymes are considered to be the first catalyst (before the occurrence of proteins) during evolution.

13.14 ENZYMES IN DIAGNOSIS

Assay of various enzyme activities in the serum gives useful information about variety of disease conditions.

Alanine aminotransferase (ALT), also known as glutamate-pyruvate transaminase (GPT) and aspartate aminotransferase (AST), also known as glutamate-oxaloacetate transaminase (GOT) are valuable diagnostic markers of heart and liver damage. Damage to heart such as myocardial infarction will lead to leakage of these aminotransferases into the blood. Activity of these enzymes in the blood can be monitored by SGPT and SGOT tests (S for serum).

Other two important enzymes which are leaked in the blood during heart disease are creatinine kinase and lactate dehydrogenase.

Liver degeneration because of disease or toxicity (e.g. toxicity of carbon tetrachloride, chloroform or other solvents used in the chemical industry) or infection also causes leakage of GOT and GPT in the blood.

Measurement of many enzyme activities is useful in the diagnosis of cancers. For example, urine β-glucoronidase in cancers of urinary bladder or pancreas; serum acid phosphatase in prostate cancer etc.

13.15 ENZYMES OF PHARMACEUTICAL AND THERAPEUTIC IMPORTANCE

Enzymes have various pharmaceutical and clinical applications. The manufacture or processing of enzymes for use as drugs is a minor but important facet of modern pharmaceutical industry. Attempts to capitalize on the advantages of enzymes as drugs are now being made at virtually every pharmaceutical research center in the world. Enzyme as drug was first realized because of observation by Emmerich and colleagues in 1902 that an extracellular secretion of *Bacillus pyocyaneous* was capable of killing anthrax bacilli and of protecting mice from lethal innoculum of the anthrax bacterium.

1. **Streptokinase:**
   A non-enzyme protein is produced by some strains of *β-hemolytic streptococci* with a molecular mass of 47-kDa protein. It does not have any intrinsic enzymatic activity.
   It forms a 1:1 complex with intrinsic plasminogen present in the fibrin clot and converts it into active plasmin. This causes fibrinolysis and dissolution of clot. Streptokinase is mainly used as a thrombolytic agent. The typical dose is 1.5 million units over one hour.
2. **Urokinase:**
   Urokinase is obtained from cultured kidney cells. It is a serine protease and contains 411 amino acids. Thrombolytic activity of urokinase is less than streptokinase. The typical dose is 1.5 million units as IV injection followed by 1.5 million units over one hour.
3. **Hyaluronidase:**
   Hyaluronidase is prepared from mammalian testes. It depolymerizes hyaluronic acid, a mucopolysaccharide, an essential component of interstitial tissue spaces which determines the permeability of the tissues. Thus, hyaluronidase increases the tissue permeability and thus helps to spread the drug or substance co-administered. Some snake-venoms and bee-venoms contain hyaluronidase.

(Hyaluronidase is usually used with local anaesthetics to enhance the anaesthesia. In radiography, IV administration is avoided by administering intended substance intramuscularly along with hyaluronidase. Hyaluronidase is also used in ophthalmic surgical procedures).

4. **Collagenase:**
   It is obtained from *Cl. histolyticum*. It acts on denatured and undenatured collagen but not on healthy or newly formed collagen and other proteins. (It is mainly used for dermal ulcers and severe burns).
5. **Papain:**
   Papain is a mixture of proteolytic enzymes derived from the latex of unripe fruit of *Carica papaya*.
   It is used in clarification of beverages and as a meat tenderiser. It is also used in cheese manufacturing.
   Clinically, it is used as an anti-inflammatory agent. Papain acts by depolymerizing the soft fibrin deposits in the inflamed areas and also enhances drainage of fluids. It is also useful in reducing obstetrical inflammation and swelling and has been used in the edema following dental surgery.
6. **Bromelain:**
   Bromelain is a mixture of proteolytic enzymes derived from the stem of *Ananas comosus* (Pineapple plants).
   Clinically, it is used in the treatment of soft tissue inflammation and oedema. Like papain, bromelain also depolymerizes the soft fibrin deposits in the inflamed areas and also enhances drainage of fluids.
7. **Trypsin:**
   Trypsin is obtained from an extract of the ox pancreas. Trypsin has therapeutic application similar to streptokinase. An aerosol trypsin can also be used to liquify excessive bronchial secretions. Combination of trypsin and chymotrypsin (chymoral) has been successfully used in the treatment of postoperative hand trauma, atheletic injuries and sciatica.
8. **Pepsin:**
   Pepsin is a proteolytic enzyme. It is obtained from the fresh stomach of hog. It is used in patients with gastric achylia (a condition characterized by defective acid and pepsin secretion probably because of stomach carcinoma or pernicious anaemia).
9. **Seratopeptidase:**
   Seratopeptidase is a proteolytic enzyme derived from the bacteria belonging to genus *Serratia*, present in the gut of the silk worm. Given orally, it enters the systemic circulation in unchanged form and can penetrate into all the tissues. Unlike chymotrypsin, seratopeptidase, being a bacterial enzyme shows rarely the allergic reactions.

It has a number of therapeutic applications like:

(a) Resolution of inflammation,

(b) Sputum liquification and lowering viscosity of sputum,

(c) Enhancement of the antibiotic effect due to removal of inflammatory barrier and increasing antibiotic transfer to infected area.

10. Brinase:

Brinase, a plasmin like proteolytic enzyme found in extracts of mold *Aspergillus oryzae*, is capable of hydrolyzing fibrin and fibrinogen as well as casein and gelatin. Brinase has demonstrated its utility in patients on chronic hemodialysis with clotted arteriovenous cannulas by restoring vessel patency.

11. Deoxyribonuclease:

Deoxyribonuclease, an enzyme that degrades nucleic acid, has recently been investigated as a mucolytic agent as a treatment for chronic bronchitis. Although, effective in producing liquefaction of secretions, it offers no advantage over standard mucolytic agents.

12. Lysozyme:

The enzyme lysozyme hydrolyzes the chitins and mucopeptides of bacterial cell walls. Thus, it has been used as an antibacterial agent usually in combination with standard antibiotics.

13. Lysostaphin:

Lysostaphin has lytic effects on coagulase-positive *Staphylococcus aureus* and is presently under extensive studies. Lysostaphin is a protease and it lyses susceptible cells in a highly efficient manner possibly by peptidase like cleavage of the glycoprotein of the bacterial wall.

Currently lysostaphin is only used topically for the reduction of staphylococcal carrier rate in the nose and throat and has been found to be effective and non-toxic.

In future, lysostaphin may prove to be useful in conditions like endocarditis. Also, as it has been found to be effective against methicillin resistant strains of *S. aureus*, lysostaphin might prove useful in the treatment of methicillin resistant staphylococcal infections, which have started appearing in Europe as well as in the United States.

14. Oncolytic enzymes:

The oncolytic enzymes fall into two major classes:

- Those that degrade small molecules for which neoplastic tissues have a requirement.
- Those that degrade macromolecules such as membrane polysaccharides, structural and functional proteins or nucleic acid.

At present, tumor-cell specificity is observed only in the former category.

L-asparaginase is a typical example of oncolytic enzyme. Certain cancerous cells are deficient in their ability to synthesize the non-essential amino acid L-asparagine and hence are dependent on asparagine of host for multiplication. Asparaginase (given parenterally) reduces asparagine levels in the host and thus interferes with the viability of tumor cells. Unfortunately, only acute lymphocytic leukaemia ordinarily responds to chemotherapy with this enzyme.

Other amino acid degrading enzymes with oncolytic activity in experimental tumors include: L-methionase (effectively dismantles L-methionine to yield methanethiol, ammonia and a keto butyric acid), L-phenylalanine ammonialyase (deaminates both L-phenylalanine and L-tyrosine yielding trans-cinnamic and trans-coumaric acids, respectively), L-arginase, L-tyrosinase, L-serine dehydratase, L-threonine deaminase and indoyl-3-alkane hydroxylase which degrades L-tryptophan.

Diphtheria toxin, a different type of oncolytic enzyme (it is still in the experimental stage) catalyzes the transfer of adenosine diphosphate ribose (ADP-ribose) moiety of nicotinamide adenine dinucleotide.

Among the oncolytic enzymes that degrade macromolecules, neuraminidase and ribonuclease are the most prominent examples. Although, currently this approach has been studied mainly in experimental trials.

Neuraminidase removes sialic acid residues from the surface of cancer cells and thus altering the immunogenicity and hence rendering the cancer cells sensitive to immune response.

Ribonucleases has shown activity against experimental tumors in mice. But the major problem is to transport these molecules into the cytoplasm, as their substrate (RNA) is in cytoplasm.

The carboxypeptidases have also been investigated as oncolytic enzymes. These enzymes cleave the carboxy-terminal residue of many peptides and are capable of hydrolyzing the L-glutamyl moiety of folic acid. Thus, they produce a state of folic acid deficiency which is deleterious to the tumor cells.

13.16 RESTRICTION ENZYMES

Restriction enzymes are DNA-cutting enzymes. These enzymes are found in bacteria, and are harvested from them for use in molecular biology and biotechnology. These enzymes cut within the molecule and hence are also called as restriction endonucleases.

H.O. Smith, K.W. Wilcox and T.J. Kelley, working at Johns Hopkins University in 1968, isolated and characterized the first restriction nuclease whose functioning depended on a specific DNA nucleotide sequence. This enzyme, called HindII, was isolated from *Haemophilus influenzae*, and it always cuts DNA molecules at a particular point within a specific sequence of six base pairs. This sequence is:

5' G T (pyrimidine: T or C) (purine: A or G) A C 3'

3' C A (purine: A or G) (pyrimidine: T or C) T G 5'

They found that the HindII enzyme always cut directly in the center of this sequence.

Now more than 900 restriction enzymes have been isolated from over 230 strains of bacteria since the initial discovery of HindII. The name of these restriction enzymes reflects their origin. The first letter of the name comes from the genus and the second two letters come from the species of the prokaryotic cell from which they were isolated. For example, EcoRI comes from *Escherichia coli* RY 13 bacteria. Numbers following the name indicate the order in which the enzymes were isolated from single strains of bacteria. Few example, of restriction enzymes are as follows:

Enzyme	Organism from which enzyme was isolated	Target sequence (* Indicates the site of cutting)
BamH1	*Bacillus amyloliquefacieris*	5' G * G A T C C 3'
EcoRI	*Escherichia coli RY 13*	5' G * A A T T C 3'
HindIII	*Haemophilus influenzae Rd*	5' A * A G C T T 3'
Kpn I	*Klebsiella pneumoniae*	5' G G T A C * C 3'
Pst I	*Providencia stuartii*	5 C T G C A * G 3'
Sma I	*Serratia marcescens*	5 C C C * G G G 3'

Restriction enzymes cut the DNA, which can then be ligated to other DNA molecule, thus forming *recombinant DNA*. Recombinant DNA is the foundation of biotechnology industry. This technology has immensely helped us to study genetics but at the same time has produced various proteins for the human therapy e.g. human insulin for diabetes, human factor VIII for hemophilia A etc.

QUESTIONS

1. Explain in detail classification of enzymes according to IUB and nomenclature.
2. Give an account of various models describing mechanism of enzyme action.
3. Write a note on the effect of substrate concentration on the activity of enzymes.
4. Enlist the different factors affecting activity of enzyme. Add a note on the role of individual factor.
5. Define inhibitor and inhibition. Describe in detail reversible inhibition.
6. Explain the irreversible inhibition with proper examples.
7. Give the salient features of allosteric inhibition.
8. Define isoenzymes, state the characteristics and significance of isoenzymes in biochemistry.
9. Explain in detail clinical significance of various isoenzymes.
10. Describe the importance of feedback regulation in biochemistry.

11. Define coenzymes. How do they differ from cofactors? Discuss the role of vitamins and non-vitamin co-enzymes in biochemical reactions.
12. Prepare a note on the mechanism of enzyme action.
13. Write short notes on:
    (a) Ribozymes
    (b) Catalytic mechanisms
    (c) Pharmaceutical importance of enzymes.
    (d) Restriction enzymes.

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